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PHYSICOCHEMICAL AND HEDONIC RESPONSE OF BARS ENRICHED WITH ENCAPSULATED FISH AND FLAXSEED OIL

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There has been a great upsurge in research era for use of natural medicines for good health and wellbeing. In this scenario, omega-3 fatty acid derived from natural sources have imperative health potential but are at great risk for use in food due to its high unsaturation and susceptibility for oxidation. To cope with this challenge, present research work has been designed to preserve these therapeutic moieties through microencapsulation. The resultant encapsulated bio actives were utilized to produce omega-3 enriched bars, which provide an efficient vehicle for delivery of useful entities in the form of snack foods. This study aims at investigating the capability of microencapsulation to prevent lipid oxidation in bars enriched with encapsulates of fish and flaxseed oil as well as to evaluate whether addition of encapsulates influenced sensory and physicochemical characteristics of the bars during storage period of 60 days. The addition of fish and flaxseed microcapsules influenced the physical attributes like color, flavor, texture, chroma and hue angle of prepared omega-3 bars significantly. The rise in concentration of microcapsules raised CIELab values except for b*, which was highest in bars enriched with fish microcapsules and lower in bars incorporated with flaxseed encapsulates, yellow color of fish oil probably contributed to this behavior which although was encapsulated but during processing imparted some color leached from the microcapsules. The L* value demonstrate lightness that was more in flaxseed oil enriched bars whereas, a* showed declining trend with increased concentration of added encapsulates in both type of encapsulated bars probably due to some grayish color imparted by encapsulated gum Arabic moiety. The formulation with 5% concentration of both powders *i.e.* fish and flaxseed encapsulate imparted higher oxidative stability and sensory score. However, it is revealed that omega-3 bars with all combinations were acceptable.
Keywords: Omega-3 bars, lipid oxidation, hedonic response, physicochemical testing, microencapsulation, fish and flaxseed oil encapsulates.

INTRODUCTION

Globally, the demand for safe and healthy foods has been escalated owing to their associated health claims, making balanced food intake a right option for prevention or remedy of health issues like diabetes, obesity, cardiopathies and malnutrition originating mainly from dietary errors. Cereal bars meet the criteria being rich source of minerals and vitamins and obtained from pleasant and sweet tasted grains (Izzo and Niness, 2001). These commodities employ variety of constituents and cater several sectors of consumers concerned with healthy life (Palazzolo, 2003).

Bower and Whitten (2000) described several products as snack comprising cakes, pizzas, popcorn and cereal bars. Commonly, the cereal bars do not come under the umbrella of functional foods certainly due to their poor nutritional profile. In previous years, interest has been enhanced in producing functional bakery bars keeping in view customers preferences in taste and ready to eat commodities. The bakery bars enriched with omega-3 rich oils offer an instant bust of energy and utilized as energy rich food.

There has been found an enhanced interest of consumers in foods fortified with omega-3 fatty acids. Recent techniques, however, have led to new tactics for stabilization, utilization and processing of omega-3 oil (Bakry *et al.*, 2016). Spray drying is among commonly employed techniques for oil encapsulation (Gouin, 2004; Gharsallaoui *et al.*, 2007). The process of spray drying eliminates water rapidly from emulsions permitting great volatiles retention (Badee *et al.*, 2012), hence shielding encapsulated moieties from environment (Gharsallaoui *et al.*, 2007; Júnior *et al.*, 2018).

In flaxseed, ALA and LA constitute about 60% of the total fatty acids, respectively ultimately considered as the richest source of ALA (Sonawane and Arya, 2014). This oil is best vegetarian source of omega-3 fatty acids with 50-60% α-linolenic acid (ALA, C18:3). Though available globally, flaxseed oil is not preferred being prone to oxidation owing to higher percentage (75%) of polyunsaturated fatty acids (PUFAs), that lead to toxic peroxides and off flavors production upon heating (Liu *et al.*, 2010; Singh *et al.*, 2011). On that account, although flaxseed oil is the greatest and dominant omega-3 fatty acids source, potentially remains

untapped for satisfying omega-3 nutritional requirements in humans. Consequently, approaches aimed at fortification and stabilization of flaxseed oil has great impact in production of omega-3 rich functional foods.

The fish oil is among significant sources of omega-3 polyunsaturated fatty acids particularly docosa hexanoic acid (C22:6n-3) and eicosapentanoic acid (C20:5n-3). Yet, these fatty acids are rapidly oxidized resulting in loss of nutritional and organoleptic properties owing to greater level of unsaturation resulting from greater level of omega-3 polyunsaturated fatty acids (Morales-Medina *etal*., 2016; Vasile *et al.*, 2016).

There has been a rising concern in consumption of snacks and fast foods in current years in response of modification in lifestyle of populations. Consumers are focusing on easy and rapidly prepared foods and comfort in gaining frozen, prepared and ready to use food commodities in market. Snacks stands out among aforementioned products and are described as small meals of substantial or small value that can be linked with sensory characteristics fun/healthy.

Bakery products comprising variety of cakes, biscuits, bakery products and pastries play a significant role in the diet of European people hence are ideal for efficient delivery of bioactive moieties to consumer in suitable food. Rising number of researchers have focused on delivery of bioactive constituents like omega-3 fatty acids in encapsulated powder form (Vitaglione *et al.*, 2012).

The novel product development necessitates adequate knowledge about consumer's likings as well as information about product composition, ingredients choice and nutritional attributes must be considered. Modification in lifestyle has shifted significant attention of people towards snacks and fast food since previous years. Consumers get a chance for access to quickly ready foods like snacks which are described as substitutes to quick meals which may or may not have nutritional significance (Constantin and Istrati, 2019).

Keeping in view the above-mentioned facts, the present research work was designed for microencapsulation of two omega-3 rich oils *i.e.* fish oil and flaxseed oil from animal and plant sources, respectively. The resultant microcapsules were, utilized for preparation of omega-3 enriched nutritional food bars using different formulations of the encapsulated oils. The main objective was probing the efficiency of microcapsules inclusion on stability and quality aspects of the developed nutritional bars.

MATERIALS AND METHODS

***Emulsion preparation*:**The encapsulating materials maltodextrin and gum arabic(30 g) were dissolved in distilled water (60 mL) and prepared solutions were left at room temperature for overnight to get full saturation of the polymer substance. Then 10 mL oil and emulsifier (5 mL each) were added under continuous stirring at 4,000 rpm for 5 min using blender (ULTRA-TURRAX T 50 basic IKA-WERKE). Since 10 mL oil was added in 90 mL distilled water containing 30 g wall materials hence the ratio of oil and encapsulating material was fixed at 1:3. The concentration of total solids (wall materials) was fixed at 30% and emulsifier (tween 20) was used at 5% concentration for each formulation.

The emulsion sample was poured into main chamber through peristaltic pump by feed flow rate of 6 mL/min. Inlet and outlet temperatures for both type of oil emulsions i.e. fish and flaxseed oil emulsion were 180 °C and 122 °C, respectively. Air at flow rate of 35 m^3/hr whereas, compressed nitrogen gas with 99.995% purity and flow rate of 40 psi pressure were used.

***Selection of fish and flax encapsulates for product development*:** Fish and flax encapsulate (one from each) based on encapsulation efficiency and peroxide value were used for product development with control for comparison purpose. Since, arabic gum encapsulated powder showed optimal performance therefore it was selected for product development phase.

***Product development (omega-3 bars)*:** In product development module, six treatments of omega-3 bars were prepared (Table 1). For omega-3 bars preparation, fish and flax microcapsules and bars were used to check their influence over product attributes. The recipe of all the treatments was same with exception of level of microcapsules. A control treatment was used for comparison without any addition of microcapsules (Table 1) High energy and nutritional bars were manufactured employing white flour, almonds, pistachio, sugar, eggs, fish and flaxseed oil enriched microcapsule and ghee. Very careful selection of ingredients was done, initially preheating of oven (150 °C) was performed and a pan lined with paper was place inside. In the meanwhile, nuts, sugar, white flour, eggs were combined in a bowl and mixed thoroughly. Then ghee and microcapsules were added and mixture was poured on pan, another layer of paper was set at top of the mixture and was pressed firmly for even layer of the mixture. Afterwards, top layered paper was removed, and mixture was allowed to bake until brown color was achieved, afterwards the baked mixture was cooled for 10 min and slicing was done with knife. The prepared bars were stored at room temperature for sensory analysis and physicochemical response assessment.

Table 1. Treatments used in product development

Treatments	Descriptions
B_0	Control
B_1	Fish microcapsule-based bars (3%)
B_2	Fish microcapsule-based bars (5%)
B_3	Fish microcapsule-based bars (7%)
B_4	Flaxseed microcapsule-based bars (3%)
B_5	Flaxseed microcapsule-based bars (5%)
B_6	Flaxseed microcapsule-based bars (7%)

***Hedonic response*:** The prepared omega-3 bars were evaluated utilizing nine-point hedonic scale according to the procedure of Jeyakumari *et al.* (2016). The products were prepared by following critical hygienic steps. At evaluation day, omega-3 bars were put into glass trays labeled with random codes. The panelists were provided with distilled water and crackers for rinsing the mouth prior to judgment of each sample.

The serving of bars was done by random order with varying time interval and it was requested to panelists to record their preference to prepared bars. The serving amount and size was maintained similar for all samples. The parameters to be determined comprised several quality attributes like taste, color, crispiness, flavor and overall acceptability all this was based on nine-point hedonic test scale. The panelists (students and staff) were the part of evaluation of sensory quality. The test was conducted by providing the panelists well ventilated cabins (compartments) with appropriate lighting facility at Sensory Evaluation Laboratory of the National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad, Pakistan.

***Physicochemical analysis*:** The prepared bars were subjected to physico-chemical analysis for a period of 60 days. During this period antioxidant potential, texture and color were measured. The protocol of Jeyakumari *et al.* (2016) was used for texture and color determination of bars whereas, antioxidant potential of omega-3 enriched bars was evaluated by using the method of Goyal *et al.* (2015).

***Color analysis*:** The color of prepared bars was evaluated by using CIE-Lab Color Meter (CIELAB SPACE, Color Tech-PCM, USA). The prepared bars were kept in transparent petri dish and directly positioned in path of light to evaluate the color parameters. The analysis of the sample was carried out for L, a and b values exhibiting brightness, redness on positive value and on negative value represents greenness and yellowness for positive & for negative blueness, accordingly. Chroma and hue values of fish and flaxseed enriched bars were calculated using the following equations.

$$\text{Chroma (C)} = [(a*^2) + (b*)^2]^{1/2}$$

$$\text{Hue angle (h)} = \tan^{-1}(b*/a*)$$

***Texture analysis*:** Texture was analyzed employing texture analyzer single arm texture analyzer TA- XT Plus, Stable Micro Systems, Surrey, UK) comprising 2 kg weight force. The measurement of forces was done against time curve attached by disk probe with 35 mm diameter that had two cycle displacement and compression speed of 10 mm per minute. It contained built in software with texture analyzer that was used for analysis of obtained data.

***Analysis of p-anisidine, peroxide*:** The extraction of oil was done by methods of Chapman *et al.* (1996). About 10 g bar sample was suspended in 25 mL solution of $CaCl_2$ (2.5%), 50 mL chloroform and methanol (25 mL). Following centrifugation for 20 min at 13,000 rpm the chloroform layer was separated.

The AOCS method (Cd 8-5319) was adopted for determination of PV. About 12 mL glacial acetic acid was added to 10 mL chloroform extract or chloroform blank followed by addition of 0.5 mL potassium iodide after swirling. The sample was swirled for 1 min followed by addition of 25 mL distilled water to end the reaction. Sample titration was performed by 0.01 N sodium thiosulphate with 0.5 mL starch as indicator until disappearance of blue color. The analysis was performed in triplicates and following equation was used for determination of PV in samples.

$$50 \times \frac{\text{Titrantsample- Titrantblank}}{\text{Sample weight (g)}} \times 100$$

The procedure already discussed was adopted for sample preparation. Afterwards the procedure of AOCS (Cd 18-90) was adopted for analysis of p-anisidine value (AOCS, 2009). The absorbance of supernatant (Ea) was determined by spectrophotometer against pure isooctane at 350 nm. Afterwards, 5 mL of each of the supernatant and isooctane was treated in Pyrex test tubes containing 1 mL solution of p-anisidine (about 0.25% in glacial acetic acid). The test tubes were stored in dark after being capped and shaken. Calculation of supernatant plus anisidine absorbance was performed at 350 nm. The measurements were repeated three times and determination of p-anisidine value was done using the formula mentioned in section 3.2.3.

***Statistical analysis*:** The obtained data for each parameter were analyzed statistically to evaluate the level of significance and comparison of means was also conducted by following the method of Montgomery (2008). ANOVA with two factors factorial under completely randomized model was utilized for product analysis because there was storage study of these parameters *i.e.* two factors (treatment and days) were involved. The data handling, graph formation and summarization of results was done by Microsoft excel (v2010).

RESULTS AND DISCUESSION

***Physico-chemical analysis of omega-3 enriched bars*:** The bars were formulated by using different formulations (3, 5, and 7%) of each of the fish and flaxseed microencapsulated powders. The resultant bars were analyzed further for texture, color, antioxidant potential and hedonic response to analyze the influence of the omega-3 addition on bars during storage period of 60 days.

***Color analysis*:** The food color is presumed as an indicator of the acceptability and quality of the product. Most customers buy and judge food through "eyes", therefore food color is often perceived as a critical acceptability and quality indicator of the product. In current years, enhanced interest of consumers in food comprising natural constituents has raised consistently. Hence the study of colored components along with their interaction holds critical position for food professionals (Galaffu*et. al*., 2015; Solymosi *et al.*, 2015).

The determination of color was carried out by CIELAB (Commission International del' Eclairage (CIE) color operating methodology for evaluation of brightness (L), redness (a) and yellowness (b) which reads these parameters as a describes greenish to reddish, b exhibits blue to yellowish color and L indicates brightness. The mean values for omega-3 bars for the L parameter are described in Table 2. It is evident from the table that the mean value of L immediately after production of bars was 62.20±0.01, 59.62±0.02, 59.04±0.02, 60.01±0.02, 59.55±0.01, 60.11±0.01, 59.21±0.01, for bar with control, 3% fish microcapsules (B_1) 5% fish microcapsules (B_2), 7% fish microcapsules (B_3), 3% flaxseed oil microcapsules (B_4), 5% flaxseed microencapsulated powder (B_5) and 7% flaxseed encapsulated powder (B_6). Whereas, after 60 days storage the L value decreased from 59.96±0.98 to 57.06±0.44.

The mean Table 3 showed highest value for B_5 (6.98±0.49) followed by B_2 (6.94±0.49), B_0 (6.77±0.49) whereas lowest value was observed for B_1 (6.59±0.71) and B_3 (6.56±0.70). As far as the storage time is concerned reduction was highest in B_4 from 7.74±0.02 to 5.60±0.02 while for L value reduction was from 59.96±0.98 to 57.06±0.44 during storage intervals. Similarly, as shown in Table 4 the value of b depicted inclining trend with passage of time due to the fact that baked bars color tends to yellow upon storage. The rise was higher for treatments containing fish oil due to yellowish color of the added microcapsules.

The highest value of b was exhibited by B_3 (38.77±0.02) with maximum percentage of fish oil (7%) followed by B_2 (38.68±0.01) with 5% fish oil, whereas lowest value was in control as 30.54±0.01. It was shown by interaction among treatment and storage that maximum b value for B_3 was

Table 2.Effect of treatments and storage on Lvalue of bar.

Storage intervals (days)	Treatments							Means
	B_0	B_1	B_2	B_3	B_4	B_5	B_6	
0	62.20±0.01a	59.62±0.02c	59.04±0.02fgh	60.01±0.02b	59.55±0.01c	60.11±0.01b	59.21±0.01de	59.96±0.98a
15	60.10±0.02b	59.03±0.02gh	59.21±0.01de	59.16±0.01de	59.12±0.02efg	59.25±0.02d	59.03±0.02gh	59.28±0.35b
30	59.16±0.01def	57.5±0.16mn	58.56±0.01j	58.43±0.02k	58.49±0.02jk	58.81±0.01i	58.57±0.01j	58.50±0.47c
45	58.93±0.01hi	55.69±0.01t	57.53±0.2m	57.03±0.01q	56.88±0.01r	57.96±0.01i	57.22±0.01p	57.32±0.92d
60	57.41±0.02mno	53.39±0.01no	57.52±0.01m	56.29±0.01op	56.39±0.01s	57.02±0.01q	56.41±0.01s	57.06±0.44e
Means	59.56±1.58a	57.05±2.28e	58.37±0.72c	58.18±1.36c	58.09±1.24d	58.63±1.06b	58.09±1.09d	

B_0 = (control bar), B_1= (bar containing 3% fish microcapsules), B_2= (bar containing 5% fish microcapsules), B_3= (bar containing 7% fish microcapsules), B_4= (bar containing 3% flaxseed microcapsules), B_5 = (bar containing 5% flaxseed microcapsules), B_6 = (bar containing 7% flaxseed microcapsules)

Table 3. Effect of treatments and storage on a value of bar.

Storage intervals (days)	Treatments							Means
	B_0	B_1	B_2	B_3	B_4	B_5	B_6	
0	7.46±0.02b	7.72±0.03a	7.76±0.02a	7.68±0.02a	7.74±0.02a	7.81±0.02a	7.38±0.01bc	7.65±0.15a
15	7.14±0.01de	6.98±0.01efg	7.18±0.02d	6.94±0.02fg	6.91±0.01gh	7.22±0.02cd	7.11±0.02def	6.93±0.11b
30	6.75±0.01hij	6.46±0.03lm	6.82±0.02ghij	6.42±0.01lm	6.73±0.02ijk	6.89±0.02ghi	6.72±0.02jk	6.68±0.16c
45	6.39±0.02mn	6.14±0.01op	6.53±0.02lm	6.11±0.04op	6.09±0.02op	6.57±0.02kl	6.33±0.03no	6.31±0.18d
60	6.10±0.13op	5.67±0.03q	6.39±0.02mn	5.63±0.03q	5.60±0.02q	6.43±0.01lm	6.01±0.02p	5.97±0.33e
Means	6.77±0.49b	6.59±0.71d	6.94±0.49 a	6.56±0.70d	6.61±0.73d	6.98±0.49a	6.71±0.50c	

B_0 = (control bar), B_1= (bar containing 3% fish microcapsules), B_2= (bar containing 5% fish microcapsules), B_3= (bar containing 7% fish microcapsules), B_4= (bar containing 3% flaxseed microcapsules), B_5 = (bar containing 5% flaxseed microcapsules), B_6 = (bar containing 7% flaxseed microcapsules)

Table 4. Effect of treatments and storage on b value of bar.

Storage intervals (days)	Treatments							Means
	B_0	B_1	B_2	B_3	B_4	B_5	B_6	
0	30.54±0.01t	31.59±0.01r	38.68±0.01e	38.77±0.02e	33.71±0.01n	33.80±0.02mn	33.92±0.02m	34.43±2.96e
15	31.11±0.04s	32.05±0.03q	39.32±0.03d	39.41±0.01d	34.30±0.22i	34.38±0.02kl	34.42±0.02kl	34.99±3.00d
30	31.23±0.02s	32.19±0.02pq	39.64±0.02c	39.68±0.02c	34.58±0.03jk	34.75±0.02ij	34.79±0.08ij	35.26±3.05c
45	31.77±0.01r	32.64±0.01o	39.97±0.01b	40.10±0.09ab	34.96±0.01hi	35.03±0.02gh	35.13±0.03fg	35.66±3.02b
60	32.39±0.02p	32.72±0.02o	40.01±0.03ab	40.21±0.02a	35.40±0.16f	35.31±0.02f	35.39±0.01f	35.92±2.89a
Means	31.41±0.63f	32.24±0.41e	39.52±0.49b	39.63±0.52a	34.59±0.57d	34.65±0.53d	34.73±0.52c	

B_0 = (control bar), B_1= (bar containing 3% fish microcapsules), B_2= (bar containing 5% fish microcapsules), B_3= (bar containing 7% fish microcapsules), B_4= (bar containing 3% flaxseed microcapsules), B_5 = (bar containing 5% flaxseed microcapsules), B_6 = (bar containing 7% flaxseed microcapsules)

Table 5. Effect of treatments and storage on chroma of bar.

Storage intervals (days)	Treatments							Means
	B_0	B_1	B_2	B_3	B_4	B_5	B_6	
0	32.41±1.08	37.57±1.32	38.43±1.38	38.45±1.44	36.98±1.35	36.87±1.41	35.54±1.36	36.61±1.95b
15	39.94±1.57	37.99±1.27	38.88±1.46	38.91±1.40	39.52±1.55	39.37±1.31	38.06±1.43	38.95±0.68a
30	34.99±1.17	38.20±1.34	39.11±1.40	39.15±1.47	39.73±1.46	39.55±1.49	38.22±1.47	38.42±1.50a
45	35.44±1.39	38.67±1.30	39.60±1.49	39.65±1.42	40.05±1.57	39.83±1.33	38.49±1.44	38.82±1.48a
60	35.99±1.21	38.85±1.36	39.80±1.43	36.86±1.39	40.45±1.49	40.19±1.51	38.84±1.49	38.71±1.57a
Means	35.75±2.43b	38.26±0.46a	39.16±0.49a	38.60±0.95a	39.35±1.22a	39.16±1.18a	37.83±1.17a	

B_0 = (control bar), B_1= (bar containing 3% fish microcapsules), B_2= (bar containing 5% fish microcapsules), B_3= (bar containing 7% fish microcapsules), B_4= (bar containing 3% flaxseed microcapsules), B_5 = (bar containing 5% flaxseed microcapsules), B_6 = (bar containing 7% flaxseed microcapsules)

Table 6. Effect of treatments and storage on hue angle of bar.

Storage intervals (days)	Treatments							Means
	B_0	B_1	B_2	B_3	B_4	B_5	B_6	
0	77.69±2.60	74.56±2.62	71.65±2.57	67.71±2.54	72.65±2.67	70.84±2.66	64.80±2.49	71.41±3.94
15	78.15±2.61	74.92±2.63	74.19±2.66	70.15±2.64	73.05±2.69	72.38±2.72	67.14±2.58	72.85±3.26
30	78.39±2.62	75.06±2.82	75.12±2.70	70.46±2.76	73.25±2.69	72.49±2.72	67.25±2.58	73.14±3.33
45	78.89±3.09	75.46±2.52	75.44±2.64	70.88±2.54	73.73±2.42	72.71±2.55	67.36±2.42	73.49±3.42
60	79.14±2.97	75.61±2.78	75.84±2.85	71.38±2.73	73.96±2.90	73.01±2.44	67.66±2.54	78.80±3.38
Means	78.45±0.52a	75.12±0.38ab	74.45±1.50b	70.12±1.27cd	73.33±0.47bc	72.29±0.75bc	66.84±1.03d	

B_0 = (control bar), B_1= (bar containing 3% fish microcapsules), B_2= (bar containing 5% fish microcapsules), B_3= (bar containing 7% fish microcapsules), B_4= (bar containing 3% flaxseed microcapsules), B_5 = (bar containing 5% flaxseed microcapsules), B_6 = (bar containing 7% flaxseed microcapsules)

observed since its initial value 38.77±0.02 at 0 day inclined to 40.21±0.02 during storage after 60 days. The overall rise in b value for 60 days was from 33.43±2.96 to 35.92±2.89. As far as mean values of chroma are concerned (Table 5) the highest value was exhibited by B_3 (38.45±1.44) that had 5% fish oil as an added ingredient. The value was minimum for control B_0 (32.41±1.08) and overall the value varied with time from 36.61±1.95 to 38.71±1.57. In the same way, mean Table 6 for hue angle values manifested minimum trend for B_6 (64.80±2.49). The value of B_5 (70.84±2.66) and B_3 (67.71±2.54) were followed by that of B_6 in decreasing trend. On the other hand, at maximum end control and B_1 with 3% fish oil were at top (77.69±2.60 and 74.56±2.62) followed by B_4 (72.65±2.67) and B_2 (71.65±2.57) with 3% fish and flaxseed microcapsules, respectively. Hue angle goes on decreasing with increasing percentage of added microcapsules. During storage time interval hue angle showed rising trend from 71.41±3.94 to 78.80±3.38 starting from 0 day to last (60) day. At the end it can be concluded that addition of fish and flaxseed oil leads to decrease in L, and a value whereas inclining trend was shown by b, hue angle and chroma. Higher value of b* was observed in treatments containing fish oil due to yellow color of the encapsulates compared to bars comprising added flax oil powder encapsulates.

In a study conducted by Nielsen and Jacobsen (2009) bars were prepared enriched with 5% fish oil as well as fish oil encapsulated with sodium caseinate and they determined sensory parameters during storage time of 0 to 11 weeks. The results showed that at 0 day the least score of aroma and flavor was exhibited by sample without fish oil due to absence of fishy flavor whereas sample with encapsulated flaxseed oil was at highest rank both at start as well as end of study period whilst, the bar prepared with fish oil addition was at lowest rank in terms of sensory attributes. Moreover, storage period influenced aroma and flavor significantly. Similarly, values obtained in present study has close trend with findings of Goyal *et al.* (2015). They characterized microencapsulated flaxseed oil through sensory and physico-chemical testing and evaluated its potential as carrying vehicle for omega-3 in vegan diet. The results exhibited cream to off white color with L value in range from 88.60 to 88.93, a value in range of 0.06 to 0.08 and b varied from 13.56 to 13.63. The huge difference regrading a and b values from present study might be attributed to difference in formulation. The formulation used for present study contained gum arabic and maltodextrin as encapsulating substances whereas, the formulation used by researchers contained sodium caseinate which contributed to its whitish color responsible for bright lightness of the microcapsule. L value however showed value close to present finding.

Results are also in harmony with the findings of Jeyakumari *et al.* (2016). They prepared cookies with different formulations and showed that microencapsulation of fat has a negative influence over lightness due to browning pigment formed in further stages of storage. They prepared cookies enriched with fish microcapsules and compared the values with formulations having milk with fish oil without

Table 7. Effect of treatments and storage on texture (kg force) of bar.

Storage intervals	Treatments							Means
	B_0	B_1	B_2	B_3	B_4	B_5	B_6	
0	0.62±0.02	0.64±0.02	0.70±0.02	0.74±0.03	0.68±0.02	0.72±0.02	0.78±0.02	0.69±0.05a
15 days	0.58±0.01	0.61±0.02	0.67±0.02	0.68±0.02	0.67±0.02	0.66±0.02	0.76±0.03	0.66±0.05b
30 days	0.53±0.01	0.57±0.01	0.62±0.02	0.64±0.02	0.63±0.02	0.62±0.02	0.73±0.02	0.62±0.06c
45 days	0.51±0.02	0.54±0.03	0.56±0.02	0.59±0.01	0.63±0.01	0.58±0.02	0.68±0.02	0.58±0.05d
60 days	0.47±0.01	0.49±0.01	0.50±0.01	0.54±0.02	0.60±0.02	0.52±0.02	0.62±0.02	0.53±0.05e
Means	0.54±0.05d	0.57±0.05d	0.62±0.07c	0.63±0.03bc	0.67±0.07b	0.62±0.07bc	0.71±0.06a	

B_0 = (control bar), B_1= (bar containing 3% fish microcapsules), B_2= (bar containing 5% fish microcapsules), B_3= (bar containing 7% fish microcapsules), B_4= (bar containing 3% flaxseed microcapsules), B_5 = (bar containing 5% flaxseed microcapsules), B_6 = (bar containing 7% flaxseed microcapsules)

encapsulation. It was found that L value was 74.81 and 70.53 whereas formulations comprising fish oil showed L value from 68.83 to 66.86 hence proving the fact that fat microencapsulation exhibit darkening impact on resultant microcapsules. On the other hand, b value which points towards yellowness of the food commodities was higher in samples with fish oil and values were found ranging from 34.58 to 35.79 compared to 28.40-30.19 in samples without encapsulated powder.

Research conducted by O' Brien *et al.* (2003) found parallel results depicting that biscuits with vegetable fat showed maximum L value in comparison to encapsulated fat. They reported that biscuits containing vegetable fat showed highest L value than the microencapsulated fat.

***Texture analysis of omega-3 bars*:** Texture is the sensation firstly perceived by consumer while touching the food commodity followed by chewing and throughout swelling of the food. The mean Table 7 shows the influence of treatments throughout storage over texture. The highest value of hardness was recorded by B_6 (0.78±0.02 kg force) followed by B_3 (0.74±0.03 kg force) and B_2 (0.72±0.02 kg force). On the other hand, minimum values of hardness were exhibited by B_1 (0.64±0.01 kg force) followed by control (0.62±0.02 kg force). Collaborative impact of studied parameter exhibited that treatments comprising less percentage of added powder were less impacted in comparison to bars enriched with more powder in storage of 60 days. The gummy and thick nature of added gum arabic might be the contributing factor in bars hardness leading to greater value for great concentration of added powder for both type of microcapsules. Overall hardness of bars has declined from 0.78±0.02 to 0.62±0.02 kg force during storage time interval. Hardness of fish microcapsule enriched bars was more impacted than flaxseed enriched powder, moreover in comparison to control bars containing microcapsules were less influenced and hardness showed less decline in bars with encapsulates than control. The fact can be supported because fat produced from vegetable source is harder in comparison to animal source fat like fish oil hence fish oil was less influenced by storage in terms of hardness compared to vegetable sourced (flaxseed) enriched microcapsules.

The results are in close agreements with findings of Jeyakumari *et al.* (2016). They produced fortified cookies using fish encapsulates against cookies fortified with fish oil emulsions. It was found that hardness decreased from 36.62 to 24.42 for cookies with fish oil emulsion and fish microcapsules, respectively exhibiting negative impact of fish microcapsules over hardness similar to present study. Moreover, Manohar and Rao (1999) exhibited parallel trend and their results showed that thickness was less in biscuits containing microencapsulated fat in comparison to biscuit with vegetable fat.

***Hedonic response of omega-3 bars*:** Hedonic response of omega-3 bars was evaluated under ambient lighting conditions. The bars were labelled ranging from 1-9 as mark for hedonic response comprising taste, color, crispiness, color and overall acceptability. The values showed momentous impact of bars sensory characteristics with exception of taste since it is not influenced by storage, whereas trend was opposite for storage where it influenced overall acceptability, crispiness and color significantly whereas interaction influence was non-momentous for all sensory parameters.

Color is crucial factor for the product success, since a product with non-uniform color will not be chosen by customer. The higher color scores were attained by treatment B_5 (7.97±0.17) followed by B_2 (7.90±0.17), B_6 (7.73±0.34) and B_4 (7.43±0.15). Regarding storage study there was decline in scores of colors from 7.57±0.33to 7.18±0.38(Fig 2). The Figure 1 representing flavor scores showed highest value for flaxseed oil enriched encapsulates B_5 (7.54±0.28) followed by B_6 (7.37±0.21) and B_4 (7.29±0.26). On the other hand, bars with fish oil encapsulates were assigned minimum scores in terms of flavor with lowest value for B_3 (7.09±0.27) followed by B_0 (6.88±0.23). The fishy flavor although was hindered by microencapsulation of fish oil but minute flavor coming out during baking of the bar might be the contributing feature for this minor decline in flavor score of resultant bars. Storage on the other hand significantly lowered flavor score from 7.24±0.19to 6.78±0.24at 60th day. As far as taste is concerned (Fig. 1) the score was greatest for treatments of B_5 (7.59±0.28), B_2 (7.46±0.27) andB_6 (7.43±0.28) and lowest for fish bars with minimum value for B_1 (7.32±0.29) followed by B_3 (7.28±0.28) and B_4 (7.04±0.26).

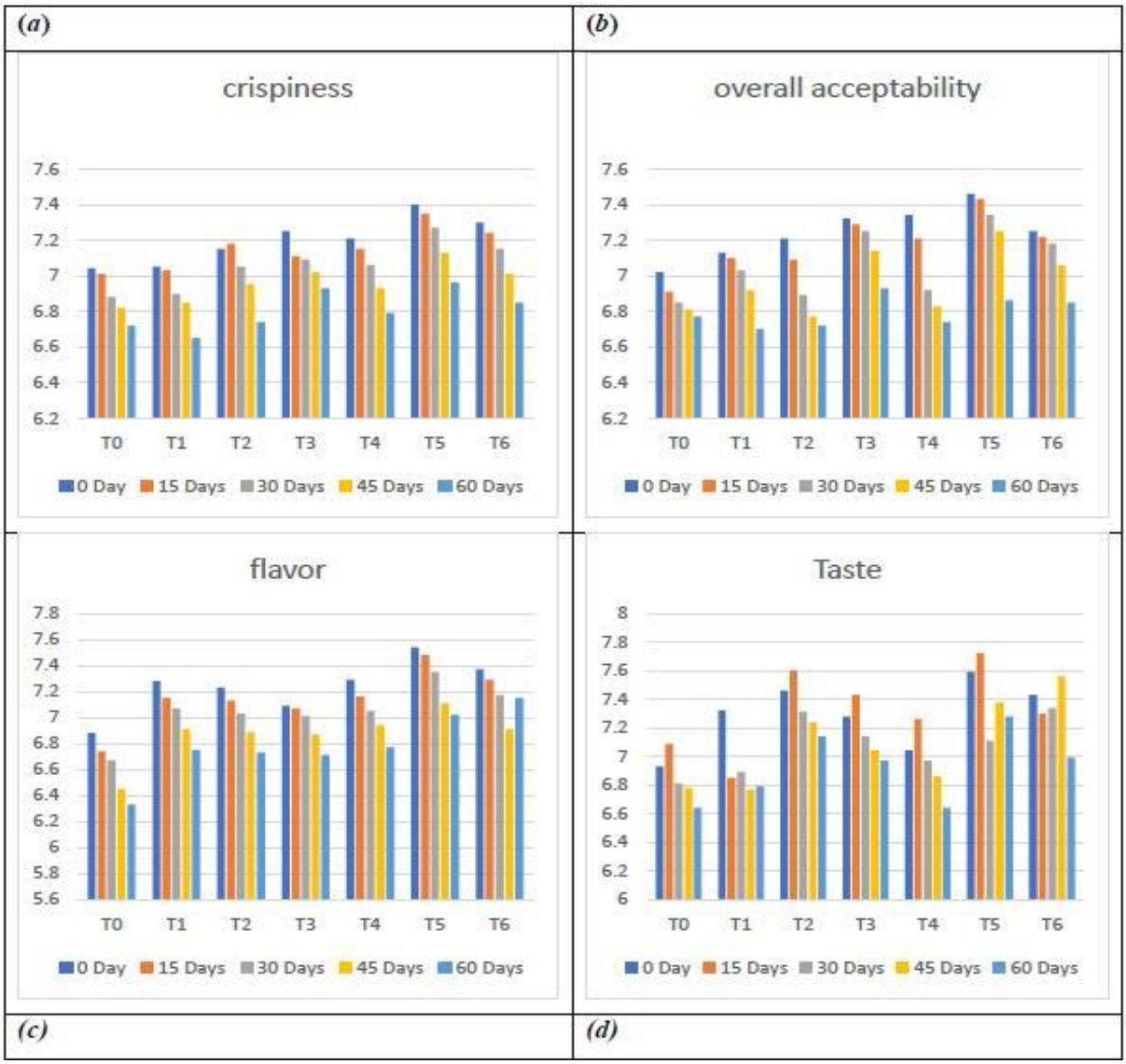

Figure 1.Effect of treatment and storage on flavor, taste, overall acceptability andcrispiness of bar

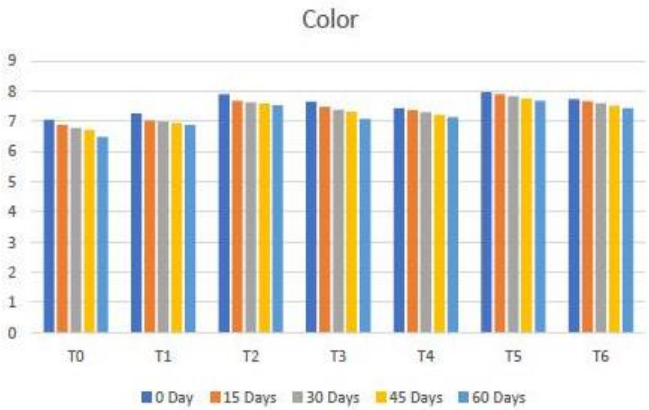

Figure 2. Effect of treatment and storage on color of bar

Storage overall has negative impact over score of taste lowering its value from 7.29±0.22 to 6.92±0.23. Coming towards crispiness (Fig. 1) values were greatest for B_5 (7.40±0.28) followed by B_6 (7.30±0.28) and B_3 (7.25±0.27) whereas values were lowest for B_0(7.04±0.24), B_1(7.05±0.24) and B_2 (7.15±0.25).

Concerning storage time, the bars have declining trend in terms of crispiness score from 7.20±0.12 at 0 day to 6.80±0.10at 60 days. The points of overall acceptability also decreased from 7.38±0.25to 6.89±0.25 (Fig 1). Concerning the scores of overall acceptability, the greatest value was assigned to treatment B_5 (7.54±0.26) followed by B_1 (7.52±0.24) and B_2 (7.51±0.25) whereas, minimum value was exhibited by B_3 (7.25±0.26) and B_0 (7.23±0.25).

The results obtained in present research are in accordance with results proposed by Conto *et al.* (2012). They prepared pan bread enriched with omega-3 fish oil and results showed decrease in flavor from 7.24 to 6.24 in flavor scores with increasing concentration of FO powder from 0.73 to 4.27. Similar decline in aroma score from 7.30 to 6.04, flavor 7.24 to 5.74 and overall acceptability points also declined from 7.30 to 5.91 with rise in FO concentration. This decline in particularly was attributed to existence of white spots upon the surface of bread probably because of microcapsules resisting bread processing. Furthermore, rising the concentration of FO contributed to bread bitter taste, hence lowering scores.

Similar behavior was shown by various other researchers including (Aliani *et al.*, 2012; Bartkiene *et al.*, 2012). They showed through their research that bread enriched with flaxseeds leads to decline in sensory characteristics. Likewise, Conto *et al.* (2012) presented similar trend and found rise in salty taste and decline in flavor and overall acceptability of bread with rising flaxseed oil and he attributed this behavior to more potassium content of the flaxseeds.

***Oxidative stability of bars*:** Assessment of anisidine value is an indication for amount of secondary oxidation products in unsaturated oils based on color estimation produced after reaction of p-anisidine and oil at 350 nm. The procedure is an approximate estimation of quantity of carbonyl or secondary oxidation compounds (Frankel, 2005). The compounds mainly comprise of aldehydes decomposed products of hydroperoxides. Fish and flax oils owing to unsaturated nature of long chain fatty acids constituents are prone to oxidation leading to high initial value of anisidine in these oils. Anisidine value (AV) can be a rough indicator of future storage capability of bars enriched with these unsaturated oils. The variation in AV was highly significant with storage, treatments as well as interaction manifested the same behavior.

Mean table (Table 8) exhibits minimum value of AV for B_5 (3.65±0.03) followed by control (3.79±0.01) and B_2 (4.25±0.02), B_3 on the other hand exhibited maximum value (6.42±0.03). During storage there was incline in AV from4.73±0.01 to 5.27±0.01. The same trend was adopted by peroxide value (PV) with B_3 exhibiting maximum value (0.466±0.288meq O_2/kg) and minimum value by B_5(0.290±0.064meq O_2/kg). Storage increased the PV from 0.248±0.03 to 0.53±0.21 meq O_2/kg of oil (Table 9).

Parallel results were obtained in preparation of meat product encapsulated with fish oil by Vasile *et al.* (2019) in order to evaluate stability, nutritional and physicochemical prospects of resultant product. The encapsulation was done in polyelectrolyte beads comprising *Prosopis alba* exudate gum and results depicted marked incline in textural and sensorial characteristics even after cooking of the product. Moreover, minimum oxidative damage as depicted by TBA value (less than 50%) was revealed in the final product.

Similar influence of storage over oxidative stability was reported by Marpalle *et al.* (2015), who evaluated the influence OF storage on oxidative stability of ALA from bread with 10 g/100 g roasted ground flaxseed and results

Table 8. Effect of treatments and storage on p-anisidine value (%) of omega-3 enriched bar.

Storage intervals (days)	Treatments							Means
	B_0	B_1	B_2	B_3	B_4	B_5	B_6	
0	3.31±0.02v	6.09±0.02hi	4.04± 0.02q	6.18±0.01fgh	5.79±0.03k	3.42±0.02uv	4.27±0.02p	4.73±0.01e
15	3.52±0.02tu	6.20±0.12efg	4.15±0.02q	6.29±0.02ef	5.91±0.02j	3.51±0.02tu	4.38±0.02nop	4.85±0.01d
30	3.61±0.02st	6.32±0.02de	4.27±0.02p	6.42±0.02cd	6.03±0.02i	3.67±0.02s	4.47±0.02mn	4.97±0.03c
45	4.12±0.03q	6.45±0.01bc	4.34±0.02op	6.55± 0.02b	6.14± 0.02ghi	3.78±0.02r	4.56± 0.01lm	5.14±0.01b
60	4.41±0.01no	6.56±0.02b	4.48±0.02mn	6.69± 0.02a	6.26± 0.01ef	3.89± 0.02r	4.63±0.01i	5.27±0.01a
Means	3.79±0.01f	6.34±0.03b	4.25±0.02e	6.42±0.03a	6.03±0.02c	3.65±0.03g	4.46±0.02d	

B_0 = (control bar), B_1= (bar containing 3% fish microcapsules), B_2= (bar containing 5% fish microcapsules), B_3= (bar containing 7% fish microcapsules), B_4= (bar containing 3% flaxseed microcapsules), B_5 = (bar containing 5% flaxseed microcapsules), B_6 = (bar containing 7% flaxseed microcapsules)

Table 9. Effect of treatments and storage on peroxide value (PV) of (meq O_2/kg of oil) omega-3 enriched bar.

Storage intervals (days)	Treatments							Means
	B_0	B_1	B_2	B_3	B_4	B_5	B_6	
0	0.286±0.002q	0.248±0.001u	0.212±0.001w	0.272±0.001s	0.263±0.002t	0.213±0.002w	0.247±0.002u	0.248±0.03e
15	0.314±0.002m	0.279±0.002qr	0.237±0.002v	0.303±0.002o	0.292±0.002p	0.244±0.001u	0.274±0.002rs	0.27±0.03d
30	0.352±0.002j	0.307±0.002no	0.273±0.002rs	0.331±0.002i	0.318±0.001m	0.272±0.002s	0.312±0.002mn	0.31±0.03c
45	0.419±0.002f	0.364±0.002i	0.338±0.001k	0.388±0.002g	0.373±0.002h	0.329±0.003i	0.379±0.004h	0.37±0.03b
60	0.512±0.002b	0.421±0.002f	0.429±0.001e	1.038±0.001a	0.436±0.001d	0.394±0.002g	0.472±0.002c	0.53±0.21a
Means	0.376±0.081b	0.324±0.062d	0.298±0.078e	0.466±0.288a	0.336±0.061c	0.290±0.064f	0.337±0.081c	

B_0 = (control bar), B_1= (bar containing 3% fish microcapsules), B_2= (bar containing 5% fish microcapsules), B_3= (bar containing 7% fish microcapsules), B_4= (bar containing 3% flaxseed microcapsules), B_5 = (bar containing 5% flaxseed microcapsules), B_6 = (bar containing 7% flaxseed microcapsules)

demonstrated rise of 6.66-13 meq O_2/kg in PV of bread, p-anisidine value on the other hand showed a rise of 10.66-13.73.

Dwyer *et al.* (2013) prepared spread by using various formulations of fish and camelina oil and resultant spread were subjected to oxidative stability analysis. Peroxide value of spread with fish oil was raised during storage due to production of hydroperoxides, lipid oxidation products in comparison to minimum value exhibited by control and spread made from combination of camelina and fish oil. It was found that rise of cameline oil in blend used for spread contributed to lowering of peroxide value. Anisidine value of spread showed inclining trend with added polyunsaturated oils. Highest AV value was exhibited by fish oil with sunflower oil at second highest level. Due to greatest amount of polyunsaturated fatty acids, fish oil exhibited greatest AV during storage as well. On the other hand, spread devoid of any added oil exhibited lowest anisidine value manifesting good stability.

***Conclusion*:** It is revealed from above results that oxidative stability of omega-3 bars was improved with addition of fish and flaxseed oil encapsulates. Formulations comprising 5% encapsulates were rated better in terms of hedonic response and physicochemical attributes. During storage incline in PV was from 0.248±0.03 to 0.53±0.21. P-anisidine value on the other hand exhibited parallel trend with lowest value exhibited by B_5 (3.42±0.02) with B_2(4.04±0.02) and B_0 (3.31±0.02) with minor fluctuation. Again, the stability is lowest in B_3 (6.18±0.01), B_1 (6.09±0.02) and B_4 (5.79±0.03). Storage inclined p-anisidine value from 4.73±0.01 to 5.27±0.01. All these variations regarding stability of the product were negligible confirming the preparation of a stable product. For future exploration it is recommended that resultant product must be analyzed for nutrikinetic, bioavailability and efficacy trial. Moreover, it suggests an opportunity for food professionals to boost encapsulated foods with acceptable sensory attributes.

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