Tuna red meat hydrolysate as core and wall polymer for fish oil encapsulation: a comparative analysis

Parvathy Unnikrishnan¹ · Binsi Puthenveetil Kizhakkethil¹ · Jeyakumari Annamalai² · George Ninan¹ · Zynudheen Aliyamveetil Abubacker¹ · Ravishankar Chandragiri Nagarajara¹

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Abstract Microencapsulation by spray drying is a well-accepted technique for fish oil stabilization. However, severe operational conditions during atomisation destabilise the emulsion, leading to capsule collapse and induction of auto-oxidation. Hence, use of food grade cross-linkers to strengthen the wall material and antioxidants to prevent lipid oxidation has been suggested. A promising option in this line is the use of bioactive peptides, which ensure oxidative stability through structural and chemical stabilisation. Present study attempted to compare the efficacy of yellowfin tuna red meat hydrolysate in protecting the core sardine oil, when used as wall and core polymer during encapsulation. Encapsulates were characterised based on morphological and physical parameters, as well as by in vitro digestibility studies. Their storage stability was also compared under accelerated (60 °C), chilled (4 °C) and ambient conditions (28 °C). Tuna protein hydrolysate exhibited significantly higher protective efficacy when used as core polymer rather than in the wall matrix of encapsulates.

Keywords Tuna red meat · Hydrolysate · Fish oil · Microencapsulation · Antioxidant · Structural stability

Introduction

Encapsulation is an effective technique to improve the stability and delivery of sensitive components like pigments, minerals, vitamins, fatty acids, phytosterols, enzymes etc. as well as living cells like probiotics into foods (Wandrey et al. 2010). These bioactive components are completely enveloped and protected by a physical barrier/wallcoating, which in turn facilitates the controlled and extended release of the core contents under specific conditions. Spray drying is one of the oldest and most common encapsulation technique adopted in the food industry on account of its flexible, continuous, economic operations and it produces particles of micro and nano scale (< 40 µm) (Zuidam and Heinrich 2010). However, one of the major constraint associated with oil encapsulation is the high operational temperature and the mechanical shearing during atomisation which destabilises the emulsion, leading to capsule collapse and oxidation of fish oil (Binsi et al. 2017a). Several authors (Serfert et al. 2009; Morales-Medina et al. 2016) have reported that microencapsulation by spray drying in the presence of antioxidants can enhance the storage stability of fish oil. It is also thought that the stabilization efficiency of antioxidants may not be the same when they are used as wall material component and as core material along with the oil. Moreover, the ordered orientation of wall and core polymers in capsular alignment is a major factor affecting the stability of emulsion during homogenisation and atomisation, and thereby the encapsulation efficiency and the integrity of the microcapsules. In this regard, employing polymers with dual properties viz., emulsifiers with antioxidative properties are advisable, which can physically stabilize the oil-in-water emulsions during atomisation and protect oil encapsulates from oxidation during storage.

✉ Parvathy Unnikrishnan
p.pillai2012@gmail.com

¹ ICAR-Central Institute of Fisheries Technology,
Kochi 682029, India

² Mumbai Research Centre of ICAR-CIFT,
Vashi, Navi Mumbai 400703, India

Protein hydrolysates are breakdown products of enzymatic conversion of proteins into smaller peptides with superior bioactivity compared to their parent proteins. Recent studies have suggested the applicability of fish protein hydrolysate as antioxidants for enhancing the stability of microencapsulated fish oil when used along with core material (Morales-Medina et al. 2016). Here, the observed stability was mainly ascribed to the antioxidant properties of hydrolysates with their superior free radical scavenging and metal chelating activities, as previously reported by several researchers (Qian et al. 2008; Sheriff et al. 2014). Owing to the smaller size of these peptides, a second possible mechanism useful in microencapsulation is the strengthening of capsular wall matrix by acting as co-wall polymers or rather as fillers. Apart from that, the superior emulsifying properties of protein hydrolysates are also thought to be helpful in the structured orientation of core and wall polymers in the emulsion droplets, thereby minimising capsular collapse during atomisation. Tuna red meat is a major process discard generated from tuna canning industry. This serves as a cheap and high-quality protein source, which could be ideally converted to hydrolysates with desirable functional and bioactive properties. Hence, with the hypothesis that tuna protein hydrolysate significantly improve the structural and oxidative stability of fish oil during both spray drying and further storage, a comparison has been made between the efficacy of yellowfin tuna (*Thunnus albacares*) red meat hydrolysate as wall and core polymer for encapsulating sardine oil. Further, the storage stability of the encapsulates under accelerated atmosphere (60 °C), chilled condition (4 °C) and ambient temperature (28 °C) were assessed. The novelty of the study lies in the aspect that tuna red meat from canning discards were utilized for the preparation of hydrolysate for stabilization of fish oil encapsulate and comparison of its efficacy both as core and wall polymer in oil encapsulate was carried out which is so far not reported. Further an indepth storage study analysis under three different conditions was done and a detailed compilation of this kind is not available in literature.

Materials and methods

Raw materials

Sodium caseinate (SRL Pvt. Ltd., Mumbai, India), maltodextrin, gum arabic, pepsin (activity 1 Anson unit/g of protein), pancreatin 4X (3 NF/USP from porcine pancreas) (Hi Media Pvt Ltd., Mumbai, India) were used for the study. Fish oil from Indian oil sardine (*Sardinella longiceps*), procured from a local seafood processing Industry in Mumbai was used for microencapsulate core preparation.

Protein hydrolysate having a molecular weight distribution of 10–30 kDa (25%), 3–10 kDa (30%) and < 3 kDa (45%) (as determined by Amicon Molecular weight cutoff centrifugal filter) and a degree of hydrolysis of $29.35 \pm 0.62\%$ (TCA method), from the red meat of yellow fin tuna (*Thunnus albacares*), optimized for antioxidant properties (DPPH radical scavenging activity: 81.9%, FRAP: 47.4 mM AA/g protein, MC: 36.01 mg EDTA/g protein, ABTS radical scavenging activity: 58.3%) was used for the study.

Fatty acid profiling

Fatty acid composition of sardine oil and sardine oil encapsulate was determined. Total lipid was extracted from the encapsulates by the method of Folch et al. (1957). The fatty acids present in the sardine oil and encapsulates were converted to fatty acid methyl esters (FAMES) using BF₃ methanol and fatty acid composition analysis was performed using gas chromatography (Varian, Guindy, Chennai; Model no: CP-3800) with a chrompack capillary column (25 m × 0.32 mm ID; 0.30 µm film thicknesses) and a flame ionization detector. Supelco 37 Component FAME Mix (Sigma-Aldrich) was used as analytical standard for FA determination.

Preparation of emulsion and spray drying

Composition of the emulsion contained fish oil to wall material in the ratio 1:5. The wall material composition included maltodextrin, gum Arabic and sodium caseinate (2:2:1) in control (SO) and sodium caseinate was partially (1:1) replaced with protein hydrolysate referred to as SPO and completely replaced with protein hydrolysate hereafter referred to as PO, for comparison. In these cases, hydrolysate acted as a wall material component. Another emulsion combination consisted of fish oil containing 1% protein hydrolysate (w/w of fish oil), where hydrolysate acted as core material component, is hereafter referred to as SOP. This particular concentration of hydrolysate was selected based on ABTS IC₅₀ value of hydrolysate from previous studies (data not shown). For emulsion, wall materials were dissolved in distilled water and kept overnight at chilled condition (4 °C) to ensure full saturation of the polymer molecules. To the saturated mixture, fish oil was added dropwise with continuous stirring at 1000 rpm for 10 min using magnetic stirrer. The mixture was then homogenized with a high speed homogenizer (Poly system PT 2100, Kinematica, AG) at 25,000 rpm for 5 min. Prior to spray drying, the emulsions were allowed to stabilize at 4 °C for 1 h.

Spray drying was done using a pilot-scale spray dryer (Hemraj Pvt.Ltd, Mumbai) equipped with a two-fluid

nozzle atomizer under the following experimental conditions viz., inlet and outlet temperature of 160 °C and 80 °C, respectively; spray flow feed nozzle diameter 1.5 mm; air pressure 2.5 bar. Fish oil encapsulates prepared by spray drying were stored in air tight containers for further analysis.

Characterization of emulsion

Emulsion stability index (ESI)

Aliquot of prepared emulsions (150 ml) were transferred to graduated test tubes and kept at 4 °C for 24 h. After this time period, bulk unseparated phase volume was measured and the stability was expressed as (Binsi et al. 2017a):

$$\text{Emulsion stability index (\%)} = \left(\frac{H_1}{H_0} \right) \times 100$$

where H_0 represented the initial emulsion volume and H_1 , the unseparated phase volume.

Characterization of microencapsulates

Scanning electron microscopy (SEM)

SEM (Philips XL 30, The Netherlands) was employed to study the surface morphology of sardine oil encapsulates. Samples were fixed onto double-sided adhesive carbon tabs mounted on SEM stubs, coated with gold in vacuum using sputter coater, and examined by SEM at 10 kV with magnification of 800×.

Differential scanning calorimetry (DSC)

Samples (about 3–5 mg, dry basis) were weighed and placed on differential scanning calorimetry (DSC) aluminum pans and equilibrated over saturated salt solutions in desiccators at 25 °C. The samples were then hermetically sealed with lids for analysis and weighed. The DSC analysis was performed in a TA-MDSC-2920(Ta Instruments, New Castle, De, USA) in an inert atmosphere (45 ml/min of N_2). Instrument calibration was carried out with Indium ($T_{\text{melting}} = 156.6$ °C). Thermo-analytical curves were obtained by heating/cooling the samples at a constant rate of 10 °C/min and a temperature ramp of 20–170 °C under nitrogen atmospheric condition. From the DSC thermogram, the temperature at onset (T_o), peak (T_p), the end (T_f), and the enthalpy (ΔH) were determined.

Fourier-transform infrared spectroscopic analysis (FTIR)

The FTIR spectra of encapsulates were recorded using a FTIR spectrometer (Shimadzu IR-Prestige-21) by KBr

method using diffuse reflectance assembly in the wavelength range of 4500–400 cm^{-1} . Encapsulate sample (about 1 mg) was mixed with potassium bromide in the ratio of 1:100 (w/w) and the reflectance spectra were recorded up to 64 scans with a resolution of 4 cm^{-1} . All the reflectance spectra were transformed to transmission spectra using the Kubelka–Munk algorithm.

Determination of encapsulation efficiency

The relationship between total oil and surface oil/free oil was used to determine the encapsulation efficiency (EE). Soxhlet method (AOAC 2012) was adopted to determine the total oil and free oil was determined as per the methodology described by Sankarikutty et al. (1988). About 2.5 g of oil encapsulate was mixed with 100 ml n-hexane with continuous stirring at ambient temperature for 15 min and then passed through Whatman No. 4 filter paper. The solvent was evaporated in a rotary evaporator and the extracted oil was dried to constant weight by using a stream of nitrogen.

$$\text{EE (\%)} = \frac{\text{Total oil} - \text{surface oil}}{\text{Total oil}} \times 100$$

Physical properties of microencapsulates

Determination of moisture content and hygroscopicity

Moisture content was estimated by oven drying method (AOAC 2012). Methodology of Cai and Corke (2000) with slight modifications were adopted for the determination of sample hygroscopicity. A known quantity of the sample (approximately one gram) was kept in desiccators containing saturated solution of NaCl (relative humidity of 75.3%) at 25 °C. Samples were re-weighed after 1 week and hygroscopicity was expressed as the amount of moisture absorbed per 100 g of dry sample.

Determination of bulk density and tapped density

The bulk density (ρ_B) and tapped densities (ρ_T) of the samples were determined (Chinta et al. 2009) to understand the flow properties exhibited by the encapsulates. A known quantity of sample powder was loosely packed through a funnel into 10 ml graduated cylinder by slight tapping to collect the powder sticking to the wall of the cylinder and the volume was recorded. Bulk density of the powder was calculated by dividing weight of the sample by its volume. Similarly, a known quantity of the sample was poured into the cylinder and tapped until a constant volume was reached to determine the tapped density. Hausner ratio and

Carr Index indicative of the flow properties were determined from the bulk and tapped densities.

Determination of colour

Colour of oil encapsulates were measured using Hunter-Lab scan XE-Spectrocolorimeter (Color Flex, Hunter Associates Laboratory, Reston, USA.) at D-65 illuminant and 10° observer. Pre-determined quantity of samples were filled in a 64 mm glass sample cup and results were expressed in terms of L*(lightness), a*(+ redness/– greenness), b*(+ yellowness/– blueness). The instrument was calibrated using white and black standard ceramic tiles and the readings were recorded in the inbuilt software.

Determination of in vitro oil release kinetics

In vitro oil release kinetics using simulated gastro-intestinal fluids were studied by the methodology described by Burgar et al. (2009). A known quantity of oil encapsulates were initially subjected to gastric digestion using simulated gastric fluid (SGF) containing pepsin at 37 °C for 2 h under acidic condition (pH 1.2). Simulated Gastric Fluid was prepared as follows: 2.0 g of sodium chloride and 7.0 ml of 36% HCl were dissolved in 900 ml of deionised water to which pepsin (3.2 g) was added and pH of the solution was adjusted to 1.2. The final volume of the solution was made up to one litre. Further, pH was adjusted to 6.8 using 1 M sodium hydroxide and simulated intestinal fluid (SIF) was added and intestinal digestion was continued under similar temperature conditions for another 3 h. For the preparation of SIF, 6.8 g of potassium hydrogen phosphate was dissolved in 900 ml of deionised water into which 77 ml of 0.2 M sodium hydroxide and 100.0 g (1 × USP) of pancreatin were added and the solution was left stirring overnight at 4 °C. The pH was adjusted to 6.8 with 1 M sodium hydroxide or with 1 M HCl and the final volume was made up to one litre with deionised water. The quantity of oil released from the microcapsules after exposure to SGF and SIF was determined separately by the method given by Millqvist-Fureby (2003). Digested samples were transferred into a 250-ml stoppered separating funnel to which petroleum ether (75 ml) was added and the solution was mixed thoroughly to separate the aqueous and organic solvent phases. The extraction was repeated twice, using 25 ml petroleum ether each time and the petroleum ether in the collected solvent phases was removed at 60 °C using a rotary evaporator. The sample was further oven dried and cooled to estimate the released oil content in sample.

Storage stability of sardine oil encapsulates

Oxidative stability of fish oil encapsulates stored under accelerated temperature of 60 °C was determined for 1 week on a daily basis by hot air oven method. At ambient (28 °C) and refrigerated (4 °C) temperature, they were analysed for up to 4 weeks at weekly interval. The sardine oil encapsulates were stored in sealed and aluminium foil wrapped glass bottles to avoid exposure to oxygen and light. Peroxide value was determined following the methodology of Shantha and Decker (1994) and thiobarbituric acid reactive substances (TBARS) were assessed as per McDonald and Hultin (1987). The variations in colour of microencapsulates were determined by Hunter-Lab scan XE-Spectrocolorimeter (Color Flex, Hunter Associates Laboratory, Reston, USA.) as described previously.

Statistical analysis

All experiments done in triplicate and the data obtained were subjected to one way analysis of variance (ANOVA) using SPSS software version 16.0 (SPSS Inc, Chicago, Illinois, USA) Duncan's multiple range test was adopted to determine the differences between the means and were considered significant at 5% levels.

Results and discussion

Fatty acid profiling

The sardine oil used for encapsulation contained palmitic acid (25.81%), DHA (11.94%) and EPA (9.18%) as the major fatty acids (Table 1). The fatty acid profile of the sardine oil encapsulates also showed prominence of these components. Among the various encapsulates, SOP samples showed comparable fatty acid profile as that of fresh sardine oil. However, the content of unsaturated fatty acids, in particular the omega-3 fatty acids were markedly lower in PO samples compared to fresh sardine oil and other oil encapsulates indicating the loss of these fatty acids to greater extent in PO sample. This might be due to severe oxidation of core oil during homogenisation and subsequent atomisation.

Characterization of emulsion

Emulsion stability index

The stability and physico-chemical characteristics of oil encapsulates during storage depends primarily on the stability of atomised emulsion. All the emulsions prepared in this study were kinetically stable at rest without any visible

Table 1 Fatty acid profile of sardine oil and sardine oil encapsulates

Major fatty acids	Sardine oil	SO	SPO	PO	SOP
Myristic acid C14:0	6.46 ^a ± 0.05	6.95 ^c ± 0.07	6.73 ^b ± 0.06	7.96 ^d ± 0.08	6.80 ^b ± 0.07
Palmitic acid C16:0	25.81 ^a ± 0.08	27.13 ^d ± 0.07	26.23 ^b ± 0.11	31.33 ^c ± 0.12	26.85 ^c ± 0.09
Palmitoleic acid C16:1	8.78 ^a ± 0.10	9.07 ^b ± 0.09	9.02 ^b ± 0.08	10.54 ^c ± 0.13	8.80 ^a ± 0.10
Stearic acid C18:0	8.22 ^a ± 0.04	8.58 ^b ± 0.14	8.30 ^a ± 0.13	9.99 ^c ± 0.11	8.60 ^b ± 0.05
Oleic acid C18:1	8.68 ^a ± 0.08	8.95 ^b ± 0.07	8.76 ^a ± 0.05	10.19 ^d ± 0.09	9.60 ^c ± 0.08
Arachidonic acid C20:4	3.36 ^b ± 0.05	3.58 ^{cd} ± 0.06	3.63 ^d ± 0.07	2.72 ^a ± 0.10	3.48 ^{bc} ± 0.04
Eicosapentaenoic acid C20:5	9.18 ^b ± 0.08	9.50 ^c ± 0.09	9.80 ^d ± 0.04	6.13 ^a ± 0.10	9.19 ^b ± 0.08
Docosa hexaenoic acid C22:6	11.94 ^b ± 0.12	12.17 ^c ± 0.07	12.39 ^d ± 0.03	7.25 ^a ± 0.03	12.06 ^{bc} ± 0.06
Saturated fatty acids	40.49 ^a ± 0.15	42.66 ^d ± 0.15	41.26 ^b ± 0.07	49.28 ^e ± 0.17	42.25 ^c ± 0.04
Unsaturated fatty acids	41.94 ^b ± 0.15	43.27 ^c ± 0.15	43.6 ^d ± 0.01	36.83 ^a ± 0.07	43.13 ^c ± 0.07
Omega-3 fatty acids	21.12 ^b ± 0.17	21.67 ^c ± 0.08	22.19 ^d ± 0.07	13.38 ^a ± 0.07	21.25 ^b ± 0.13

Values are expressed as Mean ± SD; n = 3; Different superscripts in the same row indicates significant difference ($p < 0.05$)

SO, Maltodextrin, gum Arabic and sodium caseinate as wall material (2:2:1) and fish oil as core material; SPO, Maltodextrin, gum Arabic and sodium caseinate: protein hydrolysate (1:1) as wall material (2:2:1) and fish oil as core material; PO, Maltodextrin, gum Arabic and protein hydrolysate as wall material (2:2:1) and fish oil as core material; SOP, Maltodextrin, gum Arabic and sodium caseinate as wall material (2:2:1) and fish oil containing 1% protein hydrolysate (w/w of fish oil) as core material

phase separation, even 24 h after homogenization which indicated its suitability for spray drying. The stability of emulsions depends primarily on the composition and combinations of the ingredients used as wall and core material. In general, proteins and polypeptides are considered to have superior emulsion properties, as they easily get adsorbed at the oil–water interface to stabilise both stearic and electrostatic forces (Lam and Nickerson 2013). The observed stability of the emulsions indicate that the matrix proteins used in this study viz., sodium caseinate as well as protein hydrolysate were efficient in forming a well-structured emulsion, which remained stable at rest under chilled condition. However, it should be kept in mind that the stability at rest does not guarantee the stability during atomisation under high temperature and shearing, as the emulsion structure may be altered under severe conditions. This can be further confirmed by the microstructural analysis of encapsulates.

Characterization of microencapsulates

Scanning electron microscopy (SEM)

The morphological analysis of sardine oil encapsulates revealed distinct differences in size and surface morphology both within and between the samples (Fig. 1). The microcapsules formed during spray drying showed globular capsules of varying degrees of concavities. A visible difference between the control caseinate encapsulates (SO) and those containing hydrolysates either as core or wall material were reduction in the average dimension of encapsulates in the latter. The encapsulates having protein

hydrolysate alone as wall material (PO) exhibited a distinct reduction in particle size compared to samples containing sodium caseinate (SO, SOP and SPO). This might be due to the differences in the size of micelles formed during emulsification process, owing to the superior surface-active properties of protein hydrolysates. However, the SEM image of hydrolysate containing samples showed the presence of several shrunken capsules compared to that of SO encapsulates. A possible explanation is that in these samples the emulsion might have contained several intact shorter peptides which could not be fit into the miscellar structure, and hence might have appeared as shrunken capsules in the dried powder. In the present study, among the three hydrolysate incorporated samples, higher capsular uniformity was observed when hydrolysate was used as co-wall material (SPO) compared to that as core material (SOP), or as the sole wall material (PO).

Differential scanning calorimetry (DSC)

Thermal analysis of encapsulates were carried out to predict the crystallinity and thermal behaviour of fish oil and microcapsules (Fig. 2a). The DSC analysis of pure sardine oil revealed three endothermic transitions corresponding to initial peak temperature of 25.58 °C and a second well defined melting curve with peak transition at 30.83 °C and the third minor transition at 84.27 °C. The oil encapsulates, on the other hand, showed distinctly different pattern with three different endothermic transitions. The control SO encapsulate showed a glass transition at 25.33 °C, closer to that of pure fish oil. The incorporation of hydrolysate in the wall material slightly reduced the glass transition point of

Fig. 1 SEM images of sardine oil encapsulates

encapsulates, whereas addition to core material slightly increased the glass transition point. The well-defined melting transition observed at 30.83 °C of pure fish oil could not be detected in the thermogram of any of the encapsulates. Instead, a distinct endothermic transition was observed at 71–78 °C, with the lowest temperature for SOP and the maximum for SO. This second transition is more likely associated with the destabilisation of wall polymers owing to helix-coil transformation of protein components. This was further confirmed by the higher enthalpy of transition evidenced in hydrolysate containing samples, with the highest value for PO followed by SOP and SPO. The endothermic transition observed in pure fish oil at 84.27 °C could not be detected in any of the encapsulates, as it was merged with the second transition. However, a third endothermic transition was evident at 102–118 °C with the lowest temperature for SO and the highest for SOP. Hence, it is ideal to consider this point as a critical limit above which the amorphous powders are susceptible to deteriorative changes such as, collapse, oil release, stickiness and caking affecting their quality.

Fourier-transform infrared spectroscopic analysis (FTIR)

The FTIR profile of the encapsulates in the region of 4500 to 400 cm^{-1} were analysed to comprehend the interaction behaviour of wall materials as well as the oxidation pattern

of encapsulated fish oil. The amide-I band observed at 1649.14 cm^{-1} in SO which represents C=O stretching vibrations of amide groups coupled with in-plane NH bending underwent a slight shift in band width toward higher wave number in SPO and PO, and towards a lower wave number in SOP (Fig. 2b). However, the Amide-II band that appeared at 1535.34 cm^{-1} in SO was much compressed in SPO and PO, but shifted towards a higher wave number in SOP. Moreover, an additional band at 1411.89 cm^{-1} appeared in SPO and PO. The bands between 1654 cm^{-1} and 1658 cm^{-1} were assigned to alpha-helix and the bands between 1642 cm^{-1} and 1624 cm^{-1} were assigned to beta-pleated components (Kong and Yu 2007). The characteristic band for random coil conformation can be assigned to the band located at $1648 \pm 2 \text{ cm}^{-1}$. Accordingly, in the present study, major structural rearrangement was observed with the incorporation of hydrolysate in the wall matrix, from random coil to helical confirmation. However predominance of beta-pleated conformation was evident when used along with core material. The amide-A band, which represents NH stretching vibrations appeared at 3392.79 cm^{-1} in SO shifted towards lower band width in all the other samples with major shift in SOP encapsulate. The shifting of amide-A band towards lower frequency is observed, when the N–H group of a peptide is involved in a hydrogen bond (Binsi et al. 2017c). The major shift in this band in SOP indicates

Fig. 2 **a** Thermal characteristics of sardine oil and sardine oil encapsulates by DSC; **b** infra red spectral characteristics of sardine oil encapsulates by FTIR

the interaction between oil and the hydrolysate through hydrogen bond, as expected during emulsion formation. However, amide-B band, which also represent the NH stretching vibrations remained at the similar wave length in all the samples.

The progress of oil oxidation was monitored by following the shift in the characteristic groups of secondary and tertiary oxidation products. The band at 2852.72 cm^{-1}

in SO characteristic of the symmetric stretching vibration of the aliphatic CH_2 , underwent slight shift to lower frequency in PO. Moreover, the band corresponding to symmetric stretching vibration of aliphatic CH_2 group present in pure fish oil disappeared totally in all the encapsulates. The band corresponding to ester carbonyl group of triglycerides observed at 1742.76 cm^{-1} in pure oil was shifted to 1745.58 in SO, SPO and SOP, however,

underwent minimum shift to 1743.65 in PO. This band is characteristic of the axial deformations of carbonyl bonds, present in the majority of the oxidation products, and the broadening of this band indicates the incidence of oxidation. Accordingly, in the present study, the area under ester band followed the order of SOP < SO < SPO < PO, indicating extended rate of oxidation in PO and SPO and a significantly lower value in SOP. The band at 1147.69 assigned to the vibration of the C-O ester groups in pure sardine oil shifted to 1149.57 cm^{-1} in all the encapsulates. However, the well pronounced C-H stretching vibration of the cis-double bond observed in pure fish oil at 3011 cm^{-1} completely disappeared in all the encapsulates. Similarly, the bending vibrations of the CH_2/CH_3 observed at 1464 cm^{-1} in pure fish oil underwent major shift towards lower frequency and was much condensed in all the encapsulates, indicating that all the encapsulates underwent certain extent of oxidation during spray drying process.

Determination of encapsulation efficiency

The choice of encapsulation materials and method must ensure stability of core molecule with maximum retention of its biological activity, which is directly related to the encapsulation efficiency achieved by the process (da Rosa Zavareze et al. 2014). In the present study, the encapsulation efficiency was related to the levels of protein hydrolysate in emulsion composition with higher efficiency for SPO and SOP compared to SO and PO (Table 2). The higher EE observed with the addition of hydrolysate might be related to the superior emulsifying properties of hydrolysates, which facilitated the formation of a strong emulsion stable enough to withstand the shearing forces during homogenisation and atomisation. However, the complete replacement of wall polymer with hydrolysate significantly reduced the EE. This further suggests the possibility of the formation of a weak capsule wall in PO encapsulates, supporting the hypothesis that hydrolysate primarily act as filling polymers rather than as wall polymer.

Physical properties of microencapsulates

Determination of moisture content

A major factor influencing the storage stability of dry powders is the moisture content, which enhances oxidative and microbial deterioration during storage. In the present study, the moisture content of the encapsulates ranged from 4.3 to 6.0% (Table 2). It was observed that replacement of sodium caseinate with protein hydrolysate as wall material resulted in a decrease in the moisture content. The higher moisture content observed in the case of SO encapsulates

might be as a result of larger capsular size, which in turn hindered rapid diffusion and escapement of moisture during the spray drying process. On the other hand, the hydrolysate rich in hydrophilic shorter peptides reoriented the water molecules towards the capsular surface facilitating rapid escapement of moisture during atomisation.

Determination of hygroscopicity

The absorption of water is an important factor for powder reconstitution (Fernandes et al. 2013). At the same time, hygroscopic powders easily absorb water from the surrounding environment, developing stickiness and caking during storage affecting the powder dispersibility, which is not desirable. Hence, moderate hygroscopic nature is preferred for spray-dried powders having desirable dispersibility and storage stability. In the present study, incorporation of protein hydrolysate in the emulsion increased the hygroscopicity of encapsulates (Table 2), which might be due to the hygroscopic nature of hydrolysate itself (Mohan et al. 2015). In addition to that, the smaller particle size of hydrolysate encapsulates as also confirmed by SEM, might have increased the surface area of the sample facilitating more moisture absorption from storage environment. The hygroscopicity of PO encapsulates was almost double of that of SO samples, whereas SOP and SPO showed moderate values.

Determination of bulk density and tapped density

Bulk density and tapped density are important parameters related to packing, transport, and distribution of powders. Eventhough, high bulk density is desirable from economic point of view, low bulk density imparts desirable flow properties to the powder. Both, bulk and tapped densities showed significantly higher values when protein hydrolysate was used as wall material (SPO and PO), while showing a lower value when used as core material along with fish oil (SOP) (Table 2). The bulk density includes the contribution of the inter-particulate void volume apart from true density of powder particles, hence also depends on size and shape regularity of particles, and the spatial arrangement of particles in powder bed (Binsi et al. 2017b). The increase in both bulk and tapped density values associated with incorporation of hydrolysate in the wall material indicates the formation of spherical microcapsules of smaller sizes in SPO and PO, as also confirmed by SEM images, which might have facilitated the compact packing of microencapsulates in a specific volume. Similarly, the lower bulk density coupled with almost similar tapped density values observed in SOP encapsulates indicates microcapsules of larger dimensions and lesser uniformity.

Table 2 Physico-chemical properties of sardine oil encapsulates

Parameters	SO	SPO	PO	SOP
Moisture content (%)	6.02 ^b ± 0.26	5.80 ^b ± 0.21	4.59 ^a ± 0.24	4.30 ^a ± 0.88
Hygroscopicity (%)	4.92 ^a ± 0.26	6.98 ^b ± 0.29	8.31 ^c ± 0.71	6.56 ^b ± 0.39
Bulk density (g/cc)	0.095 ^b ± 0.003	0.143 ^c ± 0.003	0.175 ^d ± 0.008	0.085 ^a ± 0.002
Tapped density (g/cc)	0.123 ^a ± 0.006	0.176 ^b ± 0.008	0.229 ^c ± 0.025	0.106 ^a ± 0.006
Colour				
L*	85.74 ^c ± 0.04	83.02 ^b ± 0.08	81.93 ^a ± 0.09	86.45 ^d ± 0.11
a*	1.07 ^b ± 0.01	1.87 ^d ± 0.01	0.68 ^a ± 0.01	1.18 ^c ± 0.02
b*	10.56 ^b ± 0.06	13.09 ^d ± 0.04	11.48 ^c ± 0.04	8.47 ^a ± 0.02
Encapsulation efficiency (%)	73.89 ^a ± 1.53	78.29 ^b ± 1.06	76.64 ^{ab} ± 1.17	78.73 ^b ± 1.94
Invitro oil release (%)				
1 h	68.2	32.1	53.1	70.4
2 h	77.7	51.2	69.7	82.4
3 h	78.7	56.4	71.8	87.31
4 h	81.5	69.0	75.8	90.8
5 h	88.7	78.0	76.3	93.6

Values are expressed as Mean ± SD; n = 3; different superscripts in the same row indicates significant difference ($p < 0.05$)

The Carr Index and Hausner ratio are used to evaluate the flow properties of powders (Fitzpatrick and Ahrné 2005). In the present study, the flow property indices revealed passable nature [1.26–1.34 (Hausner ratio); 21–25 (Carr index)] for encapsulates with either caseinate or hydrolysate as wall material, i.e. SO (1.3; 23.21) and PO (1.31; 23.36), whereas the flow properties were superior with fair flowability [1.19–1.25 (Hausner ratio); 16–20 (Carr index)] for SPO (1.23; 18.67) and SOP (1.25; 19.84). The lower hausner ratio and Carr index value exhibited SPO and SOP signifies that the powder becomes less cohesive and free flowing with the incorporation of hydrolysate in wall as well as core material. However, complete substitution of caseinate with hydrolysate might have caused the aggregation and lumping of powder in PO, owing to the higher hygroscopicity of the powder. Previously, Jeyakumari et al. (2015) have suggested the variations in the wall material composition as an influential factor in determining the flow properties of oil encapsulate.

Invitro oil release kinetics

A simulated digestion technique can be employed to understand whether the intended form and quantity of encapsulated oil is released in a controlled manner to the specific parts of the gastro-intestinal tract during digestion process (Kosaraju et al. 2009). The incorporation of hydrolysate in the wall matrix significantly retarded the release of encapsulated oil during gastric phase of digestion, with a steady state of release during intestinal phase (Table 2). The SO sample released about 78% of total oil after gastric digestion against the lowest value of 51% in

SPO and 70% in PO. Conversely, almost 82% of total loaded oil was released after gastric digestion in SOP, with a cumulative release of 94% of total loaded oil after gastro-intestinal digestion. Moreover, a spurt release of about 70% of total loaded oil was observed in the case of SO and SOP within 1 h of gastric digestion, whereas only 32% was released by SPO sample during the same duration. The higher rate of gastric release coupled with lower surface oil content, confirms the poor structural integrity of capsular wall in SO and SOP samples. On the similar ground, the lower extent of oil released during gastric digestion in SPO indicates the structural stabilisation of capsular wall in presence of hydrolysate. However, in PO samples it is possible that fish oil is tightly held by the hydrolysate owing to the superior fat binding and emulsifying properties of the hydrolysate (Parvathy et al. 2016). The smaller capsular dimensions revealed by SEM images in PO sample also suggest a similar inference.

Storage stability of oil and encapsulates under accelerated, ambient and chill storage conditions

Changes in peroxide value

The protective effect of protein hydrolysate against lipid oxidation was assessed by comparative evaluation of the oxidation pattern of sardine oil and oil encapsulates under different storage conditions (Fig. 3a, c, e). Peroxide value of the encapsulates were higher initially in comparison to pure sardine oil, eventhough was not significantly different between the encapsulate samples, except for PO. The

Fig. 3 Variations in oxidative indices of sardine oil and sardine oil encapsulates viz., **a** PV and **b** TBARS under accelerated, **c** PV and **d** TBARS under ambient and **e** PV and **f** TBARS under chill storage conditions

higher PV of encapsulates observed immediately after spray drying might be on account of the high temperature and mechanical shearing subjected during encapsulation process which in turn triggered fat oxidation. Previously, Horn et al. (2012) reported high shear during the homogenization process and the incorporation of oxygen during emulsification to be accelerators of oxidation which was also substantiated by initial high emulsion PV values by García-Moreno et al. (2016). However, during accelerated storage for 7 days, distinctly different values were observed ($p < 0.05$), with highest rate of increase in PO, followed by pure sardine oil. The SPO and SO samples showed almost similar rate of increase, whereas SOP exhibited a gradual and lowest rate of increase in PV till the last day of analysis. Similar profile was observed during storage at ambient atmosphere (Fig. 3c) and chilled conditions (Fig. 3e) as well, with higher absolute values at ambient atmosphere compared to chilled atmosphere.

The trends in PV during different storage conditions suggested that the highest rate of protection against oxidation was observed when hydrolysate was used along with fish oil as core material. On the other hand, protein

hydrolysate failed to form proper wall matrix, when used as the sole wall material giving higher PV values irrespective of storage conditions. Similarly, partial replacement of sodium caseinate did not impart additional oxidative protection to the encapsulates, compared to that with sodium caseinate alone as wall material.

Changes in TBARS values

During accelerated storage, significant variation ($p < 0.05$) was observed in the TBARS values of pure fish oil and the different encapsulates (Fig. 3b, d, f). The initial TBARS value of 0.03 ± 0.001 mg malonaldehyde/kg of fresh sardine oil increased drastically and significantly ($p < 0.05$) during storage at accelerated atmosphere (Fig. 3b). Similar trend was observed in ambient (Fig. 3d) and chilled conditions as well (Fig. 3f). However the rate of oxidation was comparatively lower in chilled conditions in comparison to other storage conditions. Similar to PV, the encapsulates also showed higher initial TBARS value compared to that of pure fish oil. Among the encapsulates, PO samples showed significantly higher ($p < 0.05$) TBARS values

Fig. 4 Variations in colour indices of sardine oil during **a** accelerated, **b** ambient and **c** chilled storage and sardine oil encapsulates during **d–f** accelerated (60 °C), **g–i** ambient (28 °C) and **j–l** chilled storage (4 °C)

throughout the storage period, irrespective of storage conditions, whose pattern was closely followed by pure fish oil. Though initially similar, a marked difference in oxidative pattern was observed between SO, SPO and SOP from first week of storage under chilled conditions (Fig. 3f) with rate of oxidation at a decreased rate in SOP whereas, a sharp increase in values were observed for SO and SPO from 2nd week onwards under ambient atmosphere (Fig. 3d). Irrespective of storage conditions, SO and SPO exhibited almost similar pattern of increase in TBARS values, whereas SOP showed much lower absolute values. The difference was much prominent during accelerated

atmosphere with similar rate of increase ($p < 0.05$) for SO and SPO, whereas the TBARS values of SOP samples did not show much variations during the first 7 days of storage (Fig. 3b).

The overall oxidative pattern of encapsulates during different storage conditions revealed the lowest TBARS formation in SOP followed by SPO and SO. Protein hydrolysates alone as wall material (PO) indicated higher PV and TBARS values as pure fish oil, suggesting least protection during storage.

Changes in colour parameters

High lipid foods undergo oxidative deterioration and associated colour changes viz., brown-colored polymers in the presence of other compounds such as amines, amino acids and antioxidants during storage (Zamora and Hidalgo 2005; Augustin et al. 2006). A sharp increase in L^* values were observed in pure sardine oil after 48 h of storage under accelerated conditions (Fig. 4a) and the difference was significant during further storage ($p < 0.05$). Similarly, a sharp increase in lightness was noted after 1 week storage of sardine oil under ambient (Fig. 4b) and chilled condition (Fig. 4c), which varied significantly ($p < 0.05$) during storage. However like sardine oil, the variations in lightness values were not so distinct in the encapsulates under accelerated, ambient and chill storage conditions (Fig. 4d, g, j, respectively). Due to compositional variations in wall material, a significant difference ($p < 0.05$) in lightness value was observed between the encapsulate samples. In the present study, the freshly prepared sardine oil encapsulates were in general, creamish white in appearance with variations in their intensity between the samples. It was observed that samples with higher proportion of sodium caseinate had lighter colour and this could be attributed to the lighter colour of this polymer itself. The colour of sample was lighter and less yellowish when protein hydrolysate was used as core material (SOP) as it masked the yellowish brown colour of the sardine oil used as core.

The chroma parameters such as a^* (redness) and b^* (yellowness) values of sardine oil and oil encapsulates showed marked changes during different storage conditions. Fresh sardine oil showed a marked decrease in redness after 48 h of accelerated storage condition (Fig. 4a) whereas an abrupt decrease in redness was noticed after 1 week of storage under ambient and chilled conditions (Fig. 4b, c, respectively). However the rate of change in redness was slightly lower for oil stored under chilled conditions. The freshly encapsulated samples indicated their nearness towards red chroma with significant variations ($p < 0.05$) between the encapsulates and PO had a comparatively lower redness value which may be on account of variations in composition. A significant decrease ($p < 0.05$) in redness value was observed for all the encapsulates during accelerated (Fig. 4e) and ambient storage conditions (Fig. 4h). However the rate of decrease in redness was comparatively less prominent in the encapsulates during chill storage conditions (Fig. 4k).

Similar to the trends in lightness, a marked increase in yellowness was noticed in sardine oil after 48 h of accelerated storage (Fig. 4a) and after 1 week of storage at ambient (Fig. 4b) and chilled (Fig. 4c) conditions. Though the variations were significant ($p < 0.05$) under accelerated

conditions, the variations were not significant at ambient and chill conditions, except during initial storage period. The b^* values indicating yellowness of samples were observed to vary significantly ($p < 0.05$) between the encapsulates and it increased significantly ($p < 0.05$) during accelerated, ambient and chill storage conditions (Fig. 4f, i, l respectively). Though initially SPO exhibited higher yellowness due to compositional variation, during accelerated storage on account of higher oxidation, the b^* values increased at a higher rate in PO which led to the formation of a homogenous group towards the end of storage and were significantly different from other samples (SO and SOP). Similar observations were made by Binsi et al. (2017a) where the oil encapsulates exhibited an increase in yellowness during storage which indicated the rapid oxidation of surface oil yielding coloured secondary and tertiary oxidation products. Studies by Carneiro et al. (2013) reported whey protein concentrate incorporated flaxseed oil encapsulate to exhibit better oxidative stability and associated colour changes being influenced by it.

Conclusion

The main objective of the present investigation was to compare the efficacy of protein hydrolysate from yellowfin tuna red meat to enhance the structural and oxidative stability of spray dried sardine oil when incorporated as wall material and core material. The results indicated higher encapsulation efficiency for the encapsulates containing protein hydrolysate either as wall or as core material. However the findings from oxidative stability studies suggested a lower rate of protection when protein hydrolysate was used as wall material, compared to that offered when used as core material along with fish oil.

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