



# Sensory profiles and oxidative stability of linseed oil microencapsulated with pea, soy, and whey proteins in high-fat food models

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## ABSTRACT

To hinder oxidation and mask flavor, linseed oil was encapsulated using maltodextrin with pea, soy, or whey protein, and incorporated in dark chocolate and shortbread cookie models. While the spray dried emulsions with whey protein and maltodextrin were most stable, this behaviour did not translate to food models. In volatiles, the fortification with pea protein capsules had closer resemblance to the control than other spray-dried samples. Storage time increased the volatiles of particularly soy and whey samples. In flavor, the pea sample, and in appearance and texture, the soy sample resembled closest the control chocolate. Addition of the spray dried emulsion increased the darkness, rancidity, roasted flavor, and hardness of the cookies. While the pea protein emerged as an intriguing choice for shell material in spray-drying of linseed oil, also fortification with non-encapsulated linseed oil resulted in favorable outcomes based on sensory perception and volatile profiles of the food models.

## 1. Introduction

The essential omega-3 polyunsaturated fatty acid (n-3 PUFA),  $\alpha$ -linolenic acid (ALA, 18:3n-3), contributes to e.g. healthy cholesterol level, blood pressure and cardiovascular health (Bork et al., 2022; Sal-a-Vila et al., 2022; Wei et al., 2018). However, the intake of ALA, and dietary n-3 PUFA in general, is universally below recommendations (Guesnet et al., 2019; Sioen et al., 2017). Main sources of ALA are plant seed oils (Wang et al., 2021; Yuan et al., 2022), among which especially linseed oil, also known as flaxseed oil, stands out with its superior ALA content (Kajla et al., 2015; Mueed et al., 2022). However, the high ALA content of linseed oil makes it susceptible to oxidation, which can lead to a reduction in both nutritional and sensory quality (Brühl et al., 2007; Frankel, 2012; Mozuraityte et al., 2016; Wang et al., 2021).

Microencapsulation, most commonly by spray-drying, may offer protection against oxidation. Sensitive core agent is encapsulated in a protective shell material, resulting in a spray-dried emulsion (SDEM) powder (Delshadi et al., 2020; Ruiz Ruiz et al., 2017). In addition to oils and fats as such, pastries and cakes serve as a source of ALA (Guesnet et al., 2019; Redruello-Requejo et al., 2023). Hence, incorporating

SDEMs into these common food products could provide convenient means of elevating the recommended daily intake of ALA. In the EU, foods containing 0.3 g of ALA per 100 g and per 100 kcal may be labeled as a source of n-3 fatty acids (FAs). Additionally, products may be labeled as a food high in n-3 FAs when they contain at least 0.6 g of ALA per 100 g and per 100 kcal. (Regulation (EC) No 1924/2006 of the European Parliament and of the Council of December 20, 2006 on Nutrition and Health Claims Made on Foods, 2014.)

Polymers typically used as coating materials include carbohydrates, dairy proteins, and their mixtures (Chang & Nickerson, 2018; Perez-Palacios et al., 2022). According to Chang and Nickerson (2018) plant-based proteins present a sustainable advancement in the field of microencapsulation. Previously, for example, soy protein isolate (Tambade et al., 2020), rice protein (Gomes & Kurozawa, 2021), lentil protein isolate (Wang et al., 2022), and pea protein isolate (Bajaj et al., 2015; Damerou et al., 2022a) have been used in encapsulation. Despite the advancements, further investigation is needed to enhance the quality of microencapsulated oils. For instance, Wang et al. (2022) observed oxidation in microencapsulated oils after an 8-week storage period. Furthermore, sensory properties have a significant importance in the

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consumer acceptance of foods (Baker et al., 2022). Regrettably, to the authors' best knowledge, there is a limited amount of research concerning the enrichment of foods with encapsulated linseed oil, especially with sustainable plant proteins.

In this study, linseed oil was encapsulated using maltodextrin, along with either pea, soy, or whey protein, and incorporated within dark chocolate and shortbread cookies. The main objectives were to study the chemical and sensory quality of the SDEMs and the food models. The hypotheses of this research were that plant proteins are suitable wall materials to replace whey protein in spray drying of linseed oil and that encapsulation will enhance the oxidative stability and preserve the sensory quality of n-3 PUFAs in fortified foods during storage.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Spray-dried emulsions

SDEMs were produced as described in Damerau et al., (2022a) using cold-pressed linseed oil, LO, (Olejarnia Świecie, Świecie nad Osą, Poland) combined with maltodextrin, M, (DE 14–22, Edpol Food & Innovation company, Łomża, Poland) and either whey protein, W, (WPC 80, Ostrowia Company, Warszawa, Poland), pea protein, P, (pea protein, Roquette, Lestrem, France) or soy protein, S, (soy protein, Barentz Food & Nutrition, Netherlands), keeping the ratio of LO, maltodextrin and protein as 2:1:1. Aqueous emulsions (2 L) were formed using Thermomix (Vorwerk, Wuppertal, Germany), and then homogenized either in two-steps (25 MPa and 5 MPa) using a high-pressure laboratory valve homogenizer (Panda 2 K, GEA Niro Soavi, Parma, Italy). The emulsions were spray dried in a Production Minor Spray Dryer (A/S Niro Atomizer, Copenhagen, Denmark) equipped with a disc spray system. The feed flow rate was maintained at 77 mL/min, while inlet and outlet air temperatures were 125 °C and 75 °C, respectively. Thus, the following SDEMs were produced: WM (whey protein and maltodextrin), PM (pea protein and maltodextrin), and SM (soy protein and maltodextrin).

#### 2.1.2. Food models

Either SDEM or non-encapsulated LO was incorporated into chocolate (Table 1), prepared according to Damerau et al. (2022a), to meet >0.3 g ALA per 100 g and 100 kcal. Thus, the following chocolate

models were produced: W (enriched with WM capsules), P (enriched with PM capsules), S (enriched with SM capsules), O (enriched with non-encapsulated LO), and a reference chocolate without LO as a control, R.

The cookie models (Table 1) were prepared as described in (Damerau et al., 2022a) with minor modifications. 34.5 g of margarine (Keiju 70%, Bunge Finland Plc, Finland), 15.2 g sugar (Aito Hienosokeri, VD-Group Plc, Belgium) and 50.2 g wheat flour (Myllyn Paras Plc, Hyvinkää, Finland) were mixed. As a result, the following cookie models were baked: W (enriched with WM capsules), P (enriched with PM capsules), S (enriched with SM capsules), O (enriched with non-encapsulated LO), and a reference cookie without LO, R. An addition of either SDEM or non-encapsulated LO to the cookies resulted in >0.6 g ALA per 100 g and 100 kcal (marked with 1) or double of this concentration (marked with 2). In the cookies fortified by SDEM, an equal proportion of flour and margarine was replaced by SDEMs, whereas in LO fortified cookies part of margarine was replaced by non-encapsulated LO. The cookies (ca. 20 g) were baked at 175 °C for 17 min. They weighed thereafter 17.8 g ( $\pm 0.13$  g).

#### 2.1.3. Storage trial of food models

The chocolate was stored at room temperature (23.6 °C) at humidity of 39.8 % for 11 weeks and the cookies at 50 °C for 31 days (Table 1). 1 g  $\pm$  0.03 g of finely cut chocolate and finely crushed cookies were weighted in triplicate in 20 mL headspace vials. The fresh samples were flushed with nitrogen to prevent further oxidation. Samples in storage trial were stored under air. For sensory analysis and lipid extraction, the samples were stored as whole; the chocolates were wrapped in waxed paper and stored in resealable plastic bags, and the cookies were stored in small aluminum foil containers with lids. After the trial the cookies were stored at -80 °C for chemical and at -20 °C for sensory analysis. Chocolate was stored at 4 °C. Prior to the sensory assessments, a new batch of chocolate was prepared for the fresh samples.

## 2.2. Methods

### 2.2.1. Scanning electron microscopy

SDEMs were attached to the microscope stage by using two-sided adhesive tape, mounted on scanning electron microscopy (SEM) tubs, and coated with palladium in a sputter coater. SEM Quanta 200 (FEI Company, Hillsboro, OR) operating an accelerating voltage of 30 kV and  $\times$  400 magnifications was used.

### 2.2.2. Total and surface oil content and encapsulation efficiency

Surface oil (easily extractable oil) and total oil contents of SDEMs were determined as previously described in Takeungwongtrakul et al. (2015) and Damerau et al. (2022b) using hexane (Sigma-Aldrich, Poznań, Poland) or a chloroform/methanol (Sigma Aldrich, Poznań, Poland) mixture (2:1, v/v), respectively. The oil content was determined gravimetrically after evaporation of solvent. Encapsulation efficiency (EE) (%) was calculated by using equation (1), where TOC indicates total oil content and SOC indicates surface oil content:

$$EE (\%) = \frac{(TOC - SOC)}{TOC} \times 100 \quad (1)$$

### 2.2.3. Fatty acid analysis

Lipids from SDEMs were extracted in triplicate as described by Damerau et al. (2022b). Lipids from the food models were extracted using a modified Folch extraction method (Folch et al., 1957). 0.3 g of food model and 1.23 mL 2-propanol (Honeywell International Inc., Germany) were mixed with an ultra turrax for 30 s at 800 rpm. 3.75 mL hexane (Honeywell International Inc., Germany) was added and mixed as previously described. The mixture was shaken with 1.5 mL 0.8% potassium chloride in MQ-water and centrifuged at 1700  $\times$ g for 2 min. The extraction was repeated with 3.75 mL hexane. The organic phases

**Table 1**

Sample materials and their codes including storage periods for collecting samples for chemical and sensory analysis.

Samples <sup>a</sup>	Chocolate models		Cookie models	
	R, O, W, S, P		R, O1, O2, W1, W2, S1, S2, P1, P2	
Time point <sup>b</sup>	Storage period (d)	Samples collected <sup>c</sup>	Storage period (d)	Samples collected <sup>c</sup>
0	0	VOC, FA, PV, GDA	0	VOC, FA, PV, GDA
1	14	VOC	7	VOC
2	35	VOC, FA, PV	14	VOC, FA, PV
3	56	VOC	24	VOC, FA, PV, GDA
4	76	VOC, FA, PV, GDA	31	VOC

<sup>a</sup> R = not fortified food model, O = fortified with non-encapsulated LO, W = fortified with whey protein concentrate and maltodextrin capsules, S = fortified with soy protein and maltodextrin capsules, P = fortified with pea protein and maltodextrin capsules, 1 = fortified with SDEM or non-encapsulated linseed oil to gain 0.6 g ALA per 100 g and 100 kcal, or 2 = fortified to gain 1.2 g ALA per 100 g and 100 kcal.

<sup>b</sup> In the sample abbreviations, the timepoint is indicated after the underscore.

<sup>c</sup> Abbreviations of conducted analyses: VOC = volatile oxidation compounds by HS-SPME-GC-MS, FA = fatty acids by GC-FID, PV = peroxide value, and GDA = general descriptive analysis.

were collected. Acid-catalyzed FA esterification method was employed (Christie, 2010). The FA methyl esters were analyzed with a Shimadzu GC-2030 with an AOC-20i autoinjector, a flame ionization detector (Shimadzu Corporation, Kyoto, Japan), and DB-23 column (60 m × 0.25 mm × 0.25 μm, Agilent Technologies, J.W. Scientific, Santa Clara, CA, USA) under previously reported conditions (Damerou et al., 2022a). Peaks were identified with Supelco 37 Component FAME mix (Supelco, Inc. St. Louis, MO, USA), 68D and GLC-490 (Nu-Check-Prep, Elysian, MN, USA) as external standards. The quantification of FAs was based on internal standard (Triheptadecanoin (Larodan Fine Chemicals AB, Malmö, Sweden)) and correction factors.

#### 2.2.4. Oxidative stability index

Oxidative stability index (OSI, expressed as hours) was determined using A 743 Rancimat (Methrom, Herisau, Switzerland) eight-channel oxidative stability instrument (Damerou et al., 2022b). 2.5 g of each SDEM or food sample was placed in a capped reaction vessel in a thermostatic electric heating block. The temperature was set at 110 °C and the airflow rate at 20 L/h.

#### 2.2.5. Physical properties

Hardness was measured using a breaking test of an Instron universal testing machine type 4301 (Canton, MA, USA) with Instron Series IX AMTS ver. 8.04 software at a temperature of 21 °C (two and four measurements for the chocolate and cookie models, respectively), using dimensions 20 mm × 15 mm × 8 mm (length × width × thickness) for chocolate and 50 mm × 10 mm (diameter × thickness) for cookies.

Measurements of color parameters  $L^*$ ,  $a^*$ , and  $b^*$  of the prepared chocolate and cookie samples were done using a digital image analysis at a temperature of 21 °C (three and four measurements for the chocolate and cookie models, respectively). The set consisted of a computer, a high-resolution, low-noise charge-coupled device (CCD) Nikon DXM-1200 color camera (Nikon Inc., Melville, USA), a Kaiser RB-5004-HF High Frequency Daylight Copy Light Set with 4 × 36 W fluorescent light tubes (color temperature 5400 K) (Kaiser Fototechnik GmbH & Co. KG, Buchen, Germany), and Laboratory Universal Computer Image Analysis (LUCIA) G v. 4.8 software (Ogrodowska et al., 2017). Additionally, total color differences ( $\Delta E$ ) of the fortified samples compared to the control sample were calculated from equation (2) (Razavizadeh & Tabrizi, 2021):

$$\Delta E = \sqrt{(L_f^* - L_c^*)^2 + (a_f^* - a_c^*)^2 + (b_f^* - b_c^*)^2} \quad (2)$$

where,  $L^*$ ,  $a^*$ ,  $b^*$  are color parameters for the fortified (f) and control samples (c).

#### 2.2.6. Peroxide value

The peroxide value (PV) of lipid extracts (equal to those used in FA analysis) was determined with a modified ferric thiocyanate method (Lehtonen et al., 2011) using iron (III) chloride standard line and expression mEq/kg food model.

#### 2.2.7. Volatile oxidation compounds

The volatile oxidation products (VOCs) of food models were analyzed with solid-phase microextraction gas chromatography-mass spectrometry (SPME-GC-MS). TriPlus RSH autosampler coupled with TRACE 1310 GC and ISQ 7000 MS detector (Thermo Scientific, Reinach, Switzerland) and equipped with DVB/CAR/PDMS-fiber 50/30 μm (Supelco, Bellefonte, PA, USA) and SPB®-624 capillary column (60 m × 0.25 mm × 1.4 μm, Supelco, Bellefonte, PA, USA), and GC-MS conditions described in Damerou et al. (2022a) were used. Identification of compounds was performed using the NIST MS Search library (version 2.4, National Institute of Standards and Technology, Gaithersburg, MD, USA).

#### 2.2.8. Sensory assessments

Two sensory panels, consisting of the students and the staff of the University of Turku and the University of Applied Sciences of Turku, were recruited to evaluate chocolates (n = 11) and cookies (n = 11) using the generic descriptive analysis (GDA). All assessors acknowledged an informed consent statement to participate in the study. They were informed about the research objectives, and participants' rights and privacy in compliance with the GDPR, the Instructions of Finnish National Board on Research Integrity, and the University of Turku. At the time of the sensory evaluation, pre-ethical permission for sensory evaluations was not required by our institution.

Three and four training sessions were held prior to the chocolate and the cookie assessments, respectively. The panel agreed on 19 attributes for cookies and 22 for chocolate (Supplementary Table 1 and Supplementary Table 2). The food models were evaluated in duplicate as fresh and after the storage period. The assessments were conducted in a sensory laboratory in accordance with the ISO 8589 standard. Cookies (ca. 6.3 g) and chocolate (ca. 5 g) were served at room temperature in covered glass bowls labeled with three-digit numerical random codes. Sample order was randomized using the Latin Squared design. Carbon filtered water and water biscuits (Carr's Table Water, Carr's of Carlisle Ltd, Great Britain) were provided for palate cleansing during forced time delays (30 s) between samples. Compusense Cloud (version 21.0, Compusense Inc., Guelph, ON, Canada) was used for data collection.

#### 2.3. Statistical analysis

The evaluation of the sensory panels and the assessors' performance was conducted using PanelCheck, version 1.4.2 (Nofima, Tromsø, Norway; Tomic et al. (2010)). Analysis of variance (ANOVA) was performed using IBM SPSS Statistics 27 (IBM Corporation, Armonk, NY, USA) with  $p < 0.05$ . One-way ANOVA was used to compare ALA content, OSI, hardness, and color while a two-way mixed ANOVA was used for PV value analysis. Two-way ANOVA models were carried out for sensory attributes (main effects and sample × assessor interactions; samples as fixed factors, assessors as random factors). Tukey's HSD tests or Bonferroni post hoc tests were conducted after the ANOVA models.

The data of the volatile compounds and the consensus sensory data, averaged over the assessors and the replicates, were subjected to the principal component analysis (PCA) executed by Unscrambler® X, version 11.0 (Camo Analytics AS., Oslo, Norway). The mean centered sensory data and auto-scaled volatile data underwent full and random cross validation, respectively, using NIPALS.

### 3. Results and discussion

#### 3.1. Characterization of SDEMs

SDEMs were of relatively spherical shape (Fig. 1). However, SM consisted of particles with higher uniformity and larger diameters, while in WM and PM, a greater number of particles with small diameters were found. Previously, the usage of soy proteins as emulsifiers in encapsulation of essential oils resulted in particles with significantly higher mean diameters than those with pea protein concentrate (Francisco et al., 2020). Higher solubility of soy proteins may promote a higher viscoelasticity of the atomized emulsion droplets, thus, greater swelling during the drying step, resulting in larger particles. In this study, some particles in SM had visible surface folds indicating softer structures and greater susceptibility to deformation. Additionally, greater shrinkage of particles has been reported in samples consisting of sunflower oil and soy proteins compared to particles containing pea proteins and the same oil (Le Priol et al., 2019). The particle shrinkage may be related to rapid formation of wall crust layer on the particle surface followed by the high amount of water evaporation throughout the drying process (Gong et al., 2016).

The total oil content of SDEMs was in the range of 45.96 (PM) to

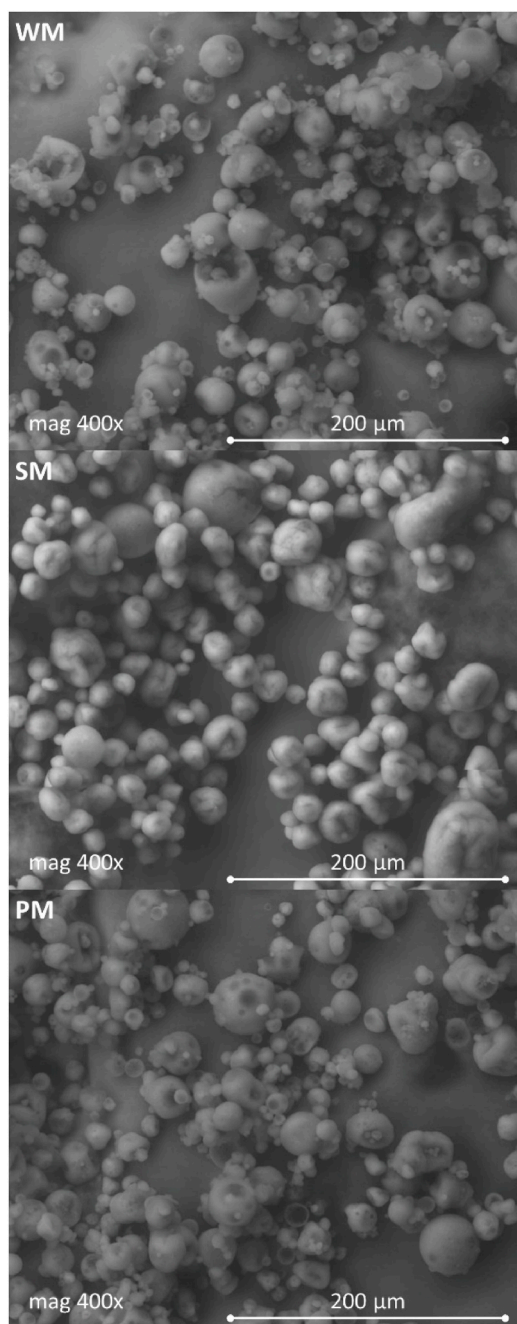


Fig. 1. SEM for spray-dried emulsions formulated with M = maltodextrin, W = whey protein concentrate, S = soy protein or P = pea protein as wall material.

49.15% (WM) (Table 2), similar as introduced in the emulsion (50%). The significantly different result of the PM sample may be related to the higher surface oil content (15.32%) compared to the WM (6.40%) and SM (8.57%) samples. Generally, low surface oil content and thus high EE

are indicators of the success of microencapsulation. [Aberkane et al. \(2014\)](#) reported EE to be influenced by the type of coating material. They did not indicate pea protein without the addition of any polysaccharide as a suitable encapsulating material. In our study, the combination of P with M resulted in the highest surface oil content of the studied LO microcapsules. [Carneiro et al. \(2013\)](#), using the same materials, obtained a much lower EE (62.3%), which is related to the total solid concentration (30%) in emulsion and wall-to-core ratio of 1:2. In our study, total solid concentration was 20% and the ratio of wall and core ingredients was 1:1. However, despite the low EE, samples showed the highest oil protection against oxidation ([Carneiro et al., 2013](#)), which is consistent with our results (Table 2). [Francisco et al. \(2020\)](#) found higher EE for encapsulated orange essential oils using soy protein, likely due to larger particle size, which is consistent with the present study. Similarly, [Le Priol et al. \(2019\)](#) reported higher EEs for sunflower oil with soy protein (91%) compared to pea protein (88%), indicating a more efficient encapsulation process with soy protein.

The FA profile of LO consisted of ALA (57.5%), oleic (19.4%), linoleic (14.8%), palmitic (5.1%) and stearic acid (2.6%), with other FAs being less than 1%, which is in comparable range with our earlier studies ([Tańska et al., 2016](#); [Ogrodowska et al., 2024](#)). ALA content of SDEMs was marginally but significantly lower than of LO (Table 2).

OSI showed that all SDEMs were more stable than LO (Table 2). WM was the most stable followed by PM. SM was least stable but still two times more stable than LO. This is consistent with the results of encapsulation of fish oil with the same wall material combinations and spray-drying conditions ([Damerou et al., 2022a](#)), indicating that wall material selection seems to have a big impact on the OSI.

### 3.2. Physical and chemical quality of food models

#### 3.2.1. Hardness and color of the food models

The texture, especially hardness, of chocolates and cookies affects their perceived quality. 30% less force was needed to break the chocolate with non-encapsulated oil than the control sample (Table 3). The addition of SDEMs, most strongly SM, increased the breaking force needed. Also, [Hadnadev et al. \(2023\)](#) reported that breaking force was higher for samples of chocolate with microcapsules, and equal to in our study, samples with whey protein were softer compared to samples with plant proteins. An opposite relationship was observed for the cookie model, where samples with whey proteins (W1, W2) were harder than those with plant proteins (Table 3). Furthermore, it was observed that the incorporation of twice the amount of each SDME into the cookies resulted in a noticeable increase in their hardness.

All fortified chocolates had a significantly lighter color, lower blueness and lower yellowness compared to control (Table 3). [Hadnadev et al. \(2023\)](#) observed differences in color parameters in chocolates with microcapsules, regardless of the coating protein type. Also, [Razavizadeh and Tabrizi \(2021\)](#) reported that chocolates with microencapsulated chia seed oil were lighter than the control. The influence of SDEM addition on cookie color was significantly lower than in the case of chocolate, as shown by the lower  $\Delta E$  values for enriched samples. The exception was only samples with twice the concentration of whey and pea proteins, which were characterized by a higher yellowness.

Table 2

Total and surface oil contents, encapsulation efficiency,  $\alpha$ -linolenic acid (ALA, 18:3n-3) content and oxidative stability index (OSI) of spray-dried emulsions (n = 2).

SDEM <sup>a</sup>	Total oil [%]	Surface oil [%]	Encapsulation efficiency [%]	ALA content of oil content [%]	OSI [h] <sup>b</sup>
WM	49.15 ± 0.95 <sup>b</sup>	6.40 ± 0.23 <sup>a</sup>	86.98 ± 0.21 <sup>c</sup>	56.22 ± 0.01 <sup>a</sup>	13.08 ± 0.22 <sup>c</sup>
SM	49.29 ± 0.30 <sup>b</sup>	8.57 ± 0.33 <sup>b</sup>	82.62 ± 0.78 <sup>b</sup>	56.93 ± 0.01 <sup>b</sup>	5.05 ± 0.23 <sup>a</sup>
PM	45.96 ± 0.58 <sup>a</sup>	15.32 ± 0.60 <sup>c</sup>	66.68 ± 0.88 <sup>a</sup>	56.06 ± 0.04 <sup>a</sup>	11.73 ± 0.22 <sup>b</sup>

<sup>a</sup> Spray-dried emulsion (SDEM) with W = whey protein concentrate, S = soy protein or P = pea protein and M = maltodextrin as wall material. Different letters on the same column indicate a statistically significant (p < 0.05) difference between samples.

<sup>b</sup> OSI – oxidative stability index at 110 °C, for non-encapsulated oil was 2.39 ± 0.11 h.

**Table 3**

Hardness (n=5), color (CIEL\*a\*b\*) (n=5), oxidative stability index (OSI) (n=2) and  $\alpha$ -linolenic acid (ALA, 18:3n-3) content (n=2) of the prepared chocolate and cookie models.

Food model	Sample type <sup>a</sup>	Hardness [N]	L*	a*	b*	$\Delta E$	OSI [h] <sup>b</sup>	ALA [g per 100 g] <sup>b</sup>	
<b>Chocolate model</b>	R	61.7 $\pm$ 2.6 <sup>a</sup>	43.9 $\pm$ 3.5 <sup>a</sup>	-5.3 $\pm$ 1.8 <sup>a</sup>	35.8 $\pm$ 1.4 <sup>b</sup>	-	77.0 $\pm$ 1.4 <sup>b</sup>	0.11 $\pm$ 0.01 <sup>a</sup>	
	O	47.4 $\pm$ 0.6 <sup>b</sup>	59.4 $\pm$ 6.2 <sup>b,c</sup>	-3.5 $\pm$ 0.2 <sup>a,b</sup>	23.0 $\pm$ 4.3 <sup>a</sup>	81.2	72.5 $\pm$ 0.7 <sup>a</sup>	2.38 $\pm$ 0.07 <sup>b</sup>	
	S	76.5 $\pm$ 0.4 <sup>d</sup>	65.1 $\pm$ 4.0 <sup>c</sup>	-1.2 $\pm$ 0.3 <sup>b</sup>	25.5 $\pm$ 2.3 <sup>a</sup>	84.3	70.1 $\pm$ 0.3 <sup>a</sup>	2.84 $\pm$ 0.08 <sup>b</sup>	
	P	73.7 $\pm$ 2.8 <sup>c,d</sup>	65.5 $\pm$ 0.6 <sup>c</sup>	-1.6 $\pm$ 0.2 <sup>b</sup>	24.6 $\pm$ 2.8 <sup>a</sup>	84.9	80.8 $\pm$ 0.4 <sup>b,c</sup>	2.36 $\pm$ 0.04 <sup>b</sup>	
	W	68.5 $\pm$ 0.6 <sup>b,c</sup>	55.8 $\pm$ 0.8 <sup>b</sup>	-1.1 $\pm$ 0.4 <sup>b</sup>	29.5 $\pm$ 0.8 <sup>a,b</sup>	76.2	82.0 $\pm$ 1.4 <sup>c</sup>	2.35 $\pm$ 0.02 <sup>c</sup>	
	<b>Average</b>		<b>65.5 <math>\pm</math> 11.0</b>	<b>57.9 <math>\pm</math> 8.8</b>	<b>-2.5 <math>\pm</math> 1.8</b>	<b>27.7 <math>\pm</math> 5.1</b>	-	<b>76.5 <math>\pm</math> 4.9</b>	-
<b>Cookie model</b>	R	10.6 $\pm$ 1.9 <sup>a</sup>	83.6 $\pm$ 0.1 <sup>b,c</sup>	-7.2 $\pm$ 0.1 <sup>c</sup>	31.6 $\pm$ 0.6 <sup>a</sup>	-	15.3 $\pm$ 0.5 <sup>b</sup>	3.64 $\pm$ 0.14 <sup>a</sup>	
	O1	16.0 $\pm$ 4.0 <sup>a,b</sup>	83.9 $\pm$ 0.3 <sup>c</sup>	-7.7 $\pm$ 0.1 <sup>a,b,c</sup>	34.1 $\pm$ 0.8 <sup>a</sup>	1.8	15.0 $\pm$ 0.0 <sup>b</sup>	4.73 $\pm$ 0.18 <sup>c</sup>	
	O2	11.9 $\pm$ 2.7 <sup>a,b</sup>	84.5 $\pm$ 0.6 <sup>c</sup>	-8.1 $\pm$ 0.1 <sup>a</sup>	34.8 $\pm$ 1.2 <sup>a</sup>	8.0	11.0 $\pm$ 0.4 <sup>a</sup>	8.16 $\pm$ 0.02 <sup>d</sup>	
	S1	14.1 $\pm$ 1.1 <sup>a,b</sup>	84.3 $\pm$ 0.6 <sup>c</sup>	-7.5 $\pm$ 0.1 <sup>b,c</sup>	34.6 $\pm$ 2.7 <sup>a</sup>	3.1	16.9 $\pm$ 0.2 <sup>c</sup>	4.37 $\pm$ 0.06 <sup>b</sup>	
	S2	20.3 $\pm$ 2.4 <sup>b,c</sup>	83.6 $\pm$ 0.7 <sup>b,c</sup>	-7.3 $\pm$ 0.2 <sup>c</sup>	36.2 $\pm$ 2.3 <sup>a</sup>	4.7	15.8 $\pm$ 0.3 <sup>b,c</sup>	8.55 $\pm$ 0.16 <sup>c</sup>	
	P1	12.2 $\pm$ 2.3 <sup>a,b</sup>	84.7 $\pm$ 0.6 <sup>c</sup>	-7.5 $\pm$ 0.1 <sup>b,c</sup>	33.2 $\pm$ 1.4 <sup>a</sup>	2.0	16.5 $\pm$ 0.5 <sup>b,c</sup>	4.78 $\pm$ 0.04 <sup>c</sup>	
	P2	27.0 $\pm$ 7.4 <sup>c</sup>	79.7 $\pm$ 1.4 <sup>a</sup>	-7.2 $\pm$ 0.5 <sup>c</sup>	48.8 $\pm$ 3.3 <sup>c</sup>	17.7	16.1 $\pm$ 0.2 <sup>b,c</sup>	8.26 $\pm$ 0.09 <sup>d</sup>	
	W1	20.2 $\pm$ 2.8 <sup>b,c</sup>	84.1 $\pm$ 1.0 <sup>c</sup>	-7.9 $\pm$ 0.2 <sup>a,b</sup>	35.9 $\pm$ 1.9 <sup>a</sup>	4.4	16.1 $\pm$ 0.1 <sup>b,c</sup>	4.41 $\pm$ 0.04 <sup>b</sup>	
	W2	30.1 $\pm$ 6.3 <sup>c</sup>	82.0 $\pm$ 0.8 <sup>b</sup>	-7.9 $\pm$ 0.2 <sup>a,b</sup>	42.4 $\pm$ 2.4 <sup>b</sup>	11.0	16.1 $\pm$ 0.7 <sup>b,c</sup>	8.09 $\pm$ 0.17 <sup>d</sup>	
	<b>Average</b>		<b>17.4 <math>\pm</math> 7.3</b>	<b>83.4 <math>\pm</math> 1.6</b>	<b>-7.6 <math>\pm</math> 0.3</b>	<b>36.8 <math>\pm</math> 5.4</b>	-	<b>15.4 <math>\pm</math> 1.7</b>	-

<sup>a</sup> R = not fortified food model, O = fortified with non-encapsulated LO, W = fortified with WM SDEMs, S = fortified with SM SDEMs, P = fortified with PM SDEMs, 1 = fortified with SDEM or non-encapsulated linseed oil to gain 0.6 g ALA per 100 g and 100 kcal, or 2 = fortified to gain 1.2 g ALA per 100 g and 100 kcal.

<sup>b</sup> Different letters (a-c) on the same column separately for each food model indicate a statistically significant ( $p < 0.05$ ) difference between samples.

### 3.2.2. ALA content and oxidative stability

The ALA level of fresh fortified chocolate ranged from 2.4 g to 2.8 g per 100 g (Table 3), which is 0.39 g–0.47 g per 100 kcal, and fulfilled the claim “source of n-3 fatty acids”. S had a significantly higher amount of ALA compared to other chocolate samples. However, this could be related to the initial nutritional calculations indicating that the required amount of SDEM addition was higher than other models. In fresh fortified cookies, the ALA concentration varied from 4.4 to 4.8 g and 8.1–8.6 g per 100 g, which is 0.89–0.97 g and 1.57–1.63 g per 100 kcal, respectively. In addition to the SDEMs, ALA originated from also the margarine. In cookies containing lower concentrations of ALA, the amount of ALA was significantly higher in P compared to S and W (Table 3). Conversely, in cookies with higher ALA concentration, the ALA level was highest in S.

PV value was initially low in both food models but increased during the storage period (Fig. 2). In the chocolates, the amount of primary oxidation compounds was slightly but significantly lower in P followed by S and W (Fig. 2A). The PV level of the stored cookies appears consistent with the findings of Srivastava and Mishra (2021) who fortified cookies with vegetable oil. The hydroperoxide level of S and P cookies was the same or lower compared to R and O, while W exhibited the least effective protection against oxidation (Fig. 2B). Surprisingly, W showed the lowest stability of all models fortified with SDEM, while of the SDEMs WM was the most oxidative stable. Lack of correspondence between the stability of SDEMs and food models has been also previously detected (Damerou et al., 2022a). The differences in behaviour for W compared to P and S might be explained by fact that amino acid composition of PM and SM is more similar than one of WM, which could have caused differences in interactions of wall material of SDEMs with the food matrix and therefore on any possible protecting effects of the food matrix.

The fortification with non-encapsulated LO and SM lowered the stability of chocolate measured by OSI while addition of PM and WM increased it (Table 3). Highest stability was observed for W. Compared to the PV the order of stability was similar as observed for SDEMs with OSI. The addition of non-encapsulated LO in the higher concentration showed a lower OSI than R (Table 3). S1 had a significantly higher OSI than other fortified cookies. This result was in line with PV findings for S1. Overall, the order of stability based on PV and OSI was dissimilar for the food models. Differences were less pronounced with OSI than with PV, possibly because PV measures primary oxidation while OSI measures time until complete oxidation. However, results of both methods combined show a clear trend that fortification with SDEMs improved

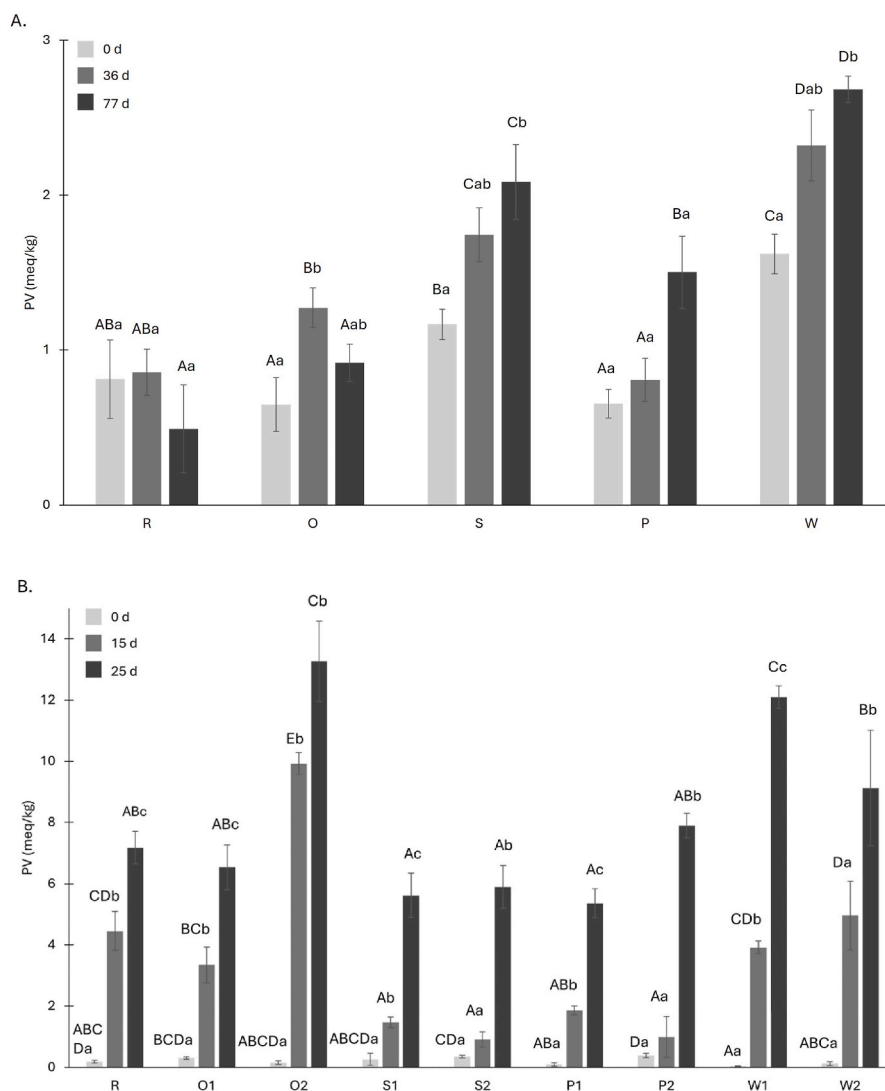
stability of food models compared to direct addition of oil. Further, the findings indicate that interactions with food matrix play a big role in stability of SDEMs in foods. Therefore, stability of SDEMs needs to be studied in foods and cannot be estimated by stability of SDEMs.

### 3.2.3. Volatile profile of the food models

A total of 73 and 96 volatile compounds were identified in the chocolate and cookie models, respectively (Table 4). The most abundant chemical groups were acids and aldehydes. Acetic acid, 3-methylbutanoic acid, and hexanal were the major volatiles in chocolate. In our previous study (Damerou et al., 2022a, Damerou et al., 2022b), acetic acid and 3-methylbutanoic acid were also the highest detected compounds in dark chocolate models. The source of acids in volatile profile of dark chocolate is mainly from the fermentation process of cocoa bean (Afoakwa et al., 2009). Among the identified compounds in cookies, hexanal, pentanal, 3-methylbutanal, and hexanoic acid dominated. They are oxidation indicators previously found from oxidized LO (Gómez-Cortés et al., 2015).

Many volatiles were only formed during storage, while some others reacted further and decreased during storage. The total amount of volatiles increased over the storage period in food models fortified with SDEMs (Table 4). In the beginning of the storage trial, the chocolate fortified with SDEMs demonstrated lower levels of volatiles compared to R and O. In comparison to chocolate, the shell material of SDEMs exhibited distinct behavior in cookies from the start of storage trial. Further, the fortification concentration influenced the content of volatiles, as noted also previously (Damerou et al., 2022a).

In the PCA model for the chocolates (Fig. 3A and B), majority of volatile compounds are clustered on the positive side of PC1 (with 53% of total variation), while only 2-acetylpyrrole is negatively loaded. PC1 predominantly discriminates the samples according to the storage period, with those stored longer positioned on the positive side of PC1. This alignment correlates with most volatiles, a pattern particularly evident in SDEM fortified chocolate compared to the others. Regarding PC2 (16% of variation), it exhibits a positive loading associated with a cluster of volatiles, including 2-methylpropanoic acid, acetic acid, 2-methylbutanoic acid, and alpha-pinene. Additionally, PC2 exhibits a moderate negative loading concerning nonanoic acid and dihydro-3-hydroxy-4,4-dimethyl-2(3H)-furanone. PC2 discriminates between the samples based on both their storage time and the fortification type. Considering the first two PCs, the fresh samples exhibit a cohesive cluster, characterized by negative loadings of PC1 and PC2. In contrast, the stored P and O samples closely resemble the references, while S and



**Fig. 2.** Peroxide values of chocolate (A.) and cookie (B.) models. For chocolate, the storage period of 0 day is marked in light grey, 36 days in grey, and 77 days in dark grey. Corresponding storage periods for cookies were 0 day, 15 days, and 25 days. Different letters indicate significant difference at  $p < 0.05$ , with capital letters (A–E) indicating distinctions between samples at a specific timepoint, and lowercase letters (a–c) denoting variations between timepoints for a specific sample. R = not fortified food model, O = fortified with non-encapsulated linseed oil, W = whey protein concentrate, S = soy protein, P = pea protein as shell material. The number refers to fortification concentration of SDEM or non-encapsulated linseed oil to gain either 1 = 0.6 g or 2 = 1.2 g ALA per 100 g and 100 kcal.

W exhibit more deviations from the Rs. Based on loadings from PC-1 the ascending order regarding volatiles secondary oxidation compounds was R, O, P, S and W. In our previous publication with fish oil (Damerou et al., 2022b) the rising order was R, O, P, W and S. The higher oxidative stability of LO, differences in FA compositions of the oils and differences in EE could contribute to different order regarding formation of volatile oxidation products during storage. The results of volatile oxidation products were in line with PV results but different from OSI result (see 3.2.2.). The OSI is most likely more affected by the food matrix itself than the PV and volatile oxidation product formation as also other compounds than lipids like proteins contribute to a total oxidation measurement.

In the PCA (Fig. 3C and D), most of the cookie samples exhibited compact clustering, with only a few extreme samples such as O2\_3, O2\_4, S2\_3, and S2\_4. Notably, most of the volatiles aligned positively with PC1 (with 60% of the total variation). This was comprehensive as PC1 primarily distinguished samples based on their storage period. Fresh samples clustered towards the negative end of PC1 axis, while stored cookies exhibit a tendency toward the positive end. PC2 (with 12 % of variation) primarily discriminated between the cookies fortified

with higher concentration of SDEMs and the remaining samples grouped closely together in moderately distinct subclusters. Based on the PCA, the oxidative stability order (lowest to highest) was R, P, O, W, and S for lower concentration and R, P, W, O, and S for the higher one. A direct comparison cannot be made with our previous publication on fortification with fish oil (Damerou et al., 2022a) because here vegan cookies were prepared. However, in both studies, the cookie model fortified with SDEM with pea protein exhibited the lowest formation of volatile compounds during storage compared to models with other SDEMs. In both studies an increased fortification concentration had a negative effect on the oxidative stability. Compared to chocolate, the results of cookie models regarding oxidative stability based on volatile secondary oxidation products were not in line with either PV or OSI. This could be based on those differences in measurement as discussed in 3.2.2.

Interestingly, the O models with direct oil additions performed better for fish oil (Damerou et al., 2022a) than for LO regarding oxidative stability. This difference may be attributed to higher EE for LO SDEMs compared to fish oil ones and dissimilarities in FA composition, which resulted in a better oxidative stability and less volatile formation. Overall, P samples resembled most of the R samples as fresh and stored

**Table 4**

The retention times and matches with NIST library of the volatile compounds identified in the chocolate and cookie models, and the peak area magnitudes in fresh references (R\_0) and in the most oxidized samples in the final timepoint, 4, in both chocolate (W\_4) and cookie models (S2\_4). Sample codes refer to Table 1.

No	Compound	RT (min)	Match with NIST20		Chocolate area <sup>a</sup>		Cookie area <sup>a</sup>	
			chocolate	cookie	R_0	W_4 <sup>b</sup>	R_0	S2_4 <sup>b</sup>
<i>acids</i>								
1	formic acid	15.59		905				++
2	acetic acid	17.18	925	959	+++	+++	++	+++
3	propanoic acid	21.80	946		+++	+++	++	+++
4	2-methylpropanoic acid	24.42	951		+++	+++		
5	pentyl ester formic acid	25.42		910				+++
6	butanoic acid	25.75	934	965	+	++	++	+++
7	2,2-dimethylpropanoic acid	26.21	863	911	+		++	+++
8	3-methylbutanoic acid	28.16	943	905	+++	+++		+++
9	2-methylbutanoic acid	28.46	913	815	+++	+++		++
10	pentanoic acid	29.88	852	931	++	+++	++	+++
11	2-pentenoic acid	31.78		882				+++
12	4-methylpentanoic acid	32.61	908		++	++		
13	hexanoic acid	33.73	952	955	+++	+++	++	+++
14	heptanoic acid	37.27		808			+	+++
15	octanoic acid	40.73	841	923	++	++	+	+++
16	2-phenylethyl ester acetic acid	43.48	865		++	++		
17	nonanoic acid	43.98	921	903	++		+	++
<i>alcohols</i>								
18	ethanol	8.17	867	935	++	+++	+	+++
19	2-propanol	9.80	899	903	+	++	+	+
20	propanol	12.99	949		+	+		
21	1,3-butanediol	14.86	805	792	+	+		+
22	butanol	18.80	889		++	+++		
23	1-penten-3-ol	19.31	918	930	+	+++	+	+++
24	pentanol	23.76		937	++	+++	++	+++
25	2-penten-1-ol	24.07	831		+	+++		
26	2,3-butanediol	26.93	904		+	++		
27	2-furanmethanol	28.84		900			+	+++
28	3-octen-1-ol (Z)	34.51		810			+	+++
29	phenylethyl alcohol	39.72	888		++	++		
<i>aldehydes</i>								
30	acetaldehyde	5.80	903	917	++	+++	++	+++
31	2-propenal	8.84		952			+	++
32	propanal	9.13	966	946	+	+++		+++
33	2-methylpropanal	12.01	956	960	+	++		++
34	2-methyl-2-propenal	12.74	960			++		
35	butanal	13.88	952	966	+	++		+++
36	3-methylbutanal	17.16		955			++	+++
37	2-methylbutanal	17.68	917	911	+	++	++	+++
38	2-butenal	17.69		913				+++
39	pentanal	19.51	890	874	++	+++	++	+++
40	2-pentenal	23.19	892	923		++		+++
41	hexanal	24.70	967	972	+++	+++	+++	+++
42	2-methyl-2-pentenal	26.32	944	934	++	+++		+++
43	furfural	27.59		946			+	+++
44	2-hexenal	27.94	912	927		++	+	+++
45	heptanal	29.39	871	940	++	+++	++	+++
46	methional	30.81		900				++
47	2,4-hexadienal	30.92	886	895	+	++		+++
48	2-heptenal (E)	31.20		864				++
49	2-heptenal (Z)	32.50	899	957	+	++	++	+++
50	benzaldehyde	33.21	903	922	+++	+++	++	+++
51	octanal	33.65	954	974	++	++	+	+++
52	2,4-heptadienal (E,E)	34.72	862	920	++	+++	++	+++
53	2-octenal (E)	36.50		962			+++	+++
54	nonanal	37.55	875	942	+++	+++	+++	+++
55	4-oxohex-2-enal	37.93	905	897		+++	+	+++
56	2,6-nonadienal	40.13		942			+	++
57	2-nonenal (E)	40.20		942			++	++
58	decanal	41.06		892			++	+++
59	2,4-nonadienal	42.47		907				++
60	2-decenal (E)	43.71		958			+	++
61	2,4-decadienal (E,E)	46.17		931			++	++
62	2-undecenal	47.56		905			+	++
<i>esters</i>								
63	methyl acetate	10.31	845	877	+	++	+	+
64	2-methyl-1-butyl acetate	24.22		798			++	+++
65	ethyl octanoate	39.96	840		++	+		
<i>heterocyclic compounds</i>								
66	furan	8.57		918			+	++
67	2-methylfuran	13.45	895	911		+	+	++

(continued on next page)

Table 4 (continued)

No	Compound	RT (min)	Match with NIST20		Chocolate area <sup>a</sup>		Cookie area <sup>a</sup>	
			chocolate	cookie	R_0	W_4 <sup>b</sup>	R_0	S2_4 <sup>b</sup>
68	2-ethylfuran	18.57	941	943	+	+++		+++
69	dihydro-2-methyl-3(2H)-furanone	25.49		916				+++
70	methylpyrazine	25.86	850	915	+	++	+	+++
71	2,5-dimethylpyrazine	29.69		825			+	+++
72	2,3-dimethylpyrazine	30.22	905		+	++		
73	acetylfuran	30.89		882				+++
74	2-pentylfuran	32.18	919	936	++	+++	++	+++
75	2-(2-pentenyl)furan	32.72		877			++	+++
76	2(5H)-furanone	33.36		922			++	+++
77	trimethylpyrazine	16.74	888		++	++		
78	5-methyl-2(5H)-furanone	34.10		919				+++
79	tetramethylpyrazine	36.56	954		+++	+++		
80	linalool oxide	37.19	830		++	++		
81	2-acetylpyrrole	37.95	860		++			
82	5-ethylidihydro-2(3H)-furanone	38.54	903	942		++	+	+++
83	dihydro-3-hydroxy-4,4-dimethyl-2(3H)-furanone	38.70	883		++	++		
84	tetrahydro-6-pentyl-2H-pyran-2-one	39.54		847			+	++
85	maltol	39.74		962			++	+++
86	tetrahydro-6-methyl-2H-pyran-2-one	40.40		945				++
87	dihydro-5-propyl-2(H)-furanone	42.12		867			+	+
88	5-butylidihydro-2(3H)-furanone	45.86		857				++
89	tetrahydro-6-propyl-2H-pyran-2-one	47.36		919				++
90	dihydro-5-pentyl-2(3H)-furanone	50.29		879			+	+
<i>hydrocarbons</i>								
91	pentane	7.84	862	918	+	++	+	++
92	2-methylpentane	10.60	950	903			+	
93	3-methylpentane	11.34	937	921	+		+++	++
94	heptane	17.42		946			+++	+++
95	1-nonene	27.06		900				++
96	2,4-nonadiene	30.30		786				+++
97	1-decene	31.39		891				++
98	decane	31.59	936		+	++		
99	2,6-dimethyldecane	35.34	901		++	+++		
100	undecane	35.47		932			++	++
101	dodecane	36.39	890		++	++		
<i>ketones</i>								
102	2,3-butanedione	14.04	883		+	++		
103	2-butanone	14.44	941	952	+	+++		+++
104	1-penten-3-one	19.05	892	870		++		+++
105	2-pentanone	19.19	980	978	+	++		+++
106	3-penten-2-one	22.32		944				+++
107	2-hydroxy-3-pentanone	26.45	873	819		++	+	+++
108	3-hexen-2-one	27.19		915				+++
109	2-heptanone	29.02	798	917	++	++	+	+++
110	3-hydroxy-3-methylbutanone	30.64		869				++
111	6-methyl-5-hepten-2-one	33.09		876			++	+++
112	2-octanone	33.21		839			++	+++
113	3-octen-2-one	35.59		905			+	+++
114	4-nonanone	36.17		861				++
115	3,5-octadien-2-one	37.48	924	919	++	+++	+	+++
116	2-nonanone	37.13	873	932	++	++	+	++
<i>terpenes</i>								
117	pinene, alpha-	29.79	938		+++	+++		
118	pinene, beta-	32.00	869		++	++		
119	3-carene	33.07	949		+++	+++		
120	D-limonene	33.79	926	849	+++	+++	++	+
121	linalool	37.25	821		++	++		

<sup>a</sup> Samples marked with "+++" belong to the top 75th percentile, samples with "++" belong to the 25th to 75th percentile, and samples with "+" belong to the bottom 25th percentile when considering all volatile compounds across all samples of either the chocolate or cookie model.

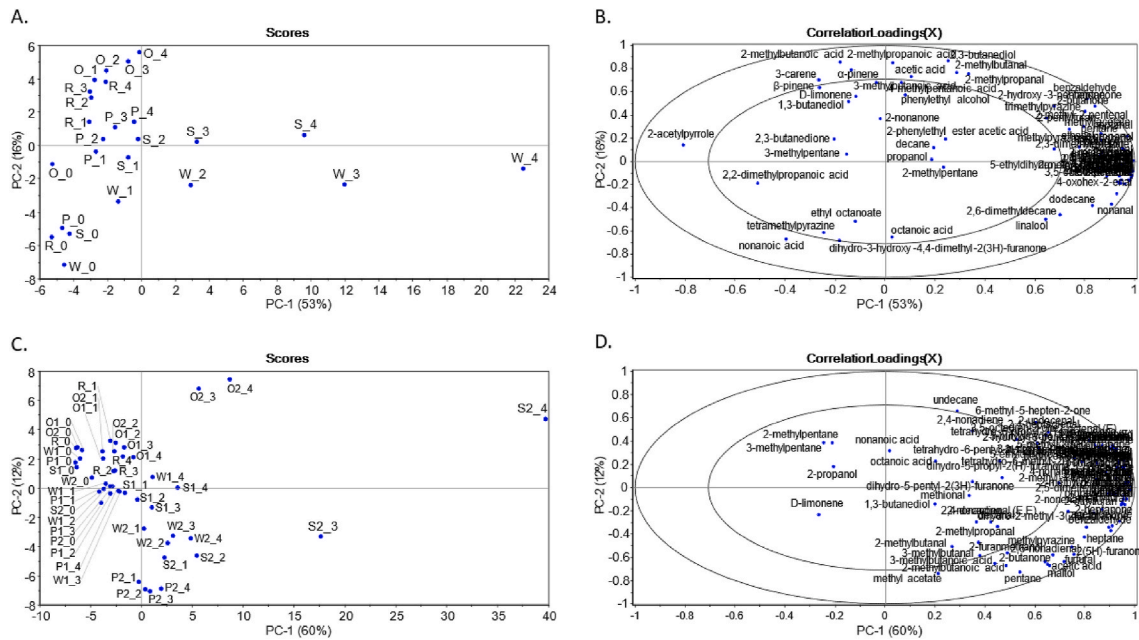
<sup>b</sup> The sample exhibiting the greatest dissimilarity from the reference sample based on the PCA model (Fig. 3).

for both food models. The P samples also performed similar or better than R samples in terms of oxidative stability by PV or OSI although PM had the lowest EE of all SDEMs. This shows again that the quality of SDEMs does not directly transfer to performances of an SDEM in the food models. More knowledge on interactions of wall material of SDEMs and food matrix is needed to predict the performance of SDEMs in food fortification.

### 3.3. Sensory quality of food models

#### 3.3.1. The performance of the assessors and the panels

Both panels effectively discriminated between the samples (Supplementary Tables 3 and 4). Strong consensus among the appearance and the texture attributes contributed to effective discrimination and repeatability. Certain assessors encountered challenges with some odor or flavor attributes, but because omitting any participants did not noticeably influence the results, all assessors were retained in analyses.

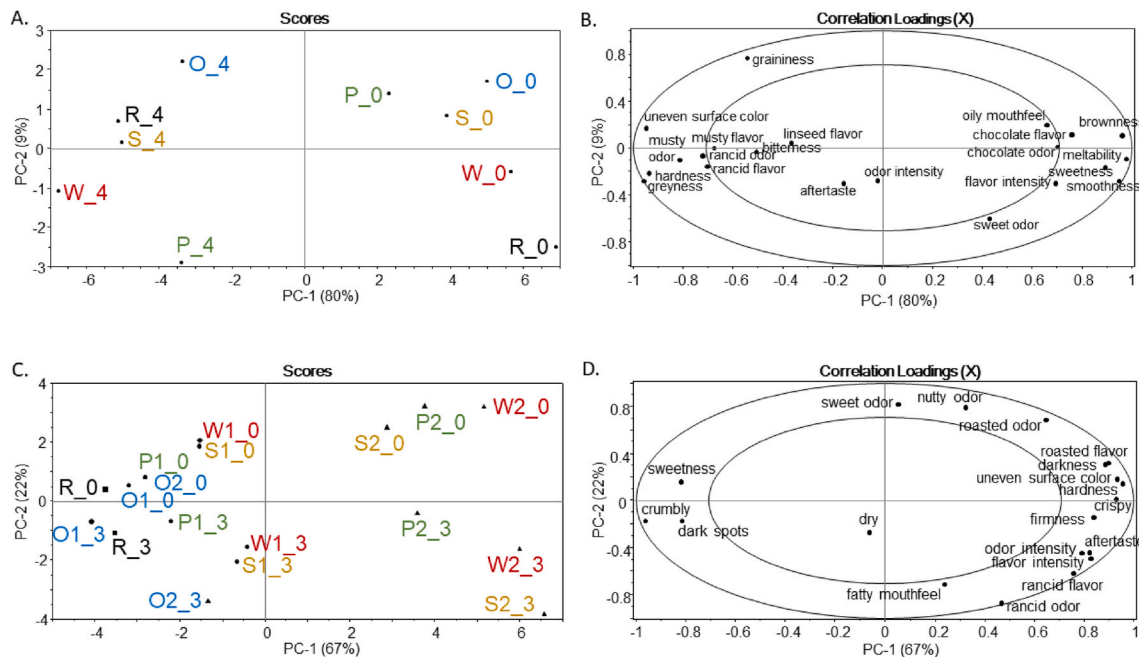


**Fig. 3.** PCAs of identified volatile compounds in chocolate (A: samples and B: volatiles) and cookie (C: samples and D: volatiles). R = not fortified food model, O = fortified with non-encapsulated LO, W = fortified with WM SDEMs, S = fortified with SM SDEMs, P = fortified with PM SDEMs, 1 = fortified with SDEM or non-encapsulated linseed oil to gain 0.6 g ALA per 100 g and 100 kcal, or 2 = fortified to gain 1.2 g ALA per 100 g and 100 kcal. The number following the underscore refers to the timepoint.

3.3.2. Sensory profile of dark chocolate model

The first principal component (Fig. 4A and B; PC1 with explained variance of 80%) separates the samples into two distinct groups according to their storage time. PC1 is positively loaded with meltability, smoothness, brownness, and sweetness, which are characteristic of the fresh samples. In contrast, the stored samples, negatively loaded on PC1, are associated with greyness, hardness, and uneven surface color.

Moreover, significant differences were observed in these attributes between fresh and stored samples (Supplementary Table 5). However, Razavizadeh and Tabrizi (2021) observed no statistical differences in the liking of sensory characteristics of milk chocolate fortified with chia seed oil encapsulated with soy protein, maltodextrin, and inulin, possibly due to the panelists' inability to discriminate the samples. The inconsistency may be due to the higher core to wall ratio of SDEMs in



**Fig. 4.** Consensus PCA scores and correlation loadings plots of the descriptive sensory profiles of dark chocolate (A: samples and B: sensory attributes) and cookie (C: samples and D: sensory attributes) models. In scores plots: R = not fortified food model (black), O = fortified with non-encapsulated LO (blue), W = whey protein concentrate (red), S = soy protein (yellow), P = pea protein (green) as shell material. In 4C, the first number refers to fortification concentration of SDEM or non-encapsulated linseed oil either 1 = to gain 0.6 g ALA per 100 g and 100 kcal (circles) or 2 = to gain 1.2 g ALA per 100 g and 100 kcal (triangles). The number following the underscore refers to the timepoint.

our study leading to noticeable sensory differences.

The influence of shell material was more evident in the stored samples (Supplementary Table 5). Sample P was rated as harder, smoother, and less grainy with a more uneven surface color than R, whereas they did not differ in the odor or the flavor attributes. At the same time, S and W exhibited closer resemblances to R in appearance and texture, but they diverged in the flavor attributes. The S and W were rated significantly higher in the LO flavor, the rancid flavor, and the musty flavor compared to P and R. Additionally, rancidity significantly increased in S and W during storage, a change not observed in the other samples. Taken together, the pea protein offers more effective protection against lipid oxidation and exhibits better flavor masking capabilities in chocolate compared to soy or whey proteins, which is in accordance with our previous research (Damerou et al., 2022a). However, soy protein emerged also as a suitable encapsulation material in the previous study.

### 3.3.3. Sensory profile of shortbread cookie model

In the PCA model (Fig. 4C and D), the samples are clustered in four groups based on the oil/SDEM concentration and the storage time. PC1 primarily separates the cookies in accordance with the concentration. The cookies fortified with lower oil/SDEM concentration are collectively linked with attributes such as crumbly, sweet, and dark spotted. Conversely, the samples with higher concentration are typified as hard, uneven color, crispy, roasted flavor, and dark. The results seem to be consistent with the findings of our previous fish oil study (Damerou et al., 2022a). The PC2 further distinguished between the two clusters, separating the samples into fresh and stored ones. Fresh chocolates correlated with sweet odor and moderately with nutty odor, while the stored were more associated with fatty mouthfeel and rancid odor.

Rancidity increased significantly in fortified cookies due to storage time and oil/SDEM concentration (Supplementary Table 6). Highest rancidity was observed in stored S2 and W2, respectively. Among the encapsulation materials, pea protein showed the most effective protection against rancid flavor and odor, followed by whey and soy, respectively. The results appear to corroborate our earlier findings in Damerou et al. (2022a) where flavor masking capacity was most prominent in pea protein samples.

## 4. Conclusions

Encapsulation by spray-drying, utilizing pea, soy, and whey protein in conjunction with maltodextrin, significantly improved the oxidative stability of LO capsules without significant loss of the n-3 PUFAs. The most effective protection against oxidation was achieved by combining whey protein with maltodextrin. However, the versatile multidisciplinary approach used in our study, spanning from chemical analyses to sensory evaluations, demonstrated that this behaviour did not translate to their use in high fat food models, dark chocolate and shortbread cookie. Volatile profiles exhibited distinguishable differences based on fortification concentration and encapsulation materials in both food models. Storage led to an increase in volatiles, particularly pronounced in soy and whey containing samples. Food models fortified with pea protein capsules demonstrated a closer resemblance to the control than the other spray-dried samples. Increasing oil content had a detrimental effect on oxidative stability. Surprisingly, the volatile profile of sample with non-encapsulated LO resembled closely that of the control sample in both food models.

The addition of LO or spray-dried emulsions in chocolate increased the graininess and decreased the meltability of chocolate models. The chocolate flavor decreased in soy and whey samples during storage while LO, musty and rancid flavors increased. The pea sample resembled most closely to the control sample. Interestingly, soy exhibited most similar appearance and texture properties to the control sample. Encapsulation influenced the appearance, odor, flavor, and texture of both fresh and stored cookies. Especially, the SDEM addition increased darkness, rancid flavor, roasted flavor, and hardness. Similarly, as in

chocolate samples, the pea sample was the closer to the control sample than other encapsulation samples.

While certain hypothesized plant protein formulations demonstrated promise, non-encapsulated LO fortification also yielded favorable outcomes based on sensory perception and volatile profiles in these high-fat food models. Pea protein emerged as an intriguing choice for shell material in spray-drying of LO. Further research is necessary to elucidate its performance in different foods with varying fat and water content.

## CRedit authorship contribution statement

**Sari A. Hakanen:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis. **Annelie Damerou:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Dorota Ogrodowska:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Conceptualization. **Annalisa Seubert:** Writing – review & editing, Investigation. **Waldemar Brandt:** Resources, Methodology. **Oskar Laaksonen:** Writing – review & editing, Supervision, Methodology. **Małgorzata Tańska:** Writing – review & editing, Writing – original draft, Investigation. **Kaisa M. Linderborg:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

## Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT 3.5 in order to improve readability and language. After using this service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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## 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.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2024.117305>.

## Data availability

Data will be made available on request.

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