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Improving Taste and Functionality of Sesame Protein Hydrolysate with Macroporous Resin Treatment

Philip John Kanu^{1,2,3*}

¹*Milton Margai Technical University, Freetown, Sierra Leone.*

²Institute of Food Technology, Nutrition and Consumer Studies, School of Agriculture and Food Sciences, Njala University, Sierra Leone.

³Centre for Development and Food Safety, Freetown, Sierra Leone.

*Correspondence:

Philip John Kanu, Milton Margai Technical University, Freetown, Sierra Leone, Tel: +232 76612050/+232 88612050.

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Introduction

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 has been reported as a result of the protein adsorption to the dialysis membranes [1].

Proteins have been desalted using either nanofiltration membranes or gel permeation chromatography using the desalting SephadexTM gels which are expensive [1].

Desalting and debittering of defatted sesame protein hydrolysate (DSPH) enhance their value-added qualities as well as processing safety into the product because of consumer sensitivity and attitude to chemicals in food formulations. Cheaper desalting options are therefore invited to lower the production costs while giving higher hydrolysate recoveries. Macroporous adsorption resins (MARs) have been used for desalting biological samples, casein non-phosphorylated peptides, and other protein hydrolysates with good hydrolysate recoveries. MAR is a non-polar adsorbent resin used mainly for adsorption of organic substances and decolorization [2,3].

It is important to select a cheaper desalting process which is simple and easy to operate. While peptide bitterness is of both academic and technological interest, no reports exist on the desalting of defatted sesame protein hydrolysate on MAR, nor are there any reports of debittering with the same. This study was intended to investigate the use of MAR in the simultaneous desalting and debittering of defatted sesame protein, analyzed for some of their functional properties, their molecular weight distribution, their amino acid content and their organoleptic properties.

Materials and Methods

The defatted sesame protein hydrolysate was got as described in Chapter 5 with DH 3. A styrene-based macroporous adsorption resin (MAR), branded DA201-C was got from Jiangsu Suqing Water Treatment Engineering Group as a kind gift (Jiang-Ying, Jiangsu, China). All other chemicals and reagents were obtained from a local manufacturer (Sinopharm Chemical Reagent Co., Ltd. (SCRC) Shanghai People's Republic of China) (Table 1) and made available at the university chemical store and were of analytical grade.

Methods

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. The DSPH was allowed to be absorbed into the MAR by stirring 1.0L of the DSPH supernatant liquid with 500 mL of MAR for 24h 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. The alcohol concentrations (ACs) varied from 25%, 50%, and 60%, followed by deionized water. The collected fractions were concentrated under vacuum and freeze-dried. The resin was regenerated by washing it with 1 mol/L NaOH followed by 1 mol/L 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 was done for it moisture and ash content of DSPH and also for the desalted and desorbed hydrolysates according to sections 2.2.2.1.2 and 2.2.2.1.3 respectively.

Molecular Weight Distribution of the Product

The molecular weight distribution of hydrolysate was determined according to the method described in section 5.2.9.

Measurement of angiotensin-I converting enzyme (ACE) inhibition activity

The ACE inhibition activity assay was performed using the method of Cushman and Cheung (40) 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, pH 8.3. A sample (50 µl for each of the samples) was added to the above reaction mixture and mixed with 1µmol Hip-His-Leu (150 µl) containing 0. 5 M NaCl. After incubation at 37 °C for 60 mi, the reaction was stopped by the addition of 1.0 N HCl (250µl). The resulting hippuric acid was extracted by the addition of 1.5 ml ethyl acetate. After centrifugation (800X 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 redissolved in 3.0 ml of distilled water and absorbance was measured at 228 nm using U3210 spectrophotometer. The IC50 value was defined as the concentration of inhibitor required to inhibit 50% of the ACE inhibitory activity.

Amino Acid Analysis

The amino acid contents for the various AC were analyzed as described in section 2.2.2.1.9.

Sensory Evaluation

In this study, the nine-point hedonic scale according to the method of Jakobsen [5] was used to evaluate the bitterness, the nutty smell and after taste in defatted sesame protein hydrolysates powder was conducted by 50 panelists.

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).

Gelation properties

Gelation properties were determined by the method of Obatolu & Cole [6] with slight modifications. The three products were determined on a 5mL 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.

Statistical Analysis

The results were subjected to statistical analysis of variance (ANOVA) as described in section 2.3.

Results and Discussion

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 [3]. 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.

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Polarity	None
Pearl Size	0.4-1.25mm
Surface area	1000-1300m2 g-1
Average pore diameter	30-40nm
Pore volume	1.0-1.1 cm3 g-1

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 desorbed fractions of the freeze dried of the DSPHs is 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%, 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% increase 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 2: Proximate analysis data for defatted sesame protein hydrolysates (DSPH) before and after desalting.

Sample	Moisture (%)	Ash (%)	Protein (%)
DSPH	$2.48 \pm 1.1.07a$	$12.4\pm1.45b$	93.89 ± 1.84
AC 25	2.64 ± 0.67	1.21 ± 1.35	$96.15 \pm 2.04a$
AC 50	1.92 ± 1.32	1.05 ± 0.46	$97.03\pm0.05a$
AC 60	3.09 ± 1.71	5.74 ± 1.13	$91.17 \pm 2.18b$

^aValues are mean \pm SEM (n=3), different letters in the same column are not significant at level (p< 0.05) but significant at p< 0.01.

Since, the interaction between the resin and the hydrolysates is regarded to be hydrophobic, the alcohol acted as detergent, while the desorption of the DSFH from MAR by the alcohol could also referred to as elution by displacement. This scenario was observed because of the presence of both the hydrophobic zone which contains hydrocarbon and at the same time the hydrophilic sites which is the hydroxyl group that is found in the alcohol.

The molecular weight distribution of defatted sesame protein hydrolysates and the three products after the debittering 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). This provided for a clear separation based on the strength of the hydrophobic interaction forces and even the size of the peptides as could be seen from the different peaks developed (Figure 1. DSPH (A), AC% (B), AC 50% (C), AC 60% (D)) as compared to the retention time utilized as shown in (Figure 2).

Table 3: Molecular weight distribution (% of total area) of defattedsesame protein hydrolysates and the three products after the debitteringprocess.

Molecular Weight (Da)	AREA 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	13.11	9.76	6.55
500-1000	0.41	9.51	24.78	27.56
<500	-	6.48	55.56	65.89

According to those peaks observed it was clear some degradation took place that will allow more protein to the released. The peaks were clearly separated from each other.

The molecular weight distribution as shown in Table 3, the results show that the short peptides moved to a stronger hydrophobic



Figure 1: Size exclusive chromatography profile during the debittering and desalting process of the sample, (A) DSPH, (B) AC%, (C), AC 50%, (D) AC 60.



group which is the AC60%. The AC60% group separated into peptides just between <500 Da to 1500 Da when AC60% was used to desorb the desalted peptides. While the other two (AC 25 %, AC 50%) exceed up to 2500Da. Since the longer peptides are less hydrophobic and are involved in weaker hydrophobic interactions, they are easily weakened and returned to the lower alcohol concentrations AC25 and AC50. 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).

Protein hydrolysates inhibiting the ACE in-vitro are potentially interesting constituents for blood pressure decreasing products. Figure 3 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 showed in the ACE inhibition (Figure 2) revealed varying contrasts between the purified peptide fractions and the unrefined hydrolysate. This shows that some amount of impurities were removed from the hydrolysates fraction by the different ACs during the desorption from MAR, similar report was made by Clemente [7]. Generally, the peptide inhibitors were reported to exert their action via specific C-terminal dipeptide [8] or tripeptide 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 [9]. The fraction product of AC60, with the highest content of hydrophobic peptides showed superior ACE inhibition (Figure 3) with the lowest IC50. The fraction AC25 followed with AC50 showing 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, eg by peptides, results in blood pressure decrease [10,11]. All the fractions were significantly (P <0.05) better inhibitors of ACE than the ordinary DSPH, probably owing to many other peptides in the DSPH that have no ACE inhibition. These result shows that MAR could be utilized to increase the production of hydrolysates with desirable functional properties. It was also observed that the fraction AC60 contained the highest amounts of Methionine, Valine Alanine, Isoleucine, Leucine, Proline, Tyrosine and Phenylalanine (Table 4), this could be the reason why that product was exceptionally bitter as all those amino acids are hydrophobic ones.





Even though AC60 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. The results show that MAR could be utilized to increase the yield of hydrolysates which could be of desirable bioactivities. The value of any protein is always seen by the type of amino acids component it displayed, in this study the content of the amino acids in the different levels of the alcohol concentration was studied, the results are summarized 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. This is so probably because of the disruption of the hydrophobic interactive forces between the hydrolysates and the resin by the higher alcohol concentration. In the case of AC25%, 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 hydrophobic amino acids in the products. The results of the relative bitterness were summarized also 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 [12] 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 [13] Bitter peptides constitute small molecular weights [13] 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. [3], and in previous works of Lalasidis and Sjoberg [14], Kanekanian et al., [15] and Cho et al., [16]. 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,

Amino acids pIb		AC 25 (g/100g protein)	AC 50 (g/100g protein)	AC 60 (g/100g protein)	EAAa (g/100g protein)		
Glycine	4.36	2.83	3.75	3.68	Infant	Adult	
Alanine	4.45	2.89	3.76	4.6			
Proline	5.68	1.98	5.69	6.85			
Cysteine	6.01	4.09	3.05	3.76			
Phenylalanine	4.05	2.6	5.56	5.86			
Tyrosine	6.52	2.11	5.96	6.31			
Serine	4.9	4.08	3.04	1.76			
Arginine	4.89	10.67	6.57	7.44			
Aspartic acid	4.58	12.75	6.45	6.74			
Glutamic acid	4.97	14.2	9.74	10.01			
nEAA (g/100g protein)		58.2	53.57	57.01			
Threonine	3.68	3.42	3.51	3.61	3.4	0.9	
Methionine	5.87	4.69	5.98	8.68	2.5	1.7	
Lysine	8.89	1.96	2.42	2.43	5.8	1.6	
Histidine	7.07	8.06	3.32	2.14	1.9	1.6	
Valine	4.82	3.59	8.66	9.88	3.5	1.3	
Isoleucine	7.42	2.85	3.92	4.16	2.8	1.3	
Leucine	5.73	5.01	7.32	8.25	6.6	1.9	
Tryptophan	5.53	1.68	2.19	3.42	1.1	0.5	
EAA (g/100g protein)		31.26	37.32	42.57			
cHydrophilic amino acids (g/100g Protein)		55.14	37.99	34.13			
dHydrophobic amino acids (g/100g protein)		25.72	48.85	54.59			
Sensory properties		No bitterness	Moderately bitter	Bitter			

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

^aSuggested profile of essential amino acid requirement for infant and adult, FAO/WHO [14]. ^bIsoelectric point adopted from Kinsella & Mohite [21]. EAA = essential amino acids. ^cHydrophilic amino acids (Histidine, Lysine, Arganine, Glutamic acid, Aspartic acid, Threonine and Serine). ^dHydrophobic amino acids (Alanine, Isoleucine, Leucine, Methionine, Phenylalanine, Proline, Tyrosine and Valine).

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. When the products were compared with the essential amino acids as recommended by FAO/WHO [17] for humans and 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 [18] and Johnson et al. [19] The supplementation of Lysine will be required if the hydrolysates are to be included in infant food formulations. Generally, the essential amino acid components were quite higher for the alcohol extracts than those reported by Kanu et al. [20]. 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. Viscosity is one of the important functional properties of food proteins. It is important for providing physical stability to emulsions [17]. The apparent

viscosity of the aqueous solutions of the three products got from the different ACs as a function of protein hydrolysates is shown in Figure 4 (a-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 Figure 4 (a-c). This might have a link with the hydrophobicity of these particular products [20]. The concentrations, molecular weight, polydispersity, hydrophobicity and conformation of each protein species affect the viscosity of protein [21].

All of these factors tend to confound the underlying inverse relationship of protein solubility and viscosity in particular [22]. Processing-induced changes in protein such as polymerization, aggregation and hydrolysis affect the viscosity of food products [22]. The 25% AC product could be useful in foods that require low viscosity.

For the one for AC50 it was observed that the viscosity increases as the concentration of the hydrolysate was added to the solution. Gelation properties of the hydrolysates from the three products are summarized in Table 5. As shown in the results, the hydrolysates



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

Hydrolysates	Protein Concentration % (w/v)									
	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

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 [23]. It could also be attributed to the enzyme used for the hydrolysis process as Alcalase 2.4L is an endopeptidase with a broad specificity to hydrophobic amino acids [24]. It resulted in peptides with different hydrophobic and charge group which thereafter involved in many kinds of interaction.

Kohnhorst and Mangino [25] 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 AC 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 deducing 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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