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A Novel high-amylose wheat-based functional cereal soup (tarhana) with low glycemic index and high resistant starch

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ABSTRACT

This study investigated the potential of high-amylose wheat flour (Svevo-HA) to enhance the dietary profile of tarhana, a traditional Mediterranean fermented cereal yogurt mixture. The moisture content of tarhana powders ranged from 7.81% to 11.64%. Color parameters varied depending on the type of flour used, with Svevo-HA samples demonstrating decreased L* values and increased a* and b* values. Mineral compositions differed significantly among tarhana samples, with higher levels of K, Mg, Mn, Fe, Cu, and Zn observed in samples prepared with Svevo-HA. Gallic acid was identified as the major phenolic compound in all the tarhana samples for free fraction, while ferulic acid was determined as the major phenolic compound for its bound form. Supplementation of tarhana soups with heat-treated Svevo-HA flour increased the resistant starch content and decreased *in vitro* glycemic index value compared to soups prepared with conventional wheat flour. These findings highlight the potential of utilizing Svevo-HA flour to develop healthier versions of traditional foods.

1. Introduction

Tarhana is a fermented yogurt and cereal mixture used in soup preparation. It holds a significant place in the diets of individuals residing in the Middle East, Asia, and certain regions of Europe. It is a popular fermented food made by combining yogurt, flour, yeast, vegetables, and spices. This mixture is fermented for up to one week, dried, ground, and used for soup preparation (Yalcin et al., 2008). In some countries, there are other products like tarhana; "tahonya/talkuna" in Finland and Hungary, "tarhana" in Albania and Croatia, "kishk" in Egypt, and "kushuk" in Iraq (Ibanoglu et al., 1995). Tarhana is a food, rich in B group vitamins, protein, calcium, copper, potassium, magnesium, zinc, and iron minerals. The formulations may differ, but flour and yogurt are the main components. Tarhana is often made using refined wheat flour. Hence, increasing the amount of dietary fiber and resistant starch is expected to improve its nutritional properties.

Englyst et al. (1992) performed *in vitro* starch digestion by mimicking gastrointestinal conditions and classified starch based on *in vitro* digestion kinetics. Starch is categorized as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) according to its digestibility. European Flair Concerted Action on Resistant Starch (EURESTA) has defined RS as "the starch or products of starch degradation that escapes digestion in the small intestine of healthy individuals and may be completely or partially fermented in the colon" (Englyst et al., 1992). RS provides health benefits because it cannot be digested by digestive enzymes like normal starch. RS, which passes undigested through the stomach and small intestine, is fermented in the colon. As a result of fermentation some short-chain fatty acids (butyrate, acetate, and propionate) are formed. Consuming RS is effective in enhancing bowel movement and reducing the time it takes for stool to pass through the gut, thus contributing to the prevention of colorectal cancer (DeMartino and Cockburn, 2020).

Foods that include RS have a relatively lower glycemic index (GI) because they slowly increase blood glucose levels. Low GI foods can be used in the diets of diabetic patients due to their positive effects on blood glucose and insulin levels (Liu et al., 2020). RS is preferred as a

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functional ingredient due to its high gelatinization temperature and lower water retention capacity compared to other dietary fiber sources (Homayouni et al., 2014).

The MEDWHEALTH project, funded PRIMA, aims to re-design a set of Med-foods and increase their healthfulness by utilizing new raw materials such as high-amylose durum wheat. The main purpose of this research was to produce a new tarhana, using a high-amylose wheat flour (Svevo-HA) and investigate its physical, chemical, technological, and nutritional properties. Svevo HA was produced through a breeding program focusing on the manipulation of starch composition to obtain durum wheat with improved nutritional value (Sestili et al., 2015). It is characterized by a higher level of amylose (58.7%) (Romano et al., 2022). The study was focused on the production of a tarhana soup with relatively higher resistant starch and lower GI, which is also a unique aspect of the study. In order to achieve this, a flour with relatively high RS content produced from high-amylose (HA) durum wheat was used for tarhana preparation, and its properties were compared with tarhana samples prepared with flours of two durum wheats (cvs. Svevo and Kiziltan), and a commercial flour.

2. Material and methods

2.1. Materials and chemicals

Svevo and Svevo-HA wheat samples were grown at the Experimental Farm "Nello Lupori" (University of Tuscia, Viterbo, Italy) in the 2021–2022 growing season and processed into flour in a Buhler laboratory mill (MLU 202, Uzwil, Switzerland) in order to obtain straight-grade flour which is abbreviated as SF (Svevo flour) and SHaF (Svevo-HA flour), respectively (Approved Method 26-21, AACC International, 2000). Kiziltan wheat sample grown in Ankara (2021–2022) was supplied by Field Crops Central Research Institute (Ankara, Türkiye). This sample was ground using a laboratory grinder (CemotecTM, CM290, Hillerod, Denmark) and sieved through a 150 μm sieve to obtain whole wheat flour (KF). Flours of durum wheat cultivars (SF, SHaF, KF) and commercial bread wheat flour (CF, Eksim Milling Co., Istanbul, Türkiye) were used in the preparation of tarhana. Yogurt, fresh baker's yeast, green and red peppers, tomato paste, onion, red pepper powder (paprika), and salt were purchased from local markets in İstanbul (Türkiye). All reagents and solvents were obtained from Sigma-Aldrich (St. Louis, MO, USA). Megazyme Resistant Starch and Glucose Assay Kits were purchased from Megazyme International (Wicklow, Ireland).

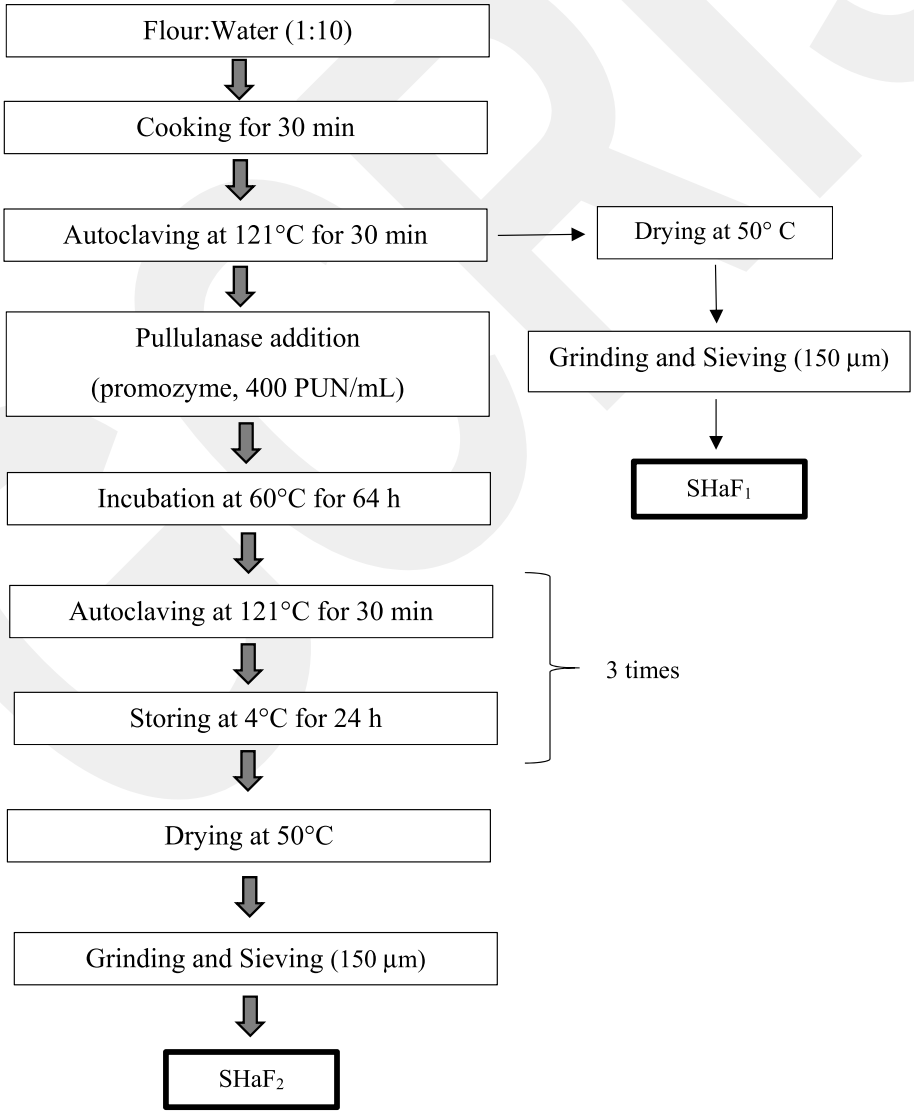

Fig. 1. Heat treatment of Svevo-HA flour for resistant starch production.

2.2. Resistant starch formation from Svevo-HA flour

For RS formation, the method by Ozturk et al. (2009) was used with slight modification (Fig. 1). Briefly, Svevo-HA flour: water mixture was cooked for 30 min and subjected to autoclaving at 121 °C for 30 min. Then, the mixture was separated into two parts, and one part was oven-dried at 50 °C, and ground to pass 150 μm sieve ($SHaF_1$). To see the impact of debranching and autoclaving-cooling cycles on RS content, the other half was incubated at 60 °C for 64 h with debranching enzyme pullulanase (Promozyme, 400 PUN/mL, 2000 U/kg starch). After the incubation, the samples were autoclaved, and stored at 4 °C for 24 h. The autoclaving-storing cycles were repeated 3 times in total. After the autoclaving-storing cycles, the sample was dried at 50 °C, and ground to pass 150 μm sieve ($SHaF_2$).

2.3. Tarhana preparation

Tarhana samples were prepared following the method outlined by Erkan et al. (2006) with some modifications. The ingredients for tarhana production are listed in Table S1. After chopping peppers and onions in a food processor (Raks-MR 1001, İstanbul, Türkiye), paprika, tomato paste, and salt were added, and the mixture was blended. Flour samples (CF, KF, SF, SHaF, $SHaF_1$, and $SHaF_2$), yogurt, and yeast were added to the mixture and blended. The mixture was incubated at 30 °C for 5 days for fermentation. For pH analysis, tarhana samples were taken at the end of the fermentation. After the fermentation, the samples were dried at 40 °C and then ground to pass 150 μm sieve (tarhana powder). Tarhana powders produced using CF, KF, SF, SHaF, $SHaF_1$, and $SHaF_2$ were abbreviated as Tar-CF, Tar-KF, Tar-SF, Tar-SHaF, Tar-$SHaF_1$ and Tar-$SHaF_2$, respectively.

2.4. Analyses of tarhana powders

The moisture contents of tarhana powders were determined according to AACC International Standard Method No: 44-15A (AACC International, 2000). The color values of the samples were measured using the L*a*b* color space (CIELAB space) with a colorimeter (Konica Minolta CR-400, Tokyo, Japan).

The method of Cankurtaran et al. (2020) was followed to determine the Ca, Fe, K, Mg, and Mn content of the samples using an inductively coupled plasma-optical-emission spectrometer (ICP-OES, Optima 2100 DV, PerkinElmer, USA). Cu, Zn, and Se content of the samples was determined using an inductively coupled plasma-mass-spectrometer (ICP-MS, 7700 Series x, Agilent, Japan). The samples were digested by a microwave oven with a mixture of 8 mL HNO_3/2 mL H_2O_2. Argon (99.95%) was the main, auxiliary, and nebulizer gas. The ICP-OES and ICP-MS operating parameters were 1300 W and 1200 W respectively, and the flow rates of nebulizer and auxiliary gas were 0.80 and 0.20 L/min, 0.70 and 0.20 L/min, respectively.

Tarhana powders were extracted for free phenolic compounds (FPCs) and bound phenolic compounds (BPCs) using the method of Tekin-Cakmak et al. (2024). Phenolic contents were determined according to the method by Tekin-Cakmak et al. (2024). with some modifications. An aliquot (100 μl) of the extract was combined with 500 μl of Folin Ciocalteu's reagent and 1.5 ml of sodium carbonate (20%). The final volume was adjusted to 10 mL with distilled water. The mixture was kept in the dark at room temperature for 2 h, then was centrifuged for 5 min at 2000×*g*. The absorbance of the supernatant was measured at 760 nm (Shimadzu 150 UV-1800, Kyoto, Japan). The DPPH radical scavenging activity and ABTS radical cation scavenging capacity of the extracts were determined according to Tekin-Cakmak et al. (2024).

Individual phenolic compounds were determined using an HPLC system coupled to a diode array detector (SPDM20A DAD, Shimadzu, Japan) according to Ozkan et al. (2022). The extracts were filtered via a membrane filter (0.45-μm). Separations were accomplished at 40 °C on a reversed-phase Athena C18-WP HPLC column (250 mm × 4.6 mm length, 5 μm particle size, CNW technologies, Shanghai, China). Solvent A (distilled water with 0.1% acetic acid) and solvent B (acetonitrile) were the mobile phase. Gradient elution was 10% B (0–2 min), 10%–30% B (2–27 min), 30%–90% B (27–50 min), and 90%–100% (51–60 min) before returning to initial conditions at 63 min. The flow rate was 1 ml/min. The chromatograms were evaluated at 278, 320, and 360 nm.

2.5. Tarhana soup preparation

Prior to the analysis tarhana soups were prepared from tarhana powders. Briefly, 6 g of tarhana powder (db) was mixed with 100 mL of water (25 °C) and stirred for 1.5 min to achieve a homogeneous suspension. The mixture was boiled for 15 min with constant stirring after the beginning of boiling (Yilmaz et al., 2010). The soup samples were freeze-dried (Martin Christ, Beta 1–8 LSCplus, Germany), ground, and kept at 4 °C.

2.6. Analyses of tarhana soups

RS contents of the samples were determined using Approved Method 32–40.01 (AACC International, 2000). The starch hydrolysis rate during *in vitro* digestion at 90 min and *in vitro* glycemic index (GI) value of freeze-dried cooked tarhana soup sample was measured according to the method of Tekin-Cakmak et al. (2024) by using a Glucose Assay Kit (Megazyme Int., Wicklow, Ireland).

The pasting properties of the tarhana samples were determined using Rapid Visco-Analyzer (Perten Inst., Sweden). Tarhana powder (2 g, dw) was mixed with distilled water to make a total mixture weight of 27.0 g and subjected to Std 1 profile (Approved Method 76-21.01, AACC International, 2000). RVA Soup Method (cooled sample profile) was used to determine the viscosity of the samples. For this purpose, 29 gr of soup sample was mixed at 80 °C for 5 min and the last viscosity values were recorded as "RVA soup index".

2.7. Statistical analysis

The data were given as the mean standard deviation for each experiment, which were all run in triplicate. Statistical analysis was performed using SPSS Statistics Software (IBM version 20, USA). Tukey's post hoc analysis was performed to compare the means of the groups, and one-way ANOVA was utilized to evaluate the differences ($p < 0.05$). Correlation coefficient was determined between RS content and *in vitro* GI values.

3. Results and discussion

3.1. Analyses of tarhana powders

The moisture contents of tarhana powders were in the range of 7.81–11.64% which is in line with the previous study (Cankurtaran et al., 2020). Color parameters of tarhana powders are presented in Table 1. The L* values were between 71.05 and 82.40, while the a* values were between 3.26 and 7.50 and the b* values were between 22.49 and 31.61 for the tarhana samples. As expected, different flours affected the color of the tarhana samples. The L* values of tarhana powders Tar-$SHaF_1$ and Tar-$SHaF_2$ were lower than those of others, probably because of heat treatments applied to these samples. Among these samples Tar-$SHaF_2$ had significantly lower L* value than that of Tar-$SHaF_1$ ($p < 0.05$) probably due to three more autoclaving storing cycles applied. The a* values of the tarhana powders were significantly ($p < 0.05$) different from each other except those of the tarhana samples produced using Svevo and Svevo-HA flours (Tar-SF, Tar-SHaF). The b* values of Tar-SF and Tar-SHaF were higher than the values of other tarhana samples as expected from good-quality durum wheat. The color results of the present study agree with Bilgicli (2009) who reported the tarhana L* and b* values as 71.59 and 32.28. Yalcin et al. (2008)

Table 1
Color and pH values of tarhana samples.

	Tarhana powders					
	Tar-CF	Tar-KF	Tar-SF	Tar-SHaF	Tar-$SHaF_1$	Tar-$SHaF_2$
L*	82.40 ± 0.18^{a}	78.25 ± 0.36^{b}	78.90 ± 0.35^{b}	78.46 ± 0.11^{b}	75.21 ± 0.05^{c}	71.05 ± 0.76^{d}
a*	5.07 ± 0.21^{c}	6.21 ± 0.17^{b}	7.50 ± 0.13^{a}	7.08 ± 0.08^{a}	3.26 ± 0.01^{e}	4.00 ± 0.11^{d}
b*	24.46 ± 0.61^{b}	22.49 ± 0.24^{c}	30.86 ± 0.92^{a}	31.61 ± 0.46^{a}	24.99 ± 0.30^{b}	23.98 ± 0.37^{bc}
Tarhana soups						
L*	62.49 ± 0.87^{a}	54.78 ± 0.48^{b}	51.89 ± 0.07^{cd}	50.20 ± 0.67^{d}	53.84 ± 0.16^{b}	53.47 ± 0.34^{bc}
a*	−2.23 ± 0.18^{f}	0.35 ± 0.04^{e}	2.01 ± 0.06^{d}	2.77 ± 0.14^{c}	5.11 ± 0.10^{a}	3.65 ± 0.18^{b}
b*	19.64 ± 0.65^{e}	25.20 ± 0.10^{d}	28.56 ± 0.48^{b}	26.94 ± 0.23^{c}	30.43 ± 0.15^{a}	26.14 ± 0.12^{cd}
pH	4.67 ± 0.01^{ab}	4.62 ± 0.02^{b}	4.75 ± 0.05^{ab}	4.80 ± 0.04^{ab}	4.82 ± 0.04^{a}	4.77 ± 0.02^{ab}

Data are expressed as mean ± S.D. of triplicate measurements. Different letters (a-d) on the same row are significantly different ($P < 0.05$) among tarhana powders or soups.
Tar-CF: tarhana produced using commercial flour, Tar-KF: tarhana produced using Kiziltan flour, Tar-SF: tarhana produced using Svevo flour, Tar-SHaF: tarhana produced using Svevo-HA flour, Tar-$SHaF_1$: tarhana produced with Svevo-HA flour heat treated without debranching and autoclaving-cooling cycles, Tar-$SHaF_2$: tarhana produced with Svevo-HA flour heat treated with debranching and autoclaving-cooling cycles.

reported that wheat, corn, and rice tarhana samples had the L* values of 79.98, 82.22, and 82.15, respectively, indicating that corn and rice tarhana samples were lighter than the wheat tarhana sample. Furthermore, they found the highest b* value in corn tarhana because of the color of corn flour.

The mineral compositions of the tarhana powders are given in Table 2. The mineral compositions of the tarhana powders Tar- $SHaF_1$ and Tar- $SHaF_2$ were not determined since they were produced from Svevo-HA flour and the treatments applied are not expected to result in major alterations in the mineral content. Utilization of different flours in tarhana production generally led to significantly different mineral contents ($p < 0.05$). Depending on the flours used, K, Mg, and Ca contents of the tarhana powders varied between 5591 and 6776 μg/g, 594–1139 μg/g, and 1533–2029 μg/g, respectively. Özdemir et al. (2007) also found that tarhana has a high mineral content in terms of K, Mg, and Ca. The range of K, Mg, and Ca values of tarhana samples were reported as 5619–9863, 644–914, and 736–1485 mg/kg by Cankurtaran et al. (2020) and 363–909, 26–86, and 119–176 mg/100 g by Tekgul et al. (2021), respectively. The K, Mg, Mn, Fe, Cu, and Zn contents of the tarhana samples produced with Kiziltan, Svevo, and Svevo-HA flours were higher than the ones produced with commercial flour. This is an expected result since it is produced from a general purpose commercial flour without bran.

Table 2
Mineral contents (μg/g) of tarhana powders.

	Tar-CF	Tar-KF	Tar-SF	Tar-SHaF
K	5591 ± 119^{b}	6487 ± 138^{a}	6510 ± 139^{a}	6776 ± 144^{a}
Mg	594 ± 10.9^{d}	1139 ± 21^{a}	761 ± 14.0^{c}	891 ± 16.4^{b}
Ca	1934 ± 53^{a}	1533 ± 45^{b}	1984 ± 54^{a}	2029 ± 55^{a}
Mn	8.56 ± 0.23^{c}	28.46 ± 0.77^{a}	12.63 ± 0.34^{b}	12.19 ± 0.33^{b}
Fe	56.91 ± 1.96^{b}	62.14 ± 2.07^{a}	41.87 ± 2.01^{c}	44.37 ± 2.13^{c}
Cu	1.72 ± 0.05^{c}	3.34 ± 0.09^{ab}	2.85 ± 0.08^{b}	3.79 ± 0.11^{a}
Zn	9.44 ± 0.27^{c}	14.80 ± 0.42^{a}	12.22 ± 0.35^{b}	16.49 ± 0.47^{a}

Data are expressed as mean standard deviation, mean values in each row with different letters (a–e) are significantly different ($p < 0.05$).
Tar-CF: tarhana produced using commercial flour, Tar-KF: tarhana produced using Kiziltan flour, Tar-SF: tarhana produced using Svevo flour, Tar-SHaF: tarhana produced using Svevo-HA flour.

The Cu and Zn levels in tarhana samples varied between 1.72 and 3.79 μg/g and 9.44–16.49 μg/g, respectively and the highest amounts were quantified in Tar-SHaF followed by Tar-KF. The Tar-KF sample was the highest or in the highest group in terms of K, Mg, Mn, Fe, Cu, and Zn, probably due to the composition of flour which was produced without bran separation. Se contents of the tarhana samples were lower than the limit of detection value of the method and equipment used. Compared to the results of the present study, lower Cu values (0.00–2.00 mg/kg) were reported by Bilgicli et al. (2006). Zn and Mn contents of wheat germ-supplemented tarhana samples were in the range of 7.2–131.2 and 13.3–80.7 ppm, respectively (Tekgul et al., 2021). Tarhana samples produced in this study can be considered a good mineral (Ca, K, Mg, Cu, Zn, and Mn) source.

The phenolic contents and antioxidant capacities of the tarhana powders in free and bound fractions are given in Table 3. The free phenolic contents of the tarhana samples were in the range of 220.50–272.97 mg GAE/100g dw, while the bound phenolic contents of the tarhana samples varied from 329.03 to 583.65 mg GAE/100g dw. Most of the phenolic compounds found in cereal-based matrices exist in forms that are covalently bound to structural components of the cell wall and not soluble (Acosta-Estrada et al., 2014). The total phenolic content (TPC) of the tarhana samples changed between 565.49 and 856.63 mg GAE/100g dw. Compared with the Tar-CF, the TPCs of the Tar-KF, Tar-SF, and Tar-SHaF were significantly higher. The TPC of Tar-$SHaF_1$ and Tar-$SHaF_2$ were lower than that of Tar-SHaF (Table 3). The loss in phenolic content in Tar-$SHaF_1$ and Tar-$SHaF_2$ samples is likely a result of the thermal treatment during the production of $SHaF_1$ and $SHaF_2$ flours. It was stated that the phenolic content has a negative correlation with the temperature applied (Biswas et al., 2020).

The TPC value of tarhana samples prepared with 100% wheat flour was found to be 2260 mg GAE/kg (226 mg GAE/100 g) by Köten (2021), which was relatively lower compared to the present study. According to Isik and Yapar (2017) as the level of tomato seed addition increased, so did the TPC and antioxidant capacity values of tarhana samples. Since tarhana consists of phenolic-rich tomatoes, peppers, and spices, these ingredients might play a significant role in its total phenolic content. The differences observed compared to the literature may be due to the formulation of tarhana samples.

ABTS and DPPH values of the free and bound fractions of tarhana powders are given in Table 3. DPPH radical scavenging capacity of free fractions varied between 24.62 and 34.75 mg TE/100 g dw, while ABTS radical cation scavenging capacity of free fractions were in the range of 18.91–49.24 mg TE/100 g dw. The DPPH values of tarhana powders in bound fraction were determined between 52.76 and 73.09 mg TE/100 g dw and their ABTS values in bound fraction were determined between 34.23 and 55.05 mg TE/100 g dw. The total antioxidant capacity values in Tar-SHaF and Tar-KF samples showed stronger DPPH and ABTS values compared to the others. In a study by Kilci and Gocmen (2014a), ABTS values of bound phenolic extracts of tarhana samples were between 134.02 and 204.72 μmol trolox/g dw.

Thermal processing methods have been used in cereal processing primarily to enhance various properties of the products such as palatability, stability, and safety. The thermal treatments also affect individual phenolics in cereal grains depending on the type of grain and severity of heat treatment (Değirmencioğlu et al., 2016).

Table 4 shows the HPLC results of fifteen individual phenolics that were screened in the tarhana samples. Gallic acid was recognized as the primary phenolic compound among the tarhana samples in its free form, while ferulic acid was identified as the major phenolic acid in its bound form. For free fraction, the gallic acid levels ranged from 39.98 mg/100g to 52.58 mg/100g while the ferulic acid content changed from 1.75 mg/100g to 3.60 mg/100 g. Although fifteen phenolic standards have been screened in tarhana samples, identity confirmation of some phenolic compounds (HPLC peaks) was not possible, specifically in the bound fractions, because of the absence of the available standards. An example of HPLC chromatogram depicting these unidentified peaks is shown in

Table 3
Free and bound phenolic contents and antioxidant capacities of powder tarhana samples.

	Phenolic Content (mg GAE/100g dw)			DPPH			ABTS		
	Free	Bound	Total[a]	Free	Bound	Total[a]	Free	Bound	Total[a]
Tar-CF	220.50 ± 2.69^{e}	355.03 ± 1.01^{c}	75.53 ± 1.68^{cd}	27.33 ± 0.74^{d}	52.76 ± 0.99^{d}	80.08 ± 0.25^{d}	19.31 ± 0.27^{c}	37.88 ± 0.45^{c}	57.19 ± 0.18^{c}
Tar-KF	255.79 ± 1.25^{b}	553.36 ± 0.83^{b}	809.14 ± 2.08^{b}	33.96 ± 0.74^{ab}	58.76 ± 0.98^{c}	92.72 ± 1.72^{b}	47.53 ± 0.22^{a}	54.63 ± 0.55^{a}	102.16 ± 0.77^{a}
Tar-SF	248.03 ± 0.82^{c}	554.84 ± 1.65^{b}	802.87 ± 2.47^{b}	30.66 ± 0.98^{c}	63.27 ± 0.49^{b}	93.93 ± 0.49^{b}	45.23 ± 0.76^{b}	49.01 ± 0.87^{b}	94.24 ± 1.64^{b}
Tar-SHaF	272.97 ± 2.44^{a}	583.65 ± 2.85^{a}	856.63 ± 0.41^{a}	34.75 ± 0.48^{a}	73.09 ± 0.48^{a}	107.84 ± 0.10^{a}	49.24 ± 0.65^{a}	55.05 ± 0.65^{a}	104.29 ± 1.30^{a}
Tar-$SHaF_1$	238.71 ± 2.08^{d}	341.44 ± 1.67^{d}	580.15 ± 3.75^{c}	31.53 ± 0.73^{bc}	55.20 ± 0.73^{cd}	86.73 ± 0.15^{c}	20.28 ± 0.55^{c}	39.02 ± 0.99^{c}	59.30 ± 0.44^{c}
Tar-$SHaF_2$	236.46 ± 0.64^{d}	329.03 ± 2.98^{e}	565.49 ± 3.62^{d}	24.62 ± 0.50^{d}	58.14 ± 1.76^{c}	82.77 ± 2.26^{cd}	18.91 ± 0.23^{c}	34.23 ± 0.68^{d}	53.14 ± 0.90^{d}

Data are expressed as mean standard deviation, mean values in each column with different letters (a–e) are significantly different ($p < 0.05$).
DPPH 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, ABTS 2,2′ -azino-bis (3-ethylbenzothiazoline6- sulphonic acid).
Tar-CF: tarhana produced using commercial flour, Tar-KF: tarhana produced using Kiziltan flour, Tar-SF: tarhana produced using Svevo flour, Tar-SHaF: tarhana produced using Svevo-HA flour, Tar-$SHaF_1$: tarhana produced with Svevo-HA flour heat treated without debranching and autoclaving-cooling cycles, Tar-$SHaF_2$: tarhana produced with Svevo-HA flour heat treated with debranching and autoclaving-cooling cycles.
[a] The sum of free and bound antioxidant capacities expressed as mg TE/100 g dw.

Fig. S1. Zhang et al. (2023) reported that in wheat flour and milling fractions, the concentration of free phenolic compounds (phenolic acids, flavonoids, stilbenes, lignans, and other phenolics) was greater compared to their bound counterparts. In the present study, the most abundant phenolics were ferulic acid, ellagic acid, gallic acid, and kaempferol for the bound fractions. Barros Santos et al. (2022) reported that the most abundant phenolics were *p*-coumaric, ferulic acid isomers, and one isomer of diferulic acid in bound extracts.

In the present study, gallic acid was found to be the most prevalent phenolic compound across all tarhana samples, followed by ferulic acid and myricetin. Quercetin is a phenolic compound measured in significant amounts in tarhana samples. A significant variation in the quantity of quercetin was observed among the samples ($p < 0.05$). The Tar-SHaF sample had the highest amount of quercetin, whereas the Tar-CF sample demonstrated the lowest concentration of quercetin. The levels of catechin were relatively low but exhibited significant variability among the samples. The Tar-Cf and Tar-KF samples exhibited the lowest concentration of catechin, however, the Tar-SHaF, Tar-$SHaF_1$, and Tar-$SHaF_2$ tarhana samples demonstrated higher concentrations of catechin. *p*-coumaric acid and chrysin were detected in trace amounts. Sinapic acid was absent in the tarhana samples, except in the Kiziltan tarhana sample. Kilci and Gocmen (2014a) reported that ferulic, caffeic, gallic, *p*-coumaric, *p*-hydroxybenzoic, sinapic, syringic, and vanillic acids were detected in the tarhana samples supplemented with steel-cut oats. In another study by Kilci and Gocmen (2014b), sinapic acid was found to be lower than other phenolic acids in the oat flour-supplemented tarhana samples. They reported that the most prevalent phenolic acids were vanillic acid, followed by ferulic and gallic acid in the samples. The differences in the results could be attributed to the variations in the extraction conditions, tarhana formulations, and utilization of different ingredients such as flour.

3.2. Analyses of tarhana soups

Color parameters of the tarhana soups are presented in Table 1. The L* values of the soups samples were in the range of 50.20–62.49. The Tar-CF sample had the greatest L* value among the tarhana samples. The a* values were −2.23, 0.35, 2.01, 2.77, 5.11 and 3.65 for the soups of tarhana samples, Tar-CF, Tar-KF, Tar-SF, Tar-SHaF, Tar-$SHaF_1$ and Tar-$SHaF_2$, respectively and their b* values were in the range of 19.64–30.43.

The pH values of the soups prepared with tarhana samples were determined in the range of 4.62–4.82 (Table 1). The pH of tarhana is lowered by the organic acids created during fermentation, and surplus moisture is eliminated by drying after fermentation (Ibanoglu et al., 1995). Due to the low pH (3.3–5.0), low moisture content (6–10%), and bacteriostatic action of organic acids generated during fermentation on harmful bacteria, tarhana has a relatively long shelf life (Özdemir et al., 2007).

RS contents of the tarhana soup samples varied between 0.36 and 5.73% (Table 5). The RS contents of Tar-SHaF, Tar-$SHaF_1$, and Tar-$SHaF_2$ were 0.84, 3.29, and 5.73%, respectively. Tar-CF, Tar-KF, and Tar-SF had significantly ($p < 0.05$) lower RS contents compared to Tar-SHaF, Tar-$SHaF_1$ and Tar-$SHaF_2$. The tarhana samples supplemented with the heat-treated flours (Tar-$SHaF_1$ and Tar-$SHaF_2$) had significantly ($p < 0.05$) higher RS content than the ones produced with other flours indicating the improvement effect of the heat treatments. Among the heat treated samples Tar-$SHaF_2$ had significantly higher RS content than Tar-$SHaF_1$. This is an expected result since Tar-$SHaF_1$ only had one cycle of autoclaving, while Tar-$SHaF_2$ had some further treatments (debranching and 3 more autoclaving-storing cycles). Effects of debranching along with autoclaving-storing cycles on RS content in high amylose (HA) corn starches were investigated by Ozturk et al. (2009). HA corn starches were subjected to autoclaving-storing cycles after debranching with pullulanase, and it was observed that RS content increased with debranching and further increases were observed with autoclaving-storing cycles. This may be because of the re-association of the starch molecules (retrogradation) during the autoclaving-storing cycles and drying process and the formation of tightly packed structures bound together by hydrogen bonds which reduces the accessibility by starch digesting enzymes.

The hydrolysis index (HI) and *in vitro* GI values of tarhana soup samples are given in Table 5. There were significant ($p < 0.05$) differences between wheat flour tarhana soups and tarhana soups prepared with heat-treated flours (Tar-$SHaF_1$ and Tar-$SHaF_2$) in terms of HI and *in vitro* GI. The HI values of all tarhana soups ranged from 27.59 to 73.65, while the *in vitro* GI values of tarhana soups ranged from 54.86 to 80.14. The foods are categorized into 3 groups according to their GI values; high-GI foods have a GI value greater than 70, while low-GI foods have a GI value lower than 55. Foods are categorized as medium GI if their GI values range from 56 to 69 (Kumar et al., 2018). According to the GI classification system, the tarhana soups, Tar-CF, Tar-KF, Tar-SF, and Tar-SHaF had high *in vitro* GI values (80.14, 75.73, 70.94, and 76.75, respectively). The tarhana samples prepared using the heat-treated flours (Tar-$SHaF_1$ and Tar-$SHaF_2$) had medium (58.12) and low (54.86) *in vitro* GI values, respectively. The decrease in the HI and *in vitro* GI is probably related to the relatively higher RS content of these tarhana soups since the correlation coefficient ($R = -0,942$) between RS content and *in vitro* glycemic index values was significant ($p < 0.01$). In a study by Simsek et al. (2014), the *in vitro* GI of the tarhana soups of homemade tarhana powders collected from different regions of Türkiye were in the range of 86.16–102.54. This is an expected result since

Table 4
Phenolic compound contents of tarhana powders (mg/100g dw).

		Free	Bound	Total
gallic acid	Tar-CF	39.98 ± 0.65^{d}	1.71 ± 0.01^{c}	41.68 ± 0.65^{e}
	Tar-KF	44.15 ± 0.01^{c}	nd	44.15 ± 0.01^{d}
	Tar-SF	47.96 ± 0.10^{b}	1.66 ± 0.01^{d}	49.62 ± 0.10^{b}
	Tar-SHaF	52.58 ± 0.20^{a}	1.63 ± 0.01^{d}	54.21 ± 0.20^{a}
	Tar-SHaF$_1$	48.86 ± 0.86^{b}	1.79 ± 0.03^{b}	50.66 ± 0.83^{b}
	Tar-SHaF$_2$	44.83 ± 0.82^{c}	2.02 ± 0.01^{a}	46.85 ± 0.82^{c}
ferulic acid	Tar-CF	2.85 ± 0.10^{b}	3.05 ± 0.05^{e}	5.90 ± 0.07^{e}
	Tar-KF	3.55 ± 0.05^{a}	3.75 ± 0.10^{d}	7.30 ± 0.11^{d}
	Tar-SF	1.75 ± 0.05^{d}	18.20 ± 0.01^{a}	19.95 ± 0.04^{b}
	Tar-SHaF	3.60 ± 0.15^{a}	18.40 ± 0.15^{a}	22.00 ± 0.30^{a}
	Tar-SHaF$_1$	1.90 ± 0.05^{c}	17.00 ± 0.30^{b}	18.90 ± 0.35^{b}
	Tar-SHaF$_2$	1.95 ± 0.03^{c}	10.80 ± 0.10^{c}	12.75 ± 0.12^{c}
myricetin	Tar-CF	nd	nd	nd
	Tar-KF	7.74 ± 0.02^{b}	nd	7.74 ± 0.02^{b}
	Tar-SF	7.65 ± 0.01^{d}	nd	7.65 ± 0.01^{d}
	Tar-SHaF	7.92 ± 0.01^{a}	nd	7.92 ± 0.01^{a}
	Tar-SHaF$_1$	7.73 ± 0.01^{b}	nd	7.73 ± 0.01^{b}
	Tar-SHaF$_2$	7.70 ± 0.01^{c}	nd	7.70 ± 0.01^{c}
ellagic acid	Tar-CF	2.55 ± 0.01^{a}	nd	2.55 ± 0.01^{f}
	Tar-KF	1.39 ± 0.03^{e}	2.84 ± 0.01^{a}	4.23 ± 0.04^{b}
	Tar-SF	1.94 ± 0.01^{c}	1.78 ± 0.01^{c}	3.73 ± 0.01^{d}
	Tar-SHaF	2.56 ± 0.06^{a}	1.99 ± 0.02^{b}	4.55 ± 0.03^{a}
	Tar-SHaF$_1$	1.81 ± 0.01^{d}	1.63 ± 0.01^{e}	3.44 ± 0.01^{e}
	Tar-SHaF$_2$	2.33 ± 0.03^{b}	1.69 ± 0.01^{d}	4.03 ± 0.02^{c}
quercetin	Tar-CF	0.46 ± 0.01^{d}	nd	0.46 ± 0.01^{e}
	Tar-KF	3.82 ± 0.03^{ab}	nd	3.82 ± 0.03^{c}
	Tar-SF	3.73 ± 0.02^{c}	nd	3.73 ± 0.02^{d}
	Tar-SHaF	3.86 ± 0.01^{a}	nd	3.86 ± 0.01^{a}
	Tar-SHaF$_1$	3.78 ± 0.01^{bc}	nd	3.78 ± 0.01^{bc}
	Tar-SHaF$_2$	3.76 ± 0.01^{bc}	nd	3.76 ± 0.01^{bc}
kaempferol	Tar-CF	1.45 ± 0.01^{bc}	1.44 ± 0.01^{b}	2.89 ± 0.02^{bc}
	Tar-KF	1.45 ± 0.02^{bc}	1.34 ± 0.01^{c}	2.79 ± 0.01^{cd}
	Tar-SF	1.44 ± 0.01^{bc}	1.41 ± 0.01b^{c}	2.85 ± 0.01^{bc}
	Tar-SHaF	1.42 ± 0.02^{c}	1.69 ± 0.06^{a}	3.10 ± 0.08^{a}
	Tar-SHaF$_1$	1.48 ± 0.01^{ab}	1.46 ± 0.02^{b}	2.95 ± 0.02^{b}
	Tar-SHaF$_2$	1.51 ± 0.01^{a}	1.24 ± 0.01^{d}	2.75 ± 0.01^{d}
caffeic acid	Tar-CF	0.73 ± 0.09^{b}	nd	0.73 ± 0.09^{d}
	Tar-KF	0.19 ± 0.01^{c}	0.91 ± 0.02^{a}	1.11 ± 0.01^{c}
	Tar-SF	0.92 ± 0.02^{b}	0.82 ± 0.03^{b}	1.73 ± 0.03^{b}
	Tar-SHaF	1.63 ± 0.04^{a}	0.98 ± 0.03^{a}	2.62 ± 0.07^{a}
	Tar-SHaF$_1$	1.38 ± 0.01^{a}	0.94 ± 0.01^{a}	2.33 ± 0.01^{a}
	Tar-SHaF$_2$	1.60 ± 0.15^{a}	0.78 ± 0.03^{b}	2.38 ± 0.19^{a}
protocatechuic acid	Tar-CF	0.46 ± 0.02^{c}	nd	0.46 ± 0.02^{c}
	Tar-KF	0.61 ± 0.01^{c}	nd	0.61 ± 0.01^{c}
	Tar-SF	1.07 ± 0.03^{b}	nd	1.07 ± 0.03^{b}
	Tar-SHaF	1.40 ± 0.06^{a}	nd	1.40 ± 0.06^{a}
	Tar-SHaF$_1$	0.93 ± 0.06^{b}	nd	0.93 ± 0.06^{b}
	Tar-SHaF$_2$	1.00 ± 0.01^{b}	nd	1.00 ± 0.01^{b}
catechin	Tar-CF	0.36 ± 0.01^{c}	nd	0.36 ± 0.01^{c}
	Tar-KF	0.35 ± 0.01^{c}	nd	0.35 ± 0.01^{c}
	Tar-SF	0.58 ± 0.01^{b}	nd	0.58 ± 0.01^{b}
	Tar-SHaF	0.87 ± 0.02^{a}	nd	0.87 ± 0.02^{a}
	Tar-SHaF$_1$	0.86 ± 0.04^{a}	nd	0.86 ± 0.04^{a}
	Tar-SHaF$_2$	0.83 ± 0.01^{a}	nd	0.83 ± 0.01^{a}
***p*-hydroxybenzoic acid**	Tar-CF	0.42 ± 0.02^{b}	nd	0.42 ± 0.02^{b}
	Tar-KF	0.98 ± 0.01^{a}	nd	0.98 ± 0.01^{a}
	Tar-SF	0.96 ± 0.02^{a}	nd	0.96 ± 0.02^{a}
	Tar-SHaF	0.41 ± 0.03b	nd	0.41 ± 0.03^{b}
	Tar-SHaF$_1$	nd	nd	nd
	Tar-SHaF$_2$	nd	nd	nd
chrysin	Tar-CF	0.12 ± 0.01^{a}	nd	0.12 ± 0.01^{b}
	Tar-KF	0.10 ± 0.01^{a}	0.10 ± 0.01^{b}	0.20 ± 0.01^{a}
	Tar-SF	nd	nd	nd
	Tar-SHaF	nd	0.19 ± 0.01^{a}	0.19 ± 0.01^{a}
	Tar-SHaF$_1$	nd	nd	nd
	Tar-SHaF$_2$	nd	nd	nd
***p*-coumaric acid**	Tar-CF	nd	0.12 ± 0.01^{d}	0.12 ± 0.01^{d}
	Tar-KF	0.14 ± 0.01^{ab}	0.21 ± 0.01^{a}	0.35 ± 0.01^{a}
	Tar-SF	nd	0.14 ± 0.02^{c}	0.14 ± 0.02^{d}
	Tar-SHaF	0.10 ± 0.01^{c}	0.16 ± 0.01^{b}	0.26 ± 0.01^{b}
	Tar-SHaF$_1$	0.13 ± 0.01^{b}	0.14 ± 0.04^{c}	0.27 ± 0.04^{b}
	Tar-SHaF$_2$	0.19 ± 0.03^{a}	nd	0.19 ± 0.03^{cd}
chlorogenic acid	Tar-CF	0.33 ± 0.01^{c}	nd	0.33 ± 0.01^{c}
	Tar-KF	0.23 ± 0.01^{d}	nd	0.23 ± 0.01^{d}
	Tar-SF	0.35 ± 0.03^{c}	nd	0.35 ± 0.03^{c}

(*continued on next page*)

Table 4 (*continued*)

		Free	Bound	Total
	Tar-SHaF	0.81 ± 0.01[a]	nd	0.81 ± 0.01[a]
	Tar-SHaF$_1$	0.62 ± 0.01[b]	nd	0.62 ± 0.01[b]
	Tar-SHaF$_2$	0.34 ± 0.02[c]	nd	0.34 ± 0.02[c]
rutin	Tar-CF	nd	0.30 ± 0.01[d]	0.30 ± 0.01[d]
	Tar-KF	0.38 ± 0.03[b]	0.84 ± 0.02[a]	1.22 ± 0.02[a]
	Tar-SF	nd	0.29 ± 0.02[d]	0.29 ± 0.02[d]
	Tar-SHaF	0.34 ± 0.01[b]	0.35 ± 0.02[c]	0.69 ± 0.01[b]
	Tar-SHaF$_1$	0.19 ± 0.03[c]	0.43 ± 0.01[b]	0.61 ± 0.03[c]
	Tar-SHaF$_2$	0.50 ± 0.06[a]	0.10 ± 0.01[e]	0.60 ± 0.06[c]
sinapic acid	Tar-CF	nd	nd	nd
	Tar-KF	0.37 ± 0.02[a]	nd	0.37 ± 0.02[a]
	Tar-SF	nd	nd	nd
	Tar-SHaF	nd	nd	nd
	Tar-SHaF$_1$	nd	nd	nd
	Tar-SHaF$_2$	nd	nd	nd

Data are expressed as mean ± S.D. of triplicate measurements. Different letters (a-f) on the same column for each phenolic fraction are significantly different ($P < 0.05$) among tarhana powders. nd: not determined.
Tar-CF: tarhana produced using commercial flour, Tar-KF: tarhana produced using Kiziltan flour, Tar-SF: tarhana produced using Svevo flour, Tar-SHaF: tarhana produced using Svevo-HA flour, Tar-SHaF$_1$: tarhana produced with Svevo-HA flour heat treated without debranching and autoclaving-cooling cycles, Tar-SHaF$_2$: tarhana produced with Svevo-HA flour heat treated with debranching and autoclaving-cooling cycles.

commercial tarhana is usually produced using general purpose flour which is expected to have relatively low RS and dietary fiber levels. The variations in GI might arise from the differences in tarhana recipes.

Viscosity is a crucial parameter for the soup quality. The RVA pasting viscosities of the tarhana samples are given in Table 5. Peak viscosity values of the tarhana samples; Tar-CF, Tar-KF, Tar-SF, and Tar-SHaF were 150, 113, 89, and 66 cP, respectively. Trough viscosity values of these samples were 109, 87, 78, and 58 cP, while their final viscosity values were 221, 198, 150, and 113 cP, respectively. Tarhana sample produced using commercial white flour (Tar-CF) had significantly ($p < 0.05$) higher peak (150 cP), trough (109 P), and final (221 cP) viscosity values among the samples. Viscosity values of Tar-SF and Tar-SHaF samples were significantly ($p < 0.05$) lower than those of Tar-CF and Tar-KF samples. The tarhana samples prepared with the heat-treated flours (Tar-SHaF$_1$ and Tar-SHaF$_2$) had the lowest viscosity values among the samples and their viscosity values were in the range of 13–17 cP during the whole heating-cooling profile of the RVA analysis. This is in line with Ozturk et al. (2009) who indicated that debranching and autoclaving-storing cycles decreased the RVA viscosity values. The RVA soup index values were 148, 123, 102, 76, 13, and 13 cP for the tarhana samples: Tar-CF, Tar-KF, Tar-SF, Tar-SHaF, Tar-SHaF$_1$ and Tar-SHaF$_2$, respectively. Similar to the RVA viscosity values, the low soup index values of RS-enriched tarhana samples might be due to the effect of the debranching and autoclaving-storing cycles. Lower soup index values might seem to be a disadvantage at first sight. Increasing the level of regular starch/flour content in tarhana formulation would result in a very thick paste which could be difficult to consume as a soup. On the other hand, increasing the RS-enriched flour in the tarhana formulation would not cause major increases in viscosity. Hence, the RS content of tarhana soups could be significantly increased without major increases in soup viscosity by adding more RS-enriched flour in formulation resulting in a healthier soup. This will probably result in a soup with similar viscosity compared to the one produced using regular white flour but having much higher RS content.

4. Conclusion

Tarhana is a popular food in the Middle East, Asia, and Europe which is rich in B group vitamins, protein, calcium, copper, potassium, magnesium, zinc, and iron minerals. It is usually produced with white wheat flour, and increasing its dietary fiber and resistant starch content will be beneficial for people following the Mediterranean Diet. This study focuses on the utilization of high-amylose wheat flour (Svevo-HA) in tarhana formulation to increase its RS content while decreasing the GI value. Svevo-HA was subjected to debranching and autoclaving-storing cycles to allow starch retrogradation, which increases the RS content of the flour samples. The tarhana soups supplemented with heat-treated flours had significantly high RS content and low *in vitro* GI value. The present study demonstrated that supplementing tarhana with high-amylose flour having a high RS content resulted in tarhana samples with enhanced nutritional properties, which might be considered as a new functional food.

CRediT authorship contribution statement

Hamit Koksel: Validation, Project administration, Methodology, Investigation, Conceptualization. **Zeynep Hazal Tekin-Cakmak:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Kubra Ozkan:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Zeynep Pekacar:** Writing – original draft, Data curation. **Sena Oruc:** Writing – review & editing, Data curation. **Kevser Kahraman:** Writing – review & editing. **Cagla Ozer:** Writing – review & editing. **Osman Sagdic:** Writing – review & editing. **Francesco Sestili:** Writing – review & editing, Project administration, Funding acquisition.

Table 5
Resistant starch content, *in vitro* GI, and RVA viscosity values of tarhana soup samples.

Sample	RS (%)	HI	GI	Peak Viscosity (cP)	Trough (cP)	Final Viscosity (cP)	Soup index (cP)
Tar-CF	0.48 ± 0.01[d]	73.65 ± 1.83[a]	80.14 ± 1.01[a]	150 ± 2.83[a]	109 ± 2.83[a]	221 ± 1.41[a]	148 ± 2.83[a]
Tar-KF	0.36 ± 0.01[d]	67.47 ± 1.15[b]	76.75 ± 0.63[b]	113 ± 0.01[b]	87 ± 1.41[b]	198 ± 1.41[b]	123 ± 0.71[b]
Tar-SF	0.39 ± 0.01[d]	65.61 ± 0.32[b]	75.73 ± 0.17[b]	89 ± 2.83[c]	78 ± 0.71[c]	150 ± 2.83[c]	102 ± 2.83[c]
Tar-SHaF	0.84 ± 0.05[c]	56.88 ± 0.39[c]	70.94 ± 0.22[c]	66 ± 2.83[d]	58 ± 2.83[d]	113 ± 2.83[d]	76 ± 2.12[d]
Tar-SHaF$_1$	3.29 ± 0.06[b]	33.54 ± 0.10[d]	58.12 ± 0.06[d]	14 ± 0.71[e]	13 ± 0.01[e]	17 ± 0.01[e]	13 ± 0.01[e]
Tar-SHaF$_2$	5.73 ± 0.09[a]	27.59 ± 0.30[e]	54.86 ± 0.17[e]	17 ± 0.71[e]	13 ± 0.01[e]	16 ± 0.71[e]	13 ± 0.71[e]

RS; Resistant starch, HI; hydrolysis index, GI; glycemic index, [a-e] Means with different letters within each column are significantly different ($p < 0.05$).
Tar-CF: tarhana produced using commercial flour, Tar-KF: tarhana produced using Kiziltan flour, Tar-SF: tarhana produced using Svevo flour, Tar-SHaF: tarhana produced using Svevo-HA flour, Tar-SHaF$_1$: tarhana produced with Svevo-HA flour heat treated without debranching and autoclaving-cooling cycles, Tar-SHaF$_2$: tarhana produced with Svevo-HA flour heat treated with debranching and autoclaving-cooling cycles.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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