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Effect of whey storage on physicochemical properties, microstructure and texture profile of ricotta cheese

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The aim of this study is to evaluate the effect of whey storage period (0, 24, 48 and 72 h) on the physicochemical parameters, color, texture and microstructure of fresh ricotta during storage. Sweet whey and acid whey were evaluated based on titratable acidity, pH, fat, cryoscopy, and density, while ricotta was based on yield, fat, protein, ash, acidity, pH, moisture, total solids, color, texture, and microstructure. This was done with analysis of variance in a completely randomized design using Tukey test at 5% probability. Whey pH values increased with storage time. Ricotta made with stored whey had average yield of 5.33%, with decreased fat content and pH, and increased acidity. There were subtle differences in color and texture of ricotta during storage; its hardness and gumminess decreased, resulting in microstructure compression. It is concluded that the production of ricotta with whey stored for up to 72 h makes the product appropriate for consumption.

Key words: Byproduct, fat, fresh cheese, ricotta, whey cheese, organoleptic properties.

INTRODUCTION

Significant milk production and consumers' acceptance of dairy products have increased the production of various types of cheeses. This has generated significant amounts of liquid waste, from 85 to 95% of the total milk volume,

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called whey (Dragone et al., 2009). In many dairy industries, this byproduct is considered waste and disposed without prior treatment, thus representing the main source of pollution in this sector (Prazeres et al., 2012). The high organic content of whey causes serious environmental impacts due to the high biochemical oxygen and chemical oxygen demand (Magalhães et al., 2011).

However, the biological treatment of whey has high costs and is therefore economically unviable for most dairy industries (Dragone et al., 2009). There is a recurring concern about the applicability of whey in milk products of added value as an effective alternative to overcome environmental issues (Silva et al., 2012). Due to the high moisture content and organic compounds, whey is very perishable, so it requires quick use or application of conservation measures such as refrigeration and/or addition of preservatives (Almeida et al., 2001). It maximum time of 72 h is recommended for the collection and industrial processing of whey, and maximum temperature of 10°C is recommended for the transport of refrigerated milk whey in isothermal tanks (Brazil, 2013).

The preparation of ricotta is a rational way to use this byproduct, as it has high nutritional value and is considered one of the most cost-effective products that use whey in its composition (Prudencio et al., 2014). Whey produced by rennet coagulation of milk casein is called sweet whey, while that obtained by lactic acid coagulation is called acid whey (De La Fuente et al., 2002). Cheeses produced by acid coagulation with heat treatment emerged as a tool for recovery of milk proteins and/or sweet whey, which have high nutritional value; examples are ricotta (Italy), anari (Cyprus) and manouri (Greece) (Fox et al., 2000). Other varieties are cottage and cream cheese (USA), white cheese (Latin America), karish (Egypt), and cokelek (Turkey). These cheeses must be consumed fresh, within 15 or 30 days (Guneser and Yuceer, 2011).

According to Brazilian law, fresh ricotta is the product obtained from cheese whey albumin with 20% of milk added. It must have cylindrical shape, weigh up to 300 to 1000 g, and unformed or unclear rough crust; it must be soft consistently, not pasty and friable; its texture should be closed with some mechanical holes, and should have white or creamy white color, with typical odor and flavor (Brazil, 2010). However, there is no technical regulation in Brazil to identify the quality of ricotta. This impairs standardization of technological procedures among the different dairy industries and control of physical and chemical, rheological, sensory and microbiological parameters during storage (Carrijo et al., 2011). As cooling helps to maintain the physical and chemical characteristics of whey and also improves the quality of the final product in the dairy industry, the aim of this study is to evaluate the behavior of the physical, chemical, and texture profile parameters of fresh ricotta made with whey stored under refrigeration.

MATERIALS AND METHODS

The whey used in this study was obtained from a dairy industry located in Rio Verde - Goiás, that is, the whey is derived from the manufacture of mozzarella cheese. The whey was stored under refrigeration in a cold chamber at temperature of 5 ± 1°C during 0 (control), 24, 48, and 72 h (treatments 1, 2, 3 and 4, respectively). At the end of the storage period, the whey was isothermally transported to the Instituto Federal Goiano - Campus Rio Verde, Goias, Brazil, for aseptic processing of ricotta cheese, according to Prudencio et al. (2014).

Whey acidity was previously standardized at 8°D for all treatments by addition of sodium bicarbonate (Behmer, 1999). Whey was heated slowly at 90°C under constant stirring. Lactic acid (0.1% v/v diluted in water 1:10) was added for it coagulate. After 10 min of rest, the supernatant mass was collected and placed in cylindrical molds with holes. It was turned for 1 h and refrigerated at 10°C for 24 h to obtain syneresis. Cheeses were sectioned into four parts, vacuum sealed and kept under refrigeration until they were analyzed. The tests were replicated during storage on 1, 8, 15, and 22 days. Yield was evaluated by the ratio between final mass of ricotta and initial mass of whey (kg), expressed as percentage, according to Zeng et al. (2007).

Sweet whey and acid whey were evaluated for acidity by acid-alkaline titration method No. 920.124 (AOAC, 2005; Brazil, 2006); pH by pHmeter PG 2000/Gehaka® (Brazil, 2006); fat by the Gerber method No. 2000.18 (AOAC, 2005; Pearson, 1976); cryoscopy by electronic microprocessor cryoscope model M90 BR/Laktron® (Brazil, 2006) and density at 15°C with Quevenne lacto-density meter (1.015 to 1.040 g.cm^{-3}) (Brazil, 2006).

Ricotta cheeses were analyzed during storage for physicochemical parameters of acidity and pH (AOAC, 2005; Brazil, 2006), and instrumental color and texture. On the eighth day of storage, dry extract composition was evaluated by gravimetric method No. 990.19 (AOAC, 2005; Brazil, 2006), ash by incineration in muffle method No. 930.30 (AOAC, 2005) fat by method No. 2000.18 (AOAC, 2005; Pearson, 1976) and crude protein by micro-Kjeldahl method No. 939.02 (AOAC 2005; Brazil, 2006).

For color and texture analysis of ricotta cheese, cubic samples of 2 cm edge were used. Color was determined with a Colorflex EZ/HunterLab® colorimeter adjusted to daylight illumination D65 and 10° angle through the CIELab system (CIE, 1996). According to the HSV color model, colorimetric space is defined by rectangular coordinates: L*, a* and b* corresponding to brightness from black (0) to white (100), chromaticity green (-)/red (+) and blue (-)/yellow (+), respectively; and cylindrical coordinates: chroma color saturation (C*) and Hue angle (°h) calculated by formulas $(a^{*2}+b^{*2})^{½}$ and arctang (b*/a*) (ABNT, 1992).

The texture profile analysis (TPA) was evaluated by texturometer with load cell of 25 kg (CT3/Brookield®) consisting of double compression test, test speed of 1.0 mm/s, compression distance of 10.0 mm, equivalent to 50% compression, contact force of 3.0 g and acrylic cylindrical probe (TA4/1000), operated by the CT Texture Pro V1.1 Build 7 software (Brookfield Eng. Labs, Inc.). Primary parameter data of hardness, cohesiveness, elasticity index, and secondary of gumminess and chewiness were collected (Buriti et al., 2005).

Microstructure was assessed at the eighth day of storage by scanning electron microscopy (SEM; JSM-6610/Jeol®), with prior lyophilization (Enterprise II/Terroni®), fat extraction in Soxhlet, No. 1122 (IUPAC, 1979) and plating in gold.

All tests were conducted in triplicate and data were analyzed by statistical software and analysis of variance. They were allocated in a completely randomized design by Tukey test at 5% probability as

Table 1. Mean titratable acidity (%), pH, fat (%), cryoscopy (° H) and density values (g /mL) of sweet whey and acid whey obtained from the processing of ricotta produced with refrigerated whey stored for zero, 24, 48 and 72 hours.

Parameter	Type of whey	Whey storage (h)			
		0	24	48	72
Acidity	SW	0.09±0.00[a]	0.10±0.02[a]	0.09±0.00[a]	0.09±0.00[a]
	AW	0.13±0.02[b]	0.14±0.01[ab]	0.14±0.00[a]	0.15±0.01[a]
pH	SW	6.18±0.06[c]	6.59±0.04[b]	6.60±0.04[b]	6.68±0.07[a]
	AW	5.13±0.10[b]	5.28±0.18[ab]	5.35±0.06[a]	5.42±0.21[a]
Fat	SW	0.57±0.17[a]	0.58±0.07[a]	0.63±0.05[a]	0.61±0.05[a]
	AW	0.12±0.24[a]	0.02±0.04[a]	0.00±0.00[a]	0.00±0.00[a]
Cryoscopy	SW	-0.520±0.003[ab]	-0.514±0.005[b]	-0.518±0.002[ab]	-0.527±0.014[a]
	AW	-0.582±0.022[a]	-0.567±0.021[a]	-0.578±0.018[a]	-0.582±0.027[a]
Density	SW	1.02±0.00[a]	1.02±0.00[a]	1.02±0.00[a]	1.02±0.00[a]
	AW	1.02±0.00[a]	1.02±0.00[a]	1.02±0.00[a]	1.02±0.00[a]

Means followed by same letter in line do not differ significantly by the Tukey test at 5% probability. SW, Sweet whey; AW, acid whey.

Table 2. Average fat, protein and ash values of ricotta produced with whey stored for zero, 24, 48 and 72 h.

Storage (h)	Parameters (%)			
	Yield	Fat	Protein	Ash
0	5.39[a]	10.95±1.05[a]	12.47±0.99[a]	1.54±0.24[a]
24	5.21[a]	10.34±1.80[a]	11.84±0.90[a]	1.47±0.11[a]
48	5.23[a]	10.08±0.83[a]	12.42±1.28[a]	1.49±0.18[a]
72	5.51[a]	8.44±1.09[b]	12.28±1.42[a]	1.36±0.18[a]

Means followed by same letter in line do not differ significantly by the Tukey test at 5% probability.

0, 24, 48 and 72 treatments of refrigerated whey. Titratable acidity, pH, fat, cryoscopy and density of sweet whey, acid whey and yield on the day of ricotta cheese processing were evaluated. Ash, fat, protein and SEM of ricotta cheese were evaluated on the eighth day. The other parameters, acidity, pH, moisture, total solids, color and texture were evaluated during storage days of 1, 8, 15, and 22.

RESULTS AND DISCUSSION

Titratable acidity, fat, cryoscopic point and density of sweet whey (0.09%, 0.60%, -0,520°H and 1.02 g/ml, respectively) did not differ with increasing refrigeration period, and unlike pH, increased from 6.18 to 6.68 (Table 1). The physicochemical parameters of sweet whey are consistent with those established by Brazilian law: acidity from 0.08 to 0.14% and pH from 6.0 to 6.8 (Brazil, 2013). In goat milk whey, pH 6.38 was found (Pizzillo et al., 2005). Sweet whey fat contents are related to the cheese manufacturing technology, which was obtained in accordance with the mixing force and milk fat content. Prato cheese whey has levels close to 0.4% (Pinto et al., 2011). For goat cheese whey, the content is higher (1.69%) (Pizzillo et al., 2005) while for fresh cheese whey, the content is lower (0.20%) (Ordóñez et al., 2005), with variations of up to 0.36% (Pescuma et al., 2010).

For the cryoscopic index, which evaluates the freezing point, values close to zero indicate addition of water; value of -0.520°H for sweet whey confirms suitability for processing, given the proximity to cryoscopy allowed for milk, from -0.530 to -0.550°H (Brazil, 2011). In the acid whey, fat content (0.04%), cryoscopy (-0.577°H) and density (1.02 g/ml) remained stable (Table 1). Acidity increased from 0.13 to 0.15%. There was an increase in pH, from 5.13 to 5.42; which remained lower than the limit of 6.0 (Brazil, 2013). There is an increase in acidity and a reduction in fat content from sweet whey to whey acid. This behavior is due to double clotting process used in ricotta production, in which the addition of organic acid increases acidity and part of the fat is inserted into the protein matrix and remains in the clot (Ordóñez et al., 2005).

The yield of ricotta cheese was not affected by whey storage period, with average value of 5.33% (Table 2). In literature, variable yields have been reported: 3.17% for ricotta with bovine whey (Hawkins et al., 2009), 7.9% for ricotta with goat and bovine milk whey (Borba et al.,

Table 3. Mean acidity (%), pH, moisture (%) and total solids (%) values of ricotta made with whey stored for zero, 24, 48, and 72 h at 1, 8, 15, and 22 days of storage.

Parameter	Storage (h)	Storage (days)			
		1	8	15	22
Acidity	0	0.19±0.05^{cB}	0.41±0.08^{bB}	0.48±0.13^{abC}	0.53±0.06^{aC}
	24	0.43±0.04^{bA}	0.47±0.08^{bAB}	0.48±0.08^{bC}	0.64±0.03^{aB}
	48	0.45±0.05^{cA}	0.45±0.06^{cB}	0.61±0.08^{bB}	0.70±0.08^{aB}
	72	0.46±0.06^{cA}	0.53±0.10^{cA}	0.77±0.13^{bA}	0.90±0.10^{aA}
pH	0	5.98±0.28^{aA}	5.88±0.26^{abA}	5.78±0.03^{bcA}	5.69±0.04^{cA}
	24	5.92±0.11^{aAB}	5.84±0.07^{abA}	5.74±0.04^{bcAB}	5.64±0.06^{cAB}
	48	5.85±0.12^{aAB}	5.75±0.08^{abA}	5.68±0.04^{bAB}	5.60±0.17^{bAB}
	72	5.81±0.13^{aB}	5.73±0.04^{abA}	5.60±0.02^{bcB}	5.52±0.08^{cB}
Moisture	0	74.60±0.76^{abB}	74.99±0.96^{abA}	75.39±0.73^{aA}	74.01±0.36^{bB}
	24	73.88±1.57^{bB}	74.67±0.75^{abA}	74.94±0.77^{aAB}	74.55±1.03^{abB}
	48	74.54±0.79^{abB}	72.62±0.81^{cB}	75.17±0.71^{aA}	74.16±1.41^{bB}
	72	75.63±0.85^{aA}	73.63±1.70^{bB}	74.02±1.43^{bB}	75.75±1.21^{aA}
Total Solids	0	25.40±0.75^{abA}	25.01±0.96^{abB}	24.61±0.73^{bB}	25.99±0.36^{aA}
	24	26.12±1.57^{aA}	25.33±0.75^{abB}	25.06±0.77^{bAB}	25.45±1.03^{abA}
	48	25.46±0.79^{bcA}	27.37±0.82^{aA}	24.83±0.71^{cB}	25.84±1.41^{bA}
	72	24.37±0.85^{bB}	26.37±1.70^{aA}	25.98±1.43^{aA}	24.25±1.21^{bB}

Means followed by same lowercase letter in line and uppercase letter in column do not differ significantly from each other according to the Tukey test at 5% probability.

2014), and 16. 88% with the addition of 5% milk (Hawkins et al., 2009). The addition of milk whey and calcium salts is favorable for ricotta yield because casein clots strengthen the protein network by improving the rheological properties of the cheese mass (Smithers, 2008). The high whey acidity accelerates clotting and negatively influences the texture, making the cheese mass softer, thus reducing yield. But, slow acidification is related to a slight increase in gel hardness (Lucey, 2004). Thus, the lack of significant differences in yield is also due to acidity correction of whey prior to processing and to the standardized protocol.

Lipids are involved with color, flavor, other sensory characteristics, yield, firmness and texture of cheese (Vargas et al., 2008). According to Brazilian ordinance No. 146/1996, cheeses are classified as extra fat or double cream with over 60% dry basis (d.b.); fat between 45 and 59.9% d.b.; semi-fat between 25 and 44.9% d.b.; low-fat between 10 and 24.9% d.b. and skim with less than 10% d.b. (Brazil, 1996).

The fat content of ricotta ranged from 8.44 to 10.95% wet basis (w.b.), corresponding to 32.15 to 43.90% d.b.. This is classified as semi-fat cheese (Table 2), which is due to the use of whole milk whey with high fat content in the processing (0.60%). The fat content of ricotta (d.b.) increased to 47.29% when 5% milk was added (Hawkins et al., 2009) and decreased to 20.31% when goat milk whey is used in the production of ricotta (Pizzillo et al., 2005). The fat content of ricotta cheese decreased when whey stored for 72 h was used. This does not affect the whey composition, being statistically equal. It may possibly be due to the weakening of the protein network with longer whey storage periods. Thus, fat globules inserted into the protein matrix were released into the exudation water; another possible factor is lipolysis caused by bacterial activity. The protein content of ricotta cheese remained unchanged with the use of whey stored for a longer period, with average value of 12.25%, in accordance with the whey composition (Table 2). Other authors have found, for ricotta with goat milk whey, values of 6.55% (Pizzillo et al., 2005), 10.3% with bovine and goat milk whey (Borba et al., 2014), and 14.92% with bovine milk whey (Prudencio et al., 2013).

The ash content did not change with treatments, with an average of 1.47% (Table 2). Ash content of 0.93% was reported for ricotta with goat milk whey (Pizzillo et al., 2005), 2.22% for ricotta with bovine and goat milk whey (Borba et al., 2014) and 2.46% when ricotta was made with whey protein concentrate (El Sheikh et al., 2011). For processed ricotta, titratable acidity in lactic acid increased both during storage, especially after the second week, when whey was stored for extended periods (Table 3). Increased acidity is due to the synthesis of metabolite by natural microflora bacteria such as *Lactobacillus* species, even in refrigerated environment, a phenomenon known as post-acidification

(Dermiki et al., 2008). Different results are found in literature. Di Pierro et al. (2011) obtained similar acidity results, from 0.26 to 0.34%, after 30 days of storage. Borba et al. (2014) found no difference up to 14 days; it remained at 0.3%. This was attributed to the absence of starter culture in the manufacture of cheese that resulted in whey.

Thus, the natural microflora of milk and whey and cheese manufacturing technology with the addition of starter cultures strongly influence the final acidity of ricotta. In this study, whey was obtained from Prato cheese manufactured with the addition of mesophilic culture of *Lactococcus lactis*, *Lactococcus* subsp. *Cremoris* and thermophilic culture of *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Lactobacillus delbrueckii* subsp. *Helveticus.*

The pH of ricotta cheeses remained relatively stable, suffering slight decrease during whey storage (Table 3). On the first day, the pH was close to the isoelectric point of whey proteins, in accordance with El-Sheikh et al. (2011), who reported pH of 5.91 and Hauschild et al. (2014), who reported pH of 5.87. Such behavior was consistent with the study of Di Pierro et al. (2011) on ricotta. It attributed this slight pH decrease to the formation of acidic amino acids and free fatty acids, resulting from proteolysis and lipolysis, respectively, and in general, to the occurrence of some buffering effect.

The total solids content of ricotta ranged from 24.25 to 27.37%, corresponding to moisture variation from 72.63 to 75.75% (Table 3). Accordingly, with moisture content above 55%, ricotta is classified as very high moisture cheese according to Brazilian ordinance 146/1996/MAPA (Brazil, 1996). A random behavior of increases and decreases in the moisture content of ricotta cheese during storage were observed. These variations can be attributed to heterogeneous syneresis in the vacuum packaging without moisture absorption (Hauschild et al., 2014). The recoverable total solids content in cheeses is quite variable according to the origin of whey, milk proportion and characteristics of manufacturing technologies that influence yield (Hawkins et al., 2009). Borba et al. (2014) found content of 24.05% for ricotta manufactured only with bovine whey and when milk is added, this content increased to 25.91%. Higher levels are found in ricotta prepared with goat milk whey (30.68%) (Pizzillo et al., 2005), or whey previously treated with calcium concentration or precipitation, which removes hydrophilic lipoproteins that reduce yield (Prudencio et al., 2014).

Variation in the physicochemical composition of ricotta during storage is linked to oxidation and degradation reactions and microbial activity. In summary, these changes become more pronounced after the first week of manufacture, with loss of nutritional and sensory quality. It shows that ricotta is prepared without salt, and is a fresh product for immediate consumption and storage of approximately seven days under refrigeration (Carminati et al., 2002). Color is extremely important in food products due to its direct influence on appearance and is one of the parameters related to consumers' acceptance (Ramos et al., 2013). There was a decrease of brightness in ricotta prepared with whey stored for 24 h and after the second week of storage (Table 4). Low L* values are assigned to darkening as a result of oxidation, enzymatic and microbiological degradation, and are undesirable for causing consumers' rejection (Dattatreya and Rankin, 2006). L* values of 93.36 are found for ricotta containing goat milk (Borba et al., 2014). This is due to the presence of smaller fat globules and conversion of β-carotene to vitamin A (Park et al., 2007).

In this study, a* coordinate showed decreased values compared to cheeses manufactured with whey on day zero and whey refrigerated for 72 h, and increased values during storage (Table 4). These values tend to be negative in relation to green color due to the presence of riboflavin, vitamin B_2 (Mestdagh et al., 2011), as observed by Borba et al. (2014) (-2.70 values). However, positive values oriented to red in the present study are the result of the addition of natural dye urucum to prato cheese that remained in sweet whey. The b* coordinate values increased when cheese was manufactured with whey from 0 to 24 h, followed by a slight decline, but did not suffer variations throughout the storage period in these treatments (Table 4).

Possibly, the predominance of the red color over the green color of riboflavin was more pronounced on the first day of whey storage, resulting in the formation of secondary yellow color, and/or transfer of carotenoids from whey to cheese (Sheehan et al., 2009). For the other storage times, compounds of brown color are likely to have prevailed, which are derived from the Maillard reaction, non-enzymatic browning by carbohydrate and protein reactions resulting from the high temperatures used in ricotta processing (Dattatreya and Rankin, 2006). Prudencio et al. (2014) found lower tendency to yellow color on ricotta with whey from fresh cheese, with average b* value of 15.42. Chroma is the degree of color saturation and low values indicate low intensity and are associated with lower purity and formation of mixed colors (Kubo et al., 2013). c* followed the same behavior of b* coordinate, which shows that saturation was more influenced by the tendency to yellow in the color of ricotta.

The color of ricotta measured by the Hue angle decreased during storage. In relation to the whey storage time, shade increased for whey of 24 h, decreased for whey of 48 h, and increased again for whey at 72 h. Kubo et al. (2013) demonstrated the effect of the addition of dye on total color variation, which occurs due to the solubility of the dye in the oil phase of protein-fat cheese matrix. Hue value close to 71° corresponds to the first quadrant of the HSV three-dimensional diagram, between 0° (red) and 90° (yellow). The use of prato cheese whey strongly influenced the color coordinates, especially the

Table 4. Mean values of the color coordinates of ricotta prepared with whey stored for 0, 24, 48 and 72 h at 1, 8, 15 and 22 days of storage.

Coordinate	Storage (h)	Storage (days)			
		1	8	15	22
L*	0	81.56±11.24^{bD}	88.03±0.78^{aB}	87.07±0.61^{aC}	87.51±0.66^{aA}
	24	87.87±1.04^{bcA}	90.82±1.10^{aA}	88.51±1.88^{bB}	87.06±0.78^{cA}
	48	86.00±0.81^{cB}	87.27±0.55^{bB}	91.11±1.22^{aA}	86.61±0.52^{bcA}
	72	83.25±2.32^{cC}	88.47±1.04^{aB}	85.62±1.06^{bD}	83.85±1.20^{cB}
a*	0	5.62±0.57^{dC}	6.14±0.35^{cB}	6.46±0.26^{bB}	6.58±0.28^{aA}
	24	6.16±0.38^{bA}	6.07±0.28^{cBC}	6.41±0.32^{aB}	6.18±0.56^{bC}
	48	6.11±0.32^{dAB}	6.29±0.38^{cA}	7.48±0.32^{aA}	6.45±0.33^{bB}
	72	6.04±0.29^{aB}	6.04±0.23^{aC}	5.98±0.26^{aC}	5.07±0.34^{bD}
b*	0	16.71±2.41^{aC}	17.29±0.48^{aB}	17.22±0.41^{aC}	17.22±0.42^{aB}
	24	19.02±0.46^{aA}	18.96±0.47^{aA}	19.22±0.54^{aB}	18.74±0.47^{aA}
	48	17.94±0.46^{cB}	18.28±0.40^{cBA}	19.60±0.73^{bA}	21.19±8.25^{aA}
	72	18.79±0.61^{aAB}	18.74±0.30^{aA}	18.83±0.36^{aB}	16.52±0.66^{bB}
Chroma	0	17.64±2.43^{aC}	18.35±0.48^{aB}	18.39±0.42^{aC}	18.44±0.47^{aB}
	24	19.99±0.50^{aA}	19.91±0.51^{aA}	20.26±0.57^{aB}	19.75±0.45^{aA}
	48	18.95±0.50^{cB}	19.34±0.46^{cAB}	20.67±0.77^{bA}	22.47±8.14^{aA}
	72	19.74±0.64^{aAB}	19.70±0.32^{aA}	19.760.38^{aB}	17.28±0.71^{bC}
Hue (º)	0	71.26±1.88^{aB}	70.45±1.09^{bC}	69.44±0.79^{cD}	69.09±0.55^{cD}
	24	72.06±0.95^{abA}	72.25±0.58^{aA}	71.56±0.75^{cB}	71.75±1.67^{bcB}
	48	71.18±0.74^{aB}	71.01±0.90^{abB}	70.56±0.53^{bC}	71.05±2.10^{aC}
	72	72.17±0.60^{bA}	72.14±0.60^{bA}	72.38±0.64^{bA}	72.95±0.74^{aA}

Means followed by same lowercase letter in line and uppercase letter in column do not differ significantly from each other according to the Tukey test at 5% probability.

tendency to be red (+a*) and yellow (+b*). The resulting yellowish coloration in the ricotta cheese can be detrimental to consumers' visual acceptance. Thus, this source of whey should be used with caution, with a previous study on sensory acceptance. According to the instrumental texture profile analysis (TPA) of ricotta during storage of 22 days (Table 5), hardness attribute was relatively the same. There were with average values of 1.02, 1.20, 1.68 and 1.01 N, respectively, for control ricotta cheese and for those manufactured with whey of 24, 48 and 72 h. These results were similar to those obtained by Borba et al. (2014), who reported average hardness of 1.94 N on the first 14 days of storage.

Hardness increased when using whey refrigerated for 48 h, and then, hardness values decreased (Table 5). Lipolysis and proteolysis reactions may have affected the stability of the protein matrix and emulsifying agents such as lipoproteins, thus contributing to the increase in hardness (Pereira et al., 2002). Denaturation or a new association among protein may occur during processing, since some whey proteins are sensitive to fluid shear (Almécija et al., 2007), which can interfere with the homogeneity of the protein network and result in reduction in hardness (Buffa et al., 2001). This attribute suffered great variation due to lack of salts in the processing of ricotta cheeses, which act as strengtheners of the protein network (Tunick et al., 2012). The cheese texture closely depends on the microstructure and chemical composition, mainly concerning fat and salt (Wendin et al., 2000) and total solids contents, pH and maturation time (Bowland and Foegeding, 2001).

Hardness is the amount of force required for compression (N); cohesiveness is the ratio between force and time for the areas of two compressions (dimensionless); elasticity is a recovery measure after the first compression (dimensionless); gumminess is the product of cohesiveness by hardness (N) and chewiness is the product of gumminess by elasticity (N) (Tunick et al., 2012). The average cohesiveness and elasticity values of ricotta did not differ significantly ($p>0.05$) throughout the whey storage. Borba et al. (2014) also observed cohesiveness and elasticity values of 0.47 to 0.74, respectively, during storage. These results show that there were no changes in the deformability of ricotta

Table 5. Average texture attribute values of ricotta prepared with whey stored for 0, 24, 48 and 72 h at 1, 8, 15 and 22 days of storage.

Attribute	Storage (h)	Storage (days)			
		1	8	15	22
Hardness (N)	0	0.86±0.16^{aC}	1.02±0.26^{aB}	1.06±0.51^{aB}	1.15±0.20^{aB}
	24	1.26±0.29^{aAB}	1.16±0.20^{aB}	1.11±0.30^{aB}	1.28±0.19^{aB}
	48	1.49±0.38^{bA}	1.62±0.48^{abA}	1.73±0.34^{abA}	1.88±0.40^{aA}
	72	0.99±0.30^{aBC}	1.05±0.15^{aB}	1.02±0.28^{aB}	0.99±0.25^{aB}
Cohesiveness	0	0.33±0.04^{abA}	0.32±0.04^{bA}	0.43±0.20^{aA}	0.33±0.04^{abA}
	24	0.36±0.05^{aA}	0.35±0.04^{aA}	0.41±0.14^{aA}	0.36±0.06^{aA}
	48	0.36±0.06^{aA}	0.35±0.05^{aA}	0.38±0.05^{aA}	0.39±0.02^{aA}
	72	0.34±0.07^{aA}	0.37±0.06^{aA}	0.45±0.15^{aA}	0.34±0.11^{aA}
Elasticity	0	0.96±0.47^{aA}	0.85±0.30^{abA}	0.76±0.18^{bA}	0.82±0.02^{bA}
	24	0.85±0.24^{aA}	0.79±0.02^{aA}	0.84±0.28^{aA}	0.89±0.23^{aA}
	48	0.82±0.02^{aA}	0.93±0.29^{aA}	0.92±0.30^{aA}	0.83±0.02^{aA}
	72	0.77±0.03^{aA}	0.87±0.16^{aA}	0.80±0.02^{aA}	0.90±0.26^{aA}
Gumminess (N)	0	0.29±0.08^{aC}	0.34±0.11^{aB}	0.38±0.19^{aB}	0.38±0.08^{aB}
	24	0.45±0.11^{aAB}	0.40±0.05^{aB}	0.43±0.08^{aB}	0.47±0.12^{aB}
	48	0.55±0.20^{bA}	0.59±0.24^{abA}	0.65±0.17^{abA}	0.73±0.15^{aA}
	72	0.32±0.08^{aBC}	0.39±0.08^{aB}	0.43±0.10^{aB}	0.33±0.12^{aB}
Chewiness (N)	0	0.34±0.19^{aA}	0.32±1±0.04^{aA}	0.29±0.13^{aA}	0.31±0.06^{aA}
	24	0.38±0.13^{aA}	0.31±0.05^{aA}	0.35±0.13^{aA}	0.42±0.20^{aA}
	48	0.40±0.16^{aA}	0.45±0.18^{aA}	0.43±0.21^{aA}	0.37±0.13^{aA}
	72	0.25±0.07^{aA}	0.33±0.06^{aA}	0.34±0.08^{aA}	0.31±0.18^{aA}

Means followed by same lowercase letter in line and uppercase letter in column do not differ significantly from each other according to the Tukey test at 5% probability.

due to changes in the chemical structure of components (Ferrandini et al., 2011).

In relation to gumminess, no significant difference ($p>0.05$) during storage was observed; it was only among treatments. This secondary attribute increased when whey refrigerated for up to 48 h was used, then, the gumminess values decreased, showing similar behavior with primary attribute of hardness. Literature shows values greater than 0.90 N when goat and cow milk whey is used (Borba et al., 2014). Although chewiness attribute is secondary, derivative of hardness, it remained stable. Ciabotti et al. (2009) reported higher chewiness value in ricotta made with mozzarella cheese whey (2.15 N). According to the texture profile analysis, ricotta cheese is defined as a viscoelastic food (Fox et al., 2000) with very soft consistency, not pasty and friable (Brazil, 1996, 2010), compressible and not too cohesive, with brittle characteristics (Tunick et al., 2012), stable texture profile during storage, required for marketing and sensory acceptability.

In scanning electron micrographs, rounded dark areas correspond to the position of fat globules, and the bright area to the protein matrix (Tunick et al., 2012). Images increase by 100x suggests a slight compression and disorder of the protein matrix when ricotta was made with whey stored for a longer period, with losses in granular and rough appearance (Figure 1). Structural changes are observed when there are changes in connections to the whey protein network according to the different manufacturing technologies (Yorgun et al., 2008). During compaction of the microstructure of ricotta with whey stored for 48 h (Figure 1C), the molecular rearrangement of proteins was likely to occur, with strengthened links, as evidenced by increased hardness and gumminess (Table 5). With whey stored for 72 h (Figure 1D), the protein matrix of ricotta loses the spongy characteristic and becomes more compressed. The decrease in interstitial spaces may be the cause and/or effect of the reduced fat content of this treatment (Table 2). There is decrease in hardness and gumminess, suggesting the occurrence of protein bonds is more weakened by proteases, and/or irreversible denaturation.

The structure and texture of acidic coagulation cheeses is closely related to heat and acid levels used during

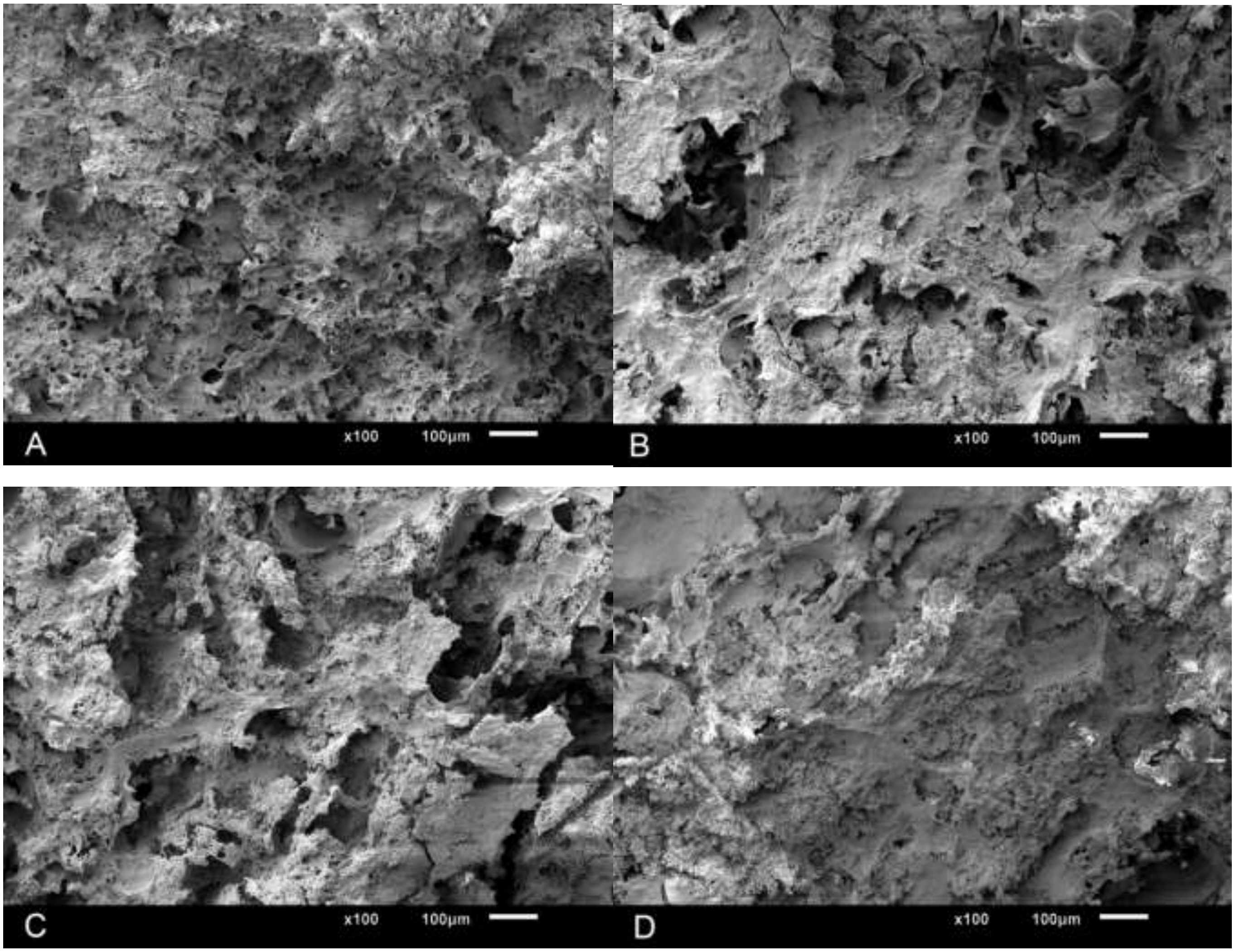

Figure 1. Scanning electronic micrographs at 100x of ricotta made with whey stored for: (A) 0 h - control; (B) 24 h; (C) 48 h and (D) 72 h.

processing as a result of the structural formation of gel and three-dimensional changes of whey protein (Guinee et al., 1993). Lower pH levels, near the isoelectric point of whey proteins, are possibly responsible for the formation of the molten matrix (Tunick et al., 2012). Possibly, immunoglobulins were responsible for this structural change because the isoelectric point (IP) ranges from 5.5 to 8.3 and because they are very sensitive to heat. They interact with β-lactoglobulin (IP 5.2) and bovine whey albumin (IP 4.7 to 4.9) via disulfide bonds (Morr and Há, 1993). Temperature above 70°C causes irreversible denaturation and polymerization of β-lactoglobulin and greater susceptibility to the action of proteases. α-lactalbumin (IP from 4.2 to 5.1) has high denaturation reversibility, around 40% after heating at 95°C due to connections with Ca^{2+} and Zn^{2+} ion (Morr and Há, 1993). Similar microstructure was observed in ricotta made with whey from fresh cheese under similar heat and acidification conditions applied to the process (Prudencio et al., 2014). In different whey concentration technologies, the authors reported compaction on the protein network.

Conclusion

The yield, protein and ash contents of ricotta cheeses were constant; however, at longer whey storage period, the fat content decreased, while acidity increased inversely proportional to pH. Coloration tends to yellow due to the manufacture of ricotta with whey from prato cheese. In texture profile analysis of ricotta cheeses during storage, there was a balance among rheological forces that make up the structure. Hardness and gumminess of the ricotta decreased with the use of refrigerated whey after 48 h. There was a subtle microstructural difference with the protein network compaction of ricotta made with whey stored for longer periods. The manufacture of ricotta cheese with whey stored for longer periods within three days under controlled refrigerated environment is a viable alternative for dairy industries, enabling better logistic use of this byproduct.

Conflict of Interests

The authors have not declared any conflict of interests.

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