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The Influence of Debittering and Desalting on Defatted Foxtail Millet (*Setaria italica* L.) Protein Hydrolysate

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ABSTRACT

In this study, we examined the influence of Macroporous Adsorption Resin (MAR) in simultaneous desalting and debittering of defatted foxtail millet protein hydrolysate (DFMPH), analyzed their amino acid content, molecular weight distribution, functional and organoleptic properties. The DFMPH were obtained by hydrolysis using Alcalase 2.4 L with a degree of hydrolysis of 27%. MAR DA 201-C was used to desalt the DFMPH. The ash was removed by adsorbing DFMPH onto MAR. Desorption was achieved by washing with alcohol at different concentrations. Ash content of the DFMPH was reduced from 10.11 to 2.11% ranges. The protein content increased from 86.84 to 96.76, 95.74 and 92.31 for the various fractions 30, 55 and 70%, respectively with a significant different ($p<0.05$). The bitter taste was reduced to slightly detectable levels. The essential amino acids were above the recommended amount of Food Agricultural organization/World Health Organization for humans. The hydrolysates and the fractions have a molecular weight between 60 and 9000 Da, maximum solubility of 91, 97, 93 and 95% at pH 12.0 and were able to form very low viscosity solutions even at high concentrations, for 30, 55 and 70%, respectively. The functional properties studied exhibited good qualities that make them acceptable for use in such applications as hypoallergenic infant formulas, sport nutrition and functional foods. DFMPH and the fractions are potential as functional food ingredient. The functional properties studied exhibited good qualities that make them acceptable for use in such applications as hypoallergenic infant formulas, sport nutrition and functional foods.

Key words: Foxtail millet, hydrolyzed protein, amino acid, molecular weight, functional properties

INTRODUCTION

Millets typically contain higher quantities of essential amino acids and are higher in fat content than maize, rice and sorghum (Kamara *et al*., 2009a). Millet contains 12.3% crude protein, 3.3% minerals 72% of carbohydrate which is thee main components of millet that include starch, protein, lipid, vitamins and minerals (Kamara *et al*., 2009a). Foxtail millet (*Setaria italica* L.) is also known as Italian millet and is one of the world's oldest cultivated crops. In the Northern area of China it has been widely used as a nourishing gruel or soup for pregnant and nursing women and has been applied in food therapy.

Functional properties of plant proteins have been exploited in a multitude of applications (for example, solubility in beverages, foaming in whipped toppings and emulsification in processed meat) resulting in an ever increasing demand for plant protein ingredients with improved processing and functional characteristics (Kamara *et al*., 2009b).

Enzymatic treatment is a particularly attractive technique to modify proteins due to the milder process conditions required, the relative ease to control the reaction and minimal formation of by-products (Mannheim and Cheryan, 1992). It has been widely used to improve the functional properties of proteins, such as solubility, emulsification, gelation, water and fat-holding capacities and foaming ability and to tailor the functionality of certain proteins to meet specific needs (Kim *et al*., 1990; Panyam and Kilara, 1996).

Extensive hydrolysis could have a negative impact on the functional properties (Kristinsson and Rasco, 2000; Qian *et al*., 2010). However, enzymatic hydrolysis also introduces undesirable attributes to the products. Among these, bitterness is one of the most objectionable characters. Bitterness has been the major limitation in utilizing protein hydrolysates in various applications, particularly in beverages. Enzymatic hydrolysis of proteins at or above neutral pH releases hydronium ions (H_3O^+) that cause a drop in pH, which if allowed to decline unabated may influence the enzyme ionization properties and consequently, its catalytic ability leading to denaturing. Likewise, the substrate susceptibility to enzyme hydrolysis is influenced by the pH. There is, therefore, need for the pH to be regulated, hence the wisdom of the pH-stat method (Adler-Nissen, 1986; Jamel, 1992).

Protein purification is an art which has been refined over the last four decades such that excellent techniques are now available that simplify or enhance the recovery and homogeneity of protein products in relatively short period of time. In protein purification, it is common to reach a desired purity acceptable for product consumption. Various techniques have been used. Traditionally, desalting of large biomolecules is performed using dialysis, which is slow besides requiring large buffer volumes. Additionally, material loses have been reported as a result of the protein adsorption to the dialysis membranes (Cuartas *et al*., 2004).

Proteins have been desalted using either nanofiltration membranes or gel permeation chromatography using the desalting Sephadex™ gels which are expensive (Cuartas *et al*., 2004). Desalting and debittering of defatted foxtail millet protein hydrolysate (DFMPH) enhances their value-added qualities as well as processing safety into the product because of consumer sensitivity.

Macroporous Adsorption Resin (MAR) have been used for desalting biological samples and protein hydrolysates with good hydrolysate recoveries. MAR is a non-polar adsorbent resin used mainly for adsorption of organic substances and decolourisation (Zhao *et al*., 2002; Wasswa *et al*., 2007; Cheison *et al*., 2007). It is important to select a desalting process which is simple and easy to operate. While peptides bitterness is of both academic and technological interest, no reports exist on desalting of defatted foxtail millet protein hydrolysate on MAR, nor are there any reports of debittering with the same sample. Selective extraction of the bitter fragments yields a product with acceptable sensory properties making it easy to use in such applications as hypoallergenic infant formulas, sport nutrition and functional foods (Meisel, 1997; Exl, 2001; Manninen, 2004; Mahmoud, 1994; Clemente, 2000). In this study, we examine the influence of MAR in simultaneous desalting and debittering of defatted foxtail millet protein hydrolysate, analyzed their functional properties, molecular weight distribution, amino acid content and organoleptic properties.

MATERIALS AND METHODS

Foxtail millet was purchased from a local market in Wuxi, People's Republic of China. The seeds were milled using a laboratory scale hammer miller and the resulting flour was sieved through a 60 mesh screen. The Foxtail Millet Flour (FMF) was dispersed in hexane at flour to hexane ratio of 1:5 (w/v) and stirred for 4 h at room temperature. The experiment was repeated twice as described above. The hexane was decanted and the DFMF was air dry for 24 h under a vacuum drier and stored at 5°C in sealed glass jars until used. This research was conducted in the School of Food Science and Technology and State Key Laboratory of Jiangnan University, Wuxi from November 2009 to January 2010.

Protein hydrolysates were made and evaluated using a range of food grade enzymes. The enzymes tested (Novo Nordisk's Enzyme Business, Beijing, China) were Alcalase 2.4 L endonuclease from *Bacillus subtilis* with specific activity of 2.4 AU g^{-1}; Favourzyme from *Aspergillus oryzae* with activity of 500 LAPU g^{-1}; Neutrase from *Bacillus subtilis* strain with activity of 1.5 AU g^{-1}; Protamex, a Bacillus protease complex with activity of 1.5 AU g^{-1} and Papain powder (Sigma, China) with 2.1 AU g^{-1} activity. The crude papain powder from papaya fruit was not totally soluble. It was extracted (16 mg mL^{-1}) with 0.05 M sodium borate buffer (pH 8.3) and the insoluble material was removed by centrifugation at 11.500x g for 10 min at 4°C with a ZOPR-52D refrigerated centrifuge (Hitachi Koki Co Ltd, Tokyo, Japan). A styrene-based Macroporous Adsorption Resin (MAR), branded DA201-C was got from Jiangsu Suqing Water Treatment Engineering Group (Jiang-ying, Jiangsu, China). All other chemicals and reagents were obtained from a local manufacturer (Sinopharm Chemical Reagent Co., Ltd. (SCRC) Shanghai, China. Table 1 was available at the university chemical store and all chemicals used in the experiments were of analytical grade.

Proteolysis with different enzymes: DFMF was hydrolyzed with five different enzymes, under the conditions given in Table 2, based on optimum hydrolysis conditions. One hundred grams of vessel immersed in a water bath maintained at appropriate temperature and 700 mL; of distilled water was added to make a suspension. The suspension was, for each enzyme, adjusted to appropriate pH and preheated to appropriate temperature; then (1%) enzyme substrate ratio was added with continuous stirring. Hydrolysis was carried out for 9 h. 75 mL aliquots were taken after 60, 120, 180, 240, 300, 360, 420, 480 and 540 min and each hydrolysate was centrifuged at 11500x g for 10 min at 4°C with a D-3756 Osterode am Harz model 4515 Centrifuge (Sigma, Germany). The supernatant was carefully decanted and immediately heated for 5 min in a boiling water bath to inactivate the enzymes. Heat inactivation followed centrifugation to prevent gelatinization of starch. The defatted foxtail millet hydrolysate was lyophilized and stored at -20°C until used. All the experiments were performed in triplicate and the results are the average of three values.

Table 1: DA201-C macroporous adsorption resin properties

Properties	Values
Polarity	None
Pearl size	0.4-1.25 mm
Average pore diameter	30-40 nm
Surface area	1000-1300 m^2 g^{-1}
Pore volume	1.0-1.1 cm^3 g^{-1}

This data is supplied with product in producer's manual manufactured from styrene based material

Table 2: Optimum conditions for hydrolysis of DFMF with different proteases

	Reaction conditions	
Proteases	pH	Temperature (°C)
Alcalase 2.4 L (AU g^{-1})*	8.0	50
Protamex 1.5 (AU g^{-1})	7.0	40
Neutrase 0.5 (AU g^{-1})	7.0	55
Papain 2.1 (AU g^{-1})	8.0	55
Flavourzyme 500 (LAPU g^{-1})†	7.0	50

*AU (Anson units) is the amount of enzyme that under standard conditions digests hemoglobin at an initial rate that produces an amount of trichloroacetic acid-soluble product which gives the same color with the Filon reagent as one milliequivalent of tyrosine released min^{-1}. †LAPU (Leucine aminopeptidase unit) is the amount of enzyme that hydrolyzes 1 μmol of leucine-p-nitroanilide min^{-1}

Degree of hydrolysis (pH-stat assay): The degree of hydrolysis (DH), defined as the percent ratio of the number of peptide bonds broken (h) to the total number of bonds per unit weight (h_{tot}), in each case, was calculated from the amount of base consumed (Adler-Nissen, 1986), as given below:

$$DDH\ (\%) = \frac{V_b \times N_b}{\alpha \times mP \times h_{tot}} \times 100 \quad (1)$$

where, V_b is base consumption in mL; N_b is normality of the base; α is average degree of dissociation of the α-NH_2 groups; mP is mass of protein (N×6.25) in g; and h_{tot} is total number of peptide bonds in the protein substrate. Approximate value of 9.2 meqv g^{-1} was used. All the experiments were performed in triplicate and the results are the average of three values

Batch debittering and desalting in a beaker: The debittering and desalting of the DFMPH was done in a beaker since this procedure is more efficient and done within a short duration. The DFMPH was allowed to be absorbed onto the MAR by stirring 1.0 L of the DFMPH supernatant liquid with 500 mL of MAR for 24 h using a mechanical stirrer. After the absorption, the content was allowed to settle and the top layer skimmed off. The MAR was washed with five-bed volumes of deionized water with stirring using a mechanical stirrer. After washing the MAR with deionized water, it was further washed with three different concentrations of alcohol in order to desorb the peptides.

Desorption with alcohol: Step-wise desorption was done by washing with alcohol at different concentrations. The Alcohol Concentrations (ALC) varied from 30, 55 and 70%, followed by deionised water. The collected fractions were concentrated under vacuum and freeze-dried. The resin was regenerated by washing it with 1 mol L^{-1} NaOH followed by 1 mol L^{-1} HCl and thoroughly rinsed with deionized water until neutral pH. This was to ensure that the peptides were properly washed of the resin.

Proximate analysis: The proximate analysis of defatted foxtail millet protein hydrolysate (DFMPH) and the desalted and desorbed hydrolysates were determined according to James (1995). The moisture content was determined by drying in an oven at 105°C until a constant weight was

obtained. Ash was determined by weighing the incinerated residue obtained at 525°C after 4 h. The crude protein was determined by the micro-Kjeldahl method and a Conversion factor of N × 6.25 was used to quantify the crude protein content (Tkachuk, 1969).

Amino acid analysis: The dried samples were digested with HCl (6 M) at 110°C for 24 h under nitrogen atmosphere. Reversed phase high performance liquid chromatography (RP-HPLC) analysis was carried out in an Agilent 1100 (Agilent Technologies, Palo Alto, CA, USA) assembly system after precolumn derivatization with o-phthaldialdehyde (OPA). Each sample (1 μL) was injected on a Zorbax 80 A C18 column (i.d. 4.6 × 180 mm, Agilent Technologies, Palo Alto, CA, USA) at 40°C with detection at 338 nm. Mobile phase A was 7.35 mM L^{-1} sodium acetate/ triethylamine/tetrahydrofuran (500:0.12:2.5, v/v/v), adjusted to pH 7.2 with acetic acid, while mobile phase B (pH 7.2) was 7.35 mM L^{-1} sodium acetate/methanol/acetonitrile (1:2:2, v/v/v). The amino acid composition was expressed as g of amino acid per 100 g of protein.

Determination of Molecular Weight (MW): Molecular weight distributions were determined by gel permeation chromatography (GPC) by using a high-performance liquid chromatography (HPLC) system (waters 600, USA). A TSK gel 2000 SW_{XL} column (7.8 i.d.×300 mm, Tosoh, Tokyo, Japan) was equilibrated with 45% acetonitrile (v/v) in the presence of 0.1% trifluoroacetic acid. The hydrolysates (100 μg μL^{-1}) were applied to the column and eluted at a flow rate of 0.5 mL min^{-1} and monitored at 220 nm at room temperature. A molecular weight calibration curve was prepared from the average retention time of the following standards obtained from (Sigma, Germany: cytochrome C (12500 Da), aprotinin (6500 Da), bacitracin (1450 Da) and tripeptide GGG.

Nitrogen solubility: Nitrogen solubility was determined according to the procedure of Bera and Mukherjee (1989), with slight modification. One hundred mg of the various samples were dispersed in 10 mL of distilled deionized water. The suspensions were adjusted to pH 2.0 to 12.0 using either 0.1 M HCl or 0.1 M NaOH. These suspensions were shaken (Lab-Line Environ-Shaker; Lab-Line Instrument, Inc., Melrose Park, Ill., USA) for 30 min at room temperature (approximately 25°C) and centrifuged at 4000x g for 30 min. The protein content of the supernatant was determined by the Kjeldahl method and percent protein solubility was calculated as follows:

$$\text{Protein solubility (\%)} = \frac{\text{PS (mg)}}{\text{PIS (g)}} \times 100 \quad (2)$$

Where:
PS = Amount of protein in supernatant
PIS = Protein in initial sample

All the experiments were performed in triplicate and the results are the average of three values.

***In vitro* digestibility by trypsin:** *In vitro* digestibility was carried out according to the method described by Elkhalil *et al.* (2001), with slight modification. Twenty milligram of protein hydrolysate samples in triplicate were digested in 10 mL of trypsin (0.2 mg L^{-1} in 100 mM Tris-HCl buffer, pH 7.6). The suspension was incubated at 37°C for 2 h. Hydrolysis was stopped by adding 5 mL

50% Trichloroacetic Acid (TCA). The mixture was allowed to stand for 30 min at 4°C and was then centrifuged at 9500 × g for 30 min using a D-3756 Osterode am Harz model 4515 Centrifuge (Sigma, Germany). The resultant precipitate was dissolved in 5 mL of NaOH and protein was measured using the Kjeldahl method. Digestibility was calculated as follows.

$$\text{Protein digestibility (\%)} = \frac{(A - B)}{A} \times 100 \quad (3)$$

Where:
A = Total protein content (mg) in the sample
B = Total protein content (mg) in TCA precipitate

All the experiments were performed in triplicate and the results are the average of three values.

Colour measurements: The colour of the hydrolysate powder was evaluated using the Hunter Lab colorimeter (WSC-S Colour Difference Meter, USA) and reported as L*, a* and b* values, in which L* is a measure of lightness, a* represents the chromatic scale from green to red and b* represents the chromatic scale from blue to yellow. The instrument was standardized to measure the colour difference with an L* value of 91.32, a* value of 0.03 and a b* value of 0.01. All the experiments were performed in triplicate and the results are the average of three values

Viscosity: Apparent viscosity of aqueous solutions of the three products got from the three levels of alcohol concentrations was estimated on a 30-40 mL of protein solution using NDJ-79 Viscometer (Shanghai, China). All the experiments were performed in triplicate and the results are the average of three values.

Gelation properties: Gelation properties were determined by the method of Obatolu and Cole (2000), with slight modifications. The fractions and DFMPH were determined on a 5 mL test tube of each hydrolysate sample suspension in deionised water at pH 7.0 and protein concentrations varying from 2 to 20% (w/v) with increments for all the three products.

Sensory evaluation: In this study, the nine-point hedonic scale according to the method of Sheppard (2006) was used to evaluate the bitterness in defatted millet protein hydrolysates powder, was conducted by 20 panelists.

Statistical analysis: Data and Statistical Analysis of Variance (ANOVA) was performed and differences in mean values were evaluated by Tukey`s test at $p<0.05$ using SPSS version 18.0 (SPSS Inc, Chicago, IL, USA).

RESULTS AND DISCUSSION

Enzymatic hydrolysis: The treatment of DFMF with Alcalase 2.4 L showed the highest increase in protein content of DFMPH during the first 300 min of hydrolysis 86.84%. The amount of proteins released decreased slightly to 76.21% for the remaining hydrolyzing period up to 540 min (Fig. 1). Flavourzyme solubilized 73.28% of protein during the first 300 min of hydrolysis and the amount

solubilized increased to 78.92% with longer hydrolyzing periods up to 540 min. Neutrase showed an increase in protein solubilization during the first 300 min of hydrolysis with 67.52% of the protein being solubilized. The treatment of DFMF with Papain showed increased solubilization of protein during the first 300 min of hydrolysis and reached 62.78% of protein. Later the amount of protein released decreased moderately to 57.74%. After 300 min, Protamex was able to solubilize 58.51% of the total protein and the amount solubilized increased to 60.74% with longer hydrolyzing periods up to 540 min (Fig. 1). The results of our study exhibited a behavior that is, similar to Betancur-Ancona *et al.* (2009). The high efficiency of Alcalase 2.4 L and Flavourzyme may be a result of a high frequency of potential cleavable sites in DFMPH which may have contributed to the high degree of solubilization. Alcalase 2.4 L was selected for the current study because of its high yield under optimum conditions, readily available, cost effectiveness and ease of handing.

Degree of hydrolysis (DH): The enzymatic hydrolysis DFMF processed with Alcalase 2.4 L (Fig. 2), exhibited a behavior that is similar to Adler-Nissen (1986). Hydrolysis with proteases at percentage 1% (enzyme to substrate ratio) developed rapidly in early reaction stage, as shown in by the rise in DH and then decreased in the rise. The reaction was asymptotic 60 min after

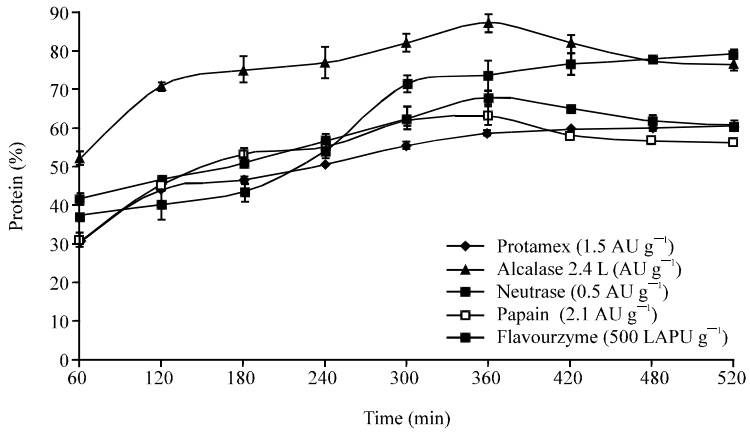

Fig. 1: Amount of protein solubilized by enzymatic hydrolysis of defatted foxtail millet flour (DFMF) by different proteases. Value represent the Mean±SD of n = 3 duplicate assays

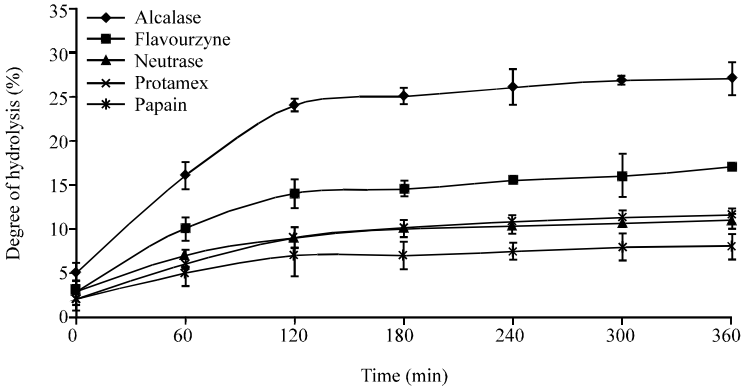

Fig. 2: Enzymatic progress curves of hydrolysis of defatted foxtail millet protein hydrolysate (DFMPH) using different enzymes. Value represent the Mean±SD of n = 3 duplicate assays

hydrolysis began. In the first 240 min it reached 26, 15.5, 10.3, 10.8 and 7.4% DH for Alcalase, Flavourzyme, Neutrase, Protamex and Papain respectively, indicating that enzymatic preparation reacted rapidly, though hydrolysis increased only gradually during the remaining reaction, eventually reaching 27, 17, 11, 11.5 and 8% DH, respectively when finished. Similar behavior was observed by Kim *et al.* (1990).

Desalting of DFMPH: The MAR properties are shown in Table 1. The peptides were desorbed from MAR using 30, 55 and 70% alcohol concentrations (ALC) following desalting meaning the peptides hydrophobicities were different. Desorption of DFMPH peptides from the MAR was achieved at all the three levels of ALC after the resin was rinsed with deionised water. The result shows that the interaction between the resin and the DFMPH is indeed hydrophobic in nature,

Table 3: Summary of total amino acids composition of desorbed fractions showing content of essential amino acid, hydrophobic and hydrophilic amino acids composition (g/100 g protein)

					FAO/WHO/UNU[a]	
					---	---
Acids	DFMPH	ALC 30%	ALC 55%	ALC 70%	Child	Adult
Essential amino acids						
Isoleucine (Ile)	3.73	3.01	3.56	3.76	3.00	3.0
Leucine (Leu)	11.62	10.42	13.64	14.04	6.00	5.9
Lysine (lys)	0.84	0.90	0.85	0.81	4.80	4.5
Methionine (Met)	2.41	3.29	3.41	3.24		
Met + Cys					2.30[b]	1.6[b]
Phenylalanine (Phe)	5.31	2.84	5.01	5.16		
Phe + Tyr					4.10[c]	3.8[c]
Threonine (Thr)	3.26	3.64	3.27	3.82	2.50	2.3
Valine (Val)	4.60	2.93	3.67	3.31	2.90	3.9
Histidine (His)	2.31	5.39	2.31	2.04	1.60	1.5
Tryptophan (Trp)	3.11	1.69	2.25	2.12	0.66	0.6
Nonessential amino acids						
Alanine (Ala)	10.22	9.12	10.90	12.99		
Arginine (Arg)	7.85	9.14	8.00	7.35		
Aspartic acid (Asp)[d]	5.92	6.92	6.25	5.70		
Cysteine (Cys)[e]	0.95	0.84	0.72	0.72		
Glutamic Acid (Glu)[f]	22.12	26.55	25.01	23.87		
Glycine (Gly)	1.95	2.39	2.36	2.06		
Serine (Ser)	6.63	6.17	7.74	7.05		
Tyrosine (Tyr)	2.88	1.37	2.46	2.64		
Proline (Pro)	10.56	4.94	6.70	9.84		
[g]Total EAA		34.11	38.17	38.29		
[h]Hydrophobic AA		37.92	49.35	53.98		
[i]Hydrophilic AA		59.71	53.43	51.29		
Sensory properties	Completely bitter	Not bitter	Slightly bitter	Significantly bitter		

[a]Suggested profile of essential amino acid requirement for infant and adult (WHO., 2007); [b]Requirements for methionine + cysteine. [c]Requirements for phenylalanine + tyrosine. [d]Aspartic acid + asparagines. [e]Cysteine + cystine. [f]Glutamin acid + glutamine; [g]Total EAA = Total essential amino acids. [h]Hydrophobic amino acids (Alanine, Isoleucine, Leucine, Methionine, Phenylalanine, Proline, Tyrosine and Valine). [i]Hydrophilic amino acids (Histidine, Lysine, Arganine, Glutamic acid, Aspartic acid, Threonine and Serine)

because even though alcohol has both hydrophobic and hydrophilic zones, the hydrophobic zone was in greater part. The non-polar amino acid residues had no contact with the water while the polar side chains pointed out towards the water molecules (Cheison *et al*., 2007). In that light, it is suffice to state that the DFMPH interacted with the resins hydrophobically to achieve a favourable configuration during the debittering, desalting and rinsing processes.

Proximate analysis: The proximate analysis data for the desorbed fractions lyophilisates are shown in (Table 5), which shows significant different ($p<0.05$) in moisture and ash contents of DFMPH and the desalted fractions. Likewise, the protein contents in the desalted fractions were enriched from 86.84% (DFMPH) to 96.77, 95.75 and 92.42% of 30, 55 and 70% ALC, respectively, which were significantly different ($p<0.05$) from each other. The results in Table 5 are within the values reported by Cheison *et al*. (2007). The increase in the protein quantity could be attributed to the mixing during the debittering and desalting process as it is likely that more protein could have been released during desalting and debittering.

Total amino acids content of the desorbed fractions: The content of amino acids in the fractions obtained from the alcohol fractionation, Table 3, showed slight different in their content of hydrophobic (and essential including tryptophan) as well as hydrophilic amino acids. Thirty percent fraction had the lowest while fraction 70% had the highest content of hydrophobic and essential amino acids and our results corroboted with Zhang *et al*. (2009) and Cheison *et al*. (2007). The separation with various alcohol concentrations for desorption was achieved owing to the different in the content of hydrophobic amino acid which make up the peptides. The 70% Fraction contained the highest amount of hydrophobic amino acids (Table 3) and hence required higher alcohol concentration to disrupt the hydrophobic interactive forces between the hydrolysates and the resin. Conversely, 30% fraction with the least hydrophobic amino acids and hence poor interaction forces was desorbed with lower alcohol concentration, the results are within the ranged reported by Wasswa *et al*. (2007) and Zhang *et al*. (2009).

Molecular weight distributions: The molecular weight distributions of the various fractions were determined by SE-HPLC. The molecular weights for all samples were calculated according to the standard equation below:

$$\text{Log Mol } 6.77\text{-}0.217T, (R^2 = 0.991642) \quad (4)$$

Results in and Table 4, show that the molecular weight distribution of different fractions (30, 55 and 70%), have similar molecular weight distributions indicating that polyptides produced from the bittering and desalting have comparatively smaller molecular weight distributions. There was significant influence of the ALC on the fractions (Table 4). Similar observation was made by Zhang *et al*. (2009). The MW distributions are between 60 and 9000 Da for the various (Table 4).

Protein solubility: An increase in the extent of enzymatic hydrolysis corresponded to an increase in the nitrogen solubility, over the pH range studied, indicating a positive relationship (Fig. 3). It has been suggested that an increase in the solubility of protein hydrolysates over that of the

Table 4: Molecular weight distribution profile of the DFMPH and desalted fractions

	Peak area (%)			
Molecular weight (Da)	DFMPH	ALC30%	ALC55%	ALC70%
>9000	1.08	-	-	-
1040-9000	10. 16	6.94	8.11	6.71
420-1040	18.11	18.25	21.52	20.99
99-420	70.66	73.82	69.31	72.32
< 60	-	0.98	1.05	0.98

ALC: Alcohol concentration; DFMPH: defatted foxtail millet protein hydrolysate

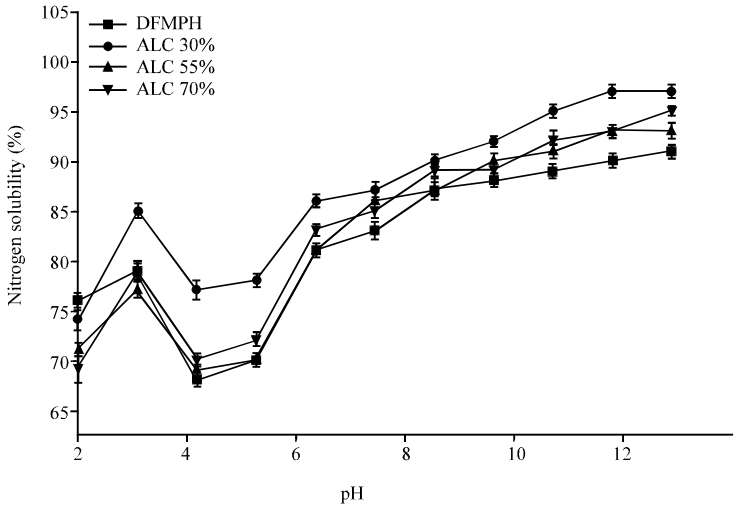

Fig. 3: Effect of pH on nitrogen solubility of DFMPH and the various alcohol concentrations fractions

original protein is due to the reduction of its secondary structure and also to the enzymatic release of smaller polypeptide units from the protein (Adler-Nissen, 1986; Chobert *et al.*, 1988).

At pH 4.0, near the isoelectric point at which the net charge of the original protein is minimized and consequently more protein-protein interactions and fewer protein-water interactions occur (Adler-Nissen, 1976; Chobert *et al.*, 1988). Above pH 6.0, the nitrogen solubilities increased rapidly with an increase in pH up to 12.0. These trends in solubilities are in agreement with Tang *et al.* (2003) and Chandi and Sogi (2007). At pH 12.0, the solubility of 30, 55 and 70% fraction reached 97, 93 and 95%, respectively and while solubility for DFMPH was 91% at pH 12.0 (Fig. 3).

***In vitro* protein digestibility:** The *in vitro* digestibility of DFMPH and the fractions were evaluated by TCA-soluble nitrogen release during digestion of trypsin. Table 5 shows a typical profile of the nitrogen release of DFMPH and ALC trypsin digestion. The fractions were more easily digested than DFMPH. The fractions and DFMPH have digestibility values with trypsin of 87.62, 85.76, 85.04 and 83.27% for 30, 55, 70% fractions and DFMPH, respectively and they where significantly different ($p<0.05$). However, our results are in agreement with Van der Plancken *et al.* (2003) and Kamara *et al.* (2009a). The unfolding of the native protein structure during the cause of hydrolysis is yet another factor that likely facilitates digestibility (Van der Plancken *et al.*, 2003; Kamara *et al.*, 2009a).

Table 5: Hunter colour parameter values of hydrolysate from the different alcohol concentration, proximate analysis and *in vitro* protein digestibility

	Sample		Code	
Hunter color parameters	DFMPH	ALC 30%	ALC 55%	ALC 70%
L*	63.13±0.13	56.79±0.17	51.38±0.54	60.47±0.28
a*	-3.98±0.02	-2.59±0.08	-1.92±0.09	-3.04±0.06
b*	22.53±0.37	29.59±0.36	27.68±0.17	23.87±0.18
Proximate analysis				
Protein content	86.84± 0.55	96.77±0.24	95.75±1.10	92.42±0.53
Ash	10.38±0.56	2.10±0.06	2.56±0.39	3.53±0.40
Moisture	2.90±0.03	2.12±0.10	1.94±0.08	2.55±0.21
In vitro protein digestibility	83.27±0.49	87.62±0.36	85.76±0.89	85.04±0.26

Values are Mean±SD of three determinations; ALC: Alcohol concentration; DFMPH: Defatted foxtail millet protein hydrolysate; L* Measure of lightness, a* Chronic scale from green (-a) to red (+ a), b* Chronic scale from blue (-b) to yellow (+ b)

Colour measurement analysis: Colour influences the overall acceptability of any food products (Papadakis *et al*., 2000). Debittering with alcohol produced protein powders that were light yellow in colour (Table 5). Thirty percent fraction was the darkest (L* = 56.79) and most yellowish (b* = 29.59) whereas 55% fraction was the lightest (L* = 60.47) and least yellowish (b* = 23.87). The L* value was significantly different ($p<0.05$) for all fractions (Table 5). Moreover, the results of this study corroboted with data reported by Wasswa *et al*. (2007).

Viscosity: Viscosity is one of the most important functional properties of food proteins. It is important for providing physical stability to emulsions (Cho *et al*., 2004). The concentrations, molecular weight' polydispersity, hydrophobicity and conformation of each protein species affect the viscosity of the solution. All of these factors tend to confound the underlying inverse relationship of protein solubility and viscosity (Schenz and Morr, 1996). Processing induced changes in proteins such as polymerization, aggregation and hydrolysis affect the viscosity of food products. The apparent viscosity of aqueous solutions of DFMPH with different alcohol concentration as a function of protein is displayed in Fig. 4.

From the results, it is obvious that the various fractions were able to form very low viscosity solutions even at high concentrations (Fig. 4). The low viscosity of protein even at high concentrations may be useful in the development of high protein soft drinks and juice-based beverages without suffering the adverse consequences of high viscosity (Frokjaer, 1994; Sekul and Ory, 1977).

Gelation properties: Gelation properties of the hydrolysates from the four products were slightly different but they have some common trend Table 6. As shown in the results, the fraction from 30% fraction did not fall from the inverted test tubes from 6 to 20% protein concentration. Similar observation was made for 55% fraction; it started slipping out from the test tube at 18% concentration. But a different scenario occurred for 70% fraction where in the sample slipped out at the lowest concentration. Present results are contrary to Yu *et al*. (2007). It could be attributed to the enzyme used for the hydrolysis, as Alcalase 2.4 L is an endopeptidase with a broad specificity to hydrophobic amino acids (Yu *et al*., 2007).

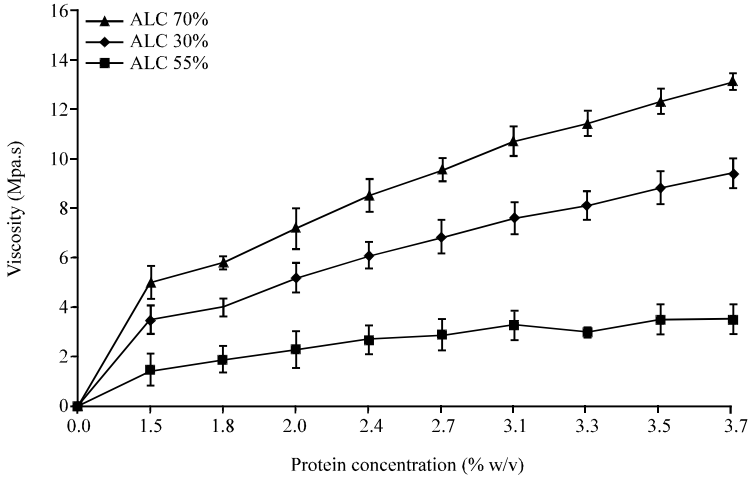

Fig. 4: Apparent viscosity of DFMPH and the various alcohol concentrations fractions. Value represent the Mean±SD of n = 3 duplicate assays

Table 6: Gelation properties of the defatted foxtail millet protein hydrolysate from different alcohol concentrations

	Protein concentration % (w/v)									
Samples	2	4	6	8	10	12	14	16	18	20
DFMPH	s	s	s	s	s	s	s	ss	ss	ss
ALC 30	ss	ss	ss	ss	ss	ss	ss	ss	s	s
ALC 55	s	s	s	ss	ss	ss	ss	ss	s	s
ALC 70	s	s	s	ss	ss	ss	ss	st	st	st

s: Slipped from inverted test tube; ss: Sample did not slip from inverted test tube; st: Slight turbidity observed; ALC: Alcohol concentration; DFMPH: Defatted foxtail millet protein hydrolysate

Sensory evaluation: A general acknowledged problem encountered in the use of enzymatic hydrolysis for modification of food proteins is the formation of bitter taste. The bitter taste can be ascribed to hydrophobic peptides and results from the degradation of the protein substrate.

The desorbtion of the hydrolysates from the MAR was done with 30, 55 and 70% fractions but 30% of ALC was observed to have extracted the bitterness from DFMPH and the final product was not bitter while 55% fraction was slightly bitter and 70% fraction was completely bitter (Table 3). Nonetheless, our results are similar to the data reported by Wasswa *et al.* (2007) and Zhang *et al.* (2009). The bitter taste in the DFMPH can be attributed to highly hydrophobic, short peptides composed largely of a good supply of essential amino acids (Kanekanian *et al.*, 2000).

CONCLUSIONS

Understanding change of foxtail millet flour complex during enzymatic hydrolysis can be useful for producing modified proteins with the desired functionality. The present results showed that the bitter and salty taste can be removed with adsorption of DFMPH on MAR followed by rinsing with deionised water to wash out the salt during which instance the peptides remained adsorb onto the MAR resins. It also provides an exciting technological manipulation to reduce bitter and salty taste. MAR, therefore, present technological importance to remove salt in protein hydrolysates. The alcohol used could be recovered and reduced cutting down the process costs. From the results

presented here, it is proposed that excellent solubility of the protein hydrolysates could be attributed to reduce size of the polypeptides obtained after debittering and desalting. The DFMPH after desalting and debittering process generally had a lower molecular weight but with no significant different between the fractions. There was also an improvement in the functional properties studied. This could be incorporated into the foods for human consumption making them potential competitors with dairy based and plant based protein hydrolysates currently being used. The results of this study could hold a prospecting future in the food industries.

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