Effect of Traditional Processing on the Nutritional Quality and *in vivo* Biological Value of Samh (*Mesembryanthemum forsskalei* Hochst) Flour

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Abstract: Roasting improved the determined protein and carbohydrate content of the flour compared to raw flour ($p < 0.05$). Baking enhanced the determined moisture and ash content of the flour compared to all treatments ($p < 0.05$). Similar amino acid content was found in both raw and treated flours with glutamic acid, glycine, arginine, and aspartic acid being predominant. Cooking reduced the total aromatic and non-essential amino acid content whereas roasting reduced the total essential amino acid content of samh flour. All treatments significantly ($p < 0.05$) decreased the antinutritional factors compared to untreated raw flour. Baking decreased the trypsin inhibitor activity by almost 98.7% whereas cooking reduced phytate and tannin content by 38.5% and 10.8, respectively. Roasting and baking significantly ($p < 0.05$) improved the *in vitro* protein digestibility of the flour. *In vivo*, the true faecal nitrogen digestibility of rats was significantly ($p < 0.05$) enhanced by all treatments. Baking and cooking increased ($p < 0.05$) the net protein utilization and biological value of the flour. Overall, the treatments improved the nutritional quality of samh flour.

Key words: Samh flour, nutritional composition, amino acid composition, antinutritional factors, *in vitro* protein digestibility, biological value

1 Introduction

Samh (*Mesembryanthemum forsskalei* Hochst) is an interesting cereal halophyte that grows naturally in the semi-arid regions of Kuwait, Saudi Arabia, and Egypt¹. Its seeds, popularly called the forskal fig-marigold by people in Al-Jouf, the northern part of Saudi Arabia, are used for several medicinal and food applications². Nutritional analysis of these seeds revealed that they are rich in protein (approximately 22%) compared to cereals like wheat (14%), triticale (12%), and rye (8%), and also contain comparable fat content (majorly unsaturated fatty acids), 10% crude fibre, and 4% ash³⁻⁶.

Samh seeds undergo many processing methods before being consumed. For instance, they can be processed into flour and used as a substitute for wheat in bread and cookies production to improve the product's appearance such as colour and nutritional quality of the products⁷⁻⁹. Moreover, owing to its high nutritional value, it could be

used as a partial replacement for corn in poultry feed¹⁰. However, the presence of anti-nutritional factors present naturally in most cereals and legumes may hinder the bio-availability of these nutrients. Further, there is low bio-availability of minerals from plant sources compared to animal sources, which is attributed to the presence of anti-nutritional factors like phytic acids and tannins that bind to these minerals¹¹. The main aim of processing is to improve the organoleptic, nutritional, and health properties of seeds as well as to reduce the anti-nutritional factors present in the seeds. Several traditional processing methods such as cooking/boiling¹², roasting¹³, soaking^{14, 15}, germination¹⁶, and fermentation^{17, 18} have been found to significantly reduce the anti-nutrient content and enhance the nutritional value of cereals and legumes. However, some processing techniques used on cereals and legume seeds have been reported to adversely affect the nutritional and potential health benefits of seeds¹⁹.

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Considerable attention had been given to studying the nutritional composition of samh seeds and their quality attributes when used in the formulation of food products; however, as samh seeds are traditionally processed in Saudi Arabia, information on how these processes affect the nutritional quality of seeds is scarce. Therefore, this study mainly aimed to investigate the effects of different thermal treatment methods (roasting, cooking, and baking) on the chemical composition, amino acid profile, anti-nutritional factors, *in vitro* protein digestibility (IVPD), and *in vivo* biological value of samh flour.

2 Experimental

2.1 Materials

The seeds of *Mesembryanthemum forsskalei* Hochst were obtained from a local market at Al-Jouf, Saudi Arabia. The samples were manually cleaned to remove impurities and stored in sealed containers at 4°C until used for processing and analysis. All other chemicals were of analytical grade and were obtained from Sigma.

2.2 Processing methods

2.2.1 Roasting

A roasting pan was heated on fire and then 5 kg of the seeds were poured onto the hot pan and shaken for about 5-6 min to develop the flavour and colour of roasted seeds. After cooling to room temperature, the roasted seeds were milled in an electric miller, sieved using 60 mesh sieves, and then stored in sealed plastic containers at 4°C until used for analysis.

2.2.2 Cooking

Raw Samh seeds were milled into a fine powder using an electric miller. The flour was mixed with water (1.5 kg flour: 3 L water) in a cooking pan and then cooked as a thick porridge for 1 h. The porridge was then freeze-dried (Freeze Mobile 12SL, The Virtis Company Inc., Garinen, NY, USA) for 24 h and then milled as described above.

2.2.3 Baking

About 30 g dried yeast was added to 1 kg of samh flour and mixed well for 5 min using a Saphan mixer model UM12 (Germany). Then, 620 mL of water was added to the mixture and mixed well for 6 min to form dough. The dough was initially incubated at 32°C for 30 min (National MFG, Co. Fincoln, Nebr. USA) and then separated into 50 g portions and incubated again for 20 min. The dough was spread and then baked in an electric oven. The bread was dried (Keraeus Hanau, Type UL5100E) at 60°C for 24 h, and then ground to a fine flour as described above and stored at 4°C.

2.3 Chemical composition

Moisture, ash, protein, fat, and carbohydrate content

was analysed as described in the AOAC standard methods²⁰⁾.

2.4 Amino acids analysis

The amino acids were assessed as described in the AOAC²⁰⁾. Prior to analysis, performic acid oxidation, acid hydrolysis, and alkaline hydrolysis treatments were conducted. For the determination of methionine, the samples were treated with cold performic acid and kept overnight at 0-5°C and thereafter cold HBr and 1-Octanol were added. After mixing on ice water bath, the samples were evaporated to dryness using rotary evaporator at 40°C. The dried samples were then hydrolyzed with 6 N HCl at 110°C for 24 h. After that, the hydrolysate was filter through Whatman No. 1 filter paper and used for analysis of Met. For determination of all other amino acids, samh flour samples were subjected to acid hydrolysis with 6N HCl as described above. Amino acids of all hydrolysates were analyzed by HPLC system (Shimadzu Corporation, Kyoto, Japan). For tryptophan determination, the flour samples were subjected to alkaline hydrolysis with 4.2 M NaOH at 110°C for 24 h. Tryptophan content in the hydrolysate was analysed spectrophotometrically (LKB, Biochem, Ultraspec II, England).

2.5 Determination of antinutritional factors

2.5.1 Trypsin inhibitor

Trypsin inhibitor activity was assessed as described by Kakade *et al.*²¹⁾. Briefly, 0.5 g samples were mixed with 40 mL of 0.05 M citrate buffer (pH 4.6) with continuous shaking for 2 h at room temperature to extract the trypsin inhibitors. The mixture was centrifuged at 4500 × *g* for 20 min. Then, 1 mL of the supernatant was mixed with 1 mL of trypsin and incubated at 37°C for 2 min. Next, 7 mL of the substrate (N-benzoyl-DL-arginine-*p*-nitroanilide hydrochloride, BAPA) was added and the reaction mixture was incubated at 37°C for 10 min. The reaction was terminated with 1 mL of 30% acetic acid and absorbance was measured at 410 nm against the blank. One unit of enzyme activity was defined as a 0.01 increase in absorbance at 410 nm.

2.5.2 Phytate analysis

The phytate content was measured as described by Latta and Eskin²²⁾ using sodium phytate as the standard.

2.5.3 Tannin analysis

The modified method of Price *et al.*²³⁾ was used for tannin content analysis in samh flour samples using Catechin as the standard, and the results were expressed as Catechin equivalents.

2.6 *In vitro* protein digestibility (IVPD)

IVPD was analysed using protein hydrolysing enzymes (trypsin, chymotrypsin, and peptidase) as described in the AOAC standard methods²⁰⁾.

2.7 In vivo study

2.7.1 Preparation of feed

Six types of feed were prepared as described by Eggum²⁴ as follows: 10% casein feed, raw samh flour feed (10% protein), cooked flour of samh seeds (10% protein), baked flour of samh seeds (10% protein), roasted samh seed flour (10% protein); and egg protein feed (4% protein). These ingredients were mixed with the other ingredients shown in Table 1.

2.7.2 Animal (rats) feeding

Thirty-six Wistar rats (4-week age, 55-75 g weight) were obtained from the animal house, Faculty of Pharmacy, King Saud University, Saudi Arabia. The rats were fed a reference feed containing casein as the main protein source for 4 d for acclimation to the laboratory conditions (12 h light/dark cycle, 20°C temperature, and 55% relative humidity). The rats were then weighed and distributed equally to 6 groups (each contain 6 rats) without variation in weight between the groups. Each group was fed the specified diet mentioned above (15 g diet/rat) and water was provided *ad libitum*. The experiment was continued for 9 d with the first 4 d was set as the initial period and the remaining five days set as the balancing period. The rats were weighed at the beginning and end of each period. Moreover, the volume of diet consumed every day was measured for each rat. During the second experimental period, the stool and urine of each rat was collected, cleaned, dried at 70°C, ground, and passed through 60 mesh sieves.

2.7.3 Estimation of biological value and true digestibility

The biological value (BV) and true digestibility (TD) of

protein were calculated as follows²⁴:

$$BV = \left[\frac{I - (F - MFN) - (U - UK)}{I - (F - MFN)} \right] \times 100$$

$$TD(\%) = \left[\frac{I - (F - MFN)}{I} \right] \times 100$$

Where; I: Uptake nitrogen, F: Faeces nitrogen, MFN: Metabolic faecal nitrogen (determined under protein-free feeding), U: Urine nitrogen, UK: Urine nitrogen metabolism

2.7.4 Protein estimation

The protein in the feed, faeces, and urine was determined using the Kjeldahl method²⁰.

2.8 Statistical analysis

The results obtained from three independent experiments were analysed using one-way ANOVA²⁵. The significant differences between variable means were assessed using a Tukey's tests at the probability level of 0.05. The results were expressed as means $\pm$ standard deviation.

3 Results and Discussion

3.1 Chemical composition

Table 2 shows the changes in chemical composition (moisture, protein, fat and ash content) of samh flour after different thermal treatments. The moisture content of raw samh flour (8.8%) was significantly ($p < 0.05$) affected by thermal treatments where roasting (5.2%) and cooking (8.1%) lowered the moisture content whereas baking (19.5%) greatly increased the determined level of mois-

Table 1 Formulations of the diets as described by Eggum (1973).

Ingredients	Diet (g/100 g)					
	Casein	Raw Samh	Cooked Samh	Roasted Samh	Baked Samh	Egg protein
Casein	11.6					
Egg						4.2
Raw flour		49.5				
Cooked flour			45.1			
Roasted flour				46.3		
Baked flour					54.6	
Corn oil	5.2	5.2	5.2	5.2	5.2	5.2
Starch	64.2	26.2	30.7	29.4	21.3	71.6
Vitamin mixture	1.0	1.0	1.0	1.0	1.0	1.0
Minerals	3.5	3.5	3.5	3.5	3.5	3.5
Choline	0.3	0.3	0.3	0.3	0.3	0.3
Methionine	0.1					0.1
Lysine		0.1	0.1	0.1	0.1	
Fibre	5.2	5.2	5.2	5.2	5.2	5.2
Sucrose	9.0	9.0	9.0	9.0	9.0	9.0

Table 2 Effect of the treatment processes on the chemical composition (%) of Samh flour.

Treatments	Moisture	Protein	Ash	Fat	Carbohydrate
Raw flour	8.8 ± 0.2 ^b	20.2 ± 0.5 ^b	2.5 ± 0.0 ^c	4.8 ± 0.0 ^a	63.7 ± 0.7 ^b
Roasted flour	5.2 ± 0.3 ^d	21.6 ± 0.6 ^a	2.6 ± 0.0 ^b	5.0 ± 0.3 ^a	65.7 ± 0.8 ^a
Cooked flour	8.1 ± 0.2 ^c	20.8 ± 0.6 ^b	2.2 ± 0.0 ^d	3.1 ± 0.2 ^b	65.9 ± 0.9 ^a
Baked flour	19.5 ± 0.1 ^a	18.3 ± 0.5 ^c	2.8 ± 0.0 ^a	2.6 ± 0.9 ^b	57.0 ± 0.7 ^c

Values represent mean ± standard deviation. Means not sharing the same superscript(s) (a-d) in a column are significantly different at $p < 0.05$.

ture. The present study agrees with the results reported by Seena *et al.*²⁶⁾ who observed that roasting significantly lowered the moisture content of *Canavalia cathartica* from 11.2 to 8.6%. This could be attributed to the exposure of samh seeds to direct heat during roasting which could remove greater amounts of moisture from the seeds. Since microbial growth thrives well in high moisture foods, lowering the moisture content of foods is desirable to extend their shelf-life²⁷⁾. The protein content of samh flour was significantly ($p < 0.05$) affected by the processing methods. Roasting significantly ($p < 0.05$) enhanced the determined protein level of samh flour; however, the protein content was significantly ($p < 0.05$) lowered after baking as compared to that in raw samh flour. No significant difference was observed in the protein content of raw and cooked flours. This finding contradicts those reported on *Sagittaria sagittifolia* L. flour where roasting had no significant effect on the protein content of the flour²⁸⁾. An increase in the determined level of protein in the roasted flour could be explained by its low moisture content, which raised the amount of organic compounds in the flour. Both the treated and untreated samh flour have protein content within the protein range of most crops such as legumes (17-30%)²⁹⁾. Unlike the protein content, baking significantly ($p < 0.05$) improved the determined ash content of samh flour as compared to raw flour and other treatments. However, cooked flour showed significantly ($p < 0.05$) lower ash content than that of other samples. Similarly, the ash content of some cereals such as sesame seeds has been reported to be significantly reduced by the cooking method¹³⁾. The fat content of samh flour was inversely related to the moisture content. This may be attributed to the oil penetrating the food after water is evaporated during the thermal treatment. A similar observation was reported in sesame seeds after undergoing thermal processing such as roasting¹³⁾. The high fat content found in roasted flour could be due to the loss of moisture from seeds resulting in the concentration of organic matter such as lipids. With regard to the carbohydrate content, there were significant ($p < 0.05$) differences among the treatments with baked samh flour having the lowest amount and this may be due to the Maillard reaction that occurs during baking.

3.2 Amino acid composition

The amino acid composition of raw and treated samh flours is presented in **Table 3**. Samh flour protein is rich in total aromatic amino acids as compared with the FAO/WHO³⁰⁾ reference. However, the levels of threonine, valine, lysine, leucine, and isoleucine were to some extent deficient in samh flour protein compared with the reference, whereas the tryptophan levels were comparable with that pattern. Treatments of samh flours did not affect the total essential amino acids in the raw samh flour. The lysine content of samh flour was decreased after roasting, which may be attributed to the exposure of seeds to direct heat during roasting, probably causing protein degradation. This contradicts the result of Khattab *et al.*³¹⁾ where thermal treatments like roasting improved the lysine content of some selected legumes (cowpea, pea, and kidney bean). However, cooking enhanced the concentration of lysine but the value was still lower than that of the reference. Furthermore, the amounts of essential amino acids were greater than those in wheat grain³²⁾. All the processing methods applied lowered the leucine:isoleucine ratios of samh flour compared to ideal ratio of 1.8:1 suggested by the FAO/WHO³⁰⁾. Similar findings were noticed in chickpea seeds processed by thermal treatment³³⁾. Deosthale *et al.*³⁴⁾ demonstrated that utilization of isoleucine and lysine in foods may be interfered by the presence of excessive leucine in the foods.

3.3 Anti-nutritional factors

The result showed significant differences in phytate, tannin, and trypsin inhibitor content in raw and processed samh flour (**Table 4**). All the thermal treatments significantly decreased the anti-nutritional factors of raw samh flour. The highest phytate reduction was noted in cooked flour (38.5%), which was significantly ($p < 0.05$) higher than that of baked flour (24.7%). No significant difference was observed in the tannin content of the treated flours but they were significantly ($p < 0.05$) lower than that in raw flour. The decrease in anti-nutritional factors may be attributed to the denaturation of anti-nutrients by heat and the leaching of anti-nutrients into cooking water during cooking process. Similar observations have been reported in linn seeds³⁵⁾, chickpea seeds³³⁾, and lentil seeds³⁶⁾. Simi-

Table 3 Effect of the treatments on the amino acid content (g/100 g protein) of samh flour.

Amino acids	Raw flour	Roasted	Cooked	Baked	FAO/WHO ²⁹⁾
Aspartic acid	9.2	9.0	9.2	9.1	—
Threonine	2.9	3.0	3.0	3.1	4.0
Serine	3.6	3.6	3.7	3.9	—
Glutamic acid	19.6	19.5	12.3	18.9	—
Glycine	18.0	18.0	17.7	17.4	—
Alanine	2.6	2.6	2.9	2.8	—
Valine	2.6	2.6	2.6	2.8	5.0
Methionine	1.9	1.8	1.8	1.5	—
Isoleucine	2.6	2.7	2.7	2.7	4.0
Leucine	4.4	4.4	4.5	4.5	7.0
Phenyl alanine	3.5	3.5	3.6	3.5	—
Tyrosine	4.1	4.0	3.4	4.0	—
Histidine	5.7	5.8	5.8	5.6	—
Lysine	2.8	1.9	3.0	2.6	5.5
Arginine	12.1	11.8	11.5	11.3	—
Tryptophan	1.0	0.9	1.0	0.9	1.0
Total aromatic AA	7.6	7.5	7.0	7.6	6.0
Total essential AA	25.7	24.8	25.6	25.7	36.0
Total non-essential AA	70.7	70.3	63.0	69.1	—
Leucine/isoleucine ratio	1.7:1	1.6:1	1.7:1	1.6:1	1.8:1

Values represent mean; AA: amino acids

Table 4 Effect of the treatments on the anti-nutritional factors of samh flour.

Treatments	Phytate (%)	Degradation (%)	Tannin	Degradation (%)	Trypsin Inhibitor (U/mg protein)	Degradation (%)
Raw flour	0.64 ± 0.01 ^a	0.0	0.074 ± 0.001 ^a	0.0	37.03 ± 0.65 ^a	0.0
Roasted flour	0.41 ± 0.06 ^{bc}	36.3	0.067 ± 0.002 ^b	9.5	30.23 ± 0.85 ^b	18.9
Cooked flour	0.39 ± 0.04 ^c	38.5	0.066 ± 0.002 ^b	10.8	26.93 ± 2.58 ^c	27.6
Baked flour	0.48 ± 0.05 ^b	24.7	0.067 ± 0.003 ^b	9.5	0.48 ± 0.05 ^d	98.7

Values represent mean ± standard deviation. Means not sharing the same superscript(s) (a-d) in a column are significantly different at $p < 0.05$

larly, the trypsin inhibitors in raw flour (37.03 U/mg protein) were significantly ($p < 0.05$) reduced after treatment to 0.48–30.23 U/mg protein, with baked flour showing the highest reduction (98.7%) rate. This was similar to the results with linn seeds³⁴⁾ and lentil seeds³⁵⁾, where thermal treatments were reported to lower the trypsin inhibitor activity by nearly half. Our findings showed that thermal treatment lowers the enzyme-inhibitory activity, thus making the treated flour nutritionally better than raw flour. Trypsin inhibitors in different food formulations have been demonstrated to be detrimental to animal performance³⁷⁾; this is reflected in the release of free amino acids caused

by decreased activity of proteolytic enzymes in the small intestine. Moreover, Pacheco *et al.*³⁸⁾ reported the occurrence of pancreatic hypertrophy in chicken caused by the presence of antinutrients in their diet. These problems could be resolved by applying a suitable thermal treatment as that used in this study, to increase the bioavailability of nutrients in samh flour.

3.4 In vitro protein digestibility

The IVPD of raw samh flour was improved by all treatments (Fig. 1). Roasting and baking significantly enhanced the IVPD of samh flour compared with that of raw flour.

Fig. 1 *In vitro* protein digestibility of samh flour under different processing treatments.

This may be due to factors such as protein denaturation, a decrease in antinutritional factors like tannins, phytic acid, and trypsin inhibitor thereby increasing protein availability (Mubarak *et al.*, 2005). However, raw and cooked samh flour did not significantly differ in their IVPD. Both the raw and treated samples had significantly ($p < 0.05$) lower IVPD values compared to casein. Similar findings were reported with mung bean seeds¹⁶⁾ and sandbox seeds³⁹⁾ following thermal treatments.

3.5 Biological value

Protein digestibility is the second most important determinant of protein quality after amino acid profiles⁴⁰⁾. Changes in rat weight and the true digestibility of all treated samples were determined in a rat bioassay (Table 5). Diets containing casein have significantly ($p < 0.05$) higher digestibility values than those containing raw and treated samh flours. The true digestibility in rats was significantly ($p < 0.05$) improved when fed with treated flours compared with raw flour, and those fed with cooked flour exhibited the highest value. This is in agreement with reports in rats fed with toasted cereal/legume (cowpea peanut toasted maize) formulations⁴¹⁾. The increase in TD following thermal treatment may be attributed to high deg-

radation of anti-nutrients by heat, thereby making the protein available for enzymatic digestion. Comparing the IVPD with TD, a lower value was obtained in IVPD compared with that from the *in vivo* measurement. Although *in vivo* studies are time consuming and with high cost of setup, they are more sensitive than *in vitro* studies. However, care should be taken for the interpretation of *in vivo* digestibility results as microbial activity in the small intestine could lead to the synthesis and uptake of dietary essential amino acids which affect the accuracy of faecal-based nitrogen digestibility assays⁴²⁾. The results of weight gained by rats fed with different diets showed no significant difference between raw and treated samh flours, but they were significantly ($p < 0.05$) lower than those fed with casein protein. This may be due to the high TD of rats fed with casein. Also, rats fed with casein demonstrated the highest net protein utilization and biological value ($p < 0.05$) compared with those fed other samples. The results also showed that the treatments (cooking and baking) of samh flour significantly ($p < 0.05$) improved the NPU of the rats fed compared with those fed raw and roasted flour, which may be due to their high TD value. The larger amount of protein digestibility in the diet resulted in a higher NPU. The nitrogen uptake of rats fed on all the diets ranged between 792.1 and 1125.7 with those fed roasted and baked flour showing the lowest and highest values, respectively. High NPU for the cooked and baked samh flours showed a higher efficiency in supporting the growth and maintenance of weaning rats compared to the raw samh flour.

4 Conclusion

The different processing methods improved the chemical composition of samh flour particularly the protein and ash content. However, essential amino acids such as lysine were reduced by the roasting method. All the processing methods were very effective in lowering the anti-nutritional factors of samh flour. Furthermore, *in vitro* and *in vivo* protein digestibilities as well as the biological value of samh

Table 5 Effect of the treatments on the biological value of samh flour proteins in model rats.

Treatments (rats fed with)	Nitrogen uptake (mg)	Changes in rat weight (g)	True digestibility (%)	Biological value (%)	Net protein utilization (%)
Casein	989.2 ± 59.4 ^b	34.5 ± 7.4 ^a	97.8 ± 3.6 ^a	93.0 ± 4.2 ^a	90.9 ± 5.0 ^a
Raw flour	1003.4 ± 81.1 ^b	12.9 ± 2.8 ^b	69.1 ± 3.5 ^d	73.9 ± 4.8 ^b	51.1 ± 5.3 ^d
Roasted flour	792.1 ± 87.7 ^c	6.6 ± 4.4 ^b	76.7 ± 4.2 ^c	69.2 ± 3.9 ^c	54.0 ± 5.0 ^d
Cooked flour	990.1 ± 121.4 ^b	10.5 ± 4.7 ^b	84.8 ± 4.7 ^b	76.5 ± 5.4 ^b	64.9 ± 6.4 ^{bc}
Baked flour	1125.7 ± 47.0 ^a	10.2 ± 2.9 ^b	78.6 ± 1.9 ^c	77.6 ± 5.4 ^b	61.1 ± 5.3 ^c

Values represent mean ± standard deviation. Means not sharing the same superscript(s) (a-d) in a column are significantly different at $p < 0.05$.

flour were enhanced by all the processing methods. Therefore, subjecting samh flour to traditional processing such as cooking, roasting, and baking may enhance the nutritional quality of the flour.

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Conflict of Interest

No conflicts of interest exist.

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