Preparation of omega 3 rich oral supplement using dairy and non-dairy based ingredients

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Abstract Fish oil is a rich source of omega 3 fatty acids, an essential fatty acid, vital for the functioning of the human body. But the undesirable flavour is an inherent limitation of fish oil which reduces its acceptability. Masking its fish flavour can increase acceptability of fish oil. The present study was focused on double encapsulation of fish oil to mask its distinct flavour. Fish oil was emulsified using soya lecithin where emulsifier to fat ratio was kept 1:4. The emulsion droplets were in the size range of 172.9 ± 1.7 to 238.2 ± 33.8 nm. The emulsion was mixed with whey protein—sodium alginate solution and converted to beads by dropwise extrusion in calcium chloride solution. Droplets were converted to soft gel beads containing fish oil. The encapsulation efficiency was 89.3%. Fish oil flavour was perceived from the dried beads. Hence, beads were further coated with high melting fat using pan coater and flavoured for making beads palatable to use as an oral supplement. Beads were free-flowing and light yellowish in colour. Beads coated with high melting fat and vanilla flavour scored more in the sensory evaluation by panelists. Beads were kept in airtight pack and stored under refrigeration.

Keywords Encapsulation · High melting fat · Whey protein · Sodium alginate · Fish oil

Introduction

The demand for functional foods is growing as they are extremely vital for prevention, control and treatment of various chronic diseases (Lee et al. 2012). Omega-3 (ω -3) and omega-6 (ω -6) fatty acids found in fish oils are among the most important functional food ingredients. They improve the cardiovascular activity, enhance long-term memory and normal brain function (Kralovec et al. 2012). However, ω -3 fatty acids are susceptible to degradation releasing unhealthy products such as secondary oxidation products of polyunsaturated fatty acids, aldehydes, ketones, alcohols, volatile organic acids, hydrocarbons and epoxy compounds reported by Shahidi and Zhong (2010). Encapsulation is an excellent approach to avoid above problems as it can provide stability and protection, confer targeted and controlled release characteristics. Furthermore, it masks unpleasant odour and taste, extends the shelf life and enhances the bioavailability and palatability of the encapsulated materials. For effective delivery of functional foods, the carrier systems should have properties such as good uptake, extended circulation time, no unacceptable clinical side effects, high biocompatibility and low immunogenicity (McClements et al. 2007). Variety of materials such as cyclodextrin, spring dextrin, chitosan, gelatin and non-gelatin globular proteins such as bovine serum albumin, egg albumin, β -lacto globulin, soy proteins, pea proteins and whey proteins have been used to develop carrier systems for functional foods (Schmitt and Turgeon 2011; Xu et al. 2013).

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Among all biodegradable polymers, alginate is one of the promising candidates for delivery matrix because gel beads can be prepared very easily in aqueous solution at room temperature without the use of any organic solvent (Kikuchi et al. 1999). Chen and Subirade (2006) documented that alginates are natural polysaccharides extracted from brown algae and have a linear chain of 1 → 4 linked β -D-mannuronic acid (M) and R-L-guluronic acid (G) residues. Encapsulation using alginates is most often carried out by drop-wise extrusion of alginate solution through a needle into a gelation medium of calcium chloride solution. Due to the replacement of sodium ion with calcium ions, alginate forms an “egg-box” structure and crosslinking for hydrogel formation occurs. Being food grade, alginate has been used for encapsulating proteins, antioxidants, polyphenols, vitamins (Chen and Subirade 2007) and probiotics (Hansen et al. 2008; Subirade et al. 2010).

Wichchukit et al. 2013 have used whey protein/alginate beads as a carrier of riboflavin as a bioactive component. They have also reported the use of tween 80 for round beads formation. Chen and Subirade (2006) developed alginate and whey protein based granular microspheres (beads) as a carrier of the bioactive compound like riboflavin.

Encapsulation technology is well known in food, pharmaceuticals, chemical and cosmetic industry. In food industry, it is used for fat, oils aroma, vitamins, colourants and enzymes. Fish oil, being a rich source of highly unsaturated long chain omega 3 fatty acids, possess a strong odour due to oxidation of unsaturated fatty acids. Encapsulation would protect fish oil from auto-oxidation of polyunsaturated fatty acids (Jafari et al. 2008). Chen et al. (2013) have encapsulated fish oil with phytosterol esters and limonene by milk proteins. Their study has provided some useful insights into the application of the co-encapsulation concept to protect spray-dried fish oil microcapsules from oxidation by introducing other lipophilic bioactive components, namely phytosterol esters and limonene also as core materials. Co-encapsulation of fish oil with phytosterol esters could effectively prevent polyunsaturated fatty acids from oxidation, and the incorporation of limonene showed good ability to mask the undesirable fishy odour.

Considering benefits and constraints of using omega 3 rich fish oils the present work is carried out with a view of encapsulating fish oil in alginate beads and coat them with high melting fat and flavour to serve as oral supplements.

Materials and methods

Materials

Sodium alginate and guar gum were obtained from SD Fine Chemicals Limited, India. Soya lecithin was procured from Sonic Biochem Extraction Limited, India. Whey protein concentrate 80 was procured from Mahaan Proteins Ltd, India. Calcium chloride was obtained from M/S SD Fine Chemicals Limited, India. Cod Liver oil (Fish oil, Sea Cod) was procured from Sanofi India Limited, India. High melting fat was procured from Mundra Enterprises, India and Flavours (International Flavour and Fragrances India Private Ltd) from the local market, Ludhiana, India.

Design of experiments

The study was designed to develop stable emulsion of fish oil using dairy protein and natural emulsifier, and convert stable emulsion to palatable beads. Based on preliminary trials, soya lecithin and whey protein concentrate (WPC) were selected for preparation stable emulsion of fish oil. Coarse emulsion was prepared by mixing fish oil, WPC and soya lecithin using overhead stirrer. Fine emulsion were prepared using two different methods viz. Ultra Turrex (High Shear Mixture) and high-pressure homogenizer. Emulsifier to fat ratio (EFR) was kept 1:4 and 1:6 in both experiments while other parameters were varied according to equipment. Rotation per minute (rpm) of high shear mixture viz., 10,000, 12,000 rpm and time for shear viz., 12, 8 min were kept as variables for Ultra Turrax while pressure viz., 10, 15, 20 Kpsi and number of pass viz., 1–4 were kept as variable for high-pressure homogenizer. The experimental range of variable was decided based on preliminary trials. Both methods were compared on the basis of particle size (in mm) and the best combination was selected further experimentation. These developed emulsions were converted into beads using sodium alginate and calcium chloride according to previous work. For pan coating of beads, wax and high melting fat were used and compared. The flavouring was done with vanilla and orange flavour to increase its palatability. The results of all the experiments were obtained in triplicate.

Preparation of beads

Fish oil (2 g) and lecithin (0.5 g) were weighed and mixed properly. 100 ml water was added and mixed using overhead stirrer (EUROSTAR, IKA, USA) with 4 blade propeller type stirrer (R 1342) of 50 mm diameter at 1700 rpm for 15 min to make a coarse emulsion. After 15 min 2 g whey protein concentrate was added gradually to the

emulsion and stirred for another 20 min. It was kept overnight for complete hydration of whey proteins. Coarse emulsion was passed four times through high-pressure homogenizer (Constant Systems Limited, UK) at 15,000 psi for fine emulsion preparation. The emulsion was kept overnight at room temperature for protein-liposome complex formation. After overnight storage, sodium alginate was gradually added to the fine emulsion @ 2% w/v. Then the mixture was stirred at 1700 rpm for complete mixing of sodium alginate. The mixture was transferred to the perforated beaker to fall dropwise in 0.2 M CaCl₂ solution (stirred at 500 rpm using magnetic stirrer) for rapid bead formation. After completion of beads formation, beads were kept for hardening in 0.2 M CaCl₂ for another 30 min. Beads were separated using a muslin cloth and washed with distilled water to remove excess of CaCl₂ adhered to the beads. Washed beads were dried in the oven at 50 °C for 3–4 h. Dried beads were stored at refrigeration temperature for further application. For coating of beads, high melting fat (HMF) was taken in the ratio of 1 g HMF: 10 g beads. Flavour was added to the HMF in the ratio of 1:5 [Vanilla Flavour: (HMF)]. Pan coating technique was used for coating beads. Dried beads were directly added to the melted and flavoured HMF and pan coating was done. Beads were enveloped by flavoured HMF upon cooling. These beads were packed airtight and kept away from direct light to check oxidation of fats. During the study of emulsion Ultra Turrax homogenizer [probe S25 N-25G (IKA T-25, USA)] was also used for making the fine emulsion in place of high-pressure homogenizer.

Particle size measurement

The particle size of the emulsion was analysed by dynamic light scattering using a nanoparticle size analyser (Zetasizer Nano series, ZEN3600, Malvern Instruments, UK). The instrument contains a 4-mW He-Ne laser operating at 633 nm wavelength. The measurement was conducted at 173° detection angle and 25 °C.

Total oil content of encapsulated beads

Two grams of microencapsulated beads were taken (before coating with HMF), crushed and fish oil was extracted using soxhlet apparatus.

Encapsulation efficiency

The amount of unencapsulated oil (free oil) was measured to calculate the encapsulation efficiency immediately after producing the beads. For this purpose, hexane (15 ml) was added to an accurately weighed amount (2 g) of microcapsule powder followed by shaking the mixture for 2 min

at room temperature. The suspension was then filtered through a Whatman No. 1 filter paper, and the residue rinsed three times by passing 20 ml hexane through each time. The filtrate solution containing the extracted oil was then transferred to an oven at 70 °C for 6 h for complete evaporation of hexane. The amount of surface oil was calculated by the difference in initial and final weights of slurry container and the encapsulation efficiency was calculated as follows (Tonon et al. 2011; Wang et al. 2011)

Encapsulation Efficiency

$$= \frac{\text{Total Oil Content} - \text{Surface Oil Content}}{\text{Total Oil Content}}$$

Colour analysis (L, a, b)

Colour analysis was done with Mini ScanTM XE plus hunter colour Lab, Virginia, USA.

Sensory analysis

Sensory analysis was carried out by nine semi-trained panellists from ICAR-Central Post Harvest Engineering and Technology. The age range was 24–56 year having both male and female on the panel. Samples were identified using alphabetical code. The ranking test was performed on the scale of 1–5. Panellists were instructed to score samples of coated beads for fish oil odour on intensity scale of 1–5. (Where 1. No fishy smell 2. Extra light fishy smell 3. Light fishy smell 4. Mild fishy smell 5. Strong fishy smell.) All the sample were evaluated in the closed chamber having maintained at 25 ± 2 °C and illuminated with fluorescent light.

Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS (ver. 20) software to evaluate the effect of three different parameters of high-pressure homogenizer viz. EFR, pressure and number of passes, and Ultra Turrax viz. EFR, rpm and time on the size of the particles. Mean of triplicate analysis with standard deviation was reported in the table and compared using ANOVA. On the basis of the critical difference (CD) means were separated. Post hoc used was Duncan. Sensory analysis was compared based on rank.

Results and discussion

Optimizing emulsion preparation

Emulsification of fish oil was tried to make oil in water emulsion using different emulsifier and natural emulsifier soya lecithin was selected for further study. Klinkesorn and co-workers have prepared emulsion using tuna oil and lecithin. They have made a coarse emulsion using high-speed blender and sonication for 2 min at a frequency of 20 kHz, an amplitude of 70%, and a duty cycle of 0.5 (Klinkesorn et al. 2005).

Emulsions were prepared with UT and HP using different combinations and compared for particle size. In case of UT method, it can be seen from Table 1 that average particle size was lower in EFR 1:4 as compared to 1:6 which indicated that EFR 1:4 ratio was adequate to envelop fish oil globules formed due to shearing in UT. A higher percentage of oil in the emulsions results in a larger mean droplet diameter for the same homogenizing conditions (Floury et al. 2000). Particle size was lower (248.5 nm) when the sample was gone for UT for 12 min duration than 8 min. Also, particle size was lower when samples are subjected to 12,000 rpm than 10,000 rpm. High rpm of UT and longer duration may have given higher shearing led to smaller droplet size. As droplets were smaller, total surface area of droplet was increased which was covered by lecithin, emulsifier. Hence sufficient emulsifier must be there to envelop the newly formed droplets otherwise these small droplets unite with each other and form bigger size globule. This may be the possible reason for having bigger sized droplet in samples having EFR 1:6. Statistical data analysis showed a significant effect of emulsifier on the mean particle size while others parameters and interaction effect was not significant which indicate that statistically rpm and time haven't any significant effect on mean particle size.

The same study was conducted with HPH method, in which EFR were kept same as previous one but levels of homogenizer pressure and levels of passes were fixed instead of rpm and time which was done in UT. As like the earlier studies, particle size was found lower (182 nm) in

EFR 1:4 than 1:6 (Table 2). Some worker also reported an increase in mean fat globule diameter with increasing oil content for the homogenization of oil in water emulsions with the APV Gaulin homogenizer. The decrease in droplet diameter was observed with increasing pressure and number of passes, which is in agreement with previous studies (Qian and McClements 2011; Tan and Nakajima 2005; Tcholakova et al. 2003). Statistical data revealed that effect of EFR and number of passes have a highly significant effect on particle size. This study also complemented results of the previous study regarding EFR. So optimum level of emulsifier was necessary to form small size emulsion. Additionally, as the number of passes in homogenizer increased, sample suffered more shear resulted in the much smaller droplet. The mean droplet diameter continued to decrease as emulsions were passed through the homogenizer an increasing number of times (Qian and McClements 2011), but the further reductions were fairly modest. According to Trotta et al. (2002), duration of processing can affect emulsion stability. Reported studies showed that the number of times the product was passed through the device affected the mean particle size and the particle size distribution. Statistical analysis proved that droplets obtained after the third pass and forth pass were not significantly different (Table 2). Also, higher pressure and the higher number of passes resulted in increased in size of droplets which can be observed when a sample having EFR 1:6 passed through HPH four time at pressure 20,000 psi. It was observed that repeating the processing or cycling resulted in a decrease in average particle size of the droplet and a narrowing of the particle size distribution, after which mean particle size and standard deviation both increased as processing continued as reported above. This observation was in harmony with reports validating that droplet size was the result of breakage and coalescence and that, for systems containing relatively high percentages of oil, increasing the operative pressure does not always lead to a reduction of emulsion droplet size. Optimum shear and optimum emulsifier were required for the fine emulsion. Both the studies gave similar result about optimum level of EFR 1:4 for good

Table 1 Particle size analysis of different combination of emulsion made with Ultra Turrax

Ratio of Emulsifier	Rpm of Ultra Turrax	Particle size (nm)	
		Time (in minutes) of Ultra Turrax	
		12 min	8 min
1:4 (Lecithin: Fish oil)	12,000	248.5 ± 12.6	266.0 ± 30.4
	10,000	262.4 ± 35.1	277.8 ± 33.6
1:6 (Lecithin: Fish oil)	12,000	318.3 ± 34.6	359.9 ± 67.7
	10,000	423.9 ± 161.6	511.4 ± 178.2

The values are given as mean ± standard deviation (n ≥ 3)

Table 2 Particle size analysis of different combination of emulsion made with High-Pressure Homogenizer

Ratio of emulsifier	Passes of high-pressure homogenizer	Particle size (nm)		
		Pressure of high-pressure homogenizer		
		10 Kpsi	15 Kpsi	20 Kpsi
1:4 (Lecithin:fish oil)	1st Pass	233.3 ± 13.0 ^a	226.2 ± 13.8 ^a	225.4 ± 10.7 ^a
	2nd Pass	203.2 ± 7.2 ^b	214.8 ± 27.1 ^b	238.2 ± 33.8 ^b
	3rd Pass	185.6 ± 6.5 ^c	183.8 ± 11.3 ^c	192.3 ± 23.2 ^c
	4th Pass	182.0 ± 4.9 ^c	172.9 ± 1.7 ^c	191.8 ± 2.7 ^c
1:6 (Lecithin:fish oil)	1st Pass	246.6 ± 11.8 ^a	251.5 ± 24.3 ^a	248.3 ± 10.6 ^a
	2nd Pass	227.3 ± 8.1 ^b	214.9 ± 12.3 ^b	213.1 ± 7.6 ^b
	3rd Pass	200.2 ± 8.0 ^c	196.5 ± 10.5 ^c	200.3 ± 8.9 ^c
	4th Pass	201.3 ± 5.0 ^c	196.7 ± 17.1 ^c	216.6 ± 36.0 ^c

The values are given as mean ± standard deviation ($n \geq 3$). Values followed by different superscripts within the same column are significantly different ($p < 0.05$) from each other

emulsion. When both UT and HPH was compared on particle size, HPH gave much finer droplets as compared to UT. Also, PDI (Poly Dispersity index) of emulsion made by HPH was quite low as compared to UT.

The mean particle size and the PDI influence the physical stability, solubility, biological performance, release rate, turbidity and chemical stability of emulsions (Tamjidi et al. 2013). High-pressure homogenization produced more stable emulsions than high-shear homogenization (Trotta et al. 2002). Based on these considerations, HPH method was selected for making emulsion and best combination pressure and pass i.e. 15,000 psi and 4 passes were selected in which smaller sized fish oil globules were reported. Particle size analysis of selected combination showed lower value for both mean particle size (163.6 nm) and PDI (0.157).

Standardization of method for production of alginate beads containing fish oil

After optimizing process parameters for emulsion formation, next objective was to optimize the process to encapsulate nano-emulsion in a matrix of alginate using sodium alginate–calcium chloride system. In first attempt, sodium alginate was dissolved in already formed emulsion and beads were formed by dropwise extrusion to 0.2 M calcium chloride solution. Encapsulated beads were harvested and dried. Visible fish oil was observed on the surface of dried beads also fish oil smell was perceived which showed that fish oil was escaped from alginate matrix. The decrease in viscosity was observed sodium alginate solution containing fish oil. Tailing was observed during beads formation due to above effect. Fine fish oil droplets were found in calcium chloride solution remain after hardening of beads and fish

oil smell perceived from calcium chloride solution. Hence, further modification in the process is required to encapsulate fish oil.

Sodium alginate droplet converted to calcium alginate bead by replacement of sodium ion with calcium ion. Crosslinking of alginate chains form beads of calcium alginate and target molecule entrapped in that matrix. When beads were dried, volume of beads reduced due to loss of water and matrix material gets contracted. As fish oil droplets were not bounded to alginate, they escaped and transferred to surface when the beads were squeezed due to drying and contraction of the bonds. Hence, retention of fish oil in alginate bond was possible only if there was any bond between fish oil droplet and alginate network and/or there was some filler material to reduce contraction effect of alginate bonds. The milk protein products viz., sodium caseinate and whey protein concentrate have excellent emulsifying and dehydration properties (Keogh and O’Kennedy 1999). The purpose of the whey protein was to emulsify and stabilise newly created fat/water interfaces. Since whey proteins are globular in nature, any adsorption to an oil/water interface will result in an unfolding of the protein molecule, stabilising the interface but denaturing the protein. Experiments were made using whey protein and skim milk powder as filler at the same level (2%, selected from preliminary studies). It was observed that sample prepared with whey protein concentrate had lower surface fish oil content and was more free-flowing than sample prepared with skim milk powder. Samples were checked for encapsulation efficiency and it was $89.3 \pm 0.5\%$. So whey protein concentrate was selected for further studies. When coarse emulsion with whey protein solution was kept overnight for hydration, drier and free-flowing beads produced then earlier. After incorporating

these process modification, standardize procedure was followed during next experiment of making whey protein-alginate beads containing fish oil.

Coating of whey protein-alginate beads containing fish oil

Sinchaipanid and co-worker have reported the application of hot-melt coating for controlled release of propranolol hydrochloride pellets (Sinchaipanid et al. 2004). Propranolol pellets containing 60% microcrystalline cellulose were prepared by using direct pelletization technique in a fluidized-bed rotary granulator. The pellets of 16:18 mesh size were collected and coated with the molten wax at various ratios and thicknesses in a fluidized-bed top spray coater. Whey protein alginate beads containing fish oil (WAF) was coated with wax as well as high melting fat using pan coating technique in which calculated amount of coating material was melted and beads were directly added to the pan. Beads coated with high melting fat were found to be more free flowing than beads coated with wax. Solidification time of wax was much faster than that of high melting fat hence it is difficult to control coating. Irregular coating and clumps found in beads coated with wax. In addition, beads coated with wax have a hard coating and gritty texture. Considering all observation high melting fat was selected for the further application. It was also observed that ratio of coating material to beads (HMF:WAF) remains almost unchanged irrespective of the initial quantity of coating material. The remaining quantity of coating material was left in the pan as uncoated material. So, 10% more HMF were taken for coating purpose as some fat would adhere to the pan also.

Flavouring of coated WAFs

Flavouring increases the palatability of product. Fish oil was encapsulated but still, there was smell of fish oil and high melting fat resulting in less acceptability. Hence, high melting fat was flavoured at the time of coating with two different flavours viz. orange and vanilla at different

concentration to increase palatability and acceptability. The idea of masking flavour was taken from US 6,235,267 (Santi and Nelson 2001) which was about taste masking of phenolic using citrus flavours. Sensory evaluation showed that vanilla flavour has more flavour masking effect than orange flavour. Finally, vanilla flavour was added in high melting fat at the rate of 20%.

Colour analysis of samples

Colour analysis of prepared beads showed a significant difference in L*, a* and b* value of four types of beads (Table 3). There was no significant difference in L* value of beads coated with HMF whether they contain fish oil or not. But the difference was significant in beads without coating. Coated beads have HMF on their outer surface hence they will show almost same lightness while lightness of uncoated samples will differ reflection due to fish oil. All sample means were significantly differed for a* value. In case of b* value, no significant difference between means of beads coated with HMF without fish oil and beads without coated with HMF with fish oil while other means were significantly differed. Probable reason could be the cumulative effect of thickness of coating as well as fish oil content.

Sensory evaluation of alginate beads samples

A panel of semi-trained sensory expert was selected for sensory evaluation of four different samples of fish oil beads. Table 3 revealed that less fish oil flavour was perceived in beads containing fish oil coated with flavoured high melting fat (Sample B) than uncoated beads containing fish oil without coating (Sample D). Acceptability of beads was increased with the coating of vanilla flavoured high melting fat. Hence, it can be concluded that use of vanilla flavour can mask flavour and increase overall perception.

Table 3 Colour parameters of samples measured by hunter colour Lab and Sensory evaluation of samples based on fishy smell on five-point ranking scale

Sample	Description	L*	a*	b*	Average perception
A	Coating with HMF and without fish oil	47.09 ± 0.56 ^b	8.65 ± 0.09 ^c	32.20 ± 0.76 ^b	1.14 ± 0.44 ^a
B	Coating with HMF and with fish oil	48.22 ± 0.26 ^c	6.68 ± 0.19 ^a	29.56 ± 0.97 ^a	2.43 ± 1.33 ^b
C	Coating without HMF and without fish oil	43.51 ± 0.12 ^a	9.30 ± 0.17 ^d	34.61 ± 0.21 ^c	1.29 ± 0.73 ^a
D	Coating without HMF and with fish oil	48.11 ± 0.59 ^c	7.04 ± 0.15 ^b	32.80 ± 0.22 ^b	3.71 ± 1.36 ^c

The values are given as mean ± standard deviation (n ≥ 3). Values followed by different superscripts within the same column are significantly different ($p < 0.05$) from each other

Score: 5—strong fishy smell, 4—mild fishy smell, 3—light fishy smell, 2—extra light fishy smell, 1—no fishy smell

Conclusion

Fish oil, rich source of unsaturated fatty acids, is prone to oxidation resulting in sharp off odour due to oxidation products. In addition, fish oil has its own distinct flavour, unacceptable to the majority of the population which creates a further problem in its use and application. Hence, a bead containing fish oil was formulated using whey protein-alginate system and further, they were coated using high melting fat and flavoured using vanilla flavour to make it more palatable. Reports of sensory evaluation support that they were sensorially acceptable. Further work in this area can be continued using other coating material, encapsulation technique and raw ingredient to produced oral supplements having more acceptability and control release.

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