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Quality parameters and oxidative stability of functional beef burgers fortified with microencapsulated cod liver oil

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ABSTRACT

Cod liver oil (CLO) has many health and dietary benefits, but is prone to rapid deterioration and developing undesirable odor. The aim of the research was to evaluate a functional beef burger fortified with CLO monolayered (ME) and bilayered (BE). CLO microcapsules were characterized for morphology (scanning electron microscopy), particle size distribution (light refractometer), thermal stability (differential scanning calorimeter), and encapsulation efficiency (*in vitro* release). Oxidative stability of the fortified burgers including pH, TBARS, and peroxide value (PV) during storage (4 ± 1 °C up to 15 days) was evaluated. The CLO microcapsules have highly uniform, droplet size was 874 nm, and accumulative release was 79.35% in 270 min in stimulated gastrointestinal. Also, found that CLO microcapsules were stable at different pH, cooking temperature, and storage at 4 ± 1 °C up to 15 days. Significant decrease in pH, TBARS, and PV in a fortified burger with microcapsules compared with unencapsulated and/or control samples. CLO-microcapsules enhanced sensory attributes of beef burger during storage and even after cooking, while burger with CLO-direct addition was rejected. Results demonstrated that CLO microcapsules as vehicles were stable at cooking temperature, keeping oil from oxidation, and improving the sensory attributes of burgers.

1. Introduction

Beef burgers are one of the most important meat products in many countries and has abundance consumed in worldwide (Antonini et al., 2020). They are a good source of proteins, fats, minerals, vitamins, and essential amino acids as well as availability, low cost, and ready to eat (RTE) (Quevedo et al., 2018). However, there is a linkage between meat products consumption and health concerns such as coronary heart diseases, hypertension, and bladder cancer (Crippa, Larsson, Discacciati, Wolk, & Orsini, 2018). These health concerns are disturbing between a variety of consumers, especially children in developed and developing countries (Afshari, Hosseini, Khaneghah, & Khaksar, 2017).

In recent years, there are growing interest and pressing demands for healthier/functional meat products by improving the fatty acids profile (Gómez, Sarriés, Ibañez, & Beriain, 2018) and incorporation of healthy ingredients such as bioactive peptides (Jiménez-Colmenero, Cofrades, Herrero, & Ruiz-Capillas, 2017), probiotics (Danza et al., 2018), fibers (Namir, Siliha, & Ramadan, 2015), natural antioxidants (López-Padilla et al., 2018), and fish oils (Aquilani et al., 2018). As the same time, the functional foods market has been increasing in Japan and USA, as well as the European countries (Zhang, Xiao, Samaraweera, Lee, & Ahn, 2010), and has created global revenue nearby U$ 299.32 billion in 2017 and is expected to increase to U$ 441.56 billion in 2022 (Statista, 2017).

Cod liver oil (CLO) has received incredible interest in food technology as a nutritional supplement (Solomando, Antequera, & Perez-Palacios, 2020), as it is a good source of ω-3 fatty acids namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Solomando, Antequera, & Pérez-Palacios, 2020). Also, has a higher content of vitamins A and D compared to fish oil (Öğütcü, Arifoğlu, & Yılmaz, 2015). CLO has many health benefits such as anti-inflammatory (Das, Sahu, Kashaw, & K Kashaw, 2017), antidiabetic (Stene et al., 2008), anticancer (Moovendhan, Seedevi, Vairamani, & Shanmugam, 2018, pp. 1–7), rickets prevention in infants (Eysteinsdottir et al., 2015), and reducing risk of cardiovascular diseases (Abeywardena & Patten, 2011; Raa, Rorstad, & Tande, 2017). In societies associated with low fish consumption, the supplementation of food products with fish oil can help promote the level of ω-3 intake (Fujita et al., 2015). However, the main challenges for the production of these foods are the undesirable odor of fish oil and instability during processing or storage (Ruiz & Campos, 2016). One alternative way to overcome these challenges is the

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use of encapsulation strategies (Chang & Nickerson, 2018).

Encapsulation is a unique approach to packaging active ingredients in the form micro-particles (Jacobsen, García-Moreno, Mendes, Mateiu, & Chronakis, 2018). Encapsulation has a number of benefits like protect, stabilize, and keep protecting nutritional, and intestinal losses (Wen, Wen, Zong, Linhardt, & Wu, 2017). Recently, different bioactive compounds were capsulated and used as enrichment in food products such as polyphenols (Liu et al., 2017), essential oils (Prakash et al., 2018), nutraceuticals (Aditya, Espinosa, & Norton, 2017), bacteria (Guerin et al., 2017), and enzymes (Ephrem, Najjar, Charcosset, & Greige-Gerges, 2018). The encapsulation methods of bioactive compounds are varied i.e. droplets, spray drying, coacervation, fluidized bed coating, extrusion, and liposome (Botelho, Canas, & Lameiras, 2017, pp. 535–586). In one study by Wrona, Nerín, Alfonso, and Caballero (2017) who found that the encapsulated green tea has inhibited the lipid oxidation and extend the shelf life of fresh minced meat. In another study by Frenzel and Steffen-Heins (2015) reported that fish oil microcapsulation using bilayer technique has boosted the oxidation stability of fish oil. However, García-Moreno et al. (2016) found that fish oil encapsulation in poly (vinyl alcohol) nanofibers more stable and high efficiency. Moreover, nano-encapsulated fish oil improved the quality characteristics of yogurt compared with direct addition fish oil (Ghorbanzade, Jafari, Akhavan, & Hadavi, 2017). The aim of this work was to (I) produce functional beef burgers fortified with micro-encapsulated CLO; (II) investigate the stability of cod liver oil microcapsules under gastrointestinal, cold, and cooking conditions and (III) the influence of cod liver oil microcapsules on quality parameters and sensory properties of beef burgers.

2. Materials and methods

2.1. Materials

Cod liver oil (CLO) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Burger ingredients including chuck steak beef cut, spices, semolina, and eggs were purchased from a local store in Cairo, Egypt. Sunflower oil (Arma Co., Egypt) was also purchased from a local store. Chitosan (CH; medium molecular weight 190–310 kDa and deacetylation degree of 75–85%, Sigma-Aldrich, Catalogue no. 448877), sodium alginate E−401 (SA; medium molecular weight, Sigma-Aldrich, Catalogue no. W201502), and soy lecithin E−322 (SL; Sigma-Aldrich, Catalogue no. 429415) all chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Calcium chloride ($CaCl_2$) and phosphate-buffered saline (PBS) were supplied from El-Naser Chemical Co. (Cairo, Egypt).

2.2. Microencapsulation of cod liver oil

The microcapsules were prepared according to the method of Liu et al. (2016) with slight modifications. Briefly, monolayer microcapsules were formulated as sodium alginate (2%, *w/v*) was dissolved in distilled water, then soy lecithin (1.5%, *w/v*) and sunflower oil were mixed with spatula in a heating bath (IKA, USA) at 50 °C, while, the chitosan (0.25%) was add to prior formula for bilayer microcapsules. Preheated cod liver oil was added drop-wise into the lecithin-oil mixture with stirring at 1000 rpm. The CLO suspension was injected through a 0.11-mm needle into 0.115 M $CaCl_2$. The beads were stirred at 300 rpm for 30 min for gasification, then filtered and washed with sterilized distilled water. The microcapsules were kept in phosphate-buffered saline (PBS) at 4 °C before use.

2.3. Preparation of beef burgers

Raw beef (19.26 ± 0.11% protein, 16.18 ± 0.24% fat, and 63.33 ± 0.58% moisture) was ground through a 4 mm plate (AW114 Model, K & G Wetter, Mississauga, ON, Canada). The burgers were produced according to the formula: 62% minced beef, 12% semolina, spices i.e. (1.75% salt, 1.25% onion powder, 0.5% garlic powder, 0.25% black pepper, 0.12% cardamom, 0.12% clove, 1.50% cubeb, 0.5% cumin, 0.5%, and 0.01% nutmeg), 7% egg, and 10% water (ice). All ingredients were combined and mixed together (~5 min) to obtain homogeneous dough and divided into 4 groups. 1st group (F1: CLO direct addition; CLO-DA), 2nd group (F2: CLO-monolayer-encapsulation; CLO-ME), 3rd group (F3: CLO-bilayer-encapsulation; CLO-BE), and the last one burgers without CLO (control; C). Beef burgers (70 ± 5 g) were shaped using (Super 54 Patty machine, Hollymatic, Countryside, IL, USA) with 9 cm diameter and 1 cm thickness. The burgers were placed into cardboard boxes with a plastic liner and stored for 15 days at 4 ± 1 °C. After that the burger samples were cooked (grilled) to an internal temperature of 73 ± 1 °C for 4 min on each side. The cooked samples were checked intervals at 0, 3, 6, 9, 12, and 15 days.

2.4. Characterization of CLO microcapsules

The morphology of CLO microcapsules was done using a scanning electron microscopy (JSM-6510- LA Ja, Japan) at an accelerating voltage of 10,000 V and 60,000× magnifications. Particle size and distribution of microcapsules were measured by a Malvern Zetasizer (ZS90, Malvern Instruments, Worcester, UK). The polydispersity index (PDI) was calculated using the cumulant analysis of the dynamic light scattering intensity auto-correlation function. When a PDI = 0 this means no variation and distribution in particle size, but PDI = 1 means large variations in size (Lawrie, Albanyan, Cardigan, Mackie, & Harrison, 2009; López-Amaya & Marangoni, 1999). All experiment was run in triplicate.

2.5. Encapsulation efficiency (EE)

The encapsulation efficiency (EE) of CLO was determined by the method described by Viriyaroj et al. (2009) with slight modifications. Initially, the microcapsules were centrifuged at 3000 rpm/10 min (Hettich Lab Technology, Germany) to remove phosphate-buffered saline film. A 10 g of CLO-microcapsules were used to extract CLO using three solvents as sequentially diethyl ether, ethanol, and petroleum ether (50 mL for each). The solvents were evaporated using a rotary evaporator (Laborota 4000, Heidolph, Germany) under conditions 35, 79, and 40 °C, respectively at 100 rpm/15–30 min. The final solution (oil extract) was filtered with filter paper (Whatman No.1) containing anhydrous Na_2SO_4, and the extracted oil was dried to a constant weight. The experiment was run in triplicate. The EE was calculated according to the equation below:

$$EE\ (\%) = 100\frac{W1}{W2}$$

Where W_1 is the oil weight in the microcapsules and W_2 is the oil initially weight added for microencapsulation.

2.6. In vitro release of CLO microcapsules

The *in vitro* release of CLO from microspheres under simulated gastrointestinal conditions of gastric juice and intestinal fluid was determined using the method proposed by (Liu et al., 2016). The 10 g of CLO microcapsules were immersed into 10 mL of simulated gastric juice (HCl buffer; pH 1.2), then incubated at 37 °C/100 rpm for 2 h. About 2 mL of samples were drawn and substituted with fresh medium to keep a stable volume during time intervals. Afterward, the microcapsules were transported to 10 mL of simulated intestinal medium (phosphate buffer at pH 6.8), then incubated at 37 °C/100 rpm for 4 h. About 2 mL of samples were drawn and replaced with fresh medium to keep a stable volume during time intervals. The CLO content of the drawn samples was determined as described in EE (section 2.5). The experiment was

run in triplicate.

2.7. GC-FID analysis of cod liver oil fatty acids profile

Fatty acids methyl esters (FAMEs) of cod liver oil were carried out according to Christie (1989, pp. 64–84). The FAMEs analysis was done by GC/FID (Shimadzu 17 A, Kyoto, Japan). The analysis conditions were; Capillary column, 50 m, 0.32, and 0.20 mm of Carbowax 20 M. Oven temperature 160 °C/20 (programmed at 10 °C min^{-1} until 240 °C). The temperature of injection 220 °C, temperature of the detector (FID) 250 °C, column flow of hydrogen 1.2 mL min^{-1}, and nitrogen 40 mL min^{-1} (split ratio; 1/100). Peaks identification was performed by retention times comparing with appropriate standards (Pure FAME mixture; Larodan, Malmoe, Sweden) at the same conditions. In this experiment, the preparation of sample was run in triplicate.

2.8. Vitamins quantification in CLO using HPLC

The CLO sample was prepared and purified using the normal-phase HPLC method according to Mattila et al. (1992). Vitamin D3 and A were analyzed using a UV/Vis HPLC. The analytical column was Ascentis® C18 (25 cm × 4.6 mm). The mobile phase was methanol/-water (98:2; v/v) with 1% of phosphoric acid solution (10% v/v); injection volume = 15 μL; flow rate = 1 mL min^{-1}; λ = 265 nm; running time: 15 min. The calibration curve was linear over a concentration range of 100–500 ng mL^{-1}. A calibration curve was plotted using the measured retention time (RT) and peak area (PA) for each concentration using which the unknown concentration of Vitamin D was determined. The experiment was run in triplicate.

2.9. Physicochemical properties of CLO and burgers

The specific gravity was measured by density meter (DMA 35, Graz, Austria) at 20 °C. The refractive index was measured using Abbe Benchtop Refractometer (BAusch & Lomb, Florida, USA) at 20 °C according to AOAC (2005). Iodine value (mg I_2g^{-1}), saponification value (mg $KOHg^{-1}$), and peroxide value (meq kg^{-1}) were determined according to AOCS (1998). pH value was measured using a digital pH-meter (model P107, Consort, Belgium) and TBARS (MDA kg^{-1}) were determined using spectrophotometric (CE 599 Universal, USA) (AOAC, 2005). All tests were run in triplicate.

2.10. Sensory evaluation of burgers

A twenty-member trained panelists aged (20–40 yrs) from the Food Technology Department in Agriculture College at Benha University. The burger samples (70 ± 5 g of each group) were placed in covered plate coded with 3-digits. The panelists were asked to evaluate color, odor, and overall acceptance for raw samples, while color, odor, taste, chewing, and overall acceptance for cooked samples. The sensory evaluation was performed based on 7-point hedonic scales (1 = dislike extremely, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor a dislike, 5 = like slightly, 6 = like moderately, and 7 = like extremely) (Pietrasik, Sigvaldson, Soladoye, & Gaudette, 2020).

2.11. Statistical analysis

All experiments were run in triplicate for CLO, while the sensory evaluation was run with twenty-panelists; two factors (microcapsules with two techniques and CLO-direct addition) were applied. For the burgers analysis (pH, TBARS, PV, and sensory properties), factorial design ANOVA with two factors as follows four treatments (control, CLO-monolayer, CLO-bilayer, and CLO-direct addition) and storage time with five points at 0, 3, 6, 9, 12, and 15 days were applied for each parameter using SPSS software (Version 18 for Windows; SPSS Inc., Chicago, IL). Tukey's multiple comparison tests at $P < 0.05$ of means were figured out (Steel, Torrie, & Dickey, 1980, p. 633).

3. Results and discussion

3.1. CLO-properties, fatty acids profile, and vitamins

Cod liver oil (CLO) is the most important food supplement/enrichment and recently used in different food products. The physicochemical properties of CLO were performed and found as refractive index 1.471 ± 0.11, specific gravity 0.922 ± 0.05, saponification value 186 ± 1.05 mg KOH g^{-1}, and iodine value 161 ± 1.12 mg I_2 g^{-1} (data not shown). Table 1 presented the fatty acids profile of cod liver oil (CLO). A total 31 fatty acids were identified and the major fatty acids were elaidic acid (16.78%), docosahexaenoic acid (13.81%), palmitic acid (10.96%), eicosenoic acid (9.63%), behenic acid (9.22%), docosanoic acid (8.92%), palmitoleic acid (5.61%), octadecenoic acid (4.87%), and myristic acid (3.72%). It was noted that the CLO is higher content in monounsaturated fatty acid (MUFA) than polyunsaturated fatty acid (PUFA). Also, the CLO is a good source of omega-3 fatty acids (DHA + EPA) which about 18.28%. These results are in agreement with those reported by Rohman (2017) who found that the properties of CLO were saponification value 186 mg KOH g^{-1}, iodine value 162 mg I_2 g^{-1}, MUFA 45.9%, and PUFA 27.2%

Although CLO is rich in fatty acids, but also a good source of vitamins soluble in fat i.e. A and D3. These vitamins have health benefits i.e.

Table 1
Fatty acids profile of cod liver oil (CLO).

Fatty acids composition (g/100 g)	Cod liver oil
Lauric acid (C12:0)	0.03 ± 0
Tridecylic acid (C13:0)	0.02 ± 0
Myristic acid (C14:0)	3.72 ± 0.07
Tetradecanoic acid (C14:1)	0.22 ± 0.01
Pentadecylic acid (C15:0)	0.33 ± 0.01
Pentadecanoic acid (C15:1)	0.14 ± 0.01
Palmitic acid (C16:0)	10.96 ± 0.06
Palmitoleic acid (C 16:1)	5.61 ± 0.09
Hexadecadienoic acid (C 16:2n-4)	0.51 ± 0.04
Margaric acid (C 17:0)	0.8 ± 0.04
Margaroleic (C 17:1)	0.56 ± 0.01
Stearic acid (C 18:0)	2.11 ± 0.04
Elaidic acid (C 18:1n-9)	16.78 ± 0.06
Octadecenoic acid (C 18:1n-7)	4.87 ± 0.04
Linoleic acid (C 18:2)	1.9 ± 0.1
Gamma-linolenic acid (C 18:3n-6)	0.23 ± 0.02
α-Linolenic acid (C 18:3n-3)	1.29 ± 0.03
Stearidonic acid (C 18:4n-3)	2.31 ± 0.04
Arachidic acid (C 20:0)	0.08 ± 0.01
Eicosenoic acid (C 20:1)	9.63 ± 0.04
Eicosadienoic acid (C 20:2)	0.35 ± 0.02
Arachidonic acid (C 20:4)	0.53 ± 0.01
Eicosatrienoic acid (C 20:3n-3)	0.23 ± 0.02
Eicosapentaenoic acid (C 20:5)	0.73 ± 0.02
Behenic acid (C 22:0)	9.22 ± 0.1
Docosanoic acid (C 22:1)	8.92 ± 0.04
Docosatrienoic acid (C 22:3n-3)	0.42 ± 0.01
Adrenic acid (C 22:4n-6)	0.25 ± 0.01
Docosapentaenoic acid (C 22:5n-3)	0.21 ± 0.01
Lignoceric acid (C 24:0)	1.31 ± 0.07
Docosahexaenoic acid (C 22:6n-3)	13.81 ± 0.1
Others	1.9 ± 0.09
Saturated fatty acid ∑SFA	28.86 ± 0.05
Monounsaturated fatty acid ∑MUFA	46.73 ± 0.01
Polyunsaturated fatty acid ∑PUFA	22.79 ± 0.04
∑PUFA/SFA	0.79 ± 0
∑MUFA/PUFA	2.05 ± 0
Omega 3	18.28 ± 0.02

SFA, saturated fatty acid.
MUFA, monounsaturated fatty acid.
PUFA, polyunsaturated fatty acid.
n = 3.

vision maintenance, immune function, growth, and bone maintenance (Wang, Vongsvivut, Adhikari, & Barrow, 2015). In Fig. 1, the results demonstrated that CLO has vitamins A and D3 were 840 and 85 I.U. mL^{-1}, respectively. These findings were agreement with Codex Standard (Codex, 2017, pp. 329–2017) which reported that vitamin A and D3 in cod liver oil should be higher than 134 and 40 I.U. mL^{-1}, respectively. As well as, the previous research by Sadeghi, Mehr, and Mirlohi (2014) found that vitamin D3 in fish oil was 70.6 I·U. mL^{-1}.

3.2. Micro-encapsulation results

3.2.1. Characterization of CLO microcapsules

The different characterizations of CLO microcapsules were evaluated. The droplet size and morphology of the CLO microcapsules were confirmed using SEM (Fig. 2). Results demonstrated that CLO microcapsules were spherical, smooth, and free from cracks. The outer layer of microcapsules could be important to protect the CLO from oxidation and undesired release (Aghbashlo, Mobli, Madadlou, & Rafiee, 2013). It was found that particle size and PDI of CLO microcapsules were 874 nm and 0.037, respectively (Fig. 2). Also, noted the CLO microspheres had a lower size distribution compared with microcapsules produced by another premix ME process (Ramakrishnan, Ferrando, Aceña-Muñoz, De Lamo-Castellví, & Güell, 2013). CLO microcapsules have the potential to keep residence time at the application site as a food supplement delivery system. Previous report found that chitosan–alginate microspheres at 7.2 μm can transmit from the gastrointestinal to the circulation system (Wei et al., 2008). Other studies confirmed that fish oil microcapsules less than 1 μm could be easily digested than a larger size (Aberkane, Roudaut, & Saurel, 2014; Aghbashlo et al., 2013; Wang, Tian, & Chen, 2011). Moreover, chitosan can enhance the bio-adhesive ability of microspheres to specific areas in the gastrointestinal i.e. stomach (Remunan-Lopez, Portero, Lemos, Vila-Jato, & Nunez, 2000), small intestine (Shimoda, Onishi, & Machida, 2001), and buccal mucosa (Remuñán-López, Portero, Vila-Jato, & Alonso, 1998).

3.2.2. Micro-encapsulation efficiency of CLO

The encapsulation efficiency is defined as the amount of core-material (cod liver oil; CLO) encapsulated inside the particles. Encapsulation efficiency rates not only the non-encapsulated CLO present on the surface of microcapsules but also the ratio of oil extracted from the capsules. Fig. 3a showed micro-encapsulation efficiency of raw CLO-monolayer-encapsulation (CLO-ME) and CLO-bilayer-encapsulation (CLO-BE) were 82.55 and 90.78%, respectively and in cooked samples were 82.29 and 90.32%, respectively. That is means about 82 and 90% of the CLO has been encapsulated within micro-liposomes and less than 18 and 10% has been free unencapsulated. However, it was noted a significant difference in efficiency level ($P \leq 0.05$) between CLO-ME and CLO-BE in both raw and cooked capsules. Based on structure of liposome, the core and bilayer wall are the hydrophilic/hydrophobic (W/O), respectively; thus, the phospholipid based bilayers act as the reservoir for CLO. One study (Heck et al., 2017) found that microencapsulation of n−3 PUFA-rich oils is effective for production of healthier burgers. The encapsulation efficiency of the active ingredients could be affected by different factors i.e. size, surface areas, layers, and liposomal method

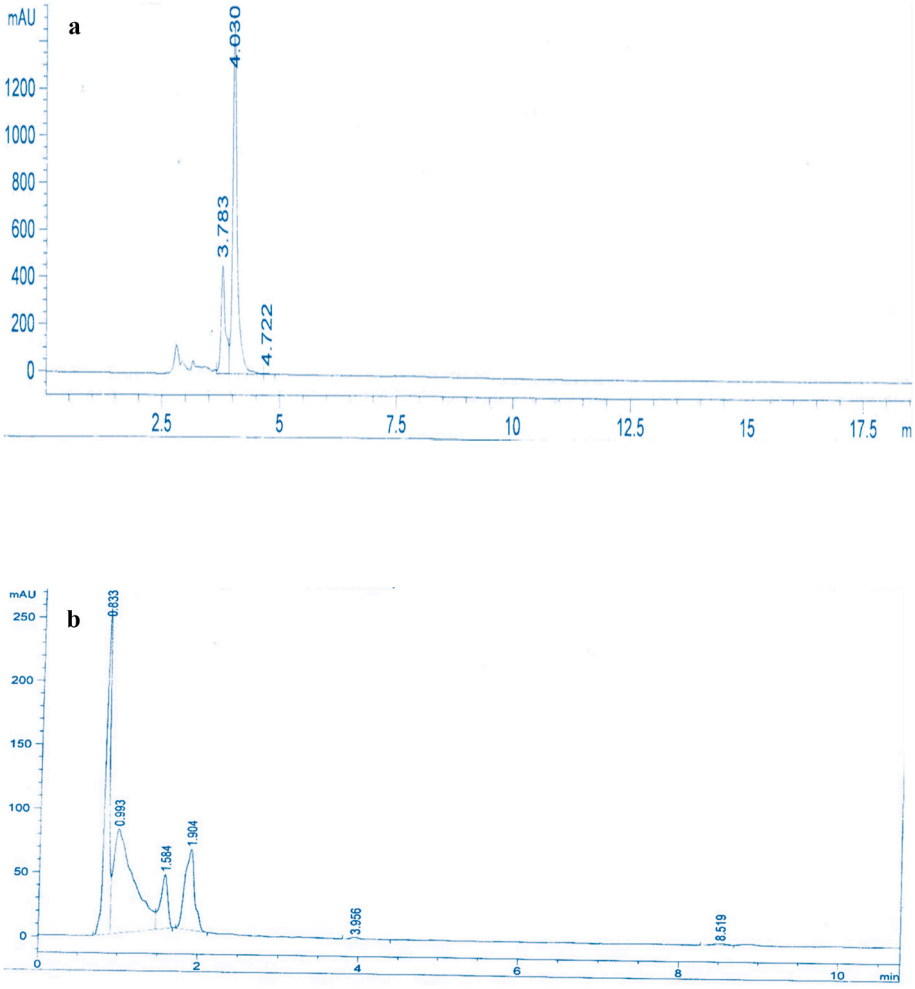

Fig. 1. Vitamins profile of cod liver oil (CLO), vitamin A (a) and vitamin D3 (b) (n = 3).

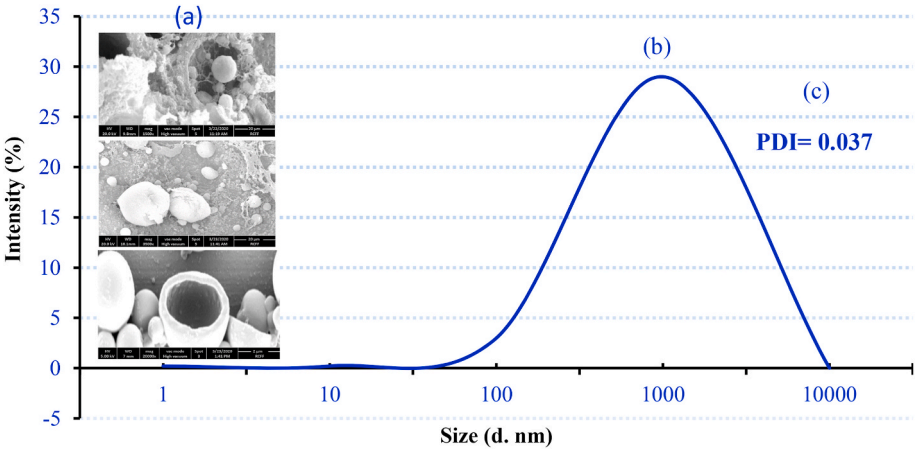

Fig. 2. Characterization of CLO microcapsules using SEM image (a), size distribution (b), and polydispersity index (PDI) (c) (n = 3).

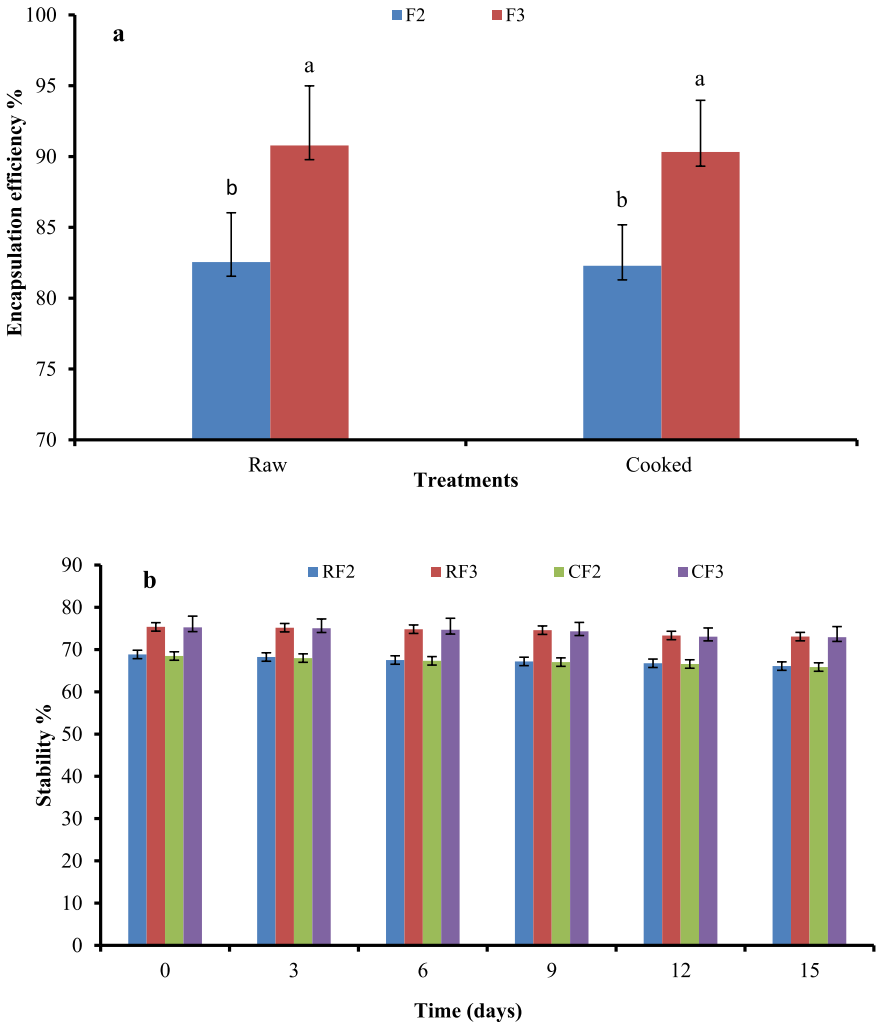

Fig. 3. Encapsulation efficiency (a) and encapsulation stability (b) of raw and/or cooked CLO microcapsules. Error bars represent standard deviation, (n = 3). F2: cod liver oil monolayer-encapsulation, F3: cod liver oil bilayer-encapsulation, RF2: raw cod liver oil monolayer-encapsulation, RF3: raw cod liver oil bilayer-encapsulation, CF2: cooked cod liver oil monolayer-encapsulation, CF3: cooked cod liver oil bilayer-encapsulation.

(Rasti, Jinap, Mozafari, & Yazid, 2012). Similar findings were reported in the previous investigations by (Carvalho et al., 2015; Ghorbanzade et al., 2017) who found that encapsulation efficiency of fish oil ranged from 85 to 95%.

3.2.3. *Stability of microcapsules containing CLO*

The stability of raw and cooked microcapsules containing CLO was evaluated (Fig. 3b). No significant difference ($P > 0.05$) in stability was recorded between microcapsules during storage for 15 days. While

observed a significant difference ($P \leq 0.05$) between CLO-ME and CLO-BE in both raw and cooked microcapsules. The results confirmed that the stability of CLO-ME and CLO-BE at time zero was 68.84 and 75.36%, respectively. However, during the storage period was noted a slight decrease in stability. That is means the proposition of the aqueous phase separated from the micro-liposome dispersion after centrifugation. On the other hand, it was noted that no significant difference ($P > 0.05$) in physicochemical, fatty acids, and vitamins (A and D3) between raw and cooked CLO-microcapsules (data not shown). Previous study found that active-liposomes stability could be affected by liposome size, layers, phospholipid structure, and techniques (Laridi et al., 2003). Another study showed that microcapsules instability was attributed to interference and eventual merging of surfaces of two or more capsules (Taylor, Gaysinsky, Davidson, Bruce, & Weiss, 2007). The high stability of micro-liposomes i.e. CLO-ME and CLO-BE may be due to the structure formula of capsules that contain phospholipids. These results are in agreement with those reported by Laridi et al. (2003) who confirmed the high stability of alginate microparticles during storage. Moreover, thermal stability of microcapsules may be due to the combination of sodium alginate and Ca^{+2} ions that form a strongly thermo-stable gel (Cheow & Hadinoto, 2013).

3.2.4. *In vitro release profiles*

The release profiles of CLO microcapsules were investigated at ambient temperature (25 °C) for 270 min. As seen in Fig. 4, the release of CLO at 120 and 270 min was 4.88% and 79.35%, respectively. The low release at simulated gastric juice at pH 1.2 could be due to a tight chitosan-alginate network formation at low pH (Coppi, Iannuccelli, Leo, Bernabei, & Cameroni, 2001; Tan et al., 2018). At low pH, the sodium hydrated alginate was shrunk and converted into a porous, as well become insoluble layer which known alginic acid skin (Ling, Wu, Neish, & Champion, 2019). However, once the microcapsules are exposed to simulated intestinal fluid at pH 6.8 the alginic acid skin is turned into a soluble layer and viscous structure that allows the higher release of CLO. These results agreement with those reported (Treenate & Monvisade, 2017) who found that chitosan alone has an inverse pH-sensitive behavior that inhibits rupturing and complete release of CLO at high pH.

3.3. *Physicochemical properties of burgers fortified with CLO microcapsules*

3.3.1. *pH value*

The pH is an important indicator of meat quality. The changes of pH in burger samples during 15 days of storage at 4 °C were evaluated. As seen in Fig. 5a, the highest pH value was observed in the control sample and the lowest was in the CLO-bilayer-encapsulated sample. The results indicated that the primary pH value in all samples was about 5.85, while this ratio was gradually increased during the storage period. A significant difference ($P \leq 0.05$) was observed in pH value between the samples containing microcapsules and the control one during the storage. No significant differences ($P > 0.05$) were recorded in pH of the different burger samples containing CLO-ME and CLO-BE because of encapsulation process protect the CLO from to degradation. Results confirmed that an increase in pH value in control and CLO direct addition compared with others treatment this may be due to the decomposition of nitrogenous compounds by microbial enzymes (Morsy, Mekawi, & Elsabagh, 2018; Solomando, Antequera, & Perez-Palacios, 2020).

3.3.2. *TBARS value*

Fig. 5b, presents the changes in TBARS contents in beef burger enriched with CLO during storage at 4 °C/15 days. At time zero, the TBARS values in C, CLO-DA, CLO-ME, and CLO-BE samples were 0.10, 0.13, 0.11, and 0.09 mg MDA kg^{-1}, respectively. While during the storage period was found rapidly increased in the CLO-DA sample up to 1.26 mg MDA kg^{-1}, followed by control (1.16 mg MDA kg^{-1}), then CLO-ME (0.76 mg MDA kg^{-1}), and CLO-BE (0.68 mg MDA kg^{-1}) after 15 days. This is in agreement with previous findings in pork burgers, chicken nuggets, and RTE meat enriched with fish oil microcapsules (Aquilani et al., 2018; Jiménez-Martín, Pérez-Palacios, Carrascal, & Rojas, 2016; Pérez-Palacios, Ruiz-Carrascal, Jiménez-Martín, Solomando, & Antequera, 2018). Generally, the storage of the burger samples led to a significant increase in the TBARS values in most treatments, enriched and not enriched CLO. Also, it was noted a higher increase of TBARS in the enriched samples with CLO-DA than in the control ones after 15 days of storage. While, the samples included CLO-BE have the lowest TBARS value. One study, Aquilani et al. (2018) found that pork burgers enriched with fish oil microcapsules not influence in TBARS value at five days refrigeration storage. Nevertheless, the different chemical composition of the meat, processing, additives, and storage conditions makes the comparison difficult. These results may be due to the microcapsule layers that act as a barrier, protect, and minimize the contact of the CLO and ω-3 PUFA with the oxidant factors in the meat products (Wenjiao, Yongkui, Yunchuan, Junxiu, & Yuwen, 2014).

3.3.3. *Peroxide value (PV)*

As shown in Fig. 5c, the initial peroxide value (PV) of burger samples

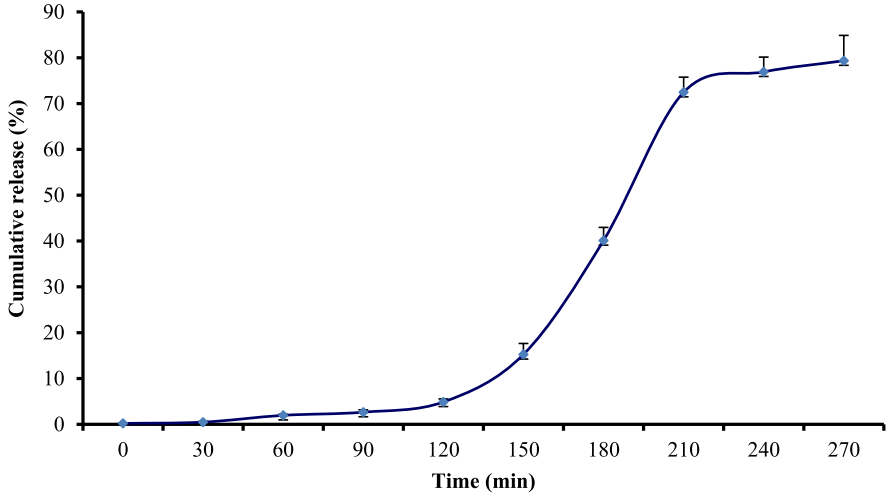

Fig. 4. *In vitro* release profile of cod liver oil microcapsules in simulated gastric juice (0–120 min) and intestinal fluid (120–270 min). Error bars represent standard deviation, (n = 3).

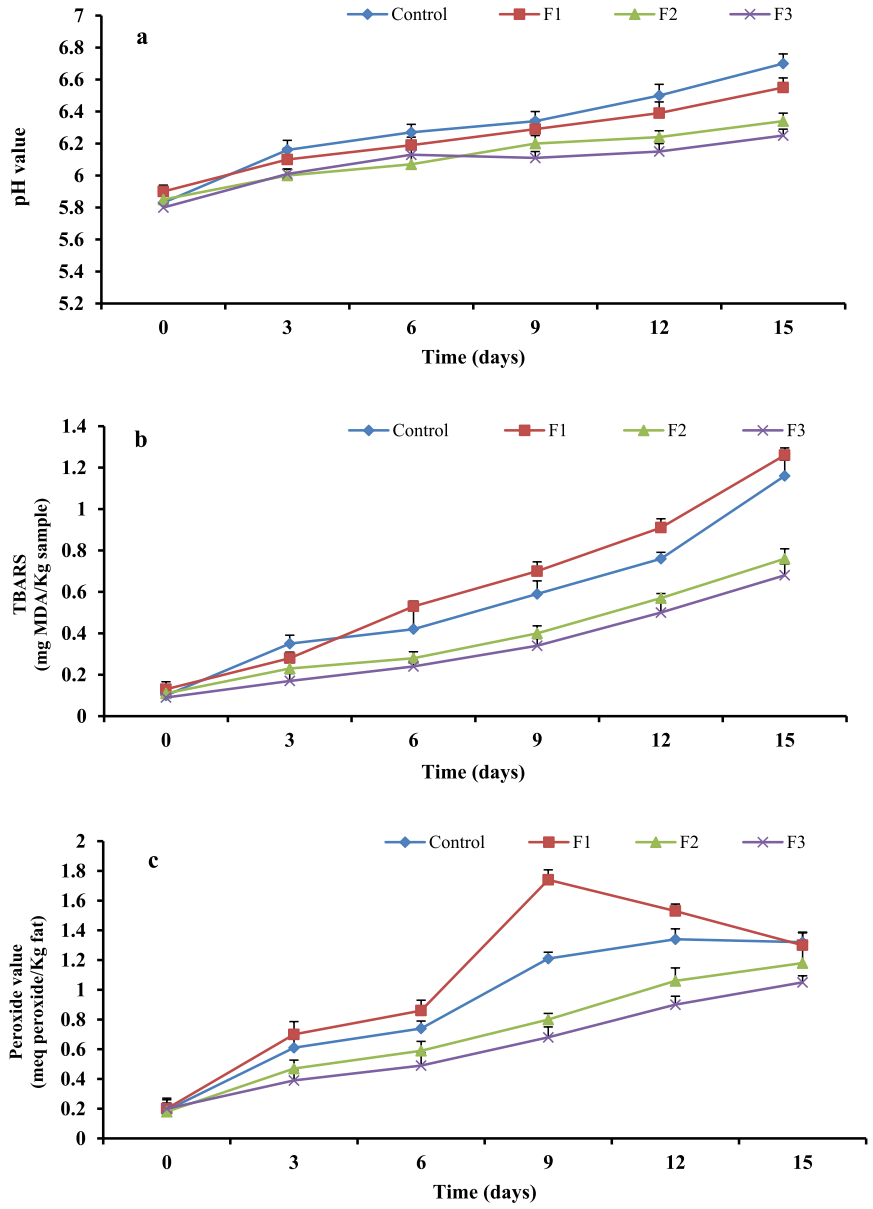

Fig. 5. Changes in pH value (a), TBARS (mg MDA Kg^{-1}) (b), and peroxide value (meq peroxide Kg^{-1} fat) (c) in burger samples incroporation CLO-microcapsules during storage at (4 °C). Error bars represent standard deviation, (n = 3). F1: cod liver oil direct addition, F2: cod liver oil monolayer-encapsulation, F3: cod liver oil bilayer-encapsulation.

containing CLO was 0.19 meq kg^{-1}, however, rapidly increased during the storage period up to 15 days. It was observed that the PV reached the threshold limit value on the 9th day of storage in control and CLO-DA samples, and then quickly decrease was recorded. However, the PV in burger samples included the CLO-ME and CLO-BE were below the limit value until the 12^{th} and 15th day, respectively during cold storage at 4 °C. The PV of burger samples C, CLO-DA, CLO-ME, and CLO-BE were 1.32, 1.3, 1.18, and 1.05 meq kg^{-1}, respectively after 15 days of storage. Results indicated that control and CLO-DA samples have a remarkable lipid-oxidation until day 9 of storage and PV maximum at the end of the primary auto-oxidation. After 9 days, the PV decreased could be due to the decomposition of hydroperoxidesto secondary lipid oxidation products (Ladikos & Lougovois, 1990). On the other hand, the samples have incorporated CLO microcapsules are less influencing oxidation. It is quite obvious that microencapsulation in monolayer and/or bilayer that protected CLO and ω-3 PUFA from deteriorating factors in the meat product. The results are in agreement with those reported by (Aquilani et al., 2018; Solomando, Antequera, & Perez-Palacios, 2020).

3.4. Sensory evaluation of burgers fortified with CLO micro-capsules

The mean scores of the sensory parameters, i.e. color, odor, and overall acceptance of raw burger samples were statistically evaluated (Table 2). In general, no significant difference ($P > 0.05$) was recorded at time zero between the treatments. However, it was observed that the color, odor, and acceptability of the burger samples decreased for all treatments up to 15 days. The control sample has scored a low value of color, odor, and overall acceptance were 4.1, 3.9, and 4.3, respectively on day 9. On the other hand, the samples enriched with CLO-DA showed a marked fishy odor the lowest acceptability scores (3.7 hedonic score) compared CLO-ME (6.1 hedonic score) and CLO-BE (6.5 hedonic score) on day 9. The sensory evaluation was conducted for 9 days for the control and CLO-DA samples because of the unpleasant odor/acceptance that probably leads to consumer rejection. While, the samples included

Table 2
Sensory responses of raw beef burgers enriched CLO microcapsules stored at 4 ± 1 °C for 15 days (mean ± SD).

Attributes	Treatments	Storage days					
		0	3	6	9	12	15
Color	Control	7 ± 0.00^{aA}	6.4 ± 0.84^{bB}	5.3 ± 0.67^{bC}	4.1 ± 0.74^{bD}	R*	R
	F1 (CLO-DA)	6.9 ± 0.32^{aA}	6.3 ± 0.67^{bB}	4.8 ± 0.79^{cC}	3.8 ± 0.92^{cD}	R	R
	F2 (CLO-ME)	7 ± 0.00^{aA}	6.9 ± 0.32^{aA}	6.5 ± 0.71^{aB}	6.1 ± 0.99^{aC}	4.3 ± 0.67^{bD}	R
	F3 (CLO-BE)	7 ± 0.00^{aA}	6.9 ± 0.32^{aA}	6.8 ± 0.42^{aA}	6.4 ± 0.52^{aB}	5.9 ± 0.32^{aC}	5.4 ± 0.7^{aD}
Odor	Control	7 ± 0.00^{aA}	6.5 ± 0.85^{bB}	4.4 ± 1.07^{bC}	3.9 ± 0.74^{bD}	R	R
	F1 (CLO-DA)	6.4 ± 0.52^{bA}	5.8 ± 0.42^{cB}	3.9 ± 0.57^{cC}	3.3 ± 0.67^{cD}	R	R
	F2 (CLO-ME)	7 ± 0.00^{aA}	6.9 ± 0.32^{aA}	6.2 ± 0.63^{aB}	6 ± 0.63^{aB}	4.1 ± 0.57^{bC}	R
	F3 (CLO-BE)	7 ± 0.00^{aA}	6.9 ± 0.32^{aA}	6.5 ± 0.71^{aB}	6.3 ± 0.67^{aB}	5.7 ± 0.48^{aC}	5.2 ± 0.74^{aD}
Overall acceptance	Control	6.9 ± 0.32^{aA}	6.2 ± 0.92^{bB}	5.5 ± 0.71^{bC}	4.3 ± 0.79^{bD}	R	R
	F1 (CLO-DA)	6.8 ± 0.42^{aA}	5.9 ± 0.88^{cB}	4.1 ± 0.99^{cC}	3.7 ± 0.82^{cD}	R	R
	F2 (CLO-ME)	7 ± 0.00^{aA}	6.8 ± 0.42^{aA}	6.4 ± 0.79^{aB}	6.1 ± 0.82^{aB}	4.2 ± 0.67^{bC}	R
	F3 (CLO-BE)	7 ± 0.00^{aA}	6.9 ± 0.32^{aA}	6.7 ± 0.67^{aA}	6.5 ± 0.71^{aB}	6 ± 0.82^{aC}	5.1 ± 0.32^{aD}

CLO: Code liver oil, CLO-DA: CLO direct addition, CLO-ME: CLO-monolayer-encapsulation, CLO-BE: CLO-bilayer-encapsulation.
[abc] There is no significant differences between any two means 'in the same column' have the same superscript small letter ($P > 0.05$).
[ABC] There is no significant differences between any two means 'in the same row' have the same superscript capital letter ($P > 0.05$).
*R: reject.
n = 20.

CLO-ME and CLO-BE conducted for day 12 and 15, respectively. Also, the cold storage seems to be the most effective on the oxidation level of enriched samples, while the additions of CLO microcapsules are less influenced. As shown in (Table 3), the sensory evaluation of cooked burger samples was performed for storing dates until off-flavor apparent. A significant difference ($P \leq 0.05$) was recorded in color, odor, taste, chewing, and acceptability scores between the treated samples. Results demonstrated that control and CLO-DA samples have low scores in all sensory parameters after cooking and during storage periods. However, CLO-DA sample a marked fishy odor and was rejected in the early stage of storage (day 6) due to unpleasant odor, taste, and acceptance. The CLO-ME and CLO-BE samples recorded high score and acceptance till day 12 and 15 of storage, respectively. This finding could be linked to the changes in the oxidation levels and the storage impact has deeply observed in control and CLO-DA samples, which could be ascribed to the high pH, TBARS, and PV values (Solomando, Antequera, & Perez-Palacios, 2020). However, the samples included microcapsules CLO-ME and CLO-BE were sensorial accepted up to 12th and 15th day, respectively. This is due to the microcapsule layers that act as a good barrier and protect the CLO from oxidation in the meat products as well microcapsule layers may avoid of ω-3 PUFA from oxidation reaction and radicals through the meat product. The results are in agreement with previous studies in enriched meat products with fish oil microcapsules, i.e. sausages (Solomando, Antequera, & Perez-Palacios, 2020), pork burgers (Aquilani et al., 2018), chicken nuggets (Jiménez-Martín et al., 2016), RTE meat (Pérez-Palacios et al., 2018), and Spanish salchichon (Lorenzo, Munekata, Pateiro, Campagnol, & Domínguez, 2016).

From the abovementioned results, microencapsulation is the best way to enhance nutrition, mask off-flavors, and facilitate storage, without negative influence on the physical, chemical or functional

Table 3
Sensory responses of cooked beef burgers enriched CLO microcapsules stored at 4 ± 1 °C for 15 days (mean ± SD).

Attributes	Treatments	Storage days					
		0	3	6	9	12	15
Color	Control	7 ± 0.00^{aA}	6.6 ± 0.55^{bB}	5.5 ± 0.42^{bC}	4.4 ± 0.54^{bD}	R*	R
	F1 (CLO-DA)	6.8 ± 0.23^{aA}	6.1 ± 0.71^{bB}	4.6 ± 0.57^{bC}	3.7 ± 0.63^{cD}	R	R
	F2 (CLO-ME)	7 ± 0.00^{aA}	6.8 ± 0.46^{aA}	6.4 ± 0.38^{aB}	6.2 ± 0.32^{aC}	4.5 ± 0.28^{bD}	R
	F3 (CLO-BE)	7 ± 0.00^{aA}	6.9 ± 0.22^{aA}	6.8 ± 0.32^{aA}	6.5 ± 0.71^{aB}	6 ± 0.25^{aC}	5.6 ± 0.43^{aD}
Odor	Control	7 ± 0.00^{aA}	6.7 ± 0.53^{bB}	4.7 ± 62^{bC}	4 ± 0.33^{bD}	R	R
	F1 (CLO-DA)	6 ± 0.37^{bA}	4.1 ± 0.75^{cB}	R	R	R	R
	F2 (CLO-ME)	7 ± 0.00^{aA}	6.9 ± 0.27^{aA}	6.5 ± 0.54^{aB}	6 ± 2.34^{aC}	4.7 ± 0.37^{bD}	R
	F3 (CLO-BE)	7 ± 0.00^{aA}	6.9 ± 0.24^{aA}	6.7 ± 0.39^{aB}	6.4 ± 0.55^{aB}	5.9 ± 0.42^{aC}	5.5 ± 0.32^{aD}
Taste	Control	7 ± 0.00^{aA}	6.6 ± 0.42^{bB}	4.8 ± 33^{bC}	4.2 ± 0.41^{bD}	R	R
	F1 (CLO-DA)	5 ± 0.22^{bA}	4.1 ± 0.32^{cB}	R	R	R	R
	F2 (CLO-ME)	7 ± 0.00^{aA}	6.9 ± 0.25^{aA}	6.6 ± 0.34^{aB}	$6.3 \pm .41^{aB}$	4.9 ± 0.32^{bC}	R
	F3 (CLO-BE)	7 ± 0.00^{aA}	6.9 ± 0.36^{aA}	6.8 ± 0.33^{aB}	6.5 ± 0.28^{aB}	6.1 ± 0.45^{aC}	5.8 ± 0.52^{aD}
Chewing	Control	7 ± 0.00^{aA}	6.7 ± 0.25^{bB}	5 ± 22^{bC}	4.4 ± 0.34^{bD}	R	R
	F1 (CLO-DA)	5.2 ± 0.34^{bA}	4.3 ± 0.55^{cB}	R	R	R	R
	F2 (CLO-ME)	7 ± 0.00^{aA}	6.9 ± 0.20^{aA}	6.7 ± 0.32^{aB}	$6.5 \pm .31^{aB}$	4.7 ± 0.38^{bC}	R
	F3 (CLO-BE)	7 ± 0.00^{aA}	6.9 ± 0.23^{aA}	6.8 ± 0.28^{aB}	6.6 ± 0.37^{aB}	6.3 ± 0.42^{aC}	5.9 ± 0.33^{aD}
Overall acceptance	Control	7 ± 0.00^{aA}	6.7 ± 0.22^{bB}	5.7 ± 32^{bC}	4.3 ± 0.42^{bD}	R	R
	F1 (CLO-DA)	5.1 ± 0.52^{bA}	4.2 ± 0.76^{cB}	R	R	R	R
	F2 (CLO-ME)	7 ± 0.00^{aA}	6.9 ± 0.33^{aA}	6.6 ± 0.26^{aB}	$6.4 \pm .41^{aB}$	4.4 ± 0.22^{bC}	R
	F3 (CLO-BE)	7 ± 0.00^{aA}	6.9 ± 0.32^{aA}	6.8 ± 0.35^{aB}	6.7 ± 0.43^{aB}	6.5 ± 0.54^{aC}	5.8 ± 0.66^{aD}

CLO: Code liver oil, CLO-DA: CLO direct addition, CLO-ME: CLO-monolayer-encapsulation, CLO-BE: CLO-bilayer-encapsulation.
[abc] There is no significant differences between any two means 'in the same column' have the same superscript small letter ((P > 0.05).
[ABC] There is no significant differences between any two means 'in the same row' have the same superscript capital letter (P > 0.05).
*R: reject.
n = 20.

properties of the food products.

4. Conclusions

This study evaluated functional beef burger fortified with mono-layered and bilayered cod liver oil (CLO) microcapsules. The characterizations of the microcapsules and quality parameters of fortified burger were done. Results demonstrated that CLO microspheres were highly uniform and have an accumulative release in stimulated gastro-intestinal. Also, noted that a significant decrease in of pH, TBARS, and PV in a fortified burger with CLO microcapsules compared with unencapsulated and/or control samples. The burger samples fortified CLO microcapsules exhibited high sensory scores, while burger with CLO-direct addition was rejected (fishy flavor). Thus, it could be indicated the viability of mono and bilayer as vehicles to enrich meat products for stability at cooking temperature, keeping oil from oxidation, and improving the sensory characteristics of burgers.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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