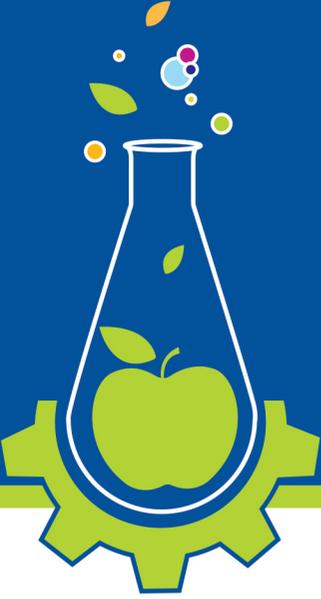




**ISTANBUL AYDIN UNIVERSITY**  
**ENGINEERING FACULTY**



# IJFER

**INTERNATIONAL JOURNAL OF  
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## **Aims and Scope**

International Journal of Food Engineering Research (IJFER) is an international , peer-reviewed journal devoted to the publication of high quality original studies and reviews concerning a broad and comprehensive view of fundamental and applied research in food science&technology and their related subjects as nutrition, agriculture, food safety, food originated diseases and economic aspects.

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## ***From The Editor***

*Istanbul Aydin University Faculty of Engineering has started to publish an international journal on Food Engineering, denoted as “International Journal of Food Engineering Research (IJFER)”. We have especially selected the scientific areas which will cover future prospective food engineering titles such as Food Processing, Food Preservation, Novel Technologies, Food Safety, Food Quality etc. and their related subjects as nutrition, food and health, agriculture, economic aspects and sustainability in food production.*

*We have selected only a few of the manuscripts to be published after a peer review process on many submitted studies. Editorial members aim to establish an international journal IJFER, which will be welcomed by Engineering Index (EI) and Science Citation Index (SCI) in short period of time.*

***Editor in Chief***  
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# International Journal of Food Engineering Research (IJFER)

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# INVESTIGATION OF LEAD, CADMIUM, ARSENIC AND MERCURY IN SOME SEAFOOD FROM BLACK SEA AND MARMARA SEA BY ICP-MASS SPECTROMETRY

Gizem İNAL ERDEM<sup>1</sup>, Haydar ÖZPINAR<sup>2\*</sup>

## Abstract

Over the last 200 year our planet has been under a threat due to industrialization and environmental pollution. Accordingly, all the species living in our environmentally polluted planet is potentially a major source of health risk. This situation is also directly leading to making the seafood and other species of waterborne is contaminated with the industrial pollutants such as heavy metals. The contaminated foods are suitable for the dissemination of these carcinogenic chemicals through the consumers. Heavy metals are the members of the third or higher rows of the periodic scale. In the present time, there are many heavy metals more than 60 such as lead, cadmium, chromium, cobalt, copper, nickel, mercury and zinc. The objective of this study was to analyze Pb, Cd, Hg, As in a total of 150 seafoods (10 shrimp, 10 calamari, 10 octopus, 10 mussel, 10 acorn, 10 mackerel, 10 coral, 10 sea bream, 10 whiting, 10 sole, 5 horse mackerel, 5 anchovy, 5 sardine, 5 bluefish, 5 gray mullet, 5 turbot, 5 red mullet, 5 coral, 5 bream, 5 butts fish) from Marmara Sea (n=75) and Black sea (n=75) by using Inductively Coupled Plasma-Mass Spectrometer ICP-MS according to the criterias by both Turkish Food Codex Declaration About Maximum Limits of Contaminants in Food Products (Declaration No: 2008/26) and NMKL 186 International method. The results revealed that the lead levels in both horse mackerel (0,385 ppm) and mullet (0,387 ppm) , and the mercury levels in both red mullet (1,707 ppm) and sea bream (1,098 ppm) from the Black sea were found to be higher, whereas the lead levels in both sardine (0,417 ppm) and butts fish (0,843 ppm) from Marmara Sea were higher than the upper limits as declared by Turkish Food Codex. In the mussels collected from Marmara Sea, the cadmium levels were found to be nearly close to the upper limit, which was 1 ppm. Also, it was provided that all the samples contained arsenic. However, no comment was performed since the Turkish Food Codex does not indicate any upper limit for itself. In Conclusion, this study provided that the seafoods collected from both Black sea and Marmara Sea significantly included heavy metals, including lead, mercury, cadmium, and arsenic. This situation leads to a significant foodborne health risk for the public health and the Turkish consumers.

**Keywords:** heavy metal, fish, blacksea, cadmium, arsenic, mercury, lead, ICP-MS

## 1. Introduction

The environmental levels of chemicals have risen steadily with human population growth, urbanization, and continued worldwide industrialization. All these toxic and inevitable pollutants adversely affect the species, threaten the human health, and lead to a harmful situation which the nature is not able to overcome [1]. The heavy metals are considered to be extremely toxic elements that have negative impacts on

chemical and physiological processes especially in aquatic organisms and humans. There is raising concern regarding the undesired health effects of different metals. Thus, their control, uptake, bioaccumulation, storage and elimination are important for the human health [2].

Heavy metal toxicity can produce serious chronic conditions such as autism, chronic fatigue syndrome, depression, multiple sclerosis, and other

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serious disorders [3]. Zinc, copper, chromium, iron and manganese are essential to body function in small amounts. But, if these metals accumulate in the body in higher concentrations, then serious damages can develop. The major heavy metals associated with poisoning of humans are lead, mercury, arsenic and cadmium. Heavy metal poisoning may occur as a result of industrial exposure, air or water pollution, and foods [4]. Excessive accumulation of dietary heavy metals such as Cd, Cr, and Pb in the human body may be a result of consumption of fishes and other seafoods [5-7]. Seafood is a valuable food in the human nutrition because it consists of protein, fats, vitamins, and minerals. Therefore, seafood has numerous health benefits. For example, recent studies have indicated that regular consumption of seafood can decrease the risk of heart attack, stroke, obesity, and hypertension. Seafood also provides essential nutrients such as omega-3 fatty acids [8].

In the recent years, the levels of heavy metals in fish have been investigated in different parts of the world. Most of these studies concentrated mainly on the heavy metals in muscles, liver, kidneys, heart, gonads, bone, digestive tract and brain [9,10]. Official regulatory institutions of many countries are responsible for inspection of seafood products for heavy metal residues, also including checks for proper labeling and documentation, sensory evaluations (e.g., visual, olfactory), and laboratory screening for other contaminants such as PCBs, toxins, and microbial pathogens [7,11]. Maximum levels have been set for certain contaminants in order to protect public health in the EU in the year 2006 (EU Commission Regulation: 852/2004/EC, 853/2004/EC, 854/2004/EC ve 882/2004/EC), as well as the guidelines set down by the Ministry of Agriculture, Fisheries and Food, the Turkish Food Codex in Turkey as of 2010 [12,13].

In this study, we investigated Pb, Cd, Hg, As in a total of 150 seafoods (10 shrimp, 10 calamari, 10 octopus, 10 mussel, 10 acorn, 10 mackerel, 10 coral, 10 sea bream, 10 whiting, 10 sole, 5 horse mackerel, 5 anchovy, 5 sardine, 5 bluefish, 5 gray

mullet, 5 turbot, 5 red mullet, 5 coral, 5 bream, 5 butts fish) from Marmara Sea (n=75) and Black sea (n=75) by using Inductively Coupled Plasma-Mass Spectrometer ICP-MS according to the criterias by both Turkish Food Codex Declaration About Maximum Limits of Contaminants in Food Products (Declaration No: 2008/26) and NMKL 186 International method.

## 2. Material and Methods

### **Sampling**

During the year 2015, a total of 150 seafoods (10 shrimp, 10 calamari, 10 octopus, 10 mussel, 10 acorn, 10 mackerel, 10 coral, 10 sea bream, 10 whiting, 10 sole, 5 horse mackerel, 5 anchovy, 5 sardine, 5 bluefish, 5 gray mullet, 5 turbot, 5 red mullet, 5 coral, 5 bream, 5 butts fish) from Marmara Sea (n=75) and Black sea (n=75) was collected. The samples were taken to the laboratory in the a sample carry case (JPB, UK) at 4°C. Then, the analysis was started in the same day (Table 1).

**Table 1.** Sampling data

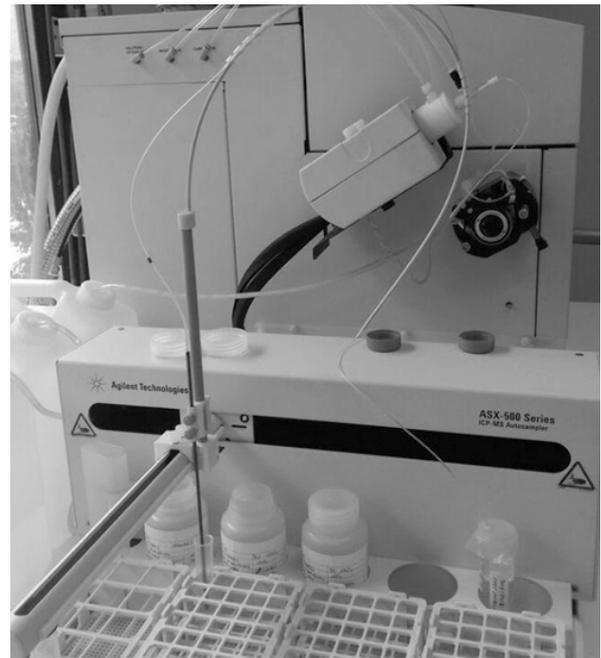
Ground fish	Surface fish	Others
Turbot	Bonito	Shrimp
Bluefish	Anchovy	Calamari
Red mullet	Horse mackerel	Octopus
Young blue fish	Sardine	Mussel
Mullet	Atlantic mackerel	
Seabream		
Picarel		
Black bream		
Grey mullet		
Haddock		
Sole		

### **Sample preparation**

The collected samples were initially homogenized using blender (Interscience, Germany). Subsequently, the homogenized sample was burned in a microwave device (CEM, USA) before the analysis in the Coupled Plasma-Mass Spectrometer (ICP-MS). A 0.2-0.5 g of the burned sample was weighed (MS104TS Mettler Toledo, Turkey) in a vessel. Then, 5 ml of Nitric acid (HNO<sub>3</sub>) (Merck, Turkey) and 2.5 ml hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (Merck, Turkey) were pipetted into the sample, respectively. The mixture was then re-burned in microwave device for 70°C/15 min, 85°C/10 min, 105°C/10 min, 110°C/5 min, 120°C/5 min, and finally 130°C/5 min, respectively. After that, the vessel was allowed for cooling in the room conditions for a while. After cooling, the solution in the burned vessel was transferred to a 25 ml flask. Then, deionized water was poured on to it till the final volume in the flask became 25 ml.

### **Analysis of Pb, Cd, Hg, and As by ICP-MS**

The analysis of Pb, Cd, Hg, and As in the seafood samples was performed by using ICP- MS Agilent 7700X (Agilent, Turkey) according to the Guidelines of NMKL 186 (2007) instructions. Before starting the analysis, calibration blank solution, calibration standards, sample blank, control standards, and control samples were all prepared accordingly. Then, a 10 ml of the solution in the 25 ml flask was put into a 10 ml propylen tube. All the solutions were placed in to the ICP-MS device. The reading was started, completed, and the results were automatically processed by the software.



**Figure 1.** ICP-MS device

### **3. Results**

In this study, a total of 150 seafood samples from Marmara Sea (n=75) and Black sea (n=75) was analysed for the presence of Pb, Hg, Cd and As by using ICP-MS device according to the Guidelines of NMKL 186 (2007) procedures.

#### **Heavy metal levels in the analyzed seafood samples from Black Sea**

The Hg level in red mullet (1.707 ppm>1 ppm) and black bream (1.098 ppm>0,5 ppm), and the Pb level in Horse mackerel (0.358 ppm>0.3 ppm) and mullet (0.387 ppm>0.3 ppm) were found to be higher than the limits declared by the Turkish Food Codex. It was also determined that all the fish samples from Black Sea included As higher than the limit given by the China (0.15 ppm).

Also, Hg was not detected in the other seafoods such as shrimp, calamari, octopus, and mussel samples, while the average levels of Cd, Pb, and As were found as 0.245 ppm, 0.347 ppm and 32.932 ppm, respectively. These data were seen to be lower than the upper limits of Turkish Food Codex, excluding the As level given as 0.15 ppm by China.

**Heavy metal levels in the analyzed seafood samples from Marmara Sea**

The Pb level in sardine (0.417 ppm>0.3 ppm) and picarel (0.843 ppm>0.5 ppm) were found to be higher than the limits declared by the Turkish Food Codex. It was also determined that all the seafood samples from Marmara Sea included As higher than the limit given by the China (0.15 ppm).

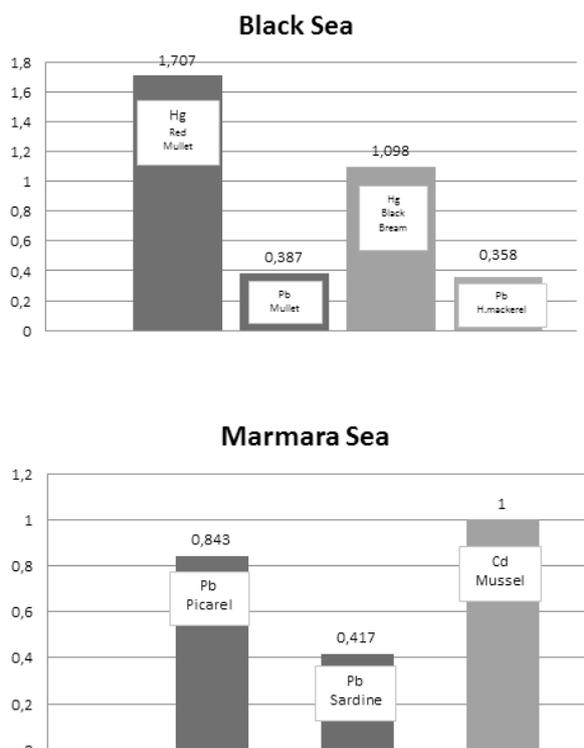
However, Hg was not detected in the other seafoods such as shrimp, calamari, octopus, and mussel samples, while the average levels of Cd, Pb, and As were found as 0.384 ppm, 0.440 ppm and 30.824 ppm, respectively. Only mussel samples contained Cd close to the upper limit by the Turkish Food Codex as 1 ppm.

**Overall results**

The results, higher than the upper limits of the Turkish Food Codex and China, belonging to the levels of heavy metals present in the analyzed seafoods were tabulated in Table 2 and Figure 2. Accordingly,

**Table 2.** The levels exceeding the upper limits in the analyzed seafoods

Origin	Ground/Surface	Type	Hg (ppm)	Cd (ppm)	Pb (ppm)	As (ppm)
Black Sea	Ground fish	Red mullet	1.707	-	-	All above Chinese limit 0.500 ppm
		Mullet	-	-	0.387	
		Black bream	1.098	-	-	
	Surface fish	Horse mackerel	-	-	0.358	
Marmara Sea	Ground fish	Picarel	-	-	0.843	
	Surface fish	Sardine	-	-	0.417	
	Others	Mussel	-	1.000	-	



**Figure 2.** Comparison of overlimit results of the seafoods from Black Sea and Marmara Sea

**4. Discussion and Conclusions**

In this study, we investigated the presence of major heavy metals such as Hg, Pb, Cd and As in a total of 150 seafood samples from Marmara Sea (n=75) and Black sea (n=75) by using ICP-MS device according to the Guidelines of NMKL 186 (2007) procedures.

The results revealed that Hg level in red mullet (1.707 ppm>1 ppm) and black bream (1.098 ppm>0,5 ppm), and the Pb level in Horse mackerel (0.358 ppm>0.3 ppm) and mullet (0.387 ppm>0.3 ppm) from Black Sea, and that in sardine (0.417 ppm>0.3 ppm) and picarel (0.843 ppm>0.5 ppm) from Marmara Sea were found to be higher than the limits declared by the Turkish Food Codex. All the fish samples were found to be contaminated with As over the upper tolerable limit as 0.15 ppm by China. The other seafoods such as shrimp, calamari, octopus, and mussel were free of Hg, and had low levels of Pb and Cd except for the mussel samples from Marmara Sea (1 ppm). The

As levels in all other seafoods were significantly higher than the upper limit set out by China.

Heavy metals are significant environmental pollutants and their toxicity is an increasing issue significant for ecological, evolutionary, nutritional and environmental reasons. Various public health measures are undertaken to control and prevent metal toxicity at various levels, such as occupational exposure, accidents and environmental factors. Thus, metal toxicity is dependent on the dose and duration of exposure, i.e. acute or chronic. [14].

Arsenic (As) is a protoplasmic poison. It affects primarily the sulphhydryl group of cells causing malfunctioning of cell respiration, cell enzymes and mitosis[15-17]. Lead (Pb) creates toxicity in living cells in the organisms following ionic mechanism and that of oxidative stress [18]. Mercury (Hg) is extremely toxic element and bioaccumulative[18]. Cadmium (Cd) is the seventh most toxic heavy metal as per ATSDR ranking [19,20].

The literature has showed that the level of As in foods ranges from 0.020 to 0.140 ppm. An adult person may be exposed to 0.050 ppm As a day. The Environmental Protection Agency of the United States of America (EPA) issued some upper limits for As in the year 2006. According to the criteria, this limit in water has been restricted with 0.010 ppm. However, there has been no limit for water in Turkey yet. Similarly, many countries have not set down any limit for major heavy metals in all types of foods. For instance, the limit for As in water in the USA was set as 0.010 ppm while it has not been made for other foods. China has determined the upper limit for As in water as 0.150 ppm. The United Kingdom ruled 1 ppm for As in infant foods (rice products) for those younger than 54 months. In the New Zealand and Australia, a WHO also known as EOS published a report dated on the year 2012 regarding the upper acceptable limits for As in fish and other seafoods as 2 ppm, respectively (<http://ecan.govt.nz/publications/Reports/heavy-metals-fish-shellfish-2012-survey.pdf>). Based on

this reference value, our fish samples from the Black Sea ranged in between 0.729-68.288 ppm whereas those for the Marmara Sea was in a range of 0.504-65.554 ppm. The highest As levels were found in shrimp, calamari and octopus samples. Among the analyzed fish samples the highest frequency for As was detected as 30.682 ppm in the red mullet samples only from the Black Sea. As compared to the international upper limits for As, our samples were highly contaminated with As.

Lead metal causes toxicity in living cells by following ionic mechanism and that of oxidative stress [21]. Our study provided that the Pb levels in the samples from Black Sea and Marmara Sea were 0-0.539 ppm and 0-0.843 ppm, respectively. According to our results, the seafoods from Marmara Sea included Pb higher than Black Sea. Among the analyzed foods, the other seafoods such as shrimp, calamari, octopus and mussel had reasonable Pb levels less than the limits as declared by Turkish Food Codex, except for As levels as compared to China's limit (0.15 ppm). On the other hand, mullet (0.387 ppm) and horse mackerel (0.385) from Black Sea, and sardine (0.417 ppm) exceed over the tolerable upper limit of Pb (0.300 ppm). Our study revealed that the Marmara Sea was exposed to a much Pb pollution worse than Black Sea. These foods are concern of a public health risk for the consumers.

Our study indicated that the Hg levels in the examined seafoods exhibited interesting differences. Among the samples from Marmara Sea the Hg levels were ranged in 0 to 0.564 ppm. The average of these measurements were higher than the acceptable level as given by the Turkish Food Codex. The fishes which contained Hg higher than the limits were determined to red mullet (1.707 ppm) and black bream (1.098 ppm). However, any Hg was not detected in shrimp, calamari, octopus and mussel samples. According to the statistical facts issued by American Food and Drug Administration (FDA), our samples had the Hg higher than the limits by FDA and other international authorities.

Our study showed that all the analyzed seafoods did exhibit any potential risk for the consumers because the Cd levels among all the samples were less than the tolerable level as declared by the International authorities, as well as the Turkish Food Codex. The Cd levels in Black sea and Marmara sea were given as 0-0.626 ppm and 0-1 ppm. The highest level was observed as 1 ppm in the mussel samples collected from Marmara sea.

In conclusion, we showed that certain fish samples and other seafoods from Black sea and Marmara sea included harmful heavy metals such as Pb, Cd, Hg and As in a various levels of contamination. Thus, the consumers are under risk if they consume them in excess amounts.

#### **CONFLICT OF INTEREST**

The authors declare that there are no conflicts of interest.

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### HAYDAR ÖZPINAR

Prof. Dr. Haydar Özpınar received his veterinary degree from Ankara University in Turkey in 1978. That same year he began to work as research assistant in İstanbul University. Soon after he received a Ph. D. scholarship from German Academic Exchange Program DAAD, and in 1984 he finished his Ph. D. at the Department of Nutrition Physiology of Ludwig Maximilians University in Munich, Germany. After that, he returned to the Department of Nutrition and Nutritional Disease at Veterinary Faculty of İstanbul University. During his scientific career he received quite a few scholarships including those from European Union, DAAD, Alexander von Humboldt Foundation and USA Fulbright. In 2004, he achieved a Fulbright scholarship, and went to University of California Davis, CA, USA. Until 2008, he studied in nutrition, immunology and genetics. He was also involved in a NIH Project. He is currently the director of Institute of Natural and Applied Sciences, a full-time faculty member at Food Engineering Department of İstanbul Aydın University, and also Chair of Food Safety Department.



# CHESTNUT FLOUR AND APPLICATIONS OF UTILIZATION

Merve METE<sup>1</sup>, Dilek DÜLGER ALTINER<sup>2\*</sup>

## Abstract

In recent years, with the increasing of people's interest in nutrition and health subjects, importance of nutrition has been increased steadily and number of studies on enrichment of foods and production of functional products have been increased. Enrichment of foods is one of the developed applications for solution of health problems which are likely to be seen in society. For this purpose, various foods having various properties are being developed with the use of different fruits and vegetables' flours.

Chestnut has a rich nutrition items, it has been used since previous times in nutrition and in daily diets due to being grown at natural conditions as well. Basically, being composed of carbohydrate, water, and a very low amount of fat, chestnut shows cereal features rather than fruit. When grinded, chestnut becomes a light-colored flour. This flour is used in puddings, bakery products, and production of bread, breakfast cereals, soup and sauces.

In food industry, there are intensive studies on the production of especially cereal products with different wheat flour and different flour additives. In the light of these studies, some Europe countries, mainly in Italy, especially use of chestnut is being widespread in commercial scale in the production of biscuit, breakfast cereal, muffin and dessert. Instead of wheat flour chestnut flour is used or added in different substitution rates. Within the scope of this review, properties of chestnut fruit, nutritional facts of chestnut, chestnut flour and properties, utilization of chestnut flour in food industry were discussed.

**Keywords:** Chestnut flour, cereal products, nutrition, enrichment

## 1. Introduction

Enrichment of foods is the addition of one or more nutrition items that are insufficient or absent and is to improve the properties. Research for the investigation of functional, nutritious and sensory quality of properties is increasing day by day. Main purpose of the production of food is to provide people with healthy and happy lives delivering safe and high nutrition value foods. Recent adaptation of people for a healthy and balanced life style and increased awareness have added strength to improvements in functional food market.

Therefore, there are studies going on for development of alternative flour additives and for usage of them in foods. Beyond supplying needs

of basic nutrition items in the body, foods or food components that provide additional benefits to human physiology and metabolic functions and that are more affective in succeeding a healthier life should be produced. In the food industry, many studies have been carried out, especially on production of cereal-based functional foods. In this review, chestnut flour, limited in production and used for product development, and studies for its usage are were discussed.

## Chestnut fruit and its properties

Chestnut is a closed-shell fruit of trees that form *Castanea* genus from the *Fagaceae* family and forming the edible seeds of this trees. Leaves of chestnut are a bit stiff, the edges are saw tooth shaped and these teeth have thorns. Being

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members of the *Fagaceae* family, chestnuts (*Castanea*) can live for many years and reach up to 30-35 meters in length (Subaşı, 2004; Atasoy and Altıngöz, 2012).



**Figure 1.** Chestnut fruit

Cool, moist and temperate place are growing areas for chestnut fruit. Therefore, Asia (China, Korea, Japan), Turkey, South Europe and North America are substantial places for production (Bodet et al., 2001). Turkey is one of the substantial areas of chestnut, and it is spread more likely in Aegean, Mediterranean and Black sea regions (Soylu, 1984; İnkaya, 2008).

Being grown completely in natural conditions and an organic food item, chestnut has been an irreplaceable part of our nutrition list. That is why chestnut tree, in some regions, is known as “the bread tree” due to high nutrition value of its fruit (Bounous et al., 2000; İnkaya, 2008). Fresh chestnut has primarily starch and various polysaccharides, fiber materials with high quality, protein, fat in low amount, various mineral materials, B1, B2 and C vitamins. It also contains potassium, phosphorus, magnesium, chlorine, iron, sodium minerals, too (Sachetti et al., 2004; Chenlo et al., 2007).

Following is the composition of chestnut fruit grown in Turkey (Table 1).

**Table 1.** Composition of chestnut fruits grown in Sinop-Erfelek Region of Turkey (in 100 gr) (Subaşı, 2004)

Analysis	Minimum Value	Maximum Value	Average Value
Shell Thickness (mm)	0.30	0.71	0.51
Protein (N. X. 5.30) g	3.43	8.27	5.683
Fat g	0.66	3.08	1.89
Starch g	29.88	63.66	47.32
Fiber g	0.06	0.29	0.129
Ash g	1.40	4.92	2.809
Phosphorus mg	47.68	229.68	133.67
Calcium mg	69.71	201.70	87.863
Magnesium mg	59.71	202.89	105.87
Zinc mg	2.63	21.87	6.970
Iron mg	1.84	16.99	6.684
Copper mg	0.33	1.29	0.738

Chestnut fruit, in general has 40-45% water, 3-6% protein, 3-5% fat, 40-45% carbohydrate, 1.3% ash. However, these values may vary based on the ecological conditions, type, genus and process (Soylu, 2004). In the following Table 2, composition of chestnut fruit in different conditions and nutrition items are illustrated (Subaşı, 2004; Atasay and Altıngöz, 2012).

**Table 2.** Composition of chestnut fruit in different conditions and nutrition items (in 100 g) (Subaşı, 2004; Atasay and Altıngöz, 2012)

Analysis	Fresh	Dried	Fried	Scalded	Flour
Carbohydrates	34	57.8	39.0	24.4	63.6
Sugars	9.6	16.1	10.7	7.5	23.6
Starch	24.4	41.7	28.3	16.9	40
Fiber	7.3	13.8	8.3	5.4	14.2
Soluble	0.6	1.1	0.7	0.6	1.0
Insoluble	6.7	12.7	7.6	4.8	13.2
Protein	3.2	6.0	3.7	2.5	6.1
Fat	1.8	3.4	2.4	1.3	3.7
Moisture (%)	52.9	10.1	42.4	63.3	11.4
Calori (kcal)	160	287	200	120	343

Even though in stiff-shell fruits the fat content is high, in chestnut carbohydrate content is high. In their study, Ertan and Kılınc (2005) found carbohydrate values between 24.53 and 31.56 g/100g. Some researchers found carbohydrate contents, based on the genus of chestnut, as in the range of 71.68-88.10 g/100g (Bounous et al., 2000, Kunsch et al., 1999, McCarthy and Meredith, 1988; İnkaya, 2008).

In another study, sugar and starch rates are found as 60-70% and 20-30%, respectively (De La Montaña-Miguel et al., 2004, Moreira et al., 2012). Most of the sugar content constitutes saccharose and saccharose contents of different varieties are found generally 8-20 g/100g (Kunsch et al., 2001; Senter et al., 1994).

Neri et al., (2010), starch content in fresh chestnut is around 71% and sugar (especially sucrose, glucose, fructose) content is varying between 9.2 and 23%. While starch in the fruit releases its unique taste when cooked, sugars provide the sensory properties to be felt (Riberio et al., 2007; Miquelez et al., 2004).

Pereira Lorenzo et al. (2006) investigated that chestnut has a lower but a high-quality fat content compared to other nuts. Yang et al. (2015) revealed that the content in 10 different chestnuts grown in China is 4.3-10.2% while Neri et al. (2010) investigated that the fat content is varying between 3.27-4.15% in a chestnut genus grown in Italy. Besides of this, chestnut has significant fatty acids in addition to low fat content. In a conducted study, researchers determined 7 different organic acids in chestnut. These are citric acid, oleic, oxalic, quinic, ascorbic, cis-aconitic and fumaric acids Ribeiro et al., 2007). These organic acids are stated as beneficial for human health due to their antioxidant capacities.

Minerals are significant in healthy and balanced nutrition. Chestnut is a significant food item in terms of potassium while it contains minerals like iron, calcium, magnesium, manganese, potassium, phosphorus and zinc. In a conducted study, it

was investigated that the amount of potassium in chestnut is between 473 and 974 mg/100g. Phosphorus is the second most important mineral in chestnut and its amount is between 104 and 148 mg/100g in chestnut. Potassium is an essential mineral for protein synthesis while it provides liquid balance in the body. Phosphorus is an important mineral in terms of development of bone and tooth (İnkaya, 2008).

Kunsch et al. (1999) determined the calcium amount as 0.017-0.033% and phosphorus as 0.050-0.068% in their study. Calcium is a co-mineral for the use of iron in the body and for the diffusion of foods across cell membrane.

Magnesium content of chestnut is varying between 63 and 93 mg/100g while calcium is varying between 41 and 51 mg/100g (Bounous ve diğ., 2000, Ferreira-Cardoso ve diğ., 1993; Neri ve diğ.,2010). Magnesium is a significant mineral playing a role in the relaxation of neural system and muscles.

Chestnut is one of the most fiber-containing nuts. Fresh chestnut contains 8-10 g/100g fiber material (Anonymous, 2016a). Dietary fibers, as well as their functional and technological properties, are the components that have protecting effects on diseases such as diverticulum, constipation, colon cancer, obesity, diabetes and cardiovascular (Dülger and Şahan, 2011).

Chestnut flour is being used widely in cake, cookie, pasta, milky pudding products, bread, breakfast cereals, soups, sauce and gravies. As a result, interest in chestnut is increasing day by day due to the potential effects of it on nutrition quality and health.

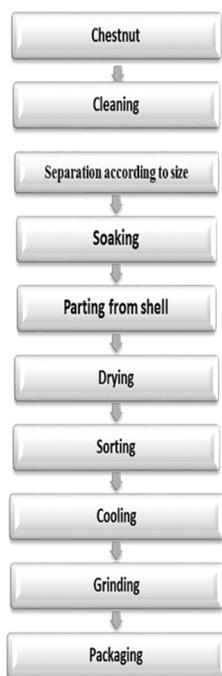
## **2. Chestnut flour production**

Chestnut flour is gluten-free flour that does not contain additive addition, is prepared naturally, by freeze drying or scalding drying methods (Seferoğlu, 2012).



**Figure 2.** Chestnut Flour

In the first step of chestnut flour production, chestnuts are cleaned and separated with respect to their sizes (Figure 2). They are immersed in water for 1 day. Therefore, they easily peeled from their shells. The grinding process is performed after softened chestnuts are separated from the shells by the steam pressure system. Drying process is performed in low-temperature drying ovens so that nutrition values in chestnuts are not affected. Following the drying process, cooling process is performed at room temperature and flour is sifted through appropriate-sized sieves and the packaging process is started. Storage is done at normal conditions (+4°C) (İnkaya, 2008).



**Figure 3.** Chestnut flour production steps

### 3. Chestnut flour and studies for utilization

Chestnut flour can be used for celiac patients due to being gluten-free as well as its nutritious properties. Some researchers investigated that chestnut flour can be used as an alternative to cow milk in the preparation of dessert and soups that are suitable for kids since lactose in cow milk has allergic effects for kids. Chestnut flour also can be used in the preparation of milky puddings, bread, baby formulas, pasta, flakes (corn flakes) (Anonymous, 2016b).

Chestnut flour contains high protein content, high amount sugar (20-32%), starch (50-60%), dietary fiber (4-10%), essential amino acid (4-7%), and low amount fat (2-4%). Also it is rich in vitamins B, C and E and potassium, magnesium and phosphorus (Sacchetti et al., 2004; Chenlo et al., 2007). The use of chestnut flour is thought to be beneficial at this stage since most of the gluten-free products are insufficient in terms of vitamin B, iron and fibers (Seferoğlu, 2012).

There are not many literature sources about chestnut flour. Mert (2012) in the study aimed to produce higher quality gluten-free wafer by mixing rice-corn flour, rice-buckwheat, and rice-chestnut flour in different rates (80:20, 60:40, and 40:60).

Chestnut flour is also used in gluten-free bread preparation due to its nutritional value enhancing effect. In a study for preparation of gluten-free bread, Demirkesen et al. (2010) investigated that chestnut flour contains 10.79% moisture, 47.80% starch, 21.51% sugar, 9.50% dietary fiber, 3.80% fat, 4.61% protein and 1.99% ash. In the study, using some gums and emulsifiers as well as chestnut and rice flour in different portions (0/100, 10/90, 20/80, 30/70, 40/60, 50/50 and 100/0), gluten-free bread was prepared. Rheological, cooking and sensory properties of the bread samples were examined. As a result, it was investigated that the sample 30%/70% chestnut/rice flour mixture has the best cooking property.

In another study, aiming gluten-free bread production, chestnut flour was used and

some physical and chemical properties were investigated during storage. Using chestnut flour in bread samples prepared using gluten-free flours, decreased the volume, and caused hardness inside of the bread but hardly enough in the bread's crust. In addition, in the both bread samples doped by 10% and 20% chestnut flour additives, the antioxidant capacity increased and especially in 20% doped sample an increased was observed in the amount of insoluble dietary fiber. Moreover, chestnut flour provides more stability during the storage protecting the color property of the bread (Pacuilli et al., 2016).

Sacchetti et al. (2004) increased the use of chestnut flour in snacks and similar products and stated that chestnut flour is appropriate for extrusion products. In addition to this, in the study it is mentioned that chestnut flour can be used as functional component in those products and addition of chestnut flour to cereal-based foods is thought to increase some nutritional contents (high dietary fiber, lysine,  $\gamma$ -amino butyric acid, E and B group vitamins), physical properties (textural, density, color) and sensory properties.

Dokic et al. (2014), in their study on the sensory effects of use of chestnut flour additive to cookies, investigated chemical, textural and color properties. They identified that texturally addition of chestnut flour to cookies increased the hardness, and about the color property they reported that luminescence (L) values decreased while red (a) and yellow (b) values increased. Moreover, it was reported that the quality properties of addition of 20% chestnut flour are similar in quality with that of control sample.

İnkaya (2008) in his study produced chestnut flour by freeze drying and scalding the chestnut samples obtained from 3 different provinces (Aydın, Bursa, Kütahya) and used it in three different portions (10, 20, 30%) in biscuit production. It was reported that spreading rate and hardness values of biscuits doped with freeze-dried chestnut flour are higher than that of scalding-dried chestnut flour doped biscuits. Moreover, in the study it was

reported that chestnut flour prevents the spreading rate in especially reduced-fat biscuits and addition of chestnut flour is appropriate flour for the production of standard and reduced-fat biscuits in terms of quality and sensory properties.

Seferoğlu (2012) examined sensory properties of gluten-free cake, bread and biscuit obtained for celiac patients by using chestnut flour in different proportions (20%, 40%, 80% and 100%). As a result of the study, it was reported that chestnut flour is a significant product for utilization in the production of cake, bread and biscuit and the acceptability of it is fairly high in terms of sensory properties. Healthy individuals and celiac patient participated in the study. In both groups, it is mentioned that the sensory scores of products obtained from chestnut flour are higher. In addition, at the end of the study, it is determined that chestnut flour is a delicious product that can be used in the production of cake, bread and biscuit and by mixing with other gluten-free flours to use it is even more appropriate for the general acclaim and cost. Celiac disease (CD, gluten enteropathy) is identified as proximal small intestine disease which is a permanent intolerance mainly against gluten in wheat and other cereals proteins similar to gluten in such cereals like barley, rye, oat in genetically sensitive individuals (In order to contribute to the treatment of this disease by diet, instead of the flours of these cereals different fruit and vegetable flours should be produced).

Dietary fibers, due to many positive effects of them on health, after determination of technological and physical properties, are lately being used to develop some desired properties in foods. Besides, dietary fibers are the main components of today's diet products due to low energy values and causing the feel of fullness. In addition to this, ability of dietary fiber to regulate blood sugar, to decrease the level of cholesterol, to be protective against constipation, obesity, bowel cancer and coronary heart diseases is thought one the positive effects on health (Budurlu and Karadeniz, 2003). Mete and Dülger Altiner (2016) used chestnut flour in order to enrich noodle samples that were

produced in conventional types in dietary fiber and some nutrition items. In the study conducted for chestnut flour-doped noodle, they examined some chemical, physical, cook and sensory properties. They added chestnut flour to the noodle in the following portions; 0%, 5%, 10%, 20%, 30% and 40%. As a result of the study, they found the dietary fiber content in chestnut flour 23.57% and parallel to this they found the content in noodle as varying between 2.54% and 11.08%. Moreover, in terms of the general acclaim, they mentioned that the highest score between chestnut flour-doped noodles is achieved by the noodles doped with 10% and 20% chestnut flour portions.

#### 4. Result

In this study, it is predicted that the use of chestnut flour in different foods especially bakery products will make contributions to the development of different product markets in terms of improvement of nutrition value and functional properties. Besides, chestnut flour is thought to be used as an alternative flour additive.

The use of chestnut flour, due to its nutritious property and appropriate sensory properties, is spreading in food industry. It is considered that chestnut flour will positively affect human health when used in food enrichment since it contains E and C vitamins, unsaturated oils like omega-3, dietary fiber components, phenolic and antioxidant components as well as the highly nutritious property content.

The use of chestnut flour in gluten-free food market and high dietary-fiber containing foods instead of wheat flour by being doped with different additives is increasing the significance of itself. Chestnut flour, which is of great significance for celiac patients, should be produced more and be added to different products. Chestnut flour can also be used in the preparation of milky pudding products, bread, flakes (corn flakes), can be used to sauce soups and gravies and for flavoring. Chestnut flour is a sought-after product in cosmetics sector as well.

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#### CURRICULUM VITAE

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# THE EFFECT OF PROCESSING TECHNOLOGIES ON LYCOPENE IN TOMATOES (*Lycopersicon esculentum L.*)

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## Abstract

Lycopene is a natural pigment of carotenoid family and recent years there is an increasing interest for the health benefits of lycopene. Tomato and tomato products are considered as one of the most important source of lycopene. The aim of this study is to determine the amount of lycopene in different tomato products such as fresh tomatoes, ketchup, tomato juice, dried tomatoes and tomato paste. It is also aimed to determine the effect of tomato paste processing on lycopene. In this study, lycopene values were found in the range of 89.14 - 581.35 mg/kg during processing at 5 different stages. The ranking of lycopene values found to be as follows: evaporator > the final product (tomato paste) > shredder > fresh tomatoes > preheating stage. Lycopene amount was low ( $89.14 \pm 1.476$  mg/kg) at preheating stage, while the highest lycopene value was found at evaporation stage ( $581.35 \pm 11.795$  mg/kg). Among all the examined tomato products, lycopene values were found in the range of 88.56 - 550.30 mg/kg in the order of : The final product (tomato paste) > 50% dehydrated tomato > 70% dehydrated tomato > ketchup > tomato juice. Tomato juice found to have low lycopene ( $88.56 \pm 2.566$  mg/kg), while the final products of tomato paste have the highest value ( $550.30 \pm 46.75$  mg/kg). In addition, some the quality properties of the products have also been examined and the following results were obtained: Soluble dry matter 28.01 – 28.27 °Brix, pH 4.06 – 4.52, total acidity 0.07 – 1.13g/100g, ash 0.41 – 13.38 %, color (a/b rate) 1.82 – 2.45, viscosity 3.57 – 4.03, invert sugar at total dry matter 42.29 – 322.97%; and black spot 0 /10g. Considering the quality parameters of all samples, it was determined that the result of chemical and physical analyses were in conformity with values defined in Turkish Standards (TS 1466 Tomato Paste - Puree, TS 5282 Ketchup, TS 1595 Tomato juice and TS 3926 Dehydrated Tomatoes)

**Keywords:** *Tomatoes, lycopene, antioxidant activity, tomato products*

## 1. Introduction

Tomato is one of the mostly produced vegetables both in Turkey and in the World [1]. Although almost all vegetables have health - protecting and healing properties for humans due to their nutritional components, some species are more prominent than others. Tomato is one of the leading species in this regard [2].

It has been reported that foodstuffs contain proteins, carbohydrates, oils, minerals and vitamins, along with one and/or many of antioxidant substances

[3]. Lycopene is the most prevalent carotenoid in tomato and constitutes about 83% of the pigments in tomato [4].

Lycopene, like all carotenoids, is derived from the acyclic  $C_{40}H_{56}$  structure [5]. Lycopene is a powerful antioxidant and main component of the red color of tomato. Lycopene level in a tomato may vary according to fruit variety, maturity stage and growing conditions [6]. Lycopene content of fresh tomato and some tomato products are given in Table 1 [7].

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**Table 1.** Lycopene content of tomato and tomato products [7]

Tomato and Tomato products	Lycopene content (µg/g wet weight)
Fresh Tomatoes	8.8 - 42.0
Cooked Tomatoes	37.0
Tomato Sauce	62.0
Tomato Paste	54.0 - 1500.0
Tomato Soup	79.9
Tomato Powder	1126.3 - 1264.9
Tomato juice	50.0 - 116.0
Pizza Sauce	127.1
Ketchup	99.0 - 134.4

Various foods may contain varying amounts of lycopene according to the agricultural methods used, processing and preservation technologies and different cooking methods. Thus, various food composition databases may define the lycopene content at different values for fresh tomatoes varieties, tomato sauce, ketchup, dried tomatoes, tomato paste, etc. [8].

Carotenoids that are locked in a food matrix are being released during food processing especially heat treatments and their bioavailability is also increased. Cooking or other heat treatments that the foods are subjected might break the cell walls of tomatoes and neutralize the binding forces between lycopene and the fruit tissue for lycopene to be more easily available [9].

Thus, the best lycopene sources are suggested to be cooked tomatoes and tomato products [10,11]. In terms of its bioavailability, lycopene in tomato paste was determined to be 2.5 times higher than the lycopene in fresh tomatoes.

Various studies reported that different antioxidant compounds in diet may be effective in preventing cancer by reducing oxidative stress. Lycopene is one of the heavily studied carotenoids in terms of reducing cancer risk [12]. Carotenoids, especially

lycopene, slow down tumor growth by increasing the communication between healthy cells. Lycopene has antioxidant effects and regularly acts against free oxygen radicals that cause DNA damage and cancer in the body [13].

Since carotenoids are transported through low density lipoproteins (LDL), they reduce cardiovascular disease risks by preventing LDL's exposure to oxidation [14]. Lutein and zeaxanthin in the eye retina protect the eye from the harmful effects of free radicals and light [15] and prevent the formation of cataracts and age-dependent macula disorders [16].

In this study the lycopene content in different tomato products were determined and discussed for a healthy nutrition. In addition, the effect of tomato paste processing on lycopene in fresh tomato was also studied through in plant applications in a tomatopaste processing plant.

## 2. Materials and Methods

### 2.1. Materials

Samples to be used as material were obtained from a national tomato paste manufacturing plant that produces commercial tomato paste. The tomato variety subjected to processing in the tomatopaste processing plant was Rio Grande grown in Bursa region, Turkey.

Totally 15 samples were taken from 3 batches of tomato paste processing stages. Two ketchup samples (manufactured under laboratory conditions), two tomato juice samples and two half-dried tomato samples (sun dried) were obtained for examination. Tomato paste and ketchup samples were taken in ~830 gr sterile glass jars, tomato juices in 1 liter glass package and dried tomato samples in 1 kg vacuum plastic packages. The samples consist of products grown and manufactured in August 2015 and the study was conducted in triplicate and the results are reported accordingly.

Samples were stored under cold conditions at +4°C until the analyses performed.

## 2.2. Methods

The methods of analyses conducted on the samples are given below.

### *Lycopene Determination:*

Three samples were taken from each sample for lycopene analysis.

Lycopene determination was conducted according to Anon.,1983 [21] and Anon., 1990 [20]. UV-VIS spectrophotometer (Optizen Pop, Korea) was used for the measurements.

The samples were prepared to be measured by the spectrophotometer after the extraction process. In extraction, water:aseton (1:1, v/v) solution was used after boiling, the solution was centrifuged and separated using petroleum ether in a separatory funnel, petroleum phase was collected.

Petroleum ether was used as blank in spectrophotometric measurements. A UV-VIS spectrophotometer (Optizen Pop, Korea) was used at 505 nm wavelength (A505), and the absorbance values were measured. The lycopene amount was calculated by using the absorbance value measured in the formula in (2.1).

Lycopene ( $\mu\text{g/g}$ ) or ( $\text{mg/kg}$ ) = (2.1)

Here;

V= The final volume of the solution (ml),

W= Amount of sample (g),

( $A_{505}$ ): Absorbance at 505 nm , (Anonim,1983; T.O.K.B., 1988).

The other analyses were performed according to the methods of analyses defined in Turkish Standards (TS 1466 Tomato Paste - Puree, TS 5282 Ketchup, TS 1595 Tomato juice and TS 3926 Dehydrated Tomatoes).

### *Statistical Methods*

The analysis was conducted in triples and the results are presented as arithmetic average  $\pm$

standard error of average. In order to statistical evaluation of the results of lycopene content, SPSS Windows software (IBM SPSS Statistics 23, 2015) with one-way ANOVA method was used. Post Hoc tests were also used in order to determine the cause of difference between the averages; Tukey multiple comparison test was used when the variances are homogenous, and Games Howel, Tamhane's T2 was used when they were not. Averages that do not show the same letter in the tables are found to be statistically different.

## 3. Results

The amounts of lycopene were determined in fresh tomato, in samples of different tomato paste processing stages and in different tomato products. The results of analyses were evaluated statistically. Lycopene values for samples that represent different tomato paste process phases are given in Table 2, Figure 1. The figure with the same letter mean that there is no statistically significant difference in terms of lycopene contents.

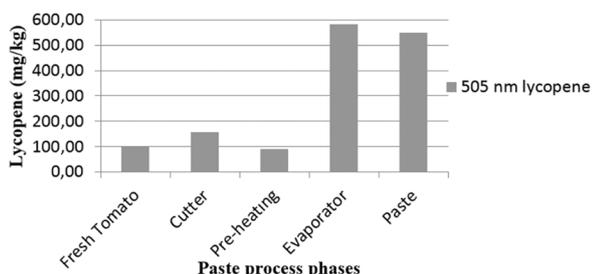
**Table 2.** Changes in lycopene content during tomato paste processing \*

Feature	
Process phases	Lycopene (mg/kg)
Fresh Tomato	102.27 $\pm$ 0.713 ab
Cutter	156.37 $\pm$ 9.042 b
Pre-heating	89.14 $\pm$ 1.476 a
Evaporator	581.35 $\pm$ 11.795 c
Final product (paste)	550.30 $\pm$ 46.753 c
Minimum	89.14 $\pm$ 1.476
Maximum	581.35 $\pm$ 11.795
Average	295.89 $\pm$ 230.302

\*: The difference between the general properties of process phases that have different letters in the same column is significant ( $p < 0.05$ ).

The lycopene values show that the pre-heating phase of tomato has a low lycopene value (89.14  $\pm$  1.476 mg/kg) and is significantly different ( $p < 0,05$ )

from other phases, except the fresh tomato phase. The highest lycopene content was determined in evaporation phase ( $581.35 \pm 11.795$  mg/kg) and it is significantly different ( $p < 0,05$ ) from other phases, except for the tomato paste. The lycopene values were determined for fresh tomato in the range of 8,8 - 42,0  $\mu\text{g/g}$ , for preheating stage at a value of 37,9  $\mu\text{g/g}$ , and for tomato paste 54-1500  $\mu\text{g/g}$  [7].



**Figure 1.** Lycopene content in tomatoes during tomato paste processing

The lycopene values for all tomato products are given in Table 3.

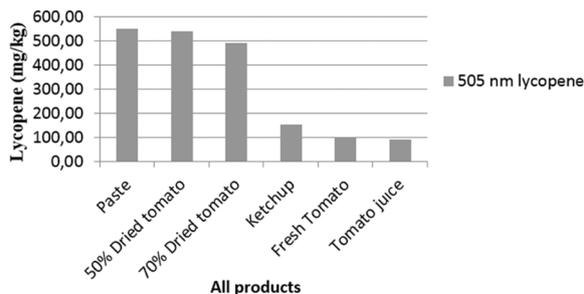
**Table 3.** Statistical results for lycopene content of all tomato products

Feature	
Product name	Lycopene ( mg/kg)
Final product (Paste)	550.30± 46.75b
Ketchup A	153.61± 11.132 a
Ketchup B	154.56± 12.903 a
Tomato Juice A	88.56± 2.566 a
Tomato Juice B	93.55± 2.933 a
50% Dried Tomato	540.020± 36.726 b
70% Dried Tomato	492.03± 51.523 b
Minimum	88.56± 2.566
Maksimum	550.30± 46.75
Avarage	296.09 ± 209.023

\*: The difference between the general properties of process phases that have different letters in the same column is significant ( $p < 0.05$ ).

The lycopene values show that tomato juice has low lycopene value ( $91.06 \pm 2.749$  mg/kg) and exhibits a statistically significant difference ( $p < 0,05$ ) from other products except for ketchup. The highest amount of lycopene was determined in the final product (tomato paste) ( $550.30 \pm 46.75$  mg/kg), and is significantly different ( $p < 0,05$ ) from other products except for 50% dried tomato and 75% dried tomato.

The lycopene contents were determined as 30.25, 159.16, 293.3, 170.08 and 96.6 mg/kg in fresh tomato, tomato paste, triple concentrated tomato paste, ketchup and tomato juice, respectively. [17]. The lycopene amounts of dried tomato products were found in the range of 500-700 mg/kg [18], while it has been determined as 659.5 mg/kg in tomato paste [19]. Lycopene contents of all tomato products are given in Figure 2.



**Figure 2.** Lycopene content of tomato products

In tomato paste processing, tomatoes at the pre-heating process have a low lycopene value ( $89.14 \pm 1.476$  mg/kg), while the evaporator phase has the highest lycopene content ( $581.35 \pm 11.795$  mg/kg).

Among all tomato products, the lowest lycopene value was determined in tomato juice ( $88.56 \pm 2.566$  mg/kg) while tomato paste has the highest amount of lycopene ( $550.30 \pm 46.75$  mg/kg).

In addition to lycopene content, some quality parameters of the products were also investigated. The statistical results of the quality specifications for all products are given in Table 4.

**Table 4.** The statistical results of the quality specifications for all products

Parameter	Product Name					
	Fresh Tomato	Tomato Paste	Ketchup	50% Dried Tomato	70% Dried Tomato	Tomato Juice
<b>pH</b>	4.41 ± 0.010	4.21 ± 0.020	4.07 ± 0.025	4.50 ± 0.020	4.40 ± 0.020	4.27 ± 0.015
<b>Brix</b>	4.78 ± 0.448	28.15 ± 0.026	26.50 ± 0.452	-	-	5.56 ± 0.030
<b>%Total Acidity</b>	0.07 ± 0.005	0.60 ± 0.017	0.33 ± 0.030	0.80 ± 0.025	1.13 ± 0.045	0.10 ± 0.015
<b>%Salt</b>	0.24 ± 0.017	0.89 ± 0.040	2.42 ± 0.120	6.24 ± 0.440	4.96 ± 0.772	1.18 ± 0.105
<b>% Ash</b>	0.46 ± 0.048	2.41 ± 0.069	2.72 ± 0.170	9.59 ± 0.246	13.38 ± 0.453	0.98 ± 0.055
<b>%Invert Sugar</b>	54.93 ± 1.845	184.51 ± 1.700	133.27 ± 1.222	322.97 ± 2.494	147.47 ± 18.504	50.21 ± 1.327
<b>%Total Sugar</b>	23.66 ± 0.380	74.85 ± 1.610	48.29 ± 1.761	103.33 ± 2.307	29.07 ± 0.170	30.08 ± 1.127
<b>Hunter lab Color (a/b)</b>	2.13 ± 0.276	2.66 ± 0.036	2.23 ± 0.015	-	-	1.82 ± 0.015

It was determined that mean values of all samples investigated were in conformity with the legal limits specified by Turkish Standards (TS 1466 Tomato Paste - Puree, TS 5282 Ketchup, TS 1595 Tomato juice and TS 3926 Dehydrated Tomatoes)

#### 4. Conclusion

The main objective of the study is determining the lycopene content in various tomato products and also the change in lycopene content during tomato paste production. It has been determined that lycopene values vary between 89.14 and 581.35 mg/kg during tomato paste production stages.

The products can be sorted for lycopene contents from high to low values during tomato paste processing: Evaporator > Final product (paste) > Cutter > Fresh Tomato > Pre-heating. Considering all the tomato products, the lycopene amounts were in the range of 88.56 and 550.30 mg/kg and . The ranking for lycopene of all tomato products: Tomato paste > 50% dried tomato > 70% dried tomato > ketchup > fresh tomato > tomato juice. In processed tomato products, heat treatment of tomato breaks down the cell walls due to heat effect and lycopene is released. Thus, the lycopene concentration in heat-treated tomato products is higher than fresh tomato. Release of lycopene occurs when it dissolved from the fruit tissue

during processing or cooking and creates a more easily available form. Thus, the best lycopene sources are suggested to be cooked tomatoes and tomato products.

Tomato is very important for humans in terms of health and nutrition. Considering the fact that the second most prevalent cause of death in the world is cancer, it is recognized that nutrition styles have a great role in cancer prevention. It is assumed that 1/3 of all cancers can be prevented through individual diet modification. Lycopene is one of the mostly studied carotenoids in terms of reducing cancer risk in human.

Lycopene protects the cells from the damages by free radicals, strengthens the connection between cells and improves cell metabolism. Its antioxidant properties provide protection against cancer, cardiovascular disorders, eye disorders, ageing, bone and skin issues, and many other disorders. In the light of the various reports on lycopene, lycopene plays an important role in preventing some chronic diseases. Since the tomato and tomato products are the important source of lycopene, tomato and products derived from tomatoes such as pastes, ketchup, tomato juice and dried tomato will help the healthy nutrition and well being of the people.

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# EXAMINATION OF SOME CHEMICAL AND FUNCTIONAL PROPERTIES OF CAROB AND SOY FLOURS AND USAGE OF THEM IN BAKERY PRODUCTS

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## Abstract

Bakery products are a nutrition group that is chosen by many people in different countries. Due to low cost, being prepared practically and nutritious, appealing to all age groups and being economical, they are preferred. Considering consumption rate of bakery products consumed by people, the enrichment by needed basic food items is inevitable.

In this study, research of some functional and chemical properties of carob and soy flour that can be used as alternatives of wheat flour, and use of them as food additives in production of bakery products such as pasta, biscuits, cracker, bread etc. is aimed.

It is investigated that carob flour (CF) and soy flour (SF) one of the materials that can be used as bakery product enrichment agent and obtain functional foods. CF has a unique smell, color and taste, has high dietary fiber content (32.87%), complex carbohydrates and a small amount of fat (0.31%). And it is also investigated that soy flour (SF) has a high protein rate ( $p < 0.05$ ) and dietary fiber content compared to wheat flour (WF). Carob flour is found to have low energy content compared to WF and SF.

Based on the functional analyses of flour samples, it is determined that CF and SF samples have a higher water absorption capacity and solubility in water compared to WF. Due to these properties, it is predicted that these flours can be used as alternative food additives and are supposed to contribute to food industry in terms of the properties of the last product and improvement of species.

**Keywords:** Bakery products, carob flour, soy flour, food additives

## 1. Introduction

Today, main reasons of health problems are inadequate and unbalanced nutrition. In order to prevent this, food enrichment method has been applied to increase the amount of foods that are consumed or taken in the body in low amounts (Aslan and Köksel, 2003).

Cereals and cereals products are commonly consumed both all around the world and in Turkey. Besides of this, cereals are an important food source in terms of vitamin, minerals and amino acids. However, cereals products have low amounts of micro nutrient and part of these micro nutrients are lost during processing (Chenge and Harby, 2003; Poletti et al., 2004).

According to researches, majority of individuals in Turkey are suffering from diseases caused by lack of vitamins and minerals. Children at growing ages, nursing mothers, babies and women are affected from food insufficiency. In a study to overcome this problem, Vitamin C, B1, B2, B6, B12, folic acid, iron, and zinc were added to 650-750 type wheat flours by encapsulation in suggested amounts in grain products. The product was delivered to consumers after required analyses performed on it. Thus, to prevent diseases caused by nutrient insufficiency is aimed (Looker, 2004).

Requirements for the success of enhancement processes in food industry are; some nutritional

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elements that customer needs to take being insufficient, standardization of nutritional elements in diet due to observing discrepancies among them, forming a controlled diet by ensuring that newly added nutritional elements will be in harmony with main elements of food, and improving physiological benefits by adding nutritional elements with various methods (Saldamlı and Sağlam, 1998).

- ✓ It is required to not cause any change of color, odor and taste with the addition of nutritious item.
- ✓ Providing necessary technological conditions and actively quality control are essential (Richardson, 1999; Salgueiro, 2002; Anonymous, 2003).

In recent years, micro nutrients such as iron, calcium, magnesium, zinc, pyridoxine, vitamins A and B vitamins were added to flour in order to enrich the flour and bakery products. These products can be obtained with direct addition of additives as well as obtaining with the addition of some floured fruits/vegetables (Ranum, 2000). Some food additives made from vegetables or legume flour are used in production of bakery products such as pasta, biscuits, cracker and bread.

In this study, it is investigated that carob flour (CF) and soy flour (SF) can be used as bakery product enrichment agent and obtaining functional foods. Based on these properties, enrichment of the bakery products with functional additives is increased the quality and nutritional properties of it.

Whereas a definite judgement on the definition of functional foods could not be formed, according to Food and Drug Administration (FDA) a functional food should contain sufficient amount of nutritional elements, should have positive impacts on health with those nutritional elements and there should be scientific studies approved by FDA that prove the assertion of the relation between diet and illness (Anonymous, 2004).

Besides, functional foods should involve the nutrients having biologically active essences like beneficial fatty acids, particular minerals or

dietary fiber, prebiotics or antioxidants (Abdel-Salam 2010). Common features of functional foods are being natural, the fact that its nutritional ingredients are non-allergic and have positive impacts on health, being reliable, being a part of diet and that their benefits to health are scientifically proven (Anonymous, 2015).

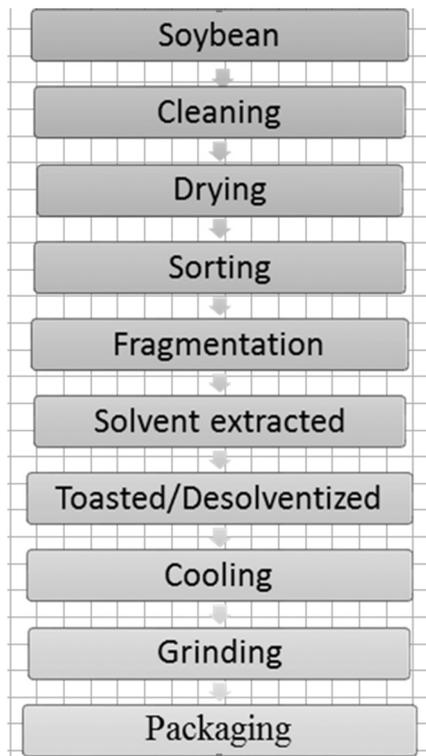
The effects of functional foods on health are listed as: To regulate basic metabolic functions (prevents from obesity and diabetes), to support antioxidant defense system against oxidative stress (prevents from some diseases like cardiac diseases, cancer, cataract, Parkinson disease and Alzheimer, and has anti-aging effect), to prevent cardiac diseases by lowering cholesterol level and blood pressure, to regulate stomach-bowel activity and to improve mental and physical development (Ashwell, 2002).

### **1.1 Soy flour and Carob flour**

Being a member of “*Leguminosae*” family, cultivated type of soybean, *Glycine max L. merrill*, is being grown annually. The seeds of soybean can be both spherical and oval. Generally being yellow, they can be purplish black, dark brown or black (Liu, 2004).

Soybeans are rich in terms of vitamins, minerals and nutrients. It contains proteins, fibers, magnesium and calcium in high proportions. Being a type of plants that provide nitrogen for the soil, soybean is categorized as oilseed crops when classification is made since soybean contains oil. Soybean contains 35-45% protein, 30% carbohydrates, 18-24% oil and 5% minerals, vitamins and some important amino acids (Anonymous, 2011).

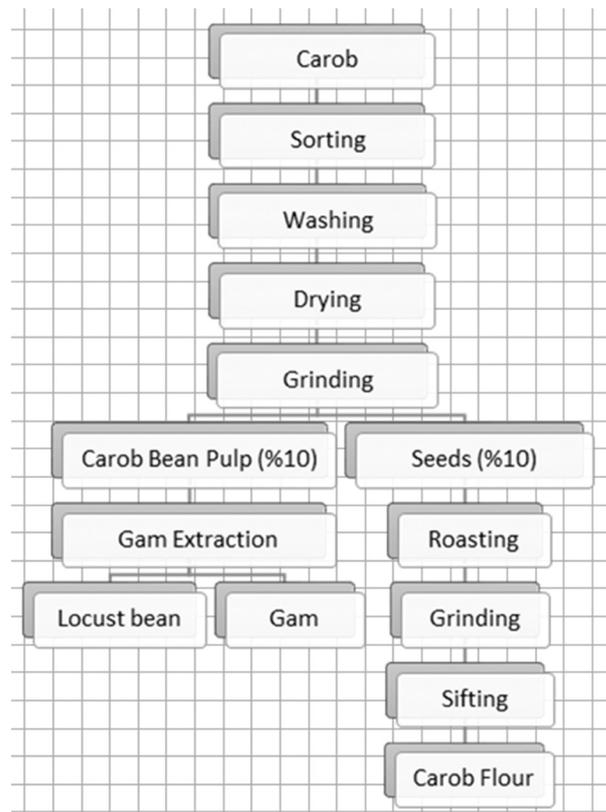
Soy flour is a rich food source in terms of proteins. At the same time, it is an excellent source of iron, calcium and B vitamin (Öner, 2006). In the process of obtaining soy flour, protein percentage that it contains remains as 55% (in terms of dry material) since no other compounds are separated except for shell and/or oil. Soy flour is used in a large range of foods in order to decrease the cost, to provide nutrition supplement for the food and to extend the shelf life. Production scheme of soy flour is shown in Figure 1.



**Figure 1.** Soy flour production steps

Being a member of *Cesalpiniaceae* family, carob (*Ceratonia siliqua L.*) is a perennial plant (Turhan et al., 2007). Carob is a valuable nutrient used in food industry, in the production of molasses and various animal feeds as well direct consumption (Race et al., 1999; El-Shatnawi et al., 2001; Pekmezci et al., 2008). Grinded and floured form of carob, can be used as an alternative of cacao in the production of ice cream, cake and sweet foods (Pekmezci et al., 2008; Urbaş, 2008).

Being rich in terms of antioxidants, carob fruit has positive effects on human health since it contains dietary fibers and minerals in high portions (Şenay, 2009). Caron contains gallic acid in high rates (Owen et al., 2003). Due to having high amounts of dietary fibers and phenolic components, carob flour is important in terms of nutrition facts (Ortega et al., 2011). Production scheme of carob flour is shown in Figure 2.



**Figure 2.** Carob flour production steps

## 2. Materials and methods

### 2.1. Materials

Among the raw materials used in this study, soy flour was supplied from Doğalsan Ltd., Ankara, carob flour was supplied from Global Gıda-Haşhaşcızade, Afyonkarahisar, durum wheat flour was supplied from Bandırma Toru Un Ltd. Şti.

### 2.2. Methods

#### 2.2.1. Chemical analyses

For the determination of chemical components AACCI methods were used. AACCI method no: 44.01 for moisture, AACCI method no: 08.01 for ash content, AACCI method no: 46.12 for the protein content, AACCI method no: 30-25.01, for the oil content and AACC method no: 02-31 titratable acidity (in terms of acetic acid) (AACC, 2000) were performed.

## Functional analyses

### Oil absorption capacity

3 gr of each flour were introduced to the sample tubes. Then, 30 mL of corn oil (Bizim corn oil) was added and samples were vortexed for 30 seconds. Samples were kept at room temperature for an hour. After keeping 1 hour at room temperature, the samples were centrifugated at 2800 rpm for 10 minutes (Hettich-Rotofix 32 A).

After centrifugation, separated supernatant part was removed and the precipitate was weighed. Oil absorption capacity of the samples were calculated using the following formula (2.2).

$$\text{Oil absorption capacity} \left( \frac{\text{g}}{\text{g}} \right) = \frac{\text{weight of precipitate (g)} - \text{weight of dry sample (g)}}{\text{weight of dry sample (g)}} \quad (2.2)$$

### Solubility and water absorption capacity

0.5 g (M1) was introduced in to the test tubes and 5 mL of pure water was added. After each 5 minutes the tubes were vortexed for 15 seconds. After vortex process, samples were centrifugated at 2100 rpm for 10 minutes. Supernatant and precipitate were separated. Wet precipitate in the tubes were weighed (M3). The samples were dried in stove overnight at 100 °C and supernatant (M2) and the precipitate (M4) were weighed. The water absorption capacities and solubility of the samples were calculated using the following formula (2.3).

$$\text{Solubility (\%)} = \frac{M_2}{M_1} * 100 \quad \text{Water absorption (\%)} = \frac{M_3 - M_4}{M_1} * 100 \quad (2.3)$$

### Total dietary fiber analysis

Total dietary fiber amount was determined enzymatically (AOAC 985.29) (using alpha amylase, amyloglycosidase and protease enzymes).

### Calculation of carbohydrate and energy

Carbohydrate and energy values determined in pasta samples are calculated with the following formula.

$$\begin{aligned} \% \text{ Carbohydrate} &= 100 - (\% \text{ Moisture} + \% \text{ Ash} + \% \text{ Protein} + \% \text{ Oil}) \\ \text{Energy} &= (9 * \% \text{ Oil}) + (4 * \% \text{ Protein}) + 4 * (\% \text{ Carbohydrate} - \% \text{ TDF}) \end{aligned}$$

### Statistical Analysis

Data obtained from the analyses were calculated using Enterprise 5.1 software and differences arising from the addition of carob and soy flours to pasta were evaluated. Least Significant Difference (LSD) was used to determine the statistical difference ( $p < 0.05$ ) when the Analysis of Variance shows a significant difference among averages. In the determination of statistically different groups among the obtained averages, LSD test was applied at the level of probability of  $p < 0.05$ . The LSD method applies standard t tests to all possible group mean pairs.

**Table 3.1.** Chemical analysis results of carob, wheat and soy flours

Sample	Moisture (%)	Ash (%)*	Oil (%)*	Protein (%)*	Titratable acidity (%)
<b>Wheat Flour (WF)</b>	14.8±0.01 a	1.15±0.01 c	2.04±0.01 a	13.9±0.01 b	0.03±0.1 b
<b>Carob Flour (CF)</b>	7,57±0.04 b	3.09±0.14 b	0.31±0.03 b	6.03±0.1 c	0,27±0.8 a
<b>Soy Flour (SF)</b>	7,04±0.04 c	7,61±0.25 a	2.04±0,3 a	59.16±0.08 a	0,36±0.00 a
<b>Min-Max</b>	<b>7.04-14.8</b>	<b>1.15-7.61</b>	<b>0.31-2.04</b>	<b>6.03-59.16</b>	<b>0.03 -0.36</b>
<b>Av±SD</b>	9.8±4.3	3.95±3.31	1.45±0.9	26.4±28.7	0.23±0.18

\* Calculated for the dry substance

\* Among the averages shown with different letters in the same column in LSD test, there is a significant difference statistically ( $p < 0.05$ )

### 3. Results and discussion

Chemical analysis results of carob and soy flours used for the enrichment process are given in Table 3.1.

According to the obtained results, the highest moisture is found in wheat flour, the highest protein and ash content were found in soy flour. Protein content that soy flour contains is higher than other raw materials.

Based on the titratable acidity (TA) values, they are found as 0.03 % in wheat flour, 0.28% in soy flour and 0.38% in carob flour. According to Turkish Food Codex Legislations, flour can have a maximum acidity value of 0.07%. The obtained results are in compliance with the legislations. Since soy and carob flours are categorized as fruit/vegetable flours, acidity contents are found to be higher.

In the study revealed by Polat (2007), in bread flour, the moisture content is found to be 14.75%, the ash content to be 0.62% and the protein content is 9.76%. Yousif and Alghzawi (2000) found moisture content in carob flour as 9%, ash content as 2.48%, protein content as 5.82% and oil content as 0.74% in their study. In the study of Ortega et al. (2011), the moisture content in carob flour is 2.3%, ash content is 3.3%, protein content is 3.8% and oil content is 0.3%. In our study, the contents for ash, oil and protein of the flours are in the ranges of abovementioned values.

Bashir et al. (2012) examined the contents of moisture, ash, protein, oil and total carbohydrate in the flours of defatted soy and wheat. They found moisture rate in soy flour as 7.14±0.11%, ash rate as 5.93±0.09%, oil rate as 0.77±0.05%, protein rate as 49.90±0.37% and total carbohydrate rate as 35.84±0.18%. They found moisture rate in wheat flour as 11.66±0.31%, ash rate as 0.80±0.01%, oil rate as 1.02±0.05%, protein rate as 12.99±0.25% and total carbohydrate rate as 73.92±0.19%. They found moisture rate in chickpea flour as 10.99±0.39%, ash rate as 2.59±0.25%, oil rate as 4.57±0.26%, protein rate as 21.61±0.37%

and total carbohydrate rate as 59.0±0.08%. The additive flours used in this study have lower moisture values than that of others in the literature. And this is advantageous in terms of shelf life. It is investigated that flours used in this study, have higher ash values and low oil value compared to chickpea flour. Considering the protein rates, protein rate of soy flour was found to be higher compared to other studies. This result is affected by the fact that soybean is a protein-rich food. Besides, the conditions of growing of soybean, the type of used soil and climate conditions and other applied processes are among the other reasons.

**Table 3.2.** Total carbohydrate and dietary fiber values of raw materials

Sample	Total Carbohydrate (%)	Total Dietary Fiber (%)	Energy (kcal)
<b>Wheat Flour (WF)</b>	68.14±0.01 b	2,93±0,01 c	334,42±0,54 a
<b>Carob Flour (CF)</b>	83.03±0.11 a	32,87±0,57 a	227,41±3,17 c
<b>Soy Flour (SF)</b>	24.14±0.06 c	18,38±0,08 b	278,04±2,33 b
<b>Min-Max</b>	24.14- 83.03	2,92-33,27	225,17-334,8
<b>Av±SD</b>	58.44±30.62	18,06±13,40	279,96±47,91

\* Calculated for the dry substance

\*Among the averages shown with different letters in the same column in LSD test, there is a significant difference statistically ( $p<0.05$ )

Total dietary fiber of carob flour used in this study is 32.87% while in soy flour it was found to be 18.38% (Table 3.2). Total carbohydrate values are 83.03% in carob flour, 24.14% in soy flour.

As the results, total carbohydrate and dietary fiber rate of carob flour was found to have significantly higher than dietary fiber rate of soy flour. Considering energy values, the highest energy values were found in wheat flour, carob flour and

soy flour, respectively. Low carbohydrate rate of soy flour is important in terms of decreasing the glycemic index in the products containing soy flour. The reason why the amount of carbohydrate is high in locust-bean flour is being carbohydrate-rich nutrient.

In the study revealed by Ahmad et al. (2012), in production of bread, grain flours that are rich in beta-glucan. Based on the results, it was investigated that beta-glucan is increasing viscosity in bread dough and is causing an increase in dietary fiber in bread. Besides of these, an increase in hardness of bread was observed.

In a study, revealed by Polat (2007), pumpkin flour doped bread was produced. Total dietary fiber amount of pumpkin flour used for production was determined as 10.13%. With the addition of apple fibers, carob and fatty soy flours to biscuits prepared with wheat flour (65%) and type 500 wheat flour, variations in total dietary fiber rates were examined. As a result of the addition of carob flour to biscuits, an increase of 42% was observed (Sebecic et al., 2007). In a study carried out by Ory and Conkerton (1983), 12.5% peanut flour was added to wheat flour and alterations on bread were examined. Based on the results, an increase of 0.1-0.3% was found in total dietary fiber rate found in bread. Total dietary fiber rates of additive flours used in this study were found higher.

Dülger and Şahan (2011), used dietary fiber for enrichment purposes which grow in importance in recent years, as components that are indigestible in small intestine and fermented in large intestine. Higher rates of total dietary fibers (TDF) of products used in this study and were determined as significant in terms of providing fiber products of grain samples used as additives and possessing beneficial properties of dietary fibers. According to Levi et al., 2001; Kahlon et al., 2001, it was reported that dietary fibers are protective against colon cancer and shortening the transport time of foods in intestines which results in preventing constipation by fecal volume.

**Table 3.3.** Functional properties of raw materials

Sample	Water Absorption Capacity (%)	Water Solubility (%)	Oil Absorption Capacity (%)
<b>Wheat Flour (WF)</b>	104,66±0,64 c	6.8±0.6 c	0.97±0.03 c
<b>Carob Flour (CF)</b>	185.5±2.12 b	44±4.24 a	0.74±0.22 b
<b>Soy Flour (SF)</b>	293±26.9 a	29±2.8 b	1.06±0.01 a

*\*Among the averages shown with different letters in the same column in LSD test, there is a significant difference statistically (p<0.05)*

Based on obtained results, water absorption capacity was determined higher in soy flour, significantly lower in wheat flour (p<0.05). Oil absorption capacity of soy flour was found to be significantly higher (p<0.05) compared to other flours (Table 3).

Functional properties are physical chemistry properties affecting behaviors of protein during preparing, process and consumption. Usage of various flour in food processing as additive is depending on the functional properties of them. Information of functional properties of proteins provides information about performances of these additives. Solubility is a result of surface active properties of proteins. Food materials having high water absorption capacity behave like functional components. With the addition of components having high water absorption capacity, viscosity and texture of the last product (soup, dough and bakery products) can be modified.

Components having high oil absorption capacity have a significant functional role in stabilization of food systems and improve viscosity and texture acting as emulsifiers. Results obtained in this study are at moderate level.

Aydın (2004), studied on pumpkin flour samples and applied different processes. It has been reported that water solubility values change in the range of 3.96-21.22% and water absorption values change in the range of 66.89-91.66%. Water solubility and water absorption capacity values of flours used in this study are higher than that of pumpkin flour.

Sahan et al. (2012) studied on oleaster flour and oleaster+peel flour produced by different processes. As a result, water solubility values were found to be in the range of 90.33-96.01% and water absorption capacity values in the range of 372.74-430.33%. Water solubility and water absorption capacity values of flours used in this study are lower than that of oleaster flour.

#### 4. Conclusion

It has been revealed that, it is possible to enlarge the using area of carob and soy flour in food industry such as using in the production of high protein and high dietary fiber and low oil and calorie containing foods. Besides, it is predicted that these flours can be used as alternative additive in different food groups as well as grain products. According to the results of the research, it can be said that CF can be used in food sector as an alternative additional matter in order to improve the nutritional and functional properties of foods.

Studies show that enrichment of different products in terms of nutrition facts and functional properties are important. Accordingly, nutritious and functional properties of foods are increased with the addition of flours obtained from fruits/vegetables and lack of nutritious items in foods is eliminated. As a result, diseases arising from malnutrition can be prevented. In order to achieve this, offering varieties to enrichment agents is necessary. Thus, production of both economical and rich foods in terms of nutrition facts will be provided. Based on this study, enrichment of different samples can be succeeded.

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