TITLE PAGE

- Food Science of Animal Resources -

Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title	**Evaluation of chemical-nutritional characteristics of whey and Ricotta obtained by ewes fed red grape pomace dietary supplementation**
Running Title (within 10 words)	Grape pomace effects on Whey and Ricotta chemical characteristics
Author	Francesca Bennato[1], Andrea Ianni[1]; Lisa Grotta[1], Giuseppe Martino[1,+]
Affiliation	[1]Faculty of BioScience and Technology for Food, Agriculture and Environment, University of Teramo, Via Renato Balzarini 1, 64100 Teramo (TE), Italy
Special remarks – if authors have additional information to inform the editorial office	
ORCID (All authors must have ORCID) https://orcid.org	Francesca Bennato (https://orcid.org/0000-0001-9030-4881) Andrea Ianni (https://orcid.org/0000-0003-3102-6804) Lisa Grotta (https://orcid.org/0000-0001-9618-4569) Giuseppe Martino (https://orcid.org/0000-0002-7878-9318)
Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	
Author contributions (This field may be published.)	Conceptualization: Martino G. Data curation: Bennato F., Ianni A. Formal analysis: Bennato F., Grotta L. Methodology: Ianni A., Bennato F. Software: Bennato F., Ianni A. Validation: Ianni A. Investigation: Bennato F. Writing - original draft: Bennato F. Writing - review & editing: Ianni A., Martino G.
Ethics approval (IRB/IACUC) (This field may be published.)	This work does not require Ethics approval

CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	**Fill in information in each box below**
First name, middle initial, last name	Giuseppe Martino
Email address – this is where your proofs will be sent	gmartino@unite.it
Secondary Email address	
Postal address	Via Renato Balzarini 1, 64100 Teramo (TE), Italy
Cell phone number	
Office phone number	+39-0861-266950
Fax number	

Abstract

This study aimed to investigate the effect on the chemical quality of whey and Ricotta obtained from ewes fed a red grape pomace dietary supplementation. The analyses were performed on whey, before and post Ricotta cheese-making, and in Ricotta after 1 (T1) and 5 (T5) d of ripening at 4°C. Moreover, fatty acid profile of whey before ricotta cheese-making and Ricotta T1 of ripening and volatile profile of Ricotta T1 and T5 were investigated. The diet did not affect whey and Ricotta lipid content, conversely, significant variations were instead observed with regard to color. A lower amount of total phenolic compounds was found in whey before ricotta cheese-making, on the contrary, an opposite trend was highlighted in Ricotta T1 although no variations in antioxidant properties were detected. Moreover, grape pomace modified fatty acid profile of whey and Ricotta but did not have any effect on protein profile of the main whey protein. The reduction of hexanal in Ricotta during the ripening suggest a better oxidative stability. The obtained results therefore suggested that the grape pomace inclusion in the ewes diet, while modifying some chemical parameters, did not induce negative effects on the characteristics and quality of dairy by-products.

Keywords: Red grape pomace, Whey, Ricotta, Fatty acid profile, Volatile compounds

Introduction

Ricotta represents a typical soft Italian cheese, mainly obtained by ewes or goat milk, and less frequently by exploiting cow and buffalo milk (Muchetti el al. 2002). Ricotta is specifically obtained by using the cheese whey deriving from breakage of the curd during cheese-making. The production process involves the direct acidification of the whey which increases the protein coagulation, the product that emerge on the surface is collected in plastic baskets characterized by small openings that allow the drainage of the liquid phase. The demand of consumers of traditional, unprocessed, and high nutritional dairy products has recently been increasing and Ricotta responds well to consumer

requests because it is a fresh product, characterized by a low percentage of fat and by a high proteins content. Dietary, genetic, milking and technological factors influence the milk composition and, consequently, the quality of cheese. Although Ricotta is a very widespread whey cheese, the literature on this product is scarce and outdated.

Vinery by-products play an important role in the small ruminant diets (Alba et al., 2019; Coreddu et al., 2020). Grape pomace (GP) is the main solid by-product of the wine industry consisting of peel, pulp, and seeds. Its storage and its disposal generate a lot of environmental and economic problems. Red GP is a matrix rich in compounds with high biological value especially polyphenols (2%-6.5%), as simple flavonoids, phenolic acids, tannins and proanthocyanidins (Yu and Ahmedna, 2013). However, the high levels of lignified fibre, tannins and anthocyanins represent a limitation for use of GP in the diet of the ruminant, since these compounds could negatively the digestive nutrient utilization (McSweeney et al., 2001; Min et al., 2002). In contrast, Moate et al. (2014) have reported that tannins, due to low rumen biodegradability, can induce an increase in the small intestine protein intake, improving rumen metabolism and decreasing methanogenesis.

Furthermore, a lot of studies have shown that the presence in winery waste of a high content of linoleic and oleic acids and phenolic compounds can have beneficial effects on animal health and consequently on the quality of products of animal origin. It has been reported that antioxidant compounds in GP, as flavonoids, can be directly transferred to the milk or after metabolic transformation by rumen microbes (Coreddu et al., 2015), causing an enrichment of the milk with substances that have health benefits for its consumers.

In the present study, the hypothesis to improve the nutraceutical and quality characteristics of milk-derived products has been assayed, supplementing the diet of lactating ewes with a GP supplementation. The presence of phenolic compounds and their effects on chemical-nutritional composition of whey and Ricotta cheeses were investigated.

Materials and Methods

59 **Experimental design and sampling**

60 Fourty-six Assaf ewes, homogeneous for days in lactation and age, were involved in the study and
61 randomly assigned into two groups of twenty-three ewes each: a control group (CTRL) and
62 experimental group (GP+) whose diet was enriched with 10% of red GP. Overall, the trial lasted 60
63 d and during this time interval all animals received isoenergetic and isoproteic diets that were
64 prepared by taking into account the nutritional needs of lactating sheeps.

65 At the end of the trial, 80 L of milk were collected for each group of ewes. The whey obtained from
66 the curd breakage during cheese-making was recovered and used to make Ricotta following the
67 procedure described by Innosa et al. (2020). For both groups, 6 Ricotta cheeses of about 450–500 g
68 were produced for each group, with a yield production equal to 8-9% and. For each group, samples
69 of whey before (WBR) and post (WPR) Ricotta cheese-making were collected. Three Ricotta cheeses
70 were sampled and aliquoted after 1 d (T1) from production, while the remaining three were left at +
71 4℃ for 5 d (T5) and then subjected to the sampling. All the collected samples were packed under
72 vaacum and stored at −20 °C until the analysis.

73

74 **Chemical analysis and color measurement of Ricotta and whey**

75 The moisture of T1 and T5 Ricotta cheeses was determined according to the AOAC methods (2000).
76 Evaluation of total lipids in Ricotta, WBR and WPR was performed by following the procedure
77 reported by Innosa et al. (2020), and **t**he amount of total fat was expressed as mean percentage on a
78 DM basis.

79 A CR-5 colorimeter (Minolta, NY, US) was used to evaluate the color of Ricotta cheese and whey,
80 by calculating the chromatic coordinates L^* (lightness), a^* (redness) and b^* (yellowness). The optical
81 system exploited an aperture size that was adjusted to 3 mm, and each measurement was performed
82 in reflectance by placing the sample into a glass petri dish (33 mm). On the contrary, WBR and WPR
83 measurements were performed in transmittance, the samples were placed into a glass rectangular cell

with optical path of 2 mm. From the already measured parameters were calculated: the total difference of color (ΔE*ab) and the Yellow Index (YI) by using the formulas listed below:

$$\Delta E^*ab = [(\Delta CIE\ L^*)^2 + (\Delta\ CIE\ a^*)^2 + (\Delta CIE\ b^*)^2]^{1/2}$$

$$YI = 142.86X(CIE\ b^*/CIE\ L^*)$$

Final ΔE^*ab values were compared with arrange by Nedomová et al. (2017).

Evaluation of total phenolic compounds and antioxidant potential in Ricotta cheese and whey

Total phenolic compounds (TPCs) of WBR, WPR and Ricotta T1 and T5 were spectrophotometrically evaluated at 765 nm by using the method described by Singleton and Rossi (1965). Fifteen milliliters of a solution composed by methanol and water (70:30, v/v) was added to 5 mL of whey and/or to 1 g of Ricotta. The mixture was shaked for 30 s, incubated at room temperature and in the dark for 40 min and centrifuged (15 min, 4000 × g), then the supernatant was removed for analysis. The calibration curve was prepared by using the gallic acid (1–100 μg/mL, $r2 = 0.994$) and results were expressed as μg equivalent of gallic acid (GAE) per mL of whey and μg equivalent of GAE per g of Ricotta.

With regard to the antioxidant potential, such parameter was evaluated by the ABTS assay according to Brahmi et al. (2012). The calibration curve was built using Trolox (1–32 μmol/mL, $r2 = 0.9991$) and the antioxidant capacity of each sample was reported as μmol equivalent of Trolox (TEAC,Trolox Equivalent Antioxidant Capacity) per mL of whey and μmol equivalent of Trolox per g of Ricotta.

Ricotta and whey fatty acid profile

Seventy milligrams of the WBR and Ricotta T1 lipid extracted as reported in paragraph (Chemical analysis and color measurement of Ricotta and whey**)** were recovered in hexane in which were added

500 µL of sodium methoxide in methanol (1:1, v/v) in order to induce the fatty acids trans-methylation. Fatty acid methyl esters (FAME) were then separated according to Bennato et al. (2019). Peaks areas of each FAME identified were analyzed by using the ChromeCard Software and the values associated to each compound were expressed as relative percentage of total FA. The value of each FA was used to calculate the sum of monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), saturated fatty acids (SFA), short chain fatty acids (SCFA), medium chain fatty acids (MCFA) and long chain fatty acids(LCFA). Furthermore, Atherogenic (AI), Thrombogenic (TI) and Desaturation indices (DI) for C14:0, C16:0, C18:0 and CLA were calculated by using the formulas reported by Innosa et al. (2020).

Whey protein extraction and separation by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Protein profile in WBR and Ricotta T1 and T5 was evaluated via SDS-PAGE, using the procedure reported by Laemmli (1970). For protein extraction, 10 g of Ricotta sample were dissolved in 10 mL of H_2O and heated at 37°C for 15 min. Then, 1 mL of 5% (v/v) acetic acid and after 10 mins 1 mL of 1 N sodium acetate were added. The samples were filtered and to 1 mL of filtered 200 µL of 100% (w/v) TCA were added. The samples were cooled at -20°C for 20 min and centrifuged at 4 °C for 20 min at 12,000 x g. Supernatant was removed, and the pellet was washed thrice with 1 mL of cold acetone at 12,000 x g rpm for 10 min. For whey protein extraction the same procedure used for Ricotta was followed even if 1 mL of whey was treated directly with 200 µL of 100% w/v) TCA.

The proteins extracted were then quantified using Bradford method (Bradford, 1976) and separated on 12% SDS-PAGE gel as reported by Bennato et al. (2020).

Densitometric analysis of the visualized bands was then performed by exploiting the ImageJ software (Rasband, 2012), and the content of proteins was expressed as relative percentage of the total protein.

135 **Determination of Ricotta volatile compounds**

136 The identification of volatile compounds (VOCs) in T1 and T5 Ricotta samples was achieved by
137 making reference to the protocol previously described by Bennato et al. (2020) and based on a solid-
138 phase microextraction (SPME) followed by a gas chromatography-mass spectrometry (GC-MS)
139 analysis performed with a gas chromatograph (Perkin Elmer, Waltham, MA, USA) coupled with a
140 mass spectrometer (SQ8S; Perkin Elmer, USA). . Briefly, 3 g of Ricotta were transferred in vials in
141 which there was the addition of 10 mL of a NaCl solution (360 g/L) and 10 μL of an internal standard
142 (4-methyl-2-heptanone). The VOCs adsorption was performed with a divinybenzene–carboxen–
143 polydimethylsiloxane SPME fiber (Supelco, Bellefonte, PA, USA) exposed for 1 h and at 60 °C in
144 the headspace. The extracted VOCs were thermally desorbed into the GC/MS and identified using
145 Kovats retention index. The data concerning each compound were expressed as relative abundance
146 on the sum of the total identified VOCs.

147

148 **Statistical analysis**

149 SigmaPlot 12.0 Software (Systat software, Inc.) was used for the statistical analysis of the obtained
150 data. Student's *t* test was applied in order to identify significant differences between the two groups
151 of data; p values lower than 0.05 were considered statistically significant. Results were reported as
152 mean ± standard deviation (SD).

153

154 **Results**

155 **Whey and Ricotta features**

156 As shown in Table 1, the diet did not affect WBR and WPR lipid content. Significant differences
157 were observed in color parameters. The colorimetric analysis carried out on WBR showed a lower
158 lightness ($p<0.01$) and a significant decrease ($p<0.01$) of CIE a^* parameter (green-red) towards to a
159 light green nuance. On the contrary, compared with the CTRL-WBR, the GP+-WBR showed higher

160 ($p<0.01$) CIE b^* parameter (blue-yellow) and YI ($p<0.01$). An opposite trend was observed in WPR.
161 In agreement with the criteria of Nedomová et al. (2017), between GP+ and CTRL was observed a
162 middle color difference (ΔE^*ab) both for WBR and WPR.
163 Ricotta T1 made from whey obtained from the rennet breakage of GP+ cheese exhibited a higher DM
164 percentage compared to CTRL ($p<0.01$) but not significant differences between the two groups were
165 observed in lipid content (Table 2). The same trend was observed in Ricotta T5. With regard to the
166 chromatic coordinates, lightness (CIE L*) and redness (CIE a*) were not affected by the diets, while
167 yellowness (CIE b*) and YI were lower in Ricotta T1 deriving from the milk obtained from the GP+
168 ($p<0.01$). After 5 d of ripening, GP+ Ricotta samples showed higher values for lightness ($p<0.05$)
169 and a* ($p<0.05$) compared to the CTRL samples.
170 Very light differences were observed both for Ricotta T1 and T5 between GP+ and CTRL.
171
172 **Total phenolic compounds and antioxidant capacity**
173 The TPC amount in WBR obtained from GP+ was significantly lower ($p<0.05$) compared to the
174 CTRL. On the contrary, no significant variations were observed for WPR in the same samples (Table
175 1). GP+ Ricotta T1 had a higher content of TPCs ($p<0.01$), after 5 d of ripening no significant
176 differences were evidenced. Furthermore, no significant variations were observed for the antioxidant
177 capacity in WBR, WPR and T1 and T5 Ricotta samples (Table 2).
178
179 **Characterization of the fatty acid profile**
180 The fatty acid profile of WBR and Ricotta T1 is reported in Table 3. No significant variations of SFA,
181 MUFA, PUFA, SCFA, MCFA and LCFA were observed in WBR and Ricotta T1. However, in whey
182 samples lower levels of odd-chain fatty acids, as pentadecylic (C15:0, $p< 0.001$) and margaric (C17:0;
183 $p< 0.05$) acids, stearic acid (C18:0, $p< 0.05$), myristoleic acid (C14:1, $p< 0.001$) and linolenic acid
184 (C18:3, $p< 0.05$), on the contrary higher levels of myristic acid (C14:0, $p< 0.05$) and vaccenic acid
185 (C18:1, t11; $p<0.05$) were observed. In Ricotta, lower levels were observed in C15:0 ($p< 0.01$), C17:0

186 ($p< 0.05$), C14:1 ($p<0.001$); on the contrary, higher level of C18:1, t11 ($p< 0.05$) were observed in
187 GP+ Ricotta samples. Furthermore, lower desaturation indices DIC14:0 and DICLA and higher
188 desaturation index DIC18:0 was observed both in whey and Ricotta of GP+ group.
189
190 **Proteolytic profile of whey and Ricotta**
191 SDS-PAGE analysis was exploited in order to characterize the protein profile of whey and Ricotta
192 (Fig. 1). WBR SDS-PAGE analysis showed the separation of the main whey proteins fraction
193 (lactoferrin, serum albumin, β-lactoglobulin and α-lactalbumin) and less intensive bands
194 corresponding to caseins residues. No significant differences in bands intensity were evidenced
195 between the two groups (Table 4).
196 In GP+ Ricotta T1 and T5 samples, a higher intensity of caseins residues was highlighted, β-
197 lactoglobulin band intensity although decrease during the ripening in both groups, a lower ($p<0.05$)
198 band intensity degradation was observed in GP+ Ricotta T5 samples compared with CTRL. No
199 significant variations were observed between the two groups in α-lactalbumin band intensity.
200
201 **Evaluation of volatile compounds**
202 Nine VOCs were identified both in T1 and T5 Ricotta samples, five aldhehydes, two ketones and two
203 carboxylic acids (Table 6). In Ricotta T1, nonanal and octanoic acid were affected by the diet,
204 resulting respectively lower ($p<0.05$) and higher ($p<0.05$) in GP+ samples in comparison to CTRL
205 Ricotta. A lower ($p<0.05$) hexanal amount was highlighted in GP+ Ricotta after 5 d of ripening.
206
207 **Discussion**
208 The dietary supplementation with red GP did not influence the lipid content of whey and Ricotta,
209 however the DM content resulted to be higher in GP+ Ricotta. Differences between the two groups
210 in moisture content of Ricotta could be correlated to a different protein concentration. As highlighted
211 by Salvatore et al. (2014), with a low protein content in the cheese whey, some challenges appear in

gel formation, conversely, with increasing protein concentrations, the number of linkages increases during heating, resulting in a more compact protein gel characterized by an improved water-holding capacity.

Whey and Ricotta color could be influenced by several factors, as the diet used for animal feeding, technological applications as heating and acidification and chemical composition of whey, such as fat, protein, Ca, and P. In the present study variations of CIE L*, CIE a* and CIE b^* components were detected in both GP+-WBR and WPR. Generally, the whey has a straw yellow color due to presence of riboflavin (vitamin B2), however, a lot of pigments contained in animal feeding could influenced a* and b* parameters (Nozière et al., 2006; Schreiner and Windisch, 2006; Solah et al., 2007). Higher CIE b* values and lower CIE a* coordinate and consequently a higher YI in GP+ WBR could be correlate to a different pigment composition compared with CTRL. Most of pigments contained in animal feeding, belonging to polyphenols family have a different affinity for protein and lipids and a different susceptibility to increased temperature like those used for the Ricotta production (Nozière et al., 2006) and this could explain the different trend in CIE b* values and YI observed in GP+ samples of Ricotta compared with WBR. However, despite the variations in color parameters observed in whey and Ricotta samples according to the table of color difference reported by Nedomová et al. (2017), it is possible to state that the difference between the two groups was very light.

Regarding the phenolic compounds, it was possible to observe a lower amount in GP+ WBR and an opposite trend in Ricotta T1. As previously stated for the different color trend between whey and Ricotta, this apparent contradiction could be explained by a different polyphenols composition that transfer differently from whey to Ricotta. The different content of TPCs was not correlated to changes in the antioxidant capacity of WBR and Ricotta T1 evaluated by ABTS assay. Recently, it has been reported that the structure as well as the position and the number of OH groups affects the antioxidant activity of phenolic compounds, producing different results of the two assays (Platzer et al., 2021). .

It has been widely demonstrated that different classes of polyphenols are able to interact with proteins, however, several factors could influence the strength of binding and affinity, such as pH, ionic strength, as well as the protein and polyphenols structures. Furthermore, changes in the temperature, may induce modifications in protein structures and ligand solubility, thus affecting the protein-phenol interactions. Several polyphenolic compounds were demonstrated to bind proteins by specific interactions with proline residues; therefore caseins, characterized by a high proline content, could represent a target of election for polyphenolic substances (Yildirim-Elikoglu and Erdem, 2017). The higher presence of caseins residues in GP+ Ricotta might explain the higher amount of TPCs in GP+ Ricotta. SDS-PAGE analysis did not show significant differences in the main protein fractions, α-lactoalbumin and β-lactoglobulin. This finding disagrees with a study of Chedea et al. (2017) that showed an increase of β-lactoglobulin but no effect for α-lactalbumin, albumin and caseins concentration in the milk of dairy cow fed a diet supplemented with 15% GP. However, the effect of polyphenol compounds on protein synthesis depends on several factors including type of compound, its concentration and the eventual presence other compounds with which competition can be generated.

Red GP supplementation modified fatty acid profile of WBR and Ricotta. To our knowledge, no research has characterized the fatty acid profile of whey obtained from the processing of cheeses produced with milk deriving from ewes fed GP. However, previous studies have highlighted an increase of linoleic acid (C18:2) and C18:1, t11 in the milk of lactating ruminants fed a diet supplementation with grape processing products, as GP and grape seed(Correddu et al., 2015; Ianni et al., 2019). Conversely to the previously mentioned studies, Manso et al. (2016) observed an increase of C18:2 concentration but no modifications for C18:1, t11 and in the relative percentages of SFA, MUFA and PUFA, by administering to ewes diets containing linseed oil and GP with different concentrations, 5 and 10 g/100 g of TMR. In our study, no differences between the two groups were observed in C18:2 but a significant increase was found in C18:1, t11 both in GP+ whey

and Ricotta samples. The differences observed in whey and Ricotta fatty acid profile compared to milk could be due to the cheese manufacturing process.

GP+ whey and Ricotta samples were characterized by a lower content of odd-chain fatty acids, C15:0 and C17:0. These acids can be produced by rumen microbial fermentation and microbial *de-novo* lipogenesis. Rumen microbial population produce odd-chain fatty acids by a pathway which utilizes the removal of the α-carbon, through the conversion of C16:0 or C18:0 to a hydroxyl fatty acid followed by decarboxylation to produce either C15:0 or C17:0, respectively. These odd-chain fatty acids are then produced in the rumen and absorbed by the mammary gland for milk fat production (Vlaeminck et al., 2006). The reduction in C15:0 and C17:0 could be a consequence of the intake of the polyphenols deriving from GP, because of the ability of these compounds to influence the rumen microbiota (Vasta et al., 2010).

In addition to this, the diet administered to the GP+ group conferred a decrease of C14:1 and an increase of C14:0 respect to the CTRL both in whey and Ricotta, this result might be mainly related to an inhibitory effect on the myristic acid desaturation by stearoyl coenzyme A desaturase (SCD), a result also supported by the decrease of C14:1/C14:0 ratio, that was considered by Mele et al. (2007) a reliable index of Δ9-desaturation in the mammary gland. No significant variations in total amount of SFA, MUFA and PUFA and consequently in AI and TI were highlighted.

The volatile profile of dairy products could be affected by the diet administered to lactating ruminants (Bennato et al., 2019; Bennato et al., 2020; Ianni et al., 2019). The analysis of VOCs has allowed to identify nine compounds, belonging to aldhehydes, ketones and carboxylic acids families, resulting by secondary lipid oxidation. In both groups the most abundant was hexanal, whose concentration was lower in GP+ samples after 5 d of ripening compared with CTRL. Furthermore, nonanal and octanoic acid were affected by the diet, resulting respectively lower and higher in GP+ samples compared with CTRL Ricotta.

Since hexanal and nonanal are typical secondary oxidation products of unsaturated fatty acids, their reduction suggest that GP supplementation may contribute to induce an antioxidant effect; at the same

288 time this may negatively influence the aromatic parameters of the Ricotta, since the presence of
289 hexanal in dairy products is strictly related to green, lemon, slightly fruity and herbal notes.
290
291 **Conclusions**
292 The present study suggests that compounds present in GP may interfere with animal biological
293 function and could be transferred in milk even following a metabolic transformation by rumen
294 microbes. After the cheese-making, these compounds could directly affect the chemical-nutritional
295 characteristic of dairy products. The results obtained in this study suggest that the supplementation
296 of GP in the ewe's diet did not induce a worsening of the quality parameters of dairy by-products and
297 a possible improvement of the oxidative stability, however further investigations are needed in order
298 to improve the characterization of the biological mechanisms responsible for these findings, without
299 neglecting sensorial evaluations useful to evaluate the acceptability of the product by the consumer.
300
301 **References**

302 Alba DF, Campigotto G, Cazarotto CJ, dos Santos DS, Gebert RR, Reis JH, Souza CF Baldissera
303 MD, Gindri AL, Kempka AP et al. 2019. Use of grape residue flour in lactating dairy sheep in
304 heat stress: Effects on health, milk production and quality. J Therm Biol 82:197-205.
305 https://doi.org/10.1016/j.jtherbio.2019.04.007
306 AOAC. 2000. Official methods of analysis of AOAC International. 17th ed. AOAC International,
307 Washington, USA.
308 Bennato F, Ianni A, Innosa D, Martino C, Grotta L, Pomilio F, Verna M, Martino G. 2019.
309 Infuence of licorice root feeding on chemical-nutritional quality of cow milk and stracciata cheese,
310 an Italian traditional fresh dairy product. Animals 9(12):1153.
311 https ://doi.org/10.3390/ani9121153

312 Bennato F, Ianni A, Martino C, Di Luca A, Innosa D, Fusco AM, Pomilio F, Martino G. 2020.
313 Dietary supplementation of Saanen goats with dried licorice root modifes chemical and textural
314 properties of dairy products. J Dairy Sci 103(1):52-62. http://doi.org/10.3168/ jds.2019-16838.
315 Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities
316 of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248-254.
317 https://doi.org/10.1006/abio.1976.9999
318 Brahmi F, Flamini G, Issaoui M, Dhibi M, Dabbou S, Mastouri M, Hammami M. 2012. Chemical
319 composition and biological activities of volatile fractions from three Tunisian cultivars of olive
320 leaves. Med Chem Res 21(10):2863-2872. https://doi. org/10.1007/s00044-011-9817-8
321 Chedea VS, Pelmus RS, Lazar C, Pistol GC, Calin LG, Toma SM, Dragomir C, Taranu I. 2017.
322 Effects of a diet containing dried grape pomace on blood metabolites and milk composition of
323 dairy cows. J Sci Food Agric 97:2516-2523. https://doi.org/10.1002/jsfa.8068
324 Correddu F, Nudda A, Manca MG, Pulina G, Dalsgaard TK. 2015. Light-induced lipid oxidation
325 in sheep milk: effects of dietary grape seed and linseed, alone or in combination, on milk oxidative
326 stability. J Agric Food Chem 63:3980-3986. https://doi.org/10.1021/acs.jafc.5b01614
327 Correddu F, Lunesu MF, Buffa G, Atzori AS, Nudda A, Battacone G, Pulina G. 2020. Can Agro-
328 Industrial By-Products Rich in Polyphenols be Advantageously Used in the Feeding and Nutrition
329 of Dairy Small Ruminants? Animals 10(1):131. https://doi.org/10.3390/ani10010131
330 Ianni A, Innosa D, Martino C, Bennato F, Martino G. 2019. Compositional characteristics and
331 aromatic profile of caciotta cheese obtained from Friesian cows fed with a dietary
332 supplementation of dried grape pomace. J Dairy Sci 102:1025-1032.
333 https://doi.org/10.3168/jds.2018-15590
334 Ianni A, Innosa D, Martino C, Grotta L, Bennato F, Martino G. 2019. Zinc supplementation of
335 Friesian cows: effect on chemical-nutritional composition and aromatic profile of dairy products.
336 J Dairy Sci 102(4):2918-2927. https://doi.org/10.3168/ jds.2018-15868

337 Ianni A, Martino G. 2020. Dietary grape pomace supplementation in dairy cows: Effect on
338 nutritional quality of milk and its derived dairy products. Foods 9(2):168.
339 https://doi.org/10.3390/foods9020168
340 Innosa D, Bennato F, Ianni A, Martino C, Grotta L, Pomilio F, Martino G. 2020. Influence of
341 olive leaves feeding on chemical-nutritional quality of goat ricotta cheese. Eur Food Res
342 Technol 246(5). https://doi.org/10.1007/s00217-020-03437-8
343 Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of
344 bacteriophage T4. Nature 227:680-685. https://doi.org/10.1038/227680a0
345 Manso T, Gallardo B, Salvá A, Guerra-Rivas C, Mantecón AR, Lavín P, De la Fuente MA. 2016.
346 Influence of dietary grape pomace combined with linseed oil on fatty acid profile and milk
347 composition. J. Dairy Sci 99:1111-1120. https://doi.org/10.3168/jds.2015-9981
348 McSweeney CS, Palmer B, Bunch R, Krause DO. 2001. Effect of the tropical forage Calliandra
349 Calothyrsus on microbial protein synthesis and ecology in the rumen. J Appl Microbiol 90:78-88.
350 Mele M, Conte G, Castiglioni B, Chessa S, Macciotta NPP, Serra A, Buccioni A, Pagnacco G,
351 Secchiari P. 2007. Stearoyl-Coenzyme A desaturase gene polymorphism and milk fatty acid
352 composition in Italian Holsteins. J Dairy Sci 90:4458-4465. https://doi.org/10.3168/jds.2006-617
353 Min BR, Attwood GT, Reilly K, Sun W, Peters JS, Barry TN, McNabb WC. 2002. Lotus
354 corniculatus condensed tannins decrease in vivo populations of proteolytic bacteria and affect
355 nitrogen metabolism in the rumen of sheep. Can J Microbiol 48:911-921.
356 https://doi.org/10.1139/w02-087
357 Moate PJ, Williams SRO, Torok VA, Hannah MC, Ribaux BE, Tavendale MH et al. 2014. Grape
358 marc reduces methane emissions when fed to dairy cows. J Dairy Sci 97(8):5073-5087. https://
359 doi.org/10.3168/jds.2013-7588
360 Mucchetti G, Carminati D, Pirisi A. 2002.Ricotta fresca vaccina ed ovina: osservazioni sulle
361 tecniche di produzione e sul prodotto. Il Latte 27:154-166.

Nedomová Š, Kilián L, Pytel R, Kumbár V. 2017. Effect of ripening time on colour and texture properties in cheese. Potravinarstvo Slovak J Food Sci 11(1):296-301.

Nozière P, Graulet B, Lucas A, Martin B, Grolier P, Doreau M. 2006. Carotenoids for ruminants: from forages to dairy products. Anim Feed Sci Tech 131:418-450. https://doi.org/10.1016/j.anifeedsci.2006.06.018

Platzer M, Kiese S, Herfellner T, Schweiggert-Weisz U, Eisner P. 2021. How Does the Phenol Structure Influence the Results of the Folin-Ciocalteu Assay? Antioxidants 10(5):811. https://doi.org/10.3390/antiox10050811

Salvatore E, Pes M, Falchi G, Pagnozzi D, Furesi S, Fiori M, Roggio T, Addis MF, Pirisi A. 2014. Effect of whey concentration on protein recovery in fresh ovine ricotta cheese. J Dairy Sci 97:4686-4694. https://doi.org/10.3168/jds.2013-7762

Schreiner M, Windisch W. 2006. Supplementation of cow diet with rapeseed and carrots: influence on fatty acid composition and carotene content of the butter fat. J Food Lipids 13:434-444. https://doi.org/10.1111/j.1745-4522.2006.00072.x

Singleton VL, Rossi JR. 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid. Am J Enol Vitic 16: 144- 158

Solah VA, Staines V, Honda S, Limley HA. 2007. Measurement of milk color and composition: effect of dietary intervention on Western Australian Holstein-Friesian cow's milk quality. J Food Sci 72:560-566. https://doi.org/10.1111/j.1750-3841.2007.00491.x

Vasta V, Yáñez-Ruiz DR, Mele M, Serra A, Luciano G, Lanza M, Biondi L, Priolo A. 2010. Bacterial and protozoal communities and fatty acid profile in the rumen of sheep fed a diet containing added tannins. Appl Environ Microbiol 76:2549-2555. https://doi.org/10.1128/aem.02583-09

Vlaeminck B, Fievez V, Cabrita ARJ, Fonseca AJM, Dewhurst RJ. 2006. Factors affecting odd- and branched-chain fatty acids in milk: A review. Anim Feed Sci Technol *131:*389-417. https://doi.org/10.1016/j.anifeedsci.2006.06.017

388 Yildirim-Elikoglu S, Erdem, YK. 2017. Interactions between milk proteins and polyphenols:
389 Binding mechanisms, related changes, and the future trends in the dairy industry. Food Rev Int
390 34:665–697. https://doi.org/10.1080/87559129.2017.1377225
391 Yu J, Ahmedna M. 2013. Functional components of grape pomace: Their composition, biological
392 properties and potential applications. Int J Food Sci Technol 48:221-237.
393 https://doi.org/10.1111/j.1365-2621.2012.03197.x
394

Table 1 Physical and Chemical evaluations of whey before (WBR) and after (WPR) ricotta cheesemaking obtained from ewes fed a standard diet (CTRL) and ewes fed red grape pomace dietary supplementation (GP+)

	WBR			WPR		
	CTRL	**GP+**	p	**CTRL**	**GP+**	p
Lipids[1], (%)	1.42 ± 0.13	1.35 ± 0.32	ns	0.09 ± 0.06	0.23 ± 0.01	ns
L*	81.01 ± 26.36	79.22 ± 19.78	***	80.09 ± 17.15	81.90 ± 19.22	***
a*	-0.37 ± 0.57	-0.53 ± 0.37	***	0.14 ± 0.34	0.18 ± 0.40	*
b*	8.23 ± 2.30	8.65 ± 2.26	***	9.59 ± 2.17	9.04 ± 2.08	***
YI[2]	14.47 ± 0.08	15.60 ± 0.15	***	17.11 ± 0.12	15.76 ± 0.13	***
ΔEab[3]	3.44			3.59		
TPCs[4] (µg GAE mL-1)	44.43 ± 1.98	37.27 ± 0.05	*	21.57 ± 0.60	26.47 ± 3.02	ns
TEAC[5] (µmol mL-1)	6.25 ± 1.11	9.48 ± 3.05	ns	9.03 ± 0.77	10.49 ± 0.84	ns

All data are reported as mean ± SD.
[1]Data are reported on as sample basis.
L*= lightness; a*= redness; b*= yellowness.
[2]YI = Yellow Index, [3]ΔEab = Color Differences, [4]TPCs = Total Phenolic Compounds, [5]TEAC = Trolox Equivalent Antioxidant Capacity.
*p< 0.05; ***p< 0.001; ns = not significant.

Table 2 Physical and Chemical evaluations of Ricotta after 1 (T1) and 5 (T5) d of ripening obtained from ewes fed a standard diet (CTRL) and ewes fed red grape pomace dietary supplementation (GP+).

	Ricotta T1			Ricotta T5		
	CTRL	**GP+**	p	**CTRL**	**GP+**	p
DM, (%)	25.13 ± 0.15	27.66 ± 0.68	**	25.61 ± 0.28	26.36 ± 0.38	*
Lipids[1], (%)	54.01 ± 2.16	46.45 ± 2.96	ns	53.43 ± 3.97	51.81 ± 4.37	ns
L*	79.97 ± 0.63	79.59 ± 1.56	ns	80.73 ± 0.30	81.15 ± 0.80	ns
a*	-1.48 ± 0.07	-1.46 ± 0.17	ns	-1.50 ± 0.05	-1.38 ± 0.06	**
b*	7.30 ± 0.19	6.84 ± 0.38	**	7.38 ± 0.32	7.47 ± 0.17	ns
YI[2]	13.04 ± 0.33	12.27 ± 0.53	**	11.26 ± 4.76	11.95 ± 3.19	ns
ΔEab[3]	0.35			0.2		
TPCs[4] (µg GAE g-1)	29.45 ± 4.12	31.43 ± 3.85	**	25.48 ± 1.31	28.83 ± 4.75	ns
TEAC[5] (µmol g-1)	18.50 ± 1.98	20.63 ± 0.75	ns	18.55 ± 5.52	24.75 ± 5.23	ns

All data are reported as mean ± SD.
[1]Data are reported on a dry matter (DM) basis.
L*= lightness; a*= redness; b*= yellowness.
[2]YI = Yellow Index, [3]ΔEab = Color Differences, [4]TPCs = Total Phenolic Compounds, [5]TEAC = Trolox Equivalent Antioxidant Capacity.
*p< 0.05; **p< 0.01; ns = not significant.

Table 3 Fatty acids composition of whey before (WBR) and Ricotta after 1 (T1) d of ripening obtained from ewes fed a standard diet (CTRL) and ewes fed red grape pomace dietary supplementation (GP+).

	WBR			Ricotta T1		
	CTRL	**GP+**	p	**CTRL**	**GP+**	p
C4:0	2.28 ± 0.20	2.36 ± 0.32	ns	3.47 ± 0.48	3.55 ± 1.07	ns
C6:0	2.46 ± 0.15	2.57 ± 0.29	ns	3.49 ± 0.49	3.63 ± 1.04	ns
C8:0	2.76 ± 0.13	2.89 ± 0.27	ns	3.84 ± 0.55	4.11 ± 1.09	ns
C10:0	9.54 ± 0.35	10.13 ± 0.75	ns	13.03 ± 1.78	13.78 ± 3.15	ns
C12:0	5.57 ± 0.10	5.92 ± 0.31	ns	6.81 ± 0.75	7.29 ± 1.10	ns
C14:0	12.46 ± 0.17	13.12 ± 0.21	*	13.31 ± 0.46	13.89 ± 0.39	ns
C15:0	1.15 ± 0.01	1.01 ± 0.02	***	1.11 ± 0.04	0.94 ± 0.08	**
C16:0	28.14 ± 0.46	28.11 ± 0.84	ns	26.10 ± 1.50	25.59 ± 3.01	ns
C17:0	0.79 ± 0.01	0.71 ± 0.03	*	0.55 ± 0.07	0.45 ± 0.06	*
C18:0	8.54 ± 0.11	7.37 ± 0.56	*	6.81 ± 0.79	5.87 ± 1.28	ns
C14:1	0.59 ± 0.01	0.50 ± 0.01	***	0.45 ± 0.03	0.31 ± 0.04	***
C16:1	1.41 ± 0.02	1.49 ± 0.06	ns	1.13 ± 0.23	1.21 ± 0.23	ns
C18:1, t11	0.28 ± 0.09	0.63 ± 0.10	*	0.62 ± 0.07	0.86 ± 0.16	*
C18:1, c9	15.01 ± 0.21	14.25 ± 0.74	ns	12.28 ± 1.49	11.65 ± 2.40	ns
C18:1, c11	0.19 ± 0.02	0.19 ± 0.03	ns	0.06 ± 0.04	0.07 ± 0.04	ns
C18:2	3.15 ± 0.05	3.25 ± 0.23	ns	2.41 ± 0.28	2.41 ± 0.43	ns
C18:3	0.66 ± 0.01	0.58 ± 0.04	*	0.53 ± 0.10	0.45 ± 0.12	ns
CLA	1.28 ± 0.12	1.25 ± 0.08	ns	0.91 ± 0.10	0.91 ± 0.17	ns
SFA	73.68 ± 0.44	74.21 ± 0.92	ns	78.53 ± 1.88	79.08 ± 3.36	ns
MUFA	17.48 ± 0.38	17.05 ± 0.68	ns	14.54 ± 1.38	14.08 ± 2.63	ns
PUFA	5.09 ± 0.12	5.07 ± 0.35	ns	3.84 ± 0.48	3.77 ± 0.72	ns
Others	3.75 ± 0.03	3.67 ± 0.03	*	3.09 ± 0.03	3.07 ± 0.02	ns
SCFA(C4-C6)	4.74 ± 0.34	4.93 ± 0.61	ns	6.97 ± 0.96	7.18 ± 2.11	ns
MCFA(C8-C15)	33.78 ± 0.47	35.34 ± 1.61	ns	40.05 ± 3.43	41.84 ± 5.76	ns
LCFA(C16-C18:3)	61.48 ± 0.75	59.73 ± 2.21	ns	52.99 ± 4.27	50.98 ± 7.86	ns
DIC14:0	0.05 ± 0.01	0.04 ± 0.01	***	0.03 ± 0.01	0.02 ± 0.01	***
DIC16:0	0.05 ± 0.01	0.05 ± 0.01	ns	0.04 ± 0.01	0.05 ± 0.01	ns
DIC18:0	0.64 ± 0.01	0.66 ± 0.01	*	0.64 0.01	0.67 ± 0.01	***
DICLA	0.83 ± 0.01	0.69 ± 0.07	**	0.59 ± 0.03	0.51 ± 0.02	**
AI	3.70 ± 0.09	3.92 ± 0.19	ns	4.73 ± 0.55	5.13 ± 1.20	ns
TI	7.81 ± 0.13	7.92 ± 0.28	ns	7.56 ± 0.12	7.70 ± 0.22	ns

Data are reported as mean percentage of total fat ± SD
SFA= Saturated Fatty Acid, MUFA= Monounsaturated Fatty Acid, PUFA= Polyunsaturated Fatty Acid, CLA= Conjugated Linoleic Acids,
SCFA= Short Chain Fatty Acids (C4:0-C6:0), MCFA= Medium Chain Fatty Acids (C8:0-C15:0), LCFA= Long Chain Fatty Acids (C16:0-C18:3)
AI= Atherogenic Index, TI= Thrombogenic Index, DI= Desaturation Index.
*p< 0.05; **p< 0.01; ***p< 0.001; ns = not significant.

433 **Table 4** Densitometric analysis of SDS-PAGE protein bands in whey before ricotta (WBR) obtained from ewes fed a
434 standard diet (CTRL) and ewes fed red grape pomace dietary supplementation (GP+).

	WBR		
	CTRL	**GP+**	p
Lactoferrin	6.39 ± 0.72	6.76 ± 0.17	ns
Serumalbumin	7.57 ± 0.05	8.36 ± 1.05	ns
A	9.60 ± 0.65	9.37 ± 0.78	ns
Caseins residues	25.41 ± 0.70	24.68 ± 1.51	ns
β-lactoglobulin	28.81 ± 0.43	28.87 ± 1.71	ns
α-lactoalbumin	8.69 ± 1.09	8.47 ± 0.66	ns
B	13.52 ± 2.09	13.50 ± 1.85	ns

435 Data are reported as mean percentage ± SD of the total proteins found in the electrophoretic profile of each sample. ns =
436 not significant.
437

438 **Table 5** Densitometric analysis of SDS-PAGE protein bands in Ricotta after 1 (T1) and 5 (T5) d of ripening obtained
439 from ewes fed a standard diet (CTRL) and ewes fed red grape pomace dietary supplementation (GP+).

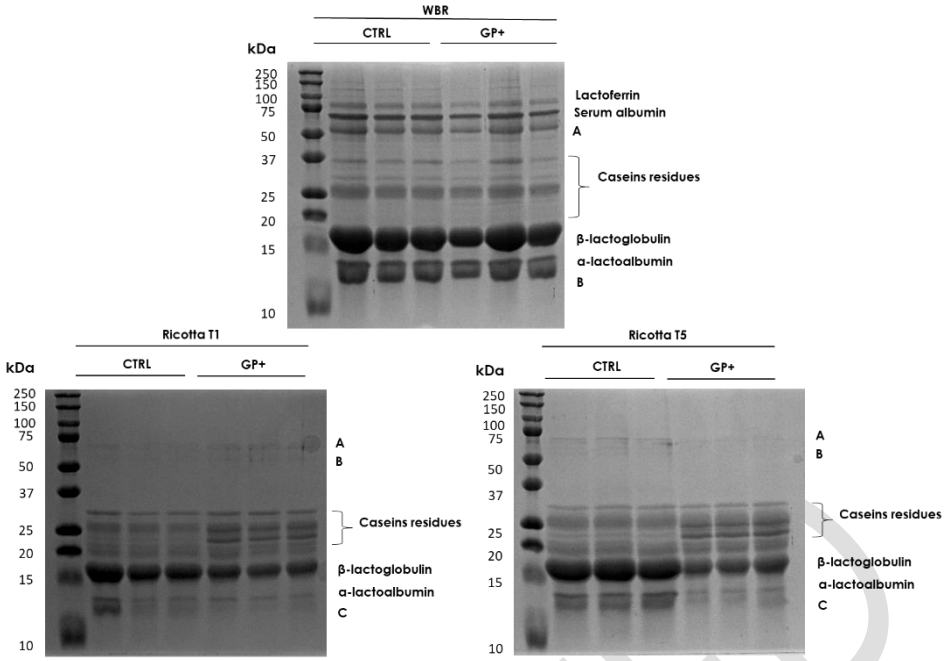
	Ricotta T1			Ricotta T5		
	CTRL	**GP+**	p	**CTRL**	**GP+**	p
A	6.80 ± 1.33	8.88 ± 1.21	ns	6.33 ± 0.69	7.37 ± 0.36	ns
B	15.61 ± 2.30	18.73 ± 2.00	ns	17.77 ± 0.29	22.42 ± 1.14	**
Caseins residues	17.88 ± 1.89	22.55 ± 1.61	*	20.16 ± 1.31	23.57 ± 0.24	*
β-lactoglobulin	40. 37 ± 1.89	38.09 ± 1.02	ns	34.20 ± 1.84	30.38 ± 0.78	*
α-lactoalbumin	8.65 ± 0.46	8.13 ± 1.78	ns	9.18 ± 0.46	8.04 ± 1.74	ns
C	10.69 ± 3.56	3.62 ± 0.98	*	12.35 ± 3.03	8.20 ± 1.31	ns

440 Data are reported as mean percentage ± SD of the total proteins found in the electrophoretic profile of each sample.
441 *p< 0.05; **p< 0.01; ns = not significant.
442

443 **Table 6** Aromatic profile of Ricotta after 1 (T1) and 5 (T5) d of ripening obtained from ewes fed a standard diet (CTRL)
444 and ewes fed red grape pomace dietary supplementation (GP+).

	Ricotta T1			Ricotta T5		
	CTRL	**GP+**	p	**CTRL**	**GP+**	p
Hexanal	24.71 ± 7.37	27.23 ± 2.85	ns	32.19 ± 4.88	22.92 ± 1.70	*
2-Heptanon	1.06 ± 0.60	1.43 ± 0.16	ns	0.77 ± 0.32	0.92 ± 0.05	ns
Heptanal	5.98 ± 1.53	8.47 ± 0.92	ns	8.56 ± 0.75	9.06 ± 3.11	ns
Octanal	3.49 ± 0.88	3.75 ± 0.37	ns	3.61 ± 0.35	3.68 ± 0.60	ns
2-Nonenal	12.00 ± 12.12	3.13 ± 0.83	ns	3.69 ± 0.23	3.12 ± 0.40	ns
2-Nonanone	2.66 ± 0.83	2.22 ± 0.51	ns	2.61 ± 0.81	2.35 ± 0.26	ns
Nonanal	13.96 ± 2.75	8.95 ± 0.77	*	12.56 ± 1.12	11.91 ± 2.18	ns
Octanoic Acid	17.54 ± 2.49	26.27 ± 3.41	*	19.26 ± 1.99	22.87 ± 3.49	ns
Nonanoic Acid	18.60 ± 4.16	18.54 ± 2.74	ns	16.75 ± 2.86	23.18 ± 3.43	ns

445 Data are reported as mean percentage of (VOCs) ± SD.
446 *p< 0.05; ns = not significant.
447



448

449 **Fig. 1** Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) pattern of whey proteins in whey
450 before Ricotta (WBR) cheesemaking and in Ricotta after 1 (T1) and 5 (T5) days of ripening obtained from ewes fed a
451 standard diet (CTRL) and ewes fed red grape pomace dietary supplementation (GP+).

452

453

454

455