Structuring functional mayonnaise incorporated with Himalayan walnut oil Pickering emulsions by ultrasound assisted emulsification

Gazalla Akhtar, F.A. Masoodi *
Department of Food Science and Technology, University of Kashmir, Srinagar 190006, India

A R T I C L E   I N F O

Keywords:
Pickering emulsions
Walnut oil
Soy protein isolate
Mayonnaise
Pectin

A B S T R A C T

Nowadays Pickering emulsions have attracted immense attention due to their enhanced stability and numerous food applications. In this context, the present study was aimed to introduce Pickering emulsions stabilized by soy protein isolate (SPI)-maltodextrin (MD)-pectin complex incorporated with Himalayan walnut oil (HWO) for development of novel mayonnaise by ultrasound assisted emulsification. The functional mayonnaise was characterised for its stability, structural, textural, rheological and morphological properties. The rheological and microstructure measurements indicated that use of SPI-pectin HWO emulsions had a viscoelastic solid behaviour ($G'' > G''$) with highly interconnected gel-like network structure leading to diffused oil droplet distribution. An increase in particle size diameter (1.86–5.09 µm) and hardness values (43.16–69.08 N) was seen with increase in the SPI-pectin wall material concentration. A significant reduction in whiteness ($L^*$ value) from 91.12 to 53.52 was noted during storage for encapsulated samples. Mayonnaise formulations containing encapsulated HWO depicted significantly lower peroxide value (2.65 meqO$_2$/kg) after extended storage period in comparison to free oil (8.33 meqO$_2$/kg). FTIR analysis of mayonnaise formulations depicted successful complexation of HWO with SPI-MD-pectin matrix. These findings would be of immense importance in designing of Pickering emulsions stabilized by protein-polysaccharide particles with aim of delivering nutraceuticals associated with myriad health benefits.

1. Introduction

Walnut oil is an omega rich oil with immense nutritional values mainly ascribed to its high content of polyunsaturated fatty acids (PUFAs) such as linoleic and linolenic acids, and monounsaturated fatty acids (MUFAs) like oleic and Petroselinic acid [1]. In addition to this, various minor components like tocopherols (mainly represented by $\gamma$-tocopherol) squalene (an unsaturated triterpenoid), phytosterols (mainly 4-methylsterols) [2], and polyphenols [3] are also present conferring health benefits on the host upon its consumption. Despite having immense health benefits, walnut oil is susceptible to lipid oxidation owing to the presence of high unsaturated fatty acid content readily producing hydroperoxides, off-flavours and off-odours compromising basic food quality, flavour and aroma. This challenge has immensely led to the development of walnut oil delivery systems such as oil-in-water stabilized Pickering emulsions which besides maintaining functional aspects can provide improved oxidative stability to the resulting fortified food.

Mayonnaise comprises of a water-in-oil emulsion prepared at low pH with a high oil content which is mainly stabilized by egg ingredients like egg yolk and egg white [4]. Traditionally a conventional mayonnaise comprises of higher oil (80%) in combination with egg yolk which acts as an emulsifier conferring stability and texture to the emulsion. However, presence of high oil concentration in addition to egg yolk limits its edibility for people prone to CVDs and egg allergy. Various studies have shown that plant protein particles and polysaccharides could stabilize mayonnaise like emulsions [5] with a significant effect on its stability and other physicochemical properties like viscosity, texture and rheology [6]. Therefore, other strategies need to be devised for incorporation of oils with high omega content such as walnut oil in emulsion-based gels to enhance solubility and stability of the lipophilic medium [7].

Numerous amphiphilic biopolymers including proteins-polysaccharide combinations form three-dimensional network structure composed of gel matrix (one or more polymers) conferring immense stabilization to the oil-in-water emulsion by adsorbing at oil droplet [8]. Complexation of oppositely charged positively charged proteins with anionic polysaccharides through electrostatic interaction is more...
environmentally friendly with improved emulsification properties [9]. These protein-polysaccharide mixtures inevitably stabilize Pickering emulsions involving inter and intra molecular adsorption features due to self-assembly of protein particles at the oil-in-water interface [10]. Amongst various features, the stability against droplet coalescence and aggregation is the key advantage of Pickering emulsions. Till recently much of the research focused on the development of novel Pickering emulsions employed for incorporation and delivery of probiotics, nutraceuticals, essential oils, fat substitute etc [11]. This has sparked its widespread interest in the food science community [12] despite very limited studies have aimed to design edible Pickering emulsions based on real application scenarios in food industry.

Pectin, an acidic polysaccharide comprises 80% galacturonic acid and is mostly used biopolymer under acidic environments. Pectin has an inherent emulsion stabilizing property and high viscosity enabling its application as a fat mimetic in complexion with proteins e.g whey protein isolate [13]. On the other hand, soy proteins represent multifunctional protein moieties representatives of the legume family with enormous protein level. Soy protein isolate (SPI) are mainly derived from soy proteins and are composed of glycinin and conglycinin [14]. Complexation between whey protein isolate (WPI) and low-methoxyl pectin, WPI-sugar beet pectin, dextran [15] has been previously reported [16]. Soy protein isolate has been utilized for encapsulation and delivery of numerous lipophilic compounds such as orange oil, palm oil, paprika oleoresin either as individual biopolymer or in combination with polysaccharides like as maltodextrin, gelatin, pectin, and others. Furthermore protein-polysaccharide combinations incorporated with bioactive compounds such as whey protein/cellulose nanocrystals, zein/ pectin and gliadin/chitosan hybrid particles have been shown to endow the final product with enhanced antioxidiant properties, oxidative stability which simultaneously opens new window for enrichment of food matrices containing bioactive lipids [17].

Ultrasonication (US) is the most important physical method involved in the encapsulation of bioactive compounds, oils etc in different plant-based biopolymers. In the industrial food applications, low frequency ultrasound in the range of 15–100 kHz is preferred because cavitation occurs within this range causing obliteration of the biopolymers accelerating its incorporation to the core [18]. The application of US in emulsification/ homogenisation promotes cost effective emulsion formation with minimal use of emulsifiers for maintenance of emulsion stability [19]. For development of mayonnaise by US assisted emulsification, desirable color attributed and viscosity alteration has been obtained with better processing attributes [20].

In the light of above references, the main objective of the present study was to investigate the possibility of developing novel functional mayonnaise by incorporation of stable oil-in-water emulsions containing encapsulated Himalayan walnut oil by US assisted emulsification. The physicochemical properties of the SPI-MD-pec Pickering emulsions stabilized with these complexes at pH 4.0 were characterized using colloidal (i.e., particle size), rheological, and microscopic techniques to determine the mechanism of emulsion stability conferred by these complexes. The novelty of this study is to clarify the usefulness of the SPI-MD-pec complex in stabilizing HWO at practical pH conditions (~4.0) encountered in mayonnaise in a cost reasonable manner.

As far as we know no study has reported the complexation of SPI-MD- pectin blend for Pickering emulsion formation of Himalayan walnut oil (HWO). In addition, it was clarified that emulsions can be stabilized at relatively low concentrations of SPI-MD-pec in an industrially feasible manner by using the method of this study, and that it was effective for stabilizing emulsions of relatively large particle size. Our study’s findings provide practical and theoretical information for using SPI-MD-pec complex as fat mimetic to partially substitute oil portion with omega rich Himalayan walnut oil and on the other hand moderately improving the rheological properties and stability with lower caloric intake. The goal of this study was to acquire further knowledge regarding SPI-MD- pec complexes and to accelerate their use in emulsified food.

2. Materials and methods

2.1. Materials

Dried Himalayan walnut kernels were procured from local market of Kashmir valley and subsequently transported to the Laboratory of Food Science & Technology. The kernels were stored in closed zip pouches at −20 °C till further analysis was carried out.

2.2. Chemicals.

High esterified (DE 60–65%) pectin was supplied by Sigma Aldrich (Milan, Italy). Maltodextrin (DE 20), and defatted soyabean meal was provided by Himedia laboratories Mumbai. Soybean oil, egg yolk, vinegar, salt and sugar were food-grade additives and purchased from local supermarket (Srinagar, Kashmir). All other chemicals and solvents used in the analysis were of analytical grade. Deionized water was used for all experiments work.

2.3. Methods

2.3.1. Ultrasound-assisted extraction (UAE) of walnut oil

Walnut kernels (100gm) were finely homogenised by employing laboratory scale polytron homogeniser (Model PT-1200C, Switzerland) at 7000 rpm for 20 min. The finely ground walnut paste was mixed with n-Hexane (1:2) and the slurry was immersed in an ultrasonic bath with frequency of 40 kHz for 10 mins for extraction. The extraction was performed in duplicate for each treatment.

2.3.2. Development of Himalayan walnut oil Pickering emulsions

The encapsulation of HWO in different concentrations of SPI, Pectin and MD for the development of Pickering emulsions has been previously described in our study Akhtar et al. [56] (Supplementary Table S1).

Briefly, the HWO Pickering emulsions were produced by the following protocol.

- SPI and MD were dissolved in distilled water, to this mixture oil was added and the emulsion was ultrasonicated (40 kHz for 10 mins) and homogenized at 20,000 rpm for 3 min
- To the primary emulsion, pectin was added according to the formulation and the primary emulsion was again ultrasonicated and homogenized at 13000 rpm for 5 min to yield the final emulsion.
- The final emulsion was frozen instantly by liquid nitrogen and freeze-dried to yield free-flowing powder.

2.4. Production of functional mayonnaise

Functional mayonnaise was enriched with free and encapsulated HWO (2.5 %). For the production of encapsulated HWO enriched mayonnaise, 100 g in total, distilled water (55 %) salt (1 %) sugar (2 %), pepper (1.5 %) and egg yolk (5%) were poured into a laboratory grade blender and dispersed for 15 s. After that, vinegar (2 %), lemon extract (1%) and soyabean oil (30 %) were simultaneously added and the mixture was further blended for more 40 s. At last, the required encapsulated HWO (2.5%) was added to the emulsion system and the mixture was blended for more 60 s. Similarly, a second mayonnaise system containing an adequate content of neat HWO oil was prepared as a control. The preparation of the second emulsion system followed the same protocol as mentioned above with a difference that the equivalent free HWO oil (1.5 wt%) was mixed to soyabean oil and then blended with the rest of ingredients. Both mayonnaise samples were stored in 100 ml capped glass bottles at ambient storage for 14 days. Samples were taken at Day 1, 7, and 14 of storage for further analysis.
2.5. Characterization of HWO enriched mayonnaise

2.5.1. Optical microscopy of HWO enriched mayonnaise

Mayonnaise structure was observed under a light microscope (Agilent systems, France). Fresh mayonnaise sample (1 mL) mixed with distilled water (1:2 sample: water ratio) was placed on a slide covered with a cover glass and subsequently observed under a light microscope (40x) at room temperature.

2.5.2. Color analysis HWO enriched mayonnaise

The color parameters of the mayonnaise samples enriched with free and encapsulated HWO was done using a Hunter Lab Colorimeter (USA). The analysis results were expressed in terms of L* (lightness), a* (redness) and b* (yellowness).

2.5.3. Rheological properties of HWO enriched mayonnaise

The rheological property of the enriched mayonnaise samples was analyzed by employing a rotational rheometer (MCR – 104, ANTON Paar, Austria). For rheological analysis, approximately 3 gm of the samples were taken and equilibrated under room temperature for 30 mins. After that, the mayonnaise samples were fed to the apparatus for analysis. The frequency sweep test evaluated the changes in storage modulus (G') and loss modulus (G'') as a function of angular frequency (0.1–100 rad/s). All the rheological tests were performed in duplicate wherein mean values were reported.

2.5.4. Emulsion droplet size measurement

The average emulsion droplet size measurement of the mayonnaise samples was performed employing particle analyser (Litesizer, 500, Anton Paar, Austria). 0.5% (w/v) of the mayonnaise samples enriched with free and encapsulated HWO was dispersed in distilled water (Merck Millipore, France) and the resulting mixture was sonicated in bath sonicator for 20 min at a frequency of 40 KHz for proper disruption of the oil globules. The measurements were performed at room temperature.

2.5.5. Texture analysis of HWO enriched mayonnaise

The functional mayonnaise was subjected to texture analysis employing a TAXT Express Stable Micro Systems texture-meter equipped with a compression probe (35 mm diameter) and a 20 kg cell. The samples was performed employing particle analyser (Litesizer, 500, Anton Paar, Austria). For rheological analysis, approximately 3 gm of the samples were taken and equilibrated under room temperature for 30 mins. After that, the mayonnaise samples were fed to the apparatus for analysis. The frequency sweep test evaluated the changes in storage modulus (G') and loss modulus (G'') as a function of angular frequency (0.1–100 rad/s). All the rheological tests were performed in duplicate wherein mean values were reported.

2.5.6. Attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FTIR)

The spectra of HWO enriched mayonnaise were determined by infrared spectroscopy (Spectrum Two, Agilent, USA). The spectra were recorded over the range of 450–4000 cm⁻¹ with a resolution of 4 cm⁻¹. The spectra of each sample were measured 2 times.

2.6. Oxidative stability of HWO enriched mayonnaise

2.6.1. Oil extraction for oxidative stability assay

Oil extraction from the mayonnaise samples was done following the method of Bligh & Dyer [21] with slight modification. Sample (10 g) was mixed with distilled water, chloroform and methanol in a ratio of (1:1:4 v/v) and the resulting mixture was subjected to magnetic stirring for 30 min at 4000 rpm. After that the mixture was transferred to a separating funnel. Bottom phase containing oil in chloroform was separated and desorbed by solvent evaporation at room temperature which was further stored in sealed vials at −20 °C.

2.6.2. Peroxide value (PV) determination

The peroxide value (PV) of the extracted HWO phase was determined following the procedure of Sun-Waterhouse et al. [22] and expressed as “milliequivalent (meq) per kg of oil”.

2.6.3. p-Anisidine value (p-AV) determination

The formation of secondary oxidation products of the extracted HWO was analysed by evaluating its p-AV. All measurements were in triplicate, and p-AnVs were calculated by the following equation:

\[ p - \text{AnV} = \frac{25 \times (1.2 \ \text{Absorbance of pure solvent} - \text{Absorbance of solvent plus oil phase})}{\text{Sample weight}} \]

(0.1–100 rad/s). All the rheological tests were performed in duplicate wherein mean values were reported.

2.7. Scanning electron microscopy

The morphological analysis of mayonnaise samples was done using scanning electron microscope (SU8010 SERIES, HITACHI, JAPAN). The semi dry mayonnaise samples were mounted on an adhesive tape connected to circular aluminium stubs en-coated with gold. The samples were then viewed at an accelerating voltage of 27 kV with 6× and 1000× magnification.

2.8. Statistical analysis

All the experiments were expressed as means with standard deviation. Results were statistically analyzed by ANOVA, using software Statistica 8.0 (Statsoft Inc., Tulsa, USA). Significant difference was determined using Duncan’s multiple range test at p ≤ 0.05.

3. Results and discussions

3.1. Color analysis, stability and visual appearance of mayonnaise samples.

The developed mayonnaise samples were characterised for color analysis through L*, a* and b* parameters over different storage intervals (Day 1, 7 & 14). Color analysis of emulsions presenting homogeneous structures was performed after 1, 7 and 14 days of storage to study the changes produced during storage. On day 1, all mayonnaise formulations presented high luminosity with low tendency to green (−a*) and yellow (−b*). When compared to control, the addition of free and encapsulated HWO does not change the mayonnaise color significantly (p > 0.05). For the mayonnaise samples containing encapsulated HWO (M1-M5), a significant influence of the wall material combination was seen on the emulsion tightness (L*) wherein at day 1, a maximum L* was depicted by samples comprising SPI-MD pec ratio (2:17.9:0.1). At
day 7, $L^*$ values of all the encapsulated samples showed an incremental increase at par with control (C), however a significant decrease in $L^*$ values of MO (mayonnaise containing free oil) were seen during subsequent storage. Mayonnaise samples enriched with encapsulated HWO showed an incremental increase in the lightness values during the storage period which can be explained by successful oil enclosure and retention by SPI-pectin complex. SPI effectively promotes the emulsion formation by enclosing the oil phase via hydrophobic interactions whereas pectin forms a stable steric layer at the interface to stabilize the protein surrounded oil phase. The electro deposition of positively charged SPI with anionic pectin molecule fabricates the close packing of oil droplets in small diameter to create strong structure of interacting aggregates [24]. MD also deciphered an intrinsic steric stabilization owing to the presence of macromolecule branch structure with conjugated compounds possessing hydrophilic nature which may impressively cover the oil droplets to eventuate an improved emulsifying activity. This enables the formation of homogenous emulsions with smaller oil droplets which results in increased light scattering thereby leading to higher $L^*$ values during the course of storage [25]. In all the analysed samples the $a^*$ values exhibited negative denominations, which implies the presence of a greenish tinge in mayonnaise. Similarly, positive $b^*$ values indicate a yellow color which increased during subsequent storage. However, mayonnaise formulations (M1-M5) containing encapsulated HWO showed lower increments in both $a^*$ and $b^*$ values than MO. As depicted in the sensory score card (Table S2) consistent results involving appearance and color were perceived by human sense with those of instrument measurements. Overall M1-M5 were generally acceptable for sensory properties including texture, color, appearance and flavor.

Fig. 1 shows the visual appearance of the mayonnaise samples enriched with free and encapsulated HWO at day 1 and day 14. At day 1 the freshly prepared emulsions depicted an intact self-standing gel with whitish appearance. At day 14, under ambient storage conditions, MO depicted emulsion instability with separate oil phase and intense microbial proliferation. As can be seen visually, M1-M5 comprised of firm gel with no phase separation or creaming attributed to the presence of three-dimensional complex network comprising SPI-MD-pectin. The emulsions (M1-M5) exhibited characteristic non-Newtonian fluid model limiting the free movement of oil droplets. These three-dimensional network structures are primary property of Pickering emulsions owing to the interaction of particles on droplet surface [26]. With increase in SPI and pectin concentration (M1-M5), a layer-by-layer deposition of soy protein enables full coverage of oil globules leading to generation of small oil droplets during the emulsification process. Once the protein is fully adsorbed on the oil droplet surface, adjustment in pH (in our case pH 4.0) and pectin will self-adsorb onto the protein layer thus forming the bilayer. The increase in adsorbed polymer layer thickness will tend to stabilize the emulsion by inhibiting droplet aggregation through enhanced electro-steric repulsions. With addition of pectin and MD, better emulsifying capacity was achieved which resulted from the formation of fibril bundles entangling the protein aggregates providing better emulsifying capacity during the shear-induced emulsification process. The phenomenon of emulsion stability during the course of storage in M1-M5 can also be attributed to the interfacial property of pectin which is dependable on various allied factors like acetyl content, protein fraction, molecular weight, degree of methylation and internal charge distribution, etc. [27]. The close integrity of pectin with SPI improved the packing effectiveness of the emulsion droplets with full coverage with its ability to stretch with SPI side chains. Pectin with its large number of backbone charges formed the more complex stable and stretched amphiphiles, leading to a more firmly packed complex enclosing oil droplets with minimum permeability to water molecules. An increase in the viscosity of continuous aqueous phase is generated with an extended thickened network slowing down oil release and droplet motion by limiting thermodynamic entropy with enhanced interfacial protein adsorption. The generation of oil globules with small diameter with narrow droplet distribution efficiently reduced the emulsion destabilisation process such as Ostwald ripening and creaming (Table 1).

3.2. Texture

As can be seen from the generated data (Table 2), the texture parameters (hardness, adhesiveness, springiness, cohesiveness and gumminess) were shown to vary significantly ($p > 0.05$) between the samples (C, MO and M1-M5). Hardness or firmness determines the primary textural attribute in food systems which refers to force required to compress the sample to a set level of deformation whereas springiness determines the ability of the food system to restore its original shape after the force is withdrawn. Cohesiveness stands for the deformation degree of the sample before fracture and is one of the secondary parameters in texture profile analysis, gumminess is a secondary TPA parameter obtained by multiplying hardness with cohesiveness respectively. As shown in the table, all the textural values increased with increase in the SPI-pectin wall material concentration in HWO Pickering emulsions. The results indicated that pectin conjugation with SPI strengthened the protein gel network around the oil phase. Since pectin is an anionic polysaccharide, it was more likely to have strong electrostatic interaction with SPI. The strengthening of the gel network thus could be associated with segregative and tangly interaction between the biopolymers leading to the formation of a three-dimensional structure around the oil globules. During protein gelation, there could be a continuous protein network with a pectin-rich phase dispersed through it resulting in lesser coalescence of oil droplets [28]. Lower values for textural characteristics in MO can be attributed with coalescence of oil droplets which leads to aggregation and flocculation of the oil phase contributing to a larger emulsion droplet size. This increase in emulsion droplet size leads to a subsequent decrease in the interface area and the contacting points between oil droplets, which could then lead to a decrease in textural values [29]. The increase in the firmness values of

Fig. 1. Cross sectional photographs depicting stability of functional mayonnaise with addition of encapsulated HWO in SPI-MD-pec matrix. (A: Day 1, B: Day 14).
permanent deformation post compression, attributed to the stable and free and SPI-MD-pectin encapsulated HWO in terms of storage modulus helical complexes of the linear chains of MD, which then altered the mechanically strong against rupture [30]. Various studies suggested the M1 in comparison to MO can be attributed to the increased stiffness of M2. The network formed in case of each gel biopolymer. The trend of G crossover of the G_0 against change in frequency (0.01′′–100 Hz) mechanism for the increased G_0 of the developed HWO emulsions. The network formed in case of each gel could not be broken even at the higher angular frequency, resulting in no crossover of the G′ and G″ over the frequency range. Therefore, the gels remained viscoelastic throughout the frequency sweep and did not transform into viscous liquids. As shown in Fig. 2, the viscosity of all the mayonnaise, especially M1-M5 gradually decreased on increasing the shear rate which confirmed the fact that all the samples possess shear thinning non-Newtonian flow behaviour. The decrease in viscosity can be interpreted by increase in the shear rate sufficient to overcome the Brownian motion which simultaneously leads to a decrease in the emulsion flow. The shear thinning behaviour may also be the result of the breakdown of the interactions between the pectin and SPI biopolymer. The trend of G′ and G″ modulus showed dependency to higher frequency values which is specified as firm gel. The proposed mechanism for the increased G′ values of the SPI-pectin complex can be attributed to the decreased mobility of protein particles adsorbed on the oil droplet surface with high molecular weight pectin molecules.

M1 in comparison to MO can be attributed to the increased stiffness of gels due to complex formation of MD with SPI which becomes mechanically strong against rupture [30]. Various studies suggested that the non-polar patches (hydrophobic tails) of SPI could bind with the helical complexes of the linear chains of MD, which then altered the properties of both protein and maltodextrin molecules. From the results shown in Table 2, an increase in SPI (2–8 g/100gm) and pectin (0.1–0.4 g/100gm) formulations increased crumb springiness and retained the permanent deformation post compression, attributed to the stable and close association of the gel network whereas the mayonnaise hardness and springiness values were seen to be dependent on one another.

### 3.3. Rheological measurements

The rheological characteristics of mayonnaise samples enriched with free and SPI-MD-pectin encapsulated HWO in terms of storage modulus (G′) and loss modulus (G″) against change in frequency (0.01–100 Hz) are provided in Fig. 2. While subjecting the gels to frequency sweep in the linear viscosity region (LVR), it was observed that the storage modulus was greater than the loss modulus for all the emulsions studied in this research. This confirmed the gel like viscoelastic behaviour of all the developed HWO emulsions. The network formed in case of each gel could not be broken even at the higher angular frequency, resulting in no crossover of the G′ and G″ over the frequency range. Therefore, the gels remained viscoelastic throughout the frequency sweep and did not transform into viscous liquids. As shown in the Fig. 2, the viscosity of all the mayonnaise, especially M1-M5 gradually decreased on increasing the shear rate which confirmed the fact that all the samples possess shear thinning non-Newtonian flow behaviour. The decrease in viscosity can be interpreted by increase in the shear rate sufficient to overcome the Brownian motion which simultaneously leads to a decrease in the emulsion flow. The shear thinning behaviour may also be the result of the breakdown of the interactions between the pectin and SPI biopolymer. The trend of G′ and G″ modulus showed dependency to higher frequency values which is specified as firm gel. The proposed mechanism for the increased G′ values of the SPI-pectin complex can be attributed to the decreased mobility of protein particles adsorbed on the oil droplet surface with high molecular weight pectin molecules.

**Table 1**

| Day | L*   | a*  | b*  | L*   | a*  | b*  |
|-----|------|-----|-----|------|-----|-----|
| 1   | 88.65 ± 1.50<sup>a</sup> | −0.31 ± 0.53<sup>a</sup> | 9.03 ± 0.43<sup>a</sup> | 78.70 ± 1.21<sup>q</sup> | −0.62 ± 1.54<sup>q</sup> | 12.48 ± 0.56<sup>q</sup> |
| 7   | 77.20 ± 1.21<sup>q</sup> | 74.48 ± 1.42<sup>q</sup> | 11.81 ± 0.98<sup>q</sup> | 69.755 ± 6.97<sup>r</sup> | 53.73 ± 0.87<sup>r</sup> | 10.41 ± 0.42<sup>r</sup> |
| 14  | 36.72 ± 0.76<sup>b</sup> | −1.72 ± 0.34<sup>b</sup> | 53.73 ± 0.87<sup>b</sup> | 73.84 ± 0.34<sup>b</sup> | −0.62 ± 1.54<sup>b</sup> | 12.48 ± 0.56<sup>b</sup> |

Data represent the mean (n = 3) ± standard deviation (SD). Different small letters (abcd…) in the same column indicate a significant difference between different concentrations, while as, different capital letters (PQRS…) in the same column indicate a significant difference between storage intervals.

**Table 2**

| Hardness | Adhesiveness | Springiness | Cohesiveness | Gumminess |
|----------|--------------|-------------|--------------|-----------|
| C        | 43.165 ± 0.874<sup>a</sup> | 24.225 ± 1.165<sup>a</sup> | 0.166 ± 0.654<sup>a</sup> | 0.175 ± 2.641<sup>a</sup> | 0.076 ± 1.247<sup>a</sup> |
| MO       | 40.935 ± 0.776<sup>b</sup> | 40.655 ± 2.448<sup>b</sup> | 0.928 ± 1.171<sup>b</sup> | 0.198 ± 0.764<sup>b</sup> | 0.216 ± 0.621<sup>b</sup> |
| M1       | 60.196 ± 1.123<sup>c</sup> | 59.432 ± 0.763<sup>c</sup> | 0.857 ± 0.548<sup>c</sup> | 0.655 ± 1.423<sup>c</sup> | 42.876 ± 1.398<sup>c</sup> |
| M2       | 64.76 ± 2.438<sup>d</sup> | 107.23 ± 1.554<sup>d</sup> | 1.21 ± 0.498<sup>d</sup> | 0.8223 ± 0.987<sup>d</sup> | 45.922 ± 0.121<sup>d</sup> |
| M3       | 69.642 ± 1.211<sup>e</sup> | 117.476 ± 1.658<sup>e</sup> | 1.488 ± 1.778<sup>e</sup> | 0.821 ± 1.543<sup>e</sup> | 48.926 ± 0.652<sup>e</sup> |
| M4       | 69.755 ± 0.437<sup>f</sup> | 124.532 ± 0.665<sup>f</sup> | 1.142 ± 0.769<sup>f</sup> | 0.866 ± 1.331<sup>f</sup> | 52.877 ± 1.594<sup>f</sup> |
| M5       | 69.805 ± 2.321<sup>g</sup> | 153.32 ± 1.678<sup>g</sup> | 1.106 ± 1.435<sup>g</sup> | 1.403 ± 1.667<sup>g</sup> | 57.61 ± 1.873<sup>g</sup> |

Average of mean values ± standard deviation mean values.

Means in a column followed by different letters are significantly different (P < 0.05).

![Fig. 2](image-url)
involved in the formation of covalent and non-covalent interactions. Practically these gels are termed as emulgels as they possess high viscosity as well as storage modulus ($G''$) which is usually higher than loss modulus ($G'$) at low strains, thus exhibiting solid-like behaviour [31].

The aggregation of SPI and the strong electrostatic interaction between the particles results in the formation of dense interfacial network thereby leading to an incremental increase in the moduli values. This hypothesis can be drawn from the charge distribution of negative and positively charged surface groups of pectin and SPI molecules which in line with theoretical calculations lead to a strong binding with each other which in case of MD is not possible due to its diffused charge distribution. This strong binding may explain the SPI-MD-pectin hybrid formation with unusual properties of a specified firm gel [32]. With increase in pectin concentration (0.1–0.4 g/100 g) an increase in the gel stiffness of the composite gel led to occupation of higher hydrodynamic volume allowing it to interact with more protein particles thereby producing more elastic gels with higher $G''$ values [33]. The SPI-MD-pectin complex encircling the HWO showed a typical shear thinning behaviour of a pseudoplastic fluid during entire shear rate range. Similar inferences were drawn from the studies of [34] for coacervates of chitosan-gum arabic and [35] for whey protein-gum arabic. We suppose that the results of these protein polysaccharide coacervates are in line with the hypothesis. Thus, the enrichment of mayonnaise with SPI-pectin complexed Pickering HWO emulsions lead to the production of strengthened gels. Well established studies have explored the effect of dispersed oil droplets on the rheological properties of the polymer stabilized emulsion systems. Although the oil concentration (10%) was kept constant in this study, the oil droplet size showed a significant effect on the rheological behaviour of the system. Smaller oil droplets tend to produce more regular and homogenous gel matrix network which successively leads to an increase in the solid behaviour of the emulsion systems [36]. Therefore, it is possible to claim that SPI stabilized and dispersed HWO droplets are significant in development of the gel structure leading to a concomitant increase of $G''$ in the emulsion systems. Indeed, protein-stabilized oil globules function as active (bond) fillers when combined with a protein gel matrix because oil droplets can connect to continuous matrix of the system and contribute to the gel strength. This aspect appears only if the filler particle modulus is higher than the gel modulus due to the Laplace pressure [37].

### 3.4. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR)

FTIR spectra of SPI, pec, MD, HWO and formulated mayonnaise (F1-F5) are depicted complex in Fig. 3. The spectrum of SPI depicted specific peaks located at 3300 cm$^{-1}$ indicated the presence of O–H groups of major free amino acid [38]. The bands observed at 2980 cm$^{-1}$ and 1400 cm$^{-1}$ corresponds to the stretching vibration of C–H [39] while as the presence of peaks at 1700 cm$^{-1}$, 1500 cm$^{-1}$ and 1360 cm$^{-1}$ corresponds to the presence of amide I, amide II and amide III attributed to the stretching vibrations of C = O, N–H, and C–H groups, respectively. The spectrum of pure pectin showed main peaks at 1025 cm$^{-1}$ and 960 cm$^{-1}$ attributed to the presence of COO$, C–O$, and C–O–C groups. The presence of peaks at 3400 cm$^{-1}$ and 3000 cm$^{-1}$ the spectrum of MD depicts typical absorption bands attributed to the presence of O–H and C–H in polysaccharides, respectively [40]. The presence of sharp peak at 1800 cm$^{-1}$ is mostly ascribed to the presence of methyl ester group (C=O-O–CH$_3$) while as the presence of peak at 1560 cm$^{-1}$ is assigned to the asymmetric stretching vibration of the carbonyl groups of the carboxylate ion (COO$-$) [12]. Peaks located between 1076 cm and 1 to 1300 cm$^{-1}$ are related to the presence of saccharide structure (C–O–C) and stretching vibrations of CH$_3$ groups [41]. FTIR spectra of mayonnaise samples containing encapsulated HWO (M1-M5) revealed the presence of strong band at 1000 cm$^{-1}$ which depicts the presence of C–O indicating SPI-Pectin complexation. Presence of a dense peak at 1650 cm$^{-1}$ indicated charge based complex coacervation of the biopolymers encapsulating HWO.

Complexation of SPI-MD-Pectin lead to shifting of the absorption bands for eg the bands located at 3300 cm$^{-1}$ shifted to 3257 cm$^{-1}$ indicating the formation of hydrogen bonds between O–H groups in SPI and C = O groups in Pec. FTIR spectrum of free HWO showed characteristic absorption peaks at 3000 cm$^{-1}$ and 2930 cm$^{-1}$ attributing to the presence of O–H and C = C–C groups, respectively. The absorption bands appearing at 2955.93 cm$^{-1}$ and 2703.28 cm$^{-1}$ correspond to the asymmetric and symmetric C–H stretching of methylene. The strong absorption band in HWO spectrum appearing at 1685cm$^{-1}$ corresponds to the presence of esters. The spectra of HWO microcapsules indicated characteristic bands appearing at 2900 cm$^{-1}$ and 2789 cm$^{-1}$ which indicated that HWO was successfully encapsulated into the SPI-MD-pectin microcapsules.

![Fig. 3. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectra of mayonnaise enriched with free (F0) encapsulated HWO (F1-F5).](attachment:image.png)
been observed that PdI is the characteristic feature to predict the average particle size and polydispersity Index (PdI) increased. It has been observed that PdI is the characteristic feature to predict the homogeneity of complex systems with values > 0.2 suggestive of homogenous particles with broad distributions [42]. From the data it was observed that all the emulsions were characterised as polydisperse emulsions (PdI > 0.2) wherein a significant variation was seen in formulations containing SPI-MD and SPI-MD-pectin combinations. Control samples and MO was seen to have comparatively lower particle size and PdI (1.86–2.14 and 2.98–5.63); however, with increasing the SPI-pectin concentration in M2-M5 the PdI increased above 10%. It is observed that PdI (1.86 ± 0.2) suggestive of homogenous particles with broad distributions [42]. From the data it was observed that all the emulsions were characterised as polydisperse emulsions (PdI > 0.2) wherein a significant variation was seen in formulations containing SPI-MD and SPI-MD-pectin combinations. Control samples and MO was seen to have comparatively lower particle size and PdI (1.86–2.14 and 2.98–5.63); however, with increasing the SPI-pectin concentration in M2-M5 the PdI increased above 10%. It is observed that the average particle size distribution of M2-M5 containing SPI-pectin encapsulated HWO was higher than M1 containing SPI-MD encapsulated HWO and this phenomenon can be attributed to the fact that emulsions which correspond to higher average particle size distributions and PdI values, were the emulsions which had the smaller droplet sizes, as seen from the results of optical microscopy, thus showing that particle size distribution does not refer to the emulsion droplets but the emulsion particles overall. It can be hypothesized that with increase in SPI and pectin wall material concentration in the dispersion medium can lead to a greater oil droplet surface area that is covered by pectin molecules, thus facilitating the formation of smaller droplet sizes. Secondly, a higher amount of pectin molecules may inhibit the re-coalescence of the droplets by increasing the viscosity of the continuous phase [43]. The above-mentioned phenomenon is consistent with the fact that decrease in the emulsion pH to 4.0 (by addition of vinegar) results in the electrostatic complexation of the cationic side chains of SPI residues (NH3+)
 with anionic carboxylic side chains (COO–) of pectin molecules. This complexation decreased the intermolecular repulsive forces leading to associative aggregation of the interacting molecules thereby increasing the particle size [44].

3.5. Particle size analysis

The average particle size distribution (PSD) of the emulsion systems is shown in Table 3. From the data it was observed that increasing the SPI and pectin concentration as wall material components in HWO Pickering emulsions for production of functional mayonnaise, the average particle size and polydispersity Index (PdI) increased. It has been observed that PdI is the characteristic feature to predict the homogeneity of complex systems with values > 0.2 suggestive of homogenous particles with broad distributions [42]. From the data it was observed that all the emulsions were characterised as polydisperse emulsions (PdI > 0.2) wherein a significant variation was seen in formulations containing SPI-MD and SPI-MD-pectin combinations. Control samples and MO was seen to have comparatively lower particle size and PdI (1.86–2.14 and 2.98–5.63); however, with increasing the SPI-pectin concentration in M2-M5 the PdI increased above 10%. It is observed that the average particle size distribution of M2-M5 containing SPI-pectin encapsulated HWO was higher than M1 containing SPI-MD encapsulated HWO and this phenomenon can be attributed to the fact that emulsions which correspond to higher average particle size distributions and PdI values, were the emulsions which had the smaller droplet sizes, as seen from the results of optical microscopy, thus showing that particle size distribution does not refer to the emulsion droplets but the emulsion particles overall. It can be hypothesized that with increase in SPI and pectin wall material concentration in the dispersion medium can lead to a greater oil droplet surface area that is covered by pectin molecules, thus facilitating the formation of smaller droplet sizes. Secondly, a higher amount of pectin molecules may inhibit the re-coalescence of the droplets by increasing the viscosity of the continuous phase [43]. The above-mentioned phenomenon is consistent with the fact that decrease in the emulsion pH to 4.0 (by addition of vinegar) results in the electrostatic complexation of the cationic side chains of SPI residues (NH3+)
 with anionic carboxylic side chains (COO–) of pectin molecules. This complexation decreased the intermolecular repulsive forces leading to associative aggregation of the interacting molecules thereby increasing the particle size [44].

3.6. Oxidative stability

Fig. 4 shows the oxidative stability in terms of formation of primary oxidation compounds (peroxide value) and secondary oxidation compounds (p-Anisidine value) of mayonnaise samples enriched with free and encapsulated HWO during storage. M1-M5 presented very low initial concentration of primary oxidation products (PV = 0.67–1.18

Table 3

| Hydrodynamic size (μm) | PDI (%) | D 10 (μm) | D 50 (μm) | D 90 (μm) |
|------------------------|---------|-----------|-----------|-----------|
| C                      | 1.861 ± 0.033a | 2.981 ± 0.228b | 1.181 ± 0.053a | 1.692 ± 0.023a | 1.955 ± 0.766a |
| MO                     | 2.164 ± 0.181b | 5.633 ± 0.217b | 1.705 ± 0.043a | 2.066 ± 0.032b | 2.717 ± 0.543b |
| M1                     | 3.727 ± 0.021c | 17.691 ± 0.763c | 1.786 ± 0.432c | 2.347 ± 0.421c | 2.813 ± 0.489c |
| M2                     | 3.861 ± 1.182c | 18.324 ± 0.048c | 2.314 ± 0.052c | 2.706 ± 0.044c | 3.036 ± 0.132c |
| M3                     | 4.472 ± 0.240a | 21.751 ± 0.014e | 2.599 ± 0.679e | 2.701 ± 0.123e | 3.083 ± 0.375e |
| M4                     | 4.561 ± 0.023d | 30.729 ± 0.361c | 2.815 ± 1.436c | 2.933 ± 0.041f | 3.265 ± 0.487f |
| M5                     | 5.09 ± 0.344f  | 32.753 ± 0.227c | 2.968 ± 0.653c | 3.015 ± 0.451f | 3.418 ± 0.655f |

Average of mean values ± standard deviation values. Means in a column followed by different letters are significantly different (P < 0.05).

Fig. 4. A) Peroxide value (PV), (B) Anisidine value of mayonnaises enriched with free (M0) and encapsulated HWO (M1-M5) during 14 days storage at 4 °C.
the possible appearance of cracks and fissures formed during lyophilization caused by the sublimation of water. The irregular and porous structure of the particles accelerates oxidation by facilitating the access of oxygen to the inside of the matrix and therefore to the encapsulated oil. The PV and p-AV values of the samples containing increasing concentration of MD were higher owing to its crystallization by moisture capture thereby increasing the rate of hydro peroxidation of the oil rapidly. This observation could be well supported by the previous report suggesting utilization of WPI-GA complex coacervates could protect tuna oil against oxidation [50].

3.7. Light microscopy

The optical images of freshly prepared mayonnaise samples enriched with free and SPI-MD-pectin encapsulated HWO are shown in Fig. 5. As shown in the Fig, MO depicted larger oil droplet diameter which can be attributed to absence of the protein polysaccharide complex and the occurrence of coagulation phenomenon. Mayonnaise samples (M2-M5) enriched with HWO microcapsules en-coated with SPI (2–8 g/100 g) and stabilized by pectin (0.1–0.4 g/100 g) depicted a well dispersed oil-in-water structure with lower droplet diameter characterised by the presence of highly packed oil droplets homogeneously distributed in the emulsion matrix. M1-M5 comprised of exactly spherical oil droplets wherein M5 displayed the highest uniformity in droplet size, while the lowest value was encountered with MO. Incorporation of micro-encapsulated HWO with increasing SPI-pectin concentration resulted in more complex particles being adsorbed at the oil–water interface thus the emulsion droplets gradually became smaller and more uniform with the increasing solid contents (M2-M5). The addition of vinegar lead to a decrease in the emulsion pH (4.0) near to the isoelectric point (pi) of SPI (4.5) thus conferring cationic properties to the amino acid side chains of the SPI molecules. In this scenario, the protonated pectin molecules can tightly interact with the positively charged SPI side chains [51] enabling the formation of a stable structure through various covalent, electrostatic force and van der Waals interactions [52] and is considered to be a driver for the complex formation at this pH. The authors also hypothesized another mechanism which involves the overlapping of protein colloidal molecules around each other layer by layer upto a certain thickness reaching the centre of mass (depletion layer) of the pectin molecules thereby increasing the solution available for pectin impregnation. This phenomenon involves an increase in phase entropy with a consequent decrease in free energy, which in turn causes an attractive interaction between the protein particles, making droplet-shaped protein inclusions disperse in a continuous phase of pectin and MD [30]. This phase interaction corresponds to the water in oil emulsions stabilised due to the formation of the gelled state of the pectin-SPI complex which prevented coalescence. The presence of increased concentration of thickening agent like pectin causes high phase viscosity and a decrease in oil droplet movement therefore the phase separation is delayed. In this context, the protein-pectin complex were capable of forming stable oil dispersions inhibiting flocculation of oil droplets and coalescence as figured in MO.

Various studies have published similar observations, Wang et al. [53] concluded that casein particles covalently attached with SPI resulted in the formation of porous honey comb-like structure due to unfolding of protein peptide chains. As a result of this bridged emulsions are formed which significantly promote the formation of emulsion network structures considered to be an ideal model for fabricating protein-stabilized emulsions [54].

3.8. Scanning electron microscopy

Morphological studies of fortified food products are very much essential for analysing embedding efficiency of bio-actives in different wall material combinations and its effect on the physicochemical, organoleptic and sensory properties of the enriched final food product. Fig. 6 shows the fractured surface of both mayonnaise types, mayonnaise enriched with free HWO (MO) and encapsulated HWO (M1-M5). From the Fig it is clear that M1-M5 resulted in the formation of flaked like structures with irregular and agglomerated particle formation. M1-M5 displayed no remarkable difference between the images which could be visually seen, however the particle diameter was roughly equivalent
to 100 µm which corresponds well with droplet size measurements. The appearance of MO shows an oily and wavy consistency with visible oil folds which corresponds to the presence of excessive free surface oil. The presence of spherical or flaked particles which correspond to the SPI-MD (M1) and SPI-MD-pec (M2-M5) complexes incorporated with HWO showed distinct oil microparticles presumably buried inside the protein-polysaccharide matrix. Furthermore, as expected these particulates were found to be absent in MO. Hence, the appearance of these particles revealed that the conjugation of SPI-pec conjugation effectively entrapped the intact HWO globules loaded microcapsules were well preserved during mayonnaise preparation limiting the process of oxidation as can be correlated with oxidative stability of the oils studied above. This clearly indicated a profound effect of pectin–protein conjugation on stability and morphological characteristics of HWO globules [55]. In addition to this, the images displayed the presence of spherical to oval voids within the matrix which implies the encapsulation of HWO within the soy protein-pectin matrix.

4. Conclusion

This study on mayonnaise enriched with n-3 PUFA containing HWO Pickering emulsions demonstrated that physical stability of the final mayonnaise was improved by the addition of SPI-MD-pec bound HWO as compared to mayonnaise enriched with free HWO. HWO microparticles encapsulated in varying concentrations of SPI-MD-Pectin were incorporated in mayonnaise at 2.5%wt basis. An increase in visco-elastic nature and textural parameters was seen in M2-M5. Increase in $L^*$ value (increase in whiteness) was most prominent in M2 and M5 samples and $L^*$ value is considered to be an important due factor because of the consumer preference for whiter color in mayonnaise. M1 had the smallest droplet size, although M2-M5 exhibited more robust solid-like properties, lower liquidity, and faster recoverability than control and MO. Besides, M2-M5 showed super stability without oil–water separation during 14-day storage under room temperature with intact organoleptic and visual properties. Thus, these results demonstrated that M1-M5 were promising substitutes for mayonnaise. Altogether this finding shows the advantage of using n-3 PUFA enriched HWO encapsulated in SPI-MD-pectin emulsifiers adsorbed at the interface to enhance the oxidative stability of mayonnaise. However, further research is required in order to improve the physical stability the organoleptic properties of hydrocolloid emulsifier stabilized mayonnaise to produce a product similar to typical commercial mayonnaise.

**CRediT authorship contribution statement**

Gazalla Akhtar: Investigation, Writing – review & editing, Formal analysis.
F.A. Masoodi: Supervision, Conceptualization, Project administration, Resources.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Acknowledgment**

Prof. F. A. Masoodi is thankful to the Indian Council of Medical Research (ICMR), Government of India for the award of Senior Research Fellowship in favour of Mrs. Gazalla Akhtar (3/1/2/136/2019-Nut)

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2022.106022.

**References**

[1] A. Ganji, I. Farahani, M.R. Pulizran, A. Ghazavi, M. Etehadifar, Ebrahimimom fared. Therapeutic effects of walnut oil on the animal model of multiple sclerosis, Nutritional Neuroscience 22 (2019) 214–222.
[2] Z.H. Zhang, X.A. Zeng, C.S. Brennan, H.L. Mu, R.M. Aadil, Preparation and characterisation of novelty food preservatives by Maillard reaction between ε-poly-lysine and reducing sugars, Int. J. Food Sci. Tech. 54 (5) (2019) 1824–1835.
[3] Y.P. Zou, Y.Y. Gao, H. He, T.K. Yang. Effect of roasting on physicochemical properties, antioxidant capacity, and oxidative stability of wheat germ oil, LWT - Food Science and Technology. 90 (2018) 246–253.
[4] C. Di Mattia, F. Balestra, G. Sacchetti, L. Neri, D. Mastrococca, P. Pittia, Physical and structural properties of extra-virgin olive oil based mayonnaise, LWT - Food Science and Technology. 62 (1) (2015) 764–770, https://doi.org/10.1016/j. lwt.2014.09. 065.
[5] E. Hoseini, A. Rajaei, M. Tabatabaei, A. Mohsenifar, K. Jahanbin, Preparation of pickering flaxseed oil-in-water emulsion stabilized by chitosan-myristic acid nanogels and investigation of its oxidative stability in presence of clove essential oil as antioxidant, Food Biophysics. 15 (2) (2020) 216–228.
