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Influence Of Cod Liver Oil As A Cocoa Butter Replacer For Shelf Life And Nutritional Enhancement.

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ABSTRACT

The product quality of a chocolate depends upon the ingredients present in them such as cocoa butter, cocoa powder, sugar, emulsifiers, fats *etc*. This paper focuses on the replacement of cocoa butter by means of cod liver oil in conventional chocolate and the evaluation of its impact on physical, biological, nutritional and microbial properties. X-ray diffraction (XRD) studies revealed that an increase in the oil concentration resulted in the crystal polymorphism attaining gradual homogeneity. The oil migration was observed using scanning electron microscope (SEM) and noticeable migration was seen as the concentration of cod liver oil and days of storage of chocolate increased. The shelf life of the chocolate formulations was evaluated by means of standard microbial spread plate method and fat bloom formation. The study revealed that vitamin A and D3 content increased with increase in concentration of cod liver oil. Hence, the fat replaced chocolates not only have notable impact on physical properties but also had biological and nutritional benefits when compared to conventional chocolate.

Keywords: Cod-liver oil; vitamin; fat bloom; shelf-life; oil migration; polymorphism

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INTRODUCTION

Dark chocolate frequently known as plain or black chocolate contains high amounts of antioxidants, cocoa solids and sugar embedded inside a complex matrix of cocoa butter. It contains comparatively less amounts of milk or no milk at all compared to other chocolate forms and has a total solid content of 65-75%, and also a higher content of cocoa butter [1] [2]. It is the cocoa butter that is responsible for the crunch, crispiness, glossy texture and melting finish of chocolate [3].

Cocoa butter is composed of six different polymorphic forms denoted as I-VI referring to the structural distribution of the crystals within it. On appropriate tempering, the cocoa butter crystallizes in form β_V. Based on the construction and the conditions under which it is accumulated, this cocoa butter can be altered from form β_V to form β_{VI}. This polymorphic alteration commonly leads to the formation of fat bloom. The transformation from form β_V to form β_{VI} arises rapidly at higher temperatures and is also accelerated by migration of oils from filling into the chocolate [4].

Fish oil is a source of polyunsaturated fatty acids (PUFAs). It is known to have high levels of omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These PUFAs exist as triglycerides (TGs) or triacylglycerols (TAGs). In previous studies, cocoa butter replacers (CBRs) like lauric fats, palm kernel oil and coconut oils have been used to replace the cocoa butter. Palm kernel oil is considered as food-grade oil of high standard [5]. In this study, cod liver oil has been explored as a main source of dietary supplement as it is regarded as a vitamin supplement containing vitamin A and vitamin D3. It has many benefits such as reducing the risk of auto immune diseases and heart diseases, lowering high blood pressure and cholesterol, which also positively impacts child development. The only limitation it possesses is its unpleasant taste and odour [6]. The traditional tempering of chocolate involves consecutive rounds of heating and cooling with an aim to obtain fat bloom free chocolate [7].

We report here the use of cod liver oil as a means for the cocoa butter replacement in dark chocolate. Though previous studies have been performed on the replacement of cocoa butter in dark chocolate using palm kernel oil, coconut oil, lauric fats *etc*., no research has yet been investigated using cod liver oil as a cocoa butter replacer. Extended studies were performed for the effective understanding of the novel cod-liver replaced chocolate characteristics with special emphasis given on shelf life and nutritional enhancement.

MATERIALS AND METHODS

Materials

Cocoa powder and cocoa mass were procured commercially from a local department store in Vellore as per the required measurements. Sugar was powdered to a fine reduced form *via* an electric grinder. Cod-liver oil was obtained from a local pharmacy store.

Chocolate Formulation

The above mentioned ingredients were mixed in a concoction in the manufacturing of dark chocolate with cod-liver oil as the cocoa butter replacer following the method described by Melo *et al*. [8] with few modifications with respect to the amount of cod liver oil added. The samples had cocoa butter replaced by incorporation of cod liver oil at levels of 0%, 5%, 10%, 15% and 20% and the ingredient quantities were devised according to the measures described by Beckett [1]. The chocolate samples were formulated by the conventional tempering process of melting the chocolate concoction at 45°C until a glossy appearance was attained. It was then cooled at 28°C for an hour followed by re-heating at 33°C to complete the tempering process [1].

Moulding and Freezing

The prepared chocolate samples were poured into moulds by varying the shape of each chocolate to differentiate between the various percentages of replaced cocoa butter samples. The thickness of each chocolate sample was approximately 3 cm. A set of samples were then stored in an environmental chamber

(Remi programmable chamber-396LAG, India) for a period of 15 days at 50 °C and at 65-70% relative humidity to equilibrate the chocolate for fat bloom and shelf life analysis while another set was subject to freezing for chocolate polymorphism studies.

Fat Bloom Analysis

The samples stored in the environmental chamber were subject to visual scrutiny for a period of 15 days at intervals of 5 days. The samples were checked on the 0^{th}, 5^{th}, 10^{th} and 15^{th} day to check for the presence of a cream/ off-white haze on the surface of the chocolate, a phenomenon commonly referred to as fat bloom [9]. The samples were also observed to visualise the colour, smoothness, texture and uprising of oil from the depths of the chocolate to the surface, which would further be determined by SEM analysis, for their comparison during the 15 day observation period.

Shelf-life analysis

Extracts of the crude chocolate sample was formulated and cultured in nutrient agar by means of spread plate method. The plates were incubated for a period of 24 hours at 37°C. Studies on microbial contamination during a period of 15 days at an interval of 5 days was performed by observation of microbial colonies for the treated chocolate samples [10].

Oil Migration

Scanning electron microscope (ZEISS-EV018, USA) was used to visualise the amount of liquid oil migration in the fat filled chocolate sample through a period of 15 days [11]. The samples included the chocolates containing 5%, 10%, 15% and 20% of cod liver oil concentrations. Each sample was analysed at varying magnifications and angles to the plane of the instrument to get a wider perspective on the migration of oil from the chocolate depth to the surface.

Chocolate Polymorphism

X-ray diffraction (XRD) was performed to determine the crystallization and phase transitions of the various polymorphic forms of the chocolate samples. The set of samples which were frozen were taken and these chocolate bars were investigated using XRD analysis by means of X-ray powder diffractometer (Bruker D8 Advance, Germany) which inculcates the use of Cukα radiation of wavelength 1.54A° [12].

Vitamin A and D3 analysis

Reversed-phase high-performance liquid chromatography (RP-HPLC) (Waters 1525, US) was used for quantification of vitamin A and D3 present in the cod liver oil containing chocolate samples. Column temperature was held constant at room temperature. 20 µl of sample was injected through column (C18, 250 mm × 4.6 mm × 5 µm) at a flow rate of 1.5 ml min^{-1} mobile phase. Methanol was used as the mobile phase. The results were monitored at 265 nm for simultaneous determination of vitamin A and D3 [13].

RESULTS AND DISCUSSION

Fat bloom visualization

As fat bloom formation is a critical factor in estimating the shelf life of any chocolate [14], it was visualized in the prepared chocolate samples in which the fat was replaced at 5%, 10%, 15% & 20% levels and subsequently stored for 0, 5, 10, 15 days were shown in Fig. 1. respectively. The fat bloom formation was not observed in any of the samples and this showed that the levels of fat replacer *viz*., cod liver oil added during the preparation of the chocolates was appropriate, which in turn reciprocates better shelf life of the prepared chocolate.

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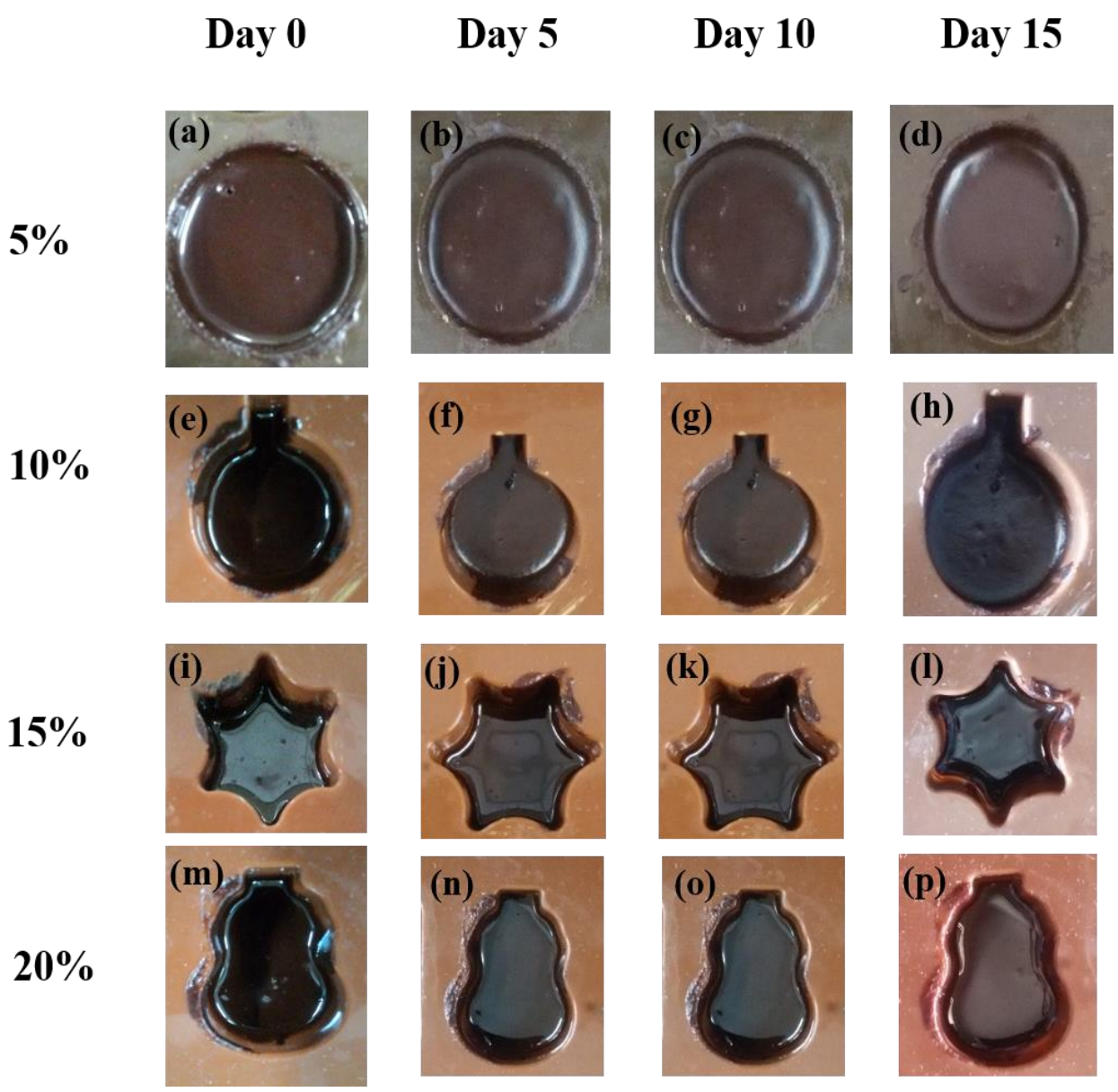

Fig. 1. Images for visualization of fat bloom in (a) Day-0, 5% fat replaced chocolate (b) Day-5, 5% fat replaced chocolate (c) Day-10, 5% fat replaced chocolate (d) Day-15, 5% fat replaced chocolate (e) Day-0, 10% fat replaced chocolate (f) Day-5, 10% fat replaced chocolate (g) Day-10, 10% fat replaced chocolate (h) Day-15, 10% fat replaced chocolate (i) Day-0, 15% fat replaced chocolate (j) Day-5, 15% fat replaced chocolate (k) Day-10, 15% fat replaced chocolate (l) Day-15, 15% fat replaced chocolate (m) Day-0, 20% fat replaced chocolate (n) Day-5, 20% fat replaced chocolate (o) Day-10, 20% fat replaced chocolate (p) Day-15, 20% fat replaced chocolate.

Microbial shelf life analysis

All the samples in which the fat bloom formation was analysed, were subject to microbial test [10]. The agar plates on the 0^{th}, 5^{th}, 10^{th} and 15^{th} day of various fat replaced chocolate samples are shown in Fig. 2. With an increase in percentage of the cod liver oil (fat replacer) in the prepared chocolates, it was observed that till 15% cocoa butter replacement showed lesser microbial colonies by the end of 5^{th} day. This proved that till 15% of cod liver oil can be used as a cocoa butter replacer in the chocolate with prolonged shelf life.

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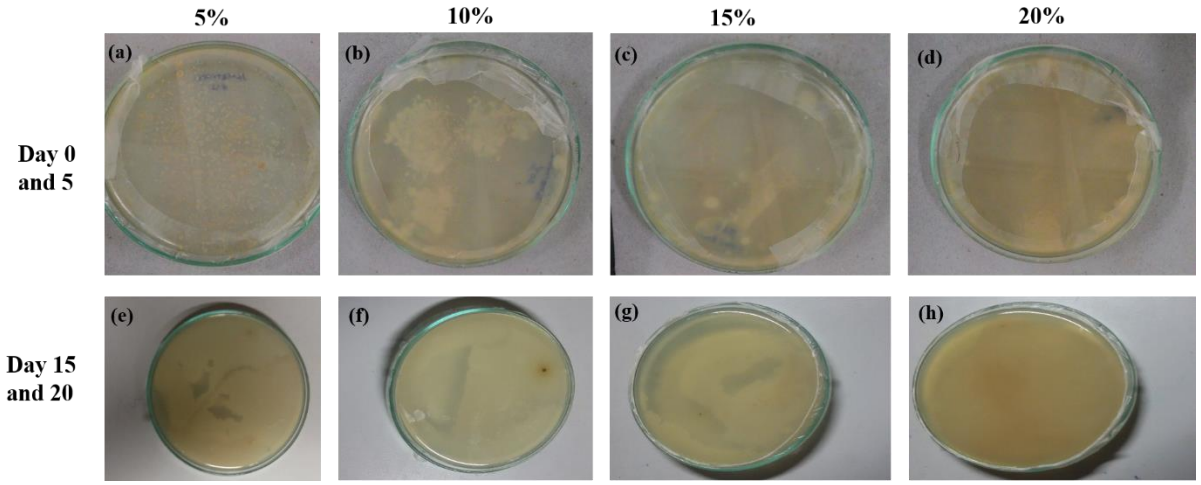

Fig. 2. (a) Day-0&5, 5% fat replaced chocolate (b) Day-0&5, 10% fat replaced chocolate (c) Day-0&5, 15% fat replaced chocolate (d) Day-0&5, 20% fat replaced chocolate (e) Day-15&20, 5% fat replaced chocolate (f) Day-15&20, 10% fat replaced chocolate (g) Day-15&20, 15% fat replaced chocolate (h) Day-15&20, 20% fat replaced chocolate.

Oil migration studies

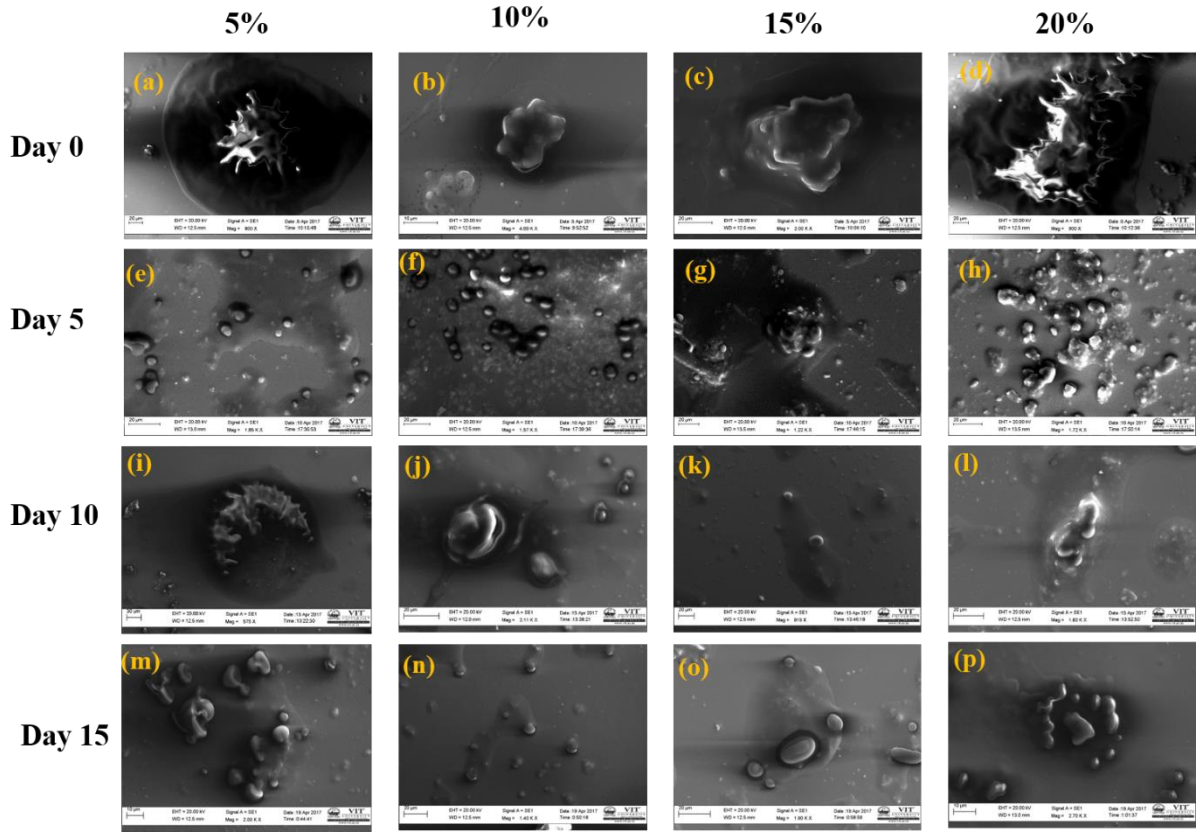

Fig. 3. SEM images of: (a) Day-0, 5% fat replaced chocolate (b) Day-0, 10% fat replaced chocolate (c) Day-0, 15% fat replaced chocolate (d) Day-0, 20% fat replaced chocolate (e) Day-5, 5% fat replaced chocolate (f) Day-5, 10% fat replaced chocolate (g) Day-5, 15% fat replaced chocolate (h) Day-5, 20% fat replaced chocolate (i) Day-10, 5% fat replaced chocolate (j) Day-10, 10% fat replaced chocolate (k) Day-10, 15% fat replaced chocolate (l) Day-10, 20% fat replaced chocolate (m) Day-15, 5% fat replaced chocolate (n) Day-15, 10% fat replaced chocolate (o) Day-15, 15% fat replaced chocolate (p) Day-15, 20% fat replaced chocolate.

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The oil migration was examined using SEM [15], in the various percentages of fat replaced chocolate samples at 0th, 5th, 10th &15th days of storage. According to the SEM micrographs (Fig 3), the oil was noticed as the black patch surrounding the chocolate particles. The area of the oil occupied around the chocolate particles was seen to gradually increase with the increase in percentage of cod liver oil and also with increase in the number of the days of storage. Oil migration follows two mechanisms *viz*., diffusion and capillary flow. If the migration of the oil is quicker, then the developing rate of fat bloom is also increased [15]. Hence, oil migration is a key factor to depict the shelf life of prepared fat replaced chocolate.

Chocolate Polymorphism

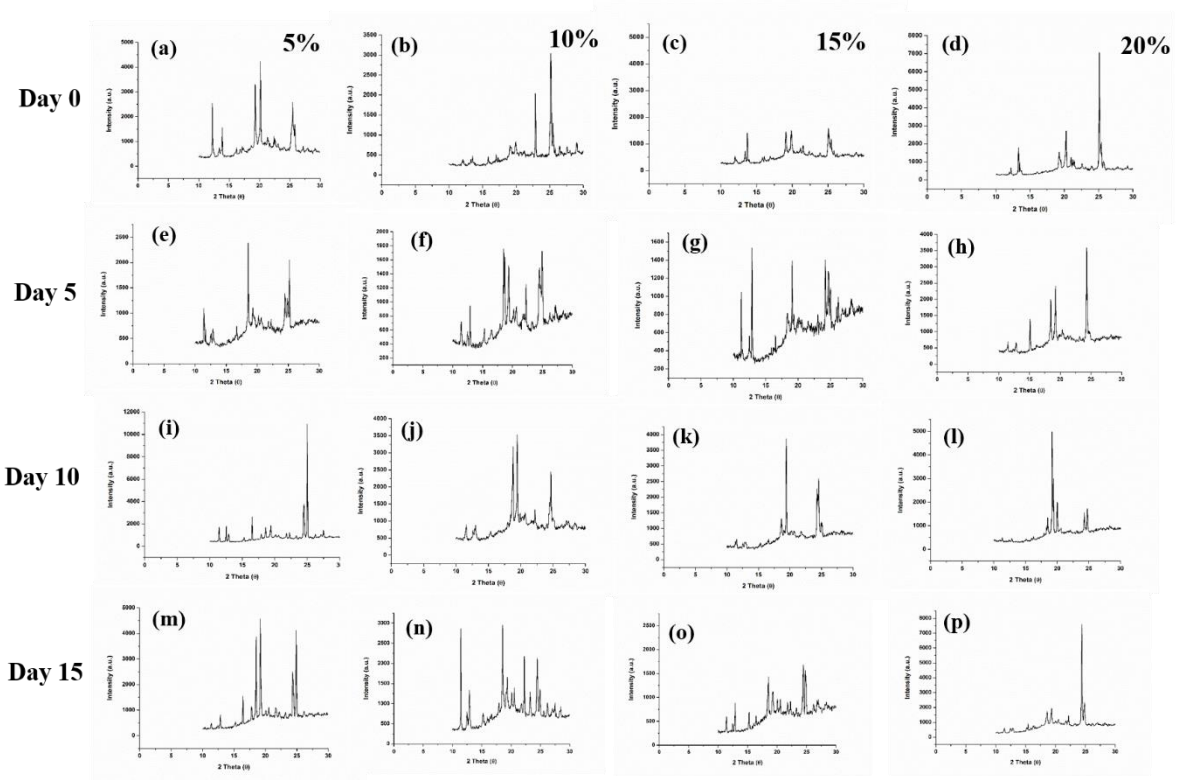

Fig. 4. X-Ray Diffractogram of: (a) Day-0, 5% fat replaced chocolate (b) Day-0, 10% fat replaced chocolate (c) Day-0, 15% fat replaced chocolate (d) Day-0, 20% fat replaced chocolate (e) Day-5, 5% fat replaced chocolate (f) Day-5, 10% fat replaced chocolate (g) Day-5, 15% fat replaced chocolate (h) Day-5, 20% fat replaced chocolate (i) Day-10, 5% fat replaced chocolate (j) Day-10, 10% fat replaced chocolate (k) Day-10, 15% fat replaced chocolate (l) Day-10, 20% fat replaced chocolate (m) Day-15, 5% fat replaced chocolate (n) Day-15, 10% fat replaced chocolate (o) Day-15, 15% fat replaced chocolate (p) Day-15, 20% fat replaced chocolate.

Typical XRD diffractograms of chocolate samples in which the fat was replaced at various levels *viz*., 5%, 10%, 15% and 20% and subsequently stored for 0, 5, 10, 15 days were shown in Fig. 4. respectively. By employing Bragg's Equation, the corresponding d-spacing values were calculated for experimentally obtained 2θ values;

$$d = n\lambda/2\sin(\theta)$$

where λ is the wavelength of the source, d is the d-spacing and h is the angle between the incident and the diffracted wave. The d-spacing value for the prominently visible highest peaks were figured out as 4.18 Å, 4.20 Å, 4.20 Å, 4.13/4.32 Å, 4.58 Å, 4.59 Å which is in compliance with the expected standard value for Form I (γ), Form II (α), Form III (β'), Form IV (β'), Form V (β), Form VI (β) peaks, stated by Benjamin [4]. Hence, the fat replaced chocolates obtained crystal homogeneity as the concentration of the cod liver oil increased as shown in Fig. 4. It was also observed that there existed a notable disappearance of desirable V (β) polymorph form

peak, not only in the higher level fat replaced chocolates but also as days of storage of chocolate was prolonged.

Determination of vitamin A and D3

Vitamin A and D3 was detected at 265nm with retention time 4 and 7 min. The results show that the percentage of cod liver oil present in the chocolate sample was directly proportional to the percentage of vitamin A and D3 present. Table. 1. shows the percentage of vitamin A and D3 present in the chocolate samples calculated from the rp-hplc chromatograms.

Table 1: Estimation of vitamin A and D3 percentage in the dark chocolates.

S. No	Cocoa butter replaced (%)	Vitamin A (%)	Vitamin D3 (%)
1	5	1.56	0.02
2	10	1.64	0.026
3	15	1.94	0.03
4	20	1.96	0.06

CONCLUSION

Our study has unveiled a novel strategy to retain the nutritive value of chocolate. Based on the study, the manufacture of dark chocolate by replacing the cocoa butter with cod liver oil at varying percentages was successful. Multiple analysis of the chocolate depicted desirable shelf life characteristics and reduced microbial invasion. XRD analysis revealed homogenous crystal polymorphism. Microbial spread plate method showed extended shelf life in the chocolate sample in spite of revealing increased oil migration in high concentrated fat replaced chocolate in successive days using SEM. In the present day scenario, cod liver oil has been acknowledged to possess superior characteristics having added health benefits and is usually consumed in the form of capsules. This unique strategy of incorporating the oil into chocolate can be seen as a way to mask the unpleasant taste and odour of the oil, thus increasing consumer acceptability and marketability of the chocolate, particularly among infants who require more amounts of vitamin A and D3 in their growing stage.

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