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Effect of microencapsulation on quality attributes of fish oil

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Abstract

The fish oil being one of the most important sources of essential fatty acids is considered as nutritional supplement for humans. It is also important to store the fish oil in such a way that it does not develop any rancid flavor and leads to its spoilage. A physical barrier is used to protect fish oil from oxygen, light and temperature difference such that the oxidation of the fish oil can be avoided. In the present study the fish oil microencapsulates were prepared using three different concentration of sodium alginate, 5% (T1), 7% (T2), 9% (T3) and the fish oil without sodium alginate T0 (C) was taken as control and the 4 samples were analyzed for its sensory characteristics and biochemical properties for 15 days at ambient temperature stored in glass bottle. According to biochemical analysis and sensory evaluation, the T1 sample of a fish oil microcapsules prepared with 5% of sodium alginate was most suitable for microcapsule preparation.

Keywords: Auto-oxidation, fish oil, microencapsulation, sodium alginate

1. Introduction

Fish oil is the mostly utilized in dietary enhancements as it is rich in Omega-3 unsaturated fat. It is essentially given as supplement to the individuals who don't consume a great deal of oily fish in their eating routine because of which they couldn't get enough of Omega-3 unsaturated fat (Wang *et al.*, 2006) [10]. As Omega-3 fatty acid are polyunsaturated fatty acid, it is prone to oxidation which degrades the nutritional value of the fish oil. To prevent this the reaction of oxygen with the oil need to be decreased which can be achieved by microencapsulation. Microencapsulation is a procedure wherein a small bead of molecule, for example, strong, fluid or even gas can be ensnared, covered or encircled with a polymeric molecule. There are two materials utilized in microencapsulation for example center/corer material and covering/coating material (Keshari *et al.*, 2016) [5]. The center material is the material which is determined to be covered and covering material is the material which is equipped for framing film with material like sodium alginate for the current research. Microencapsulation is generally used to limit the oxidation of fish oil. Fish oil characterized in a microcapsule, shields it from oxygen and metal particles and furthermore forestall fishy smell getting away from the divider material (Wu *et al.*, 2017) [12]. It likewise defers the auto-oxidation responses of lipids and improve oil solidness.

As fish oil capsule was utilized as dietary enhancement, the choice of safe covering materials is significant. Alginate and chitosan are two regular polysaccharides utilized as divider materials. As a result of outstanding biocompatibility, mucoadhesive biodegradability and mild gelation qualities of Alginate builds its utilization as the divider material. Chitosan created by deacetylation of chitin has various useful properties that makes it technically and physiologically valuable as divider material (Liu *et al.*, 2016) [6]. According to study by Mokhtari *et al.* (2017) [7] alginate is an anionic polysaccharide of (1-4)-linked β -D-mannuronic acid (M) and α -L-guluronic acid (G) widely used in bioencapsulation. Their study also stated that polysaccharides are extremely advantageous, to form filled hydrogels. As an example, sodium alginate dispersion transforms into alginate hydrogel after mixing (titration) with divalent cations (such as calcium ion). According to Wani *et al.* (2016) [2] the primary reason for encapsulating fish oil with gelling polysaccharides such as sodium alginate is to preserve its bio accessibility, control satiety and deliver essential fatty acids to a specific site in the gastrointestinal (GI) tract. Goh *et al.* (2012) [7] stated that the action of calcium ions transforms the aqueous solution of sodium alginate into a gel of variable consistency forming intermolecular crosslinks with the carboxyl groups of guluronate resulting in the well-known egg-box structure and the formation of a polymer coating or shell.

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In this experiment, the effect on the quality aspects of fish oil, when encapsulated in different concentration of sodium alginate was studied.

2. Materials and Methods

2.1 Materials

Pharma grade Fish body oil was commercially purchased. Further process of microcapsules of fish oil was done at Department of Fish Processing Technology laboratory of College of Fisheries Science, Kamdhenu University-Veraval. Sodium alginate which was used as the wall material of the fish oil microcapsule procured from Finar Limited. Different concentration sodium alginate solutions i.e., 5%, 7% and 9% respectively were prepared by mixing 5g, 7g and 9g of sodium alginate powder to 100ml of distil water. Calcium chloride used to harden the microcapsule of fish oil was procured from Thermo Fisher Scientific, India Pvt. Ltd. 3% of calcium chloride solution was prepared by mixing 3g of calcium chloride powder in 100ml of distil water. The fish oil emulsion prepared was dropped in this solution to form microcapsules.

As emulsifier, Polysorbate 20 or Tween 20 was used to prepare oil in water emulsion procured from SRL (Sisco Research Laboratories) Pvt. Ltd, India.

Vitamin E used as the anti-oxidant in the microcapsule was procured from Deve Herbes, Pvt. Ltd. 0.5ml, 0.75ml and 1ml of Vitamin E was added to the emulsion in Experiment -2 and conducted storage studies respectively.

2.2 Methodology

The direct consumption of fish oil is low in India due to its taste, odour and early spoilage. So, it can be used to form microcapsules. About 5ml of fish oil was taken with 1ml of emulsifier in four 100ml beaker. Sodium alginate was added in three beakers at concentration of 5% (T1), 7% (T2) and 9% (T3) respectively. The beaker with fish oil and emulsifier is regarded as control (T0). All the four beakers were kept overnight in dark at the room temperature. After keeping these fish oil emulsion overnight, it was mixed in a magnetic stirrer for the removal of any air bubble if present. Then the emulsion was taken in syringe of 10ml and dropped in 3% of calcium chloride solution to form microcapsules. These capsules were then taken in the petri-plates with calcium chloride solution and frozen at -4 °C. These frozen plates were then dried in a freeze dryer at 65 °C for 7-8 hrs. The formed microcapsules were then taken for sensory evaluation by using 9-point Hedonic scale method. The biochemical test for the capsules were carried out at the time span of 1st and 15th day respectively.

2.3 Observations: The sensory and biochemical analysis was carried out after 15 days of storage.

▪ **Sensory characteristics:** Sensory characteristics were evaluated for fish oil microcapsules using a 9-point Hedonic scale (Peryam and Pilgrims, 1957) [8]. The analysis was conducted on randomly selected samples. Samples were evaluated for texture, colour, taste, odour and overall quality, by 7 panelists using 9-point of Hedonic scale.

▪ **Biochemical analysis**

1. **Peroxide value:** The peroxide value is expressed in terms of mill-equivalent (meq.) free iodine per kilogram

of fat. It is determined by titrating iodine liberated from Potassium Iodine with Sodium thiosulphate solution. Thus, the determination of the peroxide value of fish oil microcapsule was done by chemical method (AOAC, 2006) [1].

$$\text{Peroxide value (meq/kg.)} = \frac{(S-B) \times N \times 1000}{W}$$

Where,

W = Weight of sample taken

S = Volume of sodium thiosulphate used for titration of a sample

B = Volume of sodium thiosulphate used for titration of blank

N = Normality of sodium thiosulphate used

2. **Free fatty acid:** The FFA value is the measure of fatty acid liberated from oil during storage due to moisture, temperature or enzymatic reaction which leads to the hydrolysis of glycerides. It can be determined directly by the titration of oil in an alcoholic medium against potassium hydroxide or sodium hydroxide solution. 10-20 g of sample was taken in the 250 ml of conical flask. To it 50-100 ml of freshly neutralised hot ethyl alcohol with 1 ml of phenolphthalein indicator was added. The mixture was boiled for 5 minutes and further titrated against potassium hydroxide solution. The value was expressed as percent of FFA calculated as oleic acid (FSSAI, 2015).

$$\text{Free Fatty Acid as \% of Oleic Acid} = \frac{28.2 \times V \times N}{W}$$

Where,

V = Titrate value of potassium hydroxide for the sample (ml).

N = Normality of potassium hydroxide solution.

W = Weight of the sample.

3. **Acid value:** The acid value is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acid present in a gram of oil/fat (FSSAI, 2015).

Acid Value = Percent fatty acid (as oleic acid) x 1.99

▪ **Statistical analysis:** ANOVA (Analysis of Variance) Statistical technique was used to find out the significant difference in samples between the treatments as per the Standard Statistical Methods (Snedecor and Cochran, 1994).

3. Results and Discussion

▪ **Sensory Evaluation:** The test was conducted after 15 days of microcapsule preparation. The score of the test stated that treatment T1 had the maximum overall acceptability followed by T3>T2>T0(C). The colour, odour, texture and taste of the T1 had the maximum score among all the treatments. The panels mean score of samples T0(C) to T3 is presented in Figure 1 and Table 1.

Table 1: Sensory evaluation of fish oil microcapsule prepared with different sodium alginate concentration after 15 days of interval.

Treatments	Sensory Evaluation				
	Colour	Odour	Texture	Taste	Overall Acceptability
T0(C)	4.3 ± 0.97 ^{bc}	4.1 ± 0.74 ^{bc}	3.9 ± 0.89 ^{bc}	4.2 ± 0.84 ^{bc}	3.1 ± 0.74 ^{bc}
T1	6.8 ± 0.45 ^a	7.1 ± 0.89 ^a	7.0 ± 0.71 ^a	7.6 ± 0.89 ^a	7.5 ± 0.87 ^a
T2	6.3 ± 0.45 ^{ab}	6.9 ± 0.74 ^a	6.2 ± 0.84 ^{ab}	7.4 ± 0.65 ^a	6.3 ± 0.45 ^{ab}
T3	6.7 ± 0.67 ^a	7.0 ± 0.79 ^a	6.1 ± 0.74 ^{ab}	6.7 ± 0.97 ^a	6.9 ± 0.89 ^a

Values are in mean ± SD, n=5, ^{a,b,c} Value with different superscripts in a column for each parameter differ significantly (*p*<0.05)

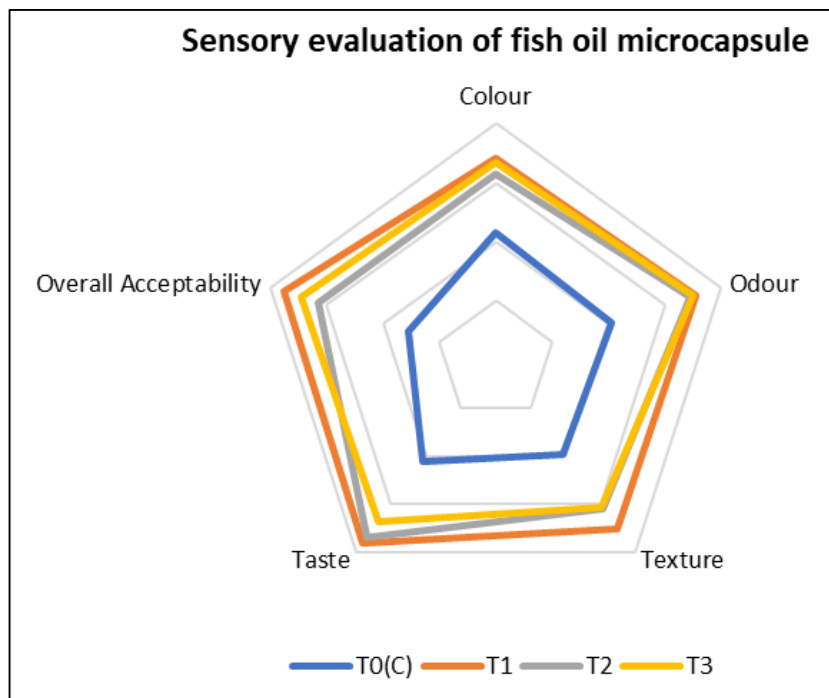
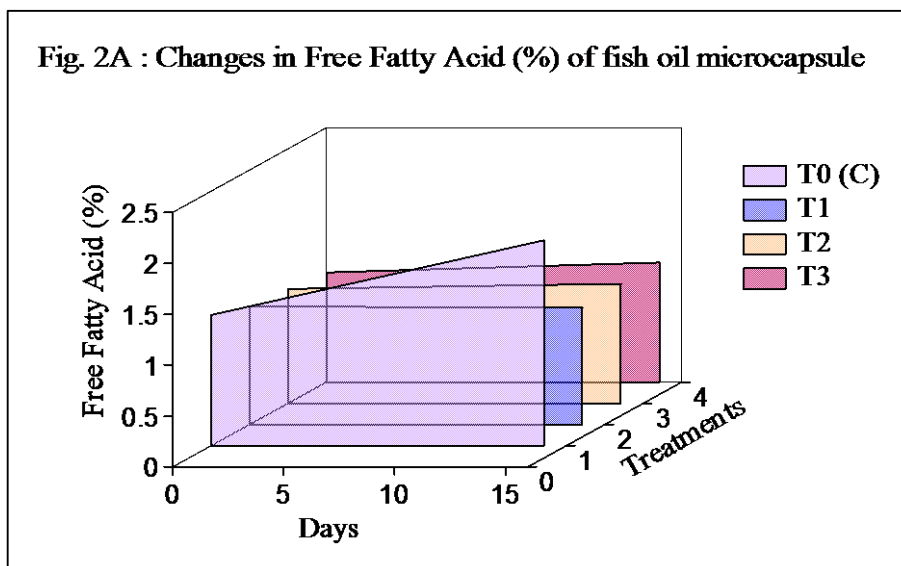


Fig 1: Sensory evaluation of fish oil microcapsule prepared with different sodium alginate concentration after 15 days of interval

▪ **Biochemical Analysis:** The treated sample were tested for free fatty acid (FFA), peroxide value (PV) and acid value (AV) from 0 day and 15 days. The treatment T0(C) was observed to have more increase in the FFA value than T1, T2 and T3. The treatment T1 was observed to have least change in the FFA value (Fig. 2A) among all treatments. According to Codex (1999) [2], the FFA level in fish oil is low i.e., 0.13-1.95% of oleic acid. All the treatment values were observed in acceptable range except T0(C). Similar trend was observed for peroxide value. The treatment T0(C) had the highest peroxide

value (Fig. 2C) among all the treatments. The treatment T1 showed the least change in the peroxide value among all the treatments. The peroxide value of oil should be less than 5 m.eq./kg (Codex, 2017) [2]. The acid value (Fig. 2B) of treatment T0(C) was highest and that for treatment T3 was observed to be lowest among all the treatment. According to Codex (2017) [2], the acceptable range of acid value for fish oils is less than 3.0 mg of KOH/kg. The treatment T0(C) had acid value be above the acceptable limit at 15th day. All the observed date are presented in Figure 2 and Table 2.



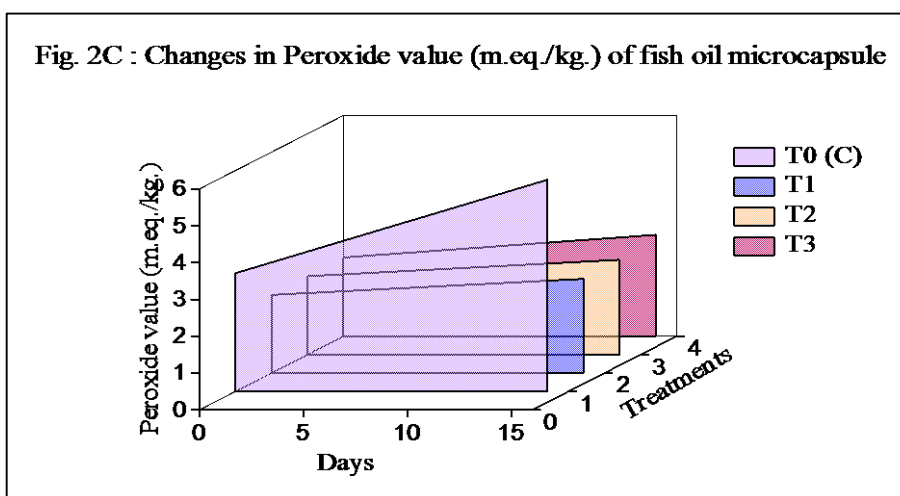
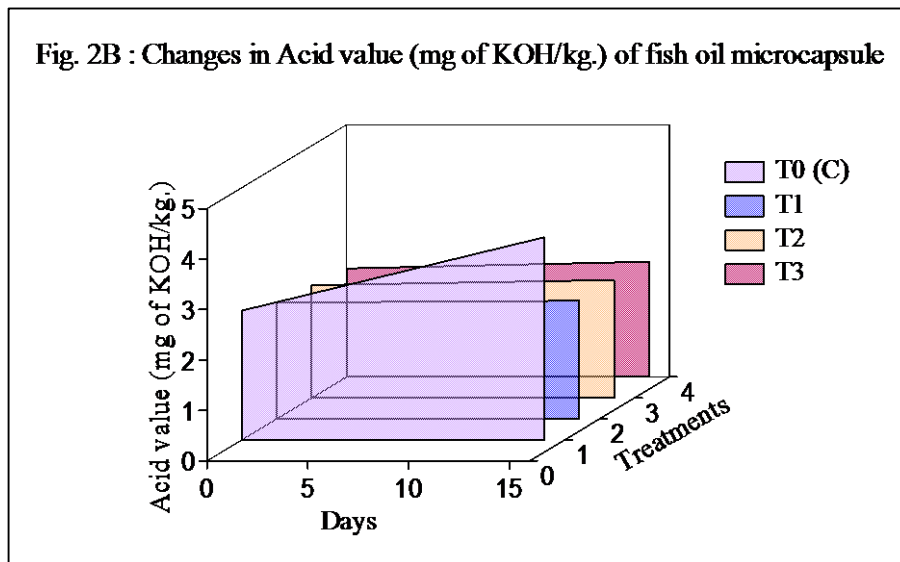


Fig 2: Changes in free fatty acid (Fig. 2A), peroxide value (Fig. 2B) and acid value (Fig. 2C) of fish oil microcapsule prepared with different sodium alginate concentration after 15 days of interval

Table 2: Changes in free fatty acid, peroxide value and acid value of fish oil microcapsule prepared with different sodium alginate concentration after 15 days of interval

Free Fatty Acid (%)				
Storage Days	Treatments			
	T0(C)	T1	T2	T3
0	1.286 ± 0.01 ^a	1.160 ± 0.01 ^a	1.124 ± 0.01 ^a	1.078 ± 0.01 ^a
15	2.020 ± 0.08 ^{ab}	1.148 ± 0.01 ^a	1.174 ± 0.01 ^a	1.176 ± 0.01 ^a
Acid value (mg of KOH/kg.)				
Storage Days	Treatments			
	T0(C)	T1	T2	T3
0	2.562 ± 0.01 ^a	2.310 ± 0.01 ^a	2.238 ± 0.01 ^a	2.146 ± 0.03 ^a
15	4.020 ± 0.17 ^b	2.342 ± 0.02 ^a	2.338 ± 0.01 ^a	2.286 ± 0.02 ^a
Peroxide value (m.eq./kg.)				
Storage Days	Treatments			
	T0(C)	T1	T2	T3
0	3.212 ± 0.30 ^b	2.122 ± 0.01 ^a	2.132 ± 0.01 ^a	2.144 ± 0.06 ^a
15	5.754 ± 0.03 ^c	2.556 ± 0.02 ^a	2.580 ± 0.05 ^a	2.760 ± 0.05 ^a

Values are in mean ± SD, n=5, ^{a,b} Value with different superscripts in a row for each parameter differ significantly ($p < 0.05$).

4. Conclusion

This study was to examine the effect of different concentration of sodium alginate on the quality attributes of fish oil. The sensory and biochemical analysis was carried out for 15 days of the storage. The sensory evaluation (Table 1) for the samples was done using hedonic scale method to analyze general texture, colour, odour, taste and overall

acceptability which stated that treatment T1 had the higher mean score for all the above characters followed by treatment T3, T2 and T0(C). The changes in the biochemical parameters were analyzed by free fatty acid (FFA), acid value (AV) and peroxide value (PV) for all the samples initially after the 15 days of storage. The free fatty acid value was observed to be in the acceptable range for all the samples except for T0(C).

The treatment T1 had the least change in the FFA value (Table 2) among all the treatments which was 1.160 ± 0.01 to $1.148 \pm 0.01\%$ respectively. The acid value (Table 2) was observed to be in the acceptable range for all the samples except for T0(C). The treatment T3 had the least change in the AV among all the treatments which was around 2.146 ± 0.03 to 2.286 ± 0.02 mg of KOH/kg. respectively. The peroxide value (Table 2) was observed to be in the acceptable range for all the samples after 15 days of storage. The treatment T1 had the least change in the PV among all the treatments which was 2.122 ± 0.01 to 2.556 ± 0.02 m.eq./kg. respectively. On the basis of result obtained, fish oil microcapsules prepared using 5% of sodium alginate was found to be most suitable for microcapsule preparation.

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