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The Debittering and Desalting of Defatted Sesame Protein Hydrolysate using a Macroporous Resin and an Assessment of its Bioactive and Functional Properties

[1,2,3]P.J. Kanu, [4]J.B.A. Kandeh, [4]P.M.P. Mornya, [2]J.B. Kanu,
[5]E.H. Sandy and [1,2]Z. Huiming
[1]State Key Laboratory of Food Science and Technology,
Jiangnan University, 1800 Lihu Avenue, Wuxi-214122,
Jiangsu Province, People's Republic of China
[2]School of Food Science and Technology, Jiangnan University,
1800 Lihu Avenue, Wuxi-214122, Jiangsu Province, People's Republic of China
[3]Milton Margai College of Education and Technology-An Affiliate of Njala University,
Goderich Campus, Freetown, Sierra Leone
[4]Njala University, Sierra Leone
[5]Department of Chemistry, Fourah Bay College, University of Sierra Leone, Sierra Leone

Abstract: The bitter and salty tastes were removed from Defatted Sesame Protein Hydrlysates (DSPH) using a macroporous resin (MAR) and different concentrations of alcohol. The MAR was used to absorb the DSPH and three levels of Alcohol Concentration (AC) (25, 50 and 60%) were used to desorb the DSPH. The DSPH desorbed with AC 25% was tasteless, DSPH desorbed with 50% AC shows mild bitterness but not salty, DSPH desorbed with 60% AC was significantly bitter but not salty. The ash content was significantly lower in the DSPH after the debittering and desalting process. But an increase in the protein content of the debittered and desalted DSPH was observed for the two lower AC levels DSPH extracted. The DSPH from the three levels of AC were analyzed for bioactivity and functional properties. The product of 60% AC, with the highest content of hydrophobic peptides showed superior ACE inhibition with the lowest IC_{50}. The 25% AC extract followed by the 50% AC showed relatively weaker ACE inhibition. Nitrogen solubility of the hydrolysates obtained from the 50 and 60% AC extracts were pH dependent over the range studied. The essential amino acids were higher than recommended by FAO/WHO with the exception of Lysine. The solubility of extracts from 25, 50 and 60% AC was pH dependent as pH increases solubility increases, viscosity and gelation properties of 50 and 60% AC increased as the concentration of DSPH was increased.

Key words: Amino acid, bitter and salt tastes, functional properties, macroporous resin, defatted sesame protein hydrolysate

INTRODUCTION

Enzymatic hydrolysis of proteins in foods frequently results in bitter and salty tastes due to the formation of low molecular weight peptides that are mainly composed of hydrophobic amino acids (Saha and Hayashi, 2001). In consumer food products, protein purification processes are often done in order to attain the desired purity standards. Various techniques have been developed to achieve this

Corresponding Author: Zhou Huiming, State Key Laboratory of Food Science and Technology, Jiangnan University, 1800 Lihu Avenue, Wuxi-214122, Jiangsu Province, People's Republic of China
Tel: +86510 84946180 Fax: +86510 85329037

aim (FitzGerald and O'Cuinn, 2006). Finishing treatment methods include processes aimed at debittering and desalting protein hydrolysates achieved through selective separations such as treatment with activated carbon, isoelectric precipitation, chromatography on silica gel, masking of bitter taste and dialysis using biomolecules, gel permeation chromatography using the debittering and desalting Sephadex™ gels etc. Most of these techniques are slow, expensive and require the use of large buffer volumes (Kanekanian *et al*., 2000; Saha and Hayashi, 2001; Cuartas *et al*., 2004; FitzGerald and O'Cuinn, 2006). In addition, there are reports of significant material losses that result from protein adsorption on the dialysis membranes (Cuartas *et al*., 2004).

Macroporous resins (MARs) have been used for debittering and desalting biological samples, casein non-phosphorylated peptides and other protein hydrolysates with good hydrolysate recoveries (Zhao *et al*., 2002; Cheison *et al*., 2007). Visser *et al*. (1975) reported that alcohol provides a suitable medium for protein hydrolysates desorption after absorption in a debittering and desalting process. Macroporous resins are non-polar adsorbent resins used mainly for adsorption of organic substances and decolourisation (Zhao *et al*., 2002; Cheison *et al*., 2007).

Desalting and debittering of Defatted Sesame Protein Hydrolysate (DSPH) enhance its added-value quality as well as addressing food safety concerns arising from consumer sensitivity and attitude towards bitter and salty tastes in food formulations. Cheaper and efficient debittering and desalting options are therefore most valuable in reducing production costs as well ensuring high hydrolysate recoveries.

Peptide bitterness has attracted both research and industrial interests (Cho *et al*., 2004; Cheison *et al*., 2007) and one of the important physico-chemical and functional properties of protein hydrolysates is their solubility over a wide range of pH, nitrogen concentration and ionic conditions (Adler-Nissen, 1986; Kester and Richardson, 1984). The breakdown of proteins into peptides generally enhances the solubility properties of the resulting product (Chobert *et al*., 1996). Numerous studies have been undertaken for analyzing the functionalities of various oil seed protein products and the enzymatic food protein hydrolysates. Currently, there is a huge amount of literature in this regard (Moure *et al*., 2006; FitzGerald and O'Cuinn, 2006; Raksakulthai and Haard, 2003), however, much has not been done with regards the debittering or desalting of defatted sesame protein hydrolysate using MAR. Thus, the objective of this study is to investigate the application of MAR as an adsorbent in removing bitter and salty tastes from DSPH and use of various concentrations of alcohol (25, 50 and 60%) to simultaneously desorb the DSPH from the MAR. In addition, an assessment is carried out on the bioactivity, amino acid content, functional and organoleptic properties of the DSPH desorbed at the three concentrations of alcohol used.

MATERIALS AND METHODS

The research was conducted in Jiangnan University, Wuxi, People's Republic of China January-April, 2009. The defatted sesame protein hydrolysate was produced in our laboratory as described by Kanu *et al*. (2009) using the enzyme Alcalase (with a known activity of 2.4 AU kg^{-1} and density of 1.18 g mL^{-1}), a bacterial endoproteinase from a strain of *Bacillus licheniformis*, Enzyme/Substrate ratio of 1.0 and a protein recovery rate of 93.98%. The production temperature was 60°C at a pH of 9.0 over a period of 80 min. The degree of hydrolysis was 14.9%. The hydrolysate produced was bitter and salty.

A styrene-based Macroporous Adsorption Resin (MAR), branded DA201-C was kindly donated by the Jiangsu Suqing Water Treatment Engineering Group (Jiang-ying, Jiangsu, China). All other chemicals and analytical reagents were obtained from a local Sinopharm Chemical Reagent Co., Ltd. (SCRC), Shanghai, People's Republic of China.

Batch Debittering and Desalting in a Beaker

The debittering and desalting of the DSPH was done in a beaker, since this procedure is more efficient and done within a short duration according to the method Cho *et al.* (2004) as modified by Cheison *et al.* (2007). The DSPH was allowed to be absorbed onto the MAR by stirring 1.0 L of the DSPH 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 used by washing with alcohol at different concentrations this was done according to Cheison *et al.* (2007) with slight modifications. The alcohol concentrations varied from 25, 50 and 60%, 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 the DSPH from the three levels of AC for protein was determined using the Kjeldahl method as described by James (1995). Moisture content was determined by placing (2 g) of the DSPH obtained from each of the AC into a preweighed aluminum dish and dried in a forced-air convection oven at 105°C until a constant weight was obtained. Ash content was determined by combusting the samples in a muffle furnace at 550°C for 12 h as described by James (1995). The experiments were done in triplicates.

Molecular Weight Distribution

This was determined using a WatersTM 600E advanced protein purification system (Waters Corporation, Milford, MA, USA). A TSK gel 2000SWXL (7.8×300 mm) column was used with 10% acetonitrile and 0.1% TFA using an HPLC grade water as a mobile phase. The calibration curve was obtained by running bovine carbonic anhydrase (29,000 Da), horse heart cytochrome C (12,400 Da), bovine insulin (5800 kDa), bacitracin (1450 Da), gly-gly-tyr-arg (451 kDa) and gly-gly-gly (189 Da). The results obtained were analyzed with the aid of millennium 32 version 3.05 software (Waters Corporation, Milford, MA 01757, USA).

Measurement of Angiotensin-I Converting Enzyme (ACE) Inhibition Activity

The ACE inhibition activity assay was performed using the method of Cushman and Cheung (1971), with slight modifications. The reaction mixture contained 5 mM Hip-Leu as a substrate, 0.3 M NaCl and 5 mU enzymes in 50 mM sodium borate buffer at a pH of 8.3. A sample (50 μL for each of the alcohol extracts) was added to the above reaction mixture and mixed with 1 μmol Hip-His-Leu (150 μL) containing 0. 5 M NaCl. After incubation at 37°C for 60 min, the reaction was halted by the addition of 250 μL 1.0 N HCl. The resulting hippuric acid was extracted by the addition of 1.5 mL ethyl acetate. After centrifugation (800 x g,15 min), 1 mL of the upper layer was transferred into a glass tube and evaporated at room temperature for 2 h in vacuum. The hippuric acid was re-dissolved in 3.0 mL of distilled water and absorbance was measured at 228 nm using a U3210 spectrophotometer, USA. The IC_{50} value was defined as the concentration of inhibitor required to inhibit 50% of the ACE activity.

Total Amino Acids Analysis

The three levels of AC 25, 50 and 60% extracted samples (20 μg each) were dried in conventional hydrolysis tubes. To each tube 100 μL of 6 mol L^{-1} HCl containing 30 mL phenol and (10 mL) 2-mercaptoethanol (6 mol L^{-1} HPME) were added and the tubes were evacuated, sealed and hydrolyzed at 110°C for 22 h. After hydrolysis, the HCl was evaporated in a vacuum bottle heated to about 60°C. The residue was dissolved in the sample buffer and subjected to amino acid analysis using a post-column Derivatizing High-Performance Liquid Chromatographic (DHPLC), system (Shimadzu, Kyoto, Japan) consisting of a Shimadzu RF 10 Axl fluorescence detector, Shimadzu SCL- 10Avp controller with thermostated column area and a Shimadzu SIL-10ADvp autosampler, operated using CLASS-VP software (version 5.03). The column was a Shim-pack ISC-o7/S1504 Na with a flow rate of 0.6 mL min^{-1}. Excitation wavelength (Ex) at 348 nm and emission wavelength (Em) at 450 nm were chosen. The column oven was maintained at 60°C. The elution solvent systems were (A) 0.2 mol L^{-1} citrate buffer (pH 3.3), (B) 0.6 mol L^{-1} citrate/0.2mol L^{-1} boric buffer (pH 10) and (C) 0.2 mol L^{-1} NaOH. Amino acids were quantified by calculation from the recorded chromatogram.

For cystine determination, samples (50 μg of DSPH from AC 25, 50 and 60%) were first oxidized with 10 μL performic acid in an ice-water bath for 4 h. The mixtures were evaporated with a vacuum pump to remove performic acid before hydrolysis.

Tryptophan determination samples (100 μg of DSPH from AC 25, 50 and 60%) were dissolved with 50 μL of 4 mol L^{-1} methanesulfonic acid containing 2 mL tryptamine (4 mol L^{-1} MSA), evacuated and tightly closed. After hydrolysis at 110°C for 22 h, the reaction mixtures were neutralized with 45 μL of 4 mol L^{-1} NaOH. An aliquot of the mixture was diluted five-folds with sample buffer before the HPLC measurements.

Sensory Evaluation

In this study, a nine-point hedonic scale according to the method of Jakobsen (1949) was used to evaluate the bitterness, the nutty smell and after taste in the three fractions (AC 25, 50 and 60%) conducted by 50 expert panelists.

Nitrogen Solubility

Solubilities of the hydrolysates of AC 25, 50 and 60% were determined according to the method of Adebiyi *et al.* (2007) with slight modifications. Samples were dispersed in distilled water (1% w/v) and the pH of the solution was adjusted to the required working pH (2-12) concentrated HCl or NaOH to limit dilution. The pH of the three samples was readjusted as required after 20 min equilibration at room temperature (23-25°C). The samples were centrifuged at 5000 rpm using a Hitachi 55P-2 Automatic Preparative Ultracentrifuge (Japan). The solubility of the nitrogen in the supernatant was determined using the Kjeldahl method. The Nitrogen Solubility (NS) was calculated according to Eq. 1:

$$\text{NS (\%)} = \frac{\text{Nitrogen content in supernatant}}{\text{Total nitrogen content in sample}} \times 100 \quad (1)$$

Viscosity

Apparent viscosity of aqueous solutions of the DSPH from the three levels of AC was estimated on a (30-40 mL^{-1}) of protein solution using NDJ-79 Viscometer (Shanghai, China)

Gelation Properties

Gelation properties were determined by the method of Obatolu and Cole (2000), with slight modifications. The DSPH from the three levels of AC were determined on a (5 mL) test tube of each

hydrolysate sample suspension in deionised water at pH 7.0 and DSPH concentrations varying from 2 to 20% (w/v).

Statistical Analysis

The results were subjected to statistical Analysis of Variance (ANOVA), using a Statistical Analysis System. The significant differences between means were determined by Duncan's Multiple Range Test (DMRT), at $p<0.05$.

RESULTS AND DISCUSSION

Debittering and Desalting

The desorption of DSPH peptides from the MAR was achieved at all the three levels of AC after the resin was rinsed with deionised water. The result shows that the interaction between the resin and the DSPH is indeed hydrophobic in nature, 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 DSPH interacted with the resins hydrophobically to achieve a favourable configuration during the debittering, desalting and rinsing. The MAR properties are shown in Table 1. The desorbtion of the hydrolysates from the MAR was done with 25, 50 and 60% AC but the 25% AC was observed to have extracted the DSPH that were not bitter while 50% AC was moderately bitter and 60% AC was significantly bitter.

This data is from the producer's manual manufactured from styrene based material (Jiangsu Suqing Water Treatment Engineering Group, Jiangying, Jiangsu, People's Republic of China.

The proximate analysis data for the absorbed and desorbed fractions of the freeze dried DSPHs are shown in Table 2. According to the results, it was observed that ash content was significantly ($p<0.05$) lower in the DSPH after the debittering and desalting process of the three levels of AC (25, 50 and 60%). But an increase in the protein content of the debittered and desalted DSPH was observed for the two lower AC levels DSPH extracted. The protein content of the DSPH obtained from AC 25% increased from 93.89 to 96.15%, AC 50% increased from the same 93.89 to 97. 03% but there was a decrease for the AC 60% extracts (93.89 to 91.17) even though the difference is not significant ($p<0.05$). 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 as a result after the salt removal or more likely that some degradation from the hydrolysates could have led to the increase protein content. However, such observations require further investigations.

Table 1: Properties of DA201-C macroporous adsorption resin

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

Table 2: Proximate analysis data for Defatted Sesame Protein Hydrolysates (DSPH) before and after desalting

	Moisture	Ash	Protein
Samples	----------(%)----------		
DSPH	2.48±1.107[a]	12.4±1.45[b]	93.89±1.84
AC 25	2.64±0.670	1.21±1.35	96.15±2.04[a]
AC 50	1.92±1.320	1.05±0.46	97.03±0.05[a]
AC 60	3.09±1.710	5.74±1.13	91.17±2.18[b]

[a,b]Values are Mean±SEM (n = 3), different superscript letter(s) in the same column are not significant at level ($p<0.05$) but significant at $p<0.01$

Molecular Weight Distribution

The molecular weight distribution of the DSPH after the debittering and desalting process was in the range 6.48-55.23 (Da) for 25%, 55.56-2.27 (Da) for 50% and 65.89-6.55 (Da) for 60% alcohol desorption (Table 3). It provided a clear separation based on the strength of the hydrophobic interaction forces and even the size of the peptides as seen from the different peaks developed (Fig. 1). According to the molecular weight distribution data in Table 3, it was observed that the process might have caused some degradation of the peptides that could have resulted to the release of more protein. The results show that the short peptides moved to a stronger hydrophobic group which were in the AC 60%. The AC 60% desorbed peptides just with a shorter range between <500 to 1500 Da while the other two levels (AC 25, AC 50%) desorbed peptides up to the peak of 2500 Da. It is rational to say that the longer peptides that are less hydrophobic show weak hydrophobic interactions and could thus be held stronger in the lower alcohol concentrations AC25 and AC50 (Cho *et al*., 2004; Cheison *et al*., 2007).

Protein hydrolysates inhibiting the ACE *in vitro* are potentially interesting constituents of blood pressure reducing products. Figure 2 shows the results of the ACE in this study. The results of this study demonstrated that the desorbed fractions showed a general dose-dependent inhibition towards ACE.

The results ACE reveals varying contrasts between the purified peptide fractions and the unrefined hydrolysate and it indicates that some amounts of impurities were removed from the hydrolysates fraction by the different ACs during the desorbtion from MAR. A similar observation was made by Clemente (2000) and Cheison *et al*. (2007). Generally, the peptide inhibitors were reported to exert their action via specific C-terminal dipeptide (Cheung *et al*., 1980) or tripeptide

Table 3: Molecular weight distribution (% of total area) of defatted sesame protein hydrolysates and the three products after the debittering process

	AREA			
Molecular weight (Da)	DSPH	AC 25 (%)	AC 50 (%)	AC 60 (%)
>2500	89.36	55.23	2.27	-
1500-2500	8.23	15.66	7.63	-
1000-1500	2.00	13.11	9.76	6.55
500-1000	0.41	9.51	24.78	27.56
<500	-	6.48	55.56	65.89

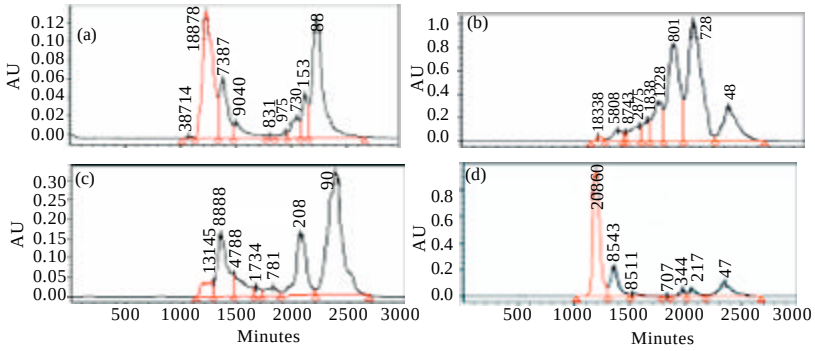

Fig. 1: Size exclusive chromatography profile during the debittering and desalting process of the sample, (A) DSPH, (B) AC 25%, (C), AC 50%, (D) AC 60%. Angiotensin-I-Converting Enzyme (ACE)

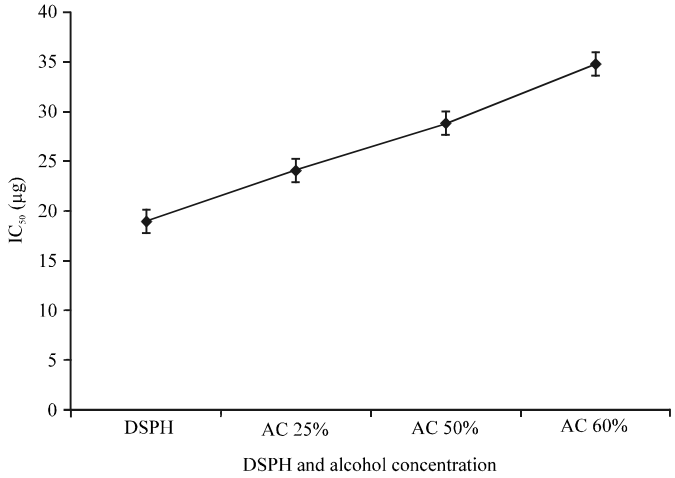

Fig. 2: The ACE inhibition activity of the hydrolysates at different AC

residues with preference for amino acid proline-rich hydrophobic residues. The most favourable C-terminal amino acids are the aromatic amino acids; tryptophan, tyrosine and phenylalanine (Meisel, 1997). The product of 60% AC, with the highest content of hydrophobic peptides showed superior ACE inhibition (Fig. 2) with the lowest IC_{50}. The AC 25 fraction followed by the AC 50 showed relatively weaker ACE inhibition. The antihypertensive effect of peptides is related to the inhibition of ACE. ACE activity results in blood pressure increase via conversion of Angiotensin I to Angiotensin II, which is a vasoconstrictive peptide and via degradation of bradykinin, which is a vasodilatative peptide. Inhibition of ACE by peptides for example, results in a decrease in blood pressure (Meisel and Schlimme, 1996; Clare and Swaisgood, 2000). All the fractions were significantly better inhibitors of ACE than the ordinary DSPH at ($p<0.05$), probably owing to the fact that many other peptides in the DSPH show little or no ACE inhibition activity. It was also observed that the fraction AC 60 contained the highest amounts of Methionine, Valine Alanine, Isoleucine, Leucine, proline, tyrosine and phenylalanine with the highest content of hydrophobic peptides (Table 4). This could be the major reason why that product was bitter as all those amino acids are hydrophobic. Even though AC 60 was bitter, it however showed the desirable property of being a better ACE inhibitor, which could make it useful in other applications especially in the pharmaceutical industry. Our resulted corroborated the findings of Chesion *et al.* (2007). The results show that MAR could be utilized to increase the yield of hydrolysates which could be of desirable bioactivities.

Total Amino Acid

The value of any protein always depends on the type of amino acids component it possesses. In this study, the content of the amino acids in the different levels of the alcohol concentration was studied and the result shown in Table 4. It reveals that the different alcohol levels show different hydrophobic and hydrophilic activities of their amino acid contents. The hydrolysate from 25% AC had the lowest while that from 60% AC had the highest content of hydrophobic amino acids (HoAA) and essential amino acids (Table 4). This is so probably because of the disruption of the hydrophobic interactive forces between the hydrolysates and the resin by the higher alcohol concentration (Cheison *et al.*, 2007). In the case of AC 25%, the low alcohol concentration may have led to a weak interaction with the HoAA and thus, resulting to the low levels of HoAA in that extract. The content of hydrophilic amino acids also revealed a general decreasing trend with increasing content of

Table 4: Total amino acids composition of desorbed products (AC 25, 50 and 60%), showing contents of essential amino acids and hydrophilic and hydrophobic amino acids composition and the sensory properties of the various products

Amino acids	pI[b]	AC25	AC50	AC60	EAA[a]	Infant adult
		----------(g/100 g protein)----------				
Glycine	4.36	2.830	3.750	3.680		
Alanine	4.45	2.890	3.760	4.600		
Proline	5.68	1.980	5.690	6.850		
Cysteine	6.01	4.090	3.050	3.760		
Phenylalanine	4.05	2.600	5.560	5.860		
Tyrosine	6.52	2.110	5.960	6.310		
Serine	4.90	4.080	3.040	1.760		
Arginine	4.89	10.67	6.570	7.440		
Aspartic acid	4.58	12.75	6.450	6.740		
Glutamic acid	4.97	14.20	9.740	10.01		
nEAA (g/100 g protein)		58.20	53.57	57.01		
Threonine	3.68	3.420	3.510	3.610	3.40	0.90
Methionine	5.87	4.690	5.980	8.680	2.50	1.70
Lysine	8.89	1.960	2.420	2.430	5.80	1.60
Histidine	7.07	8.060	3.320	2.140	1.90	1.60
Valine	4.82	3.590	8.660	9.880	3.50	1.30
Isoleucine	7.42	2.850	3.920	4.160	2.80	1.30
Leucine	5.73	5.010	7.320	8.250	6.60	1.90
Tryptophan	5.53	1.680	2.190	3.420	1.10	0.50
EAA (g/100 g protein)		31.26	37.32	42.57		
[c]Hydrophilic amino acids (g/100 g protein)		55.14	37.99	34.13		
[d]Hydrophobic amino acids (g/100 g protein)		25.72	48.85	54.59		
Sensory properties		No bitterness	Moderately bitter	Bitter		

[a]Suggested profile of essential amino acid requirement for infant and adult. FAO/WHO (1990). [b]Isoelectric point adopted from Kinsella and Mohite (1985). nEAA = non-essential amino acids. EAA = essential amino acids. [c]Hydrophilic amino acids (Histidine, Lysine, Arganine, Glutamic acid, Aspartic acid, Threonine and Serine). [d]Hydrophobic amino acids (Alanine, Isoleucine, Leucine, Methionine, Phenylalanine, Proline, Tyrosine and Valine)

hydrophobic amino acids in the products. When the products were compared with the essential amino acids as recommended by FAO/WHO (1990) for humans, all three products exhibited significantly higher ($p<0.05$) essential amino acids with the exception of Lysine for infants. In all three products, low levels of Lysine were recorded as was also reported by Krishna-Murti (1965) and Johnson *et al.* (1979). The supplementation of Lysine will be required if the hydrolysates are to be included in infant food formulations.

In generally, the essential amino acid components were quite higher for the alcohol extracts than those reported by John Kanu *et al.* (2007). This could be attributed to fact the use of the alcohol during the debittering process of the hydrolysates is responsible for higher amino acid content than reported by John Kanu *et al.* (2007).

However, they used water to extract the proteins and compared them to soybean proteins. The difference could be attributed to the fact that water could not have extracted all the proteins from the defatted sesame flour.

Sensory Evaluation

The results of the relative sensory evaluation are also shown in Table 4. According to the views of the 50 panelists, 60% AC extract was ranked the bitterest, followed by the 50% AC extract qualified as mildly bitter, while the 25% AC extract was reported to be tasteless. It was observed by the panelists that the nutty smell and salty taste that characterize sesame protein were not detected in all the three levels of alcohol extracts. The bitterness observed in AC 60% level might be due to the high AC that likely increases the hydrophobic properties of the product and hence, the crucial role of

hydrophobicity for the bitter taste is further substantiated by theoretical consideration on taste receptor chemistry (Belitz *et al*., 1979), as well as by quantitative taste studies. For bitter peptides, it is generally observed that the higher the hydrophobicity of a particular peptide, the more intense its bitter taste (Adler-Nissen, 1986). Bitter peptides constitute small molecular weights (Adler-Nissen, 1986) as displayed by the 60% AC in (Table 3). They occupy the extreme end of the theoretical hydrophobicity distribution function of all the peptides in the hydrolysate. The concentration of these peptides cannot be estimated from the average value of the hydrophobicity. The very sharp bitter taste of the 60% AC extract is entirely attributed to the high content of the hydrophobic amino acids. The organoleptic properties are significantly different ($p<0.05$) among the three products. The relationship between peptide bitterness and content of HoAA is clearly shown (Table 4), as it is also shown to be related to the content of short peptides as displayed in Table 3. Hence, the bitter taste of the 60% AC product can be undoubted related to the presence of high hydrophobic and short peptides that are largely composed of essential amino acids. This was also reported recently by Cheison *et al*. (2007) and in previous works of Lalasidis and Sjoberg (1978), Kanekanian *et al*. (2000) and Cho *et al*. (2004). The bitter extracts of 60% AC contained the highest amount of hydrophobic amino acids hence the shortest peptide chain length and its content of essential amino acids was the highest too. The 60% AC DSPH extract was recorded to contain the highest amounts of Methionine, Valine, Alanine, Isoleucine, Leucine, proline, tyrosine and phenylalanine (Table 4), which are all hydrophobic amino acids. The 25% AC hydrolysates extract was qualified to have acceptable organoleptic properties while the 50% AC extract was qualified as having mild acceptable organoleptic properties. Thus the choice of alcohol concentration as an extracting medium for hydrolysates should largely depend on the desired prioritized properties of the product. A higher alcohol concentration extract will have plenty of essential amino acids but with a sharp bitter taste (poor organoleptic properties), while a lower alcohol concentration extract will have good organoleptic properties but with lesser essential amino acids.

Nitrogen Solubility

Solubility characteristics of protein are among the most important functional properties since many functional performances of proteins depend upon their capacity to go into solution (Chobert *et al*., 1996; Moure *et al*., 2006). Figure 3 shows the nitrogen solubility curve for the three AC (25, 50 and 60%) levels over a pH range of 2 to12. As shown in Fig. 3, the solubility of the

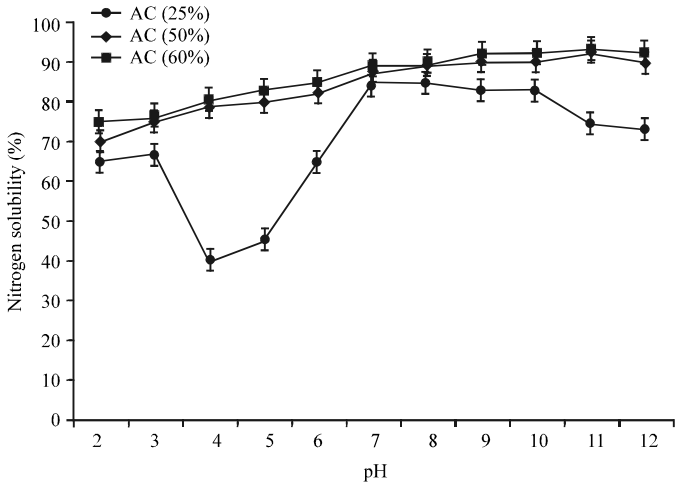

Fig. 3: Solubility Curve of AC 25, 50 and 60% over a pH range of 2-12

hydrolysates from the three alcohol extracts concentrations is enhanced significantly between pH 6 to 8 although the pattern for the hydrolysates from 50 and 60% AC is significantly different from that for hydrolysate from the 25% AC.

The 25% AC extract shows a decrease in solubility between pH 4 to 5 but the 50 and 60% AC extracts were highly soluble over the entire pH range considered. It has been suggested that an increase in the solubility of protein hydrolysate is due to the reduction of its secondary structure and also due to the enzymatic release of smaller polypeptide units from the protein (Turgeon *et al*., 1992; Cheison *et al*., 2007). This process may be more feasible at higher alcohol concentrations which may have rendered the hydrolysate more soluble across the pH range considered for the 50 and 60% AC extracts and thus resulting similar nitrogen solubility patterns. The release of proteins may have likely occurred during the debittering and desalting process for the high alcohol concentration extracts. Nitrogen solubility of the hydrolysates obtained from the 50 and 60% AC extracts were pH dependent over the range studied. This pattern could be explained by the fact that smaller, more hydrophilic and more solvated polypeptide units are produced as a consequence of the chemical properties of the alcohol (Clemente, 2000). Hence, protein aggregates could no longer be formed even at isoelectric pH. The 25% AC extracted hydrolysates had a decreased solubility at the isoelectric point but increased afterwards, with increase in pH. This could be an important feature that could expand the application of the hydrolysates obtained from this work especially in food production processes.

Viscosity

Viscosity is another important functional property of food proteins. It is important for providing physical stability to emulsions (Taha and Ibrahim, 2002). The apparent viscosity of the aqueous solutions of the three products obtained from the different ACs as a function of protein hydrolysates is shown in (Fig. 4a-c). A common trend is observed for the hyrolysates products from the three ACs although some marked differences exist in their viscosity levels. All the products exhibited single peaks that were more pronounced for the 50 and 60% AC products. The highest peak for the 25% AC was observed at 6% hydrolysates concentration comparatively lower (10 Mpa.s) than those of 50% AC (50 Mpa.s) and 60% AC (80 Mpa.s) observed at 6 and 7% protein concentrations, respectively Fig. 4a-c. This might have a link with the hydrophobicity of these particular products (Kinsella and Mohite, 1985). The concentrations, molecular weight, polydispersity, hydrophobicity and conformation of each protein species affect the viscosity of protein. All of these factors tend to confound the underlying inverse relationship of protein solubility and viscosity in particular (Schenz and Morr, 1996). Processing-induced changes in protein such as polymerization, aggregation and hydrolysis affect the viscosity of food products (Schenz and Morr ,1996). The 25% AC product could be useful in foods that require low viscosity.

Gelation Properties

Gelation properties of the hydrolysates from the three products are summarized in Table 5. As shown in the results, the hydrolysates from 25% AC did not slip out of the test tube until when the concentration of the sample was raised up to 20%. The same was observed for the sample at 50% AC; it started slipping out from the test tube at 18% concentration. But a different scenario occurred for 60% AC where in the sample slipped out at the lowest concentration. This could be attributed to the fact that the mechanism of gelation of sesame protein is similar to that of other globular proteins with an initial denaturation step flowed by an interaction to form a gel matrix, provided attractive forces and thermodynamic conditions are suitable (Mulvihill and Kinsella, 1987). It could also be attributed to the enzyme used for the hydrolysis process as Alcalase 2.4 L is an endopeptidase with a broad specificity to hydrophobic amino acids (Yu *et al*., 2007). It resulted in peptides with different hydrophobic and

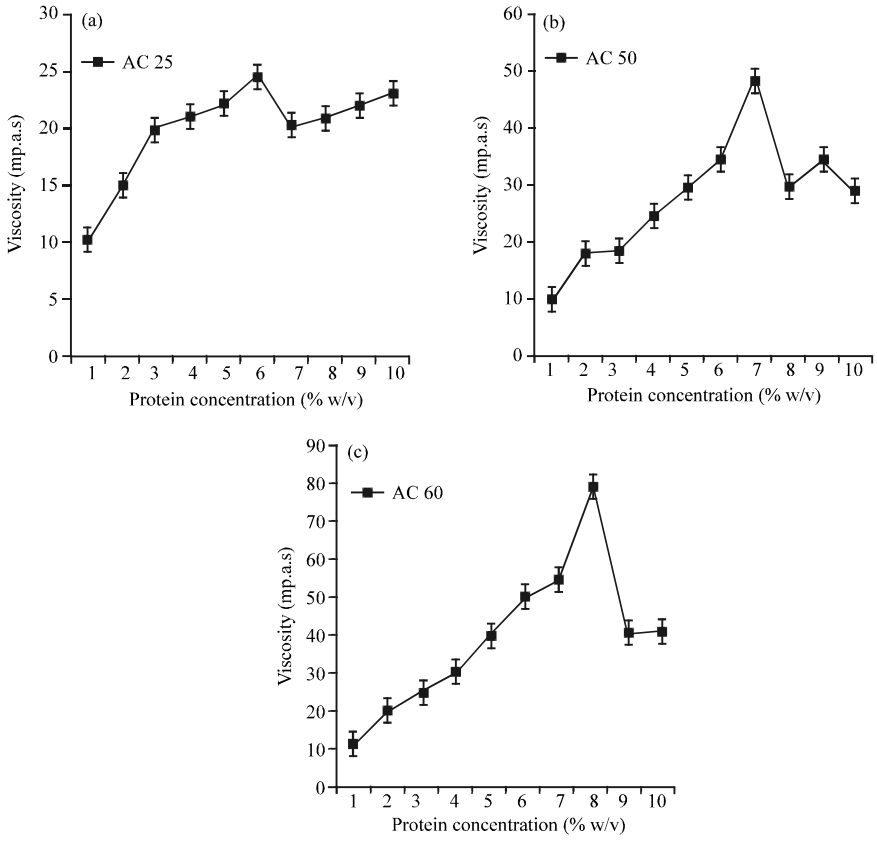

Fig. 4: (a) Viscosity (Mp.a.s) as against protein Concentration (%w/v) of the hydrolysates debittered and washed with AC 25%, (b) Viscosity of the hydrolysates debittered and washed with AC 50%, (c) Viscosity of the hydrolysates debittered and washed with AC 60%

Table 5: Gelation Properties of three hydrolysates from the different alcohol concentrations

	Protein concentration % (w/v)									
Hydrolysates	2	4	6	8	10	12	14	16	18	20
AC 25	+	+	+	+	+	+	+	+	+	++
AC 50	+	+	+	+	+	+	+	+	++	++
AC 60	++	++	++	++	++	++	++	++	++	++

+: Sample did not slip from the inverted test tube, ++: Sample slipped down from the inverted test tube

charge group which thereafter involved in many kinds of interaction. Kohnhorst and Mangino (1985) reported that although disulphide bonds contributed to the overall gel strength of the hydrolysates, the important reactions involved in cross-linking are the ionic and hydrophobic interactions. The good gel properties exhibited by 50 and 60% AC could probably be that a critical balance of their net charges was reached to attain the gel properties.

CONCLUSION

This study has demonstrated that bitter and salty taste can be removed from the DSPH through absorption-desorption mechanism using a MAR to absorb the hydrolysates and subsequently

desorbing them from the MAR with various concentrations of aqueous alcohol media. Low alcohol concentration media (AC 25%) proved to be effective extracting DSPH with virtually show no bitter or salty taste. Higher alcohol concentration (AC 50 and 60%) media are not effective in debittering or desalting DSPH but can however, be good media for extracting DSPH with high concentration of essential amino acids. Even though the hydrolysates obtained from high concentration alcohol media may have bitter taste, their high content in essential amino acids can make them useful ingredients in both food and pharmaceutical applications. The debittered and desalted DSPH obtained from the designed experimental process possess good bioactive and functional properties. After comparing the results of this study to previous ones, it can be safely deduce that alcohol media can better desorb DSPH from macroporous resins than water alone. The results of this study hold a prospecting future in the food industries especially in the area of treating bitter or salty hydrolysates obtain from oil seeds. It can also be useful in dealing with the challenges encountered in the food processing industry that require enhancing palatability of food stuffs by debittering or desalting especially if related to DSPH.

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