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Editor's Note

The Third ‘International Congress on Biological and Health Sciences’ was organized online and free of charge. We are very happy and proud that various health science-related fields attended the congress. By this event, the distinguished and respected scientists came together to exchange ideas, develop and implement new researches and joint projects. There were 28 invited speakers from 15 different countries and also more than 200 submissions were accepted. More than 50 countries contributed to the congress. We would like to thank all participants and supporters. Hope to see you at our next congress.

Best wishes from Turkey

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CONTENTS

PROCEEDINGS BOOK

Prevalence of Antibodies to Bovine alphaherpesvirus 1 in Sheep and Goats in Türkiye

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Abstract:
Bovine alphaherpesvirus 1 (BoHV-1) is a highly contagious viral disease of cattle that causes lifelong latent infection. BoHV-1 has significant economic impact on the cattle breeding industry due to respiratory and neurological problems, reduced milk production, abortions and fertility disorders. Sheep and goats are believed to be the potential hosts of virus for cattle. Molecular detection and serological evidence of BoHV-1 have been reported in Türkiye, however, little is known about the BoHV-1 situation in sheep and goats in Türkiye. Therefore, the purpose of this study was to survey the prevalence of BoHV-1 specific antibodies in sheep and goats. A total of 463 sera samples, 267 sera samples from sheep and 196 sera samples from goats were collected during the months of March 2018 and September 2019. Sera samples were tested for BoHV-1 antibodies using an enzyme linked immunosorbent assay (ELISA) kit. The overall seroprevalence of BoHV-1 in small ruminants was 3.0% (95% CI 1.5 - 4.6) with higher incidence in sheep (3.4%, 95% CI: 1.2 - 5.5) than in goats (2.6%, 95% CI: 0.3 - 4.8). No statistically significant difference in seroprevalence was observed between sheep and goats ($p = 0.78$). Animals older than 2 years old (n = 11/231) had significantly higher BoHV-1 seropositivity than in animals younger than 2 years old (n = 3/232) ($p = 0.03$). However, there was no significant difference between male (n= 5/135) and female (n = 9/328) animals ($p = 0.56$). The results of this study indicate that BoHV-1 infection is not common among the studied sheep and goat population. Future epidemiological studies are needed to better understand role of small ruminants in the transmission of the virus.

Keywords: bovine alphaherpesvirus 1, seroprevalence, sheep, goats, Türkiye

1. Introduction

Bovine alphaherpesvirus 1 (BoHV-1) is one of the important viral agents of cattle. Although subclinical form of the BoHV-1 infections is common, various clinical manifestations can be seen in infected cattle such as infectious bovine rhinotracheitis, infectious pustular vulvovaginitis and abortion (Muylkens et al., 2007). The severity of the disease can change depend on the age of the animal, virulence of the BoHV-1 strain, immun status of the host and presence of secondary bacterial infection (Kaashoek et al., 1996; Muylkens et al., 2007). After primary infection, a life-long latent infection is established in nervous sensory ganglia (Muylkens et al., 2007).

BoHV-1 is classified within the genus *Varicellovirus* in the subfamily *Alphaherpesvirinae* of family *Herpesviridae* (ICTV, 2021). The causative agent, BoHV-1, has

double-stranded linear DNA and an icosahedral capsid of about 100 nm (Maclachlan and Dubovi, 2010). BoHV-1 strains are classified into 3 subtypes: 1.1, 1.2a, and 1.2b (Muylkens et al., 2007). Strains belonged to BoHV-1.1subtype are generally responsible for infections in respiratory tract, such as respiratory disease called infectious bovine rhinotracheitis (IBR), whereas strains belonged to BoHV-1.2a subtype cause genital infections such as infectious pustular vulvovaginitis and infectious pustular balanoposthitis. BoHV-1.2b strains can cause encephalitis (Biswas et al., 2013).

Direct contact between susceptible and infected animals is the main mode of BoHV-1 transmission (Muylkens et al., 2007). Furthermore, aerosol transmission can occur over short distances. It has been reported that latently infected animals is considered to be the main source of transmission between-herd spread (Mars et al., 2000; Brock et al., 2020).

Although there are differences in prevalence and incidence, BoHV-1 infection has worldwide distribution (Ackermann and Engels, 2006). After eradication programmes Austria, Denmark, Finland, Germany, Jersey (United Kingdom), Norway, Sweden, Switzerland and Valle d'Aosta and the Province of Bolzano (Italy) are considered free of infection (Commission European, 2017).

BoHV-1 infection is endemic in Türkiye (Yesilbag and Bilge-Dagalp, 2006; Avcı et al., 2013; Yazici et al., 2021). However, there are limited data on BoHV-1 epidemiology in sheep and goats in Türkiye. Therefore, aim of this study was to determine the seroprevalence of BoHV-1 in small ruminants.

2. Materials and Methods

Sample collection

Blood samples were collected from epidemiologically independent flocks in the Afyonkarahisar Province (n = 15) in the Aegean region and Aksaray (n = 18) and Konya Province (n = 20) in the Central Anatolian region of Türkiye during the months of March 2018 and September 2019. The sample size was calculated based on expected prevalence of 50% with 95% confidence level and a precision of 6% for sheep and 7% for goats. Four to five animals were randomly selected in each selected flocks. In this study, a total of 463 blood samples, 267 blood samples from sheep and 196 blood samples from goats were collected (Table 1). Collected blood samples were centrifuged at 3000×g at 4 °C for 10 min., and then sera samples stored at -20°C until serological analysis.

Detection of BoHV-1 specific antibodies

Sera samples were heated to 56°C for 30 minutes in order to inactivate heat-labile virus inhibitors. A commercial enzyme linked immunosorbent assay kit (IDEXX, Switzerland) was used to detect BoHV-1 specific antibodies. ELISA results were assessed using ELISA reader (Epoch, BIO-TEK, USA) and were evaluated taking into account manufacturer's instructions.

Statistical analysis

The obtained data were analyses using SPSS (version21, SPSS Inc., Chicago, USA). The association between seropositivity and species, age and sex were calculated by Chi-square test. A p-value of ≤ 0.05 was considered statistically significant. The BoHV-1 seroprevalence and 95% confidence intervals were calculated by using Bayesian approach of the beta distribution.

3. Results

The overall seroprevalence of BoHV-1 in small ruminants was 3.0% (95% CI 1.5 - 4.6). BoHV-1 specific antibodies were detected in 9 sheep (9/267, 3.4%, 95% CI: 1.2 - 5.5) whereas 5 out of the 196 goats were found positive. No statistically significant difference in seroprevalence was observed between sheep and goats (p = 0.78). Animals older than 2 years old (n = 11/231) had significantly higher BoHV-1 seropositivity than in animals younger than 2 years old (n = 3/232) (p = 0.03). However, there was no significant difference between male (n= 5/135) and female (n = 9/328) animals (p = 0.56).

Table 1. Results of the ELISA

Province	**No. examined Sheep**	**Positive, (%)**	**No. examined Goats**	**Positive, (%)**
Afyonkarahisar	**82**	**2 (2.4)**	**59**	**2 (3.4)**
Aksaray	**79**	**1 (1.3)**	**54**	**-**
Konya	**106**	**6 (5.7)**	**83**	**3 (3.6)**
Total	**267**	**9 (3.4)**	**196**	**5 (2.6)**

4. Discussion

BoHV-1 causes serious economic losses in livestock due to respiratory and neurological problems, reduced milk production, abortions and fertility disorders (Nandi et al., 2009; Biswas et al., 2013). BoHV-1 can become latent following a primary infection with a field isolate, and it can be reactivated and excreted after transport, treatment with glucocorticoids and parturition (Pastoret et al., 1986; Wyler R et al., 1989). Latently infected animals have important role in the transmission of the virus between herds and flocks (Bradshaw and Edwards, 1996; Lemaire et al., 2000). This situation makes it harder to control the disease. To control the disease

eradication programmes have been applied in different countries. In IBR control programs, serosurvey studies have important impact to determine infected animals and design effective control strategies against the disease.

Molecular detection and serological evidence of BoHV-1 have been reported in Türkiye, and BoHV-1 infection is endemic in Türkiye (Yesilbag and Bilge-Dagalp, 2006; Avcı et al., 2013; Dagalp et al., 2020; Yazici et al., 2021). However, a control program against to BoHV-1 infection has not been applied in Türkiye. It has been reported that sheep and goats contribute to the transmission of the virus (Gür et al., 2019; Pourmahdi Borujeni et al., 2020). However, little is known about the BoHV-1 situation in sheep and goats in Türkiye. Therefore, in this study, seroprevalence of BoHV-1 in small ruminants was investigated.

In this study, seropositivity of BoHV-1 in sheep was 3.4%. This rate is higher than previous reported rates from different regions of Türkiye. Kadiroglu et al. (2020) reported that BoHV-1 seropositivity in sheep was 1.6% in Diyarbakır Province whereas Yilmaz and Coskun (2016) reported that seropositivity was 2.27% in sheep in Kars Province. Furthermore, Gür et al. (2019) found that 0.09% of the 1053 sheep from Afyonkarahisar Province were seropositive. Albayrak et al (2007) reported that prevalence of BoHV-1 specific antibodies in sheep was 1.74% in Northern Turkey. However, a study which was carried out in Marmara region of Türkiye found that BoHV-1 seropositivity was 9.8% in sheep (Yesilbag and Gungor 2009). The differences in the seropositivity of BoHV-1 between various provinces and regions may be related with the number of sampled animals, the number of sampled herds, the age of animals and the difference in the flock management.

In the current study, antibodies to BoHV-1 were detected in 5 goats out of the 196 goats (2.6%, 95% CI: 0.3 - 4.8). This rate is lower than previous reported rates from different regions of Türkiye. Yesilbag and Gungor (2009) found that BoHV-1 seropositivity was 38.2% in goats in Marmara region of Türkiye. Also, Gür et al. (2019) reported that 20.9% of the 277 goats from Afyonkarahisar Province were seropositive. Kadiroglu et al. (2020) reported that BoHV-1 seropositivity in goats was 50% in the Diyarbakır Province. Yazici et al. (2021) found that 5.2% of the 269 goats from the Black Sea region of northern Türkiye were seropositive. The differences in the seropositivity of BoHV-1 between various regions may be related with the age of sampled animals, the sampling strategy and the number of sampled animals, the analysis methods and the difference in the flock management.

In this study, seropositivity of BoHV-1 in sheep and goats were 3.4% and 2.6% respectively. This difference in prevalence of antibodies to BoHV-1 in sheep and goats was

statistically non-significant (p = 0.78). However, Gür et al. (2019) reported that goats had higher BoHV-1 seropositivity than sheep and this difference in prevalence of antibodies to BoHV-1 was significant. Possible explanations for this result may be the number of sampled animals and sampled flocks, and the difference in the flock management.

In the present study, age of the animals was significantly associated with BoHV-1 seropositivity. BoHV-1 seropositivity was significantly higher in animals older than 2 years old. Similarly, Mahmoud and Ahmed (2009) reported that isolation rates were higher in animals 2 years old. Also, Adeli et al. (2017) found that there was a significant relationship between age and infection. However, Danial et al. (2022) reported that there was no association between age and BoHV-1 seropositivity. Possible explanations for the differences in BoHV-1 seropositivity in different studies are the sampling strategy, the age of the sampled animals, and management conditions.

In this study, male animals had higher BoHV-1 seropositivity (3.4%) than female animals (2.7%). This difference in prevalence of antibodies to BoHV-1 in male and female animals was statistically non-significant (p = 0.77). In agreement with this study, Adeli et al. (2017) and Pourmahdi Borujeni at al. (2020) reported that there was no significant relationship between sex and infection. However, Danial et al. (2022) and reported that male animals had higher odds of infection with BoHV-1. Possible explanations for this result may be the number of sampled animals and sampled flocks, and the difference in the flock management. Furthermore, the genetic and hormonal differences between the two sexes may be related with the differences in the seropositivity of BoHV-1.

5. Conclusion

The results of this study indicate that that BoHV-1 infection is not common in the studied area. However, this study does not reflect situation of BoHV-1 infection in the whole country. Therefore, large-scale epidemiology studies are essential to determine the situation of BoHV-1 infection in small ruminants in Türkiye.

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Obtaining the Extracts from Schisandra Chinensis Fruits in Different Geographical Locations from China and Poland

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Abstract:
Schisandra chinensis is a plant whose fruits have high potential and beneficial health effects. Research has demonstrated that the pharmacological effects of *Schisandra chinensis* are attributed to its bioactive components. *Schisandra chinensis* and *Schisandra sphenanthera* both belongs to *Schisandra* genus, but the bioactive components of the two species are quite different. However, research on the bioactive components of *Schisandra* genus are still insufficient and deserve further studies, especially the factor of the geographical location of the growth on the bioactive components of *Schisandra chinensis*. Polysaccharides are the main kind of bioactive components and also are important biomacromolecules for life's activities, whose structure is closely related to biological activity. The objective of this work was to prepare *Schisandra chinensis* extracts (SCE) from fruits growing in different geographical locations in China and Poland and compare their chemical constituents and contents of the main bioactive components. The fruit of *Schisandra chinensis* were extracted with 1:10 (liquid to solid ratio) distilled water at 90 ℃ for 5 h for three times. The SCE was dried using freeze vacuum drying. Ultrasound-assisted extraction of polysaccharide from *Schisandra chinensis* using water extraction and alcohol precipitation method. The active constituents were compared and analyzed with UV detection and acid-base titration. The extraction yield of SCE in different provinces from China were 10.17%, 9.04%, 8.42%, respectively. The extraction yield of SCE from Poland was 6.30%. Results indicated the polysaccharides were the highest contents of bioactive components. The extraction yield of *Schisandra chinensis* polysaccharides was 13.12%. The chemical constituents will be tested using HPLC–UV in further experiments. These results point out the slightly differences in bioactive components between 4 kinds of *Schisandra chinensis*. All our preliminary results throw light on the relationship of bioactive components of SCE with geographical locations in which *Schisandra chinensis* grows.

Keywords: *Schisandra chinensis* extract, polysaccharides, bioactive components, extraction.

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1. Introduction

Schisandra chinensis belongs to the family *Schisandraceae* and grows in Asian countries such as China, Korea, Japan, and the far-east of Russia (Chun et al., 2014; Panossian and Wikman, 2008). *Schisandra chinensis* is a plant whose fruits have high potential and beneficial health

effects. The fruits of *Schisandra chinensis* have been used to treat many diseases in Traditional Chinese Medicine (Chen et al., 2019). They have been used in the treatment of diseases of the gastrointestinal (GI) tract, respiratory failure, cardiovascular diseases, body fatigue and weakness, excessive sweating, and insomnia (Panossian and Wikman, 2008). They were also reported to reduce hunger, delay aging, increase vitality, and improve mental health (Szopa et al., 2017). To date, many papers have shown that *Schisandra chinensis* possesses many pharmacological activities, such as antioxidant, antitumor, and hepatoprotective activities (Ranouille et al., 2018; Yang et al., 2018; Yuan et al., 2018; Zhang et al., 2013). Several reports have suggested that the major bioactive constituents of *Schisandra chinensis* are the lignans *schisandrin* A, *schisandrin* B, gomisin A, gomisin N, essential oils, and polysaccharides(Ekiert et al., 2013; Mocan et al., 2014; Szopa et al., 2017; Szopa and Ekiert, 2016).

Interestingly, *Schisandra chinensis* grown in different geographical locations become two species of plants with having different chemical composition, like *S. chinensis* (Beiwuweizi) and *S. sphenanthera* (Nanwuweizi) which grown in different regions of China (Opletal et al., 2004). There are many analytical tests have also identified significant differences in the chemical composition of the fruit of these two species(Lu and Chen, 2009). *S. chinensis* and *S. sphenanthera* both belongs to *Schisandra* genus, but the bioactive components of the two species are quite different. Little studies in the field of comparing the active chemical compositions of *Schisandra chinensis* from different regions, most of them focused on the comparative studies between *S. chinensis* (Beiwuweizi) and *S. sphenanthera* (Nanwuweizi) which are originated China. However, research on the bioactive components of *Schisandra* genus are still insufficient and deserve further studies, especially the factor of the geographical location of the growth on the bioactive components of *S. chinensis*.

Among the most studies in the field of pharmacological activity of *Schisandra chinensis* had only focused on *Schisandra chinensis* lignans, there has been little discussion about the other *Schisandra chinensis* extracts(SCE), for instance, *Schisandra chinensis* polysaccharide(SPO)(Chen et al., 2017; Ci et al., 2010; Zhou et al., 2014). Polysaccharides are a kind of component that can be widely found in different plant species, and the structures comprise a variety of monosaccharides linked by glycoside bonds. Polysaccharides are important biomacromolecules for life's activities, whose structure is closely related to biological activity. Studies indicate that SPO show multiple functional activities, mainly including immunity regulation and antitumor, antibacterial effects (Liu et al., 2014; Xu et al., 2012; Zhao

et al., 2013). Most studies mainly focused on the pharmacological functions of SPO but the exact chemical structure features are still not well described(Li et al., 2018; Liu et al., 2014). More attention should be paid to the structure characteristics of SPO. And the relationship between chemical structure and biological activity of polysaccharide is worthy of further investigation. Many biological activities of SPO have been found, but some of the interior mechanisms are still unclear.

The objective of this work was to prepare *Schisandra chinensis* extracts (SCE) from fruits growing in different geographical locations in China and Poland and compare their chemical constituents and contents of the main bioactive components.

2. Materials and Methods

Material origin

S. chinensis fruits were collected from 3 different locations in China and from one location in Poland - Botanical Garden of Lodz. 4 kinds of *Schisandra chinensis* were collected from different place located in the Liaoning(China), Shaanxi(China), Yunnan(China) and Lodz(Poland), respectively.

Preparation of SCE

The fresh fruits of S. chinensis were washed and dried at 40 ℃ for 4 days to get the dried fruits. The dried fruits were crushed and sieved by a 40 mesh (0.425mm) sieve. Then 10 g of S. chinensis powder and 100 mL of sterile water were added into a round-bottomed flask, according to the hot-water extraction method(Wang et al., 2020). The extraction condition was as follows: the ratio of material to water of 1:10, temperature at 90 ℃ and time for 5 h, 3 times. The experimental device is water bath. After extracting and centrifuging, the supernatant were collected for concentrating and drying to obtain powder. The S. chinensis extracts (SCE) were collected and stored at -20 ℃. 3 kinds of SCE were extracted in College of Biological Science and Biotechnology, Beijing Forestry University and 1 kind of SCE was extracted in Department of Environmental Biotechnology, Faculty of Biotechnology and Food Sciences, Lodz University of Technology(Figure 1).

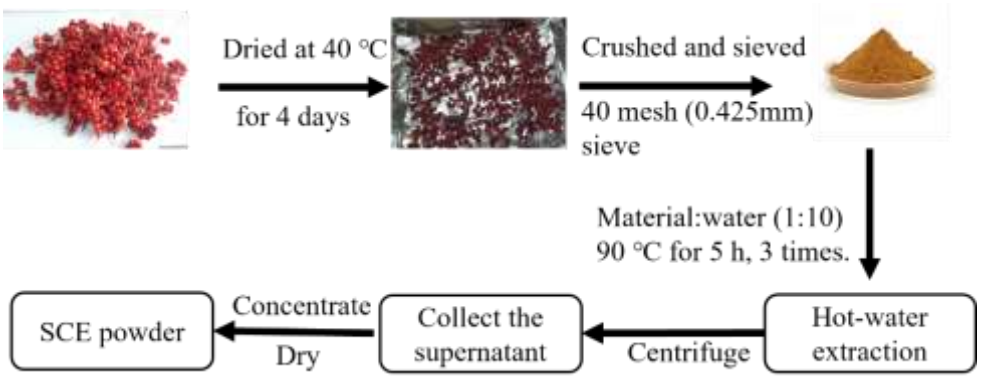

Figure 1. Flow chart of preparation of SCE

Preparation of SPO (polysaccharide)

10 g of SCE 3 powder was weighted and dissolved in 100 mL sterile water. It was pretreated using an ultrasonic cleaner at 70℃ for 40 min. The insoluble residue was removed by centrifugation (5000 rpm, 15 min). The crude polysaccharide was precipitated by adding fourfold volume of anhydrous ethanol and kept overnight. The precipitate was collected after centrifugation (5000 rpm, 15 min). The crude polysaccharide was dissolved in 100 mL of water. The proteins were removed by extraction with sevage method (water solution: chloroform: n-butanol = 25:5:1). The mixture was shake for 30 min. Then it was be allowed to stand and centrifuged to collect the water phase. The solution was centrifuged and n-butanol-chloroform phase was removed and the impurities (proteins) were removed. The water phase was collected. The process was repeated for 5 times. After adding the fourfold volume of anhydrous ethanol, it was kept overnight, centrifuged and the precipitate was dried to obtain the polysaccharide (Su et al., 2020) . The polysaccharide of SCE 3 was prepared Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Lodz(Figure 2).

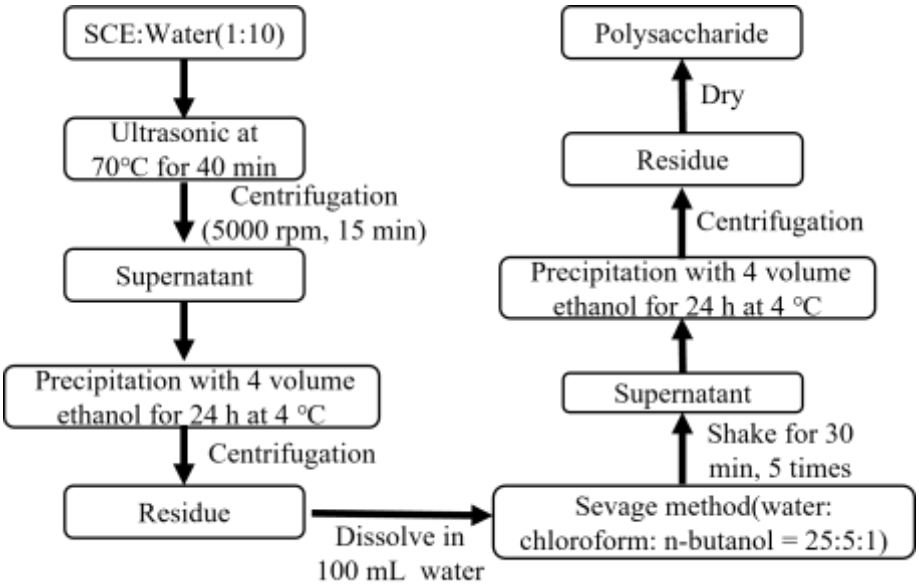

Figure 2. Flow chart of preparation of SPO

Comparing the composition of SCE

The active constituents were compared and analyzed with UV detection and acid-base titration.

3. Results and Discussion

Table 1 shows the extraction yield of SCE from different geographic origin in China and Poland. 1 kg of *S. chinensis* extracts were prepared from 3 different places in China, respectively. 63 g of *S. chinensis* extracts were prepared from Poland. The results indicated that the highest extraction yield of SCE by hot-water extraction could reach 10.17% (SCE1). The lowest extraction yield was SCE-PL, with 6.30%.

Table 1. The extraction yield of SCE from different geographic origin in China and Poland.

Extracts	**SCE/ *Schisandra* (kg)**	**Extraction yield (100%)**
SCE 1	**1.00 kg/9.83 kg**	**10.17%**
SCE 2	**1.00 kg/11.06 kg**	**9.04%**
SCE 3	**1.00 kg/11.88 kg**	**8.42%**
SCE PL	**0.063 kg/1.00 kg**	**6.30%**

S. chinensis and *S. sphenanthera* both belongs to *Schisandra* genus, but the bioactive components of the two species are quite different. As shown in Table 2, comparing the active constituent contents of SCE1-3 from different geographic locations in China with UV detection and acid-base titration. The following compounds were detected: lignans, polysaccharides, organic acids, flavones, vitamins, anthocyanins, polyphenols, and alkaloids. The amount of polysaccharide in each extract was at about 40-42%, and this compounds dominated. These results point out the slightly differences in bioactive components between 3 kinds of *S. chinensis*.

Table 2. Compositional Analysis of SCE 1-3 from different geographic locations in China.

Index	Detection method	Percentage composition(100%)		
		SCE 1	SCE 2	SCE 3
Lignans	**UV detection**	**3.2%**	**2.4%**	**2.1%**
Polysaccharides	**UV detection**	**40.9%**	**41.7%**	**41.9%**
Organic acid	**Acid-base Titration**	**10.8%**	**8.9%**	**8.7%**
flavone	**UV detection**	**9.1%**	**9.6%**	**9.3%**
Vitamin	**UV detection**	**5.3%**	**5.3%**	**5.5%**
Anthocyanin	**UV detection**	**8.4%**	**10.6%**	**10.2%**
Polyphenol	**UV detection**	**8.2%**	**7.3%**	**7.1%**
Alkaloid	**UV detection**	**3.2%**	**3.8%**	**4.8%**

As shown in Table 3, 27.54 g of polysaccharide from SCE 3 using water extraction and alcohol precipitation method. The extraction yield of *S. chinensis* polysaccharides was 13.12%.

Table 3. The extraction yield of S. chinensis polysaccharides from SCE 3 .

Extracts	SCP/ SCE3 (g)	Extraction yield (100%)
SPO	**27.54 g/210 g**	**13.12%**

The studies indicated that there was bioactive component of polysaccharide in *S. chinensis* from different places, but the content was varying because different regions varied from geographic source, harvest and storage. These studies match those observed in our results.

4. Conclusion

These results point out the differences in bioactive components between 4 kinds of *S. chinensis* from different geographic origin in China and Poland. All our preliminary results throw light

on the relationship of bioactive components of SCE with geographical locations in which *Schisandra chinensis* grows. The chemical constituents will be tested using HPLC–UV in further experiments.

These species showed an interesting chemical composition as well as obvious biological activity and will provide an important reference for the development and utilization of *Schisandra* resources in China and Poland.

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Standards of Honey Quality in Serbian Legislation

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Abstract:
According to the European Union Legislation and the *Codex Alimentarius*, honey is defined as natural sweet substance produced by honey bees from the plants nectar or from secretions of plants living parts or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honeycomb to ripen and mature. The quality of honey and bee products depends on its geographical and botanical origin, with high aspect of environment condition. Active components in plants depend on various factors and climatic conditions in different geographical locations, botanical origin etc. *Codex Alimentarius* presented that chemical characteristic and element concentration of honey must meet the criteria of honey quality, according to national and international legislation. Also, in some counties issued national legislation, decisions and guidelines which correlate with European and International standards. In Serbia honey and other honey bee products must meet criteria according to Official Gazette RS: Rulebook on quality of honey and other bee products, No. 101/2015. Official Gazette RS defined the physicochemical parameters of natural honeys, such as moisture, reducing sugars, sucrose, hydroxymethylfurfural (HMF), free acidity, diastase activity, water-insoluble content and electrical conductivity, and constitute the quality indicators which characterize individual honey varieties. In this paper, we discuss the rules governing regulation of honey in national provisions of different countries taking into account suggestion of the EU.

Keywords: honey; legislation, Codex, EU standards, quality.

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1. Introduction

Codex Alimentarius (2001) define honey as *natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature.* Different studies show that honey have a high nutritional and biological effects (Oryan et al., 2016; Tsavea et al., 2022; Ávila et al., 2022; Graikou et al., 2022). The honey is an excellent source of energy, 100 g honey supplies about 306 kcal. Similar, 20 g of

honey is the usual quantity per serving or tablespoon that provides about 61.2 kcal, which represents more or less 3% of the energy necessary per day (Bogdanov et al., 2008). The main constituents of honey are the simple carbohydrates that are used for human body energy requirements after being rapidly absorbed into the blood without previous digestion (Ajibola et al., 2012).

According to chemical composition honey content a difference sugars, predominantly fructose and glucose as well as other substances such as organic acids, enzymes, vitamins, proteins, volatile compounds, several bioactive substances (phenols and flavonoids) and micro and macroelements. The mainly sugars are carbohydrates (60–85%), predominantly fructose and glucose (Machado De-Melo et al., 2018). The water content of honey is related to different factors such as the botanical and geographical origin of nectar, season of harvesting, intensity of nectar flux, degree of maturation, manipulation by beekeepers during period of harvest, as well as extraction, processing and storage conditions (Estupinan et al. 1998; Gonzalez, 2002; Ojeda de Rodrıguez et al 2004; Pontara et al., 2012; Sabatini, 2007; Sainz-Laın and Gomez-Ferreras, 2000; Ciric et al. 2018; Ćirić et al., 2020; Ćirić et al., 2020). The physicochemical characteristics and quality are defined in different national and EU regulation. In this review paper, we discuss the different regulation of honey worldwide, primary Serbian, compere to Council directive 2001/110 EU with revised Codex Alimentarius (2001). The mail objective is to show some differences in honey quality among this regulation in order to increase their efficiency in the different countries.

2. Official Gazette RS: Rulebook on quality of honey and other bee products, No. 101/2015

Official Gazette RS (2015) (https://www.pravno-informacionisistem.rs/SlGlasnikPortal/eli/rep/sgrs/ministarstva/pravilnik/2015/101/2) defined the physicochemical parameters of natural honeys, such as moisture, reducing sugars, sucrose, hydroxymethylfurfural (HMF), free acidity, diastase activity, water-insoluble content and electrical conductivity, and constitute the quality indicators which characterize individual honey varieties. According to origin, honey is classified as:

1) flower or nectar (obtained from the nectar of plants), namely:

(1) monofloral honey,

(2) multifloral/polyfloral honey;

2) honey house;

3) baker's honey.

Monofloral honey is a product produced by honey bees from the nectar of the flowers of honey plants of a certain species. Honey with the name of a specific type of honey plant should have the taste, smell and color characteristic of that plant, with the number of pollen grains of that type of plant predominating. Monofloral honey can be labeled according to a certain plant species, if it contains at least 45% of the pollen grains of that plant species in the insoluble part (Official Gazette RS, 101/2015). Table 1 shows the minimum percentage of pollen grains in the insoluble part for certain plant species. Similar, Greece has their national legislation

regarding to chemical composition of monofloral honey (AXS, 2004). Also, German (Leitatze, 2011), Croatia (Croatia Ministry of Agricultural, 2009), Italy and Turkey (Turkish Food Codex 2012) provide a chemical parameter of different type of monofloral honey. In the most case, chemical characteristics are correlate with pollen analyses (melissopalynology).

Table 1. The minimum percentage of pollen grains in the insoluble part for certain plant species (Official Gazette RS, 101/2015)

The name of the plant species	**Proportion of pollen grains in the insoluble part**
Castanea sativa Mill.	**85%**
Brassica napus L.	**60%**
Phacelia tanacetifolia Benth.	**60%**
Tilia spp.	**25% (10%)***
Robinia pseudoacacia L.	**20%**
Mentha spp.	**20%**
Calluna vulgaris L.	**20%**
Satureja montana L.	**20%**
Taraxacum officinale Weber	**20%**
Rosmarinus officinalis L.	**20%**
Salvia officinalis L.	**15% (10%)***
Arbutus unedo L.	**10%**
Citrus spp.	**10% (5%)***
Lavandula spp.	**10% (5%)***
Helianthus annuus L.	**40%**
Medicago sativa L.	**30%**

* with characteristic sensory properties of honey for a certain type of plant (smell, taste, color)

3. Composition criteria for honey

Physico-chemical parameters of honey according to Codex Alimentarius, Serbian legislation and Directive 2001/110 EU are indicated in Table 2. Some differences exist only for the baker honey. The main composition criteria defined moisture content (%), sum of fructose and glusoce (%), water- insoluble (%), electrical conductivity (mS/cm^{-1}), free acid (mEq/kg), diastase activity (Schade units)) and HMF. Water content is an important parameter in terms of honey shelf life. The maximum allowed in Serbian honey is 20%, according to Serbian Regulations (101/15 Serbian Regulation, 2015). Electrical conductivity indicates the presence of nonorganic and organic ions in honey. The diastasis activity corresponds to the activity of

the enzyme present in 1 g of honey, which can hydrolyze 0.01 g of starch in 1 h at 40 °C, expressed as the diastase number in Göthe (Schade units) (Ahmed et al. 2013; Bogdanov et al. 1997; Codex Alimentarius 2001). The current law stipulates a minimum value of 8.00 Schade units. However, honeys with naturally lower diastase activity tolerate a minimum of 3 Schade units if honeys have up to 15 mg kg^{-1} of HMF (da Silva et al., 2016; Council Directive 2001/110/EC 2002). Several factors have been reported to influence the levels of HMF, such as temperature and time of heating, storage conditions, pH, and floral source (Fallico et al., 2006). HMF levels in our Serbian honeys were below the maximum recommended by the European legislation (maximum of 40 mg/ kg).

Table 2. Compositional criteria of honey

Parameter/ Unit	Official Gazette RS, 101/2015	Codex 2001	Directive 2001/110 EU
Moisture- %	**In general: Not more than 20%**	**In general: Not more than 20%**	**In general: Not more than 20%**
Sum of fructose and glucose- %	**Blossom honey 60 ≤** **Honeydew honey, blends of honeydew honey with** **blossom honey 45% ≤**	**> 45**	**> 45**
Sucrose- %	**In general: Not more than 5%**	**In general: Not more than 5%**	**In general: Not more than 5%**
Water- insoluble- %	**In general: Not more than 0.1%**	**In general: Not more than 0.1%**	**In general: Not more than 0.1%**
Electrical conductivity mS/cm^{-1}	**In general: Not more than 0.8** **mS/cm**	**> 0.8**	**> 0.8**
Free acid- mEq/kg	**In general: Not more than 50 meq/kg**	**In general: Not more than 50 meq/kg**	**In general: Not more than 50 meq/kg**
Diastase activity	**In general: Not less than 8**	**In general: Not less than 8**	**In general: Not less than 8**
HMF- mg/kg^{-1}	**In general: Not more than 40 mg/kg**	**In general: Not more than 40 mg/kg**	**In general: Not more than 40 mg/kg**

The Serbian legislation (Official Gazette RS, 101/2015), Codex (2001) and Directive 2001/110 EU defined limits for moisture content no more than 20%, exceptions for *Calluna* honey and baker honey. The sum of fructose and glucose are defined to exceed 45 %. The Official Gazette RS (101/2015) required the sum of fructose and glucose content for blossom honey to exceed 60%. Codex, Serbian legislation (Official Gazette RS, 101/2015) and Directive 2001/110 EU required the sucrose content, in general no more than 5%. Persano and Piro (2004) found that honey from Eucalyptus generally content less than 4,2% sucrose. In the other hand, honey from dandelion may have more than 5% sucrose (Thrasyvoulou et al., 2018).

In general, water- insoluble content are limited no more than 0.1%, exception for pressed honey (< 0.5). According to Serbian legislation (Official Gazette RS, 101/2015), Codex (2001) and Directive 2001/110 EU the electrical conductivity of blossom must be lower than 0.8 mS/cm of EC, while electrical conductivity of honeydew honey and chestnut honey must be higher than 0.8 mS/cm. Exceptions are honeys from Arbutus, Banksia, Erica, Leptospermum, Melaleuca, Eucalyptus, Tilia and blends (Machado De-Melo et al., 2017). The Serbian legislation (Official Gazette RS, 101/2015), Codex (2001) and Directive 2001/110 EU defined limits for free acid no more than 50 meq/kg.

Diastase activity, so called diastase number, used for measuring enzyme is expressed as the amount of starch solution in ml of 1% that can be hydrolyzed by the enzyme in honey within one hour at 38-40 ºC (Türk, 2012). In the other hand HMF is very important quality criterion formed as a result of dehydration of hexose in honey in an acidic environment. Also, HMF and diastase activity used to detect adultered honey (Thrasyvoulou et al., 2018). According to Serbian legislation (Official Gazette RS, 101/2015) honey should not have HMF more than 40 mg/kg^{-1}. In the other hand, Czech Republic defined that honey should not have HMF more than 20 mg/kg^{-1}, as Slovakia. Some tropical country, as Kore and India defined that honey should not have HMF more than 80 mg/kg^{-1} (Bureau of Indian Standards, 2002).

5. Conclusion

In this review manuscript, we have indicated that there are differences in legislation and standards that regulate honey in different countries. Significant differences refer to the HMF and diastase activity content in honey.

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Phenolic compounds in honey: A review

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Abstract:
Honey is a natural food, mainly composed of sugars, enzymes, amino acids, organic acids, carotenoids, vitamins and minerals, aromatic substance, flavonoids and phenolic compounds with high biological effects. The aim of this review is to describe the phenolic compounds in honey, their stability and potential as antioxidants. Phenolic compounds are defined as secondary plant metabolites with protective mechanism. Different studies presented a various profile of phenolic compounds in honey as vanillic acid, caffeic acid, syringic acid, þ-coumaric acid, ferulic acid, quercetin, kaempferol, myricetin, pinobanksin, pinocembrin, chrysin, ellagic acid, galangin, 3- hydroxybenzoic acid, chlorogenic acid, 4-hydroxybenzoic acid, rosmarinic acid, gallic acid, hesperetin, benzoic acid and others. In general, the phenolic acids are divided in two subclasses: the substituted benzoic acids and cinnamic acids. The flavones and flavanones present some flavonoids in honey, with high effects to colour, taste and flavor of honey. Behind honey, bee bread is second honey bee products rich in phenolic compounds, the mostly kaempferol, myricetin, luteolin, isorhamnetin, and quercetin. Different studies have described a higher positive correlation between total phenol content and the antioxidant activity of honey, while the color of honey has a stronger correlation with the total flavonoid content. This paper contributes to the knowledge of the phenolic compound of honey.

Keywords: honey; antioxidants; polyphenols; antimicrobial activities, phenolic compounds

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Introduction

According to *Codex Alimentarius* and EU Directive 110/2001 honey is defined, as a sweet natural substance produced by bees (*Apis melifera*) from nectar or from the secretions of some plants, which is collected by bees. Also, honey is defined very complex foods, with different chemical composition. Primary, honey is rich with sugars (approximately 80%), as dominant glucose and fructose. Honey is use in nutrition and in medicine worldwide, because honey content some biological substances as aromatic substance, flavonoids and phenolic compounds.

Table 1. Total content of phenolic compounds and flavonoids (expressed as rutin equivalent) in the different honey samples

Honey origin	Total content of phenolic compounds (μg/g)	Total content of flavonoids (μg/g)	Reference
Buckwheat	201.6 ±16.8	41.7 ±2.1	Kaškonienė et al. 2009
Heather	201.2 ±5.5	44.5 ±3.2	
Lime	153.1 ±5.5	32.0 ±1.7	
Rape	71.7 ±1.3	13.5 ±1.3	
Tualang honey 1	28.87 ± 0.41	20.52 ± 0.21	Khalil et al. 2011
Tualang honey 2	26.99 ± 0.13	21.73 ± 0.43	
Tualang honey 3	42.23 ± 0.64	25.31 ± 0.37	
Gelam honey	26.20 ± 0.56	19.47 ± 0.23	
Borneo tropical honey	15.21 ± 0.51	11.52 ± 0.27	
Honey “B”	18.59 ± 0.47	15.40 ± 0.49	
Manuka honey	52.63 ± 1.21	34.55 ± 0.45	
Multifloral	78.2 ± 2.7	/	Lianda et al. 2012
Multifloral	42.8 ± 1.9	0.25 ± 0.03	
Multifloral	57.2 ± 2.4	1.60 ± 0.16	
Multifloral	54.0 ± 2.3	4.27 ± 0.43	
Citrus sp	35.7 ± 2.4	0.30 ± 0.03	
Citrus sp	38.8 ± 3.6	0.28 ± 0.04	
Citrus sp	53.2 ± 2.9	/	
Citrus sp	40.1 ± 2.9	/	
Citrus sp	34.0 ± 1.8	0.24 ± 0.01	
Wolfberry honey	139.30 ± 14.07	ND	Cheung et al. 2019
Acacia honey	52.60 ± 6.61	ND	
Loquat honey	22.90 ± 3.22	ND	
Manuka honey	250.18 ± 14.39	14.68 ± 1.26	
Beech forest honey	188.13 ± 14.59	22.03 ± 6.03	
45° South clover honey	76.36 ± 7.31	5.56 ± 0.55	
Eucalyptus honey	175.05 ± 11.6	41.65 ± 10.35	
Orange blossom honey	74.57 ± 6.68	17.37 ± 3.91	
Wildflower honey	110.46 ± 8.39	33.66 ± 5.74	
Black forest honey	135.22 ± 12.49	2.92 ± 0.48	

*ND- not detected

In addition, honey contains about 200 other constituents, which include amino acids, organic acids, vitamins, minerals, and enzymes (Kasprzyk et al., 2018). This multitude of minor components can be added by bees or comes directly from nectar due to the ripening process (Anklam, 1998; Da Silva et al., 2016). The botanical and geographical origin, as well as the environmental conditions has a high impact on chemical composition of honey (Baroni et al., 2015). According to Serbian Legislation (Official Gazette RS, 2015 (https://www.pravno-informacionisistem.rs/SlGlasnikPortal/eli/rep/sgrs/ministarstva/pravilnik/2015/101/2), honey is classified as:

1) flower or nectar (obtained from the nectar of plants), namely:

(1) monofloral honey,

(2) multifloral/polyfloral honey;

2) honey house;

3) baker's honey.

Monofloral honey is increasingly required on the market and it is necessary to be able to determine some parameters regarding the authentication of the botanical and geographical origin, and some physico- chemical parameters (moisture, reducing sugars, sucrose, hydroxymethylfurfural, free acidity, diastase activity, water-insoluble content and electrical conductivity).

Different studies show that honey (monofloral or multifloral) has high antioxidant activity, which correlated with the presence of phenolic acids, flavonoids, ascorbic acid, carotenoids, catalase, peroxidase (Sergiel et al., 2014; Gheldof and Engeseth, 2002). Antioxidant activity and content of total phenols was confirmed for seven types of honey (Rosa et al., 2011; Al et al., 2009). Also, Estevinho et al. (2008) show that honey from Portugal were responsible for its antimicrobial effects. The composition of honey in polyphenols was found to be mostly dependent on the botanical origin but also vary with the season, climatic conditions and processing factors (Rosa et al., 2011).

Honey content a different flavonoids originate from pollen, nectar or propolis (Khalil et al., 2012). According to Bogdanov et al. (2008) the main flavonoids found in honey are pinocembrin, apigenin, campferol, quercetin, pinobanksin, luteolin, galangin, hesperetin, and isorhamnetin. Table 1 shows total content of phenolic compounds and flavonoids (expressed as rutin equivalent) in the different honey samples by botanical origin and year of honey production. Kaškonienė et al. (2009) shows that heather honey content 201.2 ±5.5 μg/g phenolic compounds, highest than buckwheat, lime and rape honey. Similar results were found in the study by Khalil et al. (2011), where total content of phenolic compounds in the Tualang honey ranged between 26.99 ± 0.13 μg/g and 42.23 ± 0.64 μg/g. Lianda et al. (2012) found that multifloral honey have highest content of o phenolic compounds compared with other examine honey type. The highest flavonoid content was identified in heather honey (44.5 ±3.2 μg/g) followed by buckwheat honey (41.7 ±2.1 μg/g), lime honey (32.0 ±1.7 μg/g) and rape honey (13.5 ±1.3 μg/g) (Kaškonienė et al. 2009). Cheung et al. (2019) not detected flavonoids in wolfberry honey, acacia honey and loquat honey. The highest total content of phenolic compounds was found in Manuka honey (250.18 ± 14.39 μg/g). In the other hand eucalyptus honey has a highest phenolic compounds (41.65 ± 10.35 μg/g) (Cheung et al. (2019).

Conclusion

In this review manuscript, we have present phenolic compounds in honey from different studies. Significant differences refer to the HMF and diastase activity content in honey. Different studies have described a higher positive correlation between total phenol content and the antioxidant activity of honey, while the color of honey has a stronger correlation with the total flavonoid content.

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Microbiological Perspective of Kokoreç, as a RTE-Street Food

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Abstract:

Kokoreç is a commonly produced and consumed traditional street food in Turkiye. In this study a total of 40 kokoreç samples (20 without spices and 20 with spices), were collected from 20 retail points located in Aydın province. The microbiological quality of kokoreç samples were determined by evaluating Total Viable Count (TVC), coliforms, *Enterococcus* spp., yeast and molds, *Staphylococcus/Micrococcus* levels and the presences of *Escherichia coli, Salmonella* spp. and sulphite reducing anaerobic bacteria. The mean TVC, *Enterococcus* spp. and *Staphylococcus/Micrococcus* levels in kokoreç samples without spices were 4.34 log cfu/g, 3.17 log cfu/g and 3.20 log cfu/g, respectively. The levels of coliforms and yeast and molds could not be determined due to limit of detection. The mean levels of TVC, coliforms, *Enterococcus* spp., *Staphylococcus/Micrococcus,* and yeast and molds were 5.14 log cfu/g, 1.96 log cfu/g, 3.43 log cfu/g, 4.31 log cfu/g and 2.65 log cfu/g, respectively. Sulphite reducing anaerobic bacteria were determined in 23 kokoreç samples (9 without spices and 14 with spices). *E. coli* and *Salmonella* spp. were not found in any of the samples analysed. When the results gathered evaluated the kokoreç samples with spices had higher microbiological load than kokoreç samples without spices. This study concluded that the microbiological quality of kokoreç samples were not good enough. The samples subjected to cooking procedures possessed pathogen and/or potential pathogen bacteria which might cause public health risks. In order to reduce these risks, intestine emptying, washing and boiling procedures should be carefully conducted and preparation of kokoreç portions should be kept as small as possible when cooking with lethal time-temperature parameters applied. In addition, the microbiological quality of spices used in service, equipment and personal hygiene should be paid attention.

Keywords: Spices, kokoreç, offals, microbiologial quality

1. Introduction

Kokoreç is one of the most popular traditional offal product in Turkiye. It is a kind of street taste produced from the small intestines of small ruminants according to the special processing technique, with a considerable food safety concerns and public health hesitations (Babaoglu et al. 2017). Kokoreç is produced by covering roasting jack with mesenterial fats, wrapped with intestines, then grilling it on open charcoal fire (Kara et al. 2013). After grilling kokoreç is

sliced and chopped, seasoned then put into bread, served as a ready to eat (RTE) food (Temelli et al. 2002).

Food cooking and consumption habits are changing day after day mainly due to some modifications in the social-life (such as migration from rural area to cities, settlement of high populations in cities, increasing trend in the numbers of employed women etc.), however hesitations regarding to the food safety issues of street foods and demands for safe foods are arising (Hampikyan et al. 2008).

The consumption of kokoreç have had an increasing trend within last years as it happened in fast-food consumption trends in large cities (Cagrı-Mehmetoglu 2018). That is because of the intestinal microbiota available in intestines to be used for kokoreç, insufficient time-temperature profiles applied in cooking/grilling and insufficient hygienic conditions applied during processing and cooking, the possibility of foodborne disease risks may increase (Temelli et al. 2002). Cross-contaminations originated from person and equipment may also contribute the elevated microbial contamination level of RTE kokoreç (Bilgin et al. 2016). Some misapplications carried out during the cooking process of kokoreç may also result in food poisoning cases. Serving remaining kokoreç not sold the day before after grilling and holding sliced-chopped kokoreç portions away from heat source for a long time before serving may increase the numbers of pathogenic microorganisms in kokoreç (Temelli et al. 2002). Studies also showed the increasing effect of seasoning on the bacterial load of kokoreç due to added contaminated spices (Cagrı-Mehmetoglu, 2018, Temelli et al. 2002). Because of these reasons given above, although it is a popular RTE street food and consumed commonly, kokoreç may possess public health risks. Studies with microbiological quality of kokoreç and other RTE foods have also been reported by several researchers (Kılıç 2016, Hampikyan et al. 2008). In this study, the microbiological quality of kokoreç samples sold in Aydın Province was investigated.

2. Materials and Methods

Material

In this study a total of 40 kokoreç samples, 20 with spices and 20 without spices, were bought from 20 retail points between January 2023 and March 2023 period and brought to the laboratory under cold chain and kept at 4ºC till analysis carried out in order to evaluate microbiological quality. For this purpose, the levels of TVC, coliforms, *Enterococcus* spp., *Staphylococcus/Micrococcus*, yeast and molds and the presence of *E. coli*, *Salmonella* spp. and sulphite reducing bacteria (SRB) were investigated.

Method

10 g of kokoreç from each sample was put in a stomacher bag aseptically and 90 ml of sterile peptone water (%0.85 NaCl + %0.1 pepton) was poured in the bag and homogenization was conducted in a stomacher (Interscience-Bag Mixer 400) for 2 min. Then decimal dilutions were carried out.

For *Salmonella* spp. isolation 25 g kokoreç for each sample was subjected to pre-enrichment and selective enrichment processes consequently. Then samples were inoculated on selective media. Biochemical and serological tests were conducted on suspected colonies (TS EN ISO 6579-1 2017).

The media and incubation conditions used at microbiological analysis are given at Table 1.

Table 1. Groups of Microorganisms and Analysis Methods

Microorganism	**Media**	**Incubation**	**Reference**
Total Viable Count	**Plate Count Agar (Oxoid CM03325)**	**30 ºC/ 48-72 h**	**ISO 4833-2 (2013)**
Coliform	**Violed Red Bile Agar (Oxoid CM0107)**	**37 ºC/ 24-48 h**	**BAM (2002)**
***Enterococcus* spp.**	**Kanamycin Esculine Azide Agar (Oxoid CM0591B)**	**35 ºC/ 18-24 h**	**Mossel et al. (1978)**
Stapyhlococcus* / *Micrococcus	**Mannitol Salt Agar (Oxoid CM0085)**	**37 ºC/ 24-48h**	**Kök et al. (2006)**
Yeast/Molds	**Potato Dexrose Agar (Merck 110130)**	**22 ºC/ 4-5 days**	**Halkman (2005)**
Escherichia coli	**TBX (Tryptone Bile X-glucuronide) (Oxoid CM945)**	**43-44 ºC/18-24h**	**ISO 16649-2 (2001)**
Sulfite reducing bacteria	**SPS Agar (Merck 110235)**	**37 ºC/ 24h**	**Labbe (2001)**
Salmonella *spp.*	**Buffered Pepton Water (Oxoid CM1049)** **RappaportVassiliadis Enrich. Broth (Oxoid CM0669)** **XLD Agar (Oxoid CM469)**	**37 ºC/ 24h** **41.5ºC/ 24h** **37 ºC/ 24h**	**TSE 6579-1 (2017)**

3. Results

The levels of TVC in kokoreç samples with and without spices were determined as 4.34±0.77 log cfu/g and 5.14±0.70 log cfu/g, respectively. None of the kokoreç samples without spices had coliforms whereas 8 kokoreç samples with spices had coliforms with a mean of 1.96 log cfu/g. The mean *Enterococcus* spp. level for samples without spices was 3.17 log cfu/g (4 samples). However, a total of 12 samples with spices had *Enterococcus* spp. with a mean of 3.43 log cfu/g. The mean levels of *Staphylococcus*/*Micrococcus* in samples without spices (17 samples) and with spices (19 samples) were 3.20 log cfu/g and 4.31 log cfu/g, respectively. Within all kokoreç samples examined yeast and molds levels were only determined in 5 kokoreç samples with spices with a mean of 2.65 log cfu/g. A total of 23 kokoreç samples (9 without spices and 14 with spices) were found to be positive SRB. *E. coli* and *Salmonella* spp. could not be detected in none of the kokoreç samples investigated.

Table 2. Microbiological level (log cfu/g) in kokoreç samples with/without spices

	Positive samples (%)		Min (log cfu/g)		Max (log cfu/g)		Mean (log cfu/g)	
	Without spices	With spices	Without spices	With spices	Without spices	With spices	Without spices	With spices
TVC	n=20 (%100)	n=20 (%100)	3	3.3	5.63	6.1	4.34	5.14
Coliform	-	n=8 (%40)	-	1	-	3.98	-	1.96
Enterococcus spp.	n=4 (%20)	n=12 (%60)	2.30	2	4.11	4.88	3.17	3.43
Staphylococcus/ *Micrococcus*	n=17 (%85)	n=19 (%95)	2.30	2.70	5.11	5.12	3.20	4.31
Yeast and Molds	-	n=5 (%25)	-	2	-	3.24	-	2.65

4. Discussion

Street foods have been defined as ‘ready-to-eat foods and beverages prepared and/or sold by vendors or hawkers especially in the streets and other similar places’ by FAO (1997). Socio-economic conditions in the countries, life styles and changes in the life styles, and urban features have increased the demand for RTE foods all over the world, which is also considered to be associated with increased risk in food infection and intoxication cases. Several studies were conducted to determine hygienic quality of kokoreç, as a very popular traditional street food, in Turkiye but no data has been found for Western part of Aegean Region.

In a study conducted by Hampikyan et al. (2008) in İstanbul showed that TVC were ranging between 5.3 log cfu/g and 7.0 log cfu/g in 15 kokoreç samples analysed. The authors also reported that 60% of kokoreç samples (9 samples) had coliforms with a maximum level of 5.84 log cfu/g. Although, 2 out 15 samples possessed *E. coli*, no *Salmonella* spp. was determined in any of the samples analyzed. In another study conducted by Temelli et al. (2005) in Bursa province of Turkiye showed that the levels of TVC, coliforms, *Enterococcus* spp. and *Staphylococcus/Micrococcus* were 10^4-10^5 cfu/g, <1.0x10^1-10^4 cfu/g, 10^2-10^4 cfu/g, and 10^2-10^4 cfu/g, respectively. Although 20% of the samples analysed for yeast and molds were found to be under the limit of detection, 80% of the samples were between 10^3 and 10^4 cfu/g. They also reported TVC, coliforms, *Enterococcus* spp., *Staphylococcus/Micrococcus*, and yeasts and molds levels in kokoreç samples with spices as 10^5-10^6 cfu/g, 10^4-10^5 cfu/g, 10^2-10^4 cfu/g, 10^3-10^5 cfu/g and 10^2-10^4 cfu/g, respectively. None of samples had *E. coli* and *Salmonella* spp.

In a study conducted on with and without spices kokoreç samples in Isparta showed that TVC was 3.72 log cfu/g in samples without spices. No coliform and yeasts and molds were determined in these samples. TVC, coliform, and yeasts and molds levels were 6.04 log cfu/g, 5.75 log cfu/g and 3.74 log cfu/g in kokoreç samples with spices (Kılıç, 2016). Kara et al. (2013) determined the levels of TVC, coliform, *E. coli*, *Enterococcus* spp., *Staphylococcus/Micrococcus*, and yeasts and molds in kokoreç samples as 6.29, 2.43, 2.10, 4.17, 2.85, and 5.89 log cfu/g, respectively.

The levels of microorganisms investigated (Table 2) were found to be varied when compared with the other researchers works (Kılıç 2016, Kara et al. 2013, Hampikyan et al. 2008, Temelli et al. 2002). This might be due to the differences in the initial loads of raw materials and the production stages of kokoreç, storage conditions, and the hygienic conditions of persons and equipment. On the other hand, similar results were found in the study reported here when compared with others (Kılıç 2016, Temelli et al. 2002) in kokoreç samples with spices with regard to added spices. The results showed that apart from contaminations occurred during processing, spices added might also have significant effects on the microbiological load of kokoreç. If spices with low hygienic quality, contaminated with pathogens used in seasoning, safety of kokoreç will affect negatively which may result in some public health consequences. The results of *Salmonella* spp. and *E. coli* incidence in kokoreç samples were in agreement with the results of Temelli et al. (2005), none of the samples contained these pathogens. However, the presence of coliform bacteria found in the samples indicated the possibility of pathogen microorganism contaminations.

Enterococcus spp. can be found in various types of foods due to their resistance and growth ability at several adverse conditions, such as pasteurization environment, availability of several inhibiting substrates, high and low temperatures, high pH and salt content etc. (Foulquié Moreno et al. 2006). *Enterococcus* spp. can survive at 63,5°C for 30 min. Therefore, its presence in heat-treated and processed foods is considered to be an indicator for a fecal contamination and/or insufficient heat application (Gökmen et al. 2017, Temelli et al. 2002, Gardin et al. 2001). Similar observations about the relevance of *Enteroccoccus* spp. and hygienic quality level of RTE food were pointed out by the work of Gökmen et al. (2017).

A total of 23 kokoreç samples (9 samples (45%) without spices and 14 samples (%70) with spices) were positive for SRB. In Turkish Food Codex (TFC 2011) limits of some microorganisms are referred. Beside this, investigating the presence of pathogen microorganisms, whatever their levels are, in the foods has of great importance (Şenses-Ergül et al. 2005). Although, the presence of SRB in foods, especially heat treated foods is an indicator of fecal contamination, it also indicates improper cleaning and sanitation of intestines for kokoreç itself, and cross-contamination. If they found suitable conditions, spore-forming SRB may switch to vegetative form, grow and cause infections and intoxications (Hallaç 2022). Considering kokoreç as a RTE, studies suggest that SRB could be associated with hygienic quality levels of food animal origin and spices (Sancak et al. 2020, Öksüztepe and Beyazgül 2014, Bostan et al. 2011). Leaving/storing unconsumed cooked kokoreç on the roasting jack under the unsuitable conditions, which increases the microbiological load and consuming it the day after may cause great health hazards.

5. Conclusion

It is thought that the microbiological quality of kokoreç samples examined is not sufficient enough to prevent public health risks and time-temperature profiles applied during grilling is not efficient to destroy pathogen and potential pathogen bacteria. The microbiological contamination level in RTE foods may be affected by mainly microbiota of raw material used in the production and the failures in hygienic conditions in the processing stages. To be able to eliminate foodborne infection and intoxication outbreak risks, good hygienic practices, HACCP and HACCP based food safety management systems should be implemented in the kokoreç production premises. At the retail level, kokoreç portions should be consumed as soon as possible, without causing temperature abuse. Cross-contamination risks should be minimized at serving. Spices should be purchased from the firms with HACCP and/or ISO 22000

certificates and contamination of spices should also be minimized and prevented at the selling points.

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As an Ethnic Food, Investigation of the Microbiological Quality of Camel Sucuk

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Abstract:

This study aimed to investigate the microbiological quality of camel sucuk, recently received geographical indication, produced in Incirliova district of Aydin province between November 2021 and February of 2022. A total of 100 samples, 50 fermented and 50 heat-treated sucuk collected from sucuk production premises and retail points, were subjected to microbiological analysis for total viable count (TVC), lactic acid bacteria (LAB), yeast-mold, *Micrococcus/Staphylococcus*, coliform bacteria, *Escherichia coli (E. coli)* levels and *Salmonella* spp.. The TVC results obtained in the study were statistically different ($p < 0.001$) for fermented (7.76 ± 0.13 log cfu/g) and heat-treated sucuk (5.73 ± 0.40 log cfu/g). The mean LAB results were significantly ($p<0.001$) higher (7.56 ± 0.17 log cfu/g) in fermented sucuk in compare to heat-treated sucuk (5.04 ± 0.52 log cfu/g). Statistically significant difference ($p<0.001$) were observed between fermented (4.90 ± 0.11 log cfu/g) and heat-treated sucuk samples (3.63 ± 0.35 log cfu/g) for yeast and molds. *Micrococcus/Staphylococcus* levels were significantly ($p<0.001$) higher in fermented samples (5.18 ± 0.05) than heat-treated samples (3.54 ± 0.43 log cfu/g). The levels of coliform bacteria were 3.69 ± 0.13 and 3.70 ± 0.91 log cfu/g for fermented sucuk and heat-treated sucuk samples. Only 3 samples containing *E. coli* with a mean of 1.87 ± 0.29 log cfu/g were found to be higher than limit of detection for heat-treated samples. However, *E. coli* was found in 24 out of 50 fermented samples with a mean level of 3.35 ± 0.14. *Salmonella* spp. was determined in 18% of fermented sucuk and 14% of heat-treated sucuk samples. It was concluded that hygienic status of raw materials, applications during processing, fermentation process and the time-temperature effect of heat application might influence the microbiological quality of sucuk. Apart from that, contaminations after processing may increase the level of microorganisms contaminated. It has been concluded that in order to prevent possible contamination, it is necessary to implement HACCP systems as well as good production and good hygiene practices in the sucuk processing premises.

Keywords: camel sucuk, *E. coli*, hygienic quality

[#] This study is summarized from the first author's MSc thesis

1. Introduction

Camel meat consumed commonly with a high preference as source of animal protein similar to beef protein, a potential alternative protein source, especially in many African and Asian countries where climatic conditions are not suitable for the cultivation of other farm animals.

Local people's taste and cooking habits, the beliefs on the health benefits of camel meat as well as economic reasons may be other factors for high consumer demand (Soltanizadeh et al., 2010; Abrhaley and Leta, 2018; Sabahat et al., 2021). The nutritional composition of camel meat differs according to the race, age, sex, condition and place on the carcass of the camel (Kadim et al., 2008; Bulbul and Koc, 2018). Camel meat consists of 66-78% water, 19% protein, 3% fat and 1.2% ash. It is a rich source of macro and micro nutrients (Kadim et al., 2008; Abrhaley and Leta, 2018; Bulbul and Koc, 2018; Baba et al., 2021; Kadim et al., 2022). Meat obtained from young camels is generally similar to beef in terms of meat quality characteristics (Baba et al., 2021).

Several methods such as drying, fermentation, salting and cooking or a combination of these are used to produce such traditional meat products to improve the flavor and shelf life of camel meat (Kok et al., 2006; Baba et al., 2021). One of the important traditional meat products produced in Turkiye is sucuk, also produced by using camel meat (Kok et al., 2006; Baba et al., 2021). Sucuk production in Türkiye is generally made by two different methods; naturally fermented and heat-treated (Kok et al., 2006; Buyukunal et al., 2016). According to Turkish Food Codex Communiqué on Meat, Prepared Meat Mixtures and Meat Products (2019) fermented sucuk are fermented meat products traditionally shaped by the local microflora with a moisture content of 40% and below, without heat treatment. Heat-treated sucuk is a heat-treated meat product with a moisture content of less than 50%. Heat treated sucuk is made by increasing sucuk dough temperature up to 72-74˚C in the ovens or conditioned rooms to reduce microbiological load of final product.

Camel meat used for the production of sucuk is provided by the animals raised for camel wrestling festivals, a series of cultural events in camel breeding culture in Turkiye. When the camels are injured or aged they are sent to the slaughterhouses to produce meat for sucuk production (Calıskan, 2009; Yilmaz and Ertugrul, 2015; Gozgec Mutlu et al., 2020).

This study aimed to examine the microbiological quality of camel meat sucuk produced in fall-winter period produced in the Incirliova district of Aydin province between November and February were investigated.

2. Material and Methods

2.1. Material

A total of 50 fermented camel sucuk produced by classical method and 50 heat-treated camel sucuk produced as stated in the geographical indication process were used as material. The sucuk samples were collected from production premises and retail points within five different occasions between November 2021 and February 2022 in order to investigate microbiological quality of sucuk samples. The ripening period was determined as 7 days for fermented samples and heat-tread sucuk samples were analyzed within 10 days of production.

2.2. Methods

2.2.1. Microbiological analyses

Total viable count (TVC), the levels of lactic acid bacteria (LAB), yeast and mold, *Micrococcus/Staphylococcus*, total coliforms and *Escherichia coli (E. coli*) in the camel sucuk

samples were determined according to the procedures stated in ISO 4833-I (2013), De Man et al. (1960), ISO 21527-2 (2008), Kok et al. (2006), ISO 4832 (2006), and Roberts and Greenwood (2003) and Halkman (2005), respectively. The presence of *Salmonella* spp. was investigated according to the ISO 6579 (2017). Serological confirmation of *Salmonella* spp. was carried out using Salmonella Latex Test (Oxoid DR1108A).

2.3. Statistical analyses

Statistical analyzes were performed using SPSS Version 22 software. Cases where the P value was below 0.05 were considered as statistically significant results. Chi-square test was used to determine whether there was a difference in the presence of *Salmonella* spp between fermented camel sucuk and heat-treated camel sucuk. Since bacterial contamination data of fermented and heat-treated sucuk did not show normal distribution, they were compared using Mann Whitney U test.

The microbiological analyses results were converted to log cfu/g for statistical analysis.

3. Result

The levels of TVC, yeast and mold, total coliform, LAB and *Staphylococcus/micrococcus in fermented and heat treated camel sucuk samples are given at Table1*.

Table 1. The mean TVC, yeast and mold, total coliform, LAB and *Staphylococcus/micrococcus* counts found in fermented and heat-treated camel sucuk (log cfu/g)

	N	**Fermented Sucuk (log cfu/g) ± SD**	**N**	**Heat-treat Sucuk (log cfu/g) ± SD**	**Sig**
TVC	**50**	**7.76 ± 0.13**	**46**	**5.73 ± 0.40**	*******
Yeast and Mold	**50**	**4.90 ± 0,11**	**15**	**3.63 ± 0.35**	*******
Total Coliforms	**50**	**3.69 ± 0.13**	**10**	**3.70 ± 0.91**	**NS**
LAB	**50**	**7.56 ± 0.17**	**46**	**5.04 ± 0.52**	*******
Staphylococcus/micrococcus	**50**	**5.18 ± 0.05**	**45**	**3.54 ± 0.43**	*******

N: number of samples, ***: $p<0.001$, NS (Not significant): $p>0.05$, SD: Standard deviation. Sig: Significance

The microbiological results revealed that the levels of almost all microorganisms investigated were significantly ($p<0.001$) higher in fermented sucuk samples when compared with heat-treated sucuk samples, except total coliforms. The levels of yeast and molds, and total coliforms could not be determined in some heat-treated sucuk samples due to limit of detection. Only 10 samples for total coliforms and 15 samples for yeast and molds were countable. TVC was almost 2 log lower in heat-treated samples in comparison with fermented samples.

The distribution of *E. coli* in fermented and heat-treated sucuk samples are given at Table 2.

Table 2. Distribution of the maximum, minimum and mean values of *E.coli* found positive in fermented and heat-treated camel sucuk samples

	N	**n**	**Max**	**Min**	**Mean ± SD**
Fermented Camel Sucuk	**50**	**24**	**4.05**	**1.0**	**3.35 ± 0.14**
Head-tread Camel Sucuk	**50**	**3**	**2.26**	**1.3**	**1.87 ± 0.29**

N: number of samples studied, n: number of *E. coli* positive samples, SD: Standard deviation.

Although 24 samples in fermented sucuk samples had *E. coli* levels over the limit of detection with a mean of 3.35 ± 0.14 log cfu/g, only 3 heat-treated samples out of 50 samples investigated reached to countable levels with a mean of 1.87 ± 0.29 log cfu/g.

Table 3. The incidence of *Salmonella* spp positive samples found in fermented camel and heat-treated camel sucuk samples

	N	Fermented Sucuk	N	Heat-tread Sucuk
***Salmonella* spp.**	**50**	**7 (14%)**	**50**	**8 (16%)**

N: number of samples studied

The incidence of *Salmonella* spp positive samples in fermented camel and heat-treated camel sucuk are given at Table 3.

4. Discussion

The microbiological characteristics of the camel meat sucuk produced by using different methods were very different each other. Only *Salmonella* spp incidence and the levels of coliforms were found to be similar. However, the incidence of *E. coli*, the levels of TVC, yeast and mold, LAB and *Staphylococcus/micrococcus were significantly higher in fermented sucuk samples.*

Although the microbiological results obtained from fermented and heat treated sucuk samples may be affected by initial load of the raw materials, the fermentation time and the characteristics of the fermentation environment, the control of air flow and humidity, changes in hygienic practices during processing and transportation, and the heat treatment applied also affect sucuk microbiological quality (Kok et al., 2006; Simsek, 2022)

Toptancıl and Ercoşkun (2017) reported significant reductions ($P<0.05$) in TVC, LAB and *Staphylococcus/micrococcus* counts after heat-treatment with 60°C (for 15 minutes), 65°C (for 10 minutes) and 70°C (as reached). Temelli et al (2005) showed that after 65°C for 30 min heat treatment, the levels of TVC, coliforms, staphylococci, yeast and molds and LAB reduced significantly ($p<0.05$) in heat-treated sucuk samples.

Therefore the differences, appr. 2 logs, between TVC levels in heat treated and fermented sucuk samples might be due to lethal effect of heating. The TVC values reported in this study were between the levels reported by Mejri et al. (2017) and Simsek (2022). Kalalou et al. (2004) reported that TVC numbers decreased from day 0 and day 21 in fermented sucuk samples. A low level of water activity and low pH over a 21-day period might cause the microbiological reduction.

LAB counts in fermented camel sucuk and heat-treated camel sucuk were found to be 7.56 ± 0.17 and 5.04 ± 0.52 log cfu/g, respectively ($p<0.001$) (Table 1). Fermentation is a natural processing technique used for enhancing the nutritional and nutraceutical content and shelf life of different meat types depending upon the starter culture in the fermented product. Lactic acid microorganisms make raw-smoked sucuk a product of long-term storage, as they prevent the development of unwanted microflora by producing lactic acid, hydrogen peroxide, organic. acids, and antibiotic substances, bacteriocins, that inhibit the development of putrefactive microflora. Bacteriocins are ribosomally synthesised peptides, or small proteins with

antimicrobial activity that are produced by bacteria. These substances actively inhibit the development of pathogens at the beginning of the stationary growth phase (Vinnikova et al., 2019). The environment of fermentation and the lenght of fermentation period are also very important factors for these features. The levels of LAB after 7 days of fermentation period was 7.56 ± 0.17 log cfu/g in the study presented here. Mejri et al. (2017) produced fermented camel sucuk, with 4.57 log cfu/g initial load of LAB at the beginning, which increased to 7.09 log cfu/g on the 14th day during ripening. Faleeha et al. (2019) reported 6.67 log cfu/g after 45 days of storage of fermented camel sucuk at 4°C. It is thought that due to the pasteurization effect of heat-treatment on the levels of LAB in heat treated samples and the adaptation of LAB in the sucuk dough and its rapid growth during fermentation process (Drosinos et al. 2005), LAB were higher in naturally fermented sucuk samples. Samelis et al. (1998) stated that the inhibition of spoilage microorganisms in foods could be achieved with low aw values and LAB being the dominant flora in the fermentation process. In the present study almost all microbiological parameters investigated were found to be very high in fermented sucuk samples. This might be due to relatively short ripening period of fermented sucuk samples which resulted lower drying and insufficient fermentation process.

In the study presented here, yeast and mold levels were detected in only 15 heat-treated sucuk, while all fermented sucuk samples were above the limit of detection. The statistically significant ($p<0.001$) differences between these 2 types of sucuk is thought to be due to water activity and hygienic quality. Ahmad and Srivastava (2007) stated that yeast and mold counts should exceed 4 log cfu/g to cause deterioration. In the study presented here, heat-treated sucuk were found to be compatible with this limit.

Simsek (2022) examined yeast and mold levels in his study with camel sucuk in Aydin region, concluded that the hygienic quality of camel sucuk was insufficient, and the current situation had the potential to pose a risk in terms of consumer health. Mejri at al. (2017) analysed yeast and mold levels during the 28-day fermentation period, and found lower results than the initial level on day 0. The decrease in yeast and mold numbers might be due to weak competition with the increasing acidic bacterial flora (Maltı and Amarouch, 2008).

In the study *Staphylococcus* and *Micrococcus* levels of heat-treated camel sucuk samples were found to be approximately 2 log lower ($p<0.001$) than fermented sucuk samples. This difference has been thought to be due to the production method.

Kok et al. (2006) and Mejri et al. (2017) reported similar *Staphylococci* and *Micrococci* levels found in this study. Malti and Amarouch (2008) observed that after 28 day ripening period the levels of *Micrococci* was higher than the initial day. Samelis et al. (1998) stated that *Micrococci* can reduce nitrate to nitrite using nitrate, and also show competitive properties against LAB. At the same time, production, personnel and tool equipment hygiene is important to prevent Staphylococcal food poisoning (Yalcin and Can, 2013). The rapid decrease in pH in fermented meat products also prevents the development of *Micrococci* and *Staphylococci*, which positively effect acid-sensitive flavor development and color formation (Erkmen, 2010; Procop et al.,2017; Ertekin and Kayapınar Kaya 2020).

The total coliform numbers obtained from the sucuk were evaluated, similar values were found in the heat-treated sucuk (3.70 log cfu/g) and the camel sucuk(3.69 log cfu/g) produced by fermentation ($p>0.05$). *E.coli* contamination level was evaluated, the difference between the fermented sucuk samples and the *E.coli* level could not be evaluated statistically, since it was

above the detectable level in only three of the heat-treated sucuk samples. Simsek (2022) detected total coliform levels as 3.10 ± 0.87 log cfu/g in fermented camel meat sucuk samples which is in agreement with results reported here. The similar incidence of coliform levels obtained in the samples produced both methods might be due to post pasteurization contamination of heat-treated samples.

Mejri et al. (2017) and Kalalou et al. (2004) found a decrease in the total coliform number as the fermentation time increased in two different studies. They noted that this reduction was due to the LAB lowering the pH and the release of bacteriocin. The presence of coliform bacteria, which we do not want to be in foods, has been detected in our study and in other studies. Since the presence of total coliform and *E. coli* in food indicates contamination, more attention should be paid to hygiene in production and contact with sources of contamination should be avoided. Only 3 heat treated samples were found to be above limit of detection, whereas 24 samples out of 50 fermented sucuk were found to be contaminated with *E. coli*. *E. coli* is one of the most important foodborne pathogenic bacterium transmitted by the feces or oral route. It is accepted as indicator bacteria for fecal contamination in food safety and hygiene, as its presence in food shows that there are insufficient hygiene conditions in the processing area (Ekici and Dumen, 2019).

Ozbey et al. (2007) found 7 (7%) *Salmonella* spp. positive samples in sucuk samples collected in Aydin region, In this study conducted in Aydin province, showed that 8 (16%) for heat-treated camel sucuk and 7 (14%) for fermented sucuk samples were positive for Salmonella *spp* . This result was higher than Ozbey et al. (2007) results, while Simsek (2022), did not detect *salmonella* spp. in fermented sucuk samples. Malti and Amarouch (2008) did not detect *Salmonella* spp. at any stage of camel sucuk production. The similar incidence of *Salmonella* spp levels obtained in the samples produced both methods might be due to post pasteurization contamination of heat-treated samples.

5. Conclusion

In this study, the microbiological quality of camel sucuk produced by classical fermentation and heat-treatment methods was investigated. Since sucuk production is not standard and fermentation occurs by natural method, various results were encountered as standard product could not be obtained.

Although the microbiological values obtained were generally in accordance with the legal limits, the presence of total coliform, *E. coli* and *Salmonella* spp. and the high levels of yeast and mold were indicating insufficient hygienic conditions and insufficient ripening period for fermented sucuk samples

While TVC, yeast and mold, LAB, *staphylococcus* and *micrococci* and *E. coli* counts were higher in fermented camel sucuk, the presence of *salmonella* spp. was higher in heat-treated camel sucuk.

It is concluded that hygienic practices applied in sucuk production and retail market should be improved, sufficient fermentation procedures and ripening period should be allowed and HACCP based food safety systems should be implemented in order to improve microbiological quality of camel meat sucuk.

6. References

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Investigation of the effect of microwave assisted extraction of broccoli (*Brassica oleracea L.* var *italica*) on quality criteria

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Abstract:

Broccoli (*Brassica oleracea* L. var. *italica* Plenck) is a popular winter vegetable belonging to the Cabbage family (*Brassicaceae*), cultivated in large areas in developed countries, and whose consumption has increased rapidly in recent years in our country. Broccoli is a vegetable rich in vitamin E (α-tocopherol), vitamin A (precursor β-carotene) and vitamin C (ascorbic acid), which are known to have antioxidant properties. For all these reasons, it was aimed to determine some quality criteria by microwave assisted extraction of broccoli vegetable. Microwave oven was used for microwave assisted extraction of phenolic substances from freeze-dried broccoli samples. Different time ranges (30, 60, 90 seconds) were applied at different power levels (360, 600 and 900 W) for the extraction. Samples were diluted 1:10 with 80% methanol (containing 1% HCl) solution, and the samples were placed in a glass beaker and extracted in a microwave oven. In order to understand the quality criteria of the samples as a result of extraction, total phenolic substance, antioxidant activity, color determination and ascorbic acid amounts were determined. According to the results, the highest amount of phenolic substance in broccoli samples was 725.52 mg GAE / kg at 360 W / 60 s, and the lowest was 225.77 mg GAE / kg at 900 W / 90 s. The DPPH method was used to determine the antioxidant activity values of the samples. The lower the EC_{50} values calculated from the graphs, the higher the DPPH radical scavenging activity. As a result of the study, the lowest EC_{50} value was found at 3.38 mg/mL and 900 W / 30 s parameters, while the highest EC_{50} was found at 900 W / 90 s at 12.29 mg/mL. When the results were examined in terms of color characteristics, differences were observed between the L^*, a^*, b^* values of broccoli samples at different times and powers. Finally, the highest amount of vitamin C was seen as 191.451 mg / 100 g at 360 W / 30 s condition, while the lowest amount of vitamin C was 85.970 mg / 100 g at 600 W / 90 s condition.

Keywords: broccoli, microwave, extraction, phenolic, antioxidant

Introduction

Plant-based foods provide people with both basic nutrition and a healthy diet with their rich bioactive compounds (Baek et al. 2021). Broccoli (*Brassica oleracea* var. *italica*) is a widely consumed vegetable from the cruciferous family known to be rich in dietary fiber, vitamins, bioactive compounds and minerals (Domínguez-Perles et al 2010). In 1999, the total production of broccoli and cauliflower worldwide was approximately 15.00 million tons, while in 2019 this amount increased to 26.92 million tons (FAOSTAT, 2021). The health benefits of broccoli have led to an increase in production quantities (Ares et al. 2013). Many previous studies have reported positive health effects of broccoli and similar products (Gigliotti et al. 2020; Le et al. 2020; Lee et al. 2019; Li et al. 2021; López-Chillón et al. 2019; Nadeem et al. 2020; Xu et al. 2020). A number of recent studies have reported that broccoli may contribute significantly to the prevention of COVID-19 symptoms (Bousquet et al. 2021).

Some cruciferous vegetables and broccoli can be eaten both raw and cooked. Broccoli is mostly consumed by steaming and microwaving at home (Yuan et al. 2009). It can affect the physical properties of foods and the levels of bioactive components due to new metabolites formed by temperature treatments applied to foods (Yuan et al. 2009). Microwave heating, one of the temperature methods, has a direct effect on the flavor of the food as a result of the uneven distribution of the heat emitted and the rapid movement of water to the surface (Salim et al. 2017). In the food industry, microwaves are used to heat food by generating heat through the vibration of water molecules in the food with microwave energy (Alajaji and El-Adawy2006). In this study, it was aimed to investigate the quality changes caused by microwave extraction of broccoli. Different time ranges (30, 60, 90 seconds) were applied at different power levels (360, 600 and 900 W) for the extraction. In order to understand the quality criteria of the samples as a result of extraction, total phenolic substance, antioxidant activity, color determination and ascorbic acid amounts were investigated.

Materials and Methods

2.1 Materials

Broccoli plants were obtained from the local market during the season and brought to the laboratories of the Department of Food Engineering, washed, and cut into pieces, dried with a freeze dryer (Scanvac Coolsafe 95-15 Pro, Denmark) to protect them from environmental conditions and used for extraction and analysis in glass containers with moisture-proof lids at -18°C (Arçelik, Turkey). All extractions were performed with this dried broccoli.

2.2 Methods

A benchtop microwave oven (LG Solar DOM, Korea) was used for microwave-assisted extraction of phenolic substances from dried broccoli samples. Different times (30, 60, 90 seconds) at different powers (360, 600 and 900 W) were applied for extraction. 80% methanol (containing 1% HCl) solution was diluted 1:10 and the samples were placed in a glass beaker and extraction was performed in a microwave oven. The extracts obtained were kept in an airtight bottle in the refrigerator at -18^{o}C until the moment of analysis (Baltacioglu et al. 2019).

Color determination

Color determination device (Konica Minolta CR400, Japan) was used to determine the color properties of broccoli extracts. After the device was calibrated with distilled water, the liquid samples were placed in the cuvette and L^{*}, a^{*} and b^{*} values were determined (Baltacioglu et al. 2019).

Determination of total phenolic content

Total phenolic matter was determined according to the Folin-Ciocaltaeu method. 100 µl sample was filled with 0.75 mL Folin-Ciocaltaeu solution (10% in water) and kept at room temperature for 5 minutes. 0.75 mL Na2CO3 (in water, 75 g/L) was added and mixed rapidly. The samples were kept in the dark at room temperature for 1.5 hours and then the absorbance values of the samples were read at 725 nm on a spectrophotometer. Gallic acid was used as standard, and the same procedure was repeated for calibration curve and applied to different concentrations of gallic acid solutions. Total phenolic matter concentration was calculated as equivalent gallic acid value (mg/kg GAE) (Baltacioglu et al. 2019).

Determination of antioxidant activity

Free radical scavenging activity assay was performed according to Blois' method using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Blois, 1958). The method is based on the ability of the extracts to give a proton or electron to lighten the color of the purple DPPH solution. A decrease in the absorbance of the reaction mixture is an indication of high free radical scavenging activity. After taking 100 µL each of the sample extracts prepared at different concentrations, 3.9 mL of 0.1 mM DPPH (80% methanol) solution was added. After vortexing, the solution was kept in the dark under room conditions for 30 minutes and the absorbance was read at 517 nm at the end of the time. Instead of the sample, 100 µL of 80% methanol was used as a control under the same conditions. The results were expressed as the EC50 value, which is the amount of antioxidant used to reduce the initial DPPH concentration by 50% (Brand-Williams et al. 1995).

Determination of ascorbic acid (vitamin C) concentration

High performance liquid chromatography (HPLC) (Shimadzu, LC-20A/ Prominence, Columbia, USA) was used to determine the concentrations of vitamin C in the samples.

Reversed phase C-18 column (5µm particle size, 4.6 mm diameter, 250 mm length) was preferred for the analyses. Firstly, the mixture of methanol and water (10:30 (v/v)) was kept in an ultrasonic bath to prevent air bubbles and this ratio was used as the mobile phase in the analysis. The calibration graph was then plotted by adjusting the solutions in the concentration ranges 10 - 80 ppm using the L-ascorbic acid (Sigma, Germany) standard. After these procedures, 5 mL of each sample was taken and 5 mL of 25% phosphoric acid was added and the samples were centrifuged at 9000g for 5 min (Nüve brand NR 800R model, Turkey). After centrifugation, phase separation occurred, and 0.5 mL of the supernatant was taken and the total volume was completed to 10 mL with 25% phosphoric acid. The samples were then passed through a 0.45µm filter and 20µL of the sample was injected into the HPLC device (Abid et al. 2014).

Statistical analysis

The data were analyzed using Minitab (version 18, Minitab Inc., State College, PA, USA) package program with 95% confidence interval and one-way ANOVA was used in the analysis of the data. Tukey's multiple comparison test was used to determine the differences between treatments. Each experiment was repeated at least three times.

Results

3.1 Color determination

Table 1 shows the results of color determination by microwave assisted extraction applied to broccoli samples. According to the results of the analysis, microwave assisted extraction process applied at different times and powers caused differences between L^*, a^*, b^* values of broccoli samples. According to the analysis results, the highest L^* value was determined in the extraction at 900 W / 90 s with 36.23 and the lowest L^* value was determined in the extraction at 360 W / 60 s with 22.34. 36 at 360 w / 60 s and the lowest a^* value was 0.43 at 60 °C / 30

min. The highest b^* value was 28.83 at 900W / 90s and the lowest b^* value was 12.18 at 360W / 60 s.

Table 1. Color determination results of microwave assisted extraction applied to broccoli samples

Extraction conditions	L^*	a^*	b^*
360 W / 30 s	$27.14^{D} \pm 0.2$	$3.41^{E} \pm 0.14$	$18.30^{E} \pm 0.72$
360 W / 60 s	$22.34^{F} \pm 0.3$	$7.36^{A} \pm 0.24$	$12.18^{F} \pm 0.17$
360 W / 90 s	$23.69^{E} \pm 0.59$	$5.53^{C} \pm 0.21$	$13.54^{F} \pm 0.23$
600 W / 30 s	$27.15^{D} \pm 0.41$	$6.77^{AB} \pm 0.35$	$20.79^{D} \pm 0.33$
600 W / 60 s	$26.70^{D} \pm 0.28$	$6.45^{B} \pm 0.22$	$20.02^{D} \pm 0.53$
600 W / 90 s	$28.80^{C} \pm 0.27$	$4.71^{D} \pm 0.18$	$22.81^{C} \pm 0.66$
900 W / 30 s	$26.69^{D} \pm 0.33$	$6.41^{B} \pm 0.21$	$20.18^{D} \pm 0.59$
900 W / 60 s	$31.45^{B} \pm 0.37$	$2.69^{F} \pm 0.36$	$27.28^{B} \pm 0.83$
900 W / 90 s	$36.23^{A} \pm 0.33$	$0.43^{G} \pm 0.08$	$28.83^{A} \pm 0.22$

The differences in letters indicate that microwave power and time are statistically effective on color values ($p \leq 0.05$).

3.2 Determination of total phenolic content

Different powers (360, 600, 900 W) and times (30, 60, 90 s) were applied for microwave-assisted extraction of phenolic substances from dried broccoli samples. According to the results, the highest amount of phenolic matter in broccoli samples was 725.52 mg GAE/kg at 360 W/60 s condition and the lowest was 225.77 mg GAE/kg at 900 W/90 s condition. The total phenolic matter amounts made by microwave assisted extraction method are given in Table 2.

Table 2. Total phenolic content (mg GAE/kg) and extraction conditions by microwave-assisted extraction method

Extraction conditions	mg GAE / kg
360 W /30 s	$351.39^{C} \pm 9.17$
360 W / 60 s	$725.52^{A} \pm 20.3$
360 W / 90 s	$486.22^{B} \pm 14.8$
600 W / 30 s	$495.42^{B} \pm 33.1$
600 W / 60 s	$474.53^{B} \pm 18.6$
600 W / 90 s	$359.60^{C} \pm 38.3$
900 W / 30 s	$469.55^{C} \pm 15.76$
900 W / 60 s	$282.74^{D} \pm 28.3$
900 W / 90 s	$225.77^{D} \pm 21.2$

The differences in letters indicate that microwave power and time are statistically effective on color values ($p \leq 0.05$).

3.3 Determination of antioxidant activity

DPPH method was used to calculate the antioxidant activity values of broccoli samples as a result of microwave assisted extraction method. Accordingly, the lower the EC_{50} values calculated from the graphs, the higher the DPPH radical scavenging activity. EC_{50} values within the scope of the study are shown in Table 3.

Table 3. EC50 values of microwave-assisted extraction

Extraction conditions	EC_{50} values (mg/mL)
360 W /30 s	$8.53^{BC} \pm 0.274$
360 W / 60 s	$6.86^{DE} \pm 0.712$
360 W / 90 s	$9.55^{B} \pm 0.404$
600 W / 30 s	$6.44^{E} \pm 0.415$
600 W / 60 s	$7.92^{CD} \pm 0.325$
600 W / 90 s	$9.44^{B} \pm 0.382$

900 W / 30 s	$3.38^{F} \pm 0.226$
900 W / 60 s	$8.83^{BC} \pm 0.376$
900 W / 90 s	$12.29^{A} \pm 0.251$

The differences in letters indicate that microwave power and time are statistically effective on color values ($p \leq 0.05$).

3.4 Determination of Ascorbic acid concentration

In our study, ascorbic acid content of broccoli samples was found to be between 85.97 and 191.45 mg/100g. When these values were examined, the highest amount was obtained in the 360W-30s process, while the lowest amount was determined in the 600W-90s process. The ascorbic acid determinations of broccoli samples are shown in Table 4.

Table 4. Ascorbic acid content of broccoli samples

Extraction conditions	mg/100 g
360 W /30 s	$191.45^{A} \pm 3.00$
360 W / 60 s	$162.42^{B} \pm 6.04$
360 W / 90 s	$135.39^{C} \pm 4.49$
600 W / 30 s	$128.22^{C} \pm 7.36$
600 W / 60 s	$99.16^{D} \pm 6.69$
600 W / 90 s	$85.97^{D} \pm 6.08$
900 W / 30 s	$134.00^{C} \pm 9.45$
900 W / 60 s	$91.33^{D} \pm 6.79$
900 W / 90 s	$86.18^{D} \pm 6.28$

The differences in letters indicate that microwave power and time are statistically effective on color values ($p \leq 0.05$).

Discussion

L^* values of broccoli samples were found to increase in direct proportion to the increase in microwave power. There is a statistical difference between the L^* values of broccoli samples treated with 360W and 900W ($p \leq 0.05$). However, there was no statistical difference in the samples treated with 600W, especially between 30 s and 60 s ($p > 0.05$). In another previous study, while the L^* value of raw broccoli samples was 39.39 ± 5.02, this value decreased to 32.11 ± 3.25 when treated with microwave (850W / 5min) and the decrease was found to be statistically significant ($p \leq 0.05$) (Czarnowska-Kujawska et al. 2022). If we compare this study with ours, it is seen that the closest L^* value to the raw broccoli sample was obtained with 900W/90s.

The difference between the a^* values of broccoli samples with different extraction power and times was found to be significant. While the closeness of the samples to the red color was the highest in this process with 360W/60s, the closeness to the green color was found to be in 900W/90s processes. Another previous study reported that the a^* value of control broccoli samples decreased gradually with MW treatment and was significant ($P \leq 0.05$) (Wei, et al. 2021).

In the study, it is seen that b^* values of broccoli samples increase in direct proportion as the microwave power increases. In this way, it was found that the tendency towards yellow color increased with increasing microwave power.

There are two conflicting views in the literature regarding the level of microwave output power and the time required for extraction. For example, data for the optimization of the MAE (microwave-assisted extraction) of total phenolics from *Ipomoea batatas* suggest that applying a high microwave power for a short period of time may be the most effective way to extract phenolics from plant material. On the other hand, high temperature can cause thermal degradation of phenolic compounds. Therefore, the obtained moderately optimal extraction

conditions are not unexpected, they were also noticed in our study. In another previous study, the total phenolic content of raw broccoli samples was 94.0 ± 3.5 mg/100 g GAE, while it decreased to 75.9 ± 0.3 mg/100 g GAE after MAE treatment (Czarnowska-Kujawska et al. 2022).

When the antioxidant activity (EC_{50}) values of broccoli samples were examined, it was concluded that DPPH radical had the highest scavenging power with the lowest amount of 900W/30s. In addition, it was concluded that the EC_{50} values of the treatments were statistically significant ($p \leq 0.05$). The effects of different cooking methods on the antioxidant activity of broccoli have been investigated in various studies (Turkmen et al. 2005; Gliszczyńska-Świgło et al. 2006; Şengül et al. 2014; Dolinsky et al. 2016; Wu et al. 2019). Previous studies have shown that the radical scavenging capacity of vegetables decreases after cooking, regardless of the treatment. In addition, it has been reported in the literature that cooked broccoli results in a higher scavenging capacity than raw samples (Faller et al. 2009).

The ascorbic acid content of fresh broccoli is highly variable, and its content depends on different maturity at harvest, growing conditions, soil condition and post-harvest storage (Podse et al. 2006). In a previous study, the amount of ascorbic acid (mg/100g FW) contained in broccoli samples was investigated by exposing them to microwave extraction (600W/30-60-90s) processes. Accordingly, the ascorbic acid amounts of broccoli samples were determined as 85.1 ± 2.5 70.2 ± 2.1 54.3 ± 2.1 mg/100 g FW (600W/30-60-90s) (Zhang et al. 2004). Similar to the above study, ascorbic acid content of broccoli samples decreased with increasing time. The results suggest that cooking affects the retention of ascorbic acid in tissues.

Conclusion

It was observed that different microwave power and time parameters applied to broccoli samples made the color values of the samples statistically significant ($p \leq 0.05$). As a result, the highest amount of vitamin C (ascorbic acid) in microwave-assisted extraction was observed at

360 W/30 s condition, while the lowest amount of vitamin C (ascorbic acid) was at 600 W/90 s condition. Total phenolic compounds of broccoli samples varied according to the process parameters. In some processes, total phenolic compounds decreased with increasing time and vice versa. Therefore, these processes were found to be statistically significant ($p \leq 0.05$). Among the EC50 values of broccoli samples, 900W / 30s parameters were found to be the best free radical scavenging conditions. It is thought that these data obtained will be a pioneer for future studies.

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A Novel Temperature Responsive Nano *In Situ* Gel For The Ocular Delivery Of Flurbiprofen

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Abstract:
The present study involves formulation and evaluation of a Temperature responsive nano *in situ* gelling system to improve the solubility and ocular residence time of flurbiprofen. This study was carried out in two phases; In first phase due to its insoluble nature it was formulated in the form of nanoparticulate system and it was evaluated and characterized. The nanoparticles obtained demonstrated an average size range of 150 to 250 nm in diameter, up to79.67% encapsulation efficiency and up to86.45 % drug release over a period of 2 h. In second phase, these particulate system were dispersed in an aqueous solutions of Pluronic F 127 (14%) and various concentrations of Carbopol 934 in combination to form a nano *in situ gel*. The prepared *in situ gel* was investigated for its physicochemical properties i.e., pH, flow ability, sol–gel transition temperature, gelling capacity and rheological properties.Carbopol did not showed any significant effect on sol-gel transition temperature in optimized concentration($\leq$ 0.3%)but altered gelling capacity,pH,transparency of the formulations in optimized *in situ* gelling formulation(NIGF3), approximately 92 % of *in vitro* drug release was observed after 6 h.NIGF3 increased precorneal residence time and high concentration in aqueous humor when compared to flurbiprofen eye drops. Higher concentration of drug in aqueous humor was due to its increased saturation solubility of drug and increased residence time was attributed due to formation of gel matrix-embedded nanoparticles. This demonstrated that nano *in situ* gels (NIGF3) containing aqueous solutions of 0.3% w/v concentrations of Carbopol 934 with Pluronic F 127 may significantly prolong the residence time and improve bioavailability of a water insoluble drug.

Key words: Nano *in situ* gel**, Flurbiprofen,** Nanoparticles,Pluronic F 127, Carbopol 934 ,Ocular Delivery.

Introduction:

Poor ocular bioavailability of drugs (>1%) from conventional eye drops (i.e., solutions, suspensions, and ointments) is mainly due to the precorneal loss factors that include rapid tear turnover, nonproductive absorption, transient residence time in the cul-de-sac, and the relative impermeability of the drugs to the corneal epithelial membrane. Frequent instillations are therefore necessary to achieve desired pharmacological effect even though result in toxicity, irritation and lack of patient compliance (Gaudanar et al., 2009)[1]But the stability of the gel formed depends on the nature and concentration of polymer used(Lee., 1990)[2].

Pluronic's(Poloxamers) are a triblock copolymer poly(ethylene oxide)-b-poly(propylene oxide)- b-poly(ethylene oxide) (PEO–PPO–PEO) showing amphiphilic behavior due to hydrophilic ethylene oxide domains and hydrophobic propylene oxide domains. Pluronics have been widely used as an ocular drug delivery system because they could prolong drug release and present satisfactory inertia for eye tissue (Dumortier et al., 2006) [3]. A major disadvantage of Pluronics is their low mucoadhesive activity and its ability to form gel at a higher concentration(>18%),due to which there might be decrease in the phase transition temperature and also corneal damage as reported previously at such higher concentration(Vadnere et al., 1984)[4].Therefore, some Pluronic-based ophthalmic formulations have been formulated with reduced concentration of Pluronics (20 to 16%) when used in combination, improving mucoadhesive property by addition of polymers such as Carbopol (Qi et al., 2007) [5].Carbopols are polymers of acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol. Carbopol 934 (Prop-2-enoic acid) was used as a viscosity enhancer (at low concentration- 0.1 to 0.4%) and possess mucoadhesive property in combination with Pluronic F 127.(Lin and Sung2000) [5].

Flurbiprofen belongs to the class of non-steroidal anti-inflammatory agents used in prophylaxis of miosis during ocular surgery and postoperative ocular inflammation..The usual adult dose of flurbiprofen sodium ophthalmic solution for the inhibition of intraoperative miosis is total of four drops- 1 drop approximately every 1/2 hour, beginning 2 hours before surgery.(AHFS Drug 2002)) [7].Nan suspension can be used to formulate such poorly water soluble drugs which offer the opportunity to address many of the deficiencies associated with such class of drug (Rainbow, 2004; Keck and Muller, 2006; Kesisoglou et al., 2007)) [8,9.10].It is reported that HPMC have the stabilizing property and hence used previously in the preparation of nanosuspensions (Dong et al., 2009) [11].In order to prevent the degradation of flurbiprofen in aqueous medium and enhance its physical stability, the prepared liquid formulation was spray-dried using spray dryer (LU 222 advanced lab) to obtain dry nanoparticles. It is reported that spray dried nanoparticles have highly porous morphologies and shown to improve the solubility of poorly soluble drugs(Chaubal and Popescu 2008)[12].

The following work involves an attempt to formulate a temperature responsive nano in-situ gel which may demonstrate improvement in the solubility of drug and provide higher precorneal residence time and improved ocular bioavailability. This work was also proposed to formulate a novel nano *in situ* gel with the low concentration of Pluronic F127 (14%) along with Carbopol (0.3%) in combination in order to provide higher precorneal residence time in ocular environment.

Materials andmethods:

Materials

Flurbiprofen was obtained as a kind gift sample from Sun Pharmaceuticals Pvt. Ltd. Vadodara. Pluronic F127 was obtained as a gift sample from BASF Co. Mumbai.Carbopol 934 was purchased from Research- Lab Fine Chem Industries; Mumbai. HPMC was obtained from Colorcon Asia, and Goa. Benzalkonium Chloride was purchased from Ranbaxy Fine Chem. Pvt., Ltd., Mumbai. All other chemicals were of analytical grade and used without any further purification.

Preparation of Flurbiprofen nanosuspension

Flurbiprofen nanoparticles were produced by antisolvent precipitation technique. Briefly, acetone and water were used as solvent and antisolvent respectively and the ratio of solvent to antisolvent was 1:20.A certain amount of drug was completely dissolved in water-miscible solvent under sonication. The obtained drug solution was then injected into aqueous phase containing the stabilizer (HPMC) with different concentration (W/V) under stirring at 1000 rpm for 30 minutes. The formulation is prepared using high-speed homogenizer (Remi Motors Ltd., Mumbai, India) at 8,000 rpm for 2 hr to obtain nanoparticles. Evaporation of the internal phase under stirring ensures the precipitations of polymeric nanoparticles.The resulting aqueous systems were sterilized by membrane filtration (0.22μ) in aseptic condition (Dong et al., 2009) [11].

Conversion of nanoparticles into Dry Powder

The prepared liquid nanosuspension formulation was subsequently spray-dried using a LU-222 advanced lab spray dryer (Labultima111/112, Mumbai, India) with a 0.7-mm nozzle, using the following standard operating conditions: inlet temperature, 105 °C, the pump was set at 10 ml/ min. Thus nanoparticles were converted into dry powders using spray dryer. The dry powder exhibited good uniformity and the yield of dry powder was approximately 86% (Chaubal and Popescu 2008; Johansen et al., 2000)[12,13]

Evaluation of flurbiprofen nanoparticles

Particle size and surface morphology

The size distributions along the volume mean diameter of the nanoparticle was measured by Dynamic Light Scattering Particle Size Analyzer (Nanotrac Particle Size Analyzer). Shape and surface morphology of nanoparticles was done by Scanning Electron Microscopy (JSM-T330A, JEOL).Small amount of nanoparticles was placed on an electron microscope brass stub. These were then coated with gold in an ion sputter. The shape and surface morphology of the nanoparticles was determined from the photomicrographs of optimized formulation (Gao et al., 2010; Pignatello et al., 2002) [14,15].

Percentage entrapment efficiency

To determine the entrapment of flurbiprofen in nanoparticles, prepared nanosuspension (20 ml) was subjected to cold centrifuge at ~ 4 °C and 14,000 rpm using Sigma 3K 30 Ultra centrifuge for 30 min. From the supernatant, 10 ml solution was taken and diluted appropriately. The resulting solutions were analyzed for flurbiprofen content using single beam UV Spectrophotometer and % Entrapment efficiency (% EE) was calculated using following equation.(Gao et al., 2010; Pignatello et al., 2002) [14, 15].

$$\% EE = \frac{\text{Total amount of drug- Free dissolved drug}}{\text{Total amount of drug}} \times 100$$

In Vitro drug release

The in vitro drug release from flurbiprofen loaded NPs was measured in triplicate by UV till 2 h.10 mg of nanoparticles were suspended in 10 mL of PBS (phosphate buffer solution, pH =7.4) containing Tween 80 at 0.1% (w/v) in a screw capped tube. The tube was placed at 37±0.5 ºC in an orbital shaker (Ecotron_, Infors AG CH-4103) at 120 rpm. At determined time intervals, the tubes were centrifuged (Sigma 202 M) at 11000 rpm for 14 min in cold. The pellet was re-suspended in 10 mL of fresh PBS/Tween 80 at 0.1% and placed back in the shaker (Zhang et al., 2008).The supernatant was collected for flurbiprofen quantification and assessed by UV analysis at 247 nm(Shimadzu UV, Japan) after suitable dilution. (Gao et al., 2010) [14].

Preparation of polymeric solutions

Aqueous solutions of Pluronic F-127 and various concentrations of carbopol 934 were prepared, in order to optimize the compositions suitable for use as *in situ gel*ling systems for ocular use as shown in Table 1. Aqueous solutions of the Pluronic F 127 were prepared by the cold method Then, nanoparticle and Carbopol 934 were respectively added into the solutions of gelling agents and stirred continuouslyuntil homogeneous solutions were obtained. stirring at 4 ˚C. solutions containing 0.3% flurbiprofen were prepared by dissolving accurately weighed flurbiprofen nanoparticles. The samples were stored at 4˚C until further use (Lin and sung 2000) [16].

Formulation code	ConcentrationofPluronicF127(%wt/vol)	Concentrationo fCarbopol-934(%wt/vol)
NIGF1	14	–
NIGF2	14	**0.2**
NIGF3	14	**0.3**
NIGF4	**14**	**0.4**

Table 1: Formulations Formulation composition of Thermo-reversible ocular gels containing Pluronic F 127 and carbopol 934.

Evaluation of in situ solution:

Rheological Studies*:*

The rheological studies of the formulations were carried out using rheometer (Brookfield model R/S CPS, USA). the temperature of the solution was increased from 25°C to 36°C. The viscosity

of the samples was recorded before and after gelling. Each experiment was performed in triplicate (Gupta et al., 2010) [17].

Measurement of Gel Strength:

50 ml of gel sample was placed in a 100-mL graduated cylinder and gelled in a thermostat at 37^0C. The apparatus for measuring gel strength, as described by was allowed to penetrate into the gel. Gels strength,*i.e.*, the viscosity which means the viscosity of the gels at physiological temperature, was determined by the time (seconds), the apparatus took to sink 5cm down through the prepared gel (Bromberg 1998) [18].

Measurement of Phase Change Temperature

An aliquot of 2 ml refrigerated tested **flurbiprofen**nano *in situ gel* formulation was transferred to a test tube and sealed with a par film. The tube was maintained in a water bath at 4°C. The temperature of the water bath was increased gradually in increments of 3°C in the beginning of the experiment and then 1°C increments in the region of sol–gel transition temperature (25–34°C) and 0.1°C when it approaches gelation. The tested formulation was left to equilibrate for 10 min at each new setting (or for 2 min when temperature was increased with an increment 0.1°C). The gelation is considered to be occurred when the meniscus of the formula would no longer move upon tilting through angle 90°. The maximum accepted gelation temperature tested was 34°C, which represents the corneal surface temperature. Each sample was measured at least in triplicate (Bromberg 1998) [18].

Drug Content:

The drug content was determined by taking 1ml of the formulation and diluting it to 100 ml with distilled water. Aliquot of 5 ml was withdrawn and further diluted to 25 ml with distilled water. Flurbiprofen concentration was determined at 247 nm by using UV spectrophotometer (Reddy et al.,2011) [19].

***In vitro Release Studies**:*

In vitro release studies of **flurbiprofen** from the formulation were performed by dialysis tubing membrane (Himedia Ltd., India) with a molecular weight cut-off of 12,000–14,000. The membrane opening was tied to the mouth of a PVC test tube (1cm diameter) and dipped in a 100 ml beaker containing STF (pH 7.4, 50 ml). The entire system was placed in beaker (250 ml) containing distilled water maintained at 37±0.5 ◦C. A small magnetic bead (Sigma–Aldrich, USA) was placed in the beaker and was stirred at 100rpm on a magnetic stirrer (Remy India Ltd.). Aliquots, each of 5 ml volume were withdrawn at hourly intervals and replaced by an equal volume of receptor medium. The aliquots were suitably diluted with the receptor medium and analyzed by UV- Vis spectrophotometer at 247 nm. (Ma et al., 2008) [20].

Ocular Irritation Studies*:*

Ocular irritation studies were performed on four male albino rabbits each weighing 2-3 kg. The sterile formulations were instilled twice a day for a period of 21 days and the rabbits were observed periodically for redness, swelling and watering of the eye(Ma et al.,2008)[20].

Stability Studies:

Stability is defined as the extent to which a product retains, within specified limits and throughout its period of storage and use (i.e. its shelf life), the same properties and characteristics that it possessed at the time of its manufacture. Stability testing is performed to

ensure that drug products retain their fitness for use until the end of their expiration dates. In optimized *in situ* gelling formulation were subjected to stability studies at ambient humidity conditions at 4±1°C , and 25±1°C for a period of one month. The samples were withdrawn after 7, 14 and 30 days and were evaluated for following parameters.

Results

Size and surface morphology of nanoparticle

Scanning electron photomicrographs of all the four formulations were taken. Average particle size of nanoparticles of flurbiprofen wasshown in Table 2. The mean particle size for formulations F1 to F4 varied in range between 140 to 250 nm, which is appropriate for ocular drug delivery. The porous nature of the spray-dried nanoparticles was observed in the SEM (Figure 1).

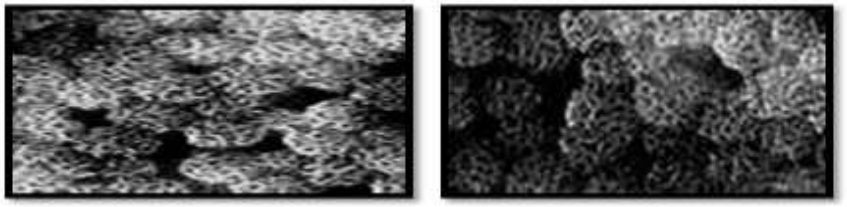

Figure 1: SEM images of spray dried drug loaded nanoparticles using HPMC

Percentage entrapment efficiency

The drug content in four batches of Flurbiprofen nanoparticles was studied. The amount of drug bound per 1 ml of nanosuspension was determined in each batch. The maximum entrapment was found in F1 (79.67%) and lowest entrapment in F3 (69.35%) entrapment efficiency of nanoparticles of flurbiprofen was shown in Table 2.

Formulations	Mean particle size (nm ± S.D.)	Polydispersity index (PI)±S.D.	% EE (%±S.D.)	Zeta potential (mv)	Cumulative % drug release (90 min)
NF1	246.90 ± 7.51	0.357 ± 0.16	79.67 ± 1.01	-21.80	84.40 ± 1.13
NF2	212.87 ± 5. 35	0.342 ± 0.08	79.35 ± 1.36	-23.78	93.42 ± 1.32
NF3	175.33 ± 3.76	0.488 ± 0.03	69.35 ± 0.69	- 20.60	85.63 ± 1.06
NF4	144.67 ± 3.32	0.442 ± 0.18	75.21 ± 1.05	-22.86	86.15 ± 2.09

Table 2: Particle size, Polydispersity index, % Entrapment efficiency, Zeta potential of spray dried nanosuspension formulations F1 to F4

Zeta potential and Polydispersity index

The zeta potential values remained in the range of negative values for all the batches (-20.60 to -23.78 mV) (Table 3). It promotes particle stability because the repulsive forces prevent aggregation with aging. The negative charge on the nanoparticles is due to the ionization of the carboxylic end groups of the surface polymer. The mean polydispersity index values for the

drug loaded formulations varied in the range of 0.357 to 0.542. It could be inferred that all the formulations showed mid-range polydispersity. PI Results are tabulated in table 2.

In Vitro drug release

In vitro drug release from the Flurbiprofen nanoparticles in phosphate buffer pH 7.4 was performed using dialysis bag diffusion technique. The profiles are biphasic, with an initial burst of drug release attributed to surface associated drug, followed by a phase of slower release as drug entrapped inside the particle diffuses out into the release medium. Particle size has a direct effect on the drug release profile from the formulations. Formulation F4 with a smaller average particle size 144.67 nm gave large initial burst release of 36.68 % after 10 min and 96.88 % drug release after 90 min. Formulation F1 with a larger average particle size 246.90 nm gave small initial burst release of 26.63 % after 10 min and 84.12 % drug release after 90 min. . A similar tendency was observed with formulation F2 (particle size 212.87) and F3 (particle size 175.33 nm) which showed initial burst release of 29.32 % and 32.07 % after 10 min while 87.07 % and 91.18% drug release after 90 min. respectively as shown in Figure 2.

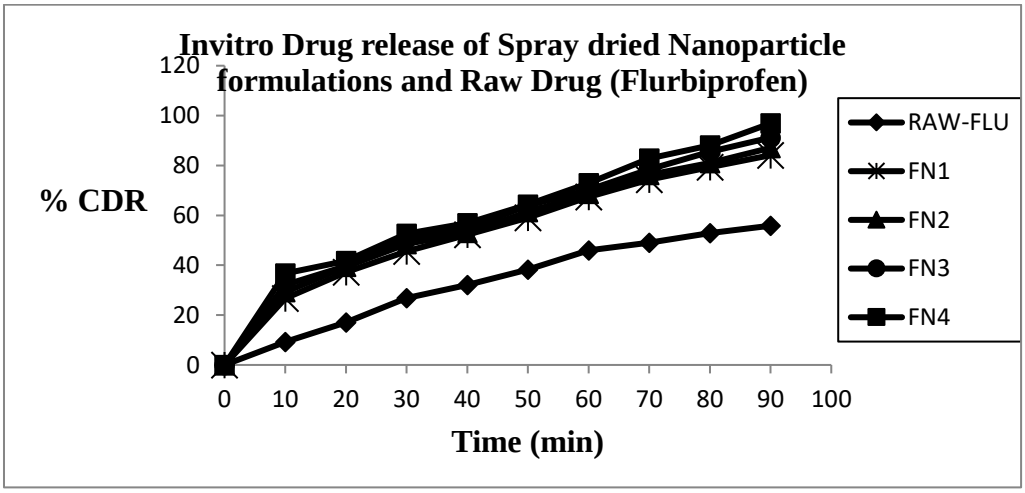

Figure 2: *In Vitro* drug release profile of raw flubiprofen and nanoparticle formulation F1, F2, F3, F4.

Rheological Studies gelling solutions

Formulations	Viscosity(CPS)	Flowabilityattemperatures(◦C) 4±1	25±1	36±1	Tsolgel(°C)
NIGF1	2190	111	111	11	36.20±0.9
NIGF2	13,428	111	111	1	35.80±1.1
NIGF3	18,340	111	111	—	35.92±2.1
NIGF4	20,917	111	11	—	31.30±0.7

Table 3: Viscosity, Flow ability and gelation temperature of various nano *in-situ* gel formulations

The viscosity was directly dependent on the polymeric content of the carbopol.The maximum viscosities of NIGF1 (without carbopol), NIGF2, NIGF3 and NIGF4 were 2190cps, 13428cps, 18340 cps,20917cps(Shown in Table 3). The formulation NIGF1 showed phase transition temperature around 36^0 C, but gave a good flowabilty without sufficient gelling strength.So carbopol was added in varying concentrations in order to increase its gelling strength which also showed slightly decrease in phase transition temperature(shown in Figure 3).

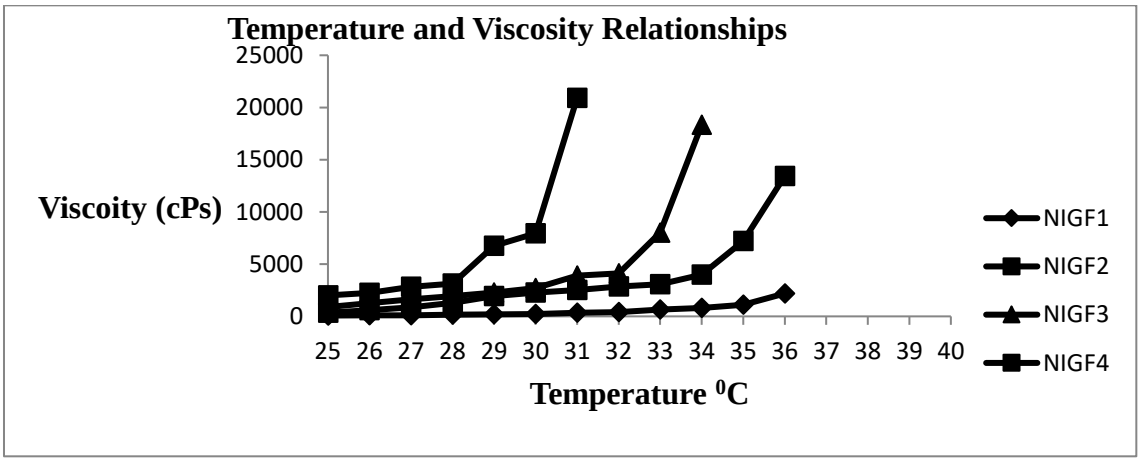

Figure 3: Temperature and Viscosity Relationships of nano in situ gel formulations containing Pluronic F127(14%) and different concentrations of carbopol 934.

Measurement of Gel Strength and Drug Content

All gel formulations exhibited good gel strength. As the concentration of carbopol increases, the gel strength decreases since the carbopol shows its pH sensitive property in the ocular pH(7.4). The drug content was found to be in acceptable range for all the formulations. The percent drug content of NIGF1, NIGF2, NIGF3 and NIGF4 formulation found to be in the range 86–98 % indicating uniform distribution of nanoparticle (shown in Table 4).

In vitro release Studies

All the formulated nano *in situ gel*s, NIGF1, NIGF3, NIGF3 and NIGF4 showed initial burst drug release of about 38%, 36%, 35%, and 30 % to the medium after 1 h respectively. The next 6 h release studies showed 95.04, 97.02 %.92.8 %, 90.5 % drug release for NIGF1, NIGF3, NIGF3, and NIGF4 respectively. (shown in table 4).

Formulations	Gel strength(sec)	Drug content(%)	Cumulative%drugrelease$^{\omega}$(6hrs)
NIGF1	132sec	90.37±0.61	95.04±0.11
NIGF2	123 sec	87.64±0.48	97.02±0.31
NIGF3	107 sec	92.21±0.59	92.80±1.40
NIGF4	89 sec	90.76±0.82	90.50±1.30

Table 4: Characteristics (Gel Strength and drug content) and *in-vitro* drug release of nano *in-situ* gel formulations

Comparative In vitro release

The in vitro release of nanoparticles and nano in situ gel of nanoparticles were studied in simulated tear fluid, pH 7.4 (Fig. 4 Cumulative percentage release vs. time). Flurbiprofen from the nanoparticles was released within 2 h . In situ gel showed high burst release during the first hour (35%) while a sustained release was maintained further release of drug from nanoparticles for 6 h.

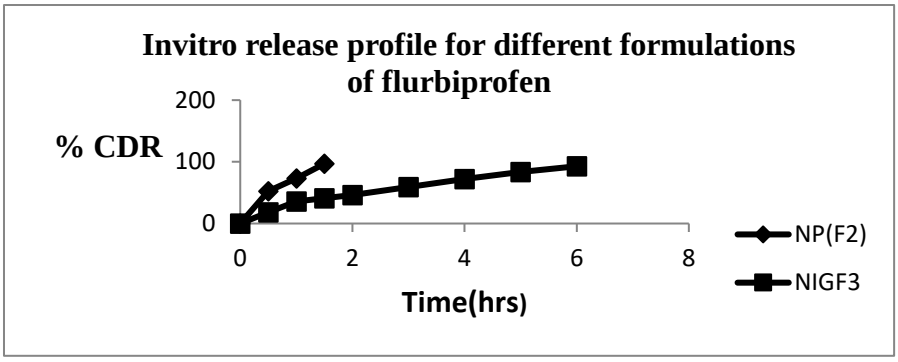

Figure 4: In vitro drug release profile of Flurbiprofen containing nanoparticles and nano in situ gel.

Ocular Irritation Studies*:*

The results of the ocular irritation studies indicated that NIGF3 were non-irritant. Excellent ocular tolerance was noticed for all the three formulations. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae were visible. No signs of redness, watering of the eye and swelling were observed throughout the study with the formulations.

Stability Studies

Optimized *in situ* gelling formulation (NIGF3) of flurbiprofen was subjected to stability studies. The observations are shown in Table 6. Formulation showed slight decrease in drug content at 25^0C (97.3%, 97.5% & 97.38% respectively) after 30 days of storage whereas at 4^0C showed significant decrease in the drug content (90.89%, 89.45% & 91.22% respectively) after 30 days of storage. This significant decrease in drug content is due to the precipitation of drug in formulations at refrigeration temperature (4^0C). The in vitro drug release from formulations was increased after stability period. The stability studies confirmed that *in situ* gelling formulations of flurbiprofen remained stable at ambient temperature 4^0C and 25^0C and humidity(shown in table5).

Stability studies of formulation NIGF3

	Parameters evaluated					
	7 days		14 days		30 days	
Storage condition	drug content	% CDR(6h)	drug content	% CDR(6h)	drug content	% CDR(6h)
4°C,	**92.13±0.21**	**95.03±0.42**	**91.91±0.11**	**94.98±0.61**	**91.61±0.23**	**94.67±0.09**
25°C,	**97.01±0.31**	**97.50±0.61**	**97.38±0.42**	**90.89±0.56**	**89.45±0.16**	**91.22±0.64**

Table 5: Stability studies: Characterization of Formulation NIGF3 Parameters evaluated.

Discussion

The result showed that high HPMC concentration in organic phase resulted in the increase of the particle size and the decrease of surface charge of nanoparticles that would slightly affect the amount of flurbiprofen adsorbed on the surface of nanoparticles. The particles with a good porous structure could be obtained by spray drying. With 0.5% concentration of HPMC was

optimized (F4) which have 144.67 nm particle size and 75.21% entrapment efficiency and gives approximately 96% invitro release. These data showed that nanosuspensions with spray drying have good affinity to improve solubility of the poorly soluble drug candidates.

The results of nano *in situ gel* demonstrate that problem with formation of gel with less concentration of Pluronic F-127 can be overcome by slight addition of carpobol in varying concentration. Concentrations of Carbopol (0.2%, 0.3% and 0.4% w/w) could slightly alter flow ability by increase in the viscosity of Pluronic gel(shown in Figure 3). The study of phase change temperature indicate that gels with different carbopol concentration show phase change temperature around 31 ^{0}C to 37 ^{0}C after dilution of the gels with the artificial tear fluid. This showed that carbopol could slightly alter sol–gel transition temperature of the product especially when it is used more than a concentration of 0.3% w/w .Formulation shows solution form in the room temperature (25^{0}C) only at minimum concentration (upto 0.3 % w/w). Pseudoplastic behavior of the formulation suggests its suitability for ocular delivery. These results indicated the viscosity of the *in situ gel* solutions is directly proportional to the concentration of carbopol and suggested that the optimum concentration for Carbopol 934P solution used in situ gel forming system as a viscosity enhancer was 0.2% – 0.3% (w/w) with 14% (w/w) of Pluronic F127.

The high burst release of the nano in situ gel was observed due to porous nature of the spray-dried nanoparticles. Since the nanoparticles were dispersed in solution form of *in situ gel*, they undergoes hydration which leads to faster dissolution of the drug in first hours (Cohen et al., 1997). The drug release from gel forming solution may occur partly via diffusion from the gels and partly due to simultaneous dissolution of the gels into the surrounding media (Costa et al., 2001). However, after 1 hours sustained release was observed because of enhancement in the strength of the *in-situ gel* by increasing the viscosity of the solution by presence of carbopol. The *in vitro* release of drugs from the gel results demonstrated that the combination of carbopol and Pluronic F127 can have major advantages for the loading of poorly soluble compounds as well as their sustained release in ocular environments. Especially, these mucoadhesive formulations based on the combination of carbopol and pluronic F127 gels would be suitable for ocular drug delivery.

Conclusion

The developed nano *insitu* gel formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability and saturation solubility by the formulation of nanosuspension. To further prolong the precorneal residence time of the flurbiprofen nanosuspension in the eye, the novel nano *in situ* gelling system was formulated in combination with Pluronic F127 (14% w/w) and carbopolpolymers. The formulation was liquid at the 25^{0}C and underwent rapid gelation upon raising the temperature to 32°C to 37^{0}C.The rheological measurements showed that the combination of Pluronic-F127(14% w/w) and carbopol polymers which had bioadhesive properties formed temperature-responsive gels with suitable gelation temperature at lower concentration of carbopol (0.3% w/v). The zero-ordered release properties were observed under experimental conditions. The rates of release of drug from such polymer gels were mainly dependent upon the gel dissolution. The in vitro release results

indicated that the developed flurbiprofen Nano *in situ* gel was able to prolong and control flurbiprofen release for more than 6 h, thus maintaining concentrations for a longer duration. *In vivo* experimental results, along with the rheological and *in vitro* drug release studies, good stability and demonstrated that *in situ* gels containing combination of Pluronic-F127(14%) and carbopol(0.3%)polymer may significantly precorneal resident prolong the time.

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Chemometric Analysis of Different Cake Additives' Chemical Composition

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Abstract:
Cake products can be found in a wide variety of forms and are one of the most important products in the bakery industry. Pumpkin (*Cucurbita pepo* L.) grown to produce pumpkin seeds does not have an option for evaluation in the food industry. Quinoa (*Chenopodium quinoa* Willd.) is an annual plant of the *Chenopodiaceae* family that originates from South America and can be cultivated in many different types of soils and climates. It has been reported to be rich in essential and semi-essential amino acids as well as high protein content. Lupine (*Lupinus albus* L.) seeds have been used in human nutrition and treatment for many years. However, in the last 20 years, quite new properties of lupine have been discovered and various uses as a functional food have begun to emerge. In this study, Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were used as multivariate analyses to reveal the chemical properties of different samples. As a result, it was determined that the chemical properties of the samples can be differentiated according to the methods. The effects of using quinoa, lupine, and pumpkin flours in cake production on chemical analyses of cake samples were evaluated using PCA score and loading charts. When the PCA score plot was examined, it was observed that the samples were grouped. According to the results, the quinoa and lupine samples differentiated from the pumpkin sample according to principal component 1. The lupine sample differentiated from the pumpkin and quinoa samples in terms of principal component 2. Accordingly, the PCA plot shows the distribution of the samples on the two principal components that make up 98.4% of the total variance. The first principal component explains 63.7% of the total variance and the second principal component explains 34.7% of the total variance. It was observed that the samples were separated by principal component analysis. According to the hierarchical clustering analysis, three different groups were formed. According to the multivariate analysis, the samples were well separated from each other in terms of chemical properties.

Keywords: pumpkin, lupine, quinoa, chemometric analysis

1. Introduction

Cucurbita pepo is an annual creeping or climbing plant with 5- angled stems up to 15 m long. The shallow root system is branched, growing from a well-developed taproot. The stems are rugged and bristle, branching 6-24 cm long often rooting at the nodes. The plant bears tendrils at 90 degrees to the leaf insertion, which are coiled and 1-6- branched (Yadav et al., 2017). Cucurbita pepo is traditionally used in many countries to treat several diseases e.g., as an anti-inflammatory, antiviral, analgesic urinary disorders, antiulcer, antidiabetic, and antioxidant (Martha et al., 2016). Pumpkin has been considered beneficial to health because it contains various biologically active components such as polysaccharides, para-aminobenzoic acid, fixed oils, sterols, proteins, and peptides (Caili et al., 2006; Murkoviz et al., 2002). These are depicted by a low content of fat (2.3% pumpkin pulp is not a rich source of oil), carbohydrates (66%), proteins (3%), and high carotenoid content with values of 171.9 to μg/g. Food value per 100 g is calories 80 kcal, crude fiber 11.46%, ash 16%. The mineral analysis indicated that pumpkin pulp contained high levels of Mn (0.5 mg/kg), Fe (1.37 mg/kg), Cu (mg/kg), Pb (0.29 g/kg), P (11.38 mg/kg), Ni (0.5 mg/kg), Ca (179 mg/kg), Mg (190 mg/kg), Na (159 mg/kg) and K (160

mg/kg) (11). The level of Pb (0.210.25 mg/kg), and Cu (25 mg/kg) is within an acceptable range (Caili et al., 20026; Elinge et al., 2012).

Quinoa has been cultivated for thousands of years in the Andean region of Bolivia and Peru. It is known by different local names, or simply quinoa or quinoa (quinoa is in Quechua). His plant was called “the mother grain” by the Incas and was considered a gii from their gods, used even for treating medical issues. Traditionally, quinoa seeds were roasted and cooked, added to soups, used as a cereal, and even fermented into beer or chichi (a traditional drink of the Andes) (Jancurova et al., 2009; Galvez et al., 2009; Cooper, 2015). In recent years, quinoa has attracted attention with its resistance to stress conditions. (Chenopodium quinoa Willd.) plant, its importance in human nutrition, and stands out for its high nutritional value. Quinoa, the plant of the Andes Mountains, is a human and animal species in the world. It is expressed as the plant of the future in its nutrition (Tan & Temel, 2019). Quinoa (Chenopodium quinoa Willd.) is a grain-like food crop traditionally used to provide nutrition and sustenance to Andean indigenous cultures for centuries. Quinoa is mainly grown in Peru, Bolivia, Ecuador, Argentina, Chile, and Colombia, although in the last years, it has been introduced in Europe, North America, and Africa with high yields. Quinoa consumption in high-income countries is increasing although is still low compared with the main producer countries of this plant. Thus, quinoa annual consumption in Bolivia and Peru was 2.37 kg/person and 1.15 kg/person, respectively, whereas the consumption was 0.03 kg/person in the US (Abugoch, 2009; FAO, 2013).

Quinoa’s superiority over these and other grains (rye, barley, and oat, among others) results from its richer protein, lipid, and ash content. The protein content (expressed as g/100 g edible matter) of quinoa seeds is ranged between 13.1% and 16.7%. These values are higher than those of rice, barley, corn, and rye and close to that of wheat. Albumins and globulins represent the major storage quinoa proteins, with percentages of 35% and 37%, respectively. However, prolamins are present in low concentrations. In addition to their high quantity, quinoa proteins are accepted as high-quality proteins because of their balanced pattern of essential amino acids. All essential amino acids are present in quinoa protein, meeting amino acid has an interesting lipid composition of about 5.5–7.4 g/ 100 g edible matter, higher than wheat (1.7 g/ 100 g edible matter) and rice (0.7 g/100 g edible matter), making quinoa be accepted as an alternative oilseed seed [13]. Palmitic acid is the major saturated fatty acid found in quinoa, constituting 10% of total fatty acids, while unsaturated fatty acids oleic (19.7–29.5%), linoleic (49.0–56.4%), and alpha-linolenic (8.7–11.7%) acids represent 88% of the total fatty acid amount of quinoa seeds, in a similar way to soybean lipid composition [14]. Fatty acids of cell membranes are well protected against damage caused by free radicals by the presence of vitamin E at a higher concentration than that of wheat. The levels of other vitamins such as riboflavin (B2), pyridoxine (B6), and folic acid are also higher than those of most other grains like wheat, rice, barley, and corn. Pyridoxine and folic acid levels in 100 g of quinoa are reported to meet adults’ daily requirements while riboflavin meets 80% of children’s and 40% of adults’ needs. High vitamin C levels have been also determined in quinoa seeds ranging from 4.0 to 16.4 mg/100 g dry matter. However, the thiamin content is lower than that of oat and barley. The mineral content of quinoa is also of great importance. The seeds have high content of calcium, magnesium, iron, copper, and zinc. Many of these minerals are present in higher concentrations than those found in common grains. Moreover, calcium, magnesium, and potassium are found in quinoa in bioavailable forms, thus their contents are considered to be adequate for a balanced diet.

The genus Lupinus, belonging to the legume family Fabaceae, includes almost 300 species. Traditional lupine species have a bitter taste because of the high content of alkaloids (from 1 to

3% of alkaloids in the dry weight of their seeds), where lupine is the main alkaloid together with other minor alkaloids (e.g., albine, hydroxylupanine, sparteine, anagyrine, lupinine, and angustifolin. The high content of quinolizidine alkaloids is associated with severe intoxication European Food Safety Authority (EFSA) considered the available data on the occurrence and consumption of these compounds still insufficient to accurately assess the risk of chronic exposure. Apart from their toxicity, alkaloids have pharmacological benefits such as alternative antibacterial and antifungal agents and agronomic benefits by contributing to the defense function against predatory herbivores and microorganisms (Direction genrale de la sante, 1998; ACNFP, 1996; Romeo et al., 2018; Yánez-Mendizábal and Falconí, 2018). In the twenties', modern breeding programs focused on selecting lupine varieties with low-alkaloid content to ensure the transition from the "wild bitter" to "cultivated sweet" lupine varieties. The mean total alkaloid content of sweet lupine seeds ranges from 0.3 to 0.5% of alkaloids in the dry weight of their seeds, depending on the species, geography, and climate. Sweet lupines demonstrated high suitability for low-input agriculture owing to their high adaptability to temperate and cold climates, low-fertile soils, harsh conditions, and high nitrogen fixation ability. For these reasons, sweet lupines are gaining traction as a more sustainable and non-genetically modified alternative to soy (Gulisano et al., 2019; Burgos-Diaz et al., 2016; Paraskevopoulou et al., 2010). Lupine crop has been recognized as a rich source of proteins similar to soy, where Lupinus mutabilis sweet had higher values than Mediterranean species. Mediterranean species were characterized by high crude fiber (21–40 g/100 g of dry matter). All lupines have a low starch content similar to soy, and provide an average energy of 309 kcal/100 g. Besides macronutrients, lupine seeds are rich in polyphenols, carotenoids, and phytosterols providing several health benefits. Therefore, lupine seeds and fours are widely used in food systems as a nutritious plant protein source since this species has similar content to that of soy and higher than peas. Nevertheless, the incorporation of lupine fours above 10% induces detrimental effects on the food quality (compact structure and hard texture particularly in bakery products as well as dark color and bitterness). Various techniques have been employed to isolate lupine proteins to be used as functional ingredients thereby avoiding the negative effects of fiber and oligosaccharides on quality (Burgos-Diaz et al., 2016; Vogelsang-O'Dwyer et al., 2020; Córdova-Ramos et al., 2020; Bähr et al., 2014; Arnoldi et al., 2015; FAO, 2017; Villarino et al., 2016).

Bakery products and their derivatives have an important place in food consumption all over the world. Bread, pasta, bulgur, biscuits, cakes, and breakfast cereals are the most consumed industrial cereal products. Bakery products constitute 65% of the food industry in Turkey (Türker et al., 2013). Bakery products such as cakes are widely consumed all over the world; hence, their enrichment with vitamins, minerals, polyphenols, and fibers is considered an effective way to produce high nutritional value foods. Today, with the increase in the labor force participation rate of women, biscuits and cake products have begun to come between the preferred products. Being practical, and delicious is a factor attracting consumers. It was found that being delicious is effective in the purchase of every age group (Gül et al., 2017).

2. Materials and Methods

Powder Production

The pumpkins containing 91 ± 1.2% (wet weight) moisture obtained from the producer in the Nevşehir region are brought to the laboratory and shredded by a food processor (Beko Robokit 2154, Turkey), then in a circulating air dryer (Termal, Turkey) until the humidity level of 10% is reached at 60°C in approximately 8 hours and ground in a laboratory mill (Ika MF10, Germany) for the production of pumpkin powder. The obtained powders were stored in a deep

freezer (Nüve FR490, Turkey) at -40°C for cake production in airtight packages. Lupine and quinoa were supplied in dry form from local suppliers. The grains brought to the laboratory in dry condition (humidity level below 10%) were turned into flour with a mill (Ika MF10, Germany) All powders obtained were used in the 150-200 micrometer range.

Analysis of Powders

The amount of oil in the powder obtained from the pumpkin was determined by Soxhlet according to AOCS Am 2-93, the amount of moisture was determined according to ICC Standard Method No: 110/1, the amount of ash was determined according to ICC Standard Method No: 104/1 (Anon, 2000; Anon, 2002).

Fiber Analysis

For the determination of fiber, 3 grams of sample powder was weighed, 50 ml of 5% sulfuric acid was added to it and the volume was completed with 150 ml of distilled water. The mixture was boiled for 30 minutes. As the water decreased during boiling, the volume was completed to 200 ml with distilled water. During boiling, the mixture was stirred with a glass rod. It was then filtered through Machereyund Nagel No: 840W filter paper. Washing was done with hot distill led water until the acid reaction disappeared completely (when the blue litmus paper was touched to the filtrate and the color remained blue). The residue remaining on the filter paper was transferred back to the beaker by washing it with a strainer over a watch glass with the diameter of the filter paper. This time, 50 ml of 5% NaOH and 150 ml of distilled water were added and boiled for 30 minutes. At the end of the boiling, the residue remaining after washing and filtering was transferred back to the beaker, dried at 110 °C, and transferred onto the filter paper given above, whose weight was determined with a precision balance. It was washed first with distilled water, then 3 times with 95% ethanol and ether, and dried in an oven at 110°C in a crucible with filter paper. After it was completely dried, it was cooled in a desiccator and weighed on a precision balance.

Statistical Analysis

The results of the physicochemical analyzes carried out in the study were analyzed with the Minitab 17 program at 95% confidence interval, and the general linear model was used in the analysis of the data. Tukey's multiple comparison test was used to determine the differences between the applications. Each experiment was repeated at least three times. The results of the analyzes are shown graphically with the Excel program. The results of physicochemical analyzes were evaluated using chemometric methods such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) using Minitab 17 (Minitab Inc., State College, USA) program. HCA analysis was performed using Euclidean distance and complete coupling algorithm.

3. Results and Discussion

The data obtained as a result of chemical analyzes were analyzed using principal component analysis (PCA), one of the multiple variable analysis methods. The effect of drying on chemical analyzes of different samples was evaluated using PCA score (Figure 1) and loading charts (Figure 2).

Table 1. Chemical compositon of pumkin powders

	Moisture	**Ash**	**Fiber**	**Oil**
Pumpkin	**3.98 ± 0.71^{c}**	**4.75 ± 0.06^{a}**	**13.39 ± 0.43^{a}**	**0.54 ± 0.03^{c}**
Quinoa	**9.57 ± 0.28^{a}**	**3.92 ± 0.10^{b}**	**8.42 ± 0.13^{b}**	**3.07 ± 0.07^{b}**

Lupin	**6.83 ± 0.02[b]**	**3.91 ± 0.12[b]**	**14.86 ± 0.13[a]**	**6.14 ± 0.34[a]**

When the PCA score plot was examined, it was observed that the samples were grouped. According to the graph, the quinoa and lupine samples differentiated from the pumpkin sample according to principal component 1. The lupine sample differentiated from the pumpkin and quinoa sample in terms of principal component 2.

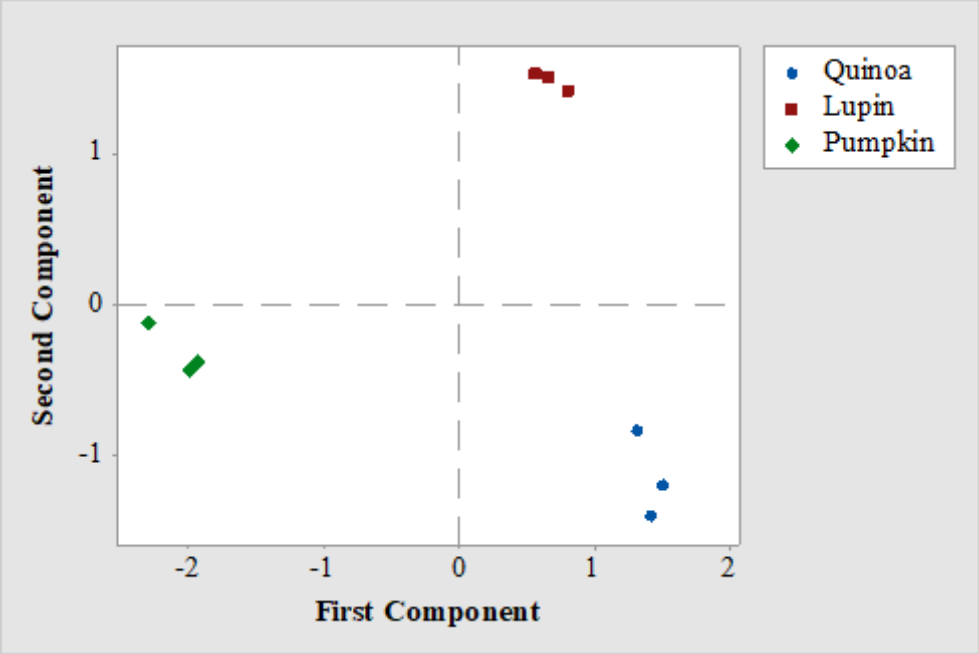

Figure 1. Score plot obtained by PCA according to the chemical analysis results of the samples

When the PCA loading plot was examined, the lupine sample was characterized by fiber and oil, while the pumpkin sample was characterized by ash and the quinoa sample was characterized by moisture.

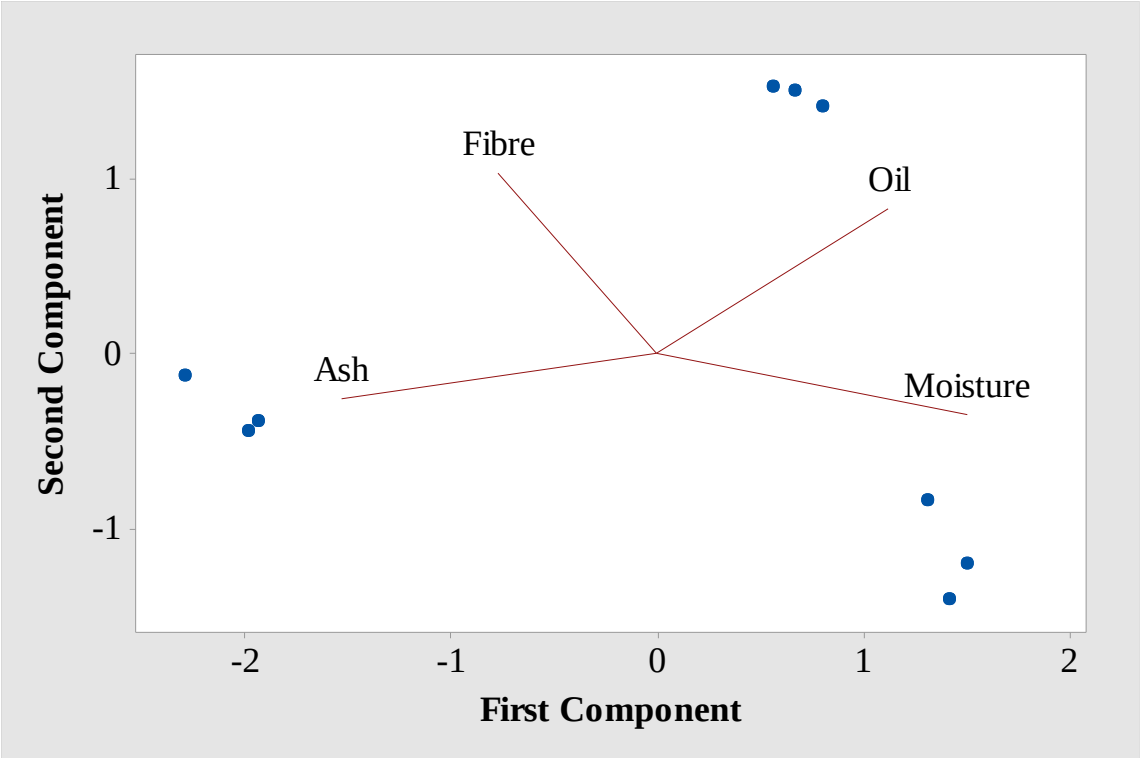

Figure 2. Loading chart obtained by PCA according to the chemical analysis results of the samples.

The eigenvalue, variance and cumulative variance values of the chemical properties of the samples as a result of PCA analysis are shown in Table 2.

Table 2. Eigenvalue, variance and cumulative variance values of the physicochemical properties of the samples as a result of PCA analysis

	Eigenvalue	Variance (%)	Cumulative variance (%)
Principal component 1 (PC1)	**2.5462**	**63.70**	**63.70**
Principal component 2 (PC2)	**1.3889**	**34.70**	**98.40**

Accordingly, the PCA plot shows the distribution of the samples on the two principal components that make up 98.4% of the total variance. The first principal component explains 63.7% of the total variance and the second principal component explains 34.7% of the total variance. The correlation coefficient values between the principal components and the chemical properties (variables) of the samples are shown in Table 3.

Table 3. Correlation coefficient values between the principal components and the chemical properties (variables) of the samples

Variables	PC1	PC2
Moisture	**0.592**	**-0.254**
Ash	**-0.604**	**-0.185**
Fibre	**0.303**	**0.739**
Oil	**0.440**	**0.596**

According to Table 3, the parameters analyzed in principal component 1 showed a positive correlation with moisture, fiber and oil, and a negative correlation with ash value. Fiber and oil values were positively correlated with principal component 2 and negatively correlated with moisture and ash values. The hierarchical clustering analysis (HCA) graph according to the chemical properties of the samples was shown in Figure 3.

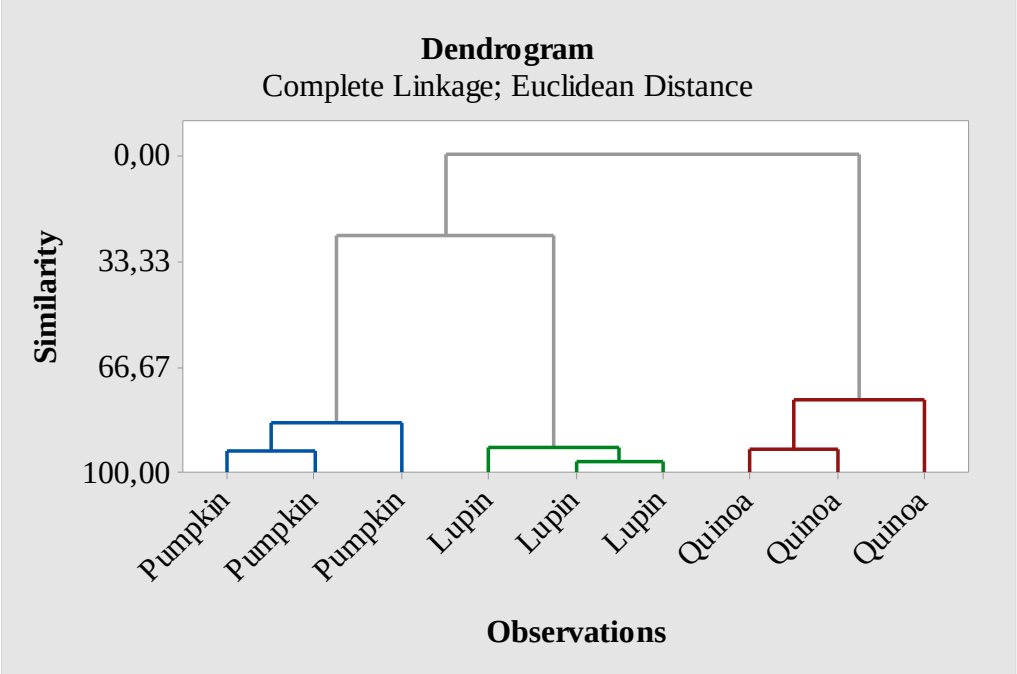

Figure 3. Hierarchical cluster analysis (HCA) plot according to the chemical properties of the samples

HCA was applied to the chemical data for further discrimination of the samples. According to the HCA dendogram graph (Fig. 3), at a similarity level of 76.71%, clusters of quinoa were mainly separated from other samples. Clusters of pumpkin were separated from other samples at a similarity level of 83.97%. Clusters of lupin were separated from other samples at a similarity level of 92.08%.

4. Conclusion

It was clearly seen that the analyzed elements of the samples were separated from each other by principal component analysis. Three different groups were formed according to the hierarchical clustering analysis. According to the multivariate analysis, the samples were well separated from each other in terms of chemical properties.

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Chips Made from Apple Pomace

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Abstract:
In this study, as a new product, apple pomace, which is a by-product resulting from the processing of fruit juice and vinegar, was used to make chips with high nutritional value. After preparing a mixture by adding maltodextrin to give the apple pomace a crisper structure and shine and cinnamon for flavor, it was shaped and placed on wax paper, and dried in the oven at 150°C for 10-15 minutes. Maltodextrin ratios were changed at 15%, 30% and 45%, and other parameters were kept constant. The effects of maltodextrin on the product were determined by analyzing the water activity, color (L*, a*, b*), total phenolic content (TPC), antioxidant activity (DPPH), texture and dry matter in the product. When the quality parameters of chips were examined, it was determined that it was a rich source in terms of nutritive properties. It has been observed that the addition of maltodextrin improves the color of the product and increases the hardness value. However, it was determined that maltodextrin did not have any positive effect on total phenolic content. In addition, it has been determined that a delicious and functional product can be obtained due to cinnamon's taste, high antioxidant activity and phenolic content. Compared with the other mixtures, it was determined that the mixture containing 45% (w/w) maltodextrin has the best color, antioxidant activity, total phenolic content, and moisture values in the products. As a result, it can be said that apple pomace chips, as a new product, is a valuable product because it is suitable for mouth taste, appeals to all ages, and is crispy and delicious.

Keywords: Antioxidant activity, apple pomace, chips, cinnamon, maltodextrin.

1. Introduction

Apple (*Malus domestica* L.) is from the Rosaceae family and generally contains 85% water, 11% carbohydrates, 2% dietary fiber, 0.6% fat, 0.5% organic acids and 0.3% protein. It is a good source of monosaccharides, minerals, dietary fiber, various biologically active compounds such as vitamin C and phenolic components known as natural antioxidants in human nutrition (Erdogan and Demirci, 2014). Apple, whose origin is known as Central Asia and the Caucasus, is mostly grown in Isparta (20.4%), Karaman (13.6%) and Niğde (12%) provinces in Turkey. Apple, which constitutes approximately 12% of the total fruit production in the world, is an important fruit that ranks second after banana. Turkey is one of the important countries in world apple production and is among the top five countries (Anonymous, 2018). Most of the apples are consumed as fresh, and some of them are used as raw materials for fruit juice and fruit concentrate production.

In the fruit juice processing industry, pomace is obtained in amounts ranging from 30-50%, depending on the fruit. While parts such as stems and leaves are separated before processing, pulp and skins are separated as pomace after processing. The wastes obtained before the process

are generally used in the production of biogas, compost, animal feed and fertilization. Wastes separated after processing can be used in different branches of the food industry and in pharmacy (Erdoğan, 2010).

The chemical composition (dry weight basis) of apple pomace is reported as follows; moisture (%) 3.90–10.80; protein (%) 2.94–5.67; total carbohydrate (%) 48.0–62.0, fat (%) 1.20–3.90, ash (%) 0.50–6.10. Apple pomace contains significant amounts of non-starch polysaccharides (35-60%), including soluble fiber (14.6%) and insoluble fiber (36.5%) on dry basis. The main components of dietary fiber on dry basis are pectin (5.5%-11.70%), cellulose (7.20–43.60%), hemicellulose (4.26–24.40%), lignin (15.30–23%, 50) and gums. In addition, simple sugars such as glucose (48.30 %), fructose (19.50–19.70 %) and sucrose (3.80–5.80 %) are found in the composition of apple pomace. Apple pomace is also good source of minerals such as phosphorus (0.07–0.076 %), potassium (0.43–0.95 %), calcium (0.06–0.10 %), sodium (0.20 %), magnesium (0.02–0.36 %), copper (1.10 mg/kg), zinc (15.00 mg/kg), manganese (3.96–9.00 mg/kg), and iron (31.80– 38.30 mg/kg) (Bhushan et al. 2008).

Apple pomace, which contains high amount of peel, has been reported a good source of phenolic compounds such as phenolic acids, flavon-3-ol, procyanidins, anthocyanins, flavonols, dihydrochalcones (phloridzin and phloretin) (Suárez et al., 2010, Rana et al., 2015). 80% of the polyphenols contained in the apple are located in the peel (Leccese et al., 2009). In an in vitro study, it was reported that the apple peel contains 6-7 times more total flavonols than the pulp and its antioxidant activity is 5-6 times higher than the pulp (Rupasinghe and Kean, 2008). In fresh apple pomace, the amount of flavonols, flavanols, dihydrochalcones, and hydroxycinnamates has been reported as 633 mg/kg DM, 406 mg/kg DM, 867 mg/kg DM, and 502 mg/kg DM, respectively (Erdoğan, 2010).

Maltodextrin is used in many different food applications due to its functional properties such as thickener, structure improver, film former, odor and oil binder, oxygen inhibitor, surface tension modifier and freezing point controller in aqueous solutions (Söbüçovalı, 2011). Cinnamon, which is obtained by drying and grinding the inner part of the cinnamon tree after the outer part of the bark is separated from the inner part, is used as a spice. Cinnamon, whose odor is sharp, strong and long-lasting, and whose taste is sweet and burning, is added to many foods to improve its flavor and aroma (Gürson ve Özçelikay, 2005).

Although there have been many studies in the literature examining the effect of different drying conditions on the production of apple chips, there has been no study on the use of apple pomace for the production of chips (Hasan et al., 2019). In this study, the possibilities of chips production from apple pomace, which is cheap, easily available and a good source of antioxidants, were investigated. For this purpose, chips were made by adding different proportions of maltodextrin and cinnamon to the pomace and its color, water activity, moisture, antioxidant activity, total phenolic content and textural properties were determined.

2. Materials and Methods

Material

In this study, apple (*Malus domestica cv.* Scarlett) was obtained from Niğde Ömer Halisdemir University Faculty of Agricultural Sciences and Technologies Research and Application Garden. Apples were stored at 4 °C until analysis.

Methods

Apple Pomace Production

The apples, which were washed, dried and the core house was removed, were divided into several pieces and kept in a container in water containing 5% citric acid. Then it was crushed in the food processor (Arnica Orbital Mix, Turkey). In order to separate the juice of the crushed apples, the mash was thoroughly pressed through four layer cheesecloths, and pomace was obtained. The pomace was shown in Figure 1.

Figure 1. Apple pomace

Chips Production from Apple Pomace

Cinnamon and maltodextrin or sugar were added to the pomace. While maltodextrin were added to the mixture at three different ratios as 15%, 30% and 45%, sugar was added in two different ratios as 15% and 30%. Cinnamon was added to the mixtures at a ratio of 1%. Mixtures was spread thinly, shaped, and put on baking trays which covered with wax paper. The shaped product was cooked at 150 °C for 30-35 minutes and left to cool at the end of cooking. The resulting apple pomace chips were shown in Figure 2.

Figure 2. Apple pomace chips

Color Analysis

A colorimeter (Chroma Meter CR-400 Konica Minolta, Sensing, Inc., Japan) was used to measure the L* (whiteness/darkness), a* (redness/greenness), and b*(yellowness/blueness) of the samples. Color measurement was performed in three regions for each sample under room conditions and the average of these values expressed the color of that sample (Baltacıoğlu et al., 2021).

Moisture Analysis

Moisture content in chips samples was made according to ICC Standard Method No: 110/1 (ICC, 2002) (Baltacıoğlu et al., 2021).

Water Activity Analysis

The water activity (A_w) of the samples was performed with A_w measuring device (Novasina, Switzerland) in the room temperature (Baltacıoğlu et al., 2021).

Texture Analysis

Hardness measurements of the chips samples (Stable Micro System TA.XTPlus Texture Analyzer, UK) were performed using the texture analyzer. SMS P/35 cylindrical Probe was set to 1 mm/s, test speed 2 mm/s, posttest speed 10 mm/s, distance 7 mm, and trigger force 10 g. The results are expressed as hardness (N) (Baltacıoğlu et al., 2021).

Total Phenolic Content Analysis

For the extraction of phenolic compounds, the samples were mixed with a methanol–water solution (80%, v/v) containing 1% hydrochloric acid (37%), according to a dilution factor of 1:10. After that, the mixture was shaken at 40 rpm over 4 h in the dark and centrifuged (Nüve, NR 800R model) at 9000 g for 15 min at +4 °C. Determination of total phenolic content was determined to the Folin ciocealtu method (Baltacıoğlu 2022). The total phenolic content results

were represented as milligrams of gallic acid equivalent (GAE) per kilogram of dry weight (mg GAE/kg DW). All measurements were carried out in triplicate.

Antioxidant Activity Analysis

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of chips was determined according to the method described by Baltacıoğlu (2022). As indicated in the method 100 µL of extracts mixed with 3.9 mL of 0.1 mM DPPH (prepared in 80% methanol) solution. After vortexing, they were soaked in the dark for 30 min at room temperature. At the end of time, the absorbances of the samples were read at 517 nm. For the blank sample preparation, 100 µL of methanol was mixed with DPPH solution, and its absorbance was determined at the same conditions. The experiment was carried out in triplicate. Radical scavenging activity was calculated using the following formula:

$$Inhibition\ (\%) = \frac{(Absorbance\ of\ the\ blank\ sample\ \ Absorbance\ of\ the\ extract)\ x\ 100}{Absorbance\ of\ the\ blank\ sample}$$

Statistical Analysis

The data were analyzed using Minitab (Version 17, Minitab Inc., State College, PA, USA) at 95% confidence intervals, and the general linear model was used in the analysis of the data. Tukey's multiple comparison test was conducted to determine the differences between applications. Each experiment was repeated at least three times.

3. Results

The color values of apple pomace chips were shown in Table 1. As the amount of sugar increased, the L* value increased. The L* value in the product containing 15% sugar was lower than the product with 30% added sugar ($p \leq 0.05$), so it became darker. While the redness (a*) value was higher in the product with 15% added sugar ($p \leq 0.05$), when the yellowness (b*) value was compared, no significant difference was found between the two samples ($p > 0.05$).

When maltodextrin-containing apple pomace chips were compared, it was determined that the L* value increased as the maltodextrin ratio increased ($p \leq 0.05$). This meant that the color became lighter depending on the increasing rate. The redness (a*) value showed a decrease ($p \leq 0.05$) as the maltodextrin ratio increased. The yellowness (b*) value was almost equal in apple pomace chips ($p > 0.05$). As the amount of maltodextrin in apple pomace chips increased, the lightness (L*) value of the chips increased, and redness (a*) values decreased so chips became brighter and less red. The addition of maltodextrin to apple pomace chips allowed the production of brighter and less red chips compared to the addition of sugar. Similar results were found in apples dried by coating with maltodextrin (Baltacıoğlu et al., 2019).

Table 1. The color values of apple pomace chips

	Color		
	L*	a*	b*
Apple Pomace (AP)	**58.56 ± 1.44^{a}**	**11.42 ± 1.08^{e}**	**42.32 ± 2.37^{a}**
AP with cinnamon	**37.11 ± 0.46^{c}**	**16.28 ± 0.85^{cd}**	**22.10 ± 0.36^{b}**
AP with cinnamon and sugar (15%)	**37.75 ± 1.10^{c}**	**23.93±1.69^{a}**	**23.71 ± 0.36^{b}**
AP with cinnamon and sugar (30%)	**40.82 ± 0.81^{bc}**	**18.59 ± 0.85^{bc}**	**24.48 ± 0.81^{b}**
AP with cinnamon and maltodextrin (15%)	**38.70 ± 4.30^{bc}**	**20.7 ± 1.50^{ab}**	**25.85 ± 3.44^{b}**
AP with cinnamon and maltodextrin (30%)	**39.46 ± 0.92^{bc}**	**16.15 ± 1.08^{cd}**	**23.01 ± 0.90^{b}**
AP with cinnamon and maltodextrin (45%)	**44.00 ± 1.73^{b}**	**14.24 ± 1.98^{de}**	**25.16 ± 0.68^{b}**

Results of physicochemical analysis was shown in Table 2. The moisture value of the samples was found to be below 5%. The water activity value of the samples was found to be below 0.5. The highest hardness value was obtained for 15% sugar-added chips. According to these results, as the amount of sugar increased, the hardness value decreased in the texture analysis. As the maltodextrin content increased, the hardness value increased up to the addition of 30% maltodextrin while there was a decrease in the use of 45% maltodextrin.

Table 2. Results of physicochemical analysis

	Moisture (%)	Water activity	Texture (Hardness, N)
Apple Pomace (AP)	**4.34 ± 0.26^{a}**	**0.307 ± 0.004^{c}**	-
AP with cinnamon	**4.00 ± 0.68^{a}**	**0.268 ± 0.021^{d}**	**79.87 ± 13.85^{de}**
AP with cinnamon and sugar (15%)	**5.24 ± 0.20^{a}**	**0.408 ± 0.001^{b}**	**384.50 ± 75.03^{a}**
AP with cinnamon and sugar (30%)	**3.90 ± 0.94^{a}**	**0.326 ± 0.001^{c}**	**216.93 ± 0.12^{bc}**
AP with cinnamon and maltodextrin (15%)	**4.32 ± 0.28^{a}**	**0.497 ± 0.014^{a}**	**177.29 ± 15.76^{c}**
AP with cinnamon and maltodextrin (30%)	**5.47 ± 0.62^{a}**	**0.475 ± 0.001^{a}**	**262.90 ± 16.48^{b}**
AP with cinnamon and maltodextrin (45%)	**4.75 ± 0.81^{a}**	**0.472 ± 0.004^{a}**	**150.67 ± 5.37^{cd}**

The effect of sugar and maltodextrin addition in different ratios on the total phenolic content and antioxidant activity of the chips was examined (Table 3). Addition of sugar increased the total phenolic content of the chips. Moreover, it was observed that the total phenolic content

increased as the maltodextrin ratio increased. In other words, it can be said that the addition of maltodextrin has a protective effect on phenolic compounds. Similarly, it was observed that maltodextrin coating increased the total phenolic content of dried apples (Baltacıoğlu et al., 2019). Similarly, addition of sugar increased the antioxidant activity of chips samples. In addition, the antioxidant activity increased as the maltodextrin ratio increased. In other words, it can be said that the antioxidant activity increased in parallel with the increase in the total phenolic content. Addition of sugar or maltodextrin increased total phenolic content and antioxidant activity. The highest total phenolic content and antioxidant activity were found in cinnamon added chips.

Table 3. Results of chemical analysis

	Total phenolic content (mg/kg DW)	DPPH (% inhibition)
Apple Pomace (AP)	**3475.70 ± 221.65^{c}**	**64.04 ± 1.48^{c}**
AP with cinnamon	**9684.30 ± 425.82^{a}**	**93.66 ± 0.55^{a}**
AP with cinnamon and sugar (15%)	**5787.53 ± 155.42^{b}**	**81.68 ± 1.11^{b}**
AP with cinnamon and sugar (30%)	**5720.86 ± 915.91^{b}**	**77.23 ± 5.43^{b}**
AP with cinnamon and maltodextrin (15%)	**2938.06 ± 175.62^{c}**	**45.38 ± 3.43^{d}**
AP with cinnamon and maltodextrin (30%)	**3793.98 ± 370.75^{c}**	**60.57 ± 3.46^{c}**
AP with cinnamon and maltodextrin (45%)	**5303.66 ±517.59^{b}**	**73.50 ± 2.29^{b}**

4. Discussion

In this study, chips were obtained by adding maltodextrin (15%, 30%, 45%) or sugar (15%, 30%) to apple pomace and the quality properties of the product were investigated. For this purpose, the color value of the apple pomace chips was compared. When the color values were examined, as the amount of maltodextrin in apple pomace chips increased, the color of the apple pomace chips became brighter. The addition of maltodextrin to apple pomace chips allowed the production of brighter, less red and yellow chips compared to the addition of sugar. Similarly, it was determined that apple slices coated with maltodextrin and dried in hot air had better color (Baltacıoğlu et al., 2019). However, it was also seen that the addition of 30% maltodextrin increased the hardness value, which was an important textural property of the chips. At the same time, it was observed that as the maltodextrin ratio increased, the total phenolic content increased. In other words, it can be said that the addition of maltodextrin has a protective effect on phenolic compounds. In addition, it can be mentioned about the antioxidant activity-

enhancing effect of maltodextrin addition. Similarly, it was observed that maltodextrin coating increased the total phenolic content of dried apples (Baltacıoğlu et al., 2019).

5. Conclusion

In conclusion, apple pomace, which is a by-product of fruit juice industry, was used to make apple pomace chips, which have high nutritional value and dietary fiber, as a new product. Chips were obtained by using maltodextrin to give the apple pomace a crisp texture and brightness, and cinnamon for flavoring. Maltodextrin gave a crispy structure and brightness to apple pomace chips. It has been determined that a delicious and consumable product can be obtained with the addition of cinnamon, which has high antioxidant activity and phenolic content.

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Isolation of Obligate Intracellular Bacteria in vivo Conditions

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Abstract:
The parathrophic bacteria such as *Chlamydia* spp. and *Rickettsia* spp. obtain the carbon and energy necessary for their life from the biosynthetic events of the host cell. Bacteria are of two types: extracellular bacteria that live freely in the environment and intracellular bacteria such as *Salmonella* spp., *Francisella* spp., *Legionella pneumophila*, *Listeria monocytogenes*, and *Yersinia* spp. that establish infections in the host cell cytoplasm or specialized vacuoles. The obligate intracellular bacteria usually require an obligate host cell for replication, including *Chlamydia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Rickettsia* spp., and *Coxiella burnetii*. The isolation of obligate intracellular bacteria for serological tests or bacterial stocks under laboratory conditions for diagnostic or experimental purposes can only be carried out in living environments. In vivo conditions such as cell culture, experimental animals, and embryonated chicken eggs are used to produce obligate intracellular bacteria. Cell cultures are more advantageous in terms of suitability for laboratory studies due to the absence of care-feeding problems, immunological reactions, sterility, etc. as in experimental animals. This review aims to present brief information about the in vivo conditions that are widely used in medical and biological research and to provide quick details about these conditions.

Keywords: obligate ıntracellular bacteria, cell cultures, experimental animals, chicken eggs embryos.

1. İntroduction

Bacteria obtain the carbon and energy they need from the energy formed because of the biosynthesis events of the host cell. Such bacteria are called paratrophic bacteria. Examples of these bacteria are *Chlamydia* spp. and *Rickettsia* spp.

Intracellular bacteria typically grow freely in the eukaryotic cytoplasm or in specialized vacuoles. Bacteria have historically been divided into two different groups; these are free-living organisms in environmental conditions, namely extracellular bacteria, and intracellular bacteria that infect and multiply inside the host cell. Examples of facultative intracellular bacteria; Salmonella spp., *Francisella* spp., *Legionella pneumophila*, *Listeria monocytogenes*, *Yersinia* spp. can be given as an example. On the contrary, obligate intracellular bacteria; They generally require an obligate host cell for replication, including *Chlamydia* spp., members of the order Rickettsiales (*Anaplasma* spp., *Ehrlichia* spp., *Rickettsia* spp., and *Orientia* spp.) and *Coxiella burnetii* (McClure et al., 2017).

The production of obligate intracellular bacteria under laboratory conditions for diagnostic or experimental purposes can only be done in living environments. Isolation and production of obligate intracellular bacteria is possible by multiplying them in vivo to obtain high titer and large amount of bacterial stock to be used in serological tests.

Media such as cell cultures, experimental animals and embryonated chicken eggs used to produce obligate intracellular bacteria are defined as in vivo systems. Cell cultures do not have care-feeding problems like in experimental animals, no immunological reaction, sterility etc. For these reasons, it also has advantages in terms of suitability for laboratory studies. In this study, it is aimed to present the in vivo environments that are frequently used in medical and biological research and to present brief information about these systems.

1. Chlamydia Infections

Although bacteria of the genus *Chlamydia* were previously thought to be viruses, they are classified among the bacterial group because they reproduce by dividing, are sensitive to some antibiotics, and have bacterial rRNA, ribosome and cell wall (Longbotttom et al., 2003). It is characterized as a unique group of obligate intracellular bacteria that carry out replication in cytoplasmic inclusion vacuoles with a biphasic developmental cycle within eukaryotic cells. There are two periods in the reproductive cycles, the infectious (elementary body form) and the reproductive form (reticular body form). The infectious form is the small, metabolically inactive form called the extracellular form, which enters the host cell via endocytosis via the receptor. After a few hours of remodeling, the elementary corpuscular form transforms into the reticular corpuscular form. The reticular body form is metabolically active and approximately 1 μm in diameter. About 20 hours after infection, the developmental cycle becomes asynchronous and some reticular bodies continue to divide, while others mature to form multiple elementary bodies. The host cell is then lysed. Elementary bodies with infective properties start a new cycle by attaching to another host, and thus the number of elementary bodies reaches millions, and these are uterine discharges, milk, urine, feces, etc. It continues to scatter in ways (Markey et al., 2013). Diseases caused by *Chlamydia* bacteria in humans and animals are shown in Table 1.

C. trachomatis	**Human**	**Ocular trachoma**
C. suis	**Pig**	**Enteritis**
C. muridarum	**Mouse and Hamster**	**Respiratory tract infection**
C. psittaci	**Poultry**	**Pneumonia and Air sac inflammation,**
	Human	**Enteritis, Conjunctivitis, Pericarditis, Encephalitis**
		Psittacosis/Ornithosis
C. felis	**Cat**	**Conjunctivitis and Pneumonia**
C. abortus	**Sheep**	**Enzootic abortion of sheep**
	Goat	**Chlamydial abortion**
	Cattle	**Chlamydial abortion**
	Pig	**Chlamydial abortion**
	Human	**Abort**
C. caviae	**Guinea Pig and Pig**	**Conjunctivitis**
C. pecorum	**Sheep, Cattle and Koala**	**Enteritis, Conjunctivitis, Polyarthritis, Encephalitis, abortion**
C. pneumoniae	**Human**	**Pneumonia, Atherosclerosis ve Conjunctivitis**
C. avium	**Birds**	**Respiratory tract infection**
C. gallinaceae	**Birds**	**Isolated from asymptomatic domestic poultry.**

Table 1: Species in the genus Chlamydia, their hosts, and the diseases they cause (Quinn., 2011).

Diseases caused by Chlamydia bacteria in domestic and wild animals are regularly reported. Chlamydial infections in swine, cattle, goats, and sheep, as well as in poultry and horses, cause major economic impacts and production losses worldwide. Factors such as *C. suis, C. psittaci, C. abortus* and *C. pecorum* are the main livestock pathogens that cause significant effects on animal health and economic loss with various clinical symptoms such as conjunctivitis, arthritis, abortion, pneumonia, etc. (Borel et al., 2018).

In a study on sheep, smear preparations were prepared from the contents of 8 placentas collected, 10 vaginal swab and 83 lungs, 97 liver, 71 spleen, 53 kidney and 76 stomach contents of 98 waste fetuses, and the preparations were stained with the modified Ziehl-Neelsen (Stamp) method and chlamydial elementary body forms were searched under the microscope. Elementary bodies were seen in 5 placenta and 2 spleen samples. To prepare inoculum from the tissue samples taken; First, it was homogenized thoroughly in a mortar using sterile sand, then 1/10 suspensions of chlamydia transport medium were centrifuged at 200 g for 5 minutes and the supernatant was collected under aseptic conditions and stored at -70 °C. The prepared inoculum was removed from -70 ºC, kept at room temperature for a while and dissolved in a water bath at 37 °C. 10% suspensions were prepared with nutrient broth containing inoculum 200 µg/ml. Inoculums were injected vertically into the yolk sac of 0.2 ml of embryonated chicken eggs. Eggs were incubated at 37 °C. The viability of the embryos was checked twice a day and deaths up to day 4 were discarded as it was thought to be due to contamination. For deaths after the 4th day, eggs were collected, and embryos were taken into sterile petri dishes

with their yellow sacs. The modified Ziehl-Neelsen (Stamp) method was stained from the samples and elementary corpuscle forms were searched under the microscope. Elementary bodies were observed in the yolk sac membranes of the samples that did not kill the embryo even 12-13 days after inoculation. BHK-21 (Puppy Hamster Kidney Cell line) and McCoy (Mouse fibroblast cell line) were used for isolation studies in cell culture. Cell lines were removed from -70 °C, inoculated into T25 flasks and incubated. After incubation, the cell growth medium was drained and washed and treated with trypsin to remove the cells from the surface. Chlamydial inclusion bodies were detected in 5 placentas, 3 vaginal swaps, and 13 fetuses by preparing preparations from cell cultures and staining with the modified Ziehl-Neelsen (Stamp) method. Samples with inclusions were considered positive. 3–5-week-old mice were used for isolation in mice. Inoculums were removed from -70 °C, kept at room temperature for a while and dissolved in a 37 °C water bath. 0.2 ml of the samples were taken and injected intraperitoneally into the mice. It was checked 7-10 days after inoculation and morbidity and mortality in mice were followed. Internal organs (lung, liver, spleen, and kidney) were removed from deceased mice, and the preparations were prepared and stained. Mice that did not die after the 10th day were killed with ether and the same sampling and staining were performed. As a result of staining, elementary bodies were observed in 5 placentas, 2 vaginal swaps, and 13 fetuses (Turutoglu, 1995).

It is stated that BGM (Buffalo Green Monkey cells) and McCoy (Mouse fibroblast cell) cell lines were used in a study to breed *Chlamydia psittaci* strains in cell culture media. In addition, the chorioallantoic membrane region of the ETY was inoculated to determine the reproduction of the agent in embryonic chicken eggs and to determine the survival time (Favaroni et al., 2021).

2. Coxiella Infections

Coxiella infections; It is a zoonotic infection caused by *Coxiella burnetti*, which is usually subclinical and rarely causes abortions in pregnant animals. *Coxiella burnetti* disease is known by different names. Due to the regions where the disease is first seen and the occupational group in which it is most frequently defined; It is also known by names such as Australian Q-fever, Slaughterhouse fever, Nine Mile fever and Balkan flu (Maurin et al., 1999).

C. burnetti is an obligate intracellular, small (0.2-0.4 to 0.4-1.0μm) Gram-negative, pleomorphic, non-motile, flagella-less, and encapsulated bacterium. Although they have an outer membrane like Gram-negative bacteria, they do not stain well by Gram staining. With Giemsa they are painted in violet. They live and reproduce in lysosomal vacuoles in living cells. They do not reproduce on synthetic media, but they reproduce in the cytoplasm of endothelial and epithelial cells and form microcolonies (Maurin et al., 1999; Woldehiwet,2004).

Ticks are recognized as the main reservoir and vector of *C. burnetii*. It has been reported that *C. burnetti* has been isolated from more than forty tick species to date. In addition, the agent can spread in nature without the need for an intermediate host. In particular, the Ixodidae (hard ticks, pasture ticks) family (Dermacentor, Hyalomma, Haemaphysalis, Boophilus and Rhipicephalus strains) and Argasidae (soft ticks, residential ticks) family, especially Ornithodorus and Argas genus, can carry C. burnetii. *Coxiella burnetti* settles and multiplies in the female genital areas and mammary glands of ruminants. Infected animals

transmit the agent to other animals and humans with uterine discharge, fetal fluid, placenta, urine, feces, milk and all kinds of contaminated products (Duron et al.,2015; Harris et al., 2000).

To produce *C. burnetii*; In-vivo media such as macrophage-like tumor cells, fibroblast cells, Human embryonic lung (HEL), tick tissue cultures or embryonic chicken eggs and laboratory animals (mouse and guinea pig) are used (Hackstadt et al., 1981; Mori et al., 2013).

The cell lines used for *Coxiella* spp. agents include epithelial, fibroblast, trophoblast, macrophage, and endothelial cells. TM3 Leydig cells (ATCC CRL-1714), TM4 Sertoli cell (ATCC CRL-1715) and GC-1 Germ cells (ATCC CRL-2053) produced from mice were used. An experimental animal model was developed using several laboratory animals, including mice, hamsters, and guinea pigs, and used to breed *C. burnetii*. However, mice have been shown to be naturally resistant to *C. burnetii*. In addition, severe combined immunodeficiency (SCID) mice have been used to effectively model different aspects of the immune response to the agent (Van Schaik et al., 2013; Andoh et al., 2007).

Since *Coxiella burnetii* is highly infectious and requires biosafety level 3 (BSL-III) laboratory conditions, it cannot be diagnosed in bacteriological diagnostic laboratories that do not carry these safety precautions. Although the infection is asymptomatic, the presence of the agent can be demonstrated by using Gimenez and Machiavello staining methods of preparations prepared from placental tissues of aborted animals. In the laboratory diagnosis of *Coxiella burnetii*, serological methods based on direct identification of the agent or demonstration of the presence of antibodies such as agent isolation from clinical samples, molecular methods and immunohistochemical techniques are used (Angelakis et al., 2010; Fournier et al., 1998).

3. Rickettsia, Ehrlichia and Anaplasma Infections

Rickettsiae are small Gram-negative, non-motile and aerobic coccobacilli that are obligate intracellular parasites of eukaryotic cells. They can be found in the cytoplasm or in the nucleus of the cell it infects. They have typical Gram-negative cell walls (peptidoglycan layer) and no flagella. The optimal breeding temperature is 33-35°C. Rickettsiae are defined as pathogenic species because they are the causative agents of various diseases in humans and animals. It is closely related to arthropod vectors that transfer the causative agent to mammalian hosts, and the zoonotic diseases it causes are among the oldest known vector-borne diseases (Paris et al., 2016; Yu et al., 2005).

Rocky Mountain Fever, caused by *Rickettsia rickettsii*, is a zoonotic disease carried by ticks of the genus Ixodid. It is clinically characterized by high fever, muscle and joint pain, anorexia, vomiting and edema in the extremities. Rickettsia infects vascular endothelial cells lining small and medium vessels in the body, causing systemic symptoms and a high mortality rate. It must be produced under laboratory conditions at the level of active biosafety level III. Therefore, in vivo environments (embryo chicken eggs, cell culture and experimental animals), serological tests, form the basis of laboratory diagnosis of rickettsial diseases (Parola et al., 2005; Krawczak et al., 2016). In their study in Slovakia, the researchers collected tick samples from this region and thought to breed *Rickettsia felis* from ticks with cell culture. For this

purpose, researchers used XTC-2 (Frog fibroblast cell line) and Vero (African green monkey kidney epithelial cell line) (Danchenko et al., 2022).

Ehrlichia canis, known as Monocytic *Ehrlichiosis* of the Dog, is a small, pleomorphic, obligate intracellular Gram-negative bacterium. The disease occurs in all dog breeds and is the primary arthropod vector of brown dog ticks (Rhipicephalus sanguineus). Ehrlichiosis infection is transmitted through saliva during the blood-sucking of the tick that carries the causative agent, and transmission occurs after blood transfusion (Rikihisa et al., 1991; Groves et al., 1975).

Ehrlichia canis are white blood cell parasites that reproduce in intracytoplasmic vesicles and have affinity for monocyte and macrophage cells on the host. In the acute phase, the disease begins within 8-21 days following the infection and progresses with laboratory findings such as fever, depression, edema, weight loss, hemorrhage, and anorexia. The subclinical phase seen in the disease can continue for years and as a result, persistent infection can occur in the host. In the chronic phase, bleeding disorders, extreme weakness, bone marrow suppression and peripheral edema are observed (Neer et al., 2006).

After a 4th passage in Dulbecco's Modified Eagle Medium supplemented with 10% inactivated fetal bovine serum, the researchers used the canine macrophage-like cell line (DH82, ATCC®-CRL -10389 TM) to propagate *E.canis* in cell culture. have been able to produce. They determined the viability rate of cells as 95% (Nambooppha et al., 2021).

Anaplasma species are obligate intracellular Gram-negative bacteria. Morphologically, it is small pleomorphic in shape and 0.3–2.5 μm in diameter, found in a membrane-enclosed intracytoplasmic vacuole in host cells. The transfer of anaplasma agents from one infected host to another affected host is carried out biologically by ticks and various blood-sucking arthropods, by surgical instruments, iatrogenically and by means of blood transfer. Infectious diseases that develop in parallel with the seasonal activities of mechanical and biological vectors can be seen. Apart from ticks, some blood-sucking arthropods, veterinary surgical materials, contaminated injectors play a mechanical role in transmission. There are 4 species in the genus Anaplasma. These; *Anaplasma marginale* is *Anaplasma ovis Anaplasma caudatum* and *Anaplasma centrale* (Markey et al.,2013; Quinn et al., 2011). Researchers used the Rhipicephalus appendiculatus cell line (RAE25) cell line to breed *Anaplasma marginale*. They detected rickettsial inclusions after 28-32 days (Bell-Sakyi et al., 2015).

CELL CULTURE

1.Cell Culture

Cell culture is a technique based on the principle of maintaining the viability of plant or animal cells in laboratory conditions by removing them from the tissue they belong to. The cell culture medium also promotes cell division and proliferation. For the cultured cells to survive and reproduce, they are placed in a medium containing various vitamins, salts, serum proteins, carbohydrates, and growth factors. In addition, cells are incubated in special CO_2 ovens to ensure optimum temperature, humidity, and pH values (Langdon, 2010; Harrison, 1907).

Thanks to the cell culture method, a model system can be obtained for the selected cell, tissue, or disease type. Various cell lines can be created to be used in many areas of life

sciences, and the biochemical and physiological processes of cells can be followed. This method plays a key role in obtaining very important findings by using the models created in this way, for example, in cell-drug interaction experiments in preclinical drug screening studies. In addition, cell culture applications are used extensively in studies such as biocompatibility tests and understanding of disease mechanisms (Hsiung, 1984; Lanza et al., 2020).

1.1. Cell Culture Types

The main source for cells to be used in cell culture is tissues obtained from living organisms. Cells are cultured by separating them from tissues by enzymatic or mechanical means and are called primary culture. Transferring the cultures to a new culture flask containing fresh medium is called passaging, and secondary cultures are obtained after the primary cultures have been first passaged, from which time these cultures are referred to as a "cell line". Primary cultures usually have a heterogeneous cell population, but as they are passaged, one cell type becomes dominant from this heterogeneous cell population and these cells are usually fibroblasts (Hsiung, 1984; Lanza et al., 2020).

Cells derived from normal tissues have limited replication ability and eventually lose their ability to divide. This phenomenon is called senescence. However, some cell lines can undergo transformation and can replicate an unlimited number of times. Transformation can occur spontaneously or by interfering with cells with viral or chemical agents. When a cell line with limited division gains the ability to divide unlimitedly by transformation, it is called a continuous cell line (Hsiung, 1984; Lanza et al., 2020).

Cells can grow in culture flasks either adherent to the base or as a suspension. Cells that develop attached to the base are called “anchorage dependent” cells, and these cells cannot continue their development without a surface to which they can adhere. The coating of the bottom of the culture flask because of the proliferation of the cells attached to the surface is called confluency. Confluence is expressed in “percent (%)” and covering the bottom of the bottle completely is called 100% confluence, while covering about half of the sample's surface is called 50% confluence. Cells derived from ectodermal and endodermal embryonic cell layers, such as fibroblast and epithelial cells, tend to grow in a sticky manner. Cells obtained from blood, bone marrow and spleen live in suspension in culture (Groves et al., 1975; Neer et al., 2006).

According to cell cultures sources;

I. Primary cell cultures

II. Secondary cell cultures

III. Continuous cell cultures

IV. Hybridomas

V. Stem cell

According to the growth form;

I. Suspension cultures

II. Cells that grow by attachment (monolayer)

The oldest and most widely used example of continuous cell lines is the HeLa cell line. This cell line was obtained in 1951 by primary culture of cells obtained by biopsy from the cervical tumor of a patient named Henrietta Lacks. Immediately after the HeLa cell line was obtained, it was used in the production of the polio vaccine. Today, studies with HeLa cell line have a great role in shaping modern medicine (Lanza et al., 2020).

Apart from the HeLa cell line, many different cell lines are used in scientific research and industrially in the pharmaceutical industry. Many of these cell lines are derived from tumor tissues and have many characteristics of the tumor type from which they were derived. For example, the Chinese hamster ovary (CHO) cell line was obtained from the ovary of a small rodent, the Chinese hamster. CHO cells are used in industrial volumes to produce recombinant protein and epidermal growth factor receptor (EGFR) studies (Lanza et al., 2020).

The nutrient requirement differs according to the type of cells, their adaptability, and the cell source depends on the type of organism. Cells may behave differently on different media. Therefore, the nutrient needs of the cell should be determined according to the purpose of the study. There are three main groups of media. These are: basal medium (basal media), medium with low serum content (reduced-serum media), and serum-free medium (Wahrheit et al., 2014). The presence of amino acids, carbohydrates, lipids, vitamins, ions, and proteins in the environment is essential for the survival and reproduction of cells. The medium is the most important component of the culture media, and besides providing the cells with growth stimulating agents such as nutrients, growth factors, hormones, they also regulate the pH and osmotic pressure of the culture medium. To meet the conditions specified in a standard medium, two basic components must be present (Price, 2017).

1. 1.1. Basal Medium

Basal media are media that contain amino acids, vitamins, inorganic salts, glucose as a carbon source and are usually supplemented with serum at a rate of 10% by volume. The essential amino acid combination required in cell cultures was first described by Eagle in 1955. The medium named Minimum Eagle's Medium (MEM), which bears his name, has survived with some modifications. MEM solution (DMEM) modified by Dulbecco is the most used medium component in somatic cell cultures today. DMEM has the glucose necessary for the cells to be fed, the appropriate osmolarity and pH to maintain their vitality, and the amino acids and vitamins necessary for them to function. However, it is not sufficient for cell development alone (Bryant et al., 2019).

Apart from these basic media, there are media with different contents that enable them to meet the basic needs of different cell types (DMEM F12, RPMI 1640, Mc Coy's Medium, Leibovitz's L-15, etc.) (Ham et al., 1963; Mather and Sato, 1977).

1.1.2. Fetal Bovine Serum (FBS)

Serum is a rich protein solution that is used for the adhesion and proliferation of cells and its content is not fully defined. This protein solution contains hormones, enzymes, growth factors that allow the cell to grow and multiply, and intercellular matrix proteins that allow it to adhere to surfaces. It also controls cell membrane permeability and acts as a carrier for the transport of lipids, enzymes, micronutrients, and trace elements into the cell. It contains

components that bind and buffer toxins. It could neutralize enzymes such as trypsin and protease. It directs cell-cell and cell-environment interactions (Verma et al., 2020).

Serum-free media are compositions in which all the serum content in the basal medium is replaced by a formulation consisting of appropriate nutritional and hormonal factors. The most important advantage of such media is the possibility of selectively adjusting the media formula for the desired cell type with appropriate growth factors. Therefore, it is possible to produce media specific to each cell type (Verma et al., 2020).

The serum ratio in the medium may vary depending on the cell type and applications. The serum ratio in a standard somatic cell culture is 10%. Media with low serum content are the compositions in which the serum content in basal media formulations is reduced and nutritional and animal factors are added instead. Thus, some undesirable effects originating from serum are tried to be minimized (Nims and Harbell, 2017; Arora, 2013).

1.1.3. Essential Ingredients of Cell Culture Media

Especially the temperature, pH value, oxygen and carbon dioxide levels and glucose concentration have a great impact on the success of cell culture studies. Basically, in a cell culture medium; inorganic salts, carbohydrates, antibiotics should be present. In addition, amino acids, proteins, peptides, hormones, and vitamins must be present for cellular metabolism to occur (Wahrheit et al., 2014; Price, 2017; Schwartz et al., 2019).

1.1.4. Fundamental Processes in Cell Culture

Media preparation includes thawing and seeding cells, changing media, and freezing cells, subculture, cell counting, passage, contamination control, maintenance, and monitoring of culture. Most media contain a pH indicator (phenol red). If the color of the medium is yellow, it indicates acidity, and purple indicates alkalinity. Yellowish acidic medium; may indicate overgrowth culture and bacterial contamination, purplish basic medium, non-growing culture, mold contamination, and a small amount of CO_2 in the environment. A cloudy image in the medium may indicate contamination or overgrown culture or excess CO_2 (Schwartz et al., 2019).

2. EXPERIMENTAL ANIMALS

Parallel to the introduction of cell cultures into laboratory studies, the field of use in routine diagnostic studies of experimental animals has remained quite limited. These systems are both more expensive and more troublesome for reasons such as maintenance and feeding. However, experimental animals are required for subjects such as hyperimmune serum, complement and amboceptor production. The most important advantage provided by experimental animals can be shown as the role of pathology, pathogenesis, and immunization studies (Festing, 1994).

Among the most used experimental animals are rabbits, rats, mice, hamsters, chicks and monkeys. In addition, these animals can be defined as "laboratory animals" from time to time, as they are suitable for working in laboratory environments. On the other hand, apart from routine diagnostic studies, large animals (cat, dog, sheep, cattle, etc.) used especially for research purposes are also included in the definition of experimental animals. Experimental animals are divided into 3 groups according to their microbiological status (Cline and Clarkson, 2015).

2.1. Conventional animals

They are experimental animals subjected to standard rearing conditions in the rearing environment and without any microbiological control. These animals can naturally contain many saprophytic or pathogenic microorganisms. Therefore, the use of these animals can be restrictive at times. However, with some routine trials, they can be used especially in the production of certain biological products (complement, amboceptor, negative or positive serum b) (Giridharan, 2021; Laaldin et al., 2020).

2.2. Specific Pathogen Free (SPF) animals

Although these animals contain different microorganisms in their bodies, they do not contain pathogenic microorganisms. Therefore, these animals should be kept under constant control and special care should be applied. Their feed and water are subjected to microbiological control and filtered air is given. Trials with SPF animals give more specific and safer results than conventional experimental animals (Mohan et al., 2021; Giridharan, 2021; Laaldin et al., 2020).

2.3. Germ Free (GF) animals

They are special experimental animals that do not contain any microorganisms in their body and do not have antibodies formed against these "microorganisms" in their blood. These animals are taken from healthy mothers by cesarean section, and they are housed in environments where all environmental conditions are kept under control. Feed and water are sterilized before consumption and filtered air is supplied. Stool and blood samples are regularly subjected to microbiological control. GF animals, which are very difficult and expensive to breed and maintain, are used in very special studies. The degree of reliability of the data to be obtained from these animals is quite high (Laaldin et al., 2020).

There are different inoculation methods that can be selected according to the purpose and the type of virus used for inoculation into experimental animals. Among the most frequently applied ones are intranasal, intratracheal, corneal, conjunctival, oral, cutaneous, subcutaneous, intradermal, intravenous, intracerebral, and intramuscular inoculations (Mohan et al., 2021).

3. EMBRYO CHICKEN EGGS

Embryo chicken eggs entered laboratory studies from the 1930s and were used as the most important tool in studies until the 1950s. Since these years, the use of ETY has started to decrease with the spread of cell cultures. Although ETY is very advantageous compared to experimental animals in terms of labor and cost, it cannot protect the same advantage against cell cultures. For this reason, the usage area of ETY has narrowed considerably (Fraguas-Sanchez et al., 2022).

To be successful in ETY applications, it is desirable that the hatcheries from which the eggs are procured are constantly monitored, that they are free from important diseases and that the fertility rate of the hatchery is preferably high. Despite all these practices, some bacterial and viral infections can be observed spontaneously from time to time in ETYs. Eggs to be planted are first checked for fertility under a light source and incubated at 35-37°C in 40-70% humidity. Eggs that are turned 2-4 times a day are checked for viability before planting, the location of the embryo and the boundaries of the air sac are marked. Living embryos are motile and there are vascularization’s in the chorioallantoic membrane. In dead embryos, the area where the embryo is located appears as a hazy black mass. Sowing should be done immediately after the markings, otherwise the embryo may be displaced (Wittig and Münsterberg, 2016).

In ETY, there are 4 regions that are frequently used for sowing. These;

I. Chorio-allantoic membrane,

II. chorio-allantoic space,

III. yellow sac and

IV. It is the amniotic sac.

Apart from these regions, inoculation can be made into the embryonal vessels on the chorio-allantoic membrane and directly to the embryo (Buddinch, 1950).

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Evaluation of The Phenolic Content of Multifloral Honey From Şanlıurfa, Türkiye

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Abstract:

Honey is a sweet and viscous liquid produced by bees by collecting nectar from flowers and processing them with the enzymes they secrete. Honey contains components such as various sugars, amino acids, minerals, vitamins and phenolic compounds that are beneficial to health. The purpose of this study was to ascertain the amounts and composition of phenolic compounds in honey samples collected from Şanlıurfa. With a liquid chromatography-tandem mass spectrometry (LC-MS/MS) instrument, 25 phenolic compounds were analyzed in the honey samples for this reason. Myricetin, protocatechuic acid, 2-hydroxy-1, 4-naphthoquinone, luteolin, kaempferol, thymoquinone, and alizarin could not be found in the honey samples among these phenolic compounds. Hydroxycinamic acid (1552.1±1556.3 ng g^{-1}) and vanillic acid (522.1±238.3 ng g^{-1}) had the greatest concentrations of phenolic compounds. Acetohydroxamic acid (3.59±0.101 ng g^{-1}) was the lowest phenolic substances of the honey samples. The levels of phenolic compounds in honey samples from Şanlıurfa city were identified as a result of this research.

Keywords: Honey, phenolic compounds, LC-MS/MS, Şanlıurfa

1. Introduction

Honey is a natural sweetener produced by honeybees from the nectar of flowers. Besides its sweet taste, honey is also known for its potential health benefits due to the presence of various bioactive compounds, including phenolic compounds. Phenolic compounds are a diverse group of secondary metabolites widely distributed in the plant kingdom, which can be transferred to honey during the nectar collection process. The phenolic compounds found in honey are mainly derived from the plants visited by bees, which vary according to the geographical location and seasonality and processing methods (Soares et al., 2017; Alshammari et al., 2022). These phenolic compounds have been reported to possess a wide range of biological activities, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, making honey a promising functional food (Zawawi et al., 2021; Ranneh et al., 2021). The concentration and composition of phenolic compounds in honey can be influenced by various factors, such as bee species, weather conditions, and soil quality (Beretta et al., 2005; Khalil and Sulaiman, 2010). The bioavailability and pharmacokinetics of phenolic compounds in honey can be affected by various factors, such as the chemical structure, food matrix, and gut microbiota (Cianciosi et al., 2018; Cardona et al., 2013).

Antioxidant activity: According to studies, honey's phenolic components have antioxidant properties that prevent oxidative stress and free radical damage. This may help lower the risk of developing chronic illnesses like cancer, heart disease, and neurological diseases. Caffeic acid, p-coumaric acid, quercetin, and kaempferol are phenolic chemicals found in honey that

have been shown in several studies to exhibit antioxidant activity (Zawawi et al., 2021; Khalil and Sulaiman, 2010; Soares et al., 2017).

Anti-inflammatory activity: Phenolic compounds in honey have also been reported to exhibit anti-inflammatory activity by reducing the production of pro-inflammatory cytokines and enzymes. This can help to alleviate inflammation and related diseases such as rheumatoid arthritis, asthma, and inflammatory bowel disease. Several studies have demonstrated the anti-inflammatory activity of phenolic compounds in honey, such as ellagic acid, quercetin, and apigenin. (Ranneh et al., 2021; Kassim et al., 2010; Khan et al., 2019)

Antimicrobial activity: It has been demonstrated that honey's phenolic chemicals have potent antibacterial properties against a variety of bacteria, fungi, and viruses. The capacity of phenolic chemicals to damage the cellular membranes of microorganisms and prevent their development accounts for this antimicrobial effect. Numerous studies have shown that the phenolic chemicals in honey have antibacterial properties, including against bacteria strains that are resistant to antibiotics (Kwakman et al., 2008; Bueno-Costa et al., 2016; Mandal and Mandal, 2011).

Anticancer activity: According to certain studies, the phenolic chemicals in honey may have anticancer properties. According to theory, the phenolic chemicals have this impact via preventing the development of cancer cells, causing cell cycle arrest and apoptosis, and lowering oxidative stress. According to studies, the phenolic chemicals in honey may have anticancer properties against a variety of cancer cell lines, such as breast, colon, and prostate cancer cells (Jaganathan and Mandal, 2009; Ahmed and Othman, 2013; Combarros-Fuertes et al., 2018).

Beekeeping gives rural residents in developing nations access to employment, money, and wholesome food. With all of these advantages, beekeeping enjoys a privileged position in farming pursuits, and Turkey is the world's second-largest producer of honey after China (Burucu and Bal, 2017). Beekeeping comes to the fore and gains importance as an activity that can be done almost anywhere from sea level to high plateaus in Turkey, which has a very large flora and the flowering times are spread throughout the year. Şanlıurfa province, which has the third largest surface area of Turkey with an area of 51 thousand decares, ranks 12th in honey production in the country, and approximately 2107 tons of honey is produced with 130 thousand hives (TEPGE, 2022). The purpose of this research was to ascertain the amounts and composition of phenolic compounds in honey samples collected from Şanlıurfa city of Türkiye.

2. Materials and Methods

All of the honey samples (n=10) were obtained from Şanlıurfa center and districts. All samples were stored in sealed falcon tubes at room temperature and in a dark environment until analysis. All chemicals used are of analytical purity and were obtained from Merck (Darmstad, Germany). The technique of Altun ve Aydemir (2020) was modified slightly for sample extraction for LC-MS/MS analysis (Durmaz, 2020). After thoroughly blending 25 g of honey sample with 5 parts of acidic water (pH 2, with HCl), the mixture was filtered through cotton to eliminate any remaining solids. After that, the filtrate was run through a glass column (50 x 5 centimeter) containing Amberlit XAD-4 resins with an average pore size of 4 nm. As a result, sugars and other polar compounds were washed with an aqueous solution while phenolic compounds stayed in the column. After washing the column with acidic water (100 mL), it was rinsed with purified water (300 mL). The entire phenol fraction was then concentrated under decreased pressure after being washed with 400 mL of methanol and concentrated under reduced pressure (40 °C). After being redissolved in 5 mL of water, the residue was filtered

with diethyl ether. (5 mL x 3). In a rotary evaporator operating at 30 °C under decreased pressure, the ether extracts were combined and concentrated. In order to prepare the desiccated residue for LC-MS/MS analysis, the sample was dissolved in 0.5 mL of methanol and passed through a 0.45 µm membrane filter. Analysis of phenolic substances using LC-MS/MS. A UHPLC instrument coupled with a double MS device was used to analyze the samples. The samples contained 25 distinct phenolic compounds, which were examined.

3. Results

Phenolic contents of honey samples are listed in Table 1.

Table 1. The phenolic components (ng g^{-1}) of the honey samples from Şanlıurfa city.

	n	Minimum	Maximum	Mean±SD
Catechin hydrate	9	23.81	70.23	42.23±5.21
Acetohydroxamic acid	3	3.79	3.679	3.59±0.101
Vanillic acid	10	189.3	904.7	522.1±238.3
Resveratrol	10	35.70	219.154	96.27±57.3
Fumaric acid	10	69.25	737.91	218.41±198.75
Gallic acid	6	44.39	95.38	61.88±25.8
Caffeic acid	10	56.98	371.82	174.17±102.01
Phloridzin dehydrate	10	73.151	171.56	111.77±26.15
Oleuropein	8	50.14	140.43	100.39±27.03
Hydroxycinnamic acid	10	351.80	4817.52	1552.1±1556.3
Quercetin	5	47.265	135.50	85.11±34.76
Ellagic acid	3	262.16	529.70	365.85±143.55
Myricetin	-	-	-	ND
Protocatechuic acid	-	-	-	ND
Silymarin	10	77.25	180.98	119.36±34.91
2-Hydroxy-1,4-naphthoquinone	-	-	-	ND
Butein	6	0.233	37.593	16.42±16.50
Naringenin	2	30.81	119.62	75.22±62.80
Luteolin	-	-	-	ND
Kaempferol	-	-	-	ND
Curcumin	5	44.87	58.85	48.51±5.81
Thymoquinone	-	-	-	ND
Alizarin	-	-	-	ND
Hydroxybenzoic acid	10	31.73	1069.32	208.12±308.26
Salisylic acid	10	23.17	1060.15	194.37±309.55

4. Discussion

The adverse effects of dangerous chemicals and artificial food additives on public health in the last few decades have encouraged people to turn to more traditional and natural techniques. The most popular of these are healing methods that use bee products to fortify and protect the immune system. Honey, which is a natural product, is also used for many purposes. In particular, the determination of the phenolic composition is also important in determining the purpose of use. Because phenolic compounds are natural antioxidant substances that are generally found in high proportions in plants and are responsible for the sensory properties of foods such as color, smell, taste, and the preservation of storage stability and nutritional value (Robbins, 2003).

In this study, 25 phenolic compounds were analyzed in the honey samples from Şanlıurfa city of Türkiye. Myricetin, protocatechuic acid, 2-Hydroxi 1-4-naphthoquinone, luteolin, kaempherol, thymoquinone, and alizarine could not be found in the honey samples among these phenolic compounds. Phenolic compounds detected in all honeys are vanillic acid, resveratrol, fumaric acid, caffeic acid, phloridzin dehydrate, hydroxycinnamic acid, silymarin, hydroxybenzoic acid, and salicylic acid. Hydroxycinnamic acid (1552.1±1556.3 ng g^{-1}) and vanillic acid (522.1±238.3) had the greatest concentrations of phenolic compounds (14.49 ng g^{-1}). Acetohydroxamic acid (3.59±0.101) was the lowest phenolic substances of the honey samples. Previous investigation have noted the presence of quercetin, which is consistent with our results (Karabagias et al., 2014). Silici et al. (2014) analyzed the phenolic components in the honeys and determined coumaric acids as major components, and stated that gallic acid and ferulic acid were the phenolic components observed in all samples. Can et al. (2015), in their study on different types of honey obtained from various regions of Turkey; They stated that caffeic, benzoic, and p-coumaric acid were in different amounts in all honey varieties, while fisetin, chlorogenic acid, and myricetin were absent in any honey variety. The results of the researcher and the findings of this study are quite different.

According to the data obtained in the study conducted by Durmaz (2020); from phenolic acids in flower honeys; fumaric acid was detected in all honeys and 2-hydroxy cinnamic acid was not found in any honey samples. Of the flavonoids, quercetin, coumarin, 6.7-dihydroxy coumarin, and kaemferol were not found in any honeysamples. The highest level in terms of fumaric acid and protocatechuic acid content was observed in Çanakkale honey, and the highest value in terms of myricetin, trans-aconitic acid, rosmarinic acid, p-hydroxy phenyl acetic acid and trans-cinnamic acid was observed in Düzce honey.

In Durmaz NE's doctoral thesis published in 2020, phenolic acids in multifloral flower honey collected from 25 different regions of Turkey; All honeys included fumaric acid, 24 honeys contained protocatechic acid, 22 honeys contained p-hydroxy phenyl acetic acid and p-hydroxy benzoic acid, 21 honeys contained vanillic acid, and none contained 2-hydroxy cinnamic acid (Durmaz NE, 2020).

Some results were similar because of the honey kinds' major pollen characteristics, but complete compatibility could not be guaranteed. This indicates that the geographic factors of the area of production affect the phenolic contents of honeys, which are not only influenced by the plant flora (Mendes, 1998).

5. Conclusion

Purity and herbal origin, which come to the fore in the world honey trade, are of great importance in the export of honey. In domestic consumption, these parameters are also used in order to prevent cheating. It is important to determine the chemical properties of honey in the

laboratory environment and to classify it according to its botanical and geographical origin. Phenolic component analysis, which are important components of honey, is also important for both food safety and medical medicine.

In conclusion, this study revealed the phenolic compounds of multifloral honey produced in Şanlıurfa, Turkiye, which has a rich plant flora and honey diversity. Finally, this study has shown once more how geographical variances, the flora from which honey is derived, and its physicochemical and biologically active characteristics all have an impact on honey.

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Epidemiological Investigation of Betanodavirus Prevalence in Sea Bass Hatcheries For All Production Stages in Türkiye#

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Abstract:
Aim: Viral Nervous Necrosis caused by Betanodaviruses is an important viral disease in sea bass farming and endemic in the Mediterranean basin. Since there are many hatcheries and sea bass culture is commonplace in Türkiye, monitoring studies are crucial for the control and prevention of the betanodavirus infections. The aim of this study was to investigation betanodavirus in sea bass hatcheries in Türkiye.

Materials and Methods: All production stages of all sea bass hatcheries actively operating in Türkiye were sampled. Samples were taken from 16 hatcheries and 135 study groups of a total of 2460 samples with each group consisting of 10-30 fish were formed. Betanodavirus was investigated by Real Time RT-PCR. Partial genome analysis and phylogenetic studies were performed according to the RNA1 and RNA2 segment of the virus.

Findings and Results: Viral RNA was detected in fingerlings in one hatchery and prevalence in Turkish hatcheries was calculated to be 6.25% (1/16). Partial genome analysis of both RNA1 and RNA2 segments of the virus revealed that it was the Redspotted grouper nervous necrosis virus genotype which is endemic in the Mediterranean basin. In the epidemiological follow-up of infected and related hatcheries, virus was not detected again.

Discussion and Conclusion: Betanodavirus was only detected in fingerling-size fish. The absence of mortality in the hatchery where the virus was detected, the healthy appearance of the sea bass and the low viral load indicate that the infection might be subclinical. Also, the fact that the virus was not found in other age groups where biosecurity measures were implemented, shows that is not active infection. As a result of this study, betanodavirus capacity of all production stages of all hatcheries in Türkiye was revealed. It can be concluded that there are no circulating betanodavirus in hatcheries and the detected virus could be of sea-water origin.

Keywords: betanodavirus, hatchery, prevalance, sea bass, Türkiye

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1. Introduction

Viral Nervous Necrosis (VNN) is a serious viral disease that causes significant losses in many marine fish species associated with vacuolizations in the central nervous system and retina (OIE 2017). Many fish species with high economic importance have proven to be susceptible to VNN

virus that have become a serious problem in aquaculture for the last thirty years (Thiery et al. 2011, Toffan et al. 2017).

Causative agent of VNN is betanodavirus which belong to nodaviridae family (Thiery et al. 2011). Betanodaviruses have a non-enveloped, rounded morphology (Johnson et al. 2003) and its genome consists of two segments, RNA1 and RNA2, which are single-stranded, positive polarity and contain three open reading frame-ORFs (Anonymous 2018b, Fields 2013, Johnson et al. 2003). RNA1 (3.1 kb) is responsible for the synthesis of RNA-dependent RNA polymerase (RdRp) with a molecular weight of 110 kDa and carries all the information necessary for autonomous replication (Gallagher and Rueckert 1988, Nagai and Nishizawa 1999). RNA1 also plays a role in regulating the temperature sensitivity of the virus. RNA2 (1410 bp) is responsible for host tropism and immunoreactivity (Johnson et al. 2003, Nishizawa et al. 1995, Ito et al. 2008, Iwamoto et al. 2004, Panzarin et al. 2016). RNA2 also contains the T4 multivariable region used in the classification of genotypes (Nishizawa et al. 1997). The third transcript, known as RNA3 (0.4 kb) originated from the RNA1 terminus during viral replication, is involved in the assembly of viral RNA in the host cell and encode non-structural B1 and B2 proteins (Fenner et al. 2006, Iwamoto et al. 2005).

Betanodaviruses are grouped into four major genotypes based on phylogenetic analysis of the T4 variable region of the RNA2 segment including striped jack nervous necrosis virus (SJNNV), tiger puffer nervous necrosis virus (TPNNV), barfin flounder nervous necrosis virus (BFNNV) and red-spotted grouper nervous necrosis virus (RGNNV) (ICTV 2017).

There are many hatcheries and sea bass culture is commonplace in Türkiye and, so, monitoring studies are crucial for the control and prevention of the betanodavirus infections. The aim of this study was to investigation betanodavirus in all production stages of all sea bass in Türkiye.

2. Materials and Methods

2.1. Sampling of hatcheries

All sea bass hatcheries registered and actively operating in Türkiye were sampled in 2016-2017. Sixteen hatcheries were sampled. Sampled hatcheries were numbered from K-1 to K-16. A total of 2460 samples were collected from all production stages of all sea bass hatcheries. Each group consists of 10-30 fish, 15 groups of fertilized eggs from 8 hatcheries, 14 groups of prelarvae (0-5 days-old) from 9 hatcheries, 22 groups of postlarvae (5-40 days-old) from 13 hatcheries, 43 groups of fry (40-80 days-old) from 13 hatcheries and 41 groups of fingerlings (80-120 days-old) from 9 hatcheries, (OIE, 2015) were sampled. Water temperatures were measured as 15 ℃ in DY tanks, 15-16 ℃ in preL tanks, 16-20 ℃ in postL tanks, 16-22 ℃ in fry tanks, and 20-24 ℃ in fingerling tanks.

2.2. Preparation of the samples

Homogenizates were obtained from whole body in FE, preL and postL, whole head in fry. Brain, spinal cord and eye were only obtained from fingerling and big size fishes. All samples were homogenized with a mortar, pestle and sterile sand (Sea sand, Merck, Germany, CAS-No:

14808-60-7) and suspended in Eagle's minimal essential medium (EMEM, Sigma-Aldrich, United Kingdom, Product No: M4655) supplemented with 2% foetal calf serum (Biochrom, Germany) and 1% antibiotic-antimycotic solution (Sigma-Aldrich, USA) at a ratio of 1/5 (w/v). The homogenates were clarified by centrifugation at 4000 g for 15 min at +4°C and percolated with 0.45 µm filtrate (Sartorius, USA) for use in molecular and virological studies. Inoculums were stored at -80 °C until used.

2.3. Molecular studies

Viral RNA extraction

Viral RNA extraction was performed using commercial extraction kit (MagNA Pure LC Total Nucleic Acid Isolation Kit, Roche, Germany) and an automatic extraction device (Roche MagNA Pure LC System, Germany).

Quantitative Real Time RT-PCR

Quantitative Real Time RT-PCR (RT-qPCR) was performed using commercial extraction kit (Real Time Ready Virus Master, Roche) and Real Time PCR device (Roche LyghtCycler ® 480 Multiwell Plate 96). Primers and probes (Panzarin et al. 2010) designed according to the T4 variable region of the RNA2 segment of the betanodavirus proposed by World Organisation for Animal Health (OIE, 2014) were used for amplification. Mastermix was prepared on ice according to kit manufacturer recommendations. Mastermix was placed in 96 well Real Time PCR plates Roche LightCycler® 480 Multiwell Plate 96, White, Germany), then 15 µl of sample, positive control and negative control were added. Plate was centrifuged at 1500 g at 4 ºC for 2 minutes and RT-qPCR test was performed.

cDNA Syntesis, PCR ve Sequencing

cDNA synthesis (Transcriptor First Strand cDNA Synthesis Kit, Roche, Germany, No. 04 379 012 001) was performed from RNA extracts of positive samples. PCR was performed from cDNAs using commercial kit (FastStart High Fidelity PCR System, dNTPack Roche, Germany, Cat. No. 04 738 292 001) (Techne TC-412, United Kingdom) for partial sequencing Primers in PCR for partial genome sequence analysis were used recommended by Toffolo et al. (2007) and Bovo et al. (2011). Partial sequencing of RNA1 and RNA2 segments was done by a commercial firm (Microsynt, Balgach, Switzerland). The correction and matching of the sequences were conducted with DNADynamo program (Blue Tractor Software Ltd., UK). The consensus nucleotide sequences obtained were compared and verified on the Basic Local Alignment Search Tool (BLAST) system in National Central for Biotechnology Information (NCBI) (Altschul et al. 1990). For phylogenetic analysis, multiple sequence alignment of RNA1 and RNA2 partial nucleotide sequences of the isolates and reference sequences obtained from the GenBank were performed with the ClustalW method using the MEGA6 program. The best Protein / DNA model was determined for both segments and the nucleotide similarity between sequences were determined. Phylogenetic trees were created with the Neighbor-Joining-NJ method using 1000 bootstrap values repetitions (Tamura et al. 2013).

3. Results

Betanodavirus was detected in 1 of 16 hatcheries (K-1) in fingerling-sized sea bass that were 115 days old, 2.5-3.74 g in weight and 6.5-7 cm in length. Isolate was named as TR.VNN.01.02. In determining the betanodavirus prevalence of the country, the hatchery in which the virus was detected in any age group, regardless of the age groups of the sea bass in the hatchery, was evaluated as positive. In determining the epidemiological units, it was aimed to reveal the situation of betanodavirus countrywide, so the whole country was considered as a unit and hatcheries as a subunit. According to epidemiological approach, betanodavirus prevalence in Turkish hatcheries was calculated as 6.25% (1/16). The K-1 hatchery, detected betanodavirus, was sampled in May when the seawater temperature started to rise, and no virus was detected in postlarvae and fry-sized fish collected in the same period. In the information obtained about the hatchery, it is stated that the facility is a closed system, biosecurity measures are applied, water supplied from the sea is filtered, ozone and UV sterilized, and older than 1.5-2 g fingerling are taken into the external system. It was learned that sea water that was not subjected was used in external system.

Positive samples were found to be RGNNV genotype with phylogenetic analysis performed according to both RNA1 and RNA2 segments (Figure 1 and 2). In the partial genome sequencing of the RNA1 segment of TR.VNN.01.02, 937 nt (between 197-1133 nt) were obtained. In the partial genome sequencing of the RNA2 segment of TR.VNN.01.02, 567 nt (between 365-931 nt), respectively, were obtained. Shorter regions with a length of 899 nt for the RNA1 segment and 480 nt for the RNA2 segment were used in order to compare more isolates in phylogenetic analyzes. The nucleotide differences of the isolates obtained in the study and the reference isolates obtained from genbank were determined according to both RNA1 and RNA2 segments.

Partial nucleotide sequences of both the RNA1 and RNA2 segments of TR.VNN.01.02 were uploaded to GenBank, and accession numbers were assigned as MT451940 for RNA1 segment, MT451942 for RNA2 segment.

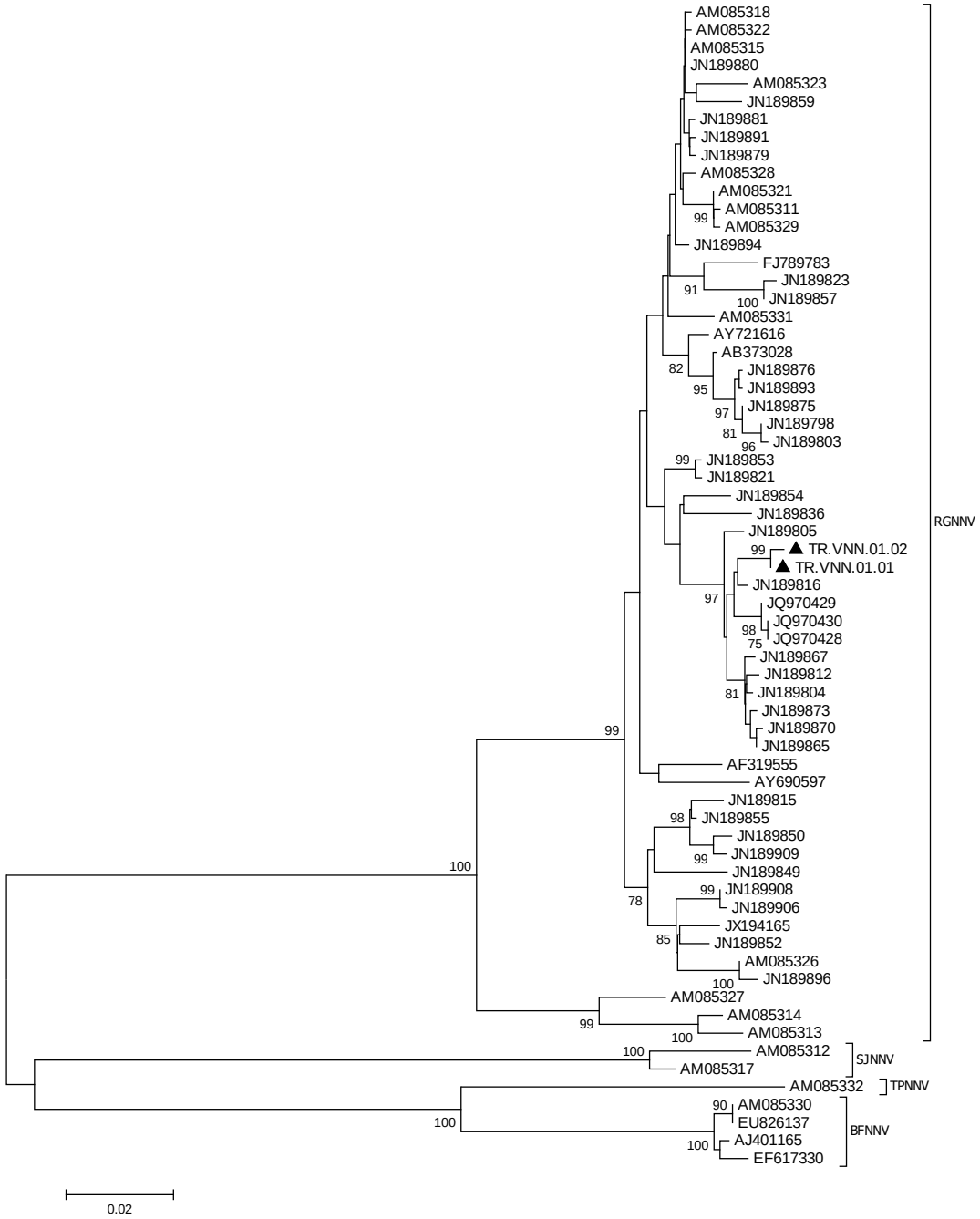

Figure 1. Phylogenetic tree according to partial genome of RNA1 segment. Phylogenetic tree was constructed based on anaylsis of 899 bp of RNA1 segment (Reference whole genom, Genbank accession number: FJ789783, was used beetwen 200-1099 nt) partial sequence of RNA1 segment using the NJ metod with TN93+G model. Confidence on tree contruction was assessed using 1000 bootstrap replicates. Only values >70% are indicated.

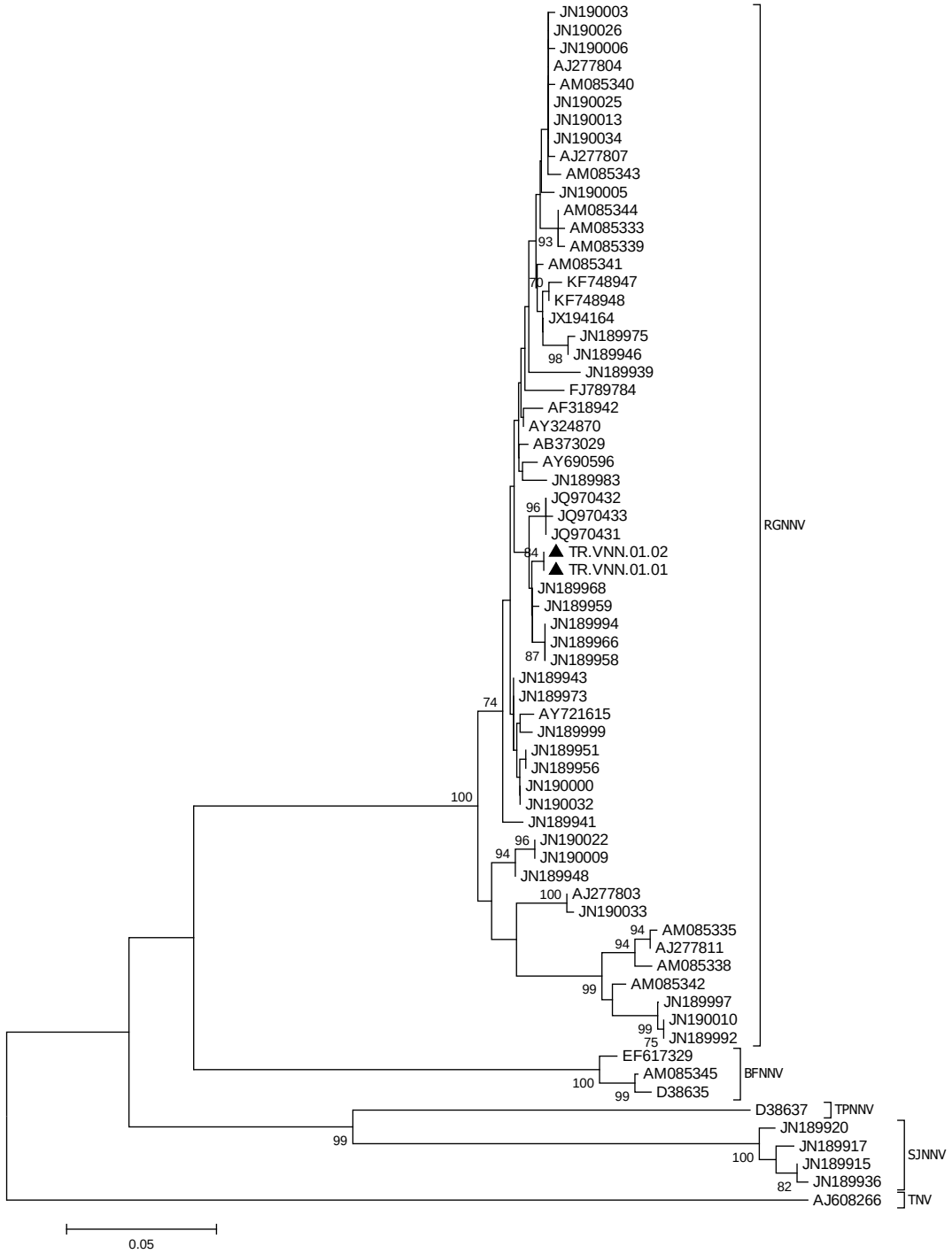

Figure 2. Phylogenetic tree according to partial genome of RNA2 segment. Phylogenetic tree was constructed based on anaylsis of 480 bp of RNA2 segment (Reference whole genom, Genbank accession number: FJ789784, was used beetwen 426-907 nt) partial sequence of RNA2 segment using the NJ metod with K2+G model. Confidence on tree contruction was assessed using 1000 bootstrap replicates. Only values >70% are indicated.

4. Discussion

Türkiye has 42% of sea bass production in the world (Anonymous 2018b) and a strategic position in terms of sea bass production both in Europe and worldwide. The sea bass production in Türkiye has high rate of 32.8% within all fish species (Anonymous 2018a).

Since VNN is not a notifiable disease in animal diseases reporting systems such as OIE and ADNS, there are difficulties in evaluating the epidemiological situation of countries. However, it is seen that the VNN is endemic in the Mediterranean basin in the light of literatures. When the few studies conducted to determine epidemiological prevalence of betanodavirus are examined, it is seen that ranges from 0.23% to 88.5% (Barker et al. 2002, Berzak et al. 2019, Gomez et al. 2004, Liu et al. 2015, Sakamoto et al. 2008). Here, we investigated betanodavirus prevalence based on epidemiological approach in all hatcheries in Türkiye. Betanodavirus prevalence in hatcheries was found to be 6.25%.

It has been reported that water temperature affects the activity of the RdRp which is necessary for the replication of the virus, and that different optimum temperatures are required for the epidemiology of different genotypes (Liu et al. 2015). It is known that betanodaviruses are highly resistant to both freshwater and seawater environment and also to be highly resistant to external environment (Vendramin et al. 2012). For this reason, larvae or fry-sized sea bass could be exposed to the virus through horizontal transmission in hatchery used contaminated sea water (Nerland et al. 2007). It is seen that the water temperatures of the hatcheries in the study are lower than the optimum growth temperature of the RGNNV genotype. However, the main purpose of the study in hatcheries is to detect the vertical transmission of the virus that can be found persistently in the spawner and the virus that can be found in the environmental water, if any. The virus has not been detected in FE, preL, postL and fry sized fish, which supports the absence of persistent virus infection in spawners. When the decontamination methods of the water used in hatcheries are evaluated, it is thought that the facilities imlement biosecurity measures by using the filter and UV method and these help the water used to be free from virus. Viruses detected in hatcheries were isolated from fish in units outside the closed system where sea water is used directly. It is thought that the detected viruses might be due to horizontal transmission from sea water where virus endemic region.

5. Conclusion

In conclusion, the presence and prevalence of betanodavirus was investigated in all age groups in both sea bass hatcheries in Türkiye. It was evaluated that there was no active infection in the juvenile sea bass and that they could be subclinically infected due to low viral load. It has been evaluated that biosafety measures prevent water-borne transmission of the virus, and it is suggested that biosafety measures implemented in hatcheries should continue and seawater should not be used without sanitation.

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Association of J Wave with Prognosis in Dogs with Congestive Heart Failure

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Abstract

Congestive heart disease is a common and inevitable condition in dogs and humans. Congestive heart failure occurs in two different forms acquired and congenital. ACVIM (American College of Internal Medicine) classifies heart failure into four stages. Electrocardiography; ıt is a valuable tool used as a prognostic method that reveals the electrical conduction of the heart, gives an early warning in terms of diagnosis, and is used as a prognostic method in rhythm disorders caused by intracardiac and extracardiac factors. It is used in conjunction with other cardiac examination methods. Waves in electrocardiography; It is a reflection of the action potentials of the atria and ventricles. In electrocardiography, the j wave (Osborn wave) is a morphological finding that protrudes between the R and ST waves as described by Osborn in 1953. The mechanism of J wave formation is not fully understood yet. It is assumed that during early repolarization, it exhibits a transient electrical variation between the ventricular endocardium and the epicardium. Changes in the J wave can be physiological and occur in conditions such as cardiac ischemia, electrolyte imbalance, and hypothermia. **Objective:** To examine the character of the J wave on the 0^{th} day and 30^{th} day of the treatment in dogs with congestive heart failure classified according to ACVIM and to reveal its relationship with the prognosis. **Methods:** 10 dogs referred to our clinic with cough, fatigue, and exercise intolerance were submitted to cardiological examination and classified according to ACVIM, On the 0^{th} day and 30^{th} day, the J wave characteristics will be determined at the 25 mm/sec derivation, 10mm/mV setting at lead II. **Results:** On the 30^{th} day, it was observed that the J wave interval and amplitude were decreased, which was positively correlated with the treatment (amplitude evaluation, $p<0.01$, interval measurements $p<0.001$). **Conclusion:** J wave is helpful prognostically in dogs with congestive heart failure, but further studies with larger samples are required.

Keywords: Electrocardiography, Congestive Failure, Dog, Osborn wave

1.Introduction

Congestive Heart Failure (CHF) is a syndrome regarding heart's inability to pump enough blood to the body, resulting in stagnant blood and causing congestion. Conditions that cause congestive heart failure are divided into acquired and congenital states. As congenital causes, arteriovenous fistulas, vascular anomalies, patent ductus arteriosus, ventricular septal defect, and atrial septal defect can be given as examples. Degenerative Mitral Valve Disease is usually the leading cause of acquired CHF in dogs. CHF, which occurs at an early age in some

predisposed breeds, is seen in 80% of geriatric patients. Apart from this disease, valvular anomalies, dilated cardiomyopathy, pericardial effusion (neoplasia, idiopathic, infective), conduction disorders (primary or secondary), Drofilaria immitis, systemic hypertension, endocarditis, neoplasia and pregnancy can cause acquired differentiation in the myocardium (Keene et al. 2019; Nelson and Richard 2019). As a result of these disorders, the heart that cannot create sufficient stroke volüme and cannot pump enough blood to the system. Hemodynamics is disturbed and edema forms. Night cough, exercise intolerance, fatigue, and inability to climb stairs are common complaints. Patients must undergo cardiological examination, also general physical test, blood pressure measurement, whole blood, and biochemistry analysis, hormone analysis (Nt-proBNP and Cardiac Troponin I), radiography, electrocardiography, echocardiography, tomography or magnetic resonance imaging if necessary Nelson and Richard 2019. ACVIM (American College of Veterinary Internal Medicine) divides heart failure patients into four stages; Stage A: breeds predisposed to heart disease, Stage B. asymptomatic heart patients, Stage C symptomatic heart patients, and Stage D heart patients in the refractory period Keene et al. 2019. In electrocardiography, the J wave (Osborn wave) is a positively deflected point with protuberant morphology located between the end of ventricular depolarization and the beginning of ventricular repolarization, that is, at the junction of the QRS wave and the ST segment. It is seen as negative deflection in avR from the limb leads and V1 from the chest leads. It is a classical finding of hypothermia, but not pathognomonic (Abbott and Cheitlin 1976; Agudelo and Schánilec 2015; Antzelevitch and Yan 2015; Hlaing et al. 2005; Yan et al. 2004).

In 1953, Dr. Osborn discovered the J wave in acidotic and hypothermic dogs, with a point he called an "injury current" that fibrillates at rectal temperatures below 25°C. Its importance in tachyarrhythmias was determined in 1996. J wave can also occur in conditions such as myocardial ischemia, hypercalcemia, brain damage, subarachnoid haemorrhage, sepsis, Brugada syndrome, acute myocardial iscischemia, prinzmetal angına and hypertrophy of the left ventricle. Although its exact pathophysiology is not described, it is thought to be caused by conduction disorders (between ventricular endocardium and epicardium during early repolarization). (Agudelo and Schánilec 2015; Osborn 1953)

2.Materials and Methods

Cavalier King Charles Spaniel and mixed breed dogs (n=10) who came to Ondokuz Mayıs University, Department of Internal Medicine with cough, fatigue, fainting, and exercise intolerance, general physical examination (palpation of lymph nodes, heart rhythm, heart rate/minute, respiratory rate/minute, dehydration rate, hepatojugular reflux examination), complete blood count, serum biochemistry analyzes, Nt-proBNP and Cardiac Troponin I hormone analyze, radiographic examination, electrocardiographic examination, and echocardiographic examination were performed. After heart failure classification according to ACVIM (American College of Internal Medicine), the patients' ECGs were obtained with the Televet V100 brand telemetric Ecg&Holter device and were evaluated with the Televet V100 PC Software. The amplitude and intervals of Day 0 J waves were measured by taking the average of 6 cycles. Telemetric ECG measurements were performed again on the 30th day after

the patients were administered conventional treatment (pimobendan 0.3 mg/kg BID, enalapril 0.5 mg/kg BID, propranolol 0.5 mg/kg BID, furosemide 1 mg/kg BID, spironolactone 0.5 mg/kg BID). Measurements of J wave characteristics on Day 0 and Day 30 were compared. KyPlot 6.0 for statistical measures. A parametric t-test was applied using Data Analysis and Visualization software. Amplitude values and interval values were compared separately.

3.Results

Diagnostic complete blood count and serum biochemistry analyses of the patients were in reference ranges. Nt-proBNP and Cardiac Troponin I value on day 0 were higher than usual and cleared the diagnosis. High vertebral heart score measurements were recorded in radiographic analyses. Electrocardiographic measurement findings were variable. Echocardiographic measurements revealed augmented left atrial size and marked mitral valve regurgitation. It was observed that the J wave measurements were high on the 0^{th} day and decreased on the 30^{th} day after the treatment. Day 0 and day 30 measurements were compared (Figure 1). It was determined that the J wave interval and amplitudes decreased on the 30^{th} day of 8 patients which demonstrated clinical improvement. Although the amplitude value decreased in 2 patients, the interval values remained the same.

In statistical analysis, amplitude (mV) and interval (ms) values of 10 dogs were evaluated by t-test. Student t-test statistical evaluation of amplitude values at day 0 and day 30 were **p<0.01; interval measurements were ***p<0.001 and were found to be significantly significant **(Table 1).**

Table1: t Test Results : **p<0.01 (amplitude evaluation), ***p<0.001 (interval evaluation)

n=10	0.Day	30. Day
Mean±Standard Deviation (interval/ms)	20,2±1,47	18,6±1,34
Mean±Standard Deviation (amplitude/mV)	0,31±0.03	0,14±0.05

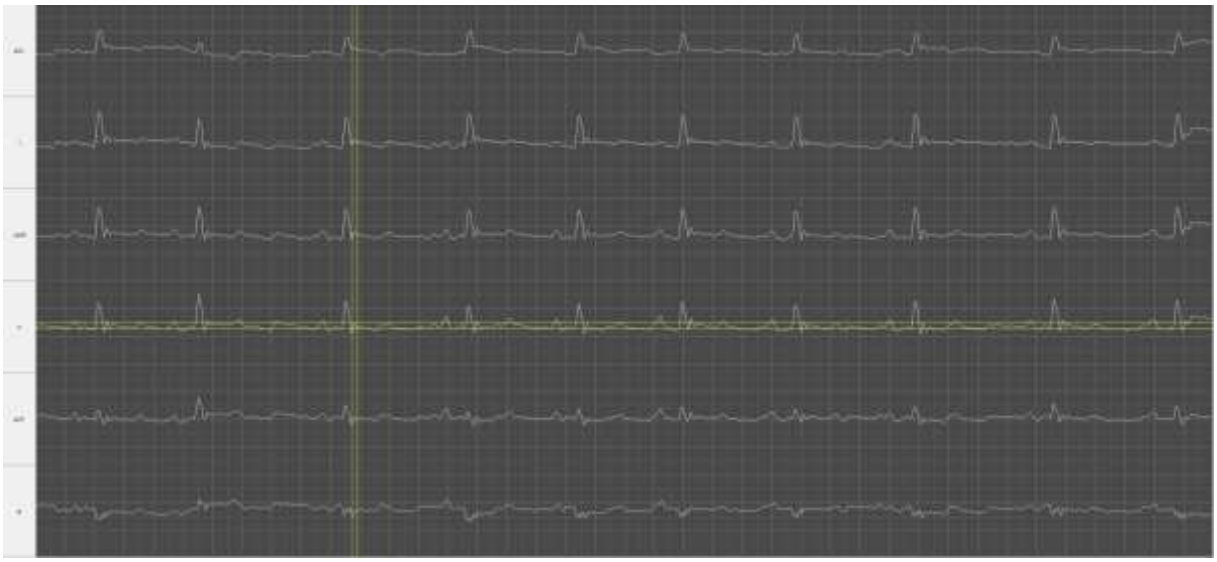

Figure 1: ECG measurement (Lead II. 25 mm/ms, 10 mm/mV) and J wave characteristic measurement with Televet V100 Telemetric ECG & Holter Device Software (Measurements are made from zoomed images)

4.Discussion

J wave may be a normal finding or may indicate a pathological result. In 2008, Haissaguerre et al. showed an association between J waves, an early repolarization pattern, and ventricular fibrillation. Accordingly, the J wave was a hallmark for the pathogenesis of ventricular fibrillation Haïssaguerre et al 2008. In the present study, no ventricular premature complex or fibrillation was observed. According to one study, a J wave was a normal finding in senior dogs without hypothermia, hypercalcemia, or other conditions leading to pathological J waves and there was no difference in the J wave regarding exercise-induced myocardial ischemia, but patients may have different electrocardiographic parameters in extensive exercises Agudelo and Schánilec. 2015. In this study, the comparison of the amplitude and interval values of the J wave at day 0 and day 30 after treatment in 10 dogs with congestive heart failure classified according to ACVIM was investigated, and it was observed that the J wave waned with the relief of symptoms by treatment. This finding may be related to increased myocardial oxygen saturation. In a study comparing Petit Basset Griffon Vende'en dogs, with different breeds, the J wave was reported as a normal electrocardiographic finding fort his spesific breed. However, it was stated that Holter follow-up studies are needed for the relationship between J waves and malignant arrhythmias. Rudling et al. 2016. A study in humans, suggested that the horizontal ST segment morphology formed after the J wave indicates arrhythmogenic conditions drawing attention to the prognostic importance of this complex Takagi et al. 2013. Another study noted that the J wave's predictive value is elucidatory in people with hypertrophic cardiomyopathy Tsuda et al 2017. In the present study, the J wave preserved its existence on the 0th and 30th days, but as we have stated, its characteristics varied. In addition, study scale was limited. Further studies with larger scales are needed.

5.Conclusion

J wave is a non-pathognomonic electrocardiographic finding that can exist in pathological and nonpathological conditions. It may also form due to myocardial ischemia in congestive heart failure. J wave is prognostically helpful in dogs with congestive heart failure, but studies with larger samples are needed.

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Determination of Antifungal and Antibacterial Activity of Cream Formulation Containing Red Pitahaya Methanol Extract

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Abstract:

Pitahaya is known as a fruit with rich bioactive compounds that benefit in many areas. Knowing the harmful side effects of chemicals in synthetic creams has increased the demand for herbal creams today. The skin is the largest organ of our body and is constantly exposed to chemical and physical threats. It is well known that probiotics are beneficial for skin. Topical application of probiotics can help maintain healthy skin and skin biome, as well as protect it from various infections. In our study, it was aimed to develop cream formulations containing extracts and probiotics, which are natural additives, for topical applications, and then to determine the antimicrobial activity of this cream formulation. For this purpose, red pitahaya flesh and peel obtained from Turkey were extracted using methanol solvent. Then, cream formulations containing red pitahaya methanol extracts and breast milk originated probiotic candidate *Limosilactobacillus fermentum* MA-7 strain have been developed. The antifungal and antibacterial activities of the developed cream formulation against *Candida albicans* ATCC 10231 and *Listeria monocytogenes* ATCC 7644 test microorganisms were investigated in vitro using well diffusion assay. The results showed that the inhibition zone diameter of the cream formulation containing red pitahaya flesh extract and *L. fermentum* MA-7 strain against *C. albicans* ATCC 10231 was determined as 6.80 mm. The higher antimicrobial activity was determined in the cream formulation containing red pitahaya peel extract and *L. fermentum* MA-7 against *C. albicans* ATCC 10231 with a 9.40 mm zone of inhibition. In addition, the inhibition zone diameters of the cream formulations containing pitahaya flesh and peel extracts and the *L. fermentum* MA-7 against *L. monocytogenes* ATCC 7644 were found to be 5.81 mm and 2.28 mm, respectively. Furthermore, the cream group (control) showed no inhibitory activity against both test microorganisms. These results indicate that the developed creams with natural ingredients may be used as a preventive and therapeutic agent against skin contaminations. The data from the study have the potential to lead to further studies in the pharmaceutical and cosmetic industries.

Keywords: Pitahaya, probiotic, skin, cream, antimicrobial.

1. Introduction

The red pitahaya fruit (*Hylocereus polyrhizus*) is of great interest due to its remarkable red-purple color and biological activities (antioxidant capacity, anti-inflammatory, anti-bacterial and anti-obesity) (Celli and Brooks 2017; Liao et al. 2020). Red pitahaya is a source of a wide range of pharmaceutically important active ingredients. Red pitahaya has anti-elastase and anti-collagenase activities (Vijayakumar et al. 2017). Consumers generally perceive products containing natural ingredients as higher quality, healthier and safer than products containing synthetic ingredients (Martins et al. 2016).

The skin is an immunogenic organ that functions as a biological sensor for us and is the first defense against external allergens (Schmidt 2004). The skin is the largest organ of our body and is constantly exposed to chemical, physical, fungal, and bacterial threats. Probiotics are well known to be beneficial for skin conditions and have been clinically studies (Roudsari et al. 2015). Bacterial infections of the skin and subcutaneous tissue are among the most common nosocomial infections in the community (Bowler 2001). The probiotics may have potential uses in the prevention and treatment of skin diseases in many areas such as skin hypersensitivity, allergic inflammation, UV-induced skin harm, wound protection. GRAS status, *L. fermentum* can be used as food, medicine and cosmetics and is not subject to pre-market approval requirements (GRAS 2020).

Skin infections caused by yeasts, especially those associated with *Candida albicans*, are quite common. In addition, *Candida* species are emerging with new strains with greater resistance to existing antifungal agents (Espinosa-Hernández et al. 2020). Plants can produce compounds with many biological activities and can act against pathogens. Thus, plant extracts are expected to be effective on drug-resistant pathogenic bacteria (Amenu 2014).

The aim of our study was to determine the usage potential of red pitahaya flesh and peel methanol extracts and cream formulation developed with human milk originated probiotic candidate *L. fermentum* MA-7 in cosmetic and pharmaceutical fields. The antifungal and antibacterial activities of the developed cream formulation against *C. albicans* ATCC 10231 and *L. monocytogenes* ATCC 7644 test microorganisms were investigated in-vitro.

2. Materials and Methods

2.1. Preparation of Red Pitahaya Extracts

The red pitahaya fruit samples were washed with distilled water. It is then air-dried at room temperature. The dried fruit samples were ground with blender (Waring). In extraction, 10 g powder from red pitahaya flesh and peel were extracted with 30 ml of methanol using a sonication device (Hielscher) on ice in 2 repetitions in 10 minutes every day (2 days) each. Red pitahaya flesh and peel extracts were dissolved using dimethyl sulfoxide (DMSO) and then sterilized with a filter (0.22 μm).

2.2. Preparation of Microorganisms

C. albicans ATCC 10231 was cultured at 30ºC in Yeast Peptone Dextrose (YPD) media and *L. monocytogenes* ATCC 7644 was cultured at 37ºC in Tryptic Soy Broth (TSB) medium for 24 hours. The test microorganisms were washed with saline solution (twice) and their concentration was adjusted to 0.5 McFarland.

2.3. Preparation of Cream Formulation Containing Red Pitahaya Methanol Extract and *Limosilactobacillus fermentum* MA-7

The antifungal and antibacterial activities of the cream formulations was obtained using the modified method used in our previous study (Asan-Ozusaglam and Celik 2023). In the

developed antimicrobial cream formulations, a cream, red pitahaya methanol extracts and a probiotic candidate strain *L. fermentum* MA-7 (Asan-Ozusaglam and Gunyakti, 2018) were used. The antifungal and antibacterial activities of the developed cream groups was determined against the microorganism strains (*C. albicans* ATCC 10231 and *L. monocytogenes* ATCC 7644) using the well diffusion assay. The culture dishes were incubated in the conditions mentioned above for the test microorganisms.

3. Results

The well diffusion assay results of cream formulations containing red pitahaya and *L. fermentum* MA-7 strain are presented in Table 1. The inhibition zone diameter against *C. albicans* ATCC 10231 and *L. monocytogenes* ATCC 7644 strains were not observed in the control (Cream) group. CE group containing cream and peel extract was determined as 1.31 mm and 1.05 mm against *C. albicans* ATCC 10231 and *L. monocytogenes* ATCC 7644 while CE group containing cream and flesh extract did not show inhibition zone diameter. Inhibition zone diameters of CEL (Cream-Flesh Extract-*L. fermentum* MA-7) group against *C. albicans* ATCC 10231 and *L. monocytogenes* ATCC 7644 were determined as 6.80 mm and 5.81 mm in the flesh extract. The inhibition zone diameter in the CEL (Cream-Peel Extract-*L. fermentum* MA-7) group was determined as 9.40 mm and 2.28 mm against *C. albicans* ATCC 10231 and *L. monocytogenes* ATCC 7644.

Table 1. Antimicrobial activity of cream formulations

Microorganism Strains	**Inhibition zone diameters of cream formulation containing red pitahaya flesh and peel extract (mm±SD)**					
			Flesh		**Peel**	
	C	**CL**	**CE**	**CEL**	**CE**	**CEL**
***C. albicans* ATCC 10231**	**-**	**6.76±0.60**	**-**	**6.80±0.10**	**1.31±0.09**	**9.40±0.32**
***L. monocytogenes* ATCC 7644**	**-**	**-**	**-**	**5.81±0.59**	**1.05±0.21**	**2.28±0.31**

*C: Cream, CL: Cream-*L. fermentum* MA-7, CE: Cream-Extract, CEL: Cream-Extract- *L. fermentum* MA-7

4. Discussion

Bacteria, acne, eczema, skin rashes, psoriasis, dermatitis, etc. it can cause many skin diseases, such as. Many of the topical medications used to treat skin-related infections are obtained through various synthetic processes that contain chemicals and have some side effects. There is a difference between pharmaceutical antibiotic and herbal antibiotic. Herbal-based antibiotics are not limited to the action of just one chemical, but they also have several components with various healing properties and produce a more synergistic effect to fight bacteria. Topical ointment containing medicinal plant extract is an alternative for treating a skin infection caused by bacteria and then preventing the use of oral antibiotics, which can develop resistance to the bacterium (Sekar and Rashid 2016).

Cream formulations prepared with *Pterocarpus santalinoides* methanol extract, the highest inhibition zone diameter against *C. albicans* was determined as 8.50 mm in P5 formulation (Okafo et al. 2023). The antimicrobial activity of *Clinacanthus nutans* leaf methanol extract of cream formulations prepared at various concentrations (25, 50 and 100 mg/ml) against *Staphylococcus aureus, Bacillus cereus, Escherichia coli and Pseudomonas aeruginosa* were determined by disc diffusion method. The antimicrobial activity of the cream formulations increased with increasing extract concentration (Sekar and Rashid 2016). The use of plant

extracts can be an alternative solution to the prevention of various skin problems by reducing the use of synthetic preservatives.
Nowadays, it is still very difficult to deal with fungal and bacterial skin infections using traditional medicines (Mlozi et al. 2023). The high antimicrobial activity of the prepared cream formulation against test microorganisms showed that it may have a potential for use in the pharmaceutical and cosmetic industry. Since studies on cream formulations containing red pitahaya extract are limited, more literature studies are needed.

5. Conclusion

The antifungal and antibacterial activities of the developed cream formulations against *C. albicans* ATCC 10231 and *L. monocytogenes* ATCC 7644 test microorganisms were investigated in vitro. The cream formulation containing red pitahaya extracts and *L. fermentum* MA-7 strain showed synergistic effect against test microorganisms. The potential for use in cosmetic and pharmaceutical fields of cream formulation containing natural ingredient red pitahaya extract and *L. fermentum* MA-7 strain instead of synthetic ingredient creams has been determined.

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Investigation of Antimicrobial Activity of Fruit Extracts from *Citrus medica* L. var. *sarcodactylis* Against Food-borne Pathogens

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Abstract:
Microbial contamination is one of the most important problems in the food industry, which leads to deterioration or contamination of food and adversely affects human health. *Staphylococcus aureus* is a major food-borne pathogen and a leading cause of foodborne illness worldwide. *Listeria monocytogenes*, another foodborne pathogenic bacterium, is thought to be responsible for serious diseases with high hospitalization and mortality rates. For this reason, protective products are needed to prevent the growth of harmful food-borne bacteria in food products. Plant extracts can be an alternative to chemical preservatives against different types of foodborne pathogens. *Citrus medica* L. var. *sarcodactylis* is a fruit belonging to the Rutaceae family, used for ornamental purposes and has medicinal importance. The aim of the study is to determine the antibacterial activity of *C. medica* L. var. *sarcodactylis* fruits against *S. aureus* ATCC 25923 and *L. monocytogenes* ATCC 7644 that cause important problems in the food industry. The antibacterial activity of water and ethanol extracts obtained from *C. medica* L. var. *sarcodactylis* fruits was obtained using disc diffusion method. The micro-dilution method was used to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts. The inhibition zone diameter of fruit water and ethanol extracts against *S. aureus* ATCC 25923 was obtained as 6.41 mm and 8.04 mm. The fruit water and ethanol extracts against *L. monocytogenes* ATCC 7644 showed an inhibition zone diameter of 6.07 mm and 7.13 mm. The results indicated that the fruit ethanol extract showed higher antimicrobial activity compared to water extract against both test microorganisms. MIC values of fruit water and ethanol extracts against test microorganisms are between 10-20 µg/µl. The lowest MBC value was determined as 10 µg/µl against *S. aureus* ATCC 25923 in fruit water extract. As a result of the study, *C. medica* L. var. *sarcodactylis* fruit water and ethanol extracts may have the potential to be used as natural antimicrobial additives as alternatives to synthetic preservatives against the food-borne pathogens.

Keywords: Antibacterial, Fruit, Extract, *Staphylococcus aureus, Listeria monocytogenes*

* The study is produced from master thesis of the first author

1. Introduction

The widespread and uncontrolled use of antibiotics leads to the development of drug resistance against pathogens. With the increase and spread of multi-drug resistant pathogens, the need for new antibiotic-containing sources is increasing (Dessen et al. 2001). Plants contain various

secondary metabolites such as flavonoid, catechin, anthocyanin, salicylate, lignan, sterols, procyanidin and glucosinolate (Hooper and Cassidy 2006). Secondary metabolites in plants are powerful antimicrobial agents against pathogen groups (Mickymaray 2019). For this reason, plants are reliable, cheap, and various research are being carried out by taking their place in alternative natural resources due to the bioactive molecules they contain (Compean and Ynalvez 2014).

Citrus medica L. var. *sarcodactylis* fruit, called Foshou in China, belongs to the Rutaceae family (Chen et al. 2022). It is a fruit with therapeutic properties with its rich nutritional content and secondary metabolites (Ma et al. 2021, Wu 2015). *C. medica* L. var. *sarcodactylis* fruit, is generally grown in Taiwan, Japan, and the southern regions of China (Peng et al. 2009).

Microbial contamination in food causes food spoilage and is a big problem for the human health, consumers and food industrial worldwide (Penha et al. 2017). Bacteria are among the microorganisms responsible for food spoilage (Lianou et al. 2016). Microorganisms multiply by using the nutrients in the environment as a result of contact with food products and produce components that can cause spoilage in food (Parlapani et al. 2017). The increase in food-borne diseases in many countries is becoming more common in recent times. Therefore, it is necessary to identify the responsible microorganisms to reduce food-borne diseases and antimicrobial agents are needed to prevent microbial growth in food products (Alahi and Mukhopadhyay 2017, Mickymaray 2019).

S. aureus is a cocci-shaped gram-positive bacterium that causes multiple infections. This bacterium is an opportunistic pathogen known for its resistance mechanism against heavy metals and antimicrobial agents (Dweba 2018). *S. aureus* strains pose a risk to the consumer by producing enterotoxins due to their proliferation and growth in foods (Bennett et al. 2013). *L. monocytogenes* is a food-borne gram-positive bacterium that causes serious infections in humans and animals (Hamon et al. 2006). It is found everywhere in nature and poses a threat to food chains (Jordan and McAuliffe 2018). *L. monocytogenes* causes listeriosis, an important infection. Listeriosis is generally seen as a result of consuming contaminated food products (Hamon et al. 2006).

In the study, the antimicrobial activity of water and ethanol extracts obtained from *C. medica* L. var. *sarcodactylis* fruit was investigated against food-borne pathogens.

2. Materials and Methods

Plant Material and Extract Preparation

C. medica L. var. *sarcodactylis* fruits used in the study were obtained from Mersin (Turkey) in November 2021. The fruits were washed with distilled water. Dried fruit were ground with Waring blender. 10 g of ground fruit was weighed for extraction and then 30 ml of solvent (water and ethanol) was added. The samples were extracted using a water bath for 2 days (12 hours). After the procedure, the remaining solvent was evaporated. The obtained extracts were dissolved with dimethyl sulfoxide (DMSO). Sterilization process was performed with a 0.45 µm filter. The extracts were preserved under suitable conditions.

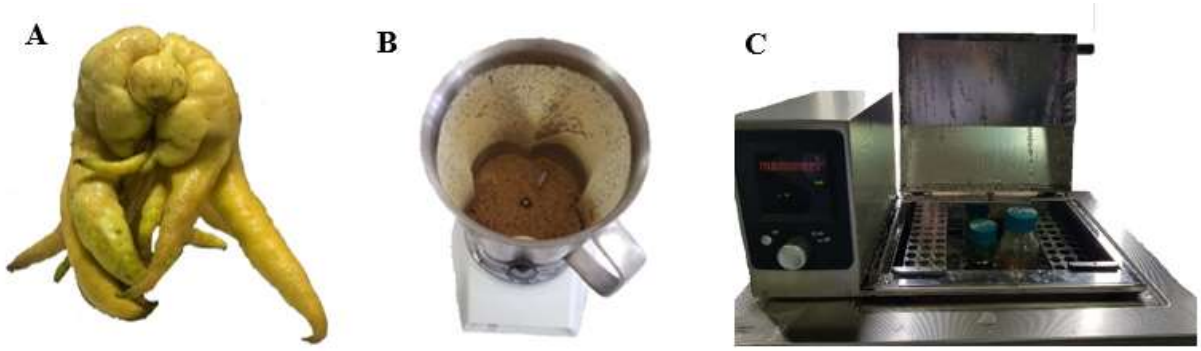

Figure 1. A: *C. medica* L. var. *sarcodactylis*, B: Grinding of dried fruit, C: Preparation of the extracts

Test Microorganisms and Culture Conditions

The test microorganisms, *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* ATCC 7644, were cultured in Nutrient Broth (NB) and Tryptic Soy Broth (TSB) at 37°C.

Determination of Antimicrobial Activity

The antimicrobial activity of *C. medica* L. var. *sarcodactylis* fruit water and ethanol extracts were determined using disk diffusion method. The bacteria concentration was adjusted to 0.5 McFarland. The prepared bacteria concentration (100 µl) was spread on the agar medium. Sterile discs (6 mm in diameter) were placed in triplicate on agar medium. 20 µl (2 mg/disc) extracts were dropped and ampicillin was used as the control group. Petri dishes were then incubated at 37°C. After incubation period, the zones of inhibition were measured with a caliper.

Micro-dilution Method

The Minimum Inhibitory (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts were determined using the micro-dilution method. The medium, extract and bacterial suspension were added to the tubes. The prepared tubes were incubated at 37°C. The concentration of the extracts in the tube that did not grow after 24 hours of incubation was determined as the MIC value. The samples from tubes were inoculated on agar medium to determine the MBC values. Petri dishes were incubated at 37°C. After 24 hours of incubation, the concentration of the extracts where bacterial growth stopped was recorded as MBC values.

3. Results

The antimicrobial activity of the extracts obtained with water and ethanol solvents from *C. medica* L. var. *sarcodactylis* fruit was determined using disc diffusion method. The inhibition zone diameters were given in Table 1. Ampicillin was used as the control group. The inhibition zones of test microorganisms against fruit water extract were 6.41 mm against *S. aureus* ATCC 25923 and 6.07 mm against *L. monocytogenes* ATCC 7644. The inhibition zones against fruit ethanol extract were 8.04 mm on *S. aureus* ATCC 25923 and 7.13 mm on *L. monocytogenes* ATCC 7644.

Table 1. Inhibition zone diameters of *C. medica* L. var. *sarcodactylis* fruit extract against pathogenic microorganisms

Test microorganisms	Inhibition zone diameter (mm)		
	CFW	CFE	Ampicillin(10µg)
***S. aureus* ATCC 25923**	**6.41±0.41**	**8.04±0.71**	**21.04±0.8**
***L. monocytogenes* ATCC 7644**	**6.07±0.06**	**7.13±1.22**	**29.57±0.1**

*CFW: *C. medica* L. var. *sarcodactylis* fruit water extract, CFW: *C. medica* L. var. *sarcodactylis* fruit ethanol extract.

The MIC and MBC values of the extracts were determined using the micro-dilution method. MIC and MBC values of the extracts against test microorganisms are given in Table 2. MIC value fruit water and ethanol extract against the test microorganisms was determined as 10-20 µg/µl. MBC values of the extracts varied from 10 µg/µl to 40 µg/µl. *C. medica* L. var. *sarcodactylis* fruit water extract with low MIC and MBC values (10 µg/µl) showed better antimicrobial activity against *S. aureus* ATCC 25923.

Table 2. MIC and MBC values of *C. medica* L. var. *sarcodactylis* fruit extracts against pathogenic microorganisms

Test microorganisms	MIC (µg/µl)		MBC (µg/µl)	
	CFW	CFE	CFW	CFE
***S. aureus* ATCC 25923**	**10**	**10**	**10**	**40**
***L. monocytogenes* ATCC 7644**	**20**	**20**	**40**	**40**

*CFW: *C. medica* L. var. *sarcodactylis* fruit water extract, CFW: *C. medica* L. var. *sarcodactylis* fruit ethanol extract.

4. Discussion

Increasing resistance to antibiotics increases the need for new antimicrobial agents. Plants have the potential to be a valuable antimicrobial agent due to the biodiversity they contain (Eloff 2019). Li et al. (2019), essential oil was obtained from the fruit of *C. medica* L. var. *sarcodactylis* and determined its antimicrobial activity by disc diffusion method. The inhibition zone diameter of the essential oil on the test microorganism (*S. aureus* ATCC 6538) was determined as 19.02 mm. The MIC-MBC values were found as 0.625-1.25 mg/ml.

Al-Kalifawi (2015), determined the antimicrobial activity of the essential oil obtained from the peel parts of *C. medica* L. var. *sarcodactylis* using the well diffusion method. The inhibition zone diameter of the essential oil obtained from the peels of the fruit against *S. aureus* ATCC 6538 was determined as 19.02 mm. The used different methods in determination of antimicrobial activity and extraction methods may cause changes in the results.

5. Conclusion

In conclusion, the fruit water and ethanol extracts of *C. medica* L. var. *sarcodactylis* had antimicrobial activity against the test microorganisms. It has been observed that the fruit water and ethanol extracts may have the potential to be used as an alternative natural an antimicrobial additive against preservatives with synthetic content in the food industry.

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Potential Antimicrobial Activity of Aronia Water Extract Against *Candida albicans* ATCC 10231 and *Aeromonas hydrophila* ATCC 19570 Strains

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Abstract:

In recent years, the increase in bacterial resistance to traditional antibiotics due to uncontrolled and unconscious consumption poses a great threat to human health. Drugs used against *Candida*-induced infections are limited and new alternative antimicrobial agents need to be developed. Infections are among the main problems of fish farms and cause serious financial losses to the producer. Plant extracts have the potential to inhibit infections by pathogens. Aronia (*Aronia melanocarpa*) is a fruit that has been used in the treatment of many diseases caused by pathogenic microorganisms in alternative medicine and has gained popularity in recent years. In this study, it was aimed to determine the antimicrobial activity of Aronia water juice extract against *Candida albicans* ATCC 10231 (clinical origin pathogen) and *Aeromonas hydrophila* ATCC 19570 (seafood-borne pathogen). The antimicrobial activity was obtained using disc diffusion and micro-dilution experiments. The disc diffusion assay results showed that the inhibition zone diameter of Aronia water extract was 7.42 mm against *C. albicans* ATCC 10231 and 9.45 mm against *A. hydrophila* ATCC 19570. MIC values of Aronia water extract were obtained as 12.5 mg/mL on test microorganisms. The extract showed MFC value as 50 mg/mL against *C. albicans* ATCC 10231. MBC value of the extract was determined as 12.5 mg/mL against *Aeromonas hydrophila* ATCC 19570. The results indicated that the fruit water extract of Aronia with good antifungal and antibacterial activities has the potential to be used as a natural alternative to synthetic drugs in the preventing or treatment of infections in pharmaceutical and feed industries.

Keywords: *Aronia melanocarpa*, Natural additive, Antibacterial, Antifungal, Infections

* The study is produced from master thesis of the first author

1. Introduction

The plant extracts have been used for medicinal purposes on humans and animals since ancient times. Today, medicinal and aromatic plants are used in modern medicine as an alternative to synthetic drugs. Medicinal and aromatic plants are offered as potential sources for new antibiotics and anticancer agents (Gurib-Fakim et al. 2005). In addition to their medicinal use in humans, medicinal plants have also been used as chemotherapeutic and food additives in aquaculture due to their potential to strengthen the fish immune system (Van Hai 2015).
Aronia (*Aronia melanocarpa*) has gained popularity in recent years with its fruits belonging to the *Rosaceae* family, which have high polyphenol content (Kokotkiewicz et al. 2010, Denev et al. 2019). The Aronia is used as a dietary supplement, and the flavonoids found in large quantities in its fruits have antimicrobial activity (Kulling et al. 2008, Bräunlich et al. 2013). Many researchers have reported that Aronia has antimicrobial (Valcheva-Kuzmanova and Belcheva 2006), antioxidant (Denev et al. 2012), anti-diabetic (Simeonov et al. 2002), anti-proliferative (Bermúdez-Soto et al. 2007) and hepatoprotective (Kowalczyk et al. 2003) effects. Pathogenic fungi cause many clinical disorders in humans such as skin infections, thrush, brain abscess, vaginitis, endocarditis and arthritis (Perumal et al. 2007). The fungi are difficult to treat, and resistance to the drug has developed with the increase in the doses of the currently used drugs. It has led to the discovery of new medicinal plants and natural materials with fewer side effects to prevent fungal infections (Jabra-Rizk et al. 2004, Kanafani and Perfect 2008). *Candida albicans* is a fungus that carries on the human skin and in the mucosal areas of the body. In addition, *C. albicans* is a pathogen that causes superficial infections and causes fatal diseases for immunocompromised patients (Todd and Peters 2019).
The World State of Fisheries and Aquaculture states that the fishing industry provides about 15% of animal protein intake (FAO 2015). Diseases caused by pathogenic microorganisms in large fish farms cause great financial losses. *Aeromonas hydrophila* is the main cause of disease outbreaks in freshwater farmed fish. It causes worldwide food problems and economic loss (Aboyadak et al. 2015; Baumgartner et al. 2017; Dash et al. 2014). *A. hydrophila* is recognized as an important foodborne bacterial zoonotic pathogen that causes death in many fish species (Samayanpaulraj et al 2020). Antibiotic use is common in fish farming in the treatment of microorganism-induced diseases. Due to the overuse of antibiotics, the incidence of resistant bacterial strains is increasing. The plants are capable of inhibiting the potential activities of fish pathogens (Direkbusarakom 2004). Since ancient times, traditional herbal medicines have been used in the treatment of diseases caused by microorganisms.
In our study, antimicrobial activity of water extract obtained from Aronia fruit on human pathogenic microorganism *C. albicans* ATCC 10231 and fish bacterial pathogen *A. hydrophila* ATCC 19570 was determined to obtain the possibilities of use in various industries as a natural antimicrobial source.

2. Materials and Methods

2.1. Plant Material
The *Aronia melanocarpa* fruit was purchased in September 2022 as fresh fruit from Aronia Food and Health I.C (Turkey).

2.2. Preparation of Aronia Water Extract
Aronia fruit was washed and dried in the sun-free environment. The dried fruits were pulverized with a Waring blender. It was extracted from Aronia fruit with water using a sonicator (Amplitude, 100%; Cycle, 1) device for 10 minutes daily (3 days). The water was then

evaporated from the extract. The Aronia water extract was dissolved with dimethyl sulfoxide (DMSO) and sterilized through a 0.22 μm filter.

2.3. Determination of Antibacterial and Antifungal Activity of Aronia Water Extract

2.3.1 Disc Diffusion Assay

Disc diffusion method was used to determine the antimicrobial activity of Aronia water extract. The test microorganism, the fungal pathogen *C. albicans* ATCC 10231 (Yeast Extract Peptone Dextrose (YPD)/Agar) and the fish pathogen *A. hydrophila* ATCC 19570 (Nutrient Broth (NB)/Agar) were used. The density of pathogenic microorganisms was adjusted to 0.5 McFarland ($1x10^8$ CFU/mL). 100 μl of the bacterial suspension was spread on solid medium. 20 μL (2 mg/disc) extract was dripped onto the sterile disc (in triplicates). *A. hydrophila* and *C. albicans* were incubated at 37°C and 30°C, respectively. After 24 hours of incubation, the zones of the zones were measured with a caliper.

2.3.2. Determination of Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal or Fungicidal (MBC or MFC) Concentration

Minimal Inhibition (MIC) and Bactericidal (MBC) or Fungicidal (MFC) Concentrations of the extracts were determined using the micro-dilution method. Aronia water extract were added to the tubes and then diluted to determine the minimum inhibition concentration. The culture (0.5 McFarland concentration) was added to tubes containing various extract concentrations. The tubes were incubated at the temperature appropriate for the test microorganisms. The non-growth concentration after incubation was evaluated as the MIC of Aronia water extract. Then, the samples in the tubes were dropped onto solid medium to determine the MBC or MFC values. The petri dishes were incubated for 24 hours. The non-growth concentration at the end of the incubation was determined as the MBC or MFC value.

3. Results

In the study, the antimicrobial effect of water extract from Aronia fruit on fungal and fish pathogens was determined using disc diffusion and micro-dilution methods. Aronia water extract exhibited antimicrobial activity on both *C. albicans* ATCC 10231 and *A. hydrophila* ATCC 19570 (Table 1). Disc diffusion assay indicated that Aronia water extract showed an inhibition zone of 7.42 mm on *C. albicans* ATCC 10231 and 9.45 mm on *A. hydrophila* ATCC 19570. In the micro-dilution method, the MIC value, which is the lowest extract concentration at which the growth of microorganisms is inhibited, was found to be 12.5 mg/mL for both test microorganisms. MBC or MFC values, which are the lowest extract concentrations where microbial growth is completely inhibited in solid media, were found to be 12.5 mg/mL and 50 mg/mL for *C. albicans* ATCC 10231 and *A. hydrophila* ATCC 19570, respectively.

Table 1. Antimicrobial activity of Aronia water extract

Test Microorganisms	**Aronia Water Extract**		
	Inhibition Zone Diameter (mm±SD)	**MIC (mg/mL)**	**MBC or MFC (mg/mL)**
***C. albicans* ATCC 10231**	**7.42±0.50**	**12.5**	**50**
***A. hydrophila* ATCC 19570**	**9.45±0.13**	**12.5**	**12.5**

4. Discussion

Today, indiscriminate, irregular and excessive use of antibiotics has caused antimicrobial resistance and rendered many drugs ineffective (Davies and Davies 2010). For this reason, new antifungal and antibacterial agents will be developed by investigating the antimicrobial effect of plant extracts.

In a study, Liepina et al. (2013) investigated the antibacterial and antifungal activities of the extract obtained from Aronia fruits on some test microorganisms (*Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *C. albicans*). The results of their study indicated that Aronia fruit extract had a high antibacterial activity with an inhibition zone diameter of 19.5 mm, but had no antifungal effect. In the current study, Aronia water fruit extract showed inhibitory activity against *A. hydrophila* ATCC 19570 as well as *C. albicans* ATCC 10231. With good antimicrobial activity could be a natural antimicrobial alternative for the pharmaceutical and feed industries.

5. Conclusion

Aronia water extract with good antimicrobial activity on all tested microorganisms could be a antimicrobial alternative for the pharmaceutical and feed industries. The extract can be used as a natural alternative to synthetic drugs in the prevention or treatment of infections caused by pathogen microorgnaisms.

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Iron (II) catalyzed Selective hydroxylation of unsaturated hydrocarbons to secondary alcohol derivatives as pharmaceutical intermediates

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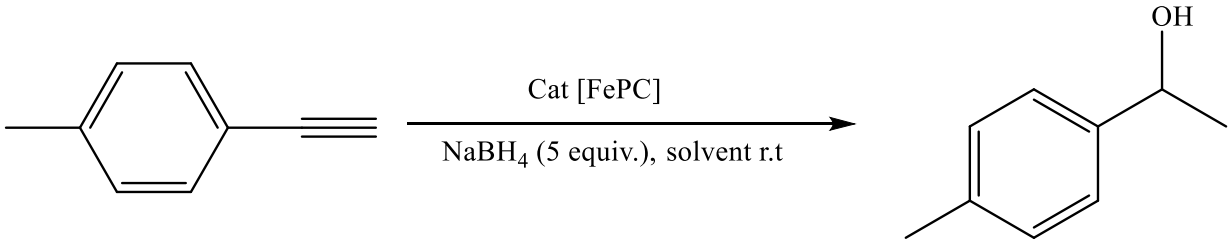
Abstract:
Transformation terminal and internal unsaturated hydrocarbons are investigated by iron (II) catalyst with excellent efficiency promotion. The production of secondary alcohols is an important intermediate fine chemical for industrials process agro-chemical and pharmaceuticals. In this work we selected alkynes as a simple of unsaturated hydrocarbons, and using Iron (II) Phthalocyanine with $NaBH_4$ catalyst, which significantly promoted the efficient catalyzed terminal and internal alkynes to secondary alcohols following addition and elimination mechanism. Adding the $NaBH_4$ to the reaction mixture is promoted the catalytic efficiency of iron (ll) in ethanol, and in the absence of $NaBH_4$, the product of secondary alcohols was no found. The hydroxylation of terminal alkynes is led the secondary alcohols formed follow anti-Markovnikov's rule. Despite its proven evasiveness, we were compiling this reaction, indirect oxidation/reduction sequence requiring iron phthalocyanine stoichiometric of alkynes. Here, we report a more direct approach that alkynes are transformed to secondary alcohols in room temperature, under oxygen of atmospheric air, in one pot. Therefore, adding the extra oxygen has assisted hydroxylated phenyl acetylene and its derivatives producing a good yield of the 1-(*p*-tolyl)ethan-1-ol with excellent regionselectivity.

Keywords: Iron catalyst, alcohol, alkynes, transformation, and hydroxylation.



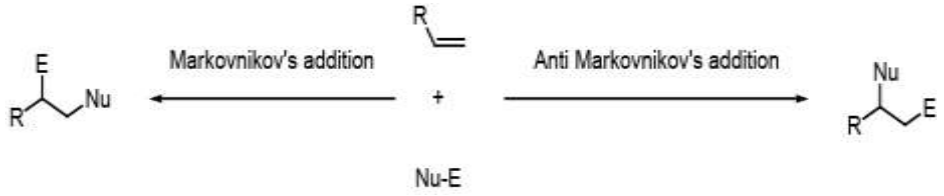
1-(*p*-tolyl)ethan-1-ol

1. Introduction

The catalytic conversion of alkynes to alcohols has become important in the particular case of metal-catalyzed hydroboration oxidation of alkynes (Park et al., (2007)(Feather Features Of Alkenes and Alcohols Antibodies ' Long and Winding Road Modulating Myelination Filling a Hole Rise of the Jellyfi shes, (2011). Alkynes have been designed reductive hydration by ruthenium (II) catalyst, introduced by Wakatsuki (Wakatsuki et al., 1991). Also, several methods for preparing alcohol from alkenes have been developed by other researchers. Many synthesis alcohol processes have been discovered by some researchers, such as: Breit and Nozaki and coworkers (hydroformylation–reduction alkenes to alcohols (Chevallier and Breit, 2006; Takahashi et al., (2010); Hintermann, (2012). Stahl and coworker reported the oxidation-reduction-sequins, Grubbs and coworker have published articles on oxidative hydration reduction using organometallic catalysis (Hintermann, (2012). Radical oxyfunctionalization has been developed using metal-catalysis of hydroxyalkylation systems in synthetic chemistry (Kano et al., (1991).

In 2012, Leli developed [Ru]-[Au] catalyzed for alkynes treatment in one step under hydrogen and water (Norinder et al., 2008, 2008). To prepare the secondary alcohols, we have seen several reports in this scope. However, researchers meet some risk of the hydrogen source such (Bu_3SnH) as a toxic reagent, and a product's purification is difficult. Using the [Ru]-[Au] is a very expensive coast; the products by this dimer catalyst were a mixture of primary and secondary alcohols. But, iron catalysts have been developed in recent years, and $FeCl_3$ was the first iron catalysis to hydration alkynes to methyl ketone as high chemical catalysts with reactivity, abundant, nontoxic, and inexpensive(Wakatsuki et al., 1991; Norinder et al., 2008).

The nature's catalysts have some limitations, and the omnipresence of olefins has exemplified transformation reaction of C-C multiple bonds such as hydroxylation, hydro carboxylation, hydrocyanation and hydro formation reactions (Roseblade and Pfaltz, 2007). Scheme 1 shows a general Markovnikov's rule in addition reaction of olefins by E-Nu which can give us non-symmetrical products (Vogel and Houk, 2022) and it has been knowingly, contrary to what you have believed, the electrophilic in addition reaction as E-H added to olefin following the Markovnikov's rule, in this case the electrophilic with hydrogen for example halogen hydro-acids react with C-C multiple bonds of alkenes or alkynes functionalization reaction (Kano et al., 1991)(Prinetto et al., 2000)(Recognition and activation by ureas and thioureas: Stereoselective reactions using ureas and thioureas as hydrogen-bonding donors, 2005)(Section, 1991)(Geib et al., 2020).



Scheme 1. Functionalization reaction of olefin

This valuable study consists of the preparation of secondary alcohols as intermediate pharmaceuticals, using alkynes derivatives as substrate with the iron phthalocyanine as a catalyst in ethanol solvent. The transformation of alkynes to 1-(p-tolyl)ethan-1-ol as intermediates fine chemicals, which known is a fragrance ingredient used in many fragrance

mixtures. This new method could improve the production of secondary alcohols with different redox metals including hydroboration oxidation of terminal and internal alkynes readily convert to secondary alcohols following Markovnikov's rule of addition mechanism.

2. Materials and Methods

2.1. Materials

1(P-methyl-Phenyl-acetylene, iron phthalocyanine and sodium bootstrap hydride were purchased from J&K (Shanghai, China). Ethanol solvents, ethyl acetate and PE were stored in laboratory storage; standard Martials were supplied by Aladdin (Shanghai, China). All solvent of extractions, GC analysis and columns was purchased from Chemicals KKDIK regulation. All other chemicals used in this study were bought at the highest grade from commercial suppliers without further purification or modification.

2.2. Methods of Synthesis

The reaction conditions of synthesis *P*-methyl-phenyl-ethan1-ol, using a flask 15 Ml, and sequentially added (0.5 mmol) scale of 68.0mg) 4-methylphenyl acetylene, 0.25 mol%, FePC (0.0125 mmol) 7.1 mg, 6 equiv (1.3 mmol) 48 mg, and ethanol solvent (3 mL) reaction mixture was stirred at room temperature further 6-24 h. TLC analysis showed the reaction results. The reaction mixture was cooled at room temperature, and diluted with CH_2Cl_2 (5 mL) and H_2O (5 ml). The organic layer was separated, and the aqueous phase was re-extracted with dichloride methyl (10 ml). The combined organic extracts were washed with H_2O (10 ml), dried over anhydrous Na_2SO_2, and purified by flash chromatography to afford the desired product of 1-(*p*-tolyl)ethan-1-ol in good yield (48 mg) 67.8 % and its colorless oil. The products were determined by TLC. However, some indirect methods were also applied to prove the performance. GC was also used with standard N–Dican (41 µl), followed by ^{1}H NMR [$CDCl_3$, 400 MHz, δ 7.11 (d, J= 8.4 Hz, ^{1}H), 6.78 (d, J= 8.4 Hz, 2H), 4.75 (q, J= 6.45 Hz, 2H), 1.44 (d, J= 6.3 Hz, 3H).

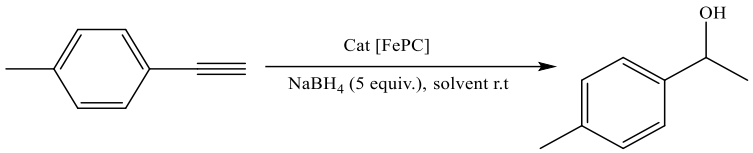
3. Results

4-Methyl-1-phenylethanol (*1-(p*-tolyl)ethan-1-ol) is a colorless liquid with a faint flowery smell, a little volatile, highly non-flammable, that is slightly soluble in water. It reacts with an acidic solution of sodium dichromate to benzoic acid.

The iron (II) compounds are readily oxidized under atmosphere air changing to iron (III) analogs, which represent the most stable and widespread iron species. Iron (II) PC is tetrahedral coordinated (Recognition and activation by ureas and thioureas: Stereoselective reactions using ureas and thioureas as hydrogen-bonding donors, 2005). This work is illustrated the aerobic synthesis method of secondary alcohols from alkynes following the Markovnikov's mechanism, in the first time by using the iron (II) phthalocyanine catalyzed transformation of alkynes to alcohols with highly regionselectivity. In the first part of this research, we would like to explain the synthesis of secondary alcohols from unsaturated hydrocarbons simply with alkynes under the optimized conditions as in Table 1. 4-Methyl 1-phenyl alcohol was produced from alkynes according to the Markovnikov and anti-Markovnikov’s rule. The reaction by direct addition of water to alkenes is likely catalyzed by Lewis/Bronsted acid to form primary and secondary alcohols (Senan et al., 2016, 2021; Lou et al., 2017)[18].

Experiments have been conducted to understand, the catalysis of security Bronsted acid by EtOH, and in some processes which have 5 mol % FePC, 5 equiv $NaBH_4$ under the mediate reaction conditions. The reaction proceeded for 18 h to ensure the completion and resulted in 75% as shown in Table 1 (entry 1), which isolated yield of the product, indicating the less catalytic effect of in situ generated Bronsted acid. The product was isolated and extracted with a mixture consisting of petroleum ether (PE) and ethyl acetate (EA) (5:1). The reaction was controlled and determined by TLC, NMR and GC spectrometry. For optimizing the reaction conditions, the scope of this new reaction is promoted with high investigation with various solvents and iron salts with 1-*P*-methyl phenyl acetylene, and the results are summarized in Table 1.

Table 1. Iron Phethalocyanyne catalyzed transformation of phenyl acetylene to 1-(*p*-tolyl)ethan-1-ol in presence of hydride metals using different solvents



entry	catalyst.mol% $NaBH_4$(6 equiv)	solvent	time	conditions	yield%
1	1mmol%FePC	EtOH	18 h	O_2	67.8%
2	1mmol%FePC	EtOH	6 h	O_2	34.6%
3	1mmol%FePC	EtOH	9 h	O_2	26.6%
4	5mmol%$Fe(NO_3)_{3.9}H_2O$	EtOH	Iday	O_2	o%
5	5mmol%$FeCL_3$	EtOH	1day	O_2	6.3%
6	5mmol%$Fe(acac)_3$	EtOH	1day	O_2	1.5%
7	2.5mmol%$Fe_2(ox)_3$	EtOH	1day	O_2	1.7%
8	2.5mmol%$Fe_2(SO_4)_{3.7}H_2O$	EtOH	1day	O_2	2.4%
9	2mmol%$C_{32}H_{16}FeN_8$	EtOH	1day	O_2	GC%
10	5mmol%FePC	EtOH	20 h	O_2	0%

Conditions:1-phenyl ethanol, using a flask 15 mL, and sequentially added (0.5 mmol) scale of (68.0mg) 4-methylphenyl acetylene, 0.1.3 mol%, FePC (0. 125 mmol) 7.1 mg, 6 equiv (1.3 mmol) 48 mg of $NaBH_4$, and ethanol solvent (3 mL) reaction mixture was stirred at room temperature further 6-24 h.

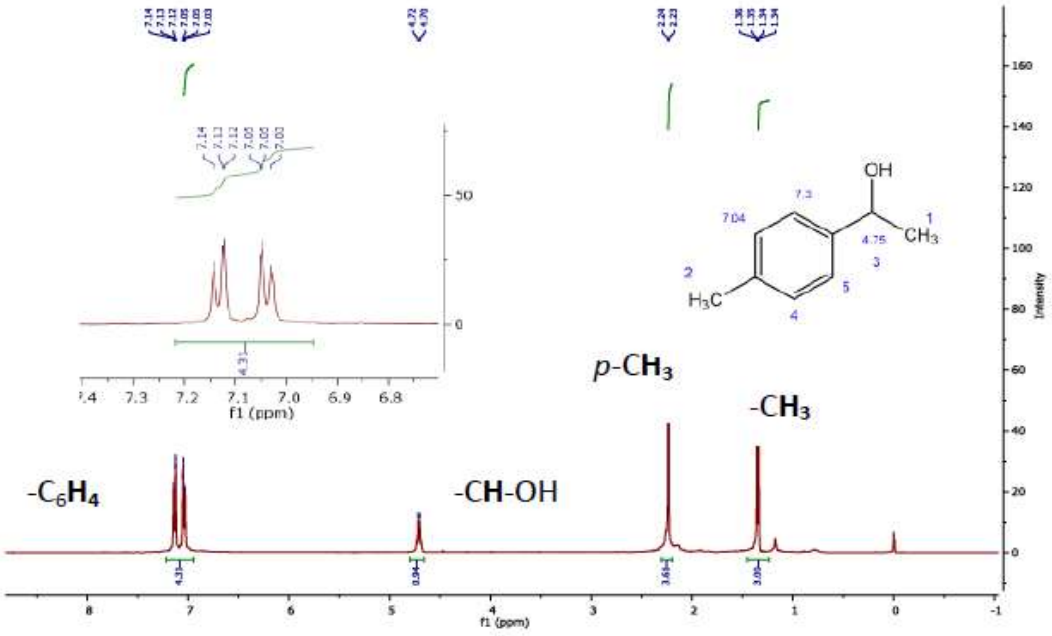
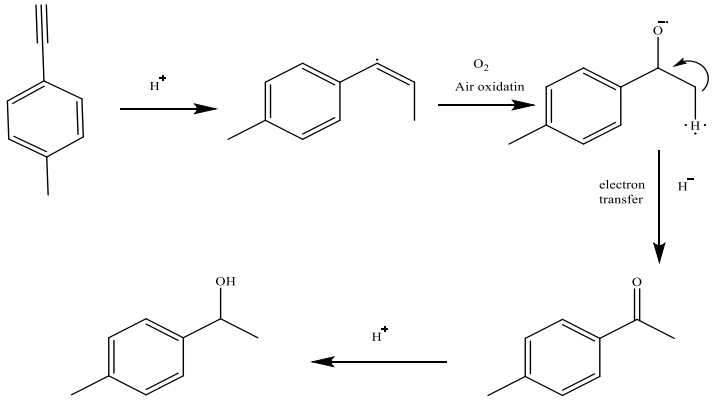


Figure 1. 1H NMR analyzed of 1-(p-tolyl phenyl) ethanol.

4. Discussion

This valuable study consists of the preparation of 1-(p-tolyl) ethan-1-ol from alkynes using the iron phthalocyanine as catalyst. The transformation of alkynes to alcohols, such as hydroboration oxidation of terminal and internal alkynes readily convert to secondary alcohols following the Markovnikov's rule of addition mechanism.



Scheme2. The mechanism reaction of anti-Markovnikov additions with Fe (ll)PC/$NaBH_4$ catalysis

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This conversion method indicates that Fe (II) has a specific role in such catalysis in absolute alcohol, and it is unlikely that another metal- catalysis which needs water to carry out reaction for hydrating (Crabtree, 2017). The phenyl acetylene was catalyzed by iron (II)PC in a typical condition and the product analysis was performed by NMR and GC with internal standard methods: (0. 5 mmol, 68.0mg) 4-methylphenyl acetylene, and (0.0125 mmol., 7 mg) FePC, reacted with (1.3 mmol,48 mg) $NaBH_4$, the mixture stirred in 5 ml ethanol at room temperature and O_2 /atmosphere air. The conditions were optimized is supported an excellent yield, as shown in Table 1, after 6- 24 h 1NHCl was added. Then, the mixture was extracted with dichloromethane, and the organic layer was dried in over using Na_2SO_4 concentration by reduced pressure and provided 1-phenyl ethanol confirmation of a high regionselectivity with very good yield, according to ^{1}H NMR. On the other hand, the reaction was controlled by TLC without caring about the air and humidity and the product was immediately extracted and isolated using a SiO_2 column, and PE /EA 5:1, at room temperature in air/O_2 atmosphere. These optimized conditions led to the concentration providing products of 1-phenyl ethanol derivatives. The determination of yield based on TLC, ^{1}H NMR, and GC results, the product [1-(*p*-tolyl) ethan-1-ol 67.8%] with high region selectivity.

5. Conclusion

The synthesis method of 1-(p-tolyl) ethan-1-ol is investigated, using *P*-methyl phenyl acetylene as substrate, which converted by iron phthalocyanine Fe (PC)/$NaBH_4$ catalyst in absolute ethanol, under atmosphere air. This work exploring the redox reaction as new strategy employed for transformation of alkynes to secondary alcohol as, providing p-tolyl) ethan-1-ol 67.8%], as desired product at room temperature. This reaction proved in general performance catalytic conversion unsaturated hydrocarbons; terminal and internal alkynes substrates scope to secondary alcohols as pharmaceutical intermediates. This project is not completed yet, and we have more modifications, additions and publications.

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Evaluation of the Relationship between the Amount and Type of Carbohydrate Consumed After the Evening Meal and Sleep Quality

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Abstract:

Aim

The aim of this study is to evaluate the relationship between the amount and type of carbohydrates consumed after evening meal and sleep quality.

Method

This study was conducted on 177 university students with an average age of 21. A general information questionnaire, 24-hour dietary recall, and Pittssburgh Sleep Quality Index (PSQI) questionnaire were applied to the participants. Body composition were determined with the TANITA DC 360 ST device using the bioelectrical impedance analysis (BIA) method. Height, waist-hip circumference was measured with a non-stretchable tape measure. Nutritional record data were recorded in BEBIS program. Final analysis of the study was done by SPSS Inc. Made with Chicago IL v21 program.

Results

The average PSQI score of the participants was 7.14. While 18.1% of the participants had good sleep quality (PSQI≤5), 81.9% had poor sleep quality (PSQI>5). No significant correlation was found between the amount of carbohydrates consumed after the evening meal and sleep quality ($p>0.05$). However, a significant correlation was observed between the percentage of carbohydrates, absorbable oligosaccharide consumption and PSQI score ($p=0.044$, $p=0.013$, respectively). Also, a significant correlation was found between protein amount, protein percentage and sleep quality ($p=0.037$, $p=0.020$, respectively). In addition, a significant correlation between the amount of water consumed after the evening meal and PSQI score was found ($p<0.05$).

Conclusion

In this study, no relationship was observed between the amount and type of carbohydrates consumed after evening meal and sleep quality, but significant difference observed with the percentage of carbohydrates suggests the possibility that food preferences may affect sleep quality. Studies using larger samples are needed.

Keywords: Sleep quality, PSQI, carbohydrate

1. Introduction

Nutrition is the use of nutrients for growth, maintenance of life and maintenance of health. There are many nutrients that provide energy to the body, and the nutrient that an adult provides the most energy is carbohydrates (Baysal, 2002). Carbohydrates are divided into simple and complex. Simple carbohydrates are carbohydrates that can be digested and absorbed in the upper gastrointestinal tract in the human body. Complex carbohydrates, on the other hand, are indigestible carbohydrates that create a fiber effect on the human body (Blaak & Saris, 1995). Carbohydrates are considered not only as a primary energy source, but also as a critical macronutrient for sleep due to hormonal regulation related to brain function and sleep (Fernstrom & Wurtman, 1971). Sleep is a complex, reversible behavior in which the individual becomes unresponsive to her/his environment and is perceptually disconnected from it (Halson, 2014). Sleep consists of rapid eye movement (REM) sleep and stages 1, 2, and 3 of non-REM (NREM) sleep. During slow wave sleep (SWS), or stage 3 of NREM, the deeper stage of non-REM sleep, brain glucose utilization and sympathetic nerve activity decrease and parasympathetic nerve activity increases relative to both wakefulness and REM sleep. Therefore, it is possible that SWS plays an important role in total body glucose regulation (Morselli et al., 2010). Good sleep quality is important for general, physical and mental health (Grandner, 2014; St-Onge, Grandner, et al., 2016). One of the widely known circadian rhythms is the sleep-wake cycle (Laposky et al., 2008). Sleep disorder is a very common disease that disrupts the normal circadian rhythm and negatively affects psychological and physical health (Pigeon, 2010). There are many studies reporting the negative effects of insufficient sleep on brain structure, activation and physiology (Lo et al., 2016). In recent years, there has been a dramatic increase in the literature on the role of diet on sleep (Zhao et al., 2020). It is predicted that diet plays an important role in regulating sleep health (St-Onge, Mikic, et al., 2016).

Diet is a behavioral factor that can affect sleep (Yogman & Zeisel, 1983). Both the timing of meals (Dollander, 2002; Roky et al., 2001) and the content of macronutrients consumed in the meal (Phillips et al., 1975; Yogman & Zeisel, 1983) affect sleep. Meals consumed close to bedtime have been associated with sleep disorders (Dollander, 2002). Some macronutrients affect sleep through tryptophan (Trp), a precursor to serotonin, a sleep-inducing agent (Hartmann & Spinweber, 1979). It is known that tryptophan (Trp), which is one of the large neutral amino acids (LNAA: tyrosine, phenylalanine, leucine, isoleucine, valine and methionine) with the effect of high glycemic index (GI) carbohydrates, insulin, has the ability to increase the plasma Trp/LNAA ratio (Berry et al., 1991). An increase in the plasma

Trp/LNAA ratio causes an increase in Trp and serotonin. This increase is thought to support sleep in the brain (Wurtman et al., 2003). Afaghi et al conducted two studies examining carbohydrate intake before sleep in healthy men. In the first study, high or low GI meals were given 4 hours and 1 hour before sleep. High GI meal consumption significantly improved sleep onset latency compared with low GI meal consumption (Afaghi et al., 2007). In the second study, Afaghi et al. compared a very low carbohydrate diet (1% carbohydrate, 61% fat, 38% protein) consumed 4 hours before bedtime with a control diet (72% carbohydrates, 12.5% fat 15.5% protein). The very low-carbohydrate diet increased SWS and all stages of NREM, while the control diet decreased REM (Afaghi et al., 2008). Porter and Horne gave six male participants a high-carb meal (130g), a low-carb meal (47g), and a no-carb meal 45 minutes before bedtime. High-carbohydrate meals resulted in increased REM sleep, decreased NREM sleep, and wakefulness (Porter & Horne, 1981).

When the studies are examined, there are few studies investigating the effect of carbohydrate intake on sleep quality. The study on the effect of the amount and type of carbohydrates consumed on or after a single meal on sleep quality is insufficient and limited. The aim of this study is to investigate the effect of the amount and type of carbohydrate taken after the evening meal on anthropometric measurements and sleep quality. At the same time, the secondary aim of this study is to investigate the effect of these data on sleep quality by calculating the 24-hour retrospective food consumption record, anthropometric measurements of total energy and nutrients, and body composition values to be used within the scope of the research.

2. Materials and Methods

This study was conducted with the students of Health Sciences University, Gülhane Faculty of Health Sciences. Within the study, there are 177 individuals, 151 women and 26 men, with an average age of 21. Students between the ages of 18-40 studying at the University of Health Sciences, Gulhane Faculty of Health Sciences, undergraduate program were briefed about the study. Consent form was presented to the volunteers and the volunteers were allowed to read it. Volunteers who signed and approved the consent form after reading it were evaluated by the researchers according to the study inclusion and exclusion criteria, and the appropriate ones were included in the study. Data were collected from the participants within the scope of the study with a questionnaire on general information, anthropometric measurements, a 24- hour retrospective food consumption record, and a Pittssburgh Sleep Quality Index (PSQI) questionnaire. Twenty four hour retrospective food consumption record method which includes writing the foods consumed by the volunteer in the last 24 hours by the researcher. The 'Food

and Nutrition Photo Catalogue' was used to determine the portion sizes of the consumed food and beverages. The energy and nutrient values were calculated by entering the recorded record into the 'Nutrition Information System (BEBIS). The sleep quality of the volunteers was evaluated with Pittsburg sleep quality index. Body weight measured in kg with the TANITA DC 360 ST body composition measuring device. Height was measured in cm with a tape measure. Body mass index (Calculated from body weight and height values. BMI=Body weight (kg)/(Height (m))^2 formula was used in the calculation of body mass index. Waist-hip circumference was measured in cm with a tape measure. Body composition were determined with the TANITA DC 360 ST device using the bioelectrical impedance analysis (BIA) method.

Statistical analysis of the study was done by Statistical Package for the Social Sciences (SPSS) Inc. Made with Chicago IL v21 program. Descriptive statistics including mean, standard deviation were calculated for each parameter. One-way ANOVA was used to determine the differences between the groups. For statistical evaluations, $p<0.05$ was determined.

3. Results

A 24-hour retrospective food record was taken of 177 undergraduate students studying at the University of Health Sciences and their anthropometric measurements were determined and their relationship with sleep quality was evaluated. Age and anthropometric characteristics of the participants are given in Table 1. According to this table, the average age of the participants is 21.18. Average height is 171.34 cm, average weight is 66.73 kg, average waist circumference is 76.99 cm, average waist/hip ratio is 1.02 cm, fat percentage is 19.7%, and average BMI is 22.57 kg/m2.

Table 1. Average values of age and anthropometric characteristics of individuals

	Mean ± SD
Age	**21,18±2,4**
Size	**171,34±5,45**
Weight	**66,73±9,95**
Waist circumference	**76,99±7,05**
Waist/hip ratio	**1,02 ±3,25**
Fat percentage	**19,77±5,9**
BMI	**22,57± 3,15**

It was observed that 32 (18.1%) of 177 participants who participated in the study had good sleep quality (PSQI≤5), and 145 (81.9%) had poor sleep quality (PSQI>5). This distribution is shown in Table 2.

Table 2. Sleep quality of the participants

	N	%
Good Sleep Quality	**32**	**18,1**
Poor Sleep Quality	**145**	**81,9**
Total	**177**	**100**

According to the analyzes made, the relationship between the anthropometric measurements of the participants and the nutrients they consumed according to the results of the food consumption record and the PSQI scores were examined. These relationships are shown in Table 3. All the nutrients shown here are after dinner. According to Table 3, no significant relationship was observed between the waist and hip ratios of the participants and their sleep quality ($p>0.05$). No significant correlation was observed between BMI and sleep quality ($p>0.05$). There was no significant relationship between the amount of energy consumed by the participants after the evening meal and their sleep quality ($p>0.05$). A positive correlation ($p=0.000$) was observed between the amount of water and the PSQI score, and therefore a negative correlation was observed with sleep quality. A positive correlation was observed between protein consumption and PSQI score ($p=0.037$), thus a negative correlation was observed with sleep quality. No significant correlation was observed between the participants' fat consumption and fat percentages and their PSQI scores ($p>0.05$). No significant correlation was observed between the amount of carbohydrates consumed by the participants and their

PSQI scores (p>0.05). A negative correlation was observed between the percentage of carbohydrates consumed by the participants and their PSQI scores (p=0.044), thus a positive correlation was observed with sleep quality. No significant correlation was observed between the amount of fiber consumed by the participants and their PSQI score (p>0.05). A positive correlation (p=0.013) between the amount of absorbable oligosaccharides consumed by the participants and their PSQI scores was observed, thus a negative correlation was observed with sleep quality.

Table 3. Relationship between anthropometric measurements and nutrients and PSQI scores

	N	R	p
Waist/hip ratio	**177**	**-0,006**	**0,935**
BMI	**177**	**0,090**	**0,231**
Energy	**177**	**0,128**	**0,091**
Water	**177**	**0,278***	**0,000**
Protein	**177**	**0,157***	**0,037**
Protein %	**177**	**0,181***	**0,020**
Fat	**177**	**0,090**	**0,233**
Fat %	**177**	**-0,043**	**0,585**
Carbohydrate	**177**	**0,091**	**0,226**
Carbohydrate %	**177**	**-0,158***	**0,044**
Fiber	**177**	**0,116**	**0,125**
Absorbable oligosaccharide	**177**	**0,187***	**0,013**

***. Correlation is significant at 0.05 level**

p<0,05 is significant

4. Discussion

Current literature shows diet can live with sleep quality (St-Onge, Mikic, et al., 2016). In particular, a relationship has been reported between macronutrients and insomnia (Tanaka et al., 2013). We aimed to evaluate the relationship between the amount and type of carbohydrates consumed after evening meal and sleep quality in this study. The main findings of our study were: i) no significant correlation was found between the amount of carbohydrates consumed after the evening meal and sleep quality; ii) a significant correlation was observed between the percentage of carbohydrates, absorbable oligosaccharide consumption and PSQI score; iii) significant correlation was found between protein amount, protein percentage and sleep quality.

iv) a significant correlation between the amount of water consumed after the evening meal and PSQI score was found.

Detailed studies examining the relationship between carbohydrate intake consumed after the evening meal and sleep quality are not included in the literature, but there are studies on the effect of whole day macronutrients (Afaghi et al., 2007; Tanaka et al., 2013; Yajima et al., 2014). One study showed that a high-carb, low-fat diet caused less sleep time than a control (Phillips et al., 1975). Another study showed that a high-carbohydrate diet reduced the time it took to fall asleep compared to control (Lindseth et al., 2013). The finding of the percentage of carbohydrates is associated with sleep quality indicates that not only macronutrients, but also the whole diet composition can be associated with sleep quality.

According to the results of a systemic review and meta-analysis, it was reported that there was no relationship between protein intake and sleep quality, but this review drew attention to the inadequacy of data and the need for randomized controlled studies on the subject (Wirth et al., 2023). In addition, a study investigating the relationship between protein intake and sleep quality reported that high tryptophan levels were positively associated with sleep duration (Sutanto et al., 2022). On the contrary, we found a negative correlation between protein consumption amounts and sleep quality in our study.

5. Conclusion

In this study, no relationship was observed between the amount and type of carbohydrates consumed after evening meal and sleep quality, but significant difference observed with the percentage of carbohydrates suggests the possibility that food preferences may affect sleep quality. Studies using larger samples and randomize controlled design are needed.

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Potential Use of Cornelian Cherry Fruit Extracts as an Alternative to Synthetic Preservatives

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Abstract:

Long-time exposure to ultraviolet (UV) ray from the sun has negative effects on skin health. The photoprotective creams are used to protect against UV-A and UV-B rays from the sun. The cream formulations with photoprotective effect cause allergenicity and toxicity as they contain various synthetic chemicals. People with high allergenicity prefer natural ingredients instead of these synthetic creams. Plant extracts with UV absorption and photoprotective properties can be used as natural protective agents. Plants are good natural resources with UV protection potential. Cornelian cherry (*Cornus mas* L.) is one of the important medicinal plants with nutritional and therapeutic properties. In this study, it was aimed to determine the potential usage of Cornelian cherry as a natural additive in sunscreens. For this purpose, water and chloroform extracts from Cornelian cherry were obtained by sonication method. Then, the solar protection factor (SPF) of the extracts was determined spectrophotometrically in the wavelength range of 290 nm-320 nm. Afterwards, the extracts were mixed with the commercial cream and the SPF value of the mixture was obtained to determine the effects of the cream on the sunscreen properties. SPF values of extract-cream mixtures prepared at various concentrations (10 ml, 5 ml and 2.5 ml) were determined. The water and chloroform extracts from Cornelian cherry showed an SPF of 7.46 and 10.67. The UV blocking capacity of the extracts is approximately in the range of 80%. The SPF value of the commercial cream (control) group was determined as 5.17 at 10 ml concentration. SPF values of the water and chloroform extract and cream mixtures were determined as 5.21 and 6.40 at 10 ml concentration. Cornelian cherry extracts increased the SPF value of the control group at a concentration of 10 ml. The water and chloroform extracts and cream mixtures have approximately 80% UV blocking capacity. The results showed that Cornelian cherry water and chloroform extracts have good UV blocking capacity. Therefore, Cornelian cherry extracts may have the potential to be used as natural sunscreen agent instead of synthetic preservatives in the cosmetic industry.

Keywords:*Cornus mas,* Sun Protection Factor, Ultraviolet, Extract, Cream

1. Introduction

Cornelian cherry (*Cornus mas* L.) is a tall deciduous shrub in the family Cornaceae, ranging from 5 to 8 m in height. It is grown in various parts of the world and popular in southern Europe (Mamedov and Craker2004). Cornelian cherry fruit has a high phenolic compound and ascorbic acid content, antioxidant activity and high biodiversity (Rop et al. 2010). In regions where Cornelian cherry is grown, its fruit is used in folk medicine, skin diseases, metabolic disorders

and diarrhea. In addition, the antiseptic properties of flowers, seeds, leaves, bark and roots are used (Ugur et al. 2020).

People are exposed to sunlight while performing their daily activities, but ultraviolet (UV) rays from the sun has harmful effects for living organisms (De Grujil 1999). UV rays from the sun are part of the solar electromagnetic spectrum. UV is solar radiation with wavelengths shorter than visible light but longer than X-rays, invisible to the naked eye. There are three main subtypes of UV rays according to their wavelengths and they are: UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (100–280 nm) (Yagura et al. 2011).UV-A tends to damage the structural proteins of the dermis (such as collagen and elastin) and is therefore responsible for premature skin aging (photo aging). UV-B can cause severe sunburn in the short term and predispose a person to skin cancer in the long term. UV-B penetration into the basal layer can damage the nitrogenous bases and protein structures of DNA and RNA, and their cumulative effects can lead to dysfunctional cellular activity. UV-C is blocked by the ozone layer in the atmosphere and cannot reach the earth (Nole and Johnson 2004). In vivo, the skin is exposed to UV-B and UV-A as UV-C is stopped by the ozone layer. Products used as sunscreen should be used to protect from UV-A and UV-B (Debacq-Chainiaux et al. 2012).

Using synthetic products to protect the skin from harmful sun rays can have harmful long-term effects (Boehm et al. 1995). These harmful effects include allergy, inflammation, sunburn cell formation, itching, photoaging, immunosuppression, and carcinogenesis (Debacq-Chainiaux et al. 2012). Plants and their extracts have UV absorption and photoprotective properties and can be used as natural sunscreen protective agents. The use of herbal extracts in sunscreen products increases the photoprotective properties. The use of flavonoids and antioxidants guarantees skin protection against free radicals, and especially flavonoids can act as sunscreen (Cefali et al. 2019). Extracts from plants can inhibit the acceleration of transcription factors of cells affected by UV rays (Rodrigues et al. 2014). Sunscreens produced with plant extracts can be used as an alternative to prevent the negative effects of synthetic products.

In this study, sun protection factors of extracts (water and chloroform) obtained from Cornelian cherry fruit and extract commercial cream mixtures were determined as an alternative to synthetic sunscreens in the cosmetic industry.

2. Materials and Methods

2.1. Plant Material and Preparation of Extracts

The Cornelian cherry fruit was purchased from Mersin. After the fruit samples were washed with distilled water, they were dried in an airy place out of the sun. The dried sample was grounded with the Waring blender. The ground fruit samples (20 g) and solvent (60 ml of water and chloroform) were homogenized by vortexing. It was then sonicated for 20 minutes per day (2 days). After extraction, the extracts were stored in dry conditions at 4°C until used.

2.2. Determination of Solar Protection Factor of Cornelian Cherry Extracts

Each extract (0.008 g) was weighed to determine the SPF value of the cranberry extracts. The weighed extracts were added with 96% ethanol (4 ml) and homogenized. Homogeneous mixtures were measured in the spectrophotometer at 5 nm intervals in the wavelength range of

290-320 nm. The obtained values were calculated using the Mansur equation (Mansur et al. 1986).

Mansur's equation:

$$SPF = CF \times \sum_{290}^{320} (\lambda) \times I(\lambda) \times Abs(\lambda)$$

2.3. Determination of Solar Protection Factor of Cornelian Cherry Extracts and Cream Mixtures

The solar protection factor of Cornelian cherry extract and commercial cream samples was determined using a modified method of Asan-Ozusaglam and Celik (2023). 1 g cream, 0.5 g fruit extract was mixed and made up to the final volume with distilled water (10 mL). The concentrations of the mixture filtered with Whatman (No: 1) filter paper was prepared as 2.5, 5 and 10 mL. Mixtures at the prepared concentration were read at 5 nm intervals using a spectrophotometer device at wavelengths of 290-320 nm.

3. Results

SPF values of Cornelian cherry extracts and extract-cream mixtures were determined in- vitro. The absorbance values found as a result of the spectrophotometric measurement were calculated using the equation of Mansur et al. (1986). The SPF value of Cornelian cherry water extract was 7.46 and the SPF value of Cornelian cherry chloroform extract was 10.67 (Table 1). According to the UV blocking percentage of the SPF values determined by Imam et al. (2015), the UV blocking percentage of the extracts was found to be approximately 80% (Table 2).

Table 1. Solar protection factor of Cornelian cherry extracts

Extract	SPF Value
CW	7.46±0.01
CC	10.67±0.18

CW: Cornelian cherry Water extract, CC: Cornelian cherry Chloroform extract

Table 2.SPF values and corresponding percentage of UV blockage

SPF	Percent of UV Blocked
2	50
4	75
5	80
10	90
15	93
25	96

SPF: Solar Protection Factor, UV: Ultraviolet

SPF values of 2.5 mL, 5 mL and 10 mL concentrations prepared with Cornelian cherry extract and cream mixtures were determined as a result of spectrophotometric measurements. SPF values of extract and cream mixtures are given in Table 3. The SPF values of the extract and cream mixtures were determined as 5.21 and 6.40 at 10 mL concentration. Cornelian cherry fruit extracts increased the SPF value of the control group at 10 mL concentration, and value decreased as the concentration decreased.

Table 3. SPF values of cream and Cornelian cherry extractmixtures

Extract	Concentration		
	10 ml	5 ml	2.5 ml
Control	5.17±0.04	0.58±0.00	0.54±0.00
CW	5.21±0.01	0.49±0.00	0.19±0.00
CC	6.40±0.04	0.78±0.00	0.17±0.01

CW: Cornelian cherryWater extract, CC: Cornelian cherry Chloroform extract

4. Discussion

Synthetic products are used to protect the skin from harmful solar rays. Long-term use of these products can cause side effects. Research and development of natural products and sunscreen effects is of great importance in the cosmetic industry (Portal et al. 2023). Studies of SPF with Cornelian cherry fruit extract are limited. In the study of Gould et al. (2010), it was found that anthocyanins in the stems of *Cornus stolonifera* have a role in the photoprotective effect. Therefore, determination the SPF values of various plant extracts can create a potential for use in the cosmetics industry. Cornelian cherry fruit also has the potential to play a role in the photoprotective effect due to the anthocyanins it contains. As a result of the studies, the determination of the SPF values of various plant extracts may create a potential for use in the cosmetics industry.

5. Conclusion

In this study, the sun protection value of cranberry fruit as a natural preservative source instead of synthetic preservatives in sunscreen creams was tested. The results showed that the cranberry juice and chloroform extracts had good UV blocking capacity. Cream formulations containing cranberry extract have the potential to be used as natural sunscreens instead of synthetic sunscreens.

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Examination of Branches of Arteria Carotis Externa in a Anatolian Wild Goat

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Abstract:
Mountain goat (Capra) is a genus that includes 9 species. The domestic goat (*Capra aegagrus hircus*) is a domesticated subspecies of the wild goat. The study material consisted of a wild goat that was brought to Kafkas University Wildlife Rescue and Rehabilitation Center but could not be saved despite all the interventions. It was observed that arteria occipitalis emerged from the division of arteria carotis communis into arteria carotis externa and arteria carotis interna. It was determined that arteria carotis externa gave branches named arteria lingualis, arteria transversa faciei, arteria palatina ascendens, ramus massetericus, arteria auricularis caudalis, arteria occipitalis, arteria temporalis superficialis and arteria maxillaris. In this study, it was aimed to determine the course of the branches of the arteria carotis communis of the Anatolian wild goat (*Capra aegagrus aegagrus*) head living in the Caucasus and Northern Anatolia Region. It should not be forgotten that these findings of the extinct Anatolian wild goat may be the first or the last study. It is thought that the presented study will contribute to scientific studies and head surgery operations on similar subjects.

Keywords: anatomy, arteria carotis externa, Anatolian wild goat

1. Introduction

Goats are mammals that make up the genus Capra from the subfamily Bovinae of the Bovidae family. Mountain goat (Capra) is a genus that includes 9 species. Domestic goat (Capra aegagrus hircus) is a domesticated subspecies of wild goat (Anonymous 1). It is possible to come across the Wild Goat, which is widespread in some countries of the Caucasus and the Middle East, in the Aegean, Mediterranean, Southeastern Anatolia, Eastern Anatolia and Black Sea regions of our country, up to 4000-4500 m above sea level (Anonymous 2).

The circulatory system consists of organs that carry all the substances necessary for life to the organs and collect the wastes formed as a result of metabolism. Arteries, one of these organs, are of vital importance for the organs to continue to function (Bahadır and Yıldız 2014).

Figure 1. Anatolian wild goat (*Capra aegagrus hircus*) in Kafkas University Wildlife Rescue and Rehabilitation Center (Kars, Turkey)

Arteria carotis communis is the vessel that carries clean blood to all formations in the head and neck. At the level of the processus transversus of the atlas or articulatio atlantoaxialis, it divides into two branches as arteria carotis externa and arteria carotis interna. The arteria occipitalis can sometimes emerge from this separation zone. Arteria carotis externa has two terminal branches, arteria temporalis superficialis and arteria maxillaris (Dursun 2008). Arteria carotis externa give these branches: arteria lingualis, arteria facialis, arteria palatina ascendens, ramus massetericus, arteria auricularis caudalis, arteria occipitalis, arteria temporalis superficialis, and arteria maxillaris. But arteria facialis is absent in sheep and goats. In these animals, the arteria transversa faciei, which separates from the arteria temporalis superficialis, vascularizes the area vascularized by the arteria facialis. The terminal branches of the arteria maxillaris are the arteria infraorbitalis and arteria palatina descendens. Other branches are arteria alveolaris inferior, arteria meningea media, arteria temporalis profunda, arteria ophtalmica externa, arteria centralis retinae, arteriae ciliares posteriores longae, rami musculares, arteria supraorbitalis, arteria lacrimalis, arteria ethmoidalis externa, and arteria buccalis (König and Liebich 2022, Popesko 2010).

It should not be forgotten that these findings of the extinct Anatolian wild goat may be the first or the last study. It is thought that the presented study will contribute to scientific studies and head surgery operations on similar subjects.

2. Materials and Methods

In order to carry out this study, necessary permissions were obtained from the Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks (288.04-9611980/E-21264211). The animal material consisted of a wild goat, which was injured for various reasons and brought to the Kafkas University Wildlife Rescue and Rehabilitation Center, but could not be saved despite all the interventions. The vena jugularis

externa was excluded from the sulcus jugularis region and the deep arteria carotis communis was reached. It was fixed with the IV tube placed inside the vein. The ligatured vessel was washed with 0.9% saline solution. After washing, latex colored with red dye (ZPK-580-S; Gerard Biological Center, Preston UK) was applied with the help of a syringe. This procedure was continued until the arteries were completely filled. If the latex applied with the injector was no longer gone, the serum tube was closed with a hemostat. After this process, the head was kept in a refrigerator (-5 degrees) for 1 week for the latex to solidify. After the latex solidified, the dissection process was started. The skin on the skull was removed. Arteria carotis communis and its continuation branches were dissected and results were obtained. A digital caliper was used for the measurements. The use of scientific terms was based on Nomina Anatomica Veterinaria (2017).

3. Results

The diameter of arteria carotis externa was 4.85 mm on the right and 5.17 mm on the left. It was observed that this vessel was a continuation of the arteria carotis communis in its course. Arteria carotis externa appeared to initially give off a thin branch, arteria occipitalis. Later, arteria lingualis and ramus parotideus were divided into arteria auricularis caudalis, ramus massetericus in the middle part. In the last part, it was determined that arteria temporalis superficialis and arteria transversa faciei, which are terminal branches, were separated from arteria carotis externa in the form of a common root (Figure 2). This root came out at the same level as the arteria maxillaris. However, it was stronger than arteria maxillaris, arteria transversa faciei, and arteria temporalis superficialis. Arteria maxillaris was first divided into arteria alveolaris inferior. Subsequently, the common root of arteria ophtalmica externa, arteria infraorbitalis, arteria sphenopalatina, and arteria palatina descendens was separating. First of all, the arteria malaris was coming out and running towards the dorsal. Then, from the mentioned common root dorsal to arteria infraorbitalis ventral, the common root of arteria sphenopalatina and arteria palatina descendens was separating (Figure 3-4). It was observed that the ramus pterygoideus originated from the ventral aspect of the arteria maxillaris and divided into two branches running in the rostral direction. It was determined that these two branches were distributed over the musculus pterygoideus medialis and musculus pterygoideus lateralis. It was observed that the arteria alveolaris inferior originates from the ventral wall of the arteria maxillaris and proceeds in the ventral direction and enters the foramen mandibula. Before entering the foramen mandibulae, musculus pterygoideus medialis, musculus pterygoideus lateralis, and musculus mylohyoideus were found to participate in arterial nutrition. It was determined that arteria alveolaris inferior gives rami dentales for mandibular

teeth during its course in the canalis mandibularis. After exiting the foramen mentale, it was observed that it formed a continuation arteria mentalis. Arteria mentalis was found to be distributed in the lower jaw region in the form of two branches. It was determined that arteria auricularis caudalis is the thickest branch originating from the caudal of arteria carotis externa, and it participates in the arterial nutrition of the ear and glandula parotis.

Figure 2. Superficial arteries in the head of wild goat (a: arteria carotis communis, b: arteria occipitalis, c: arteria carotis externa, d: arteria auricularis caudalis, e: arteria transversa faciei, f: arteria temporalis superficialis)

It was observed that arteria ophthalmica externa originated as a single root from the dorsal wall of arteria maxillaris and joined the rete mirabile ophthalmicum 3-4 cm after its origin. It was determined that arteria ophthalmica externa followed a dorsal course between musculus rectus lateralis and musculus rectus dorsalis. It was observed that arteria ophtalmica externa gave branches named arteria supraorbitalis and ramus lacrimalis.

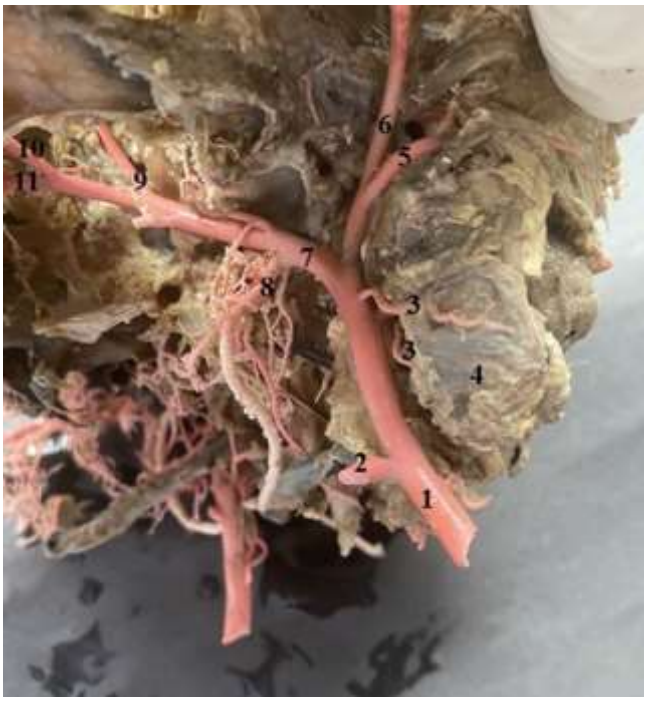

Figure 3. The course of the branches of the arteria carotis externa after removing the glandula parotis in the wild goat (1: arteria carotis externa, 2: arteria lingualis, 3: rami parotidei, 4: parotid gland, 5: arteria transversa faciei, 6: arteria temporalis superficialis, 7: arteria maxillaris, 8: arteria alveolaris inferior, 9: arteria ophtalmica externa, 10: arteria malaris, 11: common root of arteria infraorbitalis and arteria sphenopalatina and arteria palatina descendens)

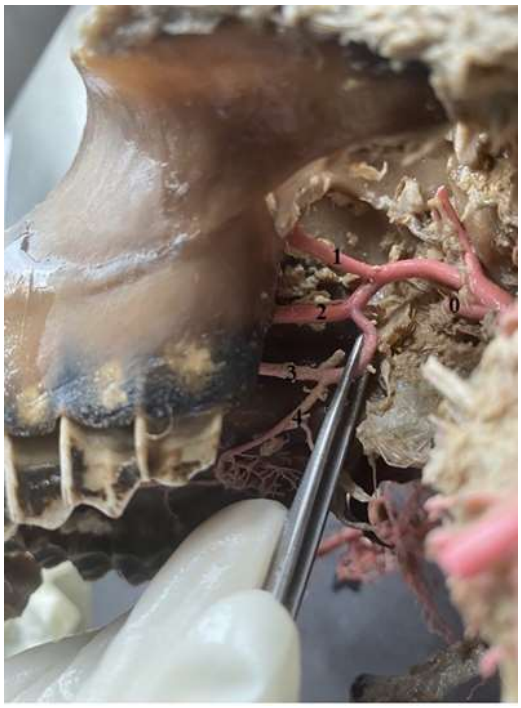

Figure 4. Branches of arteria maxillaris at the level of the fossa pterygopalatina (1: arteria malaris, 2: arteria infraorbitalis, 3: arteria sphenopalatina, 4: arteria palatina descendens)

It was determined that the arteria infraorbitalis proceeded through the canalis infraorbitalis together with the nervus infraorbitalis and provided the nourishment of the maxillary, premolar

and molar teeth. It was determined that after the arteria infraorbitalis passed through the foramen infraorbital, it first divided into two branches, and the dorsal branch divided into two branches again and participated in the nutrition of the anterior tip of the nose and the skin of the region (Figure 5).

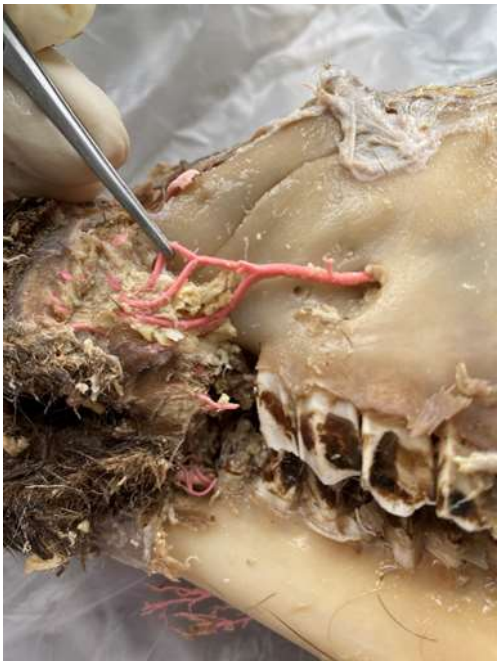

Figure 4. Arteria infraorbitalis and branches

4. Discussion

While the diameter of arteria carotis externa was reported to be 4.42 mm in Tuj sheep (Beki 2017), it was 4.85 mm on the right and 5.17 mm on the left in Anatolian wild goats. The first branch of arteria carotis externa was arteria occipitalis, as reported in giraffes (O'Brien et al. 2016). Studies in small ruminants in the literature have reported that arteria maxillaris, arteria transversa faciei, and arteria temporalis superficialis originate from the common root (Dursun 2007). In studies conducted in Akkaraman sheep, Angora goat (Nur and Dursun 1992), and Hemşin sheep (Dalga and Aslan 2016), it was reported that arteria temporalis superficialis and arteria transversa faciei originate from arteria carotis externa as a common root. It was determined that arteria temporalis superficialis and arteria transversa faciei originate at different levels from arteria carotis externa in buffaloes (Özdemir and Tıpırdamaz 2002) and Zavot cattle (Akbulut and Aslan 2013). In our study, it was determined that arteria temporalis superficialis and arteria transversa faciei were separated from arteria carotis externa in the form of a common root, in line with the findings in Hemşin and Akkaraman sheep and Angora goat. Similar to Beki (2017), it was determined that the arteria maxillaris, originating from the arteria carotis externa, proceeded in the dorsal direction and reached between the musculus pterygoideus lateralis and the musculus pterygoideus medialis, and then proceeded in the rostral direction.

As stated in the literature (Wave and Aslan 2021), arteria maxillaris was observed to be a continuation of arteria carotis externa in terms of course and thickness. It was determined that the arteria maxillaris was divided into two terminal branches named the common root of the arteria infraorbitalis and arteria malaris and the common root of the arteria palatina descendens and arteria sphenopalatina in the fossa pterygopalatina. In our study, arteria auricularis caudalis was found to be the thickest branch originating from the caudal of arteria carotis externa, similar to the literature (Akbulut and Aslan 2013, Beki 2017, Özdemir and Tıpırdamaz 2002). It was determined that the ear and glandula parotid participated in the arterial nutrition.

5. Conclusion

As a result, it should not be forgotten that these findings of the Anatolian wild goat, which is in the diversity of wildlife in our country, but which is on the verge of extinction due to illegal and uncontrolled hunting or traffic accidents in recent years, may be the first or the last study. It is thought that the presented study will contribute to scientific studies and head surgery operations on similar subjects.

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Green Syhthesis of Waste Walnut (*Juglans regia* L.) Inner Shell Based Silver Nanoparticles

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Abstract:
Nanotechnology is a developing technology to improve the physical, chemical and biological properties of matter with the size of 1-100 nm. Many sectors such as medicine, automotive, electronics, food, textile, agriculture, cosmetics, equipment and materials benefit from nanotechnology research. Silver nanoparticles (AgNP) have importance in nanotechnology applications with properties such as chemical stability, electrical conductivity, catalytic and antibacterial activity. Silver is the least toxic element compared to other elements. AgNPs could be synthesized by different methods such as physical, biological and chemical methods. The most commonly used synthesis technique is biological synthesis. Green synthesis method is the most preferred method because it is ecologically friendly, energy saving, cheap, less waste, easy scaling, and sustainability, not using toxic chemicals and being biologically compatible. In addition to the use of bacteria, yeast, algae and plants could be used as a reducing and stabilizing agent. Silver nanoparticles obtained by this method has many application area such as biosensors, medicine, etc. Walnut is a versatile fruit with its leaves, dry and green fruit, timber, inner membrane and outer peel. Walnut is a widely consumed nut because it contains valuable nutrients and oils. In this study, waste walnut inner shell was used to synthesis silver nanoparticles. WS-AgNPs were synthesized and characterized using the green synthesis technique from the shell in the inner part of the walnut. The obtained nanoparticles were characterized using UV-Vis spectrophotometer and FTIR. It was determined that the obtained nanoparticles gave maximum absorbance at 460 nm.

Keywords: green synthesis, silver, nanoparticles, FTIR, UV

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1. Introduction

Nanotechnology is a new technology that allows processing particles generally between 1-100 nm in sizes (Beykaya and Çağlar, 2016). Nanotechnology is widely used in medicine, dentistry, drug delivery systems, many biomedical applications, environment and engineering. Silver

nanoparticles (AgNP) have importance with its properties such as chemical stability, conductivity, catalytic and antibacterial activities. Compared to other elements, silver has the more antimicrobial effect and it is the least toxic element. AgNPs could be synthesized by many different methods. The most common method is the green synthesis method, also known as biological synthesis. Green synthesis method is the most preferred method because it is ecologically friendly, energy saving, cheap, less waste, easy scaling, sustainability, no use of toxic chemicals and biological compatibility. In addition to the use of bacteria, mold, yeast, algae and plants as a reducing and stabilizing agent, it is not toxic due to the absence of chemicals. Nanoparticles could be synthesized faster than other applications by green synthesis. It is the most preferred method due to its biocompatibility. Silver nanoparticles obtained by this method are used in antimicrobial application areas, separation of toxic compounds, biosensors, environment and various treatment systems. In addition to these, silver nanoparticles have an application in areas like the prevention of diseases in agriculture, the rapid elimination of existing diseases, increasing the ability of plants to absorb nutrients from the soil in agriculture, antibacterial and odor-repellent textile products, anti-scratch car paints, dirt-repellent coatings, transparent protective sunscreens, self-cleaning glass silver (Beykaya and Çağlar, 2016).

Walnut is a versatile fruit with its leaves, dry and green fruits, inner membrane and outer shell. Walnut is a widely consumed nut because it contains valuable nutrients and oils. Its fruit could be used as food but its inner membrane and outer shell are waste and not used in any application. In this study, the waste inner shell of walnut were used as electron precursor and the potential of using waste walnut was examined for nanotechnology. For this purpose, waste inner shell based silver nanoparticles were syntheses and characterized.

2. Materials and Methods

WS- AgNPs were synthesized according to Keskin (2022) with minor modifications. For this purpose Walnut inner shell extract and 0.05 M silver nitrate ($AgNO_3$) solution was mixed in a dark flask at a 1:1 ratio (v/v). The mixture was stirred for ~2 h at room temperature. The changes of color to dark brown was noted and confirmation of nanoparticle synthesis was applied by UV absorption spectroscopy between 250 and 750 nm. The formation and presence of AgNPs were determined by a UV-Vis spectrophotometer. The absorbance was recorded by scanning the wavelength in the spectrophotometer device. FT-IR analyses was also performed in order to evaluate the functional groups of the bioactive components responsible for the reduction.

At the end of the synthesis, centrifugation was performed at 9000 rpm with a high-speed centrifuge device to precipitate AgNPs from the aqueous medium.

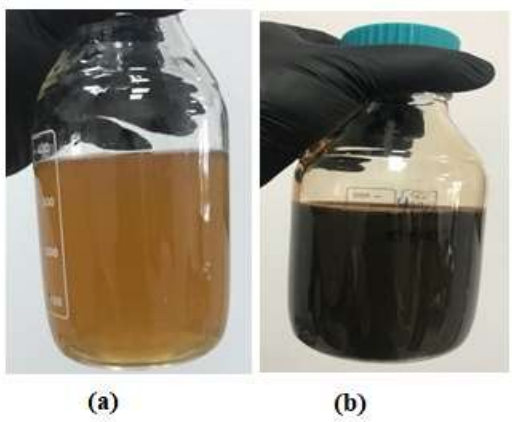

Figure 1. a) Walnut inner shell extract b) WS- AgNPs solution

3. Results

Optical properties of synthesized nanoparticles were determined using UV-Vis spectrophotometer. It was determined that the obtained nanoparticles gave maximum absorbance at 460 nm. Functional groups of Walnut inner shell extract and WS- AgNPs supernatant were determined using FT-IR and the results were shown in Figure 2 and Figure 3 respectively.

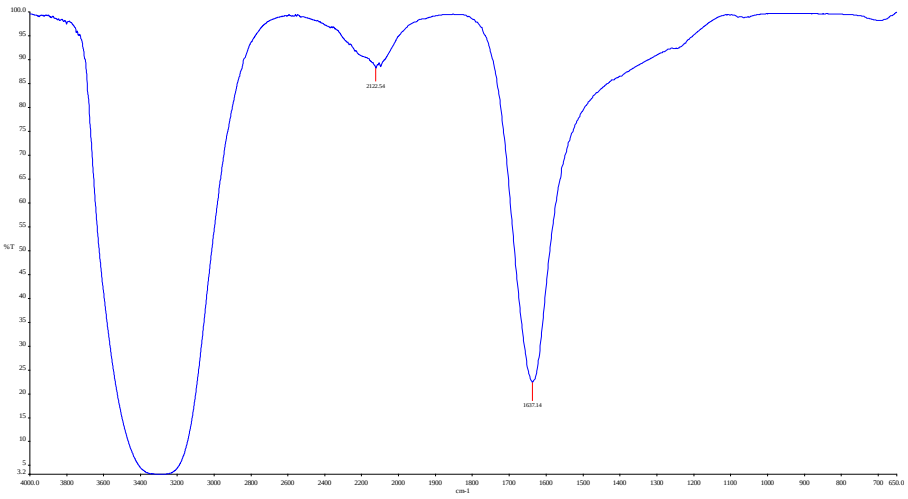

Figure 2. Walnut inner shell extract FT-IR data

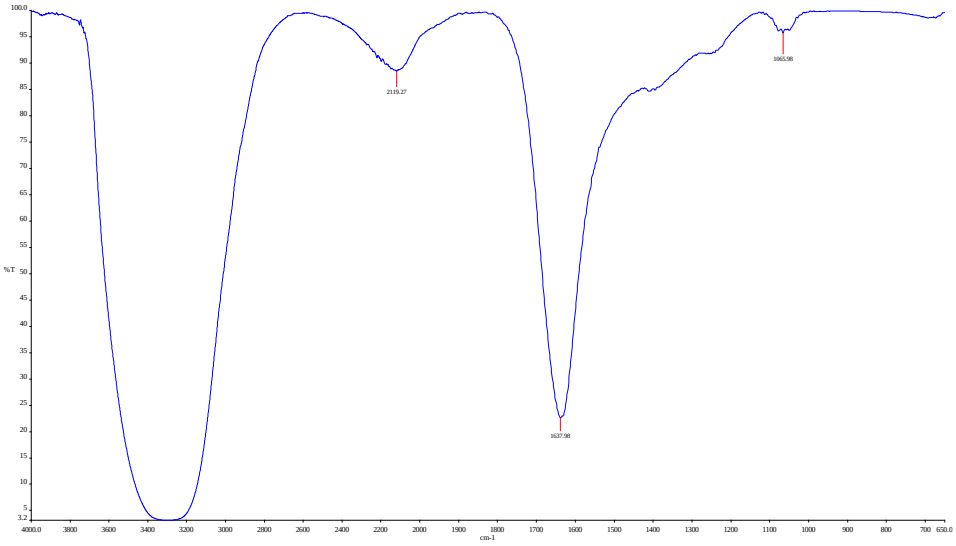

Figure 3. WS- AgNPs supernatant FT-IR data

4. Discussion

Organic solid waste management and ecological worries have been increasing in recent days (Das et al., 2019). Hence, from fruit wastes, such as fruit peels, and other organic fractions of domestic solid wastes may be used in an effective manner in nanotechnology-based applications (Ahmed et al., 2016; Reenaa et al., 2017; Omran et al., 2018).

The reduction of Ag^{+} ions to Ag^{0} was determined by intermittently measuring the absorption spectra of the reaction solution using a UV–Vis spectrophotometer. Usually, due to the excitation of free electrons, AgNPs would display a surface plasmon resonance (SPR) band at 450–550 nm (He et al., 2017; Mousavi et al., 2018). In the current study, the SPR value of WS-AgNPs was detected at 460 nm. The current result is similar to the previously reported AgNPs synthesis result (Ashraf et al., 2016). In a study, silver nanoparticles synthesized using the outer peel extract of *Ananas comosus* (L.) and the maximum absorbance was found at 485 nm (Das et al., 2019). FTIR data provides us to compare walnut inner shell extract and WS- AgNPs supernatant functional groups to determine possible reactions. As seen Figure 2 and 3, there was changes at 2122.54 cm^{-1} band and there was a new band at 1065.98 cm^{-1}. The band at 3200 to 3400 cm^{-1} represents O─H stretching groups. In a study, silver nanoparticles were syntheses from pistachio leaf extract. According to this study, AgNPs had maximum absorbance at 460

nm and had differences at functional groups of 3336.64-3334.57 cm^{-1} bands and there is no differences at 1636.82-1636.48 cm^{-1} bands (Eren and Baran, 2019). It was clear that the functional groups and maximum absorbance of AgNPs varies over a wide range and the results were obtained in this study was compatible with the literature. There are many studies showing that silver nanoparticles obtained by using different herbal sources have many effect such as antimicrobial, antioxidant etc.

5. Conclusion

In this study walnut inner shell extract was used as an electron processor to synthesis WS-AgNPs. Walnut is an important fruit and it is used in daily nutrition. Walnut inner shell is also an important walnut waste with its phytochemicals. In this study, a new product was synthesized for phytotherapy applications. After detailed characterization of WS-AgNPs, antimicrobial activity and potential for using of WS-AgNPs in phytotherapy application will be determined.

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Socıal Servıce After Dısaster

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Abstract:
Despite technological developments, it is observed that the threat areas related to biosecurity have not decreased but also increased and diversified ironically. Natural disasters, with their uncontrollability, are still close to contemporary life. Other vital risks that occur in human social environments are also experienced at an unpredictable speed and variety, just like a natural disaster. Countries that try to turn to liberal policies by avoiding the inclusiveness of social state services, on the other hand, have to turn to social policies when faced with these disasters. While the disaster of the century in Turkey, the 6 February Earthquake, first collapsed on the country with its natural and then social dimensions, the need for social service was revealed in all its aspects. The traumatic effects of disasters are diverse and have socio-economic dimensions as well as psychological ones. The earthquake has produced consequences that affect people's displacement, death and injury, disruption of health systems, scarcity of food, water and energy resources, and their ability to cope most directly with activities of daily living. Women, children, the elderly and the disabled, who are the most vulnerable segments of the society and therefore constitute the scope of the groups to which the social work field will give direct attention and support, have come to the fore in policies because they have experienced greater victimization in this process. Priorities in making choices, which became necessary in the conditions imposed by the disaster, and the shocked behavior of segments of society who were so closely confronted with the reality of death created great difficulties in carrying out their social work duties. Service conflicts of acute period officers have also been an important problem, negatively reflected on services as coordination weaknesses. In this study, emergency response policies will be discussed from the perspective of central and local governments in meeting the post-earthquake social service needs. The study, which is in compilation format, was created by scanning literature review.

Keywords: social service, earthquake, biosecurity, service conflicts, fragile groups

Introduction

Despite technological developments, it is observed that the threat areas related to biosecurity have not decreased but also increased and diversified ironically. Natural disasters, with their uncontrollability, are still close to contemporary life. Other vital risks that occur in human social environments are also experienced at an unpredictable speed and variety, just like a natural disaster (Dıjk, 2016: 244).

The security sector fed by this situation is also growing. Considering the security measures taken against natural disasters, the security measures required by metropolitan life, and the protection of buildings, borders and airports, it is stated that "an annual industry of 30-35 billion dollars is formed only for the USA" (Singer, 2009: 55).

It has not been possible for Turkey to implement an effective security policy against a disaster of unpredictable magnitude. In fact, it can be said that the 100th anniversary of the Turkish Republic is coming to an end with the country's two-decade-long test. One of them was the health and social policy services, which started on March 10, 2020 and were tested by the global

epidemic of the period, Covid 19, whose effects are still ongoing. The other one was the 6 February 2023 earthquake, which was also the disaster of the century. Both exams caused a great shock with tens of thousands of death figures.

Biohealth and Psychosocial Effects of Disasters

The traumatic effects of disasters are diverse. In addition to many physical and psychological health problems such as injury, deprivation of basic health and hygiene conditions, and psychological shock, it is observed that social balances are deteriorated. Some of the defense mechanisms developed by people and societies in dealing with disasters have dark contents. Ali (2013: 15), one of the famous writers of our literature, when he sees that a disaster has befallen our acquaintances and they are in any trouble, “we feel relief as if we have rid ourselves of these troubles, and we want to show care and mercy to those poor people, as if they have brought troubles that may come to us. ” he says. The point that the author draws attention to is that even our feelings of pity can have dark roots.

Self-questioning is another natural response. According to the French literary scholar Maurois (241), “the evil of men is largely born of envy and fear. Disaster takes their weapons from them”. They accept the fact that disasters have also hit them as a kind of "social redemption" and feel bitter pleasure.

In addition, it is possible to observe those who are attracted by the existence of a kind of "spectacular" situation in terms of social psychology. Another ironic pen of the literary world, Marquez (2014: 525, 526) exemplifies what an unprepared society can go through in his story about the earthquake that swallowed a mother and her three young children in a poor house on a wide and barren land. He states that after the disaster was reported in the radio news, those who went to the scene consisted of curious as well as aid volunteers. Unfortunately, the second slide now contains three hundred corpses, and as late as over two thousand unprepared volunteers try to offer aid to the survivors in a tremendous mess, a larger landslide and more death occurs.

The only way to reduce such psychological and sociological problems, which have many similar examples during and after disasters in real life, is to establish a society structure that can be more foresighted and organized in all its dimensions.

Coping with Disaster Impacts

Another feature of disasters is that they produce multidimensional results. In all disasters such as earthquake, flood, forest fire, tsunami, etc., people suffer not only with the loss of life and property they have, but also regardless of agriculture, industry or small business, they also lose their future economic power. Disasters are therefore long-term, devastating natural events. Foreseeing, urgent and effective policy production is required for the disaster-affected region to recover. Otherwise, the urgency of this period opens the door to all kinds of abuse, as long as the lack of foresight that feeds the disaster and the habit of greed for profit that ignores security continues.

Bringing the cities destroyed by disasters to their feet as soon as possible is important in terms of overcoming emotional as well as physical destructions and shortening the time to face pain. Futurologists point out that although natural geological disasters usually affect a city or a region, “in San Francisco after the earthquake and fires of 1906 and in these urban examples

after the great Tokyo earthquake of 1923, cities recovered in a short time" (Kurth and Easterbrook, 2015: 192).

On the other hand, the social strata's being affected by disasters includes very different dimensions. In the literature, the phenomenon defined as "social vulnerability" is the degree of vulnerability that individuals and society can be exposed to due to psychological, sociological and demographic factors.

Although it is more difficult to measure this situation compared to physical and economic vulnerability, it is accepted that groups such as women, children, the elderly and the disabled, who are vulnerable parts of the society, are more exposed to the negative effects of disasters and their ability to resist and cope is weaker.

In social vulnerability measurement, "literacy, security, basic human rights, governance, social equality, gender issues, public health, population density, livelihoods, traditional values, legal system, relationship between family and relatives, political system, ideological beliefs and institutional systems. indicators such as status are effective" (Demir, 2016: 218). In terms of these basic indicators, it is clear that vulnerable groups are more insecure.

As a matter of fact, UNICEF Turkey Representation stated that approximately 4 million school-age children were affected by the earthquakes in Turkey, and an additional 138 million dollars aid was called for for Turkey. It was also pointed out that assistance is urgent for the children and families in the region to recover and rebuild their lives after the earthquake (TRT, 2023).

Emergency response policies carried out by central and local governments and supported by many non-governmental organizations and volunteers were of great importance in meeting the social service needs after the earthquake. On the other hand, from time to time, lack of coordination emerged in the solidarity practices exhibited throughout the country.

The impact of the disaster is directly related to the vulnerabilities and capacity strengths of the societies of the country in which it occurs. The "development of the culture of taking precautions against risk" in societies such as Japan, which is known as the country of natural disasters with the body of continuous earthquake experience, also ensures that post-disaster traumas are reduced in those countries.

In the example of this country, "With the cooperation of NGO-public-private sector, there are practical courses in which survival and life-saving methods (waterless agriculture, alternative water supply methods, fire-making) and life skills are taught for children and mothers, and private schools are established for this" (Yalçın, 2020: 106) is reported.

In Turkey, it is clear that such educational elements should be given up to the primary school level. Because traumas, especially on children, can leave more permanent damage. The perceptual differences of children who are forced to make do with the information obtained through the media can create a significant problem. The coordination and synthesis of the knowledge gained in this way becomes difficult (Özdemir at all. 2002: 115-118).

These trainings should not be in the form of ordinary knowledge transfer, but should include effective teaching techniques based on research, examination, simulative, applied and participatory methods. Since aftershocks will definitely occur after major earthquakes, these direct experience conditions may also be available in post-earthquake trainings.

When we look at the literature on disasters in Turkey, it is seen that the researches made after the earthquake experiences are carried out on the basis of questioning the relations between the state and the citizen and the competences in meeting the needs and developing suggestions. Considering disasters as "laboratories where we can test the integration, resilience and healing power of social systems" of society, as well as evaluating "disasters as a social pathology" by "bracketing" this point of view (Yılmaz, 2021: 202) also constitutes a dimension of the research area.

One of the early scientific studies on the subject was the Workshop on Social Services and Social Work in Disasters held in Istanbul on March 17-18, 2023, by the Department of Social Work of the Faculty of Health Sciences of Istinye University, in cooperation with the Friedrich Ebert Foundation. Concrete suggestions were made in the workshop held with the Ministry of Family and Social Policies, municipalities, the heads of the Social Services Departments of universities and their professors, as well as non-governmental organizations and volunteers from different parts of Turkey with field experience in the earthquake zone.

Among these suggestions, important issues such as conducting studies on disaster ethics, arranging the authorities and responsibilities of Psycho-Social Support teams, respecting the professional boundaries of Spiritual Support Specialists and Mental Health Specialists and not making professional violations, and the availability of the website where non-governmental organizations can apply for accreditation, are among the important issues. has been mentioned.

Conclusion

Considering all this framework, it is important that the whole society, starting from public institutions, attain a more disaster-oriented consciousness and strengthen social resilience.

Discussions were held on the need for post-earthquake social workers in the field, as well as the need for the support of students who have reached a certain stage of their education. While the concerns discussed on this issue are justified, there may be situations where the benefit/harm debate will impose itself, depending on the magnitude of the disaster. For this reason, it is important for these students, who are health workers, to prepare for disasters and develop their skills to cope with the physical and psychological problems caused by natural disasters, both for their own health and for public health.

Considering that not only those in the earthquake area but also all the citizens of the countries with their relatives in the region came out of the process with damage, it is necessary to determine the post-earthquake trauma levels of the students who are still in education and to provide training to facilitate rehabilitation within the framework of the determinations. They will play an important role in the future in ensuring the continuation of health and social services in the event of an earthquake, participating in rescue efforts when necessary, and reinforcing the psychological health of the society.

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Instant Mixed Herbal Tea Production with Spray Drying

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Abstract:
Herbal teas are beverages that have great importance in preventing and treating diseases with their high content of antioxidant substances such as fat-soluble vitamins A and E, water-soluble vitamin C, and phenolic compounds. In this study, the production of mixed herbal tea, which is used for medicinal purposes and consumed with pleasure in our country, as instant tea by spray drying and its effect on quality parameters, especially total phenolic content, were investigated. For this purpose, the mixed herbal tea was extracted in water at different temperatures (70, 80, and 90 °C) and times (5, 10, and 15 minutes). The optimum extraction conditions were determined by considering the temperature-time combination that yielded the highest total phenolic content in the extracted samples. The extract was dried with spray drying by adding maltodextrin as a carrier material and instant herbal tea was obtained. The color, brix and total phenolic content of the teas were determined. Depending on the increase in temperature and time, an increase in L* value and brix was determined in the samples. FTIR spectroscopy was used to determine the effect of the production method on the phenolic compound. The tea sample with the highest total phenolic content (80°C for 15 minutes) was selected and dried in the spray dryer. Instant tea was dissolved in hot water (0.4g/100ml), and color, brix and total phenolic content analysis were also made in this tea. While the color and brix value of instant tea did not change as compared to the extracted tea, it was determined that the total phenolic content increased. The total phenolic content of instant tea was found to be 203.9±11.4 mg GAE/kg whereas that of mixed herbal tea extract was 168.34±4.22 mg GAE/kg. As a result of FTIR spectroscopy, it was determined that there was a change in phenolic content with spray drying in the tea samples. It is thought that this study will contribute to the widespread production of instant mixed herbal tea, rich in phenolic substances, and its practical use as an alternative to commercially produced filtered tea bags.

Keywords: FTIR, instant tea, spray drying, total phenolic content

1. Introduction

People has benefited from plants as a basic source of food and medicine since ancient times. They discovered which parts of the plants should be consumed, determined their poisonous and healing properties through trial and error methods, and not only collected but also cultivated medicinal plants (Baydar 2005). Nowadays, herbal teas are natural products of herbal origin, which are widely consumed due to their positive effects in terms of pleasure and health. Herbal teas have great importance in preventing and treating diseases with their high content of antioxidants such as fat-soluble vitamins A and E, water-soluble vitamin C and phenolic compounds (Ivanova et al., 2005). Herbal teas are mixtures based on properly preparing certain parts of plants to eliminate problems such as indigestion, colds, constipation, diarrhea, fatigue, and insomnia. Herbal teas are prepared by drying the fragrant (aromatic) parts of the roots, stems, shoots, leaves, flowers and seeds of plants and making them suitable for drinking in hot

water (Kaçar 1992). Some other herbs that are consumed by preparing infusions are sage, linden, rosehip, echinacea and clove. Many studies have shown that these plants contain high levels of antioxidant compounds (Zheng and Wang, 2001, Cavlak and Yağmur, 2016). Linden is good for colds and calming the nerves, rosehip is good for colds, sage fights viruses and bacteria and renews the body, and echinacea is used as a healing agent against burns and wounds, stomachache, sore throat and cough (Kamiloğlu Beştepe et al., 2016, Coşke Şenkal 2020).

Generally, herbal tea, obtained from plants sold in neighborhood markets or herbalists open to external influences, is not hygienic. In order to prevent these problems, plants are ground and placed in filter bags to facilitate their use. However, although this method is practical, it is reported that various materials used in the bags have a negative effect on health (Akgül 1993). As with coffee, it is possible to dry such extracts by various methods, such as spray drying, to make instant tea. Spray drying is an older and more widespread technique, whereby the extract is sprayed into hot air after pre-concentration to convert it into solid particles (Loksuwan 2007). In this study, mixed herbal tea was extracted in water at different temperatures (70, 80 and 90 °C) and times (5, 10 and 15 min). The optimum extraction conditions were determined by taking into account the temperature-time combination at which the highest total phenolic matter was obtained in the extracted samples. The extract obtained was dried by spray drying method by adding maltodextrin as carrier material and instant herbal tea was obtained. Chemical analyses were performed on the extracts and instant tea and some quality characteristics of the teas were determined. Furthermore, changes in the phenolic compounds were determined by using Fourier Transform Infrared (FTIR) spectroscopy. This research aimed to widespread production of herbal teas as instant tea instead of filter bags.

2. Materials and Methods

2.1 Material

In this study, Doğadan mixed herbal tea with Echinacea (Echinacea (33%), cinnamon, blackberry leaf, rooibos, clove, red beet, flavorings, (quince, apple), linden, turmeric, stevia, rosehip, vitamin C (1,5%), apple, sage, black pepper) was used and obtained from local markets (Niğde)

Figure 1. Doğadan mixed herbal tea with Echinacea

2.2 Tea Production

2.2.1 Herbal Tea Prepared with Infusion

In this technique, 2 g (1 sachet) of Doğadan mixed herbal tea with Echinacea was added to 200 mL of boiled water and then extracted at different temperatures (70, 80, and 90 °C) and times (5, 10, and 15 minutes).

2.2.2 Herbal Tea Prepared with Spray Dryer

40 g (20 sachets) of natural mixed herbal tea with Echinacea was added into 700 mL of hot water and brewed at 80 °C for 15 minutes and cooled to room temperature. The brix value was increased to 3.2 by adding maltodextrin to the extract with a brix value of 1.5. The obtained aqueous extract was dried in a spray dryer with an air inlet temperature of 200˚C and outlet temperature of 140˚C. Plant powder samples (Figure 2) were taken from the spray dryer chamber and stored in closed glass bottles at +4 ˚C. Powdered tea was dissolved in hot water (0.4 g/100 mL) and physical and chemical analyses were performed in the tea samples.

Figure 2. Instant mixed herbal tea with Echinacea

2.3 Physical and Chemical Analyses

Physical and chemical analyses such as color, total soluble solids and total phenolic content were done in the samples.

2.3.1 Total Soluble Solids Analysis

Total soluble solids (°Bx) of the tea samples were determined using a refractometer (Hanna Refractometer, HI96801). Measurements were carried out at 25 ± 1˚C (Baltacıoğlu 2022).

2.3.2 Color Analysis

Color analysis was performed by measuring (L*, a*, b*) values with Hunter colorimeter (Chroma Meter CR-400 Konica Minolta, Sensing, Inc., Japan). L* value indicates brightness (0-100, black-white), a* value indicates green-red color and b* value indicates blue-yellow color (Baltacıoğlu et al., 2021).

2.3.3 Total Phenolic Content Analysis

The total phenolic content was determined according to the Folin-Ciocaltaeu method. 100 µl of the sample was mixed with 0.75 ml of Folin-Ciocaltaeu solution (10% in water) and then kept at room temperature for 5 minutes. After that 0.75 ml Na_2CO_3 (75 g/L) was added to the mixture. The mixture was kept in a dark environment at room temperature for 1 hour. At the end of the incubation period, the absorbance values of the samples were read in a spectrophotometer at 725nm wavelength. Gallic acid was used as standard and the calibration curve was prepared by repeating the procedure and applied to different concentrations of gallic acid solutions. The total phenolic content results were represented as milligrams of gallic acid equivalent (GAE) per liter (mg GAE/L) (Baltacıoğlu 2022).

2.3.4 FTIR Spectroscopy Analysis

Changes in the phenolic content were determined using Fourier Transform Infrared (FTIR) spectroscopy. The absorption spectra of the samples were obtained in the 400-4000 cm^{-1} region by using FTIR spectroscopy (Bruker, Vertex 70, Germany) with ATR cell in the Central Laboratory of Niğde Ömer Halisdemir University with a resolution of 2 cm^{-1} and 128 scans. Tea samples were lyophilized to prevent interfering water bands with the spectral bands, and the dried samples were examined by FTIR spectroscopy (Baltacıoğlu 2022).

2.4 Statistical Analysis

The data were analyzed using Minitab (Version 17, Minitab Inc., State College, PA, USA) at 95% confidence intervals, and the general linear model was used in the analysis of the data. Tukey's multiple comparison test was conducted to determine the differences between applications. Each experiment was repeated at least three times.

3. Results

Mixed herbal tea with Echinacea was extracted by hot water at 70˚C, 80˚C, 90˚C for 5, 10 and 15 minutes and total soluble solids (°Bx), color and total phenolic content of the samples were determined. Brix analysis results were shown in Table 1.

Table 1. Total soluble solids of the samples (°Bx)

	Temperature (˚C)		
Time (min)	**70**	**80**	**90**
5	**0.26 ± 0.05^{b}**	**0.36 ± 0.05^{b}**	**0.58 ± 0.04^{a}**
10	**0.34 ± 0.09^{b}**	**0.32 ± 0,04^{b}**	**0.64 ± 0.09^{a}**
15	**0.4 ± 0.04^{b}**	**0.38 ± 0.04^{b}**	**0.7 ± 0.04^{a}**

Means that do not share a letter were significantly different

For each extraction time, brix values increased with temperature ($p \leq 0.05$). In the brix analysis, the best result was determined as 15 minutes at 90˚C. The results of color analysis obtained at different extraction conditions in mixed herbal tea with Echinacea were shown in Table 2.

Table 2. Results of color analysis

Temperature			
	70˚C		
Time (min)	**L***	**a***	**b***
5	**21.49 ± 0.03^{abc}**	**0.12 ± 0.04^{a}**	**2.49 ± 0.03^{a}**
10	**21.45 ± 0.01^{bc}**	**0.12 ± 0.06^{a}**	**2.38 ± 0.03^{ab}**
15	**21.36 ± 0.03^{bc}**	**0.09 ± 0.06^{a}**	**2.31 ± 0.03^{abc}**
	80˚C		
	L*	**a***	**b***
5	**21.63 ± 0.05^{ab}**	**0.03 ± 0.05^{a}**	**2.40 ± 0.03^{ab}**
10	**21.30 ± 0.06^{c}**	**0.06 ± 0.09^{a}**	**2.24 ± 0.03^{abc}**
15	**21.34 ± 0.02^{bc}**	**0.11 ± 0.02^{a}**	**2.15 ± 0.04^{abc}**
	90˚C		
	L*	**a***	**b***
5	**21.79 ± 0.32^{a}**	**-0.34 ± 0.33^{b}**	**1.95 ± 0.37^{c}**
10	**21.62 ± 0.05^{abc}**	**-0.05 ± 0.06^{ab}**	**2.20 ± 0.06^{abc}**
15	**21.64 ± 0.02^{ab}**	**-0.07 ± 0.04^{ab}**	**2.13 ± 0.02^{bc}**

Means that do not share a letter were significantly different

According to the results of the color analysis, the L* value increased depending on the increase in the extraction temperature at the extraction times of 5, 10, and 15 minutes ($p \leq 0.05$). When the color values were examined at 90°C, higher L* values were observed compared to the others, so a darker extract was obtained. At the extraction times of 5, 10, and 15 minutes, a* value decreased depending on the increase in extraction temperature ($p \leq 0.05$). Likewise, it was observed that the b* value decreased ($p \leq 0.05$) so yellowness decreased.

The amounts of total phenolic content determined in the samples as a result of extraction at different temperatures and times were shown in Table 3.

Table 3. Total phenolic content of tea samples extracted at different temperatures and times

	Total Phenolic content (mg GAE/L)		
	70°C	**80°C**	**90°C**
5 dak.	**120.42 ± 1.38^{e}**	**132.01 ± 2.77^{de}**	**95.83 ± 5.17^{f}**

10 dak.	**137.43 ± 2.14^{cd}**	**149.31 ± 6.85^{bc}**	**130.13 ± 5.86^{de}**
15 dak.	**148.20 ± 8.48^{bc}**	**168.34 ± 4.22^{a}**	**162.55 ± 8.48^{ab}**

Means that do not share a letter were significantly different

As the time for each extraction temperature increased, the total phenolic content increased ($p \leq 0.05$). The total phenolic contents of the samples extracted at 70˚C and 90˚C were lower when compared to that extracted at 80˚C. The best result was determined as 15 minutes at 80˚C so this sample was preferred for the production of instant tea. Tea was reconstituted at a rate of 0.4 g/100 ml and the color, brix, and total phenolic content of the sample prepared from instant tea was determined.

The brix analysis of instant tea was found as 0.33±0.15. This value was close to the brix value of the mixed herbal tea extract which was found as 0.38±0.04. Instant tea color values were found to be 24.2±0.13 for L*, -0.075±0.10 for a*, and 1.7±0.06 for b*. Compared to mixed herbal tea extract color values an increase in L* and a* values were observed. The reason for this increase was thought to be the high temperature applied in the spray drying method. The total phenolic content of instant tea was found to be 203.9±11.4 mg GAE/L which was higher than that of mixed herbal tea. FTIR spectra of teas obtained by different methods were shown in Figure 3.

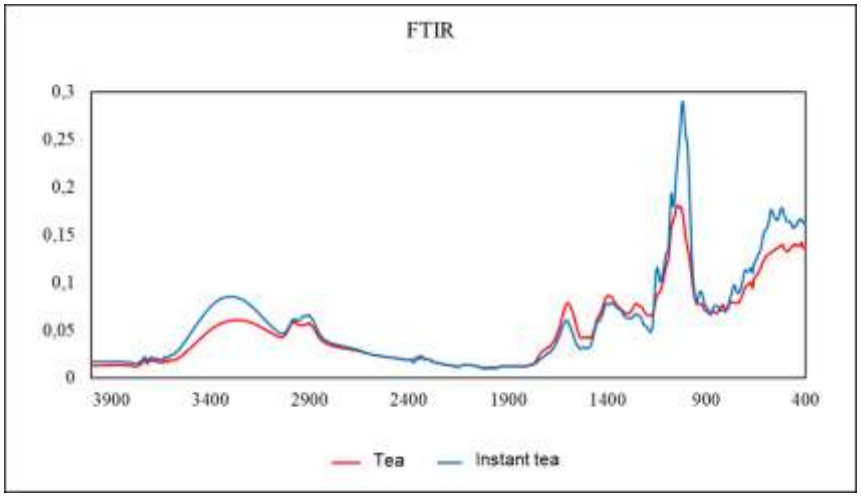

Figure 3. FTIR spectra of tea samples obtained by different methods

It was observed that the FTIR spectrum of the instant tea differed from the tea extracted with hot water. It can be said that spray drying caused an increase in phenolic compounds.

4. Discussion

In this study, instant tea was obtained by using the spray dryer from mixed herbal tea, and some physical and chemical properties were determined. As a result of these analyses, an increase in the total phenolic content and color values was observed, and a visible increase in phenolic compounds was identified as a result of FTIR spectroscopy in instant tea. Similarly, It is thought that the reason for the observed increase in the color value is the high temperature applied in

the spray drying method. Likewise, an increase in color values was observed in the samples obtained by spray drying in sage and mountain tea samples (Dinçer 2007). Similarly, an increase in total phenolic content was observed in instant tea produced from mint and thyme compared to tea obtained according to classical brewing (Akşit 2013).

5. Conclusion

It is possible to make instant tea from mixed herbal tea, which is widely consumed due to its positive effects in terms of health, by spray drying method as in coffee. According to the results, the total phenolic content of instant tea produced using the spray drying method, increased. Moreover, this change was observed from FTIR results. While no significant difference was observed in the brix values of tea products, an increase was observed in the color values. This study is thought to contribute to the practical use of instant tea as an alternative to commercially produced filtered tea bags for the production of phenolic-rich instant mixed herbal tea.

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Investigation of the Effect of a Benzimidazole Compound on Prostate Cancer Cell Line

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Abstract:

Prostate cancer, which is an important health problem worldwide, is one of the most common cancer types in men. Chemotherapy, which is one of the current methods used in cancer treatment, provides limited benefit for metastatic prostate cancer and therefore new agents that can be developed for more effective treatments are needed.

The benzimidazole core is a heterocyclic compound and is widely used in biological applications. It is among the important pharmacophores in medicinal chemistry. Benzimidazole derivative compounds play an important role in the development of many therapeutic anticancer drugs through different mechanisms of action.

In this study, a benzimidazole derivative compound was synthesized in order to obtain an anti-prostate cancer drug, and it was tested on PC3 cell line (a highly invasive human prostate cancer cell line) using MTT method. It was determined that the related compound exhibited anti-proliferative activity in the metastatic cell line. In future studies, it is aimed to comprehensively evaluate the metabolic effects of the synthesized structure as an effective anti-cancer agent candidate against prostate cancer.

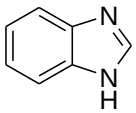
Keywords: Anticancer, benzimidazole, cytotoxic activity, prostate cancer.

1. Introduction

Prostate cancer is the second most common type of cancer in men worldwide and is one of the leading causes of cancer-related deaths (Kim et al., 2023). The vast majority of these cases are

diagnosed in men aged 65 and over. Some of the prostate cancer risk factors are age, genetics, hormones, diet, chemicals, pathology and clinical features (Waldron et al., 2023). Diagnosing prostate cancer in its early stages can make it easier to treat, while in its later stages, recurrence or metastatic prostate cancer occurs, making it somewhat more difficult to treat (McPhaul, 2008). Optional treatments for prostate cancer today include types of treatment such as surgery, radiotherapy, chemotherapy, and hormone therapy. Doxorubicin, paclitaxel and cisplatin are some important chemotherapeutic drugs that are widely used in the treatment of this cancer (Sawpari et al., 2023). There is an increasing demand and need for more effective alternative treatment modalities to the current chemotherapy treatment with limited benefit for metastatic prostate cancer.

The benzimidazole core is a heterocyclic compound that consists of a benzene ring fused with imidazole at the 4- and 5-positions of the imidazole ring and is widely used in biological applications (Satija et al., 2021). The open structure of benzimidazole is given in Figure 1.



1a

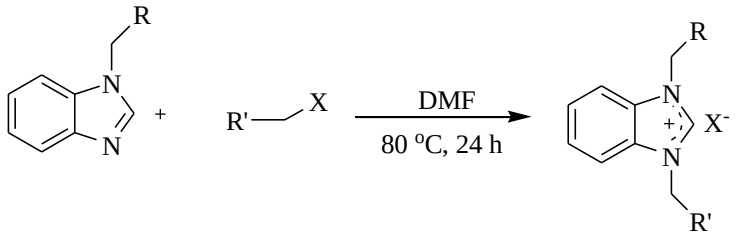
Fig 1. Benzimidazole core

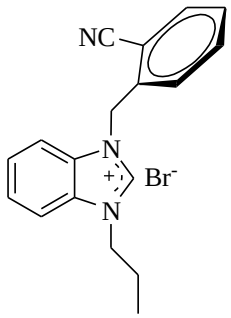
Benzimidazole derivative compounds play an important role in the development of many therapeutic anticancer drugs through different mechanisms of action (Feng et al., 2022). In the study, a benzimidazole derivative anticancer candidate compound aimed to show strong therapeutic activity against prostate cancer cells was synthesized and the cytotoxic activity of this compound on PC3 prostate cancer cell line was tested.

2. Materials and Methods

2.1. Synthesis of benzimidazolium salt of 1-(2-cyanobenzyl)-3-propyl-1*H*-benzo[*d*]imidazole-3-ium bromide

The benzimidazolium salt compound 1-(2-cyanobenzyl)-3-propyl-1*H*-benzo[*d*]imidazol-3-ium bromide was prepared in 51% yield by sequential reaction of N-alkylbenzimidazole with various alkyl or aryl halides in DMF (Fig 2).





Product 2a

Figure 2. Structure of the compound 1-(2-cyanobenzyl)-3-propyl-1*H*-benzo[*d*]imidazole-3-ium bromide

2.2. Cell Culture

Human prostate adenocarcinoma cell line (PC3) (ATCC® CRL-1435™) was purchased from ATCC (USA). Penicillin-streptomycin were purchased from Sigma-Aldrich Chemie GmbH. RPMI medium was purchased from TermoFisher Scientific. Fetal Bovine Serum (FBS) was taken from PAN Biotech (South America). GlutamaxTM-1 (100X) was purchased from Gibco by Life Technologies. Trypsin-EDTA was bought from Wisent Inc. Phosphate Buffered Saline (PBS) was bought from VWR life science. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dye were purchased from BioFrox (Germany).

2.3. Cytotoxic Activity Studies of Compound

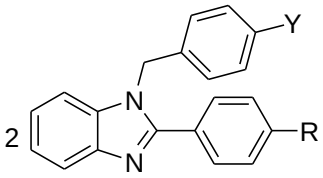
The cells were cultured in RPMI supplemented with 10% FBS, 1% GlutaMAX, and 1% penicillin-streptomycin. The cells were seeded into sterile 96-well plates at a density of 5 x 10^3 cells/well. Plates were incubated for 24 h. Following this, medium in each well was removed, and the cells were treated with the compound at six different concentrations (5, 10, 20, 50, 100 and 200 µM). The 96-well plates were incubated for 48 h. After the incubation completed, the medium was carefully aspirated and the MTT stock solution (5 mg/mL, 50 µL) was added to each well. The plates were incubated for 2 h. Then, 200 µL DMSO was added into each well and the plates were mixed on a rocker for 30 minutes. Promega reader device was used for measuring the absorbance values at 560 nm. The GraphPad Prism 5 software was used for calculating IC_{50} values.

3. Results and Discussion

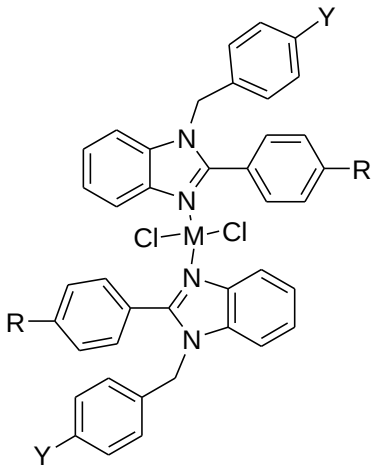
The cytotoxic activity study of compound was done against PC3 human cell line. The effects of compound on PC3 were investigated. Compound was found to be effective in prostate cancer cell line with IC_{50} value of 75 µM. The % survival rates of PC3 prostate cancer cells were calculated by the MTT cytotoxicity test. Cell viability of 200 µg/ml, 100 µg/ml, 50 µg/ml, 20 µg/ml, 10 µg/ml and 5 µg/ml the compound treated cells were obtained as 31%, 44%, 48%, 91%, 91% and 93%, respectively. It was determined that 50, 100 and 200 µg/ml of compound had only an anti-proliferative effect on PC3 cancer cells.

The cytotoxic activity of the same compound on human colon cancer (DLD-1) and human breast cancer (MDA-MB-231) cells was evaluated, with IC_{50} values >200 µM for DLD-1 and 120.96 µM for MDA-MB-231 (Akkoc et al., 2019). Some studies on the effectiveness of different benzimidazole compounds on prostate cancer cells are also given below.

Yılmaz et al. synthesized benzimidazole Co (II) or Zn (II) compounds and ligands containing 1-benzyl and 2-phenyl and measured cytotoxic activity with MTT in DU-145 human prostate cancer cell line. They found that compounds 3e, 4a and 4r showed higher cytotoxic activity against DU-145 compared to docetaxel (Fig 3). It was predicted that chloride and styrene in the structures.



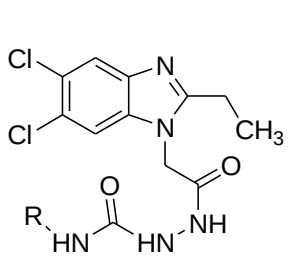
	R	Y
3a:	H	Cl
3b:	H	Br
3c:	H	Me
3d:	H	Ph
3e:	H	Styryl
3f:	Cl	Cl
3g:	Cl	Me
3h:	H	H
3i:	Cl	H



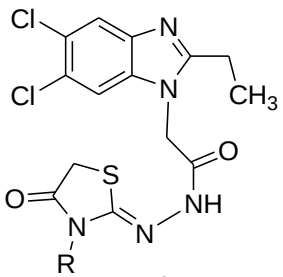
	R	Y	M
4a:	H	Cl	Zn
4b:	H	Cl	Co
4c:	H	Br	Zn
4d :	H	Br	Co
4e:	H	Me	Zn
4f:	H	Me	Co
4g:	H	Ph	Zn
4h:	H	Ph	Co
4i:	H	Styryl	Zn
4j:	H	Styryl	Co
4k:	Cl	Cl	Zn
4l:	Cl	Cl	Co
4m:	Cl	Me	Zn
4n:	Cl	Me	Co
4o:	H	H	Zn
4ö:	H	H	Co
4p:	Cl	H	Zn
4r:	H	Cl	Co

Figure 3. The open structure of synthesized compounds.

İlhan et al. synthesized benzimidazole-based compounds and determined their possible cytotoxic effects on PC3 prostate cancer cells using MTT assay. They found that 5a and 5b derivative compounds showed higher cytotoxic activity and it was reported that the highest effect was seen after 72 hours of treatment (Fig 4). The synthesized compounds were found to be active against cancer cells in the range of 25.2-88.2 μM.



5a



5b

Fig 4. Benzimidazole-based compounds.

Rashid et al. synthesized benzidazole derivatives based on the heterocyclic nucleus of Bendemustine and evaluated their cytotoxic effects on various cancer cell lines. In particular, they found that the compound 6a, which structure consisted of a methylene linker combining benzimidazole and oxadiazole, had a cytotoxic effect on leukemia, melanoma, ovarian, breast, prostate (PC3 and DU-145), colon and lung cancer cells (Fig 5).

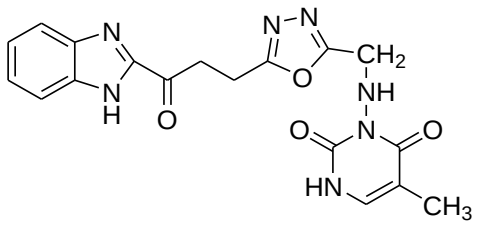
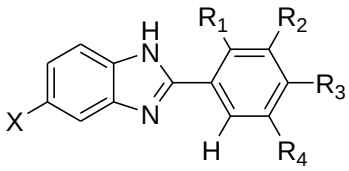


Figure 5. The open structure of compound 6a.

Huynh et al. synthesized compounds including benzimidazole core and determined their cytotoxic activities on lung (A549), breast (MDA-MB-231) and prostate (PC3) cancer cell lines using MTT assay. Compounds, especially 7a and 7b, showed significant anticancer activity in PC3 cell lines (Fig 6). They reported that the presence of electron donating groups (OH, OMe, $–NMe_2$, $–OCH_2C_6H_5$) increases the anticancer activity, while the presence of electron withdrawing groups ($–NO_2$, $–CF_3$) in the phenyl ring decreases the inhibitory ability.



	X	R_1	R_2	R_3	R_4
7a:	CH_3	H	OCH_3	OCH_3	OCH_3
7b::	CH_3	H	H	$N(CH_3)_2$	H

Figure 6. The open structure of two benzimidazole-based compounds.

As seen in the above-mentioned sample studies, it has been reported that new therapeutic-scale structures created by synthesis exhibit different activities against prostate cancer cells compared to the traditional drugs used, and that these activities are shaped according to the functional groups to which they are attached. The derivatives formed by the inclusion of various

substituents, especially in positions 1, 2 and 5 of the benzimidazole ring, exhibit pharmacological effects by taking part in various chemical and biological processes (Wadhawa et al., 2018).

5. Conclusion

With the aim of developing effective, safe, and affordable anticancer drug candidates against prostate cancer cell lines, benzimidazolium salt were designed, synthesized structurally in this study. Due to the antiproliferative effect of the synthesized compound, it is predicted that it may benefit in the design of new compounds as anticancer agents in the future.

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HORSE EVALUATION FORM TO BE USED IN HIPPOTHERAPY APPLICATIONS [#]

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Abstract:

The evaluation form of the horses used and to be used in hippotherapy application centers in private and public institutions in Turkey has been prepared with the aim of contributing to the horse trainers, health experts, veterinarians, working team in order to contribute to the healthy, safe and efficient application sessions of the disabled individual and the individual in need, and to provide direction to the academic studies that can be done with the hippotherapy horse in the future. It is known that hippotherapy is beneficial for disabled individuals and individuals in need in the world with the academic studies and the positive results of the sessions. However, positive and successful results can be achieved through the appropriate use of horses in sessions. In recent years in Turkey, there has been a noticeable increase in the opening and implementation of hippotherapy centers in private and public institutions. With the increase in the need for horses in hippotherapy application sessions with these increases, the importance of the horse to be used is through the correct horse selection and determination of its suitability. Choosing the right horse and determining its suitability can increase the efficiency of horse trainers and the working team working in hippotherapy practice centers and minimize the risks that may arise. This study was prepared as a result of field researches within the framework of the Hippotherapy Turkey project, which was accepted by the Delegation of the European Union to Turkey, with the standards determined in terms of follow-up and monitoring of the hippotherapy horse evaluation form to be used in the sessions of the horse trainers and the work team working and to be worked in the hippotherapy application centers in private and public institutions in Turkey.

Keywords: horse, trainer, hippotherapy, health, evaluation form

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1. Introduction

Horses have had an important place in life from the first time they were domesticated until today. They have made a positive contribution by being with people and have always been involved in useful work. It is known that horses have always contributed to people in the fields

of transportation, communication and transportation, and have increased people's love, loyalty and friendship towards them. Not only has the horse always been a symbol of trust and friendship in societies, but it has also represented wealth and nobility due to its majesty. The association of humanity with the horse has always had a trusting and calming effect on humans with its calm structure and energy (Ünver, 2006). Ensuring the unity of the horse with human begins with the establishment of a bond of love with the horse. Activities to be carried out with horses make today's people more attached to themselves with the formation of this bond of love. With the attachment of the human to the horse, spiritual and physical changes are seen in the human beings. These changes alone cause the horse and rider trainer to open a new window in the social life of the person who wants to learn horsemanship by improving his/her perspective on life with physical activities and positive thoughts applied on and with the horse through the appropriate horse selection. Horse coaching is the art of using a healthy and suitable horse in a good condition, and this good condition is the ability to manage the horse in the right place, to use it in a healthy and efficient way by managing the horse in the right place, in calmness, at the appropriate time, in confidence and with as little force as possible (Temurlenk, 2016). For this reason, since the selection of the right horse to be used by the horse trainer in the field of hippotherapy and determining its suitability can contribute positively to the development of disabled individuals and individuals in need, this article aims to create an evaluation form for the horse to be used in hippotherapy.

Hippotherapy Concept

Hippotherapy is practiced by occupational therapists, physical therapists and speech therapists trained in the use of horse movements to facilitate the development of disabled individuals and individuals in need. Its aim is to improve balance, coordination, posture, fine motor control, articulation and cognitive skills (Anonymous,1). Hippotherapy is a form of physiotherapy used to maintain or improve various physical qualities and characteristics such as strength, balance, agility, coordination and walking ability of various patient groups. It is a form of treatment that works well in adults and children with physical or mental disabilities (Johnson, 2009; Champagne, 2010). Animal Assisted Therapy is a form of supportive treatment applied by utilizing human and animal interaction in order to support the quality of life in individuals with chronic diseases and to help in the treatment of disorders that negatively affect mental health (Cevizci et al., 2009).

Terms Used During Hippotherapy Sessions

In order to achieve team integrity and successful work in hippotherapy application sessions, it is important for horse trainers, specialist health professionals, horse handlers, side walkers and veterinarians to know the following hippotherapy terms in multidisciplinary studies.

Coat Colors: The naming of the hairs that cover the horse's body.

Curl/tendril: The hair of horses to turn around and form a trace.

Dispatch: Taking a horse from one place to another.

Facial Markings: The white spots on the forehead of the horse's head.

Gelding: A male horse that cannot reproduce and is neutered.

Halter/bridle: It is a material made of leather or rope that is worn on the head of the horse to take it from place to place.

Horse Age: The birth year of the foals born between 01 January and 31 December in a calendar year is accepted as the birth year of that year.

Horse Holder: She is the person responsible for the care and equipping of the therapy horse, preparing the horse for the practices, and controlling the horse during the mounting , boarding and session (Anonymous,2).

Leg Markings: The white spots on the feet of horses.

Longe: Turning the horse around the circle by the trainer.

Mare: Female of the horse.

Pad: It is the material placed under the saddle, which is made of sheep's hair, which is placed on the spine and back area for the health of the horse in training applications.

Paddock: It is a fenced area where horses run freely, rest and graze.

Passport: Horse's identity card. All information is written in this document.

Race: Indicates which country the horse belongs to.

Ramp: It is a platform that helps people with disabilities and people in need get on a horse.

Rhythmical: Successive strides of equal length.

Rope: The thick rope used to take the horse from one place to another.

Saddle: It is a specially made equipment made of leather material on which the rider sits in order to practice training with the horse.
Saddle pad: It is the material that is placed under the saddle, which is made of fabric that is placed on the spine and back area for the health of the horse in training applications, and consists of a soft fabric containing fiber.

Session: It is the start and end time of the application training.

Side walker: It is the person who prepares the horse, helps the disabled and/or the person with special needs, and ensures the safety of the patient.

Stable: It is the place where horses collectively stay.

Stableman: Person who provides the vital care and nutrition of the horses.

Stall: The place where horses stay in a single chamber.
Stall Weaving: The constant movement of the horse's neck from right to left in its stall.

Stallion: A male horse that is capable of breeding and has not been neutered.

Stand: The position which four legs of the horse remain without a fixed movement.

Swallowing air: The continuous swallowing of air in the mouth of the horse by holding it anywhere with its mouth.

Tameness: It is the horse's performing the action by following the commands as desired or undesirable behavioral.

Tendon: Fibrous connective tissue that attaches muscle fibers to bone or other structure so that the horse can move its feet (Anonymous,3).

Trot: It is a light jogging gait, which is one of the natural gaits of the horse. It is a two-stroke form of walking.

Upper girth strap: It is a material made of leather or rope, which is used by wrapping it around the wither area in case the horse is operated bareback during training.

Voice assist: It is the sound of the trainer used to communicate with the horse (oho, stop, tsk tsk, get out)

Walk: It is the slowest natural gait of the horse. It is a four-stroke form of walking.

Withers: It is where the bony prominence of the horse's neck begins. This is where the horse's height is measured (Ün, A.E. ve Akbenli, F. 2020, Ün 2021, Ün 2023, Anonymous,4).

Horse Features to be Used in Hippotherapy Application Sessions

Although the horse breeds to be used in equine therapy vary according to the primary purpose of the therapy and the needs of the people to be treated, they are generally preferred according to their morphological and physiological characteristics. There are some criteria for the selection of horses to be used for hippotherapy. It is possible to list these criteria as temperament, body structure, walking and running types, health, age, special education and care (Köseman & Şeker, 2015a).Ciesla (2007) stated in his study that the horse to be used in therapy should be determined correctly and that the psychological predispositions, health status, gender and breed of therapy horses are important. In a study conducted on 34 different horses in hippotherapy training activities in five different rehabilitation centers in Poland, Ciesla stated that 71% of the therapy horses were geldings and 29% were mares, the average age of the horses was 8.4 years, the youngest was 4 years old and the oldest was 20 years old, and the shoulder heights ranged between 130 cm and 167 cm. Effective hippotherapy training sessions and contributing to the treatment of the individual can only be realized by choosing the right hippotherapy horse. Choosing the right hippotherapy horse causes the sessions to progress in a safe and healthy way. For the continuity of these processes, it is aimed to contribute more to the therapy by continuously training the horse by the hippotherapy horse trainer. Horses are extremely sensitive creatures, no matter how large they are. It increases its attention in the slightest changes that may occur in its environment and positions its movements accordingly. For this reason, the training materials to be used in hippotherapy sessions should not be suddenly presented to the horse. Before starting the hippotherapy session, it is necessary to teach the training materials that may be encountered at least one week before the hippotherapy session and to remind them again before the session starts. When the general characteristics of the therapies are examined, it is possible to list them as follows (Ün, 2020; Ciesla, 2007);

- According to the rules of the International Equestrian Federation (FEI) and ethical rules, it must be healthy in activities to be carried out with the horse,
- The horse's behavior should be normal, always close to the human, calm and harmonious in its stable, paddock and training area,
- For better communication with the individual, the horse's height should be 140 cm-160 cm,
- The horse's body proportion, that is, the head, neck, torso and leg areas should be proportional,
- The horse's hoof angles and sizes should be at equal angles and smooth, and it should move at a constant speed by taking its steps one by one at equal lengths,
- There should be no congenital or acquired defect in the anatomical structure that restricts movement,
- Always be open to communication with people and have a tendency to obey,
- Must be insensitive to environmental factors that may occur during training (wind, sudden movements, loud noises, different objects, etc.). They should be predisposed to learn what is taught and have a positive attitude towards new training programs,
- They must trust and obey the coach or the team working with him/her and be constantly involved in training,
- The horse to be used must be 7 years of age or older, have received basic training, and have passed the training stages positively through the controls of an experienced horse trainer.

The choice of horse to be used in hippotherapy applications can be from any breed, but if the horse meets the above-mentioned conditions, efficiency can be obtained from hippotherapy

applications. It has been emphasized that it is difficult to objectively determine the suitability of horses for therapeutic riding programs in terms of temperament and reactivity due to many different character traits (Anderson et al 1999). Characteristics of a therapy horse include having a calm, tolerant and harmonious temperament and not being reactive to new stimuli (Anderson et al. 1999; Gasalberti 2006).

Table 1. Behavioural Characteristics (French 1993; Anderson ve ark. 1999)

Davranış Karakteri(behavior character)		**Davranış Karakteri(behavior character)**	
Dignified	**Energetic, playful**	**Submissive against other horses**	**Dominant**
Mellow	**Timid**	**Compatible**	**Grumpy**
Calm	**Tense, Nervous**	**Reliable**	**Unreliable**
Tame	**Irritable**	**Cooperative**	**Uncooperative**
In favor with Human Contact	**Avoding**	**Brave**	**Easily Timid**
Friendly	**Combative**	**Approaching in barn**	**Avoding in Barn**
Patient	**Impatient**	**Sociable with other horses**	**Insociable with other hoses**
Gentle	**Negligent**	**Consistant, stable**	**Unstable**
Obedient	**Disobedient**	**Submissive to humans**	**Dominant**
Calm, Quiet	**Retless**	**Tolerant**	**Intolerant**

Horse Equipment in Hippotherapy Practice Sessions

Before the start of the hippotherapy application session, the horse must have a halter (leash) attached to the head for the horse's management and management, and a rope to guide it. With the recommendation of the specialist health personnel and horse/riding trainer, one or more of the materials such as saddle, upper girth, voltej girth, saddle pad, felt, sheepskin can be used on the horse before and during the session depending on the condition of the individual (Ün, 2020-2021-2023).

2. Materials and Methods

In parallel with the aim of the research, this research was conducted with the survey research model, which is one of the quantitative research methods. The suitability and evaluation form of the horses used in private and public institutions conducting hippotherapy practice sessions will be composed of three main parts. Although previous academic researches (Anderson et al., 1999; Ciesla, 2007; Sterba, 2007; Köseman and Şeker, 2015a; Köseman and Şeker, 2015b; 2015; Koca and Ataseven, Ün,2020; Anonymous,4; Anonymous,5) will form the basis of the form, the evaluation form will be revealed with the participation of research field experts.

Population and Sample

The population of the study consists of those who provide hippotherapy practice services in private and public institutions in Turkey. In the study, the "complete census method" was used

to reach the entire population, which is limited in number. The sample of the study consisted of 103 private and public institutions providing hippotherapy practice activities in private and public institutions in Turkey.

Data Collection and Analysis

Data were collected by using structured interview technique over a period of approximately 1 week by calling 103 facilities that make up the sample one by one by phone. In order to ensure content validity, the interview question was prepared based on the opinions of experts serving in the field and the relevant literature and the data collected were analyzed by the researchers (Anderson et al., 1999; Ciesla, 2007; Sterba, 2007; Köseman & Şeker, 2015a; Köseman & Şeker, 2015b; Koca & Ataseven, 2015; Ün, 2020-2022-2023; Anonymous, 4; Anonymous, 5).

Validity and Reliability

For the validity and reliability of the research in content analysis, attention is drawn to reaching the same answer by asking the same question by different researchers in repeat studies to be conducted with the same tool and the same participants (Arastaman et al., 2018; Koçak & Arun, 2006).

3. Results

When it was analyzed whether the horses of 103 public and private institutions providing hippotherapy applications had a hippotherapy certificate of conformity, it was detected that 98.05% (n=101) did not have a certificate of conformity and 1.94% (n=2) had a certificate of conformity. The density of public and private institutions that do not have a certificate of conformity for hippotherapy horses draws attention. It also is known that there is no certain standardization in the institutions that have a certificate of conformity for hippotherapy. For this reason, the certificate of conformity of the horse to be used in hippotherapy was prepared by us hippotherapy practitioners, and the conformity form of the horse used or to be used in hippotherapy applications of public and private institutions was created. The conformity form of the horse to be used in hippotherapy was put forward in the light of literature information and as a result of interviews with hippotherapy field practitioners.

Table 2. Suitability of the Horse Used in Hippotherapy in Public and Private Institutions in Türkiye

CURRENT STATUS IN TURKEY				**PUBLIC INSTITUTIONS**				**PRIVATE INSTITUTIONS**			
Holding a Conformity Certificate		**Not Holding a Conformity Certificate**		**Holding a Conformity Certificate**		**Not Holding a Conformity Certificate**		**Holding a Conformity Certificate**		**Not Holding a Conformity Certificate**	
n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
2	**1,94**	**101**	**98,05**	**2**	**6,25**	**30**	**93,75**	**0**	**0**	**71**	**100**

4. Discussion

The horse evaluation eligibility form document to be used in hippotherapy was created under three main sections. These are respectively;
Section 1: General information on the hippotherapy horse,
Section 2: Hippotherapy horse health assessment information,
Section 3: Hippotherapy horse evaluation information.

The contents of the items in the sections are explained one by one below. After the section and section descriptions are completed, the suitability form of the horse to be used in Hippotherapy will be completed.
The first chapter covers general information about the hippotherapy horse. The section is composed of ten sub-headings. It is the section containing personal information of the horse. The section created in Table 1 is shown with a diagram. The section is in order;
First subheading: Name of the horse
The horse must have a name and it is required for both communication and records.
Second subheading: Year of birth
The birth year of the horse should be known accordingly for health practices and training stages.
Third subheading: Mother's name
The horse's mother's name must be known for production and records.
Fourth subheading: Father's name
The father name of the horse must be known for production and records.
Fifth subheading: Breed
Horse breeds should be known and should be preferred in production and applications according to their breed characteristics.
Sixth sub-heading: Donu
It is necessary when communicating with the veterinarian when describing the horse's frosts should be known.
Seventh subheading: Passport number
It is the horse's identity card. It is required for health information and transportation of horses.
Eighth subheading: Jeep number
Identification of the horse and access to its information is necessary to determine which center it belongs to.
Ninth subheading: The center or club
The place name of the private or public institution where the horse is located.
Tenth subtitle: Description of the horse
To describe the horse, it is the area where the flowing, seki and cypresses are indicated.

Table 1. General Information on Hippotherapy Horse

1. GENERAL INFORMATION ON HIPPOTHERAPY HORSE			
1.1	**NAME**		**1.10 DESCRIPTION OF THE HORSE**
1.2	**YEAR OF BIRTH**		
1.3	**MOTHER'S NAME**		
1.4	**FATHER'S NAME**		
1.5	**BREED**		
1.6	**COAT COLOR**		
1.7	**PASSPORT NO**		

1.8	**CHIP NUMBER**		
1.9	**NAME OF THE CENTER/CLUB**		

The second section covers hippotherapy horse health assessment information. The section is composed of 19 subheadings. It is the section with the health information of the horse. The section created in Table 2 is shown with a diagram. Section order;

First sub-heading: General condition of the horse

It is the determination that the horse should be healthy no matter what service it performs according to animal welfare and international equine federation regulations.

Second sub-heading: Head and neck area

Since the health of the horse's head and neck region will affect the gait and rhythmic movements, it is the determination of the proportion ratio.

Third sub-heading: Body region

Since the health of the horse's body region will affect the gait and rhythmic movements, it is the determination of the proportion ratio.

Fourth subheading: Legs region

Since the health of the horse's leg region will affect the gait and rhythmic movements, it is the determination of the proportion ratio.

Fifth sub-heading: Health status of the fetlock

For the health and efficiency of the horse, if there is a visible swelling and sore in the jowl area, pain when you touch your hand, and there is a reaction restlessness in the horse, it is not healthy. If there is no evidence of what has been said, it is determined that the horse can do its duty.

Sixth sub-heading: Back health condition

For the health and efficiency of the horse, if there is a visible swelling and wound in the back area, pain when you touch your hand, and there is a reaction restlessness in the horse, it is not healthy. If there is no indication from what has been said, it is determined that the horse can do its duty.

Seventh sub-heading: Waist health status

For the health and efficiency of the horse, it is not healthy if there is a visible swelling and wound in the lumbar region, pain when you touch your hand, and restlessness in the horse's reaction. If there is no evidence of what is said, the horse can do its duty.

Eighth sub-heading: Tendon health status

For the health and efficiency of the horse, it is not healthy if there is a visible swelling and wound in the tendon area, heat pain when you touch your hand, and restlessness in the horse's reaction. If there is no evidence of what is said, it is determined that the horse can do its duty.

Ninth sub-heading: Hoof health status

For the health and efficiency of the horse, if there is a visible crack, fracture and wound in the hoof, a change in temperature when you touch your hand, if there is an odor in the nail yeast, it is not healthy, or if there is no sign, it is determined that the horse can do its duty.

Tenth sub-heading: Vaccination status

It is the determination of the vaccines made in the horse's passport with barcode numbers affixed and signed by the veterinarian.

Eleventh sub-heading: Parasite application status

Parasite treatments written in the horse's passport and signed by the veterinarian.

Twelfth sub-heading: Body temperature status

It is the instantaneous measurement of the horse in the stall and the determination of normal if the temperature is within the range of ..... degrees and not normal if it is above ..... degrees.

Thirteenth subheading: Respiratory rate status

It is the instantaneous measurement of the horse in the stall and the determination that if the respiratory rate is at ..... per minute, it is normal, and if it is above ....., it is not normal.
Fourteenth subheading: Pulse rate status
It is the instantaneous measurement of the horse in the stalland the determination that if the pulse rate is at ..... per minute, it is normal, if it is above ..... it is not normal.
Fifteenth sub-heading: Weight status
If the horse's weight ..... is normal ..... if it is above normal, it is determined that it is not normal.
Sixteenth subheading: Height
If the horse's height is above ....., it is determined to be unsuitable.
Seventeenth sub-heading: Age status
It is the determination that the horse is unsuitable if the age ..... is suitable..... above.
Eighteenth sub-heading: Breed status
This is the field where the horse's breed is written.
Nineteenth subheading: Gender status
The gender of the horse ..... mare castrated ones are suitable, ... stallion ones are not suitable.

Table 2. Hippotherapy Horse Health Assessment Information

2. HIPPOTHERAPY HORSE HEALTH ASSESSMENT INFORMATION							
2.1	**GENERAL CONDITION OF THE HORSE**		**HEALTHY**		**UNHEALTHY**		
2.2	**HEAD AND NECK REGION**		**SUITABLE**		**UNSUITABLE**		
2.3	**BODY REGION**		**SUITABLE**		**UNSUITABLE**		
2.4	**LEGS AREA**		**SUITABLE**		**UNSUITABLE**		
2.5	**CIDAGO HEALTH STATUS**		**NO PAIN**		**PAIN**		
2.6	**BACK HEALTH STATUS**		**NO PAIN**		**PAIN**		
2.7	**LUMBAR HEALTH STATUS**		**NO PAIN**		**PAIN**		
2.8	**TENDON HEALTH STATUS**		**NO PAIN**		**PAIN**		
2.9	**NAIL HEALTH STATUS**		**HEALTHY**		**UNHEALTHY**		
2.10	**VACCINATION STATUS**		**YES**		**NO**		
2.11	**PARASITE APPLICATION STATUS**		**YES**		**NO**		
2.12	**BODY TEMPERATURE S**		**NORMAL**		**NOT NORMAL**		
2.13	**RESPIRATORY RATE**		**NORMAL**		**NOT NORMAL**		
2.14	**PULSE RATE**		**NORMAL**		**NOT NORMAL**		
2.15	**WEIGHT**		**NORMAL**		**NOT NORMAL**		
2.16	**HEIGHT**		**SUITABLE**		**UNSUITABLE**		
2.17	**AGE**		**SUITABLE**		**UNSUITABLE**		
2.18	**BREED**		**DOMESTIC PONY**		**EUROPEAN PONY**		**OTHER**

2.19	GENDER		SUITABLE		UNSUITABLE		
			MARE		GELDING		STALLION

The third part of the hippotherapy horse evaluation information includes information about the horse. Hippotherapy horse evaluation information is composed of five parts. The first part is the evaluation of the horse's stall status, the second part is the evaluation of the horse's paddock status, the third part is the evaluation of the horse's training status without rider, the fourth part is the evaluation of the horse's training status with rider, and the fifth part is the evaluation of the horse's training status during the practice session. The evaluation of the sections consists of 113 sub-headings in total: first section first part 10, second section first part 19, third section first part 18, third section second part 10, third section third part 21, third section fourth part 13, third section fifth part 22.

The descriptions of the sub-headings of the sections are explained in detail in order for the users to progress easily in the creation of the sub-headings of the section sections. In the first ranking of hippotherapy horse evaluation information, the third section, the first part, the evaluation of the horse's stall status was composed of eighteen subheadings. The first part subheadings are respectively;
First sub-heading: Calmness assessment
It is the determination of the observation status of the horse to continue its daily life without any change in its behavior in the stall.
Second sub-heading: Interest assessment
It is the observation of the horse's sound and image in the stall or the observation of the horse's interest in the human being when approaching it.
Third sub-heading: Movement assessment
It is the observation and detection of the horse's continuous immobilization or movement in a part of the stall.
Fourth sub-heading: Curiosity assessment
It is the observation and determination of the horse's behavior in the stallin a state of constant curiosity.
Fifth sub-heading: Timidity assessment
It is the detection of continuous timid agitated behavior in the horse's stall.
Sixth sub-heading: Patience assessment
It is the determination of the horse's continuous patient waiting in the stall.
Seventh sub-heading: Obedience assessment
It is the determination of the horse's condition in the activities to be carried out with the horse in stall (petting, entering, etc.).
Eighth subheading: Stall situation assessment
Determination of the horse's condition of entering the stall.
Ninth sub-heading: Bite situation assessment
Determination of the biting status of the horse in the stall.
Tenth sub-heading: Double throwing situation assessment
Determination of the horse's double throwing situation in the stall.
Eleventh sub-heading: Standing up condition assessment
It is the determination of the horse's condition of getting up in the stall.
Twelfth subheading: Dervish making situation assessment
It is the determination of the horse's state of dervishizing in stall.
Thirteenth subheading: Wind swallowing situation assessment
Determination of the wind swallowing situation of the horse in the stall.

Fourteenth sub-heading: Door knocking situation assessment
Determination of the situation of the horse knocking on the door in the stall.
Fifteenth subheading: Grooming situation assessment
Determining the calmness of the horse while grooming the horse in the stall.
Sixteenth sub-heading: Feeding situation assessment
It is the determination of the horse's calmness while feeding in the stall.
Seventeenth subheading: Trust in the caregiver situation assessment
Determination of the horse's trust status to its caretaker in the stall.
Eighteenth subheading: Assessment of trust in a stranger
It is the determination of the horse's state of trust in the stranger in the stall.

Table 3.1. Horse Stable Status Assessment

3.1. HORSE STABLE STATUS ASSESSMENT	**1.**	**CALM**		**EXCITED**	
	2.	**INTERESTED**		**UNINTERESTED**	
	3.	**ACTIVE**		**INACTIVE**	
	4.	**CURIOUS**		**INCURIOUS**	
	5.	**TIMID**		**BRAVE**	
	6.	**PATIENT**		**IMPATIENT**	
	7.	**OBEDIENT**		**DISOBEDIENT**	
	8.	**SAFE TO ENTER STALL**		**NOT SAFE TO ENTER STALL**	
	9.	**NO BITING**		**BITING**	
	10.	**NO KICKING**		**KICKING**	
	11.	**NO REAR UP**		**REAR UP**	
	12.	**NO STALL WEAVING**		**STALL WEAVING**	
	13.	**NO AIR SWALLOWING**		**AIR SWALLOWING**	
	14.	**NO HITTING THE DOOR**		**HITTING THE DOOR**	
	15.	**CALM DURING GROOMING**		**TENSE DURING GROOMING**	
	16.	**CALM DURING FEEDING**		**TENSE DURING FEEDING**	
	17.	**TRUSTS TO THE CARER**		**NOT TRUSTING TO THE CARER**	
	18.	**TRUSTS TO HUMANS**		**NOT TRUSTING HUMANS**	

The second part of the third section consists of eighteen sub-headings for the evaluation of the horse's paddock situation. The second part sub-headings are respectively;
First sub-heading: Calmness assessment
It is the observation status determination of the horse continuing its life in the paddock without any change in its behavior.
Second sub-heading: Movement assessment
It is the observation and determination of the continuous movement of the horse in a part of the paddock.
Third subheading: Timidity assessment
Detection of the horse's continuously timid and agitated behaviors in the paddock.
Fourth sub-heading: Adaptation status assessment

It is the determination of the horse's harmony with other horses in the paddock.
Fifth sub-heading: Approach situation assessment
Detection of approaching the horse in the paddock.
Sixth sub-heading: Biting assessment
Detection of approaching the horse in the paddock.
Seventh sub-heading: Double throwing assessment
Detection of double throwing when the horse is approached in the paddock or by other horses.
Eighth sub-heading: Dispatching assessment
Determination of whether the horse is taken or brought to the paddock.
Ninth sub-heading: Assessment of trust in the caretaker
Determination of the horse's trust in its caretaker in the paddock.
Tenth sub-heading: Assessment of trust in a stranger
The horse is the determination of trust in the stranger in the paddock.

Table 3.2. Horse Paddock Situation Assessment

3.2. HORSE PADDOCK SITUATION ASSESSMENT	**1.**	**CALM**		**EXCITED**	
	2.	**ACTIVE**		**INACTIVE**	
	3.	**INTERESTED**		**UNINTERESTED**	
	4.	**ADAPTABLE TO OTHER HORSES**		**NOT ADAPTABLE TO OTHER HORSES**	
	5.	**EASY TO APPROACH**		**UNEASY TO APPROACH**	
	6.	**NO BITING**		**BITING**	
	7.	**NO KICKING**		**KICKING**	
	8.	**EASY TO LEAD**		**UNEASY TO LEAD**	
	9.	**TRUSTS TO THE CARER**		**NOT TRUSTING TO THE**	
	10.	**TRUSTS TO HUMANS**		**NOT TRUSTING HUMANS**	

The third part of the third chapter consists of twenty-one sub-headings. The sub-headings of the third part are respectively;
First sub-heading: Calmness situation assessment
It is the determination of the horse's calm progress in riderless training.
Second sub-heading: Movement situation assessment
Determination of the horse's mobility in riderless training.
Third sub-heading: Curiosity situation assessment
Determination of the horse's state of curiosity in riderless education.
Fourth sub-heading: Timid situation assessment
Determination of the horse's timidity in riderless training.
Fifth sub-heading: Patience situation assessment
Determination of the horse's patience in riderless training.
Sixth sub-heading: Obedience situation assessment
Determination of the horse's obedience in riderless training.
Seventh sub-heading: Upper girth condition assessment
Determination of the horse's reaction to the upper girth in riderless training.
Eighth sub-heading: Hash and felt situation assessment
Determination of the horse's reaction to the haşe and felt in riderless training.
Ninth sub-heading: Saddle condition assessment
Determination of the horse's reaction to the saddle in riderless training.
Tenth sub-heading: Post situation assessment

The horse's post reaction in riderless training is the determination of the situation.
Eleventh subtitle: Lonj situation assessment
Determination of the situation of the horse in lonj work in riderless training.
Twelfth sub-heading: Referral status assessment
Determination of the horse's riderless dispatch status.
Thirteenth subheading: Ramp condition assessment
Determination of the horse's condition in front of the ramp without rider.
Fourteenth subheading: Object condition assessment
Determination of the horse's condition in different objects without a rider.
Fifteenth subheading: Material condition assessment
To determine the situation of the horse in riderless educational materials.
Sixteenth sub-heading: Follow-up situation assessment
Determination of the horse's riderless trainer following status.
Seventeenth subheading: Step situation assessment
Determination of the regularity of the horse's steps without rider.
Eighteenth sub-heading: Learning status assessment
Determining the situation of the horse's training to learn the work taught without a rider.
Nineteenth sub-heading: Biting situation assessment
Determination of the horse's biting status in riderless progression or in training.
Twentieth subheading: Double situation assessment
It is the determination of the horse's condition in riderless progression or in pairs in training.
Twenty-first subheading: Trainer trust assessment
It is the determination of the horse's state of confidence in riderless progression or in training.

Table 3.3. Riderless Training Situation Assessment

3.3 RIDERLESS TRAINING SITUATION ASSESSMENT	**1.**	**CALM**		**EXCITED**	
	2.	**ACTIVE**		**INACTIVE**	
	3.	**INTERESTED**		**UNINTERESTED**	
	4.	**CURIOUS**		**INCURIOUS**	
	5.	**PATIENT**		**IMPATIENT**	
	6.	**OBEDIENT**		**DISOBEDIENT**	
	7.	**ALLOWING UPPER GIRTH**		**NOT ALLOWING UPPER GIRTH**	
	8.	**CAPARISON AND FELT CAN BE USED**		**CAPARISON AND FELT CANNOT BE USED**	
	9.	**SADDLE CAN BE USED**		**SADDLE CANNOT BE USED**	
	10.	**PELTRY CAN BE USED**		**PELTRY CANNOT BE USED**	
	11.	**SUITABLE FOR LUNGING**		**NOT SUITABLE FOR LUNGING**	
	12.	**FOLLOWS IN REPLACEMENT POSITION**		**NOT FOLLOWS IN REPLACEMENT POSITION**	
	13.	**STANDS BY THE RAMP**		**NOT STANDS BY THE RAMP**	

	14.	**NOT AFRAID OF OBJECTS AT FIRST SIGHT**		**AFRAID OF OBJECTS AT FIRST SIGHT**	
	15.	**NOT AFRAID OF TRAINING MATERIALS**		**AFRAID OF TRAINING MATERIALS**	
	16.	**FOLLOWS CONSISTATLY**		**NOT FOLLOWS CONSTANTLY**	
	17.	**KEEPS STRIDING CONSTANTLY**		**NOT KEEPS STRIDING CONSTANTLY**	
	18.	**MOTIVATED TO LEARN**		**NOT MOTIVATED TO LEARN**	
	19.	**NO BITING**		**BITING**	
	20.	**NO KICKING**		**KICKING**	
	21.	**TRUSTS TO THE TRAINER**		**TRUSTS TO THE TRAINER**	

Fourth part of the third section is the situation assessment of equestrian training consists of twenty-one sub-headings. Fourth part sub-headings respectively;
First sub-heading: Calmness situation assessment
It is the determination of the horse's calm progress in rider training.
Second sub-heading: Movement situation assessment
Determination of the horse's mobility in rider training.
Third sub-heading: Curiosity situation assessment
Determination of the state of curiosity in equestrian education.
Fourth sub-heading: Timid situation assessment
Determination of the horse's timidity in rider training.
Fifth sub-heading: Obedience status assessment
Determination of the horse's obedience in riderless training.
Sixth sub-heading: Riding situation assessment
Determination of the horse's riding situation in rider training.
Seventh sub-heading: Dismounting situation assessment
Determination of the horse's dismounting situation in rider training.
Eighth sub-heading: Sound condition assessment
Determination of the horse's sensitivity to the trainer's voice in rider training.
Ninth sub-heading: Training situation assessment
Determining the status of the horse in continuous education in equestrian education.
Tenth sub-heading: Step situation assessment
The horse's steps in rider training are equal and rhythmic.
Eleventh sub-heading: Learning status assessment
Determination of the learning situation of the horse in rider training.
Twelfth sub-heading: Follow-up situation assessment
It is the determination of the situation of the horse following the dispatcher in rider training.
Thirteenth sub-heading: Stance condition assessment
Determination of the horse's posture in rider training.
Fourteenth sub-heading: Gait condition assessment
It is the determination of the horse's walking situation in equestrian education.
Fifteenth sub-heading: Rapid gait condition assessment
Determination of the horse's fast walking condition in rider training.
Sixteenth sub-heading: Assessing the situation as if from a standstill

It is the determination of the horse's walking situation from the posture in rider training.
Seventeenth sub-heading: Assessment of the situation from posture to gait
The horse's posture in equestrian education is to determine the situation.
Eighteenth sub-heading: Assessment of the situation in the pair in training
It is the determination of the horse's situation of throwing in the double in rider training.
Nineteenth subheading: Bite situation assessment in training
Determination of the horse's biting situation in rider training.
Twentieth subheading: Trainer situation assessment
Determination of the situation of trainer confidence in horse rider training.
Twenty-first subheading: Foreign person situation assessment
The horse's rider is the determination of the situation of trust in the stranger in the training.

Table 3.4. Training Situation Assessment with Rider

3.4. TRAINING SITUATION ASSESSMENT WITH RIDER	**1.**	**CALM**		**EXCITED**	
	2.	**ACTIVE**		**INACTIVE**	
	3.	**INTERESTED**		**UNINTERESTED**	
	4.	**TIMID**		**BRAVE**	
	5.	**OBEDIENT**		**DISOBEDIENT**	
	6.	**MOUNTABLE**		**UNMOUNTABLE**	
	7.	**EASY TO DISMOUNT**		**UNEASY TO DISMOUNT**	
	8.	**ANSWERS TO VOICE COMMAND**		**NOT ANSWERS TO VOICE COMMAND**	
	9.	**INVOLVED IN TRAINING CONSTANTLY**		**NOT INVOLVED IN TRAINING CONSTANTLY**	
	10.	**STRIDES RHYTMICALLY**		**NOT STRIDES RHYTMICALLY**	
	11.	**MOTIVATED TO LEARN**		**NOT MOTIVATED TO LEARN**	
	12.	**FOLLOWS WHEN A RIDER ON TOP**		**NOT FOLLOWS WHEN A RIDER ON TOP**	
	13.	**CAN STOP AT THE SPOT**		**CANNOT STOP AT THE SPOT**	
	14.	**CAN WALK**		**CANNOT WALK**	
	15.	**CAN TROT**		**CANNOT TROT**	
	16.	**CAN WALK AFTER STOP**		**CANNOT WALK AFTER STOP**	
	17.	**CAN STOP AFTER WALK**		**CAN STOP AFTER WALK**	
	18.	**NOT KICKING DURING TRAINING**		**KICKING DURING TRAINING**	
	19.	**NOT BITING DURING TRAINING**		**BITING DURING TRAINING**	

	20.	**TRUSTS TO THE TRAINER**		**NOT TRUSTS TO THE TRAINER**	
	21.	**TRUSTS TO STRANGERS**		**NOT TRUSTS TO STRANGERS**	

Fifth part The third part consists of twenty-two sub-headings. The sub-headings of the fifth part are respectively;
First sub-heading: Calmness status assessment
Determination of the horse's calmness during the practice session.
Second sub-heading: Movement status assessment
Determination of the horse's movement status during the practice session.
Third sub-heading: Timid state assessment
Detection of the horse's skittish state during the practice session.
Fourth sub-heading: Communication situation assessment
Determination of the communication status of the horse during the practice session.
Fifth sub-heading: Ramp condition assessment
Determination of the ramp status of the horse during the practice session.
Sixth subheading: Progress status assessment
Determination of the horse's progress during the practice session.
Seventh sub-heading: Disabled car situation assessment
Determination of the horse's approach to the disabled cart during the practice session.
Eighth subheading: Ramp riding situation assessment
Determination of the horse's ramp riding status during the practice session.
Ninth subheading: Ramp dismounting condition assessment
Determination of the horse's ramp descent status during the practice session.
Tenth subheading: Exercise condition assessment
Determination of the exercise application status of the horse during the application session.
Eleventh subheading: Work status assessment in the session
Determination of the working status of the horse during the practice session.
Twelfth subheading: Sound condition assessment
Detection of the horse's condition from the environment during the practice session.
Thirteenth subheading: Material condition assessment
Material condition assessment of the horse during the practice session.
Fourteenth subheading: Condition assessment in the session
Determination of the horse's status of working alone in the practice session.
Fifteenth subheading: Rhythmic situation assessment in the session
It is the determination of the horse's equal and rhythmic stride status during the practice session.
Sixteenth subheading: Posture status assessment
Determination of the horse's stance during the practice session.
Seventeenth subheading: Gait condition assessment
It is the determination of the horse's walking condition during the practice session.
Eighteenth subheading: Assessment of the condition almost from the stance
It is the determination of the horse's gait from the stance in the practice session.
Nineteenth subheading: Stance status assessment from almost
It is the determination of the horse's stance during the practice session.
Twentieth subheading: Assessment of the double situation in the session
It is the determination of the situation in the pair to the patient or the team during the application session of the horse.
Twenty-first subheading: Bite status assessment in the session
Determination of the situation of the horse biting the patient, the team or the referral rope during the application session.

Twenty-second subheading: Session team situation assessment
The horse's confidence in the team during the application session is to make a due diligence.

Table 3.5. Hippotherapy Session Training Assessment

3.5. HIPPOTHERAPY SESSION TRAINING ASSESSMENT	**1.**	**CALM**		**EXCITED**	
	2.	**ACTIVE**		**INACTIVE**	
	3.	**BRAVE**		**TIMID**	
	4.	**COMMUNICATIVE**		**NON-COMMUNICATIVE**	
	5.	**STANDS BEFORE THE RAMP**		**NOT STANDS BEFORE THE RAMP**	
	6.	**FOLLOWS DURING LEADING**		**NOT FOLLOWS DURING LEADING**	
	7.	**NOT AFRAID OF WHEELCHAIR**		**AFRAID OF WHEELCHAIR**	
	8.	**MOUNTABLE ON THE RAMP**		**NOT MOUNTABLE ON THE RAMP**	
	9.	**DISMOUNTABLE ON THE RAMP**		**NOT DISMOUNTABLE ON THE RAMP**	
	10.	**SUITABLE TO EXERCISE WHILE ON TOP**		**NOT SUITABLE TO EXERCISE WHILE ON TOP**	
	11.	**NOT AFRAID OF TRANSITION ACTIVIRIES**		**AFRAID OF TRANSITION ACTIVITIES**	
	12.	**NOT DISTRACTED BY NOISE**		**DISTRACTED BY NOISE**	
	13.	**MATERIALS CAN BE USED ON TOP**		**MATERIALS CANNOT BE USED ON TOP**	
	14.	**CAN BE WORKED ALONE DURING THE THERAPY WITHOUT ANOTHER HORSE**		**CANNOT BE WORKED ALONE DURING THE THERAPY WITHOUT ANOTHER HORSE**	
	15.	**STRIDES RHYTMICALLY CONSTANTLY**		**NOT STRIDES RHYTMICALLY CONSTANTLY**	
	16.	**CAN STOP ON SPOT**		**CANNOT STOP ON SPOT**	
	17.	**CAN WALK**		**CANNOT WALK**	
	18.	**CAN WALK AFTER A STOP**		**CANNOT WALK AFTER A STOP**	
	19.	**CAN STOP AFTER WALK**		**CANNOT STOP AFTER WALK**	
	20.	**NOT KICKING DURING TRAINING**		**KICKING DURING TRAINING**	
	21.	**NOT BITING DURING TRAINING**		**BITING DURING TRAINING**	
	22.	**TRUSTS TI HIPPOTHERAPY TEAM**		**NOT TRUSTS THE HIPPOTHERAPY TEAM**	

5. Conclusion

By creating an evaluation form for the horses used and to be used in hippotherapy application centers in private and public institutions in Turkey, it will qualitatively and quantitatively support the academic studies that can be done with the hippotherapy horse in the future to

contribute to the horse trainers, health experts, veterinarians, and the working team in order to ensure that the application sessions of the disabled individual and the individual in need are healthy, safe and efficient. In addition, the standards of determining the correct horse selection and suitability in hippotherapy application sessions have been put forward by the horse trainer and the study team.

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Effects of different thickening agents on the colour, texture and sensory characteristics of jelly kelle paça (khash)

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Abstract:
Kelle paça is a traditional Turkish soup whose main ingredient is the head and feet of lamb, goat or beef. The other ingredients are onions, water, salt and spices. Jelly kelle paça is a product obtained by cleaning the head and feet of lamb, goat or beef and boiling them in salt water, then filling them into casings. In recent years, due to the increasing demand for fast food, jelly kelle paça has been produced to facilitate the preparation of the popular kelle paça soup. In the production of jelly kelle paça, starch is generally used to ensure gelation. The aim of this study was to determine the effects of different thickening agents on the colour and texture properties of jelly kelle paça samples. Seven different jelly kelle paça samples were produced: T1: starch (control group); T2: gelatin; T3: pectin; T4: starch and gelatin; T5: starch and pectin; T6: gelatin and pectin; and T7: starch, gelatin and pectin. Different thickeners decreased the L^* and b^* values of the samples compared to T1 (control group) ($P < 0.05$). T4 had higher a^* values than T3 ($P < 0.05$). While gelatin (T2) increased the hardness of the jelly kelle paça samples, the lowest hardness values were found in samples T5, T6 and T7 ($P < 0.05$). Pectin decreased the springiness values of the samples ($P < 0.05$). The different thickeners had no influence on the cohesiveness of the samples ($P > 0.05$). It was found that the T4 group had the highest consistency score on the sensory panel ($P < 0.05$). In terms of general acceptability, panellists gave higher scores for T2, T4, T6 and T7 than for the control sample (T1) ($P < 0.05$).

Keywords: hardness, meat industry by-product, traditional Turkish soup, thickener.

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1. Introduction

Edible meat consisting not of carcasses but of the internal organs of slaughtered animals is called "offal". Some of them (e.g. liver, kidney, heart, brain) are consumed fresh, while others (tripe, trotters, small intestine) are offered for consumption after processing (Öztan 2005). Although offal contains more water and carbohydrates than red meat, it has a lower fat content. The protein content of offal (with the exception of brain) is about the same as that of muscle tissue. Head meats and trotters of slaughtered animals are among the important offal.

Kelle paça soup is a traditional Turkish soup whose main ingredient is the head and trotters of lamb, goat or beef. The other ingredients of kelle paça soup are onions, water, salt and spices. It is also known by other designations, namely khash, pacha and kalle-pache. In recent years, due to the increasing demand for fast food, kelle paça is processed into "jelly kelle paça" and

put on the market, consumed mainly in restaurants and households in the form of pre-made food raw materials in the form of kelle paça soup.

Starch is a good source of carbohydrates and also has many different functions in food. Starch reduces cooking losses, increases the water-holding capacity and stability of meat products and is also used to improve the textural properties (Ölçer and Akın 2008).

Gelatin is a structurally irreversible, pure protein obtained by partial hydrolysis of collagen from the connective tissue and bones of slaughtered animals such as cattle, sheep, goats and pigs. Gelatin has important properties such as strong formability, transparent gelation, flexible film, easy digestibility and meltability in hot water. For this reason, it is used in the food industry (Yetim 2011).

Pectin is a natural polysaccharide found in the cell walls of plant tissues (Ngouémazong et al. 2012). Pectin is used in the food industry as a gelling agent (in products such as jam, marmalade, jelly), emulsifier and stabiliser (Basanta et al. 2012, Ngouémazong et al. 2012).

There is limited information in the literature about the kelle paça and generally the studies focused only on the kelle paça soup (Özkan 2019, Yüce 2020). However, to our knowledge, there are no previous reports on the jelly kelle paça. Therefore, this is the first study to evaluate the potential of gelatin, starch, pectin and different combinations of these substances as thickening agents in the production of jelly kelle paça samples by investigating colour and texture properties.

2. Materials and Methods

2.1. Materials

The heads and trotters of lambs were obtained from a slaughterhouse in Konya (Yılet, Konya, Türkiye). Starch (from potato, Smart Kimya, İzmir, Türkiye), gelatin (from beef, Zag Kimya, İstanbul, Türkiye), and pectin (from apple, Tito, İzmir, Türkiye) used in the production of jelly kelle paça purchased.

2.2. Production of kelle paça samples

Production of jelly kelle paça is described step by step as follows: (1) Tongues and tendons are boiled for 2.5 hours (2) Tongues and tendons are cleaned (3) Tongues, tendons and head meat are minced (4) Water is added to this mixture in a ratio of 1:1 and boiled for 3 hours (121 ^{o}C and 5 atm) (5) 1.5% salt and 1.5% starch are added and mixed (6) This mixture is filled into 60-calibre casings and cooled. Traditionally, potato starch is used in the above-mentioned production method. In this study, the starch was replaced by gelatin, pectin and a combination

of these thickening agents. Therefore, seven different jelly kelle paça samples were produced: T1: jelly kelle paça samples produced with 1.5% starch (control group); T2: jelly kelle paça samples produced with 1.5% gelatin; T3: jelly kelle paça samples produced with 1.5% pectin; T4: jelly kelle paça samples produced with 0.75% starch and 0.75% gelatin; T5: jelly kelle paça samples produced with 0.75% starch and 0.75% pectin; T6: jelly kelle paça samples produced with 0.75% gelatin and 0.75% pectin; and T7: jelly kelle paça samples produced with 0.50% starch, 0.50% gelatin and 0.50% pectin.

2.3. Colour measurements

A chroma metre (CR -400, Konica Minolta, Inc., Osaka, Japan) with illuminant D65, 2◦ observer, diffuser/O mode was used for colour determination. The lightness (L^*), redness (a^*) and yellowness (b^*) of the samples were determined. The measurements were carried out on the outer surfaces of the jelly kelle paça samples (Hunt et al., 1991).

2.4. Texture profile analysis

Texture profile analysis (TPA) was performed on the samples using a method described by Herrero et al. (2007). The jelly kelle paça samples were cut into 1.5 cm high slices for TPA and the analyses were performed on 5 parallel slices for each group. TPA was carried out using a texture analyser (TA-HD Plus Texture Analyser, UK) at room temperature and the following parameters were determined: Hardness (N), Adhesiveness (N.s), Cohesiveness, Springiness and Chewiness (N). A cylindrical plate with a diameter of 36 mm and a load cell with a weight of 50 kg were used. The sample was compressed with a delay of 0.1 s between downward movements (pre-test speed: 1 mm/s, test speed: 5 mm/s, post-test: 5 mm/s, and compression: 50%).

2.5. Sensory evaluation

The sensory analysis was carried out on the samples of kelle paça soup. The kelle paça soup samples were prepared from the jelly kelle paça samples produced with different thickeners. The sensory panel consisted of seven semi-trained panellists. The samples were coded with three-digit random numbers and seven samples of kelle paça soup were randomly distributed to the panellists in one session. A 9-point hedonic scale was used for the sensory analysis (9 - very good, 1 - very bad). The panelists were asked to rate the different characteristics (colour, smell, taste, consistency and general acceptability) of the kelle paça soup samples.

2.6. Statistical analysis

This study was performed in two replicates. A completely randomized design was employed. For statistical analysis of the data, MINITAB version 16.0 (Minitab Inc., State University,

USA) was used. Significant differences were confirmed at $P < 0.05$ and differences were compared with Tukey test.

3. Results

3.1. Colour properties of jelly kelle paça samples

*L**, *a** and *b** values of jelly kelle paça samples are given in Table 1. The highest *L** value was found in the control group sample produced with starch (T1), while the gelatin, the pectin and the combination of the different thickeners reduced the lightness values of the samples ($P < 0.05$).

Table 1. *L**, *a** and *b** values of jelly kelle paça samples produced with different thickening agents

Sample group	***L****	*a**	*b**
T1	**55.15 ± 1.77^{a}**	4.44 ± 0.26^{ab}	13.19 ± 0.21^{a}
T2	**44.13 ± 0.76^{c}**	4.98 ± 0.26^{ab}	11.07 ± 0.03^{b}
T3	**48.06 ± 0.65^{b}**	4.11 ± 0.25^{b}	10.24 ± 0.65^{b}
T4	**46.06 ± 0.27^{bc}**	5.17 ± 0.19^{a}	11.64 ± 0.60^{b}
T5	**46.58 ± 1.01^{bc}**	4.65 ± 0.45^{ab}	10.89 ± 0.25^{b}
T6	**46.56 ± 0.61^{bc}**	4.42 ± 0.08^{ab}	10.69 ± 0.38^{b}
T7	**45.59 ± 0.22^{bc}**	5.06 ± 0.06^{ab}	10.90 ± 0.12^{b}

$^{a-c}$ Within the same column, values with different lowercase superscripts indicate significant differences ($p < 0.05$). T1: starch (control group); T2: gelatin; T3: pectin; T4: starch and gelatin; T5: starch and pectin; T6: gelatin and pectin; and T7: starch, gelatin and pectin.

The combination of starch and gelatin (T4) had higher redness values than kelle paça samples produced with pectin (T3) ($P < 0.05$). It was found that the *a** values of samples T1, T2, T5, T6 and T7 did not differ from those of samples T3 and T4 ($P > 0.05$).

Thickeners other than starch and their various combinations reduced the b* values of the samples compared to control group ($P < 0.05$).

3.2. Textural characteristics of jelly kelle paça samples

Hardness, adhesiveness, springiness, cohesiveness and chewiness values of jelly kelle paça samples are given in Table 1. Gelatin significantly increased the hardness values of the samples (T2), while T5, T6 and T7 had the lowest hardness values ($P < 0.05$). Pectin decreased the springiness values of the samples compared to the other groups ($P < 0.05$), except for T5 ($P > 0.05$).

Different thickening agents did not affect the adhesiveness and cohesiveness of the jelly kelle paça samples ($P > 0.05$). T3 and T5 had significantly lower chewiness values than T2 ($P < 0.05$). It was determined that the chewiness values of T1, T4, T6 and T7 did not differ from those of samples T2, T3 and T5 ($P > 0.05$).

Table 2. Textural characteristics of jelly kelle paça samples produced with different thickening agents

Samples	**Hardness** (N)	Adhesiveness (N.s)	Springiness	Cohesiveness	Chewiness (N)
T1	**105.89 ± 0.34^{bc}**	1.80 ± 0.33^{a}	0.81 ± 0.00^{a}	0.24 ± 0.13^{a}	20.36 ± 8.03^{ab}
T2	**125.89 ± 4.93^{a}**	4.94 ± 0.83^{a}	0.86 ± 0.02^{a}	0.30 ± 0.56^{a}	32.38 ± 3.47^{a}
T3	**96.21 ± 3.10^{cd}**	4.67 ± 1.50^{a}	0.70 ± 0.05^{b}	0.18 ± 0.25^{a}	12.22 ± 2.45^{b}
T4	**113.30 ± 0.33^{b}**	2.03 ± 1.04^{a}	0.82 ± 0.02^{a}	0.20 ± 0.50^{a}	18.69 ± 4.15^{ab}
T5	**94.96 ± 1.64^{d}**	4.21 ± 0.24^{a}	0.77 ± 0.01^{ab}	0.18 ± 0.14^{a}	12.71 ± 3.69^{b}
T6	**94.81 ± 2.06^{d}**	2.54 ± 0.41^{a}	0.80 ± 0.02^{a}	0.24 ± 0.06^{a}	18.06 ± 4.26^{ab}
T7	**89.75 ± 1.67^{d}**	2.97 ± 0.50^{a}	0.83 ± 0.00^{a}	0.21 ± 0.02^{a}	16.08 ± 2.28^{ab}

$^{a-d}$ Within the same column, values with different lowercase superscripts indicate significant differences ($p < 0.05$). T1: starch (control group); T2: gelatin; T3: pectin; T4: starch and gelatin; T5: starch and pectin; T6: gelatin and pectin; and T7: starch, gelatin and pectin.

3.3. Sensory scores of kelle paça soup

Figure 1 shows the sensory evaluations of the kelle paça soup samples. The different thickeners had no influence on the colour, smell and taste of the samples ($P > 0.05$). www

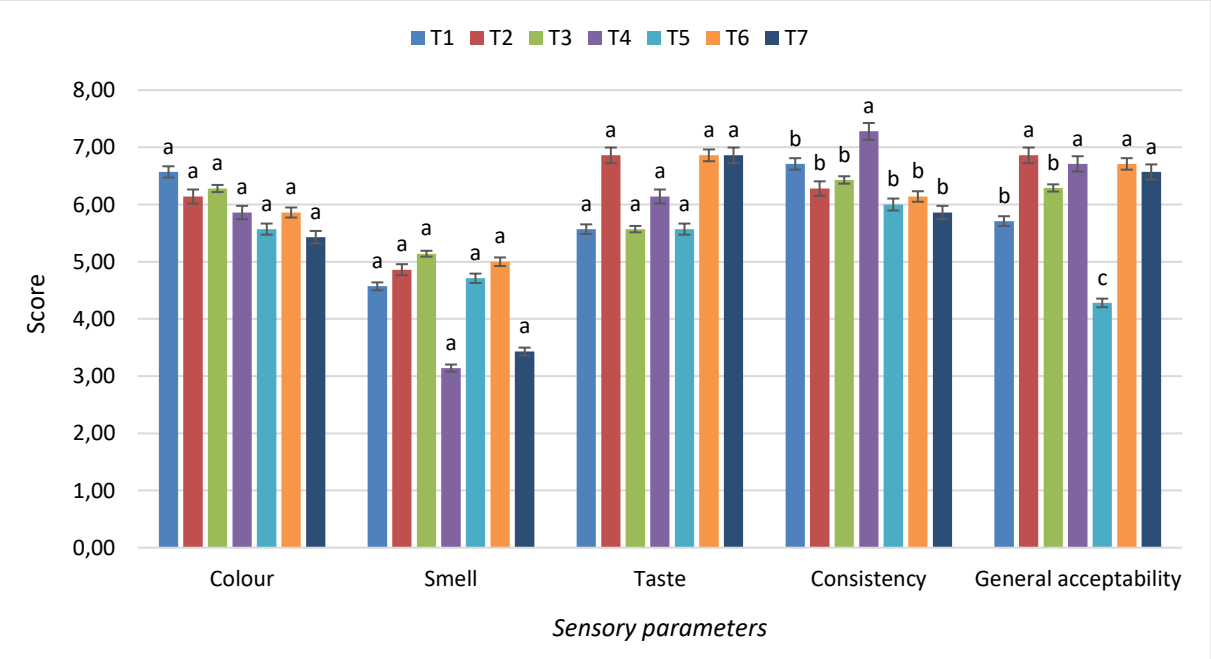

Figure 1. Sensory scores of the samples of kelle paça soup

4. Discussion

In this study, gelatin improved the textural properties of jelly kelle paça samples compared to the other thickening agents. It was reported that the functional properties of gelatine can be divided into two groups. The first group includes properties related to gelling, e.g. gel strength, gelling time, setting and melting temperature, viscosity, thickening, texturisation and water binding. The second group relates to the surface behaviour of gelatine, e.g. emulsion formation and stabilisation, protective colloid function, foam formation and stabilisation, film formation and adhesion/cohesion. None of the hydrocolloids currently on the market is able to cover all of the above properties in all applications (Schrieber and Gareis 2007). In the present study, it is thought that the increase in hardness values of samples including gelatine could be due to its better gel formation compared to starch and pectin. In parallel, the highest consistency score of kelle paça soup samples containing a combination of starch and gelatin in the sensory analysis supports this situation.

5. Conclusion

Starch, gelatin, pectin and various combinations of these substances affected the colour and some textural properties of the jelly kelle paça samples, and they also affected some sensory parameters of the kelle paça soup samples. Gelatin and pectin as well as various combinations of thickening agents decreased the lightness and yellowness values of the samples compared to samples including starch. Gelatin improved the textural properties of jelly kelle paça samples, while pectin reduced the hardness of the samples compared to the starch. Consequently, the current study showed that gelatin, pectin and their combination could be used as a thickening agent. However, in the absence of information in the literature, more studies should be conducted on kelle paça.

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Potential for Application of Encapsulation Technology in the Meat Industry to Improve Effectiveness of Polyphosphates on Quality and Safety of Ready to Eat Meat Products

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Abstract:
Improving meat safety and quality to protect consumer health has been an important issue for both meat processors and researchers. Meat product processing is vital stage for microbial contamination in a ready-to-eat (RTE) meat products. Lipid oxidation is one of the main factors limiting the quality and acceptability of RTE meat products. Lipid oxidation also causes quality problems such as discoloration, drip losses, off-flavor and off-odor developments. Lipid oxidation also leads to loss of consumer confidence in purchasing RTE meat products due to the formation of carcinogenic and mutagenic substances. Contamination of RTE meat products with pathogens such as *Listeria monocytogenes*, *Escherichia coli* and *Salmonella spp*., or spoilage microorganisms like *Pseudomonas spp*. may also cause serious foodborne illnesses for consumers. Furthermore, contamination of RTE meat products with spoilage microorganism can create sensory quality problems and reduce shelf life of RTE products. Therefore, foodborne outbreaks cause recalls and negative publicity which can result in a decrease in purchase of RTE meat products by consumers. Thus, meat processors and researchers are constantly searching for strategies to control potential bio-hazards in RTE meat products. Many food additives are used in the product formulations to control the growth of undesirable microorganisms in muscle foods. Polyphosphates (PP) are commonly used in various meat product processing for their beneficial effects such as improved water binding capacity and cooking yield, accelerated curing process, reduced lipid oxidation and improved textural attributes. PP also is able to inhibit the growth of several Gram-negative, Gram-positive bacteria and yeast. Phosphatase enzymes naturally found in raw meat material have a ability to hydrolyze PP into shorter-chain length PP or orthophosphates. As a result of this reaction, PP may loss some of their antioxidant and antimicrobial properties. Encapsulation is very promising technology for protecting PP from enzymatic hydrolysis caused phosphatases by enrobing PP into capsules. Previous studies demonstrated that encapsulated (e) PP maintained antioxidant capabilities of PP in muscle foods. Therefore, this review study summarizes previous studies about utilization of ePP to improve antioxidant and antimicrobial properties of PP in meat and meat products.

Keywords: Polyphosphates, meat, safety, quality, encapsulation.

1. Introduction

The meat industry is searching to develop strategies to reduce economic losses and increase the shelf life and storage stability of RTE meat products. Muscle foods are highly susceptible to

microbiological and chemical deterioration when preservation methods are not used because they are rich in essential nutrients and have high pH and water activity (Jayasena and Jo, 2013). Many methods such as the use of natural or synthetic additives, thermal processes, cooling, freezing, vacuum or modified atmosphere packaging are used in order to ensure safety and to maintain quality in muscle foods (Gould, 1996). Aim of the meat industry by using antimicrobial and antioxidant additives is to protect the products against microbiological and chemical deterioration. PP are widely used food additives in the meat industry due to their antimicrobial and antioxidant properties as well as they provide many beneficial effects such as improved water binding capacity and cooking yield, accelerated curing process and improved textural attributes (Gadekar et al., 2014).

PP can also be used to control the growth of microorganisms during meat processing. PP are not additives that have antimicrobial effects specifically, but are chemical components that have a suppressive effect on the growth of microorganisms under certain conditions (Moon et al., 2011). Antimicrobial effects of PP occur by changing the chemical structure of the environment by acidification, by binding metal ions such as calcium, magnesium and iron, which must be present in the environment for microbial growth, or as a synergist effect when used with certain antimicrobial additives such as nisin, ethylenediamine tetraacetic acid (EDTA) and nitrite (Akhtar et al., 2008). Gram-positive bacteria are more affected by PP than gram-negative bacteria. The inhibitory effect on gram-positive bacteria depends on the chain length of PP and the preventive effect increases with increases of chain length (Palmeira-de-Oliveira et al., 2011).

Lipid oxidation is a reaction that causes discoloration, formation of toxic compounds, nutrient losses and reduction of shelf life (Falowo et al., 2014). This reaction is affected by many intrinsic and extrinsic factors such as unsaturation of their fatty acids, low molecular weight metal ions, pH, oxidative enzymes, storage temperature, light, oxygen, water activity (Shahidi and Zhong, 2010). PP exhibit their antioxidant effects by binding metal ions which catalyze oxidation reaction (Kılıç et al., 2014). The antioxidative activities of PP depend on the type and concentration of PP (Kılıç et al., 2016a). It has been stated that PP such as sodium tripolyphosphate (STP), sodium acid pyrophosphate (SPP), sodium hexametaphosphate (HMP) and tetrasodium pyrophosphate (TSPP) have antioxidant effects in meat products and they show synergistic effect when used with vacuum or modified atmosphere packaging (Kılıç et al., 2014; Kılıç et al., 2016b; Kılıç et al., 2018). Lee (1993) indicated that tripolyphosphates form stronger complexes with metal ions (especially iron and copper) than those of pyrophosphates. Researcher noted that the best ion-sequestering agents are long chain PP, and the ion-

sequestering ability increases with increases of chain length. The powerful antioxidant effect of long chain PP can be reduced by the phosphatase enzymes before cooking (Kılıç et al., 2014). In addition, current studies showed that this effect can be increased by using encapsulation technology (Kılıç et al., 2014; Du and Claus, 2015; Kılıç et al., 2016a,b). Encapsulation is described as a technology to entrap solids, liquids, or gaseous materials within capsules that can release their contents under controlled rates and certain conditions (Nedovic et al., 2011). Encapsulation technology in food processing contains the coating of tiny particles of food components such as flavors, sweeteners, colorants, acidulants, vitamins and enzymes (Desai and Jin, 2005). This review is aimed to present information about current studies regarding usage of ePP to improve antioxidant and antimicrobial properties of PP in muscle foods.

2. Antioxidative Effectiveness of Encapsulated Polyphosphates

Besides the many beneficial effects of PP in muscle foods, PP exhibits antioxidant effects (Kılıç et al., 2014). Recent studies indicated that antioxidant effects of PP can be improved with the use of encapsulation technology (Kılıç et al., 2014; Kılıç et al., 2016a,b). Sickler et al. (2013a) evaluated that the effects of the use of encapsulated (e) and (u) unencapsulated PP (STP and SPP) in cooked ground beef patties, researchers stated that the lowest cooking loss and TBARS values were obtained in uSTP usage, and all PP treatments had also lower TBARS values than control samples. Sickler et al. (2013b) also reported that eSTP reduced the TBARS by 77% (0.3% eSTP) and 80% (0.5% eSTP) compared to the same amount of uSTP. Kılıç et al. (2014) tested ePP (STP, HMP and SPP) at two different coating levels (30% and 50%) on lipid oxidation in ground chicken and beef during raw and cooked storage. Researchers reported that encapsulated or unencapsulated forms of STP and SPP were the most effective PP types in delaying lipid oxidation in both meat species. Authors also stated that the coating level had no impact on the lipid oxidation inhibition level (Kılıç et al., 2014). Xie et al. (2014) reported that the eSTP demonstrated an enhanced antioxidant effect and the antioxidant effect provided by eSTP was more significant as the storage time increased. Kılıç et al. (2015) stated that the application of higher end-point cooking temperature decreased TBARS values in cooked ground beef, whereas increased LPO values in cooked ground beef and chicken. Authors suggested that using lower end-point cooking temperatures provided more benefits when using ePP. Du and Claus (2015) stated that STP, SPP and HMP are significantly effective in limiting the lipid oxidation in ground turkey, as well as PP degradation due to phosphatases are reduced by encapsulation technology. Kılıç et al. (2016a) indicated that the antioxidant effect of eSTP or eSPP can be improved with increasing added ePP level in product formulation. Furthermore,

Kılıç et al. (2016b) also revealed that eSTP and eSPP were the better then eHMP for inhibiting oxidative changes in cooked ground beef and chicken. Furthermore, researchers stated that the antioxidative effectiveness of these ePP can be improved with a higher melting release point of the encapsulation material. Claus et al. (2016) indicated that pH difference between two sets (high, 6.4 to 6.7; low: 5.9 to 6.2) of turkey breasts had a little effect on lipid oxidation inhibition by ePP. Another study (Kılıç et al., 2018) revealed that the antioxidative effectiveness of STP and SPP can be improved with 0.25% ePP usage combined with cohort uPP in patty formulation. Researchers suggested that meat industry should consider adding 0.25% additional ePP to their product formulations in order to achieve more effective inhibiting the lipid oxidation in RTE meat products.

3. Antimicrobial Effects of Encapsulated Polyphosphates

PP do not exhibit strong antimicrobial effects, but exhibit synergistic effects when they are combined with other preventive techniques (Bunkova et al., 2014). PP may inhibit microbial growth (1) by forming complexes with metal ions which are necessary for cell division, (2) by lowering pH with acidic PP (3) by disrupting the cell wall integrity (4) by increasing oxidative stress and (5) by causing changes in cell morphology (Bunkova et al., 2014). In addition, PP reduce the heat resistance of the most bacteria. It has been reported that the inhibitory effect on gram- positive bacteria depends on PP chain length, and the long chain PP have a better inhibitory effect than shorter-chain PP (Bunkova et al., 2014). Shorter-chain PP or orthophosphates are released from PP when PP are degraded by phosphatases in the meat system. Therefore, antimicrobial activity of PP may be decreased. Encapsulation is an alternative technology that can be used to protect PP from phosphatases by enrobing PP into capsules (Kılıç et al., 2014). Tenderis et al. (2020) investigated the effects of sodium lactate (SL), eSTP or eSPP forms, and their combinations on *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Staphylococcus aureus* growth in cooked ground beef during 30 days storage at 4 or 10 °C. Researchers indicated that STP or SPP usage in formulation had some inhibitory effect on *S.* Typhimurium, *E. coli* O157:H7 and *S. aureus* growth in cooked ground beef during 30 days storage at 4 or 10 °C. In addition, researchers indicated that antimicrobial efficiency of PP is not affected by encapsulation, and the usage of PP and SL combinations have synergistic effect on reducing the growth of *S.* Typhimurium, *E. coli* O157:H7 and *S. aureus* in cooked ground beef. Tenderis et al. (2021) also evaluated the effectiveness of using sodium lactate, eSTP, eSPP and various combinations of these additives on growth of *Listeria monocytogenes* and *Pseudomonas fluorescens* in cooked ground beef during 30 d refrigerated storage. This

study demonstrated that STP had no inhibitory effect and SPP had some inhibitory effect on the growth of *L. monocytogenes* and *P. fluorescens* in cooked ground beef during refrigerated storage. In addition, no further inhibitory effect was provided by encapsulated forms of these PP. Hovewer, the combination of SL with uPP or ePP or uePP increased synergistically the antimicrobial efficiency against *L. monocytogenes* and *P. fluorescens*.

4. Conclusion

Previous studies have been performed to test the effectiveness of ePP. Results of these studies revealed that ePP usage can be an effective strategy to control oxidative changes in RTE meat products. In addition, these studies indicated that ePP has not been contributed on antimicrobial activity. However, when PP are used with other antimicrobial agents like sodium lactate, PP or ePP can demontrate synergistic effects to inhibit the growth of microorganisms.

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The Effects of *Malva Neglecta* on The Probiotic Bacterium *L. rhamnosus* GG

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Abstract:

Traditional herbs are products that are used as folk medicine. Worldwide, medicinal plants and plant products have been widely used. One of these herbs is *Malva neglecta* (common mallow), which is used to treat a variety of ailments including stomachaches, wound healing, muscle pain, respiratory system inflammation, and diarrhea. Probiotic microorganisms, which are important in the intestinal flora, have been shown to prevent intestinal disorders. They can prevent pathogen colonization and reproduction, boost the immune system, and have antimicrobial properties. Probiotic-enriched foods are products that, when consumed in sufficient quantities, benefit the host. *Lacticaseibacillus rhamnosus* GG is the most extensively studied probiotic strain. Thus, the aim of the present study is to investigate antibacterial and antioxidant properties, as well as auto-aggregation of *L. rhamnosus* GG when grown with *Malva Neglecta* extract. The results showed that *L. rhamnosus* GG probiotic bacteria grown with *Malva Neglecta* extract demonstrated better auto-aggregation and antibacterial activity but not antioxidant activity.

Keywords: Antioxidant, Auto-aggregation, Antibacterial Activity, Plant extract, Probiotics

1. Introduction

Traditional medicinal plants are defined as medicinal products derived from the plant's roots, stems, leaves, bark, seeds, fruits, and flowers that can be used to promote general health and treat diseases (Tang and Halliwell, 2010). Herbal ingredients have been used in almost every country on the planet (Robinson and Zhang, 2011). *Malva neglecta* (Common mallow) is an annual herbaceous plant in the *Malva* genus. The leaves are circular with shallow lobed edges. Flowers are produced in clusters of leaf axils. *Malva neglecta*, which is widely considered a weed, is used as a food and herbal medicine for a variety of ailments including stomach pain, wound healing, muscle pain, respiratory system inflammation, and diarrhea (Dalar et al.,2012).

Recent studies indicated that it has crucial pharmacological properties such as antimicrobial, antioxidant, anti-inflammatory (Al-snafi, 2019). Hasimi et. al (2017) studied that it has strong antioxidant activity and antimicrobial effects of *Malva neglecta* against *Escherichia coli*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* (Hasimi et al., 2017).

According to the definition of the World Health Organization (WHO), probiotics are live microorganisms that provide health benefits to the host when given in adequate amounts (Fuller, 1999). Positive health benefits include the reduction of gastrointestinal infections and inflammatory bowel disease and modulation of the immune system (Fuller and Gibson, 1997). *Lactobacilli*, *bifidobacteria,* and *enterococci* are common known and studied probiotic bacteria. It has been determined that probiotic microorganisms, which are important in the intestinal flora, have preventive effects on intestinal disorders. They can prevent colonization and reproduction of some pathogens, strengthen the immune system, and can have anticholesteric, antigenotoxic, antimicrobial and antimutagenic effects. Lactic acid bacteria (LAB), which are the best known of probiotics, constitute a diverse group of Gram-positive, non-spore-forming, catalase-negative organisms found in a variety of habitats (Carr et al., 2002). The health effects of *Malva neglecta* are well documented. The aim of the present study is to investigate antibacterial and antioxidant properties, as well as auto-aggregation of *L. rhamnosus* GG when grown with *Malva Neglecta* extract.

2. Materials and Methods

2.1.Preparation of *Malva neglecta* plant extract

The whole plants were collected from Bartın, Turkey. 5 g plants were weighed and crushed in mortar. 50 mL of ethanol (containing 10% glycerol) was added and mixed for 24 h. Extract was filtered through Whatman No. 1 filter and stored at -20°C until studies start.

2.2.Growth of Probiotic Bacteria

Lacticaseibacillus rhamnosus GG (LGG) bacteria were divided into groups, one group being the control, without *Malva neglecta* extract, and the other groups contained concentrations of 50 µg/mL, 100 µg/mL, 250 µg/mL, 500 µg/mL, 1000 µg/mL and grown in semi-synthetic lactic acid bacteria medium (LABSEM) without shaking at 37ºC.

2.3.DPPH Scavenging Assay

L. rhamnosus GG grown with *Malva neglecta* extract were centrifuged at 3500 rpm for 15 minutes. The supernatants obtained after centrifugation were used for DPPH scavenging activity. Bacterial supernatants were placed in the wells of 96-well plate as undiluted, ½, or ¾ diluted with ethanol, followed by addition of 100 µL DPPH to each well. The prepared plate was incubated for 30 minutes at room temperature in the dark. Spectrophotometric measurements were made at 517 nm. Decrease in the absorbance was used to assess relative DPPH scavenging activity.

2.4.Auto-Aggregation Assay

To measure auto-aggregation, bacterial cells were harvested at stationary phase (3200xg, 15 min), washed with PBS and re-suspended with PBS having OD_{600} of 0.5. Auto-aggregation was determined by adding 4 milliliters of bacterial suspensions to falcon tubes. Every hour, 0.1 milliliter of the suspension will be added to the tube containing 0.9 milliliter of PBS and the absorbance at 600 nm was measured (Kos B. et al., 2003). From here, the percentage of auto-aggregation was calculated using the formula:

$$\%Autoaggregation = (1 - \frac{A_t}{A_0}) \times 100$$

2.5.Antibacterial Activity of *Malva neglecta*

In order to investigate antibacterial activities of the *Malva neglecta*, Broth micro-dilution Assay was used (Brandt et al., 2010), with some modifications. Briefly, Gram (−) *Escherichia coli* and Gram (+) *Staphylococcus aureus* bacterial cultures were inoculated into Nutrient Broth (NB) cultures from frozen stocks for 24 h at 37 °C. In total 200 µL of microtiter plate wells, 20 µL of bacterial cultures were inoculated with NB containing different concentrations of the *Malva neglecta* (0 – 1000 µg mL^{-1}). Negative controls were prepared using LB without bacteria. Positive controls did not contain any *Malva neglecta*. Absorbance of microtiter plates were read at 600 nm using an ELISA reader before and after incubation (24^{th} h) at 37 °C. Bacterial viability was measured as percentage of compound-treated bacterial groups to the positive control (bacterial viability of positive control was taken as 100%).

2.6.Statistical Analysis

All the experiments were performed as three biological replicates. The data were represented with mean and standard deviations. Either Student's *t*-test or one-way ANOVA were used for statistics and $p<0.05$ was considered as statistically significance.

3. Results

3.1.Effects of *Malva neglecta* on auto-aggregation property of *Lactocaseibacillus rhamnosus* GG

Auto-aggregation demonstrates the adhesion of probiotic bacteria of the same species to epithelial cells in the human gastrointestinal tract in clusters. The attachment of microorganisms to epithelial surfaces is related to both aggregation and hydrophobicity of the cell surface. To exert beneficial effects, probiotics should reach a certain mass by aggregation (Y. Sui *et al.*, 2020).

Figure 1 shows the auto-aggregation of *L. rhamnosus* GG exposed to various concentrations of *Malva neglecta*. When we look at the results, we notice the auto-aggregation feature. When we look at the *Malva neglecta* concentrations, 1000 µg/mL at the first hour and 100 µg/mL, 250 µg/mL, 500 µg/mL, and 1000 µg/mL at the second, third, and fourth hours are statistically significant.

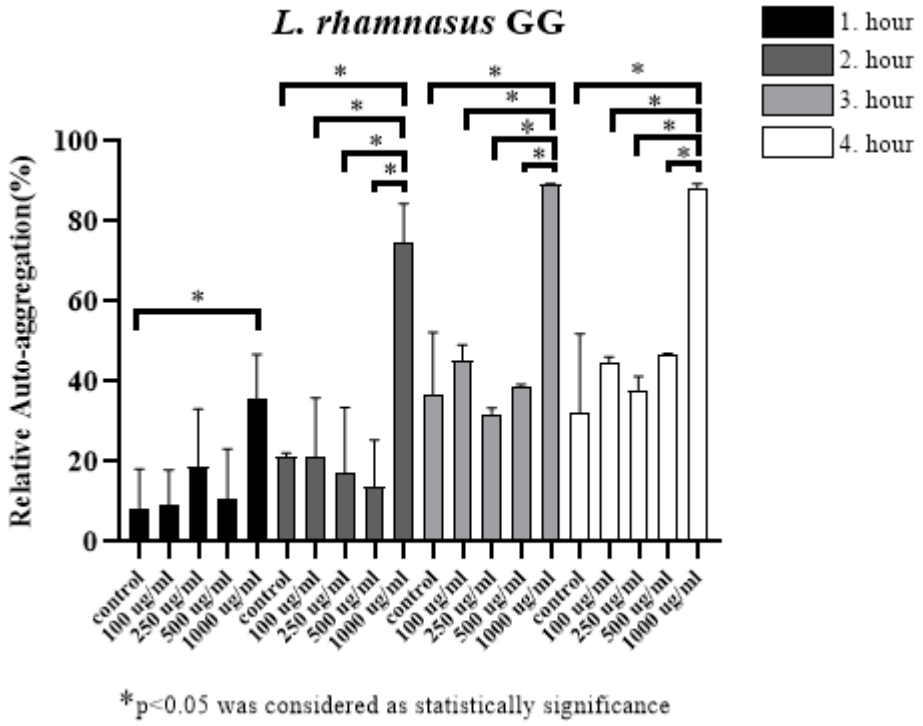

Figure 1. The effect of *Malva neglecta* extract on auto-aggregation of *L. rhamnosus* GG

3.2.Effects of *Malva neglecta* on antibacterial property of *Lactocaseibacillus rhamnosus* GG

The antibacterial properties of *L. rhamnosus* GG grown with *E. coli* and *Malva Neglecta* are shown in Figure 2. While the plant extract had antibacterial properties against *E. coli*, it had no antibacterial properties against LGG bacteria grown with it. Concentrations of 250 µg/mL with the control, 500 µg/mL with the control, 250 µg/mL with 1000 µg/mL, and 500 µg/mL with 1000 µg/mL were statistically significant in the plant extract.

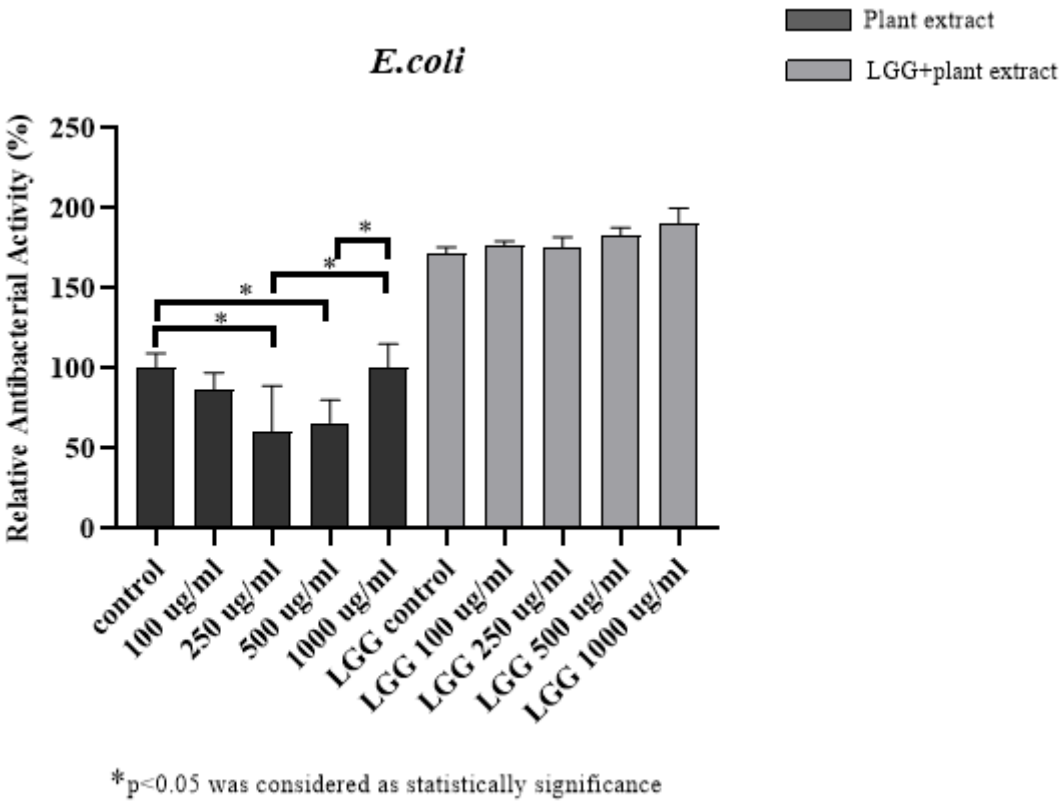

Figure 2. Antibacterial properties of *L. rhamnosus* GG grown with *Malva Neglecta with E. coli*

The antibacterial properties of *L. rhamnosus* GG grown with *S. Aureus* and *Malva Neglecta* are shown in Figure 3. *L. rhamnosus* GG and *Malva neglecta* both demonstrated antibacterial activity. When we look at the plant extract, there is statistical significance between control and 250 µg/mL, 100 µg/mL and 1000 µg/mL, and 250 µg/mL and 1000 µg/mL for the control. LGG bacteria concentrations were statistically significant between control and 500 µg/mL, control and 1000 µg/mL, 100 µg/mL and 1000 µg/mL, 250 µg/mL and 1000 µg/m Lconcentrations.

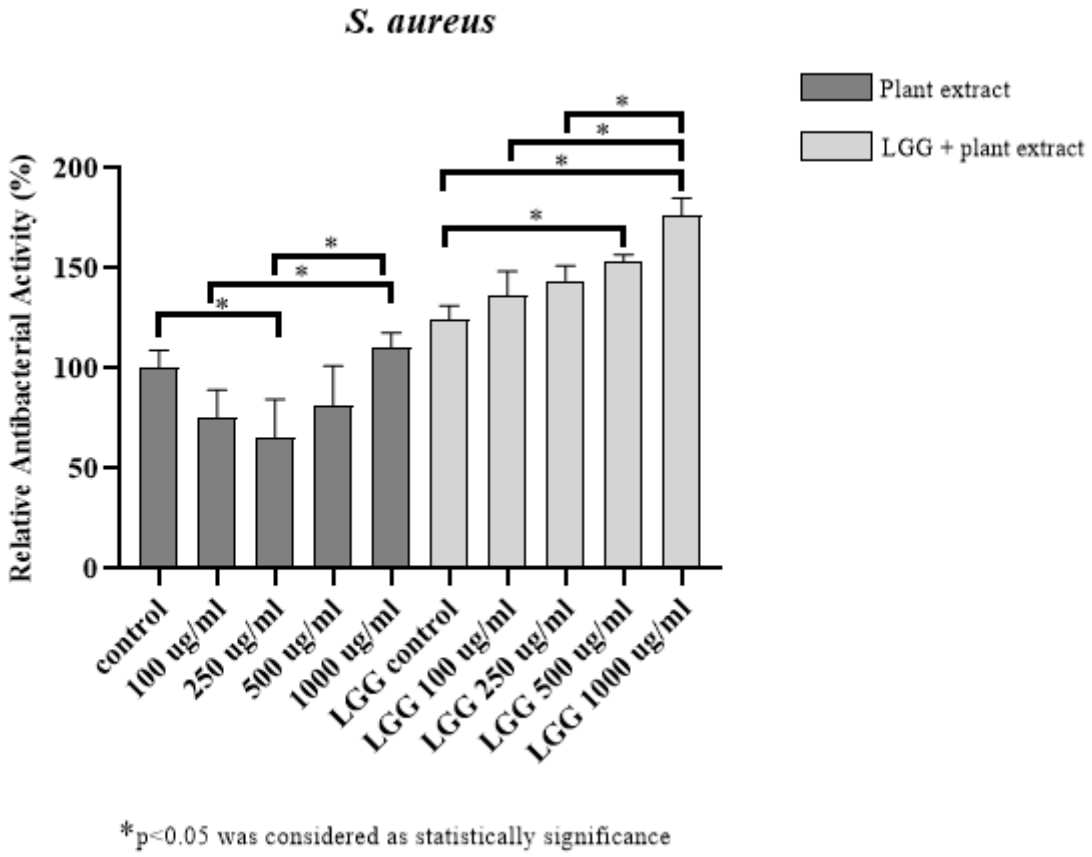

Figure 3. Antibacterial properties of *L. rhamnosus* GG grown with *Malva Neglecta with S. Aureus*

3.3.Effects of *Malva neglecta* on antioxidant property of *Lactocaseibacillus rhamnosus* GG

The antioxidant properties of *L. rhamnosus* GG grown with *Malva Neglecta* are shown in Figure 4. When we look at the results, we can see that as the concentration of *Malva Neglecta* extract increased, so did the concentration of *L. rhamnosus* GG grown with *Malva Neglecta*. *Malva Neglecta* at 1000 µg/mL lacked antioxidant properties.

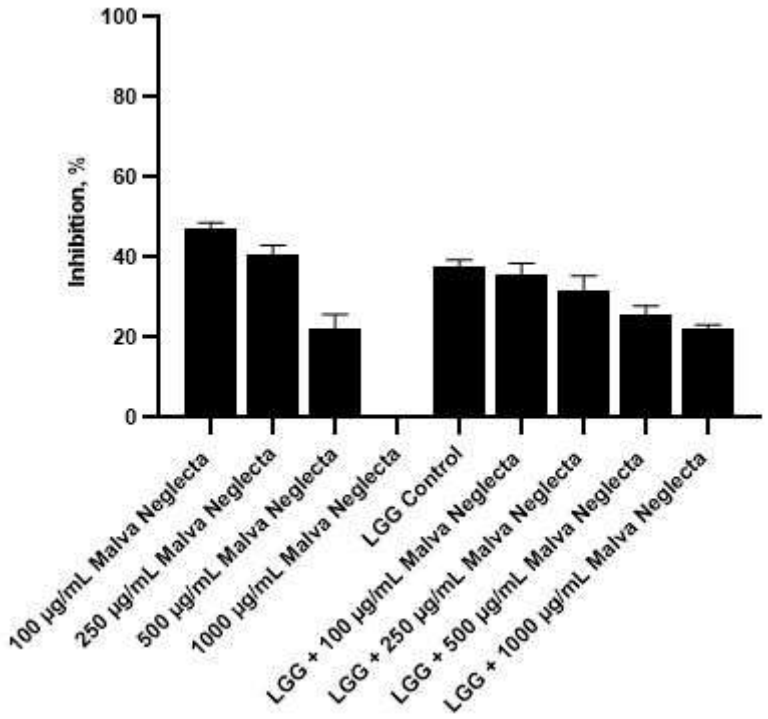

Figure 4. Antioxidant properties of *L. rhamnosus* GG grown with *Malva Neglecta.*

4. Discussion

The community formed by microorganisms belonging to the same species by binding to each other is called as auto-aggregation. Auto-aggregation is an important feature for probiotic bacteria in their ability to attach to the mucosa in the human body (Kos B. et. al. 2003). In this study, we observed an increase in the auto-aggregation when the plant extract was added. This could mean that probiotic bacteria can exhibit a potential to reside longer in the GIT and to exert the probiotic activities on the host when *Malva neglecta* extract is supplemented to the growth.

Antioxidants are considered important nutraceuticals because of their many health benefits (W. Droge 2002, M. Valko et. al. 2007). 1,1-Diphenyl-2-picryl-hydrazil (DPPH) is a stable free radical with an unpaired valence electron at one atom of the nitrogen bridge (P. C. Eklund et. al. 2005). DPPH radical scavenging is the basis of the popular DPPH antioxidant assay (M. H. Alma et. al. 2003, S. Kordalı et. al. 2005). In the present study, we observed that addition of plant extract did not have any effects on the antioxidant property of *L. rhamnosus* GG, even though this probiotic bacterium has itself antioxidant activity.

5. Conclusion

In this study, the effects of *Malva neglecta* extract grown with on auto-aggregation, antibacterial and antioxidant properties of *L. rhamnosus* GG probiotic bacteria were investigated. Plants have long been used in traditional medicine, and their effects on human

health are being researched. *Malva neglecta* is used in traditional medicine to treat gastrointestinal problems. A symbiotic relationship can exist between the plant extract and the probiotic. Auto-aggregation increased at concentrations of 100 μg/mL, 250 μg/mL, 500 μg/mL and 1000 μg/mL. The plant extract demonstrated antibacterial properties against *E. coli* and *S. aureus*. *L. rhamnosus* GG grown with *Malva neglecta* extract has no antibacterial properties against *E. coli* but does have antibacterial properties against *S. aureus.* At increasing concentrations, *L. rhamnosus* GG grown with *Malva Neglecta* extract and *Malva Neglecta* plant extract show no antioxidant properties.

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Determination of Zearalenone in Liver by LC-MS/MS Method, in the Republic of Albania

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Abstract:

Zearalenone (ZEA) is frequently produced by Fusarium species growing on maize but also on barley, oat and wheat. Animals could be at high risk of being exposed to ZEA through the intake of contaminated feed. They can cause a variety of adverse health effects and pose a serious health threat to humans and livestock. ZEA has anabolic properties and estrogenic resulting in severe disorders of the reproductive system of mammalian species, despite its relatively low acute toxicity. The liver is one of the target organs for the accumulation and metabolism of ZEA. As no data exist on the occurrence of Zearalenone in liver, in Republic of Albania we have analyzed them by LC-MS/MS. Nineteen (n=19) ovine, caprine and swine liver samples were taken in the study during the year 2022. The samples were taken from different regions of Albania. All samples were collected using official sampling methods. The compound was extracted from liver tissue using ethyl acetate. The purification was performed by solid-phase extraction (SPE) with sorbents C18, primary secondary amine and magnesium sulphate. Using an isotope labelled internal standard ($^{13}C_{18}$-Zearalenone), high recovery were achieved and the overall recovery was 99%. The results are not corrected for recovery, because the compound ZEA is quantified using an internal standard and a matrix matched calibration curve. The Limit of Quantification (LOQ) value for ZEA was determined 25 µg/kg. No carry-over factors from feed to liver were determined. The Zearalenone contents in liver were demonstrated that was below the LOQ for all the samples analyzed. The levels of Zearalenone found in this study indicate that the presence of this compound in liver is not hazard for public and animal health.

Keywords: zearalenone (ZEA), liver, LC-MS/MS

1. Introduction

Mycotoxins are toxic secondary metabolites produced by fungi. They are contaminants in foods and feeds, causing harmful effects upon animal and human health. The most important mycotoxins in naturally contaminated foods and feed are aflatoxins, zearalenone, T-2 toxin, deoxynivalenol, ochratoxins and fumonisins. These compound contaminated feed are a severe threat to both livestock productivity and human health and cause significant worldwide economic losses every year. (Magnoli AP, 2017; Bintvihok A, 2002)

The climatic conditions during plant development prior to harvest are the major determinants for the ZEA contamination level of feed. Zearalenone it is mainly formed pre harvest but its synthesis might continue under poor storage conditions. (Dänicke et al., 2015).

Zearalenone accumulation before the harvest time, is typically detected in high levels in samples of natural animal feed because of their improper storage. (Zhang et al., 2018; N. D. Krout-Greenberg, 2013).

Zearalenone is a mycotoxin compound which is present in cereals cultivated all over the world. The liver cells are exposed to zearalenone activity, this compound has hepatotoxic influence to this organ. (Stadnik A, 2009)

Zearalenone (ZEA) is a kind of mycotoxin that poses great threat to the liver of livestock and human due to its toxicity however, its toxicity mechanism on prepubertal gilts liver function and development is not known. (Wu, 2021)

Zearalenone was known to be a toxic substance of extensive concern to livestock. Decades later it was found that this compound is an estrogen agonist. (Jana N, 2018). This compound causes estrogenic disorders such as infertility, testicular atrophy, miscarriage, vaginal prolapse, uterine hypertrophy, feminization or breast enlargement. (Nurshad et al., 2019; Paula LC, 2022)

The aim of this study is to contribute to the knowledge disposition of zearalenone and to give an accurate method for the detection of this compound. We describe in this paper a procedure for the analysis of ZEA in liver and report the levels of it in ovine, caprine and swine liver.

The objective of this study was to assess the risk of mycotoxin exposure posed to Albania livestock from Zearalenone. Nineteen samples of liver were collected and analyzed for Zearalenone. The method is based in a sensitive liquid chromatography with tandem mass spectrometry method using negative ionization mode. High recovery was achieved and the overall recovery was 99%.

2. Materials and Methods

Nineteen liver samples were collected during year 2022. Were randomly sampled, collected using official sampling methods. The samples were taken from different regions of Albania as Tirana, Durres, Vlora, Berat, Diber, Elbasan, Fier, Gjirokaster, Korça, Kukes, Lezhe, Shkoder.

Figure 1. The map of coordinates' from which the samples were taken

The distribution of the samples is presented in the Table 2:

Table 1. Sampling by location, liver samples.

Country	Ovine	Caprine	Swine
Diber	**1**		
Durres			
Elbasan	**1**		
Fier	**1**	**1**	**1**
Gjirokastra	**1**	**1**	
Korça	**3**	**1**	
Lezhe			
Shkoder	**1**	**1**	
Tirana	**1**	**2**	
Vlora	**2**		
Berat	**1**		
Kukes			
Total	***12***	***6***	***1***

Sampling

The liver samples were packed in sterile plastic bags, were labeled with a specific code. A form was filled out, which contains all the data about the animal from which the sample was taken, such as age, registration number, sampling date, sampling time, sampling method, reason for

sampling, transport conditions and farm address. Then the samples were transported in refrigerated boxes. The samples were stored at -20°C until the analysis was performed.

Reagents and Standards

Zearalenone CAS Nr.17924-92-4 (Sigma Aldrich)

$^{13}C_{18}$ Zearalenone CAS Nr.911392-43-3 (Sigma Aldrich)

H_2O (LC-MS Grade) LiChrosolv®

Ethyl acetate (Titol Chimica)

$MgSO_4$ (Titol Chimica)

C18 sorbent (Macharey-Nagel)

PSA primary secondary amine (Macharey-Nagel)

n-Hexan (Titol Chimica)

Methanol (Titol Chimica)

Sample Extraction

3 g of sample was weighted into 50 ml propylene centrifuge tube. Then 7 ml H_2O was added to each tube, the content was homogenized. After that 10 ml ethyl acetate was added, each tube was vortex 60 second. Then a QuEChERS containing 400 mg NaCl, 1600 mg $MgSO_4$ was added to every tube. The tubes were shaken on vortex for 60 seconds. The contents of the tubes were centrifuged for 10 min at -4°C at 8000 rpm. The ethyl acetate supernatant was transferred into clean tubes 15 ml. After a new QuEChERS containing 400 mg C18, 400 mg PSA, and 1.2 g $MgSO_4$ was added to every tube. The tubes were shaken on vortex for 60 seconds. The contents of the tubes were centrifuged for 10 min at -4°C at 8000 rpm. The ethyl acetate was transferred into 15 ml tubes and dried under a stream of nitrogen at 55±5°C. The dry residues were dissolved in 0.5 ml H_2O and 3 ml methanol. After the samples were defatted twice with 5 ml n-hexane, the methanolic phase was evaporated under a stream of nitrogen at 55±5°C. In the end the residues was dissolved in 300 µl methanol: H2O (50:50 v/v) and filtered through 0.2µm PTFE. Then the extract was transferred to vials and 10 µl was injected to LC-MS/MS.

Chromatographic analysis

Calibration curve in matrix in the range 0 - 400 µg/kg was used to calculate the concentration in µg/kg of ZEA. The analyzes were carried out in LC-MS/MS Agilent Technologies 6440

Triple Quadrupole. Chromatographic separation on Synergy 4 µm- Hydro-RP 80A (150 x 2mm) (Phenomenex) column was achieved using isocratic elution of mobile phase A: 0.05mM ammonium acetate in 10% methanol (LC-MS/MS grade) and mobile phase B: methanol LC-MS/MS grade.

MS conditions

Flow rate 0.5 ml/min

Column temperature 50°C

Injection volume 10µl

Analysis time 17 minute

Ion transitions are reported in table 2:

Table 2. Ion transition

Retention time (min)	**Analyte**	**Transitions [m/z]**	**Collision energy [eV]**	**Polarity**
8.0 – 8.1	**ZEA**	**317.1->130,1**	**25**	**[M-H]**
		317.1->175,1	**20**	**[M-H]**
	$^{13}C_{18}$ ZEA	**335,2->185,2**	**26**	**[M-H]**
		335,2->291,2	**20**	**[M-H]**

Below is shown the Zearalenone peak from LC-MS/MS Agilent Technologies 6440 Triple Quadrupole:

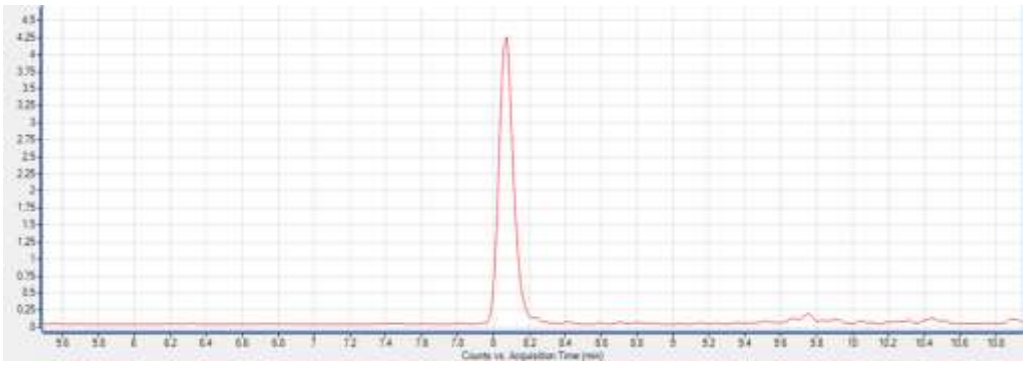

Figure 2. The Zearalenone peak area.

Statistical analyses

The data were statistically analyzed using Agilent Mass Hunter Quantitative Analysis and Excel 2013. Analysis of variance and simple regression analysis were also applied.

Validation procedure

The linearity, selectivity, accuracy, precision and sensitivity evaluated refer to the guideline COMMISSION REGULATION (EC) No 401/2006. (401/2006). Using an isotope labelled internal standard ($^{13}C_{18}$-Zearalenone), high recovery were achieved and the overall recovery was 99%. The Limit of Quantification (LOQ) value for ZEA was determined 25 µg/kg.

3. Results

In this study we analysed nineteen (n=19) ovine, caprine and swine liver samples during the year 2022. The samples were taken from different regions of Albania as shown in the table 3.

All samples were collected using official sampling methods.

Table 3. Liver sampling by location

Country	Ovine	Caprine	Swine	Compliant ≤ LOQ	Noncompliant ≥ LOQ
Diber	**1**				
Elbasan	**1**				
Fier	**1**	**1**	**1**		
Gjirokastra	**1**	**1**			
Korça	**3**	**1**		**19**	**0**
Shkoder	**1**	**1**			
Tirana	**1**	**2**			
Vlora	**2**				
Berat	**1**				
Total	***12***	***6***	***1***		

The results are not corrected for recovery, because the compound ZEA is quantified using an internal standard and a matrix matched calibration curve.

None of the samples were found to be above the LOQ 25 µg/kg.

The Zearalenone content in the liver was demonstrated that was below the LOQ for all the samples analyzed as show in figure 3.

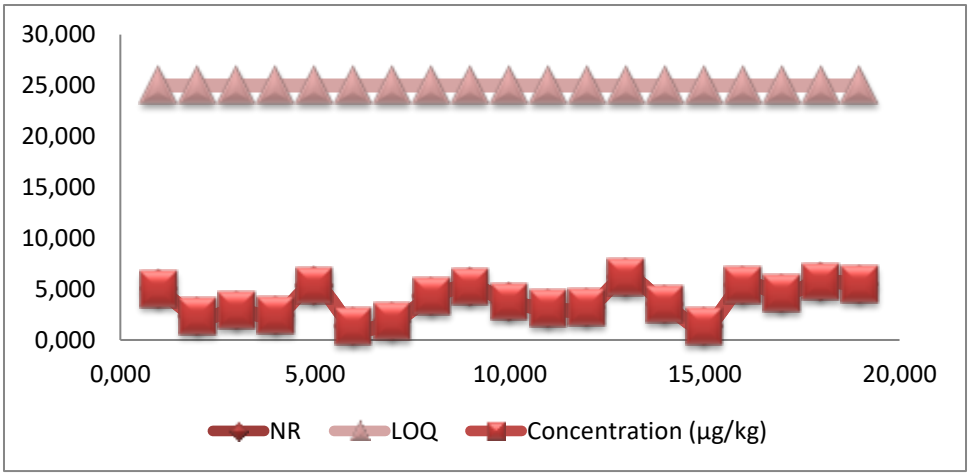

Figure 3. Concentration of Zearalenone in ovine, caprine and swine liver during 2022

4. Discussion

Nineteen ovine, caprine and swine liver samples were taken in the study during the year 2022. This LC-MS/MS method allows the selective determination of Zearalenone in animal liver. In combination with a one-step solid-phase extraction with sorbent C18, primary secondary amine, sodium chloride and magnesium sulphate sample clean-up it enables high sample through-put along with the possibility to identify the analyte and to quantify it.

Most international organizations and countries have set regulations for permissible levels in cattle origin foods, to protect consumers from these contaminants.

Guidance values for ZEA, for animal feed intended for sheep are 0.5 mg/kg, it has been recommended under Commission Recommendation 2016/1319/EC (EFSA 2017).

ZEA contamination in cereal based food and cereals, pose a significant health risk to animals worldwide and humans, possibly contribute to considerable economic losses (Rai et al., 2019).

Considering the consumer health risk, specific regulations for Zearalanone in food stuffs have been establish by European Union (EU). EU legislations defines that maximum permitted limits for ZEA should be 20 µg/kg in processed cereal foods, 100-200 µg/kg in unprocessed cereals, 75 µg/kg for processed cereals and 50 µg/kg in cereal snacks (EFSA 2011).

In previous studies, very limited reports have been documented for the presence of ZEA contaminating ovine, caprine and swine liver.

Based on recent data the most sensitive animal species the pig and taking into account comparisons between pigs and humans (Iqbal SZ, 2014), the Panel on Contaminants in the Food Chain has established a tolerable daily intake (TDI) of 0.25 ZEA mg/kg body weight per day was assumed for the consumer (EFSA 2011).

In cases where the samples were being cooked and eaten by consumers, the exposure would be small because if it is calculated as the daily intake, the value will be below 0.25 mg/kg the TDI reported by EFSA. (Paula LC, 2022)

Based on the current knowledge on carryover of Zearalenone from feed to liver, it can be concluded that foodstuffs of animal origin do not pose a significant risk for the consumer (Dänicke et al., 2015).

5. Conclusion

The method that we have developed in our laboratory was effective for the detection and quantification of Zearalenone residues in liver and could also be potentially used for detecting of this compound in other animal tissues with high sensitivity and precision.

The levels of Zearalenone found in this study indicate that the presence of this compound in liver is not hazard for public and animal health. The low levels of Zearalenone concentrations found in liver indicate that animal feed was not contaminated. But further studies should be carried out by analyzing a larger number of samples in order to have a continuous monitoring if there are concentrations of Zearalenone in liver. No carry-over factors from feed to liver were determined for Zearalenone. The Zearalenone contents in liver were demonstrated that was below the LOQ for all the samples analyzed.

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In sight on nutrition patterns and attitudes regarding coronary heart disease patients in Misurata

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Abstract

A healthy nutrition pattern and lifestyle modification should be an important part of therapy for cardiovascular diseases (CVD). A greater effort is required to manage by lifestyle, eating behavior modification and good nutrition practices with or without medication. A hospital-based study conducted in some Misurata hospitals to evaluate the dietary patterns and eating behavior among coronary heart diseases (CHD) patients, in addition to correlate the relationship between dietary practices, and eating habit with laboratory tests. The study has consisted of 100 (CHD) patients, who selected randomly during follow-up in 2019-2020. The data collection techniques are involved the food frequency questionnaire, patient file data, and anthropometric measurements. Pearson correlation used to examine variation and relationship between variables. The results revealed that, highly significant variation ($P<0.001$) of CHD patients whose drink full fat milk, soft beverage, fresh fruit juice, consume sweets, adding sugar to diet, eating fruit wholesome and non-followed a diet regimen that recommended by a dietitian. The result indicated that a significant correlation ($P<0.05$) of total cholesterol and high-density lipoprotein with olive oil intake, that according laboratory test. Majority patients found to be ignorant foods that reduce risk of diseases related to CHD, no therapeutic lifestyle change, poor dietary habits and insufficient application among food regimen towards reduce disease complications. The appropriate nutrition education in hospitals and patients must be referring to nutritionist for dietary counselling and advice.

Keywords: *Attitudes, Coronary, Dietary, Heart disease, Misurata.*

I. INTRODUCTION

Coronary heart disease is a type of heart disease that develops when the arteries of the heart cannot deliver enough oxygen-rich blood to the heart. It is the leading cause of death in the United States. Coronary heart disease often caused by the buildup of plaque, a waxy substance, inside the lining of larger coronary arteries, [1]. CHD is the leading cause of death in the United States (U.S.) indicates that every 25 seconds, an American will have a coronary event related to CHD, and every minute someone will die from CHD [2]. However, little information is known about their CHD knowledge and CHD risk factors despite estimations proclaiming that heart disease deaths will increase between the years 2010 and 2030 [3].

Non-communicable diseases forecasted to increase substantially in developing countries because of lifestyle transitions associated with increasing urbanization, economic development, and globalization. The Global Burden of Disease study cites diet as a major factor behind the rise in hypertension, diabetes, obesity, and other CVD components [4]. There are an estimated >500 million obese and close to 2 billion overweight or obese individuals worldwide. Furthermore, unhealthy dietary patterns have negative environmental impacts, notably on climate change [5].

Despite significant advances in our understanding of optimal dietary patterns to prevent CVD, additional research including large cohort studies of dietary patterns are needed in different regions of the world to address existing knowledge gaps. This includes evaluation of the impact of specific fruits and vegetables, types of dairy foods, type and amount of carbohydrate, optimal cooking oils, region-specific dietary patterns, and cooking methods [6].

Despite the growing numbers of Libyan in Misurata City, it considered a "hidden minority" because of the lack of research-based information on their health. It estimated that CHD kills more than half of Libya s in the Misurata city. Despite this, little known about their baseline knowledge, healthy dietary consumption patterns, therapeutic lifestyle and good eating behavior. Research shows individuals who are not aware of healthy dietary pattern for developing a disease are less likely to adopt preventive behaviors. Awareness of CHD and its risk factors are significant in preventing and reducing CHD deaths [7]. The aim of this study is to assess the socio-demographic characteristics, dietary pattern and eating behavior of CHD patient in addition to discover the correlation between eating habits and nutrition practices with blood lipid profile.

II. MATERIAL AND METHODS

A. Area of study

This is study was carried out in some hospitals of Misurata in Libya. Misurata is a city in the Misurata District in northwestern Libya, situated 187 km (116 mi) to the east of Tripoli and 825 km (513 mi) west of Benghazi on the Mediterranean coast near cape Misurata. With a population of about 550,000, it is the third-largest city in Libya, after Tripoli and Benghazi. It is the capital city of the Misurata District and it called the trade capital of Libya. It has lied at a longitude is 32 º.377533" N and Latitude is 15º.092017" E. It is located is at 7 meters' height, which is equal to 23 ft. above sea level.

B. Research Design

A hospital-based and cross-section study used to discover the relationship between dietary pattern, attitudes and eating behavior of coronary heart disease patients. The data collected used FFQ and patient file data most likely of CHD patient's investigation method.

C. Study population

All of the participants informed about the study purpose and signed the study consent forms. The study proposal approved by the ethical committee board of the department, and all procedures followed by the ethical standards of the Misurata University. The study interviewed about 100 (CHD) patients, who selected randomly from some public in Misurata City, Libya. The study was targeted all age groups and respected the gender issue.

D. Study duration

The study conducted from December 2019 up to April 2020. The duration distributed among designing of questionnaire, data collection, analysis and interpretation, and report writing.

E. Data collection procedures

1. Questionnaire

Well-designed questionnaire according to objectives of the study. Three parts of the questionnaire were compiled and face-to-face interviews of CHD patients in selected hospitals in Misurata. The first part of the questionnaire was included socio-demographic characteristics, the second part was included laboratory tests and the third part was included dietary assessment.

2. Anthropometric data

The anthropometric evaluation had done through BMI calculation using the procedure of anthropometric measurements and evaluation [8]

3. Laboratory tests

The blood lipid level have been recorded according to news test as mentioned in patient file. This to identify who would be included in the study.

F. Data quality management

A well-structured questionnaire was prepared according to study purposes and aims. A pre-test of the questionnaire done before actual data collection just to check its accuracy, response to analysis, and estimate which time needed.

G. Statistical analysis

SPSS (version 18) program used for data analysis. Descriptive statistical methods represented in the frequency and percent as well as Pie charts and histograms. Pearson Correlation used to study the relationship between variables. The relationship between the two variables is significant if P-value is less than 0.05.

III. RESULTS AND DISCUSSION

The study conducted to dietary pattern and eating behavior regarding coronary heart disease (CHD) patients, those were attended primary care services in selected hospitals at Misurata City, Libya. In addition, it was described the relationship between socio-demographic characteristics variables that influence attitude and eating practices.

A. The socio-demographic characteristics

The result of present study revealed that CHD are more prevalent within males 55% than females, 45% (table 1). This is finings similar to recent study stated that, CHD has be considered a disease of men. However, CHD is the leading cause of death both in men and in women [9, 10]. Meanwhile, the CHD reasons for gender variation is not clear; it might have attributed to the protective effect of estrogen [11]. The majority of patients fall within the age group 51-70 years old. This finding was closed to the study reported that 85% of CHD patients were at range 51-70 years [9]. While It is estimated that 82 percent of people who die of coronary heart disease are 65 and older at the same time, the risk of stroke doubles every decade after age 55 [12].

The education level of CHD patients, the majority patients were illiterates and primary education. That means, patients in low education level have facing constrains regarding health

care issues rather than educated people, who have more chance to reduce the causes and complications of CHD, throughout health diet and fellow therapeutic lifestyle.

The physical assessment of CHD patients is very important; the patients at normal range lower than overweight and obese. Obviously, the majority of patients were overweight and obese. Obesity is an independent risk factor for CHD. It is burden for hypertension, hyperlipidemia, diabetes, and impaired glucose tolerance. Those with central obesity have over twice the risk of heart attack [13].

TABLE 1. The socio-demographic of CHD patients

Variables	Groups	Percent
Age groups (year)	30 - 50	17%
	51 - 70	52%
	>70 years	31%
Gender groups	Male	55%
	Female	45%
Education level	Illiterate	41%
	Primary	21%
	Secondary	18%
	University	20%
Anthropometric measurements	*Normal range*	22%
	Overweight	56%
	Obese	22%

B. Dietary assessment of CHD patients

The study investigated about how many meals per day that ate by CHD patients (figure 1). The result found with irregular food intake, who eating one, two and more than three meals per day. The result of this study closed to that reported, the majority of CHD patients ate three meals per

day, and most of patients after discharge took their meals at home, because it was prepared according to their food habits [9].

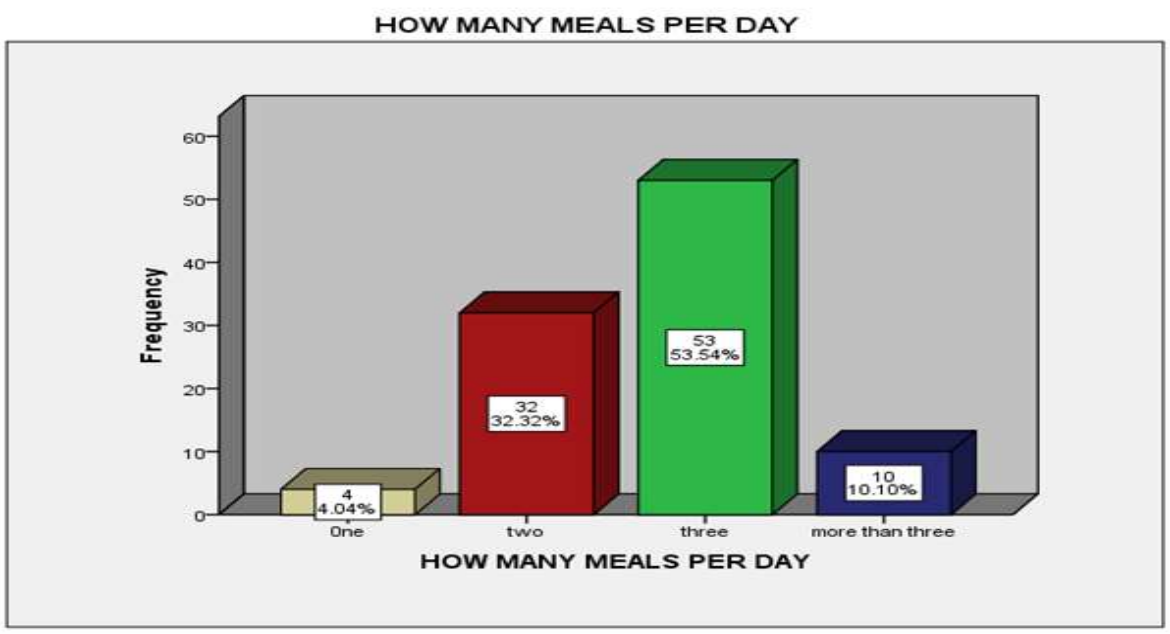

Figure 1. How many meals ate by CHD patients per day?

The cooking methods practices by CHD patients play importance role in causes and complications of CHD. The steaming methods for cooking more used by CHD patients in Misurata whereas, lower proportion of patients were used boiling and grill method for cooking (figure 2). The patient should be aware about risks of fry's foods, which increased risk of CHD. Cook food with healthy cooking method such as grilling, baking, roasting, steaming or stir-frying to reduce total fat (oil) intake in our diet [14].

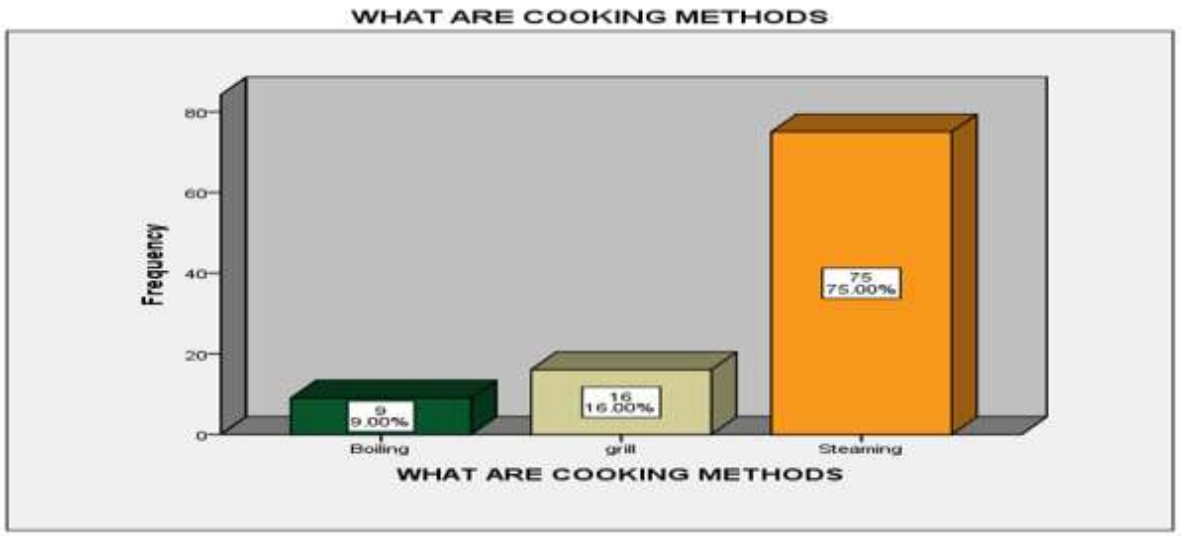

Figure 2. Food cooking methods by CHD patients?

The table (2) shows that the kinds of meat did ate by patient, the result shows that the higher proportion of patients were ate lean meat compared to unhealthy kinds of meat turned out. The patients who eat low-fat meat lower risk of coronary heart disease, and this good indicates of awareness about healthy diet. More than half patients consumed lean meat but high percentage of CHD patients consumed fatty meat containing high saturated fat and cholesterol. Meat is rich in protein, iron, zinc, and B vitamins, but can also contain significant amounts of cholesterol and saturated fatty acids, which raise LDL-C and lower triglyceride [15]. In more recent analyses, red meats, particularly processed red meats, were associated with increased CVD, CHD, stroke, and cancer mortality, whereas poultry was not [16].

Table 2. Kinds of meat do you eat?

Types of meat do you eat	Frequency	Percent
Fatty meat	24	24%
Lean meat	52	52%
Both (lean& fatty)	19	19%
Organs	5	5%
Total	100	100%

How many times per week do you eat fresh vegetable? This question gets different answers among CHD patients (table 3). Majority of patients had taken a fresh vegetable on a daily basis. As recommended for cardiovascular diseases to intake high fiber diet like fruit and vegetable to increase daily intake of fiber, that should be at least three serving of vegetables on daily bases [13]. A higher dietary fiber intake was associated with a lower risk of both coronary heart disease and all-cause mortality. For long-term intake, the strength of the association between dietary fiber and all-cause mortality decreased with increasing age [17]. Meta-analysis of 20 prospective cohort studies (16,981 stroke events) found that fruit and vegetable consumption was associated with decreased stroke risk [18].

Table 3. How many times do you eat fresh vegetable?

Eat fresh vegetable	Frequency	Percent
Daily	53	53%
4-6 times/week	7	7%
3-1times/week	31	31%
Occasionally	8	8%
None	1	%1
Total	100	100%

In table 4. The CHD patients were investigated about drink full fat milk, soft beverages, fresh juice, eating sweets, fresh fruit, adding sugar to diet and fellow up with dietitians, the result revealed higher significant difference ($p \leq 0.001$) between variables. It is bad dietary habits to know that majority of CHD patients drinking full fat milk, soft beverage and eating sweets, it seen these dietary practices should be raised blood cholesterol and increased risk factors for CHD. Meta-analyses reported that, increased low-fat dairy consumption is associated with lower LDL-C, triglycerides, plasma insulin, insulin resistance, waist circumference, body mass index, possibly blood pressure; and reduced diabetes risk [19]. While the positive dietary habits of those who eating fresh fruit wholesome, drink fresh juice and no adding sugar to their diet. The epidemiological relationships between sugar-sweetened beverages consumption, overweight, obesity, hypertension, and T2DM are strong [20]. In a meta-analysis of prospective studies of sugar sweetened beverages and hypertension, CHD, and stroke, the relative risk for a 1-serving increase in sugar sweetened beverages per day was significant for CHD, incident hypertension, but no clear effect was seen for total stroke [21]. The result demonstrated, the higher significant variation ($p \leq 0.001$) on almost patients do not followed a diet regimen with dietitian, whereas, little patients Followed a diet regimen with dietitian. It is seen that majority of CHD patients had not get advice and follow-up with dietitian.

Table 4. Dietary assessment about some ate by patients

Variables	Answers	Frequency	Percent	P-value
Drink full fat milk	No	13	13%	0.000
	Yes	85	85%	
	N/A	2	2%	

Drink soft beverages	No	45	45%	0.000
	Yes	51	51%	
	N/A	4	%4	
Eating sweets	No	50	50%	0.000
	Yes	49	49%	
	N/A	1	%1	
Eating fresh fruits wholesome	No	2	2%	0.000
	Yes	97	97%	
	N/A	1	%1	
Drink fresh fruit juice without sugar	No	36	36%	0.000
	Yes	62	62%	
	N/A	2	%2	
Adding refine sugar to diet	No	88	88%	0.000
	Yes	10	10%	
	N/A	2	2%	
Followed a diet regimen with dietitian	No	92	92%	0.000
	Yes	8	8%	

In table (5) shows the relationship between blood lipid profile and other variables, it is clear that no significant correlation ($p>0.05$) of blood lipid profile with eating chicken, eggs, fish and butter. A meta-analysis showed that eggs increase TC, HDL-C, and TC:HDL-C, but five recent studies subsequently reported that egg consumption did not significantly alter these parameters or endothelial function [22]. CHD risk factor related to cholesterol levels. Some studies suggested that 45% of heart attacks in Western Europe are due to abnormal blood lipids. People with low levels of HDL cholesterol have an increased risk of CHD [23]**.** That also indicated no significant correlation between blood fat levels with eggs, fish and butter consumption. While, there is a significant correlation ($P<0.05$) between total cholesterol level and high-density lipoprotein (HDL) of CHD patients with consumption of olive oil. Whereas, the p-value for other blood fat levels are greater than 0.05. The result of current study agreed to the findings suggested that the daily intake of extra virgin olive oil in hyperlipidemia patients could reduce

the susceptibility of low-density lipoprotein. A Mediterranean dietary pattern characterized by a high intake of olive oil (rich in oleic acid and antioxidants), fish [(rich in N-3) long chain polyunsaturated fatty acid)], vegetables and fruits has been associated with a lower coronary heart disease incidence and total mortality [24]. A replacement of saturated fat by monounsaturated fatty acid and increase in the consumption of fruits and vegetables to achieve proper antioxidant and folate status lead to decrease of coronary heart disease risk [12].

Table 5. Correlation between blood fat profile & other variables

Variables	Blood fat profile	(r)	P-value	Design
Eating chicken (with skin, without skin)	Total cholesterol	-0.07	0.493	No significant correlation
	HDL	0.122	0.228	
	LDL	0.023	0.820	
	Triglycerides	-0.053	0.630	

Eating egg	Total cholesterol	0.035	0.734	No significant correlation
	HDL	-0.074	0.469	
	LDL	0.147	0.150	
	Triglycerides	0.091	0.374	
Eating fish	Total cholesterol	0.011	0.913	No significant correlation
	HDL	0.060	0.560	
	LDL	-0.130	0.205	
	Triglycerides	0.125	0.222	
Add olive oil to the diet	Total cholesterol	-0.269	0.003*	Significant
	HDL	0.39	0.035*	
	LDL	-0.188	0.064	No-significant correlation
	Triglycerides	-0.031	0.759	
Add butter to the diet	Total cholesterol	-0.036	0.729	No significant correlation
	HDL	0.074	0.474	
	LDL	0.119	0.248	
	Triglycerides	.0150	.8850	

r= correlation, * P≤0.05, ** P≤0.001

IV- CONCLUSION

Obviously, many CHD patients consume foods that increase risk for CHD, no therapeutic lifestyle change; poor dietary habits and insufficient application among food regimen towards reduce disease complications. Their intake of full fat milk, soft beverages, food high in fat such as egg yolk, fatty meat, butter, sweets is a bad dietary habit. In this study, the majority of CHD patients 76% were overweight and obese, this indictor they are under the risk factor for hyperlipidemia, diabetes and impaired glucose tolerance. The result revealed that no significant correlation between blood lipid profile with consumption of chicken, eggs, butter and fish. Whereas, significant correlation between blood lipid profile and olive oil intake.

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