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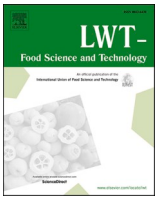

Encapsulating capacity of ultra-high-pressure homogenization (UHPH): Replacement of milk fat by vegetable oils using buttermilk as a functional ingredient in yogurt processing

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ABSTRACT

This study investigated the characteristics of yogurts produced by substituting dairy fat with polyunsaturated fatty acids (PUFA)-rich oils encapsulated with buttermilk (BM) in spray-dried emulsions (SDE). Two homogenization methods, conventional (CH) and ultra-high-pressure homogenization (UHPH), were compared to obtain the emulsions for spray drying. Recombined milks (RMs) were formulated using two different concentrations (4 g/100 g and 6 g/100 g) of SDE, followed by fermentation. Yogurt characteristics were evaluated during cold storage using various parameters, including coagulation properties, texture and rheology, microstructure, physicochemical characteristics (color, pH, total acidity, and water holding capacity), oxidative stability, main fatty acid profile, microbial assessment, and sensory evaluation. During cold storage, several parameters significantly influenced the yogurt characteristics. The CH yogurts exhibited higher textural parameters (firmness and consistency) and viscoelastic parameters (G′ and G′′) compared to the UHPH yogurts at the same SDE concentration. However, UHPH yogurts generally showed better water holding capacity (WHC) values. UHPH yogurts also demonstrated superior stability to oxidation and higher PUFA content. The observed differences between the CH and UHPH treatments can be attributed to the structuring of fat-protein-BM into colloidal particles based on the homogenization system employed in this study. Neither of the homogenization systems nor the SDE content impacted yogurt flavor.

1. Introduction

Functional foods have gained significant popularity in international markets over the past two decades due to their diverse health promoting benefits (Liu et al., 2019). Omega-3 fatty acids (FA) are essential for various biochemical processes, cell membranes, brain development, and physiological functions in the human body. The global market for omega-3 FA supplements reached a value of USD 5,580,000 in 2020, with an expected annual growth rate of 8.6% from 2020 to 2028 (Grandview Research, 2020).

To meet the demand for fats and oils rich in polyunsaturated FA (PUFA), fortification of commonly consumed foods with these fatty acids has become prevalent in the food industry. However, during processing, distribution, and handling, PUFA are susceptible to oxidation, resulting in off-flavor and a decrease in quality and health promoting benefits (Uluata, McClements, & Decker, 2015). Encapsulation strategies have been extensively developed to protect PUFA from oxidation during processing and storage, focusing on the emulsification system and compounds employed to shield them. Various compounds, such as proteins, carbohydrates, and low molecular weight emulsifiers are commonly used to create stable emulsions for further drying and encapsulation of oil droplets into microcapsules (Ruiz, Ortiz, & Segura, 2017).

Buttermilk (BM), a co-product of butter manufacturing, is an inexpensive and readily available ingredient in the food industry. It possesses emulsifying capacity and functionality, attributed to the polar lipids and proteins present in the milk fat globule membrane (MFGM) (Barry, Dinan, & Kelly, 2017). These compounds have also been associated with notable health promoting benefits, including improved brain cognitive development and immune system support for optimal growth in infants (Hernell, Timby, Domellöf, & Lönnerdal, 2016; Singh & Gallier, 2017). Moreover, commercial BM has demonstrated excellent

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encapsulating properties, surpassing skim milk powder when used to encapsulate fish oil (Augustin et al., 2015). It also mimics the natural milk fat globule membrane due to its polar lipid composition, potentially enhancing the digestion and utilization of encapsulated omega-3 FA (Zhang et al., 2020).

The successful encapsulation of lipophilic compounds, such as PUFA-rich oils, requires the formation of stable emulsions prior to dehydration. Emulsions are thermodynamically unstable colloidal systems, prone to creaming, aggregation, and coalescence. High-energy mechanical devices such as high-pressure homogenizers or ultrasonic generators are commonly employed in the industry to produce stable emulsions by reducing droplet size (Floury, Desrumaux, & Lardières, 2000; Weiss, Takhistov, & Julian, 2006).

Ultra-high-pressure homogenization (UHPH) is a versatile technology operating at pressures up to 350 MPa, compared to conventional homogenization (CH) typically ranging between 20 and 50 MPa. UHPH can inactivate microorganisms and enzymes, produce submicron emulsions with excellent physical stability, and induce changes in colloidal structures. The composition of emulsifying agents used in UHPH can influence the restructuring of the protective layer of droplets, impacting the techno-functional properties (Dumay et al., 2013; Sato, Matsumiya, Kaneko, Okazaki, & Matsumura, 2021).

Spray drying is a cost-effective method widely used in the food industry to convert emulsions into easy-to-handle, transport, and to preserve powder form. Encapsulation through spray drying enables the production of small-size particles resistant to chemical and physical damage, ensuring protection against oxidation, light, and temperature (Di Giorgio, Salgado, & Mauri, 2019; Geranpour, Assadpour, & Jafari, 2020).

Yogurt, a popular dairy product, is known for its natural composition rich in nutrients and numerous health promoting benefits attributed to fermentation (Donovan & Rao, 2019; Gumus & Gharibzahedi, 2021; Kok & Hutkins, 2018). It is an ideal candidate for nutrient fortification. Substituting milk fat with PUFA-rich oils emulsified with MFGM components from buttermilk presents an opportunity to develop a balanced functional food. The production of spray-dried emulsions (SDE) containing PUFA-rich oils stabilized by UHPH has been recently investigated (Varela et al., 2022), demonstrating superior properties compared to SDE produced by CH. This study aims to evaluate the influence of homogenization type (CH and UHPH) used to produce SDE and the concentration of SDE added to stirred yogurts on the relevant quality characteristics of the final product.

2. Material and methods

2.1. Materials

Materials used for yogurt manufacturing, UHT skim milk, and skim milk powder (SMP), were purchased from a local supermarket. According to the supplier, SMP contained 32.5 g/100 mL protein, 1 g/100 mL fat, and 54.5 g/100 mL carbohydrate. Lyophilized yogurt culture, composed of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* Y0-MIX 300 LYO 10 DCU, was purchased from Danisco (Buxières, France). Refined sunflower was purchased from Gustav Heess Company (Barcelona, Spain). The characteristics and composition according to the producer were: acid value = 0.1 g/100 mL (mg KOH/g); peroxide value (meq O_2/kg) = 0.02, fatty acid composition (15–85 g/100 mL C-18:1, 50–72 g/100 mL C-18:2). Crude Chia oil was as follows: acid value = 0.27 g/100 mL (mg KOH/g), peroxide value = 1.5 (meq O_2/kg). Fatty acid composition (6.35 g/100 mL C-18:1, 19.29 g/100 mL C-18:2, 64.85 g/100 mL C-18:3) according to the specifications was obtained from Interfat Natural Oils (Barcelona, Spain). All other chemicals used were of analytical or better grade.

2.2. Emulsion and yogurt preparation

Preparation of emulsion and spray-dried emulsion (SDE) were previously and fully described (Aghababaei, Cano-Sarabia, Trujillo, Quevedo, & Ferragut, 2021). The SDE composition used for yogurt manufacturing was: 7 g/100 g BM, 30 g/100 g maltodextrin (MD), 10 g/100 g oil, 47 g/100 g total solids, and 3 g/100 g moisture. Four different recombined milks (RMs) to produce yogurts were prepared by varying the percentage of SDE added as well as the type of homogenization used to obtain the SDE. Thus, the nomenclature of yogurts indicates these two variables as follows: CH4 (4 g/100 g SDE treated by CH); CH6 (6 g/100 g SDE treated by CH); UH4 (4 g/100 g SDE treated by UHPH); and UH6 (6 g/100 g SDE treated by UHPH). To prepare the RM for further preparation of yogurts, UHT skim milk was mixed with 3 g/g SMP and 4 or 6 g/100 g SDE. RMs were stirred with a blade stirrer for 10 min and were further pasteurized in batch at 82 °C for 20 min. After cooling of the mixes, they were stored at 4 °C overnight to complete powder hydration. The next day, RMs were heated to 45 °C, inoculated with 0.02 g/100 g of the starter culture Y0-MIX 300 LYO 10 DCU (Danisco, Buxières, France) containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus,* and incubated at 43 °C until pH 4.6 was reached. Yogurts were stored at 4 °C for 24 h and further stirred with a Jata FP500P rod blender (Tudela, Navarra, Spain) for 1 min clockwise to breakdown the gels. Then, stirred yogurts were transferred to sterile media bottles and stored at 4 °C until analysis.

2.3. Evaluation of coagulation properties

The coagulation process was monitored on inoculated RM (10 mL) at 43 ± 2 °C using an Optigraph System (Ysebaert Dairy Division, Frépillon, France) which is a measure based on the attenuation of near-infrared region (NIR) signal. From the coagulation curves (optical signal *vs* time), three parameters were obtained (Fig. 1): onset of gelation (OG) obtained from the maximum of the second derivative; aggregation rate (AR), calculated from the slope of the linear part of the curve; and final gel firmness (FGF) at the end of the coagulation, obtained by the difference between the final and initial values of the optical signals.

The acidification curves of RM during the fermentation process were performed with a Cinac32 equipment (Ysebaert Dairy Division, Frépillon, France), by continuous monitoring of pH. Inoculated RMs (100 mL) were poured into 250 mL Erlenmeyer flasks at 43 °C in a water bath until yogurts reached pH 4.6.

2.4. Texture, rheology, and microstructure

Textural characteristics were evaluated by using a Texture Analyzer TA.TX2 (Stable Micro Systems, Surrey, UK) equipped with a flat cylindrical probe 35 mm diameter, applying a constant speed of 1 mm/s up to a depth of 5 mm. A back-extrusion test was performed to determine

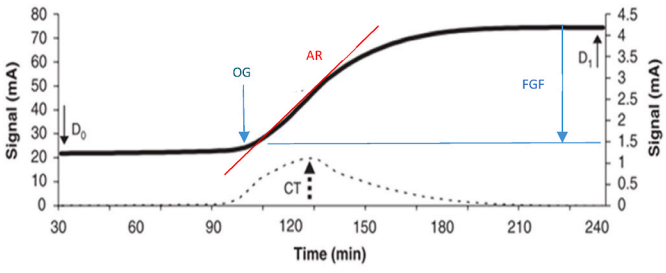

Fig. 1. Coagulation curves as a function of time. CT is the time at the maximum of first derivative value (dotted line). AR is the aggregation rate, which is the slope of the plot over the coagulation period. Final gelation firmness (FGF) was calculated as D1–D0.

firmness (maximum force in compression) and consistency (area under the compression curve) (Liu, Xu, & Guo, 2007). Briefly, 60 g of yogurt at 4 °C was poured into an 80 mm height, 45 mm diameter plastic container. The analysis was conducted in yogurts at the refrigeration temperature, about 4 °C.

Dynamic oscillatory testing of stirred yogurts was performed using a rheometer Thermo Haake RS1 (Thermo Electron Corporation, Karlsruhe, Germany) with 20 mm diameter parallel serrated plates probe with 2 mm gap at 4 °C. One spoonful of the sample was carefully loaded onto the plate of the rheometer, and once the measure position was reached, the sample was left to stand for 5 min before the test started. Frequency sweep was performed in the range of 0.1–10 Hz, in the viscoelastic linear region previously determined. The viscoelastic parameters, storage modulus (G′) and loss modulus (G′′) were recorded.

Confocal laser scanning microscopy (CLSM) observations were performed in a Leica TCS SP2 AOBS microscopy (Leica, Heidelberg, Germany). The protein matrix was stained using 10 mg of fluorescein isothiocyanate (FITC) (Fluka, Sigma- Aldrich, Germany) completely dissolved in ethanol. Then, it was added to 5 mL of preheated and inoculated RMs. After incubation at 43 °C for 4 h, yogurts were kept overnight at refrigeration temperature and further stirred. Microscope observations were made with × 63 oil immersion objective at wavelength of 488 nm and with 1024 × 1024 format.

2.5. Physicochemical characteristics of yogurts: color, pH, total acidity, and water holding capacity (WHC)

The color of yogurts was assessed with a colorimeter Konica Minolta CR-410 (Konica Minolta, Osaka, Japan), with a reference D65 light source and a 10° observer angle. The CIE L* a* and b* color coordinates were obtained. The following formulas were utilized to determine the Yellowness Index (YI) and Whiteness Index (WI), respectively.

$$YI = 142.86 \times b^* \times L^{*-1}$$

$$WI = 100 - [(100 - L^*) + a^{*2} + b^{*2}]^{0.5}$$

The pH of yogurts was measured using Crison Basic 20 pH-meter (Crison Instruments S.A., Alella, Spain). Titratable acidity (TA) was determined according to the ISO/TS 11869:2012 method.

The WHC was determined by centrifuging 40 g of yogurt at 5000 *g* for 20 min at 22 °C to separate the supernatant (whey) in triplicate on days 1, 14, and 28. The following equation was used to calculate WHC g/100 g:

$$WHC\ g/100\ g = (W_1 - W_2)/W_1 \times 100$$

Where: W_1 =Initial yogurt weight, W_2 = Weight of whey after centrifugation.

2.6. Stability to oxidation

Quantification of hydroperoxides and malondialdehyde in yogurts was performed by the methods proposed respectively by Hu, Julian, and Decker (2003) and Papastergiadis, Mubiru, Van Langenhove, and De Meulenaer (2012) which were carried out with slight modification and were fully described previously (Varela et al., 2022).

2.7. Fatty acid composition of yogurts

Oleic, linoleic, and α-linolenic acid content of yogurts fortified with 4 and 6 g/100 g of SDE was determined on days 1 and 28 of storage by using a gas chromatograph HP-6890 Series GC System (Hewlett-Packard, Waldbronn, Germany) equipped with a flame ionization detector and a HP-6890 Series Injector. A capillary column VF-5 ms (Agilent Technologies, California, U. S.), 30 m × 0.25 mm with 0.25 μm film thickness containing 5% phenyl-methylpolysiloxane was used. The procedure reported by Bondia-Pons, Moltó-Puigmartí, Castellote, and López-Sabater (2007) was used with a slight modification. Gas chromatography conditions were as follows: separation of fatty acid methyl esters (FAMEs) was carried out, the carrier gas was helium with a head pressure of 220 kPa, the split ratio was 50:1 and the injection volume were 1 μL. The oven and detector temperature were 250 °C and 260 °C, respectively. The temperature program was as follows: initial temperature of 100 °C was increased at 8 °C/min to 180 °C and held at this temperature for 9 min and further increased at 1 °C/min to 230 °C and held at this temperature for 10 min (total run time: 84 min). Detector gas flow: H_2, 40 mL/min; make-up gas (He), 25 mL/min; air, 400 mL/min.

2.8. Microbiological analysis

Mesophilic aerobic bacteria, aerobic spores, coliform bacteria, and *E. coli* were determined on RM samples. Samples were serially diluted in peptone water (Oxoid, Basingstoke, UK) and plated on Plate Count Agar (PCA, Oxoid) to enumerate mesophilic aerobic bacteria. Viable counts of spores were enumerated after the serial dilutions of samples were heated at 80 °C for 10 min, quickly cooled, poured into PCA plates, and incubated at 30 °C for 48 h. For the enumeration of coliforms/*E. coli*, serial dilutions were plated on the surface of Chromogenic Coliform Agar (CCA) and incubated at 37 °C for 24 h. *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*) and *Streptococcus thermophilus* were enumerated according to ISO 7889:2003. For each treatment, samples were analyzed during storage at 4 °C on days 1, 14 and 28. Yogurt samples were serially diluted from 10^{-1} to 10^{-6} in peptone water. *L. bulgaricus* and *S. thermophilus* were counted in the selective media MRS and M17, under anaerobic conditions, at 37 °C for 72 h and 37 °C for 48 h, respectively. All samples were analyzed in duplicate.

2.9. Sensory evaluation

A panel of 20 university faculty and staff members who were familiar with yogurt (mean age = 31 years, with an age range between 22 and 61 years), were asked to identify differences. The sensory evaluation consisted of triangle, descriptive, and preference tests. Two triangle tests were carried out on days 7 and 14 after yogurt manufacture to assess whether the judges could find significant differences between the two treatments of CH4 *vs* UH4 and CH6 *vs* UH6 or not. Once the results of the first triangle test had been analyzed, the effect of the concentration incorporated into the yogurts (UH4 *vs* UH6) was compared using another triangle test on day 14. A descriptive test based on a 7-point intensity scale (1: dislike extremely; 7: like extremely) was conducted to identify the different attributes (creaminess, consistency, acidity, strange aroma, and lactic aroma) in the stirred yogurt. Finally, the judges were asked to rank the samples of yogurt between treatments UH4 and UH6 in order of preference.

2.10. Statistical analysis

Effects of different levels of SDE (treated with CH and UHPH) addition, and storage time on physical, chemical, and sensory parameters were analyzed based on one-way and two-way analysis of variance (ANOVA) test using the Minitab Express™ version 1.5.3 (Minitab, State College, PA, USA). Significant differences between means were determined by the Tukey test. A confidence level of 95% ($P < 0.05$) was used. At least three individual productions of each formulation and treatment were performed.

3. Results and discussion

3.1. Acid coagulation process

During the acidification process of yogurt, the milk caseins become unstable and coagulate, forming a protein matrix that entraps the

aqueous phase and oil droplets, with varying degrees of interaction between them (Alexander & Dalgleish, 2004).

Table 1 shows the acid coagulation parameters of samples incubated at 43 °C. Evolution of pH during the coagulation process is depicted in Fig. 2. The onset of gelation (OG) occurred significantly earlier in UHPH-RMs compared to CH-RMs. Additionally, increasing the percentage of added SDE (spray dried emulsion) resulted in earlier OG. These results could be attributed to the smaller size of oil droplet aggregates in UHPH-RMs, which had a larger effective surface area compared to the individual oil droplets in CH-RMs (Varela et al., 2022). The smaller aggregates in UHPH-RMs contribute to the earlier onset of gelation, as observed in previous studies (Serra, Trujillo, Quevedo, Guamis, & Ferragut, 2007). However, once gelation started, the aggregation rate (AR) and the final gel firmness (FGF) were both higher in CH-RMs. In previous studies (Aghababaei et al., 2021; Varela et al., 2022), liquid emulsions treated by the two different homogenization systems showed different morphology of colloidal structures. The colloidal structures from CH treatments presented larger spherical droplets, whereas those treated with UHPH, especially those treated at 200 MPa, presented a greater number of asymmetric aggregates of smaller size than the previous ones. It is to be expected that when reconstituting the SDEs to form the RMs, these colloidal structures were maintained to a greater or lesser extent. In yogurt, it is known that the predominant interactions in gel formation are of the casein-casein type, so it could be hypothesized that the higher AR and FGF observed in CH yogurts could be due to the predominance of these interactions, since there would be more continuity of casein for their interaction. In contrast, in UHPH yogurts, the greater amount of particle aggregates would act as disruptors of the continuity of these interactions.

The acidification curves of the yogurts did not show significant differences between treatments, indicating similar kinetics of fermentation ($P < 0.05$). The overlapping acidification curves in Fig. 2 demonstrate that pH 4.6 was reached after approximately 198 ± 3 min. This aligns with the findings of previous studies, which reported incubation times of about 183–240 min to reach the desired pH (Horiuchi et al., 2009; Soukoulis, Panagiotidis, Koureli, & Tzia, 2007).

3.2. Texture, rheology, and microstructure

Texture parameters, specifically firmness and consistency, of the yogurts were assessed using back extrusion testing during storage at 4 °C. Firmness refers to the maximum force applied during compression, while consistency indicates the thickness of the sample (Ciron, Gee, Kelly, & Auty, 2010). Back extrusion testing provides insights into the behavior of the sample as it flows back under applied compression, leading to the destruction of the gel microstructure.

On the first day, there were no significant ($P > 0.05$) differences in texture parameters between the various yogurt samples. At this early stage, when the stirred gel has not settled yet, the main factor influencing yogurt texture is likely the crystallized fat under refrigerated conditions (Serra et al., 2007), which was consistent across all yogurts. However, as the gel structure settled on days 14 and 28, some similarities and differences emerged between the yogurts during storage. CH6 and UH4 yogurts appeared to exhibit similar firmness and consistency, suggesting that the flow during the compression of the gel resulted in the formation of yogurt flocs of similar size. However, UH6 yogurts showed the lowest values of both firmness and consistency. Although it may seem contradictory that increasing SDE concentration in UHPH yogurts displayed the lowest texture parameter values, this could be explained by the previous studies performed in the emulsions and SDE with the same compositions as in the present study (Varela et al., 2022). In this study, the oil was present in the UHPH-treated emulsions in the form of aggregated droplets through proteins. These colloidal structures in higher concentrations in UH6 yogurts could act as disruptive particles, interrupting the continuity of the casein network. As a result, more open spaces were present in the stirred yogurts, as depicted in Fig. 3, which ultimately led to reduced firmness and consistency of those yogurts. Ciron et al. (2010) in low-fat stirred yogurts also described the formation of fat globule aggregates when comparing conventional homogenization and microfluidization at 150 MPa. Microscopic observations revealed that microfluidized milk-based yogurts exhibited more open spaces compared to those made with conventionally treated milk, impacting the texture parameters obtained

Table 1
Coagulation parameters of yogurts containing different content (4 and 6%) of CH and UH SDE.

Sample	OG (min)	AR (mA min^{-1})	FGF (mA)
CH4	115±1^{a}	0.35 ± 0.01^{b}	41.3 ± 0.4^{b}
CH6	110.4 ± 0.2^{b}	0.372 ± 0.001^{a}	45.3 ± 0.3^{a}
UH4	106.5 ± 0.3^{c}	0.332 ± 0.001^{c}	37.8 ± 0.5^{c}
UH6	102±1^{d}	0.346 ± 0.003^{b}	40.5 ± 0.4^{b}

Means in each column with different superscript letters were significantly different ($P < 0.05$).
OG: onset of gelation; AG: aggregation rate; FGF: gel firmness at the end of gelation (n = 3).

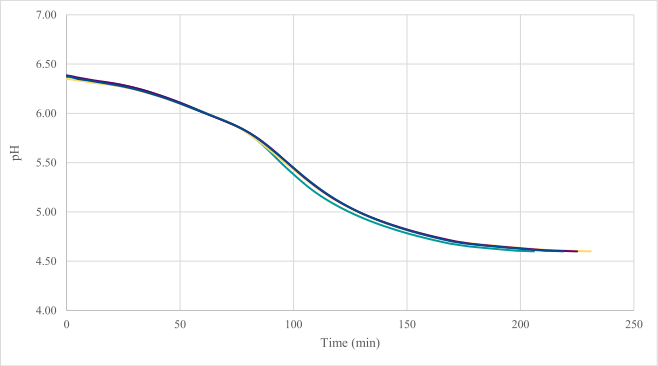

Fig. 2. pH curves as a function of time during fermentation of the different types of yogurts. Green: CH4, Yellow: CH6, Purple UH4, and Blue: UH6.

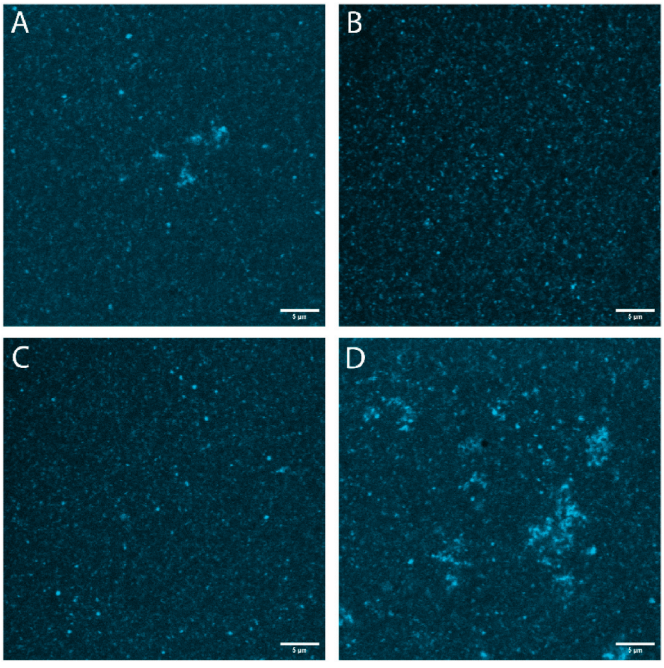

Fig. 3. CLSM images of the different types of yogurts: CH4 (A), CH6 (B), UH4 (C) and UH6 (D).

through back extrusion testing.

Dynamic oscillatory testing with small amplitudes within the linear viscoelastic region allowed the assessment of interaction strength responsible for the network formation in semi-solid and solid foods without disrupting their structure. G' (storage modulus) represents the intensity and/or number of interactions contributing to network formation, while G'' (loss modulus) relates to the viscous nature and interactions that do not directly affect the three-dimensional network (Tabilo-Munizaga & Barbosa-Cánovas, 2005). All yogurts exhibited characteristic solid behavior, with higher G′ values than G'' values, as shown in Table 2. CH yogurts consistently displayed significantly higher ($P < 0.05$) G′ values than UHPH yogurts throughout the storage period. Moreover, the increase in viscoelastic parameters with the addition of higher percentages of SDE aligned with this difference, as expected. Furthermore, all yogurts showed a significant increase in both G′ and G'' from day 1 to day 28 of cold storage, indicating the settling of the gel structure produced by increased protein-protein interactions, favored, among other factors, by pH decreasing over storage.

3.3. Evaluation of color, pH, acidity and WHC

Consumer preference is influenced by the immediate perception of color, which is a fundamental characteristic. The color coordinates (L*, a*, b*) of the yogurts were measured during storage, with L* values representing luminosity, the white achromatic attribute. L* values for all yogurts and storage periods were similar, ranging from 82.9 to 83.9. Stirred yogurt, characterized by a rough surface due to the gel particles formed during beating, exhibited a slightly lower diffuse reflection than expected. However, neither the type nor the content of SDE added to the RM had a significant influence on luminosity. The a* (green-red) and b* (blue-yellow) coordinates indicated positive values, with red and yellow contributing to the chromatic component of the color, respectively. The a* values ranged from 1.51 to 1.83, while the b* values ranged from 2.55 to 3.34, with the latter indicating a predominant yellow contribution to the yogurt color. The calculated values of whiteness index (WI) and yellowness index (YI) (Table 3) demonstrated the prevalence of whiteness over yellowness, with a slightly higher yellowness observed in UHPH yogurts.

There were no significant differences in pH values between any of the yogurt samples. A significant decrease ($P < 0.05$) in pH was observed from day 1 to day 14 of storage due to the high metabolic activity of the yogurt starters during the initial stages. The pH remained constant from day 14 until day 28 when yogurt production ceased in refrigeration (Lucey, 2004).

The titratable or total acidity of yogurts should ideally be within the range of 0.5–1.6 g/100 mL according to quality standards for fermented milks (WHO/FAO, 2003). Table 3 displays the total acidity values for each type of yogurt during the 28-day storage at 4 °C. Initially, CH yogurts exhibited higher total acidity compared to UHPH yogurts. This initial difference persisted during storage, although it was not significantly different ($P > 0.05$) from day 14 onwards. A study by Serra, Trujillo, Guamis, and Ferragut (2009) on physicochemical characteristics of yogurts made from CH and UHPH-treated milks also reported higher total acidity in CH yogurts during storage. In the present study, lactose primarily derived from UHT milk and skim milk powder used in RM formulations remained constant across all yogurts. Therefore, there were no significant differences ($P > 0.05$) in total acidity between yogurts with 4 g/100 mL or 6 g/100 mL added SDE. Furthermore, differences in the perceived sensory acidity between the samples was deemed insignificant, as will be shown in the corresponding section. It is worth noting that the total acidity in this study was within the higher end of the recommended range. This may be attributed to an over-acidification experienced on day 0, as the fermentation process was stopped below pH 4.6.

Syneresis, the expulsion of the aqueous phase from the yogurt gel, is a common issue that affects product quality (Lucey, 2004). The amount of expelled aqueous phase is inversely related to water holding capacity (WHC). Hence, greater expulsion results in lower WHC. To minimize syneresis, skim milk powder (SMP) and/or milk proteins are commonly added in yogurt production. In this study, 3 g/100 g SMP was added to all RMs. Centrifugation was used to induce syneresis and compare the WHC of the different yogurt types produced. It is important to note that centrifugation-induced syneresis does not fully represent the real network contraction of yogurts but rather indicates the gel structure ability to retain water. Results of WHC (Table 3) showed that CH6 and

Table 2
Mean values ± SD of textural and rheological parameters of yogurts during storage at 4 °C.

Sample	d1	d14	d28
Firmness (N)			
CH4	$0.23 \pm 0.04^{a,A}$	$0.214 \pm 0.004^{ab,B}$	$0.183 \pm 0.003^{b,C}$
CH6	$0.24 \pm 0.03^{a,A}$	$0.22 \pm 0.01^{ab,AB}$	$0.200 \pm 0.005^{b,B}$
UH4	$0.26 \pm 0.03^{a,A}$	$0.233 \pm 0.007^{a,A}$	$0.22 \pm 0.01^{a,A}$
UH6	$0.21 \pm 0.02^{a,A}$	$0.190 \pm 0.008^{b,C}$	$0.183 \pm 0.004^{b,C}$
Consistency (N*s)			
CH4	$0.9 \pm 0.1^{a,A}$	$0.82 \pm 0.02^{a,AB}$	$0.78 \pm 0.01^{a,B}$
CH6	$0.9 \pm 0.1^{a,A}$	$0.84 \pm 0.04^{a,A}$	$0.80 \pm 0.01^{a,AB}$
UH4	$1.0 \pm 0.1^{a,A}$	$0.87 \pm 0.03^{a,A}$	$0.85 \pm 0.03^{a,A}$
UH6	$0.8 \pm 0.1^{a,A}$	$0.77 \pm 0.01^{a,B}$	$0.76 \pm 0.03^{a,B}$
G' (Pa)			
CH4	$67 \pm 1^{c,B}$	$99 \pm 1^{b,B}$	$128.5 \pm 0.5^{a,B}$
CH6	$87.1 \pm 0.9^{c,A}$	$120.9 \pm 0.8^{b,A}$	$158 \pm 1^{a,A}$
UH4	$60.7 \pm 0.9^{c,D}$	$66 \pm 1^{b,D}$	$97.2 \pm 0.5^{a,D}$
UH6	$65 \pm 1^{c,C}$	$86.8 \pm 0.08^{b,C}$	$100 \pm 1^{a,C}$
G'' (Pa)			
CH4	$18.7 \pm 0.6^{c,B}$	$25.7 \pm 0.3^{b,B}$	$33 \pm 1^{a,B}$
CH6	$24,7 \pm 0.2^{c,A}$	$32.0 \pm 0.5^{b,A}$	$40 \pm 1^{a,A}$
UH4	$17.2 \pm 0.4^{b,C}$	$17.4 \pm 0.3^{b,D}$	$26 \pm 1^{a,C}$
UH6	$18.6 \pm 0.3^{c,B}$	$22.8 \pm 0.2^{b,C}$	$26.1 \pm 0.3^{a,C}$

Different capital letters in each column indicate significant differences ($P < 0.05$).
Different small letters in each row indicate significant differences ($P < 0.05$) ($n = 3$).

Table 3
Color parameters (WI, YI), WHC, TA and pH values of different yogurt samples during storage at 4 °C.

Sample/days	1	14	28
WI			
CH4	$94.86 \pm 0.01^{ab,B}$	$94.89 \pm 0.03^{a,A}$	$94.81 \pm 0.01^{b,B}$
CH6	$94.81 \pm 0.01^{b,B}$	$94.72 \pm 0.01^{c,C}$	$94.916 \pm 0.005^{a,A}$
UH4	$94.74 \pm 0.01^{b,C}$	$94.776 \pm 0.004^{a,B}$	$94.68 \pm 0.01^{c,C}$
UH6	$94.50 \pm 0.01^{b,D}$	$94.488 \pm 0.005^{b,D}$	$94.64 \pm 0.02^{a,D}$
YI			
CH4	$4.68 \pm 0.05^{a,D}$	$4.45 \pm 0.06^{b,D}$	$4.39 \pm 0.07^{b,B}$
CH6	$4.83 \pm 0.04^{b,C}$	$4.91 \pm 0.01^{a,C}$	$4.365 \pm 0.009^{c,B}$
UH4	$5.05 \pm 0.02^{a,B}$	$5.018 \pm 0.007^{b,B}$	$5.08 \pm 0.01^{a,A}$
UH6	$5.68 \pm 0.03^{b,A}$	$5.85 \pm 0.01^{a,A}$	$5.14 \pm 0.05^{c,A}$
pH			
CH4	$4.50 \pm 0.04^{a,A}$	$4.30 \pm 0.02^{b,A}$	$4.25 \pm 0.01^{b,A}$
CH6	$4.47 \pm 0.06^{a,A}$	$4.29 \pm 0.01^{b,A}$	$4.23 \pm 0.01^{b,A}$
UH4	$4.52 \pm 0.01^{a,A}$	$4.28 \pm 0.03^{b,A}$	$4.226 \pm 0.005^{c,A}$
UH6	$4.52 \pm 0.01^{a,A}$	$4.30 \pm 0.03^{b,A}$	$4.23 \pm 0.02^{b,A}$
TA (mmol/100g)			
CH4	$16.6 \pm 0.1^{b,A}$	$17.1 \pm 0.6^{ab,A}$	$17.5 \pm 0.2^{a,A}$
CH6	$16.8 \pm 0.1^{a,A}$	$17.1 \pm 0.2^{a,A}$	$17.1 \pm 0.2^{a,AB}$
UH4	$16.01 \pm 0.07^{c,B}$	$16.7 \pm 0.2^{b,A}$	$17.0 \pm 0.1^{a,AB}$
UH6	$15.81 \pm 0.07^{b,B}$	$16.4 \pm 0.4^{a,A}$	$16.6 \pm 0.3^{a,B}$
WHC (%)			
CH4	$31.0 \pm 0.2^{b,C}$	$31.1 \pm 0.1^{b,D}$	$31.8 \pm 0.3^{a,C}$
CH6	$35.20 \pm 0.07^{a,A}$	$35.2 \pm 0.4^{a,B}$	$35.9 \pm 0.7^{a,A}$
UH4	$33.1 \pm 0.1^{a,B}$	$33.1 \pm 0.3^{a,C}$	$33.5 \pm 0.4^{a,B}$
UH6	$35.7 \pm 0.5^{a,A}$	$36.0 \pm 0.1^{a,A}$	$36.3 \pm 0.6^{a,A}$

Different capital letters in each column indicate significant differences ($P < 0.05$). Different small letters in each row indicate significant differences. ($P < 0.05$). Each value is expressed as mean ± SD ($n = 3$).

UH6 yogurts had significantly ($P < 0.05$) higher WHC compared to CH4 and UH4 yogurts. Furthermore, UHPH yogurts exhibited significantly higher WHC compared to CH yogurts. Thus, in this study, the SDE content and UHPH treatment were responsible for increasing water retention in yogurts. The positive contribution of UHPH treatment may be attributed to the higher water retention in the interior and at the interface of the oil-protein aggregates in UHPH-treated yogurts, as observed by Varela et al. (2022) in SDE obtained through UHPH treatment. It has been reported that acid-induced gelation of protein-based emulsions with high fat content leads to higher WHC (Gyawali & Ibrahim, 2016). This could be due to the oil droplets, bound by proteins at the oil-water interface, acting as protein particles in the case of UHPH SDE, thereby increasing immobilized water. Moreover, the WHC remained stable during the storage of each sample. Although water expulsion is a time-dependent phenomenon due to progressive compaction of the yogurt network over time (Li, Ye, & Singh, 2021; Gilbert & Turgeon, 2021), the values obtained through centrifugation also included water expelled by spontaneous syneresis, which was observed in small quantities and could not be measured accurately.

3.4. Stability to oxidation

Chia and sunflower oils are rich in PUFA, thus they are prone to oxidation during processing and storage, which may produce off-flavors (Estrada, Boeneke, Bechtel, & Sathivel, 2011). Oxidation of yogurts containing chia and sunflower oils (50:50) were evaluated for primary (hydroperoxide concentration) and secondary oxidation (malondialdehyde, MDA, concentration) at days 1 and 28. The effect of type of homogenization system to obtain SDE and the percentage added to RMs (4 and 6 g/100 g) on the primary and secondary oxidation of yogurts is shown in Fig. 4. Hydroperoxide concentration (Fig. 4A) of CH yogurts was significantly higher ($P < 0.05$) than UHPH yogurts on day 1 as well as on day 28 of storage. Moreover, the increase of those values of UHPH-yogurts from day 1 to day 28 was much less pronounced than those observed in CH yogurts. Varela et al. (2022) observed similar results in SDE containing 7 g/100 g BM, like in the present study, i. e. 7CH-SDE was more prone to primary oxidation than 7UHPH-SDE. However, they performed the study of oxidation stability in forced conditions at 50 °C for one month and observed a maximum value of hydroperoxide concentration at day 15, which was followed by a reduction at day 31, meaning that the secondary oxidation was in progress by the degradation of hydroperoxides. In the present study, the oxidation was analyzed in yogurts at 4 °C, thus the further degradation of hydroperoxides was not produced during the storage.

One of the final products of polyunsaturated fatty acid oxidation is MDA, which is indicative of secondary oxidation (Fig. 4B). With the exception of UH4, which on day 1 of storage showed higher MDA values than CH4, this parameter showed a resembling behavior as primary oxidation, with higher MDA values in CH compared to UHPH-yogurts, both on days 1 and 28. As was observed in 7SDE by Varela et al. (2022), the encapsulation efficiency of UHPH-SDE, in general and, in 7UHPH-SDE, in particular, were higher than those SDE obtained by CH. Probably, the small droplets which were well covered by phospholipids and proteins forming aggregates were responsible for keeping the oil well retained in those colloidal structures, and therefore, protecting oil against oxidation.

3.5. The composition of the main unsaturated fatty acids

The main composition of unsaturated fatty acids (oleic, linoleic, and α-linolenic acids) in yogurts on days 1 and 28 of storage, is presented in Table 4. Linoleic acid was found to be the most abundant polyunsaturated fatty acid (PUFA) in all yogurts due to the 50:50 ratio of chia and sunflower oils in their composition. This oil mixture was selected to mitigate the strong green aroma of pure chia oil and contribute to a balanced ratio of omega-6 to omega-3 fatty acids in yogurts. Sunflower oil is rich in oleic and linoleic fatty acids, while chia oil primarily contributes to the α-linolenic acid content, and to a lesser extent the linoleic acid content, of the mixture. As anticipated, increasing the amount of SDE added to RMs resulted in higher levels of fatty acids in yogurts. In all cases, UHPH treatment significantly increased the content of unsaturated fatty acids in yogurts compared to CH. UHPH yogurts contained higher amounts of oleic, linoleic, and α-linolenic acids. As mentioned earlier (Aghababaei et al., 2021; Varela et al., 2022), the encapsulation efficiency of UHPH-treated SDE was higher than that of CH, providing better protection against oxidation, as

Table 4
Fatty acid composition (mg/100g) of the different yogurts on days 1 and 28 of storage at 4 °C.

Sample/ FFAA	Oleic acid		Linoleic acid		Linolenic acid	
	d1	d28	d1	d28	d1	d28
CH4	107.80 ± 3.87^{a,B}	84.16 ± 4.36^{b,B}	201.92 ± 2.99^{a,C}	146.89 ± 2.03^{b,C}	190.01 ± 3.12^{a,C}	112.30 ± 3.74^{b,D}
CH6	185.61 ± 5.73^{a,A}	149.53 ± 7.85^{b,A}	256.26 ± 8.29^{a,A}	233.84 ± 2.34^{b,A}	227.73 ± 4.10^{a,A}	174.80 ± 3.64^{b,B}
UH4	118.61 ± 6.81^{a,B}	94.47 ± 3.16^{b,B}	216.77 ± 2.97^{a,B}	189.86 ± 3.81^{b,B}	210.79 ± 8.08^{a,B}	158.66 ± 1.99^{b,C}
UH6	194.91 ± 2.05^{a,A}	155.463 ± 1.420^{b,A}	268.167 ± 1.419^{a,A}	242.23 ± 6.38^{b,A}	240.73 ± 4.94^{a,A}	193.573 ± 1.615^{b,A}

Different capital letters in each column indicate significant differences for each sample and for each FA in the same day ($P < 0.05$).
Different lowercase in each row for each FA indicate significant differences for each sample in days 1 and 28 ($P < 0.05$) ($n = 3$).

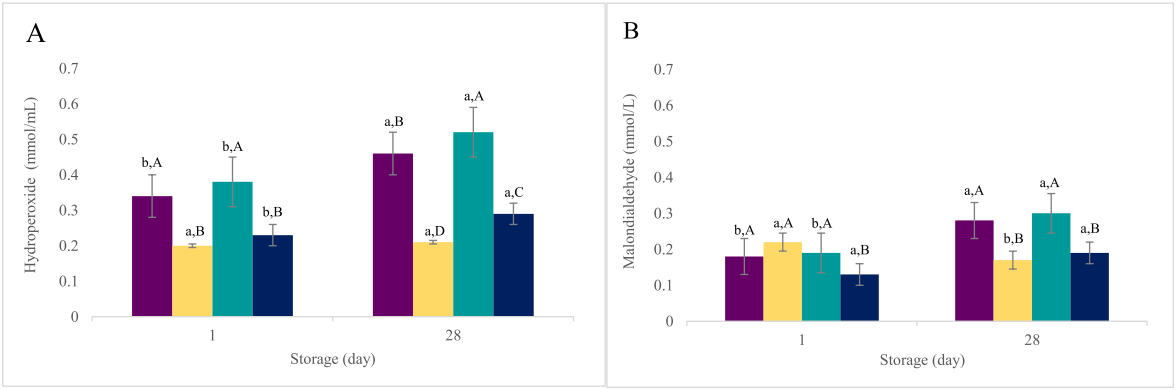

Fig. 4. Hydroperoxides (A) and malondialdehyde (B) concentration of yogurts at days 1 and 28 of storage at 4 °C. Purple: CH4, Green: CH6, Yellow: UH4, Blue: UH6.

observed in section 3.4. During storage, a significant decrease in FA concentrations was observed in yogurts. In addition to the high susceptibility of unsaturated FA to oxidation, hydrolysis of triglycerides with the liberation FA, which was observed in the over-acidification during storage, may have also contributed to the oxidation (Table 3). Despite the reduction in FA levels during storage, the PUFA content of yogurts makes a valuable contribution to the diet. Yogurt is a food consumed daily by many people and the intake of these essential fatty acids contributes to a balanced diet.

3.6. Microbiology

Initial microbial counts of RMs for further production of yogurts were performed in selective media for coliforms/*E. coli*, mesophilic aerobes, and their spores. No coliform/*E. coli* colonies were detected in any of the samples, and, in the case of mesophilic aerobes and their spores, the mean values were 1.70 and 1.34 log CFU/mL, respectively.

European legislation (WHO/FAO, 2003) states that the lactic acid bacteria in yogurt (*L. bulgaricus* and *S. thermophilus*) must be viable and present in the finished product in a minimum amount of 1×10^7 CFU/g or mL. Table 5 shows the microbial counts of each microorganism in yogurts after 0, 1, 14, and 28 days in MRS and M17 media (selective for *L. bulgaricus* and *S. thermophilus*, respectively). Counts after yogurt production corresponded to the RM inoculated and analyzed prior to the fermentation process, which explains the significant difference between the counts on day 0 and those on subsequent days. Once the fermentation process was completed, bacterial growth was, in general, around 7 log CFU/g, as required by legislation. Yogurts made with SDE treated by CH or UHPH showed no significant differences ($P > 0.05$) between them and no effect related to the percentage of SDE added to yogurts was observed. Counts of L. *bulgaricus* remained constant during the storage of yogurts while *S. thermophilus* showed a significant increase ($P < 0.05$) in counts on day 28, which could be related to a greater adaptation of *S. thermophilus* to the formulations used.

3.7. Sensory evaluation

Sensory evaluations were conducted using triangle testing, followed by descriptive and preference testing. Twenty judges participated in different sets of triangle tests to determine if the addition of different percentages of SDE (4 g/100 g or 6 g/100 g) and the type of homogenization (CH or UHPH) were detectable in the yogurts. The sets of triangle tests were carried out sequentially, comparing two different samples in each set, based on the results of the previous tests. The first set of triangle testing (Fig. 5A) compared CH4 vs UH4 yogurts, and no significant differences were detected. Therefore, the next set of triangle tests (Fig. 5B) compared CH6 vs UH6 yogurts, and again, no significant differences were observed. Subsequently, a third set of triangle tests (Fig. 5C) compared UH4 vs UH6 yogurts, based on the superior quality characteristics observed in this study compared to CH yogurts. The results of this set of triangle testing did not show any significant differences ($P < 0.05$) between UH4 and UH6, indicating that none of the pairs of samples were distinguished by the judges. This is particularly important because the addition of 6 g/100 g SDE, which contains a higher concentration of PUFA, did not result in any unusual flavors in the yogurts.

For the descriptive test, five parameters associated with the sensory profile of the yogurts were evaluated: consistency, creaminess, acidity, lactic aroma, and off-flavors. Fig. 5D illustrates that UH6 obtained higher scores than UH4 in all parameters except "lactic aroma" and "acidity." "Consistency" and "creaminess" were the only parameters that showed a significant difference ($P < 0.05$) between the two samples. UH6 yogurts obtained scores of 4.3 ± 0.4 for consistency and 4.43 ± 0.43 for creaminess on a scale of 0–5, while UH4 yogurts obtained scores of 3.8 ± 0.4 and 3.75 ± 0.35, respectively, for those attributes. Additionally, although not significantly different ($P > 0.05$), the preference test conducted between UH4 and UH6 showed that yogurts with 6 g/100 g SDE received higher scores, likely due to the better texture perceived in the descriptive test.

Table 5
Microbiological counts (mean log CFU/g ± SD) of recombined milks inoculated with yogurt starters (day 0) and yogurt samples during storage at 4 °C (days 1, 14 and 28).

Sample/ days	d0	d1	d14	d28
L. bulgaricus				
CH4	$4.56 \pm 0.22^{b,A}$	$7.01 \pm 0.62^{ab,A}$	$7.85 \pm 1.74^{a,A}$	$7.53 \pm 0.49^{a,A}$
CH6	$4.60 \pm 0.30^{b,A}$	$6.91 \pm 0.42^{a,A}$	$6.96 \pm 1.50^{a,A}$	$7.28 \pm 0.56^{a,A}$
UH4	$4.55 \pm 0.23^{b,A}$	$7.24 \pm 0.30^{a,A}$	$6.78 \pm 0.81^{a,A}$	$7.41 \pm 0.70^{a,A}$
UH6	$4.58 \pm 0.21^{b,A}$	$7.36 \pm 0.40^{a,A}$	$6.81 \pm 0.86^{a,A}$	$7.45 \pm 0.50^{a,A}$
S. thermophilus				
CH4	$4.17 \pm 1.63^{b,A}$	$8.50 \pm 0.71^{a,A}$	$8.44 \pm 0.96^{a,A}$	$9.15 \pm 0.00^{a,B}$
CH6	$3.69 \pm 0.65^{b,A}$	$8.58 \pm 0.65^{a,A}$	$8.41 \pm 0.96^{a,A}$	$9.19 \pm 0.00^{a,A}$
UH4	$3.17 \pm 0.39^{b,A}$	$8.79 \pm 0.47^{a,A}$	$8.51 \pm 0.98^{a,A}$	$9.14 \pm 0.00^{a,C}$
UH6	$3.79 \pm 0.59^{b,A}$	$8.58 \pm 0.67^{a,A}$	$8.46 \pm 0.92^{a,A}$	$9.13 \pm 0.00^{a,C}$

Different capital letters in each column indicate significant differences ($P < 0.05$). Different small letters in each row indicate significant differences ($P < 0.05$) ($n = 2$).

4. Conclusions

The choice of homogenization system (CH or UHPH) influenced the quality parameters of the yogurts. CH treatment produced yogurts with higher firmness and consistency compared to UHPH treatment. However, UHPH yogurts improved WHC and offered better protection against oxidation, preserving PUFA content during storage compared to CH yogurts. Importantly, sensory evaluation indicated that neither the homogenization system nor the SDE content significantly impacted yogurt flavor, allowing for the use of higher SDE concentrations without altering sensory attributes. UHPH technology, with its ability to simultaneously homogenize and modify colloidal structures, has the potential for broad applications in the food industry. It offers opportunities for improved emulsion stability and interaction between emulsifiers and the oil phase.

Statement

During the preparation of this work the authors used Chatgpt in order to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

CRediT authorship contribution statement

Fatemeh Aghababaei: Investigation, Writing – original draft. **Antonio J. Trujillo:** Validation, Project administration. **Bibiana Juan:** Validation, Methodology, Data curation. **Marta Capellas:** Validation, Methodology. **Victoria Ferragut:** Conceptualization, Methodology, Data curation, Writing – review & editing, Supervision.

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.

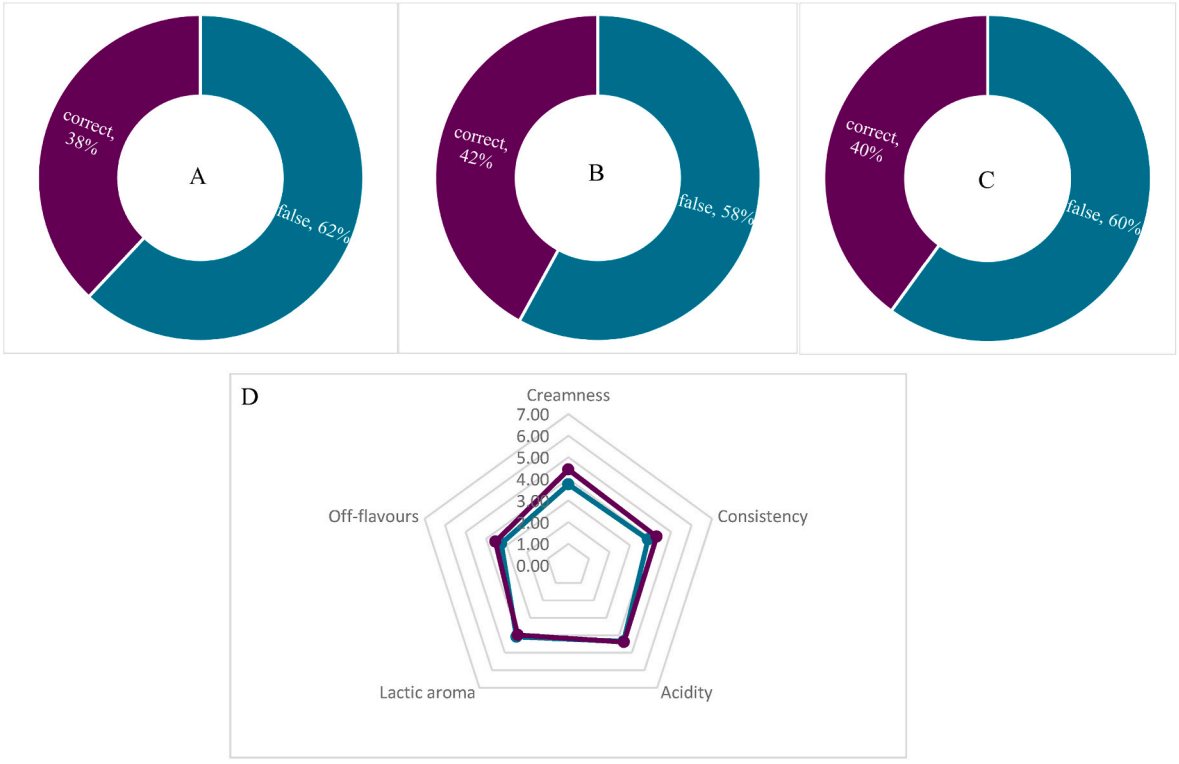

Fig. 5. Sensory analysis of yogurts. Triangular testing of the different pairs of yogurts: CH4 *vs* UH4 (A), CH6 *vs* UH6 (B), UH4 *vs* UH6 (C). Descriptive test of yogurts UH4 (green line) and UH6 (purple line) (D).

Data availability

Data will be made available on request.

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