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Double emulsions fortified with plant and milk proteins as fat replacers in cheese

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1. Introduction

Fat is one of the major macronutrients of food products (Martin & Issanchou, 2019). However, high consumption of fat has been associated with increased risk of heart disease, type-2 diabetes and stroke risks (Mozaffarian, 2016). The vast majority of countries have established guidelines to reduce fat consumption, and studies have shown that buyers seek reduced fat or fat-free products (Fenko, Nicolaas, & Galetzka, 2018). Cheese is a complex, multiphase system, whose fat content varies between 11 and 47% (Widdowson, 2015). One of the popular cheese types that is consumed worldwide is Cheddar cheese (Fox & McSweeney, 2004). In the UK, cheese consumption is 1.7 kg per capita, with 54% being Cheddar cheese in 2016, with the vast majority being consumed during summer. Taking these facts into account, food companies and cheese producers, aspire to manufacture fat reduced products to augment their market share (Pinto et al., 2016).

A major pitfall of low fat cheese is its stiff and rubbery texture due to changes developed in the protein structure, as fewer fat droplets are immersed in the protein matrix (Gunasekaran & Ding, 1999). Additional drawbacks of low-fat cheese are its poor sensory characteristics (e.g. umami flavor) and its higher meltability and free oil. The ability of a cheese to retain the free oil upon heating, is a functional property that affects the use of the cheese as topping ingredient (Rowney et al., 2004). These characteristics are connected with the biochemical alterations during maturation and with the low ratio of fat to moisture (Fenelon & Guinee, 2000; Guinee & O'Callaghan, 2013).

Hence, there is a need to look for ingredients that act as an active filler and are embedded in the cheese protein matrix, in order to produce high quality low-fat cheese (Lobato-Calleros et al., 2008; Ramel & Marangoni, 2018). Several approaches have been evaluated for their capability to produce high quality low-fat cheese, for example homogenization of the cheese milk, which led to microstructure and flavor improvements but did not affect the free oil (Emmons, et al., 1980; Madadlou et al., 2007). Several studies reported the use of oleogels for reducing fat content and improving the cheese quality, although in their vast majority they are using ingredients which are not approved to be included in cheese recipes (Bemer et al., 2016; Ramel & Marangoni, 2018).

A plausible approach to overcome these limitations is the use of double emulsions. Water-in-oil-in-water ($W_1/O/W_2$) emulsions comprise of water-in-oil emulsion dispersed in an external water phase. Double emulsions have been recommended as an approach to reduce the fat content of various food products, such as cheese (Lobato-Calleros et al., 2008), yogurt (Lobato- Calleros et al., 2009), and meat systems (Faraji et al., 2004). The inner water phase is able to reduce the fat content

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in an equal oil-in-water (o/w) emulsion. The main limitation of double emulsions is their high susceptibility to destabilization due to phase separation, droplet flocculation or diffusion of the inner water droplets to the outer aqueous phase. The stability of double emulsions is affected by the emulsifiers used to stabilize the interfaces, the size of the oil droplets, and the emulsification method. Although several studies have been performed in order to produce low-fat cheese with the aid of double emulsions, to the best of the authors' knowledge, they all use liquid oils that are not approved to be added in cheese recipes (Giroux et al., 2013; Karahan et al., 2011; Lobato-Calleros et al., 2007).

For the formulation of low-fat cheese, the ingredients used ought not to interact or be embedded in the protein matrix, thus reaching optimal functionality from each ingredient. This will lead to achieving the desired properties, such as texture and sensory. Protein has been extensively used as a fat replacer and fortification method in cheese products (Rinaldoni et al., 2014; Talbot-Walsh et al., 2018). Milk proteins, such as whey proteins and caseins, are the most broadly used ingredient for the fortification of food products (Khouryieh et al., 2015; Xu et al., 2016). What is more, rice, wheat, corn, pea, canola, and potato proteins are the major plant-based proteins used as alternatives to animal-based fortification proteins (Day, 2013). Rice protein is known for being hypoallergenic (Watanabe, 1993), and for its capability of lowering the cholesterol level. Rice has also higher biological value and protein digestibility compared to milk proteins (Eggum, 1979) and it is an affordable raw ingredient (Agboola, et al., 2005).

Pumpkin seed protein is available in pumpkin seed cake, after the extraction of the pumpkin seed oil (Ozuna and León-Galván, 2017). This oil is known for its high content of unsaturated fatty acids and other bioactive compounds (Rabrenović et al., 2014). Due to the large quantities of pumpkin seed oil that are produced, especially in Austria and Slovenia (Fruhwirth and Hermetter, 2008), a great amount of pumpkin seed cake is subsequently created, which contains up to 60% protein. Until now, these proteins are mainly being utilized in livestock feed (Caili, Huan, & Quanhong, 2006). Therefore, there are opportunities to incorporate these proteins into food products in order to increase their functionality. However, there is limited research using these proteins in food systems (Bučko et al., 2015; Bučko et al., 2016). Therefore, the food industry needs to explore alternative plant-based protein sources.

The objective of the present study is to produce low-fat cheese using double emulsions, as well as to study the effect of the addition of whey protein isolate (WPI), rice protein (RP), or pumpkin seed protein (PSP) in the properties of primary and double emulsions as fat replacers in cheese.

2. Materials and Methods

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2.1 Materials

Anhydrous milk fat (AMF) (99.88% fat, 0.12% moisture) was obtained from Spear Associates Ltd. (Bala, UK). Whey protein isolate (WPI) (Lacprodan- DI-9224 with protein content of 92 ± 2% and 0.3% moisture content) was provided by Arla Foods Ingredients (Viby, Denmark). Rice protein (RP) (68% protein, 0.5% moisture) and pumpkin seed protein (PSP) (65% protein, 0.8% moisture) were both purchased from Indigo Herbs (Glastonbury, UK). The lipophilic surfactant sorbitan monooleate (Span 80) was purchased from Sigma- Aldrich (St Louis, USA), while surfactant polyglycerol polyricinoleate (PGPR) was kindly donated by Palsgaard (Juelsminde, Denmark). Unhomogenised milk (2.4% fat) was purchased from a local dairy and used as such. Mesophilic and thermophilic starter culture (Choozit RA21, Danisco, France). All aqueous solutions were prepared with tap water.

2.2 Preparation of primary emulsions

The WPI, RP and PSP stock solutions were prepared by dissolving the appropriate amount of powder in water under continuous stirring at room temperature (18^{o} C) for 60 min. The solutions were stored in the refrigerator at 4^{o}C overnight to ensure complete hydration. The protein concentration of the dispersions was between 10, 15, 20, and 25% wt. The fat phase contained two lipophilic surfactants (PGPR and Span 80) in a 1:1 ratio. The total surfactant content of the primary emulsions was 2% wt. After heating the AMF to 45^{o} C, the lipophilic surfactants were added while stirring with a propeller stirrer (Eurostar 60, IKA, Staufen, Gernany) at 2000 rpm. Primary water-in-oil (w_1/o) emulsions were produced by adding 40% wt. of the aqueous phase to the fat phase in a 2 L beaker while mixing. The emulsion was then homogenized with the aid of a rotor-stator homogenizer (Magic lab, IKA, Staufen, Gernany) at 45^{o} C for 20 min at 25000 rpm. The samples were immediately cooled to room temperature after homogenization. The different proteins gave similar dispersed-to-continuous phase viscosity ratio of the of the primary emulsion (λ= 0.1 - 1.0). The final primary emulsions contained 60%wt. fat.

2.3 Preparation of double emulsions

Double emulsions were prepared by dispersing 5% wt. of the primary emulsions (w_1/o) into 95% wt. of the external aqueous phase (milk) using a rotor-stator homogenizer (L5M-A, Silverson, Chesham, UK) at 8000 rpm for 10 min. The temperature of the double emulsions was 38^{o} C. All emulsions were prepared in duplicate.

2.4 Cheese making

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The double emulsions with each protein content that gave better stability and smaller droplet size were selected in order to make 3 low fat cheeses enriched with proteins. At the same time, a standard full fat (FF) and a low fat (LF) Cheddar cheese were produced and used as references. 10 L vats (FT-20 cheese vat, Armfield, Ringwood, UK) were filled with 9.5 kg milk and 0.5 kg w/o/w emulsions and preheated to 32°C. 0.1 g of the starter culture was added to the vat and allowed to ripen for 60 min, for all the produced samples. The pH of the samples after the ripening, was recorded and kept at statistically same values. The rennet (CHY-MAX powder Extra NB, Chr. Hansen) was diluted was diluted 1:5 with water and was then added and the milk was allowed to stand for 40 min. When a firm coagulum was achieved, the curd was cut with 1.6 cm wire knives, then allowed to rest for 5 min. The way to ensure that the firm coagulum was successful was when the knife could not cut the coagulum.

At this point, stirring and heating began until the temperature reached 38°C after 35 min. It was then held at 38°C until the pH reached 6.30, at which time the whey was drained. The cheese was formed into one slab and it was then Cheddared by flipping the pieces every 10 min and stacking until the pH of the curd reached 5.40. The curds were milled and salted (3% salt). The last step was to place the curds in molds and pressed at 10 psi for 1 h, followed by 40 psi for 18 h. Finally, the cheese was removed, vacuum packed and left to mature at 12^{o} C for at least 14 days. The cheeses were sampled after 1 and 14 days by removing a slice from each cheese using a knife. All samples were produced in triplicate. The same procedure was followed to make the FF cheddar (using 10L whole milk) and LF (using whole milk and skim to 2.4% wt. fat).

2.5 Characterization of emulsions

2.5.1. Droplet size analysis of emulsions

The droplet size distribution of the primary emulsions was determined using static light scattering (Malvern Mastersizer 3000, Malvern Instruments Ltd, UK) equipped with a Hydro-dispersion unit. Before starting the experiment, the formulations were diluted in sunflower containing 1% wt. Span 80. The refractive indices of fat and water were taken as 1.467 and 1.330, respectively, and the Mie theory was used for the analysis. The size distribution was expressed as the median diameter (d_{50}). The polydispersity index was also evaluated used the following equation:

$$PDI = \frac{D_{90} - D_{10}}{D_{50}} \quad \text{(Eq. 1).}$$

The same apparatus was used to measure the droplet size of the double emulsions. In that case, double emulsions were diluted to water, while the refractive index of

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milk was taken as 1.345. At least three measurements were performed on freshly prepared primary and double emulsions, and the results are expressed as the average value.

2.5.2. Emulsion stability

The stability of emulsions at ambient temperature was measured by centrifugation. Emulsion samples (approximately 30 g) were put into test tubes and centrifuged (Megastar 1.6, VWR, USA) for 20 min at 3005 g, at 38^{o}C. The height of the serum layer of centrifugation after storage was recorded using a ruler for all the tested emulsions. The stability is presented in the serum Index (SI), which is calculated using following equation:

$$SI\% = \frac{H_s}{H_e} * 100 (Eq. 2)$$

where Hs is the height of the serum layer and He is the total height of the emulsion. A lower SI, therefore, represents a more stable emulsion.

2.5.3. Structure of double emulsions

Samples of freshly prepared double emulsions were observed using an optical microscope (Olympus BX51, Essex, UK) at 25^{o} C. The emulsion was being diluted with water (1:6 volume) and a drop was placed on the glass slide and gently covered with a cover slip. Several pictures were taken from random sample positions and 300 droplets were measured per repetition representing the overall appearance of the emulsions.

2.5.4. Rheological properties of solutions and emulsions

Rheological measurements of the emulsions were performed on a stress-controlled rheometer (Physica MCR 301, Anton Paar, Graz, Austria) equipped with a double-gap geometry with 26 mm diameter (DG-26.7). The temperature was kept constant (32.0 ± 0.1^{o} C) using a water bath. To achieve thermal and structure equilibrium, 7 ml of the sample was left at rest in the measurement system for 5 min prior to analysis. The apparent viscosity of the internal aqueous phase and the double emulsion was determined versus the imposed shear rate from 0.1 to 1000 s^{-1}. 10 points per decade were measured while the whole measuring time was 10 min. The viscosity measurements are reported as the average of at least three different samples. All rheological measurements were conducted with freshly prepared emulsions. The calculation of the viscosity ratio (λ) of each emulsion was based on the following equation:

$$\lambda = \frac{\eta_d}{\eta_c} (Eq. 3)$$

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where η_d is the viscosity of the dispersed phase at shear rate of 100 s^{-1} and η_c is the viscosity of the continuous phase at the same shear rate.

2.6. Cheese analyses

2.6.1. Composition of cheeses and yield

The pH of the cheese was evaluated with a pH-meter (Jenway 550, Fisher Scientific, Loughborough, UK) after 14 days of maturation. The cheeses were also tested for compositional analyses at 14 days. The moisture content of the cheeses was measured using a moisture balance with halogen heat source (MB25, Ohaus, China). 1 g of cheese sample was grated and put in a sample pan in the balance. The temperature was set at 102^{o}C. The evaluation of the cheese's nutritional properties (fat, salt, and protein content) had been performed with a FOSS Food Scan using a near infrared transmittance (Hilleroed, Denmark). For that purpose, a 20 g sample of cheese were put in a petri dish and added in the equipment, where the fat, protein and salt content of the cheese were evaluated.

The cheese yield was calculated from the following equation:

$$cheese\ yield(\%) = \frac{Weight\ of\ cheese(kg)}{Weight\ of milk(kg)} (Eq. 4)$$

2.6.2. Textural properties

A Texture profile analyzer (Stable MicroSystems, Texture Technologies, USA) was used to measure the textural properties of the cheeses. Before the measurement the cores of cheeses were taken from the inner part of the cheese and were cut in 3-cm cubes with a razor. The cubes were individually wrapped in plastic film to avoid surface drying and equilibrated for about an hour at room temperature (20^{o} C) before testing. The cubes were compressed in two cycle test at a speed of 1.2 mm/s with 30% deformation from their initial height. Hardness (H) was recorded as the maximum force during the first compression cycle.

2.6.3. Melting profile

Schreiber's test with minor modifications (Altan, Turhan, & Gunasekaran, 2005) was used to determine the meltability of the cheeses. Cores of the cheese were cut (20 mm cubes) and the cubes positioned at the centre of a glass petri dish. Reference figures of concentric cylinders with 10 mm diameter increments were prepared and placed at the bottom of the dish. The dishes were then covered and refrigerated (4^{o} C) for 10 min. The dishes were then transferred to a preheated oven and left there for 3 min at 100^{o} C. The samples were left to cool down to room temperature (20^{o} C) for 20 min and then the diameter of the cheese was measured at 4 different places.

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After creating the average of these readings, the meltability was calculated as the % increase of the diameter from the initial diameter.

2.6.4. Oil loss

The evaluation of oil loss was done by using a modified filter paper analysis (Ramel & Marangoni, 2018). Core of the cheeses (20 mm cubes) were cut and weighed. Whatman filter papers (Grade 4, VWR, Lutterworth, UK) were weighted separately. Cheese samples were positioned on top of the paper and stored in a refrigerator (4^{o} C) for 1 week. The papers were weighted again after removing the cheeses following 1 week of storage. Oil loss was calculated using the following equation:

$$oilloss(\%) = \frac{(W_f - W_i) - (W_{cf} - W_{ci})}{W_i} (Eq. 5)$$

where W_f is the final weight of the paper after 1 week of storage (when all the oil has migrated), W_i is the weight of the paper before adding the cheese, W_{cf} is the final weight of the empty paper, and W_{ci} is the initial weight of the empty paper.

2.7. Statistical analysis

Statistical analysis of the results was performed with Statgraphics Centurion XV (Statgraphics, Rockville, MD, USA) and a one-way ANOVA with Tukey test was applied in order to estimate the significant differences at a 95% level of confidence.

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3. Results & Discussion

3.1 Characteristics of W_1/O primary emulsions

In this study, different protein types (WPI, PSP, RP) were added to the aqueous phase of the primary emulsions. It is known that every protein has a different stabilizing mechanism. It is a key requirement to achieve small droplet sizes and emulsions that are stable against coalescence, in order to increase the yield of the inner droplets in the final double emulsion. Physical properties of the primary emulsions were evaluated to gain insight into the adsorption and stabilizing mechanisms. Fig. 1 depicts the mean droplet size (d_{50}) of emulsions based on number prepared with different protein types and concentrations from 10-25% wt.

From Fig. 1 shows that the droplet size is decreasing as protein concentration increases, until it reaches a critical point from where it remains stable. That trend applies in all emulsion samples, regardless of the protein type. This indicates that even the lowest protein concentration (20% wt.), efficiently covers the droplets' surface. After the critical concentration of 20% wt., the excess protein remains in the aqueous phase. This phenomenon was previously mentioned (Paximada, et al., 2016). In all the tested primary emulsions, the droplet size shows monomodal distribution for all protein type and concentrations used (data not shown).

WPI emulsions achieved the lowest d_{50} values in the range of 0.128 um (at 25%wt. protein), with their saturation concentration achieved at 20% wt. A probable explanation is that whey proteins have enhanced emulsifying properties compared to vegetable proteins such as rice and pumpkin seed proteins, which favours the better stabilization of WPI emulsions (Richert, 1979; Sahagún et al., 2018). Addition of WPI shows more efficient surface coverage and quicker diffusion of the protein on the interface, which leads to smaller droplet sizes (Gulseren & Corredig, 2013). Another explanation is the poor solubility of proteins from vegetable origin in water (Xu et al., 2016). In fact, native proteins, such as vegetable ones, consist of a rigid polypeptide structure, which is joined with several subunits by hydrophobic interactions and intermolecular bonds (Paraman et al., 2007).

The term emulsion stability refers to its ability to resist changes in its properties through time (McClements, 2005). The stability of emulsions is an important property since it contributes to the shelf-life of the products. The time during which the emulsion is stable depends mainly on the nature of the ingredients added, such as stabilizers and thickeners (Dickinson, 1992). Hence, the stability of the primary emulsions have been recorded and presented as the serum index (SI) in Table 1.

A decrease of the SI is evident by increasing the concentration of protein. Emulsions containing more than 20% wt. protein were stable (SI= 0%), regardless of the protein type. These results are in accordance with the droplet size measurements, which show that any addition of protein above 20% wt. does not have a significant effect

on the emulsion. Similar values for the SI have been found previously for emulsions stabilized with WPI, where SI values of 12% were reported (Kaltsa et al., 2014).

Another property that significantly affects the emulsion's structure and stability is the viscosity. The viscosity of the emulsions as a function of different protein concentrations and types is presented in Table 1. All the emulsions, independent of the protein concentration or type, exhibit a nearly Newtonian-like behavior as their viscosity is practically independent from the shear rate (data not shown). Viscosity values increase as the emulsifier concentration increases.

It is known that the emulsion droplet breakup is affected by the deformability of the water droplets and the forces that are applied at the water/oil interface. Droplet breakup is associated with the capillary number. Capillary breakup of a droplet occurs when the droplet has no time to adapt its shape to the rapidly varying flow field. This then results in a highly elongated shape on which perturbing ripples develop. If the capillary number exceeds a critical value, the droplets tend to develop into unstable drops and break up (Schröder et al., 2012). The Capillary number relies on the viscosity ratio as summarized in Eq. 3. As shown, when the viscosity of the continuous phase increases, the viscosity ratio reduces, and lead to a higher droplet breakup rate. Many studies have shown that viscosity ratios between 0.1 and 1 facilitate the droplet break up (McClements, 2015; Oppermann et al., 2018; Schröder et al., 2012).

Taking all the above into account, it is important to gain information on the effect of viscosity ratios on the droplet size of the emulsions. In Table 2 the viscosity ratios of the emulsions versus their size are summarized. This graph is divided into two areas: the first being for protein concentrations up to 15%. In that first area, the droplet size is significantly reduced as the viscosity ratio increases. This has been confirmed by other researchers (Chen et al., 2015; Pal, 2007; Schröder et al., 2012; Tan et al., 2020) . Above a critical viscosity ratio, there are no significant alterations on the droplet size. It is worth noting that the protein type plays a major role on the droplet size. It is shown in Table 2 that the interfacial properties of the proteins are crucial, since the droplet size differs for the same viscosity ratio when using different types of protein.

Overall, the protein type and concentration highly affect the physical properties of the fat-based emulsions. Primary emulsions were used to prepare the double emulsions. The best concentration of each protein type was selected in order to produce the double emulsions. Based on the results from this section, it is concluded that 25%wt. addition of protein resulted in stable (SI=0%) primary emulsions with the lowest particle sizes (0.128 – 0.90 nm). Hence, the 25% wt. protein concentration was selected for the following experiments.

3.2 Characteristics of $W_1/O/W_2$ double emulsions

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Milk was chosen as the external aqueous phase to produce the double emulsions. It is noted that the energy input that is being used for the production of double emulsions need to be relatively low, in order to avoid the movement of the inner water droplets to the outer aqueous phase. The average droplet size of the double emulsions produced using 5% of the primary emulsion has been evaluated and presented in Fig. 2.

The protein type significantly affects the droplet size of the double emulsions, with WPI giving the smallest size. The results show that average droplet size of the double emulsions is in the range of 1.8 - 4.2 μm, which is similar to the milk fat globules found in whole milk (3-5 μm). Since they have the same size, we assume that the double emulsions substitute the milk fat in the cheese without affecting its micro-structure. A study using double emulsions in order to substitute milk fat in cheeses, however they only achieved larger droplet sizes (8-30 μm) (Giroux et al., 2013).
Samples of freshly prepared double emulsions were observed using an optical microscope and presented in Fig. 3. Double emulsions containing WPI exhibited smaller droplets and less flocculation phenomena compared to those containing PSP or RP. This is in accordance with the droplet size measurements and is attributed to the differences at the size of the primary emulsions.

Internal phase composition had major consequences on the serum index and viscosity of the double emulsions (Table 3). For emulsions containing WPI, the SI was significantly lower (0.6%), compared to those formulations that contain PSP or RP (1.7 and 5.2% respectively). The lower stability of PSP and RP double emulsions is explained by the flocculation phenomena that are evident in Fig. 3. Several studies have described that flocculation of droplets is being augmented when having a high fat concentration, such as more than 15% fat (Giroux et al., 2013).

3.3 Incorporation of double emulsions in cheeses

Model cheeses were produced using milk with 3 different double emulsions (containing WPI, PSP or RP in the inner aqueous phase). In order to compare with the model cheeses, two control cheddars (FF and LF) were also made. The effect of the substitution of milk fat with double emulsions on their properties was evaluated. The composition of the model and control cheeses is presented in Table 4

The yield of the model cheeses is not affected by the protein used (approximately 11%). The yield is being increased compared to the LF. It is well established that LF cheeses end up with lower yields. This is attributed to the fact that the casein, whey, and milk fat concentrations are decreased during the production of LF cheddars (Romeih et al., 2002). The exhibited higher yield is of utmost importance for the food industry, this leads to the reduction of their costs. These results are in accordance with other studies dealing with the production of low-fat cheese using fat mimics. pH

of the cheeses is not affected by the emulsion addition or by the reduction of the fat (Table 4), as their values remain statistically the same, for all the tested samples.

Our model cheeses produced with WPI exhibit a decrease of the fat content. Specifically, the cheeses with double emulsions yield fat contents between 16 and 20%, while the FF cheese yields 31% fat and the LF yields 17%. The fraction of the fat phase that was added to the LF and the model cheeses was the same. However, the fat phase of the WPI cheeses had a significant amount of water droplets in it, leading to the reduction of the fat content (Lobato-Calleros et al., 2008). With respect to the type of protein used, WPI reaches lower fat content compared to both PSP and RP. The reason behind this phenomenon is that the WPI forms a layer on the surface of the inner water droplet which leads to the retention of the protein matrix of the cheese. Protein content, on the other side, is higher for the WPI cheeses. Given that the protein concentration added to all the samples was the same, it is assumed that WPI had higher retention rates in the cheese matrix.

Fig. 4 depicts the hardness of model cheeses and the control cheeses. It is evident that FF cheese statistically exhibits the lowest hardness (20 N). All emulsion cheeses show statistically the same hardness ranges (29-35 N), while the LF cheese exhibits the highest hardness (41 N). It has been recorded that the use of o/w emulsions as a substitution of fat globules, leads to the reduction of the hardness of the final cheeses, due to the low melting point of the o/w emulsion (Lobato-Calleros et al., 2007). However, studies show an increase of the hardness when making low fat cheese or substituting milk fat with double emulsions (Sharma Khanal et al., 2019). This phenomenon is attributed to the augmented degree of calcium-induced cross-linking, which mitigate the higher concentration and volume fraction of the casein network, thus producing firmer cheeses (McCarthy et al., 2015).

An essential functionality of cheese and cheese products is their meltability. Meltability was calculated as the % of the increase in cheese diameter and is summarized in Fig. 4 for the model cheese and the controls. The emulsion cheeses did not have any significant difference in their meltability, while FF cheddar exhibits the lowest increase in cheese diameter. LF cheese, on the other hand, exhibits statistically the highest increase in its diameter. (Ramel & Marangoni, 2018) have identified that the total meltability of the system is affected by the protein network, rather than the fat.

Oil loss of model and control cheeses was calculated and is depicted in Fig. 4. It is evident that the highest oil loss values are observed in LF cheese (20%), and the lowest in FF cheese (5%). As far as emulsion-based cheeses are concerned, PSP and RP do not exhibit any statistical difference of the oil loss values. In these cases, the protein macromolecules reduce the interactions between fat and the protein network (Rogers et al., 2010). In such a case, reduced protein is available for emulsification and leads to destabilization of the oil and subsequent oil leakage in the final cheese.

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4. Conclusions

The aim of this study is to determine the effect of various proteins as emulsifiers of the inner aqueous phase of double emulsions. Double emulsions consisted of WPI, PSP or RP dissolved in the inner aqueous phase, anhydrous milk fat with PGPR and Span 80 as the oil phase and milk as the outer aqueous phase. It was observed that the protein type and concentration highly affected the physical properties of the fat-based emulsions: an increase of the protein concentration led to larger viscosity ratios and emulsions with smaller droplet size. This study shows that the nutritional, physical and mechanical properties of low-fat cheese are tailored by incorporating double emulsions. The results show that the authors' approach to producing food grade double emulsions is an effective technique for the production of dairy products which have specific low-fat attributes. These emulsions have significant future applications in the food industry, particularly in a healthy diet conscious society.

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Table 1. Physical properties (serum index, apparent viscosity) of primary emulsions (W_1/O), made with milk fat, PGPR, and Span 80 as the continuous phase and whey protein isolate (WPI), pumpkin seed protein (PSP), or rice protein (RP) dissolved in water as the dispersed phase. The concentration of the protein in the emulsions varied between 10% wt. (WPI10, PSP10, RP10) and 25%wt. (WPI25, PSP25, RP25).

Emulsions	% wt. protein	SI (%)	Viscosity at 1000 s^{-1} (mPa s)
WPI10	10	2.3^{c} (0.5)	10^{A} (0.5)
WPI15	15	1.6^{b} (0.3)	15^{B} (1)
WPI20	20	0.0^{a} (0.0)	70^{C} (8)
WPI25	25	0.0^{a} (0.0)	120^{E} (9)
PSP10	10	7.6^{f} (0.9)	87^{C} (8)
PSP15	15	3.2^{d} (0.2)	110^{D} (5)
PSP20	20	0.0^{a} (0.0)	330^{G} (10)
PSP25	25	0.0^{a} (0.0)	750^{I}(103)
RP10	10	8.9^{g} (0.7)	230^{F} (53)
RP15	15	5.4^{e} (0.6)	360^{G} (23)
RP20	20	0.0^{a} (0.0)	530^{H} (27)
RP25	25	0.0^{a} (0.0)	910^{J} (98)

In parenthesis standard deviation values.

Mean values followed by the same letters are not significantly different ($P > 0.05$).

Table 2. Average droplet size of primary emulsions (W_1/O) emulsified with whey protein isolate, pumpkin seed protein, or rice protein as a function of apparent viscosity ratio.

WPI		PSP		RP	
λ_{1000} (-)	**d_{50} (um)**	**λ_{1000} (-)**	**d_{50} (um)**	**λ_{1000} (-)**	**d_{50} (um)**
0.033	1.3^{d}	0.29	3.8^{D}	0.767	5.5^{d}
0.051	0.904^{c}	0.348	1.62^{C}	0.787	2.51^{c}
0.247	0.386^{b}	1.103	0.98^{B}	1.757	1.04^{b}
0.4	0.128^{a}	2.9	0.69^{A}	3.033	0.91^{a}

Mean values followed by the same letters are not significantly different ($P > 0.05$).

Table 3. Physical properties (serum index, apparent viscosity) of double emulsions ($W_1/O/W_2$), containing 95% wt. milk and 5% wt. of the whey protein primary emulsion (wow WPI), pumpkin seed protein primary emulsion (wowPSP) or rice protein primary emulsion (wowRP).

Emulsions	SI (%)	Viscosity at 1000 s^{-1} (Pa s)
wowWPI	0.6^{a} (0.0)	0.5^{A} (0.0)
wowPSP	1.7^{b} (0.5)	1.4^{B} (0.3)
wowRP	5.2^{c} (0.8)	1.8^{B} (0.7)

Standard deviation values in parenthesis.

Mean values followed by the same letters are not significantly different ($P > 0.05$).

Table 4. Composition of full fat cheese (FF), low-fat cheese (LF) and reduced fat cheese products containing 5% of whey protein double emulsion (WPIcheese), pumpkin seed protein double emulsion (PSPcheese) or rice protein double emulsion (RPcheese) after 14 days of production.

Cheese	Yield (%)	Moisture (%)	Fat (%)	Protein (%)	Salt (%)	pH
WPI	11.2^{b}	40.9^{B}	15.8^{a}	33.4^{D}	1.7^{a}	5.27^{A}
PSP	11.3^{b}	42.7^{C}	19.3^{c}	29.3^{C}	1.7^{a}	5.31^{A}
RP	11.6^{b}	43.5^{C}	20.1^{c}	25.1^{B}	1.8^{a}	5.33^{A}
FF	13.5^{c}	36.2^{A}	31.2^{d}	21.3^{A}	1.8^{a}	5.24^{A}
LF	9.3^{a}	40.4^{B}	17.3^{b}	29.7^{C}	1.8^{a}	5.30^{A}

Mean values followed by the same letters are not significantly different ($P > 0.05$).

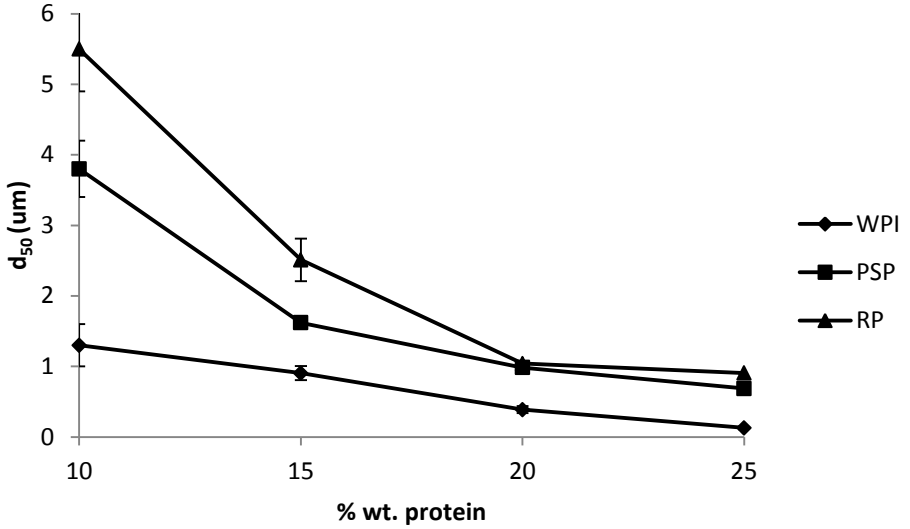


Fig. 1. Average (n=3, i=3) droplet size of primary emulsions (W_1/O), made with milk fat, PGPR, and Span 80 as the continuous phase and whey protein isolate (WPI-◆), pumpkin seed protein (PSP-■), or rice protein (RP-▲) as the dispersed phase. The concentration of the protein in the emulsions varied between 10% wt. and 25%wt. Bars indicating standard deviations.

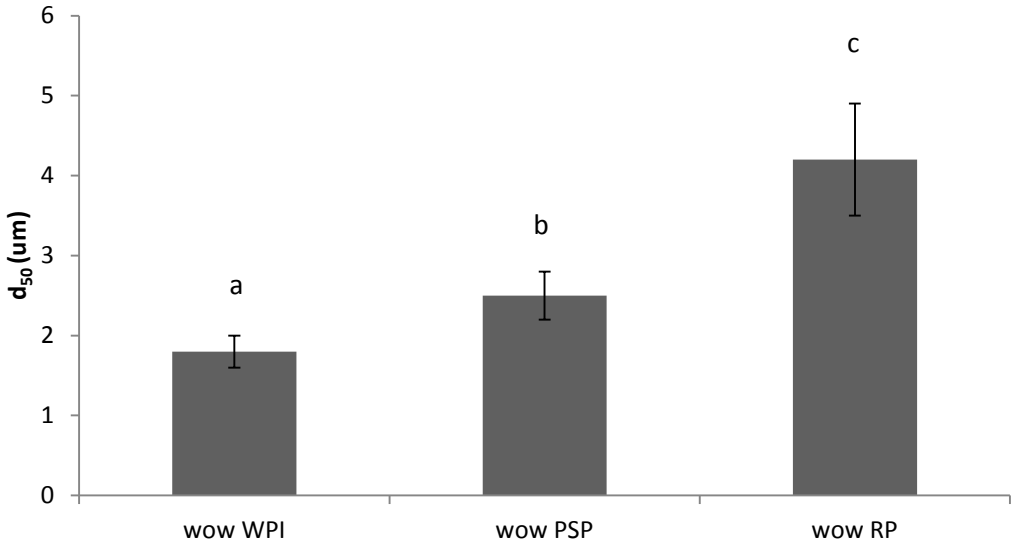
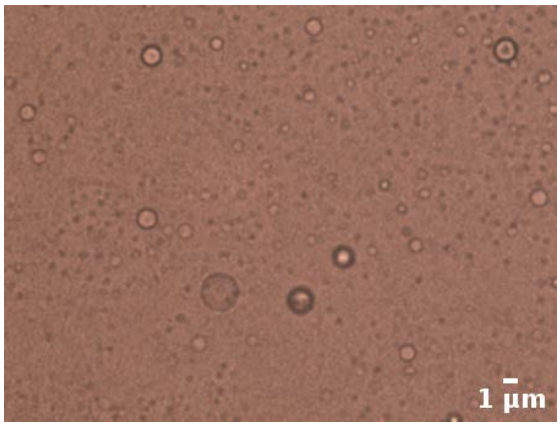
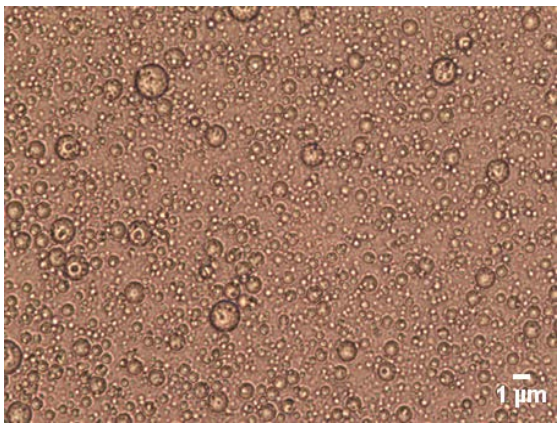


Fig. 2. Average (n=3, i=3) droplet size of water-in-oil-in-water emulsions ($W_1/O/W_2$) emulsions containing 95% milk and 5% wt primary emulsion with whey protein isolate (WPI), pumpkin seed protein (PSP), or rice protein (RP). Bars indicating standard deviations. Mean values followed by the same letters are not significantly different ($P > 0.05$).

wowWPI



wowPSP



wowRP

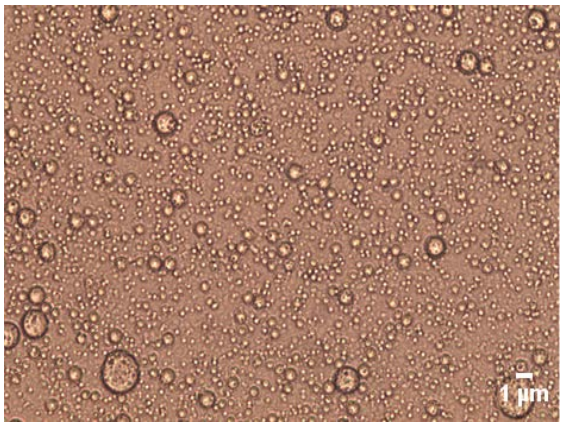


Fig. 3. Optical microscopic images of the water-in-oil-in-water emulsions prepared with 95% milk and 5% wt primary emulsion with whey protein (wowWPI), pumpkin seed protein (wowPSP), and rice protein (wowRP).

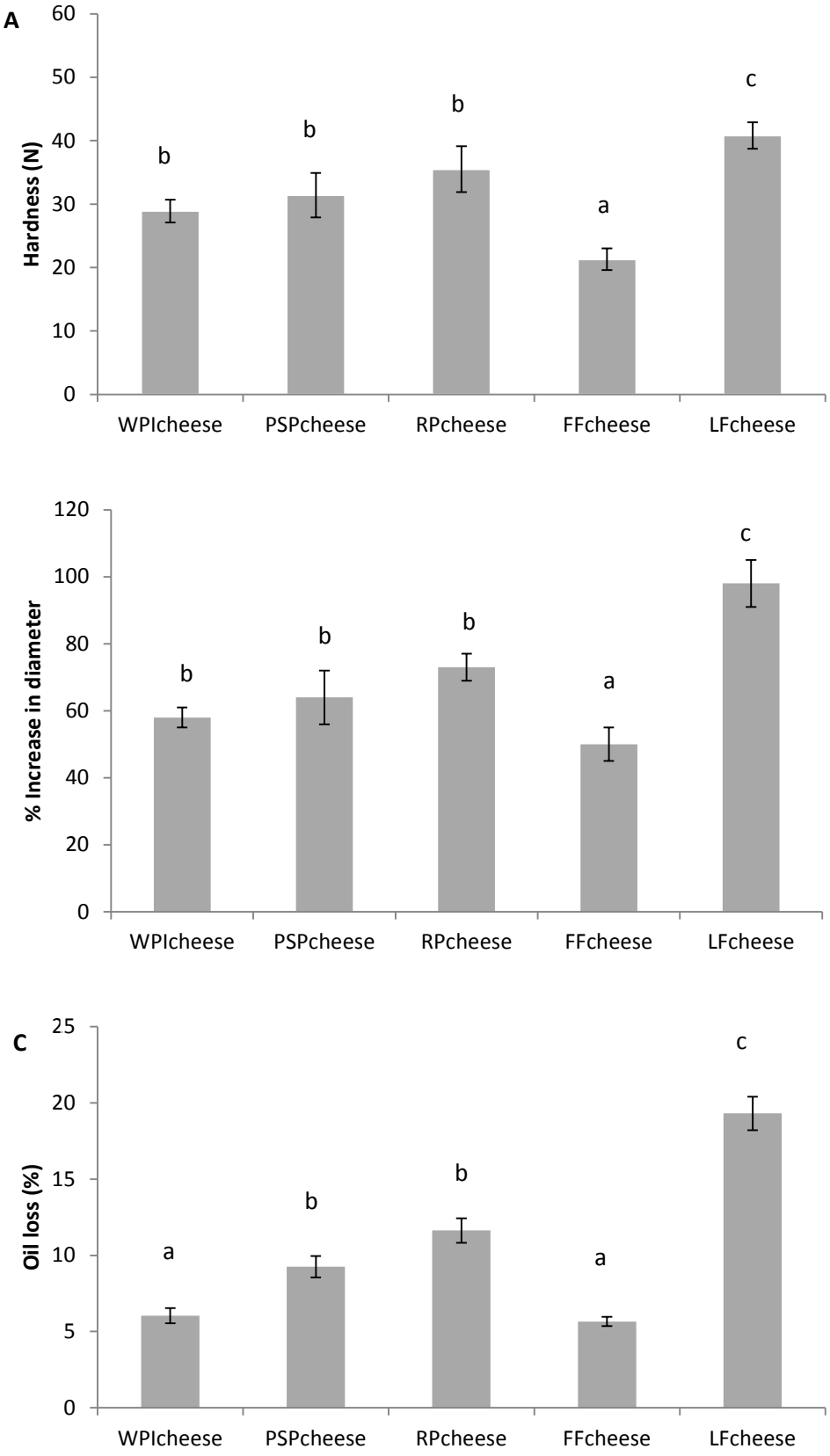


Fig. 4. Texture characteristics of full fat cheese (FF), low-fat cheese (LF) and reduced fat cheese products containing 5% of whey protein double emulsion (WPIcheese), pumpkin seed protein double emulsion (PSPcheese) or rice protein double emulsion (RPcheese) after

14 days of production: hardness (a), meltability (b), and oil loss (c). Bars indicating standard deviations.

Mean (n=3) values followed by the same letters are not significantly different ($P > 0.05$).