Evaluation of ultrafiltrated dairy by-products as fat replacers in the production of reduced fat washed curd cheese

Ana Raquel Borges
Polytechnic of Coimbra
Arona Figueiroa Pires
Polytechnic of Coimbra
Natalí Garcia Marnotes
Polytechnic of Coimbra
David Gama Gomes
Polytechnic of Coimbra
Marta Fernandes Henriques
Polytechnic of Coimbra
Carlos Dias Pereira (cpereira@esac.pt)
Instituto Politecnico de Coimbra https://orcid.org/0000-0003-1705-2301

Research

Keywords: Whey, Buttermilk, Second cheese whey, Ultrafiltration, Reduced-fat cheese

Posted Date: April 14th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-19081/v1

Abstract

In the present study different dairy by-products were used as ingredients in the production of reduced-fat (RF) washed curd cheeses. Whey, buttermilk and sheep's second cheese whey, previously concentrated y ultrafiltration (UF), were used envisaging the improvement of texture and flavour of the RF cheeses. UF concentration, is a technique that can be easily available to small scale dairy plants, allowing for the recovery of those dairy by-products. Conventional full-fat (FF) cheeses presented more than 45% fat (d/b) while RF cheeses presented values in the range 20-30%, being in most cases classified as low-fat cheeses according to national standards. The ratio protein in dry matter/fat in dry matter was lower than 1 in FF cheeses and in the range 1.8-2.7 in RF cheeses. The paste of FF cheeses presented a more pronounced yellow colour at the 60th and 90th days of ripening, indicating that fat plays a major role regarding this parameter. The different by-products showed different performances when added to milk used in the production of cheeses. After the 60th day of ripening, FF cheeses and RF cheeses with added buttermilk presented lower values for the hardness of the paste (5.0-7.5 N) when compared to the remaining cheeses. At the end of ripening, chewiness of the paste was also significantly lower in these cheeses. RF cheeses with 5% incorporation of UF concentrated buttermilk presented the best results both concerning texture and sensory evaluation. This fact can be related to the specific composition of buttermilk, namely to its richness in phospholipids.

Introduction

Characteristics of reduced-fat (RF) and low-fat cheeses (LF)

The firm texture observed in RF and LF cheeses is one of the major problems resulting from fat reduction (Rogers et al. 2009). Cheese structure can be described as a continuous protein network interrupted by dispersed fat globules which originate weak points in the protein network. As reported by Zalazar et al. (2002) and McCarthy et al. (2016), in RF/LF cheeses the para-casein network becomes denser, originating the development of a firm and rubbery texture that does not break down during mastication.

Fat also plays an important role in the development of flavour and appearance of cheese. Banks (2004), indicates that the loss of flavour in RF/LF cheeses results from the lack of precursors from the fat, the lack of fat as a solvent for flavour compounds, or to differences in the physical structure of RF/LF cheeses that inhibit certain enzymatic reactions which are essential for the formation of flavour compounds. Drake et al. 2010, report that flavour differences observed between FF and RF Cheddar cheeses are not solely due to differences in the cheese matrix and flavour release, but also to differences in ripening biochemistry, which lead to an imbalance of many flavour-contributing compounds.

Despite the significant advances in understanding the biochemical and physicochemical characteristics of RF and LF cheeses and the introduction of technological developments, there is still a need to evaluate solutions with the potential to improve the flavour, texture and sensory properties such cheeses.

Strategies to improve the characteristics of RF/LF cheeses

The general approaches that have been used to improve RF or LF cheese texture involve decreasing protein concentration, promoting greater hydrolysis of proteins, or creating a bigger filler phase in order to limit the density of the para-casein network (Rogers et al. 2010). These strategies can be divided in three categories: (i) manipulation of process parameters to enhance moisture level; (ii) starter culture selection and use of adjunct cultures (*i.e.* non-starter lactic acid bacteria); (iii) use of stabilizers and fat mimetics to improve cheese texture (Mistry 2001).

The process parameters can be modified in order to increase water retention in the curd, which influences texture properties. This can be achieved by using lower coagulation temperatures, by increasing curd grain size, by lowering curd scalding temperatures or by increasing the surface area of fat globules through milk homogenization (Banks 2004; Mayta-Hancco et al. 2019).

The use of exopolysaccharide (EPS) producing starter cultures can also improve the textural characteristics of LF cheeses by changing the microstructure and proteolysis (Di Cagno et al*.* 2014) and has the potential to improve the flavour (Oluk et al*.* 2014a,b).

The use of fat replacers, alone or in combination with the manipulation of process parameters, is perhaps the most promising alternative to improve the sensory properties of RF/LF cheeses. These ingredients are water-soluble compounds used to replace the functional characteristics of fat. They improve texture and yield (Koca & Metin 2004), as well as the sensory and functional properties, by binding water and by providing a sense of lubricity and creaminess. Polysaccharides (PS) and whey proteins (WP) are the fat replacers most commonly used.

Lashkari & Khosrowshahi (2014), tested the effects of incorporating guar and arabic gums in milk with various fat contents on the chemical and rheological properties of Iranian white cheese. Diamantino et al. 2014 report that waxy maize starch used as a fat replacer increased the moisture content and the water holding capacity, improving the overall quality of RF cheeses. Wydrych et al. (2015), report that partial replacement of milk fat with inulin increased meltability, density, cohesiveness and viscosity, while it decreased hardness and adhesiveness of acid casein processed cheeses. Wang et al. (2016) indicate that the addition of carrageenans improved the textural and rheological properties of LF cheeses by increasing moisture in non-fat substance (MNFS). Palatnik & Herrera (2017) compared cheeses containing agave fructans with FF and RF samples without fructans and demonstrated the texturing role of the carbohydrates. Dai et al. (2018), report that konjac glucomanan ca be a potential fat replacer to be used in Mozzarella. Sharma Kanal et al. (2018) report that the addition of alginate significantly improved the textural, microstructural and colour properties of LF Cheddar cheeses. Li et al. (2019), tested waxy rice starch, sodium carboxymethyl cellulose (CMC) and glutamine transaminase (TG) as texturizers and crosslinking agents in Mozzarella.

Native WP can be aggregated to obtain colloidal microparticulated whey protein (MWP). MWP is formed by mixtures of native whey proteins and protein aggregates. These particles can be manufactured in diameters ranging from 0.1 to 100 μm (Torres et al. 2016; Zhang et al. 2016). Their ability to enhance creaminess is based on a "*ball bearing mechanism*" originally reported by Cheftel & Dumay (1993) and confirmed by Liu et al. (2016).

According to Henriques et al. (2018), several aspects have to be considered when using WP as ingredients in cheese production: (i) the importance of WP denaturation, which allows for their contribution to the protein matrix of the cheese; (ii) the increased water-holding capacity of cheese curds; (iii) the lower acidification of the cheeses as a result of the higher buffering capacity of WP; (iv) the occurrence of differences in flavour of the modified products, which tend to be more pronounced over ripening.

When using WP as a fat replacer in cheese it is recommended a low ratio of native/denatured WP to ensure its function as inert filler. Hinrichs (2001) reports that particle size should be in the range 1-10 µm in order to avoid disturbance of the para-casein network. However, other authors refer that higher particle sizes (20-100 µm) do not impart negative effects to cheese properties, namely taste, flavour or consistency (Frusch & Kokx 2008; Giroux et al. 2018).

Whey proteins and MWP are commercially available as powders. However, small and medium-scale cheese plants can concentrate whey by ultrafiltration and, after appropriate treatments, use the liquid whey concentrates in cheese production. The same methodology can be applied to other dairy by-products, envisaging their in-plant valorization.

In the present work, three liquid by-products of the dairy industry, namely buttermilk, whey and second cheese whey (the whey resulting from the production of whey cheese), all previously concentrated by ultrafiltration (UF), were tested as ingredients in the production of experimental RF/LF cheeses.

Materials And Methods

Three types of reduced-fat cheese with different dairy by-products as ingredients were compared with conventional full-fat cheese (FF) and reduced-fat cheese (RF). The tested fat replacers were, buttermilk (CB), whey (CW) and sheep´s second cheese whey (CS). Second cheese whey or *Sorelho* is the by-product resulting from the manufacture of *Requeijão*, the Portuguese whey cheese. All the by-products were previously concentrated by ultrafiltration (UF) with a volumetric concentration factor of *ca.* 15 (VCF=VFeed/VRetentate). The five cheese products were coded as: i) conventional full-fat cheese (FF); ii) reduced-fat cheese (RF); iii) reduced-fat cheese with concentrated buttermilk (RF+CB); iv) reduced-fat cheese with concentrated whey (RF+CW) and; v) reduced-fat cheese with concentrated sheep's second cheese whey (RF+CS).

UF concentrates were obtained using the procedure reported by Henriques et al. (2013) with small adjustments, namely the ultrafiltration process temperature (40-45 ºC) and the smaller membrane cut-off (10 kDa). In the case of CW, after concentration, the retentate was submitted to thermal denaturation (90 ºC for 20 min) prior to homogenization at 10 MPa, in a homogenizer Rannie™ model Blue Top (Denmark), being kept frozen (-25 ºC) until the moment of use. Buttermilk and *Sorelho* were concentrated by UF using the same conditions and pasteurized at 75 ºC for 5 min, being kept frozen at -25 ºC prior to their incorporation into the milk.

The bovine milk (Quinta da Cioga, Portugal) was delivered to the dairy pilot plant at *Escola Superior Agrária de Coimbra* (ESAC). Part of the milk was skimmed in a Westfalia™ type ADB centrifuge (Germany) and standardised to 3.5% (v/v) of fat (for the production of FF cheese) and to 1.5% (v/v) of fat (for the production of reduced-fat cheeses). At this stage, according to the different batches to be produced, the fat replacers (5% v/v) were added. Each batch was made up of 40 L.

Milk pasteurization took place at 74±1 °C for 30 s in Pasilac Therm™ (France) plate and frame heat exchanger. After temperature stabilization (29.5±0.5 °C), 0.2 $mL.L^{-1}$ $CaCl_2$ solution (51% w/v) (supplied by Tecnilac, Portugal), starter culture (Mesófilo Plus Starter, Enzilab, Portugal) (10 $mg.L^{-1}$, containing *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. c*remoris* and *Streptococcus thermophilus*), KNO_3 (25 $mg.L^{-1}$) and 20 $mg.L^{-1}$ animal rennet (> 92 g/100 g chimosin, supplied by Tecnilac, Portugal) were added to the milk formulations and mixed thoroughly. The coagulation of the mixtures was performed for approximately 45 min at 30±1 ºC. When coagulation was completed, grids were used to cut the curd into small pieces (2 cm^3), in order to promote whey drainage. After drainage of half of the whey, the same amount of salted hot water (1% w/v salt, 30 ºC) was added to the curd and the mixture was thoroughly agitated prior to the final whey drainage. The recovered curd was then moulded into plastic moulds before being pressed and stored in a refrigerated chamber at 8-9 ºC, for 24 h. After this period, cheeses, weighing approximately 250 g were immersed in a brine solution (18-20 ºBaumé) for 1.5 h and finally transferred to the ripening chamber (10±2 ºC) being kept there for 90 days.

The chemical composition of cheeses, colour, texture, pH and titratable acidity were assessed on the 1st, 30th, 60th and 90th days of ripening. Each physicochemical parameter was evaluated in triplicate.

Cheese moisture was determined by drying the cheese sample in an oven at 105 ºC for 24 h according to AOAC method 248.12 (1997). Dried samples were tested for ash content in a muffle furnace at 550 °C for 4 h (AOAC 935.42, 1997).

The fat content was determined using the Van Gulik method (ISO 3433, 2008). Cheese protein was determined by multiplying the total nitrogen content of the samples, obtained using the Kjeldahl procedure (AOAC 920.132, 1997), by a factor of 6.38.

The pH was measured directly in cheeses, using a pH meter (PHM61 Laboratory pH Meter, Denmark) equipped with a probe for reading solids and the titratable acidity was expressed as g of lactic acid/100 g cheese (AOAC 920.124, 1997).

According to the physicochemical composition of each cheese sample, moisture in non-fat substance (MNFS) and fat in dry matter (FDM) were calculated.

Colour was expressed by the individual three coordinates of CIEL*a*b* system using a Chroma Minolta CR-200B colorimeter (Japan). For each cheese type, three readings for colour were performed on the rind and on the paste of two cheeses (n=6).

Cheese hardness (N), adhesiveness (g.s), chewiness and cohesiveness were determined by means of texture profile analysis (TPA) using a Stable Micro Systems Texture Analyzer (model TA.XT Express Enhanced, Stable Micro Systems LTD, UK). The cylindrical test probe used for the test was TA-24 (1/4" diameter, acrylic, 35 mm tall) under a pre-test speed of 1.0 mm/s and the test speed of 2 mm/s. while for texture. Three penetrations were performed on the surface (without rind) of two cheese samples (n=6).

Non-trained members of staff and students performed sensory analysis, at the 30th, 60th and 90th days of ripening. Each of the sensory evaluation tests involved 30 members which, individually, expressed their consent. The tests involved evaluation of the cheese samples according to the following parameters: external and sliced aspect, aroma, taste and texture. Overall impression was also evaluated through a ranking test. Each cheese category was coded and the tasters were asked to evaluate both the visual and gustatory aspects using a 1-9 scale (1=dislike extremely; 2=dislike very much; 3=dislike moderately; 4=dislike slightly; 5=neither like nor dislike; 6=like slightly; 7=like moderately; 8=like very much; 9=like extremely).

One-way ANOVA tests, included in StatSoft Statistica 8.0, were performed to compare the means of the physicochemical properties of the cheeses and attributes used for the sensorial evaluation. The Tukey HSD post-hoc test, with a 95% confidence level was applied to assess differences between treatments.

Results And Discussion

Table 1 presents the composition of the different ingredients added as fat replacers to the 1.5% (v/v) fat milk batches. Significant differences were observed in the protein, fat and ash contents of those products. Concentrated buttermilk presented the lower level of protein, while CS presented the highest value. The lowest fat content was observed in CS. CB and CW presented similar dry matter values, while CS showed a significantly lower content.

Table 1. Proximal composition of the different ingredients used for cheese production.

	DRY MATTER	±	PROTEIN	±	FAT	±	ASH	±
CB	13.78^{a}	0.42	3.65^{a}	0.07	1.41^{a}	0.01	0.75^{a}	0.09
CW	13.19^{a}	0.68	5.49^{b}	0.04	2.81^{b}	0.01	0.92^{b}	0.03
CS	10.35^{b}	0.14	6.56^{c}	0.05	0.41^{c}	0.01	0.54^{c}	0.02

CB: UF concentrated buttermilk; CW: UF concentrated whey; CS: UF concentrated sheep’s second cheese whey. Means within the same column with different superscripts are significantly different ($p < 0.05$).

The differences in the composition of the ingredients are reflected in the dry matter and fat content of the mixtures used for cheese production (Table 2). The mixtures containing CW and CS presented the lower levels of fat, while the mixture used for the production of FF cheese presented a higher level of fat and lower levels of protein, lactose and minerals. The protein, lactose and ash contents did not show significant differences between the mixtures used for the production of RF cheeses.

Table 2. Composition of the different milk batches used for cheese production.

	DM	±	F	±	P	±	L*	±	M	±
FFM	11.45^{a}	0.04	3.35^{a}	0.01	3.04^{a}	0.02	4.40^{a}	0.03	0.66^{a}	0.00
RFM	9.91^{b}	0.08	1.41^{b}	0.03	3.18^{b}	0.02	4.63^{b}	0.03	0.69^{b}	0.00
RFM+CB	10.03^{b}	0.04	1.43^{b}	0.02	3.22^{b}	0.01	4.68^{b}	0.02	0.70^{b}	0.00
RFM+CW	9.63^{c}	0.16	1.17^{c}	0.03	3.17^{b}	0.05	4.61^{b}	0.07	0.69^{b}	0.01
RFM+CS	9.84^{bc}	0.14	1.22^{bc}	0.05	3.23^{b}	0.03	4.69^{b}	0.05	0.70^{b}	0.01

FFM: full-fat milk; RFM: reduced-fat milk; RFM+CB: reduced fat milk plus UF concentrated buttermilk; RFM+CW: reduced fat milk pus UF concentrated whey; RFM+CS: reduced fat milk plus UF concentrated sheep's second cheese whey; DM: dry matter; F: fat; P: protein; L*: lactose; M: minerals). Means within the same column with different superscripts are significantly different (p< 0.05) (*calculated by difference).

As it can be observed in Fig.1A, the FF cheese presents a significantly higher (p<0.05) level of solids in all stages of ripening, when compared to all other samples. Both the type of cheese and ripening time have significant effects on the dry matter content. Concerning moisture in non-fat substance (MNFS) FF cheeses also present higher values (Fig.1B). Exceptions are the values of MNFS of RF, RF+CB and RF+CW at the 30th day, and of RF+CB at the 60th day of ripening, which present values similar to FF.

According to the Portuguese standard, NP-1598 (IPQ, 1983), at the 30th day of ripening all cheeses can be considered as semi-soft (61-69% MNFS). At the 60th day, only FF and RF+CB cheeses maintain this classification, being all others classified as semi-hard (54-63% MNFS). At the end of the ripening period (90th days), all the cheeses are classified as hard (49-56% MNFS).

The protein content is significantly lower in the FF cheeses (Fig.2A). In all other cheese samples, protein represents more than 50% of the solids, being the highest values observed in RF+CW and RF+CS. Concerning fat content (Fig. 2B), the FF cheese presents more than 45% fat on dry basis (being classified as a full-fat cheese according to NP-1598), whereas all other cheeses present values in the range 20-30%, being, in most cases, classified as low-fat (10-25% dry basis). However, in the cases of RF at the 1st, 60th and 90th days of ripening, RF+CB at the 1st and 60th days of ripening and of RF+CW at the end of ripening, the cheeses can be classified as half-fat according to NP-1598 (25-45% dry basis).

The ratio protein in dry matter/fat in dry matter (Pdm/Fdm) (Fig. 3A) shows clear differences between FF and RF cheese samples. This value is lower than 1 in the FF cheese, whereas in the case of the remaining samples is in the range 1.8-2.7, being the highest values observed in RF+CS. Although CS presents significantly higher protein content when compared to CB or CW, the mixtures used for cheese production did not show significant differences regarding this parameter. Hence, the protein content of CS cannot justify, on its own, this occurrence. Second cheese whey normally presents a high proportion of denatured whey protein aggregates resulting from the drastic heat treatment (*ca.* 90 ºC 10 min) to which whey is submitted during the production of whey cheeses. The better retention of such aggregates in the cheese curd, as compared to native proteins, may explain the higher protein content of RF+CS and, to some extent, of RF+CW (in which protein was also denatured). With regard to the ratio protein in dry matter/moisture (Pdm/M), the maximum value attained by the FF cheeses is around 1.15, at the end of the ripening period, whereas in the case of the reduced fat cheeses is in the range 1.2-1.4 (Fig. 3B). Higher values of Pdm/Fdm and of Pdm/M are expected to promote a harder texture, associated to lower sensory scores of the reduced-fat cheeses. RF+CB cheeses present ratios of Pdm/M lower than 1.0, both at the 30th and 60th days of ripening, with values slightly higher than those of the FF cheese. This fact had positive repercussions on the textural and sensory properties of these cheeses.

On the first day of ripening, the pH values of the cheeses are in the order of 5.5-5.9, being significantly lower ($p<0.05$) in the case of RF+CW and significantly higher in the case of RF+CS (*ca*. 5.9) (Fig. 4A). After the 30th day of ripening, the values decreased to 5.0 in the case of FF and RF, being significantly higher in the cases of cheeses with added fat replacers (*ca*. 5.2). After this moment, the pH increased steadily until the end of ripening, being the increase more pronounced in the case of cheeses with added fat replacers. RF+CS cheeses showed higher pH values at the 60th day of ripening, being significantly higher at the 90th day. The titratable acidity (TA) shows the inverse tendency (Fig. 4B). The highest values were observed for FF and RF cheeses at the 60th and 90th days of ripening. Overall, the reduced-fat cheeses containing fat replacers presented lower TA values, being the lowest values observed for RF+CW in all stages of ripening.

Concerning the colour parameters of the cheeses (Fig. 5), the luminosity (L*) of the rind of RF+CW cheeses is significantly lower at the first day of ripening. At the 30th day, the L* value of the rind is significantly higher in RF and RF+CB when compared to all other samples. After the 60th day of ripening all the reduced-fat cheeses show significantly lower L* values when compared to FF, being the cheeses with added fat replacers clearly darker when compared to FF an RF. The luminosity of the paste showed a tendency to increase between the 30th and the 60th day, exception made to the case of RF+CS. At the end of the ripening period all cheeses showed significantly lower L* values when compared to the initial values, indicating the darkening of the paste. By the end of ripening, all the reduced-fat cheeses showed significantly lower L* values of the paste, being the paste of RF+CW and RF+CS significantly darker than the ones of RF and RF+CB. Thus, it appears that both those fat replacers significantly impaired the colour of the paste of cheeses. Concerning the a* parameter of the rind, the initial values were very similar, reflecting the white colour of the products, but shifted towards the green colour (*ca.* -3.5) after 30 days. Then, these values increased until the 60th day, being the increase more pronounced in RF and RF+CB. By the end of ripening the a* values of the rind decreased again in all reduced-fat cheeses, with the exception of RF+CW. At the end of ripening RF+CS showed a significantly lower a* value. The a* values of the paste were very similar until the 30th day, then decreased at the 60th day and finally increased slightly at the end of the ripening period. This increase was more pronounced in RF+CW and RF+CS. The FF cheese presented significantly lower a* values at the 60th and 90th days of the ripening period. From the 1st to the 30th days of ripening, the b* value of the rind shifted from 0 to values around 20 in the case of the FF cheese, while in the cheeses with fat replacers, at the 30th day, the b* values were in the order of 15-17. This evolution indicates the shift from white to yellow. Then, the b* values were maintained, or slightly reduced, until the end of ripening. The same pattern was observed with the b* value of the paste. However, the change only was evident from the 60th day onwards. The paste of the FF cheese presented a more pronounced ($p<0.05$) yellow colour at the 60th and 90th days of ripening, indicating that fat has a significant impact on this parameter. RF+CW and RF+CS presented significantly lower values of b* at the 60th and 90th days of ripening.

With regard to the texture (Fig. 6), hardness values show significant differences ($p<0.05$) at the 30th day of ripening, presenting RF+CW and RF+CS higher values. From the 60th to the 90th day of ripening, a sharp increase of the hardness values was observed in the cases of RF, RF+CW and RF+CS, while RF+CB showed values similar to those of FF cheese. FF and RF+CB maintained values of hardness in the order of 5.0-7.5 N all over ripening. In the case of adhesiveness, RF+CW and RF+CS also present significantly lower values by the end of the ripening period, whereas RF+CB presented the highest values. In all cases this parameter significantly decreased between the 60th and the 90th day of ripening. At the end of ripening, chewiness values are significantly higher in RF, RF+CW and RF+CS being the values of RF+CB similar to those of FF. It is evident that after the 60th day of ripening, with the exception of RF+CB, all the reduced-fat cheeses show clear differences in texture when compared to FF cheeses. Concerning these parameters, it can be considered that the use of liquid buttermilk was the best option for the replacement of fat, since RF+CB cheeses are very similar to FF cheeses. Diamantino et al. 2014 reported that fat reduction increased the hardness of *Minas* fresh cheeses, promoting a denser microstructure and less proteolysis. Henriques et al. 2018, report values of the order of 3.6 N for the hardness of RF cheeses with addition of 10 (v/v) liquid whey protein concentrates (LWPC) plus 0.25-0.5% (m/v) Simplesse™, while conventional RF cheeses showed values in the order of 8 N. The cheeses with addition of LWPC produced by those authors also presented significantly higher levels of MNFS, when compared to conventional RF cheeses. Kavas et al. (2014) report that cheese fracturability, cohesiveness and hardness increased with decreasing fat, while elasticity and adhesiveness decreased. They also report that cheese lightness and red and yellow indexes decreased with decreasing fat content.

The sensory evaluation results are depicted in tables 3 and 4. At the 30th day of ripening no differences between cheeses could be detected, regarding their appearance. RF+CW and RF+CS cheeses obtained significantly lower scores for texture and taste. This fact is also reflected by the lower ranking obtained by both samples (Table 4). At the 60th day of ripening the defects in texture and taste of RF+CW were not evident, while RF+CS showed significantly lower scores for these parameters. However, at the end of the ripening period RF+CW presented significantly lower scores for appearance, texture and taste. The FF cheese presented the highest scores for aroma and taste at the 60th day of ripening and for appearance and taste at the end of the ripening period. RF+CB presents similar results to the FF cheeses in all stages of ripening. It has to be highlighted that, until the 60th day of ripening, for several sensory parameters, RF+CB cheeses obtained the highest scores, although not significantly different from the ones obtained by FF cheeses.

Table 3. Sensory evaluation of the cheese samples at the 30th, 60th and 90th days of ripening.

	AP 30	±	AR 30	±	TE 30	±	TA 30	±
FF	7.00^{a}	1.17	6.63^{ab}	1.85	7.03^{a}	1.22	7.23^{a}	1.63
RF	7.10^{a}	1.71	6.63^{ab}	1.56	6.67^{ab}	1.73	6.57^{ab}	1.83
RF+CB	7.50^{a}	1.17	6.87^{a}	1.38	7.33^{a}	1.49	7.27^{a}	1.26
RF+CW	6.80^{a}	1.65	6.00^{ab}	1.60	5.77^{b}	1.45	5.73^{b}	1.98
RF+CS	6.63^{a}	1.50	5.73^{b}	1.48	5.57^{b}	1.65	5.57^{b}	1.94
	AP 60	**±**	**AR 60**	**±**	**TE 60**	**±**	**TA 60**	**±**
FF	7.53^{a}	1.20	7.23^{a}	1.10	7.53^{ab}	1.20	7.50^{a}	1.28
RF	7.30^{a}	0.95	6.70^{a}	1.42	7.40^{ab}	1.35	7.27^{ab}	1.55
RF+CB	7.53^{a}	0.94	7.03^{a}	1.19	7.67^{a}	1.18	7.10^{ab}	1.27
RF+CW	7.23^{a}	1.04	6.77^{a}	1.38	7.00^{ab}	1.39	6.90^{ab}	1.32
RF+CS	7.20^{a}	1.19	6.60^{a}	1.48	6.63^{b}	1.73	6.43^{b}	1.89
	AP 90	**±**	**AR 90**	**±**	**TE 90**	**±**	**TA 90**	**±**
FF	7.77^{a}	1.28	7.13^{a}	1.61	7.37^{a}	1.69	7.50^{a}	1.59
RF	7.40^{ab}	1.04	6.70^{a}	1.58	6.90^{ab}	1.54	6.93^{ab}	1.53
RF+CB	7.43^{ab}	0.94	6.90^{a}	1.40	6.97^{ab}	1.63	7.00^{ab}	1.51
RF+CW	5.93^{b}	1.66	6.17^{a}	2.02	6.00^{b}	1.82	6.33^{b}	1.63
RF+CS	6.77^{ab}	1.36	6.10^{a}	1.90	6.63^{ab}	1.52	6.63^{ab}	1.52

AP=appearance; AR=aroma; TE=texture; TA=taste. (FF) full-fat; (RF) reduced-fat; RF+CB: reduced-fat with concentrated buttermilk; RF+CW: reduced-fat with concentrated whey; RF+CS: reduced-fat with concentrated second cheese whey. Means within the same column with different superscripts are significantly different ($p< 0.05$).

Table 4. Ranking of the cheese samples at the different periods of ripening. Lower values indicate higher positioning in the ranking.

	RANK 30	±	RANK 60	±	RANK 90	±
FF	2.23^{a}	1.30	2.23^{a}	1.22	2.17^{a}	1.49
RF	2.80^{a}	1.32	2.97^{ab}	1.30	2.60^{ab}	1.35
RF+CB	2.23^{a}	1.07	2.60^{ab}	1.28	2.90^{ab}	1.18
RF+CW	3.83^{b}	1.23	3.50^{b}	1.46	3.87^{b}	1.31
RF+CS	3.90^{b}	1.18	3.70^{b}	1.37	3.47^{b}	1.14

FF: full-fat; RF: reduced-fat; RF+CB: reduced-fat with concentrated buttermilk; RF+CW: reduced-fat with concentrated whey; RF+CS: reduced-fat with concentrated second cheese whey. Means within the same column with different superscripts are significantly different ($p < 0.05$). AP=appearance; AR=aroma; TE=texture; TA=taste. Means within same column with different superscripts are significantly different ($p < 0.05$).

MWP and buttermilk added to the cheese milk improved the texture of cheeses whereas the flavour was improved by selected *Lactobacillus* ssp. isolated from good-quality cheese (Skeie et al. 2013). The results presented by these authors show that it was possible to produce a 10% fat Dutch cheese with an improved texture compared with the regular cheese without any additional ingredients. Stankey et al. 2017, also report that MWP improved Cheddar cheese yield due to the water-binding ability of denatured whey protein and improved the textural properties by decreasing firmness. Hence, similar results could be expected with the addition of liquid CW, but this was not observed, particularly after the 60th day of ripening. At the 60th day of ripening the ratio Pdm/M of RF+CB showed values for similar to FF cheeses while in all other cases this ratio presented significantly higher values, which adversely affected texture. Perreault et al. (2017) assessed the effect of denatured whey protein concentrate (DWPC) and its fractions on cheese yield, composition, and rheological properties of cheeses. For cheeses with the same moisture content, the use of DWPC had no direct effect on rheological parameters. The protein aggregates were primarily responsible for the increase in cheese yield while moisture content explained to a large extent the variation in cheese rheological properties. Olivares et al. (2019) evaluated the fat mimicking mechanism of MWP in milk-based systems using rheological and tribological techniques reporting that friction levels attained with MPW proteins and dairy fat at typical speeds involved in oral processing were comparable, demonstrating therefore the capability of MWP dispersions to imitate dairy fat in milk-based systems from a lubrication point of view. Romeih et al. (2012), used buttermilk powder in LF Cheddar cheese and refer that cheese made with BM addition had an homogeneous protein network with small voids and a smoother and less coarse structure. Bahrami et al. (2015) also used BM in cream cheese. As the percentage of BM increased, the total solids, fat, protein, fat in dry matter and ash of cheese milk decreased significantly, leading to a softer and moister curd. However, samples prepared with more than 25% BM were not acceptable to the taste panel. Hickey et al. (2017), evaluated the effect of BM powder addition post-curd formation, or liquid BM addition to cheese milk, on the characteristics of Cheddar-style cheese. Addition of 10% buttermilk powder resulted in higher phospholipid content, moisture, pH and salt levels, and lower fat in dry matter. BM addition also originated a more porous cheese microstructure with higher fat globule coalescence and increased free fat, while increased moisture and decreased protein, fat and pH levels. Hickey et al. (2018), report that liquid BM addition to cheese milk resulted in a softer cheese compared to other cheeses, while BM powder addition had no influence on cheese firmness compared to the control cheese. However, significant differences in sensory profiles associated with off-flavour were also observed with the addition of liquid BM to cheese milk. Addition of 10% BM powder to cheese curds resulted in cheese comparable to the control Cheddar with similar structural and sensory characteristics, although with differences in overall cheese flavour. In the case of our products no adverse effects resulted from the addition of BM to cheese milk.

Conclusion

The reduction of fat in cheeses often affects negatively their sensory properties. Therefore, several approaches are normally used to minimize those negative effects. In the present study, UF concentrated liquid buttermilk, whey protein concentrate and sheep's second cheese whey were used for the purpose. From the results obtained, it is evident that UF concentrated liquid buttermilk improved significantly the properties of RF/LF cheeses, which showed good overall sensory assessment and compared well to FF cheeses. It is recommended that further work should compare the fat replacing properties of such products, both in the liquid and dry form. Optimization of mixtures of such by-products should also deserve further research.

Abbreviations

BM: buttermilk; CB: concentrated buttermilk; CW: concentrated whey; CS: concentrated second cheese whey; DWPC: denatured whey protein concentrate; FF: full-fat; LF: low-fat; LWPC: liquid whey protein concentrates; MWP microparticulated whey protein; MNFS: moisture in non-fat substance; RF: reduced-fat; UF: ultrafiltration.

Declarations

Acknowledgements: The authors are gratefull to Adélia Vaz, Lurdes Pires and Jorge Arede for their help in the production of cheeses in pilot plant.

Availability of data and materials: all data generated or analyzed during this study are included in this published article and can be provided by the corresponding uthor upon request.

Ethics approval: not applicable; informed consent to participate in sensory tests was provided to panel members.

Consent for publication: not applicable.

Competing interests: the authors declare that they have no competing interests.

Authors' contributions: ARB, AFP, NGM, DG conducted the study (UF concentration of dairy by-products, cheese production, preparation and analysis of samples); CDP supervised the experiments, analyzed the data, and drafted the manuscript; MFH reviewed and edited the article. All authors read and approved the final manuscript.

Funding: this research was funded by by national funds through the ministry of Agriculture and Rural Development and co-financed by the European Agricultural Fund for Rural Development (EAFRD), through the partnership agreement Portugal2020-PDR, under the project PDR2020-101-030768: LACTIES-*Inovação, Ecoeficiência e Segurança em PME´s do Sector dos Lacticínios;* and through FCT-Foundation for Science and Technology-project UID/AMB/00681/2019.

References

AOAC (1997) Official methods of analysis of Association of Official Analytical Chemists. 16th ed. Volume II. 33 Dairy Products USA.

Bahrami M, Ahmadi D, Beigmohammadi F, Hosseini F (2015) Mixing sweet cream buttermilk with whole milk to produce cream cheese. Irish J. Agric. & Food Res. 54 (2):73-78. https://doi.org/10.1515/ijafr-2015-0008

Banks JM (2004) The technology of low-fat cheese manufacture. Int. J. Dairy Tech. 57:199-208.

Cheftel J C & Dumay E (1993) Microcoagulation of proteins for development of creaminess. Food Reviews Int. 9:473-502.

Dai S, Jiang F, Corke H and Shah N P (2018) Physicochemical and textural properties of mozzarella cheese made with konjac glucomannan as a fat replacer. Food Res. Int. 107:691-699. https://doi.org/10.1016/j.foodres.2018.02.069

Diamantino V R, Beraldo F A, Sunakozawa T N, Lúcia A and Penna B (2014) Effect of octenyl succinylated waxy starch as a fat mimetic on texture, microstructure and physicochemical properties of Minas fresh cheese. LWT-Food Sci. and Tech. 56:356-362. https://doi.org/10.1016/j.lwt.2013.12.001

Di Cagno R, De Pasquale I, De Angelis M, Buchin S, Rizzello C G and Gobbetti M (2014) Use of microparticulate whey protein concentrate, exopolysaccharide-producing Streptococus thermophiles, and adjunct cultures for making low-fat Italian Caciotta-type cheese. J. Dairy Sci. 97:72-84. http://dx.doi.org/10.3168/jds.2013-7078

Drake M A, Miracle R E and Mcmahon D J (2010) Impact of fat reduction on flavour and flavour chemistry of Cheddar cheeses. J. Dairy Sci. 93:5069-5081. https://doi.org/10.3168/jds.2010-3346

Frusch JAH & Kokx JJMP (2008) Cheese with added whey protein agglomerates. European Patent Application. EP1917861 A1.

Giroux H J, Veilllete N, Britten M (2018) Use of denatured whey protein in the production of artisanal cheeses from cow, goat and sheep milk. Small Rum. Res. 161:34-42. https://doi.org/10.1016/j.smallrumres.2018.02.006

Henriques M, Gomes D, Brennan K, Skryplonek K, Fonseca C, Pereira C (2018) The use of whey proteins as fat replacers for the production of reduced fat cheeses. In: Marta Henriques, Carlos Pereira (Eds.). Cheese Production, Consumption and Health Benefits. Nova Science Publishers. New York. ISBN: 978-1-53612-841-3

Hickey C D, Diehl BWK, Nuzzo M, Millqvist-Feurby A, Wilkinson MG, Sheehan JJ (2017) Influence of buttermilk powder or buttermilk addition on phospholipid content, chemical and bio-chemical composition and bacterial viability in Cheddar style cheese. Food Res. Int. 102:748-758. https://doi.org/10.1016/j.foodres.2017.09.067

Hickey C D, O'Sullivan M G, Davis J, Scholz D, Kilcawley K N, Wilkinson M G, Sheehan J J (2018) The effect of buttermilk or buttermilk powder addition on functionality, textural, sensory and volatile characteristics of Cheddar-style cheese. *Food Research International*, *103*: 468-477.

https://doi.org/10.1016/j.foodres.2017.09.081

ISO 3433, (2008) Cheese-Determination of fat content-Van Gulik method. International Organization for Standardization (ISO).

Koca N & Metin M (2004) Textural, melting and sensory properties of low-fat fresh kashar cheeses produced by using fat replacers. Int. Dairy J. 14:365-373. https://doi.org/10.1016/j.idairyj.2003.08.006

IPQ-Portuguese Institute of Quality (1983) NP-1598. Cheese Definition, classification, packaging and marking. Lisbon, Portugal.

Lashkari H & Khosrowshahi A (2014) Chemical composition and rheology of low-fat Iranian white cheese incorporated with guar gum and gum arabic as fat replacers. J. Food Sci. Tech. 51:2584-2591. https://doi.org/10.1007/s13197-012-0768-y

Li H, Liu Y, Sun Y, Li H and Yu J (2019) Properties of polysaccharides and glutamine transaminase used in mozzarella cheese as texturizer and crosslinking agents. LWT-Food Sci. Tech. 99:411-416. https://doi.org/10.1016/j.lwt.2018.10.011

Liu K, Stieger M, van der Linden E and van de Velde F (2016) Effect of microparticulated whey protein on sensory properties of liquid and semi-solid model foods. Food Hydrocol. 60:186-198. https://doi.org/10.1016/j.foodhyd.2016.03.036

Mistry V V (2001) Low fat cheese technology. Int. Dairy J. 11:413-422. https://doi.org/10.1016/S0958-6946(01)00077-2

McCarthy C M, Wilkinson M G, Kelly P M and Guinee T P (2016) Effect of salt and fat reduction on proteolysis, rheology and cooking properties of Cheddar cheese. Int. Dairy J. 56:74-86. https://doi.org/10.1016/j.idairyj.2016.01.001

Olivares M L, Shahrivar K, de Vicente J (2019) Soft lubrication characteristics of microparticulated whey proteins used as fat replacers in dairy systems. J. Food Eng. 245:157-165. https://doi.org/10.1016/j.jfoodeng.2018.10.015

Oluk A C, Güven M and Hayaloglu A A (2014) Proteolysis texture and microstructure of low-fat Tulum cheese affected by exopolysaccharide-producing cultures during ripening. Int. J. Food Sci. Tech. 49:435-443. https://doi.org/10.1111/ijfs.12320

Oluk A C, Güven M and Hayaloglu A A (2014) Influence of exopolysaccharide-producing cultures on the volatile profile and sensory quality of low-fat Tulum cheese. Int. J. Dairy Tech. 67:265-276. https://doi.org/10.1111/1471-0307.12118

Palatnik D R, Herrera P A, Rinaldoni A N, Basurto R O and Campderrós M E (2017) Development of reduced-fat cheeses with the addition of Agave fructans. Int. J. Dairy Tech. 70:212-219. https://doi.org/10.1111/1471-0307.12334

Perreault V, Rémillard N, Chabot D, Morin P, Pouliot Y, Britten M (2017) Effect of denatured whey protein concentrate and its fractions on cheese composition and rheological properties. J. Dairy Sci. 10(7):5139-5152. https://doi.org/10.3168/jds.2016-12473

Rogers N R, Drake M A, Daubert C R, Mcmahon D J, Bletsch T K and Foegeding E A (2009) The effect of aging on low-fat, reduced-fat, and full-fat Cheddar cheese texture. J. Dairy Sci. 92:4756-4772. https://doi.org/10.3168/jds.2009-2156

Rogers N R, McMahon D J, Daubert C R, Berry T K and Foegeding E A (2010) Rheological properties and microstructure of Cheddar cheese made with different fat contents. J. Dairy Sci. 93:4565-4576. https://doi.org/10.3168/jds.2010-3494

Romeih E A, Moe K M, Skeie S (2012) The influence of fat globule membrane material on the microstructure of low-fat Cheddar cheese. Int. Dairy J. 26 (1):66-72. https://doi.org/10.1016/j.idairyj.2012.03.008

Sharma Khanal B K, Bhandari B, Prakash S, Liu D, Zhou P and Bansal N (2018) Modifying textural and microstructural properties of low fat Cheddar cheese using sodium alginate. Food Hydrocol. 83:97-108. https://doi.org/10.1016/j.foodhyd.2018.03.015

Skeie S, Alseth GM, Østlie H, Abrahamsen R K, Johansen A G, Øyaas J (2013) Improvement of the quality of low-fat cheese using a two-step strategy. Int. Dairy J. 33 (2):153-162. https://doi.org/10.1016/j.idairyj.2013.04.003

Torres I C, Mutaf G, Larsen F H and Ipsen R (2016) Effect of hydration of microparticulated whey protein ingredients on their gelling behaviour in a non-fat milk system. J. Food Eng. 184:31-37. http://dx.doi.org/10.1016/j.jfoodeng.2016.03.018

Zhang Z, Arrighi V, Campbell L and Lonchamp J (2016) Properties of partially denatured whey protein products: Formation and characterisation of structure. Food Hydrocol. 52:95-105. https://doi.org/10.1016/j.foodhyd.2015.06.009

Zalazar C A, Zalazar C S, Bernal S, Bertola N, Bevilacqua A and Zraitzky N (2002) Effect of moisture level and fat replacer on physico-chemical, rheological and sensory properties of low fat soft cheeses. Int. Dairy J. 12:45-50. http://dx.doi.org/10.1016/j.jfoodeng.2016.03.018

Wang F, Tong Q, Luo J, Xu Y and Ren F (2016) Effect of carrageenan on physicochemical and functional properties of low-fat Colby cheese. J. Food Sci. 81:1949-1955. https://doi.org/10.1111/1750-3841.13369

Wydrych J, Gawron A and Jeli T (2015) The effect of fat replacement by inulin on the physicochemical properties and microstructure of acid casein processed cheese analogues with added whey protein polymers. Food Hydrocol. 44:1-11. https://doi.org/10.1016/j.foodhyd.2014.08.022

Figures

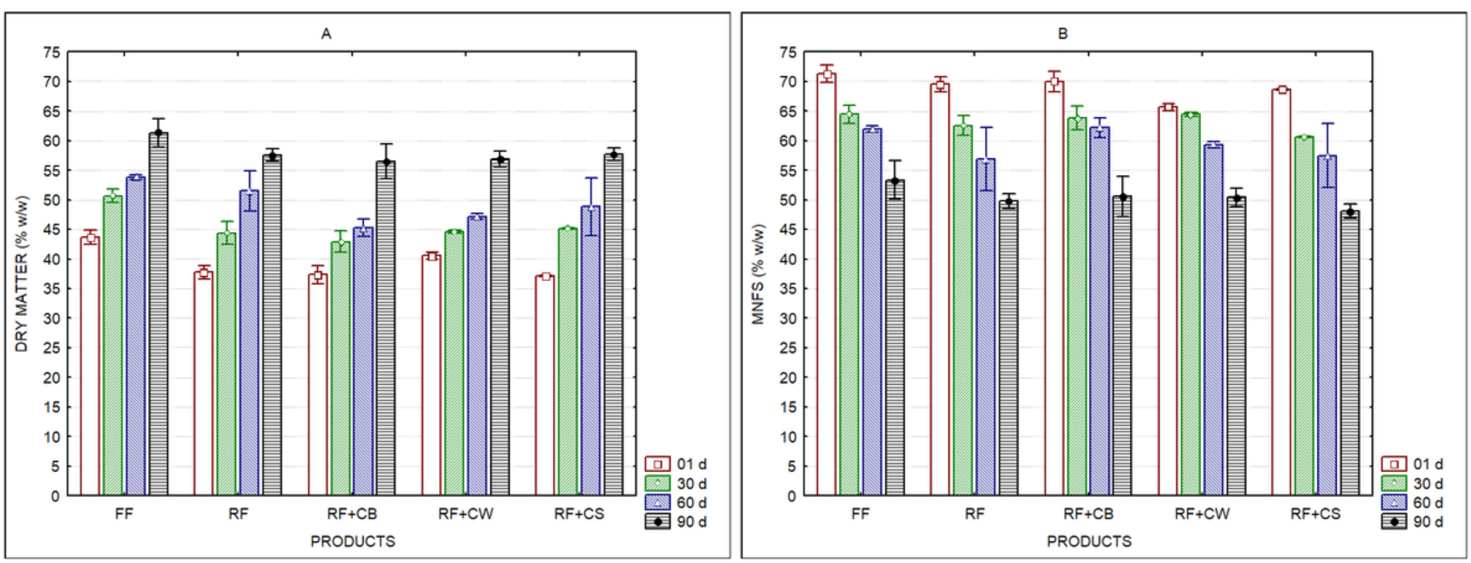

Figure 1

Dry matter (A) and moisture in non-fat substance (MNFS) (B) of tested cheeses over ripening.

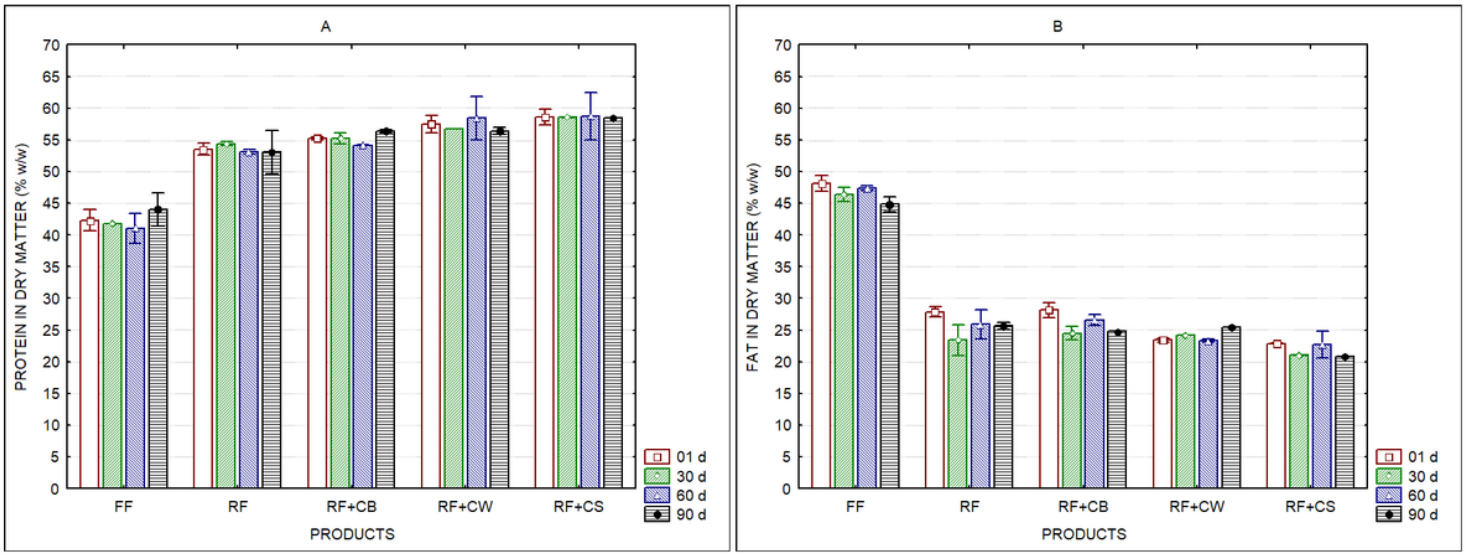

Figure 2

Protein in dry matter (A) and fat in dry matter (FDM) (B) of tested cheeses over ripening.

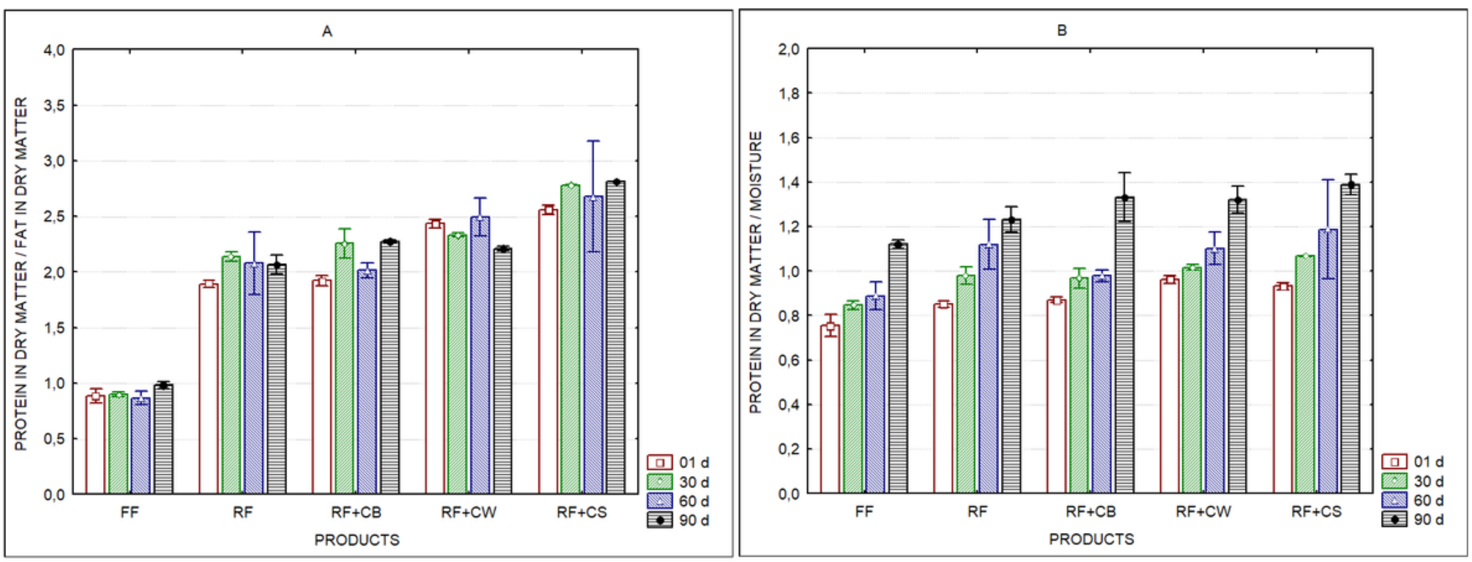

Figure 3

Ratio protein in dry matter/fat in dry matter (Pdm/Fdm) (A) and protein in dry matter/moisture (Pdm/M) (B) of tested cheeses over ripening.

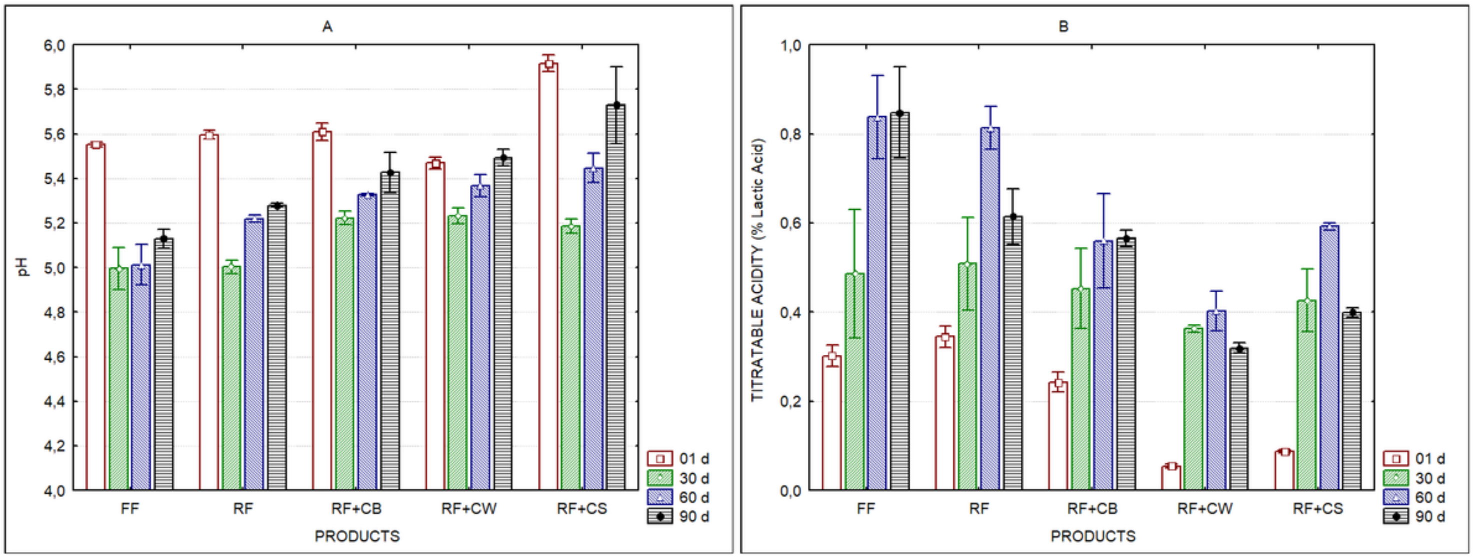

Figure 4

(A) pH and (B) titratable acidity (TA) of tested cheeses over ripening.

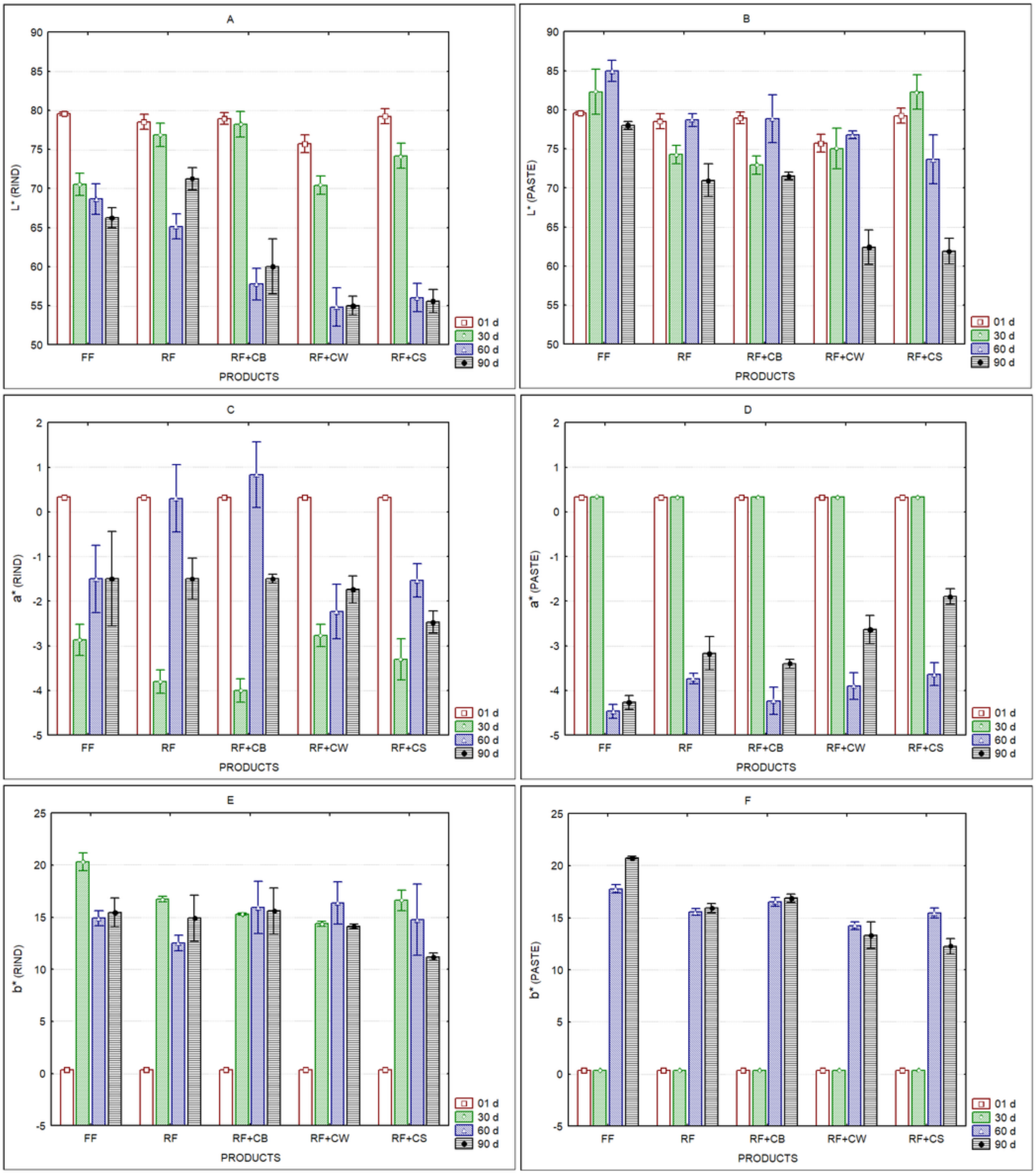

Figure 5

Colour parameters of the rind (A) and of the paste (B) of tested cheeses over ripening.

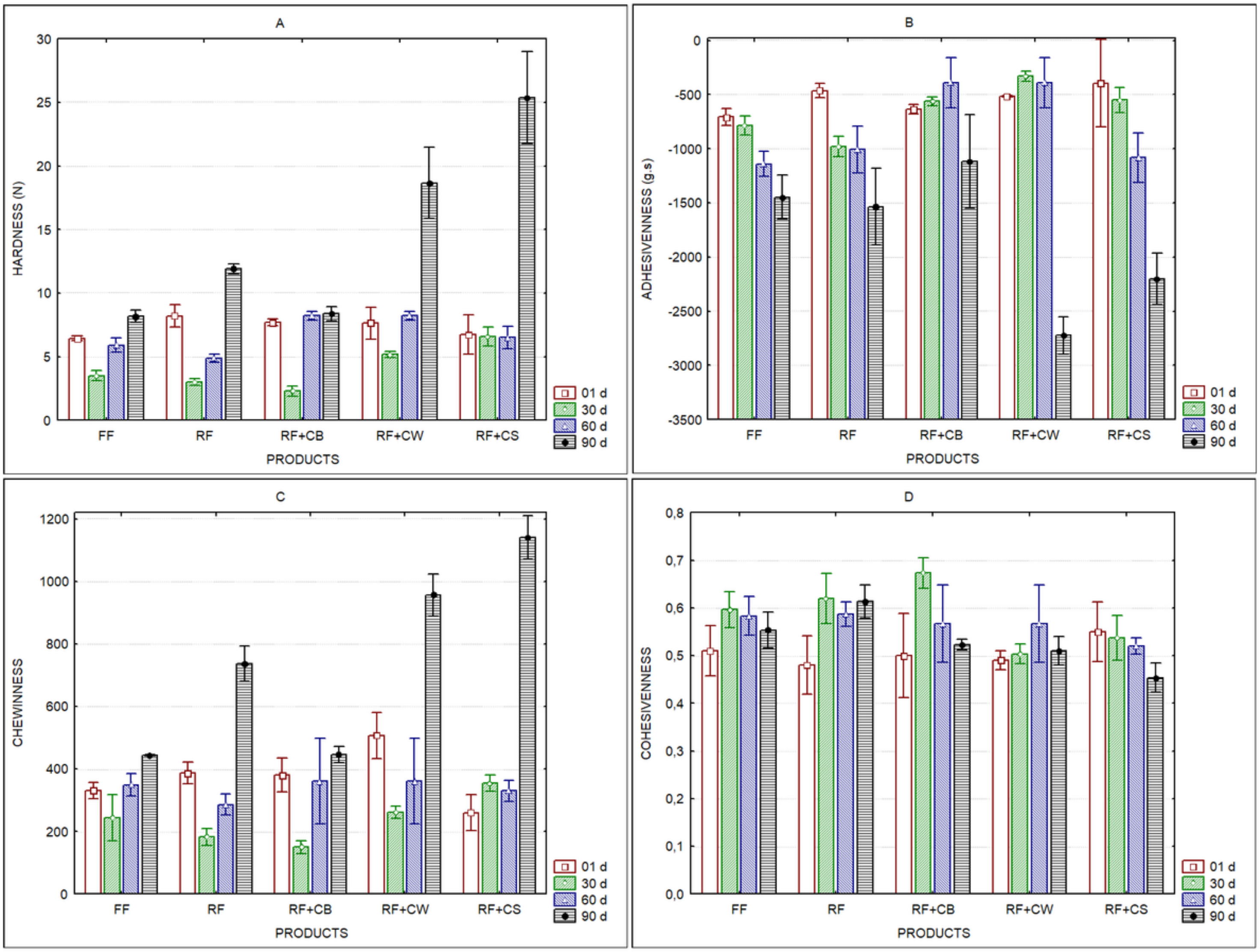

Figure 6

Texture parameters of the tested cheeses over ripening. (A) Hardness; (B) Adhesivenness; (C) Chewinness; (D) Cohesivenness.