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STABILIZATION OF EMULSIONS BY *Monodora myristica* SEED PROTEIN HYDROLYSATE: EFFECT OF MOLECULAR WEIGHT DISTRIBUTIONS ON SURFACE, EMULSIFYING AND FOAMING PROPERTIES

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ABSTRACT

Development of sustainable benign emulsifiers from bioresource for industrial applications has continued to dominate research frontiers. Therefore, this research aimed to investigate the effect of varied molecular weights of *Monodora myristica* seed protein on the surface, emulsifying and foaming properties of emulsion systems. *M. myristica* seed protein was extracted via dialysis and fluxed into > 12, 000 kDa (MPF1), 12, 000-10, 000 kDa (MPF2), 10, 000-8, 000 kDa (MPF3), 8, 000-4, 000 kDa (MPF4) and < 4, 000 kDa (MPF5) molecular weights distributions. The results showed that the lower molecular weights *M. myristica* seed polypeptides fluxes were more distributed with aliphatic, hydrophilic and polar amino acids, whereas aromatic, hydrophobic, non-polar and sulphur-based amino acids residues were inherently found in the higher molecular weights variant. The surface tension of the amphoteric polypeptides increased with molecular weights, but decreased with protein flux concentration, consistent with their refractive index (1.2835-1.4612) and surface hydrophobicity index (17.55-48.22). The interfacial protein concentration and adsorbed protein, increased with molecular weight distributions of the protein fluxes. The emulsifying stability index (55.38-78.44 min.), average particle size of droplets (63.92-188.50 µm) and protein solubility (58.48-78.44 %), revealed that the higher molecular weights *M. myristica* seed protein fluxes has a greater tendency to form viscous stable emulsion with reduced foaming properties. The physicochemical properties exhibited by *M. myristica* seed polypeptide fluxes, were largely influenced by their amino acids residues and molecular weight distributions and present these specialty biopolymers with auspicious potential as suitable emulsifiers in food, pharmaceutical and chemical formulations.

Keywords: Polypetides, *Monodora myristica*, Emulsifier, Molecular weight, Functional properties

INTRODUCTION

Protein, an agricultural bioresource with proven techno-functionalities is increasingly being demanded for use to improve the texture and longtime stability of industrial products (Samuel *et al.*, 2025). Most Plausible, the growing concern, by consumers for healthy diet and biocompatible health-care products has driven the search for plant and animal-based emulsifiers, as surrogates to their synthetic analogues employed in water-in-oil (W/O), oil-in-water (O/W) and multiple emulsion systems (Yang *et al.*, 2022), frequently used in food, biomedical and chemical industries for the distribution of micronutrient, preservatives, vitamins and formulation of beverages (Ricardo *et al.*, 2021). Moreover, thermodynamic instability of emulsion systems has necessitated their compounding with suitable amphoteric macromolecules, as emulsifier to achieve dynamic stability of their heteromolecular chains (Boonlao *et al.*, 2022).

Empirical studies, have demonstrated that molecular weight distributions of protein hydrolysates play important role on the absorption character of this biopolymer at the oil-water interface and the physicochemical properties of the interfacial layers of emulsion systems (Cao *et al.*, 2021). Nevertheless, despite the upsurge for plant-based protein as emulsion stabilizers for a range of industrial products, significant fraction of plant biomass is still under-utilized, owing to their poor techno-functionalities and proteinase-types inhibitors with antinutritional cofactors, which could cause allergies and limit their applicability in industrial formulations (Igartus *et al.*, 2024). Thankfully, to overcome these drawbacks, membrane separation technology has been adapted to flux protein and other biomacromolecules on the basis of size, shape, density, charge, solubility and binding character (Pan *et al.*, 2023), and ramp-up amongst many other things, the emulsifying and functionality indices of the macromolecule (Gabriela *et al.*, 2021). Furthermore, technologies such as ultrafiltration and nanofiltration processes are conducted under mild conditions (Tan and McClements, 2021), which comes with flexibility of system design, preservation of the pristine molecular structure and speciation of molecular weights of protein and free amino acids to develop refreshingly new products with enhanced functional properties (Ricardo *et al.*, 2021).

Studies on fractionation of protein hydrolysates for stability of emulsion systems have been reported; recent advances in the utilization of natural emulsifiers to form and stabilize emulsions (McClements *et al.*, 2017), application of advanced emulsion technology in food industry (Tan and McClements, 2021), effect of molecular weight on the emulsion properties of microfluidized gelatin hydrolysates (Cao *et al.*, 2021), peptide-protein hydrolysate and their derivatives: their role as emulsifying agents for enhancement of physical and oxidative stability of emulsions (Lin *et al.*, 2022), effect of molecular weight on the interfacial and emulsifying characteristics of rice glutelin hydrolysates (Yang *et al.*, 2022) and effect of molecular weight on the structural and emulsifying characteristics of bovine bone protein hydrolysate (Zhu *et al.*, 2023). Clearly, from the foregoing studies, emulsifying and other physicochemical properties of proteins are largely influenced by structural alterations of the amino acids monomer residues, molecular weight distributions of the polypeptide chain and extraneous factors notably; pH, ionic strength, temperature and pressure.

Monodora myristica also known as calabash nutmeg is a tropical tree of the Annonaceae family and native to tropical

Africa (Adewole *et al.,* 2013). In time past, its seeds were widely sold as an inexpensive nutmeg substitute and milled as spice for soup to relieve constipation. Local names of *M. Myristica* include; Gyada miya, Ehuru, Ariwo, Awerewa, Ehiri and Airama (Okechukwu *et al.,* 2022). Premise on the ambiguity, of the specificity of lower and-higher molecular weights protein fluxes as stabilizers for emulsion systems. We theoretically conceived that adopting, a novel extraction protocol for *M. myristica* seed protein hydrolysate and fractionation of the amphoteric macromolecular hydrolysate, into narrow molecular size distributions could preserve the structural integrity of the biopolymer with a consequential effect, on the physicochemical properties of dual and multiple emulsion systems. Thus, this research study aimed at extracting protein from *M. myristica* seed, fractionate the protein hydrolysate into narrow molecular weight fluxes and assess the effect of the molecular weight distributions on the surface, emulsifying and foaming properties of oil-in-water emulsion system.

MATERIALS AND METHODS

Materials

All reagents/chemicals used in this study were supplied by Merck and Sigma-Aldrich and used without further purification.

Collection and Pretreatment of *M. myristica* Seeds

M. myristica seeds were purchased from Zuru market, Zuru Local Government Area, Kebbi State. The seeds were decorticated, washed under tap water and sun-dried for two weeks. The dried seeds were milled (Binatone food blender BLG-650), sieved (< 20µm), packed into polyethylene bags and stored in a refrigerator.

Extraction of *M. myristica* Seed Protein Hydrolysate

M. myristica seed protein was extracted via dialysis as outlined by (Samuel *et al.,* 2025) with slight modification. 5g of defatted *M. myristica* seed powder was aggregated with deionized water at a ratio of 1:10 and the solution dialyzed at 4^0C (to maintain the structural integrity of the protein macromolecules), using a Thermos scientific slide-A-lyzer dialysis cassette semi permeable membrane (5,000- 17,000 kDa MwCO) for 24 h. The pH was then adjusted to 9.0 using 0.2 M NaOH solution to precipitate *M. myristica* seed protein. The precipitated protein hydrolysate was centrifuged (16,000 rpm for 10 minutes), weighed and freeze-dried (SFDQ 3000).

Fractionation of *M. myristica* Seed Protein Hydrolysate

The fractionation of *M. myristica* seed protein hydrolysate into various molecular weight distributions was achieved via size exclusion chromatography (SEC) performed with a Shimadzu (Shim-Pack Bio Diol Japan) size exclusion chromatography column coupled with 280 nm SPD-M40 UHPLC inert cell. The column system consists of proteema precolumn micro column series (4.6 mm x150 mm) with porosity of 100Å and a particle size of 2µm polymer standards service (PSS), with separation in the range 150-160,000 kDa. Isocratic elution was used for the chromatography, with a mobile phase made up of 15% acetonitrile in 100 $mmolL^{-1}$ Sodium phosphate buffer (pH 6.9) at a flow rate of 0.2 $mLmin^{-1}$ and 25^0C column temperature. After filtration via 0.35 µm syringe filters, 5 µL of filtrate and pullulan standard (256-452,000 kDa) were injected and separated through ultrafiltration membrane with protein fluxes of > 12, 000 kDa (MPF1), 12, 000-10, 000 kDa (MPF2), 10, 000-8, 000 kDa (MPF3), 8, 000-4, 000 kDa (MPF4) and < 4, 000 kDa (MPF5) molecular weights fractions collected and lyophilized in a freeze-dryer (SFDQ 3000).

Measurement of Amino Acid Distribution of *M. myristica* Seed Protein Fluxes

The sample fluxes were derivatizatized with 6-aminoquinoyl-N-hydroxysuccinimidyl carbamate (Adewumi *et al.,* 2022), and their amino acid distributions determined with a High Performance Liquid Chromatography (HPLC model 2350, ISCO, Lincoln, NE) reticulated with photo diode array detector (Refracto Monitor IV; Thermo Separation Products, Riviera Beach, FL), Spherisorb ODS 2 column (250 4.6 mm, 80 mm pore size, 5 mm particle size; Waters, Milford, MA). The temperature of the column was maintained at 25ºC by a Brinkman RC 6 temperature bath (Westbury, NY). Mobile phase A consists of 30 mML^{-1} potassium dihydrogen phosphate buffer with 0.2 % Tetrahydrofuran adjusted to pH 7.0 with 4.0 M KOH, and mobile phase B consists of 15 % HPLC grade acetonitrile mixed with 20 ml milli-Q- water. The mobile phase flow rate was 0.5 mL/min Data collection and analyses were performed using Hewlett-Packard (Wilmington, DE) Chemstation software. All analyses were done in triplicates.

Preparation of Emulsion Solutions

2 g of *M. myristica seed* protein flux sample was weighed and added to a 250 mL volumetric flask containing 1 g of soybean oil and 100 mL deionized water. The flask and its content were shaken in an orbital mechanical flask shaker (Innova 2000) for 3 h; homogenized (12, 000 rpm for 30 minutes) and 0.2 mL 0.01 % Sodium azide was added to inhibit microbial growth (Cao *et al.,* 2021).

Determination of Surface Hydrophobicity Index of *M. myristica* Seed Protein Flux Stabilized Emulsion

The surface hydrophobicity index of the emulsions was determined by florescence spectroscopy as described by (Ya'ara *et al.,* 2022: Samuel *et al.,* 2025). Emulsions with concentrations; 5.0, 7.5, 10.0, 12.5 and 15.0 µg mL^{-1} were prepared in 0.01 M (pH 7.0) phosphate buffer. 5.0 µL aliquot of each concentration was aggregated with 50.0 µL 4.0 mM 8-anilinonaphthalene-1-sulfonic acid in a vial. Fluorescence intensity was determined with an alamar blue fluorochrome at 370 nm and 490 nm for excitation and emission wavelengths respectively using a spectrofluorometer with grating monochromator (FP-8200). The slope of the Fluorescence intensity against emulsion concentration plots was recorded as the surface hydrophobicity index.

Determination of Refractive Index of *M. myristica* Seed Protein Flux Stabilized Emulsion

A precision refractometer (Shimadzu KPR-300 Japan) was used to determine the refractive index of M. *myristica* seed protein flux stabilized emulsions at an average room temperature of 18 ^{0}C. 5 mL of freshly prepared emulsion solution was measured into a 20 mL centrifuge tube, homogenized (4000 rpm for 5 minutes) and allowed to equilibrate for 1 h. Three drops of the hydrocolloid were dropped on the surface of the lower glass prism previously cleaned with methanol and closed. Monochromatic light was passed through the glass prism and the image produced observed through a telescope focused in the direction of the refracted ray. The prism box was adjusted until the two sections coincided. The observed refractive index was then read through the scale of the eyepiece. The corrected refractive index of the protein fluxes was evaluated using equation 1 (Schoftstall *et al.,* 2000).

$$\eta c = \eta o + 0.00045(t - 20) \quad (1)$$

Where ηc = the corrected refractive index, ηo = the observed refractive index and t is the ambient temperature.

Measurement of Surface Tension of *M. myristica* Seed Protein Flux Stabilized Emulsion

The surface tensions of the emulsions were determined using the pendant drop method at ambient temperature (25±2^{0}C) as described by (Ndagana *et al.,* 2015) with slight modification. Emulsion solution containing 0.1, 0.2, 0.3, 0.4 and 0.5 wt. % protein flux concentrations were prepared in a 100 mL volumetric flask and stopped with aluminum foil; the flasks were equilibrated at 1000 rpm for 5 minutes, the density of the hydrocolloids were determined (Odejobi, 2019) and the solution fed into a clamped 10 mL burette to capacity. The burette tap was adjusted to efflux one drop of emulsion solution, after every 3 seconds into a 10 mL beaker. The time taken to collect 2 mL of *M. myristica* seed protein flux stabilized emulsion solution was recorded with a stop clock. Similarly, the time taken to collect equal volume of deionized water (reference standard) was measured for each experimental run. The surface tension of the emulsions was calculated;

$$Surface\ tension\ (N/m) = \frac{\gamma 1 \eta 1\ \rho 2}{\eta 2\ \rho 1} \quad (2)$$

Where γ1 is the surface tension of deionized water at 25^{0}C, ηl is the number of drops of deionized water, ηl2 is the number of drops of emulsion sample, ρ1 is the density of deionized water and ρ2 is the density of emulsion sample.

Determination of Particle Size of *M. myristica* Seed Protein Flux Stabilized Emulsion

The particle size of emulsions prepared with the *M. myristica* seed protein fluxes were determined as described by (Zhu *et al.,* 2023), with the refractive indices of 1.53, 1.33 and 0.10 for the dispersion part (distilled water) of the medium, the real part of the medium and the imaginary part respectively. The particle sizes of the emulsion stabilized by *M. myristica* seed protein fluxes, were measured at d_{15}, d_{55}, d_{95} for cumulative packed particle volume at 15, 55 and 95 % and D connotes the average particle size of the emulsion droplets.

Determination of Percentage Adsorbed Protein and Interfacial Protein Concentration of *M. myristica* Seed Protein Flux Stabilized Emulsion

The percentage of adsorbed protein of *M. myristica* seed protein fluxes stabilized emulsion and its interfacial concentrations were determined as described by (Zhu *et al.,* 2023). 2 mL of freshly prepared emulsion sample was measured into a 20 mL centrifuge tube, homogenized (10,000 rpm at 10 minutes) and the oil and water phases were separated. The bottom transparent liquid portion was withdrawn with a syringe and filtered through a 0.35μm membrane. The protein solution was centrifuged (12,000 rpm at 5 minutes) and supernatant protein content determined. The protein absorption (%) and interfacial protein concentration (mg/m^2) of the hydrocolloids were evaluated, equations 3 and 4 respectively;

$$Protein\ Absorption\ (\%) = \frac{C3-C2}{C1} \times 100 \quad (3)$$

$$Interfacial\ protein\ concentration\ (\mathrm{mg/m2}) = \frac{(C3-C2)\ \mathrm{D}}{6\varphi} \quad (4)$$

where, C_1 is the protein concentration (mg/mL) in the original emulsion; C_2 is the protein concentration (mg/mL) of the water phase after centrifugation of the emulsion and C_3 protein concentration of the emulsion after centrifugation (mg/L), D is the average particle size (μm) of the emulsion and φ is the oil phase volume fraction (0.2).

Determination of Emulsifying Properties of *M. myristica* Seed Protein Flux Stabilized Emulsion

Emulsifying properties of *M. myristica* seed protein flux stabilized emulsion were measured using spectrophotometry technique as outlined by (Wang *et al.,* 2023). 50 μL of freshly prepared *M. myristica* seed protein flux stabilized emulsion solution was diluted 100 times with 10% (w/v) sodium dodecyl sulfate in distilled deionized water (SDS) solution and it absorbance determined at 500 nm (A_0) using a double beam UV-Visible spectrophotometer (UV-1280, Shimadzu, Japan), a second absorbance was measured again after 10 minutes (A_{10}). The emulsifying activity index (EAI) in (m^2/g) and emulsifying stability index (ESI) in (min) were evaluated using equations 5 and 6 respectively;

$$EAI = \frac{2\ x\ 2.303\ x\ \mathrm{A0\ x\ N}}{c\ x\ \varphi\ x\ 10{,}000} \quad (5)$$

$$ESI = \frac{\mathrm{A0}}{\mathrm{A0-A10}} \mathrm{\ x\ 10} \quad (6)$$

N = dilution factor, φ is the volume fraction of the oil phase taking as (0.2) and 2 and 2.303 are constants.

Determination of Protein Solubility of *M. myristica* Seed Protein Flux Stabilized Emulsion

The solubility of protein in protein flux stabilized emulsions was measured as described by (Ya'ara *et al.,* 2022). 5 mL of sample was weighed into 50 mL centrifuge tube, containing 20 mL deionized water and the pH was adjusted to 5.5 using 1.0 N HCl. The solution was centrifuged at 5000 rpm for 20 minutes, the supernatant was withdrawn and its absorbance measured at 595 nm. A reference calibration curve in the concentration range 1.0- 00.0 mg/mL prepared using Bovine Serum Albumin (Analar molecular grade) was used to determine the protein composition in the supernatant and residue after centrifugation respectively. The protein solubility was calculated from equation 7;

$$Protein\ solubility\ (\%) = \frac{P_s}{P_{ti}} \times 100 \quad (7)$$

Where P_s = protein in the supernatant and P_{ti} = total protein in the emulsion.

Determination of Foaming Properties of *M. myristica* Seed Protein Flux Stabilized Emulsion

The foaming properties (foam-forming capacity and foam stability) were measured as described by (Gita *et al.,* 2022). 10 mL of freshly prepared emulsion was measured into a 50 mL centrifuge tube and homogenized (2500 rpm for 5 minutes). The colloidal suspension was allowed to stand for 45 minutes at room temperature (25 ±2^{0}C). The foam-forming capacity (FFC) and foam stability (FS) were evaluated;

$$\mathrm{FFC\ (\%)} = \frac{K_f - K_i}{K_i} \times 100 \quad (8)$$

$$\mathrm{FS\ (\%)} = \frac{K_s}{K_i} \times 100 \quad (9)$$

Where K_i is the initial foam volume of the emulsion, K_f is the final volume of emulsion after homogenization and K_s is the volume of foam after standing for 45 minutes.

RESULTS AND DISCUSSION

The distributions of amino acid residues in *M. myristica* seed protein fluxes are represented in table 1.

Table 1: Amino acid distribution of *M. myristica* seed protein fractions

Amino acid class	Protein Fraction (%)				
	MPF1	**MPF2**	**MPF3**	**MPF4**	**MPF5**
Aliphatic	15.55±0.43	15.92±0.33	16.14±0.51	45.10±0.47	52.15±1.21
Aromatic	35.25±1.22	31.22±0.96	29.61±0.74	14.74±0.52	11.21±0.34
Sulphur-based	1.15±0.03	0.84±0.02	0.72±0.03	0.45±0.01	0.36±0.01
Hydrophobic	24.94±0.77	19.60±0.82	17.92±0.66	15.55±0.57	10.62±0.32
Hydrophilic	4.64±0.44	6.19±0.51	8.74±0.48	11.22±0.84	13.45±0.92
Non-polar	18.44±0.22	16.12±0.32	13.66±0.41	10.15±0.23	7.91±0.31
Polar	16.44±0.44	20.18±0.30	26.22±0.88	33.13±0.96	41.32±1.34

The results showed that lower molecular weights *M. myristica* seed polypeptide fractions were more distributed with aliphatic, hydrophilic and polar amino acid residue on the biopolymer chains, whereas the stereochemistry of the higher molecular weights protein fluxes were replete with aromatic, hydrophobic, sulphur-based and non-polar amino acids monomer units. This suggests diamino-monocarboxylic acid (lysine and arginine), monoamino-dicarboxylic amino acids (aspartic acid and glutamic acid) and monoamino-monocarboxylic amino acids (glycine, serine and alanine) with hydrophilic aliphatic linear chain (Lin *et al.*, 2022) and polar moieties, predominantly detected in the lower molecular weights variants of *M. myristica* seed protein fluxes constitutes an ampholytes amalgam (Cao *et al.*, 2021), which permeates the membrane, and can readily separate in an electric field by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS PAGE), as the anionic surfactant SDS micelles (Kutzli *et al.*, 2021), could covalently binds to the amphoteric molecule (Gabriela *et al.*, 2021), thereby facilitating the unfolding and sieving through of these biopolymers, with better biological, chemical and hydrodynamic properties. The higher score for hydrophobic, aromatic, sulphur-based and non-polar amino acid residues in the higher molecular weights fractions, corroborated the preponderance of amino acids with aromatic rings notably; histidine, proline, tryptophan, phenylalanine and disulphide-bridge linked amino acids (cystein and metheonine) (Yang *et al.*, 2022), in these fractions. In addition, the amino acid distributions showed that the proportion of hydrophobic moieties on the biopolymers increases with molecular weight, which suggest the α-helix and β-sheet of the ordered secondary structure (Zhu *et al.*, 2023), was significant with increase in molecular weights of *M. myristica* seed protein fluxes.

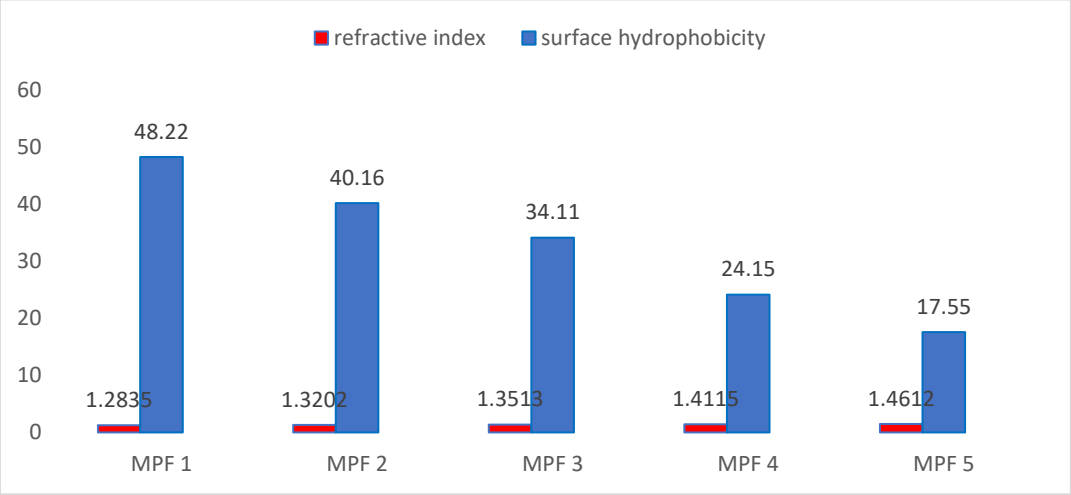

Figure 1: Refractive Index and Surface Hydrophobicity of *M. Myristica* Seed Protein Fluxes Stabilized Emulsion

The surface hydrophobicity and refractive index are significant indicators to assess the level of molecular alteration of the amino acid monomers on a polypeptide chain and the bulk property of hydrocolloid suspensions respectively (Yao *et al.*, 2020; Wang *et al.*, 2023). Figure 1 shows that the refractive index increased marginally, with decrease in molecular weight of *M. myristica* seed protein fluxes, in contrast, to the remarkable increased in surface hydrophobicity, with increasing molecular weight of the fluxes. The insignificant increase, in the former and the direct proportionate increased of the latter, with respect to the molecular weights of the biopolymer fluxes, indicate, a better inter-molecular interactions of the preponderant non-polar and hydrophobic amino acids residues content of higher molecular weight fractions, exposed on these biopolymer fluxes and the triglyceride molecules of the lyophobic colloid (McClements *et al.*, 2017; Zhu *et al.*, 2023). Conversely, the higher hydrophilic polar scores of amino acid residues in the lower molecular weights fraction, weaken their hydrophobic interactions within the lipid phase (Schroder *et al.*, 2017), but increases their presence more in the aqueous phase, forming monomodal polypeptide chains (Cao *et al.*, 2021), which resulted in increased, bulk density and colloidal properties of the aqueous phase (Zhu *et al.*, 2023), accordingly, alter to a greater degree the route of the monochromatic light rays, consistent with optical refraction in two medium of varied densities. These characteristics shown by the higher molecular weights *M. myristica* seed protein fractions connote their suitability as natural emulsifiers for consistency required for food and personal care products and in emulsion systems targeted for efficient delivery of surface active ingredients.

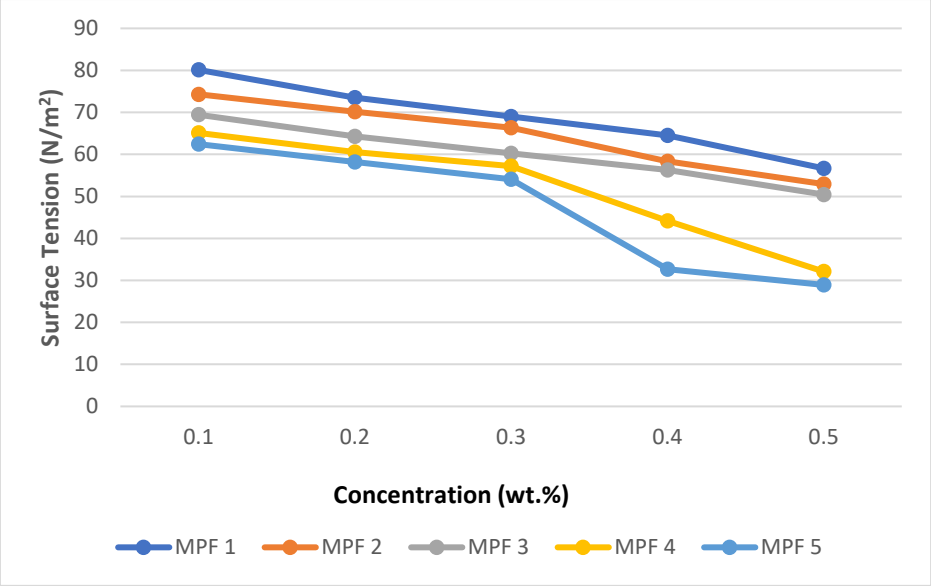

Figure 2: Surface Tension of *M. Myristica* Seed Protein Fractions

The surface tension of *M. myristica* seed protein fluxes are shown in figure 2. The surface tension of polypeptides macromolecules, demonstrated an inverse proportion with concentration of the biopolymer stabilized emulsion. MPF5 flux presents with the lowest surface tension, which could be attributed to its highest content of hydrophilic and polar amino acid residues of 13.45±0.92 % and 41.32±1.34 % respectively (table 1), accrued largely from lysine, arginine, histidine, aspartic acid, glutamic acid and serine, these bio-based monomers, were preferentially retained, in the aqueous phase (Samuel *et al.*, 2025) and decreased the tendency of the hydrocolloid to shrink into the possible minimum surface area (Yang *et al.*, 2022). The acute surface tension within the minimum aerial space for emulsion stabilized by the higher molecular weights *M. myristica* seed protein fluxes, suggest the relative migration and retention of the preponderant hydrophobic monomer species present in these fluxes at the constricted surface area (Samuel *et al.*, 2025). The noticeable decreased in surface tension in the hydrocolloids, beyond 0.3 wt. % flux concentration, more pronounced for MPF5, corroborated the preponderance of hydrophilic moieties in the lower molecular weights variants of *M. myristica* seed protein. The elevated surface tension as exhibited by higher molecular weights variant of *M. myristica* seed polypeptide fluxes, qualifies these amphoteric molecular species, as emulsifiers for stable blended products notably; mayonnaise, salad dressings and dairy products to create smooth textures, prevent syneresis and extend shelf-life.

Table 2: Particle Size of *M. myristica* Seed Protein Fractions

Protein fraction	Particle size (µm)			
	d_{15}	d_{55}	d_{95}	D
MPF1	35.41	58.12	103.82	63.92
MPF2	53.04	92.11	142.50	98.21
MPF3	72.11	115.52	168.14	136.30
MPF4	86.46	134.22	184.51	172.11
MPF5	102.53	151.20	202.44	188.50

Particle size of droplets is a major determinant for stability of dual or multiple reactive emulsion systems. Table 2 represents the particle sizes for *M. myristica* seed protein fluxes, which indicate that d_{15}, d_{55}, d_{95} and D of the emulsions decreased significantly, with increase in molecular weight of *M. myristica* seed protein fractions. Comparatively, the average molecular size (D) 172.11µm for MPF4 is higher than 98.21µm for MPF2, this 42.94% decrease in particle size, implied the latter polypeptide flux is in good stead to reduce the propensity of the oil droplets to coalesce into a lipid mass and connote better emulsion stability by the latter (Ricardo *et al.*, 2021; Zhu *et al.*, 2023). Generally, d_{15}, d_{55}, d_{95} and D for the protein fluxes increased with decrease in molecular weight of the amphoteric molecular fractions. This provides useful insight, and substantiates the readily mechanistic adsorption, of the oil droplets onto the larger hydrophobic surface area of higher molecular weight protein fluxes (Tan and McClements, 2021); thereby confer enhanced thermodynamic stability to the dual emulsion system prepared with these amphiphilic macromolecules.

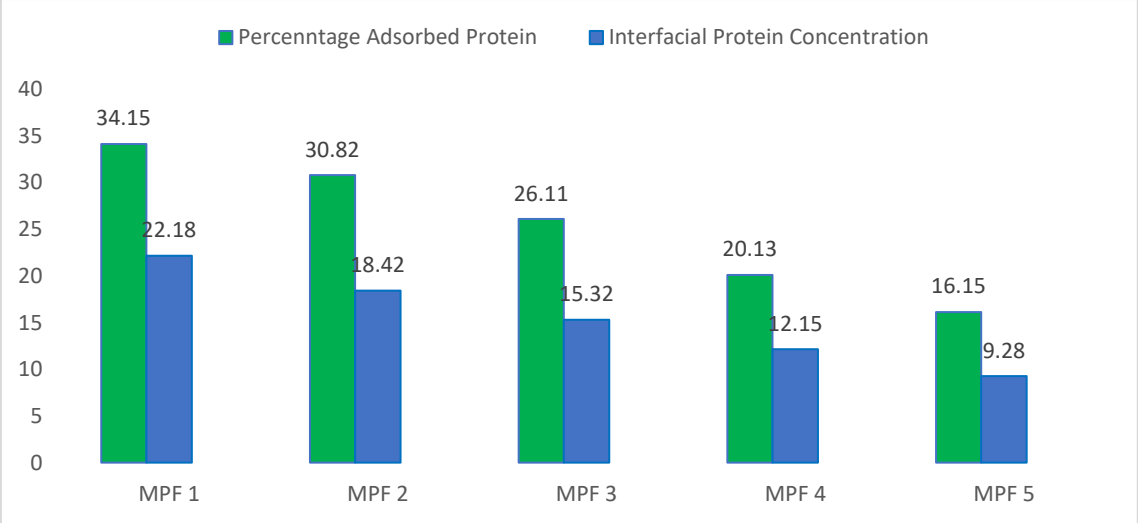

Figure 3: Percentage Adsorbed Protein and Interfacial Protein Concentration

Figure 3 showed that *M. myristica* seed protein adsorbed and their interfacial concentrations decreased concurrently with molecular weights of *M. myristica* seed protein fluxes. MPF1 protein flux, with the highest values of 22.18 mg/m^2 and 34.15 %, for percentage adsorbed protein and interfacial protein concentration respectively, could be ascribed to the following; greater formation of film thickness on the oil droplet surface (Wang *et al.*, 2023), the presence of higher hydrophobic groups in the biopolymer kinetic chain (Kutzli *et al.*, 2021) and increased stronger adsorption capacity of the polypeptide molecule at the oil-water interface of the dual emulsion system (Cao *et al.*, 2021). In consequence, all these, did provide better spatial repulsion, of the oil droplets, which culminated in greater stability index of the emulsion system. The low protein adsorption and interfacial concentration, shown by the lower molecular weights protein fluxes, coupled with their relatively higher hydrophilic and polar amino acid residues, more pronounced for MPF5 (table 1), endowed these biomacromolecules with better encapsulating properties for vitamins and flavor for enhanced bioavailability and stability index of emulsion systems used in pharmaceutical formulations for drug delivery therapies.

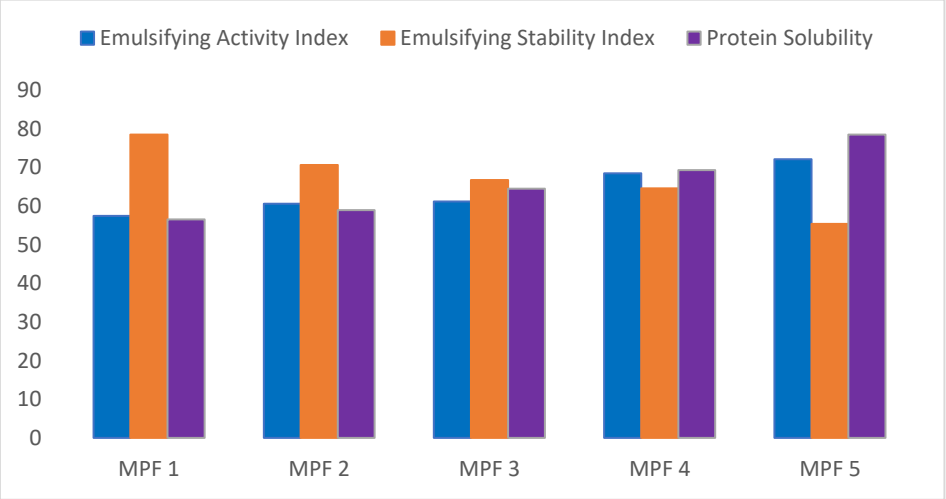

Figure 4: Emulsifying Properties and Protein Solubility of *M. myristica* Seed Protein Fluxes

The EAI and ESI are important indicators, which promotes emulsion formation and the capacity to maintain a stable emulsion over time respectively (Wang *et al.*, 2023). The emulsifying activity index (EAI) and emulsifying stability index (ESI) exhibited contrasting trend, the latter decreased, while the former increased, with molecular weight of protein fluxes. The lowest EAI and protein solubility, with a corresponding highest ESI values for MPF1, suggest that the more hydrophobic and non- polar characteristics of this bio-based macromolecule, makes it more susceptible to rapidly adsorbed at the oil-polypeptide interface (Kutzli *et al.*, 2021), with lower solubility in the aqueous phase (Lin *et al.*, 2022). Consequently, this amphoteric biomacromolecule, showed the greatest propensity, to reduce droplet particle size and a correspondence superior emulsion stability index. The higher emulsifying stability index and lower protein solubility, demonstrated by higher molecular weight *M. myristica* protein fluxes, is instructive, as it predisposes these polypeptides to preventing counter stabilization issues especially; coalescence, Ostwald ripening and flocculation (Ricardo *et al.*, 2021). Thus, presents these biopolymers as suitable emulsifies for preparation of alkyd resin from seed oil derived monoglycerides for paint formation and polymer synthesis via emulsion polymerization.

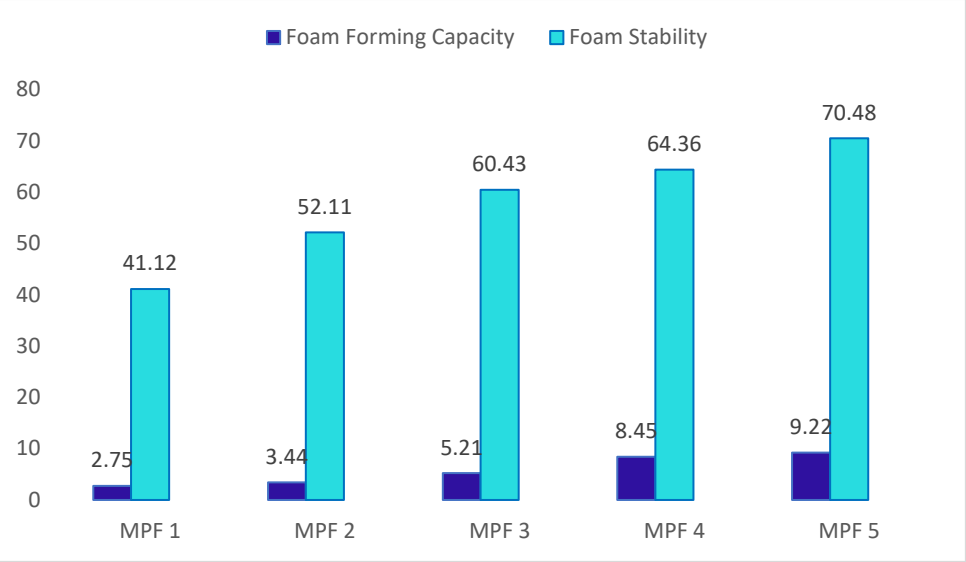

Figure 5: Foaming Properties of *M. myristica* seed Protein Fractions

The foaming properties of *M. myristica* seed protein fluxes stabilized dual emulsion system are represented in figure 5. The tendency for an emulsifier to foam is imperative for its industrial application (Gita *et al.,* 2022). Higher molecular weight *M. myristica* seed protein fluxes demonstrated lower foaming properties; this implied the higher hydrophobic surface character, of these amphoteric biopolymers, most plausibly, showed a superior peptide-lipid interaction at their interface. However, the lower hydrophobic amino acid recorded for the lower molecular weight protein fluxes suggest, the more hydrophilic moieties in these amphiphilic polypeptides interacted more at the water-air interface (Deotale *et al.*, 2020), which resulted in the production of a continuous stable elastic membrane and interfacial films with encapsulated air mass over time (Wang and Vardhanabhuti, 2024). This remarkable foaming behavior exhibited by lower molecular weights *M. myristica* seed protein, certify these macromolecules as emulsifiers in stable systems used in solid and solvent-based products, where minimal emulsification and separation into distinct phases is desired.

CONCLUSION

The effect of varied molecular weight of *M. myristica* seed protein hydrolysates on physicochemical properties imperative to measure the stability of oil-in-water emulsion system has been investigated. The Higher molecular weight variants of the polypeptide molecules form a thicker layer with the lipid phase, which remarkably reduced the droplet particle size with improved viscosity and better interaction with the emulsion system. This study did provide baseline information, within these molecular weight ranges for the utilization of *M. myristica* seed protein hydrolysates as sustainable emulsifiers for dual emulsion systems used in industrial processes.

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