



Characterization and quality evaluation of Foxtail Millet (*Setaria Italica*) protein

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Abstract

The present study was conducted to extract and evaluate the foxtail millet (FM) protein using four extraction techniques: (i) 7% α -amylase (A₁), (ii) 10% α -amylase (A₂), (iii) 7% α -amylase+0.1% glucoamylase (A₃), and (iv) 10% α -amylase+0.1% glucoamylase (A₄). The extracted protein was enriched and then employed for cooking with different temperatures (100, 120, 130, and 140°C) and times (10, 20, 30 and 40 min). Results revealed that prolamin-assisted extraction increased the protein level, however the yield of concentrated protein was very low. The flour treated with 7% α -amylase yielded reasonable amounts of concentrated protein and protein level. Non-essential amino acid cysteine was found only in uncooked extracted enriched (UCEE) FM protein, which was entirely absent in cooked extracted enriched (CEE) FM protein. Nutritional compositions especially protein, ash, fat and crude fiber was decreased with the advancement of cooking temperatures and time except moisture, carbohydrate and energy. Conclusively, use of 7% α -amylase yielded reasonable protein and cooking at 100°C for 30 min retained higher nutritional content compared to others.

Keywords: millet protein, protein extraction, nutrition, sensory evaluation

1. Introduction

The millets, including sorghum, proso, Japanese, and foxtail millet are a valuable source of human food in Africa and Asia. Approximately 762.712 metric tons of millet has been reported to produce worldwide where India is the top millet producing and China is the top yield contributing country in the world ^[1]. In South-East Asian region especially India, China and Bangladesh, foxtail millet is produced mainly and is used as human food where it has high content of protein, fiber and lipid content compared to common consumed grains such as rice, wheat, corn and other millets ^[2]. The millet has favorable effect on health benefits where it improves insulin sensitivity and cholesterol metabolism through an increase in adiponectin concentration. Furthermore, its protein serves as another beneficial food component in obesity-related diseases such as type 2 diabetes and cardiovascular diseases (CVDs).

Extraction of protein using prolamin and a number of methods including alcoholic, methanol and enzymatic is the most common that have been reported by previous studies ^[3-8]. Although several methods are available, each having particular advantages and disadvantages such as some research methods are very old, unclear and produced low yield of protein while the protein content is high but commercially and economically is not feasible. Hence, the modification of method for protein extraction is needed. Cooking is a common consumed method used for rice to improve nutritional quality and yield ^[9, 10]. But commercially available cooking techniques such as boiling,

roasting, cooking time and temperature have not been successfully employed on nutritional quality of millets. Therefore, the study has been undertaken to modify the different methods and find out the optimum cooking temperature and time to minimize the nutritional losses of the millet protein during cooking.

2. Material and methods

The foxtail millet (Dong Fang Liang) was collected from the Shanxi Province of China. Then it was grinded and subjected for protein extraction, cooking, amino acid and nutritional analysis.

2.1 Extraction of protein

The protein was extracted according to the method of Nishizawa *et al.* ^[5] with some modifications. Under the modification, the flour was treated with different treatments *viz.* 7 % α -amylase (A₁), 10 % α -amylase (A₂), 7 % α -amylase+0.1 % glucoamylase (A₃), 10 % α -amylase+0.1 % glucoamylase (A₄). The treated flour was dispersed into distilled water at flour to water ratio of 1:5 (w/v). Then the prepared mixture was stirred with magnetic for 15 min for proper mixing. The pH was adjusted at 7.0 before shaking in water bath. Then the mixture was shaken in water bath at 50°C for 36 h. During shaking the pH was checked and adjusted 7.0 at 3 h interval. Then the extract was centrifuged by high-speed refrigerated centrifuge at 11000 rpm for 15 min at room temperature. The residues were collected by removing the liquid from the upper surface of the centrifuge

tube. The process was repeated twice as described above. After getting the resultant material (concentrate protein), it was defatted into n-hexane at the resultant material to hexane ratio of 1:5 (w/v). Then the mixture was stirred in water bath at 60°C for 10±1 hours. The solid was collected from lower surface by dropping of n-hexane from the conical flask. After that it was air-dried for 24 hours under a vacuum drier to entirely remove the hexane from the solid. The concentrated protein was compared with prolamin extraction (A5) and kept in deep freeze (-40°C) for 24 hours. Then the concentrated protein was dried in hot air oven dryer at 50°C for 36 hours and the moisture content was maintained below 10%.

2.2 Cooking

The extracted protein was cooked at protein to water ratio of 10:15 (v/w). There were two factors namely; factor A: cooking time (10 min, 20 min, 30 min and 40 min), factor B: Cooking temperature (100±1°C, 120±1°C, 130±1°C and 140±1°C). The temperature higher than 100°C were maintained using the oven (Ferranini, FEA7616X, Italy). Then the cooked protein was subjected for proximate and sensory analysis.

2.3 Nutritional analysis

The analysis of amino acid compositions [11], crude protein [12], crude fat [13], crude fiber [14], ash [15], and moisture content [16], were done in duplicate according to the method of 'National Standards of People's Republic of China. Carbohydrate content was determined by differences of total contents (crude protein, crude fat, crude fiber, ash and moisture content) from 100. Energy value available from FM protein was calculated multiplying the number of grams of crude protein, crude fat and carbohydrate by 17, 37 and 17, respectively.

2.4 Sensory evaluation

It was done according to the standard method of Joshi [17], using a 9-point hedonic scale, i.e. 9= Like extremely, 8= Like very much, 7=Like moderately, 6= Like slightly, 5=Neither like or dislike, 4=Dislike slightly, 3= Dislike moderately, 2=Dislike very much and 1=Dislike extremely. The different preferences as indicated by scores were evaluated by statistical methods.

2.5 Statistical analysis

Data analysis was performed by One-way ANOVA with post-hoc using Turkey Multiple Comparisons Test using software SPSS 17.0 (IBM INC., New York). The significance was defined at the 95 % confidence level. All data were expressed in duplicate as means ± standard deviation.

3. Results & Discussion

Yield of concentrated protein and protein level getting from different procedures are shown in Table 1. It was found that treatment A₄ showed highest quantity of concentrated protein (10.00±0.71 g) but the protein level was lowest (19.4±0.40 %) followed by others. Treatment A₅ given highest level of protein (68.60 %) but the yield of concentrated protein was lowest (1.50±0.41 g) compared to others. For the treatment A₁, the protein level was 44.07±1.01 % whereas the yield of concentrated protein was 7.17±0.33 g per 100 g. However, on comparison of different

treatments and considering the reasonable yield and protein level, A₁ performed better for protein extraction as low cost technique. These findings were supported by Nishizawa *et al.* [4], Choi *et al.* [7], and Nishizawa *et al.* [6], who followed a mixture of α-amylase and glucoamylase for the extraction of concentrated protein from proso millet, Korean foxtail millet and Japanese millet and received protein content 40.3 %, 52.4 % and 39.3 % respectively.

Table 2 show 7 essential and 10 non-essentials amino acids of the UCEE and CEE FM protein. The UCEE FM protein was found to contain high content of essential and non-essential amino acids than CEE FM protein (Table 2). It is notable that non-essential amino acid cysteine was only present in UCEE FM protein where it was entirely absent in CEE FM protein (Table 2). An achieving higher amino acid composition by the UCEE FM protein may be because of enriched with lysine and threonine. On the other hand, the lower presence of CEE FM protein may be due to loss of nutritive value during cooking. The loss of nutritive value may be attributed to decrease of digestibility, destruction and/or biological inactivation of amino acids, inhibition of proteolytic and glycolytic enzymes, and interaction with metal ions [18]. Other reasons may be due to heat treatment of amino acid residues of protein generates a mixture of L- and D-racemic forms after protonation, and that racemization of the essential amino acids residues of protein reduces their nutritional value [19, 20].

Table 1: Yield of concentrated protein and protein level using different treatments

Treatment	Protein content (%)	Concentrated protein (g/100g)
A ₁	44.07±1.01 ^d	7.17±0.33 ^b
A ₂	37.60±0.95 ^c	8.10±0.90 ^{bc}
A ₃	23.90±1.10 ^b	9.19±0.51 ^{cd}
A ₄	19.40±0.60 ^a	10.0±0.71 ^d
A ₅	68.60±1.40 ^e	1.50±0.41 ^a

A₁=7 % α-amylase, A₂=10 % α-amylase, A₃=7 % α-amylase + 0.1 % glucoamylase, A₄= 10 % α-amylase + 0.1 % glucoamylase, A₅= Prolamin extraction; values with different superscript letters are significant in row wise.

Table 3 represents the proximate composition of foxtail millet protein cooked under different time and temperatures. The results show that the protein content was significantly changed with advancement of time and temperature during cooking. The decreased protein content could be attributed to leaching of nutrients into the cooking water for long time cooking with high temperature and may also have resulted from Maillard reaction [21, 22], or protein denaturation [23]. Fat content was insignificant at 100±1 °C, 120±1°C, 130±1°C and 140±1°C for 10, 20 and 40 min cooking but it was decreased by the temperature. For 30 mins cooking, there was insignificant difference between 100±1°C and 120±1°C temperature although it was decreased by the temperature. But a significant difference was observed between 130±1°C, 140±1°C temperature for 30 min cooking. However, the fat content was decreased with increasing the time and temperature (Table 3). These decreases might be attributed to their diffusion into cooking water [24], due to physical and chemical changes during heating [25], elevated temperature and metallic ions may facilitate fats oxidation and degradation also during cooking [26]. Other reason might be due to variation in loss of volatile components resulting due

to fat hydrolysis during cooking [27]. In case of crude fiber content, insignificant differences were observed among the temperature of 100±1 °C, 120±1 °C, 130±1 °C and 140±1 °C for 20, 30 and 40 min cooking but the fiber content was decreased by the temperature. For 10 min cooking, there were insignificant differences among the temperature of 120±1 °C, 130±1 °C and 140±1 °C but they (120±1 °C, 130±1 °C and 140±1 °C) were significantly decreased than 100±1 °C temperature (Table 3). However, the fiber content was decreased gradually during 10, 20 and 30 min but during 40 min cooking, it was drastically decreased. The decrease in crude fiber content during cooking could be attributed to solubilization as increase in temperature leads to breakage of weak bonds between polysaccharide and glycosidic linkages in dietary fiber polysaccharides [28]. These result in a decreased association between fiber molecules and/or depolymerization of the fiber bringing about solubilization. In case of ash content, there was insignificant difference between 100±1 °C and 120±1 °C temperature where it was significant between 130±1 °C and 140±1 °C for 10 min cooking. For 20 and 30 min cooking, the ash content was insignificantly decreased at 100±1 °C, 120±1 °C and 130±1 °C temperature. But cooking at 140±1 °C for 20 and 30 min was significantly decreased than other temperatures although there was insignificant difference between 20 and 30 min cooking. However, the ash content was decreased by increasing time and temperature during cooking. These may be due to leaching of minerals in boiling water [29, 30]. Moisture content was insignificantly increased at 100±1 °C, 120±1 °C, 130±1 °C and 140±1 °C temperature during cooking for 10, 20 and 30 min. But it was significantly increased during 40 min cooking than 10, 20 and 30 min. It is noteworthy that the moisture content was increased with increasing time and temperature of cooking. This may be due to enhancement water holding capacity [31], as well as water absorption during cooking [29, 30].

In case of carbohydrate, the changes were not significantly differed at 100±1 °C, 120±1 °C and 130±1 °C but significantly differed by the 140±1 °C temperature during cooking for 10 min. For 20 min cooking, the changes were insignificant between the temperature of 100±1 °C and 120±1 °C but significantly differed by the 130±1 °C and 140±1 °C temperature of cooking. For 30 min cooking, there were significant changes between 100±1 °C and 120±1 °C temperature but insignificant difference was observed between 130±1 °C and 140±1 °C temperature. For 40 min

cooking, no significant changes were observed by the temperature of 100±1 °C, 120±1 °C, 130±1 °C and 140±1 °C. However, the carbohydrate content was increased with increasing time and temperature of cooking. These results may be due to decreasing crude protein, crude fat, crude fiber and ash content with increasing cooking time and temperature as the carbohydrate values was calculated.

Table 2: Amino acid compositions of extracted protein under different conditions

Amino acids		Different conditions	
		UCEE FM protein	CEE FM protein
Essential	Lysine	1.848	0.995
	Threonine	1.186	0.754
	Isoleucine	1.307	0.646
	Leucine	2.764	2.347
	Methionine	1.307	0.282
	Phenyl alanine	2.184	1.781
	Valine	1.486	0.892
Non-essential	Alanine	1.930	1.593
	Arginine	1.246	1.118
	Aspartate	1.459	1.326
	Cysteine	1.166	0.00
	Glutamate	7.00	5.347
	Glycine	1.748	1.230
	Histidine	1.735	0.889
	Proline	1.781	1.696
	Serine	1.982	1.875
	Tyrosine	1.645	1.125

The estimated energy values were significantly increased by the temperature of 100±1 °C, 120±1 °C, 130±1 °C and 140±1 °C although the changes were insignificant between 120±1 °C and 130±1 °C temperature for 10, 20 and 30 min cooking. For 40 min cooking, the changes were insignificantly differed among the temperature of 100±1 °C, 120±1 °C, 130±1 °C and 140±1 °C. Here, it is noted that the energy values at 130±1 °C and 140±1 °C temperature for 40 min cooking was decreased where it was increased for other conditions. The reason may be due to tremendously increased estimated carbohydrate value and decreased crude protein and crude fat content at 130±1 °C and 140±1 °C temperature during 40 min cooking. Therefore, the study show that cooking time and temperature affect the nutritional composition of the extracted protein but the cooking at 100±1 °C temperature for 30 min contributed to minimize the nutritional losses of the extracted protein (Table 3).

Table 3: Cooking time and temperature effect on nutritional composition of extracted protein

Para meter	Cooking time (min)	Cooking temperature (°C)			
		100±1	120±1	130±1	140±1
Protein (g/100 g)	10	23.7±0.20 ^c	23.0±0.10 ^c	22.6±0.30 ^b	21.7±0.30 ^a
	20	23.8±0.15 ^c	23.2±0.13 ^c	22.8±0.20 ^b	22.0±0.10 ^b
	30	23.9±0.21 ^c	23.4±0.18 ^c	22.9±0.15 ^b	22.30±0.12 ^b
	40	22.6±0.13 ^b	22.1±0.13 ^b	21.5±0.25 ^a	20.7±0.11 ^a
Fat (g/100 g)	10	5.26±0.13 ^c	5.20±0.10 ^c	5.06±0.12 ^c	4.98±0.12 ^c
	20	4.77±0.07 ^b	4.75±0.15 ^b	4.53±0.13 ^b	4.37±0.17 ^b
	30	4.67±0.06 ^b	4.56±0.06 ^b	4.31±0.10 ^{ab}	4.07±0.02 ^a
	40	4.25±0.10 ^a	4.15±0.04 ^a	4.07±0.07 ^a	4.03±0.06 ^a
Crude fiber (g/100 g)	10	3.81±0.13 ^d	3.51±0.19 ^c	3.33±0.13 ^c	3.04±0.16 ^c
	20	3.35±0.15 ^c	3.24±0.16 ^c	3.10±0.10 ^c	2.99±0.19 ^c
	30	2.66±0.16 ^b	2.41±0.11 ^b	2.28±0.12 ^b	2.01±0.15 ^b
	40	1.89±0.09 ^a	1.74±0.04 ^a	1.63±0.13 ^a	1.51±0.11 ^a
Ash (g/100 g)	10	3.64±0.14 ^c	3.48±0.14 ^c	3.12±0.05 ^b	1.71±0.11 ^a

	20	3.21±0.06 ^b	3.13±0.13 ^b	2.64±0.12 ^b	1.59±0.09 ^a
	30	3.19±0.07 ^b	2.99±0.10 ^b	2.50±0.15 ^b	1.57±0.07 ^a
	40	1.77±0.06 ^a	1.65±0.05 ^a	1.56±0.03 ^a	1.52±0.02 ^a
Moisture content (g/100 g)	10	5.41±0.11 ^a	5.51±0.10 ^a	5.63±0.13 ^a	5.80±0.10 ^a
	20	5.49±0.09 ^a	5.58±0.12 ^a	5.70±0.10 ^a	5.91±0.11 ^a
	30	5.61±0.11 ^a	5.67±0.17 ^a	5.77±0.13 ^a	5.96±0.04 ^a
Carbohydrate (g/100 g)	40	6.04±0.04 ^b	6.19±0.11 ^b	6.59±0.09 ^b	6.91±0.09 ^b
	10	58.18±1.18 ^a	59.30±1.0 ^a	60.26±1.26 ^a	62.77±1.0 ^b
	20	59.38±1.38 ^a	60.10±1.0 ^a	61.23±1.23 ^{ab}	63.14±1.14 ^b
Energy (KJ)	30	59.97±1.97 ^a	60.97±1.97 ^{ab}	62.24±1.24 ^b	64.09±1.09 ^b
	40	63.45±1.45 ^b	64.17±1.17 ^b	64.65±1.65 ^b	65.33±1.33 ^b
	10	1586.58±1.02 ^a	1591.50±1.50 ^b	1595.84±1.30 ^b	1620.25±1.25 ^d
	20	1573.55±1.45 ^a	1591.85±1.85 ^b	1596.12±1.12 ^b	1609.07±1.07 ^c
	30	1598.58±1.42 ^b	1603.01±2.0 ^c	1606.85±1.15 ^c	1619.22±1.22 ^d
	40	1620.10±1.10 ^d	1620.14±1.14 ^d	1615.14±1.60 ^d	1611.62±1.50 ^d

All values are means of triplicate determinations ± SD. Means within columns with different letters a, b, c, d indicates significant result (p<0.05). Same letter means no significant difference.

The sensory evaluation viz. appearance, color, flavor, texture and overall acceptability show that the highest score was gained by the appearance at 100±1 °C for 30 min cooking than others. Cooking for 30 min at 100±1 °C, 120±1 °C, 130±1 °C and 140±1 °C also performed better than 10, 20 and 40 min. The acceptability scores for color were obtained high by the 30 min cooking at 100±1 °C temperature than other time and temperature (Table 4). Cooking at 100±1 °C for 30 min gained the highest score for flavor where the lowest score was gained by the 120±1 °C,

130±1 °C and 140±1 °C temperature for 10 and 40 min cooking. The preference score for texture was obtained higher also by the 100±1 °C for 30 min cooking whereas it was lower at 120±1 °C, 130±1 °C and 140±1 °C for 10 and 40 min. However, cooking of extracted protein at 100±1 °C temperature for 30 min has highest score of overall acceptability (8.51) than others. Therefore, 100±1 °C temperature for 30 min may be considered for cooking of extracted foxtail millet protein in terms of good appearance, color, flavor, texture and overall acceptability.

Table 4: Sensory evaluation of extracted protein based on cooking time and temperature

Parameter	Cooking time (min)	Cooking temperature (°C)			
		100±1	120±1	130±1	140±1
Appearance	10	4.55±0.31 ^a	4.38±0.20 ^a	4.21±0.09 ^a	4.08±0.05 ^a
	20	7.37±0.06 ^c	6.35±0.05 ^b	6.19±0.01 ^b	6.12±0.02 ^b
	30	8.93±0.03 ^d	7.29±0.03 ^c	7.19±0.04 ^c	7.08±0.02 ^c
	40	6.29±0.07 ^b	6.07±0.04 ^b	4.13±0.03 ^a	4.05±0.04 ^a
Color	10	4.52±0.11 ^a	4.13±0.03 ^a	4.12±0.04 ^a	4.03±0.04 ^a
	20	7.30±0.07 ^c	6.25±0.05 ^b	6.17±0.04 ^b	6.09±0.01 ^b
	30	8.93±0.03 ^d	7.19±0.02 ^c	7.14±0.03 ^c	7.06±0.04 ^c
	40	6.36±0.09 ^b	6.26±0.06 ^b	4.14±0.03 ^a	4.06±0.04 ^a
Flavor	10	4.38±0.20 ^a	4.08±0.11 ^a	4.07±0.04 ^a	4.03±0.05 ^a
	20	7.30±0.23 ^c	6.33±0.07 ^b	6.20±0.03 ^b	6.09±0.05 ^b
	30	8.90±0.02 ^d	7.33±0.04 ^c	7.25±0.04 ^c	7.17±0.04 ^c
	40	6.30±0.09 ^b	4.17±0.03 ^a	4.13±0.03 ^a	4.04±0.04 ^a
Texture	10	4.41±0.09 ^a	4.30±0.09 ^a	4.16±0.04 ^a	4.06±0.04 ^a
	20	7.51±0.08 ^c	6.23±0.03 ^b	6.14±0.03 ^b	6.09±0.05 ^b
	30	8.74±0.19 ^d	7.43±0.07 ^c	7.35±0.05 ^c	7.22±0.02 ^c
	40	6.36±0.08 ^b	6.27±0.10 ^b	4.34±0.13 ^a	4.07±0.06 ^a
Overall acceptability	10	4.47±0.17 ^a	4.21±0.15 ^a	4.47±0.17 ^a	5.69±0.20 ^b
	20	7.19±0.20 ^c	5.65±0.30 ^b	5.69±0.20 ^b	7.15±0.03 ^c
	30	8.51±0.04 ^d	7.21±0.04 ^c	7.18±0.01 ^c	7.10±0.04 ^c
	40	6.15±0.14 ^b	4.15±0.14 ^a	4.15±0.14 ^a	4.11±0.07 ^a

All values are means of triplicate determinations ± SD. Means with different letters a, b, c, d indicates significant result (p<0.05). Same letter means no significant difference.

4. Conclusion

Results suggest that prolamin extraction (A₅) contributed high protein content but its concentrated protein was very low where it is commercially not feasible. Apart from prolamin, simply use 7% α-amylase produced high protein content with reasonable concentrated protein. Therefore, 7% α-amylase may be recommended for extraction of protein from cereal crops. In case of cooking, 100±1 °C for 30 min cooking retained more amino acid and other nutrient compositions than others. This technique may apply by the animal experimental researchers those would like to conduct

research on cereals protein and their effects on liver injury, type-2 diabetes, coronary heart disease, cancer as well as all kinds of CVDs. Side by side agro-processors may apply to make the value add products as well as protein enrich products especially for the bakery and dietary products. The high protein products may contribute to reduce the body weight and biochemical markers such as serum enzyme activities AST, ALT and LDH and Cholesterols (LDL-C, TC and TG).

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