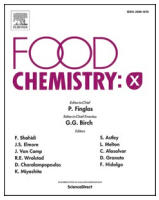
Food Chemistry: X 17 (2023) 100583

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



# Fortification of chocolate with microencapsulated fish oil: Effect of protein wall material on physicochemical properties of microcapsules and chocolate matrix

Miroslav Hadnađev [a,*], Marina Kalić [b], Veljko Krstonošić [c], Nataša Jovanović-Lješković [b], Tamara Erceg [d], Dubravka Škrobot [a], Tamara Dapčević-Hadnađev [a]

[a] University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[b] University Business Academy Novi Sad, Faculty of Pharmacy, Trg Mladenaca 5, 21000 Novi Sad, Serbia
[c] University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Hajduk Veljkova 3, 21000 Novi Sad, Serbia
[d] University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

ARTICLE INFO

*Keywords:*
Chocolate
Microencapsulation
Fish oil
Proteins
Texture
Rheology

ABSTRACT

Protein stabilized fish oil microcapsules were incorporated into chocolates in order to design fortified product which could bear the nutritional claim "source of or high omega-3 fatty acids". Protein wall material (soy, whey and potato) influenced microcapsules and chocolate performance. Soy protein resulted in the smallest microcapsules with the lowest content of surface oil. Peroxide values were low even after 14 days of microcapsules storage. Incorporation of microcapsules into chocolate led to increase in Casson viscosity and breaking force as well as decrease in melting enthalpy, due to prevalence of particle–particle over fat-fat interactions. Increase in microcapsules concentration resulted in chocolate with poorer snap and higher tendency to fat bloom formation. Whey protein microcapsules, having the largest diameter, resulted in chocolate with the lowest breaking force and melting enthalpy and the highest whitening index. In general, microcapsules addition did not require chocolate production modification and led to sensory acceptable product.

## 1. Introduction

Fish and aquatic invertebrates are rare and significant sources of long chain omega-3 polyunsaturated fatty acids such as docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3). Due to DHA and EPA ability to exert a positive influence on human health, European Commission has authorized the health claims related to normal function of the heart and maintenance of normal blood triglyceride levels and blood pressure for food providing daily intake of 250 mg to 3 g of EPA and DHA (EU, 2012). However, fish oil, together with omega-3 fatty acids isolated from it, is susceptible to oxidation which reduces its palatability and safety (Davidov-Pardo, Roccia, Salgado, León, & Pedroza-Islas, 2008).

In order to protect fish oil against lipid oxidation microencapsulation technology has been successfully applied (Barrow, Nolan, & Holub, 2009). One of the most widely used techniques in microencapsulation is spray-drying, due to its simplicity, continuous operation, low cost and possibility to be easily scaled-up compared to other techniques (Bakry et al., 2016). Moreover, it has been shown that the bioavailability of omega-3 fatty acids from microencapsulated fish oil as delivered by spray-dried emulsions is high and equivalent to the ones delivered as soft-gel capsules (Barrow et al., 2009). Stability of microcapsules and bioavailability of encapsulated material depend greatly on microcapsules' wall material. Various polymers are being used as a wall material for production of fish oil microcapsules by spray drying, including combination of maltodextrin, sodium caseinate and whey protein (Ramakrishnan et al., 2014), sodium caseinate/carbohydrates of different DE (Hogan, O'Riordan, & O'Sullivan, 2003), different milk-originated wall materials (Aghbashlo, Mobli, Madadlou, & Rafiee, 2012), etc. Recently, there has been increased interest in using proteins as encapsulating agents due to their amphiphilic and gelling properties, and susceptibility to enzyme degradation in gastrointestinal tract (Wang, Tian, & Chen, 2011). The major concern for using proteins over previously reported biopolymers as a wall material is due to their poorer solubility in water and higher viscosities at higher concentration. Proteins that have been proved to be suitable for encapsulation process

* Corresponding author.
*E-mail address:* miroslav.hadnadjev@fins.uns.ac.rs (M. Hadnađev).

https://doi.org/10.1016/j.fochx.2023.100583
Received 3 October 2022; Received in revised form 16 January 2023; Accepted 19 January 2023
Available online 23 January 2023

could be derived from animal sources such as whey proteins, casein, gelatin, etc. and also from plant sources (soybean proteins, cereal proteins, pea proteins, tuber proteins, etc.). Whey proteins exhibit very good emulsifying and dehydration properties and therefore were successfully utilized as wall material in microencapsulation processes of different bioactive compounds (Ramakrishnan et al., 2014). Recently, there is an increasing trend of using plant proteins in microencapsulation due to so called "green" approach in different industries, vegan nutrition, safety aspects of animal-derived products, less allergenic effect of plant derived proteins in comparison to animal, etc. Soybean proteins have been shown to be suitable for microencapsulation process due to their solubility, emulsifying ability, good film-forming, gelation and water and fat absorption properties (Nesterenko, Alric, Silvestre, & Durrieu, 2013). Potato derived proteins are generally considered as nonconventional protein source usually obtained in starch processing industry as by-product. Although potato protein application in microencapsulation is limited, it could be promising due to its high nutritive value and good functional properties (Wang, Chang, Zhang, Ma, Chen, & Li, 2020).

Fish oil microcapsules have been successfully incorporated into ice cream (Nawas, Yousuf, Azam, Ramadhan, Xu, & Xia, 2017), bread (Davidov-Pardo et al., 2008), low-fat mayonnaise (Rahmani-Manglano, González-Sánchez, García-Moreno, Espejo-Carpio, Jacobsen, & Guadix, 2020) without significantly affecting their physiochemical and/or sensory properties. To the best of our knowledge, there is no comprehensive study following the whole process from the development of protein-stabilized fish oil microcapsules to their incorporation into dark chocolate.

Over the past few decades, chocolate has become one of the most popular foods across the globe with Europe accounting for 45 % of the global consumption (approximately 9 kg/year/person in Western European countries) (Afoakwa, 2016; Beckett 2008). Considering its high consumption, it seemed as a reasonable option to fortify chocolate with different bioactive compounds as a delivering agent. Previous studies showed that fortifying chocolate with high oleic peanut oil, phytosterols, cinnamon bark oleoresin, chia seed oil microcapsules can provide a stable and acceptable final product (Agibert & Lannes, 2018; Tolve, Condelli, Caruso, Barletta, Favati & Galgano, 2018; Praseptiangga, Invicta, & Khasanah, 2019; Razavizadeh & Tabrizi, 2021). Moreover, the knowledge gained from the study of Toker et al. (2018) showed that incorporation of encapsulated forms of EPA/DHA (commercial microencapsulated powder based on fish gelatin) in dark chocolate represents the best option in terms of product quality compared to other forms (free-flowing powder, nutritional oil derived from algae, triglycerides).

Therefore, the aim of this study was to investigate the efficiency of fish oil microencapsulation, using different proteins (soy, whey and potato) as wall material, as well as the morphology and stability of obtained microcapsules. Moreover, prepared microcapsules were used to fortify dark chocolate in an amount to carry the nutritional claim "source of omega-3 fatty acids" as well as "high omega-3 fatty acids", according to Commission Regulation (EU) No 432/2012 (EU, 2012), i.e. 40 mg and 80 mg of the sum of eicosapentaenoic acid and docosahexaenoic acid per 100 g and per 100 kcal of chocolate. Effect of fish oil microcapsules with different proteins as wall materials on quality properties of the chocolate (rheological properties, melting behaviour, texture analysis, colorimetric indices, liking study) was also evaluated.

## 2. Materials and methods

### 2.1. Materials

Fish oil containing high content of omega-3 fatty acids (65 % of ω-3 fatty acids of which 33 % of eicosapentaenoic acid (EPA) and 22 % docosahexaenoic acid (DHA)) was provided from Pharmatech AS (Rolvsø, Norway). Three different proteins were used as a coating material in microcapsule preparation: soy protein isolate (82.77 % protein and 5.06 % moisture content) (Soypro 950IJ, Qingdao CPI Enterprise Co., ltd., Qingdao, China), whey protein concentrate (74.82 % protein and 5.21 % moisture content) (Olimp Laboratories Sp.z.o.o., Debica, Poland) and potato protein isolate (83.11 % protein and 5.79 % moisture content) (Solanic® 200, Avebe, Veendam, The Netherlands). Dark chocolate couverture "Donau" 60/40/38 (Kondima, Engelhardt GmbH & Co KG, Germany, 60 % cocoa solids, 40 % sugar, 38 % total fat content, emulsifier: sunflower lecithin) was donated from Eugen Chocolate (Glożan, Republic of Serbia). Other chemicals used in this study were p. a. grade and all solutions were prepared using demineralized water.

### 2.2. Preparation of microencapsulated fish oil powder

Fish oil microcapsules were prepared using emulsification as a prestep to spray drying. Briefly, wall material solutions (4 % (w/w) protein aqueous solutions, calculated on dry matter) were prepared one day before emulsification and stirred overnight to allow full hydration. Core material, consisted of fish oil and soy lecithin in ratio of 1:0.134, was added to protein wall material solution in order to maintain the core to wall ratio of 1.134:4. Subsequently, 1 % fish oil-in-water emulsions were prepared using a rotor–stator homogenizer IKA T25 ULTRA-TURRAX® (IKA®-Werke GmbH & Co. KG, Germany) at 10,000 rpm for 10 min in an ultrasonic bath (ATU, Model ATM40-3LCD, Barcelona, Spain) operating at ultrasonic power of 100 W (Krstonošić, Kalić, Dapčević-Hadnađev, Lončarević, & Hadnađev, 2020). Subsequently, the microcapsules were prepared using a Nano Spray Dryer B-90 in an open loop mode with air as drying gas (Büchi Labortechnik AG, Flawil, Switzerland) based on preliminary trials at following operating conditions: inlet temperature 110 °C, outlet temperature 45 °C, the drying gas flow rate = 110 L/min, relative spray rate of 100 % and the 7.0 μm spray nozzle.

### 2.3. Microcapsule Characterization

#### 2.3.1. Microencapsulation yield and efficiency

The yield of microencapsulation was determined as the ratio of the mass of microcapsules collected at the end of the spray drying process to the mass of the wall and the core of the microcapsules (protein and fish oil).

In order to determine the efficiency of microencapsulation, i.e., the amount of encapsulated oil, the method described by Tonon, Grosso, & Hubinger (2011) has been employed, with certain modifications. A sample containing 1 g of microcapsules was placed in a test tube and vortexed with 10 mL of petroleum ether for 2 min at room temperature. The resulting solution was filtered through quantitative filter paper (CHMLAB, grade F2042), and the powder remaining on the filter paper was washed three more times with 10 mL of petroleum ether and each time filtered. In the end, all the filtrates were collected and combined (Jafari, Assadpoor, Bhandari, & He, 2008).

The filtrate was then transferred to a clean evaporation bottle (mass previously measured), and the sample was evaporated at 60 °C (until constant weight). The mass of oil was obtained from the difference between a bottle with surface oil after evaporation and a clean bottle (Jafari et al., 2008).

Microencapsulation efficiency was calculated using Eq. (1):

$$Microencapsulation\ efficiency = \frac{T_o - S_o}{T_o} \times 100 \quad (1)$$

where $T_O$ represents total mass of oil used for microencapsulation and $S_O$ is the surface oil determined using the previously explained procedure.

#### 2.3.2. Scanning electron microscopy (SEM)

Characterization of microcapsule morphology as well as their particle size was carried out using JEOL JSM 6460LV scanning electron microscope (Tokyo, Japan). The samples of microcapsule powder were previously mounted on scanning electron microscope stubs using

double-sided adhesive tape and afterwards coated with gold using Sputter Coater SC 005 (Bal-Tec GmbH, Schalksmühle, Germany). Subsequently, they were analysed under high vacuum conditions at an accelerating voltage of 25 kV and at magnification of ×5,000 and ×50,000.

The size of the microcapsules and wall thickness was determined from SEM images with the use of the ImageJ software (National Institutes Health, Bethesda, MD).

#### 2.3.3. Microcapsules oxidative stability

The oxidative stability was obtained by determining the peroxide value of the oil, as described by Tonon et al. (2011), including certain modifications. In order to extract the oil from the microcapsules (Partanen, Raula, Seppänen, Buchert, Kauppinen, & Forssell, 2008), 0.5 g of the microcapsule sample was suspended in a test tube in 5 mL of distilled water and shaken in a vortex until completely dispersed (about 2 min). Then 900 μL of the solution was mixed in a test tube with 4.5 mL of cyclohexane:isopropanol (2:1). Phase separation was achieved by centrifugation for 4 min at 1000*g* and the top layer was taken for analysis.

The determination of the peroxide value of the oil was performed spectrophotometrically (Kellerby, McClements, & Decker, 2006; Partanen et al., 2008). A 600 μL sample volume was mixed with 2.8 mL of chloroform:methanol (7:3). To develop the colour, 30 μL of ammonium thiocyanate / iron (II) chloride solution (Kellerby et al., 2006) was added, the sample was briefly vortexed, left to react for 20 min in the dark, and the absorbance was measured at 500 nm.

A solution of iron(III)-chloride in a mixture of chloroform:methanol (7:3) was used to make the calibration curve, diluted so that there is a linear dependence of the absorbance on the concentration of iron(III)-chloride.

The peroxide value of the oil (expressed in milliequivalents of peroxide per kilogram of sample) was calculated according to the Eq. (2) (Shantha & Decker, 1994):

$$Peroxide\ value = \frac{(A_s - A_b) \times m}{55.84 \times m_0 \times 2} \quad (2)$$

where $A_s$ is the absorbance of the sample, $A_b$ is the absorbance of the blank, m is the slope of the curve (in this case, m is 0.02), $m_0$ is the mass of the sample in grams, and 55.84 is the atomic mass of iron. Dividing by 2 the results are expressed in milliequivalents of peroxide instead of milliequivalents of oxygen.

### 2.4. Preparation of chocolate bars

Fish oil microcapsules were added into previously melted (at 50 °C) dark chocolate couverture at the amount of 1.97 % and 3.94 %, which corresponded to a sum of 40 mg of EPA and DHA per 100 kcal as well as a sum of 80 mg of EPA and DHA per 100 kcal of chocolate sample. Microcapsules were incorporated into melted dark chocolate couverture in previously heated Farinograph mixing bowl (Brabender, Germany) set to a speed of 90 rpm for 30 min which was previously determined as a time needed to observe uniform particle distribution. The mixture was then manually tempered on a cold marble surface, moulded into 7.5 cm length, 2.5 cm width and 0.6 cm height chocolate bars and cooled at 4 °C for 90 min to ensure complete solidification. Cooled samples were demoulded, packed in aluminium foil and stored at 20 °C. Control sample was prepared using the same conditions, but without microcapsule addition. Each sample had a constant mass of 15 g. Colour parameters and melting behaviour was determined in chocolate samples stored for 120 days, while rheological and textural measurements were performed after 48 h of storage.

### 2.5. Physiochemical analysis of chocolate

#### 2.5.1. Rheological properties

Rheological measurements were performed using a Haake MARS rheometer (Thermo Scientific, Karlsruhe, Germany) equipped with a cylinder Z20 DIN at 40 ± 0.1 °C.

Hysteresis loop tests were carried out according to IOCCC method (IOCCC, 2000) by increasing the shear rate from 2 to 50 $s^{-1}$ within 180 s, then it was kept constant at a maximum shear rate of 50 $s^{-1}$ during 60 s, and finally decreased linearly from 50 to 2 $s^{-1}$ for 180 s. The obtained experimental data were fitted by the Casson's (Eq. (3)) model (Lohman & Hartel, 1994):

$$\sqrt{\tau} = \sqrt{\tau_{CA}} + \sqrt{\eta_{CA}\dot{\gamma}} \quad (3)$$

where τ is the shear stress (Pa), $\tau_{CA}$ – Casson yield stress value, $\eta_{CA}$ - Casson plastic viscosity and $\dot{\gamma}$ – shear rate.

In-shear structural recovery measurements were performed according to Achayuthakan and Suphantharika (2008). Briefly, the sample was subjected to three stepped shear flow test under the following conditions: a constant shear rate of 1 $s^{-1}$ was applied for 120 s, followed by a shear rate of 300 $s^{-1}$ during 60 s and repeated shear rate of 1 $s^{-1}$ for 180 s. The in-shear recovery value was calculated as the ratio of average apparent viscosity obtained during the first 120 s of the third step to the average apparent viscosity value determined in the first step.

#### 2.5.2. Melting properties

Melting behaviour of stored (14 days) chocolate samples was investigated by differential scanning calorimetery (TA Q20, TA Instruments, New Castle, USA). Approximately 6 mg of each sample was placed in aluminium pan and sealed. Samples were heated under a nitrogen atmosphere, in temperature range from 5 to 60 °C at heating rate of 5 °C/min in one cycle. Indium was used as a calibration reference. Onset ($T_{onset}$), peak ($T_{peak}$), end ($T_{end}$) temperature as well as energy required for the complete melting ($\Delta H_{melt}$) were determined from corresponded thermograms.

#### 2.5.3. Colour measurement

The effect of chocolate fortification with fish oil microcapsules on colour and appearance was determined by Minolta Chroma Meter CR-400 colorimeter (Konica Minolta Sensing Inc., Osaka, Japan) (8 mm Ø contact area). Prior the measurement, the instrument was calibrated using a standard light white reference tile and the measurements were performed under standard illuminant D65. The obtained results were presented according to the CIELab colour system and they were expressed in terms of $L^*$ (lightness/brightness), $a^*$ (greenness to redness) and $b^*$ (blueness to yellowness). The whiteness index value, that according to Lohman & Hartel (1994) combines lightness and yellow-blue into a single term, was determined by the Eq. (4):

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (4)$$

In order to determine whether there were differences between the tested samples, results were also expressed as the total colour difference (ΔE) using Eq. (5):

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (5)$$

where $\Delta L$ is the lightness difference, $\Delta a$ is the redness difference and $\Delta b$ is the yellowness difference values.

If $\Delta E < 1$, colour differences are not obvious to the human eye; $1 < \Delta E < 3$, colour differences cannot be appreciated by the human eye; and $\Delta E > 3$, colour differences are obvious to the human eye (Francis & Clydesdale 1975). Colour measurements were conducted in 6 replicates per batch.

#### 2.5.4. Texture analysis

Breaking force and brittleness of the chocolate bars (7.5 cm length, 2.5 cm width and 0.6 cm height) were evaluated using a TA.XTPlus Texture analyser (Stable Micro Systems, Godalming, UK) using a 3-Point Bending Rig (HDP/3 PB) and 5 kg load cell in a compression mode at 2 mm/s test speed and a gap distance of the base plate of 55 mm. The maximum force in N required for breaking a chocolate sample referred to as sample breaking force, whereas, distance (displacement) at which chocolate breaks referred to brittleness. Textural measurements were conducted in 6 replicates per batch at 20 °C 14 days after chocolate preparation.

#### 2.5.5. Liking study

A total of 75 consumers (31 men and 44 women, aged 23 – 54 years), regular chocolate consumers (at least 2 days per week) were included in the liking study. All participants reported that they are not on a special diet or intolerant to any ingredient in chocolate sample, including fish oil. Samples were delivered as chocolate bars (7.5 × 2.5 × 0.6 cm) wrapped in aluminum foil for packaging and marked with three-digit numbers. Participants were instructed to rinse their mouth with water between samples evaluation. They evaluated overall liking, appearance liking, odour liking, taste and aroma liking and texture liking for each sample on 9-point hedonic scales (1 = I don't like it at all, 9 = I like it very much).

The study was conducted according to Helsinki Ethical Guidelines and adapted for food sensory analysis at the Institute of Food Technology in Novi Sad, University of Novi Sad, Serbia. The study was approved by the Ethics Committee of the Institute of Food Technology in Novi Sad, University of Novi Sad, Serbia (Ref. No. 175/I/15-3).

### 2.6. Statistical analysis

Data obtained in this study were expressed as means ± standard deviations of replicate analyses. A one-way analysis of variance (ANOVA) with Tukey's test was performed using Statistica 10.0 (StatSoft Inc., Tulsa, OK, USA). The significance of differences among the mean values was indicated at the 95 % confidence level.

## 3. Results and discussion

### 3.1. Microcapsules appearance, size and surface and inner morphology

The microstructures of protein stabilized microcapsules observed by SEM are presented in Fig. 1 with magnification from 5000 to 50,000 times. The diameter of the microcapsules as well as the wall thickness was analysed by direct measurement from the SEM images of >50 microcapsules. In general, spray-drying process results in particle sizes between 1 μm and 50 μm (Nesterenko et al., 2013). Since during the emulsification process drops of different sizes were formed (Krstonošić et al., 2020) it resulted in high polydispersity of microcapsules obtained in this study. In all samples, the diameter of the most of the microcapsules ranged from 1.0 to 8.0 μm, while the smallest particles were 100–700 nm. This is in agreement with the size of fish oil microcapsule stabilized with barley protein (1 to 5 μm) and typical for microcapsules intended for food applications (Wang et al., 2011). The fish oil microcapsules, produced in this study, were generally spherical in shape with a few dents along the surface. Microcapsules produced with potato protein showed smooth structure, while whey protein capsules were characterized with the presence of surface dents and higher ratio of larger particles. Soy protein microcapsules had high ratio of small particles with a few cracked capsules. While potato and whey protein produced microcapsules of 212–314 nm wall thickness, these values were higher (257– 364 nm) for soy protein capsules (Fig. 1, data not shown for potato protein wall material). As expected, the properties of wall building material used during the production of microcapsules and emulsion characteristics influenced the thickness of the microcapsules wall and appearance of the microcapsules. Production of somewhat smaller microcapsules with thick walls when using soy protein may be related to the smaller droplet size of fed emulsion and high molecular weight and solution viscosity of soy protein (Krstonošić et al., 2020). However, lower viscosity of potato stabilized emulsion (Krstonošić et al., 2020) increased internal circulation of sprayed droplets and resulted in reduced size of finished microcapsule (Aghbashlo et al., 2012) and their smooth surface.

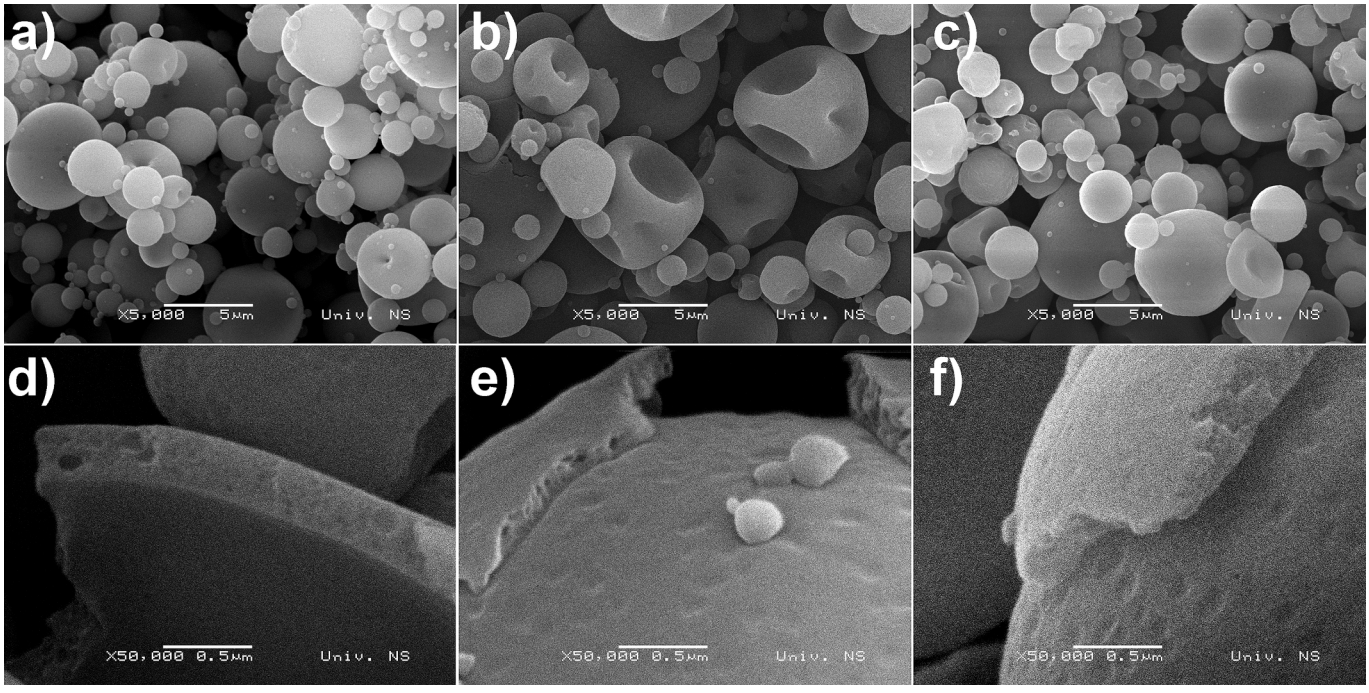



**Fig. 1.** The microstructures of potato (a), whey (b) and soy (c) protein stabilized fish oil microcapsules at magnification of × 5,000 and the morphology of the microcapsule wall prepared using potato (d), whey (e) and soy (f) protein observed by SEM at magnification of 50,000.

### 3.2. Microencapsulation yield and efficiency

Microcapsulation yield along with surface oil content and the amount of encapsulated oil (encapsulation efficiency) are presented in Table 1. Whey protein stabilized microcapsules demonstrated slightly higher yield compared to other protein microcapsules probably due to low consistency index of whey protein stabilized fish oil-in-water emulsions (Krstonošić et al., 2020). Encapsulation efficiency, an important quality attribute for microcapsules storage, varied from a minimum value of 68 % to a maximum value of 73 %. In the study performed by Davidov-Pardo et al. (2008) encapsulation efficiency of soybean protein isolate was 65 %, while whey protein concentrate utilization resulted in 87.2 % fish oil enclosing.

As reported by other authors (Jafari et al., 2008), fine and monodisperse emulsions have been found to produce microcapsules with lower surface oil content, and consequently high oil encapsulation efficiency. Therefore, soy protein emulsions which were characterized with the smallest and the most uniform-sized droplets compared to other emulsions (Krstonošić et al., 2020) resulted in microcapsules with the lowest content of surface oil. Moreover, low solubility of soy protein (Krstonošić et al., 2020) also contributed to low diffusivity of microcapsules wall material (Kim, Chen, & Pearce, 2003)). Although it would be expected that dense, smooth surface of potato protein microcapsules together with the small emulsion droplet size will result in potato protein ability to encapsulate higher content of oil, potato stabilized microcapsules demonstrated lower encapsulation efficiency than whey protein microcapsules. Likely petroleum ether, that was used to extract surface oil, extracted both surface and encapsulated oil in the case of potato protein wall material, since this protein had the greatest surface hydrophobicity (Krstonošić et al., 2020).

### 3.3. Oxidative stability of the microcapsules during storage

The oxidative stability of encapsulated fish oil is presented as the change in the peroxide value of the sample during 14 days of storage at 8 °C (Table 1). Short stability study was selected due to previous investigations which have shown that most of the oxidative changes in spray dried oil happen within the first two or three weeks since the spray drying. This is due to the fact that even though the number of peroxides formed increases after the first two weeks of storage, it also decreases over time as a result of simultaneous decomposing in oxidation process. This leads to the same results in terms of oxidative stability after 9 or 12 weeks of storage as the ones after two weeks (Partanen et al, 2008). In addition, microcapsules produced in this study were incorporated in chocolate matrix which is considered shelf-stable food, mainly due to its low unsaturated fatty acid content and high content of polyphenols (Afoakwa, 2016), which protect the product from oxidation. Therefore, besides protein wall material, chocolate matrix provided additional protection from fish oil oxidation.

In this study, the obtained peroxide values ranged from 0.8826 to 0.9892 meq peroxide/kg oil 24 h after the production. During storage, peroxide values showed a permanent but non-significant increase regardless of the nature of the protein wall material.

As can be seen from Table 1, the microcapsules produced using soy protein were less oxidized. This was expected since previous studies showed a positive correlation between surface oil content and peroxide values due to the direct contact of exposed oil with the oxygen (Tonon et al., 2011). However, oxidative stability of protein coated fish oil cannot be explained just on the basis of extractable oil; being consistent with work of Hogan et al. (2003). In spite of being characterized with higher content of surface oil compared to soy protein (Table 1), potato protein stabilized capsules exhibited non-significantly higher peroxide values. There are several factors that could contribute to this phenomenon: (i) abundance of hydrophobic amino acids in the potato protein which may bind surface oil (Krstonošić et al., 2020); (ii) higher content of amino acids (Met, Tyr, Lys, Asp, Ser, Phe, Ala) having antioxidant activities which could confer protection against oil oxidation (Krstonošić et al., 2020; Wang et al., 2016) and (iii) protective effect of dense, cracked free surface wall material (Fig. 1) for further oil diffusion.

### 3.4. Rheological behaviour of chocolate with fish oil microcapsules

Rheological measurements represent useful tool in chocolate manufacturing process since they correlate to the final product quality in terms of texture and sensory properties. The obtained results (Table 2) revealed that values of Casson plastic viscosity were significantly higher for all tested samples in comparison to control sample. These results are in accordance with the findings of Agibert and Lannes (2018) who concluded that the addition of microcapsules with a high oleic peanut oil resulted in Casson plastic viscosity increase. Similar values for Casson plastic viscosity of dark chocolate system determined at 40 °C were obtained by Fernandes, Müller, and Sandoval (2013). However, Casson yield stress values were slightly, but not significantly higher for systems containing 80 mg EPA + DHA in comparison to control sample. According to Beckett (2008) the fat has more pronounced effect on plastic viscosity than on the yield stress value meaning that higher amount of free moving fat would act as a lubricating material on the surfaces of solid particles thus affecting the decrease in plastic viscosity value. However, the yield stress values are mostly influenced by the forces between the solid particles that are related to the distances between them. Therefore, the increase in fat content has lower impact on yield stress value in comparison to plastic viscosity value.

The increase in plastic viscosity as well as yield stress values upon microcapsule addition could be due to increased solid particles content which was not followed by the increase in fat phase. Samples containing microcapsules were characterized by lesser content of free moving fat phase as well as more intensive particle–particle interactions due to lower content of free fat phase which was entrapped in crystal network between the particles. Consequently, it impedes the flow of the tested samples resulting in increased viscosity (Agibert & Lannes, 2018). Moreover, there is a significant difference between the samples containing different amount of microcapsules. Increase in both yield stress and especially plastic viscosity values upon microcapsule addition could be attributed to higher solid particle content and decreased content of fat phase, as previously explained. However, no differences were noticed in the samples containing microcapsules with different proteins as a coating material.

In-shear structural recovery measurements (Table 2) have shown slight decrease in recovery values with the increase in microcapsules content pointing to the discontinuity in system structure upon presence

**Table 1**
Encapsulation yield, content of free oil, encapsulation efficiency and oxidative stability of fish oil microcapsules prepared with different types of protein used as a coating material.

| Protein wall material | Yield of encapsulation (%) | Free oil content (%) | Encapsulation efficiency (%) | Peroxide value (meq peroxide/kg oil) | | |
|---|---|---|---|---|---|---|
| | | | | After 24 h | After 7 days | After 14 days |
| Potato | $72 \pm 3.1^{a}$ | $39 \pm 0.38^{b}$ | $61 \pm 0.48^{a}$ | $0.989 \pm 0.010^{a}$ | $1.095 \pm 0.001^{a}$ | $1.246 \pm 0.091^{a}$ |
| Whey | $85 \pm 2.4^{b}$ | $32 \pm 0.66^{ab}$ | $68 \pm 0.62^{ab}$ | $0.903 \pm 0.031^{a}$ | $1.006 \pm 0.084^{a}$ | $1.107 \pm 0.072^{a}$ |
| Soy | $70 \pm 2.7^{a}$ | $27 \pm 0.66^{a}$ | $73 \pm 0.12^{b}$ | $0.883 \pm 0.041^{a}$ | $1.004 \pm 0.091^{a}$ | $1.027 \pm 0.045^{a}$ |

Values represent the means ± standard deviation; n = 3. Values in the column followed by the same superscripts are not significantly different ($P > 0.05$).

**Table 2**
Rheological and melting properties of chocolate samples containing different amount and type of fish oil microcapsules.

| Sample | | Rheological properties | | Melting profile | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Casson yield stress (Pa) | Casson plastic viscosity (Pas) | In-shear recovery value | $T_{onset}$ (°C) | $T_{peak}$ (°C) | $T_{end}$ (°C) | $\Delta H_{melt}$ (J/g) |
| Control sample | | $4.69 \pm 0.194^{ab}$ | $1.44 \pm 0.012^{a}$ | $1.05 \pm 0.003^{a}$ | $28.85 \pm 0.127^{c}$ | $34.46 \pm 0.113^{b}$ | $36.61 \pm 0.131^{a}$ | $45.6 \pm 0.42^{d}$ |
| *Content of EPA + DHA 40 mg per 100 g and per 100 kcal* | | | | | | | | |
| Protein wall material | Potato | $4.72 \pm 0.037^{ab}$ | $1.74 \pm 0.036^{b}$ | $1.02 \pm 0.004^{bd}$ | $29.54 \pm 0.198^{d}$ | $36.05 \pm 0.141^{c}$ | $40.38 \pm 0.185^{c}$ | $42.5 \pm 0.49^{c}$ |
| | Whey | $4.61 \pm 0.030^{a}$ | $1.74 \pm 0.020^{b}$ | $1.04 \pm 0.008^{ac}$ | $28.80 \pm 0.126^{bc}$ | $34.20 \pm 0.170^{ab}$ | $39.49 \pm 0.157^{b}$ | $39.9 \pm 0.55^{b}$ |
| | Soy | $4.65 \pm 0.067^{a}$ | $1.74 \pm 0.015^{b}$ | $1.03 \pm 0.006^{cde}$ | $29.71 \pm 0.155^{d}$ | $36.11 \pm 0.198^{c}$ | $40.41 \pm 0.174^{c}$ | $43.0 \pm 0.56^{c}$ |
| *Content of EPA + DHA 80 mg per 100 g and per 100 kcal* | | | | | | | | |
| Protein wall material | Potato | $4.84 \pm 0.054^{b}$ | $2.12 \pm 0.024^{c}$ | $1.01 \pm 0.010^{b}$ | $28.16 \pm 0.212^{b}$ | $33.77 \pm 0.156^{a}$ | $39.58 \pm 0.162^{b}$ | $39.5 \pm 0.73^{b}$ |
| | Whey | $4.84 \pm 0.093^{b}$ | $2.09 \pm 0.023^{c}$ | $1.03 \pm 0.004^{c}$ | $27.24 \pm 0.170^{a}$ | $34.46 \pm 0.162^{b}$ | $39.49 \pm 0.175^{b}$ | $32.5 \pm 0.62^{a}$ |
| | Soy | $4.77 \pm 0.078^{b}$ | $2.09 \pm 0.035^{c}$ | $1.02 \pm 0.006^{be}$ | $28.43 \pm 0.184^{bc}$ | $33.92 \pm 0.184^{ab}$ | $39.62 \pm 0.156^{b}$ | $40.0 \pm 0.71^{b}$ |

Values represent the means ± standard deviation; n = 3, Values in the column followed by the same superscripts are not significantly different ($P > 0.05$).

of additional particles. However, these changes were not large enough to influence chocolate production process and system recovery after molding.

### 3.5. Melting properties

Chocolate samples containing fish oil encapsulated by potato, soy and whey protein wall material showed similar melting profiles (curves not shown) as control sample. The differences between samples reflected in onset ($T_{onset}$), peak ($T_{peak}$) and end ($T_{end}$) temperature as well as melting enthalpy ($\Delta H_{melt}$) values presented in Table 2. The melting profiles demonstrated the existence of single endothermic peak at about 33–36 °C which corresponded to the melting profiles of chocolate fortified with microcapsulated chia seed oil observed by Razavizadeh & Tabrizi (2021). Compared with the control chocolate, addition of low concentrations of potato and soy protein microcapsules led to increase in onset and peak temperatures of the fortified chocolate, while further addition decreased both temperatures. The lowest alterations in values of both onset and peak temperatures were observed when whey protein microcapsules were incorporated in chocolate matrix probably due to the low specific surface of whey protein stabilized microcapsules which reduced the possibility for particle–particle interaction. The values of $T_{end}$ increased with microcapsules incorporation into the chocolate formulation, suggesting higher temperature requirements to complete melting, which could be attributed to the existence of the eutectic effect by introducing additional components in the chocolate formulation (Wang, Liu, Shan, Jin, Wang & Li, 2010).

According to the results presented in Table 2 and Supplementary Fig. 1, control sample had a higher value of melting enthalpy ($\Delta H_{melt}$) than the samples containing microcapsules. Microcapsule incorporation led to uniform reduction in melting enthalpy values from 45.6 to 32.5 J/g proportional to the amount of microcapsules. Similar behaviour was reported by Razavizadeh and Tabrizi (2021) who concluded that higher amount of energy for perfecting fat melting in control sample relates to more stable structures in the control chocolate compared to the one containing microcapsules. Moreover, according to Afoakwa, Paterson, Fowler, and Vieira (2008a) fat has direct impact on the value of the melting enthalpy. Namely, enthalpy of melting is reduced in chocolate samples with lower fat content. In this study, a part of cocoa butter in couverture was replaced with protein stabilized fish oil which slightly reduced saturated fatty acids content and increased an amount of solid particles and polyunsaturated fat. The highest reduction in melting enthalpy was noticed in whey protein stabilized microcapsules which were characterized with the largest diameter (Fig. 1). In general, microcapsules addition did not have a large influence on chocolate melting profile, suggesting that the melting behaviour of the fortified chocolate in the oral cavity will not be altered (Toker et al., 2018).

### 3.6. Appearance and colour of chocolate with fish oil microcapsules

Beside texture, appearance and colour (Fig. 2A, Table 3) represent very important parameters in initial product acceptability perceived by the consumers.

The addition of microcapsules significantly increased the $L^*$ value (Table 3), i.e. the samples containing microcapsules were brighter in comparison to control sample, regardless of the protein type used as a coating material. This could be related to the fact that fish oil was encapsulated with proteins which were yellowish in colour, therefore obtained microcapsules had lighter colour and their incorporation in chocolate matrix resulted in increase in $L^*$ value. The obtained behaviour was in agreement with findings of Agibert and Lannes (2018) who also concluded that addition of microcapsules into dark chocolate increased $L^*$ value and thus led to production of brighter chocolate.

All studied samples were characterized with positive $a^*$ and $b^*$ values thus control as well as samples containing different types and amount of microcapsules were in the chromatic range of red and yellow which was in agreement with findings of Praseptiangga et al. (2019) who added cinnamon bark oleoresin microcapsules into dark chocolate. The incorporation of fish oil microcapsules significantly increased $a^*$ value and the colour of all chocolate samples became redder compared to control sample, whereas it did not have significant impact on $b^*$ value in samples containing 40 mg EPA + DHA. However, there are evident differences in all colour parameters by further increase in microcapsules content regardless of protein type used as coating material. Tolve et al. (2018) showed that the addition of microencapsulated phytosterols resulted in $L^*$, $a^*$ and $b^*$ values increase which was in accordance with the results presented in this study. Type of the protein wall material did not have significant impact on colour parameters.

In order to measure the intensity of colour difference between samples, difference colour index ($\Delta E$) was calculated. All sample having 40 mg EPA + DHA expressed colour differences that cannot be appreciated by the human eye ($1 < \Delta E < 3$), whereas the rest of the investigated samples showed colour differences compared to control sample which were obvious to the human eye i.e. the values of total colour differences

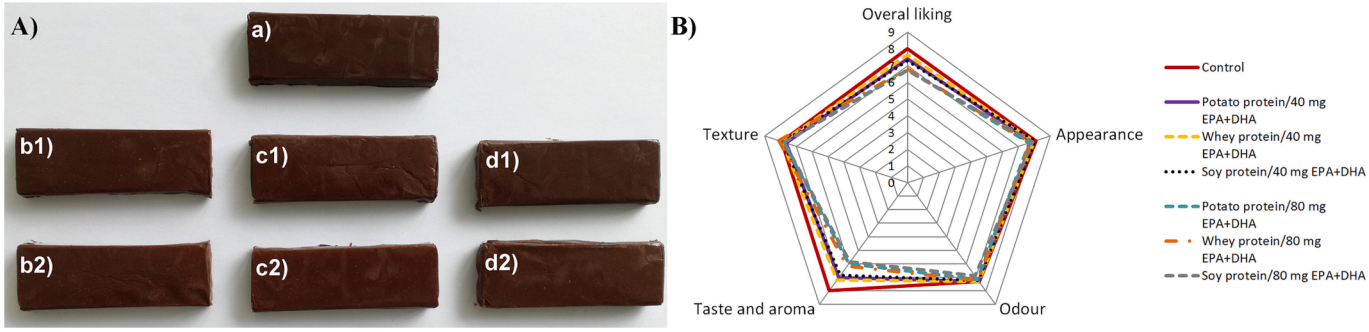



**Fig. 2.** Appearance of chocolate samples containing different amount and content of protein stabilized fish oil microcapsules (A): a) control sample; b) potato protein; c) whey protein; d) soy protein; b1, c1, d1) content of EPA + DHA 40 mg per 100 g and per 100 kcal and b2, c2, d2) content of EPA + DHA 80 mg per 100 g and per 100 kcal and results of liking study (B).

**Table 3**
Colour and texture parameters of chocolate samples containing different amount and type of fish oil microcapsules.

| | | Colour parameters | | | | | Texture | |
|---|---|---|---|---|---|---|---|---|
| Sample | | *L** | *a** | *b** | WI | ΔE | Breaking force (N) | Brittleness (mm) |
| Control sample | | $29.64 \pm 0.389^{a}$ | $7.64 \pm 0.143^{a}$ | $5.09 \pm 0.185^{a}$ | $29.04 \pm 0.284^{a}$ | n.a. | $9.85 \pm 0.653^{a}$ | $78.38 \pm 0.441^{a}$ |
| *Content of EPA + DHA 40 mg per 100 g and per 100 kcal* | | | | | | | | |
| Protein wall material | Potato | $31.08 \pm 0.335^{b}$ | $8.10 \pm 0.124^{b}$ | $5.17 \pm 0.181^{a}$ | $30.41 \pm 0.114^{b}$ | $1.51 \pm 0.19$ | $14.52 \pm 0.020^{b}$ | $79.71 \pm 0.269^{b}$ |
| | Whey | $31.98 \pm 0.519^{c}$ | $8.07 \pm 0.098^{b}$ | $4.81 \pm 0.158^{a}$ | $31.34 \pm 0.205^{c}$ | $2.39 \pm 0.31$ | $12.85 \pm 0.743^{c}$ | $79.16 \pm 0.238^{b}$ |
| | Soy | $31.94 \pm 0.766^{c}$ | $8.196 \pm 0.250^{b}$ | $5.24 \pm 0.449^{a}$ | $31.25 \pm 0.074^{c}$ | $2.36 \pm 0.28$ | $13.78 \pm 1.156^{bc}$ | $79.02 \pm 0.297^{ab}$ |
| *Content of EPA + DHA 80 mg per 100 g and per 100 kcal* | | | | | | | | |
| Protein wall material | Potato | $33.57 \pm 0.529^{d}$ | $8.74 \pm 0.234^{c}$ | $6.05 \pm 0.470^{b}$ | $32.72 \pm 0.544^{d}$ | $4.18 \pm 0.51$ | $13.67 \pm 0.277^{b}$ | $79.46 \pm 0.228^{b}$ |
| | Whey | $33.72 \pm 0.540^{d}$ | $9.00 \pm 0.078^{d}$ | $6.25 \pm 0.205^{b}$ | $32.82 \pm 0.072^{d}$ | $4.46 \pm 0.64$ | $11.89 \pm 1.610^{c}$ | $79.24 \pm 0.358^{b}$ |
| | Soy | $33.18 \pm 0.616^{d}$ | $9.03 \pm 0.103^{d}$ | $6.24 \pm 0.281^{b}$ | $32.28 \pm 0.145^{d}$ | $3.98 \pm 0.42$ | $13.02 \pm 0.229^{bc}$ | $79.37 \pm 0.233^{b}$ |

Values represent the means ± standard deviation; n = 6, Values in the column followed by the same superscripts are not significantly different ($P > 0.05$).

(*ΔE*) were higher than 3.

Whiteness index (*WI*) is often used as a measure of fat bloom effect which can be caused by fat migration and recrystallization, affecting changes in light scattering and consequently having negative effect on appearance attributes (Afoakwa, 2016). Higher *WI* values indicate lighter chocolate surface and fat bloom formation (Silva et al., 2017; Son et al., 2018). Cocoa butter polymorphic form V is the most desirable crystalline form, giving the chocolate with good resistance to contraction and bloom as well as good snap ability and improved shelf life (Afoakwa, Paterson, Fowler, & Ryan, 2008b). This form can be transformed in more stable form VI (34–36 °C) as a result of prolonged storage.

Silva et al. (2017) concluded that upon 120 days of storage whiteness index of chocolate enriched with probiotics increased due to fat bloom formation developed in tempering procedure. Chocolate tempering is one of the most important and critical step in development of fat bloom. Namely, it was revealed that the addition of probiotics during the tempering procedure influenced recrystallization of lipids resulting in higher fat bloom formation and thus *WI* value increase in comparison to control sample. In this research, the addition of microcapsules into dark chocolate has also resulted in whiteness index increase which was even more pronounced by further increase of microcapsule content to 80 mg EPA + DHA probably due to white-yellowish colour of added microcapsules. Moreover, the increase in *WI* values could be attributed to fat recrystallization caused by microcapsule addition (Silva et al., 2017). The obtained results are in accordance with findings of Razavizadeh and Tabrizi (2021) and Agibert and Lannes (2018) who also found that the addition of the microcapsules to the chocolate significantly increased whiteness index (*WI*). The similar trend of whiteness index increase was determined by Erdem et al. (2014) who included maltodextrin and lemon fibre into dark chocolate and obtained no negative effects on the colour properties of chocolate samples. Kumara, Jinap, Man, and Yusoff (2003) determined the values of *WI* of chocolate samples in the range of 35–45 which was in agreement with this study. Although there were no significant differences between proteins used for microcapsule preparation, it can be observed that samples containing whey protein microcapsules had the highest *WI* values which could be related to the fact that they were the lightest in comparison to potato and soy protein and with the highest content of large microcapsules.

### 3.7. Texture of chocolate with fish oil microcapsules

Brittleness and breaking force are the most important textural properties of chocolate. Chocolate brittleness or snap can be related to the distance to break i.e. the force versus distance (displacement) graph. Chocolate samples having longer breaking distance are characterized by poorer snap, they are less brittle and tend to bend instead of sharp snap (Beckett, 2008). Breaking force or fracturability represents the maximum force needed for a material to be fractured.

Addition of microcapsules into chocolate matrix resulted in breaking force as well as brittleness increase (Table 3), i.e. the samples were characterized with poorer snap, and they were less brittle in comparison to control sample. Breaking force values were significantly higher for all samples with microcapsule addition, whereas brittleness was not significantly higher only for the sample containing 40 mg EPA + DHA with soy protein used as a coating material. Moreover, in most of the cases there were small differences regarding breaking force as well as brittleness value between the samples containing microcapsules. The obtained results are in agreement with findings of Praseptiangga et al. (2019) who found that the cinnamon bark oleoresin microcapsule in dark chocolate resulted in hardness (breaking force) increase. Moreover, Afoakwa, Paterson, Fowler, and Vieira (2009) also revealed that the

breaking force of dark chocolate increased with a decrease in solid fat phase which could be attributed to increasing particle–particle interactions that were stronger than fat-fat links.

Regarding the protein type it can be concluded that samples containing whey protein had the lowest breaking force value. This could be related to weaker particle–particle interactions which could be a result of the highest content of large microcapsules prepared with whey protein (Fig. 1) characterized by smaller specific surface areas and thus decreased amount of particle surface–surface contacts. According to Afoakwa et al. (2009) breaking force is inversely related to particle size as well as solid fat content which was confirmed in this investigation.

### 3.8. Liking study

According to the results of the liking study, presented in Fig. 2B, consumers preferred the control chocolate over fortified types. However, all chocolate samples were considered acceptable since their mean scores for overall liking were above 6.0. Botelho et al. (2014) have also noted that scores above 6.0 (on a 9 point hedonic scale) represent a good acceptability for "dark chocolates". Statistical analysis showed that there were no statistical differences ($p < 0.05$) in the mean values for chocolate appearance, odour and texture. The appearance and texture scores of all samples ranged from 7.69 to 8.11 and from 7.65 to 8.12, respectively. Thus, the colour and texture differences detected using colorimeter and texture analyser (Table 3) were not perceived by the consumers, or these differences did not impact the chocolate liking. However, the incorporation of encapsulated fish oil in chocolate affected ($p < 0.05$) the product overall acceptability, mostly because of the lower scores for taste and aroma of fish oil enriched chocolate. Among enriched samples, taste and aroma of chocolates containing whey protein microcapsules was rated with the highest scores, probably due to milky taste of whey protein which is more associated with chocolate consumption than potato or soy taste. In general, when microencapsulated fish oil was added to chocolate, the sensory quality of the product was not strongly affected due to the masking properties of microencapsulation technique. This was in agreement with the study performed by Toker et al. (2018) who concluded that microencapsulation of EPA/DHA is preferred over other forms of omega-3 fatty acids in terms of chocolate sensory properties.

## 4. Conclusions

Results obtained in this study revealed that it is possible to produce fish oil fortified chocolates using proteins as microcapsules' wall material with similar quality characteristics compared to conventional ones. Samples containing protein stabilized fish oil microcapsules had lower content of free moving fat phase and thus more intensive particle–particle interactions in comparison to control sample which influenced increase in Casson viscosity value, breaking force and brittleness, while decreasing melting enthalpy. Microcapsule size influenced chocolate breaking strength and melting enthalpy, as well as microencapsulation efficiency of fish oil where systems with higher ratio of large-size microcapsules exhibited lower breaking strength and melting enthalpy, while microcapsules with large population of small-sized particles had lower content of surface oil and thus higher microencapsulation efficiency. However, these changes were not large enough to influence chocolate processing and system recovery after moulding. Moreover, enriched chocolate samples were considered sensory acceptable.

## CRediT authorship contribution statement

**Miroslav Hadnađev:** Conceptualization, Investigation, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Marina Kalić:** Formal analysis, Investigation, Data curation, Writing – original draft. **Veljko Krstonošić:** Conceptualization, Resources, Supervision, Project administration. **Nataša Jovanović-Lješković:** Formal analysis, Visualization, Investigation. **Tamara Erceg:** Investigation, Resources, Data curation. **Dubravka Škrobot:** Investigation, Data curation. **Tamara Dapčević-Hadnađev:** Investigation, Resources, Writing – original draft, Funding acquisition.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

### Acknowledgement

This work was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia under the Agreement on the Implementation and Financing of Research of the Institute of Food Technology [Grant Number: 451-03-68/2022-14/200222].

### Ethics statement

The study does not involve animal experiments. The liking study which involved consumers was conducted according to Helsinki Ethical Guidelines and adapted for food sensory analysis at the Institute of Food Technology in Novi Sad, University of Novi Sad, Serbia. The study was approved by the Ethics Committee of the Institute of Food Technology in Novi Sad, University of Novi Sad, Serbia (Ref. No. 175/I/15–3).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100583.

## References

Achayuthakan, P., & Suphantharika, M. (2008). Pasting and rheological properties of waxy corn starch as affected by guar gum and xanthan gum. *Carbohydrate Polymers, 71*(1), 9–17. https://doi.org/10.1016/j.carbpol.2007.05.006

Aghbashlo, M., Mobli, H., Madadlou, A., & Rafiee, S. (2012). The correlation of wall material composition with flow characteristics and encapsulation behavior of fish oil emulsion. *Food Research International, 49*(1), 379–388. https://doi.org/10.1016/j.foodres.2012.07.031

Agibert, S. A., & Lannes, S. C. D. S. (2018). Dark chocolate with a high oleic peanut oil microcapsule content. *Journal of the Science of Food and Agriculture, 98*(15), 5591–5597. https://doi.org/10.1002/jsfa.9102

Afoakwa, E. O., Paterson, A., Fowler, M., & Vieira, J. (2008). Relationship between rheological, textural and melting properties of dark chocolate as influenced by particle size distribution and composition. *European Food Research and Technology, 227*(4), 1215–1223. https://doi.org/10.1007/s00217-008-0839-5

Afoakwa, E. O., Paterson, A., Fowler, M., & Ryan, A. (2008). Flavor formation and character in cocoa and chocolate: A critical review. *Critical Reviews in Food Science and Nutrition, 48*, 1–18. https://doi.org/10.1080/10408390701719272

Afoakwa, E. O., Paterson, A., Fowler, M., & Vieira, J. (2009). Microstructure and mechanical properties related to particle size distribution and composition in dark chocolate. *International Journal of Food Science & Technology, 44*(1), 111–119. https://doi.org/10.1111/j.1365-2621.2007.01677.x

Afoakwa, E. O. (2016). *Chocolate science and technology*. John Wiley & Sons.

Bakry, A. M., Abbas, S., Ali, B., Majeed, H., Abouelwafa, M. Y., Mousa, A., & Liang, L. (2016). Microencapsulation of oils: A comprehensive review of benefits, techniques, and applications. *Comprehensive Reviews in Food Science and Food Safety, 15*(1), 143–182. https://doi.org/10.1111/1541-4337.12179

Barrow, C. J., Nolan, C., & Holub, B. J. (2009). Bioequivalence of encapsulated and microencapsulated fish-oil supplementation. *Journal of Functional Foods, 1*(1), 38–43. https://doi.org/10.1016/j.jff.2008.09.006

Beckett, S. T. (2008). *The science of chocolate (2nd edition)*. Royal Society of Chemistry.

Botelho, P. B., Galasso, M., Dias, V., Mandrioli, M., Lobato, L. P., Rodriguez-Estrada, M. T., & Castro, I. A. (2014). Oxidative stability of functional phytosterol-enriched dark chocolate. *LWT-Food Science and Technology, 55*(2), 444–451. https://doi.org/10.1016/j.lwt.2013.09.002

Davidov-Pardo, G., Roccia, P., Salgado, D., León, A., & Pedroza-Islas, R. (2008). Utilization of different wall materials to microencapsulate fish oil. *American Journal of Food Technology, 3*(6), 384–393. https://doi.org/10.3923/ajft.2008.384.393

Erdem, Ö., Gültekin-Özgüven, M., Berktaş, I., Erşan, S., Tuna, H. E., Karadağ, A., … Cutting, S. M. (2014). Development of a novel synbiotic dark chocolate enriched with Bacillus indicus HU36, maltodextrin and lemon fiber: Optimization by response surface methodology. *LWT-Food Science and Technology, 56*(1), 187–193. https://doi.org/10.1016/j.lwt.2013.10.020

EU (2012). Commission Regulation (EU) No 432/2012 of 16 May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health, 02012R0432-EN-17.05.202-010.001-2, available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02012R0432-20210517&from=EN.

Fernandes, V. A., Müller, A. J., & Sandoval, A. J. (2013). Thermal, structural and rheological characteristics of dark chocolate with different compositions. *Journal of Food Engineering, 116*(1), 97–108. https://doi.org/10.1016/j.jfoodeng.2012.12.002

Francis, F. J., & Clydesdale, F. M. (1975). *Food colorimetry: Theory and applications*. Avi Publishing.

Hogan, S. A., O'Riordan, E. D., & O'Sullivan, M. (2003). Microencapsulation and oxidative stability of spray-dried fish oil emulsions. *Journal of Microencapsulation, 20*(5), 675–688. https://doi.org/10.3109/02652040309178355

IOCCC (2000). International Office of Cocoa, Chocolate and Sugar Confectionery. Viscosity of chocolate and chocolate products. Analytical method. CABISCO (Eds.). Brussels, Belgium. p. 152.

Jafari, S. M., Assadpoor, E., Bhandari, B., & He, Y. (2008). Nano-particle encapsulation of fish oil by spray drying. *Food Research International, 41*(2), 172–183. https://doi.org/10.1016/j.foodres.2007.11.002

Kellerby, S. S., McClements, D. J., & Decker, E. A. (2006). Role of proteins in oil-in-water emulsions on the stability of lipid hydroperoxides. *Journal of Agricultural and Food Chemistry, 54*(20), 7879–7884. https://doi.org/10.1021/jf061340s

Kim, E. H. J., Dong Chen, X., & Pearce, D. (2003). On the mechanisms of surface formation and the surface compositions of industrial milk powders. *Drying Technology, 21*(2), 265–278. https://doi.org/10.1081/DRT-120017747

Krstonošić, V. S., Kalić, M. D., Dapčević-Hadnađev, T. R., Lončarević, I. S., & Hadnađev, M. S. (2020). Physico-chemical characterization of protein stabilized oil-in-water emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 602*, Article 125045. https://doi.org/10.1016/j.colsurfa.2020.125045

Kumara, B., Jinap, S., Man, Y. C., & Yusoff, M. S. A. (2003). Note: Comparison of colour techniques to measure chocolate fat bloom. *Food Science and Technology International, 9*(4), 295–299. https://doi.org/10.1177/108201303036045

Lohman, M. H., & Hartel, R. W. (1994). Effect of milk fat fractions on fat bloom in dark chocolate. *Journal of the American Oil Chemists' Society, 71*(3), 267–276. https://doi.org/10.1007/BF02638052

Nawas, T., Yousuf, N. B., Azam, M. S., Ramadhan, A. H., Xu, Y., & Xia, W. (2017). Physiochemical properties and sensory attributes of ice cream fortified with microencapsulated silver carp (Hypophthalmichthys molitrix) oil. *American Journal of Food Science and Nutrition Research, 4*(3), 79–86. http://www.openscienceonline.com/journal/fsnr.

Nesterenko, A., Alric, I., Silvestre, F., & Durrieu, V. (2013). Vegetable proteins in microencapsulation: A review of recent interventions and their effectiveness. *Industrial Crops and Products, 42*, 469–479. https://doi.org/10.1016/j.indcrop.2012.06.035

Partanen, R., Raula, J., Seppänen, R., Buchert, J., Kauppinen, E., & Forssell, P. (2008). Effect of relative humidity on oxidation of flaxseed oil in spray dried whey protein emulsions. *Journal of Agricultural and Food Chemistry, 56*(14), 5717–5722. https://doi.org/10.1021/jf8005849

Praseptiangga, D., Invicta, S. E., & Khasanah, L. U. (2019). Sensory and physicochemical characteristics of dark chocolate bar with addition of cinnamon (*Cinnamomum burmannii*) bark oleoresin microcapsule. *Journal of Food Science and Technology, 56*(9), 4323–4332. https://doi.org/10.1007/s13197-019-03901-8

Rahmani-Manglano, N. E., González-Sánchez, I., García-Moreno, P. J., Espejo-Carpio, F. J., Jacobsen, C., & Guadix, E. M. (2020). Development of fish oil-loaded microcapsules containing whey protein hydrolysate as film-forming material for fortification of low-fat mayonnaise. *Foods, 9*(5), 545. https://doi.org/10.3390/foods9050545

Ramakrishnan, S., Ferrando, M., Aceña-Muñoz, L., Mestres, M., De Lamo-Castellví, S., & Güell, C. (2014). Influence of emulsification technique and wall composition on physicochemical properties and oxidative stability of fish oil microcapsules produced by spray drying. *Food and Bioprocess Technology, 7*(7), 1959–1972. https://doi.org/10.1007/s11947-013-1187-4

Razavizadeh, B. M., & Tabrizi, P. (2021). Characterization of fortified compound milk chocolate with microcapsulated chia seed oil. *LWT-Food Science and Technology, 150*, Article 111993. https://doi.org/10.1016/j.lwt.2021.111993

Shantha, N. C., & Decker, E. A. (1994). Rapid, Sensitive, Iron-Based Spectrophotometric Methods for Determination of Peroxide Values of Food Lipids. *Journal of AOAC INTERNATIONAL, 77*(2), 421–424. https://doi.org/10.1093/jaoac/77.2.421.

Silva, M. P., Tulini, F. L., Marinho, J. F., Mazzocato, M. C., De Martinis, E. C., Luccas, V., & Favaro-Trindade, C. S. (2017). Semisweet chocolate as a vehicle for the probiotics Lactobacillus acidophilus LA3 and Bifidobacterium animalis subsp. lactis BLC1: Evaluation of chocolate stability and probiotic survival under in vitro simulated gastrointestinal conditions. *LWT-Food Science and Technology, 75*, 640–647. https://doi.org/10.1016/j.lwt.2016.10.025

Son, Y. J., Choi, S. Y., Yoo, K. M., Lee, K. W., Lee, S. M., Hwang, I. K., & Kim, S. (2018). Anti-blooming effect of maltitol and tagatose as sugar substitutes for chocolate making. *LWT-Food Science and Technology, 88*, 87–94. https://doi.org/10.1016/j.lwt.2017.09.018

Toker, O. S., Konar, N., Palabiyik, I., Pirouzian, H. R., Oba, S., Polat, D. G., … Sagdic, O. (2018). Formulation of dark chocolate as a carrier to deliver eicosapentaenoic and docosahexaenoic acids: Effects on product quality. *Food Chemistry, 254*, 224–231. https://doi.org/10.1016/j.foodchem.2018.02.019

Tolve, R., Condelli, N., Caruso, M. C., Barletta, D., Favati, F., & Galgano, F. (2018). Fortification of dark chocolate with microencapsulated phytosterols: Chemical and sensory evaluation. *Food & Function, 9*(2), 1265–1273. https://doi.org/10.1039/C7FO01822C

Tonon, R. V., Grosso, C. R., & Hubinger, M. D. (2011). Influence of emulsion composition and inlet air temperature on the microencapsulation of flaxseed oil by spray drying. *Food Research International, 44*(1), 282–289. https://doi.org/10.1016/j.foodres.2010.10.018

Wang, C., Chang, T., Zhang, D., Ma, C., Chen, S., & Li, H. (2020). Preparation and characterization of potato protein-based microcapsules with an emphasis on the mechanism of interaction among the main components. *Journal of the Science of Food and Agriculture, 100*(7), 2866–2872. https://doi.org/10.1002/jsfa.10277

Wang, F., Liu, Y., Shan, L., Jin, Q., Wang, X., & Li, L. (2010). Blooming in cocoa butter substitutes based compound chocolate: Investigations on composition, morphology and melting behavior. *Journal of the American Oil Chemists' Society, 87*(10), 1137–1143. https://doi.org/10.1007/s11746-010-1604-z

Wang, R., Tian, Z., & Chen, L. (2011). A novel process for microencapsulation of fish oil with barley protein. *Food Research International, 44*(9), 2735–2741. https://doi.org/10.1016/j.foodres.2011.06.013

Wang, L., Yang, J., Wang, Y., Zhang, J., Gao, Y., Yuan, J., … Ju, X. (2016). Study on antioxidant activity and amino acid analysis of rapeseed protein hydrolysates. *International Journal of Food Properties, 19*(9), 1899–1911. https://doi.org/10.1080/10942912.2015.1085397