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Physicochemical Properties of Starch from a Cereal-Based Fermented Food (Tarhana)

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Abstract

Tarhana is a traditional fermented food product in Turkey. There is limited research on starch characteristics of tarhana. In this study, starch properties of various home-made tarhana samples have been investigated. Starch content ranged from 59.64 to 69.95 and there were significant (P<0.05) differences in starch damage (3.78-10.84%). The starch pasting and gelatinization of the tarhana samples also showed significant (P<0.05) differences. Estimated glycemic indexes ranged from 86.16 to 102.54 and are all considered high glycemic index. Amylose content of the tarhana also showed significant (P<0.05) variation (20.70-29.03%). There were significant (P<0.05) differences in the molecular mass of the tarhana starch. The molecular mass of the high molecular weight amylopectin ranged from 2-15 million daltons and the amylose ranged from 300 thousand to 2.4 million daltons. Overall, there were significant (P<0.05) differences in chemical composition and starch characteristics of tarhana samples.

Keywords: Tarhana; starch; Amylose; Amylopectin; Glycemic index

Introduction

Tarhana, a traditional fermented cereal product is widely consumed in Turkey in the form of thick soup. Tarhana is prepared using wheat flour, yogurt, yeast, vegetables and spices [1,2]. Methods for tarhana preparation vary from one place to another, but cereals and fermented milk are always the main components in the recipe [1]. After mixing of all ingredients, the fermentation process is usually carried out by yogurt bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophiles* and baker's yeast [3] for a period of one to seven days [3,4]. After fermentation, the mixture is sun-dried and ground. Most tarhana consumed in Turkey is home-made but there is also production at the industrial level [2]. The amount and type of ingredients used in tarhana preparation may affect its nutritional and sensory attributes [4]. For example, it has been reported that tarhana is a good source of minerals, B vitamins, organic acids, free amino acids and phenolic compounds; and these are some reasons why tarhana is widely consumed in Turkey [5]. However, tarhana is produced with ingredients that have high starch content, such as white-wheat flour, whole meal flour, or semolina [5]. It is important to understand the role these ingredients play in the functionality (cooking, viscosity, texture) and nutritional properties of tarhana soup.

Starch is comprised of two polymers, amylose and amylopectin. Amylose is essentially linear (long linear chains) formed by α-(1,4) linked glucose units with a few branches; while amylopectin, with high molecular weight and highly branched structures, consists of α-(1,4) and α-(1,6) glucosidic linkages [6]. Most starches contain 20%-30% amylose and 70%-80% amylopectin, and the ratios vary with the starch botanical source. The amylose/amylopectin ratio has an effect on the functionality of starch [6,7].

The amylose/amylopectin ratio influences the granule size distribution, starch crystallinity, organization of the molecules within the granule and the chemical nature of these both polymers [7]. Amylose/amylopectin ratio also affects the nutritional quality of starch gauged by its digestion rate and resultant glycemic response, which is represented by the glycemic index (GI) and used as an indicator of carbohydrate quality [8]. In several reports, it is mentioned that the fine structure of amylopectin plays an important role in the functionality of starch [6-9]. Because of this, it is important to understand starch structure to explain the functional properties and digestibility of starch in tarhana soup. Current studies on tarhana focus on the analysis of protein digestibility, reduction of anti-nutritional factors, increasing availability of proteins and phenolic compounds. However there is a lack of information about the role of starch on the physicochemical and digestibility properties of tarhana soup, which are important parameters in the final quality of this food. The objective of this work was to analyze the chemical composition, gelatinization and pasting properties, estimated glycemic index, amylose/amylopectin ratio and molar mass of amylose and amylopectin in home-made tarhana soup prepared and collected from different regions of Trakya, Turkey.

Materials and Methods

Materials

The ingredients used in tarhana preparation were commercial white wheat flour, semolina, yogurt, tomato paste, fermented white wheat bread dough, lentil flour, onion, table salt and sunflower oil. All tarhana samples were home-made and collected randomly from four cities (Tekirdag, Canakkale, Edirne and Kirklareli) of Trakya region of Turkey.

Sample preparation

Samples were ground using a UDY mill (UDY Corp., Fort Collins, CO) with a 0.8 mm screen to reduce the particle size to a uniform size for all samples. The ground samples were blended and placed in zip-top plastic bags and stored at 4°C before further analysis.

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Proximate analysis

Moisture and protein were determined according to AACCI approved methods 44-15.02 and 46-30.01, respectively [10]. The moisture was measured by calculating the percentage weight lost after heating at 130°C for 1 hour. A Leco nitrogen combustion analyzer (FP-528, Leco Corp, St. Joseph, MI) was used to determine protein content and 5.7 was used as conversion factor.

Total starch and starch damage were both determined using Megazyme assay kits (Megazyme, Bray Ireland). For determination of total starch, samples (100 mg) were hydrolyzed with thermostable α-amylase in MOPS buffer (50 mM, pH 7.0, 5 mM $CaCl_2$, 0.02% NaN_3) at 100°C for 6 minutes. The samples were then incubated at 50°C for 30 minutes with amyloglucosidase (AMG) in sodium acetate buffer (200 mM, pH 4.5, 0.02% NaN_3). The amount of glucose released was measured by adding glucose oxidase peroxidase (GOPOD) and reading the absorbance at 492 nm [10]. Damaged starch was measured by incubating the sample with α-amylase at 40°C in sodium acetate buffer (100 mM, pH 5.0, 5 mM $CaCl_2$) for exactly 10 minutes. The samples were centrifuged (2000 g, 10 minutes) and a portion (100 µl) of the supernatant was incubated with AMG for thirty minutes at 40°C. The samples were then incubated with GOPOD and the absorbance was read at 492 nm [10].

Pasting properties

The pasting properties of the samples were measured using a Rapid Visco Analyzer (RVA) (Perten Instruments, Springfield, IL). The analysis was done according to AACCI approved method 76-21.01 [10], with some modification. Five minutes of stirring at 50°C was added to the beginning of the test for equilibration of the samples. After the 5 minutes of stirring the sample was heated to 95°C and held for 2.5 minutes and then cooled to 50°C.

Thermal properties of starch

The starch gelatinization of the samples was measured using Differential Scanning Calorimetry (DSC) according to the method of Kim et al. [11] as modified by Ovando-Martinez et al. [12]. The samples (3.5 mg) were weighed into aluminum sample pans and 8µl deionized water was added. The samples were allowed to stand at room temperature (≈ 25°C) overnight. The samples were heated along with an empty reference pan from 10-120°C at a rate of 10°C per minute.

Estimated Glycemic Index

The hydrolysis index and estimated glycemic index (eGI) were measured according to the method of Ovando-Martinez et al. [12] using the Englyst *in vitro* assay method for starch hydrolysis [13]. The samples were incubated at 37°C with an enzyme mix (amyloglucosidase, invertase and pancreatin) for 180 minutes. Aliquots of the digest were taken every 20 minutes to determine the amount of glucose released by reaction with glucose oxidase/peroxidase (GOPOD). A sample of commercial white bread (purchased from a local grocery store and air dried at room temperature) was analyzed as a reference. The hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of the sample by the area obtained for commercial white bread (hydrolysis curve 0 min to 180 min). The estimated GI (eGI) of the samples was calculated using the equation described by Ovando-Martinez et al. [12]: eGI=8.198+0.862*HI.

High performance size exclusion chromatography – multi angle light scattering

The ratio of amylopectin to amylose in the samples was determined using High Performance Size Exclusion Chromatography (HPSEC) with a refractive index detector (RID) according to the method of Ovando-Martinez et al. [14]. The samples were defatted by boiling in methanol (2 ml) for 30 minutes. Then, the starch was extracted by dissolving the samples in potassium hydroxide: urea (2 ml, 9:1) while heating at 100°C for 15 minutes. The starch was precipitated by adding 2 aliquots of 3 ml of absolute ethanol while vortexing. After drying in the oven at 50°C overnight the samples were prepared for HPSEC analysis by re-dissolving in potassium hydroxide:urea (5 ml, 9:1) at 100°C for 90 minutes. The dissolved samples were neutralized using 1M HCl and filtered through a 0.45 µm nylon syringe filter into vials for analysis. The samples were injected (100µl) onto an Agilent 1200 High Performance Liquid Chromatography (HPLC) system. The mobile phase was water and the flow rate was set to 0.5 ml/minute. The separation was done using Waters ultra-hydrogel 1000 and ultra-hydrogel linear columns with a guard column in sequence. The columns and RI detector were kept at 30°C. The ratios of High Molecular Weight (HMW) amylopectin, Low Molecular Weight (LMW) amylopectin and amylose were determined by the area under the RI signal curve.

The molecular mass of the starch was also determined using HPSEC-MALS. The dn/dc value for calculation of the starch molecular mass was 0.146 [15-17]. The Debye model with a fit degree of one was used for calculation of the molar mass. The results were fitted to a first order polynomial model.

Statistics analysis

All the analysis was done in duplicate. The analysis of variance (ANOVA) was conducted with Microsoft Excel. Least significant differences (LSD) with α=0.05 was determined for mean separation.

Sample[a]	Protein	Starch	Starch Damage
	% DWB[b]	% DWB[b]	% As Is
A1	13.87	69.95	6.58
A2	14.46	65.61	3.78
A3	11.74	67.53	5.50
B1	12.96	67.32	7.65
B2	14.75	59.64	10.84
B3	12.16	65.36	6.33
C1	11.07	62.73	6.66
C2	15.28	66.18	5.15
C3	14.40	68.34	4.95
D1	14.81	69.50	7.09
D2	13.29	66.59	4.78
D3	14.02	69.27	6.55
E1	11.81	67.14	7.62
E2	13.08	65.90	5.54
E3	13.12	61.83	3.79
LSD[c] (P<0.05)	0.19	1.40	0.67

[a]A1, B1, C1: Edirne; D1, E1, D3, E3: Tekirdag; A2, B2, C2, D2, E2: Canakkale; and A3, B3, C3: Kirklareli; all cities are from the region of Trakya, Turkey
[b]DWB: Dry Weight Basis; [c]LSD: Least Significant Difference (P<0.05)

Table 1: Socio demographic characteristics of Arada Sub- city high school students, Addis Ababa Ethiopia, 2013 (n=800).

Results and Discussions

Proximal analysis of tarhana soup samples

Table 1 shows the proximate analysis of tarhana samples. The protein content varied from 11.01% to 15.28% showing significant differences among samples (P< 0.05). It has been reported that the type and amount of yogurt used in tarhana preparation affects the protein content in the soup. However, Erkan et al. [5] reported that the main factor affecting this parameter is the type of flour used. Since starch is one of the main components of tarhana soup and is responsible for its physicochemical properties, the total starch content of the tarhana samples was determined (Table 1). The starch content ranged from 59.64% to 69.95%. B2 sample had the lowest starch content (59.64%), high protein or high content of other non-starch materials compared to all the soup samples. The differences among samples in starch content could be due to the different type of flour or the white wheat flour/semolina ratio used in the tarhana soup preparation among places where the samples were collected. However, the 1-7 day fermentation process during the tarhana preparation [4] could affect its starch content. The wheat flour and semolina don't have the enough fermentable sugars to convert into carbon dioxide and ethanol; and so, during this process food for yeast (low molecular weight starch fractions) is formed by the action of amylases on available starch [18]. Since the samples were home-made and collected from different parts of Trakya, Turkey, the fermentation process could vary, and accordingly each sample would have variations in the starch content. The starch damage analyzed in the samples was between 3.78%-10.84%. This type of starch may have an effect on the psychochemical properties of the tarhana soup, affecting the cooking time, viscosity, texture and palatability.

Pasting properties

Viscosity is a very important parameter of tarhana soup in terms of palatability and consumer acceptability. The pasting properties of tarhana samples are presented in Table 2. The peak time did not showed significant differences among samples (P<0.05) indicating that starch granules present in the tarhana samples hydrate and swell in approximately the same amount of time. During heating at high temperature the peak viscosity (PV) is determined. This indicates that the majority of the starch granules are fully swollen but intact and physically interacting with each other, causing increased viscosity. The PV was in the range of 67.7 RVU-167 RVU. The lowest PV value of the B2 sample could be attributed to its lower starch content and higher starch damage (Table 1). The hot paste viscosity (HPV) values varied from 36.5 RVU to 106.6 RVU and showed significant (P<0.05) differences among samples. The differences observed in the PV and HPV could be attributed to the swelling of starch granules presented in each tarhana sample, which is influenced by the amylose-lipid complex, amylose/amylopectin ratio and contents, interaction between starch chains within the amorphous and crystalline region of the granule and the fine structure of the amylopectin [19].

Samplea	Peak Time	Peak Viscosity	Hot Paste Viscosity	Breakdown	Cold Paste Viscosity	Setback
	(min)	RVU[b]				
A1	10.7	157.5	100.0	57.5	226.7	126.7
A2	10.6	167.0	104.3	62.7	235.1	130.8
A3	10.8	133.3	91.5	41.8	206.2	114.7
B1	10.4	113.0	61.8	51.3	143.6	81.8
B2	10.4	67.7	41.0	26.7	95.0	54.0
B3	10.2	88.9	36.5	52.4	87.0	50.6
C1	10.7	135.3	91.0	44.3	225.5	134.6
C2	10.6	162.6	106.6	56.0	278.7	172.1
C3	10.4	136.2	62.8	73.3	156.8	94.0
D1	10.7	149.0	89.9	59.1	234.3	144.4
D2	10.6	121.6	79.7	41.9	160.4	80.8
D3	10.6	121.0	66.9	54.1	154.8	87.9
E1	10.6	137.8	78.6	59.2	178.1	99.5
E2	10.7	153.8	91.9	61.9	174.7	82.8
E3	10.6	112.0	64.5	47.6	162.8	98.3
LSD[c] (P<0.05)	0.1	5.7	4.6	3.0	7.7	5.6

[a]A1, B1, C1: Edirne; D1, E1, D3, E3: Tekirdag; A2, B2, C2, D2, E2: Canakkale; and A3, B3, C3: Kirklareli; all cities are from the region of Trakya, Turkey
[b]RVU: Rapid Visco Units: [c]LSD: Least Significant Difference (P<0.05)

Table 2: Pasting properties of tarhana soup samples.

After starch granules swell completely a disruption occurs, and the starch molecules (amylose and amylopectin) release into solution. Then, the molecules form random and the viscosity decreases. This disruption is known as breakdown (BD), which showed significant differences among samples (P<0.05). C3 sample had the highest value (73.3 RVU), while B2 sample had the lowest value (26.7 RVU). It has been reported that a high value of BD indicates the starch granule is more susceptible to shear and it is disrupted more easily, while a low BD value indicates high shear stability of the sample [20]. After the cooling stage begins, the viscosity starts to increase because of the reorganization of the amylose and amylopectin which form a gel. Such reorganization and increase in the viscosity is measured as cold paste viscosity (CPV) which ranged from 278.7 RVU to 87 RVU and presented significant differences (P<0.05). Among samples, the highest CPV was observed for C2 and the lowest was observed for B3. The trend observed in the CPV could be attributed to the rate of molecular entanglement and interaction between the chains of amylose and amylopectin during the reorganization of these molecules in the cooling step. Setback (SB), a measure of the starch retrogradation tendency, is defined as the difference between the HPV and CPV [19,21]. The SB varied significantly (P<0.05) among samples and ranged from 50.6 RVU to 172.1 RVU. It has been mentioned that the variations in the SB are due to differences in the amylose fraction, amylose/amylopectin ratio, molecular size, temperature and pH of the sample [19-21]. In our case, the amylose content is not likely affecting the SB viscosity, but perhaps variations in the pH of each sample could affect the starch retrogradation rate between amylose and amylopectin. In general, the differences in the pasting properties of tarhana samples can be attributed to the type of flour (affecting starch concentration and amylose amylopectin ratio), amylose-lipid complexes (inhibition of granule swelling), and length of fermentation (causing starch damage and changes in pH) during its preparation.

Gelatinization properties of starch in tarhana samples

Gelatinization is a thermal disordering of crystalline structure of native starch granules. The most common method to measure starch gelatinization is using differential scanning calorimetry (DSC); which reveals an endothermic melting process related to the loss of crystalline order [22]. The gelatinization parameters of tarhana samples measured with DSC are presented in Table 3. The gelatinization transition temperatures T_o (onset), T_p (peak) and T_c (end) were in the range of 59.93-66.38°C, 65.89-71.54°C and 72.13-77.09°C, respectively. Sample C1 had the highest T_o, T_p and T_c transition temperatures indicating that this sample had a high thermo-stable granular structure that required more energy to destabilize the amylose and amylopectin molecules. On the other hand, the lower gelatinization transition temperatures of tarhana showed that starch could have more amorphous and less

Sample[a]	T_o [b] (°C)	T_p [c](°C)	T_c [d](°C)	ΔH_{gel} [e](J/g)
A1	61.24	68.23	74.94	5.57
A2	61.93	67.21	73.24	5.77
A3	65.77	71.22	76.81	4.33
B1	61.18	67.56	74.04	6.38
B2	59.93	67.32	73.65	3.97
B3	63.09	69.30	75.53	5.11
C1	66.38	71.54	77.09	6.49
C2	60.30	65.89	72.13	6.91
C3	61.58	67.89	74.61	6.73
D1	61.60	67.87	74.81	5.17
D2	60.32	67.14	73.16	6.63
D3	61.17	66.99	74.59	8.18
E1	61.96	68.72	76.09	9.00
E2	60.64	66.65	72.96	7.38
E3	62.63	68.47	74.57	6.57
LSD[f] (P<0.05)	0.81	0.72	0.99	1.83

[a]A1, B1, C1: Edirne; D1, E1, D3, E3: Tekirdag; A2, B2, C2, D2, E2: Canakkale; and A3, B3, C3: Kirklareli; all cities are from the region of Trakya, Turkey
[b]To= Onset temperature
[c]Tp= Peak temperature
[d]Tc= End temperature
[e]ΔH= Enthalpy
[f]LSD: Least significant difference (P<0.05)

Table 3: Gelatinization properties of starch in tarhana soup samples.

crystalline material resulting in less resistance to gelatinization [11]. These differences could be attributed to the variation in fermentation period used to prepare the samples among locations which can affect the starch structure. Elsewhere, the variation observed in the transition temperatures of tarhana samples can depend of the starch concentration causing a dilution effect; granule size, heterogeneity within the granules population, amylose-lipid complexes, amylose/amylopectin ratio and fine structure of amylopectin affecting the rate and extend of the swelling process and loss of the crystallinity. The gelatinization enthalpy (ΔH_{gel}) (Table 3) varied from 3.97°C to 9°C. This parameter is a measure of the molecular order in form of double helices and crystalline structure of amylopectin in the granule [7]. The higher ΔH_{gel} values were observed in E1, D3 and E2, indicating that these samples may have highly ordered crystalline structure of amylopectin (high crystallinity), meaning that more energy was required to disrupt the double-helical order and disorganize the starch structure. It has been reported that the study of starch gelatinization in tarhana samples is a good indicator of the degree of cooking [23]. The gelatinization transition temperatures and gelatinization enthalpy observed among samples could indicated that different tarhana soup may require different cooking times to achieve the desired consistency for the soup.

Estimated glycemic index

The glycemic index (GI) is a ranking of carbohydrates based on their immediate effect on blood glucose levels. The GI can be an indicator of starch digestion of carbohydrate-based food products. Because the *in vivo* evaluation of GI in humans can be difficult and costly, there are studies that measure the *in vitro* starch digestibility of starchy foods in order to predict *in vivo* effects [13]. The hydrolysis index (HI) and estimated glycemic index (eGI) determined in tarhana samples are presented in Table 4. The HI represents the rate of starch hydrolysis and was used to determine the eGI. The HI and eGI of the tarhana samples ranged from 90.45-109.44 and 86.16-102.54, respectively; also it was observed that HI and eGI showed the same trend. Samples B2 and D2 had significantly (P<0.05) lower eGI values compared to the rest of the samples. In the case of B2, its low starch content and high protein content could explain its low eGI. However, D2 sample did not present this trend. It has been reported that proteins, lipids, polyphenols and their interaction with the starch molecules during cooking process, as well as the structural properties of starch influence the *in vitro* starch digestibility [9]. According to the GI classification system, tarhana samples had a high GI (>70); the high GI is because the starch in the tarhana soup has been gelatinized, which make the starch more available for hydrolysis by digestive enzymes. Also, the small starch granules of wheat [9], the A-type X-ray diffraction pattern [24] and the presence of pores on the surface [25] make wheat starch less

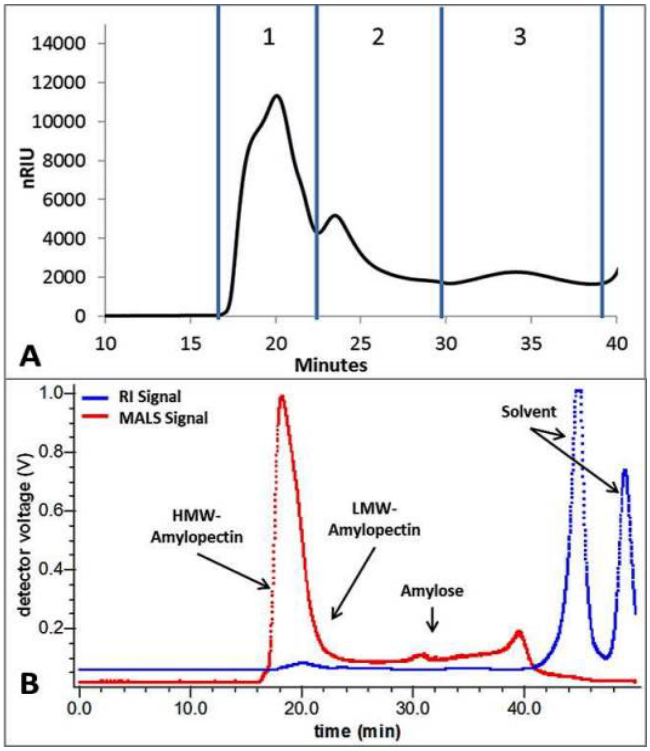

Figure 1: Reference chromatograms of (a) refractive index signal and (b) multi angle light scattering with refractive index signals for extracted starch. 1=High molecular weight amylopectin, 2=Low molecular weight amylopectin, 3=Amylose, nRIU=Nano refractive index units. MALS: Multi Angle Light Scattering; HMW: High Molecular Weight, LMW: Low Molecular Weight.

Sample[a]	HI[b]	eGI[c]
A1	98.84	93.40
A2	102.23	96.32
A3	96.74	91.59
B1	101.41	95.61
B2	90.45	86.16
B3	98.19	92.84
C1	103.30	97.24
C2	101.69	95.85
C3	109.44	102.54
D1	99.31	93.80
D2	92.45	87.89
D3	107.32	100.70
E1	105.67	99.28
E2	101.96	96.09
E3	99.25	93.75
LSD[d] (P<0.05)	4.95	4.26

Table 4: Hydrolysis index and estimated glycemic index of tarhana soup samples.

Sample[a]	Amylopectin HMW[b] %	LMW[c] %	Amylose %	Amylopectin HMW[b] M_w^d (Da^e)	LMW[c] M_w^d (Da^e)	Amylose M_w^d (D_a^e)
A1	50.76	24.77	24.45	$1.06x10^7$	$2.74x10^6$	$1.67x10^6$
A2	50.65	23.82	25.53	$9.32x10^6$	$4.29x10^6$	$8.65x10^5$
A3	48.67	24.57	26.75	$8.92x10^6$	$5.29x10^6$	$6.61x10^5$
B1	42.30	28.67	29.03	$2.06x10^6$	$1.22x10^6$	$3.02x10^5$
B2	49.98	26.64	23.38	$9.56x10^6$	$4.45x10^6$	$9.94x10^5$
B3	52.33	25.29	22.39	$1.04x10^7$	$3.92x10^6$	$9.71x10^5$
C1	50.67	25.45	23.89	$9.47x10^6$	$4.50x10^6$	$9.72x10^5$
C2	47.22	27.03	25.75	$1.55x10^7$	$7.10x10^6$	$2.44x10^6$
C3	47.33	28.15	24.52	$1.16x10^7$	$5.09x10^6$	$1.51x10^6$
D1	48.80	27.66	23.54	$1.23x10^7$	$6.10x10^6$	$1.33x10^6$
D2	43.97	28.08	27.94	$6.28x10^6$	$4.76x10^6$	$6.79x10^5$
D3	49.04	25.33	25.64	$1.07x10^7$	$6.63x10^6$	$1.08x10^6$
E1	52.83	26.47	20.70	$1.08x10^7$	$4.58x10^6$	$9.13x10^5$
E2	49.22	25.36	25.42	$1.19x10^7$	$6.48x10^6$	$1.15x10^6$
E3	54.41	22.88	22.70	$1.21x10^7$	$5.51x10^6$	$1.16x10^6$
LSD[f] (P<0.05)	1.19	1.17	1.17	$4.10x10^5$	$7.33x10^4$	$6.04x10^4$

[a]A1, B1, C1: Edirne; D1, E1, D3, E3: Tekirdag; A2, B2, C2, D2, E2: Canakkale; and A3, B3, C3: Kirklareli; all cities are from the region of Trakya, Turkey
[b]HMW= High Molecular Weight; [c]LMW= Low Molecular Weight; [d]Mw=Weight Averaged Molecular Weight; [e]Da= Dalton; [f]LSD= Least Significant Difference (P<0.05)

Table 5: Amylopectin and amylose ratio in tarhana soup samples.

resistant to amylase hydrolysis compared to starch from other sources like tubers and legumes [9]. Although tarhana soup presented high GI, it has a high content of slowly digestible starch from the point of view of digestion rate. While the slowly digestible starch is completely digested before reaching the large intestine, it is slowly hydrolyzed along the entire digestion process and results in a more even and slow release of glucose [24].

Amylose, amylopectin and molecular mass of starch

It has been mentioned that the physical organization of amylose and amylopectin in the granular structure of starch, as well as the chain length distribution and organization of the clusters of amylopectin molecules, have a relationship with the functionality of starch including its digestibility properties [8]. For this reason, it is important to understand the nature of the starch structure in starchy foods like tarhana soup. The chromatograms of amylose and amylopectin of tarhana samples obtained with HPSEC-RID are depicted in Figure 1a. The representative chromatogram obtained after starch extraction presented three peaks corresponding to 1) high molecular weight (HMW) amylopectin, 2) low molecular weight (LMW) amylopectin and 3) amylose, as was previously reported in various food systems [14]. The area under the RI signal curve in the chromatogram was used to determine the amylose and amylopectin content (Table 5). The HMW and LMW amylopectin content ranged from 54.41%-42.30% and 28.67%-22.88%, respectively. The amylose content ranged from 20.70% to 29.03%. The variation observed in the HMW and LMW amylopectin and amylose ratios may be attributed to changes in the starch content caused by the variations in the fermentation process of the dough. During fermentation the amylases in the flour may start to hydrolyze the starch to produce fermentable sugars at different levels based on the specific length and conditions of fermentation for each tarhana sample. Wani et al. [21] determined that final viscosity is affected by the amylose content. There are some significant (P<0.05) differences in amylose content (Table 5) among some but not all of the samples. Due to these differences, the weight averaged molecular weight of each fraction .was determined with HPSEC-MALS, where it was possible to identify the molecular mass of each starch fraction seen in the chromatogram (Figure 1b, reference chromatogram). The molecular mass of HMW and LMW amylopectin and amylose in tarhana samples is presented in Table 5, where the molecular masses varied from 2.06×10^6-1.55×10^7, 1.22×10^6-7.10×10^6 and 3.02×10^5-2.44×10^6, respectively. Significant differences (P<0.05) in the molecular mass of all the three fractions in tarhana soup were observed. This confirmed that fermentation process in tarhana preparation affected the molecular mass of starch and which caused effects on the functional properties of tarhana. It has been mentioned that molecular weight of amylose and amylopectin is involved in the functional properties of starchy foods and plays an important role in the digestibility of starch. The physicochemical properties and *in vitro* starch digestibility of tarhana soup could depend of the amylose and amylopectin content and their molecular mass.

Conclusions

The physicochemical and digestibility properties of tarhana soup were investigated. Because of the different ingredients used during tarhana preparation among locations where the samples were collected, there were significant (P<0.05) differences in the protein and starch content. The fermentation time and conditions used during the tarhana preparation could affect the starch structure and result in differences in gelatinization, pasting and *in vitro* starch digestibility properties of each sample. Also, differences in the amylose and amylopectin content were observed. Although, in this study the differences in the molecular mass could have multiple causes, such as fermentation time, fermentation conditions and type of cereal used. To better understand the functionality of starch in tarhana it may be beneficial to analyze the chain length distribution of amylose and amylopectin and their distribution in the starch granule after the fermentation process, one step in the tarhana preparation.

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