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"Future of Agriculture, Agriculture of Future"

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# AGRICULTURAL ECONOMICS



# GENERATION Z SHAPING URBAN AGRICULTURE DEVELOPMENT IN EMERGING MARKETS – INSIGHT FROM BOSNIA AND HERZEGOVINA

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### Abstract

Strong urbanisation trends followed by periodical food supply chain shortages/failures motivate (peri)urban population and initiatives to produce, process and deliver enough quantity and quality food. Besides, high-level policy processes and events/initiatives such as UN-HABITAT, FAO, Food for the cities, MUFPP, RUAF, PURPLE create enabling environment for urban agriculture initiatives, resulting in strong development and high attractiveness of urban agriculture and short food supply chains. One question arises, is UA policy-led development sustainable? The sustainability of UA future will strongly depend on the attitudes of young people, future consumers and activists which are facilitators of consumers' perception change that is necessary for stronger urban agriculture development. The youth population tends to be more productive, as well as more open to new ideas, they are greater risk-takers and therefore more innovative. Therefore, this study aims to identify attitudes, behavior and intention of youth people by using the Theory of Planned Behavior. Understanding the attitudes and motivation of this specific group will enrich the knowledge base and provide evidence to estimate urban agriculture opportunities and challenges shaped by consumers/market behaviour. At the same time, it will provide insights necessary to create better public policy that can give stronger impetus for urban agriculture development in emerging markets such as Bosnian and Herzegovinian.

**Keywords**: Urban Agriculture, Generation Z, Theory of Planned Behavior, Transitional Countries, Bosnia and Herzegovina



# IS THE DATA "NEW OIL" TRANSFORMING GLOBAL AGRIFOOD SYSTEM?

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### Abstract

Food value chains are currently very unsustainable systems that are recognized as responsible for ongoing air, land, and water pollution, with a high environmental footprint. It globally accounts for 70% to 90% of freshwater consumption, for a quarter of total GHG (greenhouse gas) emission, and 75% of global ammonia emission mainly from the use of fertilized land and animal waste. Agrifood emission is likely to increase 15% to 20% by 2050. Such unsustainable food production patterns are responsible for 70% of the reduction of biodiversity by 2050 and the loss of 10 to 15 million hectares of cropland annually caused by fertilization, pesticides, deforestation, and overirrigation. In addition, more than 30% of food is wasted across the food value chain, reaching 179 to 290 kg per capita annually in developed countries. All these negative impacts/outcomes of AgriFood system are driven by digitally-obsoleted organization and governance, which rely on linear "extract-makedispose" relations that do not promote information exchange, collaboration, and harmonization decreasing its capacity to face interconnected, very complex challenges. Consequently, regardless of digital/technology solutions, a timely, reliable, trustful, and accurate "product life cycle" information/data flow between and across all stakeholders is broken or even missing, which limits the capability of society and customers to push so needed transformation toward sustainable and resilient food value chains. Therefore, the main objective of this paper is to draw attention towards the importance of product life cycle information flow, as a key factor driving transformation toward Technology Enriched Agrifood System capable to open up business opportunities for both rural and urban actors providing environmental services and nutritious and affordable food for all.

**Keywords:** Information Flow, Transformation, Agrifood System, Improvements, Sustainable and Resilient Food Value Chain



### WHAT ARE THE MAIN DRIVERS OF CONSUMERS CONSUMING TRADITIONAL FOOD PRODUCTS? THE CASE OF GÜMÜŞHANE PESTIL

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### Abstract

Turkey is a country with a high potential for local or traditional food products and the utilization of this potential may provide significant gains to the Turkish economy. Traditional food production, which is a guarantee for consumers in terms of natural and healthy products and supports rural development and the development of local culture, is accepted as a global phenomenon today. This study investigates the antecedents of traditional food production consumption. Therefore, the aim of this paper is to clarify the main drivers for consuming traditional food production in Gümüşhane province which is in northeast of Turkey. The traditional food product included in this paper is "pestil" (dried mulberry pulp). A framework lean on characteristics of consumers purchasing traditional pestil and understanding their motives for it, is proposed. We can say that the traditional pestil speciality has a vital appeal that could be better reinforced with the drivers of consumer. Considering the data obtained from a survey conducted with 166 households in Gümüşhane, Turkey were used. According to findings of the data, the two most important factors influencing the consumers' decision to purchase Gümüşhane Pestil were the content of the pestil and its aroma. We also found that there were two different consumer segments to buy Gümüşhane Pestil. One of the consumer segments was the conscious traditional food consumers, who gave more importance to the variables that reflect the characteristics of the pestil. The other segment consumers were typical buyers who did not have enough awareness about the properties of traditional pestil. It can be concluded that conscious consumers of pestil, who attach importance to product value, spend more for it.

Keywords: Consumer, Drivers, Pestil, Traditional foods, Gümüşhane

### Introduction

Presently, the food sector is regarded as one of the most important in the current global economy (Guine et al., 2021). One of the important issues of the food sector is traditional foods. Traditional food requires a production system that preserves the use of original raw materials and sensory quality at every stage of production, without harming the characteristics of it. It can be said that,



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traditional products are the economic values that emerge as a result of a production process. In addition, traditional products are not only economic values, but also reflect the culture of that region. Understanding the main drivers and consuming behaviour with respect to traditional food is of significant importance. Depending on consumers' increasing consumption, the determination of their traditional food motives as well as their demands and expectations is very important in terms of monitoring and directing the development of the markets for these products. Some recent studies (Nystrand and Olsen, 2020; Garcia-Barron et al., 2021; Zhang and Jakku, 2021; Başaran, 2020; Guine et al., 2021; Roselli et al., 2018) highlight knowledge about nutrition and quality, perceptions, attitudes, willingness to pay, product characteristics and socio-economic factors as important for consumer's choices regarding traditional food. Consumers usually associate traditional food products with quality and associate them with tradition (Guine et al., 2021). At the same time, consumers also would like to buy healthier, more featured, more nutritive products. These attributes seem to be the most widespread attributes of traditional food products in general. Traditional foods' attributes or values are important to food choice in general (Steptoe et al., 1995). It is also critical to understand consumers' attitudes and opinions towards it (Funk et al., 2021). Since, consumers' preferences for traditional food attributes are complex, dynamic and multidimensional (Zhang and Jakku, 2021).

Turkey has a rich texture in terms of traditional food diversity and production. Economical production and marketing of local foods are also increasing in size. Among the traditional products such as sucuk, pastrami, yoghurt, tarhana, that are estimated to be around 2500 in Turkey (Onurlubaş and Taştan, 2017). This study aimed to present the main drivers of consumers towards traditional food product is called "pestil" (dried fruit pulp). In the past, pestil was consumed as a snack only by the local people in Gümüşhane province which is in the northeast of Turkey. Today it plays a vital role as an industrial product in the production sector creating added value in the province's economy and providing employment for many people (Dogan and Adanacioglu, 2021). We investigate for the first time the main drivers of consumers living in Gümüşhane province consuming pestil which is a traditional food product. We have identified the following hypotheses that needed to be tested among Gümüşhane consumers.

H1: Factors reflecting traditional product characteristics have a greater influence on consumers' decision to purchase Gümüşhane Pestil.

H2: Consumers who purchase the traditional Gümüşhane Pestil consist of different consumer segments.

H3: Consumers who care the product value are willing to pay or spend more for traditional foods such as Gümüşhane Pestil.

As far as we know, these hypotheses were not tested before. Therefore, it would be useful to find out if they are still valid to pestil as well, to consumers.



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### **Materials and Methods**

An online survey was addressed to Gümüşhane province consumers in June-September 2020. The sample consisted of 166 inhabitants of Gümüşhane, Turkey. The main material of the research consists of consumers over the age of 18 who voluntarily participate in the survey and consume pestil. National and international literature on the subject was also used. The sample was representative for the general population, regarding: age, gender, education, household size, income and occupation. The survey was conducted with the use of google form. Before the survey was applied, a pilot survey was conducted via mail system, the necessary arrangements were made and the survey form was reshaped.

Demographic characteristics information and purchasing behaviour of the consumers were presented with simple descriptive statistics. A 5-point Likert scale was used to determine the factors that affect consumers' decision to purchase traditional pestil. The reliability of the data was measured by conducting a reliability analysis for the answers given by the consumers. Cronbach's Alpha coefficient, which is the general reliability coefficient, was used to assess the reliability.

Clustering analysis was conducted by considering the Likert scale scores for each of the variables that may be effective in purchasing decisions. Consumer segments were determined by cluster analysis. For this purpose, the K-Means clustering method, which is one of the non-hierarchical clustering methods, was applied. In this method, similar observations are clustered one by one around the typical observation. In this clustering method, ANOVA type tests are used to look at the averages of the observations that make up each cluster according to the variables. This method is considered quite reliable. (Kalaycı, 2006).

### **Results and Discussion**

### **Demographic information of participants**

The research sample included consumers of different genders, ages, education levels, family size, family incomes and working status (Table 1).



Variable	Abbreviation	Explanation
Education Level	Education (edu)	Illiterate(1), Primary school(2), Secondary(3), High school (4), Pre
		degree (5), Undergraduate (6),
		Postgraduate (7)
Respondent's Age	Average Age (age)	Number of years
Working Status	Respondent's employment (emp)	1) working, 0) not working
Size of household	Household size (number of person) (hsize)	Number of persons in the house
Gender	Respondent's gender (gend.)	1) female, 2) male
Family income	Family monthly income (TRY)	≤ 2500 (1), 2501-3000 (2),
		3001-4000 (3), 4001-5000 (4),
		5001-6000 (5), 6001-7000 (6),
		7001-8000 (7), 8001-9000 (8),
		9001-10000(9)

Table 1. Variables used in the study

The demographic information of the consumers is given in Table 2. According to the table, the average age of the respondents was 35.19 years, the mean of education was 6.31 and this value corresponds to the undergraduate level as seen in Table 1 and the average number of the person in the household was 3.28 people. The same finding was obtained in the study by Wang et al. (2020). They found that the majority of the Chinese consumers (% 28.4) in their study was in undergraduate level. On the other hand, in the study of Başaran (2016), the majority of the consumers (% 48.7) was in high school level. In these studies, when we consider the variables of the average age and the household size, similar findings were identified as 30 years and 3 persons. In our study, the average gender was found as 1.76 which means nearly 2 and this value matches with the male variable. According to Table 2, most of the respondents were the working people and the monthly income of the respondents was between 7001-8000 TRY.

Table 2. Demographics of consumers

Items	Min.	Max.	Mean	Standard Dev.
edu	3.00	7.00	6.31	0.686
age	23.00	55.00	35.19	7.470
emp	0.00	1.00	0.98	0.134
hsize	1.00	7.00	3.28	1.265
gend	1.00	2.00	1.76	0.431
Monthly Income (Turkish Lira)	1.00	9.00	7.08	1.919



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### The underlying incentives for buying Gümüşhane pestil

The majority (75%) of respondents reported that the pestil they buy is as a souvenir or to consume in the household as it is shown in Table 3. The local description of pestil is a mixture of mulberry, honey, milk, and flour spread on cloth, and after drying, a high-nutrient fruit pulp is obtained (Gümüşhane Governorship Publications, 2010). In the past, pestil was consumed as a snack only by the local people in Gümüşhane province. According to this result it can be said that pestil was only used as a snack like in the past in the household by local people, but presently consumers buy it as a souvenir. Therefore, pestil can be also thoroughly recognized for local tourism.

Reason	n	%
Domestic consumption	9	5.4
Souvenir	30	18.1
Both	125	75.3
Other	2	1.2
Total	166	100.0

Table 3. The underlying incentives for buying Gümüşhane pestil

### Purchasing frequency of pestil in the examined consumers

Purchasing frequency of pestil is given in Table 4. It was seen that majority of the respondents (42.2%) bought pestil once a month and approximately 28% of them bought pestil twice a year. Based on this finding, it could be said that people living in Gümüşhane have a habit of buying pestil as souvenir or domestic consumption. It is clear that increasing the market of the pestil may trigger more willingness to buy it. Oraman et al.'s (2010) study stated the similar finding that majority of the participants (36%) bought Tekirdag Kofte which is a traditional food product once a month.

Items	n	%
Once a month	70	42.2
Once a year	22	13.3
Twice a year	46	27.7
Three or four times a year	6	3.6
Five times a year	1	0.6
Six or seven times a year	1	0.6
Other	20	12.0
Total	166	100.0

#### The amount and the expenditure of the pestil consumption

The questions "how many kilos of pestil do you buy in a year" and "how much do you spend for it in total" were asked to find out the pestil consumption of people who live in Gümüşhane province (Table 5). In considering the amount and the expenditure of pestil consumption of the respondents, it was seen that they purchased minimum 1 kg and maximum 60 kg in a year, the average amount



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of the pestil consumption in a year was found as nearly 12 kg. According to the information obtained from these consumers, they spent in total minimum 50 TRY and maximum 3000 TRY for the pestil in a year and the average expenditure was found in total as 472 TRY.

Statements	Min.	Max.	Mean	Standard Dev.
How many kilos of pestil do you buy in a year?	1.00	60.00	11.70	10.773
How much do you spend for the pestil in total in a year? (TRY)	50.00	3000.00	472.34	458.383
NA	4			
Total	166			

Table 5. The amount and the expenditure of the pestil consumption

### Place of purchase for the pestil

The interviewed consumers stated their place of purchase for pestil in Table 6. Concerning the purchase places, 55.4% of the respondents bought pestil from the factory outlets and approximately 40% of these consumers bought it from the shops which sold local food products in the research area. Third place in purchasing place was delicatessen with a share of 2.4%, while only small part bought pestil from supermarket and bazaar. According to this finding, it can be noted that local shops that sell local products or shops that sell pestil factory's products are the most popular places for the consumers to buy pestil. Thus, these places may positively affect consumers' pestil purchase. Contrary, in a similar study by Duru and Seçer (2012); Taştan et al., (2014), it was reported that the most important traditional food purchasing place of consumers was the supermarkets.

Table 6.	Purchasing	place	of pestil
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Items	n	%
Supermarket	3	1.8
Delicatessen	4	2.4
Local shops	66	39.8
Factory outlet	92	55.4
Bazaar	1	0.6
Total	166	100.0

### Factors affecting consumers' pestil consumption

In order to determine the factors that may be effective in consumers' pestil consumption decisions, the participants were asked about the importance of some possible variables. These are price, content, flavour, brand, transparency or colour of the product. The reliability of the data was measured by conducting a reliability test for the answers given by the consumers to these variables. In the analysis, the Cronbach's Alpha value, which is the general reliability coefficient, was found



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to be 0.714. Since this value is between  $0.60 \le \alpha < 0.80$ , the scale was found to be quite reliable (Table 7).

Table 7 Delighility statistics

	Table 7. Reliability statistics	
Cronbach's Alpha	Cronbach's Alpha based on standardized items	N of Items
0.714	0.724	5

The level of importance given by the consumers to the possible variables that may be effective in the consumption decisions are shown in Table 8. It was seen that the most important variable which was effective in the consumers' decision to consume pestil was the content. The fact that consumers gave more importance to the content of the pestil was due to the some features that distinguish Gümüşhane Pestil from the pestil produced in other regions. Unlike other regions, honey and milk are added to the content of Gümüşhane Mulberry Pestil. In addition, walnuts and hazelnuts are added in much larger amounts. At least 20 kg of honey, 15 kg of milk, 20 kg of walnuts or hazelnuts are used for 100 kg of Gümüşhane Pestil. (Turkish Patent and Trademark Office, 2004). The second and third most important variables affecting the consumers' decision to buy pestil were determined as the aroma and transparency of the product, respectively. Brand is the fourth variable in influencing consumers' decision. Price, on the other hand, was determined as the least affected factor.

Variables	Mean*	Std. Deviation
Price	2.90	1.052
Ingredients	4.15	.857
Flavour	4.07	.909
Brand	3.76	1.074
Transparency	3.83	1.112

Table 8. The importance level of variables in the consumers' purchasing decisions

\* mean importance scores given by respondents (1: not important-5: very important)

Consumer segments were determined by cluster analysis, taking into account the scores given by consumers for five variables. As a result of the cluster analysis, two segments appeared for the 166 consumers. The first segment includes 45 consumers and the second segment includes 121 consumers. ANOVA results revealed that there was a significant and important difference between the clusters (Table 9).



Variables	Cluster	•	Error			
	Mean Square	df	Mean Square	df	F	Sig.
Price	4.147	1	1.087	164	3.814	0.053**
Ingredients	32.753	1	0.54	164	60.707	0.000*
Flavour	51.203	1	0.519	164	98.712	0.000*
Brand	73.667	1	0.712	164	103.531	0.000*
Transparency	99.533	1	0.637	164	156.355	0.000*

Table 9. ANOVA Results

\* denotes statistical significance at 1% level, \*\* denotes statistical significance at 10% level.

The importance levels of the consumer segments to the variables in their decision are shown in Table 10. Accordingly, significant differences were found between the two consumer segments in terms of variables other than price. Consumers in the second segment gave more importance to the variables that reflected the characteristics of traditional pestil compared to the consumers in the first segment. Consumers in the second consumer segment represented a group of consumers who placed more value on the content of the pestil, its flavor, its transparency and its brand. For this reason, consumers in the second consumer segment are called as "consumers of traditional pestil". Consumers in the first consumer segment, on the other hand, can be named as "those who have little awareness of traditional pestil" or "those who tend to consume less traditional food".

Variables	Cluster 1 (45) Mean*	Cluster 2 (121) Mean*
Price	2.64	3.00
Ingredients	3.42	4.42
Flavour	3.16	4.40
Brand	2.67	4.17
Transparency	2.56	4.30

Table 10. The importance level of variables by consumer segments

\* mean importance scores given by respondents (1: not important-5: very important)

Traditional pestil consumers buy 11.93 kg per year on average, while those with low awareness about traditional pestil buy 11.06 kg. Although traditional pestil consumers consumed slightly more pestil, there was no statistically significant difference between the two consumer segments. On the other hand, there was a statistically significant difference between the two consumer segments in terms of spending on traditional pestil. Those who were less aware of traditional pestil spent 396.74 TRY per year, while this amount of expenditure was 499.66 TRY for traditional pestil consumers (Table 11). This result reveals that traditional pestil consumers are willing to pay or spend more because of the value they give to this product.



Statements	Cluster 1 (45)	Cluster 2 (121)	Mann-Whitney
	Mean	Mean	P value
How many kilos of pestil do you buy in a year?	11.06	11.93	.506
How much do you spend for the pestil in total in a year? (TRY)	396.74	499.66	.073*

Table11. The amount and the expenditure of the pestil consumption by consumer segments

\* denotes statistical significance at 10% level

### Conclusions

This study was carried out to determine what are the main factors affecting the consumption of traditional food products. The answer to this question was given through the example of "Pestil" produced in Gümüşhane province. Survey conducted with consumers in Gümüşhane province revealed that two important factors are more effective in consumers' decision to purchase pestil. These are the content and the aroma of the pestil. The survey conducted with the consumers shows us that the consumers who buy pestil are composed of two different consumer segments. One of the consumer segments is the conscious traditional food consumers who give more importance to the variables that reflect the characteristics of the traditional pestil. Consumers in the other segment are typical buyers who do not have enough awareness about the characteristics of pestil. However, it is possible to say that a significant part of the consumers interviewed in this study consists of conscious traditional food consumers. One of the most important results of this study is to reveal the purchasing behaviours of two different consumer segments of Pestil. According to results of this study, although there is not a significant difference between the consumption amounts of conscious consumers and the consumers who have low awareness, there is a significant difference in terms of spending amount. It has been determined that conscious traditional pestil consumers spend more for the pestil. This is due to the fact that consumers of traditional pestil attach great importance to product value. The result shows that consumers who attach high importance to product value are willing to pay or spend more for traditional foods.

The results of this study reveal that it is important to create marketing mix strategies that emphasize customer value in the marketing of traditional food products such as Gümüşhane Mulberry Pestil. In this context, the fact that Gümüşhane Mulberry Pestil is a product that receives geographical indication registration can be considered as an important advantage.

The survey results of this study lead us to the conclusion that there is a consumer segment that has little awareness about traditional mulberry pestil. As a matter of fact, it can be mentioned that an important part of the society does not have detailed information about traditional or local food products. Although geographical indication is taken for many of these products, it should be taken into account that there is a consumer community that does not know exactly what it means. In this



context, it is considered important to raise awareness of consumers about traditional food products such as Gümüşhane Mulberry Pestil.

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### ECONOMIC EVALUATION OF CORN SILLAGE PRODUCTION IN BOSNIA AND HERZEGOVINA

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### Abstract

Various fodder plants are used for the production of silage, and the best quality is obtained from corn, which is also the most common nutrient in animal production. Corn silage production in BiH is mainly concentrated on large agricultural holdings focused on cattle production, with a strong increase in production in the past period. Total sown area increased by 39.7%, silage production by 78%, and yield per ha by 27.4% in BiH for the period 2011-2020, but domestic needs for silage have not yet been reached. The study aimed to determine the economic effects of corn silage production in the BiH. Two farms were selected: (i) farm A - the private company "Farma Spreča" d.o.o. Kalesija, (ii) and farm B - public company "Poljoprivredno dobro Butmir" d.o.o. Sarajevo -Ilidža. Primary data for the survey were collected based on semi-structured interviews with farm representatives. For each farm, production and economic indicators of corn silage production in 2016 and 2017 were determined and analyzed. By making analytical calculations, the volumes and costs of corn silage production were determined for the total area (400 and 170 ha) and the area per ha, then the production values and indicators of financial result and profitability were determined. Research results indicate that even with a decrease in production volume, from 29.03t/ha to 25.03t/ha at farm A, and from 34.39t/ha to 30.23t/ha at farm B, the positive financial result was achieved (and increase) on both farms, from 583EUR to 732EUR at farm A, and from 1.030EUR to 1.053EUR on farm B. Efficacy indicator increases on farm A from 1.40 to 1.71, while at farm B slightly increases from 1.66 to 1.69. The results of the research show that the production of corn silage achieves positive financial results during the observed period giving the impetus to farmers to produce their inputs, and therefore better manage their agricultural holdings.

Keywords: Corn Silage, Farm Management, Economic Evaluation, Livestock Production

## **ANIMAL PRODUCTION**



### DETERMINATION OF NUTRITIONAL COMPOSITION, METABOLIZABLE ENERGY, DRY AND ORGANIC MATTER DIGESTIBILITY WITH NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIRS) IN CORN SILAGE

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### Abstract

This study aims to determine the nutritional composition, metabolizable energy (ME), in vitro dry and organic matter digestibility (DMD-OMS) in maize silage materials based on the measurements obtained from Near Infrared Reflectance Spectroscopy (NIRS) device. For this purpose, the corn silage samples used in the study were collected ten different dairy and beef farm in İzmir, were analyzed by conventional wet chemistry (CWC) and near infrared reflectance spectroscopy (NIRS). The relationship between estimates obtained from NIRS device and CWC values was statistically evaluated. The prediction values for the corn silage samples were satisfactory for the following characteristics; dry matter (DM), ash, ether extract (EE), crude protein (CP), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), sugar, starch, ME, in vitro DMD and OMD. The coefficient of multiple determination (R2), the value of the root mean square error of cross validation (RMSECV) and the value of the residual prediction deviation (RPD) of some FFPs were compatible. NIRS prediction of DM, ash, EE, CP, CF, NDF, ADF, sugar, starch, ME, in vitro DMD and OMD contents were more precise, with high R2 (0.89, 0.99, 0.90, 0.97, 0.99, 0.99, 0.99, 0.98, 0.94, 0.99, 0.99 and 0.98 respectively); low RMSECV (0.32, 0.06, 0.13, 0.04, 0.12, 0.18, 0.16, 0.01, 0.89, 0.08, 0.29, and 0.67 respectively); and high RPD (3.57, 14.6, 3.96, 6.19, 21.3, 23.8, 31.1, 7.99, 4.15, 7.19, 7.79, and 9.66 respectively). There was no statistical difference between WCM and NIRS for the same parameters. Statistically, there is no significant difference between the all parameters obtained by both methods and they are similar to each other. (P < 0.05). It has been demonstrated that the NIRS method is a fast, reliable and good estimation method for quantitatively determining the chemical composition, ME, in vitro DMD and OMD in corn silage.

### Introduction

Animal performance is affected by forage quality which in itself is determined by the chemical composition of the forage material. Knowledge of the chemical composition of different forages allows for the formulation of feeds to provide the correct balance of nutrients to meet an animal's nutritional requirements. The gross chemical composition indicates the amount of nutrients (principally energy and protein) that are available in both digestible and metabolisable form. In



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ruminants, this has traditionally been determined by regression models, where nutrient digestibility is predicted from gross composition data (Daccord et al., 2016), in vitro digestibility assay (Roughan and Holland, 1977) or in situ techniques using porous bags suspended in the rumen of fistulated animals (Huntington and Givens, 1995).

Roughages are the most abundant and inexpensive source of feed that are extensively used in ruminant feeding. The quality of the roughages is primarily indicated by the amount and composition of their structural carbohydrate contents. Specifically, the NDF and ADF fractions that form the cell wall of plants are important parameters determining their intake and digestibility. Various analyzes such as Weende analysis, Van soest analysis, in vitro ruminal and enzymatic techniques are used to determine the nutrient matter content of roughages. However, since chemicals are used in all these techniques, time and cost calculations are required to be made. However, these wet-chemical based methods can be time-consuming and expensive, therefore more rapid methods to estimate feed composition and digestible nutrient content have been developed. Studies continue to explore more economically efficient and less time consuming alternative methods in analyzing the chemical composition of the feeds and forages. Near infrared reflectance spectroscopy (NIRS) is one of the important alternatives studied on this subject. The NIRS method is an analysis method that calculates the nutrient content of feeds based on mathematical modeling without using chemicals (Goldman et al., 1987; Parrini et al., 2019; Ünal, 2005; Kellems and Church, 2010). Nearinfrared (NIR) spectroscopy has been widely used to predict gross chemical composition since the work of Norris et al. (1976) on nutrient digestibility, and the following publication of Barber et al. (1990). Most of the studies conducted have focused on general quality evaluations such as ADF, NDF and CP content. To best of our knowledge, there is no study on the calculation of the indexes used in the measurement of feed quality such as DMD and OMD with the values determined by NIRS and their association with the indexes calculated over the values obtained with reference analysis. In the present study, the use of (NIRS) spectroscopy to predict both corn silage chemical composition and in-vitro digestibility is investigated. Therefore, the aim of this study is to compare the wet chemical analysis results of DM, ash, CP, EE, NDF, ADF, starch, sugar, ME<sub>nc</sub>, DMD and OMD values of corn silages with NIR analysis results, and to determine whether the chemical composition and digestibility of corn silage can be determined by NIRS.

### **Materials and Methods**

Ten different corn silage samples were taken from different dairy and beef cattle farms in İzmir. The samples were taken fresh and weighed 0.5 kg from farms and brought to the laboratory in airtight bags and kept at -20°C until analysis. Wet corn silage samples with known weights were dried in the oven for 48 hours at 60°C until their weight did not change and the dry matter level was determined. Air-dried and weight known feed samples were dried in the oven at 105 ° C for 4 hours and the dry matter level was determined. The chemical analyses were carried out at the Ege University, Faculty of Agriculture, Department of Animal Science and Animal Feed Chemical Analysis Laboratory. Chemical analyses were performed in triplicate. Dried feed samples were



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ground to pass through a 1-mm screen using Cyclone Mill Twister (RETSCH, Haan, North Rhine-Westphalia, Germany) grinder. The experimental samples used in the study were analyzed according to the methods reported in AOAC (1997). All samples were analyzed for dry matter (DM) (method 934.01), ash (method 942.05), crude protein (CP) (method 990.03), ether extract (EE) (method 920.39), and crude fiber (CF) (method 962.09). The sugar content of the materials was determined by the Luff-Schoorl method (EC, 2009), and starch was determined by the polarimetric method. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined as described by Van Soest et al. (1991). Estimates for ruminant metabolic energy (ME) as kcal/kg in DM were based on crude nutrients (protein, fiber, and fat levels) determined from the samples using a prediction equation (TSI-961, 1991); CP, EE, and CF quantities in organic matter (g kg<sup>-1</sup>).

### ME kcal kg<sup>-1</sup>:3260+(0.455xCP+3.517xEE-4.037xCF)

Dry matter digestibility (DMD), and organic matter digestibility (OMD) in vitro were determined according to cellulase technic method (De Boever et al 1986 modifed from Tilley and Terry, 1963).

NIRS analysis was done with a Bruker MPA (Multi-Purpose Analyzer; Bruker Optics, Germany) using OPUS spectroscopy software statistic version 7. Reflectance data was recorded over the wavenumber range of  $1200 - 2000 \text{ cm}^{-1}$  with 64 scans per spectrum and a spectral resolution of 4 cm<sup>-1</sup>. The acquisition time for a single spectrum was 66 s. Background spectra were collected with no samples present on the crystal, and under the same experimental conditions. Beam alignment was checked before collection. For every sample three spectra were collected to determine repeatability and to determine if there were issues from the finite particle size of the sample. The average of these three spectra for each sample were subsequently used for the statistical analysis.

The statistical analysis of the results included T-test to compare CWC and NIRS values using the SPSS 15 (IBM SPSS Statistics 15.0, 1997). The optimum number of components in the regression model was determined based on the values of root-mean-squared error of cross-validation (RMSECV). The optimum number of components was the smallest value that gave a RMSECV within one standard error of the lowest RMSECV value. RMSECV was calculated using equation (Chen et al., 2008). The selected model's performance was determined for the calibration, crossvalidation and external validation datasets. A robust model should produce similar values of evaluation metrics between the calibration, cross-validation and external validation settings. Prediction of CWC values from RMSEE were done using regression after correlation analysis to include the correlated parameters into the estimated model. Calibrations and predictions sets were developed by means of partial least-squares (PLS) regression (OPUS/QUANT software, Bruker, Germany). PLS is the appropriate multivariate calibration technique to avoid the problem of the very high intercorrelation between absorbances. Differences were considered to be significant based on the 0.05 level of probability.

### **Result and Discussion**

Two general types of feed assay, chemical sometimes called "conventional wet chemistry", and near infrared reflectance spectroscopy (NIRS) were compared in this study for corn silage. Table 1



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showed that the values of corn silages samples obtained from NIRS were similar to CWC values for DM, ash, EE, CP, CF, NDF, ADF, starch, sugar, DMD, OMD, and ME<sub>nc</sub> parameters.

	WCM			NIRS			
%	Min	Max	Mean±SD	Min	Max	Mean±SD	Р
DM	93.77	96.21	95.16±0.22	93.60	96.57	95.20±0.29	0.396
ash	4.97	7.43	$6.26 \pm 0.20$	4.99	7.23	6.23±0.19	0.927
EE	2.24	3.63	$2.80\pm0.11$	2.24	3.64	$2.82 \pm 0.11$	0.940
СР	8.42	9.67	9.09±0.10	8.38	9.61	8.99±0.11	0.666
CF	19.32	28.01	$23.72 \pm 0.67$	19.21	28.00	$23.19 \pm 0.70$	0.896
NDF	43.42	57.76	51.31±1.11	41.38	56.75	49.49±1.23	0.711
ADF	28.28	44.53	$36.45 \pm 1.28$	27.27	43.71	35.12±1.31	0.869
Starch	17.07	28.88	$20.88 \pm 1.06$	20.50	30.22	$24.80 \pm 0.94$	0.935
Sugar	0.71	1.05	$0.91{\pm}0.02$	0.56	0.96	$0.78 {\pm} 0.03$	0.799
DMD	52.53	65.38	$58.10{\pm}1.17$	51.98	66.79	$58.45 \pm 1.30$	0.838
OMD	42.19	61.09	50.71±1.65	43.44	61.28	51.33±1.53	0.844
MEnc kcal/kg	2006	2448	2239±34	2001	2496	2256±38	0.788

Table 1. Chemical composition and nutritive value of corn silages, dry matter basis (%)

The average DM content (%) were obtained 95.16 for WCM, 95.20 for NIRS; ash contents (%) were obtained 6.26 for WCM, 6.23 for NIRS; EE contents (%) were obtained 2.80 for WCM, 2.82 for NIRS; CP contents (%) were obtained 9.09 for WCM, 8.99 for NIRS; CF contents (%) were obtained 23.72 for WCM, 23.19 for NIRS; NDF contents (%) were obtained 51.31 for WCM, 49.49 for NIRS; ADF contents (%) were obtained 36.45 for WCM, 35.12 for NIRS; starch contents (%) were obtained 20.88 for WCM, 24.80 for 24.80; sugar contents (%) were obtained 0.91 for WCM, 0.78 for NIRS. Dry matter digestibility of corn silage samples was obtained 58.10 % for WCM; 58.45% for NIRS. ME<sub>nc</sub> value of corn silage samples were obtained 2239 kcal/kg for WCM, 2256 kcal/kg for NIRS.

The findings obtained from our study were similar to the findings of other studies performed with NIRS (Pehlevan and Özdoğan 2015, Atalay and Kahriman 2020; Despal et al.2020). In the study conducted by Pehlevan and Özdoğan 2015; it was determined that there is a statistically significant difference between the crude protein, ether extract and NDF values obtained in cotton leaves according to NIRS and chemical analysis methods. It was observed that there is a statistically significant difference between the crude protein, ether extract, ash, NDF, ADF values obtained according to NIRS and chemical analysis methods in carob (Pehlevan and Özdoğan, 2015). With the NIRS method, it was emphasized that maize fractions in different physiological maturity gave acceptable estimates of low and medium crude protein and net energy level with acceptable accuracy, but did not give an acceptable estimation about the continuous high level of crude protein and net energy level (Volkers et al., 2003). Different studies (Lovetta et al., 2004; Lovetta et al.,



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2005) showed that the NIR method can only be as good as the reference method used, and that Nearinfrared (NIR) spectroscopy also has significant potential in the rapid assessment of maize silage, but the predictive accuracy of the model depends on the accuracy of reference samples, the selection of the regression technique used and the sample preparation procedures. Although our study is not a calibration development study, the findings suggest that similar situations may occur in calculations based on the results obtained from a commercial NIRS calibration.

Spectral data had significant differences according to sample type (Atalay and Kahriman 2020). This may be attributed that the sample composition and moisture content. Spectral plot has higher peaks region around at 1450 nm and 1940 nm. These regions are well-known wavelengths related to moisture content in biological samples within scanning interval (Manley, 2014). On the other hand, physical and chemical composition of the sample has important effect on the spectral data/plot obtained from the NIR measurement (Pasquini 2003). The same samples can give different spectroscopic information due to differences in their particle size, moisture content as well as other biochemical compounds such as protein, ether extract and starch content. In the study used roughage samples in the forms of "dry basis" and its biochemical composition show high stability. We must notice that this case must have strong effect on the obtained results from NIR measurements and of course prediction results.

	Calibration (n=30)			Valida	tion (n=30)	
	$\mathbb{R}^2$	RMSEE	RPD	$\mathbb{R}^2$	RMSCEV	RPD
DM	0.99	0.01	223.00	0.89	0.32	3.57
ash	0.99	0.01	137.00	0.99	0.06	14.6
EE	0.99	0.04	22.50	0.90	1.13	3.90
СР	0.99	0.01	297.00	0.97	0.04	6.19
CF	0.98	0.56	8.32	0.99	0.12	21.30
NDF	0.99	0.29	19.80	0.99	0.18	23.80
ADF	0.95	0.26	4.93	0.99	0.16	31.10
Strach	0.99	0.01	239.00	0.94	0.89	4.15
Sugar	0.99	0.02	11.1	0.98	0.01	7.99
DMD	0.90	0.54	3.21	0.99	0.29	12.90
OMD	0.99	0.03	132.00	0.98	0.67	8.63
MEnc kcal/kg	0.99	16.50	11.80	0.99	9.13	12.40

Table 2. Coefficients of determination and calibration  $(R_c^2)$  and validation  $(R_v^2)$ , RMSEE, RMSCEV, and RPD index obtained by PLSR to predict the chemical composition and the nutritive value in corn silages

An effort to improve the accuracy of measurement by adjustment of NIRS values was made by using a prediction model regression from the correlated parameters. The results of correlation test between CWC and NIRS data are shown in Table 2. The correlation coefficients between related calibration and validation parameters were high ( $R \ge 0.90$ ) for DM, ash, EE, CP, CF, NDF, ADF, starch, sugar, DMD, OMD, and MEnc parameters. The results of this study have revealed that NIRS



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method can be used in the quantitative determination of DMD, OMD and ME<sub>nc</sub> values of corn silage. It was determined that two general types of feed assay (WCM and NIRS) used determination corn silage nutrition composition had not a significant effect on estimation results, and both methods gave the same results. Similar results were reported a previous study (Vander Schaaf, 2013) focused on the comparing the prediction performances of desktop and portable (the same instrument in here) NIR instruments for analyzing the components of corn silage and alfalfa hay. This study showed that two instruments gave similar results for dry matter (r=0.86) but no strong correlation for other components such as protein, ash, and fat content. Study results in agreement in this finding and we could associate this result with the measurement capacity of the devices and the type of sample used. The portable device used in this study has a short scanning interval compared to desktop NIRS instrument and it has also low scanning intensity. Therefore, desktop instrument may give similar results to ref analyses.

### Conclusions

The results indicated that the NIRS method can be used to quantitatively determine the chemical composition, DMD, OMD and  $ME_{nc}$  values of corn silage. It was determined that the type of analysis method used did not have a significant effect on the estimation results.

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### IMPORTANCE OF FLEA CONTROL IN GOAT FARMS: THE CASE OF BORNOVA DISTRICT OF IZMIR PROVINCE

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### Abstract

In this study, it was tried to determine the damage caused by fleas to both humans and animals in sheep-goat breeding enterprises in five villages (Kurudere, Kayadibi, Yaka, Eğridere, Karaçam) in Bornova district of İzmir province. Among those who are registered with Bornova District Directorate of Agriculture and who want to take part in this project on voluntary basis, they have been selected to be at least 10-20% of the total number of livestock enterprises in the village. For this purpose, in addition to a survey study containing approximately 71 questions, possible situation determination was made with photography and sampling. It has been determined that 71.79% of the enterprises are family type and 61.54% have a closed shelter. Goat and sheep are bred together in 69.23% of the enterprises. It was determined that 84.61% of the sheep and goat farms had flea problems, and 94.87 % of them caused animal deaths from fleas. Fleas were mostly seen in kids (33.33%) and adults (28.21%). The first place among the symptoms of fleas; itching + hair loss (20.51%). Body condition score (48.72% and 41.03%, respectively) in the herd is poor in both adult and young animals. Flea control was the most common practice by removing manure from the shelter (38.46%), followed by liquid pesticides (17.95%). In most of the enterprises (89.74%), flea problems were also detected in cats or dogs. As a result, studies of external parasites, which have negative effects on both human and animal health, should be given importance especially epidemiologically in sheep breeding. In the case of small cattle breeding practices, hygiene and sanitation, animal welfare, and health protection practices, the damage caused by external parasites will be relatively reduced. Effective control methods to be selected by accurately determining the ectoparasite species and their hosts will also make significant contributions to the control of some zoonotic diseases.

Keywords: Flea Prevalence, Goat Farms, Control Methods, Host, Flea Symptoms



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### Introduction

Fleas are blood-sucking arthropods, infecting a variety of mammals and birds, including humans (Joseph et al. 1984; Halos et al. 2014; Hastriter et al. 2018). The most important ones in the field of medicine and veterinary medicine are some species belonging to the Pulicidae (Pulex irritans, Xenopsylla cheopis, Ctenocephalides canis, Ctenocephalides felis), Ceratophyllidae (Nosopsyllus consimilis, Nosopsyllus fasciatus), and Leptopsyllidae (Leptopsylla segnis) family (2008). Although various studies have been conducted on flea species in goats raised in different parts of the world (Kusiluka et al. 1995; Yakhchali and Hosseine, 2006; Gracia et al. 2012), there is little research on this subject in our country (Yılmaz et al. 2017). An intense flea infestation can cause significant anemia as well as lamb and kid mortality (Fagbemi, 1982). Sheep and goat production has an important place in the Turkish economy (Ertuğrul et al, 2010). In the absence of necessary health controls, many parasitic diseases occur in animals and cause economic losses. Among these parasitic diseases, external parasites have an important place. It has been reported that severe itching, hair loss, acanthosis, hyperpigmentation, and hyperkeratinization in advanced cases occur in animals due to the bites of fleas, while pulicosis is generally formed in humans (Unat et al. 1995; Yeruham et al. 1996, 1997, 1999). External parasites; It causes skin diseases, especially in sheep and goat farms engaged in extensive breeding, an increase in mortality in young and adult animals, a decrease in yields, and some reproductive defects. These diseases and skin defects also mean significant economic losses for some African and Asian countries exporting sheep and goat leather due to quality defects in leather (Yacob, 2013). This study, it is aimed to investigate the causes of flea density in goat breeding enterprises in Bornova district of Izmir province, to develop solutions and to determine the economic losses caused by fleas.

### Material and Method Farm chosen

Five villages located in the rural areas of Bornova district of İzmir province and from the enterprises in these districts registered with the District Directorate of Agriculture (Darm Record System). They want to take part in the project on a voluntary basis; the number of enterprises will be selected to constitute at least 15-20% of the total livestock farms in the district. In addition, the locations (latitude, longitude, and elevation values) of these sample business centers will be determined with the help of a GPS device.

#### Survey study

In the study, in the enterprises determined based on the breeder records of the Bornova District Directorate of Agriculture; A questionnaire study consisting of questions including land, animal wealth and care-feeding-housing-health-protection practices, and pasture and manure management will be conducted by face-to-face interviews. There are 71 questions in total in different categories in the survey.



### Sample size

There is approximately 8400 head of sheep and goats in the Bornova district of İzmir province. Among the five villages, only the enterprises where these animals are intensively reared have been determined, and homogeneous material will be collected by sampling at least 10-20% of the total animal assets from each farm.

### Results

In the research, the findings of the farms, the owner, herd management, and flea control methods were discussed in order and summarized in tables.

### **Results for farms**

The frequency distributions of the enterprises are given in Table 1. 71.79 % of the enterprises are family type and 61.54% have closed shelter. A significant part of the enterprises produces combined (64.10 %) and sacrificial (35.90 %) production. As a species, mainly sheep and goats (69.23 %) are raised. While a significant part of the farm owners does not own a land (82.05 %), the existing lands are also capable of doing a significant dry farming (79.48 %).



Traits	Groups	n	%
Earran tarra a	Family	28	71.79
Farm type	Commercial	11	28.21
	Family	34	87.17
Labor force	Temporary	5	12.83
Labor force	Permanent	-	-
	Other	-	-
	Closed	24	61.54
	Open	2	5.13
Housing type	Semi-open barn	9	23.08
	Shelter	4	10.25
	Other	-	
	Milk	-	
	Slaughering	-	
Branch of	Breeding	-	
production	Combine	25	64.10
1	Sacrificial	14	35.90
	Other	-	
	Goat		
	Sheep	5	12.82
Livestock	Cattle	7	17.95
LIVESTOCK	Sheep +Goat	27	69.23
	Poultry	-	-
	Other	-	-
	Own capital	30	76.92
Source of	Bank credit	-	-
	Own capital +bank credit	9	23.08
capital	Incentive credit	-	-
	Other	-	
	No	32	82.05
L and accept(da)	Ownership land	7	17.95
Land assset(da)	Rental	-	-
	Other	-	-
L and true -	Irrigable (da)	8	20.52
Land type	Dryland (da)	31	79.48

Table 1. Frequency table for farm characteristics

### Farm owner knowledge

In the study, information about animal farms is given in Table 2. In the research, a significant portion of the farm owners (87.17 %) is male. As an age group, 56.412 % are in the 41-60 age range. When the education level is examined, 43.59 % of them are primary school graduates. In general, the owners of the farms answered that they are farmers (43.59 %) and the main reason for keeping livestock is their main source of income (76.92 %).



Traits	Group	n	%
Gender	Male	34	87.17
Gender	Female	5	12.83
	<20	-	-
A	21-40	12	30.77
Age	41-60	22	56.41
	>60	5	12.82
	Illiterate	2	5.13
	Primary school graduate	17	43.59
	Primary school dropout	5	12.82
	High school	6	15.38
Education level	High school dropout	5	12.82
	Vocational school	3	7.69
	University graduated	1	2.56
	Farmer	17	43.59
Occurry	Self-employment	11	28.21
Occupy	Retired	10	25.64
	Other	1	2.56
	Basic income source	30	76.92
	Additional income	9	23.08
Reason for	Hobby	-	-
farming	Hobby + additional	-	-
	income	-	-
	Other		
	<5	4	10.26
Year for	6-10	9	23.08
	11-20	19	48.72
farming (year)	21-30	5	12.82
	31 >	2	5.12

Table 2. Frequency disturbance for ownership (%)

### **Flock management practises**

Practises for herd management in goat farms are given in Table 3. Animals (sheep and goats) are kept together (84.61 %) in the enterprises. When the way the breeders market their animal products is examined, the dairy farmer (58.98 %) has priority, followed by their marketing method with 25.64 %. Since irrigated farming, opportunities are limited and they do not have enough lands to produce animal feed, they supply both roughage (43.59 %) and concentrated feed (38.46 %) in the form of purchase. In a significant part of the farms, dogs (48.72 %) are raised in terms of grazing and security. There are other farms (76.92 %) near the goat farms and their distance (less than 500 m) is less (74.36 %).



Traits	Group	n	%
Are sheep and goets housed together?	Yes	33	84.61
Are sheep and goats housed together?	No	6	15.39
	Owni	10	25.64
Animal product marketing method	Cooperative	6	15.38
	Dairy	23	58.98
	Completely own	8	20.51
Poughaga supply method	Some own+some purchase	12	30.77
Roughage supply method	Comletely purchase	2	5.13
	No feed	17	43.59
	Completely own	3	7.69
Concentrate feed supply method	Some own+some purchase	12	30.77
Concentrate feed supply method	Comletely purchase	9	23.08
	No feed	15	38.46
	Own	-	-
Vaccines	Special veterian	12	69.23
	State veterian	27	30.77
	Dogk	19	48.72
Type of animal raised in the farms	Cat	13	33.33
	Poultry	7	17.95
Is there a livestock farm near the farm?	Yes	30	76.92
is more a nyestock farm hear the farm?	No	9	23.08
If yes, is this farm near you?	Close	29	74.36
	Very close	10	25.64

Table 3. Some practices of herd management in goat farms

### Flea control

The findings regarding the fight against fleas in the study are given in Table 4. Significant parts of the enterprises (84.61 %) have a problem with fleas. Fleas are mostly seen in summer (48.72 %) and spring (33.33 %) seasons of the year. There are animal deaths caused by fleas and their rate is (94.87 %). While the age group of animals in which fleas were most common was juveniles with 33.33 %, adults with 28.21 % followed it. The incidence of fleas in animals of all age groups is 7.69 %. The region where fleas are most common from the animal body is the back with 23.08 %. This is followed by the feet with 17.95 % and the rate of incidence in the whole body was 15.38 %. The least common area is the genital area. Itching+hair loss was the first among flea symptoms with 20.51 %. This in order; Itching was followed by 17.95 % and weakness 17.95 %. The most commonly used method in controlling fleas was removal of manure from the barn (38.46%), followed by the application of liquid pesticides with 17.95 %. The rate of those who use more than one method is 10.26 %. In a significant part of the enterprises (76.92 %), fleas were seen again after the struggle. The body color of the animals with the most fleas was black (33.33 %), followed by brown with 25.64 %. The incidence of fleas in animals of all colors is 12.82 percent. Both in livestock enterprises with fleas and in young animals (48.72 %). In the study, the fight against fleas in livestock enterprises and the answers to the questions asked about this subject are given in Table 4.



Traits	Group	n	%	Traits	Group	n	%
Is there a flea problem in the flock?	Yes No	33 6	84.61 15.39	Do cats or dogs have flea problems??	Yes No	35 4	89.74 10.26
In which season of the year are fleas most common??	Summer Autum Winter Spring Every season	19 5 2 13 -	48.72 12.82 5.13 33.33	Flea control method	Spray Powder Gas or diesel dripping painting board equipment Manure dispoasal Injection Using multipky methods	7 4 2 1 15 6 4	17.95 10.26 5.13 2.56 38.46 15.38 10.26
Do you have a dead animal caused by fleas?	Yes No	37 2	94.87 5.13	Does the flea problem reappear after spraying?	Yes No	30 9	76.92 23.08
In which age group are fleas most common?	Kids Milking Young goat Pregnant goat Goat All	13 3 4 5 11 3	33.33 7.69 10.26 12.82 28.21 7.69	Body color of the animal with fleas	White Grey Brown Black Every color	3 8 10 13 5	7.69 20.52 25.64 33.33 12.82
The area where the flea is seen on the animal's body	Foots Back Ear Barrel Genital organ Udder and its circumference Armpit Tüm vücut	7 9 2 5 1 4 5 6	17.95 23.08 5.13 12.82 2.56 10.26 12.82 15.38	Body condition of young animals in the herd	Bad Moderate Good	19 16 4	48.72 41.03 10.25
Flea symptoms	Itching Hair loss Lesion Anemia Zayıflık Itching+hair loss Hair loss +lesion	7 6 5 3 7 8 3	17.95 15.38 12.82 7.69 17.95 20.51 7.69	Body condition of goats in the herd	Bad Moderate Good	16 20 3	41.03 51.28 7.69
Is there a cat or dog in the farm?	Yes No	33 6	84.62 15.38	Do you take a deep or foot bath for fleas?	Yes No Sometimes	5 32 2	12.82 82.05 5.13

Table 4. Frequency distribution of flea control methods (%)

### Manure management

In the study, information on manure management of goat farms is given in Table 5. It was determined that a significant part of the enterprises (82.05 %) had litter material. As litter material, mostly soil (23.08 %), straw (20.51 %) and straw (17.95%) constitute the other important bedding material used. The main reason why the specified material is not used as bedding; it is cheap (71.29 %). In goat farms, manure is generally made by hand shoveling (94.87 %). Manure management is



generally done daily (51.28 %) and weekly (35.89 %). In the enterprise, manure is stored in the form of heaps (53.85 %) on the soil. All of the manure obtained is sold (69.23 %).

Traits	Group	n	%
Is there any litter material?	Yes	32	82.05
	No	7	17.95
Used as litter material?	Cut straw	8	20.51
	Straw	7	17.95
	Wood shaving	3	7.69
	Burned manure	5	12.82
	Soil	9	23.08
	Concrete	4	10.26
	Rocks	3	7.69
Reason for choosing litter material	Cheap	28	71.79
	Easy supplied	11	28.21
	Other	-	-
Manure collecting system	Handle scraping	37	94.87
	Otomatic system	-	-
	Tractor	2	5.13
Manure collecting frequency	Daily	20	51.28
	Weekly	14	35.89
	Montly	4	10.26
	Seasonaly	1	2.57
Manure storaging method	Hiping on the soil	21	53.85
	Open manure pit	12	30.77
	On the field or farm near	6	15.38
Manure evaluating type	I am all selling	27	69.23
	I use on the my field	12	30.77

Table 5. Barn manure management frequnces disturbance for goat farms (%)

### **Discussion and Conclusion**

In this study, the findings of the importance of flea, an external parasite in sheep and goats, and the methods of controlling them were examined. In the study, the incidence of fleas in goat farms was 89.74%. This rate is 52.2% in enterprises where both species (sheep and goats) are together. When the characteristics of determining the flea problem of the enterprises are examined, the findings are similar to the findings of Kandemir et al (2015) in the İzmir region, Koyuncu et al (2006) in the Çanakkale region, and the results of the goat farming operations in the provinces of Taşkın et al. Similarly, the fact that Madeira et al. (2000) found higher values in their study in Sao Paulo-Brazil as well as Abdullahi et al. As in this study, as the herd size increases and the housing and pasture conditions deteriorate in livestock holdings with the extensive production system, the incidence of external parasites increases relatively (Kusiluka and Kambarage, 1996). The incidence of fleas in family-type businesses was higher especially in young/puppies (33.33%) than in adults



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(28.21%). However, the incidence of fleas in adult animals in commercial enterprises is higher than in relative family-type enterprises. The reason why fleas are more common in young animals in family-type businesses; grooming or self-cleaning behavior is less than adults (Pegram et al. 2004). On the other hand, Lehman (1993) stated that young animals have more parasite problems than adults do, which is due to the low licking/grooming behavior as well as the relatively high ratio of total body surface area to body weight. It has been stated that it is more common when the aforementioned harsh climatic conditions are combined with insufficient pasture (Hoskind et al. 1991). Moreover, the fact that the skin thickness is higher in adult or old animals than in young animals reduces the susceptibility to external parasites relatively. In the study, the effect of physiological periods/age of animals was not significant. This finding is inconsistent with the Fagbemi (1982) report, which is consistent with Ogbe (1998). However, contrary to the findings in the study, Makelesh (2010) found that in a study conducted in the Tigray region of Ethiopia, goats have a higher ectoparasite load than sheep, and Sertse and Wessone (2007) found that goats have higher ectoparasites than sheep. It is seen in pregnant or lactating sheep and goats with a high rate of external parasites, especially fleas. The most important reason for this is; it is the physiological sensitization of the immune system as well as herd management (Silagi et al. 2012). In addition to female animals, male animals carrying ectoparasites have a significant effect on the transmission of parasites to other females in the herd during the mating period (Davis et al. 2006). Seasonal differences play an important role in the development of ectoparasite populations such as fleas, and the differences in climatic factors such as temperature and humidity in geographical regions play an important role in this regard (Tavasoli and Rahbari, 1998).

The contribution of sheep and goat breeding to the economy of the region and the country is substantial compared to other animal species. For this reason, studies of external parasites, which have negative effects on both human and animal health, should be given importance especially epidemiologically. External parasites can cause significant damage to the skin as well as animal losses directly or indirectly in enterprises. This is an extremely important issue for businesses that export sheep and goatskins. However, if breeding practices, hygiene and sanitation, animal welfare and health-protection practices are done correctly, the damage caused by external parasites will be relatively reduced (Akgül et al. 2019). In addition, effective control methods to be selected by determining the ectoparasite species and their hosts will make important contributions to the control of some zoonotic diseases. More attention should be paid to studies on external parasites in terms of both animal and human health. The subject should not be limited to fleas, but rather to determine the incidence of ticks, lice and other external parasites, but also to determine their direct or indirect effects on their hosts. The importance of flea damage for small cattle breeders should be explained well and control methods that will minimize animal deaths or economic losses should be determined.



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# VARIATION OF MILK YIELD IN SAANEN GOATS ACCORDING TO LACTATION PERIODS

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# Abstract

In this study, milk yields of Australian origin Saanen goats in different lactation periods were estimated. For this purpose, the Wood lactation curve model was adapted to total 500 milk yield measurements measured at 14 days intervals of 40 pure Saanen goats reared in Ege University Faculty of Agriculture Department of Animal Science Farm Animal Experiments Research, Education and Practice Unit. Information on milk yield trends was obtained by estimating the milk yields of goats in the first trimester of lactation in January-March and in the middle period of April-June. The milk yield of the last 3 months was not considered due to continued lactation. According to the goodness of fit of Wood's model, the determination coefficient values (R<sup>2</sup>) of the individual estimation results in the first and middle periods of lactation were 0.837 and 0.874, respectively. The root mean square error (RMSE) of Wood's model was 0.91 for the first and 0.81 for the middle periods of lactation curve in Saanen goats. However, it would be beneficial to conduct similar studies with larger herd and yield records.

Keywords: Saanen, Milk Yield, Lactation Curve, Wood Model



# PHOTOPERIODIC LIGHTING COMBINED WITH MILD THERMAL MANIPULATION DURING INCUBATION MAY IMPROVE CHICK QUALITY AND EARLY POST-HATCH GROWTH IN BROILERS

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# Abstract

Research on manipulation of incubation environment, such as lighting and cyclic changes in temperature, has received increased attention regarding to improve hatching performance and produce better adaptive broiler chicks to post-hatch environment. The current study aimed to investigate the effect of photoperiodic lighting together with a mild thermal manipulation of broiler embryos on chick quality, hatching and post-hatching performance. There were two treatments in the experiment. 1. Photoperiodic lighting in which eggs were exposed to a 16h of light and 8h of darkness (16L: 8D) along with optimal EST of 37.8°C (Light-Control); 2. Photoperiodic lighting along with a mild thermal manipulation (TM) protocol (1°C higher eggshell temperature (EST, 38.8°C) for 6 h/day from 11 to 16 embryonic day than optimal EST of 37.8°C) (Light-TM). Each treatment was replicated in two incubators using a total of 420 Ross broiler breeder eggs. After hatch, a total of 180 chicks (90/ treatment) were raised on 10 floor pens (1.4 m<sup>2</sup>) with 5 replicates per treatment under standard management conditions for broiler chicks till 32 days of age. Although there was no significant difference between the treatments regarding the chick weight at hatch, a significant improvement in chick length in Light-TM group was observed as an indication of improvement in chick quality. Broilers from Light-TM group had higher body weights at 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> weeks as compared with Light-Control group. However, final body weight of broilers, feed intake and feed conversion ratios did not differ with the treatments. It can be concluded that photoperiodic lighting along with a mild TM during incubation can have a positive impact on chick quality and improve the early growth performance.

**Keywords:** Incubation, Photoperiodic Lighting, Thermal Manipulation, Chick Quality, Broiler Performance.

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# OPPORTUNITIES AND RESTRICTIONS OF PINE HONEYDEW HONEY IN THE WORLD HONEY MARKET

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# Abstract

Turkey has very high beekeeping potential with about over 8.128.360 honeybee colonies and 109.330 tons of annual honey production according to recent national statistics. About 25.000 tons of this production consists of pine honey which produced only in southwest of the country and this production is about the 92% of world pine honey production. Pine honey is of particular importance for Turkey as a "geographical sign". So that makes Turkey the biggest pine honey producer and exporter of pine honey in the world. Turkey exports a big part of the total production (approximately 10.000 tons/year) of pine honey to EU countries and earns 7-10 million EURO from this trade. The biggest pine honey importer country is Germany. However, there are some factors that limit this advantageous situation. There is an incompatibility between the criteria regarding the honeydew honey within the framework of the laws of the EU member states in their own countries. In other words, apart from pine honey, since there is no common legislation on pine honeydew honey, important problems are experienced during the export of this honey and in the laboratory inspection phase. The fact that pine honey is special to Turkey and almost all of the produced amount is exported reveals the necessity of a separate codex definition specific to this honey. In order to ensure the continuity of production of pine honey, which is a very important export product of Turkey and to ensure its presence in the world market

Keywords: Pine Honey, Honeydew Honey, Honey Sector, World Market.



# GREEN LASER IRRADIATION EFFECTS ON CAMEL SPERMATOZOA QUALITY DURING INCUBATION

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# Abstract

The objective of this study is to develop a more sensitive and less costly technique of green laser irradiation of spermatozoa with short-wavelength 532 nm and continuous wave from a diode laser light with a total output power of 3 mW at different exposure times of 0 (control, non-irradiated), 2, 4, 6, 8, and 10 min for improvement of camel spermatozoa quality. A total number of forty testes from twenty Sudani camels (Camelus dromedarius) were used in the present study (5-10 years old and 500-600 kg body weight). Following laser irradiation, the percentages of motile spermatozoa, viability and acrosomal damage of spermatozoa were assessed during incubation at 37°C for 8 hours. The obtained results showed that the highest (P<0.05) value of the percentage of motile spermatozoa was recorded with spermatozoa exposed to 6 min of laser irradiation and the lowest (P<0.05) value was recorded with the control group. Otherwise, the highest (P<0.05) value of the percentages of dead and acrosomal damage of spermatozoa were recorded with spermatozoa exposed to 10 min and the lowest (P<0.05) value was recorded with 2 min. The prolongation of incubation time at 37°C decreased (P<0.05) the percentage of motile spermatozoa, while increased (P<0.05) the percentages of dead and acrosomal damage of spermatozoa during incubation at 37°C for 8 hours. In conclusion, there is a possibility of adopting green laser irradiation as an easy, time saving, less coastly, and straightforward technique for improvement of the camel spermatozoa quality to optimize the artificial insemination program.

Keywords: Camels; Spermatozoa; Laser Irradiation; Quality; Incubation.

# Introduction

The camel (*Camelus dromedarius*) is an important livestock species uniquely adapted to hot and arid environments. With increasing human population pressure and decline of food production in Africa there is an urgent need to develop previously marginal resources and optimize their utilization through appropriate livestock production systems of which camel production is certainly the most suitable one. However, plentiful and complicated natural restrictions can adversely affect the reproductive capacity of the one-humped camel (El-Hassanein et al., 2004).



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Artificial insemination (AI) is considered as one of the most important and the fastest way in the modern technology for the application of genetic improvement through the breeding programmes of farm animals. The progress in AI, semen preservation and related techniques in camels has been slow in comparison to other animals due to the difficulty of semen collection, little information in semen characteristics, semen dilution and storage of semen. During preservation, several factors may be responsible of the possible decrease in fertilizing ability of semen during storage under different conditions (Anand, 1979). Therefore, it is necessary to evaluate other strategies which aim to improve sperm mitochondrial function.

The term laser stands for light amplification by stimulated emission of radiation. During the last two decades of the 20th Century, the effects of laser on biological tissues have been studied widely. It has been clearly revealed that laser irradiation, has declared biological effects. Irradiation of spermatozoa has been shown to improve sperm motility with a low intensity helium-neon (He-Ne) laser (Karu, 2012). The first application of laser to improve sperm function was shown in 1969 (Goldstein, 1969), which has been applied in various species like human (Lenzi et al., 1989), mouse (Cohen et al., 1998), sheep (Zan-Bar et al., 2005), dog (CorralBaqués et al., 2009), and rabbit (Iaffaldano et al., 2010) spermatozoa. Thus, the current study aimed to define the effect of green laser irradiation with short-wavelength 532 nm, 3 mW with different exposure times of 0, 2, 4, 6, 8, and 10 min on the epididymal camel spermatozoa quality during incubation at 37°C for 8 hours.

## **Materials and Methods**

The current study was carried out at Embryo Transfer Laboratory, Artificial Insemination and Embryo Transfer Department, Animal Reproduction Research Institute, Giza, Egypt, in cooperation with the Laser Atomic Spectroscopy Laboratory, Department of Laser Applications in Measurements, Photochemistry and Agriculture, National Institute of Laser Enhanced Sciences, Cairo University, Giza, Egypt.

## **Experimental animals**

A total number of twenty clinically healthy Sudani camels (*Camelus dromedarius*) with a total number of forty testes aged from >5 to 10 years or more with live body weights of 500-600 kg were used in the present work and provided from automated El-Bassatein slaughterhouse, Cairo, Egypt.

#### Epididymal spermatozoa collection

## Transportation of the samples

The genitalia (epididymides connected to the testes) were removed from the carcass and transported in a thermos flask including sterile physiological saline (0.9%) with 100  $\mu$ g/ml streptomycin at 25°C according to the method described by Goto et al. (1989) through 2-3 hours after slaughtering.



# Sperm recovery

Forty testes were fully cleaned then the blood was wiped off by puncturing the superficial blood vessels of the cauda. By using a sterile scalpel and forceps, the epididymis was sectioned into three respective parts, caput, corpus, and cauda in three sterile Petri dishes of 100 mm diameter containing saline solution.

# Semen extension

Epididymal camel spermatozoa were collected, pooled, and evaluated for each camel and then diluted with lactose-yolk-citrate dilution (2.9 g sodium citrate dihydrate, 0.04 g citric acid anhydrous, 1.25 g lactose and 10 ml egg-yolk, per 100 ml distilled water, 500 I.U/ml penicillin and 500 µg streptomycin sulphate were also added to the extender) according to Musa et al. (1992).

# Incubation of semen at 37°C

Semen was divided into 6 groups, a control group (non-irradiated group) and 5 groups of irradiated semen with green laser irradiation for (2, 4, 6, 8, and 10 min). All groups were stored by incubation at 37°C for up to 8 hours. Motile, dead and acrosomal damage (%) of spermatozoa exposed to different exposure times of laser irradiation (0, 2, 4, 6, 8, and 10 min) were recorded following incubation. Moreover, enzymatic activity (AST, ALT and ALP enzymes) were also determined during incubation at 37°C for up to 8 hours.

## Semen evaluation

## Epididymal sperm motility (%)

Motile spermatozoa (%) were detected as an oscillatory movement of the flagellum due to the highly viscous nature of camel semen according to Tibary and Anouassi (1997).

## Dead spermatozoa (%)

The eosin/nigrosin staining procedure was carried out by dissolving 1.67 gm eosin and 10 gm nigrosin in distilled water up to 100 ml according to Hackett and Macpherson (1965).

## Acrosomal damage of spermatozoa (%)

The percentage of acrosomal damage was assessed according to Watson (1975). Spermatozoa were stained with 2% of trypan blue (T-0887 Sigma) for assessment of sperm viability then for 40 min with a 10% solution of Giemsa (Merck, Darmstadt, Germany) in distilled water prepared immediately before use.

## Laser specifications and irradiation parameters

Green laser ( $\lambda$ =532 nm) from a Diode Pumped Solid State (DPSS) laser [LSR-PS-II] with an output power of 3 mW was used for irradiation of spermatozoa with different exposure times (0, 2, 4, 6, 8, and 10 min). The irradiance of 3 mW/cm<sup>2</sup> was calculated according to Calderhead (1990) using the following equation:



$$Irradiance = \frac{Power \ output \ (mW)}{Application \ surface \ (cm^2)}$$

## Statistical analysis

Two-way ANOVA was used to analyze data statistically, using the procedure of General Linear Model (GLM) of SAS (SAS, 2000). Duncan's Multiple Range Test (Duncan, 1955) was used to detect significant differences among means. Percentage values were transformed into arc-sin values before being statistically analyzed. The statistical model used in the experimental work was as follows:

$$Y_{ijk} = \mu + L_i + I_j + (L_i \times I_j) + e_{ijk}$$

Where,

 $Y_{ijk}$  = the observed value of the dependent variable determined from a sample taken of spermatozoa.

 $\mu$  = the overall mean.

 $L_i$  = the fixed effect of laser irradiation durations (min), i= 0, 2, 4, 6, 8, and 10.

 $I_j$  = the fixed effect of incubation time (hours), j= 0, 1, 2, 4, 6, and 8.

 $L_i \times I_j$  = the interaction between laser irradiation durations (min) and incubation time (hours).  $e_{ijk}$  = the residual error.

# **Results and Discussion**

## Sperm motility (%)

Fig. 1 revealed that the effect of green laser irradiation (532 nm, 3 mW) for 6 min (P<0.05) increased the percentage of motile spermatozoa of the dromedary camel during incubation at  $37^{\circ}$ C than the control group. These results were in agreement with Nicolae et al. (2015) who found that exposure to helium neon (He-Ne) laser irradiation led to enhanced ram sperm motility, viability, mitochondrial function and hypo-osmotic swelling response at dose of 6.12 J/cm<sup>2</sup> comparing to the dose of 3.96 J/cm<sup>2</sup> which reduced the quality of seminal attributes than the control sample. Similarly, laffaldano et al. (2016) found that irradiated frozen-thawed ram semen by He-Ne laser irradiation with fluencies ranged from 3.96 to 9 J/cm<sup>2</sup> resulted in a significant increase in sperm motility and viability at 6.12 J/cm<sup>2</sup>. It could be attributed to the interaction between the mitochondria and laser irradiation which in turn enhance semen quality (Lone et al., 2018), in which higher levels of cytochrome C oxidase activity (COX) and adenosine-5-triphosphate (ATP) were observed in irradiated spermatozoa comparing to non-irradiated spermatozoa and a positive correlation between COX activity and ATP levels was observed; also COX activity and ATP levels were positively correlated with sperm motility.



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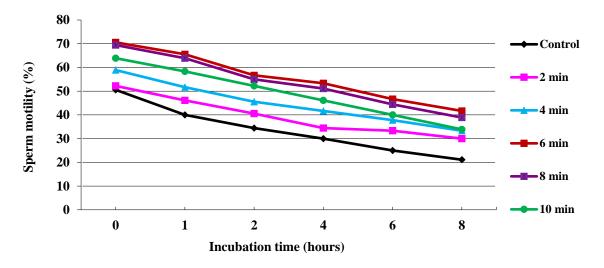


Figure 1. Effect of different exposure times of green laser irradiation on motile camel spermatozoa (%), during incubation at 37°C for up to 8 hours.

The present study showed the prolongation of incubation time at 37°C for 8 hours decreased (P<0.05) the percentage of sperm motility at all exposure times of laser irradiation and the control group (Fig. 1). These findings may be due to the high metabolic activity of spermatozoa which leads to a toxic effect on the sperm by increasing the production of lactic acid. A similar trend was observed by Abd El-Salaam et al. (2012) who found that the percentage of sperm motility of camel spermatozoa was significantly (P<0.01) decreased with advancement of incubation time at 37°C for up to 12 hours. Furthermore, the current study revealed that the interaction effect between incubation time at 37°C for 8 hours and green laser irradiation on motile spermatozoa was insignificant.

#### Dead spermatozoa (%)

The dead spermatozoa (%) during incubation at  $37^{\circ}$ C was higher (P<0.05) of spermatozoa exposed to 10 min of laser irradiation than other exposure times and the control group (Fig 2). Comparing to the control group (57.96%), the dead spermatozoa (%) decreased (P<0.05) with spermatozoa exposed to 2, 4, and 6 min of laser irradiation with an average value of 47.41, 49.07, and 54.44%, respectively. A similar trend was reported by Iaffaldano et al. (2010 & 2016), who found that the viable spermatozoa (%) was higher (P<0.05) at 24 hours of storage with 6.12 and 9.0 J/cm<sup>2</sup> than the control in rabbit semen. Similarly, Nicolae et al. (2015) reported that the percentage of viable spermatozoa in ram semen was significantly (P<0.05) increased for both of control and irradiated spermatozoa with a dose of 6.12 J/cm<sup>2</sup> than those irradiated with a dose of 3.96 J/cm<sup>2</sup>.



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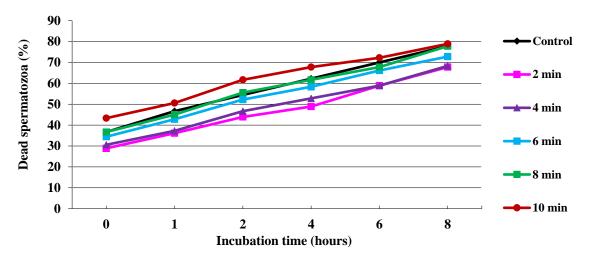


Figure 2. Effect of different exposure times of green laser irradiation on dead camel spermatozoa (%), during incubation at 37°C for up to 8 hours.

The present study showed that the prolongation of incubation time at 37°C for 8 hours increased (P<0.05) the dead spermatozoa (%) of the dromedary camels at all exposure times of laser irradiation and the control group (Fig. 2). A similar trend was observed by Abd El-Salaam et al. (2012) in the camel spermatozoa, in which the advancement of incubation time at 37°C for up to 12 hours increased significantly (P<0.05) the percentage of dead spermatozoa. The interaction effect between incubation time and green laser irradiation on dead spermatozoa was insignificant.

#### Acrosomal damage of spermatozoa (%)

The percentage of acrosomal damage was significantly (P<0.05) higher of spermatozoa exposed to 10 min of laser irradiation than other exposure times and the control group (Fig. 3). In respect to the control group (25.76%), acrosomal damage (%) decreased significantly (P<0.05) with spermatozoa exposed to 2 and 4 min of laser irradiation with an average value of 23.22 and 24.07%, respectively. Similar trends were observed by Yeste et al. (2016) who reported that there was no change in acrosomal integrity and viability in liquid stored boar semen, while an increase in sperm motility was observed in which there was a higher reduction in semen quality parameters in non-exposed spermatozoa than irradiated spermatozoa after incubation for 90 min at  $37^{\circ}$ C.



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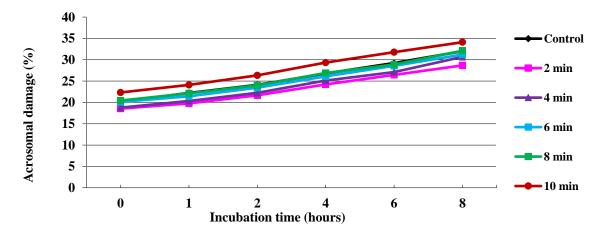


Figure 3. Effect of different exposure times of green laser irradiation on acrosomal damage (%) of camel spermatozoa, during incubation at 37°C for up to 8 hours.

The present study showed that the prolongation of incubation time at  $37^{\circ}$ C for 8 hours increased (P<0.05) the acrosomal damage (%) at all exposure times of laser irradiation and the control group (Fig. 3). The current results agreed with Abd El-Salaam et al. (2012) in the camel spermatozoa, in which the advancement of incubation time at  $37^{\circ}$ C for up to 12 hours increased significantly (P<0.05) the percentage of dead spermatozoa. The interaction effect between incubation time at  $37^{\circ}$ C for 8 hours and green laser irradiation on acrosomal damage of spermatozoa was insignificant.

#### Conclusion

In conclusion, laser in the field of semen biology may be regarded as an easy, time saving, less costly, effective and more sensitive technique which can be used for enhancing the artificial insemination program of the dromedary camel due to the beneficial effects of laser on semen quality which included the increase in sperm motility, livability and acrossomal integrity which consider an indicator to improve mitochondrial function which in turn lead to enhanced sperm survival.

It could be recommended for collection and short-term storage of the epididymal spermatozoa of the dromedary camels at 37°C for artificial insemination by being exposed to 6 min of green laser irradiation with short-wavelength (532 nm, 3 mW) to reinforce the fertilizing ability of she-camel, especially in the desert regions where liquid nitrogen may not be available for freezing of semen for a long time.

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# STATE AND PERSPECTIVES OF AQUACULTURE IN THE MUNICIPALITY OF BUŽIM

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# Abstract

Based on natural resources, Bosnia and Herzegovina has a significant potential for aquaculture, especially for the production of freshwater fish species. The municipality of Bužim occupies the far northwestern part of Bosnia and Herzegovina and belongs to the Una-Sana Canton. The significant wealth of the Canton is natural beauty, because this area abounds with rivers, smaller and larger watercourses, warm springs, caves, forests and cultural and historical monuments. This paper analyzes the existing state of fish diversity in five water streams the Una-Sana Canton, as well as the prospects of this Canton for increasing production. The objective of the ichthyological research is to examine structure of fish populations, distribution of fish populations along the longitudinal profile of the investigated catchment area of the river Baštra, its tributaries and standing waters in the territory of the cadastral municipality of Bužim. For this research work, 1.429 pieces of fish with a total weight of 168,847.45 grams were caught, a total of 15 different fish species were caught. Also, this paper deals with physical and chemical parameters of water. From physical parameters, the analysis included: water and air temperature, pH value and conductivity. The analysis of chemical properties of water at the investigated sites included next parameters: the concentration of dissolved oxygen, hydrogen carbonate, nitrite, ammonia, sulfates, etc. This research has shown that in the fishing region belonging to the municipality of Bužim, both in terms of environmental conditions and the composition of the mixed fish settlement, there are favorable conditions for maintaining species valuable for sport fishing, the most important of which are: rainbow trout, brown trout, chub, carp, marble and pike.

Keywords: Fish Diversity, Water Streams, Parameters, Bužim.

# Introduction

Bužim municipality is located in the far northwestern part of Bosnia and Herzegovina, in the area of Peripannonian physiognomic region. More specifically, the municipal territory belongs to the immediate transition zone between the extreme southern parts of the northern temperate landscape zone to the northern parts of the northern subtropical landscape region. In terms of sectorality, the



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municipal area is located in the area of the Atlantic western landscape sector of the northern temperate zone in Bosnia and Herzegovina (Vuković, 1966).

The basis of all ichthyological research is to get to know the fish populations of the researched area. The primary task of ichthyological research is to ascertain the existing fish populations, ichthyological analysis, and to obtain relevant data on the presence of certain fish species in the localities of the researched area. The objective of this scientific research is to examine the structure of fish populations, ie distribution of fish populations along the longitudinal profile of the investigated catchment area of the river Baštra, its tributaries and standing waters in the territory of the cadastral municipality of Bužim. Also, the paper deals with physical and chemical parameters. The first and larger sub-basin belongs of the river Bužimica and its tributaries, whose waters flow into the Sava River Glina. Approximately 1/3 of the municipal territory belongs to the sub-basin of Baštra, the Una River Basin (Muhamedagić et al. 2010).

The results of ichthyological analyzes also provide data on the state of fish populations, their structure, ie data from which it can be determined whether a population is declining, stagnant or rising. Therefore, such research has a special, both fundamental and applied significance for applied fisheries, given that, in the final analysis, the aim is to protect the wildlife of the area, which will certainly be achieved by controlling a certain aquatic ecosystem and bringing fish populations exploitation, ie. fish stock, within the limits of reasonable exploitation (Aganović, 1979., Bogut et al. 2006., Vuković 1977).

## **Material and Methods**

To assess the structure of fish populations along the longitudinal sequence of the Baštra river basin, as well as its tributaries and standing water in the cadastral municipality of Bužim, 4 characteristic areas were treated - sites with a total length of 450 meters and cultivated watercourse area of 1,800 m<sup>2</sup>. Locals that have been cultivated: the river Baštra - the village of Baštra, the river Pašinac - the village of Pašin brod, the lake - the village of Vrhovska and the river Bužimnica - the village of Begovići. Four sites were selected for analyzing: the Baštra River (L / 1), the Pašinac River (L / 2), Lake Vrhovska (L / 3) and the Bužimnica River (L / 4).

The analysis of physical and chemical parameters was performed by the Laboratory for Water Control of the Health Institution of the Institute of Public Health of the Una-Sana Canton Bihać. Sampling of water for analysis was performed at 4 sites. The analysis included 18 parameters. Of the physical parameters, the analysis included: water and air temperature, pH value and conductivity. The analysis of the chemical properties of water at the investigated localities included a number of parameters: the concentration of dissolved oxygen, hydrogen carbonates, nitrites, ammonia, sulfates, etc.

Based on the main facts, the aim of these ichthyological research was to consider the structure of fish populations, and distribution of fish populations along the longitudinal profile of the investigated catchment area of the river Baštra, its tributaries and stagnant waters in the municipality Bužim.



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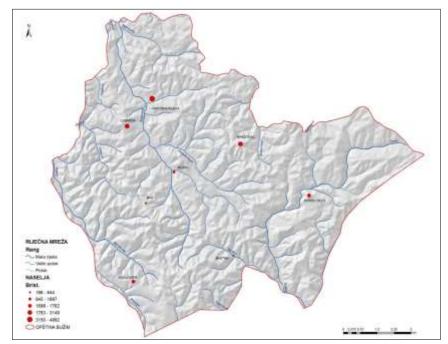


Figure 1. The river network of the municipality Bužim (Source: TK 1:200.000, List: Bihać)

The river Bužimica is the largest watercourse and the backbone of the largest part of the river network of the municipality of Bužim. The majority of springs are located in the foothills zone around the settlement of Konjodor, at altitudes between 450 and 500 m. The second area where the source head is formed is the western and northwestern foothill morphostructure in the zone of the settlements Mrazovac, Varoška river and Zaradostovo. The total length of the stream that belongs to the municipal territory, and which has the rank of a small river is about 12.6 km.

## **Results and Discussion**

According to the Law on Waters of the F BiH (Official Gazette of the Federation of BiH 70/06), the river Baštra belongs to the I category of water quality (Art. 5 classification of surface waters). At the first locality (Baštra settlement), the water temperature of the Una River was  $9.5 \degree$  C, and the pH was 7.43.

Dissolved oxygen was 10.77 mg / l, while oxygen saturation was 93.98%, which is a consequence of good direct aeration of water above this locality. The electrical conductivity was 397  $\mu$ S / cm, which is in the category of water of the first quality because for this category of water the electrical conductivity is <500  $\mu$ S / cm. According to water hardness, Una in this locality belongs to hard waters (from H 15.01), which corresponds to an average value of 255 mg / 1 CaCO3.

Analysis of Pašinac river water taken at the Pašin Brod site shows the following important values: water temperature was 9.2 ° C, water pH was 7.49, dissolved oxygen was 10.68 mg / l, while oxygen saturation was 92.55%, the electrical conductivity is 422  $\mu$ S / cm and the total hardness is 16.24° dH



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The analyzed sample of Bužimica water taken at the Jabukovac site shows the following values: water temperature is 9.6 ° C, pH 7.51, dissolved oxygen is 10.12 mg / l, while oxygen saturation is 88.15%, electrical conductivity is 416  $\mu$ S / cm, and the water hardness is 16.8 ° dH.

The water of Lake Vrhovska shows the following physical and chemical characteristics: water temperature was 8.7 ° C, pH was 7.40, dissolved oxygen was 8.79 mg / l, oxygen saturation 74.62%, while conductivity was 325  $\mu$ S / cm. The total hardness was 13.77 ° dH, which classifies it as moderately hard water.

Nitrite concentrations in all four tested water samples show very low values (from 0.0006 to 0.001 mg / 1), which classifies them as water and quality. The ammonia content in the tested water samples shows different values. Thus, the amount of ammonia present at the Pašinac site (0.11 mg / 1) classifies this water in I quality class, as well as water from the Vrhovska Lake site (0.13 mg / 1). Waters from the localities Baštra (0.25 mg / 1) and Jabukovac (0.29 mg / 1) according to this parameter are classified in the II quality group of waters.

The assessment of the quality waters of the river Baštra, Pašinac, Bužimnica and Vrhovska lakes was done on the basis of physical and chemical characteristics of water on the tested profiles. Physico-chemical properties of water are one of the determining factors in assessing the quality of certain watercourses. They are also of crucial importance for the development of populations of animal and plant organisms in aquatic ecosystems, because by changing some water quality factors, the conditions of life change, which has an ecological significance for aquatic organisms, and these parameters are followed in this research. Also, the peak waters are classified in 5 classes according to the Law on Waters of the Federation of Bosnia and Herzegovina and according to the Classification of Water and Categorization of Watercourses pH values of the tested waters are within the limits of neutral and slightly basic solutions, which corresponds to the values of natural waters. The pH value of the Baštra river was 7.43, Pašinci 7.49, Bužimica 7.51, and the lake 7.40. The tested rivers belong to the pH values of the I class. The value of dissolved oxygen concentrations in the Baštra river (Baštra locality) was 10.77 mg / l, which corresponds to saturation and oxygen saturation of oxygen of 93.98%, while in the Pašinac river (locality Pašin Brod) 10.68 mg / l, and saturation with oxygen was 92.55%. The water of the Bužimnica River (Jabukovac site) had a slightly lower concentration of dissolved oxygen of 10.12 mg / 1, as well as oxygen saturation of 88.15%, while the water of

Vrhovska lake had the smallest value of dissolved oxygen (8.79 mg / l) and saturation (74.62%). Water samples of Baštra, Pašinac and Bužimnica, according to the oxygen saturation values, belong to the first class of water quality, while the samples from the Vrhovska Lake belong to the second class of water quality. The unit of measure for electric conductivity is  $\mu$ S / cm, ie (micro-Simens per centimeter). Electrical conductivity is a useful physical indicator since it is proportional to the concentration of total dissolved solids in dilute solutions. The investigated water of the Baštra river (Baštra locality) has a value of 397  $\mu$ S / cm, Pašinac (Pašin Brod) 422  $\mu$ S / cm, Bužimnica (Jabukovac site) 416  $\mu$ S / cm, and the Vrhovska lake 325  $\mu$ S / cm, which according to them this parameter, is classified in the first class of surface water quality. In Bosnia and Herzegovina, it is most often expressed in German degrees - ° dH (German Deutsche Härte). All measured values



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correspond to the requirements for I class of surface water quality. According to the obtained results, the water of Lake Vrhovska has a total hardness of 13,77  $^{\circ}$  dH, which is classified as moderately hard water. Water Baštra from the Baštra site shows the total water hardness of 15.01  $^{\circ}$  dH, water Pašinac (location of Pašin Brod) 16.24  $^{\circ}$  dH, and water Bužimnica (locality Begovići) 16.8  $^{\circ}$  dH.

# Locality L / 1 Rijeka Baštra - village Baštra

At the locality of the river Baštra - the village of Baštra, 6 species of fish were registered, caught with an electric generator 172 pieces of fish, with a total weight of about 6.6 kg. During fishing at the L / 1 site, the air temperature was 14 ° C, the water temperature 6.5 ° C, and the oxygen content in the water 10.7 mg / 1.

Fish species	Fish species Number		Body weight (g)				Standard lenght (cm)			Total lenght (cm)		
1 Isli species	of pieces	max	min	Х	Σ	max	min	х	max	min	х	
Hucho Hucho	4	62.0	62.0	62.0	248.00	16.5	16.5	16.5	19.0	19.0	19.0	
Salmo trout	6	108.0	8.0	58.0	348.00	19.0	8.0	13.5	22.0	9.0	15.5	
Chub	63	127.5	28.5	78.0	4.914	19.0	12.0	15.5	22.0	14.5	18.25	
Barbel	4	13.1	7.4	10.25	41.00	9.0	7.5	8.25	11.0	8.5	9.75	
Alburnus - Scheneider	89	16.5	3.9	10.2	907.80	10.0	6.0	8.0	12.0	7.0	9.5	
Comon Gudgeon	6	18.6	18.2	18.4	110.40	10.5	10.0	10.25	12.0	11.5	11.75	
Total:	172				6.569,2							

Table 1. Ichtiopopulation of river Baštra

# Locality L / 2 Rijeka Pašinovac - village Pašin Brod

At the locality L / 2 of the river Pašinovac - the village of Pašin Brod, nine species of fish were registered, caught with an electric generator 324 pieces of fish, with a total weight of about 79 kg.



Fish species	No of		Body w	veight (g	g)	Standa	ard lengt	h (cm)	Total length (cm)		
I ish species	pieces	max	min	Х	Σ	max.	max	min	Х	min.	Х
Salmo trout	3	123.0	11.0	67.0	201.00	21.0	9.5	15.2 5	23.0	11.0	17.0
Comon dace	204	716.0	43.0	379. 5	77.418	33.5	13.5	23.5	38.0	16.5	27.25
Plotica	4	145.0	122. 0	133. 5	534.00	20.0	18.0	19.0	22.0	19.5	20.75
Barbus balcanicus	3	22.0	14.0	18.0	54.00	11.5	9.5	10.5	13.0	11.5	12.25
Alburnus - Scheneider	66	10.0	3.0	6.5	429.00	8.5	6.5	7.5	10.0	7.5	8.75
Gaga	17	4.0	2.0	3.0	51.00	6.0	5.0	5.5	7.0	5.5	6.25
Comon Gudgeon	4	19.0	14.0	16.5	66.00	14.0	10.0	12.0	12.5	11.5	12.0
Bulhead	16	13.7	2.7	8.2	131.20	8.5	5.0	6.75	10.0	6.5	3.25
Barbus	7	6.5	1.2	3.85	26.95	8.0	6.0	7.0	9.0	6.5	7.75
Total	324				78.911						

# Locality L / 3 Jezero - village Vrhovska

Seven species of fish were registered at this locality L / 3 Jezero - village Vrhovska, 797 pieces of fish, with a total weight of about 80 kg, were caught with an electric generator and fishing nets.

	No of	Body weight (g)			Standard length (cm)			Total length (cm)			
Fish species	pieces	max	min	X	Σ	max.	max	min	X	min	Х
Comon dace	15	2.100	800.0	1.450	21.750	42.0	32.5	37.25	48.0	38.5	43.25
Carp	12	823.00	12.0	417.5	5.010	28.5	7.0	17.75	35.5	8.5	22.0
Golden Carp	672	148.0	7.5	77.75	52.248	16.0	6.5	11.25	20.5	8.0	14.25
Barbus balcanicus	1	77.0	77.0	77.0	77.00	16.5	16.5	16.5	19.5	19.5	19.5
Comon Gudgeon	94	11.0	1.0	6.0	564.0	9.0	4.0	6.5	10.5	5.0	7.75
Pike	2	36.0	21.0	28.5	57.00	14.5	12.5	13.5	16.0	14.0	15.0
Barbus	1	3.0	3.0	3.0	3.00	6.0	6.0	6.0	7.5	7.5	7,5
Total	797				79.708						

Table 3. Ichtiopopulation of river Jezero - village Vrhovska



# Locality L / 4 Rijeka Bužimnica - village Begovići

At the locality L / 4 Rijeka Bužimnica - village Begovići, nine species of fish were registered, caught with an electric generator 136 pieces of fish, with a total weight of 3.66 kg.

Fish spacios	No of		Body	weight (g)		Standa	rd leng	th (cm)	Tota	l length (	cm)
Fish species	pieces	max	min	Х	Σ	max.	max	min	х	min.	Х
Salmo trout	4	215.0	19.0	117.0	468.00	25.0	11.5	18.25	28.0	13.0	20.5
Comon dace	21	24.0	8.7	16.35	343.35	11.0	8.0	9.5	14.0	10.0	12.0
Plotica	9	321.5	217.0	269.25	2.423.25	26.5	23.5	25.0	32.0	28.0	30.0
Barbus balcanicus	5	15.5	5.0	10.25	51.25	9.5	6.5	8.0	10.5	8.0	9.25
Alburnus - Scheneider	74	15.0	2.5	8.75	647.50	9.0	6.0	7.5	11.0	7.5	9.25
Gavčica	2	5.0	5.0	5.0	10.00	6.0	6.0	6.0	7.5	7.5	7.5
Gaga	11	3.0	2.5	2.75	30.25	6.0	5.5	5.75	7.0	6.5	6.75
Comopn gudgeon	4	5.0	2.0	3.5	14.00	7.0	4.5	5.75	8.5	5.5	7.0
Bulhead	6	4.0	1.0	2.5	15.00	6.0	3.5	4.75	7.0	4.5	5.75
Total	136				3.659.25						

Table 3. Ichtiopopulation of river Bužimnica - village Begovići

The results of ichthyological analyzes also provide data on the state of fish populations, their structure, ie data from which it can be determined whether a population is declining, stagnant or increasing. Therefore, such research has a special, both fundamental and important significance for applied fisheries, given that, in the final analysis, the aim is to protect the wildlife of the area, which will certainly be achieved by controlling a certain aquatic ecosystem and bringing fish populations, fish stock, within the limits of reasonable exploitation (Vuković and Kosorić 1978., Zukić Žujo 2009).

# Conclusions

From physical and chemical parameters that were examined it is easy to say that waters we used in this research work belong into first or second class of water. It means they are very clean and possibilities for fishing and fish farming in them is very good. Small problem was with some of examined stream pollution with organic material but nothing that could be bigger problem. This examination showed us that this part of Bosnia and Herzegovina has a great potential for fish farming some of indigenous fish species for European fish market or as a fingerling for farming in other country.



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# FOOD TECHNOLOGY



# **BIOCOMPOSITE FOOD PACKAGING FILMS BASED ON PROTEINS AND CARBOHYDRATES: PHYSICAL AND BARRIER PROPERTIES**

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# Abstract

Protein-based films have good mechanical properties and stronger intermolecular covalent bonds which provide good barrier properties against oxygen. However, protein-based films have high water vapor permeability due to their hydrophilic character. In order to reduce the water vapor permeability of protein films, it is advantageous to use them together with carbohydrates to form a bicomposite structure. The present study aimed to develop the carbohydrate and protein based biocomposite films for food packaging applications. By using sodium alginate, pectin, carboxymethyl cellulose as carbohydrates; and gelatin, sodium caseinate as proteins, biocomposite films were produced with a carbohydrate: protein ratio of 10:90. In order to improve the film properties, different dry matter content (5-10%) and glycerol ratios (10-20%) were studied. Mechanical properties, water vapor permeability and opacity values of biocomposite films were determined. Among the composite films, alginate-gelatin composite films showed highest water vapor permeability as 1.49x10<sup>-10</sup>g/m.s.Pa. Compared to gelatin-based composite films, sodium caseinate-based composite films have lower water vapor permeability ranging from 0.91x10<sup>-10</sup> to 1.09x10<sup>-10</sup>g/m.s.Pa. The tensile stress values of composite films vary between 14.29 and 42.93MPa. The highest tensile stress was found to be 42.93 MPa in pectin-gelatin composite films with 5% dry matter and 15% glycerol content. The tensile strength decreased when the glycerol ratio was increased to 20% in order to improve the brittle structure of sodium caseinate based composite films. Gelatin alginate composite films have the highest elongation ability among all composite films.Sodium caseinate based composite films were found to have poor textural properties at low concentrations. Opacity values of sodium caseinate based composite films were found to be higher than gelatin based composite films, but light transmittance of gelatin-based composite films is improved at low dry matter content. In terms of all film properties, carbohydrate and protein type was selected as pectin and gelatin, respectively, for preparing biocomposite food packaging film.

Keywords: Biocomposite Film, Food Packaging, Gelatin, Sodium Caseinate, Carbohydrates.



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#### Introduction

The food packaging area increasingly demands biodegradable and environmentally friendly packaging materials that can indicate required physical, mechanical and barrier properties. The efficiency of edible films and coatings in food packaging is determined by their mechanical, physical and sensory properties. Among these properties, the film tensile strength, elongation percentage and film thickness constitute its mechanical properties, while the film's oxygen, carbon dioxide and water vapor permeability also constitute its physical properties (Doğangün Bahtimur, 2018).

In the preparation of films basically, hydrocolloid (carbohydrate and protein) compounds or lipids are used. Film properties differ according to the type of materials used in the film composition. Carbohydrate-based films have- low moisture barrier as they are composed of hydrophilic polymers. These films are formed as a result of the remodeling of the polymer chain by evaporation of the solvent, forming hydrophilic and hydrogen bonds in a film matrix. Protein films are formed by partial denaturation of polypeptide chains by adding a solvent, changing the pH, adding an electrolyte to crosslink, and applying heat if required. The formation of a protein matrix occurs due to the partially denatured peptide chains being linked together via hydrophilic and hydrogen bonds. For this reason, proteins provide advantages with their networking and elasticity properties in obtaining biodegradable packaging (Rhim and Perry, 2007).

It is essential to optimize the film formulation according to the film properties, considering the foods to be applied in the development of film materials. However, contrary to the use of a single type of raw material, current studies have focused on developing films made of biocomposite materials. Films obtained by using different protein and carbohydrate compositions show better barrier properties and improve their mechanical properties. In general, each type of film-forming hydrocolloid (protein or polysaccharide) or some plasticizer additives has advantages and disadvantages. An alternative to this is to design composite films composed of various components to combine the advantages of each and eliminate the disadvantages (Debeaufort et al., 1998). The functional properties of composite films depend on their composition and film-forming procedure.

#### Materials and methods

The present study aimed to develop carbohydrate and protein-based biocomposite films for food packaging applications. Sodium alginate, pectin, carboxymethyl cellulose as carbohydrates, and gelatin, sodium caseinate as proteins, were used to produce biocomposite films. The carbohydrate: protein ratio was kept as 10:90 in all film trials. The amount of glycerol used to reduce the fragility of the films was kept constant at 15% by weight on a dry basis in the first trials. In cases where it was found to be insufficient, 20% glycerol was produced. Since glycerol will increase the permeability properties of the films, the amount of glycerol in the film formulation was not increased above 20%.



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## **Preparation of films**

The amount of protein was determined to form 10, 7.5 and 5% dry matter in total and was transferred into distilled water. Gelatin solution was prepared by keeping at 70°C for 10 minutes. Sodium caseinate solution was prepared by keeping at 80°C for 1 hour. Alginate, pectin, carboxymethyl cellulose solutions were homogenized by mixing at 4000 rpm with a homogenizer. The solutions were degassed in an ultrasonic bath. Then composite film solution was left to stir for 1 hour at 700rpm in a magnetic stirrer. 15% glycerol was added on a dry basis into the homogeneous composite film solution and left to mix again at 700rpm for 1 hour. The film solution obtained was poured into 9 cm diameter aluminum cups in equal proportions and dried in a tray dryer at 40°C at an air speed of 1 m/s until it reached a constant weight.

# Film thickness

Film thickness was measured with a manual micrometer. Thickness measurements were randomly made from 6 different points for each film and average thickness values were used in the calculations.

# Water vapor permeability

The composite films' water vapor permeability (WVP) was determined gravimetrically using the modified standard ASTM E96 method. Discs with a diameter of 3.5 cm were cut from the films for permeability measurement. Film discs were placed on vessel cups containing silica gel. Water vapor permeability was calculated by taking the weights of the containers, which were kept at 25 °C for 24 hours under the ambient conditions of 75% relative humidity provided with saturated NaCl solution and the film layer was exposed to 0% relative humidity.

## **Mechanical Properties**

Tensile stress (MPa) and elongation at break (%) values of composite films were measured with TA.XT texture analyzer using ASTM D882 standard method. Tensile stress (MPa) and elongation at break (%) values were determined from the graphs obtained. The films' tensile strength (MPa) was calculated by dividing the highest force that the film could withstand until rupture by the cross-sectional area of the film. The elongation (%) values of the films at break were calculated by dividing the length of the film by the initial length

# **Opacity Analysis**

The opacity value of the films was determined using the method applied by Dou et al. (2018). Each film to be tested was cut at  $10\times45$  mm dimensions to fit the dimensions of the spectrophotometer cuvette. Absorbance values were measured at 600nm by using a spectrophotometer (Schimadzu, UV-1601, Japan). Air (blank measurement) was used as a reference. The opacity values of the films were calculated using the equation below.



Opacity =  $(A_{600}) / X$ 

A600: Absorbance value at 600nm, X: Film thickness (mm)

#### **Results and Discussion**

#### Water Vapor Permeability of Composite Films

Water vapor permeability values of gelatin-based composite films were found in the range of  $0.96 \times 10^{-10}$  and  $1.37 \times 10^{-10}$  g/m.s.Pa (Table 1). Since the dry matter content of the films (10%) and the pouring amount are kept constant, the fact that the thickness values of the films are quite close to each other is an indication of the effectiveness of the differences in water vapor permeability from factors other than the film thickness. The low water vapor permeability can be explained by the film matrix's interactions between carbohydrates and protein. Among the gelatin-based composite films containing 10% dry matter, alginate-gelatin composite films showed the highest water vapor permeability. After alginate-gelatin composite films, there are pectin-gelatin composite films with high water vapor permeability. Alginate, one of the hydrophilic polymers, has a high water holding capacity. Therefore, water vapor permeability is higher than pectin-based films. Although edible films obtained from cellulose, pectin, alginate and carrageenan have more substantial structure, it is stated that the water vapor barrier properties are weak due to their hydrophilic properties (Tanaka et al., 2001). In the composite films formed with alginate and pectin, a decrease in water vapor permeability was detected due to the decrease in the dry matter content (<10%) without changing the glycerol ratio. The lowest value for water vapor permeability was determined as 0.59 x10<sup>-10</sup> g/m.s.Pa in pectin-gelatin films with 5% dry matter and it was followed by alginate gelatin films as 0.72 x 10<sup>-10</sup> g/m.s.Pa. When comparing alginate and pectin-based composite films, alginate binds water molecules due to differences in hydrophilic structure, and that allowing more water vapor to pass through compared to pectin.

The water vapor permeability values of the sodium caseinate-based composite films were found to be  $0.91 \times 10^{-10}$  and  $1.09 \times 10^{-10}$  g /m.s.Pa. Compared to gelatin-based composite films, sodium caseinate-based composite films have lower water vapor permeability. Especially pectin-sodium caseinate composite films had the lowest water vapor permeability among all samples. When alginate and pectin-based films are compared among composite films, it is seen that alginate binds water molecules, allowing more water vapor passage compared to pectin due to the differences in hydrophilic structure. In composite films obtained with both types of protein, gelatin and sodium caseinate, pectin-based films provided lower water vapor permeability than alginate-based films. An increase in water vapor permeability was observed as the amount of glycerol increased in alginate-sodium caseinate composite films. Similarly, an increase in water vapor permeability was observed as the amount of glycerol increased in pectin-sodium caseinate composite films. Since low water vapor permeability is preferred, composite films formed by alginate or pectin carbohydrates and gelatin in low dry matter amounts have the expected properties.



 Table 1. Thickness and water vapor permeability values of gelatin and sodium caseinate based composite films

 obtained with different dry matter and glycerol content.

Type of film	Dry matte	CHO:PRO,	Film thickness	Water vapor permeability			
	r	Glycerol (%)	( <b>mm</b> )	(g/m.s.Pa) x 10 <sup>-10</sup>	(g.mm/m <sup>2</sup> .h.kPa )		
Aljinat-Jelatin	10 %	10:90, 15%	$0.29\pm0.01$	$1.49\pm0.15$	$0.54\pm0.05$		
	7.5 %	10:90, 15%	$0.23\pm0.08$	$0.91 \pm 0.12$	$0.33\pm0.04$		
	5 %	10:90, 15%	$0.16\pm0.01$	$0.72\pm0.04$	$0.26\pm0.02$		
Pektin-Jelatin	10 %	10:90, 15%	$0.28\pm0.01$	$1.37\pm0.01$	$0.49\pm0.01$		
	7.5 %	10:90, 15%	$0.22\pm0.03$	$0.75\pm0.06$	$0.27\pm0.02$		
	5 %	10:90, 15%	$0.15\pm0.02$	$0.59\pm0.07$	$0.21\pm0.02$		
Pektin-NaK	10 %	10:90, 15%	$0.26\pm0.05$	$0.91\pm0.04$	$0.33\pm0.01$		
	10 %	10:90, 20%	$0.29\pm0.01$	$0.97\pm0.05$	$0.35\pm0.02$		
Aljinat-NaK	10 %	10:90, 15%	$0.30\pm0.03$	$1.09\pm0.09$	$0.39\pm0.01$		
	10 %	10:90, 20%	$0.25\pm0.03$	$1.30\pm0.11$	$0.47\pm0.04$		
Carboxymethyl cellulose-gelatin	5 %	10:90, 15%	$0.16 \pm 0.01$	$0.96\pm0.08$	$0.35 \pm 0.03$		

## **Mechanical Properties of Composite Films**

Mechanical properties of composite films obtained with different dry matter and glycerol content are presented in Table 2. It was observed that gelatin alginate composite films had slightly higher tensile stress and elongation at break. In addition to the film strength, the films' elongation ability (%) shows flexibility. Among all the composite films produced, gelatin alginate composite films have the highest elongation ability. Sodium caseinate-based composite films had lower tensile stress and percent elongation at break compared to gelatin-based composite films. The effects of different dry matter amounts (10, 7.5, 5.0%) on the film properties were investigated by keeping the glycerol ratio constant (15%) of the composite films formed with gelatin-based alginate and pectin. The decrease in dry matter content in alginate-gelatin composite films resulted in a slight decrease in film tensile stress. In pectin-gelatin composite films, the decrease in dry matter increased the film tensile stress.

The film structure is quite fragile and the tensile stress was determined as 22.25 and 22.82 MPa for pectin-sodium caseinate and alginate-sodium caseinate films, respectively. In pectin-sodium caseinate films, the highest tensile stress was 22.25MPa in films with 10% dry matter and 15% glycerol. When the glycerol ratio was increased to 20% to improve the films' brittle structure, the tensile strength decreased.



 Table 2. Mechanical properties of gelatin and sodium caseinate based composite films obtained with different dry matter and glycerol content.

Type of film	Dry matter	CHO:PRO, Glycerol (%)	Tensile stress (MPa)	Elongation at break (%)
Alginate-Gelatin	10 %	10:90, 15%	$32.21\pm4.42$	$111.50\pm2.65$
	7.5 %	10:90, 15%	$31.56\pm3.24$	$96.62\pm5.43$
	5 %	10:90, 15%	$28.86 \pm 4.16$	$103.69\pm5.72$
Pectin-gelatin	10 %	10:90, 15%	$28.37 \pm 1.07$	$98.09 \pm 9.37$
	7.5 %	10:90, 15%	$36.74\pm3.92$	$99.60\pm3.82$
	5 %	10:90, 15%	$42.93\pm2.14$	$82.27\pm3.20$
Pectin-NaK	10 %	10:90, 15%	$22.25\pm3.43$	$75.46 \pm 4.66$
	10 %	10:90, 20%	$14.29\pm1.85$	$81.15 \pm 1.96$
Alginate-NaK	10 %	10:90, 15%	$22.82\pm1.95$	$71.10\pm3.57$
	10 %	10:90, 20%	$12.81\pm2.16$	$73.45\pm4.75$
Carboxymethyl cellulose-gelatin	5 %	10:90, 15%	$42.99 \pm 4.70$	$90.49 \pm 4.75$

## The Opacity of Composite Films

A low opacity value is expressed as a high transmittance indicator for films. The opacity values of the composite films range from 0.229 to 0.580 (Table 3). Opacity values of sodium caseinate composite films were higher than gelatin composite films. Gelatin is used in various food products with its gel-forming feature as well as its clarity and shine-giving function. The smooth gel structure formed due to the denaturation of gelatin has a bright and clear appearance (Erge and Zorba, 2018). For this reason, it is expected that the opacity values of the gelatin films are low; in other words, the light transmittance is high.

Opacity values of sodium caseinate composite films were higher than gelatin composite films. Decreasing dry matter content in the pectin-gelatin films increased the opacity, which may be associated with the clarity from the gelatin. The same situation was observed in alginate-gelatin composite films. Among gelatin-based composite films, alginate-gelatin composite films with 5% dry matter showed the highest opacity. The relationship between concentration and opacity in gelatin-based films is also reported in the literature. Soy protein and gelatin-based composite films were developed by Cao et al. (2007) and it was found that when the soy protein/gelatin ratio in the films changed from 8:2 to 0:10, the opacity of the composite films decreased due to the increasing amount of gelatin. When the gelatin content of the composite films is increased, there are fewer soy



protein particles in the films and the films become more transparent due to the clear structure of the gelatin.

Table 3. The opacity of gelatin and sodium caseinate based composite films obtained with different dry matter and glycerol content.

Type of film	Dry matter	CHO:PRO, Glycerol (%)	Opacity value
Alginate-Gelatin	10 %	10:90, 15%	$0.262\pm0.006$
	7.5 %	10:90, 15%	$0.344\pm0.019$
	5 %	10:90, 15%	$0.519\pm0.012$
Pectin-gelatin	10 %	10:90, 15%	$0.283\pm0.019$
	7.5 %	10:90, 15%	$0.459\pm0.074$
	5 %	10:90, 15%	$0.498\pm0.034$
Pectin-NaK	10 %	10:90, 15%	$0.580\pm0.037$
	10 %	10:90, 20%	$0.534\pm0.050$
Alginate-NaK	10 %	10:90, 15%	$0.535\pm0.032$
	10 %	10:90, 20%	$0.695\pm0.050$
Carboxymethyl cellulose-gelatin	5 %	10:90, 15%	$0.356\pm0.017$

## Conclusion

In composite films obtained with protein, gelatin and sodium caseinate, pectin-based films provided lower water vapor permeability than alginate-based films. Sodium caseinate-based composite films were found to have poor textural properties at low concentrations. Gelatin alginate composite films have the highest elongation ability among all composite films. Opacity values of sodium caseinate-based composite films were higher than gelatin-based composite films, but the light transmittance of gelatin-based composite films is improved at low dry matter content. For all film properties, carbohydrate and protein types were selected as pectin and gelatin, respectively, for preparing biocomposite food packaging film.



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# EMERGING TRENDS IN NON-DAIRY PLANT-BASED PROBIOTIC FOODS

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# Abstract

Probiotics are living organisms that have various beneficial health effects for humans and animals. Although most of the commercial probiotic foods are dairy based, there is an increasing consumer and commercial interest for non-dairy probiotic foods. Consumer concerns such as vegetarian/vegan nutrition, lactose intolerance, cholesterol, and milk allergies for dairy products play important role in this trend. Plant based probiotic products such as vegetable, fruit and cereal based foods present suitable media and a good substrate for growth and viability of probiotic microorganisms. The purpose of this work is to discuss recent studies for various non-dairy plant based food products and understand the current status. Terms such as probiotic strain type, pH, sensory characteristics, temperature, storage conditions, viability, oxygen viability and shelf life are of great importance in the development of non-dairy probiotics. It seems that fruit based non-dairy probiotic foods such as fruit juices, ready to eat fruits, dried fruits and fermented tables have been studied more comprehensively than others such as vegetable, cereal and legume based ones. In the initial years, limited probiotic strains belonged to Lactobacillus and Bifidobacterium genus obtained by human sources were used. During years, various probiotic strains obtained from fermented food environments were also included. Beside common health effects, new specific beneficial health claims can be targeted for non-dairy probiotic foods. Consumer acceptance and organoleptic properties seem main limitations for industrial productions. In the future, new and diverse commercial non-dairy probiotic foods are expected in the global market.

Keywords: Non-dairy, Probiotics, Fermentation, Lactobacillus.



# MICROBIOLOGICAL CONTAMINATION OF FRESH MILK

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# Abstract

Due to its chemical composition, milk is an extremely suitable substrate for the growth and development of many microorganisms including pathogens. So, the cases of diseases associated with the consumption of milk and dairy products in different countries are placed in a special place in the scientific and professional interest. In order to control fresh milk and milk products, the control of microbiological correctness of the samples taken in two cities at local markets and shopping centers that sell such products was done. A microbiological analysis of samples taken in the summer and winter months of the year was performed to make a comparison of these two annual periods. For research purposes, a total of 112 samples of milk and dairy products were taken: fresh milk, sour cream, yogurt, cottage cheese, cheese in a slice, whole cheese. The results of microbiological analysis of samples taken from shopping centers in the summer months showed that 13 samples or 43.3% did not comply with the regulations and 17 samples or 56.7% did. Samples taken from local markets at the same time proved to be much safer because 8 or 26.7% of them did not meet, and 22 samples or 73.3% met the requirements of microbiological safety. Repeated analysis in the winter period showed, out of a total of 36 samples of dairy products taken from shopping centers, only 7 or 19.5% did not meet the requirements of 29 samples or 80.5% met the microbiological requirements. Of the 16 samples of dairy products taken from the local markets, 5 or 31% did not meet the requirements for microbiological safety, while 11 or 69% met the requirements. In conclusion, looking at the overall results of microbiological analysis in these two periods, 79 samples or 70.5% met the requirements of microbiological correctness while 33 or 29.5% did not meet.

Keywords: Fresh Milk, Milk Products, Microbiological Analyses, Good Hygiene Practices, Monitoring

# Introduction

Raw milk is material extremely suitable for the growth and development of many microorganisms, including pathogens such as S. *aureus, Bacillus spp., Clostridium botulinum, L. monocytogenes, E. coli, Shigella spp., Salmonella spp., Campylobacter spp., and Brucella spp.* Therefore, cases of disease associated with cheese consumption in different countries are placed in a special place in the scientific and professional literature (Panday et al., 2000; Hegarty et al., 2002; Espie et al., 2006, Markov et al., 2009). The primary sources of pathogenic bacteria in milk are a



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sick milking animal, an infectious udder, and a sick man. However, the cause of the spread of pathogenic bacteria in milk is always the result of insufficient hygiene, ignorance or negligence of workers (Samaržija, 2014).

Contamination of milk and the other dairy products with pathogenic bacteria can occur at any time: during production, processing and marketing. The primary sources of pathogenic bacteria in milk and dairy products are: sick milking animals, infectious udder (mastitis) and sick man. Spoilage of raw milk by lactic acid bacteria is possible if the milk is not stored at a temperature of 4 ° C after milking. This most often occurs in underdeveloped countries where milk is still transported in buckets or tanks without refrigeration, especially in warmer weather (Tratnik and Božanić, 2012).

*Lactobacillus, Lactococcus, Leuconostoc and Enterococcus spp.* are the most common causes of spoilage of unrefrigerated milk, and the error is manifested by a change in the taste and appearance of milk. The bacterium *Lactococcus lactis subsp. lactis* is the main cause of spoilage of raw milk if stored at a temperature of 10-37 ° C, it is able to form lactic acid and small amounts of propionic acid. *Mesophilic bacteria* and most species of *Thermophilic lactic acid bacteria* destroy medium pasteurization temperatures of 72 ° C / 15 seconds (Regulation (EC) No 853/2004., Walstra et al., 2006).

In order to minimize the possibility of contamination of milk and dairy products, it is necessary to conduct hygiene control, which includes raw materials, especially fresh milk, finished products, employees, equipment, auxiliary devices and means of transport on delivery, distribution and sale (Karahmet et al., 2017). The aim of this research was to gain insight into the microbiological picture of milk and other dairy products sold in the markets and shopping centers.

## Material and method

For the purpose of analyzing and checking the level of health safety of milk and milk products, sampling and microbiological analysis of raw milk and milk products for the presence of *Aerobic mesophilic bacteria and Coagulase positive staphylococci* was performed, which proves the acceptability of raw milk for further heat treatment. Sampling was conducted twice, in the summer and winter time. Summer sampling took place in July and August, winter sampling was carried out in November and December. Total number of taken samples was 112. In the summer period, 60 samples of milk and dairy products were taken, 30 from two local markets and 30 from shopping centers. In the winter, 52 samples were taken, 36 samples of dairy products from shopping centers and 16 samples from local markets. Microbiological analyzes has been performed according to Ordinance on the organization of official controls of products of animal origin intended for human consumption (Official Gazette, No. 103/12), and Rulebook on microbiological criteria for food (Official Gazette of B and H No. 11/13). After taking samples, they were subjected to microbiological analysis in the laboratory for microbiology at the Faculty of Agriculture and Food in Sarajevo.



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#### **Results and Discussion**

After performed microbiological analyzes of samples of fresh milk and other milk products, the following results were obtained. During the summer sampling there were taken 60 samples thirty in shopping centers and thirty in the local markets. Collected analyzes showed that out of 30 samples of dairy products taken from shopping centers, 13 of them or 43.3% did not meet the requirements of the Ordinance on the microbiological safety of food. In this analysis 17 or 56.7% of them met the requirements of the Rulebook on microbiological criteria for food (Official Gazette of Bosnia and Herzegovina No. 11/13).

Situation in the local markets was a bit different than in the shopping centers. Out of 30 used samples milk and the other dairy products 22 or 73.3% of them were satisfied. Eight analyzed samples or 26.7% weren't meet microbiological criteria for food (Official Gazette of Bosnia and Herzegovina No. 11/13). It means that dairy products from local markets had been more safety than milk products from shopping centers in same period even situation was really bad in both places.



Figure 1. Results of shopping centers versus market places microbiological analysis of dairy products (summer samplings)

In the winter sampling, 36 samples of milk and various dairy products were taken from the shopping centers. After the performed microbiological analysis, we obtained very poor results. Out of a total of 36 samples of dairy products taken from shopping centers, only 7 or 19.5% did not meet the requirements of the Ordinance on the microbiological safety of food. In this analysis of 36 samples 29 or 80.5% of them met the requirements of the Rulebook on microbiological criteria for food (Official Gazette of Bosnia and Herzegovina No. 11/13) (Annexes 1 and 2). The most common causes of unsatisfactory findings were the following groups of microorganisms: *Yeasts and Molds* in 13 cases, *Escherichia* in 2 cases and *Enterobacteria* in 2 cases. It is certainly important to note that it is "common" to find yeasts and fungi in fermented milk products, but what is worrying is that they should never have been found there: *Escherichia and Enterobacteria*.



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The obtained results show that good hygienic and good manufacturing practice was not conducted during the manipulation or storage of products in the refrigeration system. The assumption is that even during the manipulation, not much care was taken about the good hygienic practice among the employees in the shopping centers, because there were yeasts and *Escherichia coli and Enterobacteria*, which is by no means the usual microflora of these products (Karahmet et al. 2017, Samaržija 2014).

From the results obtained by microbiological analysis of milk samples and the dairy products, presence of *yeasts and molds* (67%), *Enterobacteriacae* (22%), *Escherichia spp* (11%) were found, while *Staphylococae* were not found. The number of total colonies forming units of microorganisms formed ranged from  $2.5 \times 10^2$  CFU / ml), in *Escherichia coli* to  $170 \times 10^2$  CFU / ml), in *Enterobacteria*. (Figure 1).

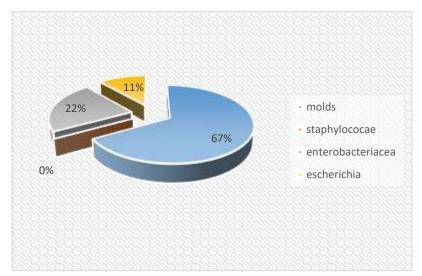


Figure 2. Presence of microorganisms in shopping centers samples (Winter sampling)

Although twice less samples were taken from local markets than from shopping centers, in the end, quite bad results were achieved. Namely, out of 16 samples taken for microbiological analysis, 5 (31.5%) of them didn't met the requirements, 11 samples (68.5%) met requirement of the Ordinance on microbiological safety of food Official Gazette of Bosnia and Herzegovina No. 11/13. These data tell us what the risk of buying and consuming dairy from the local markets was in the city zone. As can be seen from the Figure 3, four samples of homemade sour cream met the microbiological criteria out of a total of six, it was also confirmed with samples of hard fat cheese ratio was 4 to 1 for satisfying samples, while for cottage cheese the ratio was 3 to 2 for satisfying samples. If we look at the results of this analysis, it was necessary to react urgently in order to fundamentally change the situation, otherwise there would be a more serious incident of foodborne illness like intestinal and other diseases in the narrower or wider area of the city.

The results of the percentage of positive (unsatisfying) and negative (satisfying) analyzed samples of dairy products from the local markets was 5 versus 11 which is a really bad finding that



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required a broader action by the inspection authorities. As can be seen from the figure below of the 16 sampled products, 5 or 31.5% of them did not meet the requirements of the Ordinance on microbiological safety, while 11 or 68.5%. Numerically, the number of forming colonies of individual microorganisms in samples taken from the local markets ranges from  $66 \times 10^2$  CFU / ml to  $400 \times 10^2$  CFU / ml that is in accordance with Clays et al., 2013., De Reu et al., 2004. Gillespie et al., 2003).

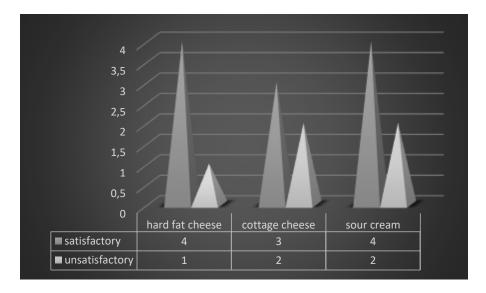


Figure 3. Results of market places analysis of dairy products (Winter samplings)

Due to everything obtained in this control and despite the demand for "peasant" products to which consumers give certain advantages over products from industrial production, the sale of these products poses a potential danger to consumer health and a real public health problem. Restrictive measures should therefore presuppose well-organized veterinary and sanitary control of the production and sale of the mentioned products on the markets of larger cities (Karahmet et al., 2017; Samaržija, 2015; De Reu et al., 2004; Gillespie et al., 2003).

The obtained results showed that the samples of raw milk and milk products do not meet all the provisions of the Ordinance. Microbiological analysis gave worrying results, given the fact that the presence of non-desirable microorganisms was found in raw milk samples. Also, statistical analysis of the obtained results proved that there are statistically significant differences in the quality of the tested samples in terms of sampling location.

After this control, it can be stated that the unsatisfactory health safety of milk and dairy products is obviously related to insufficient maintenance of hygiene in milk production and processing. However, the presented data together with the data from the available literature indicate the fact that the most common problems can be found in insufficient attention in the part of hygiene of raw material and finished product handling (Samaržija 2015; Šolaja et al., 2007; Tratnik and Božanić, 2012; Božanić et al., 2012). Although the health safety situation of dairy products should be significantly improved, it can still be said that more frequent product controls should be performed



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on the market and also in shopping centers until sufficiently reliable results of microbiological analyzes of fermented dairy products are achieved.

Data from domestic and foreign literature and our practical experience in Veterinary - sanitary control indicate the fact that dairy products in general, and even home - made products, in most cases do not meet the prescribed requirements of hygiene. On the other hand, in professional and administrative circles, the question of how to ensure purposeful administrative and other measures to protect the health safety of these products with clear indications of the implementation of restrictive measures in their sale in the markets is constantly relevant.

If we want to have quality and safe milk, we should take all possible precautions on the way to raw milk from the farm to the sale. In this fight against microorganisms, several important conditions must be met: impeccable hygiene of milking and further handling; it is important to cool the milk to (4 °C) as soon as possible; provide a closed milk handling system; as short as possible cold storage of milk (4 °C); as short as possible transport of milk with refrigeration; impeccable cleanliness of equipment; heat the milk by pasteurization or sterilization (Lejeune, J. T., & Rajala-Schultz, P. J. 2009., Karahmet et al., 2017., Samaržija 2015., De Reu et al., 2004. Gillespie et al., 2003).

#### Conclusions

In accordance with the obtained analysis results, we can conclude the following. By comparing the results of microbiological analyzes of shopping centers and samples from the local markets, devastating results were obtained. Namely, out of 112 samples of dairy products, 79 or 70.5 % met the requirements of the Ordinance on microbiological safety of food Official Gazette of Bosnia and Herzegovina No. 11/13. From the total samples taken for microbiological analysis, 33 or 29.5 % of them of them did not meet requirements. What is worse, in 7 cases Enterobacteria were identified, in 5 cases Staphylococae and in 8 cases Escherichia coli. By the analysis of the results of microbiological findings of samples of dairy products taken from the market revealed the presence of Yeasts and Molds (43%), Enterobacteriacae (9%), Escherichia spp (26%), while Staphylococae (22%) were present. The presence of pathogenic species was shown in all sales facilities which clearly indicates that the main risk bearer in the facilities is a worker, so the basic recommendation would be the education of employees, which would contribute to increasing general personal hygiene. At the end of this research, inspection bodies were activated to more often control and sanction such bad practice. Considering the high rate of raw milk and dairy products contamination with the above isolated bacteria, sanitary practice during collecting, transporting and vending is recommended since the consumption of unpasteurized milk may inflict an important public health risk.

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## PACKAGING SOLUTIONS TO REDUCE BREAD WASTAGE

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#### Abstract

The wastage of bread, which is one of the most consumed foodstuffs in the world, is one of the important social problems today the most important causes of bread wastage are staling or microbiological deterioration of bread quickly, that is, bread is not a foodstuff having a long shelf life. One of the most effective methods to prevent bread wastage is to use appropriate packaging technology that increases the shelf life of the bread. However, it is stated that approximately 70% of the bread sales in the world are unpackaged, 30% are packaged. While the market growth rate of packaged bread in the last five years was 5%, this value was 2% for unpackaged ones. As a result, unpackaged bread is more consumed, but growth is in favor of packaged bread. The fact that the shelf life is longer and the amount of waste is reduced accordingly, and that it is hygienic, naturally causes the packaged bread market to grow. When we look at the geographical distribution of bread market, the packaged bread is mostly consumed in North America, Western Europe, and Australia, while unpackaged bread is preferred in South America, Eastern Europe, and developing countries. Nowadays, the world is faced with the Covid-19 pandemic, and the demand and thus the supply for people to consume bread and other foodstuffs in packaged forms has increased. However, to protect the environment and reduce the amount of food waste, it is necessary to use appropriate packaging technologies in bread, as in other foodstuffs. For this reason, in this review study, the packaging applications that are effective in extending the shelf life of different bread types are mentioned to reduce the wastage of bread and to be beneficial to the bakery products sector.

Keywords: Bread, Shelf Life, Packaging, Wastage

## Introduction

Among foods, bread is one of the basic cereal food and has great religious, cultural, or even social importance. Having a neutral taste and aroma, being cheap, the ease of obtaining the materials used in production, having high nutritional value, and can be consumed with several foods make bread the most primary food item (Adıgüzel et al., 2019; Demir, 2010).

However, social changes that have occurred over time have strongly modified the way bread is produced, purchased, and consumed, increasing the need to extend the shelf life of this perishable product. The waste of bread, which is one of the most consumed foodstuffs in the world, is one of the important social problems today.



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The most important causes of bread wastage are staling of bread or microbiological deterioration, that is, bread does not have a long shelf life. The shelf life of bread is mainly affected by two mechanisms. One of them is staling phenomenon which is a complex of chemical and physical changes occurring during storage, involving a progressive decrease of consumer acceptance. (Licciardello et al., 2017; Pateras, 2007; Rodri'guez et al., 2000; Avital et al., 1990). Staling involves structural changes, such as an increase in the firmness, and dryness of the crumb, due to moisture loss and starch retrogradation, as well as aroma and flavor reductions, due to oxidative phenomena. Also related to loss of crust crispiness, due to the relocation of water from crumb to crust. The other shelf-life limiting factor for bread is microbiological growth (Ding et al., 2019; Curti et al., 2017; Licciardello et al., 2017; Amigo et al., 2016; Giannone et al., 2016; Alhendi and Choudhary, 2013; Fik et al., 2012; Novotni et al., 2011; Ronda et al., 2011; Pateras, 2007; Kotsianis et al., 2002; Rodri'guez, 2000). There are three kinds of microorganisms that affect bread spoilage. First is mold which is the main cause of bread spoilage. Bread is especially susceptible to mold growth due to its high humidity, water activity between 0.94 and 0.98, pH around 6 (Schmidt et al., 2020; Arvanitoyannis., 2012; Pateras., 2007). The most common bread spoilage molds are Penicillium spp., Aspergillus spp. Bacteria are a second factor, especially bacteria which produces ropes cause rope disease. This is caused mainly by Bacillus subtilis but other species of Bacillus are also capable of causing rope disease. including B. licheniformis, B. megaterium and B. cereus (Thompson et al., 1998). The third group is yeasts. The most important yeast in bread is Endomyces fibuligera (Upasen and Wattanachai, 2018; Alhendi and Choudhary, 2013; Celik, 2008; Avital, 1990; Nielsen and Rios, 2000). Limiting spoilage and deterioration reactions of bread are of great importance at the industrial level when the product is sold far from the place of production and significantly needs an extended shelf life.

As bakery products are becoming an essential part of the international food market, the baking industry should develop and optimize the technology to increase the value and enhance the safety of these products (Heras-Mozos et al., 2019; Licciardello et al., 2017; Gray and Bemiller, 2003; Kotsianis et al., 2002).

One way to reduce this economic loss and bread wastage is the use of proper packaging systems. Bread shelf-life can be improved utilizing various packaging solutions, essentially aimed at retarding mold growth and staling. Packaging technology affects the shelf life of food, intended as the time during which the decline of sensory and microbiological quality remains at a tolerable level. The main aim of packaging is to protect the bread from environmental effects, such as contaminants, microbial spoilage, oxygen transmission, water vapor, and insects (Heras-Mozos et al., 2019; Upasen and Wattanachai, 2018; Licciardello et al., 2017; Alhendi and Choudhary, 2013; Fik et al., 2012).

The conventional packaging procedure applied in the baking industry involves atmospheric packaging of bread by using polymeric films having high gas transmission rate. However, modern packaging is performed under a modified atmosphere or allowing packaging to interact with food which means active and intelligent packaging technology. Active and intelligent packaging, are



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made of functional materials deliberately interacting with food for extending or monitoring the shelf-life.

In this review, various types of packaging already used or recently proposed to extend the shelf life of bread are presented along with their main active functions.

#### **Common Types of Packaging Materials for Bread**

The most commonly used material for bread packaging was paper, waxed paper, or glazed imitation parchment, impregnated on both sides with a paraffin wax containing PE-LD and other alternative additives, or cellulose film. Currently, bread is usually packaged in bags made of polyolefin films, such as polypropylene (PP) or low-density polyethylene (PE-LD) bags in which the end is twisted and sealed with a strip of adhesive tape. This form of packaging makes it possible to retard a mode of deterioration of the bread, namely the loss of moisture (Galić et al., 2009).

However, these types of packaging represent a system of protecting bread against environmental contamination but can achieve only a very slight improvement in shelf life in terms of maintaining its softness. Therefore, these packaging types are suitable only for fresh bread, intended for immediate consumption, and are appropriate for consumers used to an approximately daily purchase in short food supply chains (directly from a local retailer).

#### **Modified Atmosphere Packaging**

Modified atmosphere packaging (MAP) is the modification of the packaging atmosphere to provide an optimum atmosphere in a way that will extend the shelf life of the food and preserve its quality characteristics for a long time. MAP for bread mainly involves removing or reducing the oxygen gas concentration and supplying carbon dioxide gas having fungistatic effect in-package atmosphere to inhibit the growth of aerobic microorganisms (Upasen and Wattanachai, 2018; Licciardello et al., 2017; Giannone et al., 2016; Hasan et al., 2014; Licciardello et al., 2014; Alhendi and Choudhary, 2013; Fik et al., 2012; Muizniece-Brasava et al., 2012; Değirmencioğlu et al., 2011; Kotsinais et al., 2002; Rasmussen and Hansen, 2001; Rodriguez et al., 2000; Nielsen ve Rios, 2000; Farber, 1991; Avıtal et al., 1990). Most of the MAP applications for bakery products is composed of carbon dioxide alone or in combination with nitrogen in different concentrations (20% CO2 + 80% N2, 50% CO2 + 50% N2, 60% CO2 + 40% N2, 100% CO2).

Many researchers have been studied the optimal concentrations and types of gases for MAP as shown in Table 1. In all these studies, the main aim was to reduce the oxygen content in order to delay the firming of the bread and increase the shelf life. Hasan et al. (2014) studied different concentrations of carbon dioxide and showed that the mold-free shelf-life of pita bread was extended to more than 3 weeks. In addition, Muizniece et al. (2012) examined the active packaging in combination with modified atmosphere and showed that the shelf-life of wheat bread samples packed in (MAP) was extended from 7 days up to 11 days. In another study, calcium-enriched wholemeal bread was packed by MAP (60% CO2, 40% N2), and no microbiological changes were observed after 24 days at  $20 \pm 1$  °C (Fik et al., 2012). Sliced bread containing 0.15% of potassium



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sorbate delayed mold growth for up to 21 days with MAP (Degirmencioglu et al., 2011). Sourki et al. (2010) indicated that the shelf life of traditional Iranian flatbread was prolonged from four days to about 21 days by using MAP with high carbon dioxide concentration and high-barrier laminated and vacuum bags packages. On the other hand, Rasmussen and Hansen (2001) found no significant differences in the firming rate of wheat bread, compared to sample bread packaged in atmospheric air, but MAP had a positive effect on the microbial shelf-life of bread. With no added preservative, MAP composed of 50% CO2 and 50% N2 extended the shelf-life of bread from 2 to 15 days at 22-25 °C (Rodri guez et al., 2000).

Breads	Gas combination		Shelf life		Reference
Dieaus	%CO <sub>2</sub>	%N <sub>2</sub>	MAP	In air	Reference
Pita bread	40-100	60-0	More than 3 weeks	2 days	Hasan et al., 2014
Sliced wheat bread	60	40	11 days	7 days	Muizniece et al., 2012
Wholemeal bread (calcium- enriched)	60	40	Up to 24 days	6-12 days	Fik et al., 2012
Sliced bread	0-100	100-0	21 days	7 days	Degirmencioglu et al., 2011
Iranian flat bread	50-70	50-30	21 days	4 days	Sourki et al., 2010
Wheat bread	50-100	50-0	24 days	8 days	Rasmussen and Hansen., 2001
Sliced wheat flour bread	0-50	100-50	15 days	2 days	Rodrı´guez et al., 2000

Table 1. Studies related with the modified atmosphere packaging of different types of breads

## **Active Packaging**

In active packaging technology, the active ingredients are integrated into the package and can be extended the shelf life of the food by providing a targeted change in the packaging by direct contact of the package with the food or the controlled release of the volatile active substance (Pasqualone, 2019; Upasen and Wattanachai, 2018; Ahmed et al., 2017). Although MAP technology removes oxygen from the package, some oxygen remains in products that contain pores, such as bread, cakes, and cookies. This oxygen may cause the growth of molds and other spoilage bacteria or oxidation that results in undesirable odors and flavors (Üçüncü, 2007).

To prevent mold growth, the oxygen rate in the atmosphere should be below 0.4% (Upasen et al., 2018). However, this problem can be solved by active packaging, which can be more efficient



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than classical MAP technology. Active packaging includes some active ingredients such as oxygen and ethylene scavengers, carbon dioxide scavengers and emitters, and moisture absorbers. For example, oxygen scavenger sachets absorb oxygen inside the package and reduce the oxygen concentration in hours to less than 0.01% (Ahmed et al., 2017; Alhendi and Choudhary, 2013; Muizniece-Brasava et al., 2012). Sachet-based AP effectively reduces the trapped oxygen in the package and can extend the bakery product shelf life.

Breads	Packaging conditions	Shelf life	Reference
Chinese steamed bread	AP: oxygen absorber combined with ethanol emitter	6 days to 11 days	Sheng et al., 2015
Ciabatta bread	AP: active ethanol emitters + MAP	5 days to 16 days	Hempel et al., 2013
Sliced wheat bread	AP: oxygen scavenger MAP: CO <sub>2</sub> 60% and N <sub>2</sub> 40%	7 days to 14 days	Muizniece et al., 2012
Bun and bread	AP: linear low-density polyethylene (LLDPE) film was modified by blending with zinc, iron, or ascorbic acids as oxygen scavenger	Extended from 2 to 5 days	Matche et al., 2010
Sliced wheat bread	AP: an ethanol emitter combined with an oxygen absorber	4 days to 30 days	Latou et al., 2010
Wheat and rye bread	Different MAP AP: oxygen absorbers AP: Mustard oil	2 days to 30 days	Suhr and Nielsen, 2005
High moisture/ph bakery products	AP: water-ethanol and mastic oil- ethanol emitters	3 days- > 28 days	Koukoutsis et al., 2004
Pre-baked buns	AP: LD-PE bags with a commercial ethanol emitter	From 4 to 13 days	Franke et al., 2002
Sliced rye bread	AP: ethanol and oxygen absorbers	From 8-12 to 42 days	Salminen et al., 1996

Table 2. Studies related with active packaging technology in different types of breads

The most widely studied active packaging systems for extending the shelf life of bread are oxygen absorbers and/or ethanol emitters as shown in Table 3. Muizniece et al. (2012) showed that oxygen absorbers are effective to prevent microbial growth during storage and enhance the shelf life of wheat bread up to 14 days. Moreover, oxygen scavenging agents can also be incorporated into the packaging film matrix in active packaging applications. Matche et al., (2010) studied with oxygen scavenging film of LLDPE (linear low density polyethylene) for packaging of bread and extended the shelf life from 2 to 5 days, because of less oxygen availability and control of moisture inside the package. Another study showed that the use of ethanol emitters alone extended the microbiological quality of Ciabatta bread to 16 days without the need for additional modified atmosphere gas application (Hempel et al., 2013). Pre-baked buns, packed into gamma sterile low-



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density polyethylene bags with different amounts commercial ethanol emitter, named Ethicap (Freund Corporation, Tokyo, Japan), retarded mold growth for 13 days (Franke et al., 2002).

Combination of oxygen scavengers and ethanol emitters is more effective to extend the shelf life of bread. In a study, oxygen absorbing and ethanol emitting sachets were used in combination for extending the microbial stability of wheat-based Chinese steamed bread, and oxygen absorber alone extended the shelf life to 6 days whereas the combination of oxygen absorber and ethanol emitter extended to 11 days (Sheng et al., 2015). Latou et al., (2010) showed that the combined form of oxygen absorbing and ethanol emitting active sachet was more effective in inhibiting the growth of molds, yeasts, and bacteria in sliced wheat bread in comparison to the use of chemical preservatives, such as calcium propionate and potassium sorbate. Shelf life was extended to 30 days for samples containing the combined active sachets whereas it was 6 days for bread with preservatives. Salminen et al., (1996) also reported that the microbial shelf life of sliced rye bread allowed to prolog up to 42 days by packaging with commercial oxygen absorber ATCO and ethanol emitter.

Agents having antioxidant capacity such as essential oils, plant extracts can also be used in active packaging technology to maintain the oxidation properties of the food or inhibition of microbial growth. This active packaging method can also be used for bread. A combination of MAP and active packaging of mustard oil added into the package to a piece of 1x1 cm sterile filter paper inhibited the mold growth of wheat and rye bread for 30 days (Suhr and Nielsen, 2005). Koukoutsis et al., (2004) have been evaluated water-ethanol (WE) and mastic oil-ethanol (ME) emitters to control the growth of microorganisms in high-moisture and high pH bakery products and they found that growth of most microorganisms was inhibited for more than 28 days.

Nano-additives such as nano cellulose can be used the food industry a stabilizing agent, emulsifier and can also be used in food packaging, including active packaging applications.

Breads	Packaging materials	Shelf life	Reference
Sliced wheat bread	A new nanocomposite film and coating based on chitosan-carboxymethyl cellulose-oleic acid (CMC-CH-OL) incorporating zinc oxide (ZnO) nanoparticles	shelt-life of sliced wheat	Noshirvani et al., 2017
Bread	A nanofibrous membrane of montmorillonite- nylon 6 (MMT-N6) was deposited, by electrospinning technique, over PP films	This packaging solution inhibit fungal growth for 5 days of storage.	Agarwal et al., 2014
Bread	The active packaging was prepared by coating an ethanol suspension of $Ag/TiO_2$ nanocomposite on a PE layer.	No yeasts and molds observed after 6 days of storage.	Mihaly Cozmuta et al., 2014
Sliced bread	Methylcellulose films incorporating nanoemulsions (droplet diameter 180-250) of essential oil of Clove Bud (Syzygium aromaticum) and Oregano (Origanum vulgare)	Both essential oils lessened the counts of yeasts and molds in sliced bread during for 15 days.	Otoni et al., 2014

Table 3. Studies related with use of nanotechnology in active packaging



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A new nanocomposite coating based on chitosan-carboxymethyl cellulose-oleic acid (CMC-CH-L) incorporated with different concentrations of zinc oxide (ZnO) nanoparticles, allowed to extend the shelf-life of sliced wheat bread from 3 to 35 days compared to control without coating (Noshirvani et al., 2017). In another research, a nanofibrous membrane of montmorillonite-nylon 6 (MMT-N6) was deposited, by means of an electrospinning technique, on polypropylene films used for bread packaging. This packaging solution displayed better oxygen barrier properties than PP alone, and therefore inhibited fungal growth during 5 days of storage (Agarwal et al., 2014). Mihaly Cozmuta et al. (2014) proposed an active packaging system based on Ag / TiO2 nanocomposite in ethanol suspension coated onto a high-density polyethylene layer. Ag/TiO2-HDPE significantly extended the shelf life and the microbiological stability of bread compared with neat HDPE bags and no yeasts and molds were observed after 6 days of storage. Methylcellulose films incorporated with nanoemulsions of essential oil of clove bud (Syzygium aromaticum) and oregano (Origanum vulgare) decreased yeasts and molds counts in sliced bread stored for 15 days (Otoni et al., 2014).

## **Intelligent Packaging**

Intelligent packaging systems are the packaging systems that provide information to producers, retailers, and consumers in the context of food safety and quality and give them early warnings about product quality. Simple packaging may not be enough to maintain quality, there should be an intelligent system that can constantly monitor the packaging environment and provide the correct information at the right time regarding the quality characteristics of the food products. For this purpose, different intelligent devices can be integrated into food packaging systems in order to make the environment intelligent and to communicate with users regarding the changing form of the packaging (Sohail et al., 2018).

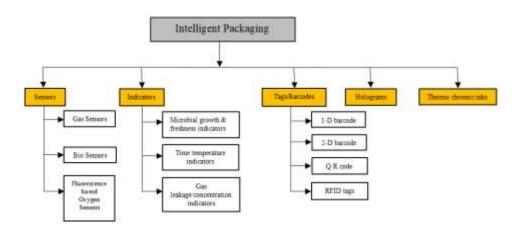


Figure 1. The structure of the intelligent packaging system, including various kinds of substances and devices

For example, Eye Oxygen (Mitsubishi Gas Chemical Co., Tokyo, Japan) acts as a gas sensor is a self-adhesive label whose color changes from purple to pink when oxygen in the package falls below 0.1%.



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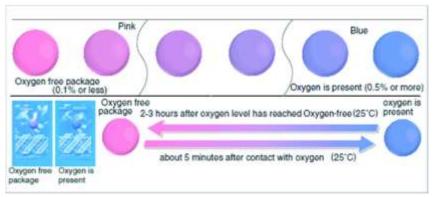


Figure 2. Oxygen sensor indicating the oxygen concentration in the food package.

Table 4. Studies related	with the intelligent	packaging of	different types of bread
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Breads	Packaging conditions	Smart technologies	Reference
Chinese steamed bread	AP: oxygen absorber combined with ethanol emitter	Eye oxygen indicator	Sheng et al., 2015
Ciabatta bread	AP: active ethanol emitters + MAP	Oxygen sensor	Hempel et al., 2013

The studies related to the intelligent packaging of bread are presented in Table 4. Eye oxygen indicators, in particular, was used to monitor oxygen levels to extend the microbial stability of Chinese steamed bread using a combination of an ethanol emitter and an oxygen absorber (Sheng et al., 2015). The oxygen indicator was attached to the inside of commercial Ciabatta bread packs before gas flushing (for MAP) and sealing. The package also contained an ethanol emitting sachet. This sensor allowed continuous and non-destructive monitoring of the amount of oxygen inside the pack over time (Hempel et al., 2013).

## **Edible Films and Coating**

Environmental issues make sustainability become another important requirement for packaging. Edible coatings are thin layers of edible material applied directly on the product surface providing a barrier to moisture and oxygen or exert an antifungal activity.

A study conducted by Bun<sup>\*</sup> ková et al. (2010) showed that monoacylglycerols such as 1monocaprin, 1-monocaprylin, and 1-monolaurin have antifungal activity against fungi isolated from contaminated bread. Also, spraying aqueous solutions of these monoacylglycerols in the form of microfilm on the surface of freshly baked bread had a significant effect on preventing spoilage: bread remained free of molds for fourteen days of storage even in a contaminated environment. This



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system seems to be a suitable procedure for the treatment of bakery products with a prolonged storage period.

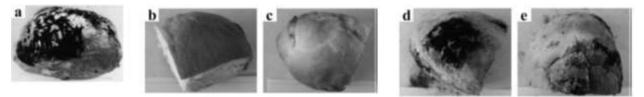


Figure 3. Antifungal effect of monoacylglycerol (MAG) on bread only contamination from environment after 14 days. (a) Control: bread without treatment of MAG, (b) treatment with 1-monocaprylin after baking, (c) treatment with 1-monocaprylin before baking, (e) treatment with 1-monocaprin before baking, (e) treatment with 1-monocaprin before baking (Bun<sup>×</sup> ková et al., 2010).

In another research conducted by Mladenoska et al., (2013), monolaurin has been investigated as an antimicrobial agent also by incorporating it in a lipid-based (such as sunflower oil) edible coating for bread. Coated bread had approximately two times longer shelf-life than uncoated.

#### Conclusion

It is seen that from the studies that it is possible to provide an extended shelf life of bread by modified atmosphere packaging or novel packaging methods such as active and intelligent packaging or functional edible films and coatings. Most of these methods will not cost much and these are easy-to-apply methods. Increasing the shelf life of bread is a traditional challenge for especially industrial producers. Considering that the industrialization of bread production has increased recently, we think that we will see these packaging is an effective way to reduce bread wastage and allow extended shelf life which in turn allows the product to be delivered far from the production site and reach new markets.

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## FOOD PACKAGING TRENDS WITHIN SUSTAINABLE DEVELOPMENT

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### Abstract

Food packaging is playing an increasingly important role in maintaining the added value of products by ensuring that food can be delivered with minimal waste. Packaging plays an important role in the safe distribution of products in today's society and supply chains. Packaging is a sustainable practice for the food industry on its own, but the increasing environmental impact of the disposal of widely used plastic packaging materials as waste and the limited resources cause concern in societies. For this reason, sustainable packaging concepts are constantly evolving. The food industry, non-governmental organizations, scientists and even consumers are becoming more aware of these concepts. With this awareness reaching global dimensions, sustainable packaging concepts and food packaging materials suitable for these concepts continue to be developed today. In this review, the concepts of biodegradable packaging materials, recyclable packaging, reducing of packaging waste and reusable packaging have been investigated and recent academic and industrial trends related to these concepts have been examined.

**Keywords:** Sustainable Develepment, Sustainable Packaging, Food Packaging, Biodegredability, Reducing Packaging Waste, Recycable, Reusable.

## Introduction

In addition to the limited resources of our world, the increasing needs of humanity due to population growth increase the future concern of humanity. Although awareness has increased in recent years, this concern is not new. This awareness, which emerged at the beginning of the 20<sup>th</sup> century, introduced us to the definitions of sustainability and sustainable development. Although it is expressed in many different ways, the most accepted definition of Sustainable Development is the definition put forward by the World Environment and Development Commission in 1987. According to this definition, sustainable development is the planning of the life of today and the future in a way that will allow the needs of future generations to be met and developed by ensuring the conscious consumption of resources without harming natural resources, by establishing a balance between human and nature. (Robertson, 2009; Atmaca, 2018).

Packaging materials are seen as waste by societies and are thought to have a negative impact on the environment. Therefore, the packaging industry has been under pressure to reduce packaging waste, prevent overuse of packaging and improve recyclability, especially during the last decade. In



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order to change this perception, packaging concepts and packaging materials have also started to be discussed under the scope of sustainability. Several organizations and firms have defined sustainable packaging (Lewis et al., 2007; Robertson. 2009). These definitions generally specify how sustainable packaging should be, rather than stating the meaning of sustainable packaging in a sentence. If we gather the most known definitions under one roof, sustainable packaging can be described as in Figue 1, according to the Sustainable Packaging Alliance (SPA) of Australia and the Sustainable Packaging Coalition (SPC) of the United States.

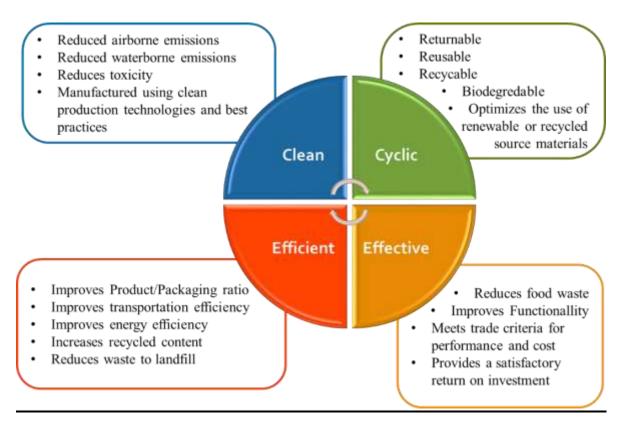


Figure 1. Definition of sustainable packaging due to according to SPA and SPC

It is known that toxic components emitted into the environment during the disposal of packaging wastes endanger the life of living things (Petkoska et al., 2021). Therefore, in the long run, there are increasing concerns that it will be effective in global warming, which will cause climate change. It is thought that future generations will also be adversely affected by this situation. Another reason is the inefficient use of limited resources, leading to a shortage of raw materials in the future, and the resulting increase in production costs (Emblem, 2012).

Because of such long-standing concerns, global community organizations and public institutions are shaping the industry with various legal and voluntary directives, contracts and regulations. There are regulations on the collection, storage, disposal of packaging waste, and the terms of use of recycled material. (Emblem, 2012). It is seen that consumers, as well as organizations, tend to prefer sustainable packaging consciously or unconsciously. The design, color, and general appearance of



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the packaging play a role in the preference of the product. Expressions such as "eco-friendly", "recyclable packaging", "recycled packaging", "Bio-packaging" or designs for these definitions tend to be more inclined in recent years (Barmaki, 2012; Magnier and Schoormans, 2015; Martinho et al., 2015; Boz et al., 2020; De Marchi et al., 2020).

Sustainable food packaging studies are widely studied in both industry and academia. In particular, the negative impact of petroleum-based plastics on the environment is becoming increasingly evident (Petkoska et al., 2021). Studies on the use of biodegradable materials as an alternative to petroleum-based plastic materials, reducing the use of packaging materials, and circularity in food packaging such as recyclable packaging, recycled packaging, reusable packaging have become popular in recent years. This study reviews both the main topics within the scope of sustainable packaging and the academic and industrial application trends in recent years.

#### **Biodegredable Polymers**

Petroleum-based polymers are widely preferred due to their ease of processing, being a light packaging material, preserving the food well, transportation efficiency, and low cost (Attaran et al., 2017). But, there is an increasing demand for food packaging that does not increase environmental pollution and for products that are produced efficiently with sustainable processes. As a result, this situation has led to an awareness and increase in research and industry focusing on the development of sustainable, biodegradable materials that can improve food safety and improve food quality. Biodegradable polymers are generally examined in two groups as polymers obtained from renewable and fossil resources. Polymers obtained from renewable resources are also known as biopolymers and are divided into subclasses as agrobiopolymers, synthetic biopolymers, and microbial biopolymers according to their method of production. Almost all biopolymers find their place in food packaging studies (Attaran et al., 2017; Zhong et al., 2020).

#### **Development of Biodegradable Packaging Alternatives**

Polylactic acid (PLA) is obtained by the polymerization of lactic acid monomers (mostly Llactide) and is a widely studied biopolymer in academia and industry (Arrieta et al., 2014a; Arrieta et al., 2014b). It can be processed by film extrusion, thermoforming, and blowing extrusion methods and has high processability. In addition, energy and carbon dioxide emissions are low. It is known that PLA can be successfully mixed with plasticizing agents such as Polyethylene glycol (PEG), Oligomeric lactic acid (OLA), and citrate esters like triethyl citrate and acetyl tributyl citrate (Garcia-Garcia et al., 2020). Although it has a good moisture barrier property, its gas barrier property is low. In order to improve the barrier and mechanical properties, it is generally coextruded with other biopolymers or petroleum-based polymers to improve the targeted properties, and the composites are formed with nanoparticles (Söğüt and Seydim, 2017). The barrier properties, thermal properties, and tensile strength of PLA can be improved by forming organonanoclay, montmorillonite nanoclay (MMN) composites. However, as the nanoclay ratio rises above a certain level, it becomes hard, brittle and the elasticity begins to decrease (Villegas et al., 2019; Alves et



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al., 2020). Silicon oxide coatings significantly increase the oxygen and water vapor barrier properties and improve their thermal properties (Garcia et al. 2018; Zuo et al., 2020). In addition, by forming composites with cellulose nanocrystals, stretchability, thermal properties, workability, and oxygen barrier properties are improved. At the same time, cellulose nanocrystals generally increase the biodegradability of the material and decrease the surface wettability (Montes et al. 2018; Karkhanis et al., 2018; Pal et al. 2019; Karkhanis et al., 2021) and good mechanical and oxygen barrier properties, and good biodegradability are obtained. In recent years, the use of nanofillers is widespread and studies have increased to increase the flexibility, stretchability, and biodegradability of the biopolymer to which it is added (Sanchez-Garcia and Lagaron, 2010; Peelman et al., 2013; Karkhanis et al., 2021)

The polyhydroxyalkanoate (PHA) family are biodegradable thermoplastic polymers produced by a wide variety of microorganisms such as bacteria and algae (Darani and Bucci, 2015; Costa et al., 2019). This family of biopolyesters is heat treatable and flexible. PHAs have found applications in the form of packaging materials including films, cans, coatings, fibers, and foam materials, or in other materials such as biofuels, medical implants, and drug delivery carriers (Sharma et al., 2021). Their use is limited due to their high cost. In addition, some unfavorable properties of PHA, such as hardness, brittleness, low gas barrier properties, and thermal instability; make it disadvantageous for its wide use (Tan et al., 2020). Its physical and barrier properties can be improved by modifying its surface or combining PHA with other polymers, inorganic materials, and enzymes. In addition, it is a good potential biodegradable material due to its durability, moisture barrier properties, and good biodegradability. Polyhydroxy butyrate (PHB) is also in the PHA family and has good machinability and high oxygen barrier properties. Therefore, it is widely used in studies and the properties of the composite material can be improved with other polymers and nanoclays such as MMN (Tarrahi et al., 2020).

Although it does not reach global scales in food packaging applications, the use of bioplastics is becoming increasingly common. When it is researched for industrial applications of biodegradable packaging materials, it is notified that especially PLA has various applications as rigid packaging materials such as beverage cups, salad, fruit and vegetable containers, yogurt cups, sandwich bowls, and water bottles (Peelman et al., 2013; Jabeen et al. 2015). Today, applications in flexible packaging have increased more, and examples are also encountered in multi-layer composite materials. It is possible to come across the use of cellulose, metalized cellulose, and cellophane especially as interlayers in compostable flexible packaging (Jabeen et al. 2015). The packaging material of teabags produced by Numi Organic Tea Company consists of compostable outer bag envelopes of 3-layers of paper/cellophane/PLA. Cast cellophane film is used as the barrier layer, whose oxygen barrier property is between 0.03 - 0.06 cm3 / 100 sq. day, and it is stated by the company that it provides a good oxygen barrier property thanks to the crystal structure in the nature of cellophane (Reynolds, 2021a).

Bacardi Company stated that by 2023, 100% biodegradable bottles will replace the 80 million plastic bottles produced annually under the company's roof. Nodax PHA (polyhydroxyalkanoate), produced by Danimer Scientific, is obtained from the oils of palm canola and soybean seeds, and it



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is stated that unlike the 400-year degradation period of petroleum-based plastics, it disappears in nature within 18 months without leaving harmful microplastics behind. (Reynolds, 2021b). DalterFood Group in Italy uses a compostable and biodegradable film made by processing residues of agricultural products and other renewable resources for single-dose sachets used for shredded and cut cheese (Mohan, 2021).

#### **Edible Packaging Options for Shelf-Life Prolongation**

Edible packaging is commonly involved in studies as films and coatings, and it is widely studied to extend the shelf life of foods, as well as to improve sensorial, physical, and nutritional properties of food. Studies in this context generally aim to protect food by turning one or more edible biomaterials into a coating solution or film together with a functional component and gaining an active packaging feature. Essential oils, plant extracts, metal oxide particles, monoterpene phenols, minerals are frequently used as functional agents (Falcó et al. 2019a; Wang et al., 2019; Olivo et al., 2020; Xiong et al., 2020).

Chitosan is a type of polysaccharide that is biodegradable, has antibacterial and film-forming properties, is non-toxic, and does not cause natural pollution. Barrier properties can also be improved with chitosan coating. Although chitosan has good mechanical, and gas barrier properties, but it has high moisture sensitivity. It is widely studied scientifically as it shows antimicrobial and antifungal activity (Pinzon et al., 2020; Yadav et al 2020).

Alginate is renewable, abundant, biodegradable, biocompatible, and water-soluble materials. The addition of the divalent cation to the alginate solution leads to the formation of a gel via ion exchange. It is among the most researched basic biopolymers in recent years and attracts attention in edible packaging as a film-forming material. Alginate-based edible coatings and films can improve the textural properties, quality and extend the shelf life of fruit, vegetables, meat, seafood, and dairy products by reducing dehydration and controlling respiration. However, studies indicate that alginate/calcium chloride alone is not sufficient to inhibit microbial growth. Microbial inhibition can be achieved when used together with antimicrobial additives such as essential oils, nisin, lactoperoxidase enzyme in the film formulation (Parreidt et al. 2018; Falcó et al. 2019b; Theagarajan et al., 2019).

#### **Circularity of Food Packaging**

While designing the packaging, environmental sustainability should be considered as well as cost, food safety, shelf life and ease of use. (Ingrao et al., 2015). In addition, food packaging plays an important role in reducing the amount of consumer food waste and thus food waste (Williams et al., 2012). While applying innovative packaging design, the basic function of food packaging should also be considered and the design should be done in a way that reduces the losses of food and the waste of packaging. In this context, concepts such as packaging recycling, recycled packaging, recyclable packaging, waste reduction, reusable packaging appear or are being developed.



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#### **Recycling of Packaging and Reducing Packaging Waste**

Plastic material production in the World and Europe in 2018 has been 359 million tons and 61.8 million tons, respectively (Plastic Europe, 2020). Most of the plastics are designed for single-use and their service life is short. The largest share in the use of produced plastic materials is in packaging applications with 39.9%. According to Plastics Europe data, in 2016, the recycling of packaging waste collected after consumer use was equal to that of disposal at landfills, and in the following years, the ratio of recycled packaging waste to disposed waste increased gradually (Plastic Europe, 2020).

Rigid plastics are the focus of recycling in plastics. However, the situation is different for multilayer and monolayer flexible packaging. Flexible packaging materials are not widely accepted by recycling companies due to their low bulk density, technical difficulties in recycling lines, and low economic efficiency (Horodytska et al., 2018). Multilayer packaging material aims to combine the superior properties of various materials so that the forming, adhesion, pressure holding, protection, and barrier properties of the material can be improved (Mckeen, 2017). Although there are recycling methods such as post-delamination recycling (Fávaro et al., 2013; Cervantes-Reyes et al., 2015) and bulk recycling (Uehara et al., 2015), they are still not sufficient for industrial-scale applications (Horodytska et al., 2018; Tartakowski, 2010). These types of packaging wastes (especially postconsumer wastes) are collected and incinerated in landfills (Bayus et al., 2016). Minimizing the amount of material usage and the number of layers will increase the contribution of packaging to sustainable development (Barlow and Morgan, 2013).

Aluminum foil is widely used as a barrier layer with a thickness of 7  $\mu$ m - 9  $\mu$ m in multi-layer food packaging since its permeability properties are very low (Bayus et al., 2016; Emblem and Emblem, 2012). However, due to disadvantages such as fossil fuel, high metal consumption, and high costs, it has been replaced by metalized films in the following years (Decker et al., 2004). However, the barrier properties of metalized films are lower than that of aluminum foil, and it cannot be said that they are a complete alternative in terms of this feature. In recent years, studies on the use of metal oxide as a coating or nanofiller have been gaining popularity.

Nanocomposite materials reinforced with titanium dioxide, silicon dioxide, zinc oxide, and aluminum oxide gain the properties such as antimicrobial, moisture barrier, oxygen barrier, and flexibility, depending on the function of the metal oxide it contains. Aluminum oxide coatings significantly improve the moisture and oxygen barrier properties of the packaging (Hirvikopi et al. 2010). With the 50 aluminum oxide film coating of PLA, a permeable film that can be used even in the packaging of moisture-sensitive dry foods can be obtained (Hirvikopi et al., 2010). The studies related to the effect of aluminum oxide coating on the film barrier properties are presented in Table 1.



Table 1 Effect of aluminium öxide coating application on barrier properties of polymeric packaging materials

Film Structure	OTR	WVTR	Reference
	(cm3/m2.day.bar)	(g/m2.day)	
BOPP 18 μm	≈500	≈4.5	
18 μm BOPP /AlOx	$0.89\pm0.01$	$2.19\pm0.06$	Struller et al., 2014
12 μm PET	≈120	≈40	
12 μm PET/AlO <sub>x</sub>	$0.54\pm0.05$	$0.56\pm0.03$	Struller et al., 2014
12 μm PET/AlOx	1.0-1.5	0.5–1.0	
12 μm PET /AlOx/ink	1.5-8	1–3.5	
12 μm PET/AlOx/Adhesive/ 40 μm CPP	$1.34 \pm 0.23$	$0.85 \pm 0.10$	Struller et al., 2019
30 μm PP	1250	*	
30 μm PP/ 50 nm AlOx	109	*	Hirvikopi et al., 2010
25 μm PLA	315	93	
25 μm PLA/ 50 nm AlOx	32	3,3	Hirvikopi et al., 2010
15g/m2 paper coated with PE	>20.000	8,5	
15g/m2 paper coated with PE /50 nm AlOx	818	4,6	Hirvikopi et al., 2010
PLA	*	$53 \pm 4$	
PLA /25 nm AlOx		$33 \pm 6$	Hirvikopi et al., 2011
40 μm PLA	$470 \pm 1$	$39\pm5$	
40 μm PLA/25nm AlOx	$0.4 \pm 0.1$	$10 \pm 1$	Hirvikopi et al., 2014
127 μm PI (Polyimide)	$30\pm0.1$	3±1	
127 μm PI (Polyimide) /25nm AlOx	$2.2\pm1.9$	2±1	Hirvikopi et al., 2014
27 μm PI Cellophane	$4.0\pm0.1$	$144\pm19$	
27 μm PI Cellophane/25nm AlOx	$2.6 \pm 2.3$	$15\pm9$	Hirvikopi et al., 2014

#### **Packaging Design**

Sustainable packaging should not only be considered as an alternative to the traditional materials used. In addition, the design of the packaging material should be optimized according to the food. All packaging materials must function with minimum resources (Białek, 2014). The industry has the responsibility to review and optimize the environmental performance of the packaging material for all relevant lifecycle stages. Optimization should be performed according to the impact of using the higher amount of packaging material as well as product losses that may result from using less packaging material as is seen in Figure 2 (Consumer Goods Forum, 2011). From this point of view, while reducing the amount of packaging in food packaging, It should also be taken into account that the food should provide the required shelf life. The reduced packaging material should be designed to be balanced with the life cycle of the food.



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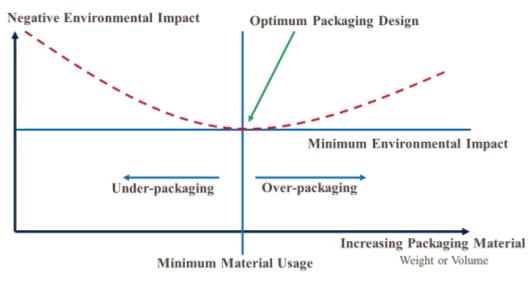


Figure 2. Innventia AB model- optimum pack design (Consumer Goods Forum, 2011)

When it is examined that the industrial application of recycled or recyclable food packaging materials, it is thought that they can be widely used in rigid packaging such as packaging materials in the form of glasses, or bottles of big brands. In order to convey the importance of recycling to the consumers of Coca-Cola products in Sweden, product labels include information that recycled packaging is used and that the packaging can be recycled again (Reynolds, 2021c).

Due to the insufficient recycling of multi-layer flexible packaging, the use and development of single-layered packaging materials in the packaging industry has become more interesting. Flexopack company has been developed a polyolefin-based single-layered film as a flexible food packaging (Packaging Europe, 2021). Gualapack has been using 100% recyclable single-layer packaging material for baby food from the German company Frucht Bar (Mohan, 2021).

#### **Reusable Packaging**

Reusable packaging is more economical and more environmentally friendly than the disposable packaging concept. This concept has been used in certain sectors in the past, but due to the lack of strict legislation, it has been replaced by disposable packaging in some areas (Golding, 1999; Coelho et al., 2020). The refillable packaging is also seen as an effective solution to prevent plastic pollution (Ellen MacArthur Foundation, 2019). Moreover, it has environmental advantages such as optimizing the use of materials, protecting fossil fuels, minimizing the consumption of carbon dioxide and nitrogen oxides and the need for raw materials and energy, and reducing the environmental impact of various elements (Babader, 2019; Mahmoudi and Parviziomran, 2020). Reusing products and materials for as long as possible reduces the need for virgin (or primary) materials. When loops are closed in sustainable ways (for example: with minimal energy use to close loops), the environmental footprint of the material used is reduced. Therefore, reusable packaging materials present a great opportunity to preserve the functionality of the material and



product, and to achieve major reductions in material use and environmental impacts (Coelho et al., 2020). Table 2 shows the industrial applications of reusable packaging in recent years.

Packaging Type	Application	Company	Reference
Refillable Bulk Dispenser	Development of marketing system integrated with mobile application	MIWA	Coelho et al., 2020
Returnable Cup	Alternatives to single-use cups for offices, restaurants, cafes, events and festivals has been growing.	CupClub, Revolv, Globelet, ReCup, Meu Copo Eco	Coelho et al., 2020
Returnable Food Containers	Alternative for single-use takeaway containers,	GoBox, reCIRCLE	Coelho et al., 2020
Reusable Bottles	Home appliance that makes sparkling water	SodaStream	Ellen Macarthur Foundation, 2019
Reusable metal ready to meal boxes	Ready-made meal service using reusable packaging	DabbaDrop	Ellen Macarthur Foundation, 2019
Reusable glass jars	Vending machine serving fresh meals stocked daily and sold in reusable glass jars.	Fresh Bowl	Ellen Macarthur Foundation, 2019

Table 2.	Recent	applications	of reusable	packaging
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## Conclusions

For each packaging system, the optimum amount of each packaging material to be used in that packaging combination becomes important. The reasons for this can be shown as the risk factors that may have negative effects on the environment (deterioration of food and damage to the environment) if it is used in a very small amount than it should be, and that it may pollute the environment by creating unnecessary waste if it is used more than necessary.

Although it is seen that biodegradable materials began to take more place in industrial food packaging production, it does not seem possible to replace plastics on a global scale in today's conditions due to the high cost of these materials, insufficient properties, and difficulties in processability, However, like almost every technology output, the idea that such problems can be overcome in the future should not be seen as delusional.

Therefore, we will continue to use traditional packaging materials for many years. Increasing the recycling rates of traditional packaging materials is a must. However, focusing on recycling alone is not enough for sustainable packaging. The sustainable packaging design should also be given importance and packaging designs should be optimized. Although no material has a clear advantage over the others in ecological terms, it should be taken into account that each of these materials can have both positive and negative effects on the environment.



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# HEALTH BENEFITS OF SOME TRADITIONALLY CONSUMED MEDITERRANEAN GREENS

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## Abstract

The Mediterranean diet is recognized by UNESCO as the "Intangible Cultural Heritage of Humanity" that defines many Mediterranean countries. In the Mediterranean diet, plants (greens such as blessed thistle, wild radish, tangle grass, chicory, asparagus, criste marine etc.)) and herbs are traditionally considered an integral part of the diet. Plants are usually boiled, cooked with meat, or consumed as stuffing in pastries. Studies have shown that wild and cultivated plants grown in the Mediterranean region are rich in phenolic compounds. The effects of phenolic compounds on human health have attracted great interest recently. Oxidative stress, which is defined as the formation of reactive oxygen species (ROS) or the formation of free radicals and an imbalance in antioxidant levels, is associated with various diseases such as degenerative nervous system diseases, aging, and diabetes. With their antioxidant properties, phenolic compounds can protect cells against oxidative damage and thus reduce the risk of various diseases associated with oxidative stress. Many studies have shown a relationship between phenolic compounds and antioxidant, anti-inflammatory effects. On the other hand, some phenolic compounds may support beneficial intestinal microflora, which may positively affect the risk of chronic disease. At the same time, its anti-inflammatory, antiangiogenic, and anti-cancer properties have already been described for various phenolic compounds. Many enzymes catalyze the production of ROS in different metabolic pathways. Xanthine oxidase is responsible for oxidative damage responsible for various pathological diseases. Therefore, inhibition of this enzyme is important for the prevention of many inflammatory diseases. However, the consumption of plants rich in phenolic compounds grown in the Mediterranean basin and also included in the Mediteranean diet is limited due to several reasons such as seasonal restrictions, lack of cultivation, fresh consumption imperatives, and therefore unavailability of them for everyone. Therefore, the traditional consumption pattern of Mediterranean greens should be reevaluated with a more modern approach (i.e., their fermented products) that allows consumers to adapt them into their diets.

Keywords: Mediterranean Diet, Phenolic Compounds, Plants, Anti-inflammatory, Antioxidant



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#### Introduction

In recent years, the effect of dietary patterns on diseases has attracted considerable attention. One of the dietary patterns is the Mediterranean diet consumed around the Mediterranean basin (Lăcătuşu, 2019). In general, the Mediterranean diet is a plant-based dietary pattern that includes vegetables, fruits, grains, nuts and legumes, moderate fish, seafood or dairy products, limited amount of meat and alcohol, most often cooked with the addition of significant amounts of olive oil (Davis, 2015). The concept of the Mediterranean diet as a healthy diet was first publicized in 1945 by Ancel Keys, an American scientist, he had been impressed by the healthy state of the population, which was attributed to the diet, when he was stationed in Salerno, Italy (Arvantikis, 2014). On the other hand, the Mediterranean diet is recognized by UNESCO as the "Intangible Cultural Heritage of Humanity", which defines the dietary pattern of many Mediterranean countries. This unique dietary pattern, which includes olive oil, fruit, and vegetables is currently attracting considerable interest because of its health benefits and has become a valuable medical tool in the world today (Martins, 2011; Lăcătuşu, 2019). Studies have confirmed the health benefits of the Mediterranean diet in the prevention of cardiovascular disease, type 2 diabetes, metabolic syndrome, cancer, and neurodegenerative diseases.

Besides olive oil, fruits, and vegetables, wild edible plants have always played a significant role in the folk traditions of the Mediterranean region (Qneibi, 2018). In rural areas across the Mediterranean region, it was a usual practice to gather wild plants for many purposes including the preparation of food recipes and folk medicines (Chatzopoulou et al., 2020).

In this article studies on the health benefits of wild edible plants traditionally consumed in the Mediterranean diet were reviewed.

#### Wild edible plants as part of the Mediterranean diet

According to the Food and Agriculture Organization, the wild edible plant is defined as the "plants that grow spontaneously in self-maintaining populations in natural or semi-natural ecosystems and can exist independently of direct human action." The Mediterranean basin is characterized by a huge abundance of wild edible species, so the Mediterranean basin can be referred to as a biodiversity hotspot of them (Ceccanti et al, 2018). In ancient Mediterranean cultures, wild edible plants had been used for different aims such as food, production of goods, magic and religious rituals, etc. (Bennett et al.,2006). Over the years, the use of these plants in traditional recipes for the Mediterranean diet has increased, and in parallel, their medicinal properties have been discovered. Recent studies have shown that wild edible plants are constitutively rich in secondary metabolites which are also known as bioactive compounds (Bennett et al,2006; Dresler et. al, 2017; Zlatíc and Stankovíc, 2017; Ceccanti et al, 2018; Guerriero et al, 2018).

Montefusco et al. (2015), investigated antioxidant activities, total phenols, flavonoids, tocochromanol (tocopherol and tocotrienol), and carotenoid contents of the edible part of wild and cultivated varieties of chicory (*Cichorium intybus* L.) and in the leaves of the wild species of poppy (*Papaver rhoeas* L.). The samples were collected in the countryside of Salento, Italy (Figure 1).



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They found that the hydrophilic antioxidant activity of all analyzed plants was higher than that of lipophilic antioxidant activity. In addition, wild poppy leaves exhibited the highest antioxidant activity, the highest content of total phenols and flavonoids among the other studied plants. The carotenoid contents of wild species were found to be higher than those of cultivated ones.



Figure 1. Photos of the analyzed plants: "Catalogna" chicory (a), "Otrantina" chicory (b), wild chicory from San Pietro Vernotico (c), wild chicory from Statte (d), and wild poppy (e); sampling sites (green spots) in the Salento Peninsula, Southern Italy (f) (Montefusco et al., 2015)

Morales et al. (2011) analyzed tocopherols composition and antioxidant activity of eight wild plants (*Apium nodiflorum* L. Lag, *Foeniculum vulgare Mil.*, *Montia fontana* L. *Silene vulgaris* (*Moench*) *Garcke*, *Asparagus acutifolius* L, *Bryonia dioica Jacq*, *Humulus lupulus* L *and Tamus communis* L) which are traditionally used in Spain. The results showed that the leafy vegetables *Apium nodiflorum* and *Montia fontana* presented the highest antioxidant capacity in all the antioxidant assays performed. They reported that the higher antioxidant capacity of *Apium nodiflorum*, could be attributed to its high total phenols and flavonoids contents. In the case of *Montia fontana* high antioxidant activity was most probably due to both its high total phenols and flavonoids, as well as its tocopherols content, especially  $\alpha$ -tocopherol. Among the four species in the second group, *Humulus lupulus* presented the highest antioxidant capacity, again most probably due to its highest phenolics and total tocopherols content. Samples in the second group had lower antioxidant activity than the leafy plants in the first group (Figure 2 and 3).



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Figure 2. First group of plants; (A) *Apium nodiflorum (L.) Lag.;* (B) *Foeniculum vulgare Mill.;* (C) *Montia fontana L.;* (D) *Silene vulgaris (Moench) Garcke* 



Figure 3. Second group of plants; (A)*Asparagus acutifolius L.*;(B) *Bryonia dioica Jacq*; (C) *Humulus lupulus L.*; (D) *Tamus communis L.* 

Disciglio et al (2017), investigated the qualitative characteristics of several edible wild plant species, including those most consumed in Foggia Province (southern Italy) and some of which have not been previously analyzed before. The edible parts of 11 wild species and 3 cultivated species have been used for analysis. Total polyphenols and antioxidant activities of wild *C. intybus* and *B.officinalis* were higher than those of their cultivated equivalents.

Qneibi et al (2017), examined the taste, total phenols and antioxidant activities of fresh, roasted, shade dried and boiled leaves of edible *Arum palaestinum*. In Palestinian folk food and medicine, *Arum palaestinum* is one of the notable wild plants which have been used since historical times. They reported that the cooking and drying of *A. palaestinum* caused a lower numbing taste. In addition, there was a relationship between the antioxidant activity and total phenolic content of *A. Palaestinum*. The authors also stressed that drying of *A. Palaestinum* in an oven was the most effective method both for consumption and for the preparation of nutraceuticals and pharmaceuticals.

De Falco et al. (2019), analyzed the nutritional properties of 16 spontaneous wild plants. They reported that these wild edible plants were good sources of minerals as well as polyphenols.

#### Conclusion

The Mediterranean diet has known as a healthy diet, and it has become a valuable medical tool in the world today. The main foodstuffs of this plant-based diet are olive oil, vegetables, and fruits. Besides these foodstuffs, wild edible plants have always been an important part of this diet because of the huge abundance of them in the Mediterranean basin. The researchers who are interested in the health benefits of the Mediterranean diet have also given importance to wild edible plants. As a



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result, it has been discovered that wild edible plants are constitutively rich in secondary metabolites which are also known as bioactive compounds. These compounds have an important role in the competition, defense, attraction, and signaling for plants and these compounds can elicit pharmacological effects as an antioxidant, anti-inflammatory, and anti-carcinogenic in humans and animals. Their other beneficial effects, especially in the prevention and delay of chronic diseases, have also been identified.

Although beneficial effects of wild edible plants rich in bioactive compounds grown in the Mediterranean basin have been proved, their consumption are limited due to several reasons such as seasonal restrictions, lack of cultivation, fresh consumption imperatives, and unavailability of them for everyone. Therefore, the traditional consumption pattern of the Mediterranean greens should be reevaluated with a more modern approach (i.e., their fermented products) that allows consumers to adapt them into their diets.

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# TESTING OF PHYSICO-CHEMICAL AND SENSORY PROPERTIES OF PATE

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## Abstract

Production and consumption of minced meat products requires special care for using of these products. For this reason, in this study approached those basic items that should be followed in order to satisfy such a product search and consumer demands regulations on these products and rules on labeling of minced meat products, including pate. The aim of this study was to examine the quality of chicken pate, determine whether there are statistically significant differences for the acceptability of sensory properties after storing in the refrigerator, and compare whether certain conditions are met by the valid rulebook. In this paper, first of all has been checked declaration of products and their conformity to legal requirements. Subsequently, measurements were made of aw, pH, moisture content, salt content of the product. Fifteen trained panelists sensory evaluated pate samples from the same batch for the following sensory effects: dough appearance, color, texture, aroma and taste. the assessment showed that the declaration does not meet the requirements of the Rulebook on Minced meat, Semi-finished Products and meat Products (Official Gazette of BiH, No 82/13). The mean pH in the pate tested was 6.35. The moisture content was 57.42 %. In the sample of the tested chicken pate, aw was 0.956. The content of salt in the chicken pate was 1.36 %. Based on the sensory evaluation, it can be concluded that the tested samples only differ significantly in taste (p = 0.027).

Key words: Pate, Texture, Physical And Chemical Parameters, Sensorial Assessment

## Introduction

Nowadays, there are fewer and fewer foods that man takes directly from nature for food, but most of them are obtained by various processing and finishing processes, and they often lose much of their original value. There is a small number of industrially obtained foods that, in addition to the required amounts of basic components (proteins, fats, etc.), also contain the necessary trace elements, vitamins and other biologically valuable substances in sufficient quantities, which should be taken into account in the technological production process (Potparić et al., 1979., Operta, 2017a.).

Production of pate is based on one of the oldest, safest and healthiest ways of canning food with heat, which dates back to the early 19th century. The production process itself is time consuming and involves many phases and steps. It is extremely important to choose a good and quality raw



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material before the very beginning of the production process, which is the main prerequisite for a quality product. Pate is a product that we traditionally consume in our country. They produce with the help of the latest technology, from carefully selected raw materials, fresh spices and the best parts of meat. Pate is a rich source of high-value proteins and minerals and in this sense is of high nutritional value (Barbić, 2016., Operta 2017., Morales et al., 2011). Pate products are related to emulsified meat products made primarily with liver, lard, meat and species. Examples of these kinds of products are pate (cooked in casings), terrine (hot-molded in recipients), mousse (including eggs to form a foamy texture) and rillete (made with meat and liver). Since the meat must be added precooked, this reduces the meat proteins functionality making the product less thermostable, where fat can be released affecting color and flavor Morales et al., 2011; Kolbabek et al. 2019).

In our country, pate is produced from pork, beef and chicken meat with the addition of liver, and such products have recognizable sensory properties. However, pate can also be made from the meat of the other slaughter animals or fish. In the available literature, we find data on research by other authors describing pate produced on the basis of liver of ducks, chickens, goats, fish, ostriches, emphasizing their sensory properties and nutritional specificities (Amaral et al., 2013., Kritchevski, 2002). According to the Rulebook on Minced Meat, Semi-finished Products and Meat Products (Official Gazette of Bosnia and Herzegovina, No. 82/2013), meat pate is a product of various types of meat, mechanically separated meat (MSM), fatty and connective tissue, offal, blood and products from blood, broth, soup and additional ingredients. In poultry, poultry and game products, the quantity of meat after which the pate is named must be at least 60% of the total quantity of meat in the product. The protein content of meat in the product must not be less than 8%, and the relative content of connective tissue protein in meat proteins (collagen content) must not exceed 25%. The basic procedures in the production of pate are homogenization of adipose tissue, meat and other ingredients, and binding of water and emulsification of fats with the help of meat proteins and added proteins of plant or animal origin (Amaral, 2011., Operta, 2017., Zlatarević, 2015).

Each food is represented by basic organoleptic properties, such as color, consistency, smell, taste, etc. then, packaging, design, etc. Often, these properties are not an adequate reflection of the chemical composition, nutritional value, content components and their optimal ratio, etc., which are essential for quality food. These properties must be proven by physico-chemical, microbiological and other tests. Canned meat are products made of different types of meat, fatty tissue, offal, skin, connective tissue and additional ingredients or meat products, which after processing are heat treated by pasteurization and sterilization in hermetically sealed packaging (Morales et al. 2011; Kolbabek et al. 2019., Martin-Sanches et al., 2017., Kritchevski, 2002).

Ternes et al. (1991) by investigating the viscosity and consistency of liver pate, found that the pate filling achieves the best viscosity at 68 °C and the strongest consistency at 80 °C. The dependence of the viscosity of the liver suspension on the amount of added salt was also determined, so that suspension 5 shows the optimal viscosity at a salt concentration of 1.2 - 1.6% meat suspensions can withstand up to 6% salt.

In this type of emulsified meat products, there is a relatively high proportion of fat that affects the sensory, physico-chemical and nutritional properties, which has a direct impact on the texture:



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cutting and spreading. Since the meat must be added pre-cooked, this reduces the function of the meat proteins making the product less thermostable, whereby fat can be released which affects the color and taste. (Totosaus, 2005; Totosaus, 2010).

## **Material and Methods**

"Chicken pate" from our well-known manufacturer was taken as the material for this research. The composition of this pate was as follows: cooked chicken meat 35%; water; chicken liver 5%; soy proteins; kitchen salt; corn starch; spices; thickeners (E412, E415); flavor enhancer (E621); preservative (E250).

First of all, the declaration and its compliance with the Ordinance and its requirements were checked. After that, tests of water activity (aw) with an aw-meter, pH measurement with a pH meter, determination of moisture according to BAS ISO 1442/2007 and determination of NaCl were performed.

The analyses were performed according to the required specifications. Pate were served at room temperature, and unsalted crackers and knives were provided to spread the samples. The assessors evaluated the intensity of different attributes on a scale up to 20 points for all attributes (external appearance – max 2 points, smell – max 3 points, color – max 3 points, texture – max 3 points, aroma – max 4 points and taste – max 5 points). A group of 15 educated panelists from the Faculty of Agriculture and Food in Sarajevo performed a sensory evaluation of the pate from the same batch and evaluated it for the following sensory effects: dough appearance, color, texture, aroma and taste. The pate is served at room temperature, and unsalted crackers are provided to spread the samples. Sensory analysis of samples was performed in the analytical laboratory of the Institute of Technology of the Faculty of Agriculture and Food in Sarajevo according to the standard ISO 8589: 2007.

Based on the evaluations of the evaluators, who evaluated the appearance, color, smell, texture, aroma and taste of the pate test, in the IBM SPSS software package intended for statistical data processing, an analysis of the obtained sensor evaluations was performed.

#### **Results and the Discussion**

According to the Rulebook on minced meat, semi-finished products and meat products (Official Gazette of Bosnia and Herzegovina, No. 82/13), in poultry, poultry and wild animal meat, the amount of meat by which the pate is named must be at least 60% of the total amount of meat in the product. On the declaration of chicken pate, the producer was 35%. According to the Ordinance, the content of meat protein in the product must not be less than 8%, and the relative content of connective tissue protein in meat proteins (collagen content) must not exceed 25%. The declaration of the tested chicken pate states only soy proteins but not the total protein content. Also, according to the Rulebook mentioned above (Official Gazette of Bosnia and Herzegovina, No. 82/13), the relative content of connective tissue protein in meat proteins (collagen content) in poultry meat pate must not be higher of 15%.



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In the tested samples of chicken pate, the relative protein content was not stated at all on the product declaration. The average nutritional value per 100 g of product is not stated even if it is one of the basic parts of the declaration advocated by the EU declaration regulations. The content of water, salt, starch and spices is not stated on the declaration. The net quantity as an integral part of the chicken pate declaration is stated. Shelf life is stated but not the date of packaging. The origin of the chicken pate is stated. Unfortunately, the data from the declaration of chicken pate products do not meet the requirements of the Rulebook on minced meat, semi-finished products and meat products (Official Gazette of Bosnia and Herzegovina No. 82/13).

The water activity (aw) in meat is the part of water that is available for biochemical reactions and the growth of microorganisms. Along with temperature and acidity (pH), aw is a key factor in the sustainability of meat and meat products. It is used to measure the activity of free water in meat. A very important parameter for monitoring the growth of microorganisms in meat that develop at 0.91 (min. for bacteria) - 0.71 (min. for fungi).

- In the sample of tested chicken pate aw value was 0.956.

The mean pH in the tested pate was 6.35 and the water content was 57.42.

Salt (NaCl) is one of the oldest ingredients used in canning meat. NaCl is generally added in an amount of 1.4 - 3%, depending on the type of meat product. Addition below 1.2% is not recommended, as protein activity begins at this concentration. Salt has several important functions in the production of meat products;

□ helps in dissolving and extracting proteins;

- $\Box$  improves taste;
- □ has partial antimicrobial properties;
- $\Box$  reduces the aw value of the product;
- $\Box$  increases water and fat retention capacity;
- $\Box$  improves emulsifying properties (Operta, 2017).

The salt content in the tested chicken pate was 1.36%.



						95% Confidence Interval for Mean			
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
Appearance	1	5	1.7000	.27386	.12247	1.3600	2.0400	1.50	2.00
	2	5	1.5000	.50000	.22361	.8792	2.1208	1.00	2.00
	3	5	1.4000	.65192	.29155	.5905	2.2095	.50	2.00
	Total	15	1.5333	.48058	.12408	1.2672	1.7995	.50	2.00
Collor	1	5	2.7000	.27386	.12247	2.3600	3.0400	2.50	3.00
	2	5	2.5000	.61237	.27386	1.7396	3.2604	1.50	3.00
	3	5	2.2000	.44721	.20000	1.6447	2.7553	1.50	2.50
	Total	15	2.4667	.48058	.12408	2.2005	2.7328	1.50	3.00
Smell	1	5	2.4000	.54772	.24495	1.7199	3.0801	2.00	3.00
	2	5	2.1000	.41833	.18708	1.5806	2.6194	1.50	2.50
	3	5	1.9000	.65192	.29155	1.0905	2.7095	1.00	2.50
	Total	15	2.1333	.54989	.14198	1.8288	2.4379	1.00	3.00
Texture	1	5	2.6000	.41833	.18708	2.0806	3.1194	2.00	3.00
	2	5	2.5000	.50000	.22361	1.8792	3.1208	2.00	3.00
	3	5	2.4000	.82158	.36742	1.3799	3.4201	1.00	3.00
	Total	15	2.5000	.56695	.14639	2.1860	2.8140	1.00	3.00
Aroma	1	5	3.3000	.67082	.30000	2.4671	4.1329	3.00	4.50
	2	5	3.1000	.41833	.18708	2.5806	3.6194	2.50	3.50
	3	5	2.5000	.79057	.35355	1.5184	3.4816	1.50	3.50
	Total	15	2.9667	.69351	.17906	2.5826	3.3507	1.50	4.50
Taste	1	5	4.4000	.41833	.18708	3.8806	4.9194	4.00	5.00
	2	5	3.9000	.82158	.36742	2.8799	4.9201	3.00	5.00
	3	5	2.9000	.96177	.43012	1.7058	4.0942	1.50	4.00
	Total	15	3.7333	.96115	.24817	3.2011	4.2656	1.50	5.00

Table 1. Descriptive statistical analizys

After descriptive analysis, an analysis of variance homogeneity was performed. If sig. > 0.05 satisfies, ie the repeats of the samples are sufficiently homogeneous. In this analysis, sig. > 0.05 for each of the tested parameters, which means that the results are homogeneous and as such give a realistic picture of the condition of the tested chicken pate product. The results of this analysis are given in the following table.



		Sum of Squares	df	Mean Square	F	Sig.
Appearance	Between Groups	.233	2	.117	.467	.638
	Within Groups	3.000	12	.250		
	Total	3.233	14			
Collor	Between Groups	.633	2	.317	1.462	.270
	Within Groups	2.600	12	.217		
	Total	3.233	14			
Smell	Between Groups	.633	2	.317	1.056	.378
	Within Groups	3.600	12	.300		
	Total	4.233	14			
Texture	Between Groups	.100	2	.050	.136	.874
	Within Groups	4.400	12	.367		
	Total	4.500	14			
Aroma	Between Groups	1.733	2	.867	2.080	.168
	Within Groups	5.000	12	.417		
	Total	6.733	14			
Taste	Between Groups	5.833	2	2.917	4.930	.027
	Within Groups	7.100	12	.592		
	Total	12.933	14			

Table 2. ANOVA

Based on the analysis of variance, it can be concluded that the tested samples only differ significantly in taste, because the taste is p = 0.027, which is less than the significance threshold of 0.05.

The concentration of hydrogen ions (pH value) is significant both for meat (eg meat ripening control, water binding assessment) and for meat products (eg proof of spoilage of meat products). The pH scale ranges from 0 to 14. For meat and meat products, the pH value is between 4.5 - 8.0. For pâtés and canned meat, pH values range from 5.8-6.4 (Zlatarević 2015).

Water activity, which is expressed as aw-value, is important for meat products, both for production technology and for product maintenance. A scale of 1.0 - 0.0 is used for the aw value. Raw meat has an aw-value of 0.99 if it is lean. Meat products have a lower aw-value than raw meat due to the addition of salt and fat or for drying (Zlatarević 2015; Morales et al. 2011; Barbić 2016).

The moisture content of meat and meat products is the quotient of the loss of mass caused under working conditions and the mass of a part of the test sample. Water plays an important role in heat-treated sausages: it helps extract protein and makes the product juicy. Protein extraction is best done at low temperatures, so water is often added in the form of ice to keep the meat temperature low (Barbić 2016; Amaral et al. 2013). A low temperature is also required, to avoid overheating caused by the high speed of rotation of the knives when cutting. High temperature would reduce the ability



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of water to bind by proteins and gelling. Depending on the sausages, water is added in the amount of 15-35%. It is added together with salts and phosphates (Operta, S. 2017).

#### Conclusions

In accordance with the obtained analysis results, we can conclude the following. First of all, information from the declaration of chicken pate products does not meet the requirements of the Rulebook on minced meat, semi-finished products and meat products (Official Gazette of Bosnia and Herzegovina No. 82/13). The average of pH value in the tested pate was 6.35, water content was 57.42. In the samples of tested chicken pate aw value was 0.956, the salt content in the chicken pate samples was 1.36%. Based on the sensory evaluation, it can be concluded that the tested samples of chicken pate differ only significantly in taste, because the taste is p = 0.027, which is less than the level of significance 0.05.

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# THE INVESTIGATION OF TOTAL PHENOLIC, FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY VALUES OF OAK TREE FRUIT AS AN IMPORTANT ENDEMIC PLANT OF TURKEY AND IRAN

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#### Abstract

With increasing the world's population, alongside the importance of the economic standpoint, the use of limited food resources should be systematically monitored. Additionally, expectancy in modern societies have been steeply risen owing to developments in the sciences of Medicine and Nutrition. People deeply care about the quality of food consumption affecting their health and welfare. Factors such as globalization, trade developments, population growth, and urbanization are the main reasons to seek the significant modifications in the patterns of the production and consumption as well as demands for the food products. Since 1990, functional foods have gained popularity to improve health and wellness. Functional foods are never considered as same as the medicine, pills, nutritional supplements, etc. They are mainly expressed as a part of the normal daily diet e.g. fruit and vegetables, cereals, beverages, supplementing foods, fortified, and improved food. The phenolic, flavonoid content, and antioxidant activities of acorns have been well-known for good nutrition. Therefore, in this study, these were investigated as a functional food. The type of oak tree is Quercus ithaburensis subsp. mocrolepsis, an important endemic plant in Turkey and Iran. According to the results, the highest total phenolic and flavonoid were measured in the acorns grown in Turkey is  $386,75 \pm 1,06$  mg GAE/100 g DM, and  $61,95 \pm 1,20$  mg CAE/100g DM respectively, and about antioxidant activity in DPPH and ABTS<sup>+</sup> analysis methods, the maximum value was obtained  $324,01 \pm 01 \text{ mg TE}/100 \text{g DM}$  and (ABTS<sup>+</sup>)  $386\pm 12 \text{ mg TE}/100 \text{g DM}$ . However, in the acorns cultivated in Iran, the lowest amount of phenolic, flavonoid content, and antioxidant activities were measured  $323,16 \pm 2,10$  mg GAE/100 g DM,  $57,32 \pm 2,24$  mg CAE/100g DM,  $276,67 \pm 3,50$ mg TE/100g DM correspondingly.

**Keywords**: Functional Food, Acorn Fruit, Total Phenolic Content, Flavonoid Content, Antioxidant Activity

## Introduction

Oak is the common name of wood-resistant forest trees, including the evergreens in summer and winter, among about 600 species of the genus *Quercus* of the *Fagaceae* family. The oak species,



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which have a wide distribution are in the world, are specified as Turkey, Iraq, Iran, Syria, Lebanon, Pakistan, Afghanistan, and Greece, which are the homeland of the Middle East and West Asian countries. This tree, however, grows in countries around the world, including the United States, England, France, Germany, Latvia, Poland, Serbia, and has been designated as their national plant (Hashemi et al., 2019). The leaves are lobed, toothed, or straight-edged in most cases. Oak trees are extremely adaptive. They can live in a wide variety of climates elevations, and because the acorn has such a long shelf life as a viable food. Agroforestry systems that utilize forest products have been demonstrated to be more likely to survive and thrive the challenge now is to alert foresters farmers and the food industry to the possible uses of acorns. Oak trees are generally divided into three groups as White Oaks, Red, and Evergreen oaks according to the ripening time of their fruits, leaf, and bark characteristics. Furthermore, oak wood is used in the construction, dyeing, ink, and leather industries. The cylindrical fruits called "Acorn" are in a goblet, some species have large and clawed acorns.

The acorns of the oaks in these groups also show differences in terms of both their physical and chemical structures (Anonim., 2020; Szabłowska, Tańska., 2020). The mainly used parts of these trees have been wood and bark as well as acorns for human or animal feeding and leave to prepare infusions with medicinal or nutritional purposes (Vinha, Barreira, Costa, & Oliveira, 2016; Gamboa-Gomez et al., 2017; Costa, Lourenço, Oliveira, & Pereira, 2019). The chemical component contents of acorn fruits vary depending on the species. An oak fruit contains an average of 31 to 59% starch, 2.75 to 8.44% protein, and 0.7 to 9 % fat. Oak fruits are used in a variety of industrial applications due to their high levels of unsaturated fatty acids especially oleic acid and essential fatty acids such as linoleic acid and  $\alpha$ -linolenic acid, which plays an important role in eicosanoid synthesis, promoting a decrease in blood serum triglyceride levels and an increase in HDL-cholesterol levels (Taib et al., 2020). The oil extracted from acorns has a similar taste to olive oil and contains high levels of unsaturated fatty acids (over 80%), which provide balance by lowering the elevated cholesterol level (Korus et al., 2015; Makhlouf et al., 2018; Vinha et al, Szabłowska, Tańska., 2020). Furthermore, acorns are a nutritive, social, and rich in minerals such as potassium, as well as vitamins A, Thiamine (B1), Riboflavin (B2), and Niacin (B3) (Rybicka, Gliszczyńska-Swiglo, 2017). Nevertheless, this work evidenced the lack of studies investigating the use of highlevel extraction technologies to improve not only phenolics but further bioactive lipids, polysaccharides, and totally different molecules using supercritical solutions, pressurized fluids additionally as microwave- or pulsed electric fields-assisted extractions that have been widely utilized to obtain bioactive fractions from natural sources (Charef, Yousfi, Saidi, Stocker., 2008; Daștan et al., 2012; Al-Rousan et al., 2013; Korus et al., 2015; Li et al., 2015; Patra et al., 2018; Makhlouf et al., 2018; Gil-Ramirez, Smiderle, Morales, Iacomini, & Soler-Rivas, 2019; Tejedor-Calvo et al., 2020; Zia, Khan, Shabbir, Maan, & Khan, 2020; Vinha et al, Szabłowska, Tańska., 2020).

According to researches, acorns contain a high concentration of bioactive components such as tannins, Gallic acid, ellagic acid, galloyl or Hexa-hydroxy diphenyl derivatives, tocopherol, phenolic, and flavonoid compounds (Vinha et al., 2016; Makhlouf et al., 2018; Özünlü et al., 2018;



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Xu et al., 2018; Oliveira, Pereira, 2018; Hidalgo et al., 2021). It has been shown in various studies that the vitamins, minerals, and bioactive components contains and it's used in bakery products add flavor and taste to bakery products. Acorn fruits have had a wide range of uses from past to present. While it is consumed as food in human nutrition with different uses (nuts, dried tea, cake, biscuit, ice cream, and roasted oak coffee) it is also consumed as animal feed in many countries. Furthermore, because of its antioxidant, antitumor, and antimicrobial properties, it has been used as traditional medicine by some societies since ancient times. Furthermore, oak wood is used as a building material in a variety of applications (USDA.,2007; Umachigi et al., 2008; Claudia.,2013; Xu et al.,2018; Makhlouf et al.,2019; Anonim.,2020). As consumers seek out enriched and healthy foods, the demand for functional foods is rapidly increasing in the twenty-first century. Acorns are added to wheat bread, which has an important place in people's nutrition, because of their high bioactive and fiber content, allowing it to be enriched in terms of nutrition and quality. The US Department of Agriculture (USDA) recommends a dietary fiber Reference Intake of 25 g/day for women and 38 g/day for men (Martins et al., 2020). The dietary fiber intake rate of those fed according to the Turkish Nutrition Guide-TUBER (2016) being determined to be 68.2% (Değerli & El., 2019). In children, when the nutritional values taken is examined, it is seen that it reaches 54.6% in terms of fiber and is below the Daily Reference Intake Value (GRD) The addition of flour obtained from oak, fruits to the bread formulation in order to increase fiber content in the Daily Reference Intake Value (GRD) (Ministry of Health, 2014; TÜBER, 2016).



Figure1.Chemical component contents of acorn



Figure 2. Usage of acorn fruit in food industry

## Materials and Method Materials Sample preparation

The acorns used in the study came from the Turkish province of Izmir (Seferihisar) and the Iranian province of Tabriz (Shabestar). Both acorn samples were collected in the same year (2020) and dried using the same method at 40 °C for 17 hours (tray dryer).



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## Method Total phenolic contents

The total phenolic content had been measured by the FolinCiocalteu method, as suggested by Kumar et al. (2012) with slight modifications. Initially, the beer or plant extract were diluted with ratio of 1:10 (v:v). Then the omount of 0.5 mL of the Folin–Ciocalteu reagent were added to 0.1 mL of diluted extract. After 2 min the omount of 2 mL of sodium carbonate (%7 w/v) were added. The final volume was adjusted to 7.5 mL within distilled water. The mixture was kept in dark for 1 hour. The absorbances were measured at 760 nm using a spectrophotometer in Shimadzu 1240 (Japan). Calibration curve of the gallic acid standard solution was adopted to determine the total polyphenols concentration (GAE mg /L).

## **Total Flavonoid Contents**

The total flavonoid contents had been determined by using aluminum chloride colorimetric method described by a previous report (Singleton & Rossi, (1965); Jia et al., (1999); Sánchjez-Moreno, 2002; Liu, Lin, Wang, Chen, & Yang, 2009). Briefly, 250  $\mu$ L sample extract solution, was mixed with 1.50 ml distilled water and 75  $\mu$ L of 5% sodium nitrite and the mixture was stored for 5 min. Then 150  $\mu$ L of 10% AlCl3 and 125  $\mu$ L of 1M NaoH were added. The absorbance were measured at 510 nm by using spectrophotometer after a reaction for 10 min. The standard curve was plotted by using +(-) catechin (50–600 mg/L), and total flavonoid contents were calculated as mg catechin equivalent (Catechin mg /L) extract.

## Antioxidant Activity ABTS<sup>+</sup> assays

The ABTS+ radical scavenging activity had been performed according to Re et al., (1999). The ABTS+ radical cation, ABTS<sup>+</sup> stock solution (7mM). Prepared by mixing with 2.45 mM potassium persulfate solution and incubating the mixture in the dark at room temperature for 12-16 hours. For the measurements, the stock solution was diluted with distilled water until it provided an absorbance value of 0.700 at 734 nm. After adition of 2 ml of ABTS<sup>+</sup> solution to 0.1 ml of extract, it was kept in the dark for 6 minutes and the absorbance was recorded at 734 nm (Shimadzu 1240). For the ABTS<sup>+</sup> test, a Trolox calibration curve was prepared and the results were expressed as (Trolox mM/L).

# Antioxidant Activity DPPH assays

The antioxidant capacity of the extracts obtained from the samples had been measured according to the DPPH (2,2-difenil-1-pikrilhidrazil) radical reducing activity method (Singleton & Rossi, 1965; Brand-Williams et al., 1995; Sánchez-Moreno, 2002). The amount of 6×10-5 M DPPH was



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prepared methanol. The amount of 4.9 ml of methanolic DPPH solution was added to 0.1 ml of sample or beer diluted. The resulting mixture was kept in the dark for 30 minutes. The absorbance against the blank at 517 nm was recorded by using spectrophotometer (Shimadzu 1240). A calibration curve of (1 - 5 mM) Trolox was prepared for analysis. The results were expressed as mg Trolox equivalents (TE)(Trolox mM /L).

## Result

According to Table 1, Turkish acorn has a total phenolic content of 386.751.06 mg GAE/100g, while Iranian acorn has a lower Gallic acid equivalent of 323.162.10 (mg GAE/100g DM). In terms of total flavonoids, there is a significant difference (p<0.05) between the oak fruits of Turkey and Iran. In terms of total flavonoid substance content, Turkish acorns have 61.951.20 (mg TE/100g DM), while Iranian acorns have 57.322.24 (mg KE/100g DM). The results show that Turkish oak fruits had higher antioxidant activity than Iranian samples (p<0.05).

Table 1. Total amount of phenolic and flavonoid substances and antioxidant activity values in the acorn samples from Iran and Turkey

	Phenolic (mg GAE/100)	Flavonoid (mg CAE/100)	DPHH Antioxidant Activite (mg TE/100g DM)	DPPH Antioksidan inhibisyon (%)	ABTS Antioxidant Activite (mg TE/100g DM)	ABTS Antioksidan inhibisyon (%)
IRAN	323,16±2,10	57,32±2,24	276,67±3,50	30,16	311,42±18	42,63
TURKEY	386,75±1,06	61,95±1,20	324,01±01	34,11	367,93±12	51,16

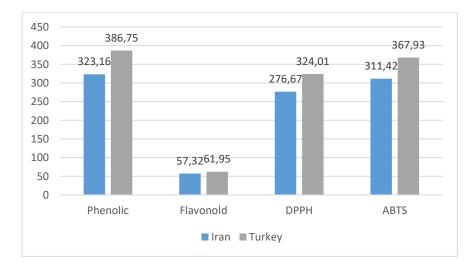


Figure 3. Total phenolic substance and total flavonoid contents and antioxidant activity of acorn fruit extracted in different solvent systems significant difference (p<0.05)



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According to the results of the analysises, a significant difference (p<0.05) had been found in the total amount of phenolic, flavonoid substances and antioxidant activity values in the acorn samples from Iran and Turkey. Conditions, process, different drying methods, solvent and analysis methods used in the extracts may differ.

Makhlouf et al. (2019) provided basic information about the presence of bioactive compounds and antioxidant capacity in two different acorn berries (*Quercus ilex L.* and *Quercus* suber *L.*) to evaluate them as functional food components and potential natural antioxidant sources in their study in Algeria. In this study, total phenolic content was determined using spectrophotometry and calculated as gallic acid equivalents (GAE) using the Folin-Ciocalteu procedure. The flavonoid content of the extracts studied was also estimated using aluminum chloride methods. Antioxidant capacity was determined using two different methods (DPPH• and ABTS•+). The results showed that antioxidant properties, total phenolic, and total flavonoid contents varied significantly between selected species and extracts. The flour samples had the highest total phenolic contents (1101–1464 mg GAE/kg dry weight) and antioxidant capacities, with average values of 52.62–40.78 mol TE g<sup>-1</sup> dry weight for the DPPH and 36.19–44.50 mol TE g<sup>-1</sup> dry weight for the ABTS assays, respectively.

In another study, Özünlü et al..(2018) conducted a study on this topic the effects of the extract of three different species of Valonia oak (*Quercus ithaburensis*), Ordinary oak (*Quercus petraae*), Holy oak (*Quercus ilex*) on stored chicken meat had been investigated. The results of analysis proved that the extracts of different species oak fruits can effectively prevent protein and lipid oxidation during storage (2 °C/14 days) due to their high phenolic content. The acorn treated group had higher total phenolic content and DPPH radical scavenging activities than the control group. The phenolic content of samples was found to be significantly higher in *Quercus ithaburensis*, *Quercus ilex*, and *Quercus Petraae* samples compared to the control sample at each evaluation period (P<0.05). The difference in total phenolic content could be described by the concentration of various polyphenols in the acorn extracts. The total phenolic content of all samples decreased during the storage period. At the end of storage, *Quercus ithaburensis* and *Quercus ilex* samples had the highest total phenolic content, while control samples had the lowest, indicating that they could be used as natural antioxidant sources in meat products.



Table 2 Total phanolic contant	(mg/100 g) values of chicken mea	t complex during storage at 2°C
Table 2. Total phenone content	(IIIg/100 g) values of chicken lifea	t samples during storage at $Z = C$

Total Phenolic (mg GAE/100g)	Control (mg GAE/100g)	Quercus ithaburensis (mg GAE/100g)	Quercus petraae (mg GAE/100g)	<i>Quercus ilex</i> (mg GAE/100g)
0	46.90±1.72	82.02±0.59	77.57±1.51	82.02±2.68
1	43.83±1.77	76.05±3.00	67.51±2.17	74.36±1.45
4	37.83±0.89	61.84±1.87	55.01±0.81	61.26±1.25
7	32.28±0.49	55.13±0.52	47.93±1.31	55.64±2.70
14	25.75±0.88	48.85±1.39	42.56±0.84	48.87±0.44

Table 3. DPPH radical activity values of chicken meat samples during storage at 2  $^{\circ}\mathrm{C}$ 

Antioksidan activity (DPPH (%))	Control (µmol TE/ g <sup>-1</sup> )	Quercus ithaburensis (µmol TE/ g <sup>-1</sup> )	Quercus petraae (µmol TE/ g <sup>-1</sup> )	<i>Quercus ilex</i> (μmol TE/ g <sup>-1</sup> )
0	29.11±0.28	47.35±0.91	57.47±0.62	52.34±1.88
1	26.07±0.28	47.07±0.91	46.04±2.29	46.32±0.46
4	23.85±0.47	33.89±0.52	40.39±0.43	39.19±0.82
7	23.12±0.48	27.12±0.22	31.80±0.34	31.41±1.11
14	9.72±1.38	25.32±2.1	29.53±1.12	28.91±0.60

#### **Discussion and Conclusion**

Today, innovations in the food industry's production of functional foods have given a lot of weight to healthier products that contain more grains, fiber, prebiotics and probiotics, or antioxidant substances. Consumers are increasingly demanding foods that are high in bioactive components, satiating, or make them feel full after eating them. Although Turkey has a rich potential in terms of oak species and the fruit of this tree has beneficial effects on human health, it is unable to profit by the full benefits of its content. Factors such as globalization, trade developments, population growth, and urbanization are the main reasons to seek the significant modifications in the patterns of the production and consumption as well as demands for the food products. In conclusion, although the acorns have not commercially used in food matrices so far, it considered that the acorns might be use in the food products as both a natural antioxidant source and a flavor enhancer.



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## DIETARY HABITS OF ADULTS PRIOR AND DURING THE COVID-19 LOCKDOWN IN BOSNIA AND HERZEGOVINA'S COVIDiet STUDY

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## Abstract

The aim is to evaluate dietary habits of adults living in Bosnia and Herzegovina prior and during the COVID-19 lockdown as the part of international project COVIDiet INT which was conducted in 19 European and 4 non-European countries. Participants (N = 1.507) completed a 44-items online survey designed for the assessment of general food habits and consumption frequency of selected foods using the validated 14-items Mediterranean diet as a reference of a healthy diet with the addition of socio-demographic characteristics, weight gain and physical activity. Survey was distributed using social media during the first lockdown, april 2020. Respondents were mostly: females (76.71%), people living in a family home (89.91%), with higher education degree (76.38%), aged between 21 to 50 years (82.35%). Consumption of olive oil (81.95%), vegetables (70.07%), red meat (68.75%), carbonated drinks (63.17%), legumes (85.47%), fish (80.16%), non-homemade pastries (62.24%), wine (74.72%), intake of fried foods (69.61%) remained the same as usual. Chicken and turkey meat were proferred over red meats (70.94%). Cooked vegetables were consumed by 49.04% (2 and more servings). Prior lockdown 52.62% had one meal outside the home and 53.88% cooked more during the lockdown. Proffered cooking method was mostly the stew (72.06%). Around 54% decreased the intake of fast food during the lockdown while 47.05% ate more than usual. However, snacking remained the same for near 60% of participants. 37.49% maintained and 31.59% decreased physical activity during the lockdown. 54.68% did not gain any weight. Regarding BMI, 54.35% had normal weight, 33.51% had overweight and 10.68% were obese. We assume that intake of the most of food remained as usual because survey was conducted when pandemic has just started and prior to lockdown people bought the food they usually consume and has a longer shelf life. Recommendation is to conduct survey again, one year after pandemic started.

Keywords: COVID-19, Lockdown, Dietary Habits, Bosnia and Herzegovina.



## **BEE BREAD AND BEE POLLEN**

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## Abstract

Physical, chemical, and biological properties of bee bread and bee pollen, their effects on health and studies on these products were investigated. Bee pollen is the reproductive units in the male organs of flowering plants and is used by bees to supply the basic protein needs of the colony and larvae. Bee bread is produced by fermenting pollen with various bee secretions and its bioavailability is higher than bee pollen. These products contain various components such as amino acids, carbohydrates, fatty acids, phenolic compounds, and vitamins. Bee bread and bee pollen have high antioxidant and antimicrobial properties depending on these bioactive components. It has been determined that bee bread and bee pollen, which are highly nutritious products with their rich composition and beneficial effects, contribute to the development and improvement of new products and can be used as functional food and supplementary products. According to the compiled literature results, it was determined that bee bread and bee pollen are promising products for the medicine and food industry.

Keywords: Bee Pollen, Bee Bread, Composition, Biological Activity

## Introduction

Natural products have been used by people in various fields since ancient times, especially playing an important role in the protection of health and the treatment of diseases. Bee products have been very important in different fields such as nutrition and treatment of diseases since ancient times. As a result, apitherapy centers were established in China, Russia, Romania and some eastern European countries where bee products are used in the treatment of diseases. These studies are also carried out in our country, and it takes its place in the first place in beekeeping activities (Sorucu, 2019; Mayda, 2019).

Bee products are examined in two groups. The first is honey, bee pollen, bee bread and propolis, which are products produced by bees from their body secretions or added to their secretions. The other group consists of royal jelly, beeswax, bee venom and apilarnil, which are products produced from the bee itself (Mayda, 2019).

Bee bread and bee pollen have a very important place among bee products because of their bioactive components. These products are used as functional food in the field of nutritional support



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and alternative medicine, and in the development and improvement of new products (Silici, 2015; Karlıdağ and Keskin, 2020).

Bees collect pollen from plant anthers, mix it with the secretion from salivary glands or nectar, and place it in specific baskets which are situated on the tibia of their hind legs. Pollen consists of two protective layer; the first layer is called exine. This layer has a very complex structure and is very difficult to digest when consumed as food. The exine layer consists of carbohydrates, glycoproteins, proteins, lipids, terpenoids, long chain aliphatic and phenolic substances. It also provides storage area for secondary products such as volatile compounds, surface lipids, pigments, phenolic substances and flavonoids produced in tapetum cells (Mayda, 2019; Canli, 2014).

Bee bread is a very valuable bee product whose main ingredient is pollen. It is seen that it is more valuable and beneficial compared to pollen, because of the unique and abundant beneficial bacteria it contains. Bee bread, which is the main food item for baby bees and queen bees, is a fermented product that worker bees mix their own secretions with pollen and nectar and store in honeycombs. Part of the pollen exine layer is broken down with this fermentation. In this way, bee bread becomes an easily digestible food item in the human body, which makes it a very valuable food. While the digestion of pollen in the stomach is around 60%, bee bread, which is a fermented product, can be completely digested in the stomach (Karaman et al., 2017; Keskin and Özkök, 2020). In this study, it was aimed to examine the composition of bee bread and bee pollen, their effects on health, and to investigate the studies on bee bread and bee pollen.

#### The Composition of Bee Bread and Bee Pollen, Their Effects on Health

Although bee bread and bee pollen are highly nutritious products with the components they contain, they can be used as functional food in the field of alternative medicine and as a food supplement due to their biological properties (Mărgăoan et al., 2019; Silici, 2015).

Pollen is used by bees to meet the protein needs of the colony and to feed the larvae (Silici, 2015; Sorucu, 2019). Beekeepers collect pollen used for food consumption with pollen traps placed in front of the hives. Collected pollen is stored by drying or freezing (Silici, 2015). The color, shape and content of bee pollen depend on factors such as the type of the plant from which it is collected, the geographical characteristics of the region and production techniques (Aydın, 2016). Pollen is a distinct product in terms of various amino acids, carbohydrates, saturated/unsaturated fatty acids, lipids, sterols, vitamins, terpenes, phenolic substances, enzymes and minerals, with the highest amount of protein. Its chemical composition consists of an average of 10-40% protein, 15-50% carbohydrates and 1-13% lipids (Mayda, 2019; Sorucu, 2019).

Bee bread is an important bee product whose main component is pollen. It is formed by mixing pollen with honey and other bee secretions and undergoing lactic acid fermentation for about two weeks. Due to being a fermented product, bee bread can be stored in the hive for a long time. Bees use bee bread as a source of protein, fat and vitamins, and bee bread is the raw material of royal jelly. It contains a large amount of beneficial bacteria in its composition and with this feature, it shows more bioactive properties compared to pollen. Although it contains proteins, amino acids, fatty acids, lipids, sterols, enzymes, minerals, vitamins and phenolic substances in its composition,



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when its chemical composition is examined, it contains approximately 20% protein, 3% lipid, 24-35% carbohydrates, 3% vitamins and minerals. It contains less protein and fat compared to pollen, while it has more carbohydrates and lactic acid (Karlıdağ and Keskin, 2020; Mayda, 2019; Silici, 2015).

Bee bread and bee pollen have positive effects on human health due to their high nutritional value and bioactive compounds. Their properties cause these products to have positive effects on the immune system, and enable the body to fight bacteria and perform tissue repair (Mărgăoan et al., 2019).

Depending on its source, pollen has many beneficial properties. Bee pollen shows biological effects such as antibacterial, antifungal, antitumoral (prostate, colorectal, breast cancers), radioprotective, antioxidant. immunomodulator. antiaging, antiosteoporosis, antianemia. antidiarrheal, memory enhancer, probiotic, regenerative, performance enhancing, aphrodisiac properties (Sorucu, 2019). In recent years, bee pollen, which is a supportive food, has been used as a supplement in the nutrition of athletes, children with growth retardation, anemic patients and people with hepatitis (Yıldız, 2011). Bee bread and bee pollen have positive effects on human health because they contain essential amino acids that the human body cannot synthesize. Especially, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of these essential amino acids create cardioprotective, anti-arrhythmic, antithrombotic and anti-inflammatory effects with their ability to lower cholesterol and triglyceride levels in the blood (Silici, 2015). At the same time, bee bread shows good antioxidant properties. Bee bread shows positive effects on reproductive hormones and provides positive development in increasing muscle strength and volume. Due to the acetylcholine it contains in its composition, bee bread is good for blood pressure and chronic constipation disorders and is used in their treatment (Sorucu, 2019). Considering all these features, bee bread and bee pollen have been described as a good food supplement and their use has become widespread becaues of their positive contributions to human health.

#### **Studies on Bee Bread and Bee Pollen**

The investigations conducted on bee bread and bee pollen were summarized in Table 1.



Table 1. Be	e Bread	and Bee	Pollen	Studies
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Product	Investigated properties	References
Bee pollen	Vitamin $B_1$ , vitamin $B_2$ , vitamin $B_3$ , vitamin $B_6$ , vitamin $B_{12}$ and	Konar et al., 2010
Bee pollen	folic acid Botanical, physical and chemical properties of pollen from the Black Sea region and its role in preventing liver damage	Yıldız, 2011
Bee pollen	Amount of reduced glutathione and oxidized glutathione	Birișik et al., 2011
Bee pollen	Fatty acid composition	Karagözoğlu et al., 2012
Bee pollen	Antioxidant and antimicrobial properties	Borycka et al., 2015
Bee pollen	Palynological spectrum, composition and fatty acid composition	Kaplan et al., 2016
Bee pollen	Microbiological quality	Sandıkçı-Altunatmaz and Yılmaz-Aksu (2016
Bee pollen	Antioxidant activity and phenolic compounds	Aydın (2016)
Bee pollen	Total phenolic and flavonoid content and antioxidant activity, enzyme inhibitory potential	Özparlak ve ark. (2017)
Bee bread	Antioxidant and antimicrobial properties	Akhir et al. (2017)
Bee bread	Phenolic profile	Sobral et al. (2017)
Bee bread	Functional properties characterization	Kowalski and Makarewicz (2017)
Bee bread	Potential use as a food supplement and source of nutraceutical	Tomás et al., 2017
Bee pollen	Use as a natural and functional ingredient in gluten-free bread and its effect on the physical, chemical, technological and sensory properties of the obtained breads.	Conte et al., 2018
Bee bread	Sugar, fructose/glucose ratio, total phenolic and flavonoid content	Urcan et al., 2018
Bee pollen	Fatty acid, amino acid, protein, crude fat, reducing sugar, carotene, calcium, iron, zinc and selenium content	Wu et al., 2018
Bee pollen	Lipid, vitamin C, $\beta$ -carotene and lycopene content	Estevinho et al., 2018
Bee pollen	Nutritional value, phytochemical composition and botanical origin	Gardana et al., 2018
Bee pollen	Antioxidant activity	Karkar et al., 2018



Table 1. Bee Bread and Bee Pollen Studies

Bee pollen	Physicochemical properties, phenolic profile and antioxidant activity	Okumuş et al., 2018
Bee pollen	The effect of drying with hot air on the quality characteristics of bee pollen	Isık et al., 2019
Bee bread	Flavonoid content and antimicrobial activity	Hudz et al., 2019
Bee bread	Nutritional value, chemical composition, antioxidant activity, antibacterial activity	Bakour et al., 2019
Bee bread	Physicochemical properties and composition	Othman et al., 2019
Bee pollen	Antimicrobial, antioxidant properties, overall acceptability, biogenic amine content	Bartkiene et al., 2019
Bee pollen	Nutritional differences depend on pollen wall	Yan et al., 2019
Bee pollen	Antioxidant activity	Anjos et al., 2019
Bee pollen	Antimicrobial effect	Velásquez et al., 2019
Bee pollen	Antioxidant activity, phenolic compounds	Belina-Aldemita et al., 2019
Bee pollen	Phenolic content, antioxidant, vitamin composition (C and E) and antioxidant capacity	Kanar and Mazı, 2019
Bee pollen	The effect of enzymatic hydrolysis on the structure of bee pollen and the release of compounds	Zuluaga-Domínguez et al., 2019
Bee pollen	Effects of ultrasonic and ball milling on nutrient release and antioxidant effects of rose (Rosa rugosa) bee pollen in vitro and in vivo	Yang et al., 2019
Bee pollen	Effect of adding bee pollen to beef burger on oxidative stability	Heldt et al., 2019
Bee pollen	Water activity, diastase number, proline and 5-hydroxymethyl-2- furfural content	Kanar and Mazı 2019
Bee pollen	Antioxidant capacity, morphometry, color parameters and FTIR spectroscopy	Bleha et al., 2019
Bee pollen	Physicochemical properties and chemical compositions	Belina-Aldemita et al., 2019
Bee pollen	Phenolic profile, antioxidant properties	Kostić et al., 2019
Bee pollen	Functional ingredient in milk powder	Thakur and Nanda 2019
Bee pollen	Antioxidant activity and ability to prevent DNA damage	Şahin and Karkar 2019



Table 1. Bee	Bread and	Bee Pollen	Studies
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Bee pollen, bee bread	Effect on microfungi growth and buckwheat, wheat, oat and corn grain	Keriene et al., 2019
Bee pollen, bee bread	Plant origin, chemical composition and antioxidant capacity	Mayda, 2019
Bee pollen	Protein, ash, mineral, color, moisture, carotenoid and oil content, some bioactive properties and phytochemical components	Küçüktürk et al., 2019
Bee bread	Sterol content	Kaplan et al., 2019
Bee pollen	Moisture, ash, protein, fat, fiber and sugar profile	Başdoğan et al., 2019
Bee pollen	Moisture, water activity, pH value, aflatoxin content	Arslan and Durmaz 2019
Bee bread	Element content and fatty acid composition	Ciric et al., 2019
Bee pollen, bee bread	Antiviral effect of bee products and ability to stimulate the immune system against the COVID-19 epidemic	Lima et al., 2020
Bee pollen, bee bread	Element analysis	Pohl et al., 2020
Bee bread	Composition and phytochemical properties	Wahyuni et al., 2020
Bee pollen, bee bread	Plant sources, fatty acid composition, total phenolic-flavonoid contents and element profiles	Mayda et al., 2020
Bee bread	Total phenolic and flavonoid content, antioxidant activity, vitamin C and E	Sawicki et al., 2020
Bee pollen	The effects of lyophilization and oven drying techniques on the biochemical properties of pollen	Keskin and Özkök, 2020
Bee bread	Macro and micro elements, phytochemicals, bioactive compounds, aroma profile	Conte et al., 2020
Bee pollen	Phenolic compounds	Oroian et al. 2020
Bee pollen	Antioxidant activity	Şahin and Kemal, 2020
Bee pollen, bee bread	Total phenolic compound, flavonoid, antioxidant activity	Keskin and Özkök, 2020



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#### Conclusion

In this study, the chemical, physical, biological properties and health effects of bee bread and bee pollen, which are very valuable bee products, and studies in various fields on these products were investigated. Due to the bioactive components they contain, bee bread and bee pollen offer the opportunity to be used in different areas and provide various benefits. Bee bread and bee pollen, which are very rich products in terms of amino acids, carbohydrates, fatty acids, lipids, sterols, terpenes, phenolic substances, enzymes, minerals and vitamins, are seen as good alternatives in terms of nutrition because of the valuable components they contain.

Bee pollen and bee bread were regarded as a valued distinct food. Bee pollen and bee bread are valuable source of natural nutrients and considering it as a favorable product for human nutrition and health. Due to nutritional, chemical and bioactive characteristics of bee pollen and bee bread, these products have positive effects on human health and are good products for the food and pharmaceutical industries.

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# SULFORAPHANE EXTRACTION FROM CRUCIFEROUS GROUP VEGETABLES AND SEEDS

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## Abstract

The sulforaphane compound, which has a natural, strong anticancer and antimicrobial effect, ensures that the uncontrolled cell proliferation caused by cancer can be stopped. In this study, it was aimed to determine the sulforaphane compound, which has many effects on health, and the cruciferous group vegetables and seeds (cabbage, broccoli, black radish and kohlrabi) that give the highest sulforaphane content. First, the vegetables were dried in a tray dryer (60 °C), then the seeds and vegetables were ground. Before the extraction process, the samples were subjected to hydrolysis with the aid of water (35 °C, 2 hours, 1:2 solid/water (w:v)) in order to ensure the formation of sulforaphane. Afterwards, the samples were extracted by microwave assisted extraction method (150 W, 2 min), using 50-50% ethyl acetate-water (v:v) solvent, and the sulforaphane content was determined by HPLC-DAD. At the same time, it is thought that sulforaphane does't show antioxidant properties directly, but indirectly exerts its antioxidant effect with the help of other antioxidant compounds. For this reason, antioxidant (DPPH and % inhibition) and phenolic content analyzes were carried out in the extracts obtained. As a result of the study, the amount of sulforaphane in the range of 5.36 to 875.18  $\mu$ g/g DM was found in the vegetables and seeds of the cruciferous group. As a result of the analyzes, the amount of antioxidant capacity was calculated between 32.36 and 186.5 TEAC/ 100 g DM, as well as the inhibition antioxidant activity between 4.34% and 89.78%, and the total amount of phenolic substance between 20.11 and 253.3 mg GAE/ 100 g DM was calculated. As a result, higher sulforaphane content, total phenolic substance content and antioxidant capacity (DPPH and % inhibition) amount were found in the seeds of cruciferous group vegetables, while the highest sulforaphane content was determined in cabbage seed, the highest antioxidant capacity (DPPH and % inhibition) and total phenolic substance content were determined in kohlrabi seed.

Keywords: Sulforaphane, Cruciferous Group Vegetable, Microwave Assisted Extraction, HPLC.



# USE OF PARAPROBIOTICS AND POSTBIOTICS IN DAIRY TECHNOLOGY

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## Abstract

Probiotics are defined as live microorganisms that have various health benefits when taken into the host cell at a certain level. Probiotics have many health effects on conditions such as diarrhea, lactose intolerance, irritable bowel syndrome, insulin resistance, weight control, regulation of blood pressure and cholesterol levels. In recent years, it has been reported in many studies that the effects of probiotics can also be achieved by dead probiotics and their metabolites. In the literature, non-living probiotic microorganisms have been defined as paraprobiotic and their metabolites as postbiotic. However, ambiguity has emerged between concepts such as probiotic, paraprobiotic and postbiotic. For this reason, in this study, probiotic, paraprobiotic, postbiotic and other terms that can be used in this sense will be examined and literature information related to their use in the field of dairy technology will be compiled.

Keywords: Probiotic, Paraprobiotic, Postbiotic, Dairy Technology



# EFFECT OF ULTRASOUND TREATMENT ON FUNCTIONAL AND SENSORIAL PROPERTIES, AND PROTEIN PROFILES OF TURKEY MEAT

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#### Abstract

Poultry is one of the good quality sources of protein that can meet needs of people and is relatively cheaper when compared to other alternatives. However, its consumption is low due to its sensory properties. Recently, the use of ultrasound, which is one of the novel food processing technologies, to improve the texture of meats has come to the fore. This study aimed to investigate the effect of ultrasound treatment on turkey meat proteins in terms of functional properties, protein hydrolyses and as well as sensorial properties of turkey meat. The sensory evaluation is scoring test and score range is between 1-5. The parameters are appearance, tissue, flavor, and general acceptance. The tissue score (TS) obtained for ultrasound treated turkey meat (USM) was significantly higher (4.23  $\pm$  0.09) than the score obtained for non-treated turkey meat (CM) (3.38  $\pm$  0.49). In terms of the taste  $(4.11 \pm 0.26)$  and general acceptability  $(3.75 \pm 0.09)$  scores of USM were higher than those of CM  $(3.63 \pm 0.23 \text{ and } 3.42 \pm 0.22, \text{ respectively})$ . Although there was no significant difference between the TS parameters, USM had a lower hardness value and a higher chewiness value. In addition, the cooking loss of USM (14.70%) was lower (p>0.05) than those of CM (15.28%). At the end of invitro digestion, the total amount of free amino acids released from CM (21.15 g/100g) is higher than USM (18.84 g/100g). The total amount of amino acids is 39.18 g/100g in CM and 37.18 g/100g in USM. The free/total amino acid ratio is 54% in CM and 51% in USM. The results showed that ultrasound treatment improved the sensory properties of turkey meat, but not affected the protein digestibility. Therefore, ultrasound treatment can be recommended as an effective method to improve the sensory and some functional properties of turkey meat.

Keywords: Protein Hydrolyses, Texture, Sensorial Properties, Tenderness

#### Introduction

Meat is valuable animal food and is the first option for animal protein by many consumers (Lafarga et al., 2014). Compared to vegetables and cereals, it has a higher protein content. It is a quality source of protein for humans due to its presence of all essential amino acids and the high digestibility (95%) of the proteins it contains (Iseri and Erol., 2009; Bax et al., 2013; Sayd et al.,



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2016; Caron et al, 2016). Many essential amino acids (phenylalanine, histidine, isoleucine, leucine, threonine, valin) are found in turkey meat in higher quantities than red meat (TURKOMP, 2021). Poultry meat contains a low amount of fat and cholesterol, in addition to containing full-quality protein and many micronutrients. Turkey meat contains an average of 8% fat and the fat content in the breast meat is < 5%. Linoleic acid, one of the polyunsaturated fatty acids needed in human nutrition, is found in turkey meat (2.6 mg/g fat) in higher amounts than chicken meat (0.9 mg/g fat) (Masiero L., 1993; Iseri and Erol., 2009). They have also valuable components with various health benefits such as glutathione, taurine, and anserine (Bordoni et.al., 2017).

Turkey meat consumption in Turkey is quite low compared to other animal sources (red meat, chicken meat, fish, eggs) (Association of Poultry Meat Producers and Breeders, BESD-BİR, 2021). Turkey meat production and consumption can be significantly increased by processing and marketing turkey meat into different products, changing the policies applied in favor of turkey making, and better promoting turkey meat (Koyuenbe et al., 2010). Nowadays, meat is offered to customers as parts or advanced processed products instead of carcasses. So, many properties of meat such as color, water retention capacity or texture have gained great importance (Bihan-Duval et al., 2006). In addition, texture and meat color are the significant criteria that play a role in consumer preference (Grow et al., 2008). Differences in process characteristics, how products are produced is one of the issues that consumers have focused on for the last 10-15 years (Karaca et al., 2005). For this reason, new and safe technologies have been explored in order to increase the consumption of turkey meat with high nutritional value and to present the texture to the consumer for improved flavor.

Increasing consumer awareness and consumer preferences towards more reliable, high quality, healthy, and environmentally friendly foods has brought up environmentally friendly food processing methods called green technology. In recent years, supercritical fluid extraction, extrusion, pulsed electromagnetic field, high pressure, and high-power ultrasound technologies are recognized as technologies that have significant advantages in ensuring food safety without heat treatment of food, thus improving product quality, energy efficiency, and developing new products (Kang et al., 2021). In agriculture and food processing, high-power ultrasound is recognized as a green physical and non-thermal technology to improve the safety and quality of food (Kang et al., 2021). Ultrasound is a form of energy produced by waves with a vibration frequency above the audible limit (20kHz) for humans (Peña-Gonzalez et al., 2018). In studies on the effects of ultrasound in meat processing, US application increases the permeability of muscle tissue, positively affects the curing, marinating, drying, and softening stages, increases the water holding capacity of the meat, improves the texture, salting process. (Zhao et al., 2014; Alarcon-Rojo et al., 2015; Peña-Gonzalez et al., 2018). It has also been used successfully in the inactivation of microorganisms (Alarcon-Rojo et al., 2018).

This study aimed to investigate the effect of ultrasound treatment on turkey meat proteins in terms of functional properties, protein hydrolyses/digestibility, and as well as sensorial properties of turkey meat.



## Material and Methods Materials and food processing

Turkey meat (*Pectoralis major*) used in the project was supplied by Pinar Integrated Meat and Flour Industry Inc. Ultrasound at 35 kHz frequency was applied to vacuum packed turkey meat in an ultrasonic bath for 40 minutes. Turkey meat is lined with baking paper and cooked in this way in a convection oven at 180 °C for 20 minutes.

## Methods

All analyses were performed 2 parallels and 3 replicates. The pH of the samples, which were homogenized by blender, were measured by immersing pH meters. At least two measurements were made, and the average value was taken. Moisture was determined according to the method of AOAC 950.46. Protein Analysis was carried out using NIR (Near InfraRed) technology. Total fat was determined by Soxhlet method according to the method of AOAC 960.39. Protein solubility was determined by the method recommended by Warner et al., (1997). Protein concentration in the supernatant was tested by Bradford method (Bradford, 1976). Water holding capacity (WHC) was determined with minor modification by the method proposed by Hughes et al., (1997). Cooking loss was determined according to the method proposed by Bertram et al., (2003). *In vitro* digestion was carried out according to the methods proposed by Brodkorb et al., (2019). The protein profile of the all samples (raw, cooked, gastric digested and intestinal digested) was determined by SDS-page analysis (Laemmli,1970). Raw and cooked samples were lyophilized for this analysis and TEM images were obtained by Ege MATAL. Free and total amino acid after *in-vitro* digestion were determined using HPLC by Ege MATAL.

Tissue profile analysis of samples was obtained using a uniform analyzer (TA-XT Plus, Texture Analyzer Stable, Micro System United Kingdom). Parameters calculated from the resulting force and time curves were hardness, adhesiveness, springiness, cohesiveness, chewiness. Scoring tests were performed with 6 semi-trained panelists for each sample (Altug et al., 2011). The criteria were appearance, tissue, flavor, and general acceptance, and the range was in between 1-5.

## **Results and Discussion**

## **Chemical analysis**

Table 1 shows the moisture, protein, fat, and pH values of the samples. There was no statistically significant difference between the control and US treated raw samples (P>0.05).



ANALYSIS	СМ	USM
Moisture (%)	$67.87\pm0.24^{\rm a}$	$67.72\pm0.61^a$
Protein (%)	$27.47\pm0.38^{\rm a}$	$27.58\pm0.64^{\rm a}$
Fat (%)	$0.83\pm0.20~^{\rm a}$	$0.70\pm0.42^{\rm a}$
pH	$6.03\pm0.06^{\text{ a}}$	$\pm 0.04$ <sup>a</sup>

#### **Protein solubility**

Figure 1 shows the amount of soluble myofibrillar protein of the samples. The solubility of myofibrillar protein of USM was lower than that of CM (p<0.05).

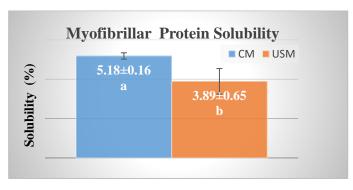


Figure 1. Solubility of myofibrillar protein

The relationship between the US power and myofibrillar protein solubility was reported in many studies. The results of these studies indicated that an increase in US power caused a significant increase in myofibrillar protein solubility as reported by Zhang et al., 2017 (from 22.89% to 88.49%) and by Wang et al., 2017 (from 6.55% to 9.02% in 6 minutes). The other factor that affects US efficiency is the duration of US application. Amiri et al., (2018) reported that samples treated with 300 W US for 30 minutes displayed the greatest increase in protein solubility when compared to other samples treated with 300 W US for 10 and 20 minutes. In our study, the applied US power (215 W) and the material (turkey meat) we used were different from the studies mentioned. Therefore, the applied US power may not have been sufficient to increase the solubility of turkey meat myofibrillar proteins. In addition, forms of meat such as fillet, tenderloin, or cubed meat are also likely to be the factors that affect protein solubility. This is supported by the study of Cárcel et al (2012), which reported that the effect of US increases as the particle size decreases.



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#### **Cooking loss**

Cooking loss is one of the important characteristics of meat. Cooking loss is expected to explain part of the variation in juiciness and affects the appearance of meat. A high cooking loss gives an expectation of less optimal eating quality. Also, the cooking loss is of great economic importance to the catering industry (Aaslyng et al.,2003). There was no statistically significant difference between the cooking losses of USM and CM (Table 2).

Cooking loss (%)			
СМ	USM		
$15.29 \pm 1.66^{a}$	$14.71\pm0.35^{\rm a}$		

Table 2. Cooking losses of turkey meat samples

Similarly, in a recent study, no difference between the cooking losses of US treated, marinated, and control chicken samples were reported (Inguglia et al., 2019). Xiong et al., (2012) reported that the use of US (24 KHz, 12 W/cm2, 4 min) did not cause a significant decrease in the cooking loss. The combined treatments of US and exogenous proteolytic enzyme inhibitors also could improve chicken meat tenderness and decrease cooking loss.

#### **Protein profile**

The protein profiles of raw, cooked, gastric digests and gastrointestinal digests of turkey meats were determined by SDS-Page analysis (Figure 2).

Although 10 protein bands were observed for raw USM and CM their  $M_w$  were different. Protein bands of the raw sample marked as 1, 3, 5, 7, 8 and 9 on the SDS-page gel correspond to the same proteins of cooked sample marked as 1, 2, 3, 4, 5, 6 and 7 on the SDS-page gel, respectively. After cooking, 11 protein bands were observed in USM sample. These proteins may be actin (43.97 kDa), tropomyosin (37.66 kDa), myosin light chain I (24.60 kDa), troponin -I (~22.74 kDa), and myosin light chains II (22.74) respectively according to their  $M_w$ . The SDS-page profiles of the samples showed that the bands with  $M_w>37$  kDa were not hydrolyzed in gastric digestion, and the proteins with  $M_w>50$  kDa were hydrolyzed in the small intestine.



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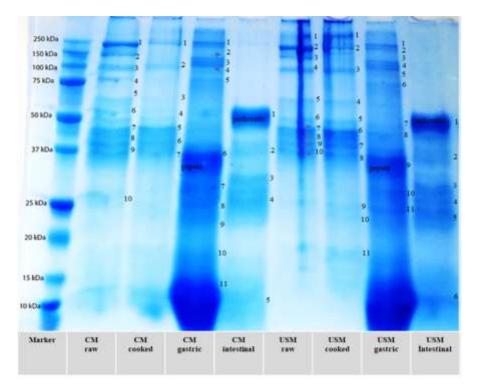


Figure 2. CM raw: Raw control sample, CM cooked: Cooked control sample, CM gastric: Gastric digested sample, CM intestinal: Gastrointestinal digested sample; USM raw: Ultrasound-treated raw sample, USM cooked: Ultrasound-treated cooked sample, USM gastric: Gastric digested ultrasound-treated sample USM intestinal: Gastrointestinal digested ultrasound-treated sample

## Free and total amino acids

Table 3 shows the free and total amino acid amounts, and free/total amino acid ratio of the samples that represent the digestibility/hydrolysis.

Amino acid	CM	USM
Free amino acid (g/100g)	21.15	18.84
Total amino acid (g/100 g)	39.18	37.17
Free/ Total amino acid ratio (%)	54	51

Table 3. Free and total amino acid amounts, and free/total amino acid ratio of the samples

In both samples, it was observed that more than 50% of the total amino acids in the protein were liberated as free amino acids due to hydrolysis by gastrointestinal proteases. Apart from the free amino acids released, the remaining total amino acids (18.03 g/100 g for CM and 18.33 for USM)



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can be peptides and/or non-hydrolyzed proteins with a molecular weight <10 kDa when evaluated together with the SDS-page profile. It seems that US treatment has no remarkable effect on protein hydrolysis. Although protein hydrolysis is expected to increase with US treatment, Li et al., (2021) reported that the reasons why protein hydrolysis does not increase maybe that free amino acids cannot be released due to protein aggregation that occurs during US treatment, and/or that free amino acids enter the Maillard reaction with reducing groups during heat treatment. Therefore, results showed that US treatment improved the sensory properties of turkey meat, but not affected the protein digestibility.

Li et al., (2021) examined the effects of heat treatment, ultrasound treatment, and the combinations of heat-ultrasound and ultrasound-heat treatments on the hydrolysis and flavor properties of scallops (*Aloididae aloidi*). Seventeen types of free amino acids were detected in all samples and the highest amount of free amino acids was detected in the sample treated with US. However, heat-treated and ultrasound-heat treated samples exhibited the lowest free amino acids (Li et al., 2021).

#### Scanning electron microscope (SEM)

SEM images of raw CM and raw USM, showed that the mechanical damage in USM was noticeable (Figure 3, marked with a red arrow). Especially fracture is apparent in connective tissue fibers. The SEM image of the cooked sample showed that the integrity of the tissue was disrupted, and some fractures/fragments occurred (Figure 3). The loosening and change in texture were much more apparent in cooked USM and a break in a myofibril due to acoustic energy was noticeable (Figure 3).



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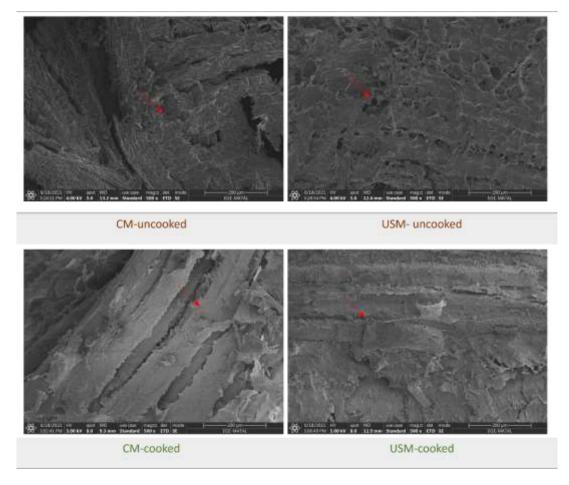


Figure 3. Scanning Electron Micrographs of cooked and uncooked CM and USM

# Texture profile analysis

The results of tissue profile analysis of the samples are given in Table 4. Although there was no significant difference between the tissue parameters, USM had a lower hardness value and a higher chewiness value.

Table 4. Tissue profile analysis	Table 4.	Tissue	profile	analysis
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	Hardness(N)	Adhesiveness	Springiness	Cohesiveness	Chewiness
СМ	43.38±2.70 <sup>a</sup>	-1.83±2.70 <sup>a</sup>	0.109±0.016 <sup>a</sup>	$0.124 \pm 0.012^{a}$	0.70±0.13 <sup>a</sup>
USM	42.04±1.79 <sup>a</sup>	-2.22±2.98 <sup>a</sup>	$0.131 \pm 0.019^{a}$	$0.170 \pm 0.049^{a}$	1.50±0.98ª

Pena-Gonzalez., (2019) applied high-intensity US (40 kHz, 11W/cm2, 60 min, 4°C) on beef (*M. longissimus dorsi*) and stored the samples at 4°C. After14 days of storage, the US-treated meat was more tender and juicier than that of control. The application of US increased the sensation of tenderness without changing other sensory attributes.



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#### Sensory analysis

Table 5 shows the sensory analysis' results of CM and USM. There was no statistically significant difference between the appearance scores of the USM and CM (p>0.05). However, the texture, flavor, and general acceptance score of the USM sample was significantly higher than those of CM (p<0.05).

Sensory parameters	СМ	USM
Appearance	$4.33\pm0.35^{\rm a}$	$4.57\pm0.10^{\rm a}$
Tissue	$3.38\pm0.49^{\rm a}$	$4.23\pm0.09^{b}$
Flavour	$3.63\pm0.23^{\rm a}$	$4.11\pm0.26^{\text{b}}$
General Acceptance	$3.42\pm0.22^{\rm a}$	3.75±0.09 <sup>b</sup>

#### Table 5. Sensory analysis results of turkey samples

The improved sensory properties in terms of taste, flavor, color, and texture of US-treated beef were reported in the study of Peña-Gonzalez et al., (2019).

#### Conclusion

In conclusion, US treatment did not have significant effects on water holding capacity, protein solubility, and cooking loss of turkey tenderloin. USM sample displayed the lowest protein solubility (3.89 mg/ml) (p<0.05). There was no difference between the protein digestibility of USM and CM. The molecular weights of the proteins in the raw USM samples were different from the CM. SEM images showed that mechanical damage in the tissue of the USM sample was clearly visible. In addition, tissue disturbance, and some fractures are present in the SEM image of cooked USM. Although there was no significant difference between the tissue parameters, USM had a lower hardness value and a higher chewiness value. The appearance of USM and CM was similar. However, the USM sample was preferred in terms of other sensory criteria (texture, flavor, and general acceptance score).

Therefore, ultrasound treatment can be recommended as an effective method to improve the sensory and some functional properties of turkey meat.

#### Acknowledgment

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# THE EFFECTS OF CHITOSAN ON QUALITY PROPERTIES OF SEAFOOD PRODUCTS

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# Abstract

Seafood and seafood products are often preferred by today's consumers in a healthy diet because they are among the food groups with high nutritional value. Seafood and seafood products are known as rich sources of functional and health-supporting components that are involved in many important metabolic activities in the human body, such as vitamins that enhance the immune system, lipids with a significant amount of valuable polyunsaturated fatty acids that prevent degenerative diseases and easily digestible and high quality proteins. However, seafood and its products are highly perishable so they can deteriorate very quickly under inappropriate storage conditions. Since seafood products are susceptible to spoilage due to microbiological, enzymatic and biochemical reactions, various quality losses can occur during storage of these products. Chitosan is one of the biodegradable materials obtained from natural sources and used for the preservation of foods. In addition, chitosan is a biocompatible, environmentally friendly, allergen-free, non-toxic and lowcost biopolymer. Also, chitosan and its derivatives have antimicrobial activities on food-borne pathogens, and a strong antioxidant effect on the oxidation of lipid and protein in seafood and its products. Therefore, chitosan and its derivatives are mainly used to retard biochemical changes, enzymatic reactions and microbiological spoilage and to extend the sensory acceptability in seafood and its products. Chitosan and its derivatives have also beneficial properties for health promotion and disease prevention such as having anti-inflammatory and anti-tumor effects with wound-healing activity. In this study, the use of chitosan as a food preservative, applications of chitosan and its derivatives on seafood and seafood products, the protective effect against microbial spoilage and oxidative deterioration and sensory quality of these products during the storage were investigated.

Keywords: Chitosan, Seafood, Seafood Products



# ISOLATION AND IDENTIFICATION OF CAROTENOID PRODUCING YEAST

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# Abstract

Carotenoids are naturally occurring lipid-soluble pigments that are synthesized by plants and microorganisms. They exhibit yellow, orange, red and purple colors. Carotenoids have found applications in different industries including pharmaceuticals, nutraceuticals, food and feed, as well as in cosmetics. Carotenoids act/ function as antioxidents by neutralizing harmful free radicals resulting from normal cellular activity (in human and animals) therefore, they are used as supplements to enhance- promote health. Moreover, the addition carotenoids in aqua feed improves the color in the flesh and skin of fish as well as in the shells of arthropods. While their addition to poultry feed contributes to the skin color and to the characteristic orange – yellow color of egg yolks. Therefore, the current study aimed to isolate and identify carotenoid producing yeasts that can be used as supplements in animal feeds. 13 yeast isolates were obtained from 9 soil samples taken from various regions. 5 isolates, which revealed red, orange and pinkish colorations, were selected for molecular characterization. The amplification of the 26S rDNA allowed the identification of 4 isolates as Rhodotorula mucilaginose and one isolate as Cystobasidium slooffiae. Chemical synthesis of carotenoids is one of the most important production methods. However, recent studies have demonstrated the toxic, carcinogenic and teratogenic properties of synthetic-derived pigments. The process is also challenged by high production cost, by-product formation, and harmful environmental effects. Therefore, there is an increased interest in microbial carotenoids as a safe alternative. Pigmented yeasts have an advantage over algae, plants, and bacteria due to their unicellular and relatively high growth rate using low-cost fermentation media.

Keyword: Yeast, Carotenoid, 26S rDNA, Food

# PLANT PRODUCTION



# A PRELIMINARY STUDY ON THE EFFECT OF DIFFERENT NITROGEN LEVELS ON THE FORAGE YIELD AND SOME AGRONOMICAL COMPONENTS OF TRITICALE (*X TRITICOSECALE* WITTMACK)

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# Abstract

Triticale (x Triticosecale Wittmack), the first successful human-made cereal grain, was consciously hybridization by crossing wheat with rye. This hybrid plant combined wheat's qualities for making various food products with rye's strength for adaptability to hard soils, drought tolerance, cold endurance, disease resistance and low-input requirements. Nowadays triticale is accepted global with its area expanding significantly, particularly in stress-prone ecologies. Winter-type cultivars, a considerable amount of biomass is useable for grazing, cut forage, dual-purpose cultivation, and silage and hay production. The performance of mineral fertilizer in forage depends on the association of rate, source, place, and time of application. Nitrogen fertilizers play a vital role in increasing forage yields however, one of the main constraints in achieving proven crop potential is an unbalanced use of nutrients. The current research aimed to establish the effect of different nitrogen levels (0-40-80-120-160-200-240-280 kg·ha<sup>-1</sup>) on triticale forage yield and some yield characteristics. This study encompassed the results of a nine-months (from October 2018 to June 2019) pot experiment conducted at the research area in Field Crops Department, Faculty of Agriculture, Ege University, Izmir, Turkey. Data were recorded for forage yield and yield components like plant height, number of plant, fresh herbage yield, dry matter (DM) yield and DM content. The application of nitrogen has led to a great and momentous increase of yield compared to the control. Finalized from the findings of this study that application of nitrogen at 200 kg N·ha<sup>-</sup> <sup>1</sup> gave maximum DM yield per pot under the Mediterranean agro-ecological conditions with the cultivar 'Ege Yıldızı' in Turkey.

Keywords: x Triticosecale, Nitrogen, DM Yield

# Introduction

In the group of other small grain cereals and forage crops, triticale receives a very critical place because it has a large potential use for several purposes. Designed genotypes from "man-made" grain species the most important is a hybrid of wheat (*Triticum* spp.) and rye (*Secale cereale* L.) called triticale (*x Triticosecale* Wittmack) in agricultural production. Crop is well considered to be an interesting species that can be grown even in unfavorable stress conditions (limited water



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availability, acid soils, nutrient deficiency or toxicity and high disease pressure, etc.) or in marginal environments (Varughese et al., 1996; Liu et al., 2017). Winter varieties of high and solid stems, triticale is a very important fodder plant whose biomass is used in the feeding of ruminants as fresh, hay or silage. As a forage plant, triticale has a higher yield than other real cereals. Ruminants and non-ruminants easily digest and absorb triticale protein, as well as other grain cereals (Đekić et al., 2009).

Triticale has an important extensive root system, and can mine the soil more efficiently in conditions where fertility is poor, make it an ideal crop for managing fertilization. As it well known, optimum fertilizer plays a critical role in increasing yield and quality of plants. Compared to other mineral nutrients, N is required in relatively high quantities for model vegetative and reproductive growth in cereals (Eyupoglu, 1986). On the other hand nitrogen fertilizers are a major expense for producers, thus optimizing the use of fertilizers will decrease this cost. Therefore, the objectives of this study were to quantify the variation in forage yield of triticale across different N-fertilization levels in order to improve triticale production under agro ecological conditions of Izmir.

# **Materials and Methods**

This study was conducted as a pot study in outdoor conditions on the experimental area of Field Crops Department, Faculty of Agriculture, Ege University, Izmir, Turkey from October 2018 to June 2019 with typical Mediterranean climate characteristics. Some meteorological data from the experimental area in Bornova-Izmir and some characteristics of the experimental soil are presented in Table 1 and Table 2, respectively.

	2018-2019		Long Year Average	
	Temperature (°C)	Precipitation (mm)	Temperature (°C)	Precipitation (mm)
October	19.4	40.4	19.1	43.2
November	15.1	58.1	13.8	109.7
December	8.7	83.5	10.5	137.9
January	8.7	369.3	9.0	112.2
February	9.8	106.3	9.2	99.7
March	13.2	37.8	11.8	82.9
April	16.3	55.2	16.1	46.4
May	21.9	2.3	21.0	25.4
June	26.1	2.9	26.0	7.5
Total-Mean	15.4	755.8	15.1	664.9

Table 1. Some meteorological data of experimental area in Bornova, Izmir, Turkey in 2018-19



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Sand (%)	80.2	<b>CaCO3</b> (%)	0.82
Clay (%)	1.8	<b>Organic material (%)</b>	1.27
Silt (%)	18.0	Total N (%)	0.092
Texture	Loamy	P (ppm)	1.14
рН	5.83	K (ppm)	40
Total salt (%)	0.03	Ca (ppm)	1450

Table 2. Some characteristics of the experimental soil

Ege Yıldızı cultivar of triticale was used as crop material. The seeds were sown in a plastic pots filled with 17 kg loamy experimental soil on  $24^{\text{th}}$  October, 2018. Eight rates of nitrogen (0, 40, 80, 120, 160, 200, 240 and 280 kg·ha<sup>-1</sup> N) were used as randomized complete block design with four replications. Half a dose of nitrogen fertilizer (urea), 80 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> (triple superphosphate) and 100 kg·ha<sup>-1</sup> K<sub>2</sub>O (potassium sulphate) were applied with sowing, and the rest of nitrogen [(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>] was applied at the end of tillering stage. There were no problems with pests and diseases during the experiment. Weeds were manually removed from the pots; no herbicide was used to control weeds.

The plants were harvested when the triticale reached milk dough stage of seeds. Morphological characteristics (structure element of the yield) were measured: Plant height (cm); 5 plants were measured from the soil surface to the top level of the plant before harvest. Number of plant per pot; the plants in each pot was counted. Fresh herbage yield ( $g \cdot pot^{-1}$ ); after the harvesting of plants in pot, total above-ground biomass was measured. DM content (%); harvested fresh crops were weighed and dried to a constant weight at 105°C during 48 h and then was calculated (Bulgurlu and Ergül, 1978). DM yield ( $g \cdot pot^{-1}$ ); DM yield was calculated by multiplying the fresh herbage yield with the DM content. The obtained data were statistically processed by analysis of variance (ANOVA) with the Statistical Analysis System (SAS, 1998). If ANOVA indicated differences between treatment means, a LSD (Least Significant Difference) test (0.01) was performed to separate them (Stell et al., 1997).

#### **Results and Discussion**

Data presented in Table 3 shows that nitrogen levels had a significant effect on the all of tested characteristics in the experiment except DM content trait.



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N levels	Plant height (cm)	Number of plant per pot	Fresh herbage yield (g <sup>.</sup> pot <sup>-1</sup> )	DM content (%)	DM yield (g·pot <sup>-1</sup> )
NO	72.4 e	11.0 f	23.8 g	44.4	10.6 f
N40	84.9 d	12.8 f	58.8 f	41.0	24.1 e
N80	90.0 d	17.5 e	83.8 e	41.4	34.7 d
N120	99.9 bc	20.8 d	102.5 d	41.4	42.4 c
N160	106.6 ab	31.8 b	141.3 b	41.1	58.1 b
N200	111.7 a	37.0 a	158.8 a	40.5	64.3 a
N240	106.3 ab	29.0 c	119.5 c	40.0	47.8 c
N280	90.8 cd	20.8 d	112.5 cd	39.4	44.3 c
Mean	95.3	22.6	100.1	41.2	40.8
LSD (0.01)	9.3	2.6	10.7	ns	6.1

Table 3. Effect of different N levels on the forage yield and some yield characteristics of triticale

ns: not significant

N levels had significant effect on the plant height of triticale (Table 3). Numerically, the highest plant height (111.7 cm) was recorded at N200 level, whereas the lowest plant height (72.4 cm) was observed at N0 level. And also, there were no statistical difference among N160, N200 and N240. Graham et al. (1983) applied five levels of nitrogen fertilizer (0, 35, 70, 105 and 140 kg·ha<sup>-1</sup> N) on triticale plants. They stated that plant height increased with the application of up to 70 kg·ha<sup>-1</sup> N but greater amounts of N then this resulted in significant lodging in triticale. Gerdzhikova (2014) found that nitrogen application increases with 7.07% the stem height compared to no fertilization (control) with the height of stem average is 103 cm with variation from 78 (min) to 125 (max) cm. The application of nitrogen promotes plant taller because cell protein content and size increase on leaf area and photosynthesis rate rises (Wysocki et al., 2007).

Results presented in Table 3 showed that all N levels significant increased the number of plant per pot as compared with control (N0). N200 gave the highest number of plant per pot (37.0). And also, there was no statistical difference between N120 and N280. The lowest number of plant per pot (11.0) was measured in N0. Bielski et al. (2020) conducted an experiment with five different N levels (0, 40, 80, 120 and 160 kg  $\cdot$  ha<sup>-1</sup> N) on the triticale plants. Researchers found that the increased number of ears per square meter at high level of N application in their study (N0:427, N40:450, N80:472, N120:486 and N160:511). In another study by Takıl and Olgun (2020) significant differences in the number of spike per square meter (254, 322, 383, 393 and 356, respectively) were reported depending on the different N levels (0, 50, 100, 150 and 200 kg  $\cdot$  ha<sup>-1</sup> N, respectively) in triticale. The application of N fertilizer enhance the number of tiller, as it increases the cytokine content within tiller nodes and the germination of the tiller primordium (Liu et al., 2011).

ANOVA exposed significant effect of N application at different levels on fresh herbage yield of triticale (Table 3). Increasing N levels enhanced fresh herbage yield up to N200 level, but after that, the fresh herbage yield noticeably reduced. The maximum fresh herbage yield was recorded at fertilizer level of N200 (158.8 g·pot<sup>-1</sup>) whereas the lowest fresh herbage yield was recorded from N untreated pots (N0:23.8 g·pot<sup>-1</sup>). Buhedma et al. (2016) reported similar trend in increase biological yield due to N fertilization. In their study, three increasing N levels (80, 100 and 120 kg·ha<sup>-1</sup> N) led



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to significant enhanced in biological yield (N80:2.62, N100:3.85 and N120:4.02 t  $\cdot$  ha<sup>-1</sup>) on triticale plant. Another study in wheat (Ullah et al., 2018), a significant increase in the biological yield from dose of 0 kg  $\cdot$  ha<sup>-1</sup> N (10 kg m<sup>-2</sup>) noticed up to the nitrogen dose of 203 kg  $\cdot$  ha<sup>-1</sup> N (16.1 kg  $\cdot$  m<sup>-2</sup>). Physiologically the ability to more produce biological yield is the photosynthetic efficiency of the canopy, owing to more nitrogen taken up (Shewry, 2007). Our findings are in parallel with those results.

The results indicate that nitrogen application had not significant effect on DM content (Table 3). Numerically, N0 gave highest DM content (44.4%) but the lowest DM content (39.4%) was obtained in N280. Nitrogen fertilization increase cell volume and moisture content in this way the DM content of forage trend to decline (Capstaff and Miller, 2018). Some researchers (Gullap and Cerit, 2021) found that significant effect of different N levels on triticale with decreased DM content with increased N level (N0:41.5%, N40:41.3%, N80:41.98% and N120:40.45%). On the contrary these results, Yun and Ataku (1998) were reported that significant effect of different N level (N120:33.9%, N160:35.3% and N200:36.2%). Our findings are not totally parallel with those results.

There were statistically significant differences in DM yield among the different nitrogen levels (Table 3). The highest DM yield ( $64.3 \text{ g} \cdot \text{pot}^{-1}$ ) was recorded in N200 level whereas the lowest DM yield ( $10.6 \text{ g} \cdot \text{pot}^{-1}$ ) was recorded in N0 level in our study. Moreover, there were no statistical differences among N120, N240 and N280 levels. McDonald (2002) supported that during pollination high levels of N raised the total DM that helps to get more forage yield. Cazzato et al. (2011) indicated that total DM yield increased significantly with 100 kg·ha<sup>-1</sup> nitrogen fertilization ( $10.7 \text{ t} \cdot \text{ha}^{-1}$ ) compared to the control ( $0 \text{ kg} \cdot \text{ha}^{-1} \text{ N}$ ) supplied ( $5.8 \text{ t} \cdot \text{ha}^{-1}$ ) treatment on triticale plants. In another study by You et al. (2020) not significant differences in the hay yield of triticale were reported depending on the different N levels (0, 60, 120, 180, 240 and 300 kg·ha<sup>-1</sup> N). Our findings are not totally accordance with those results.

#### Conclusions

Using the results of this research of the effect of different nitrogen fertilization of triticale forage yield and some yield components, the following conclusions can be drawn:

These results were obtained from the pot trial conducted on loamy type of soil using triticale Ege Yıldızı cv., application of the higher rates of N levels increased the above-mentioned traits compared to the control treatment, except DM content.

All traits amount increased with the application of up to 200 kg ha<sup>-1</sup> N, but greater amounts of N then resulted in significant decreasing in mentioned traits of triticale.

It can be recommended from the experimental traits of triticale grown at the Mediterranean ecological conditions that maximizing forage yield could be obtained by adding 200 kg N per hectare. However, field experiments in the coastal part of the Aegean region under Mediterranean climate still need to confirm practical recommendations to producers.



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# ASSESSMENT OF SOIL EROSION INTENSITY USING EROSION POTENTIAL METHOD: CASE STUDY – BRKA RIVER BASIN

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# Abstract

The aim of this study is to assess the intensity of erosion processes (Z) in the Brka River Basin (Bosnia and Herzegovina). At the same time, the paper takes into account the current conditions of climate, soil, hydrology, using modern hardware and software solutions. The method being used is erosion potential method (EPM) or Gavrilović's method supported by GIS techniques. Using EPM, the erosion intensity (Z) was obtained, as the result of the empirical equation that combines numerical values for soil resistance to erosion (Y), protection of soil from atmospheric influence (X), erosion type ( $\phi$ ) and slope ( $\sqrt{Jsr}$ ). The Brka River Basin covers an area of 184 km<sup>2</sup>. Therefore, for a precise observation of the basic characteristics of the basin, as well as for a more accurate calculation of the erosion intensity (Z), in this paper the Brka basin area is divided into 5 following sub-basins: the Rašljanska river (27.9 km<sup>2</sup>), the Maoča river (23.5 km<sup>2</sup>), the Rahić river (24.2 km<sup>2</sup>), the Zovičica (75.3 km<sup>2</sup>), and the immediate Brka basin (32.9 km<sup>2</sup>). The highest erosion intensity is in the central part of the basin (Zovičica sub-basin) in the area around Štrepci, Gornji Rahić, Šatorović, Gornji and Donji Zovik. Based on the categories of erosion, 17.40% of the territory is affected by excessive erosion, and 7.23% of the area is without erosion. Intense erosion covers 6.70% of the basin, medium erosion 7.20%, light erosion 12.70% while very light erosion present in 48.75% of the Brka river basin. The erosion process in the Brka basin has a medium erosion intensity, with an average erosion coefficient of Z = 0.47.

Keywords: Gavrilović Method; EMP, GIS, Erosion Intensity (Z); Brka River Basin

# Introduction

Soil erosion is still a global problem that causes damage to the environment, thus affecting the economy of human communities. American professor Hugh Bennett, known in professional circles as the "father of land conservation", said: "Erosion is one of the most complex problems and also one of the most insidious enemies of the human economy." Recently, erosion has become a rising problem when it comes to environmental protection and biodiversity in the Balkans (Spalević et al., 2015). Bosnia and Herzegovina is very sensitive to the destructive processes of erosion and flooding, especially in the northern parts of the country (Čadro et al., 2019; O. Žurovec et al., 2017). The quantities of stripped material affect agricultural producers throughout Bosnia and Herzegovina. As much as 83% of the total area of BiH is endangered by water erosion (Lazarević, 1985). In addition



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to local research at the plot level (Žurovec and Čadro, 2008), Gavrilović's method was also used to analyze erosion on larger areas. The erosion map of SR Bosnia and Herzegovina was made in the period 1980 - 1985. (Lazarević, 1985), later in 2012 the erosion map of the Bosnian-Herzegovinian entity Republika Srpska was made (Radislav Tošić et al., 2012) at a scale of 1: 25,000, and in 2018 the erosion map of the Vrbas River Basin at a scale of 1: 25,000 (Lovrić and Tošić, 2018). Aside from that, the map of erosion of the Brka river basin was made in 2020 (Kaloper et al., 2020). Authors used a simple erosion potential method modified and adjusted by Lazarević (1985) and all required erosion coefficients (Y, X,  $\varphi$  and slope) were generalized for larger areas.

Based on this research the intensity of the erosion process in the Brka basin has a medium erosion character, with an average erosion coefficient of Z = 0.46. Excessive erosion takes up 16.68%, intensive 7.24%, and 7.72% is without erosion (Kaloper et al., 2020). Apart from this, no serious research related to soil erosion has been conducted in the Brka River Basin since the end of the last war in BiH (1992 - 1995).

# **Materials and Methods**

#### Analyzed area and data collection

The Brka River Basin, located in the northeastern part of Bosnia and Herzegovina, encompasses the northern slopes of Mount Majevica and part of Bosnian Posavina. Figure 1 shows the Brka River Basin in relation to the Brčko District and Bosnia and Herzegovina. It covers an area of 184 km<sup>2</sup> and is divided into five sub-basins (Figure 2).

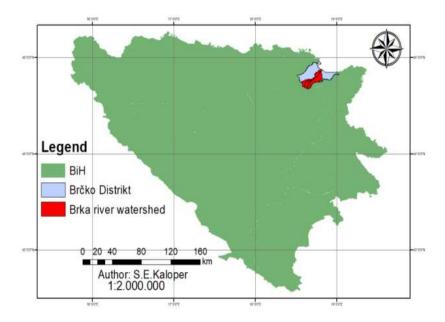


Figure 1. Position of the Brka river basin in relation to BiH

The surface of the Rašljanska river sub-basin is 27.9 km<sup>2</sup>, the Maočka river 23.5 km<sup>2</sup>, the Rahić river 24.2 km<sup>2</sup>, the Zovičica river 75.3 km<sup>2</sup>, and the Brka river itself 32.9 km<sup>2</sup>. The highest point is Okresanica, which is located at 815 meters above sea level, while the lowest point is the delta of the



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river Brka at 84 meters above sea level. Most of the basin area is located within the Brčko District, and a smaller part is located to the south towards Federation of Bosnia and Herzegovina (FBiH) entity, i.e. the municipalities of Srebrenik and Čelić (Figure 3).



Figure 2 and 3. Basin, sub-basins and administrative map of the Brka river

# Erosion intensity calculation method

The erosion potential (EMP) method, also known as the Gavrilović's method (Gavrilović, 1972), modified according to Lazarević (1985) and adapted for use in the GIS (geographic information system) (Dragičević et al., 2013), was used to estimate erosion intensity. EMP was used to map and calculate erosion intensity (Z). This method has been used for more than 40 years in Bosnia and Herzegovina, but also in the countries of the region (Serbia, Montenegro, Croatia, Slovenia, North Macedonia) and around the world (Italy, Iran, Iraq, Chile, etc.). Soil erosion coefficient or erosion intensity (Z) was calculated by the analytical method through the equation:

$$Z = Y \times X \times (\phi + \sqrt{Jsr})$$

Where:

Y - the coefficient of soil erodibility (soil resistance to erosion)

X - the coefficient of vegetation protection (protection of soil from atmospheric influence)

 $\boldsymbol{\phi}$  - erosion type coefficient

 $\sqrt{Jsr}$  - the average slope in percentages

Quantitative values of the erosion coefficient (Z) were used to group the erosion intensity in 5 classes according to Lazarević (1985):

Type of erosion	Class	Ζ
Excessive erosion	Ι	>1.00
Intense erosion	II	0.71 - 1.00
Medium erosion	III	0.41 - 0.70
Mild erosion	IV	0.21 - 0.40
Very mild erosion	V	0.01 - 0.20

Table 1. Types of erosion intensity according to Lazarević (1985)



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The boundary of the catchment area was determined using a digital terrain model (DEM: 25 m x 25 m) and a map of the hydrographic network of BiH; the soil protection coefficient (X) was determined using land cover map CORINE 2018 (100 m x 100 m) based on the proposed values according to Lazarević (1985), Globevnik et al. (2003) and Ali Saeedu (2016).

Soil erodibility (Y) was based on Pedological Map of BiH (1:50,000). Reconnaissance of erosion processes in the areas of the Brka River basin was made by field trips in the period from March to July 2020. Open-source satellite imagery was used to specifically select points and determine the type and degree of erosion ( $\phi$ ). The field part was preceded by marking potential soil erosion points on orthophotos. Field trips and determination of the ( $\phi$ ) coefficient were performed from August 31st to September the 4th, 2020 using GPS devices (Garmin, eTrex Touch 35), while an unmanned aerial vehicle: UAV (DJI Phantom 4 pro) was used for inaccessible locations. The coefficient ( $\phi$ ) was determined at the spot using values given by the Gavrilović method.

Esri® ArcGIS 10.2.1 software was used to determine the average slope in percentages ( $\sqrt{Jsr}$ ), while the GIS tool raster calculator was used to create the Z map.

#### **Results and Discussion**

The altitude of the Brka river basin in the lowland or north part ranges from 84 to 210 meters above sea level, when it gradually overflows into the hilly-mountainous region of the Majevica mountain to the south with altitude up to 815 meters above sea level (Figure 4).

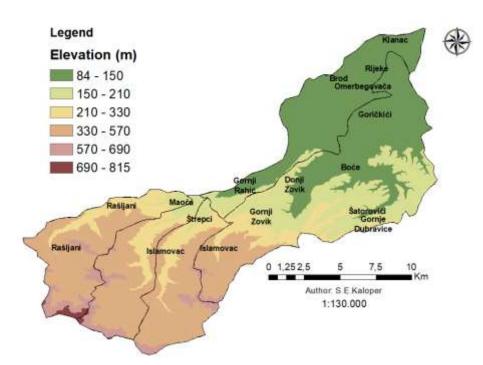


Figure 4. Altitude of the Brka river basin



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The part of the basin that extends from 84 to 150 meters above sea level occupies the largest area of 61.03 km<sup>2</sup> or about 33.29% of the basin. Second to that, is an area of 32.80 km<sup>2</sup>, ie about 17.89%, that is 150 to 210 meters above sea level. Next is an area at 210 - 330 m above sea level which takes up 12.66% of the basin (23.21 km<sup>2</sup>), and an area of 330 to 570 m above sea level which takes up about 31.58% (57, 89 km<sup>2</sup>). That makes a total of 95.42% of the Brka river basin area. The rest, about 4.58% of the Brka basin, covers the highest points of the area on the Majevica mountain itself.

In the upper part of the Brka river basin on the Majevica mountain or south side of the basin, watercourses abound in a larger amount of larger sediment (fragments of rock material, large pebbles, etc.). Moving towards the middle and rear part of the basin in watercourses mainly finer eroded material (gravel, sand, parchment etc.) can be found. On the river terraces of the river Brka there are several villages, Gornji Rahić, part of Palanka, Brka, as well as part of the town of Brčko.

The largest number of watercourses is located in the upper part of the basin. In the upper part of the Brka river basin, numerous streams are visible, as well as cuts caused by negative anthropogenic impact such as heavy rains and intensive snow melting. There are clearly visible traces of neglected forest roads, some of which are very likely from the period of "Karavlah" deforestation, when these areas were inhabited by the minority people of Karavlah, whose basis of "rural" economy was deforestation and primitive wood processing. Almost all roads in the upper part of the Brka river basin are in a rather poor condition, and in certain sections, are not paved at all. The petrographic structure of the Brka river basin, in the upper part around the flows of the Rašljanska, Maočka and Islamovačka rivers, is mainly represented by clays, sandstones, marls, shales, serpentines, limestones and various carbonate debris, gravels and sands. The lowland part of the basin is covered mainly by accumulations of clay minerals that form pseudogleyic soils, with some minority regions where clays, sandstones and shales can be found.

#### Hydrography and water resources

The Brka river basin flows into the Sava River, which is a part of largen Danube River basin and the Black Sea basin (Figure 5).



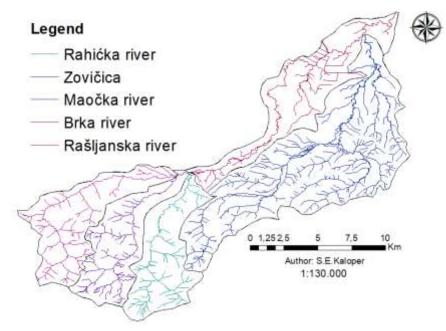


Figure 5. Hydrological map of the Brka river basin with sub-basins

The total length of all watercourses is 446.54 km, and the river Brka has the longest watercourse which is 26.5 km long (Table 2).

Name of	F	0	Max.A	Min.A	D	lp	la	L	Dd
the basin	km <sup>2</sup>	km <sup>2</sup>	m	m	km	km	km	km	km/k m²
Rašljanska river	27.99	30.764	815.0	160.0	0.655	16.461	40.712	57.172	2.043
Zovičica	75.31	53.527	698.9	88.2	0.611	24.391	185.087	209.478	2.782
Brka directy	32.95	44.341	270.0	84.8	0.185	26.575	48.456	75.030	2.277
Rahićka river	24.27	28.416	699.6	153.8	0.546	13.938	37.970	51.908	2.139
Maočka river	23.58	29.058	799.7	153.6	0.646	12.419	40.539	52.957	2.246
Brka	184.1	77.389	815.0	84.8	0.730	26.575	419.971	446.545	2.426

Table 2. Hydrological and spatial characteristics by sub-basins

Note: F - Area; O - Perimeter basin, Max.A - Maximum altitude; Min.A - Minimum altitude; D - Average height difference of basin; lp - Length of main watercourse; la - Length of secondary watercourses; L - Total length of all watercourses; Dd - River network density

The total perimeter of the basin is 77.38 km<sup>2</sup>, the largest being near the Zovičica river basin, (53.52 km<sup>2</sup>). The maximum altitude is 815 meters and is located in the basin of the Rašljanska river on Majevica, while the lowest point is at the confluence of the rivers Brka and Sava and is 84 meters above sea level. In addition to Brka, which represents the longest watercourse, the river Zovičica follows with 24.3 km than Rašljanska river 16.4 km, Rahićka river 13.9 km and Maočka river 12.4 km. The highest density of all watercourses is in Zovičica (209.4 km), and the lowest in Rahićka



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River (51.9 km). Brka, as the largest watercourse, originates in the village of Maoča by the confluence of the Maočka River and the Rašljanska River, and not far from the junction, the Rahićka River flows into it.

# **Pedological characteristics**

The Brka River Basin area is covered by different types of soil. According to the national soil classification of Bosnia and Herzegovina, eight different soil types can be found (Figure 6).

These are district cambisols, eugleys, eutric cambisols, fluvisols, humofluvisols, luvisols, pseudogleys and very few rendzinas. In the area of the upper – south part of the Brka basin, the hilly/mountainous part, there are mainly district cambisols and luvisols. In smaller areas, along the flows of smaller tributaries, there are eutric cambisols and humofluvisols, and the share of eugley is small. District cambisols in the Brka River Basin are mainly characterized by lithochromatogenous red / purple soils and brown soils on clays and sandstones. Luvisoli, are characterized by brown degraded soils on clays and marls. Eutric cambisols are manifested as brown soils on shales and serpentines, brown carbonate soils on carbonate debris and limestone, brown carbonate / non-carbonate soils on marls, and brown non-carbonate soils on gravel or sand. Humofluvisols are present mainly on gray-brown swampy and meadow (charcoal) soils. The lowland (plain - north) part of the Brka river basin is covered mainly by pseudogleys, followed by humofluvisols and district cambisols, and a smaller part by luvisols, eutric cambisols and fluvisols. Pesudogleys are represented by podzolic pseudogley terraced and valley soils, and fluvisols by alluvial carbonate soils on loams and sands.

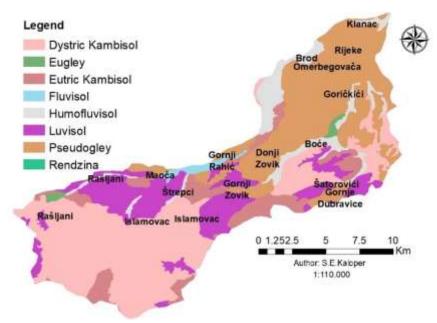


Figure 6. Pedological map of the Brka river basin



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The most common soil type in the Brka River Basin are the District cambisols, which are covering 68.1 km<sup>2</sup>, or 37% of the basin (Table 3). In Bosnia and Herzegovina, they belong to the three most common soil types (Tvica and Čustović, 2010).

Soil type	Area (km²)	Percentage of total basin land (%)
Distic cambisols	68.1	37.0
Eutric cambisols	17.4	9.4
Eugley	1.2	0.6
Fluvisol	1.9	1.0
Humofluvisol	13.0	7.0
Luvisol	29.7	16.1
Pseudogley	52.5	28.5
Rendzina	0.003	0.001

The second most represented soil is Pseudogley (52.5 km<sup>2</sup>) that takes up 28.5% of the basin. These are followed by Luvisoli (29.7 km<sup>2</sup>) at 16.1%, Eutric cambisoli (17.4 km<sup>2</sup>) 9.4%, and Humofluvisoli (13 km<sup>2</sup>) 7%. The smallest areas are consumed by Fluvisoli (1.9 km<sup>2</sup>) or 1% of the basin, Eugley (1.2 km<sup>2</sup>) ie 0.6% and a negligible amount of Rendzina of 0.001%.

District cambisols are also called brown forest soils. Distributed in the areas of Majevica around Rašljani, Maoča, Gornja Maoča, Islamovac, Štrepci, but we can also find them around the villages of Šatorović, Donje Dubravice and Boderište. Eutric cambisols are essentially brown soils with over 50% bases. Smaller areas are located around Palanka along the river Brka, in Rašljani and Štrepci, around Velina Sela and Repina Brda (Šatorovići). Euglej can be found between Boće and Boderište along the river Zovičica and around Gornji Rašljan along the Rašljanska river. Fluvisol is located around Gornji Rahić along the river Brka and along smaller tributaries. Humofluvisols are located in the urban part of the town of Brčko, the settlements of Brod and Brka, along the river Brka and, furthermore, around Omerbegovača and Boderište all the way to Zovik along the river Zovičica. Smaller areas are represented along the Rašljanska, Maočka and Rahićka rivers. Luvisols are located in the area of Šatorović, Gornji Zovik, Maoča and part of Rašljan. Pseudogleys cover an area of 52.5 km<sup>2</sup>. These are heavy soils primarily used for agricultural production, but most of the urban part of the city of Brćko and the area of Omerbegovača, Brka, Boća, parts of Boderište, Palanka, Donji Zovik, Gornji Rahić and smaller parts on the "edges" of Šatorović and Maoča is settled on it as well.

#### Land use

Using the European land cover map - CORINE (CLC, 2018), it was determined that 89.2 km<sup>2</sup> (48.5%) of the basin area is under deciduous forests (Figure 7). About 0.4 km<sup>2</sup> (0.2%) are covered by mixed forests, and 1.9 km<sup>2</sup> (1%) by shrubs. The most represented forest crops are oak (medunac and sessile oak), hornbeam, poplar, maple, beech, linden and willow. Mixed agricultural areas cover about 42.8 km<sup>2</sup> (23.2%), areas of mostly agricultural production cover about 17.1 km<sup>2</sup> (9.3%), and



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apple and berry fruits cover about 2.7 km<sup>2</sup> (1.4 %). Plums, cherries, sour cherries, apples, pears, walnuts, peaches, quinces, etc. mainly grow in this area. Urban areas cover about 9.9 km<sup>2</sup> (5.3%) and 0.2 km<sup>2</sup> (0.1%) are industrial areas (Table 4).

The largest forest areas are located in the southern part of the basin around Rašljani, Gornja Maoča and Islamovac. The central part of the basin is occupied by agricultural areas where intensive production takes place. Orchards are distributed over smaller areas, mostly along the entire basin.

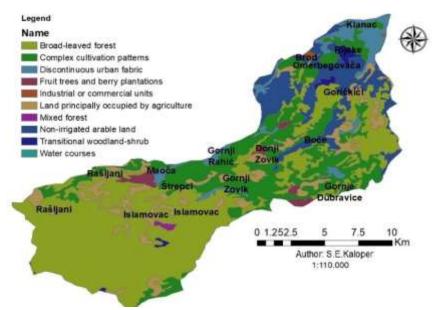


Figure 7. How to use the Brka river basin (According to: CLC2018)

In this area, there are also the highest quality pedunculate oak forests (Bosanski Šamac, Bosanska Gradiška, Brčko and Bijeljina, about 14,000 ha) (Bašić et al., 2007; Ballian and Memišević-Hodžić, 2016). In the past, floodplain pedunculate oak forests occupied large areas in the Sava River area, and occurred disjunctively. Today they are often alternated with pedunculate and hornbeam forests, and we find them within agricultural areas (Ballian and Memišević-Hodžić, 2016).

Types of soil use	km <sup>2</sup>	Percentage (%)
Urban (built) areas	9.9	5.3
Industrial areas	0.2	0.1
Field crops without irrigation	19.5	10.6
Apples and berries	2.7	1.4
Mixed agricultural areas	42.8	23.2
Areas mostly under agriculture	17.1	9.3
Deciduous forests	89.2	48.5
Mixed forests	0.4	0.2
Shrubby vegetation	1.9	1.0
Waterways	0.03	0.01

Table 4. T	ypes of	land and	surface use
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On the cultivated agricultural soil, farmers grow mainly cereals (wheat, barley, oats, corn...), industrial plants (soybeans, oilseed rape, medicinal and aromatic plants, sunflower...), vegetable crops (potatoes, carrots, onions, garlic, beans, peas, cabbage, tomatoes, peppers, cucumbers, melons, watermelons, etc.), fodder plants (clover, alfalfa, fodder corn, grass clover mixtures, etc.), berry fruits (strawberries, raspberries, blackberries), apple fruits (apple, pear, quince) and stone fruits (plum, cherry, sour cherry).

# Soil erodibility coefficient -Y

Soil erodibility coefficient (Figure 8) was determined on the basis of the Pedological Map of BiH (1: 50.000). Appropriate values have been assigned to different soil classes based on lazarević methodology (1985). The values of the soil erodibility coefficient in District cambisols range from 0.2 to 0.5. Eutric cambisols from 0.2 to 0.6. Fluvisols have a value of 0.2, Humofluvisols from 0 to 0.2, Luvisols from 0.4 to 1, Pseudogleys from 0.5 to 1.1, Rendzina 1.2, while Eugleys have a value of 0 since they are always on completely flat surfaces.

Higher values of soil erodibility coefficient (Y) are located in the lower (lowland) and central part of the Brka river basin. The most resistant soils are located in the upper part of the basin, but also along the rivers Brka and Zovičica, ie in flat areas.

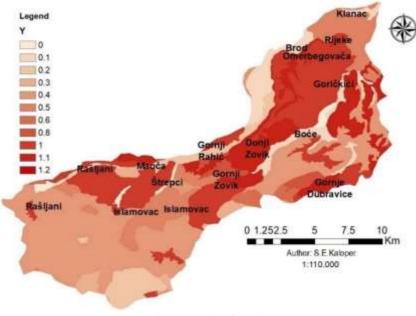


Figure 8. Y basin value

# Soil protection coefficient - X

Soil protection coefficient X (Figure 9) was determined using a map of land cover of European countries (CORINE, 2018, 100 m x 100 m) based on X values selected by comparing the proposed values according to Lazarević (1985), Globevnik et al. (2003) and Ali Saeedu (2016). The values are given in Table 5.



Types of soil use	km <sup>2</sup>	X values
Urban (built) areas	9.90	0
Industrial areas	0.20	0
Field crops without irrigation	19.50	0.90
Apples and berries	2.70	0.70
Mixed agricultural areas	42.80	0.40
Areas mostly under agriculture	17.10	0.40
Deciduous forests	89.20	0.05
Mixed forests	0.40	0.05
Shrubby vegetation	1.90	0.50
Waterways	0.03	0

Table 5. Land use and X values

The values of the coefficient X in the area of the Brka river basin range from 0 to 0.9 (Figure 9).

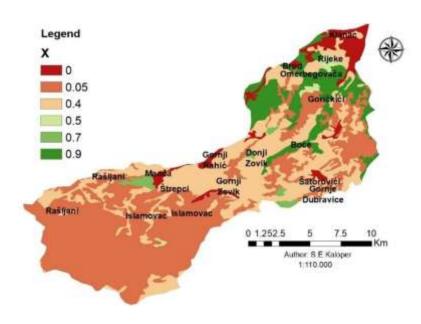


Figure 9. X basin value

In the basin area, for the agriculture land is used mostly in the lowland part around Omerbegovača, Brod, Brka, Boća (around the river Zovičica) and in the central part around Šatorović, Donji Zovik and Maoča. The upper part of the basin, as well as the industrial and urban zones, are generally represented by very low X values.

#### Determination of the coefficient of visible and clearly expressed erosion processes ( $\boldsymbol{\Phi}$ )

Open-source satellite imagery was used to determine the value of the  $(\phi)$  coefficient and 29 sites in the Brka River Basin were visited. At most of the sites, terrain was reconnoitered, except for a



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couple of sites where, due to the impossibility of access, the inspection was performed with the help of an unmanned aerial vehicle / drone (DJI Phantom 4 pro). Each locality was assigned an appropriate value.

Certain areas in the part of the basin where the slope is less than 2% are often affected by floods and high groundwater, as a result of which the water carries particles from the soil surface. Examples are most often seen on corn and soybean plots along the Brka River and its tributaries. At higher altitudes of the Brka river basin, an interesting phenomenon related to the cultivation of fruit crops is noticeable, especially in Šatorovići and Zovik. It is often the case that the orchards were not contoured, but were cultivated down the slope. This unconsciously "stimulates" soil erosion, which leads to the deterioration of the physical, chemical and mechanical properties of the soil. The area of the Brka river basin is also characterized by the presence of several open pits, some of which have been inactive for several years. They are located around Štrepci and Rašljani. Since these are permanently destroyed areas with constant erosion processes, they were assigned the highest value  $(\phi)$ .

The values of the  $\phi$  coefficient in the area of the Brka river basin in the localities from the terrain range from 0.1 to 1 (Figure 10).

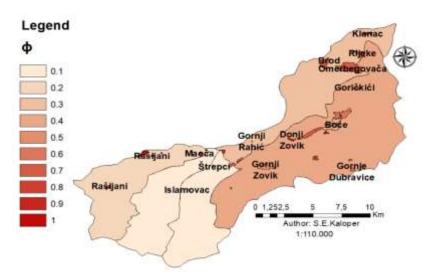


Figure 10. Values  $\phi$  of the coefficient in the area of the Brka river basin

The sub-basins of the Rahićka and Maočka rivers have the lowest values, followed by the Rašljanska and the immediate Brka river basin. The Zovičica sub-basin has the highest value.

#### Actual erosion intensity (Z)

Once all the necessary maps for the calculation and spatial representation of the erosion intensity (Z) have been obtained, the next step was to use a raster calculator tool to calculate and create an erosion intensity map (Z) of the Brka River Basin, as shown in Figure 11.



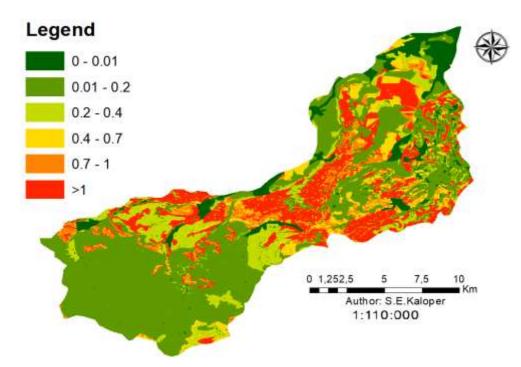


Figure 11. Erosion intensity (Z) in the Brka river basin area

The spatial distribution of erosion intensity shows the highest erosion intensity in the central part of the basin in the area around Štrepci, Gornji Rahić, Šatorovići, Gornji and Donji Zovik. Although the upper part of the basin has a higher slope of the terrain and it would be expected that the area is greatly affected, most of that area is covered with forests, which largely protects the soil from erosion. This is not the case in the central and lower part of the basin, which are indeed characterized by smaller slopes, but where intensive agricultural production is carried out on soils with poor physical water characteristics. This essentially means that soil characteristics and land use have a influence on the intensity of erosion processes dominant in the Brka basin Based on the categories of erosion, 17.40% of the territory is affected by excessive erosion, and 7.23% of the area is without erosion (Table 6).

Erosion category Erosion intensity range		Basin area (km <sup>2</sup> )	Percentage of basin (%)	
No erosion	0-0.01	13.30	7.23	
Very weak	0.01 - 0.2	89.66	48.75	
Weak	0.2 - 0.4	23.36	12.70	
Medium	0.4 - 0.7	13.24	7.20	
Intense	0.7 - 1	12.33	6.70	
Excessive	>1	32.00	17.40	

Table 6: Categories of soil erosion in the Brka River Basin

Intense erosion covers 6.70% of the basin, medium erosion 7.20%, weak erosion 12.70% while very weak erosion is present in 48.75% of the Brka river basin.



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According to the results, the intensity of the erosion process in the Brka basin has a medium erosion character, with an average erosion coefficient of Z = 0.47 (Table 7). The most intense erosion occurs in the sub-basin area of the river Zovicica (Z = 0.59), while the sub-basins of the Maočka and Rahićka rivers, located in the southern part of the Brka basin, which with higher altitude and forest vegetation, have much lower values (Z = 0.30 - 0.32).

Basin name	Area	Erosion intensity	
Dasin name	F (km <sup>2</sup> )	Z	
Rašljanska river	27.99	0.45	
Zovičica	75.31	0.59	
Brka directly	32.95	0.45	
Rahićka river	24.27	0.30	
Maočka river	23.58	0.32	
Brka	184.10	0.47	

Table 7. Erosion intensity (Z)

To compare, the average value (Z) for the Vrbas basin is significantly lower Z = 0.18 (Lovrić and Tošić, 2018), as well as for most of the other basins in the BiH entity of RS: Bosnia Z = 0.20; Drina = 0.45; Sana = 0.15 (Radislav Tošić et al., 2012). In the Ekbatan Dam basin (Iran), which is approximately the same size (218 km<sup>2</sup>), and has significantly higher altitude with a maximum of 3580 m above sea level, the average erosion coefficient is Z = 0.76 (Amini et al., 2010); in the Vranje-Banjska basin (150 km<sup>2</sup>) Z = 0.50 (Kostandinov, 2008). This indicates pronounced erosion processes in the Brka basin. This is especially true for the Zovičica river basin (Z = 0.59) and the Brka river basin (Z = 0.45). This is probably the result of high soil erodibility (pseudogley and luvisol) which are mainly actively used for agricultural production.

# Conclusion

The average Z value, that is the average erosion intensity of 0.47, and 44% of the territory threatened by water erosion, of which 17.40% of the area is affected by excessive erosion indicates the fact that in the Brka basin certain soil protection measures are more than necessary. The upper part of the basin is covered with forest vegetation and therefore is well protected from erosion processes. This is especially true of the Maočka and Rahićka river sub-basins where mainly smaller areas are affected. These are actively cultivated in the immediate vicinity of watercourses and populated areas (Rašljani, Maoča and Štrepci). In such conditions, it is necessary to pay attention to the surfaces that are processed on the steepest slopes and to legally disable processing. Use these areas exclusively as meadows and pastures or for similar purposes. Give attention to illegal deforestation and rehabilitation of torrents, but also local roads that are constantly eroded due to heavy rainfall.

Most of the agricultural production in this basin takes place in the central part of the basin, mainly on pseudogleys and luvisols, that is soils with poor water-physical characteristics. Since land use is a significant factor that people can control to some extent, it is necessary to act primarily in the



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direction of changing the way of use. Changing arable land by a combination of fruit and field production could help reduce the territory threatened by water erosion. In addition to this measure, it is necessary to approach this problem with a whole set of measures to achieve even better results.

Apart from the above, plots that are actively used for the purpose of agricultural production should not be left bare – unsown. Applying such a crop rotation that allows the presence of vegetation cover as long as possible, in orchards and crops with a wide range of grass. This would reduce the impact force of the raindrops to a minimum. Special attention should be paid to the length of the plots located on the slopes. Contouring and sowing / planting are recommended whenever the size and shape of the plot allow, especially in the central part of the basin. Broad-rooted crops should be avoided. The most endangered areas should be approached with measures of restoration and remediation of land for the purpose of soil remediation.

In order to repair the properties of the soil, especially pseudogley, terrain rigging with potential drainage of excess groundwater should be applied, increasing soil roughness to slow down runoff and generally repair the physical and chemical properties.

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# EFFECTS OF SOME BIOLOGICAL FOLIAR APPLICATIONS ON FRUIT CHARACTERISTICS OF 'CHANDLER' WALNUT VARIETY

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# Abstract

In Turkey, the orchards are being established with the 'Chandler' walnut variety in different ecologies, nowadays. In the current study carried out in Demirci/Manisa district, it was aimed to determine the effects of some biological foliar applications (effective microorganism (EM.5), vermicompost, amino acid, seaweed, stimulant and control) in two different periods on some fruit characteristics in this variety. Consequently, amino acid application took the first place for nut weight (12.68 g). All foliar applications had positive effect on shell thickness. Among the treatments, the effect was not determined in terms of fruit sizes, but color parameters were changed based on applications. Thus, L\*, a\*, h<sup>0</sup> and C\* values decreased, b\* values increased. In general, amino acid application was effective for the investigated properties.

Keyword: Juglans regia, Foliar Spray, Biological Applications, Fruit Characteristics

# Introduction

Walnut, which has a high nutritive value, is one of the most important nut fruit species. Depending on the awareness of healthy nutrition, the interest in this species has increased today.

In Anatolia, which is the origin center and natural spread of many fruit species, walnut is widely cultivated (Akça et al., 2014). Turkey is ranked the fourth place with 225.000 tons of walnut production in the world (Anonymous, 2021). The orchards are being established with the 'Chandler' walnut variety in different ecologies.

Recently, despite the high demand, there has not been a significant increase in both the yield per tree and the amount of production, and even serious problems have been observed (Oguz et al., 2016). It is noteworthy that there are a limited number of studies on practices aimed at increasing the quality and yield of varieties in Turkey. As known, since plant nutrient deficiency causes low yield and quality, appropriate fertilization applications can be effective in perennial species such as walnuts. (Şen, 1986). However the intensive use of fertilizers to meet the food needs of the increasing population negatively affects human health and causes significant damage to the ecosystem. Because productivity depends on adequate intake of nutrients by plants (Zaman et al.,



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2014). In this context, environmentally friendly agricultural practices are widely used in terms of sustainability.

Beneficial microorganisms, known as biological fertilizers against chemical fertilizers, have gained importance in recent years (Dede, 2013; Welbaum et al., 2004). They have effective use for agricultural purposes. Biologically active substances that have an effect on growth and development are produced by these microorganisms. Effective microorganisms are not a single species, but consist of a mixture of different groups of microorganisms (Higa and Parr, 1994). This group includes photosynthetic and lactic acid bacteria, yeasts, fungi and effective enzymes.

Vermicompost, which is an organic fertilizer, is obtained as a result of passing food wastes through the digestive systems of worms. It is important because it contains nutrients in dissolved and usable form by plants. Thanks to vermicompost, resistant to stress factors and high quality products can be obtained (Abacıoğlu et al., 2020).

Another environmentally friendly application is amino acid applications. The amino acid used in agricultural practices increases plant growth, blooming, yield and quality as well as the resistance of plants to adverse climate and soil factors (Anonymous, 2020a).

Another similar application, seaweed, enhancing photosynthesis capacity, resulting in high fruit set. In this way, an increase is observed in the production of sugar, protein and organic acids necessary for plants. Quality production occurs due to healthy plant development (Bender and Şen, 2017).

Stimulants are widely used in agriculture. Stimulants activate the biochemical and physiological resources of plants. It is easily absorbed by the plant and accelerates cell sap movements. It has a positive effect on the germination, rooting, development and maturation. Especially significant effect on fertilization is also reported (Anonymous, 2020b).

In perennial plants such as fruit trees, the effects of all these applications were revealed on vegetative development, fruit characteristics, yield, nutrient content and disease resistance. Considering the explanations above, in the current study, it was aimed to determine the effects of some biological foliar applications on some fruit properties of 'Chandler' walnut variety in Manisa/Demirci region.

#### **Material and Methods**

The current study was conducted in Manisa/Demirci location (39°02'39"N 28°35'56"E, altitude 740 m) in 2019 year. Chandler walnut variety (7-years-old) was used as plant material. This variety is foliar and blooms in the late period. Fruits ripening in the middle season are large, oval and smooth. The shell breaks more quickly and easily (Özçağıran et al., 2014). For biological applications effective microorganism (EM.5 300 ml/10lt), vermicompost (50 ml/10lt), amino acid (120 cc/da), seaweed (100 gr/da) and stimulant (10 ml/10lt) as foliar sprays were treated to plant material. The applications were carried out 2 times such as after the blooming male flowers and hazelnut-sized fruits for all applications. The content of the applied fertilizers is given below (Table 1).



Fertilizers	Content				
Effective microorganisms	Lactic acid bacteria (Lactobacillus delbrueckii, Lactobacillus plantarum, Lactobacillus rhamnous, Lactobacillus casei), Yeast (Saccharomyces cerevisiae) Phototrophic bacteria (Rhodopseudomonas palustris)				
Amino acidTotal Organic Matter 20% Organic Carbon (C) 9% Organic Nitrogen (N) 3% Water Soluble Potassium Oxide (K2O) 3% Free Amino Acids 14% Ph 3-5					
Seaweed	Organic matter 453% Alganic Acid 2.50% Dissolved in water (K <sub>2</sub> O) 15% Ph 7-9 EC(dS/m) 24,4				
Stimulant	1 g/L Sodium 5-nitroguaiacolate 2 g/L Sodium orto-nitrophenolate 3 g/L Sodium para-nitrophenolate				

Table 1. Microbial fertilizers and their contents.

The harvested fruits were separated from green peels and dried in the shade. Average nut and kernel weight were determined on precision electronic scale (0.01 g) then the kernel ratio (%) was calculated. The widths, height, length of the nut and shell thickness were state dusing a digital caliper sensitive to 0.01 mm (Şen, 1980). Fruit color was measured by a CR400 model Minolta Colorimeter in CIE L\* a\* b\*, hue (hue° = tan–1 (b\* / a\*)) and croma (C\* = (a\*2 + b\*2)  $\frac{1}{2}$ ).

The experiment was carried out according to the design of the random blocks, with 3 replications and 3 trees per replication. 10 samples were evaluated for each replication. The data were subjected to analysis of variance using SPSS 20 statistical package program. Significant differences between averages were defined by Duncan test at the P<0.05 significant level.

#### Results

The data on some fruit parameter are given in Table 2. The statistical differences were not detected in fruit sizes. However, vermicompost application had a positive effect on fruit sizes in general. Statistically, for nut weight the highest value was determined by amino acid (12.68 g) application. The lowest value occurred on trees treated with effective microorganism (10.73 g). Shell thickness is an important parameter for nut fruit species. This character has also changed positively depending on the applications. Walnuts with less shell thickness were obtained with effective microorganism (1.53 mm), amino acid (1.58 mm) and vermicompost (1.58 mm) applications, while a thicker shell was observed in untreated trees (1.83 mm). In other applications, it remained between these two groups.



Applications	Width (mm)	Length (mm)	Height (mm)	Nut weight (gr)	Kernel weight (gr)	Kernel ratio (%)	Shell thickness (mm)
Effective microorganism	32.48	34.09	39.69	10.73 b	5.27	49.62	1.53 a
Vermicompost	33.60	34.06	41.18	11.85 ab	5.45	46.17	1.58 a
Amino acid	32.92	34.22	40.09	12.68 a	4.06	46.83	1.58 a
Seaweed	32.94	34.14	40.68	11.26 b	5.17	45.98	1.73 ab
Stimulant	32.31	33.75	39.93	11.65 ab	5.24	45.17	1.66 ab
Control	32.89	34.36	39.38	12.08 ab	5.54	45.81	1.83 b

Table 2. Some fruit quality parameters in Chandler variety

The differences between the averages were determined by the Duncan test at P≤0.05 significance level.

There was a statistical difference between the applications with regard to color parameters (Table 3). L\* values, which express light color in fruit, changed within the limits of 48.09 (stimulant) - 55.81 (control), and lighter colored walnuts were detected in the control group. On the other hand, it is noteworthy that a\* value is higher in the treated trees compared to the control group. In this respect, stimulant application took the first place. In terms of b\* value, vermicompost application was low, while other applications formed the same group. Vermicompost application was not effective on light yellow kernel formation, which is an indicator of quality in walnuts. In addition, control was found to be high in  $h^0$  and C\* values.

Applications	L*	a*	b*	h <sup>0</sup>	C*
Effective microorganism	50.41 bcd	8.71 ab	28.98 a	73.27 bc	30.26 ab
Vermicompost	49.66 cd	8.55 ab	27.53 b	72.76 c	28.83 b
Amino acid	53.20 abc	7.55 bc	29.17 a	75.52 ab	30.15 ab
Seaweed	54.49 ab	7.71 abc	29.75 a	75.45 ab	30.74 a
Stimulant	48.09 d	9.28 a	28.94 a	72.22 c	30.39 ab
Control	55.81 a	6.90 c	29.83 a	76.97 a	30.62 a

#### Table 3. Color parameters in Chandler variety

The differences between the averages were determined by the Duncan test at P≤0.05 significance level.

#### **Discussion and Conclusion**

Fertilization is extremely important for sustainable agriculture and the environment. Considering this situation, vermicompost application does not harm ecosystems and does not have a toxic effect on plants (Bellitürk, 2019). It has a positive hormonal effect thanks to the synthesis by worms of substances such as auxin, cytokinin and gibberellin, which play a major role in plant development. If this ecological fertilizer is applied to plant leaves, it accelerates photosynthesis and metabolism (Abacıoğlu et al., 2020; Yılmaz, 2017). In a study on strawberries, an increase in fruit sizes was noted as a result of the combined application of inorganic fertilizer and vermicompost (Yavic, 2019). A similar finding was obtained in this study conducted on walnuts. Wani and Bhat (2016), found that the quality parameters of four selected walnuts improved with conjoint application of vermicompost and inorganic fertilizers. Parallel to the findings of these researchers, in our study,



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quality parameters such as nut width and height, kernel ratio, shell thickness and a\* value were improved with this application compared to the control.

Amino acids, which are the building blocks of proteins, directly or indirectly play a role in many metabolic activities of plants. When the plant is sprayed with amino acids through the leaves, it increases plant growth, blooming, yield and quality as well as the resistance of plants to adverse climate and soil factors. In a research in Romania, the effect of different amino acid leaf fertilizers on the production and fruit quality of 'Redix' apple variety was investigated. The data indicate that all the applied treatments were effective in increase yield and fruit quality than control tree (Ilie et al., 2018). In another study conducted in Iran, the combination of amino acid and calcium chelate foliar spray application was applied to "Golden Delicious" and "Granny Smith" apple trees. Accordingly, it is emphasized that applications significantly increase fruit quality (Arabloo et al., 2017). The increase in fruit weight with this application in apples is parallel to our study. Because the highest fruit weight in walnut was determined by the same application. On the other hand, it was recorded that foliar application of some amino acid compounds was not effective on quality characteristics on Momtaz and Ohadi pistachio cultivars (Molaie et al., 2013).

Beneficial microorganisms, which are among environmentally friendly agricultural practices, have a positive effect on fruit quality characteristics and yield thanks to the active substances produced by them (Shakeel and Hassan, 2018). In the study conducted by Acarsoy Bilgin et al., (2020) on the same walnut variety for two years, it was noted that the shell thickness was positively affected. In support of this, this study also shell thickness in effective microorganism application was less than control. This feature is considered as an important quality feature for nut fruit.

Environmentally friendly agricultural practices are becoming more and more important in terms of human and environmental health. Today, when this awareness comes to the fore, biological fertilizer applications, which are among the solution alternatives against chemical fertilizers, gain great importance. Today, walnut cultivation is encouraged in large areas with the support of the government. In particular, the amount of trees of the Chandler variety is increasing. Moreover, this variety is also of great interest to the consumer. In this study carried out in the 'Chandler' walnut variety in Manisa/Demirci location, where cultivation is widespread, vermicompost and amino acid applications improved nut width and height, shell thickness properties. In addition, nut weight increased with amino acid application. In the light of these findings, it is thought that it would be beneficial to continue the research in detail, since fruit quality characteristics change depending on many factors.

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## EFFECTS OF DIFFERENT TREATMENTS ON LETTUCE PRODUCTION BY FLOATING WATER CULTURE TECHNIQUE

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## Abstract

In this study, the effects of nutrient solution depth and the cleaning levels of seedling roots before planting were investigated on plant development and product quality of Lollo rossa type lettuce (cv. Carmesi RZ, 85-49) grown by floating system in greenhouse. Plastic (HDPE) tanks with internal dimensions of 44x114x29,5 cm were used as growing pools. Two different nutrient solution depths (10 cm and 20 cm) were tested and for this, 50 or 100 L nutrient solution was filled in each plastic tank. Cleaning of the seedling roots before planting was done in 3 different ways: (1) fully cleaned, (2) roughly cleaned or (3) not cleaned. Plants were grown on foam plates floating on the nutrient solution. Seedlings obtained from the seedling company were transferred to the water culture on October 17, 2019. The trial was established with 3 replications. During the growing period, pH and EC values of the nutrient solution were measured regularly. Temperature and relative humidity in greenhouse and temperature of nutrient solution were recorded. Plants were harvested on 28 November 2019. As a result, the effect of nutrient solution depth on plant growth was found to be significant, and if the depth of the nutrient solution was 20 cm, plant growth increased compared to 10 cm. Average marketable lettuce weight was 206 g at 20 cm nutrition solution depth and 167 g at 10 cm nutrition solution depth. It was concluded that cleaning seedling roots before planting affects negatively plant growth.

Keywords: Lolla rossa, Soilless Culture, Floating System, Seedling Root Cleaning, Depth of Nutrient Solution

## Introduction

Hydroponic production of leafy vegetables has been increasing in the last years (Gül et al., 2020). These products are not contaminated with soil or substrate, they are cleaner and microbiologically safer. Therefore, they require less washing and ensure water saving. For this reason, they are highly demanded by ready-to-eat industry as well as home consumption (Tomasi et al., 2015; Kotsiras et al., 2016; Petropoulos et al., 2016). Floating system and nutrient film technique (NFT) are the main hydroponic techniques used for leafy vegetables. In floating system, which is modified from deep water culture, plants are grown on a light material (e.g. high density polystyrene or Styrofoam) that floats on nutrient solution. The depth of the nutrient solution pools is 20-30 cm in the classic floating



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system. On the other hand, NFT is based on recirculating thin layer of nutrient solution (less than 1 cm) on watertight channels (Savvas et al., 2013).

It is reported that floating system is advantageous in terms of buffering the root zone temperature due to large volume of nutrient solution. It can be adapted to simple growing conditions with the low set up and management cost and the little automation required for monitoring and adjusting the nutrient solution (Savvas et al., 2013).

Lettuce can be produced more than eight cropping cycles by using hydroponic techniques in greenhouses (Savvas et al., 2013). Due to the high temperatures almost half of the year, floating system seems more suitable to the Mediterranean Basin for year-round lettuce production. In Turkey, floating systems have been used in very limited greenhouses particularly for production of Batavia type lettuces (Gül et al., 2020). More research is needed for adapting this technique to the local conditions. This study aimed to investigate the effects of nutrient solution depth and the cleaning levels of seedling roots before planting on plant development and product quality of Lollo rossa type lettuce (cv. Carmesi RZ, 85-49) grown by floating system in greenhouse.

#### **Material and Methods**

The experiment was conducted in an unheated polyethylene tunnel (38° 27′ 16.2" N, 27° 13′ 17.8" E) between 17 October and 28 November 2019. The tested lettuce type was Lollo rossa and cultivar was Carmesi RZ (85-49) (Rijk Zwaan).

Plastic tanks with dimensions of 114x44x29.5 cm were used as nutrient solution pools. Nutrient solution in each tank was aerated by a compressor worked continuously. Elemental composition of nutrient solution as mg per L was 150 N, 50 P, 150 K, 150 Ca, 50 Mg, 5 Fe, 0.5 Mn, 0.5 B, 0.03 Cu, 0.05 Zn and 0.02 Mo. Seedlings purchased from the commercial nursery were transplanted to the floating system on 17 October 2019. Each tank had 12 plants (24 plants per m<sup>2</sup>).

Experiment was set up according to the completely randomized design with 3 replicates. Two factors, namely nutrient solution depth and cleaning of the seedling roots before planting were compared. 10 cm and 20 cm were tested as nutrient solution depth and for this, 50 or 100 L nutrient solution was filled in each plastic tank. Cleaning of the seedling roots before planting was done in 3 different ways: (1) fully cleaned, (2) roughly cleaned or (3) not cleaned.

Nutrient solution temperature, greenhouse air temperature and relative humidity were recorded by Onset Hobo data recorders every 15 minutes. EC and pH of nutrient solution were measured by a portable device twice weekly.

Harvest was made on 28 November 2019. Before harvest, thickness of the second external leaves in 5 plants in each experimental unit were measured by micrometer (Mitutoya, Japonya); and chlorophyl index was measured by chlorophyl meter (SPAD- 502 Plus, Konica Minolta, Japan). Then, plants were harvested, total and marketable head weights, and number of unmarketable leaves were recorded. Height and diameter of the heads, length of the roots were measured, marketable leaf number was determined. Leaf colour was mesured by Konica Minolta CR-400 Chroma Meter, Japan) as L, a\*, b\*; hue (h°) and ve chroma (C\*) were calculated (McGuire, 1992). Fresh and dry weights of leaf and root samples were recorded.



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Nutrient solution consumption was determined as L plant-1 by taking into account the quantities of nutrient solution in the each tank at the beginning and end of the trial.

The collected data were analysed by analysis of variance with IBM SPSS Statistics 20 statistical software. Duncan's test was used to assess significant differences at P<0.05.

#### Results

During the experiment, inside the greenhouse, daily average temperature and relative humidity changed between 14.4 and 22.4°C, and from 59 to 69%, respectively. Daily maximum and minimum temperatures varied from 37.5 to 16.0°C and from 17.2 to 7.4°C, respectively (Figure 1).

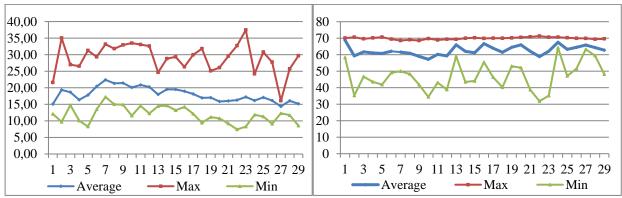


Figure 1. Temperature (left) and relative humidity (right) in the greenhouse.

Figure 2 shows the daily average, maximum and minimum temperatures of nutrient solution of which the depths of 10 and 20 cm. Average daily temperatures varied between 24.5 and 17.4°C, and 23.6 and 17.2 in the 10 cm and 20 cm nutrient solution depth, respectively. Daily maximum temperatures were between 28.8 and 18.8°C, and 25.9 and 18.6°C in the 10 cm and 20 cm nutrient solution depth, respectively. Daily minimum temperatures varied from 22.0 to 15.4°C, and from 21.9 to 15.4°C in the 10 cm and 20 cm nutrient solution depths, respectively.

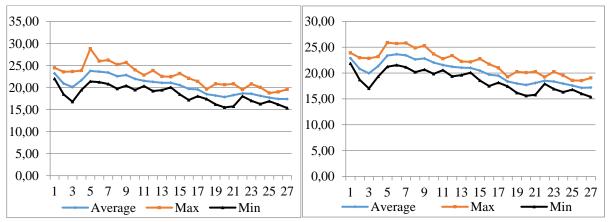


Figure 2. Temperatures of nutrient solution with a depth of 10 cm (left) and 20 cm (right).



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During the experiment, nutrient solution pH varied as in the Table 1. The mean values in 10 and 20 cm nutrient solution depth were 6.67 and 6.34, respectively. Cleaning the seedling roots before planting gave rise to increase nutrient solution pH in both depths.

Treatments	Weeks						
Nutrient solution depth * Cleaning level of seedling roots before planting	1	2	3	4	5	6	Average
10 cm * Fully cleaned	5.91	6.78	7.27	7.01	6.96	6.98	6.82
10 cm * Roughly cleaned	6.08	6.62	6.99	6.72	6.83	7.03	6.71
10 cm * Not cleaned	5.81	6.37	6.61	6.57	6.56	6.97	6.48
20 cm * Fully cleaned	5.66	6.54	6.95	6.36	6.25	6.61	6.39
20 cm * Roughly cleaned	5.61	6.31	6.55	6.63	6.25	6.57	6.32
20 cm * Not cleaned	5.71	6.39	6.56	6.35	6.32	6.59	6.32

Table 1. Changes in nutrient solution pH

Variation nutrient solution EC was summarized in Table 2. Average EC values in 10 and 20 cm nutrient solution depth were 1610 and 1632  $\mu$ S/cm.

Treatments		Weeks				
Nutrient solution depth * Cleaning level of seedling roots before planting	1	2	3	4	5	Average
10 cm * Fully cleaned	1552	1594	1616	1627	1621	1602
10 cm * Roughly cleaned	1549	1549	1569	1579	1538	1557
10 cm * Not cleaned	1569	1686	1698	1708	1700	1672
20 cm * Fully cleaned	1623	1627	1640	1649	1625	1633
20 cm * Roughly cleaned	1623	1658	1659	1673	1661	1655
20 cm * Not cleaned	1594	1613	1607	1612	1616	1608

Table 2. Changes in nutrient solution EC (µS/cm)

It was determined nutrient solution depth had significant effect on head weight and marketable head weight (Table 3). Nutrient solution depth 20 cm increased head weight compared to 10 cm.



Treatments		Head Weight (g)	Number of Discarded Leaf (pcs/head)	Weight of Discarded Leaf (g/head)	Marketable Head Weight (g)
Nutrient solution	10 cm	172,3 b	2,1	9,4	166,5 b
Nutrient solution depths	20 cm	210,4 a	2,1	12,2	206,3 a
deptils	Р	0,0222	1,0000	0,0797	0,0063
	Fully cleaned	175,6	2,1	9,6	170,1
Cleaning of the seedling roots	Roughly cleaned	188,1	1,9	10,8	190,1
	Not cleaned	210,4	2,2	12,0	199,0
	Р	0,1815	0,5158	0,4093	0,1764
10 cm * Fully clear	ned	157,6	2,2	7,5	147,3
10 cm * Roughly c	leaned	180,5	1,8	11,2	178,3
10 cm * Not cleane	ed	178,9	2,2	9,7	173,8
20 cm * Fully clean	ned	193,6	2,0	11,7	192,8
20 cm * Roughly c	leaned	195,7	2,0	10,5	201,8
20 cm * Not cleane	ed	242,0	2,2	14,3	224,2
Р		0,4281	0,7462	0,2772	0,6365

Table 3. Mean head weight and marketable head weight, discarded leaf number and weight

Nutrient solution depth had significant effects on stem diameter, number and thickness of leaves (Table 4). These properties were higher in the 20 cm nutrient solution depth. Cleaning level of the seedling roots had significant effect on leaf number which was higher when the seedling roots were not cleaned. On the other hand, there was no significant difference between the completely and roughly cleaned roots in this respect.

Table 4. Effect of nutrient solution depth and seedling root cleaning on Lollo rossa plant growth

		Plant	Plant	Stem	Root	Number	Leaf
Treatments		Height	Diameter	Diameter	Length	of Leaf	Thickness
		( <b>cm</b> )	(cm)	( <b>mm</b> )	(cm)	(adet)	( <b>mm</b> )
Nutrient	10 cm	20,4	16,2	11,6 b	71,6	18,0 b	0,179 b
solution	20 cm	20,7	17,1	14,0 a	75,9	19,3 a	0,200 a
depths	Р	0,7529	0,2782	0,0043	0,1012	0,0012	0,0060
	Fully cleaned	20,8	16,3	12,5	70,6	18,1 b	0,195
Cleaning of	Roughly cleaned	20,0	16,9	12,7	72,9	17,8 b	0,187
the seedling roots	Not cleaned	20,8	16,8	13,1	77,7	20,1 a	0,186
10013	Р	0,7332	0,8506	0,7769	0,0928	<,0001	0,4375
10 cm * Fully	cleaned	20,4	15,6	10,9	70,7	17,6	0,177
10 cm * Roug	hly cleaned	20,3	16,7	12,1	71,3	17,2	0,182
10 cm * Not c	leaned	20,5	16,3	11,9	72,7	19,3	0,178
20 cm * Fully cleaned		21,1	17,1	14,2	70,5	18,7	0,213
20 cm * Roug	hly cleaned	19,8	17,1	13,3	74,5	18,3	0,192
20 cm * Not c	leaned	21,1	17,3	14,3	82,7	20,9	0,193
Р		0,8516	0,8693	0,4471	0,2615	0,8095	0,2111



Main and interaction effects of treatments on chlorophyl index and leaf colour parameters except b, were not significant (Table 5).

Tuesta		Chlorophyll Index			Colour		
Treatments		(SPAD)	L	a*	b*	h°	C*
Nutrient	10 cm	16,75	33,91	-6,32	20,79	-1,28	21,83
solution	20 cm	16,97	36,85	-6,82	21,52	-1,27	22,69
depths	Р	0,5470	0,0623	0,6979	0,3446	0,8030	0,4187
	Fully cleaned	16,76	35,94	-7,12	21,12	-1,25	22,35
Cleaning of the seedling	Roughly cleaned	16,92	34,46	-706	21,96	-1,27	23,21
	Not cleaned	16,90	35,73	-5,54	20,38	-1,32	21,21
roots	Р	0,9250	0,6662	0,5208	0,2529	0,5031	0,3140
		·					
10 cm * Fully	y cleaned	17,05	35,29	-8,01	21,94 a	-1,23	23,41
10 cm * Roug	hly cleaned	16,76	33,04	-7,54	21,79 a	-1,24	23,07
10 cm * Not c	leaned	16,45	33,40	-3,43	18,67 b	-1,39	19,00
20 cm * Fully	cleaned	16,48	36,60	-6,24	20,31 ab	-1,28	21,30
20 cm * Roug	hly cleaned	17,08	35,89	-6,58	22,15 a	-1,30	23,36
20 cm * Not c		17,36	38,07	-7,65	22,10 a	-1,24	23,41
Р		0,2743	0,6421	0,1464	0,0465	0,1453	0,0664

Table 5	Chlorophyl	index a	and leaf	colour	narameters
rable J.	Children and a second se	much t	and rear	colour	parameters

L indicates the brightness from black:0 to white:100; negative a\* indicates green; positive b\* indicates yellow; hue angle (h°) indicates the basic components of color (0°:red, 90°:yellow, 180°:green and 270°:blue); chroma (C\*) indicates the saturation and vividness of the color.

Nutrient solution depth had significant effect on shoot dry weight which was increased in the 20 cm nutrient solution depth compared to 10 cm. Seedlings with no cleaned roots increased shoot fresh weight and roots dry weight. These properties were higher in the plants obtained by the seedlings with no cleaned roots (Table 6).

		Leav	ves	Ro	ots
Treatments		Fresh Weight	Dry Weight	Fresh Weight	Dry Weight
		( <b>g</b> )	(g)	(g)	(g)
Nutrient	10 cm	192,4	7,5 b	46,1	2,04
solution	20 cm	233,9	9,3 a	53,7	2,41
depths	Р	0,0729	0,0287	0,1634	0,0671
Classica of	Fully cleaned	176,0 b	7,4	44,2	1,91 b
Cleaning of the seedling	<b>Roughly cleaned</b>	214,6 ab	8,3	46,7	2,04 b
	Not cleaned	248,9 a	9,5	58,8	2,71 a
roots	Р	0,0470	0,1016	0,0816	0,0083
10 cm * Fully	cleaned	172,5	7,5	45,5	1,87
10 cm * Roug	hly cleaned	195,5	7,5	39,2	1,73
10 cm * Not c	leaned	209,4	7,6	53,5	2,51
20 cm * Fully	20 cm * Fully cleaned		7,4	42,9	1,95
20 cm * Roug	hly cleaned	233,7	9,1	54,1	2,35
20 cm * Not c	leaned	288,5	11,5	64,1	2,92
Р		0,4054	0,1190	0,3814	0,5072



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Nutrient solution consumption was 17.5 L/12 plants in all cleaning levels of the seedling roots. It was determined that nutrient solution consumption was higher in the 20 cm nutrient solution depth compared to 10 cm, and it was 24.2 and 22.5 L per 12 plants obtained by the seedlings with no cleaned roots and completely or roughly cleaned roots, respectively (Figure 3).

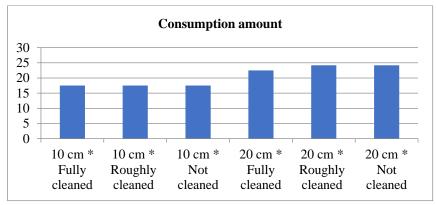


Figure 3. Nutrient solution consumption.

#### **Discussion and Conclusion**

Lettuce plants grow well at greenhouse temperature between 12 and 21°C and it is ideal to keep relative humidity at 50-70%. Since the greenhouse had no climate control, ideal temperatures could not provide in the greenhouse throughout the growing season, especially maximum greenhouse temperatures were higher than 21°C up to the last week of growing season. Our results showed that the temperatures of the nutrient solution were averagely 6.5°C lower than the greenhouse air temperatures during daytime, and averagely 7°C higher during night time. This result support the findings of previous studies (Cortella et al., 2014; Gül et al., 2020). Thompson et al. (1998) report that if nutrient solution was kept at optimal temperature, lettuce plants could be grown well at air temperatures outside the normal range, and particularly at high air temperature.

Harvesting was made 42 days after transplanting. In the studies realised in Antalya, Lollo rossa type lettuce plants grown by water culture were ready to harvest 48 days after planting (Uygunsoy, 2016; Okudur, 2018). The differences may be resulted from the environmental conditions. It is known that shoot and root temperature affect lettuce growth in floating hydroponic system (Thompson et al., 1998).

Average marketable head weight varied between 147 and 178 g in 10 cm nutrient solution depth, and between 193 and 224 g in 20 cm nutrient solution depth. Okudur (2018) reported that head weight of Lollo rossa type lettuce (cv. Carmesi) plants grown by water culture was between 240 and 302 g. Average head weight of hydroponically grown lollo rossa lettuce was found 133 g by Uygunsoy (2016). The differences between the results may be attributed to the environmental conditions and treatments tested.

Nutrient solution depth had significant effects on head weight, number and weight of leaves. These properties increased in nutrient solution depth of 20 cm compared to 10 cm. Increase in plant growth in 20 cm nutrient solution depth may be due to the fact that the larger volume of nutrient



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solution had higher buffering capacity on temperature and chemical properties. It was concluded that 20 cm nutrient solution depth was more suitable for simple growing conditions that the nutrient solution is not replenished, and its properties are not automatically monitored and adjusted.

Cleaning level of seedling roots before planting had significant effect on number of leaves. This property decreased by cleaning of the roots. It is thought that cleaning seedling roots before planting affects negatively plant growth. This result may be attributed to the fact that cleaning of the roots makes adaptation of seedlings difficult after planting.

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## CFD USAGE MODELS IN AGRICULTURAL PRODUCTION

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## Abstract

Computational fluid dynamics (CFD) is a sub-branch of fluid mechanics that provides the determination of fluid behavior. CFD, which is defined as a method of simulation used to determine fluid behavior depends on parameters such as fluid type, flow rate and flow geometry. The precision and accuracy of the simulations created with the help of developing technology and increasing computer processing capacity are increasing day by day. This method is also used in order to solve the problems that occur in agricultural production and to improve the existing machinery and systems which is a work area where the flow is always present. This method, in which the interactions of solid, liquid and gaseous environment with fluids can be examined, can be used for all steps from the beginning to the end of agricultural production. These steps can be listed as tillage, seeding-planting, irrigation, spraying, harvest-threshing, product processing, storage and air conditioning. In tractors, which are used as the power source of the machines that perform these processes, this method enables the improvement of ergonomic features. With this method, it is possible to examine the effects of unmanned aerial vehicles on agricultural areas in smart agriculture applications that have become widespread with the increasing use of technology. In this article, it is aimed to be a example for future studies by compiling the data obtained from the studies carried out for the various machines and systems that are considered to be examined in the existing studies reported in the literature.



# THE EFFECTS OF SODIUM NITROPRUSSIDE (SNP) APPLICATIONS AGAINST SALINITY STRESS ON MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN GRAPE SEEDLINGS

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#### Abstract

Salinity is among the major abiotic stresses negatively affecting the growth and productivity of plants. Sodium nitroprusside (SNP) an external nitric oxide (NO) donor has been found effective to impart salinity tolerance to plants. Grapevine (V. vinifera L.) is widely cultivated around the world; however, salinity stress hampers its growth and productivity. In this study, the effects of SNP applications on improving the morphological, physiological and biochemical properties of grapevine seedlings under salinity stress were investigated. The research was carried out in the cold storage, plant growing greenhouse and research laboratories available in Yozgat Bozok University Faculty of Agriculture in 2020–2021. Grape seeds of the Cardinal cultivar were stratified at +4°C for 90 days and then treated with sodium nitroprusside in an aqueous solution at concentrations of 0, 0.5, 1.0 and 1.5 mM for 24 and 48 h. After the plants reached the seedling stage with 3-5 leaves, they were exposed to NaCl stress, which was initiated with 25 mM and increased by 25 mM every 10 days, reaching a concentration of 150 mM. Morphological, physiological and biochemical analyses were performed on seedlings after a total development period of 120 days. The results obtained from statistical analysis showed that SNP was effective for the morphological (Root and shoot length, fresh and dry weight), physiological (chlorophyll content, membrane injury index, stomatal conductance and leaf temperature values) and biochemical (malondialdehyde "MDA" contents, superoxide dismutase "SOD", catalase "CAT" and ascorbate peroxidase "APX") properties of the grapevine in terms of reducing salinity stress, and there were significant differences between the applied SNP concentrations. The results obtained from this study reveal that SNP can be used to reduce the losses in the transformation processes of seeds obtained from hybridisation studies, especially for grapevine breeding.

Keywords: Sodium Nitroprusside, Salinity, Abiotic Stress, V. vinifera L., Antioxidant Enzymes



# A RESEARCH ON THE COMPARISON OF COLD PRESSED OLIVE OIL PRODUCTION AND CONTINUE TYPE EXPRESSING TECHNIQUES AND THE ECONOMIC ANALYSIS OF THE OIL AMOUNT AND QUALITY

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## Abstract

In this research supported by Ege University Scientific Research Projects Coordinatorship, a farm type cold-pressed olive oil processing facility was established. It is aimed to examine the efficiency parameters between the continue type pressing process and the cold pressing oil process. Approximately 5000 olive trees belonging to Ege University in Mordoğan, Bornova and Menemen were used. The oils obtained by both methods were compared proportionally in terms of oil amount, oil yield, acidity values, fatty acids obtained by two different processes. In addition, it has been determined whether the efficiency in cold-pressed oil production varies according to the production regions, in which regions the continue type and in which regions the cold-pressed olive oil process is more suitable. In addition, economic gains were obtained for the three olive production locations mentioned as a result of the research. When the quality parameters in the research are examined, it can be seen that values close to cold-pressed olive oil can be obtained on the condition that "no high temperature treatment is applied in the continue system", that the nutritional content and quality properties can be packaged without deterioration, but that it differs from the continue system with the high linoleic and linolenic acid values obtained in cold-pressed olive oil. It was concluded that to the fore.

Keywords: Olive oil, Oil composition

## Introduction

Olive oil, unlike other vegetable oils, can be obtained and consumed in its natural state without any chemical treatment. Olive oil is divided into three classes: natural, refined and riviera type. Natural olive oils are also divided into three groups as extra natural (<1%), natural first (<2%) and natural second (<3%) according to their free fatty acids content (Anonymous, 2010).



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Phenolic compounds in olives affect the color of the olive fruit, its nutritional value, the stability of olive oil obtained from olives, and its resistance to microorganisms (Alkın, 2003). The formation of phenolic and volatile components in natural olive oil varies depending on many factors (Bayrak et al., 2010; García-Vico et al., 2017). These components have been reported to contribute effectively to the prevention of cardiovascular and thrombotic diseases. The main phenolic compounds found in olives; Tyrosol (p–hydroxy–phenyl ethanol), hydroxytyrosol (HT) (2, (3, 4– dihydroxyphenyl)–ethanol), are the conjugated products of these phenols and elenolic acid and oleuropein. These factors that determine the formation; The climatic conditions of the region where the olive is grown, its variety, the altitude of the region where it is grown, cultivation techniques, harvest time, processing techniques and storage conditions are (Bozdogan et al., 2003; Kaula et al., 2007; Karagöz et al., 2017).

Total Energy	885 kcal
Carbohydrates and Protein	0 g
Yağ	100 g
Saturated	14 g
Mono unsaturated	73 g
Poly Unsaturated	11 g
Omega 3 – Omega 6 oil	<1.5 g – 3.5-21 g
Vitamin E	14 mg
Vitamin K	62 µg

Table 1. Nutritional values for 100 g of olive oil (~109 mL)

Recommended fatty acid contents for olive oil (extra virgin and refined) according to international standards; 56-83% oleic, 7.5-20% palmitic, 3.5-20% linoleic, 0.5-3.5%, stearic, 0.3-0.5% palmitoleic, 0-1.5% linolenic, 0-0.5% myristic acid and much less other oil acids should consist of (Armutcu, et al., 2013). In olive oil, the ratios of monounsaturated, polyunsaturated and saturated fatty acids are on average 73%, 11% and 14%, respectively (Table 1).

Two-phase production systems were developed in Italy in the early 1990s in order to minimize the disadvantages of three-phase continue plants without giving up their advantages. The process stages are similar to three-phase systems. After the olives are washed, they are crushed with the help of metal crushers, kneaded for a while in kneading vats called "malaxor", then the olive paste enters the decanter. The difference starts at this point. In two-phase systems, no external water is added to the decanter. Again, this separation system based on density difference has two different outputs: Olive oil and wet pomace, which also includes the black juice of the olive. He finds the reason why these systems are called two-phase. Since the olive paste is not heated and hot water is not added, the olive oils produced in these systems are often called "cold pressed".



Advantages:

\*Since it is a sequential production method, there is no risk of holding or accumulating, contact with air is limited. Oxidation and contamination risks of olive oil during processing are minimized.

\* Since no water is used and olive oil is not heated during the process, the antioxidants, vitamins and natural flavors it contains remain in the olive oil. Olive oil has high flavor and nutritional value.

\* It does not produce environmental waste as there is no black water outlet.

Disadvantages:

\* Its efficiency is low, the remaining oil in the pomace is higher than the three-phase systems.

\* Pomace is more difficult to dispose of. Therefore, this system is not suitable for high volume production. Many pomace factories do not want to buy fresh pomace due to transportation and storage difficulties.

These systems, which have the ability to produce high quality olive oil when suitable processing conditions are provided, are safer for the consumer than the traditional pressing method, which can also produce high quality olive oil, but carries the risks of oxidation and contamination (Tokusoglu, 2008).

Three-phase continue (sequential) systems have recently started to be used in olive oil production. These systems have been developed with the development of machinery technologies to increase efficiency by facilitating the workflow in olive oil production, to minimize contact with air and reduce the possibility of oxidation, and thus to obtain longer-lasting olive oil. The most important feature of these systems, which have been used since the 1960s, is the separation of olive oil by a centrifuge system called a "decanter", unlike the traditional pressing method. In continue systems, after the olives are washed, they are crushed with the help of metal crushers, kneaded for a while in kneading vats called "malaxers", then the olive paste enters the decanter (centrifuge system). Meanwhile, some water is added to the system from outside to ensure separation. This separation system based on density difference has three different outputs: Olive oil, black water (olive juice and water added to the system) and dry pomace (olive pulp). He finds the reason why these systems are called three-phase. Since the olive oil obtained with this system comes out with the black water of the olive, a second centrifuge system is needed to purify the oil from water.

Advantages;

\* Since it is a sequential production method, there is no risk of holding and accumulating, contact with air is limited. Oxidation and contamination risks of olive oil during processing are minimized.

\* It is a fast and efficient method.

Disadvantages;

\* Since a large amount of water (mostly hot) is used during the process, some water-soluble antioxidants, vitamins and natural flavors contained in olive oil are separated from the olive oil with water. The nutritional value of olive oil decreases (Kayahan and Tekin, 2006).

\* The black water that emerges during the process has an environmental effect. It should be disposed of in a way that is not harmful to nature. This issue is one of the important problems of both our country and other olive oil producing countries today.



Table 2. Major and minor components in olive oil (Armutcu et al., 2013)

Major Components	Minor Components		
Fatty acids (Oleic acid, palmitic acid,	Non-glyceride Esters and Waxes		
linoleic acid, stearic acid, palmitoleic acid) Triglycerides	Aliphatic Alcohols		
Glycerides	Triterpene Alcohols: Erythrodiol and Uvaol		
	Sterols: β-sitosterol, campesterol, stigmasterol		
	Hydrocarbons (squalene, volatile hydrocarbons		
	(phenanthrene, pyrene, fluorantrene), carotenoids (β-carotene and lycopene))		
	carotenoids (p-carotene and rycopene))		
	Pigments: chlorophylls and pheophytins		
	Volatile Compounds		
	Phenolic Compounds		

One of the methods used to extract the oil from various seeds and seeds is the Cold Pressing method, while the seeds are compressed to release their oil, it is called the cold pressing method because no heat is given from the outside. As no heat is given from the outside, special shapes have been made on the machine parts in order to remove the heat caused by friction from the press. The molecules in the oil, which are very beneficial for human health, can be preserved thanks to the low temperature during the squeezing process. For this reason, the benefits of oils obtained by cold pressing method for human health are gaining great importance day by day as a result of researches. Refined oils have begun to leave their place on the shelves in markets and restaurants to cold pressed oils.

The primary purpose of this project is to compare the oils obtained from the olive groves of Ege University Faculty of Agriculture in the Mordoğan region in terms of quality parameters and to determine which method is more effective. With this project, E.U. Cold pressed oil production has started with the farm type cold pressed olive oil machine and equipment to be installed in the Menemen Research and Application Farm of the Faculty of Agriculture. In addition, the olive oils obtained from the olives harvested from our Mordoğan region, which is the region where the ancestors of olives are located, especially in olive oil production, were compared in terms of quality and squeezing methods.



## Material and Method Material

For the purpose of research, the infrastructure of Ege University Faculty of Agriculture Menemen Application, Research and Production Farm and the olive groves of our Faculty of Agriculture in Mordoğan were used.

Mordoğan is located on the Karaburun peninsula. It is a region where olive oil and palm olive production are at the forefront in terms of agricultural production.

## Method

## Continue system method of obtaining olive oil

The production of fabricated extra virgin olive oil, known as the continue system, was obtained from the olives obtained from the Mordoğan production areas in the form of service procurement. For this purpose, the olives collected daily without wasting time were sent to the oil factory when sufficient amount was reached, and only after the processing of the Faculty products, they were labeled as Mordoğan region olive oils and taken to rest.

The following steps are similar in both methods.

- 1. Reception of olives to the factory
- 2. Leaf separation and washing
- 3. Crushing Crushing process
- 4. Kneading (Malaksation)
- 5. Phase separation process (with the addition of hot water in the continue system)
- 6. Separation of oil by centrifuge

## Method of obtaining cold pressed olive oil

Within the scope of the project, a Miniolive brand, domestically produced farm-type cold-pressed olive oil machine, filtration system, steel lances to rest and store the oil, and a bottling unit were installed in the Menemen Application, Research and Production Farm. Production was carried out in Menemen facilities.

- 1. Reception of olives to the factory
- 2. Leaf separation and washing
- 3. Crushing process
- 4. Kneading (Malaksation)
- 5. Phase separation process
- 6. Separation of oil by centrifuge

## Crushing

The olives are crushed by the hammer mill on the machine. With this technique used, it is ensured that the best dough is obtained. Crushing is done by rotating blades at high speed (3000 rpm). The



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blades break the olives in a very short time, forcing them to pass through the small holes around them. The dough obtained in this way (passing through small holes) is directed to the kneading process.

#### Kneading

The kneading process of the olive paste is carried out by mixing the dough from the hammer mill for 45 minutes. The machine has a closed system vertical nitrogen gas inlet and mixing unit. Thanks to the fact that this unit is closed and suitable for nitrogen gas compression, air contact with the olive paste is minimized, preventing oil oxidation and, accordingly, peroxide increase (increase in the acidity of olive oil). There is a heating system on the kneading unit to facilitate oil separation from the olive paste (26°C max).

In the washing unit that comes as an additional part of the machine, the harvested product is washed and separated from its stems and leaves.

#### Separating (Separation of oil and pomace water)

Obtaining the oil from the pulped olives takes place in the decanter. The plant juice, pomace and oil are separated from each other in the decanter. The separation principle is based on the centrifugal force principle. The high rotation speed of the unit (3200-4200 rpm) causes the formation of three different layers within the unit. The first outer layer consists of pomace, the second layer consists of plant sap, and the third layer consists of oil.

The olive oil coming out of the decanter is transferred directly to the aging tank to be rested in the tank for a few days. A 50-70 lt oil tank was added to the olive oil outlet of the machine, and the oil accumulated here was taken to the resting tank for a few days, and after the particles formed in the olive oil were precipitated, filtration was carried out with a paper filter.

## Oil quality analyzes

Before the analysis of fatty acids, the crude oil samples obtained are subjected to esterification process. In this process; 0.5 g crude oil sample is placed in a 15 ml screw cap falcon tube, 1 ml of 2 N methanolic KOH solution and 7 ml of n-hexane are added and centrifuged at 4500/min for 30 minutes (ThermoScintific SL 16 R, Thermo Electron LED GmbH, Germany). Thus, the resulting upper phase is clarified. 1-2 ml of sample taken from the upper phase containing fatty acid methyl esters is placed in special glass bottles and made ready for injection into gas chromatography. With the help of the automatic sampling apparatus (HP7683 B), 1  $\mu$ l is automatically taken from the samples and injected into the device. The oil composition was determined by the "Agilent Technologies 6890N" brand gas chromatograph device (Agilent, CA, USA) at Ege University Faculty of Agriculture, Department of Horticulture, and a 60-meter-long "Spelco 2380" brand capillary clone (60 mx 0.25 mm id, 0.20  $\mu$ m film thickness; Supelco, Bellefonte, PA, USA). The resulting peaks will be identified using the fatty acid standard (SupelcoTM 37 Component FAME mix, Supelco, Bellefonte, PA, USA). The peaks that emerged during the analysis were determined



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by calculating the time and area of the peak using the standard, and the results were given as % fatty acid.

#### Results

The olives harvested from the Aegean University Faculty of Agriculture's facility in İzmir-Mordoğan and the olives harvested from the Aegean University Faculty of Agriculture's Menemen Application, Research and Production Farm were processed separately and half of the products were sent to the oil factory for the continue system, while the other half was produced in the farm with this project. It was brought to the farm type cold pressed oil plant. Within the scope of the project, the equipment, whose images are presented below, was brought to the cold press oil processing facility of our faculty and used for the purpose of this project.

The necessary legal permissions were obtained in our olive oil plant, which started to be used in practice for students and in research for academic staff, and all documents were obtained and brought into operation according to the criteria of the Ministry of Agriculture and Forestry.

Our olives produced were grown according to organic farming conditions and organic farming certificate was given by the Organic farming certification body authorized by the Ministry of Agriculture and Forestry. Likewise, organic product certificates were obtained for our olives and oils produced in our own facility.

#### Oil quality analysis results

Olive oil consists of 98-99% glycerides. This ratio varies depending on whether the oil is natural or refined. Glycerides are formed by the esterification of long chain (C16 - C18) fatty acids with glycerol containing three hydroxyl groups. Biologically synthesized oil is neutral. However, free fatty acids are formed due to the poor condition of the fruit (especially wormy, fly-infested) or due to improper processing or prolonged waiting before processing.

This method is one of the best methods to be recommended in terms of seeing the quality of olive oil. With this method, it is possible to detect oils such as deodorized oil (colon oil), refined olive oil and natural primary olive oil mixed with virgin olive oil for cheating purposes.

A certain internal standard is added to the sample, then it is separated into fractions by passing it through a column of active diluted silica gel. The appropriate fraction with less polarity than the triglycerides under the test conditions is collected, then analyzed directly by a capillary column gas chromatograph. GC Operating conditions are generally as follows;

Oven temperature: Programmed operation is performed. Detector temperature: 350 °C, Injector Temperature: on-column Column: 15 m long SPB 5



Table 3. Oil quality analysis results of natural extra virgin olive oil and cold-pressed olive oil (%)

Fatty acid	Continue ekstra virgin Olive oil	Cold pressed Olive oil
Palmitic acid (C16:0)	13.31	15.03
Palmitoleic acid (C16:1)	1.04	0.98
Margaric acid (C17:0)	0.15	0.11
Heptadesanoic acid (C17:1)	0.13	0.23
Stearic acid (C18:0)	0.70	0.66
Oleic acid (C18:1)	72.0	68.64
Linoleic acid (C18:2)	11.24	12.79
Linolenic acid (C18:3)	0.75	0.80
Arachidic acid (C20:0)	0.38	0.51
Exocenoic acid (C20:1)	0.30	0.25
Total saturated fat (TSF)	16.00	17.66
Total unsaturated fat (TUF)	83.90	82.22
Total monounsaturated fat (TMSF)	71.91	69.27
Total polyunsaturated fat (TPUF)	11.99 *	12.95
$\Sigma TUF / \Sigma TSF$	5.24	4.66
$\Sigma$ TPUF / $\Sigma$ TSF	0.75	0.73

Palmitic acid contents were found to be 13.31% and 15.03% in both continuous extra virgin and cold pressed olive oil, respectively, and these values are between the values (7.5-20%) determined by the Ministry of Agriculture and Forestry in the Turkish Food Codex (TGK). The results we obtained show parallelism with the results of other researchers examining palmitic acid (Toplu, 2000; Konuskan, 2017). Stearic acid contents were determined between 0.70% (continue) and 0.66% (cold pressed). These rates are between the values reported by the TGK (0.5-5.0%). Linoleic acid contents ranged from 11.24% (continue) to 12.79% (cold-pressed), and it was determined that linoleic acid was higher in cold-pressed Z. oil. Linoleic acid ratios were within the limits (3.5-21%) reported by the TGK. The findings obtained were similar to those of Munğan (2015). Palmitoleic acid composition was determined between 1.04% and 0.98%. Linolenic and arachidic acids were determined in the range of 0.75%-0.80% and 0.38%-0.51%, respectively. The aforementioned features are within the specified limits of the TGK. The percentage distribution of fatty acids differed according to the cultivars.

The main fatty acids found in olive oil are oleic (C18:1), linoleic (C18:2), palmitic (C16:0) and stearic (C18:0) acid (Wiesman, 2009). Fatty acid composition has an important place in terms of the nutritional value of olive oil. The positive effect of olive oil on health depends on the polyunsaturated fatty acids in its structure (Haddam et al., 2014). Olive oil contains two basic pigments. These; The chlorophylls give the green color and the carotenoids give the yellow and orange color (Kocaayan, 2013).



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When the quality parameters of both methods are examined; It has been concluded that values close to cold-pressed olive oil can be obtained, provided that "no high temperature treatment is applied in the continue system", that the nutritional contents and quality properties can be packaged without deterioration, but it differs from the continue system with the high linoleic and linolenic acid values obtained in cold-pressed olive oil.

#### **Discussion and Conclusion**

There was no statistically significant difference in the fatty acid compositions examined. Growing conditions, maintenance processes and harvest time are more effective in oil composition. However, one of the total polyunsaturated fatty acids, cold pressed olive oil was found to be higher with 12.95% compared to the continue system (11.99%). This parameter is an indication that cold pressed olive oils are of higher quality.

When the quality parameters of both methods are examined; It has been concluded that values close to cold-pressed olive oil can be obtained, provided that "no high temperature treatment is applied in the continue system", that the nutritional contents and quality properties can be packaged without deterioration, but it differs from the continue system with the high linoleic and linolenic acid values obtained in cold-pressed olive oil.

#### Acknowledgement

With this project numbered 2017-ZRF-039, supported by the Ege University Scientific Research Projects committee, a farm-type cold-pressed olive oil machine and other equipment was installed in the Menemen Research and Application Farm of the Faculty of Agriculture. Thanks to this facility, which can also be used in future projects, new devices have been added to the faculty inventory.

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# INFLUENCE OF REPEATED POLLINATION ON FRUIT AND SEED SET IN CROSSES BETWEEN HYBRID TEA ROSE CV. MYRNA AND OIL– BEARING ROSE

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## Abstract

Crossbreeding is one of the most widely used methods in the development of new cut rose varieties. The fruit and seed set are critical to the effectiveness of crossbreeding. To increase fruit and seed set, the Hybrid Tea rose cv. Myrna was pollinated three times at 24 h intervals with the Oil-Bearing Rose (*Rosa damascena* Mill.) in this study. In total, 45 flowers were pollinated in the greenhouse which is located in the Department of Horticulture, Faculty of Agriculture, Ankara University in Ankara, Turkey, in May 2019. 15 of the flowers were pollinated one time, 15 were pollinated two times and 15 were pollinated three times. The number of seeds per fruit, fruit weight, and seed weight were examined in the experiment. Compared with once pollination, three times pollination was most effective in terms of all traits examined. The number of seeds per fruit in three times pollination was 2 times more than once pollination (23.00). Moreover, fruit (10.64 g) and seed (0.27 g) weight in three times pollination were 2.5 times more than in once pollination (4.39 g and 0.11 g). In the study, it was concluded that repeated pollination in roses seems to be effective in increasing the number of seeds per fruit and fruit weight.

Keywords: Rosa sp., Hybridization, Seed Formation, Fruit Set, Repeated Pollination



# INFLUENCE OF GROWTH REGULATORS (IBAs) OF DIFFERENT CONCENTRATIONS ON QUALITY ROOTING OF HARDWOOD CUTTINGS OF SELECTED VARIETIES OF CURRANT (*Ribes* L.)

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## Abstract

The basis of successful and modern fruit production is adequate production of planting material. Currant is a species that is easier to propagate than other berry species, but the rooting capacity differs between species. Rooting also depends on climatic and technological factors. In this paper, the research investigates the influence of growth hormone (IBA) of different concentrations, from 500 ppm and 1000 ppm on the ability to form roots in mature cuttings of different currant varieties such as Pink Champagne, Rovada, Junifer, Jonkers, and including the control variant (without hormone application). The study of the influence of growth hormone (IBA) of different currant varieties showed that there was no statistically significant difference between control cuttings and cuttings treated with 500 ppm IBA concentration, while the use of 1000 ppm IBA concentration showed statistically significant poorer root development in cuttings of red currant varieties Rovada, Junifer, Jonkers. In the case of the Pink Champagne variety, the use of growth hormone IBA with a concentration of 500 ppm is justified, because the cuttings treated with this concentration achieved better results in the total number of roots and had the highest height of the above ground system.

Keywords: Currant Varieties, Rooting, Pink Champagne, Rovada, Junifer, Jonkers, Growth Hormone



## APPLICATION OF DIFFERENT UREA CONCENTRATIONS IN PROTECTION OF GREENHOUSES FROM SNOW

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## Abstract

During the winter period, greenhouses in Bosnia and Herzegovina are often exposed to excessive snowfall. To prevent damage to the greenhouse structure due to the heavy weight of the snow, it is necessary to implement appropriate preventive measures. To achieve this, various methods are used in practice, one of these methods is based on the application of different concentrations of UREA. This research aims to determine the rate of snow melting using different concentrations of UREA (5%, 10%, 15%, 20% and 30%) at different temperatures. Based on temperatures from 0 to 5 °C, the results of the research show that the fastest snow melting in hour time was achieved by using UREA 10% and 30% concentrations, while at 20% concentrations, snow melting is the slowest. UREA concentrations of 15% and 30% showed the best effect in the rate of snow melting during 24 hours at the positive temperatures, while the concentration of 20% showed the worst result. On the other side, at negative temperatures from 0 to -5 °C, the fastest snow melting was achieved with UREA concentration of 10% within 1 and 24 hours and the slowest snow melting with a concentration of 15%. In this study, all tested urea concentrations showed significantly better results at positive temperatures compared to negative, on average by two to three times. Under the given conditions, our recommendation is to use UREA concentration of 10%, because, the use of higher concentrations does not achieve a significant effect and can only affect the reduction of the profitability of using this method.

Keywords: Greenhouses, Snow, UREA, Melting, Damage Reduction



## MORPHOLOGICAL CHARACTERISTICS AND YIELD OF CUCUMBER DEPENDING ON GRAFTING

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## Abstract

Talking about a widespread type of vegetable cucumber (*Cucumis sativus* L.) means that we should immediately emphasize how widespread it is in our country, both in food and production. Based on that, there is an urgent need for quality and disease-resistant varieties/ hybrids of technological production, which reduces the possibility of disease. When it comes to the method of growing cucumbers, in recent years in our areas there is a grafted method of cultivation as a relatively successful method of cultivation. The example of the grafted method will be comparatively analyzed with the non-grafted one, in order to, on the basis of morphological and productive differences, successfully record the main characteristics- both of them. The experimental analysis was based on the CHARTA hybrid. The examined parameters are the number of lateral roots, number of roots hairs, root mass, leaf length and yields. The results of the examined parameters of the grafted cucumber are as follows: (18.00 ± 2.65), (3.58 ± 0.29), (10.60 ± 1.48), (20.00 ± 1.00) and (161.00 ± 17.56). The results of this study show that the cultivation of grafted cucumber in relation to non-grafted achieves a higher yield compared to non-grafting morphological characteristics of statistically significant differences were observed only in the number of lateral roots.

Keywords: Cucumbers, Grafting, Non-Grafting, Root, Yield



# INFLUENCE OF PYROPHYLLITE AS A SOIL CONDITIONER ON PRODUCTIVITY AND ACCUMULATION OF NITRATE IN LETTUCE (Lactuca sativa L.)

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## Abstract

Lettuce (Lactuca sativa L.) is leafy vegetable rich in vitamins, especially of vitamin C, minerals and other biologically active substances with high antioxidative properties. On the other side lettuce considered to be a high source of nitrate. Nitrate by itself is relatively nontoxic but it may be endogenously transformed to nitrite, which can react with amines and amides to produce N-nitro compounds which have been related to an increased risk of diseases. It can grow year round and produce large quantity of yield. However, lettuce produced receives large applications of mineral fertilizers. Nowadays consumers are more concerned about food quality than yield alone. Besides, fertilization related environment problems are also big concerns of the society. The aim of this study was to evaluate whether the application of pyrophyllite could reduce the use of mineral fertilizers in lettuce production without adverse effects on its yield and quality. The research was conducted in winter 2019/2020 in greenhouse without heating system, in region of Srebrenik. The experiment was set on splitplot method with three repetitions with substitution of fertilizers with pyrophyllite in amount of 25%, 50% and 75% of recommended fertilizer compared to the control treatment i.e. 100% recommended fertilizer rate. The results of this study showed that the substitution of fertilizers with pyrophyllite in amount of 25% and 50% of recommended fertilizer rate under experimental conditions increase lettuce yield and quality compared to the control treatment i.e. 100% recommended fertilizer rate.

Keywords: Lettuce, Pyrophyllite, Nitrate, Fertilizers, Yield



# EFFECT OF APPLICATION OF DIFFERENT AMOUNTS OF NITROGEN AND STAGES OF PLANT DEVELOPMENT AT CUTTING ON YIELD AND FORAGE VALUE OF GRASSLANDS

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## Abstract

Grasslands in Bosnia and Herzegovina (natural and sown) account for over 50% of the total agricultural land. However, although grasslands occupy large areas they are not optimally used. Inadequate use without the application of nitrogen fertilizer as well as cutting in the late stages of plant development have a negative impact on producing good quality forage and achieving high and stable yields. As the quality of biomass and grassland productivity largely depend on soil fertility, botanical composition, and stage of plant development at cutting, the aim of the research was to determine the influence of the application of different amounts of nitrogen fertilizer and the stage of plant development at cutting on dry mass yield, crude proteins and grassland microbial activity. The results of the research show that the stage of plant development at cutting and the application of different amounts of nitrogen have a significant impact on the dry mass and crude protein yields. The dry matter yield ranged from 7.54 t/ha<sup>-1</sup> in the N<sub>0</sub> variant (grass earing stage) to 13.19 t/ha<sup>-1</sup> in the N<sub>90</sub> variant (grass flowering stage). Cutting at the grass earing stage can produce a considerably higher amount of good quality forage mass from the grassland, as well as the application of 50 to 70 kg/ha<sup>-1</sup> of nitrogen. The examined parameter of soil microbial activity showed dependence on the development stage of plant and the amount of N fertilizer applied. A higher number of bacteria and actinomycetes was found in the flowering stage compared to the earing stage, while the number of fungi was higher in the earing stage compared to the flowering stage in all variants of the experiment.

Keywords: Grasslands, Nitrogen, Plant Development Stage, Dry Mass Yield, Crude Protein Yield.



## PHENOLOGICAL CHARACTERISTICS OF SWEET CHERRIES VARIETIES ON GISELA 6 ROOTSTOCK IN HERZEGOVINA REGION

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## Abstract

The purpose of this study was to determine dynamics and energy flowering and harvest time of sweet cherry (*Prunus avium* L.) cultivars Early Lory, Kordia and Regina grafted on rootstock Gisela 6. The research was conducted in the private plantation near Mostar (Bosnia and Herzegovina) with characterized colluvium soil type, during the period of two years (2020–2021). Obtained results showed significant impact of cultivar and year on researched parameters. The earliest period of blossoming as well as the duration was registered with Early Lory cultivar while the latest blossoming and maturation time were registered with Kordia in both year of study. The impact of meteorological conditions in different years was evident, so the earlier beginning and shorter duration of blossoming in all cultivars was recorded in 2020. In terms of maturation time, the earliest beginning of maturation was recorded in the cultivar Early Lory in both years, while the latest in Kordia cultivar. The shortest maturation duration was recorded in the Regina, while the longest maturation duration in the Kordia in both observed years. Based on the obtained results, it may be concluded that investigated cultivars showed the great adaptability to agro–ecological conditions in Herzegovina region and showed no significant oscillation in relation to dynamics of blossoming and maturation period and that it should be further on investigated in terms of other characteristics.

Keywords: Cultivar, Sweet Cherry, Blossoming Time, Maturation.

## Introduction

The production of sweet cherry in the world, even in Bosnia and Herzegovina, has been constantly increasing for the last 20 years. However, this type of production is still organized on smaller areas and the production potentials of this area have not been sufficiently used.

The largest producers of sweet cherry were Turkey, USA, and Chile in the world in 2019 year. In Europe, 775,983 t were produced on the area of 169,445 ha (average 4.6 t/ha) in 2019. In the same period, in Bosnia and Herzegovina, sweet cherry were grown on 5,097 ha and were produced



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9,379 t (average 1.84 t/ha) (FAOSTAT, 2021). The production of sweet cherry in our country is characterized by low yields. The reason for that is: insufficient knowledge of the adaptation of cultivars and rootstocks to agro–ecological conditions, as well as the low degree of production intensity.

Until the beginning of this century, production of sweet cherry in Bosnia and Herzegovina was based on the old cultivars, as well as and generative rootstocks which characterized by high vigor, difficult harvesting and high costs of production (Drkenda et al., 2012).

To intensify sweet cherry production, it is necessary to use rootstocks of low to moderate vigor, new cultivars of large fruit, firmer skin, from early to late ripening time, and the application of modern agrotehnical and pomotehnical measures. According to *Garcia–Montiell* (2010) the quality of sweet cherry fruit is defined by fruit weight, color, taste, firmness, sweetness, aroma with significant differences between cultivars. The rootstock as well as type of soil directly affects the vegetative growth, yield, phenological phase and fruit quality (*Usenik et al.*, 2010). The use of rootstocks of low and moderate vigor, allows the lower planting distance, faster fruiting, easier application of agro–technical and pomotehnical measures (Vercammen et al., 2008; Pall et al., 2017). According to Pal et al. (2017) one of the key factors for production of sweet cherry is the combination of cultivars/rootstocks and adaptation to climatic and soil growing conditions.

In the last 20 years, in Herzegovina area, has been working to increase the intensity of sweet cherry production by introducing new cultivars in combination with low to medium vigor rootstocks, such as Gisela 5 and Gisela 6, Colt, et cetera. However, these cultivars/rootstocks combinations have not yet been sufficiently researched in this area.

The phenological characteristics (flowering and harvest date) of sweet cherry depend on the combination of cultivars/rootstocks, type of soil and agroecological growing conditions (Šupljeglav–Jukić et al., 2020). Knowledge of phenological characteristics is important for the correct choice of species, cultivras and pollinators (Lisandru et al., 2017).

Adaptability to climatic and soil conditions is an important criterion when evaluating a new rootstock. This topic has been the subject of research by several authors (Radunić et al., 2008; Aliman and Drkenda, 2009; Usenik et al., 2010; Gadže et al., 2011; Milatović et al., 2013; Aliman et al., 2013; Hadžiabulić et al., 2017; Lisandru et al., 2017; Hasanbegović et al., 2020; Šupljeglav–Jukić et al., 2020; Corneanu et al., 2021).

The aim of this study was research the phenological characteristics (flowering and harvest date) of sweet cherry (*Prunus avium* L.) cultivars Early Lory, Kordia and Regina grafted on rootstock Gisela 6 colluvium soil type in Submediterranean Herzegovina (the southern part of Bosnia and Herzegovina).



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#### **Material and Methods**

A field experiment was conducted in commercial field "Jaffa komerc" Blagaj ( $43^{\circ}16' 58.44$ " N 17°50' 45.16" E; 45 m above mean sea level) in Herzegovina during the period of two years (2020–2021). The orchard was planted in 2014. All tested cultivars (Early Lory, Kordia and Regina) were grafted on rootstock GiSelA 6 with planting distance of 4 x 3.60 m. Trees were established in a random block design with two trees in three repetitions for each cultivar. The trees were trained as free pyramid system, with support for irrigation. Conventional orchard management practices are implemented (pruning, fertilization, pests and diseases control).

Flowering was recorded by recommendations of the International Working Group for pollination: start of flowering -10% open flowers, full bloom -80% open flowers, end of flowering -90% of the petal fall (*Wertheim*, 1996). The maturation period (start and end of maturation) was considered when fruits developed adequate quality characteristics (color parameters, soluble solids contents, firmness) for the consumption.

### Results and Discussion Meteorological data and characteristics of soil

The average of multi-year (1971–2000) and average monthly temperature and precipitations (January–June (2020–2021)) are shown in Figure 1.

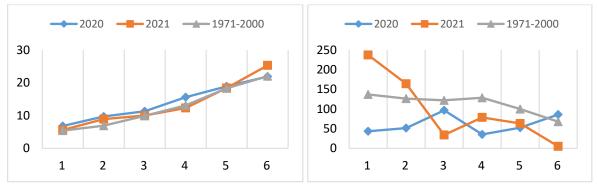


Figure 1. The average of multi-year (1971–2000), average monthly temperature (left) and precipitations (right) (January–June (2020–2021)).

The weather conditions (average monthly temperature and precipitations) have been recorded by the meteorological station in Mostar, (Federal Hydrometeorological Institute Bosnia and Herzegovina). The Herzegovina area is characterized by the average of multi-year (1971–2000) temperature of 14.8 °C and 1439.3 mm of the multiannual sum of precipitations. During the study period of flowering and harvest period of sweet cherry (January–May, 2020–2021), the average temperature of air was warmer in 2020 than in 2021 as well as compared to average multi-year temperature (Figure 1). The largest difference of average monthly temperature in April (3.3 °C warmer in 2020 than in 2021). The total amount of precipitation was highest in January 2021 (237.3



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mm), while in March (33.9 mm) and June (5 mm) in 2021 was deficit of precipitations. Significant differences regarding the average temperature and amount of precipitation were registered in the flowering period in the years of research, what had an impact on the beginning and duration of flowering and harvest date of sweet cherry fruits.

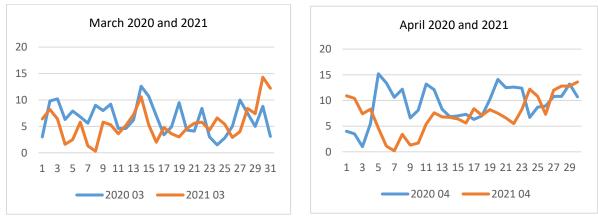


Figure 2. Absolute minimum daily temperatures of air for March and April (2020-2021).

The first two decades of March 2020 were significantly warmer than in March 2021, while the end of March and the beginning of April 2020 had lower absolute minimum daily temperatures compared to the same period in 2021 (Figure 2). From 5. April 2021 there is a sudden drop in temperature. The air temperature of 0.2 °C was recorded 6. April 2021 and this trend of lower temperatures compared to 2020 continues until towards the end of this month. All this affected the phenophase of flowering.

The soil at the Blagaj site belongs to the alluvial-colluvial soils with a predominance of colluvial material. They are water-permeable, well aerated with stable microaggregates. They do not hold water well, so irrigation is a necessary agro-ameliorative measure. Results of textural composition of the soil at the experimental orchard in Blagaj are shown in table 1.

Donth (am)	Textura	Texture mark		
Depth (cm)	Sand	Clay	Silt	(USDA)
0-30	69	23	8	Sandy Loam
30-60	66	25	9	Sandy Loam

Table 1. Textural composition of the soil at the experimental orchard in Blagaj

According to USDA classification, the soil experimental orchard belongs to sandy clay loam (table 1). Table 2 shows the chemical properties of the soil of the experimental orchard.



Soil type	Colluvial soil				
Depth (cm)	0–30	0–60			
pH in H <sub>2</sub> O	8.04	8.16			
pH in KCl	7.35	7.39			
Total CaCo <sub>3</sub> (%)	42.32	39.73			
Active Ca(%)	4.25	4.25			
Humus (%)	5.12	3.78			
Total N(%)	0.34	0.31			
Active $P_2O_5$ (mg/100 g)	9.49	9.21			
Active K <sub>2</sub> O (mg/100 g)	47.76	44.90			

Table 2. Chemical properties of soil at the experimental locality Blagaj

According pH values in H<sub>2</sub>O and KCl solution, the tested soil is moderately alkaline reaction (Soil Survey Manual, 1993) (Table 2). The content of total CaCO<sub>3</sub> (42.32 % and 39.73%, respectively) indicates a strong carbonate soil. The active calcium content was 4.25%. The humus content was from 3.78% (30–60 cm) to 5.12% (0–30 cm). According Gračaninu (1947), the land in the orchard belongs to the class of quite humus soils. Soil analysis showed a high to very high amount of available potassium (44.9–47.76 mg K<sub>2</sub>O/100 g), while it was poor in available phosphorus content (9.21–9.49 %) (Giandon, 2000; Vukadinović and Vukadinović, 2011).

#### Phenological phase of flowering

Data of phenological phases of flowering of the examined sweet cherry cultivars are shown in table 3.

Phenological phases of	Early Lory		Kordia		Regina	
flowering	2020	2021	2020	2021	2020	2021
Start	18.3.	20.3.	6.4.	1.4.	10.4.	3.4.
Full	21.3.	24.3.	9.4.	5.4.	13.4.	6.4.
End	28.3.	1.4.	21.4.	17.4.	22.4.	18.4.
Duration (days)	10	12	15	16	12	15

Table 3. Phenological phases of flowering of sweet cherry cultivars in Herzegovina area (2020–2021)

The start of flowering depends on the cultivar and climatic characteristics of the growing area, and can vary from year to year. The beginning of flowering is also influenced by air temperatures before flowering, as well as temperatures during the period of winter dormancy. According Štampar (1966), flowering of sweet cherry begins at an average daily temperature of 8 °C to 15 °C, depending on the cultivar.

The start of flowering in 2020 was from 18.3. (Early Lory) to 10.4. (Regina), while in 2021 it was from 20.3. to 3.4. The variation of the start of flowering between the cultivars in 2020 was 23 days, and in 2021 was 14 days. The variations by years were 2–7 days. Based on the phenophase of the start of flowering, we can conclude that this phenophase is influenced by the cultivar and meteorological conditions in the years of study.



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The full of flowering occurred in three–four days in cultivar Early Lory and Kordia, depending on the year of study, while in the cultivar Regina full of flowering occurred within three days of the start of flowering in both years of study (table 3). In accordance with the start and full flowering, there was and the end of flowering. Namely, the earliest end of flowering was in the cultivar Early Lory (28.3., respectively 1.4.), then the cultivar Kordia (21.4., respectively 17.4.), while the latest end of flowering was in the cultivar Regina (22.4. respectively 18.4.).

The shortest duration of flowering was in the cultivar Early Lory (ten days in 2020), while the the longest flowering duration was in cultivar Kordia (16 days in 2021).

Our results for period from start to full of flowering, as well as duration of are in agreement with the results of the author Nyéki and Soltész (1996). Previous authors reported that the sweet cherry is characterized by a short period between the start and full of flowering, and the duration of flowering from 6 to 15 days. On the other hand, our results for the cultivar Kordia are in agreement with the results of the authors Corneanu et al. (2020).

Compared to the results of Milatović et al. (2013), Radičević et al. (2016) and Serbezova (2019) the beginning of flowering of the cultivar Early Lory was earlier in agroecological conditions of Herzegovina area.

Results of start of flowering in the cultivar Kordia are in accordance with the previous findings (Radičević et al., 2011; Milatović et al., 2013; Milić et al., 2019; Serbezova, 2019; Corneanu et al., 2020). The obtained results of start of flowering in the cultivar Kordia are not in accordance with the results of Stojanović et al. (2012) and Blažková et al. (2010) which record later date the start of flowering this cultivar. These variations are due to the mild climatic characteristics of the areas in which our research was conducted.

The obtained results of start of flowering in the cultivar Regina are in accordance with the previous findings (Radičević et al., 2011; Milić et al., 2019). On the other hand, we recorded the earlier date the start of flowering in cultivar Regina compared to previous research authors Stojanović et al. (2013) and Serbezova (2019).

#### Phenological phase of maturation

Data of phenological phases of maturation of the examined sweet cherry cultivars are shown in table 4.

Phenological phases of	Early Lory		Kordia		Regina	
maturation	2020	2021	2020	2021	2020	2021
Start	1.5.	12.5.	15.5.	27.5.	18.5.	29.5.
End	19.5.	29.5.	2.6.	12.6.	6.6.	18.6.
Duration (day)	18	17	18	16	19	20

Table 4. Phenological phases of maturation of sweet cherry cultivars in Herzegovina area (2020–2021)



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The start of maturation in 2020 was from 1.5. (Early Lory) to 18.5. (Regina), while in 2021 it was from 12.5. to 29.5. The differences in the start of maturation between the years were 11–12 days. Namely, a significant influence of the year was established, because the weather conditions were significantly different in the years of the study. According to the time of maturation, the cultivar Early Lory is the early cultivar, and the Kordia and Regina are late cultivars (Bassi, 2010). The cultivar Early Lory had the earlier date of maturation in regards to previous findings (Radičević et al., 2011; Milatović et al. 2013).

The date of start of maturation in cultivar Kordia varied depending on the year, even 12 days (15.5.2020. and 27.5.2021.). The cultivar Kordia had the earlier date of maturation compared to the results of the authors (Blažkova et al., 2010; Milatović et al., 2013; Stojanović et al., 2013; Milić et al., 2019).

The cultivar Regina had the latest date of maturation and was recorded differences of 11 days between test years (18.5., respectively 29.5.). The obtained results of time of maturation in the cultivar Regina are not in accordance with the results of Radičević et al. (2011), Stojanović et al. (2013), Milić et al. (2019) and Serbezova (2019) which record later date the maturation this cultivar.

The earlier date of maturation of cultivar covered by the research, compared with the results of other authors may be due to the climatic characteristics of the study area, as well as the type of soil because it is a warm, skeletal soil (colluvium).

## Conclusions

Based on two-year studies of the phenology of flowering and maturation of sweet cherries, we can conclude the following:

- Sweet cherry is a species that is very sensitive to temperature of ait in the period before flowering and during flowering;
- The phenosase of flowering was influenced by cultivar and meteorological characteristics in the years of study;
- These cultivars have not yet been sufficiently researched in Herzegovina area, so it is recommended to investigate their adaptation to the Herzegovina area, as well as compatibility with different rootstocks;
- Also, climate change is evident, which includes warmer winters and a greater possibility of late spring frosts, and knowledge of the phenology of flowering of the introduced cultivars in the given ecological conditions is of exceptional importance.

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