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Preparation and Assessment of Surface and Functional Properties of Protein Blends from *Moringa oleifera* and *Monodora myristica* Seeds

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ABSTRACT: Protein, an amphoteric macromolecule has proven to play important role in enhancing nutritive standard and product quality. Recently, research attention is been focused on the protein fraction implicit in underexploited biomass to address the increasing demand for this agro-based resource with exceptional attributes. Consequently, the objective of this paper was to prepare and assess the surface and functional properties of protein blends compounded from *Moringa oleifera* and *Monodora myristica* seeds using appropriate standard techniques. The protein blends showed thixotropic behavior at elevated concentration and presented 1MOI:1MMI blend with the highest values of 9.75%, 22.47%, 32.11%, 60.21% and 72.09% for polar, basic, acidic, hydrophilic and non-essential amino acid distributions respectively. In contrast, 1MOI:2MMI and 2MOI:1MMI blends showed higher essential, aliphatic, aromatic, hydrophobic and non-polar amino acid distributions *albeit* lower in the former. The surface tension for the protein blends is in inverse proportion with concentration, consistent with their refractive indices (1.42-1.51), η_{sp} (1.95-5.92 cP), η_{int} (128.14-257.81 cP) and E_{η} (5.51-14.40 KJmol^{-1}). The protein blends exhibited foaming and solubility properties which were dependent on pH, whereas their swelling characters were influenced by temperature. The 1MOI:1MMI and 2MOI:1MMI polypeptide blends showed better emulsifying properties and surface hydrophobicity respectively. Based on the surface and functional characteristics of these biopolymer blends, they could find application as suitable hydrocolloids for food, pharmaceutical and allied industrial products.

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Moringa. oleifera is an illustrious plant of the family moringaceae widely cultivated for its numerous medicinal and nutritive benefits, owing to its high valued amino acids, phytochemicals and antioxidants Vergara-Jimenez *et al.,* (2017). The seed finds uses as recipes for soup, tradition herbal formulation and water purification. Sultana (2020), and have been established to contain all essential amino acids Vaknin *et al.,* (2021). Studies have shown techniques for protein extraction, functional properties and amino acid profile of *M. oleifera* seed protein isolates which comes with distinctive features notably; quality, colour and sensory properties suitable for diverse applications Aja *et al.,* (2014); Rao *et al.,*

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(2016); Mune *et al.,* (2016); Jain *et al.,* (2019); Javed *et al.,* (2021).

Monodora Myristica also known as calabash nutmeg is a tropical tree of the Annonaceae family. It is native to tropical Africa from Sierra Leone in West Africa to Tanzania Adewole *et al.,* (2013). In time past its seed were widely sold as an inexpensive nutmeg substitute and milled as spice for soup to relieve constipation and control passive uterine hemorrhage in women immediately after child birth Ezenko *et al.,* (2017); Samuel *et al.,* (2024a). Other names of *M. Myristica* are Jamaican nutmeg, Ehuru, Ariwo, Awerewa, Ehiri, Airama, African orchid nutmeg Okechukwu *et al.,* (2022). Essential aliphatic amino acids content of *M. myristica* seed protein, which constitute the hydrophobic regions of protein molecules were low compared to that derived from other spice plants and present *M. myristica* protein with poor emulsifying property *albeit* rich source of acidic amino acids Aremu *et al* (2011); Ekeanyanwu, (2013). In addition, the total content of sulphur and essential- amino acids in *M. myristica* seed reported by Ekeanyanwu, (2013), was lower than their respective recommended thresholds for infants (FAO/UN/WHO. 1991).

Plant proteins are proven source of indispensible healthy protein in human diet, imperative for the control of food texture, consistency and cost Mune *et al.,* (2016). The amino acid composition and distribution in the polypeptides chain length, inherent in this bio-based molecule, is the major determinant for utilization of plant protein-types as functional ingredients to increase the nutritional quality of food and as emulsifier and stabilizers for hydrocolloids Javed *et al.,* (2021). Moreover, the amino acid residue of the protein macromolecule plays important roles in proper functioning of organisms, resistance to infection, healing for wound and fracture Ekeanyanwu, (2013). Protein are biomacromolecules whose physicochemical properties are characterized by molecular size and orientation, concentration, shape and flexibility, concentration, molecular weight, charge of the protein, intermolecular interaction and degree of hydration Laiho *et al.,* (2017). Extraneous factors notably; ionic strength, pH and temperature can also influenced the solution properties of this amphoteric macromolecule Du *et al.,* (2018). Proteins are of commercial interest because of their high nutritional value and excellent functionality in food and non-food formulations Javed *et al.,* (2021). However, even with the plethora of plant protein sources, only cowpea, soybean and kidney bean has been extensively exploited for their industrial applications Wang *et al.,* (2008); Gorissen *et al.,* (2018); Jain *et al.,* (2019). Studies on Evaluation of Crude Protein and Amino Acid Composition of Nigerian *Monodora Myristica* Ekeanyanwu, (2013) and Characterization of *Moringa oleifera* Leaf and Seed Protein Extract Functionality in Emulsion Model System Ya'ara *et al.,* (2022) have been reported. Recently, Adewumi, *et al.,* (2022), reported the Functional Properties and Amino Acids Profile of Bambara Groundnut (*Vigna subterranean*) and *Moringa oleifera* Leaf Protein Complex.

The resurgence of interest for cheap sources of protein with novel characteristics have continue to steer research attention on the potentials constitutional in underutilized plant biomass as veritable matrix to developing a pleasingly different protein blend with specialty properties. Moreover, from a technical standpoint, surface and functional attributes of protein admix are prerequisite parameters to designing unit operation, provide standard operating procedure for quality assurance in product development and offer very unique opportunity to import desirable attributes for development of health-promoting foods. Therefore, the objective of this paper is to prepare and assess the surface and functional properties of protein blends compounded from *Moringa oleifera* and *Monodora myristica* seeds.

MATERIALS AND METHODS

Collection and pretreatment of M. oleifera and M. myristica seeds: M. oleifera seeds were kindly supplied by local farmers within the premises of the Federal University of Agriculture, Zuru and *M. myristica* seeds were purchased from Zuru market. Both seeds vouchers were authenticated as mounted and systematically classified in the herbarium of the Department of Crop Science, Federal University of Agriculture, Zuru. The seeds were decorticated, washed under running water and sun-dried for two weeks. The dried seeds were milled separately and sieved (< 30µm) to obtained their respective powdered. The powdered biomass was packed into polyethylene bags and stored in a refrigerator.

Isolation of M. oleifera and M. myristica seed proteins: M. oleifera and *M. myristica* seed proteins were isolated as described by Javed *et al.,* (2021) with slight modification. 100 g of seed powdered was defatted Houde *et al.,* (2018) and stored in a refrigerator before protein isolation. Protein was isolated from the defatted sample via dialysis using a Thermo scientific slide-A-lyzer dialysis cassette semi permeable membrane (10.0 to 17.0 kDa MwCO). 5g of sample was aggregated with double distilled water at a ratio of 1:5 and the solution dialyzed at 4^0C (to

maintain the structural integrity of the protein macromolecules) for 24 h. The pH was then adjusted to 9.0 using 0.5 M NaOH solution to precipitate the protein isolates. The precipitate (protein dialysate) was centrifuged (12000 rpm for 30 minutes), weighed and lyophilized in a freeze-dryer (SFDQ 3000).

Preparation of M. oleifera and M. myristica seed protein blends: 10 g of lyophilized *M. oleifera and M. myristica* seed protein isolates were weighed and added separately to 20 ml milli-Q- water in a 250ml volumetric flask, stopped and equilibrated in a thermostatic oil-bath set at 10^0C for 30minutes, after which three protein blends; 1MOI:1MMI, 1MOI:2MMI and 2MOI:1MMI, were prepared on a weight/weight bases. MOI and MMI represent *M. oleifera* and *M. myristica* seed protein isolates respectively.

Determination of amino acid content of M. oleifera and M .myristica seed protein blends: The Amino acid content of the protein blends, after derivatization with 6-aminoquinoyl-N-hydroxysuccinimidyl carbamate Adewumi *et al.,* (2022) were determined with a High Performance Liquid Chromatography (HPLC model 2350, ISCO, Lincoln, NE) and a photo diode array detector (Refracto Monitor IV; Thermo Separation Products, Riviera Beach, FL) was used to analyze the products. A Spherisorb ODS 2 column (250 4.6 mm, 80 mm pore size, 5 mm particle size; Waters, Milford, MA) was used for the separation. The temperature of the column was maintained at 25ºC by a Brinkman RC 6 temperature bath (Westbury, NY). Mobile phase A consists of 30 mM/L potassium dihydrogen phosphate buffer with 0.4 % Tetrahydrofuran adjusted to pH 7.0 with 4.0 M KOH, and mobile phase B consists of 50 % HPLC grade acetonitrile mixed with 50 ml milli-Q- water. The mobile phase flow rate was 0.5 mL min^{-1} Data collection and analyses were performed using Hewlett-Packard (Wilmington, DE) Chemstation software. All analyses were done in triplicates.

Determination of surface tension of M. oleifera and M. myristica seed protein blends: The pendant drop method Ndagana *et al.,* (2015) was adopted with slight modification for surface tension measurements of the protein blends at ambient temperature. protein solution; 1, 2, 3, 4 and 5 wt. % concentrations was prepared by weighing the appropriate mass of protein blend into 250 ml volumetric flask, containing the required volume of distilled water and stopped; the flask was equilibrated at 150 rpm for 30 minutes at room temperature. The solution density was determined Odejobi, (2019), and the solution fed into a clamped burette. The burrette tap was adjusted to efflux one drop of protein solution into a 10 ml beaker after every 5 seconds. The time taken to collect 5 ml of protein solution was recorded with a stop clock. Similarly, the time taken to collect 5 ml of distilled water used as reference standard was equally measured for every experimental run. The surface tension of the protein blends was evaluated

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

Where γ1 is the surface tension of distilled water at 25^0C, Ƞ1 is the number drops of distilled water, Ƞ2 is the number of drops of protein sample, ρ1 is the density of distilled water and ρ2 is the density of protein sample.

Determination of refractive index of M. oleifera and M. myristica protein blends: A refractometer was used to determine the refractive index of *M. oleifera* and *M. myristica* seeds protein blends. Protein solution was prepared by dissolving 0.1 g of protein sample in 10 ml distilled water in a 50ml centrifuge tube and homogenized (2500 rpm for 15 minutes). Two drops of protein solution was dropped on the surface of the lower glass prism previously cleaned with acetone and closed. Monochromatic light ray 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 refractive index was then read of on the scale through the eyepiece.

Viscometry of M. oleifera and M. myristica seed protein blends: The specific and intrinsic-viscosities of the blends were determined with an Ostwald viscometer. The viscometer was rinsed with distilled water and then with little protein solution to initialized the apparatus before mounting in an oil-bath. The upper bulb of the viscometer was filled with protein solution (1 wt. %) and the capillary flow time of the solution from the upper to lower bulb was recorded with a stop clock. The efflux time for equal volume of distilled water was also determined. The same procedure was repeated for 2, 3, 4 and 5 wt. % concentrations. The specific viscosity of protein samples were evaluated (equations 2);

$$Specific\ viscosity = \frac{t_2 - t_1}{t_1} \quad (2)$$

The intrinsic viscosity of the protein blends was determined (equation 3) Samuel *et al.,* (2024b)

$$\eta_{\text{int}} = \frac{\eta_{sp}}{C} \quad (3)$$

Where t_1, t_2, c, η_{sp} and η_{int} denote the flow time for distilled water, flow time for protein solution, concentration of protein solution, specific and intrinsic- viscosities respectively.

Measurement of activation energy of flow for M. oleifera and M. myristica protein blends: The modified form of Arrhenius equation Samuel *et al.,* (2024b), with respect to intrinsic viscosity was applied to evaluate activation energy of flow for the protein blends at 30, 40, 50, 60 and 70^0C.

$$In\,\eta_{\text{int}} = InA - \frac{E_\eta}{RT} \quad (4)$$

E_η was deduced from the slope of ln Π_{int} against 1/T plot and R= 8.314 J mol^{-1} K^{-1} used as molar gas constant.

Determination of water and oil absorption capacity of protein blends: The water and oil absorption capacities of the protein blends were measured according to Adebowale and Lawal, (2004); Adewumi *et al.,* (2022). 1.0 g of protein sample was added to 10 ml distilled water in a 100 ml volumetric flask, the flask was agitated manually, its content fed into a 50 ml centrifuge tube, weighed and homogenized (3000 rpm for 60 minutes). The supernatant was separated and excess water removed. The centrifuge tube containing the residue was reweighed and the water absorption capacity calculated;

$$\text{Water absorption capacity (g/g)} = \frac{W_3 - W_2}{W_1} \quad (5)$$

To determine the oil absorption capacity, the same experimental protocol was used with distilled water replaced with soybean oil and oil absorption capacity evaluated;

$$\text{Oil absorption capacity (g/g)} = \frac{W_3 - W_2}{W_1} \quad (6)$$

Where W_1 = weight of blend protein isolate, W_2 = weight of centrifuge tube and protein blend and W_3 = weight of centrifuge tube and water/oil absorbed protein sample.

Determination of swelling power of protein blends: The method as described by Adeleke *et al.,* (2017) with slight modification was used to measure the swelling power of the protein blends. 1.0 g protein sample was weighed into a 50 ml previously weighed conical flask containing 10 ml distilled water and stopped. The flask was agitated manually, heated in a thermostatic oil-bath at 30^0C for 30 minutes, allowed to cool to ambient temperature and homogenized (2000 rpm for 15 minutes) in an orbital mechanical flask shaker (Innova 2000). The experiment was repeated at 40, 50, 60 and 70^0C. Swelling power was evaluated (equation 7);

$$\text{Swelling power (\%)} = \frac{W_2 - W_1}{W_1} \times 100 \quad (7)$$

W_1 = weight of experimental sample of protein blend, W_2 = weight of experimental sample after homogenization.

Determination of foaming properties of protein blends: The foaming behavior of the protein blends were assessed Ya'ara *et al.,* (2022) and Adewumi *et al.,* (2022) with slight modification. 1.0 g each of protein blend was dissolved into five centrifuge tubes each of 50 ml containing 10 ml distilled water and centrifuged at 10,000 rpm, the tubes were withdrawn after 15, 30, 45, 60 and 75 minutes and the volume of foam length in the tube measured. The effect of pH on foaming properties was studied within the range 2-10, in each instance adjusting the pH with 1.0 M HCl or 1.0 M NaOH and experimental duration of 75 minutes. Foaming capacity (FC) and Foaming stability (FS) were evaluated using;

$$FC(\%) = \frac{V_2 - V_1}{V_1} X100 \quad (8)$$

$$FS(\%) = \frac{V_t}{V_0} \times 100 \quad (9)$$

Where V_1 = volume of foam length before centrifugation, V_2 = volume of foam length after centrifugation, V_t = volume of foam length after time t and V_o = initial volume of foam length.

Measurement of the solubility of M. oleifera and M. myristica seed protein blends: The solubility of the protein blends was determined as described by Adeleke *et al.,* (2017) and Ya'ara *et al.,* (2022). 1.0 g of protein sample was added to 20 ml distilled water contained in 50 ml centrifuge tube and the pH adjusted to 2.0 using 1.0 M HCl. The solution was centrifuged at 2500 rpm for 30 minutes, supernatant was withdrawn and its absorbance measured at 595 nm. A reference calibration curve in the concentration range 2.0 to 20.0 $mgmL^{-1}$ prepared using Bovine Serum Albumin (Analar molecular grade) was use to deduce the protein content in the supernatant and residue after centrifugation. This experimental protocol was repeated for pH 4, 6, 8 and 10 in each run adjusting the pH with 1.0 M HCl or

1.0 M NaOH accordingly. The solubility of the protein blends were evaluated;

$$Protein\ solubility\ (\%) = \frac{P_s}{P_{ti}} \times 100 \quad (10)$$

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

Determination of emulsifying properties of M. oleifera and M. myristica seed protein blends: Emulsifying characteristics of the protein blends were determined as outlined by Arise *et al.,* (2015). 1.0 g of protein blend was added to 20 ml distilled water contained in a 50 ml centrifuge tube and homogenized (250 rpm for 5 minutes), 2 ml of the solution was drawn into a 20 ml centrifuge tube containing 2 ml of soybean oil and centrifuged at 2500 rpm for 10 minutes and the emulsifying layer measured. The emulsion was then heated at 70^0C for 20 minutes in a thermostatic oil-bath and centrifuged (2500 rpm for 10 minutes) and the emulsified layer measured. The emulsifying capacity and emulsifying stability were evaluated from equations 11and 12 respectively;

$$Emulsifying\ capacity\ (\%) = \frac{H_e}{H_t} \times 100 \quad (11)$$

$$Emulsifying\ stability\ (\%) = \frac{H_h}{H_0} \times 100 \quad (12)$$

H_e= height of emulsified layer in the centrifuge tube, H_t = height of total content in the centrifuge tube, H_h = height of emulsified layer after heating and H_o = height of emulsified layer before heating.

Determination of surface hydrophobicity of M. oleifera and M. myristica seed protein blends: Surface hydrophobicity of the protein blends were measured by florescence spectroscopy technique as outlined by Ya'ara *et al.,* (2022). Sample protein blends with concentrations; 100, 150, 200, 250 and 300 µg mL^{-1} were prepared in 0.01 M (pH 7.0) phosphate buffer. 100.0 µL aliquot of each concentration was aggregated with 10.0 µL 8.0 mM 8-anilinonaphthalene-1-sulfonic acid in a vial. Fluorescence intensity was measured with an alamar blue fluorochrome at 530 and 590 nm for excitation and emission wavelengths respectively using a spectrofluorometer with grating monochromator (FP-8200). The surface hydrophobicity was evaluated from the slope of the fluorescence intensity versus protein content.

RESULTS AND DISCUSSION

The protein blends showed thixotropic behavior and their amino acids composition and representative class distributions are presented in tables 1 and 2 respectively.

Table 1: Amino acid composition of *M. oleifera* and *M. myristica* seed protein blends

Amino acid	1MOI:1MMI (mg/g)	1MOI:2MMI (mg/g)	2MOI:1MMI (mg/g)
*Leucine (Leu)	19.33±0.62	22.33±1.11	26.16±1.43
*Valine (Val)	12.96±0.84	14.14±1.34	16.03±1.08
*Isoleucine (Ile)	9.55±0.31	12.12±0.78	14.66±1.21
*Phenylalanine (Phe)	9.11±0.55	12.06±0.95	17.71±1.66
*Threonine (Thr)	8.44±0.71	5.01±0.08	8.31±0.86
*Lysine (Lys)	6.08±0.53	8.44±0.93	10.22±1.21
*Histidine (His)	5.32±0.18	6.78±0.81	8.99±0.44
*Metheonine (Met)	4.26±0.45	6.14±1.07	7.66±0.65
*Tryptophan (Typ)	1.04±0.03	1.51±0.13	0.63±0.02
*Cysteine (Cys)	0.57±0.01	0.82±0.04	0.26±0.01
Total essential amino acid	76.66	89.35	110.63
+Glutamic acid (Glu)	74.11±2.72	76.31±2.22	78.84±2.83
+Arginine (Arg)	49.31±1.66	54.05±1.62	58.11±1.88
+Proline (Pro)	15.41±1.07	19.04±1.03	21.67±1.53
+Glycine (Gly)	14.97±1.22	18.81±1.54	20.08±1.42
+Aspartic acid (Asp)	14.05±0.93	16.22±1.23	20.01±1.05
+Alanine (Ala)	12.33±0.87	15.22±1.33	18.91±1.55
+Serine (Ser)	10.98±0.91	10.66±1.05	12.63±1.76
+Tyrosine (Tyr)	6.77±0.51	6.93±0.77	8.08±0.64
Total Non-essential amino acid	197.93	217.24	238.33
Total amino acid	274.59	306.59	348.96

**Essential amino acid +Non-essential amino acid*

The content of essential amino and non-essential amino acids range of (27.91-31.70%) and (68.80-72.08%) respectively, are higher than those for hemp, oats and soybean Srinivasan and Chadrasekhara, (2004) and in range with required daily allowance (RDA) for infants, children and adults (FAO/UN/WHO/1991). Leucine, valine, isoleucine and phenylalanine which are the preponderant amino acids in all the blends are higher than those reported for yellow pea, chickpea and lupin Ekeanyanwu,

(2013). The acidic amino acid distribution (28.32-32.11%), which is higher than (22.16-22.60%) for basic amino acid content, attest to the acidic character of the blended isolates and stand them in good stead as replacement for serum albumin in zone electrophoresis, basic ion exchangers in protein chromatography Zhu *et al.,* (2023) and as antioxidants owing to their metal chelating character Odekanyin *et al.,* (2024). The hydrophilic amino acid distribution (56.48-60.21%) for these protein amalgams, which exceeds those for their hydrophobic score, suggests better digestibility and bioavailability of these protein blends. The sulphur amino acids, accounted for by metheonine and cysteine, in these protein blends *albeit* higher in 2MOI:1MMI, readily forms disulphide-bridge with enzymes Wagoner and Foegeding, (2017), explicate these amphoteric admixes as cofactors, their suitability in functional foods and as important buffers for glucose formation. The high essential amino acid in all the protein blends largely accrued from leucine and valine contents, characteristics of plant protein Aremu *et al.,* (2011); Ekeanyanwu, (2013) are vital markers for growth and development of red blood cells, thereby accentuates their inclusion in cereal-based infant formula.

Table 2: Amino acid distribution of *M. oleifera* and *M. myristica* seed protein blends

Amino acid	1MOI: 1MMI (%)	1MOI: 2MMI (%)	2MOI: 1MMI (%)
Essential	27.91	29.14	31.70
Non-essential	72.09	70.84	68.30
Aliphatic	27.98	29.03	30.12
Aromatic	7.74	8.91	10.15
Acidic	32.11	30.18	28.33
Basic	22.47	21.60	21.16
Sulphur	1.76	2.27	2.58
Hydrophilic	60.21	57.89	56.48
Hydrophobic	30.07	39.76	41.21
Polar	9.75	8.39	7.64
Non-polar	37.48	39.09	40.94

Glutamic acid, arginine and aspartic acid in the blends isolates, are acidic hydrophobic non-essential amino acids residues that have been reported to help address coronary diseases Gorissen *et al.,* (2018); Odekanyin *et al.,* (2024) and stabilization of hydrocolloids, due to intra and inter-dipole moment of COO^- and NH_3^+ groups in the amino acid side chain with the aqueous phase Jain *et al.,* (2019); Javed *et al.,* (2021). The score (0.26-0.57 mg/g) and (0.63-1.51mg/g) for cysteine and tryptophan respectively, *albeit* only the former was reported in *M. myristica* seed protein Ekeanyanwu, (2013), detection of the latter amino acid, a precursor for repair of body tissues and hormonal balance Ya'ara *et al.,* (2022), indicate the alkaline dialysis prevented the characteristic structural modification of this hydrophobic essential amino acid with aromatic side group constitutional in both seed mass.

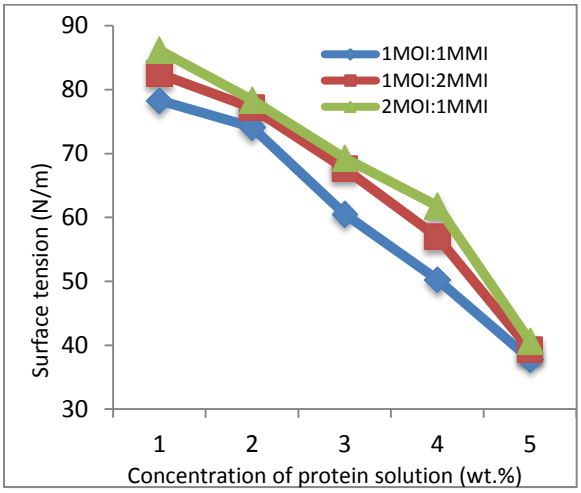

Fig 1: Surface tension of *M. oleifera* and *M. myristica* seeds protein blends

The surface tension of *M. oleifera* and *M. myristica* seed protein blends are shown in figure 1. The amphoteric macromolecules demonstrated a surface tension which is inversely proportional to their concentration. The 2MOI:1MMI blend presents with the highest surface tension within the concentration range (1-5 wt.%), this could be attributed to its highest content of hydrophobic amino acid residue of 41.21% (table 2), accrued largely from leucine, proline, glycine alanine and phenylalanine, preferentially migrates to the surface of the protein solution and shrink the minimum area. Thereby, increases the intermolecular forces Ndagana *et al.,* (2015), with an acute interfacial tension within the minimum space relative to those for 1MOI:1MMI and 1MOI:2MMI. Besides, the lowest surface tension recorded for 1MOI:1MMI could be attributed to its highest hydrophilic amino acid distribution, which necessitated the retention of more hydrophilic amino acid residues in the aqueous phase and reduced their migration towards the surface of the solution. However, as concentration increases from 2 to 5 wt. %, the remarkable decrease in surface tension of 32.90%, 44.81% and 52.00% for 1MOI:1MMI, 1MOI:2MMI and 2MOI:1MMI respectively, suggest the thixotropic behavior of the protein blends, orchestrated by the rearrangement and reorganization of their hetero-amino acids monomers into a dangling oligomers in the aqueous phase, profoundly in 2MOI:1MMI, resulted in significant reduction of these moieties at the surface of the colloidal solutions Muhammad and Dianne, (1995); Ya'ara *et al.,* (2022). Obviously, this concentration-dependent

surface tension demonstrated by these proteins admixes, is instructive and indicates better interfacial surface properties of these hydrocolloids at low concentration. The highest surface tension shown by 2MOI:1MMI protein blend at the lowest and highest concentrations *inter alia* qualifies it more as ingredient for surfactants and marination of food surfaces before stir-frying or cooking to retain flavor and other sensory properties, while in contrast 1MOI:1MMI protein admix with lowest surface tension over the concentration-gradient, will be most preferred for preparation of transdermal patch and matrix for hydrocolloid strip to enhance drug therapeutics.

Table 3: Refractive indices of *M. oleifera* and *M. myristica* protein blends

Blend protein isolate	**1MOI: 1MMI**	**1MOI: 2MMI**	**2MOI: 1MMI**
Refractive index	1.51±0.02	1.46±0.01	1.42±0.04

The refractive indices of the seed protein blends are shown in table 3. The 1MOI:1MMI protein blend shows the highest refractive index of 1.51, consistent with its low surface tension. The elevated refractive index for this protein blend relative to those for 1MOI:2MMI and 2MOI:1MMI protein admixes, could be attributed to the aggregate molar residue refractivity; 23.82 cm^3, 34.62 cm^3, 34.10 cm^3, 39.47 cm^3, 26.06 cm^3, 19.16 cm^3 and 30.37 cm^3 for threonine, histidine, lysine, arginine, aspartic acid, serine and glutamic acid respectively Hauying *et al*., (2011), in 1MOI:1MMI protein blend exceeds those for 1MOI:2MMI and 2MOI:1MMI protein blends. Furthermore, the highest hydrophilic amino acid distribution of 60.21% for 1MOI:1MMI (table 2), implies the amino acids residues in the aqueous phase, are monomodal and alter the course of light ray more in this colloidal suspension. This high refractive index, reflective of a high intrinsic bulk property stands this hydrocolloid in good stead for application as optical coatings in organic light emitting diodes and as antireflective coatings for lenses. The refractive indices of 1.42 to 1.51 for *M. oleifera* and *M. myristica* seeds protein blends are in range with 1.33 to 1.60 for protein solutions Hayakawa *et al.,* (1995); Hauying *et al*., (2011) and compares well with 1.48 for whey protein solution Jessica *et al.,* (2021).

Table 4: Viscometry and energy of flow for *M. oleifera* and *M. myristica* protein blends

Blend concentration (wt. %)	**η_{sp} (cP)**			**η_{int} (cP)**			**E_η (KJmol^{-1})**		
	1MOI: 1MMI	**1MOI: 2MMI**	**2MOI: 1MMI**	**1MOI: 1MMI**	**1MOI: 2MMI**	**2MOI:1 MMI**	**1MOI: 1MMI**	**1MOI: 2MMI**	**2MOI: 1MMI**
5	2.43	2.19	1.95	136.07	132.51	128.14	9.87	7.63	5.51
10	2.73	2.31	2.07	145.04	139.30	131.32	12.15	10.02	8.72
15	3.72	3.32	2.91	184.73	176.20	168.08	12.92	11.20	9.11
20	4.49	4.06	3.56	231.30	206.62	186.44	13.86	11.74	9.46
25	5.92	4.84	4.12	257.81	228.62	217.09	14.40	12.08	10.71

Viscosity indicates the internal molecular friction and connotes the intrinsic bulk properties of Newtonian and non-Newtonian solution Samuel *et al.,* (2024b). Table 4 shows 1MOI:1MMI protein blend with the highest viscometry (specific and intrinsic viscosities) and activation energy of flow in all measured concentrations. This could be ascribed to the highest acidic and basic amino acids distributions recorded for this protein blend. The carboxylic (-COOH) groups in the former distribution readily forms polyacrylamide (polypeptides linkages) with primary (NH_3^+) and secondary (NRH_2^+) amine groups in the latter Laiho *et al.,* (2017). The preponderance of this amphoteric biopolymer in an interlaced isotactic chain in 1MOI:1MMI protein solution as concentration increases, resulted in increased internal molecular friction and consequential retardation of chain mobility Ndagana *et al.,* (2015). In addition, the increase in isotropic behavior in proportion with concentration is more pronounced for this colloidal suspension and enables it to retain mechanical properties of the macromolecules equally in all direction of flow. The viscosities of the protein blends are in consonance with their activation energies of flow, at low concentration amino acid residues could easily interchange within clusters Jessica *et al.,* (2021), and resulted in low E_η. However, as the concentration of the solution increases, the amino acid monomers are more localized and interlaced, with a corresponding decreased in entropy owing to macromolecular and hetero-oligomeric amino acids residues formations, which implies a greater energy to activate their mobility. The values for activation energy of flow (5.51-14.40 KJmol^{-1}) for the protein blends, with optimum value < (15.2-33.3 KJmol^{-1}) for whey protein concentrates Muhammad and Dianne, (1995), present these colloidal solutions as Newtonian fluids Muhammad and Dianne, (1995), and indicates these hydrocolloids are endowed with rheological characteristics that qualifies them for ultra-filteration, spraying and pumping Ulloa *et al.,* (2011), within a

temperature range 30 to 70^{0}C without been denatured and retaining their structural integrity.

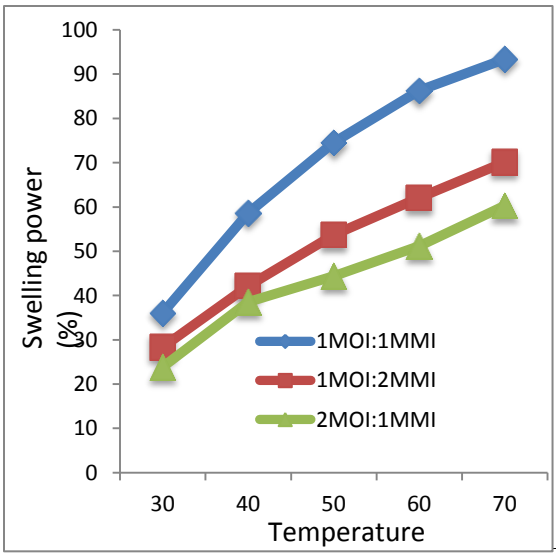

Fig 2: Swelling power of *M. oleifera* and *M. myristica* seeds protein blends

The swelling behaviors of the protein admixes are presented in figure 2. The protein blends swell with increment in temperature and present 1MOI:1MMI protein blend with the highest swelling at the measured temperatures. This higher swelling for 1MOI:1MMI protein blend over those for 1MOI:2MMI and 2MOI:1MMI protein admixes may be attributed to the highest content of 9.75 % polar amino acid residues, characterized by threonine, serine, cysteine and tyrosine. The increase in swelling capacity in proportion with temperature, suggests, thermal energy facilitated the hydration of the polar functional side groups in 1MOI:1MMI hydrocolloid than those in 1MOI:2MMI and 2MOI:1MMI protein blends.

Table 5: Water and Oil absorption capacities for protein blends

Protein blend isolate	Water absorption capacity (gg^{-1})	Oil absorption capacity (gg^{-1})
1MOI:1MMI	6.02±0.24	3.52±0.10
1MOI:2MMI	4.85±0.09	3.91±0.05
2MOI:1MMI	5.64±0.07	4.02±0.03

Hence, enlist it inclusion in food pastries as its constituent preponderant hydrophilic amino acids connote improve moisture sorption and could presents this protein mass with better dough swelling consistency and bioavailability Javed *et al.,* (2021). The swelling capacities 93.36%, 70.14% and 60.33 % for 1MOI:1MMI, 1MOI:2MMI and 2MOI:1MMI amphoteric blends respectively at 70^{0}C is higher than those for Bambara groundnut and *Moringa oleifera* leaf protein blend at the same temperature Adewumi *et al.,* (2022).

The water and oil absorption capacities for *M. oleifera* and *M. myristica* seeds protein blends are represented in table 5. The water absorption capacity 6.02 gg^{-1} for 1MOI:1MMI protein blend which is higher than those for 1MOI:2MMI and 2MOI:1MMI polypeptides blends, is also slightly higher than 5.82 gg^{-1} reported for *Moringa oleifera* leaf protein isolate Adewumi *et al.,* (2022). This suggests the 9.75% content of polar amino acid distribution in 1MOI:1MMI protein blend as evidenced in table 2 corroborate a grater interaction of polar moieties with water molecules in the aqueous phase relative to the latter blends. The water absorption capacities for the protein blends substantiate their hydrophilic amino acids distributions and swelling capacities. The oil absorption capacities of the protein blends depict an inverse relation compared to their water absorption capacities. The values of 3.52 gg^{-1}, 3.91 gg^{-1} and 4.02 gg^{-1} of oil absorption capacities for the blends are in concordance with their non-polar amino acid distributions of 37.48%, 39.09% and 40.94% for 1MOI:1MMI, 1MOI: 2MMI and 2MOI:1MMI protein blends respectively. The relative marginal proportion of hydrophobic hydrocarbon side chain in the 2MOI:1MMI protein blend predisposes this biopolymer blend more to readily bind with hydrophobic fatty acid triglyceride molecules in soybean oil, thereby presents this protein blend with superior underlying properties to minimize rancidity and affirm its inclusion in food products targeted for better mouth feel with improved shelf-life. The 4.22 gg^{-1} for 2MOI:1MMI *M. oleifera* and *M. myristica* seeds protein blend is slightly higher than 3.87 gg^{-1} reported for *Moringa oleifera* leaf protein complex Ya'ara *et al.,* (2022), but two, three and four-folds that reported for protein fractions from mulberry Sun *et al.,* (2017), Berley protein concentrates Houde *et al.,* (2018) and Bambara groundnut and *Moringa oleifera* leaf protein complex Adewumi *et al.,* (2022) respectively. The foaming properties of *M. oleifera* and *M. myristica* seeds protein blends are presented in table 6, while figures 3 and 4 represents the effect of pH on the foaming capacity and stability of the protein blends respectively. The 2MOI:1MMI protein blend showed better foaming properties compared to 1MOI:1MMI and 1MOI:2MMI protein admixes. The 2MOI:1MMI amphoteric molecular-blend presents with more significant decrease of 11.15% in foaming stability after 15 minutes, relative to 1.25% and 1.72% for 1MOI:1MMI and 1MOI:2MMI protein blends respectively, within the same period. Similar trend was also demonstrated for their foaming capacities.

Table 6: Foaming properties for *M. oleifera* and *M. myristica* protein blends

Time (min.)	Foaming capacity (%)			Foaming stability (%)		
	1MOI:1MMI	1MOI:2MMI	2MOI:1MMI	1MOI:1MMI	1MOI:2MMI	2MOI:1MMI
15	11.41±0.17	14.12±0.21	19.45±0.01	75.44±2.22	88.19±3.33	95.21±2.54
30	10.71±0.05	12.44±0.19	15.22±0.21	74.19±2.90	86.47±3.51	84.06±2.66
45	6.07±0.08	9.73±0.04	14.78±0.08	66.64±1.74	73.36±2.07	83.13±1.92
60	4.56±0.02	7.80±0.15	13.65±0.17	58.32±1.53	64.18±3.00	82.55±2.43
75	3.90±0.04	6.24±0.03	12.55±0.14	50.11±1.70	61.30±2.06	81.40±2.70

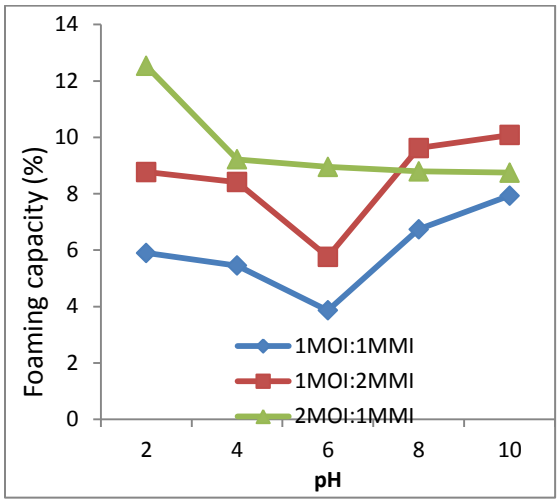

Fig 3: Effect of pH on foaming stability of *M. oleifera* and *M. myristica* protein blends

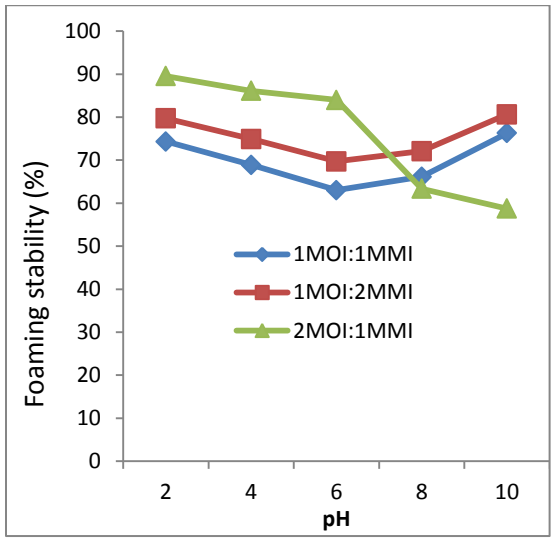

Fig 4: Effect of pH on foaming stability of *M. oleifera* and *M. myristica* protein blends.

This could be attributed to the propensity of 2MOI:1MMI protein blend to rapidly form and maintained an elastic membrane with encapsulated air mass as foaming time increases Aderinola *et al.*, (2020); Ya'ara *et al.*, (2022). Moreover, the highest distribution of hydrophobic amino acid and surface tension, consistent with its low molar refractive index (table 3), for this protein blend are excellent indicators enhancing good foaming properties. The foaming stability for 2MOI:1MMI after 75 minutes is in range with 80.47% for glutelin in mulberry protein Sun *et al.*, (2017) and 80-90% for globulin in *Moringa oleifera* seed protein Aderinola *et al.*, (2020). The effect of pH on foaming capacity and stability are shown in figures 3 and 4 respectively. The foaming properties for the protein amalgams are pH dependent with the 2MOI:1MMI protein solution presenting with the highest foaming properties within the acidic pH (2-6) region. However, the 1MOI:2MMI and 1MOI:1MMI protein solutions shows elevated foaming properties within the alkaline pH (8-10) region *albeit* lower for the latter with the minimum foaming properties recorded for 2MOI:1MMI protein admix solution within the alkaline region. This suggest in alkaline medium, the force of cohesion between amino acids residues in the air-impregnated elastic membrane is less than the force of adhesion between amino acids in the membrane and the aqueous phase for the 2MOI:1MMI protein blend whereas, the reverse physicochemical occurrence predominate for 1MOI:1MMI and 1MOI:2MMI protein blends even though higher in the latter. Accordingly, present 2MOI:1MMI with better foaming properties in acidic medium but in contrast, 1MOI:2MMI and 1MOI:1MMI blends will perform better in alkaline medium. These better foaming properties with variation in time and within the acidic pH region for 2MOI:1MMI, from a technical standpoint underscores it as ingredient in; froth-beverages, polysaccharide based starter-cultures for creamy consistency in yogurt and whipping heavy cream (ice-cream). These results affirmed those for Mung bean protein Du *et al.* (2018), *Moringa oleifera* leaf protein isolates Jain *et al.*, (2019) and Bambara Groundnut and *Moringa oleifera* leaf protein complex Adewumi *et al.*, (2022). The solubility of *M. oleifera* and *M. myristica* seeds protein blends are presented in figure 5. The protein blends demonstrated a pH dependent solubility within the range 2-11, which presents 1MOI:1MMI and 1MOI:2MMI protein solutions with a remarkable decrease in solubility between pH 2 and 5, relative to a marginal increase for 2MOI:1MMI protein solution. This suggest, that below the isoelectric point (pH 4-5), autoprotolysis (self-ionization) of the amphoteric blends produces a cocktail of ionic species with a net

resultant repulsion of charged protein molecules, which enhances the insubstantial solubility of amino acid residues in 2MOI:1MMI, but resulted in decreased solubility in 1MOI:1MMI and 1MOI:2MMI protein blends.

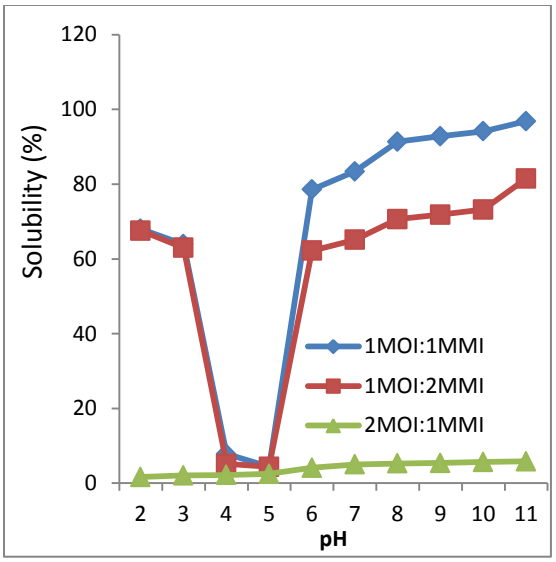

Fig 5: Effect of pH on the solubility of *M. oleifera* and *M. myristica* protein blends

Furthermore, the considerable increase in solubility for 1MOI:1MMI and 1MOI:2MMI protein blends beyond the isoelectric point implies the preponderance OH^-, anchored a negative charge on the macromolecule and dissociated the congregated protein amalgam into amino acid residues with a corresponding increase in dipole-dipole attraction with the aqueous phase. However, for the 2MOI:1MMI protein blend with the highest distribution of hydrophobic amino acid content, the aforementioned chemical transformations resulted in marginal solubility of its amino acid monomers. Moreover, the low solubility of the latter could be attributed to the following; deprotonation of the preponderant hydrophobic and sulphur-based amino acid residues and increased molecular weight occasioned by crosslinking of disulphide molecules and collection of insoluble polysaccharide fibres and other neighboring biopolymers into macromolecular aggregates Du *et al.,* (2018); Ya'ara *et al.,* (2022). Similar trend was reported for *Moringa oleifera* leaf protein Ya'ara *et al.,* (2022) and Bambara Groundnut and *Moringa oleifera* leaf protein complex Adewumi *et al.,* (2022). The contiguous and convoluted characteristics of the curves; for 1MOI:1MMI and 1MOI:2MMI protein blends between pH 2 and 5, presumably may be ascribed to similarity of dipole-dipole inter-play of charged amino acid molecules in their aqueous phases. The solubility of 96.84% for 1MOI:1MMI at pH 11 is slightly higher than 92.72% at pH 9 reported for *Moringa oleifera* leaf protein concentrate Gopalakrishnan *et al.,* (2021), but compares well with those for commercial soy protein concentrate Lakemond *et al.,* (2000), protein isolate from sunflower Ulloa *et al.,* (2011) and Mung bean protein Du *et al.,* (2018). In the near future, this esoteric protein adjunct could be a surrogate for conventional protein hydrocolloid suspension.

Table 7: Emulsifying properties and surface hydrophobicity of protein blends

Parameter	Blend protein isolate		
	1MOI:1MMI	1MOI:2MMI	2MOI:1MMI
Emulsifying capacity (%)	54.34±2.11	51.22±3.09	46.83±2.33
Emulsifying stability (%)	67.46±4.20	66.19±5.42	50.49±1.75
Surface hydrophobicity	121.44±2.40	139.22±4.56	156.14±3.34

The emulsifying properties and surface hydrophobicity of the polypeptides blends are represented in table 7. The emulsifying power and surface hydrophobicity of protein molecule are bulk properties inherent in this amphoteric macromolecule, and determines its ability to form and maintain stable emulsion Jessica *et al.,* (2021). The high emulsifying capacities of 51.22% and 54.34%, with corresponding emulsifying stabilities of 66.19% and 67.46% for 1MOI:2MMI and 1MOI:1MMI respectively, with values above fiftieth-percentile, implies these protein blends are endowed with better emulsifying activity and emulsifying stability indices Zhu *et al.,* (2023), *albeit* higher in the latter and confirm that these physically blended protein molecules will demonstrate better properties to decrease the migration rate of oil droplets, sedimentation speed and reduce micelle particle size in colloidal suspensions. In addition, these protein blends maintained and prevented the flocculation and coalescence of the soybean oil globules more than the 2MOI:1MMI protein blend. The emulsifying capacity of 54.34% for 1MOI:1MMI is in range with 56.36% reported for Bambara groundnut protein isolate Adewumi *et al.,* (2022). However, the emulsifying stability of 66.19% and 67.46% for 1MOI:2MOI and 1MOI:1MMI protein blends respectively, are less than 90.89% for bovine bone protein hydrolysate Zhu *et al.,* (2023), but higher than 47.28% and 56.37% reported for *Moringa oleifera* leaf protein isolate and Bambara groundnut and *Moringa oleifera* leaf protein complex Adewumi *et al.,* (2022). Table 7 showed 2MOI:1MMI protein blend with the highest surface hydrophobicity score of 156.14, which is in agreement with its highest hydrophobic amino acid

distribution. This indicate, the amino acid in this protein blend are globular in structure, occasioned by conglomeration of its aliphatic and aromatic groups Ya'ara *et al.,* (2022), accrued from its 10.15% and 30.12 % aromatic and aliphatic amino acid distributions (table 2), relative to a rod and random coiled-liked structural orientations in 1MOI:2MMI and 1MOI:1MMI protein blends. The latter morphological orientation which is expected to be more balanced in proportion for the 1MOI:1MMI protein blend, judging from its lowest values of 7.74% and 121.44 for aromatic amino acid distribution and surface hydrophobicity respectively, implies more interlaced isotactic macromolecules are retained in the aqueous phase of this protein blend. The value of 156.14 for surface hydrophobicity reported for 2MOI:1MMI protein blend is slightly higher than those reported for *Moringa oleifera* seed protein isolate Mune *et al.,* (2016) and *Moringa oleifera* seed protein Ya'ara *et al.,* (2022).

Conclusion: In conclusion the protein blends from *M. oleifera* and *M. myristica* seeds showed remarkable compositions of amino acids and representative class distributions of amino acids. The distinguishing features of these physically blended protein amalgams, specifically, surface tension, viscometry, solubility, swelling, surface hydrophobicity, foaming and emulsifying properties are empirical and indicate their suitability as surrogates for conventional proteins used in industrial processes.

Declaration of Conflict of Interest: The authors declare no conflict of interest

Data Availability Statement: Data are available upon request from the corresponding author

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