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The effect of fermentation and drying on the water-soluble vitamin content of tarhana, a traditional Turkish cereal food

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Abstract

Tarhana is a popular and widely consumed traditional Turkish fermented wheat-flour-yoghurt mixture. The effects of fermentation (30 °C for 4 days) and drying (50, 60 and 70 °C) on the contents of several water-soluble vitamins (ascorbic acid, niacin, pantothenic acid (vitamin B_5), pyridoxine (vitamin B_6), thiamine (vitamin B_1), folic acid and riboflavin (vitamin B_2)) in tarhana, a traditional Turkish cereal food, have been studied. The contents of water-soluble vitamins was analyzed by HPLC. Statistical analysis of the data showed that a 4-day fermentation and drying had a significant effect ($p < 0.05$) on the contents of water-soluble vitamins of tarhana. The fermentation resulted in significant increases of riboflavin, niacin, pantothenic acid, ascorbic acid and folic acid contents of the samples, but no significant differences, with thiamine and pyridoxine. Highest losses of the water-soluble vitamins were at 70 °C for the 35 h drying period.

Keywords: Tarhana; Fermentation; Drying; Vitamin; HPLC

1. Introduction

Tarhana is dried soup prepared through lactic acid fermentation, initiated by the presence of yoghurt or sour milk. Fermentation is usually carried out by yoghurt bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and yeast (Özbilgin 1983). After fermentation, the mixture is sun-dried and ground to a particle size of <1 mm (Ibanoğlu, Ainsworth, Wilson, & Hayes, 1995; Ibanoğlu & Maskan, 2002). Most of the tarhana consumed in Turkey is homemade and therefore sun-dried. However, there is a great commercial potential for the production of tarhana on an industrial scale using modern drying techniques (Ibanoğlu & Maskan, 2002).

Tarhana is prepared by mixing wheat flour, yoghurt, yeast and a variety of cooked vegetables (tomatoes, onions, green pepper), salt and spices (mint, paprika), followed by fermentation for one to seven days (Dağlıoğlu, 2000). In the central and eastern part of Turkey, one or more of certain ingredients, such as milk, soybean, lentil, chickpea, corn flour and egg, are also added (Dağlıoğlu, 2000; Türker & Elgün, 1995). Tarhana has an acidic and sour taste with a strong yeast flavour and is also a good source of proteins, vitamins and minerals and is therefore used largely for feeding children and elderly people (Dağlıoğlu, 2000; Hamad & Fields, 1979). Vitamins can be classified into two main groups: water-soluble and fat-soluble. Among the B group of water-soluble vitamins, both thiamine (B_1) and pyridoxine (B_6) are important vitamins. They play different specific and vital functions in metabolism, and their lack (or excess) produces specific diseases (Moreno & Salvado, 2000). However, vitamins are relatively unstable, affected by factors such as heat, light, air, other food components and food processing conditions (Machlin, 1991; Ottaway, 1993). Because of the critical role of vitamins in nutrition and their relative instability, qualitative and quantitative analyses are important issues as well as a challenging task for food manufacturers. HPLC is the preferred technique for vitamin separation because of its high selectivity (Leenheer et al., 1985).

The effects of fermentation on the thiamine, riboflavin and vitamin B_{12} content of tarhana has been studied

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using microbiological assays (Ibanoğlu, Ibanoğlu, & Ainsworth, 1999). However, there is no published research study on the HPLC determination of all the water-soluble vitamins of tarhana during fermentation. Also, to the best of our knowledge, no information is published about the effect of drying on the water-soluble vitamins of tarhana. The objectives of this study were: (a) to determine the water-soluble vitamins changes during a typical 4-day tarhana fermentation, (b) to determine the effects of different drying temperatures (50, 60 and 70 °C) on the water-soluble vitamin contents of tarhana and (c) to help the tarhana manufacturing industry in determining the optimum drying temperatures for the least loss of water-soluble vitamins of tarhana.

2. Materials and methods

2.1. Materials

The ingredients (wheat flour, yoghurt, yeast, onions, pure tomato, salt, paprika, mint) used in tarhana preparation were purchased from local markets in Ankara, Turkey. The crude protein content of white wheat flour, based on total weight, was 12.2%. The yoghurt used was full fat set yoghurt (pH 3.7) made from cow's milk and had a fat content of 3.0% (wet basis). Tomato puree was double concentrated (30% dry solids). Yeast was baker's yeast in active dry form. The spices used were in powder form.

2.2. Preparation of tarhana dough for fermentation

The composition of tarhana dough, based on total weight (wet basis), was as follows: wheat flour 1000 $g\,kg^{-1}$; yoghurt, 500 $g\,kg^{-1}$; onions, 120 $g\,kg^{-1}$; tomato puree, 120 $g\,kg^{-1}$; salt, 40 $g\,kg^{-1}$; yeast, 10 $g\,kg^{-1}$; paprika, 20 $g\,kg^{-1}$; mint, 20 $g\,kg^{-1}$. To prepare tarhana dough, onions were chopped and blended for 10 s with 100 ml of tap water by means of a waring blender at the highest speed. The tomato puree, salt, paprika and mint were added, blended for 30 s, brought to boil and simmered for 10 min. The mixture was left to cool to room temperature and then yoghurt, wheat flour and yeast were added and the mixture was kneaded (Arçelik Food Processor, İstanbul, Turkey) for 5 min at low speed after the addition of a further 200 ml of tap water. The resulting dough was spread over a stainless steel tray to a depth of 10–12 mm and incubated at 30 °C for 4 days to ferment the tarhana dough (Ibanoğlu et al., 1999). During the 4-day fermentation, triplicate samples were taken every day for the pH, acidity, moisture and water-soluble vitamin analyses. The mixture was manually blended, prior to sampling, to ensure homogeneity of the samples taken. All the results are means of three determinations with two replicates.

2.3. Drying

After a 4-day fermentation, the mixture was dried in a hot air oven (Ehret, KMB 6/Görschr, Emmendingen, Germany) at 50 °C for 48 h, 60 °C for 40 h and 70 °C for 35 h and finely ground (Lionhill Mill 14920, Copenhagen, Denmark) to a particle size of <400 μm for the analyses of pH, acidity, moisture and water-soluble vitamins. Drying time of the samples was determined by the moisture analysis until a 10% moisture content was observed

2.4. Standards

Methanol (HPLC grade) and K_2HPO_4 (extra pure) were obtained from Merck (Darmstadt, Germany). Water used in all the experiments was doubly-distilled and deionised. The vitamin standards (ascorbic acid, niacin, pantothenic acid, pyridoxine, thiamine, folic acid and riboflavin) were of analytical-reagent grade and obtained from Sigma (Sigma–Aldrich Chemie GmbH, Deisenhofen–Germany). Stock and standard solutions of water-soluble vitamins were prepared in water. For preparing calibration curves, five different concentrations of each standard were used. Thus, a calibration curve was prepared for each vitamin. Correlation coefficients of ascorbic acid, niacin, pantothenic acid, pyridoxine, thiamine, folic acid and riboflavin, based on the concentration (μg/ml) versus peak area (mAU), were found to be >0.999.

2.5. Sample preparation (solid phase extraction = SPE)

Tarhana consists of many components that cause chromatographic interferences with vitamins. For that reason, the Sep-Pak C_{18} (500 mg) catridges were used to remove most of the interfering components. Four parts of deionized water were added to one part of tarhana (dilution factor, $F = 5$). The mixture was homogenized using a homogenizer at medium speed for 1 min. The homogenized samples were centrifuged for 10 min at 14×10^3 g (Sigma, Bioblock Scientific 2–16). The solid phase extraction method of Cho, Ko, and Cheong (2000) was used for the extraction of water-soluble vitamins. The stationary phase was flushed with 10 ml methanol and 10 ml water adjusted at pH 4.2 to activate the stationary phase. Then, 10 ml of homogenized and centrifuged tarhana were loaded. The acidified water was prepared by adding a 0.005 M HCl solution drop by drop with stirring until pH reached the predetermined value. The sample was eluted with 5 ml water (pH 4.2), followed by 10 ml methanol, at a flow rate of 1 ml/min. The eluents were collected in a bottle and evaporated to dryness. The residue was dissolved in mobile phase. Prior to HPLC analysis, all

samples were filtered using FP 30/45 CA-S filters (Schleicher and Schuell, Darmstadt–Germany) with 0.45 μm (7 bar max) pore size.

2.6. Methods

Detection was performed at 220 for all the water-soluble vitamins. The elution solvents used were A (KH_2PO_4, pH 7) and B (100% methanol). The samples were eluted according to the following gradient: 1% B for 5 min, 1–30% B (linear gradient) over 15 min, then 30%B for 5 min (SUPELCO, 2000). The chromatographic data on the peaks were integrated up to 25 min. The flow-rate was 1 ml /min. The column was operated at room temperature (25 °C). The sample injection volume was 20 ml. Identification of compounds was achieved by comparing their retention time values and UV spectra with those of standards stored in a data bank. Concentrations of the water-soluble vitamins were calculated from integrated areas of the sample and the corresponding standards.

2.7. Apparatus for HPLC

For the analysis, a Discovery C-18 150×4.6 mm column (Cat. No: 504955), a Shimadzu model HPLC (Shimadzu corporation, Kyoto, Japan) system consisting of a column oven (Shimadzu, Model CTO-10ASVP), a UV–Vis diode array detector (Shimadzu, model SPD-M10 Avp) set at 220 nm, a degasser (Shimadzu, Model DGU 14A), and a liquid chromatography pump (Shimadzu, Model LC-10AT-VP), and a software programme (Shimadzu) were used. The sample (20 μl) was injected with a syringe (Hamilton Co., Reno, NV, USA) into the HPLC. A typical chromatogram of the tarhana on a discovery C-18 (150×4.6 mm I.D.) column using KH_2PO_4–methanol as the mobile phase with a flow rate of 1.0 ml/min is illustrated in Fig. 1.

2.8. Further determinations

Acid concentration of the samples were determined by a titrimetric method using 0.1 M NaOH and expressed as percent lactic acid (AOAC, 1980). The pH (potentiometric) was measured with a pH meter (WTW GmbH and Co., Model 537, Weilheim-Germany) and the moisture content was measured by drying the samples at 130 ± 3 °C for 1 h in an air oven (AOAC, 1980)

2.9. Recovery of water-soluble vitamins

Tarhana samples, containing known amounts of ascorbic acid, niacin, pantothenic acid pyridoxine, thiamine, folic acid and riboflavin, were spiked with the different levels (25, 50, 75, 100 and 200 μg l^{-1}) of ascorbic acid, niacin, pantothenic acid, pyridoxine, thiamine, folic acid and riboflavin to determine the srecovery of the extraction procedure. The average percentage recoveries of ascorbic acid, niacin, pantothenic acid, pyridoxine, thiamine, folic acid and riboflavin in tarhana were 93.8%, 95.5%, 102%, 97.6%, 94.7%, 93.4% and 92.9%, respectively, for five different concentrations. The levels of the same vitamins in tarhana samples were corrected for the average percent recoveries.

2.10. Statistical analysis

Statistical analysis of the data was performed using the system developed by SAS Institute, Inc. (SAS, 1985). When analysis of variance (ANOVA) revealed a significant effect ($p < 0.05$, $p < 0.01$), data means were

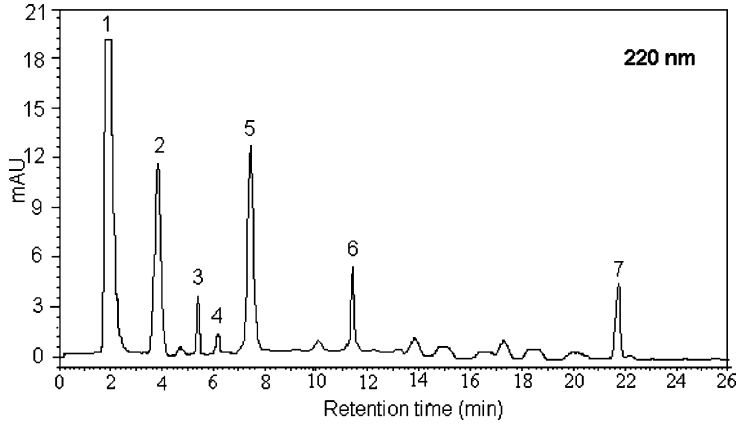

Fig. 1. Separation of water-soluble vitamins of tarhana by HPLC at 220 nm. Peaks: 1, ascorbic acid; 2, niacin; 3, pantothenic acid; 4, pyridoxine; 5, thiamine; 6, folic acid; 7, riboflavin.

compared with the least significant difference (LSD) test.

3. Results and discussion

To the best of our knowledge, there are no published research studies about the HPLC determination of the effect of fermentation and drying on the water-soluble vitamin contents of tarhana.

Fig. 1 illustrates the separation of six water-soluble vitamins of tarhana by HPLC. As shown in Fig. 1, a good separation can be achieved in 22 min. Ascorbic acid, niacin, pantothenic acid, pyridoxine, thiamine, folic acid and riboflavin are separated well. Identification of compounds was achieved by comparing their retention time values and UV spectra with those of standards stored in a data bank. The average percentage recoveries of ascorbic acid, niacin, pantothenic acid, pyridoxine, thiamine, folic acid and riboflavin in tarhana were found to be 93.4%, 96.3%, 93.5%, 98.6%, 95.7%, 93.1% and 96.2%, respectively, for five different concentrations. The contents of vitamins in tarhana samples were corrected for the average percent recoveries.

The effects of a 4-day fermentation and drying (50, 60 and 70 °C) on pH, acidity and moisture content of tarhana are shown in Table 1. While the pH content of the tarhana samples decreased significantly ($p < 0.05$) during the fermentation, acidity of the samples increased significantly ($p < 0.05$). The moisture values of the tarhana samples were not affected by the fermentation and were similar. Only very slight decreases in moisture values ($p > 0.05$) were observed compared to the initial moisture values in the tarhana samples. Statistical analysis of the data showed no significant differences ($p > 0.05$) in the pH and acidity contents of the tarhana samples between the tested treatments (drying at 50, 60 and 70 °C). Drying treatments can slightly effect increments in the pH and acidity contents of the tarhana samples. The 50, 60 and 70 °C drying treatments resulted in a slight increase in the pH and acidity contents of the tarhana samples. The 50, 60 and 70 °C drying treatments resulted in 0.87 %, 1.30% and 1.73% increases in acidity contents of tarhana samples, respectively. Drying times of the tarhana samples for 10% moisture content at 50, 60 and 70 °C were found to be 48, 40 and 35 h, respectively.

The effects of a 4-day fermentation on the ascorbic acid, niacin, pantothenic acid, pyridoxine, thiamine, folic acid and riboflavin contents of tarhana were investigated and the results of vitamin analyses are shown in Table 2. Statistical analysis of the data showed that there were significant differences ($p < 0.05$) in the water-soluble vitamins of the tarhana samples between the fermentation and drying. Ascorbic acid, pantothenic acid, folic acid, thiamine and riboflavin concentrations of tarhana samples did not change during the first day of fermentation. Ascorbic acid, pantothenic acid, folic acid, thiamine and riboflavin concentrations of tarhana samples increased significantly ($p < 0.05$) after the first day of fermentation. However, niacin and pyridoxine concentrations of tarhana samples increased significantly ($p < 0.05$) during the four days of fermentation. Ascorbic acid, niacin, pantothenic acid, pyridoxine, thiamine, folic acid and riboflavin contents of the tarhana samples during fermentation were measured. As seen from Table 2, as the fermentation period increased ascorbic acid, niacin, pantothenic acid, folic acid and riboflavin contents of the samples increased ($p < 0.05$).

Table 1
The effects of a 4-day fermentation and drying (50, 60 and 70 °C) on pH, acidity and moisture contents of tarhana

	Fermentation period (day)		Drying			
	0 (Control)	4	Control	50 °C	60 °C	70 °C
pH[A]	4.6 ± 0.01a	4.0 ± 0.01b	4.0 ± 0.01	4.30 ± 0.01	4.38 ± 0.01	4.45 ± 0.01
Acidity[A]	7.8 ± 0.2a	22.7 ± 0.2b	22.7 ± 0.1	22.9 ± 0.1	23.0 ± 0.2	23.1 ± 0.1
Moisture (%)[A]	70.0 ± 0.3	69.3 ± 0.3	69.1 ± 0.2	10.0 ± 0.3	10 ± 0.2	10.0 ± 0.3

Different letters in the same line are significantly different at $p < 0.05$.
[A] Values are the means of three determinations with two replicates.

Table 2
The effects of a 4-day fermentation period on the water-soluble vitamin content (mg kg^{-1}) of tarhana samples

Fermentation period (day)	Ascorbic acid[A]	Niacin[A]	Pantothenic acid[A]	Pyridoxine	Thiamine[A]	Folic acid[A]	Riboflavin[A]
(0) Control	15.5 ± 0.3a	10.2 ± 0.3a	4.2 ± 0.2a	0.32 ± 0.1	4.3 ± 0.2	0.38 ± 0.1a	1.7 ± 0.2a
1	15.9 ± 0.3b	12.8 ± 0.3b	4.5 ± 0.2a	0.29 ± 0.1	4.3 ± 0.2	0.44 ± 0.1b	2.0 ± 0.2a
2	16.1 ± 0.2c	14.3 ± 0.2c	4.7 ± 0.1a	0.30 ± 0.1	4.3 ± 0.1	0.46 ± 0.1c	2.2 ± 0.1b
3	16.3 ± 0.3d	14.5 ± 0.3d	4.8 ± 0.2b	0.32 ± 0.1	4.4 ± 0.1	0.48 ± 0.2 d	2.3 ± 0.2c
4	16.3 ± 0.3d	14.60 ± 0.4d	4.8 ± 0.1b	0.30 ± 0.1	4.4 ±	0.47 ± 0.1d	2.3 ± 0.2c

Different letters in the same column are significantly different at $p < 0.05$.
[A] Values are the means of three determinations with two replicates.

Table 3
The effect of drying temperature (°C) on the water-soluble vitamin content (mg kg^{-1}) of tarhana

Temperature (°C)	Time (h)	Ascorbic acid[A]	Niacin[A]	Pantothenic acid[A]	Pridoxine[A]	Thiamine[A]	Folic acid[A]	Riboflavin[A]
(Control)	0	16.3 ± 0.3a	18.4 ± 0.4a	4.8 ± 0.2a	0.30 ± 0.1a	4.4 ± 0.1a	0.48 ± 0.2a	2.9 ± 0.1a
50	45	16.1 ± 0.3a	17.9 ± 0.4b	4.7 ± 0.1a	0.29 ± 0.1a	3.6 ± 0.1b	0.45 ± 0.1b	2.7 ± 0.1a
60	40	15.9 ± 0.3b	17.2 ± 0.3c	4.4 ± 0.1ab	0.25 ± 0.1b	3.1 ± 0.2c	0.43 ± 0.3c	2.4 ± 0.1b
70	35	14.0 ± 0.3c	16.1 ± 0.3d	4.0 ± 0.2b	0.23 ± 0.1b	2.6 ± 0.1d	0.40 ± 0.2c	2.1 ± 0.1c

Different letters in the same line are significantly different at $p < 0.05$.
[A] Values are the means of three determinations with two replicates.

In contrast, no significant differences were found in thiamine and pyridoxine contents with the increase of fermentation period. Also, no significant differences were determined in thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, ascorbic acid and folic acid contents of the third and fourth day fermentation tarhana samples. But, thiamine, riboflavin, pantothenic acid, ascorbic acid and folic acid contents of third and fourth day fermentation tarhana samples were higher than those of control, first and second day fermentation tarhana samples. However, the niacin and pyridoxine contents of the samples were not significantly affected by the fermentation period. Ibanoğlu et al. (1999) reported that fermentation activity of tarhana was high during the first day of fermentation. The highest increments in ascorbic acid, pantothenic acid, thiamine, folic acid and riboflavin contents of tarhana samples were determined at the end of the first day fermentation of tarhana. Research is now focussed on the relationship between vitamins and lactic acid bacteria during the fermentation of tarhana.

The effects of drying on the water-soluble vitamin contents of tarhana are shown in Table 3. The water-soluble vitamin content of control sample was decreased by the drying treatment at 50, 60 and 70 °C. Increasing the drying temperature decreases the water-soluble vitamin level in tarhana. As the drying period increased, water-soluble vitamin content of tarhana samples also decreased. The highest losses of ascorbic acid, niacin, pantothenic acid, pyridoxine, thiamine, folic acid and riboflavin in tarhana were found to be 13.5%, 12.6%, 18.0%, 23.3%, 39.8%, 16.3% and 26.7%, respectively, with the 70 °C drying temperature, when compared with the control sample. On the other hand, the lowest losses of ascorbic acid, niacin, pantothenic acid, pyridoxine, thiamine, folic acid and thiamine in tarhana were 1.35%, 2.93%, 3.69%, 3.33%, 16.3%, 5.86% and 5.90%, respectively, with the 50 °C drying temperature, when compared with the control sample.

4. Conclusion

The results of this study showed that both fermentation and drying temperature had significant effects on the water-soluble vitamin concentrations of tarhana. It can be concluded that fermentation had an increasing effect on the water-soluble vitamin contents of tarhana while drying had a decreasing effect. Oven-drying of the samples at 70 °C, after fermentation, caused important losses in water-soluble vitamins of the samples when compared with the 50 and 60 °C oven-drying treatments.

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