UTILIZATION OF HAZELNUT MEAL AS AN INGREDIENT FOR PROTEIN ENRICHED DRINK

by
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to my beloved family…

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ABSTRACT

UTILIZATION OF HAZELNUT MEAL AS AN INGREDIENT FOR PROTEIN ENRICHED DRINK

Hazelnut is one of the most popular nuts in the world since it has an impressive nutritional profile due to its protein and fat availability and high contents of fiber and minerals. Hazelnut meal obtained as an industrial by-product from extraction of oil from hazelnuts contains high amount of protein, dietary fibers and minerals. Recently, fortified foods has become a market all around the world because of increasing nutritional awareness among consumers, especially who exercise regularly, and in this market, the demand for high protein food products is increasing day by day. Within this context, hazelnut meal can be a sufficient alternative that meets protein need. The purpose of this study is to determine the suitability of hazelnut meal which is an industrial waste as an ingredient in protein enriched beverage, especially for consumers who exercise regularly. Protein concentrate was obtained depending on the isoelectric point which was pH 4.5 of the hazelnut meal protein. Compositional and functional analysis showed that hazelnut meal protein can be used as a food ingredient. After compositional and functional analysis of hazelnut meal and hazelnut meal protein, comprehensive study for beverages including the protein concentrate in two different concentration (2 percent and 4 percent) had been applied. The quality of these protein enriched beverages had been evaluated by physicochemical and sensory analysis. According to sensory analysis, protein drink with 4 percent of protein was chosen as the most preferred drink after reference protein drink.

ÖZET

FINDIK KÜSPESİNİN PROTEİNCE ZENGİNLEŞTİRİLMİŞ İÇECEK YAPIMINDA DEĞERLENDİRİLMESİ

Fındık, protein ve yağ bileşenleri ile yüksek miktardaki lif ve mineral içeriğiyle etkileyici bir besin profiline sahip olduğundan dünyadaki en popüler çerezler arasında yer almaktadır. Fındıktan yağ elde etme işlemi sonrasında endüstriyel yan ürün olarak çıkan fındık küspesi, yüksek miktarda protein, besinsel lif ve mineralleri içermektedir. Son zamanlarda özellikle düzenli olarak spor yapan tüketiciler arasında beslenme konusunda oluşan farkındalık, takviye edici gıdaların tüm dünya çapında bir pazar haline gelmesine sebep olmuştur ve bu pazarda proteince zenginleştirilmiş gıdalara olan talep günden güne artış göstermektedir. Bu bağlamda, fındık küspesi proteine olan talebi karşılamak için nitelikli bir yol olabilir. Bu çalışmanın amacı, fındık küspesinin özellikle düzeli olarak egzersiz yapan tüketiciler için proteince zenginleştirilmiş içeceklerde bir bileşen olarak kullanımının uygunluğunu araştırmaktır. Fındık küspesi ve küspeden elde edilen proteinin kimyasal ve fonksiyonel analizleri, fındık küspesinden elde edilen proteinin değerli bir gıda bileşeni olduğunu ortaya koymaktadır. Fındık küspesi ve küspeden elde edilen proteinin kimyasal ve fonksiyonel analizlerinden sonra, küspeden elde edilmiş proteini farklı konsantrasyonlarda (yüzde 2 ve 4) içeren içecek için kapsamlı bir çalışma gerçekleştirilmiştir. Proteince zenginleştirilmiş bu içeceğin kalite parametreleri uygulanan fizikokimyasal ve duyusal analizler ile değerlendirilmiştir. Duyusal analiz sonuçlarına göre, yüzde 4 oranında protein içeren içecek, panelistler tarafından referans içeceğinden sonra en çok tercih edilen içecek olmuştur.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS/ABBREVIATIONS

°C	Degree centigrade
µL	Microliter
AOAC	Association of official analytical chemists
ANOVA	Analysis of variance
EAA	Essential amino acids
FAC	Fat absorption capacity
FAO	Food and agriculture organization
g	Gram
h	Hour
HM	Hazelnut meal
HMF	Hazelnut meal flour
HMPl	Hazelnut meal protein pellet
HMPP	Hazelnut meal protein precipitate
l	Liter
M	Molarity
m	Meter
mg	Milligram
ml	Milliliter
mm	Millimeter
NEAA	Non-essential amino acids
PUFA	Polyunsaturated fatty acids
rpm	Revolutions per minute
WAC	Water absorption capacity
WHO	World health organization

1. INTRODUCTION

Hazelnut (*genus Corylus*) belongs to birch family *Betulaceae* and subfamily *Corylaea*, is cultivated in northern hemisphere (Korea, Japan, China, Tibet, Northern Iran, Turkey, Europe and Northern America) which has mild climate [1, 2]. It is one of the major nuts together with peanut, almond, walnut, cashew and pistachio. Among the hazelnut cultivars, *Corylus avellana Linnaeus* is the most commercial cultivar, followed by *Corylus maxima* and *Corylus colurna* also called as Turkish Hazel [3]. Although their nutritional composition changes from one cultivar to another, in general terms, hazelnut has an impressive nutritional value because of its fat, protein, minerals and vitamins content. Turkey is the leader in the market based on hazelnut production amount (70 percent of total production amount), production area (77 percent of total production area) and trade, and also has a lot of important commercial hazelnut cultivars [4]. In Turkey, almost all of hazelnut produced is from Black Sea region and among 33 provinces, Ordu, Giresun and Samsun are three of the highest production areas. Although Turkey is the main hazelnut producing country, consumption rate is very low where it ranks as the third country. Hazelnut is not only consumed as a snack, but also is used as an ingredient, especially in chocolate industry. Additionally, hazelnut kernels, due to high fat content, is utilized in oil process as a raw material. After hazelnut oil extraction, hazelnut meal is obtained as an industrial waste with high protein content, approximately 40 percent [5]. According to investigation of amino acid composition of hazelnut meal, it is shown that glutamic acid, aspartic acid and arginine are the three of highest amino acids (8.81, 3.28 and 4.17 g/100 g, respectively) and it contains all essential amino acids with 80-90 percent digestibility [6, 7]. There is an increased interest about variety of protein sources because of world population growth, ethic and environmental concerns and also healthy lifestyle relevance in modern society. In other words, people prefer plant protein instead of animal protein. Day by day, legume, nuts, grains, seeds and vegetable varieties are consumed as plant protein sources. Ready-to-eat products like functional snacks or beverages are demanded by urban consumers. Most people who need additional protein in their daily diets prefer to consume it in a drink [8]. Also, people who exercise regularly meet hydration needs besides protein with this kind of drinks.

The purpose of this thesis is to produce protein concentrates with high quality and high concentration in a powder form, which was not denatured due to aqueous extraction and lyophilization for the production of vegetable protein from hazelnut meal which is an industrial waste, and afterwards, to apply the obtained hazelnut meal protein as an ingredient in protein-enriched beverage at different concentrations.

Within this project, chemical and physical composition of hazelnut meal that is an industrial waste have been well established. Functional properties of this protein concentrate constituted important part of this thesis because functional characteristics of protein concentrate determined its compatibility in a beverage. Therefore, utilization of hazelnut meal protein concentrate was investigated by analyzing the solubility, water and fat absorption capacities of protein. The utilization of hazelnut meal protein in a protein-enriched drink has been done and, rheological and sensory properties have been analyzed.

2. LITERATURE REVIEW

2.1. HAZELNUT

Hazelnut is a fruit of tree nuts and it is one of the major nuts together with peanut, almond, walnut, cashew and pistachio. Its natural growing area is in the temperate zone of Northern Hemisphere that has humid and mild climate [2]. The origin of hazelnut is known as Asia Minor and it is mentioned that the history of hazelnut culture dates back 5000 years [9]. Hazelnut (*genus Corylus*) belongs to birch family *Betulaceae* and subfamily *Corylaea* (1). There are two different important *Corylus* species among the other 25 species, approximately. They are *C. avellana L* known as the European hazel which has a wide cultivation area (Turkey, Italy, Spain, USA and Greece) and *C. colurna L.* known as Turkish hazel which is limited with Balkans, Romania and northern Turkey [1, 10]. These types of nuts have an economic importance, and they are produced commercially. These cultivars of hazelnuts are grown as shrub and having a leafy husk which is shown in Figure 2.1.

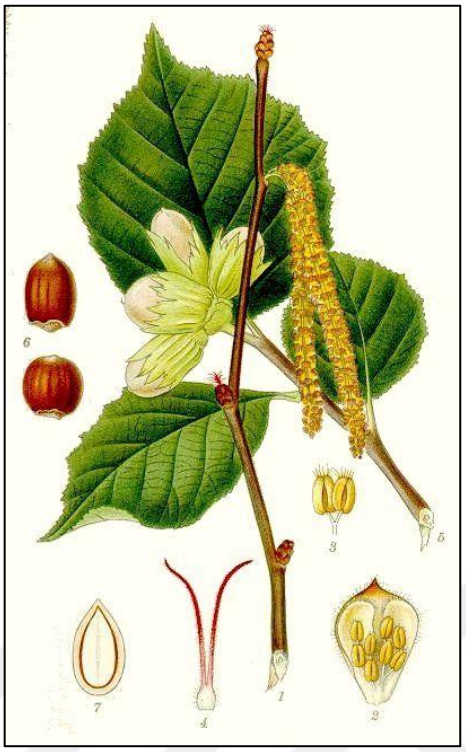

Figure 2.1. Hazelnut (*Corylus avellana L)* [4]

Hazelnuts are considered to be important for human diet due to their impressive nutritional value. They are a good source of fat, carbohydrate, protein, minerals and vitamins. It is a well-known fact that, physical and chemical composition of hazelnut shows differences according to their varieties. There are 18 cultivars of hazelnut (Acı, Cavcava, Çakıldak, Foşa, Ham, İncekara, Kalınkara, Kan, Karafındık, Kargalak, Kuş, Mincane, Palaz, Sivri, Tombul, Uzunmusa, Yassı Badem, and Yuvarlak Badem) cultivated in Turkey and also they are characteristically classified in 3 groups which are rounded, pointed and long [11-13]. On an average, 100 gram of hazelnut has 600-650 calories [13-15]. As mentioned before, hazelnut is rich in fat content and this rate changes between 50-73 percent. Due to the high incidence of unsaturated fatty acids, which are oleic, linoleic, palmitic, linolenic and stearic acid, it is considered to be beneficial for human health [14]. In Table 2.1, moisture and fat contents of 16 varieties of hazelnut are listed which show that fat content is between 56.07-68.62 percent, and the moisture is from 2.49 percent to 5.25 percent [3].

Table 2.1. Moisture and fat content of sixteen different kinds of hazelnut [3]

Variety	Moisture%	Total Oil%	Fatty Acids (g/100g)					
			Palmitic C16:0	Palmitoleic C16:1	Stearic C18:0	Oleic C18:1	Linoleic C18:2	Linolenic C18:3
Cavcava	5.25	56.07	5.87	0.22	2.37	78.8	12.7	0.069
Cakıldak	4.86	60.67	4.89	0.32	2.15	80.7	11.9	0.059
Fosa	4.46	59.5	5.62	0.37	1.7	79	13.2	0.074
Incekara	4.27	60.75	5.67	0.32	1.76	79.5	12.7	0.073
Kalınkara	4.14	68.52	5.71	0.42	2.42	79.5	11.9	0.067
Kan	3.41	63.05	5.72	0.32	2.3	81.8	9.82	0.053
Karafındık	2.49	67.75	5.62	0.28	2.37	78.9	12.8	0.058
Kargalak	4.39	59.57	4.89	0.42	0.86	81	12.7	0.067
Kus	4.41	61.25	5.69	-	0.87	79.9	13.5	0.076
Mincane	4.71	57.95	5.02	0.38	1.9	82.8	9.89	0.029
Palaz	4.76	57.65	4.87	0.34	2.13	77.6	15	0.076
Sivri	4.78	63.89	4.72	0.42	2.49	79.2	13.2	-
Tombul	4.63	64.6	5.17	0.48	1.75	77.8	14.8	0.054
Uzunmusa	4.17	61.75	5.7	0.46	1.41	78.8	13.6	0.069
Yassı Badem	3.56	63.48	4.87	0.28	1.43	81.1	12.2	0.046
Yuvarlak Badem	4.61	58.3	5.66	0.36	0.87	74.2	18.73	-

Hazelnut kernel protein content is reported to vary from 11 to 24 percent and this is almost 22 percent of daily protein intake [16-18]. Within that, protein content and amino acid structure of hazelnut varieties are given in Table 2.2 and the results change between 11.7 percent and 20.08 percent [3]. It is shown that Arginine and Leucine are dominant essential amino acids among these cultivars. These two amino acids compose 48 percent of the total amino acids and the highest contents are found in Yassı Badem and Kargalak, respectively.

Table 2.2 Protein and essential amino acid (mg/100g) content of seventeen different kinds of hazelnut [3]

Variety	Protein (%)	Essential amino acid (mg/100g)								
		Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Acı	16.6	2127	542	567	1124	424	148	582	434	684
Cavcava	20.8	1763	385	318	1169	389	146	576	474	618
Cakıldak	19.4	1864	521	627	1187	378	124	568	425	657
Fosa	15.8	2306	393	674	1025	474	189	718	474	768
Incekara	16.3	2265	512	567	1217	479	182	542	467	618
Kalınkara	11.7	2218	398	689	1197	447	149	749	517	807
Kan	17	2178	367	618	1269	481	159	767	502	624
Karafındık	15.6	1979	413	568	1215	402	178	724	423	616
Kargalak	15.2	2184	389	624	1271	469	148	557	432	642
Kus	16.8	1187	392	497	1179	427	148	561	448	671
Mincane	20	2249	393	548	1165	519	149	765	484	627
Palaz	18	1274	590	573	924	386	179	578	416	785
Sivri	18.7	2148	382	483	1085	489	163	728	468	617
Tombul	17.5	2146	377	519	1030	468	150	678	497	629
Uzunmusa	17	1867	382	624	1249	514	189	563	478	618
Yassı Badem	17.9	2322	348	565	1149	517	171	579	427	656
Yuvarlak Badem	20.8	1965	315	492	1093	395	169	598	492	633

Protein contents of Tarragona and Italian hazelnut are nearly same and Turkish hazelnut varieties (Ordu, Trabzon, Giresum, Akcakoca) have 12.9 g/100g, 13.2 g/100 g, 13.5 g/100 g

14.2 g/100g protein, respectively. Protein fractionation for different 22 varieties are also shown in Table 2.3 [16, 19, 20].

Table 2.3. General protein fraction of hazelnut varieties (g/100g dry matter) [16]

Varieties	Protein content (g/100g dry matter)			
	Fraction			
	Alb+Glo	Glu	Pro	Total
1	10.9	1.46	0.1	12.46
2	10.6	1.28	0.09	11.97
3	12.5	2.07	0.19	14.76
4	11.6	1.74	0.18	13.52
5	13.2	1.92	0.17	15.29
6	12.7	1.64	0.15	14.49
7	11	1.75	0.2	12.95
8	11.2	1.51	0.12	12.83
9	12.8	1.8	0.16	14.76
10	13	1.58	0.22	14.8
11	12.5	1.65	0.18	14.33
12	13.7	2.08	0.16	15.94
13	13.2	1.73	0.12	15.5
14	10.2	1.33	0.11	11.64
15	11.1	1.6	0.16	12.86
16	15.2	2.24	0.23	17.67
17	13.1	1.45	0.17	14.72
18	13.6	1.68	0.16	15.44
19	15.8	2.43	0.22	18.45
20	15.5	2.11	0.2	17.81
21	14.2	2.08	0.19	16.47
22	11.1	1.51	0.12	12.73

Due to high content of potassium, magnesium, calcium, vitamins B and E, iron and zinc, hazelnut consumption can help to reduce the risk of cancer, to improve bones, to increase muscle mass and to protect heart and digestive system [21, 22]. Ash and mineral contents of seventeen Turkish hazelnut cultivars are illustrated in Table 2.4. Results show that potassium is the most abundant mineral among the others and this content presents 55 percent of the total mineral. The highest potassium content is found in Cakıldak. In addition, carbohydrate content of seventeen hazelnut cultivars is also reported and results present that carbohydrate content ranged between 7.57-10.9g/100g that are cultivated in 2013 and between 6.47-

12.7g/100g that are cultivated in 2014 [3]. Researches show that carbohydrate composition of Turkish hazelnut includes pentose (2.62 percent), reducing sugars (0.12-0.18 percent), sucrose (4.79-5.57 percent) and starch (3.54-11.1 percent) [23, 24].

Table 2.4. Mineral content (mg/100g) of seventeen different kinds of hazelnut [3]

Variety	Total ash (%)	Mineral elements (mg/100 g)								
		K	P	Ca	Mg	Fe	Cu	Mn	Zn	Na
Acı	2.22	1036	340	204	208	4.3	2.2	3.6	2.7	2.16
Cavcava	2.72	886	331	161	152	3.7	2.8	7.7	3.2	2.63
Cakıldak	2.6	1470	335	224	224	5.1	2.6	10	4.4	2.42
Fosa	2.25	1052	339	172	176	4.8	2.6	8.4	3.1	2.22
Incekara	2.41	506	246	175	152	3.9	1.8	4.3	2.9	2.04
Kalınkara	1.87	914	233	65	144	4	2	2.4	2.2	2.62
Kan	2.13	750	285	101	168	3.3	2.2	3.5	2.3	2.79
Karafındık	1.9	776	325	194	160	5.1	2.5	7.5	3	2.66
Kargalak	2.37	928	202	158	144	3.6	1.7	2.5	2.4	2.26
Kus	2.3	706	239	180	176	3.8	2.3	3.1	2.4	2.71
Mincane	2.43	1002	285	214	184	5	2.5	4	3.3	2.37
Palaz	2.61	1014	370	328	200	4.9	3.2	7.7	3.4	2.32
Sivri	2.3	920	270	129	184	4	2.2	3.4	2.6	3.81
Tombul	2.43	814	288	217	168	4.2	2.3	7.7	2.7	3.19
Uzunmusa	2.34	872	288	234	160	4.2	2.3	7	3.6	2.31
Yassı Badem	2.42	382	228	174	144	3.2	2	4.8	2.2	2.42
Yuvarlak Badem	2.46	640	272	230	192	3.6	2.2	7.6	2.7	2.72

Like chemical composition, physical composition also depends on cultivar of hazelnuts. There are several physical properties like nut and kernel size, nut and kernel weight, shell thickness, kernel ratio, color *etc*. Some physical properties like dimensions (length, width, thickness), mass, diameter and surface area of nuts and kernels which varied in the range of 18.91-25.47 mm, 15.09-21.2 mm, 12.76-21.2 mm, 1.8-4.15 g, 16.15-22.41 mm, 8.21-15.82 cm^2 fot nuts, and 14.789-21.08 mm, 11.27-16.33 mm, 8.91-16.06 mm, 0.99-1.82 g, 13.05-16.64 mm, 5.36-8.74 cm^2 for kernels, respectively, are presented in Table 2.5 [25].

Table 2.5. Physical properties of hazelnut cultivars [25]

Hazelnuts	Lenght (mm)	Width (mm)	Thickness (mm)	Mass (g)	Diameter(mm)	Surface area (cm^2)
Nuts						
Allah verdi	20.76	18.07	18.07	2.49	18.92	11.26
Fosa	20.52	18.61	18.59	2.37	19.21	11.61
K-1/1	21.11	20.45	20.11	2.94	20.55	13.28
K-19/6	22.28	20.2	20.04	2.84	20.81	13.64
K-24/2	21.7	20.25	20.19	2.72	20.7	13.48
Kargalak	25.08	21.2	21.2	4.15	22.41	15.82
Kus	21.74	16.59	16.58	2.33	18.13	10.39
Mincane	19.04	17.2	17.06	2.02	17.74	9.9
Sivri	20.53	15.09	13.62	1.84	16.15	8.21
Uzun Musa	18.91	17.11	16.99	1.8	17.64	9.79
Yassi badem	25.05	16.91	12.76	2.61	17.52	9.67
Yuvarlak badem	25.47	15.32	13.85	2.3	17.53	9.67
Kernels						
Allah verdi	16.45	14.25	14.25	1.18	14.94	7.03
Fosa	16.25	14.39	14.38	1.28	14.97	7.06
K-1/1	16.85	15.14	14.77	1.5	15.55	7.61
K-19/6	17.29	16.11	15.84	1.46	16.39	8.48
K-24/2	16.89	16.33	16.06	1.5	16.42	8.48
Kargalak	18.99	15.62	15.62	1.82	16.64	8.74
Kus	17.59	13.76	13.76	1.25	14.92	7.02
Mincane	14.798	14.05	13.59	1.05	14.13	6.29
Sivri	16.47	12.44	10.87	0.99	13.05	5.36
Uzun Musa	15.71	14.43	14.11	1.11	14.72	6.83
Yassi badem	20.74	12.35	8.91	1.21	13.14	5.43
Yuvarlak badem	21.08	11.27	10.46	1.27	13.52	5.76

For color, L (lightness-darkness), a (red-green) and b (blue-yellow) are measured and results are represented in Table 2.6 [25].

Table 2.6. Color properties of hazelnut cultivars [25]

Hazelnuts	L	a	b
Nuts			
Allah verdi	27.17	11.04	19.48
Fosa	23.7	12.01	18.18
K-1/1	27.2	11.38	19.89
K-19/6	24.97	8.67	13.23
K-24/2	17.33	10.46	16.29
Kargalak	33.35	14.33	23.82
Kus	26.58	13.65	22.1
Mincane	21.07	9.53	13.81
Sivri	34.95	13.15	21.35
Uzun Musa	27.36	10.29	15.74
Yassi badem	26.87	10.75	17.45
Yuvarlak badem	29.41	13.23	21.14
Kernels			
Allah verdi	28.83	12.63	22.55
Fosa	28.61	12.16	22.21
K-1/1	28.55	10.14	20.38
K-19/6	24.31	9.44	18.08
K-24/2	26.02	11.96	20.32
Kargalak	24.35	11.46	19.41
Kus	30.8	12.29	22.22
Mincane	24.28	10.52	19.96
Sivri	32.01	10.64	23.22
Uzun Musa	29.85	10.91	20.93
Yassi badem	34.96	12.06	24.01
Yuvarlak badem	33.59	11.7	22.02

2.2. RATES OF PRODUCTION AND CONSUMPTION OF HAZELNUT

Hazelnut has an economic and ecological importance for most of the countries, especially for Turkey with about 70 percent of the total global production which makes leader country in hazelnut production amount. Italy (over 13 percent), USA (4.1 percent), Azerbaijan, Georgia and Spain follow the ranking, and Iran, China, France and Greece are other producers that are shown in Figure 2.2 [4].

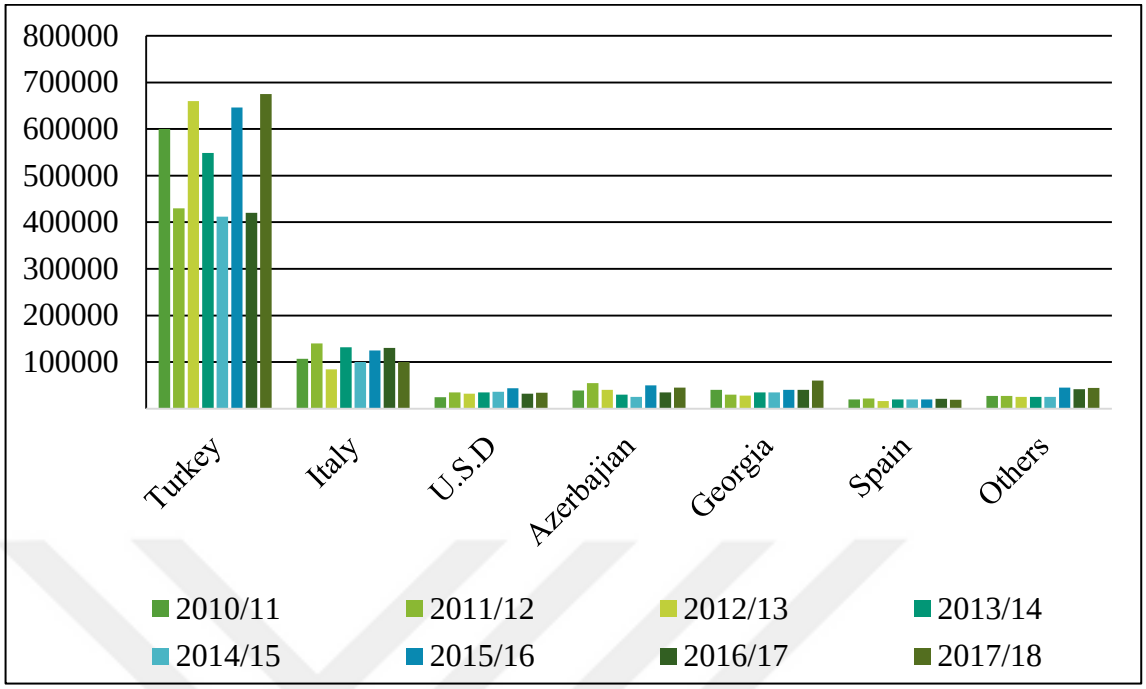

Figure 2.2. Production rates (in tones) of hazelnut [4]

According to Worldwide hard-shell fruit production rate, hazelnut has the third rank after almond and walnut with having 903.864 hectares production area and Turkey which has the widest cultivation area has around 703.000 hectares (77 percent of total production area). Italy has the second rank, followed by Azerbaijan, Georgia, Iran, USA, Spain, Chile and China (Figure 2.3).

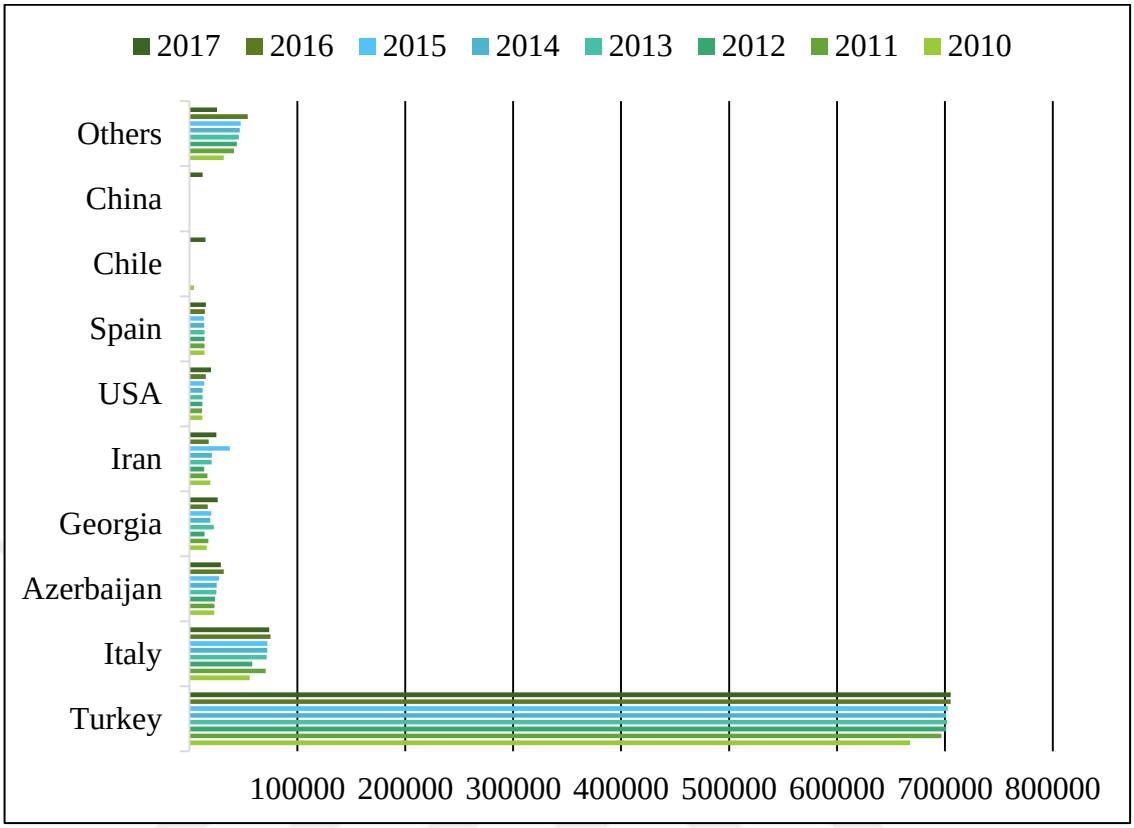

Figure 2.3. Production area (in hectares) of hazelnut [4]

In Turkey, hazelnut is generally produced in Black Sea region due to its very favorable ecological condition for good quality hazelnut. According to statistical record of TUIK, there are 33 provinces where hazelnut is produced in Turkey. Ordu (32 percent), Giresun (17 percent), Samsun (13 percent) are provinces that lead the production, followed by Sakarya, (10 percent), Trabzon (9 percent) and Duzce (9 percent).

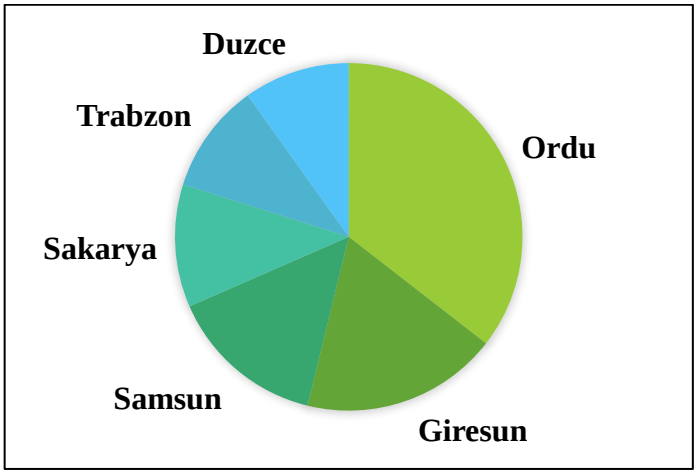

Figure 2.4. Production area of hazelnut in Turkey [4]

Among the leading producing countries, Italy has the highest consumption rate, followed by Greece. Although Turkey is main hazelnut producing country, consumption rate is very low that makes it third country. Worldwide, Switzerland is the greatest consumer country and consumption rate (per person in a year) is four times more than Turkey's. As mentioned before, hazelnut is very important fruit economically, because all parts of hazelnut are used in wide range of industrial areas, especially in food industry. Besides being suitable for fresh consumption as snack, hazelnuts are used as an ingredient in 80 percent of chocolate industry as sliced, blanched, roasted, chopped and powder, and used in confectionery products and biscuit, cake and ice-cream manufacturing.

There are three hazelnut by-products which are hazelnut green leafy cover, hazelnut hard-shell and hazelnut kernel. Hazelnut green leafy cover surrounds hazelnut hard-shell and kernel and rarely found with hazelnut tree leaf. They are removed mechanically. There is hazelnut hard-shell under the green leafy cover and contain kernel which is consumed commercially. Hazelnut green leafy cover is mostly used as fertilizer, hazelnut hard-shell is used for energy production as a fuel due to its flammable structure and lastly, kernel that is edible seed that has huge utilization area in food industry as an ingredient. In addition, because of the high fat content, hazelnut is also used to produce hazelnut oil as flavor and cooking oil for the food industry.

2.3. HAZELNUT OIL

Hazelnut oil is obtained from the hazelnut fruit which has a high fat content, approximately 65 percent. Due to unsaturated fatty acid content, particularly monounsaturated fatty acids (mainly oleic acid) which generates 70-82 percent of it, it has a significant role in human health and also researches showed that high oleic acid content improves digestibility of total fat [14, 26]. Besides useful fatty acids composition, hazelnut oil contains abundance of phytosterols that have antioxidant properties. It is known that phytosterols reduce blood cholesterol and risk of cancer [27-30]. In Table 2.7, chemical composition of hazelnut oil is shown.

There are basically two hazelnut oil production processes that are physical and chemical. In physical process, hazelnut oil is obtained by using mechanical power. Hydraulic pressing and screw pressing are physical process examples for gathering oil from kernel. In addition, chemical processes are also used to remove oil with extraction method using conventional solvent materials. Hazelnut oil process which is shown in Figure 2.5 starts with mechanical cleaning. In cleaning step, hazelnuts are properly prepared for oil process by removing undesired materials that can damage the process. After that, kernels are dehulled and hard-shells are removed by cracking. Before pressing steps (prepressing or final step), hazelnut kernels are mostly conditioned by heating. The reason of prepressing step, before the extraction, is efficiency. Oil in pressed kernel is dissolved by hexane and oil is released as a form of oil-hexane solution which is called miscella. In distillation step, hexane is removed from both hazelnut residue and miscella and is collected for reuse. In this way, crude oil is separated from hexane and meal is produced. Furthermore, after crude oil is produced, refining process is applied to improve odor, color, flavor, and stability of oil [32].

Table 2.7. Composition of hazelnut oil [31]

Compound	Hazelnut oil
Fatty acids (%)	
Palmitic acid (C16:0)	5-7
Stearic acid (C18:0)	1-3
Oleic acid (C18:1)	70-82
Linoleic acid (C18:2)	8-17
Linolenic acid (C18:3)	0.1
Tocopherols (ppm)	
α-Tocopherol	329-448
β- Tocopherol	2-6
γ-Tocopherol	5-47
δ-Tocopherol	0.3-4.5
Phytosterol classes (ppm)	
4-desmethylsterols	
Sitosterol	1050-1700
Campesterol	50-95
Stigmasterol	10-18
Δ5-Avenasterol	20-80
Total	1200-2000
4-monomethylsterols	
Obtusifoliol	Tr-18
Gramisterol	Tr-17
citrostadienol	17-122
4,4'-dimetylsterols	
β-Amyrin	12-192
Butyrospermol	Tr-27
Cycloartenol	Tr-96
24-Methylenecycloartanol	Tr-72
Wax esters (ppm)	
C36	42-186
C38	21-97
C40	18-80
C42	Tr
C44	1-16
C46	3-17
Aliphatic alcohols (ppm)	
C23	-
C24	4-34
C25	6-34
C26	5-59
C27	-

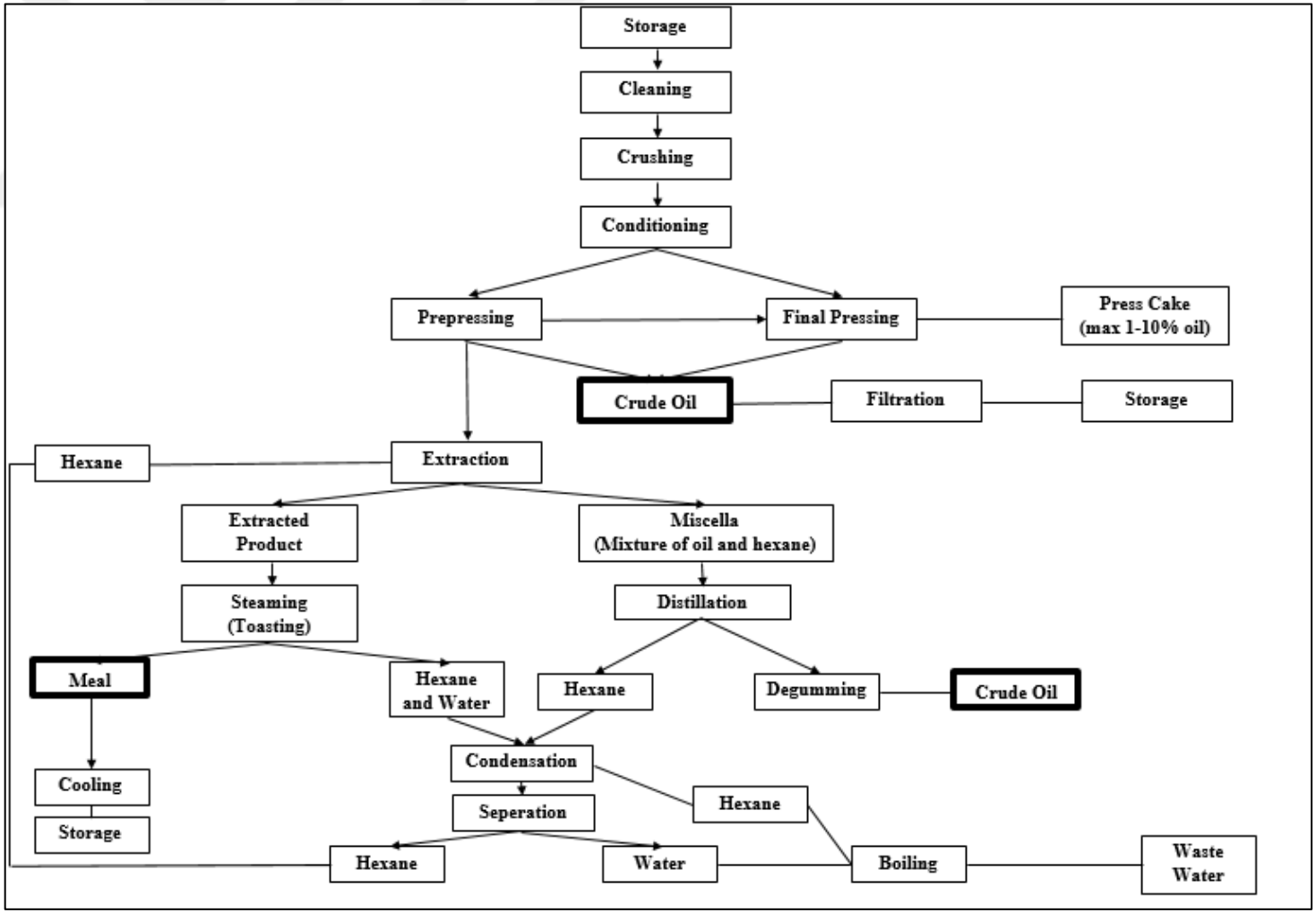

Figure 2.5. Hazelnut oil and hazelnut meal production process [32]

2.4. FUNCTIONAL PROPERTIES OF HAZELNUT MEAL

As mentioned above, hazelnut meal is a main by-product of industrial hazelnut oil production process. Table 2.8 shows the nutritional composition of different types of hazelnut meal. It is clear that hazelnut meal is rich in protein which could be extracted and used in the food industry.

Table 2.8. Composition of hazelnut meal obtained from different processes [5]

Type of Meal	Solid Content (%)	Ash (%)	Protein (%)	Crude Oil (%)
Extraction	91.3	8.2	42.1	1.8
Expeller	92.2	7.1	39.4	9
Press	92.3	3.7	40.4	11.2

Detailed investigation of hazelnut meal (Tabel 2.9) revealed that glutamic acid, aspartic acid and arginine are the main amino acids (8.81, 3.28 and 4.17 g/100 g, respectively) and also it contains all essential amino acids with 80-90 percent digestibility [6, 7, 33].

Hazelnut meal is used as plant protein source in animal feed industry, especially used in aquaculture as fish-feed in Turkey, in Black Sea region where is almost all hazelnut production is made [34-36]. In addition to that a research shows that hazelnut meal is highly digestible product with in results of effective protein degradability and digestion coefficient of organic matter and crude protein [33]. Therefore it can be used as an ingredient for food industry even if it is mostly used for animal feed.

Table 2.9. Composition of defatted hazelnut flour [7].

Composition	Hazelnut Meal
Proteins	32.2
Fat (%)	9.86
Carbohydrates (%)	25.7
Fibre (%)	20.7
Moisture (%)	6.03
Ashes (%)	5.56

Phosphorus (%)	0.78
Potassium (%)	1.78
Iron (mg / 100 g)	6.83
Calcium (%)	0.41
Sodium (%)	1.76
Magnesium (%)	0.37
	g/100g
Aspartic acid	3.28
Glutamic acid	8.81
Alanine	1.92
Arginine	4.17
Phenylalanine	1.35
Glycine	1.49
Hydroxyproline	0.13
Isoleucine	1.11
Histidine	0.57
Leucine	2.07
Lysine	0.95
Proline	1.18
Serine	1.5
Tyrosine	0.84
Threonine	0.99
Valine	1.42
Cystein and Cystin	0.62
Methionin	0.4
Tryptophan	0.35

2.5. PROTEIN-ENRICHED DRINKS

In recent years, consumer interest towards functional food and drinks has been increasing. The reason is that these functional products provide additional nutrients in daily diets. Beyond satisfying the basic nutritional needs of the body, it is also defined as foods that provide additional benefits on metabolic functions, thereby protecting body from diseases and achieving a healthier life. It is known that the nutrients that provide these benefits can be found in foods naturally or can be added from outside. The simplest method to produce functional foods is enriching them in vitamin, minerals, proteins, fatty acids or antioxidant for specific purposes which are seen as a key for healthier lifestyle. For providing this

healthier lifestyle, easy and quick alternatives like functional snacks or beverages are demanded. According to research that made in 2017, 65 percent of adults looked for vitamin and minerals-enriched food or beverage, 63 percent of them needed more fiber foods and 60 percent of them demanded protein added products [37]. According to a research, 60 percent of consumers in the USA indicated that, when they bought a food or beverage, health and wellness had an important effect on purchasing decision and these consumers define healthy food as if it contains high amount of nutrients [38]. There is a new customer category and people in this category exercise in their daily routine and they are interested in protein consumption. This is because people who are aged 18-34 believe that protein consumption helps to maintain healthy bones and one-third of adults believe that protein is a very important nutrient for immune system [39]. In addition, 64 percent of adults think that protein consumption supplies energy during all day and 50 percent of protein drink users consume it because of this reason [40-42]. Among the other ones, protein is the most popular functional food ingredient, nowadays. It has a huge utilization area and because of this, a large variety of protein sources are demanded. The sales of nutritional bars indicate increase as 18 percent whilst premier protein drinks show plus 84 percent [8, 43].

According to a report about trends of ingredients usage in the beverages, there are 20 categories and alcoholic drink, hot drink, soft drink and dairy drink are the major ones [44]. Soft drinks include bottled water, carbohydrates, fruit/vegetable juice, RTD coffee, RTD tea, sports and energy drinks and Asian specialists drink [44]. Also protein enriched drink is in this category and health and wellness is the most focused demand among consumers. Protein is mostly used in beverages for increasing textural properties. However it has a huge role in sports recovery, satiety and weight management. All things considered, protein enriched drinks, especially with vegetable proteins, have been gaining interest among the consumers and this rising interest generates a huge demand.

Since interest in protein beverages is recently beginning to increase, it is a new market. Therefore, there is no statistical study about that but they are categorized as fortified/functional beverages. Euromonitor has estimated sales of fortified/functional beverages was 717.1 TRY million with 94.8 percent current growth, in 2017 [45]. In Turkey, Red Bull, Coca-Cola, Dogadan, Unilever, Nazli Gida, Nestlé and Sirma are major local companies that produce fortified/functional beverages. Coca-Cola has Zico Protein Smoothie and Sirma has Sırma Protein and Sırma Kolajen [45]. In addition to that, Zeroshot

Dumbbell Therma Burn, Protein2o, Reneva Collagen protein drink Fit and Reneva Collagen protein drink Beauty are protein beverage products that are sold in Turkey.

3. MATERIALS AND METHODS

3.1. MATERIALS

Hazelnut meal (HM) (Figure 3.1) was kindly provided from Çotanak/Altaş (Ordu, Turkey) and it was stored at -20°C until used. Catalyst tablets (5g Potassium Sulphate (K2SO4)- 0.5g Copper(II) Sulphate (CuSO4.5H2O)) obtained from Gerhardth (Königswiner, Germany), sulfuric acid (95-97 percent) from Fluka Chemie (Buchs, Switzerland), boric acid (≥99.5 percent) from Sigma-Aldrich (Steinheim, German), bromocresol green indicator ($C_{12}H_{14}Br_4O_5S$) from Merck (Darmstadt, Germany), methyl red indicator from Sigma-Aldrich (Steinheim, German), ethanol (≥99.8 percent) from Sigma-Aldrich (Steinheim, German), hexane (95 percent) from Sigma-Aldrich (Steinheim, German), Yudum sunflower oil, Bradford reagent from Sigma-Aldrich (Steinheim, German), albumin from bovine serum (BSA) from Sigma-Aldrich (Steinheim, German), sucralose, citric acid, vitamin mix, lemon emulsion, ascorbic acid, sodium benzoat, potassium sorbat, fruitmax starfruit bright WS, capcolors orange 058 WSS were used.

Figure 3.1. Hazelnut meal

3.2. METHODS

3.2.1. Protein Extraction from Hazelnut Meal Flour

Approximately, 100 gram of HM was ground around for 45 seconds by a mixer. Grounded hazelnut meal flour (HMF) was passed through the 2.24 mm screen and stored at 4°C until used (Figure 3.2).

Figure 3.2. Hazelnut meal flour

HMF was used directly in protein precipitation procedure and it was mixed with water in a ratio of 1/12 (w/w). The pH of the solution was set to 12 by 5 M NaOH and stirred at room temperature with magnetic stirrer for 1 h at 400 rpm (Figure 3.3).

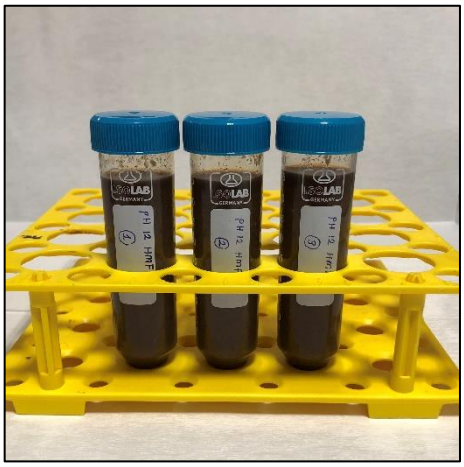

Figure 3.3. Hazelnut meal solution at 12 pH

Solutions were centrifuged (Sigma, 3-30K) at 3.000 g for 15 min at 4°C. The pellet which is called hazelnut meal pellet (HMPl) was freeze dried and stored at 4°C for determining chemical compositions and protein whereas the remaining protein in the obtained supernatant was further precipitated at pH 4.5 by 2M HCl (Figure 3.4).

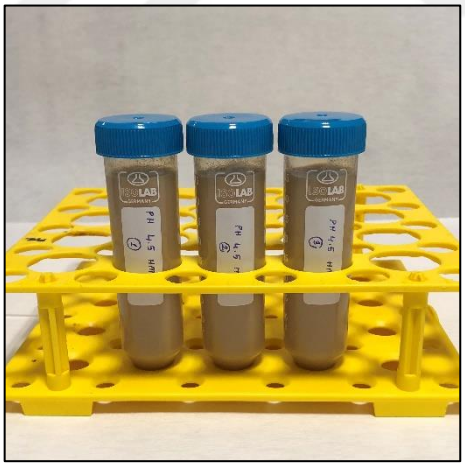

Figure 3.4. Hazelnut meal solution at 4.5 pH

Afterwards, the solutions were centrifuged at 3.000 g for 15 min at 4°C. The supernatant was removed and the obtained precipitate was suspended in distilled water at 10 percent w/v, frozen at -80°C overnight and freeze-dried (CHRIST, Alpha 2-4 LD Plus, Germany) for 5 days. Finally, hazelnut meal protein precipitate (HMPP) was obtained (Figure 3.5).

Figure 3.5. Hazelnut meal protein precipitate (HMPP)

3.2.2. Proximate Composition

3.2.2.1. Moisture Content

Moisture content of HMF, HMPP and HMPl were determined with rapid moisture analyzer (OHAUS® MB 45). 3 g of samples were weighed on aluminum plate which was on the digital balance of analyzer and then heated. Temperature program was arranged as fast, drying temperature was 180°C and measurement time was 4 min.

3.2.2.2. Ash Content

AOAC Official Method 923.03 was used for determination of ash content in samples. One gram of samples were weighed and put in crucibles then they were placed in muffle furnace (Nabertherm). Ignite was done at 550°C for 24 h. After that, crucibles were taken and put in desiccator until they reached to room temperature. Then, ash contents of samples were weighted.

3.2.2.3. Protein Content

The Kjeldahl procedure was used to determine the protein content of samples and the conversion factor was used as 6.25 [46]. Approximately 0.5 gram samples were put in the

Kjeldahl digestion tubes and added two catalyst tablets (Kjeltabs CX) with 20 ml concentrated sulfuric acid. All digestion tubes were placed on the digestion block (BUCHI SpeedDigester K-425) and samples were heated at 400°C for 6 h. When the samples were allowed to cool to room temperature, 50 ml distilled water and 80 ml NaOH (33 percent, w/v) were added to each tube in distillation unit (BÜCHI Distillation Unit, K-355). Nitrogen was trapped in 4 percent boric acid solution and total nitrogen concentration was determined by titration with 0.1 N standardized HCl solution. Titration was done until the endpoint of indicators (0.1 percent bromocresol green and 0.1 percent methyl red in ethanol) which is pH 4.2.

3.2.2.4. Amino acid Composition

Amino acid profile of HMPP was provided by Sinop University using liquid chromatography-tandem mass spectrometry (LC-MS/MS) according to method described by Scientific and Technological Research Application and Research Center (SÜBİTAM) [39]. Jasem amino acid kit was used as an analysis kits. The concentration of target amino acids were analyzed in electrospray ionization (ESI) mode and multiple reaction monitoring (MRM) method was used. Amino acid analysis was done after acid hydrolysis. 0.5 g HMPP sample and 4 ml Reagent-2 was added into a glass tube. Tube was kept at 110°C for 24 h until all amino acids were hydrolyzed. When hydrolysate reached to room temperature, it was centrifuged at 4.000 rpm for 5 min. Then, 100 µL of supernatant was transferred and mixed 900 µL distilled water. Solution was diluted once again. 50 µL of diluted hydrolysate was pipetted into vial and 50 µL internal standard solution and 700 µL Reagent-1 were added onto sample. Solution was vortexed for 5 s and 3 µL of all samples were injected to LC-MS/MS system. Chromatographic separation was completed by using A and B mobile phases with a flow rate of 0.7 ml/min in a gradient program for 7.5 min. Settings of electrospray source were as described: capillary voltage +2000 volt, gas temperature 150°C and gas flow 10L/min.

3.2.2.5. Fat Content

Fat was extracted with hexane in automatic Soxhlet unit (BUCHI E-812). One gram of sample was weighed in a thimble with glass wool and thimble was placed in Soxhlet extraction unit. 80 ml hexane was added in beakers and they were then put in heating panel. Hexane was chosen as a solvent in program settings and after 12 cycles extraction was done. Hexane was removed by heating and at end of the process, oil was weighed.

3.2.3. Functional Properties

3.2.3.1. Water Absorption Capacity (WAC)

One gram of HMF and HMPP were mixed with 10 ml water and vortex for 2 min. After that solutions were incubated at room temperature for 30 min and then centrifuged at 3.000 g for 20 min at 20°C. Supernatant was decanted and after 10 min drainage, sediment was weighed.

Water absorption capacity (3.1) (WAC);

$$WAC = \frac{weight\ of\ sediment\ (g)}{weight\ of\ dry\ sample(g)} \quad (3.1)$$

3.2.3.2. Fat Absorption Capacity (FAC)

One gram (w_0) of HMF and HMPP were mixed with 10 ml (v1) (sunflower oil and vortexed for 2 min. After that solutions were incubated at room temperature for 30 min and then centrifuged at 3.000 g for 20 min at 20°C. Supernatant (v2) was decanted into 10 ml graduated cylinder and volume of oil was recorded. According to volume, FAC was calculated.

Fat absorption capacity (3.2) (FAC);

$$FAC = \frac{v_1 - v_2}{w_0} \quad (3.2)$$

3.2.3.3. Protein Solubility

Protein solubility of HMPP was assessed with different pH valuess between pH 3.2 (pH of hazelnut meal protein beverage) and pH 12. Soluble protein content of precipitate was determined by Bradford method and as a standard albumin from bovine serum was used. 10 mg HMPP was suspended in 10 ml distilled water and pH was adjusted by using 0.01 N NaOH and 0.01 N HCl. For clarification of solution, centrifugation was done at 4500 g 15 min at 4°C and soluble protein content was determined in this supernatant. For preparing standard solution, 20 mg BSA was mixed with 10 ml distilled water and the standard solution was diluted in a range between 0.1-1.4 mg/ml. 0.1 ml of supernatant was mixed with 3 ml Bradford reagent and vortexed for 2-4s. Solution was incubated at room temperature for 45 min. Sample was transferred to plastic cuvette and absorbance was measured at 595 nm. Soluble protein concentration was determined according to standard curve with Equation (3.3).

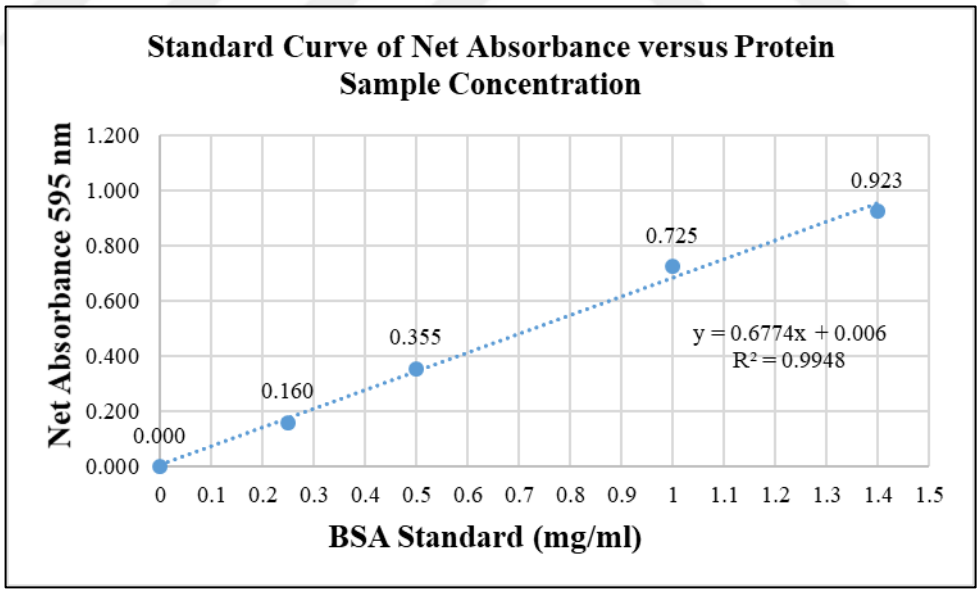

Figure 3.6. Standard curve of net absorbance versus protein sample concentration

Protein Concentrate Equation (3.3) (Pc);

$$Pc\ (mg/ml) = \frac{(abs\ value\ of\ sample - abs\ value\ of\ blank) - 0.006}{0.6774} \qquad (3.3)$$

Protein solubility (Ps) was determined at different pH values between 3.2 and 12 and results were calculated according to Equation (3.4).

Protein Solubility Equation (3.4) (Ps);

$$Ps\ (g/g_{Pc}) = \frac{Pc_t}{Pc_{Total}} \tag{3.4}$$

Pc_t : Protein concentration after hydrolysis, (g/L)

Pc_{Total} : Total protein concentration, (g/L)

3.2.4. Production of Hazelnut Meal Protein-Enriched Drink

3.2.4.1. Beverage Formulation

The basic industrial ingredients were used for protein enriched drink with two different protein concentrations. These ingredients were sucralose, citric acid, vitamin mix, lemon emulsion, ascorbic acid, sodium benzoate, potassium sorbate and HMPP and the formulation was shown in Table 3.1. for 250 ml protein enriched drink.

Reference drink was chosen as ZeroSHOT® Dumbbell BCAA which contains 0.21 percent taurine, 0.42 percent glutamine and 1.05 percent BCAA (branched-chain amino acid).

Table 3.1. Protein enriched drink formulation for 250 ml drink

HMP for 2 percent	5g
HMP for 4 percent	10g
Sucralose	0.025 g
Citric acid	0.1 g
Vitamin Mix	0.0375 g
Lemon Emulsion	0.3 g

Ascorbic acid	0.05g
Sodium Benzoat	0.0375g
Potassium Sorbat	0.0625g
Fruitmax Starfruit Bright WS	10 µl
Capcolors Orange 058 WSS	6 µl

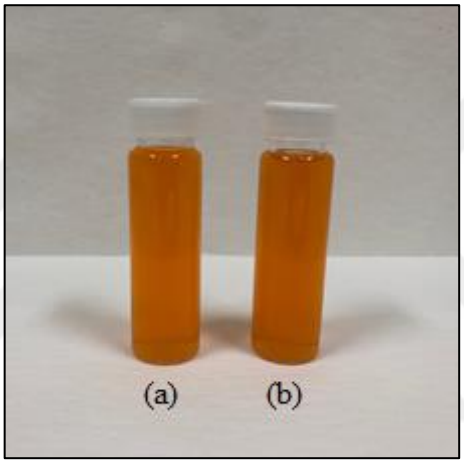

Figure 3.7. Hazelnut meal protein-enriched drink with 2 percent protein (a) and 4 percent protein (b)

Both formulated beverages were pasteurized at 80°C. After 2 minutes they filtered in to the bottles. The sealed bottles were pasteurized at 80°C for 15 minutes again and stored at 4°C until they were analyzed.

3.2.4.2. pH

pH measurement of two different formulated beverages was determined with the use of a calibrated pH metre (MeterLab, PHM210 STANDARD pH METER).

3.2.4.3. Color

Lightness (whiteness, L*), green to red color (negative-to-positive scale, a*), and blue to yellow color (negative-to-positive scale, b*) values of 2 percent protein and 4 percent protein drinks were measured using a colorimeter (Konica Minolta CM-5).

3.2.4.4. Turbidity

The turbidity of protein enriched beverages was measured using a spectrophotometer at 600 nm (GENESYS 10S UV-VIS Spectrophotometer) with plastic cuvettes and calibrated with distilled water.

3.2.4.5. Titratable acidity

To determine titratable acidity, organic acid in beverages was titrated with NaOH and titratable acidity in this drink measured according to end point of indicator phenolphthalein. For analysis, CO_2-free water was prepared. 500 ml distilled water was boiled in a flask. After that, ascarite in syringe was attached to the flask and water was cooled to room temperature. 1 percent phenolphthalein solution was used as an indicator. One gram phenolphthalein was dissolved in 100 ml ethanol. For standardized 0.1 N NaOH solution, KHP solution was used. 2 g KHP was dried in an oven at 120 °C for 2h. 0.4 g KHP was mixed with 25 ml CO_2-free water, 3 drops of 1 percent phenolphthalein solution were added and solution was titrated with 0.1 N NaOH until end point of indicator that was pH 8.3. After that, 10 ml sample was pipetted and mixed with 25 ml CO_2-free water. 3 drops of 1 percent phenolphthalein solution were added and solution was titrated with 0.1 N NaOH until end point of indicator that was pH 8.2.

3.2.4.6. Total soluble solid content

Total soluble solid content of the hazelnut meal protein enriched-drink was determined as °Brix at 25 °C using a digital refraction meter (Bellingham+Stanley DR103L). Calibration was done against 0 percent, 30 percent and 60 percent w/w sucrose.

3.2.4.7. Viscosity

Viscosity was determined using a rheometer (Malvern, Kinexus Pro) Measurements were performed over a shear rate range of 1–100 s^{-1} at 20^{o}C. The sample was placed between the cone and plate and the viscosity was determined according to shear rate parameters of the samples.

3.2.5. Sensory Analysis

The protein enriched drinks were analyzed for appearance, taste, sweetness, bitterness, sourness, texture, aroma and overall acceptability. 75 untrained panelists opinions' were measured in different scale. In this thesis nine-point hedonic scale from "1 = Dislike Extremely" to "9 = Like extremely" was used and briefly information about survey (Appendix A) was given panelists. Each panelist tested three beverages that identified with random three digit numbers (374, 785, 962) that were designed randomly . Water and cracker were given as a neutralizer between each sample.

3.2.6. Statistical Analysis

All analyses were carried out for three replicates and the data were reported as means ± standard deviation. Statistical analyses were performed using t-test and one-way analysis of variance (ANOVA, $p < 0.01$) by Minitab® v. 18 (Minitab Inc., USA). When ANOVA analysis revealed significant difference, Tukey post hoc test was applied to identify the statistically different groups ($p < 0.01$).

4. RESULTS AND DISCUSSIONS

4.1. PROTEIN EXTRACTION FROM HAZELNUT MEAL FLOUR

Protein extraction was done according to isoelectric point of hazelnut meal protein which was pH 4.5. According to result, 24.57 g of 100 g HMF was precipitated and this amount generated approximately 60 percent of whole hazelnut meal protein.

4.2. PROXIMATE COMPOSITIONS

Proximate compositions of hazelnut meal samples are represented in Table 4.1. Results show that moisture content of HMF (8.33±0.07) is higher than other samples and is in agreement with results in Table 2.8. [5]. Moisture content of HMPP and HMPl are slightly the same and they have low moisture content. Lyophilization is the main reason of the low moisture content for these samples. Results indicated that 40.73 percent of HMF is protein. Approximately, 60 percent of hazelnut meal protein was precipitated at 4.5 pH and this precipitate had 89.13 percent protein. In addition to that there are different researches performed with similar hazelnut meal. In these researches protein solubility was analyzed at pH 9.5 [47-49]. According to total protein content of these hazelnut meal from oil extraction determined as 44.8, 46.8 and 54.4 percent in dry weight basis, respectively [47-49]. These results demonstrated that hazelnut meal is quite suitable for protein extraction. Moreover, total protein content that was searched in this thesis higher than other protein sources like sunflower (32 percent) and rapeseed (35 percent) meals, and very similar to soybean meal between 48.5 and 58.1 percent [50, 51].

The protein content of HMPP was almost same with a research that was investigated with same sample [38]. The obtained protein is called as a concentrate because protein content of HMPP is higher than 65 percent and lower than 90 percent dry weigh basis [39]. Also, after protein solubilisation, protein content of HMPl was investigated and found as 14.67 percent. Within that, high protein content of this precipitate is the most conclusive proof that HMPP is a potential ingredient for food industry. Results show that approximately 40 percent of

HM is hazelnut meal protein and 24 percent of HM was precipitated as HMPP and non-precipitated part which was 14 percent of HM was in HMPl.

Table 4.1. Proximate compositions of hazelnut meal samples.

	HMF	**HMPP**	**HMPl**
Moisture (%)	8.33±0.07[a]	2.58±0.08[b]	2.92±0.03[c]
Ash (%)	5.99±0.12[a]	3.85±0.39[b]	9.41±0.50[c]
Protein (%)	40.73±0.13[a]	89.13±0.96[b]	14.67±0.74[c]
Fat (%)	10.02±0.13 [a]	2.53±0.03 [b]	6.31±0.31[c]
Carbohydrate (%)	34.92±0.12 [a]	1.92±1.02 [b]	66.70±0.89 [c]

Data represent average of three independent samples ± standard deviation. Different superscript in rows represent statistically significant differences (p<0.01).

Fat content of samples showed that HMPP has the lowest fat content and it was followed by HMPl and HMF. HMF has 10.02±0.10 percent fat content and this results was mostly higher than the other results that were analyzed with same samples [34-36]. In addition to that in hazelnut oil production process, after extraction, oil content of meals are in a range between 1-10 percent so results are in agreement according to this process. Reason of some differences in results is the proximate composition of hazelnut meal samples were affected in many ways like by type of hazelnut, climate effect and environmental factors.

It is known that dose of essential amino acids (EAA) is in a correlation with muscle protein synthesis [52, 53]. In addition to that, it is a significant fact that EAA content of protein source must be identified for growth of muscle or maintain muscles weight [53]. Totally 18 essential and non-essential amino acid were identified for HMPP (Table 4.2). Among the other EAA (cystine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine and valine), arginine has the highest content and it accounts for approximately 28 percent of all essential amino acids. Leucine follows it with 5.96±0.06 g/100 g amino acid. Arginine and leucine results in agreement with that both of them are two of the most abundant essential amino acid in hazelnut meal protein [3]. Also all essential amino acids in HMPP generate 47.11 percent of total amino acids. According to results, among the non-essential amino acids (NEAA) (alanine, aspartic acid, glutamic acid, glycine, ornithine, proline, serine and taurine), glutamic acid is determined in a highest percentage value that is approximately 40 percent of all NEAA and this finding was clearly in accordance with the literature studies [6, 7, 17]. In addition to that, all NEAA in HMPP accounts for 52.89 percent of total amino acids. As a result of this composition, HMPP seem

to be a good protein and amino acids source. Therefore, use of this protein concentration should be considered.

Table 4.2 Amino acid composition of hazelnut meal protein precipitate (HMPP)

	Amino acids	**g/100g**
EAA	Arginine	10.30 ± 0.07
	Cystine	0.99 ± 0.01
	Histidine	0.53 ± 0.01
	Isoleucine	2.95 ± 0.06
	Leucine	5.96 ± 0.06
	Lysine	2.46 ± 0.02
	Methionine	0.77 ± 0.01
	Phenylalanine	4.08 ± 0.02
	Threonine	2.82 ± 0.02
	Tyrosine	2.38 ± 0.02
	Valine	3.42 ± 0.09
	BCAAs	**12.33**
	Total EAA	**36.65**
NEAA	Alanine	4.29 ± 0.03
	Aspartic Acid	9.79 ± 0.09
	Glutamic Acid	16.59 ± 0.05
	Glycine	3.09 ± 0.10
	Ornitine	0.04 ± 0.00
	Proline	3.21 ± 0.04
	Serine	4.12 ± 0.04
	Taurine	0.00 ± 0.00
	Total NEAA	**41.14**

Data represent average of three independent samples ± standard deviation.

EAA profile of HMPP was compared with defatted soybean meal (DSM) because soybean is one of the most common plant protein source in human diets and also with whey protein (WP) which is a widely used animal based protein (Figure 4.1) [52, 54-56].

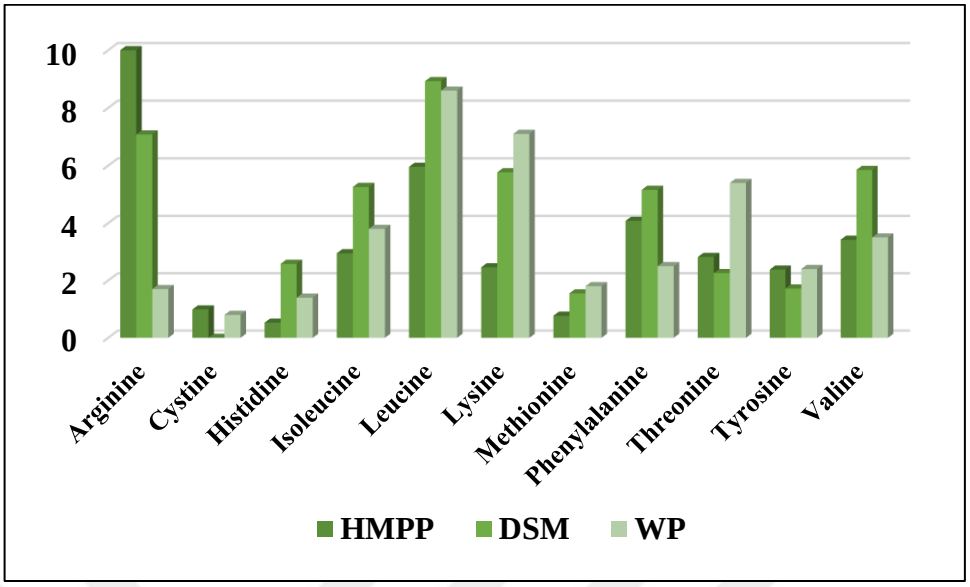

Figure 4.1. Essential amino acid profile of HMPP, DSM and WP

It was observed that HMPP has relatively low EAA content than DSM and WP and all protein isolates or concentrates were low in sulfur-containing amino acids which are methionine and cysteine. Arginine had the highest level in HMPP and less obvious variability was determined between HMPP and WP in cysteine, isoleucine, methionine, tyrosine and valine. The content of branched chain amino acids (BCAA) in HMPP was almost the same with WP and lower than DSM. BCAA are an important group for increased muscle growth because leucine in BCAA affects the reaction of responsible for muscle protein synthesis [57, 58]. It was proven in a study, BCAA alone increases muscle growth [59]. Additionally, when people consume 5.6 grams of BCAA in a drink after exercise, their muscle protein synthesis increase 22 percent than others [59]. During exercise muscles use BCAA in blood and decreasing BCAA level in blood leads to increased tryptophan which is responsible of fatigue during exercise [58-61]. According to WHO/FAO/UNU, EAA requirement is given as at least 24 percent of total amino acids and EAA content of HMPP was higher than this requirement [62]. Lysine content of HMPP was also higher than the WHO/FAO/UNU requirement but lower than WP and DSM.

4.3. FUNCTIONAL PROPERTIES

4.3.1. Water and Fat Absorption Capacities

There are some important functional properties that affects utilization area of proteins. Water and fat absorption capacity (WAC and FAC) are important parameters for the food processing within protein solubility, emulsifying properties, foaming capacity, and color are these functional properties. These functional properties gain importance in terms of food products that is used. Proteins demonstrate the lowest solubility at their isoelectric point and the reason is that, at this point protein-protein interaction level is the highest. These ion interaction affects most of functional properties of proteins that are solubility, fat and water absorption capacities [63, 64]. WAC and FAC of hazelnut meal samples were shown in Table 4.3.

Table 4.3. Water and Fat Absorption Capacities (WAC and FAC) of Hazelnut Meal Samples

	HMF	**HMPP**
WAC (g/ g protein)	2.94 ± 0.28^{a}	2.21 ± 0.03^{a}
FAC (ml oil/g protein)	4.95 ± 0.13^{a}	4.85 ± 0.28^{a}

Data represent average of three independent samples ± standard deviation. Different superscript in rows represent statistically significant differences ($p<0.01$).

FAC is based on electrical charge, hydrophobicity and area of the protein surface and results show that both HMPP and HMF had high FAC values and they were not significantly different ($p > 0.01$). Results demonstrated that they may be used good emulsifying agents in food industry. Also this result is in a correlation with literatures' [65, 66].

WAC is a consequence of hydrophobic and hydrophilic interaction of protein molecules and it influences some rheological properties like texture and viscosity of food. Even though high WAC of protein is significant parameter for high viscous food like sauce, soup or bakery product, in beverage production it is an undesirable property because it affects liquidness of product. Both HMF and HMPP had low WAC which were not significantly different ($p > 0.01$) and results are almost matching with similar studies [65, 66]. In addition, they readily show that this protein concentrate is suitable for use in drink formulation.

4.3.2. Protein Solubility of Hazelnut Meal Protein Precipitate

Protein solubility of precipitate was determined by Bradford method with BSA in different concentration and BSA was used a standard and determination was done according to standard curve of BSA which was shown in Figure 3.6. Total protein concentration (Pc) in protein solution was measured with spectrophotometer at 595 nm and according to absorbance (abs) value protein concentration was calculated with Equation (3.1)

Protein solubility (Ps) was determined at different pHs between 3.2 and 12 and results were calculated according to Equation (3.2) and given in Figure 4.2.

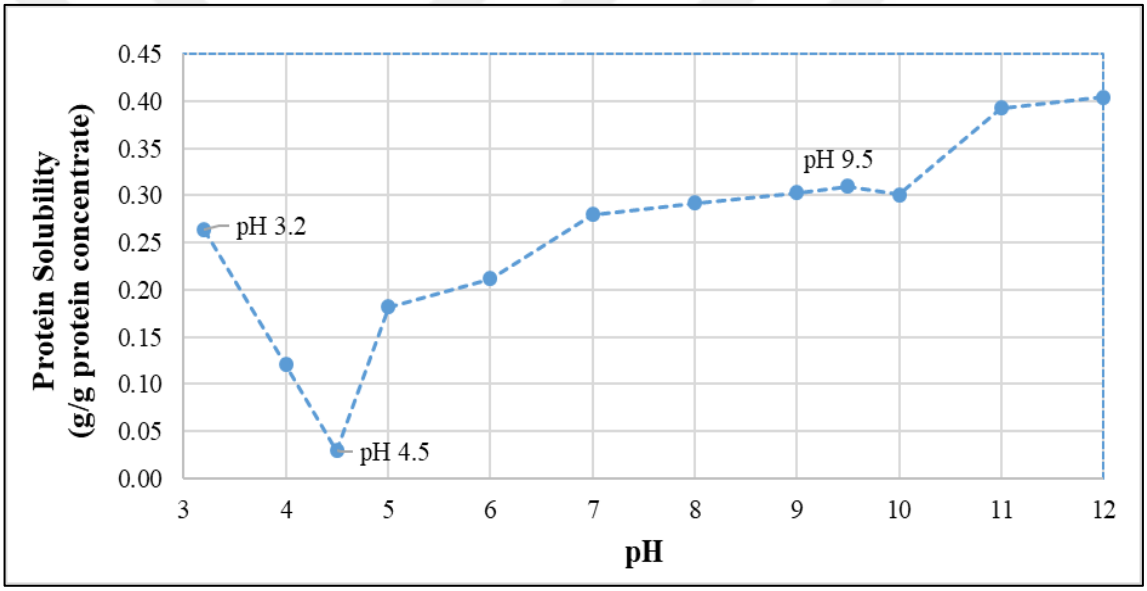

Figure 4.2. Soluble protein content in different pHs

The minimal solubility was identified at pH 4.5 which was used in precipitation of protein with isoelectric point and the maximum solubility was at pH 12 which was also employed for high solubility of HMP. At pH 3.2, solubility was found as 0.26 g/g protein concentrate. This pH and protein solubility at this point has an important impact on beverage formulation. Also at neutral pH, solubility (0.27 g/g protein concentrate) content was slightly similar with soluble content at pH 3.2. On the other hand, Figure 4.2 clearly demonstrated that solubility profile of HMPP has a U shape and this profile has a correlation with the results of another hazelnut meal protein solubility assay [47]. In addition to that this profile of protein solubility show similarity with other nut proteins that were concentrated from walnut, cashew and brazil nut [67-69].

4.4. UTILIZATION OF HAZELNUT MEAL PROTEIN IN PROTEIN DRINK

Two different protein-enriched drinks with 2 percent and 4 percent HMPP were prepared with same formulation and reference drink were compared with each other according to main properties.

Reference drink was chosen as ZeroSHOT® Dumbbell BCAA because this beverage is one of the most popoular protein drink and target market of this beverage is people who do sports like running, biking, CrossFit or triathlon in their daily life for recovery of muscle weight and need to take additional nutrients. This beverage contains 0.21 percent taurine, 0.42 percent glutamine and 1.05 percent BCAA (branched-chain amino acid).

pH, turbidity, titratable acidity and total solid content measurements of the drinks were given in Table 4.4.

Table 4.4 pH, turbidity titratable acidity and brix results of protein enriched drinks

	2% HMPP Protein Drink	**4% HMPP Protein Drink**	**Reference Protein-Enriched Drink**
pH	3.23 ± 0.04^{b}	3.29 ± 0.02^{b}	3.54 ± 0.02^{a}
Titratable Acidity (%)	0.15 ± 0.03^{b}	0.16 ± 0.02^{b}	0.89 ± 0.02^{a}
Turbidity (%)	56.73 ± 0.63^{a}	58.12 ± 0.45^{a}	61.03 ± 0.12^{a}
Brix (%)	1.7 ± 0.06^{b}	3.8 ± 0.06^{c}	2.10 ± 0.00^{a}

Data represent average of three independent samples ± standard deviation. Different superscript in rows represent statistically significant differences ($p<0.01$).

pH is an important parameter for beverage quality because it is known that pH value between 2.5 to 5.5 extend shelf life of some soft drinks and inhibit microorganism growth. In addition to that, pH is the simplest way to check the quality of end product. While pH is related with microbial stability, titratable acidity is related with acid taste of beverage. In general, when pH decreases, titratable acidity or total acidity (TA) increases. However there is no relationship between pH and TA directly, because pH measures concentration of free hydrogen ions and TA measures amount of acid ions. In this thesis results show that pH of samples increases with TA and both of them did not significantly differ based on protein concentration ($p > 0.01$).

There is a slight increase in pH value and turbidity of HMPP protein enriched drink when protein concentration is increased. Also, pH values of both formulations were similar with reference drink and all of them were lower than 4. TA of two beverages (2 percent protein and 4 percent protein) are almost same with each other. However TA of reference drink was higher than the others. Brix results show that soluble solids were almost two times higher in beverages contain 4 percent protein than 2 percent protein. Also, soluble solid content of reference protein enriched drink which has 1.47 percent protein, is between those two beverages. Brix results indicated significant difference in all drinks ($p < 0.01$).

According to color measurements (Table 4.5), L* value results, which gives the lightness or whiteness of the sample, shows that L* value of 2 percent protein and 4 percent protein enriched drinks are very similar with each other and lower than reference drink's results. The common reason of low L* value is the Maillard reaction of drying process which is done at 50 ^{o}C. However, in this study drying process was done with freeze drier so the reason of low L* value might be the inherent color of protein concentrates. a* value indicates the scale of color from green to red of the sample and results demonstrated that redness of 2 percent protein and 4 percent protein enriched drinks were higher than reference drink. Lastly, b* value shows the range of color from blue to yellow color and yellowish color is very high in 2 percent protein and 4 percent protein drinks according to reference drink. Differences in color values were indicated between formulated drinks and referenced drink analytically, and also this differences were recognized by panelists with sensory evaluation and formulated drinks have highest liking. This part was explained on the following pages.

Table 4.5. Color properties of protein enriched drink

	2% Protein Drink	**4% Protein Drink**	**Reference Protein Drink**
L*	76.47±0.37 ^{b}	76.06±0.05 ^{b}	85.13±0.05 ^{a}
a*	23.98±0.27 ^{a}	24.31±0.02 ^{a}	10.48±0.01 ^{b}
b*	86.48±0.07 ^{b}	87.38±0.03 ^{a}	44.78±0.04 ^{c}

Data represent average of three independent samples ± standard deviation. Different superscript in rows represent statistically significant differences ($p<0.01$).

4.4.1. Viscosity

Viscosity is another important concern that describe beverages because product processing, packaging and also customer acceptance are based on viscosity of beverage. The viscosity of protein-enriched drinks at constant temperature (20 ^{o}C) are given in Figures 4.3-4.5.

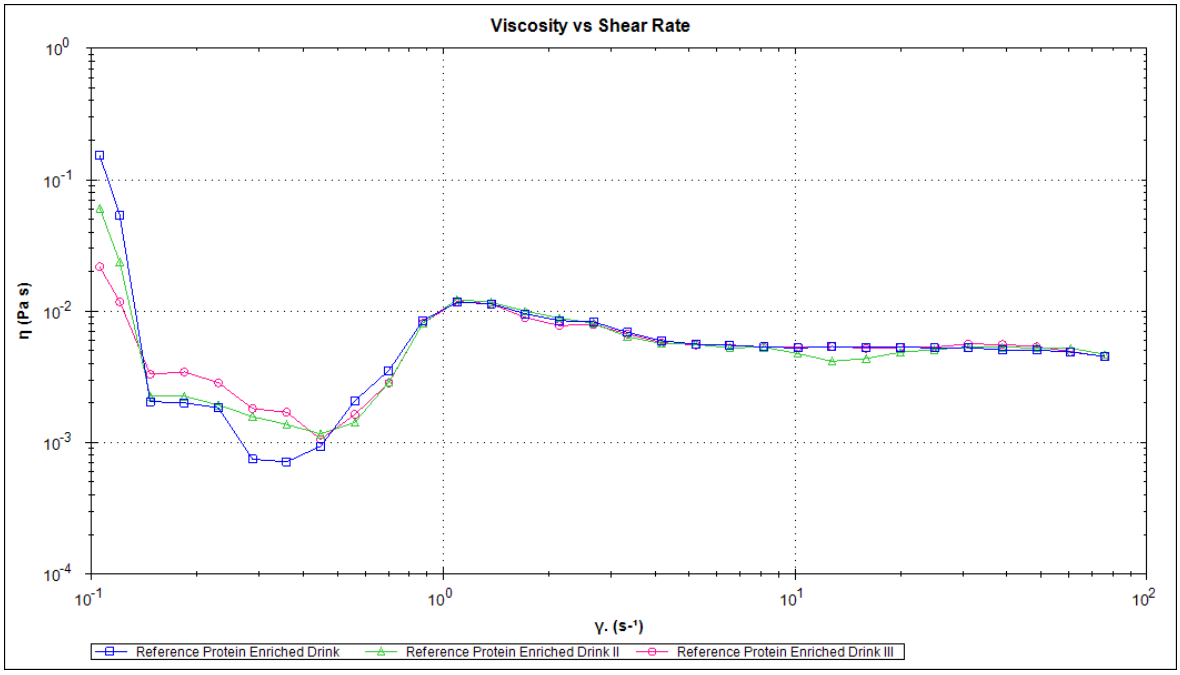

Figure 4.3. Viscosity vs shear rate of reference protein drink

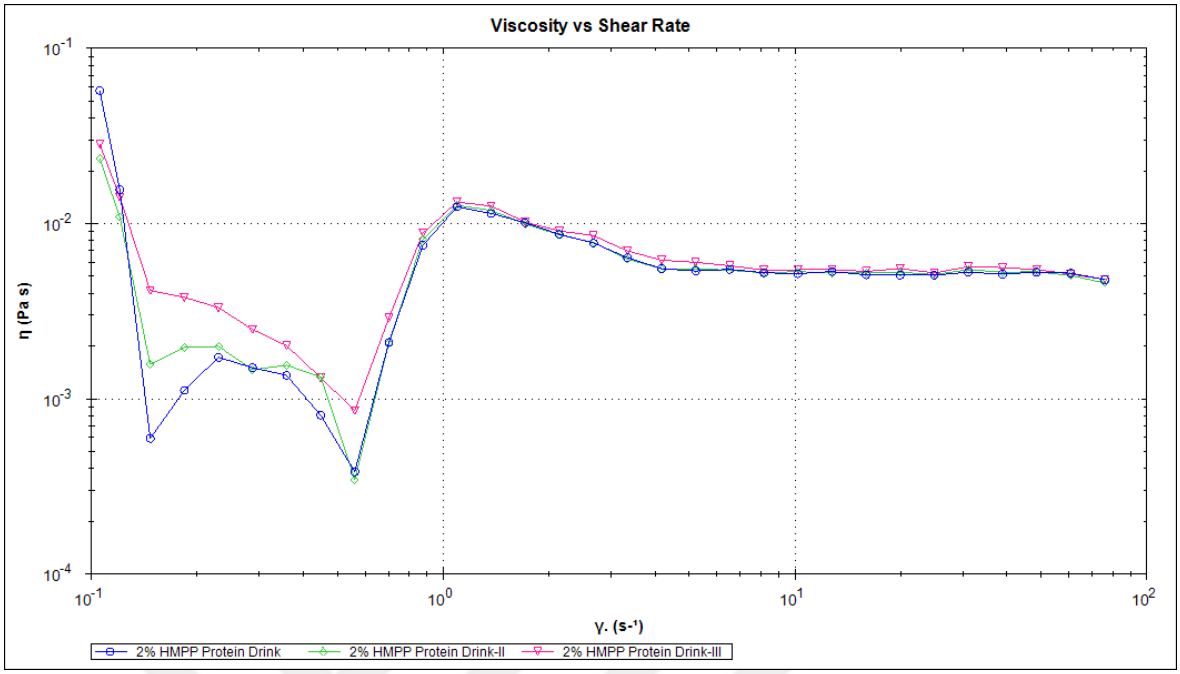

Figure 4.4. Viscosity vs shear rate of 2 percent HMPP protein drink

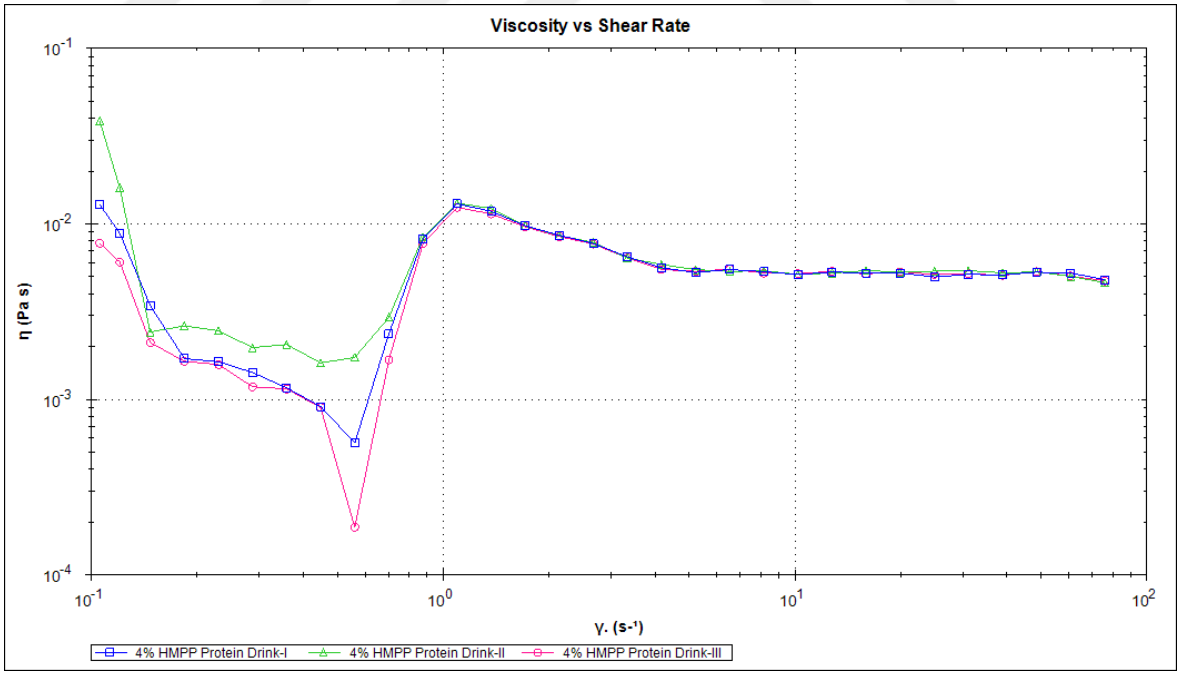

Figure 4.5. Viscosity vs shear rate of 4 percent HMPP protein drink

According to viscosity-shear rate graphs, flow behavior of HMPP drink samples show changes with time and shear rate. As illustrated in figures, at low shear rate, all samples displayed non-Newtonian shear thinning flow behavior and in this shear rate range viscosity

of samples decreased with increasing shear rate. An increased viscosity was expected with increasing protein concentrate. However there was not any significant differences in viscosity for different formulations. The reason of this might be WAC of HMPP. Protein concentrates had low WAC which affects viscosity of samples. In general, shear rate of mount is approximately $55s^{-1}$ and results show that viscosity was measured approximately 0.06 Pa.s for both formulations and reference drink [70]. It is illustrated that viscosity of formulated two drinks are very close to reference drink which is consumed as a commercial beverage.

4.4.2. Sensory Analysis

The acceptability of protein enriched drinks with 2 percent and 4 percent protein as well as the reference drink was investigated with sensory attributes like appearance, taste, sweetness, bitterness, sourness, texture and aroma using 9-point hedonic scale by 75 untrained panelists. The overall acceptability was also evaluated for these products. Results of sensory analysis were indicated in Figure 4.6. Appearance, taste, sweetness and texture were found to be statistically significant in the evaluation of sensory analysis of protein drinks prepared with 2 percent and 4 percent HMPP ($p < 0.01$).

49 percent of panelists indicated that they did not consume protein drink, 27 percent of them consume less than once per month, 7 percent once per month, 13 percent two to three times per month and 4 percent consume one to two times per week. In addition, 17 percent of the participants did not do any sports, 15 percent of them do sports once per month, 35 percent of them 2 to 3 times per month, 21 percent of them 1 to 2 times per week, 11 percent more than 3 times per week and 1 percent of every day sports.

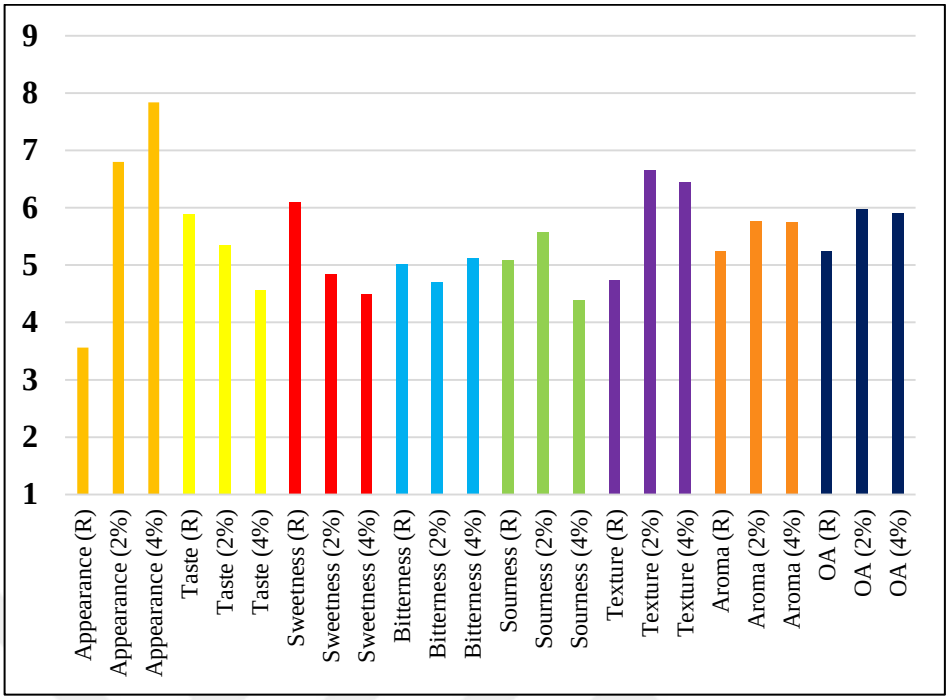

Figure 4.6. Sensory Analysis of Protein Drinks

Table 4.6 Sensory analysis results of protein enriched beverages.

	4% Protein Drink	**2% Protein Drink**	**Reference Protein Drink**
Appearance	7.84 ± 1.37^{a}	6.80 ± 1.35^{b}	3.56 ± 1.85^{c}
Taste	4.56 ± 2.01^{b}	5.36 ± 2.02^{ab}	5.89 ± 2.48^{a}
Sweetness	4.49 ± 2.15^{b}	4.84 ± 1.99^{b}	6.11 ± 2.31^{a}
Bitterness	5.13 ± 2.43^{a}	4.71 ± 2.36^{a}	5.03 ± 2.39^{a}
Sourness	4.39 ± 2.06^{b}	5.57 ± 2.30^{a}	5.08 ± 2.55^{ab}
Texture	6.45 ± 2.62^{a}	6.65 ± 1.99^{a}	4.73 ± 2.69^{b}
Aroma	5.75 ± 2.33^{a}	5.77 ± 2.51^{a}	5.24 ± 2.73^{a}
Overall	5.91 ± 2.39^{a}	5.99 ± 2.47^{a}	5.24 ± 2.56^{a}

Data represent average of three independent samples ± standard deviation. Different superscript in rows represent statistically significant differences ($p<0.01$).

Appearance has a significant role for identification, purchase and consumption decision of a food product because it is the first characteristic sense that is perceived by human for final selection. This includes optical properties like color, look, gloss or semiprimality; or physical properties like size or shape of food as a visual perception. Appearance score of

protein drinks 4 percent, 2 percent and reference were 7.84±1.37, 6.80±1.35 and 3.56±1.85, respectively (Table 4.6) and there are significant differences between results ($p < 0.01$). Most of the panelists indicated that 4 percent protein drink was more acceptable according to appearance because of clarity of the product.

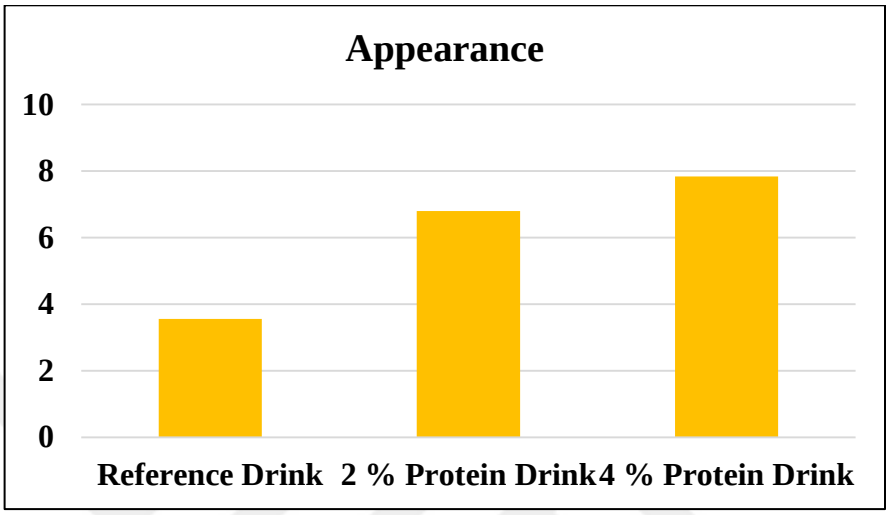

Figure 4.7 Sensory analysis-Appearance

Turbidity of reference drink was higher than other two drinks and 2 percent - 4 percent protein drinks were close to each other. In addition to that color results (L*,a* and b*) of beverages in Table 4.5 indicates that reference drink results were quite different from protein drinks with 2 percent and 4 percent concentrates. It may be concluded that 4 percent protein enriched drink was preferred than 2 percent and reference drink according to appearance of products.

Taste is another important attributes of food product that includes sweetness, bitterness, saltiness or sourness. In this sensory analysis evaluation besides overall acceptability of taste, sweetness, bitterness and sourness parameter of products were also evaluated and results were illustrated in Figure 4.8.

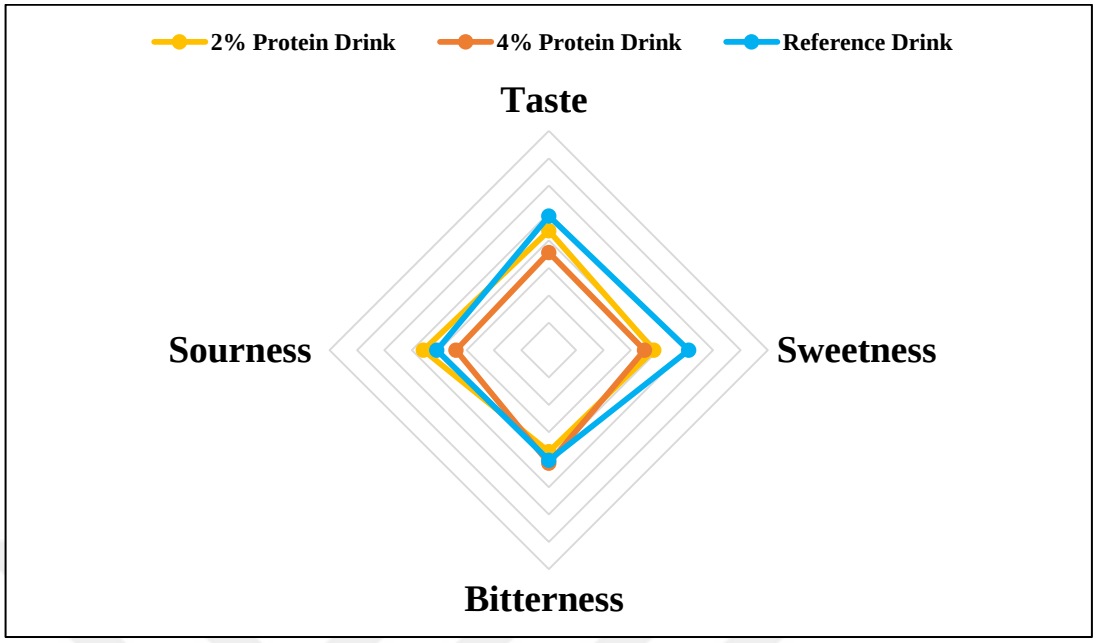

Figure 4.8. Taste, sweetness, bitterness and sourness results of protein drinks

Taste result shows that there are statistically significant differences between overall taste, sweetness and sourness ($p < 0.01$). Observation of taste and sweetness results indicate that 2 percent protein drink has the highest liking after reference drink, and 2 percent protein drink has the highest sourness mean score among other drinks. Although formulation of both 2 percent and 4 percent protein drink were same, ıt was observed that there were some differences according to sensory analysis. The reason of this high protein content changes overall taste of product and panelists might be affected from this reason because reference drink has almost 1.47 percent protein content which was the lowest protein content.

After visual evaluation, texture which is the combination of touch, mouthfeel or sight is important perception for determination of food product. Results demonstrated that there is no significant differences between 2 percent and 4 percent protein drink ($p < 0.01$) because of having same formulation but there are significant differences between protein concentrate drinks and reference drink. Also, texture of reference drink had lowest mean score which was 4.73±2.67. Aroma also plays important role for identification and consumption decision of a food product and results show that there is no significant differences between three products.

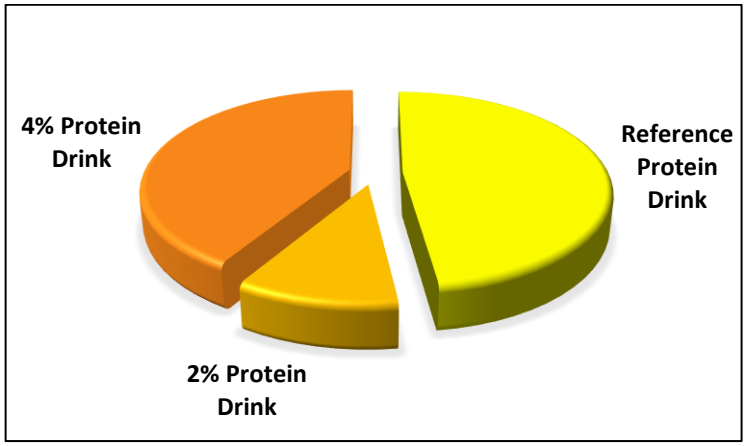

Figure 4.9. Preference percentage of protein drinks

According to the results of the preferred sample (Figure 4.9), the panelists preferred reference drink, which constituted 36 percent of the panelists. The closest value to the drink taken as a reference belongs to the 4 percent protein containing beverage, which constitutes 31 percent of the panelist. 83.87 percent of the participants who prefer 4 percent protein drink do sports in their life and 34.62 percent of these participants were female and the rest of them were male. It may be concluded that people who do sports prefer 4 percent protein drink rather than the other drinks.

5. CONCLUSION

Hazelnut oil is consumed because of its potential significant role on human health and also its taste and aroma. The studies carried out to utilize the hazelnut meal, a by-product of the hazelnut oil process, has gained importance in recent years. Within this thesis, a detailed study was performed on the extraction of plant-based protein from hazelnut meal and utilization of these proteins in beverages with different protein concentrates. The results illustrated that hazelnut meal as an industrial waste is sustainable for protein extraction and this protein concentrate has important functional properties. According to the results of amino acid composition of HMPP, this final product and whey protein which is animal-based were comparable. This data suggest that hazelnut meal is an alternative source for a plant based protein and could meet the demand of the increased interest about variety of plant protein sources instead of animal protein because of world population growth, ethic and environmental concerns and also healthy lifestyle relevance in modern society. Formulated beverages with HMPP were compared with reference drink which attracts young consumers' attention and one of the most preferred protein drink in fortified/functional beverage industry. It was observed that the formulated product containing 4 percent protein was chosen as the most preferred drink by untrained panelists.

Thus, this study showed that protein concentrate from hazelnut meal has a good amino acid composition as a plant protein source. From the results of functionality study of hazelnut meal protein concentrate it can be concluded that this protein concentrate is applicable as an ingredient for food industry, especially for protein-enriched beverages.

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APPENDIX A : SENSORY ANALYSIS EVALUATION FORM

Age: Gender:

Department: Education:

I. There are three samples labeled as **374**, **785** and **962**, to be tested. Rank each sample for the following quality parameters after tasting it. Use a ranking of 1-9 in your evaluations. To cleanse your mouth and prevent the last sample you drunk from making an objective evaluation, please chew a cracker and take a sip of water between each sample that is to be tasted.

1. Dislike Extremely
2. Dislike Very Much
3. Dislike Moderately
4. Dislike Slightly
5. Neither Like Nor Dislike
6. Like Slightly
7. Like Moderately
8. Like Very Much
9. Like Extremely

a. Please, focus on the appearance of the samples. How much do you like or dislike the **APPEARANCE** (color, look) of these beverages?

374	**785**	**962**

b. Now, please taste as much of the sample as you need to form your opinion. How much do you like or dislike overall **TASTE/FLAVOUR** of these beverages?

374	**785**	**962**

c. How much do you like or dislike **SWEETNESS** of these beverages?

374	**785**	**962**

d. How much do you like or dislike **BITTERNESS** of these beverages?

374	**785**	**962**

e. How much do you like or dislike **SOURNESS** of these beverages?

374	**785**	**962**

f. How much do you like or dislike **TEXTURE/CONSISTENCY** of these beverages?

374	**785**	**962**

g. How much do you like or dislike **AROMA/SMELL** of these beverages?

374	**785**	**962**

h. How much do you like or dislike **OVERALL ACCEPTABILITY** of these beverages?

374	**785**	**962**

II. **How often** do you drink protein beverages?

None	
Less than once per month	
Once per month	
2 to 3 times per month	
1 to 2 times per week	
More than 3 times per week	

III. **How often** do you exercise?

None	
Once per month	
2 to 3 times per month	
1 to 2 times per week	
More than 3 times per week	
Everyday	

IV. Which sample did you **prefer**?

374	**785**	**962**

Thank you for your time. Please make sure all the questions are answered.

APPENDIX B : STATISTICAL ANALYSIS TABLES

Table B.1. One-way analysis of variance (ANOVA, $p < 0.01$) for three HM samples for moisture content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Type	2	62.5951	31.2975	8383.27	0.000
Error	6	0.0224	0.0037		
Total	8	62.6175			

Table B.2. One-way analysis of variance (ANOVA, $p < 0.01$) for three HM samples for ash content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Type	2	47.1950	23.5975	171.68	0.000
Error	6	0.8247	0.1375		
Total	8	48.0198			

Table B.3. One-way analysis of variance (ANOVA, $p < 0.01$) for three HM samples for protein content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Type	2	8565.25	4282.63	8687.88	0.000
Error	6	2.96	0.49		
Total	8	8568.21			

Table B.4. One-way analysis of variance (ANOVA, $p < 0.01$) for three HM samples for fat content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Type	2	84.2927	42.1463	1116.05	0.000
Error	6	0.2266	0.0378		
Total	8	84.5192			

Table B.5. One-way analysis of variance (ANOVA, $p < 0.01$) for three HM samples for carbohydrate content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Type	2	6294.94	3147.47	5131.46	0.000
Error	6	3.68	0.61		
Total	8	6298.62			

Table B.6. Analysis of two sample t-test for WAC

Descriptive Statistics: WAC				
Type-C	N	Mean	StDev	SE Mean
HMF	3	2.936	0.284	0.16
HMPP	3	2.2094	0.0326	0.019
Estimation for Difference				
Difference			Pooled StDev	99% CI for Difference
0.726			0.202	(-0.035; 1.487)
T-Value			DF	P-Value
4.39			4	0.012

Table B.7. Analysis of two sample t-test for FAC

Descriptive Statistics: FAC				
Type-C	N	Mean	StDev	SE Mean
HMF	3	4.952	0.132	0.076
HMPP	3	4.853	0.280	0.16
Estimation for Difference				
Difference			Pooled StDev	99% CI for Difference
0.099			0.219	(-0.724; 0.922)
T-Value			DF	P-Value
0.55			4	0.609

Table B.8. One-way analysis of variance (ANOVA, $p < 0.01$) for three different protein drinks for pH value

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Drink	2	0.154822	0.077411	122.23	0.000
Error	6	0.003800	0.000633		
Total	8	0.158622			

Table B.9. One-way analysis of variance (ANOVA, $p < 0.01$) for three different protein drinks for titratable acidity

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Drink	2	1.07582	0.537911	1052.43	0.000
Error	6	0.00307	0.000511		
Total	8	1.07889			

Table B.10. One-way analysis of variance (ANOVA, $p < 0.01$) for three different protein drinks for turbidity

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Drink	2	28.89	14.445	8.43	0.018
Error	6	10.28	1.714		
Total	8	39.17			

Table B.11. One-way analysis of variance (ANOVA, $p < 0.01$) for three different protein drinks for brix

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Drink	2	6.32667	3.16333	355.87	0.000
Error	6	0.05333	0.00889		
Total	8	6.38000			

Table B.12. One-way analysis of variance (ANOVA, $p < 0.01$) for three different protein drinks for color (L*)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Drink	2	157.492	78.7459	1660.53	0.000
Error	6	0.285	0.0474		
Total	8	157.776			

Table B.13. One-way analysis of variance (ANOVA, $p < 0.01$) for three different protein drinks for color (a*)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Drink	2	373.351	186.676	7696.20	0.000
Error	6	0.146	0.024		
Total	8	373.497			

Table B.14. One-way analysis of variance (ANOVA, $p < 0.01$) for three different protein drinks for color (b*)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Drink	2	3553.61	1776.80	723585.35	0.000
Error	6	0.01	0.00		
Total	8	3553.62			

Table B.15. One-way analysis of variance (ANOVA, $p < 0.01$) for three different protein drinks for appearance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Drink	2	747.4	373.720	157.56	0.000
Error	222	526.6	2.372		
Total	224	1274.0			

Table B.16. One-way analysis of variance (ANOVA, $p < 0.01$) for three different protein drinks for taste

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Drink	2	67.56	33.778	7.11	0.001
Error	222	1054.91	4.752		
Total	224	1122.46			

Table B.17. One-way analysis of variance (ANOVA, $p < 0.01$) for three different protein drinks for taste

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Drink	2	108.2	54.093	11.64	0.000
Error	222	1032.0	4.649		
Total	224	1140.2			

Table B.18. One-way analysis of variance (ANOVA, $p < 0.01$) for three different protein drinks for bitterness

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Drink	2	7.40	3.698	0.65	0.525
Error	222	1272.16	5.730		
Total	224	1279.56			

Table B.19. One-way analysis of variance (ANOVA, $p < 0.01$) for three different protein drinks for sourness

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Drink	2	53.31	26.653	4.98	0.008
Error	222	1187.65	5.350		
Total	224	1240.96			

Table B.20. One-way analysis of variance (ANOVA, $p < 0.01$) for three different protein drinks for texture

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Drink	2	167.1	83.560	15.48	0.000
Error	222	1198.2	5.397		
Total	224	1365.4			

Table B.21. One-way analysis of variance (ANOVA, $p < 0.01$) for three different protein drinks for aroma

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Drink	2	13.55	6.773	1.06	0.348
Error	222	1419.01	6.392		
Total	224	1432.56			

Table B.22. One-way analysis of variance (ANOVA, $p < 0.01$) for three different protein drinks for overall acceptability

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Drink	2	25.21	12.604	2.08	0.127
Error	222	1343.01	6.050		
Total	224	1368.22			