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In vitro protein digestibility and content of thiamin and riboflavin in extruded tarhana, a traditional Turkish cereal food

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Tarhana, a traditional Turkish yogurt-wheat flour mixture, was extruded using a twin-screw extruder. The effects of extrusion variables and post-extrusion process on the *in vitro* digestibility (PD) of the protein and contents of thiamin and riboflavin in the extruded samples were measured and the results were compared with those of an unextruded control sample. The changes in barrel temperature (60–120°C), feed rate (10–20 kg/h, wb) and screw speed (10–300 rpm) did not have any significant ($p > 0.05$) detrimental effect on the PD and thiamin and riboflavin contents of tarhana at constant moisture content (43%, wb). However, oven-drying at 55°C for 48 h after extrusion caused approximately 30% thiamin losses in the samples while the *in vitro* protein digestibility and riboflavin content of the samples did not change significantly ($p > 0.05$). Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Fermented milk–cereal foods form an important part of the diets of many people in the Middle East. One such product, tarhana, is widely consumed in Turkey and has an important role in the diets of infants and young children, as well as the elderly (Siyamoglu, 1961). Traditionally, tarhana is prepared by mixing yogurt, wheat flour, yeast and a variety of cooked vegetables and spices followed by fermentation for one to seven days (Campbell-Platt, 1987). The resulting material is then air-dried and used in soup making, giving a product with high nutritional contents of protein and vitamins. The low pH (3.8–4.2) and low moisture content (6–9%) make the tarhana a poor medium for pathogens and spoilage organisms; tarhana is not hygroscopic and it can be stored for 2 to 3 years without any sign of mould grow (Wang & Hesseltine, 1981).

The traditional batch method of tarhana manufacture is not automated with manufacturing capacities being low and highly labour intensive. Extrusion cooking is a process that is being used in the food industry for precooking and forming, mainly cereal-based products such as breakfast cereals, bread products and soup bases (Cheftel, 1986). A model system has been described for the production of instant tarhana soup powder by extrusion cooking (Ibanoglu *et al.*, 1996), which would allow tarhana to be produced at a lower cost due to more efficient use of energy and a greater process control with greater production capacities (Harper, 1979). Extrusion cooking may improve the digestibility of proteins by inactivation of proteolytic inhibitors and opening the protein structure through denaturation in some foods (Dahlin & Lorenz, 1993). However, it is known that heat processing can also cause a decrease in the digestibility of proteins and availability of essential amino acids through non-enzymic browning reactions and thermal cross-linking (Tannenbaum *et al.*, 1985). Also, as far as vitamins are concerned, depending on extrusion conditions, mainly destructive effects are reported (Killeit, 1994). The vitamin concentrations in some extruded products such as snacks and candies are not important since they generally contribute little to overall nutrient intakes (Camire *et al.*, 1990; Cheftel, 1986). However, vitamin levels are important in such foods as infant foods and similar products, which may form a major part of an individual's daily diet (Bjorck & Asp, 1983; Camire *et al.*, 1990). Therefore, this investigation was undertaken to study the effect of extrusion process variables (barrel temperature, feed rate, and screw speed) on protein *in vitro* digestibility, thiamin and riboflavin contents of tarhana. The results were compared with those observed for an unextruded control sample.

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MATERIALS AND METHODS

Materials

The ingredients used in tarhana preparation were purchased from local markets in Manchester, UK. The composition of extruded tarhana, based on total weight (wet basis), was as follows: wheat flour 50%, yogurt 25%, onions 12%, tomato puree 6%, salt 4%, yeast 1%, paprika 1%, lactic acid solution 0.6%, dill 0.2%, and mint 0.2%. The crude protein content of the wheat flour used was 12.4%. Yogurt was made from cow's milk with a fat content of 3.6%. Yeast was baker's yeast in active dry form. Tomato puree was double concentrated. The spices used were in powder form. Food grade lactic acid solution was used in the experiments (86%, w/w).

Experimental design

A full factorial three-variable, three-level experimental design with 6 replications at centre point (Gacula & Singh, 1984) was used with barrel temperature (60, 90, and 120°C), screw speed (100, 200, and 300 rpm) and feed rate (10, 15, and 20 kg/h) being the process variables. This range of operating variables was found to be feasible for tarhana extrusion. The lower, upper, and centre point of the design were coded as −1, +1, and 0, respectively (Table 1).

Extrusion

Extrusion of tarhana was performed in a co-rotating twin-screw extruder (Continua 37, Werner and Pfleiderer, Stuttgart, Germany) with a screw diameter of 37 mm and L/D = 27. A screw profile which is a standard design for processing cereals and flour-based products was used throughout this study, which was made up of self-wiping elements except for a section consisting of short reverse and forwarding elements (Ibanoglu *et al.*, 1996). The barrel was heated using circulating oil from an independent heating unit with the feed zone being cooled by tap water. Two circular dies each of 4 mm diameter 19 mm length were used.

To accomplish tarhana extrusion, the ingredients other than the wheat flour were processed using a bowl chopper (Kilia Type 201, Kiel, Germany) to obtain a slurry which could be fed to the extruder using a peristaltic pump (Watson Marlow, Falmouth, UK). The wheat flour was fed into the extruder using a volumetric twin-screw metering feeder (Rospen, Gloucester, UK). The overall moisture content and pH of tarhana (wheat flour plus the slurry) before extrusion were 43% (wet basis) and 4.2, respectively.

When the extrusion system reached a steady state as indicated by constant percentage torque, pressure and constant material temperature, samples were collected, oven-dried (55°C, 48 h), finely ground (Retsch GmbH, Haan, Germany) to a particle size of < 250 μm, and stored in air-tight glass containers at room temperature until analysis.

Controls

In order to evaluate the effect of extrusion and post-extrusion process (i.e. oven-drying) on *in vitro* protein digestibility and thiamin and riboflavin content of the samples, freeze-dried unextruded and freeze-dried extruded samples were used as control samples.

Analytical methods

Determination of protein in vitro *digestibility (PD)*
The pepsin-pancreatin method of Saunders *et al.* (1973) was used. The sample (250 mg) was suspended in 15 ml of 0.1 N HCl containing 1.5 mg pepsin (porcine stomach mucosa, Sigma, St Louis, USA), gently shaken at 37°C for 3 h, neutralised with 0.5 N NaOH and treated with 4 mg of pancreatin (porcine pancreas, Sigma, St Louis, USA) in 7.5 ml of 0.2 M phosphate buffer at pH 8.0. The mixture was shaken in a water bath at 37°C for 24 h, solids separated by centrifuging at 1500*g* for 10 min and the solution filtered (0.45 μm, Millipore Corporation, Bedford) before analysis for nitrogen by the Kjeldahl method (AOAC, 1984) using a Buchi apparatus (Models 430 and 320, Buchi Laboratoriums-Technic AG, Flawil, Switzerland). Percent PD was calculated as

$$\text{PD}(\%) = [(\text{Nitrogen} \in \text{supernatant})/(\text{total nitrogen})] \times 100$$

Determination of vitamins
The thiamin and riboflavin contents of the samples were determined by microbiological assays (AOAC, 1990). The test organisms were *Lactobacillus fermentum* NCIMB 6991 and *Lactobacillus casei* NCIMB 6375 for thiamin and riboflavin, respectively. Culture medium, inoculum broth and maintenance agar were of Difco Laboratories, Detroit, MI, USA. Thiamin and riboflavin were extracted from the samples as follows. A 5 g sample was mixed with 50 ml of 0.1 N H_2SO_4 and autoclaved for 30 min at 121°C. After cooling, the contents were adjusted to pH 4.5 with 2 M sodium acetate. To the solution were added 1 ml of 2% α-amylase (*Aspergillus oryzae*, Sigma, St Louis, USA) and 5 ml of 10% papain (papaya latex, Sigma, St Louis, USA). The solution was incubated for 12 h at 35°C followed by filtration. The error limit in the microbiological analysis of thiamin and riboflavin was ± 10%.

RESULTS AND DISCUSSION

Protein *in vitro* digestibility

In vitro digestibility of the protein is an important aspect which defines the nutritional quality of a protein

Table 1. Experimental design used and protein *in vitro* digestibility (PD) and vitamin contents of oven-dried extruded tarhana and freeze-dried unextruded samples on dry basis (results are average of three replicates ± SD)*

Experiment no.	Extrusion process variables **			PD (%)	Thiamin (μg/g)	Riboflavin (μg/g)
	A	B	C			
1	−1	−1	−1	80.1 ± 0.5[a]	1.5 ± 0.2[a]	0.7 ± 0.2[a]
2	−1	−1	0	81.2 ± 0.6[a]	1.7 ± 0.1[a]	0.8 ± 0.1[a]
3	−1	−1	1	80.6 ± 0.8[a]	1.5 ± 0.2[a]	0.8 ± 0.2[a]
4	−1	0	−1	80.3 ± 0.7[a]	1.5 ± 0.2[a]	0.8 ± 0.1[a]
5	−1	0	0	79.7 ± 0.7[a]	1.5 ± 0.1[a]	0.8 ± 0.1[a]
6	−1	0	1	81.2 ± 0.4[a]	1.5 ± 0.2[a]	0.7 ± 0.1[a]
7	−1	1	−1	79.8 ± 0.7[a]	1.5 ± 0.1[a]	0.7 ± 0.1[a]
8	−1	1	0	80.5 ± 0.6[a]	1.5 ± 0.2[a]	0.8 ± 0.1[a]
9	−1	1	1	80.7 ± 0.9[a]	1.5 ± 0.1[a]	0.8 ± 0.1[a]
10	0	−1	−1	80.3 ± 0.7[a]	1.7 ± 0.1[a]	0.9 ± 0.1[a]
11	0	−1	0	80.8 ± 0.4[a]	1.5 ± 0.2[a]	0.9 ± 0.1[a]
12	0	−1	1	80.7 ± 0.4[a]	1.5 ± 0.1[a]	0.8 ± 0.1[a]
13	0	0	−1	80.6 ± 0.4[a]	1.6 ± 0.1[a]	0.7 ± 0.1[a]
14	0	0	0	80.0 ± 0.5[a]	1.6 ± 0.1[a]	0.8 ± 0.1[a]
15 (R)	0	0	0	81.1 ± 1.0[a]	1.6 ± 0.1[a]	0.7 ± 0.1[a]
16 (R)	0	0	0	80.4 ± 0.6[a]	1.5 ± 0.1[a]	0.7 ± 0.1[a]
17 (R)	0	0	0	80.7 ± 0.4[a]	1.5 ± 0.2[a]	0.8 ± 0.1[a]
18 (R)	0	0	0	79.5 ± 0.6[a]	1.5 ± 0.2[a]	0.8 ± 0.2[a]
19 (R)	0	0	0	80.1 ± 0.5[a]	1.6 ± 0.1[a]	0.8 ± 0.1[a]
20 (R)	0	0	0	81.2 ± 0.4[a]	1.5 ± 0.1[a]	0.7 ± 0.1[a]
21	0	0	1	81.6 ± 0.8[a]	1.6 ± 0.1[a]	0.9 ± 0.2[a]
22	0	1	−1	80.1 ± 0.5[a]	1.5 ± 0.1[a]	0.7 ± 0.2[a]
23	0	1	0	80.2 ± 0.4[a]	1.4 ± 0.2[a]	0.8 ± 0.1[a]
24	0	1	1	81.0 ± 0.9[a]	1.6 ± 0.2[a]	0.7 ± 0.1[a]
25	1	−1	−1	81.2 ± 0.3[a]	1.4 ± 0.2[a]	0.8 ± 0.1[a]
26	1	−1	0	80.9 ± 0.4[a]	1.6 ± 0.1[a]	0.8 ± 0.2[a]
27	1	−1	1	80.5 ± 0.5[a]	1.5 ± 0.1[a]	0.8 ± 0.1[a]
28	1	0	−1	80.0 ± 0.9[a]	1.5 ± 0.1[a]	0.8 ± 0.2[a]
29	1	0	0	80.1 ± 0.9[a]	1.4 ± 0.2[a]	0.8 ± 0.1[a]
30	1	0	1	79.9 ± 0.6[a]	1.6 ± 0.1[a]	0.8 ± 0.1[a]
31	1	1	−1	80.5 ± 0.4[a]	1.5 ± 0.1[a]	0.7 ± 0.2[a]
32	1	1	0	81.3 ± 0.4[a]	1.5 ± 0.2[a]	0.8 ± 0.1[a]
33	1	1	1	80.6 ± 0.4[a]	1.5 ± 0.1[a]	0.8 ± 0.1[a]
Unextruded tarhana				80.7 ± 0.5[a]	2.2 ± 0.1[b]	0.8 ± 0.1[a]

*Mean values with the same superscripts within the same columns are not significantly different at 0.05 level (TUKEY HSD multiple comparison test).
**A, barrel temperature at heating zone of the extruder (°C); B, feed rate (kg/h, on wet basis); C, screw speed (rpm). Moisture content 43% (wet basis).
(R): Replication points of the design.

along with its amino acid composition and bioavailability (Dahlin & Lorenz, 1993). An *in vitro* method, using proteolytic enzymes for protein hydrolysis, was used to evaluate the effect of extrusion conditions on *in vitro* digestibility of the proteins in tarhana. Protein *in vitro* digestibility (PD) values for tarhana samples extruded at different extrusion conditions were found to be in the range of 79.5–81.3%, which were not significantly different from each other at 0.05 significance level (Table 1). Also, the PD of unextruded control tarhana (80.7%) was not significantly different ($p > 0.05$) from the extruded counterparts. The results given in Table 1 might suggest that extrusion and post-extrusion processing (i.e. oven drying at 55°C for 48 h) did not change the *in vitro* digestibility of the proteins significantly ($p > 0.05$). Low moisture extrusion (i.e. high shear) (Bjorck & Asp, 1983) and high product temperatures (> 180°C) (Cheftel, 1986) have been associated with decreased protein *in vitro* digestibilities in extruded products. It may be possible that the low viscosity of tarhana, due to its high moisture content (43%, wb) and relatively mild extrusion temperatures (< 120°C) prevented such adverse effects on PD of tarhana during extrusion.

Thiamin and riboflavin content

Cereal products are among the most important sources of B-group vitamins (Killeit, 1994). The effect of extrusion conditions on the thiamin and riboflavin contents of tarhana were investigated. The thiamin and riboflavin contents of samples extruded at different conditions were not significantly different from each other at 0.05 level (Table 1). Therefore, it can be concluded that extrusion processing itself did not cause any significant

Table 2. Thiamin content of freeze-dried unextruded and freeze-dried extruded samples on dry basis (results are average of three replicates ±SD)*

	Freeze-dried unextruded	Freeze-dried extruded
Thiamin (μg/g)	2.2±0.1 [a]	2.1±0.2 [a]

*Mean values with the same superscripts within the same row are not significantly different (P >0.05, TUKEY HSD multiple comparison test).

losses in thiamin and riboflavin contents of the samples under the process conditions used in this study. It is reported that increasing moisture content generally improves the retention of vitamins (Killeit, 1994). Also, thiamin and riboflavin are known to be stable in acidic conditions (Camire *et al.*, 1990). Therefore, it appears that the high moisture content (43%, wb) and low pH (4.2) of tarhana prevent possible destruction of the vitamins during extrusion.

It is seen from Table 1 that changing extrusion conditions did not cause any significant loss of thiamin in tarhana. However, by referring to the thiamin content of the unextruded control sample (Table 1) it can be seen that there is a significant ($p > 0.05$) loss in thiamin content between this sample and extruded samples. Therefore, in order to evaluate whether this loss in thiamin content was due to the extrusion process itself or due to the oven-drying, an additional extrusion run was performed. The selection of extrusion conditions in this run was based on the conclusion that changes in extrusion conditions would not affect the thiamin content of tarhana. Therefore, an arbitrary choice of extrusion conditions in this additional run should not have affected the result significantly. The extrusion conditions were 60°C barrel temperature, 20 kg/h feed rate and 200 rpm screw speed. The extruded sample obtained from this run was freeze-dried, ground and analyzed for thiamin content as before (Table 2).

The results of this additional run (Table 2) suggest that the apparent loss in thiamin content of extruded tarhana given in Table 1 could result from the oven-drying rather than the extrusion processing itself. Although the pH of tarhana was low (4.2), the drying time seems to exert a predominant effect in this case (Cheftel, 1986). Millauer *et al.* (1984) observed approximately 10% loss of thiamin during the final drying of crispbread. Thiamin losses up to 25% have been reported during baking (Linko *et al.*, 1981).

Tarhana had to be dried after extrusion before grinding, due to its high moisture content (43%, wb). This brought the necessity of using a drying system. Therefore, when considering the effect of extrusion process on thiamin content of tarhana, drying should be considered as a part of the extrusion and the effect of drying should be taken into account.

CONCLUSIONS

The results of this research showed that tarhana can be extruded using a twin-screw extruder without any significant loss in *in vitro* digestibility of the protein and riboflavin content. However, oven-drying of the samples after extrusion caused approximately 30% loss in thiamin content of the samples.

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