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Effect of α_{S1}-casein genotype on phenolic compounds and antioxidant activity in goat milk yogurt fortified with *Rhus coriaria* leaf powder

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ABSTRACT

The aim of this work was to evaluate the phenolic compounds and antioxidant activity of goat milk yogurt characterized by different α_{S1}-casein genotypes and fortified with *Rhus coriaria* leaf powder. The α_{S1}-casein genotype was determined by isoelectric focusing, total phenol content was determined by the Folin–Ciocalteu method, phenolic compounds were identified and quantified by HPLC-UV analysis, and antioxidant activity was measured using 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid and ferric-reducing antioxidant power. The statistical analysis showed a significant effect of the studied factors. Comparing different genotypes it emerged that yogurt from goats with weak alleles at *CSN1S1* loci (*FF*) showed the lowest phenolic compounds and therefore a lower antioxidant activity compared with yogurt from goats with strong alleles at *CSN1S1* loci (*AA*, *BB*, *AB*). *Rhus coriaria*-fortified yogurt showed a significant increase in total phenolic compounds and antioxidant activity in comparison with plain yogurt. The *FF*-fortified yogurt presented the lowest total phenol content and antioxidant activity. This could be due to a greater capacity of proteins and peptides in this yogurt to form stable complexes with phenols. The different total phenol content detected in *R. coriaria*-fortified yogurt indicates that the α_{S1}-casein genotype influenced the amount of added phenols that are bound to the caseins and, therefore, the part that remains free and that affects the biological capacity of the final product.

Key words: goat yogurt, α_{S1}-casein genotype, sumac, antioxidant activity, phenolic compound

INTRODUCTION

In the last decade, the development of functional foods with natural ingredients that promote health has increased (Granato et al., 2017). Yogurt, a very popular fermented milk product, is considered an important functional food for its high nutritive and therapeutic values. Pattorn et al. (2012) highlighted the numerous health benefits due to the nutraceutical quality of yogurt. The antioxidant activity of yogurt is mainly due to whey and casein proteins that have a high tendency to chelate metals and to donate electrons and atoms (Colbert and Decker, 1991; Tong et al., 2000; Rival et al., 2011), and to bacterial fermentation that leads to the release of several bioactive peptides (Kudoh et al., 2001; Virtanen et al., 2007; Gómez-Ruiz et al., 2008). In a recent report, Perna et al. (2013) showed that the antioxidant activity of cow milk yogurt was significantly influenced by the casein haplotype probably because of the specific amino acid sequence of the milk protein variants that may influence the release of peptides during proteolysis. Goat milk yogurt is a healthy alternative to cow milk yogurt, containing more vitamin A and B; in particular, vitamin B_3 content in goat milk is almost double that of cow milk. Furthermore, due to the α_{S1}-CN characteristics, goat milk has a lower allergenic potential than cow milk; thus, people intolerant to cow milk yogurt often are able to consume goat milk yogurt with no adverse effects. In the goat species, high polymorphism was found at the 4 casein genes with some alleles associated with null or reduced expression of the specific protein. From a quantitative point of view, the *CSN1S1* gene, coding for α_{S1}-CN, is the most variable casein gene. In fact, according to the α_{S1}-CN content, *CSN1S1* alleles are sorted into 4 groups: strong alleles (*A*, *A′*, *B1*, *B2*, *B3*, *B4*, *B′*, *C*, *H*, *L*, and *M*), producing almost 3.5 g/L of α_{S1}-CN each; intermediate alleles (*E* and *I*), each producing 1.1 g/L; weak alleles (*F* and *G*), contributing 0.45 g/L; and null alleles (*01*, *02*, and *N*), producing no α_{S1}-CN (Caroli et al., 2007; Park et al., 2007). Fortification of yogurt with polyphenol-rich foods is a widely used technique to improve both sensory characteristics (Muniandy et al., 2016) and antioxidant capacity (Gallo et al., 2013; Perna et al., 2014) of the product. Many kinds of polyphenol-rich fruits are frequently added in yogurt as flavorings, even if today nontraditional additives such as vegetable powders, pulps, and natural extracts ob-

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tained from row vegetable vegetables are often used in the production of fermented dairy products. Vegetables are a valuable source of nutrients, are rich in dietary fiber, minerals, and bioactive components such as vitamins, carotenoids, and polyphenols, and are also low in calories (Srivastava et al., 2015). Consequently, the fortification of yogurt with selected vegetables is a method to enhance health properties and to develop novel functional dairy products. Several authors have shown a strong correlation between antioxidant capacity and phenol content (Beretta et al., 2005; Meda et al., 2005; Blasa et al., 2006). *Rhus coriaria* L. (sumac), belonging to the Anacardiaceae family, is a plant with antioxidant properties that grows in Mediterranean countries, North Africa, southern Europe, Afghanistan, and Iran (Nasar-Abbas and Halkman, 2004). It is considered a valid remedy in traditional medicine for its analgesic, antidiarrhetic, antiseptic, anorexic, and antihyperglycemic properties (Rayne and Mazza, 2007). In the last decade, several studies have been published on the antibacterial properties of *R. coriaria* preparations (Ahmadian-Attari et al., 2008; Motaharinia et al., 2012), which may be used for food production (Bozan et al., 2003; Candan and Sokmen, 2004; Kosar et al., 2007). *Rhus coriaria* contains various substances including polyphenols such as gallic acid, methyl gallate, kaempferol, quercetin (Shabana et al., 2011), and hydrolyzable tannins, which have a strong antioxidant effect (Kosar et al., 2007). Also, gallic acid possesses excellent antioxidant (Yen et al., 2002), antiobesity (Hsu and Yen, 2007), hepatoprotective (Jadon et al., 2007), and anticancer (Sun et al., 2016) activities. In the scientific literature, no reports are available on the antioxidant capacity of *R. coriaria*-fortified yogurt made from goat milk with different α_{S1}-CN genotypes. The aim of the present study was to evaluate the phenolic compounds and antioxidant activity of goat milk yogurt characterized by different α_{S1}-CN genotypes and fortified with *R. coriaria* leaf powder.

MATERIALS AND METHODS

Chemicals and Apparatus

The 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic-acid; **ABTS**), 2,4,6-tripyridyl-s-triazine (**TPTZ**), potassium persulfate, hydrochloric acid, ferric chloride, sodium phosphate, ethyl acetate, acetonitrile, *n*-hexane, sodium hydroxide, phosphoric acid, acetic acid, 2-mercaptoethanol, urea, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, ammonium persulfate, potassium phosphate monobasic, sodium acetate, gallic acid, chlorogenic acid, caffeic acid, vanillic acid, *p*-coumaric acid, syring acid, ferulic acid, (−)-epicatechin, (+)-catechin, rutin, narirutin, naringin H, quercitin, rosmarinic acid, kaempferol, luteolin, and HPLC-grade methanol were purchased from Sigma-Aldrich (Milan, Italy). Acrylamide, bis-acrylamide, and ampholine buffer were purchased from GE Healthcare Amersham Bioscience (Buckinghamshire, UK). Coomassie Brilliant blue G250 was purchased from Bio-Rad (Richmond, CA). Folin–Ciocalteu reagent was purchased from Carlo Erba (Milan, Italy). All chemicals and solvents used were of analytical grade. *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* were purchased from Insao s.r.l. (Liscate, Milan, Italy). Turkish *R. coriaria* leaf powder was purchased from Terza Luna (http://www.terzaluna.com/prodotto/sumach-o-sommaco/). Equipment included a UV-VIS Spectrophotometer 1204 (Shimadzu, Kyoto, Japan), isoelectric focusing Multiphor II Electrophoresis System (Pharmacia LKB, Uppsala, Sweden), and an HPLC equipped with Varian ProStar Pump model 210, Rheodyne injector with a 20-μL loop, UV–VIS detector Varian ProStarmodel 325, and Galaxie chromatography software (Varian Inc., Walnut Creek, CA).

Milk Sample

This study was conducted on 32 Maltese goats raised on the same semi-extensive farm in Potenza province (southern Italy). The feeding system was conducted under the typical grazing and management conditions of herds in southern Italy; namely, natural pasture ad libitum plus 500 g/head per day of concentrate (260 g of barley, 85 g of chickpeas, and 155 g of wheat bran). Before starting the test, about 150 animals in lactation were identified by isoelectric focusing (**IEF**) to define their genotype at the α_{S1}-CN locus (*CSN1S1*), at the same genotype at *CSN1S2* (*AA*), *CSN2* (*AA*), and *CSN3* (*AA*) loci (Figure 1). After the definition of individual phenotypes, the goats were split into 4 groups by *CSN1S1* genotype: 3 strong groups, composed of 8 goats homozygous for strong (*AA*) alleles, 8 goats homozygous for strong (*BB*) alleles, and 8 goats heterozygous for strong (*AB*) alleles, and 1 weak group, composed of 8 goats homozygous for weak (*FF*) alleles. From each animal, 3 L of individual milk was collected to manufacture 2 yogurts: 1 plain yogurt and 1 *R. coriaria*-fortified yogurt, for a total of 32 plain yogurts and 32 *R. coriaria*-fortified yogurts.

Genetic Variants of α_{S1}-CN by IEF

Individual milk samples were kept at 4°C and defatted by centrifugation (3,000 × *g* for 30 min at 4°C); the fat layer was solidified at −20°C for 20 min and removed. Casein was prepared by isoelectric precipitation

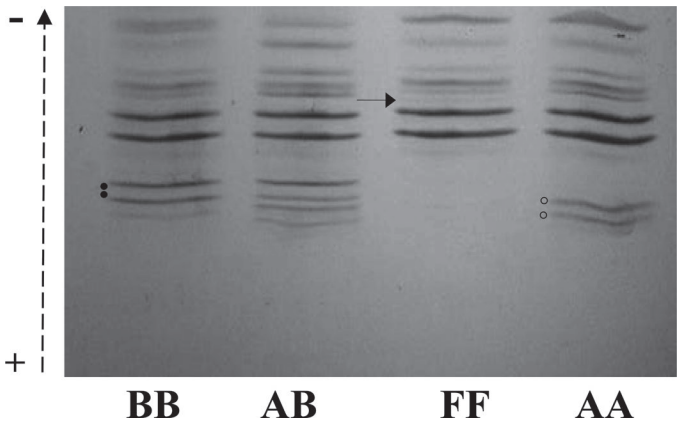

Figure 1. Isoelectric focusing patterns of same Maltese goat milk samples separated in a pH range of 2.5 to 10. *CSN1S1 B* (●); *CSN1S1 A* (○); *CSN1S1 F* (→).

at pH 4.6 with 10% (vol/vol) acid acetic and 1 *M* sodium acetate at room temperature. After centrifugation at 3,000 × *g* for 10 min at 4°C, the casein pellet was washed twice with distilled water and stored at −20°C. The whole casein was dissolved in 9 *M* urea and 1% 2-mercaptoethanol for IEF analysis, according to Aschaffenburg and Drewry (1959). The genetic variants of the different α_{S1}-CN by IEF were determined according to the method of Trieu-Cuot and Gripon (1981). The IEF analysis was performed on polyacrylamide gel (5% acrylamide and 0.15% bisacrylamide) with a thickness of 1 mm and 2% carrier ampholytes to create a gradient of pH 2.5 to 10.0. Gel was prefocused at a constant value of 0.35 W/mL of gel and at the maximum limit of 1,200 V. The gel was stained in Coomassie Blue G-250 according to Blakesley and Boezi (1977).

Preparation of Yogurts and R. coriaria Water Extract

Yogurt samples with added *R. coriaria* leaf powder and plain yogurt (without any addition) were prepared. Briefly, after heat-treating at 95°C for 15 min followed by cooling to 45°C, all individual milks (2 L for each sample) were inoculated at the same time with 1% (vol/vol) *S. thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus*. Fermentation was carried out at 45°C until the pH reached a value between 4.5 and 4.7 (∼8 h). Once the desired pH was reached, the *R. coriaria* leaf powder (20%, wt/vol) was added and incorporated by mechanical stirring; consequently, the prepared product was a stirred type yogurt. A control (named *R. coriaria* water extract) was also prepared using the same protocol of yogurt manufacture but without milk. Samples were cooled at 4°C for 24 h and finally stored at −20°C until analyzed.

Sample Preparation for Analysis

Yogurt samples and *R. coriaria* water extract were placed in an ultrasound water bath apparatus (Elma Transsonic 460/H, Singen, Germany) for 10 min at 25°C and centrifuged at 5,000 × *g* at 4°C for 20 min. The supernatant was separately filtered through a 0.45-μm cellulose acetate membrane filter (Sigma-Aldrich, Milan, Italy) and was used to measure the total phenolic compounds, individual phenolic acids, and antioxidant activity.

Determination of Total Phenolic Content

Quantification of total phenolic compounds was carried out with the Folin–Ciocalteu method as reported by Citta et al. (2017) with some modifications. Briefly, 100 μL of clear supernatant was added to 100 μL of Folin–Ciocalteu. After 2 min, 3 mL of 10% Na_2CO_3 was added and the samples were incubated for 15 min at room temperature. Absorbance was measured at 765 nm and gallic acid (0–200 mg/L) was used as reference standard. Results were expressed as milligrams of gallic acid equivalents (**GAE**) per gram of water extract or yogurt.

HPLC-UV Analysis of Phenolic Compounds

Extraction procedure of phenolic compounds for HPLC analysis was carried out as described by Dekdouk et al. (2015). Briefly, the clear supernatant obtained as previously described was lyophilized and the phenolic compounds were extracted by maceration using ethyl acetate. The solvent was replaced 3 times and obtained extracts were dried using a rotary evaporator. Finally, the dried ethyl acetate extracts were defatted by acetonitrile/*n*-hexane partition and the acetonitrile fractions were used for analysis. The phenolic compound analysis was performed in liquid chromatography as described by Perna et al. (2013). The phenolic profile was detected at 280 nm, the identification was carried out by comparing retention time and spectral characteristics of unknown analytes with those from commercial standards (Yao et al., 2003), and the results were expressed as micrograms of phenolic compound per gram of yogurt or water extract.

Antioxidant Activity

The ABTS radical scavenging and ferric-reducing antioxidant power (**FRAP**) assays were carried out according to Perna et al. (2014). Results were expressed as milligrams of Trolox equivalents (**TE**) per gram of

water extract and micrograms of TE per gram of yogurt.

Statistical Analysis

Data were analyzed according to the following linear model (SAS version 7, SAS Institute Inc., Cary, NC):

$$y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk},$$

where y_{ijk} is the kth observation at the ith level of factor α and the jth level of factor β; μ is the overall mean; α_i is the fixed effect of the ith genotype ($i = 1, 2, 3, 4$); β_j is the fixed effect of the jth yogurt ($j = 1, 2$); and ε_{ijk} is the random error. Before setting the values, expressed in percentage terms, they were subjected to arcsine transformation. Student's t-test was used for all variable comparisons. Results are presented as mean ± standard deviation. Differences between means at the 95% ($P < 0.05$) confidence level were considered statistically significant.

RESULTS AND DISCUSSION

Phenolic Compound Content and Antioxidant Activity of R. coriaria Water Extract

Rhus coriaria plant is a rich source of phenolic acids, condensed and hydrolyzable tannins, anthocyanins, gallic acid derivatives, flavonoid glycosides, and organic acids (Abu-Reidah et al., 2015). Total phenols and individual phenolic compounds content of water extract from *R. coriaria* leaf powder, prepared using the same protocol of yogurt manufacture but without milk, is reported in Table 1. The total amount of phenolic compounds extracted from *R. coriaria* leaves was 15.87 mg of GAE/g of water extract, in line with what was reported by Romeo et al. (2015). *Rhus coriaria* water extract showed a wide variety of the phenolic components detected by HPLC analysis (Table 1), in agreement with what was reported by other authors (Jakobek et al., 2007; Kosar et al., 2007; Abu-Reidah et al., 2015). In particular, 11 phenolic compounds were identified and quantified: 4 flavonoids and 7 phenolic acids (Figure 2). The presence and content of phenolic compounds detected in our study were in line with what was reported by several authors (Kosar et al., 2007; Al-Boushi et al., 2014) in the *R. coriaria* plant. As for the phenolic acids, gallic acid was particularly high (924.36 μg/g of water extract), confirming what was reported by Ferk et al. (2007) and Kosar et al. (2007), who defined gallic acid as the active principle of the *R. coriaria* plant. The total amount of phenolic acids identified and quantified in the *R. coriaria* water extract was 1,213.49 μg/g of water extract, corresponding to the 7.65% of total phenol content; in particular, the gallic acid content corresponds to 5.82% of total phenolic compounds. The total amount of individual flavonols identified and quantified in the *R. coriaria* water extract was 510.12 μg/g of water extract, corresponding to the 3.21% of total phenol content. Among these, epicatechin is the most represented flavonol found in *R. coriaria* water extract at a concentration of 193.08 μg/g of water extract, whereas narirutin was found at a lower concentration (93.99 μg/g of water extract) with respect to the other detected individual flavonoid compounds. Closely related to the polyphenol content is the antioxidant activity. *Rhus coriaria* water extract showed a high antioxidant capacity (Table 2). In particular, the antioxidant activity was 725.75 and 41.27 mg of TE/g of water extract when the ABTS and FRAP assays were applied. Ferk et al. (2007) estimated the antioxidant power of *R. coriaria* to be 50 times greater than that of vitamin E and C. Chakraborty et al. (2009) highlighted that the antioxidant capacity of the *R. coriaria* is closely related to the presence and content of flavonoids and gallic acid.

Phenolic Compound Content and Antioxidant Activity of R. coriaria-Fortified Yogurt

The total phenol content of the studied yogurt samples is reported in Table 3. Regardless of the considered genotype, total phenol content in plain yogurt was 3.31 mg of GAE/g of yogurt. It is necessary to specify that the Folin–Ciocalteu reactivity of plain yogurt is due to the presence of both phenols deriving from the animal feeding and nonphenolic milk compounds such as low molecular weight antioxidants, free amino acids, peptides, and proteins (Helal and Tagliazucchi, 2018). Many authors (Besle et al., 2010; Sepe et al., 2011; Di Trana et al., 2015) highlighted the influence of diet on the polyphenol content in milk. De Feo et al. (2006) showed a positive correlation in goat milk between ingestion of fodder and antioxidant components, mainly flavonoids, such as rutin and quercetin. Comparing different genotypes, α_{S1}-*FF* plain yogurt showed lower total phenol content compared with the strong α_{S1}-CN yogurt ($P < 0.05$). The differences observed among the genotypes could be due to lower efficiency of feed utilization, lower capacity of feed intake, worse ability to select feed, and lower efficiency of nutrient transfer to the milk of goats with α_{S1}-*FF* than those with strong genotypes (Avondo et al., 2009; Pagano et al., 2010; Bonanno et al., 2013). *Rhus coriaria*-fortified yogurt showed a significant ($P < 0.01$) increase in total pheno-

Table 1. Total phenolic and individual phenolic compound content of water extract from *Rhus coriaria* leaf powder

	R. coriaria water extract	
Item	Mean	SD
Total phenolic (mg of acid gallic equivalent/g of water extract)	15.87	0.55
Phenolic acid (μg/g of water extract)		
Gallic acid	924.36	53.81
Rosmarinic acid	217.02	29.64
Chlorogenic acid	21.22	5.21
Vanillic acid	22.27	6.38
Caffeic acid	8.63	1.23
p-Coumaric acid	9.57	2.01
Syring acid	10.42	1.98
Flavonoid (μg/g of water extract)		
Epicatechin	193.08	18.94
Catechin	112.02	11.87
Rutin	111.03	12.34
Narirutin	93.99	9.87

lic compounds in comparison with plain yogurt (Table 3). Regardless of the considered genotype, the total amount of phenolic compounds in fortified yogurt was 11.18 mg of GAE/g of yogurt, which resulted in a value of 7.87 mg of GAE/g of yogurt when corrected for the contribution of plain yogurt (3.31 mg of GAE/g of yogurt). The comparison with total phenol content detected in the *R. coriaria* water extract highlighted that the amount of total phenolic detectable with the Folin–Ciocalteau method in *R. coriaria*-fortified yogurt was 53.94%, whereas the remainder remains bound to milk proteins. Statistical analysis showed a significant effect of casein genotype on the phenolic content and antioxidant activity of fortified yogurt ($P < 0.001$). In particular, total phenolic content was higher in yogurt from goats with strong alleles at *CSN1S1* loci (*AA*, *BB*, *AB*) than yogurt from goats with weak alleles at *CSN1S1* loci (*FF*). It is known that polyphenols have a high binding affinity for proteins, which leads to the formation of soluble or insoluble complexes (Papadopoulou and Frazier, 2004; Helal and Tagliazucchi, 2018). Numerous hydrophobic interactions occur particularly between polyphenols and proline-rich proteins, such as casein (Richard et al., 2006; Soares et al., 2007; Frazier et al., 2010). Kartsova and Alekseeva (2008) reported that the catechin binds to proteins, according to the order β-CN > α-CN > κ-CN > whey protein. The milk protein polymorphism affects the amino acid composition of protein and, consequently, the ability to bond with phenols. Korhonen and Marnila (2013) found that the A^2 variant of β-CN shows a greater ability to interact with phenolic compounds compared with A^1 variant because of its Pro residue in the chain. In this study, *FF*-fortified yogurt presented the lowest total phenol content ($P < 0.05$). As the nitrogenous fraction of yogurt is characterized by the simultaneous presence of intact lactoproteins (caseins and whey proteins) and peptides of various molecular weights, these results could be due to a greater capacity of proteins and peptides in *FF* yogurt to form stable complexes with phenols. Leroux et al. (1992) found that several phenotypes of α_{S1}-CN are associated with the *F* allele. It is known that *F* variant, compared with *A* variant (199 amino acids), originates from internal deletions due to the outsplicing of 3 exons during pre-mRNA processing. Moreover, in addition to the major transcript form having 3 skipped exons, the above-mentioned authors identified multiple forms of variant *F* that originate by a dysfunction of the splicing machinery in which large multicomponent complexes, the spliceosomes, are involved. These multiple forms could have a different primary structure, which could lead to the formation of peptides with sites that have different reactivity with the phenolic molecules. Moreover, in *FF* goat milk, the lower α_{S1}-CN content may be partially compensated by other caseins (Greppi and Roncada, 2008; Valenti et al., 2012). Specifically, Tziboula (1997) observed high α_{S2}-CN content in goat milk homozygous for weak or null alleles at *CSN1S1* locus; this could result in a further increase in the ability to form complexes with phenols. The most representative monomeric phenolic compounds in the supernatant of *R. coriaria*-fortified yogurt were identified and quantified using HPLC (Table 4). All detected phenols were present in all samples of studied yogurts and, as found in the *R. coriaria* water extract, gallic acid was the individual phenolic compound found at the highest concentration (321.99 μg/g of yogurt). As expected, no phenolic acids and flavonoids were found in the plain yogurt. The comparison with the amount of phenolic compounds detected in the *R. coriaria* water extract revealed that only a part of phenolic compounds was recovered in the

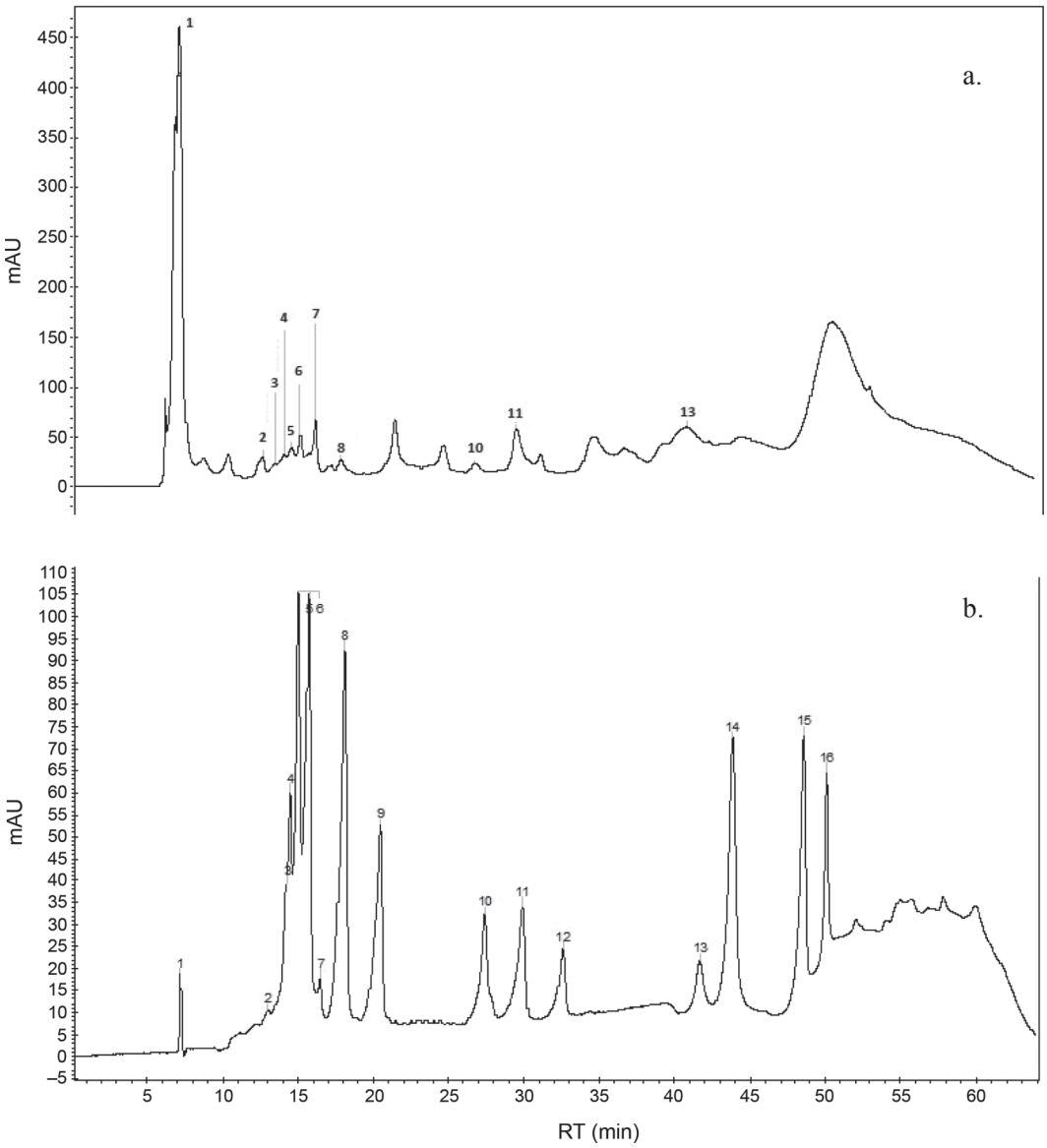

Figure 2. High-performance liquid chromatograms (detected at 280 nm) of *Rhus coriaria* water extract (a) and standard mixture of polyphenols (b). Peaks: 1, gallic acid; 2, catechin; 3, vanillic acid; 4, chlorogenic acid; 5, caffeic acid; 6, syring acid; 7, epicatechin; 8, *p*-coumaric acid; 9, ferulic acid; 10, rutin; 11, narirutin; 12, naringin H; 13, rosmarinic acid; 14, kaempferol; 15, quercetin; 16, luteolin. RT = retention time.

supernatant of *R. coriaria*-fortified yogurt (Table 4). These findings were in line with what was detected by Helal and Tagliazucchi (2018) in cinnamon-fortified yogurt. The recovery yield was different among the different monomeric compounds; in particular, among the phenolic acids, gallic acid, coumaric acid, and vanillic

Table 2. Antioxidant activity[1] of water extract from *Rhus coriaria* leaf powder

	R. coriaria water extract	
Item[1]	Mean	SD
ABTS (mg of Trolox equivalents/g of water extract)	725.75	55.21
FRAP (mg of Trolox equivalents/g of water extract)	41.27	2.12

[1]ABTS = 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical scavenging assay; FRAP = ferric-reducing antioxidant power assay.

Table 3. Total phenolic content (mg of gallic acid equivalents/g of yogurt) in plain yogurt and *Rhus coriaria*-fortified yogurt separately for α_{S1}-casein genotype

	Plain yogurt (32 samples)		*R. coriaria*-fortified yogurt (32 samples)	
α_{S1}-CN genotype	Mean	SD	Mean	SD
AA	3.72[a,A]	0.28	11.49[a,B]	1.50
AB	3.42[a,A]	0.41	11.39[a,B]	1.55
BB	3.37[a,A]	0.48	12.01[a,B]	2.23
FF	2.71[b,A]	0.34	9.83[b,B]	1.16

[a,b]Different lowercase superscripts depict the statistical difference within a column ($P < 0.05$) among means for the same yogurt batches at different α_{S1}-casein genotype.
[A,B]Different uppercase superscripts depict the statistical difference within a row ($P < 0.01$) between means for different yogurt batches.

acid showed a recovery yield higher than 33%, whereas among the flavonoids, a higher recovery yield was detected for catechin and rutin (>20%). Rosmarinic acid showed the lowest recovery (<6%). Statistical analysis showed a significant effect of the genotype on the amount of monomeric phenolic compounds in the supernatant of *R. coriaria*-fortified yogurt ($P < 0.01$). In particular, comparing different genotypes it emerged that *FF* goat yogurt showed the lowest content of all detected phenolic compounds (Table 5). In addition, *FF* yogurt also showed a lower recovery yield for all detected phenols compared with strong α_{S1}-CN yogurt (Figure 3), highlighting, however, a recovery of more than 25% for coumaric acid, vanillic acid, and gallic acid (27.11, 27.10, and 24.90%, respectively). The observed variations in the recovery of the different compounds are attributable to the binding affinity between individual phenols and protein. In support of this, Helal et al. (2014) found that caseins had high binding affinity with kaempferol and low with syringic acid; Kanakis et al. (2011) reported that β-LG had a high binding affinity with epigallocatechin, whereas with catechin it presented a low binding energy. The ABTS and FRAP activities of plain and fortified yogurt are shown in Table 6. Regardless of the considered genotype, fortified yogurt exhibited significantly higher antioxidant activity than the plain yogurt both in the ABTS and FRAP assay (7,884.90 and 1,105.28 vs. 860.60 and 67.12 μg of TE/g of yogurt, respectively), in line with what was reported by several authors (Najgebauer-Lejko et al., 2011; Chouchouli et al., 2013; Frumento et al., 2013) who highlighted a positive correlation between yogurt fortification with vegetables and antioxidant activity. In particular, the values of ABTS and FRAP in fortified yogurt were about 9 and 19 times, respectively, higher than the value of the plain yogurt. Antioxidant activity of yogurt is influenced by the casein genotype ($P < 0.01$), in agreement with what was

Table 4. Monomeric phenolic compounds in the *Rhus coriaria* water extract and *R. coriaria*-fortified yogurt supernatant[1] determined by HPLC

	R. coriaria water extract (μg/g of water extract)		*R. coriaria*-fortified yogurts (μg/g of yogurt)		Recovery[2] (%)
Item	Mean	SD	Mean	SD	
Phenolic acid					
Gallic acid	924.36	53.81	321.99	31.92	34.83
Rosmarinic acid	217.02	29.64	12.09	2.72	5.57
Chlorogenic acid	21.22	5.21	5.40	0.85	25.45
Vanillic acid	22.27	6.38	7.41	1.73	33.27
Caffeic acid	8.63	1.23	1.67	0.41	19.42
p-Coumaric acid	9.57	2.01	3.33	0.78	34.77
Syring acid	10.42	1.98	2.30	0.62	22.05
Flavonoid					
Epicatechin	193.08	18.94	36.92	7.41	19.12
Catechin	112.02	11.87	26.28	6.32	23.46
Rutin	111.03	12.34	30.20	7.34	27.20
Narirutin	93.99	9.87	11.28	2.51	12.01

[1]Monomeric phenolic compounds in *R. coriaria*-fortified yogurt supernatant was defined as the mean of each monomeric compound independent of α_{S1}-casein genotype.
[2]The recovery yield was defined as the percentage ratio between the concentration in the *R. coriaria*-fortified yogurt and the concentration in the *R. coriaria* water extract.

Table 5. Monomeric phenolic compounds in *Rhus coriaria*-fortified yogurts supernatant determined by HPLC separately for α_{S1}-casein genotype

	α_{S1}-CN genotype							
	AA (8 samples)		*AB* (8 samples)		*BB* (8 samples)		*FF* (8 samples)	
Item	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Phenolic acid (μg/g of yogurt)								
Gallic acid	358.91[a]	46.02	356.89[a]	27.75	342.01[a]	30.21	230.15[b]	23.69
Rosmarinic acid	11.69[a]	1.76	13.86[a]	5.14	13.02[a]	2.30	9.79[b]	1.70
Chlorogenic acid	5.33[a]	0.78	5.83[a]	0.90	5.73[a]	1.42	4.72[b]	0.31
Vanillic acid	6.96[a]	0.40	8.67[a]	3.96	7.97[a]	1.79	6.03[b]	0.75
Caffeic acid	1.61[a]	0.14	2.00[a]	0.97	1.73[a]	0.25	1.36[b]	0.29
p-Coumaric acid	3.47[a]	0.55	3.89[a]	1.03	3.35[a]	0.66	2.59[b]	0.89
Syring acid	2.23[a]	0.34	2.62[a]	0.90	2.50[a]	0.75	1.84[b]	0.51
Flavonoid (μg/g of yogurt)								
Epicatechin	37.89[a]	3.71	38.30[a]	13.44	42.48[a]	7.42	29.02[b]	5.06
Catechin	26.17[a]	2.84	30.16[a]	11.29	26.88[a]	7.11	21.90[b]	4.04
Rutin	30.99[a]	4.28	32.97[a]	10.18	35.53[a]	9.95	21.32[b]	4.95
Narirutin	11.67[a]	2.34	11.73[a]	3.27	13.16[a]	1.95	8.57[b]	2.48

[a,b]Different superscripts depict the statistical difference within a row ($P < 0.05$) among means for the same yogurt batches at different α_{S1}-casein genotypes.

detected by Perna et al. (2013). Comparing different genotypes, *FF* plain yogurt showed the lowest radical scavenging activity and FRAP ($P < 0.05$). The antioxidant capacity of plain samples is mainly due to the proteolytic activity of the starter lactobacilli used in yogurt production that leads to the formation of bioactive peptides with antioxidant activity (Virtanen et al., 2007; Gómez-Ruiz et al., 2008; Rutella et al., 2016). The genetic polymorphism affects the type of bioactive peptides released from milk proteins (Minervini et al., 2003; De Noni et al., 2009); the peptides released from the *A* variant of β-LG are small (3 kDa) and they are responsible for antioxidant activity compared with the *AB* variant (Hernández-Ledesma et al., 2005). Moreover, antioxidant activity of yogurt is affected by heat treatment undergone during manufacture (Galleher et al., 2005), by the production of organic acids during fermentation and after acidification (Correia et al., 2004), and by enzymatic hydrolysis of whey protein and casein that leads to the possible aggregation of peptide processes (Adt et al., 2011). The lower antioxidant capacity found in *FF* plain yogurt could also be explained by the lower content of fat and protein, as reported by Havemose et al. (2006). Chiang and Chang (2005) and De Marchi et al. (2007) highlighted a positive correlation between protein content and antioxidant activity. The antioxidant capacity of *R. coriaria*-fortified yogurt is closely associated with the α_{S1}-CN genotype ($P <$

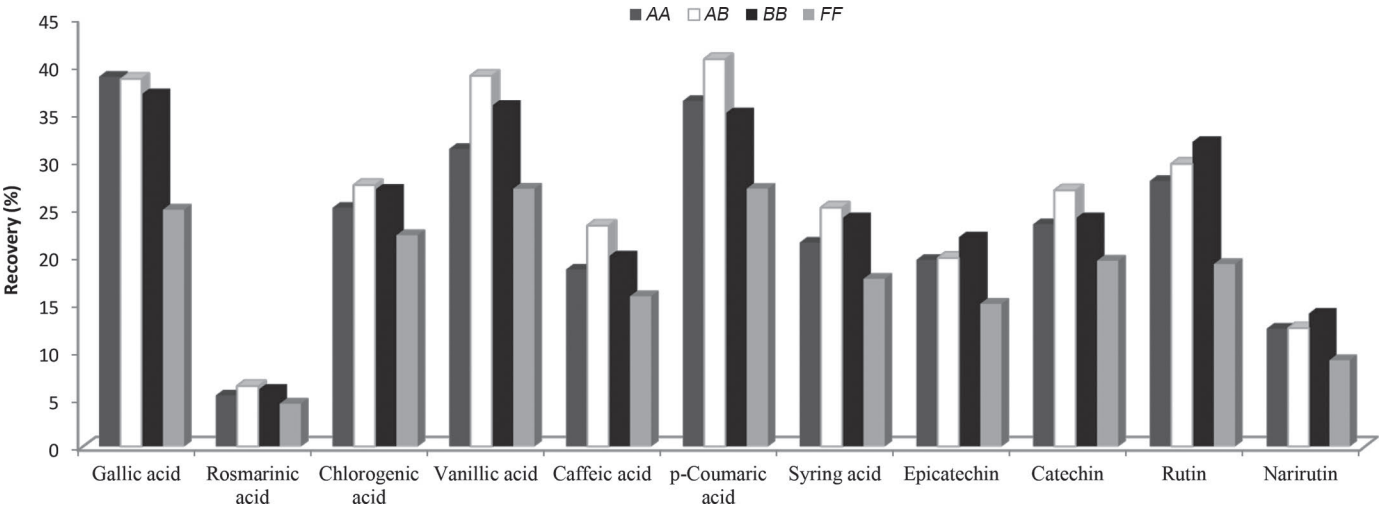

Figure 3. Recovery (%) of individual phenolic compounds, defined as the percentage ratio between the concentration in the *Rhus coriaria*-fortified yogurt supernatant and the concentration in the *R. coriaria* water extract, separately for α_{S1}-casein genotypes.

Table 6. Antioxidant activity (μg of Trolox equivalents/g of yogurt) of plain yogurt and *Rhus coriaria*-fortified yogurt supernatant separately for α_{S1}-casein genotype[1]

	ABTS				FRAP			
	Plain yogurt (32 samples)		*R. coriaria*-fortified yogurt (32 samples)		Plain yogurt (32 samples)		*R. coriaria*-fortified yogurt (32 samples)	
α_{S1}-CN genotype	Mean	SD	Mean	SD	Mean	SD	Mean	SD
AA	954.11^{a,A}	149.83	8,162.74^{a,B}	1,306.79	82.99^{a,A}	13.70	1,452.70^{a,B}	247.96
AB	903.37^{a,A}	101.61	8,128.08^{a,B}	1,242.91	72.34^{a,A}	7.76	1,394.44^{a,B}	278.82
BB	896.38^{a,A}	135.32	8,322.10^{a,B}	1,301.23	72.074^{a,A}	8.03	1,477.95^{a,B}	324.27
FF	688.54^{b,A}	93.96	6,926.69^{b,B}	850.87	41.09^{b,A}	7.80	960.13^{b,B}	169.89

^{a,b}Different lowercase superscripts depict the statistical difference within a column ($P < 0.05$) among means for the same yogurt batches at different α_{S1}-casein genotypes.

^{A,B}Different uppercase superscripts, for each assay, depict the statistical difference within a row ($P < 0.001$) between means for different yogurt batches.

[1]ABTS = 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical scavenging assay; FRAP = ferric-reducing antioxidant power assay.

0.01), in agreement with that detected by Perna et al. (2014) who studied the effect of casein haplotype on antioxidant activity of cow yogurt fortified with sulla and chestnut honey. Prigent and Dimitrov (2003) showed that the effect of the interaction between proteins or peptides (or both) and phenolic compounds on antioxidant activity depends on amino acid composition of the proteins and type of phenols. The *FF*-fortified yogurt showed the lowest values for ABTS and FRAP, confirming the results obtained by the Folin–Ciocalteu method. The different behavior detected among fortified yogurts led us to hypothesize that the effects of protein-polyphenol complex on antioxidant capacity are interactive, in agreement with Arts et al. (2002). On the other hand, the interaction between polyphenols and milk proteins could have a protective effect because this interaction may provide a physical trapping and therefore increase the stability of polyphenols during digestion. Whether a fraction of added polyphenols will remain unbound and exert antioxidant activity depends greatly on the proteins/added polyphenols ratio, as detected by Najgebauer-Lejko et al. (2011) in yogurts fortified with catechin-rich tea infusions.

CONCLUSIONS

The possibility of using goat milk for the production of fortified fermented products can allow development of new nutraceutical foods. The results obtained in this research highlights the role of milk protein polymorphisms and polyphenols on antioxidant capacity of fortified goat milk yogurt. In particular, in this study α_{S1}-*FF* fortified yogurt showed the lowest phenol content and therefore a lower antioxidant activity compared with the strong α_{S1}-CN yogurt. The different total phenol content detected in *R. coriaria*-fortified yogurt indicate that the α_{S1}-CN genotype influenced the amount of added phenols that are bound to the caseins and, therefore, the part that remains free and that affects the biological capacity of the final product. However, to better evaluate the effects of goat yogurt-*R. coriaria* combination, future investigations on bioavailability of polyphenols after digestion of *R. coriaria*-fortified yogurt are needed.

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