Neuigkeiten für die MS-basierte Lebensmittelanalytik – SCIEX 2021 Live Webinar Serie

Datum und Zeit: Donnerstag, 20. Mai 2021, 09:45 a.m. – 12:00 p.m. CET
Vortragssprache: Deutsch

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Willkommen zum lebensmittelanalytischen Webinar 2021 von SCIEX und Phenomenex

In unseren kontinuierlichen Bemühungen zur Entwicklungen neuer Workflows und massenspektrometrisch basierter Analysentechnologien möchten wir Ihnen heute die neuesten Innovationen und damit verbundenen Methoden zur Erfüllung Ihrer Anforderungen in der Lebensmittelanalytik vorstellen. Dieses Webinar widmet sich den neuen Möglichkeiten, die täglichen Herausforderungen zur Erfüllung regulatorischer Vorgaben in komplexen Matrices zu meistern. Und es sind gerade solche Entwicklungen, die zukünftige, neue analytische Anforderungen der Lebensmittelindustrie erst ermöglichen.

Themen

Zeit	Themen
09:45 a.m.	**Einführung** *André Schreiber, Senior Sales, Germany, SCIEX*
09:50 a.m.	**Chromatographische Ansätze in der Lebensmittelanalytik** *Jörg Baute, European Food/Environmental/Cannabis Business Development Manager, Phenomenex Ltd, Germany*
10:20 a.m.	**SCIEX Technologie Update** *Axel Besa, Manager Business Development Central Europe, SCIEX, Germany*
10:50 a.m.	**Effiziente und robuste Pestizid-Analytik: Verbesserung der Lebensmittelsicherheit durch neue Level der Empfindlichkeit** *Dr. Jianru Stahl-Zeng, Senior Technical Marketing Manager EMEAI, SCIEX, Germany*
11:20 a.m.	**Die Anwendung der Kapillar-Elektrophorese in der Lebensmittelanalytik** *Karsten Hendricks, Sales Representative CE Central North und Netherlands, SCIEX, Germany*
11:50 a.m.	**Diskussion und Fragen** *André Schreiber, Senior Sales, Germany, SCIEX*

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HEALTH, NUTRITION, AND FOOD

In vitro phenols bioaccessibility and antioxidant activity of goat milk yogurt fortified with *Rhus coriaria* leaf powder

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Abstract: Goat yogurt samples fortified with 20% (w/v) *Rhus coriaria* leaf powder were *in vitro* digested in order to evaluate the total phenolic content (TPC), antioxidant activity (AA), and bioaccessibility of phenolic compounds in the digestate. After digestion, TPC and AA values of the *R. coriaria*-fortified yogurts increased compared to the undigested yogurts ($P < 0.001$). In particular, TPC has increased about twice; whereas, AA values have increased about 10 and 6 times, for ABTS and FRAP assays, respectively. The bioaccessibility index was well above the 100% for all identified phenols; except for (-)-epicatechin (82.04%), rutin (51.51%), and gallic acid (5.42%). This different behavior highlighted that the bioaccessibility was modulated by both the yogurt-polyphenol complexes and phenol stability under digestion system. These findings can contribute to elucidate the influence of *in vitro* digestion on antioxidant capacity and polyphenols recovery infortified yogurts, and may help in the design of dairy products with better functional quality

Practical Application: *Rhus coriaria L.* (Sumac) is a polyphenol-rich Mediterranean plant that may be used as functional ingredient to enrich fermented food such as yogurt. However, in fortified yogurts the evaluation of bioaccessibility, that is, the compounds released from the yogurt and stable in the digestive environment, thus able to exert their biological effects on the gastrointestinal system, is more important than the content of these compounds in the corresponding food. This study highlighted the phenolic content, antioxidant activity, and bioaccessibility of phenolic compounds in goat milk yogurt fortified with *R. coriaria* leaf powder after simulated gastro-pancreatic digestion.

KEYWORDS
antioxidant activity, bioaccessibility, goat yogurt, *in vitro* digestion, sumac

1 | INTRODUCTION

Today the fortification of yogurt, already perceived by consumers as a functional food for its health promoting ingredients, with polyphenol-rich vegetables is a widely used technique to enrich these fermented foods with the therapeutic effects of phenols (Granato et al., 2018; Perna et al., 2014; Perna et al., 2019). *Rhus coriaria* L. (Sumac), belonging to the Anacardiaceae family, is a plant that grows in Mediterranean countries, it has numerous biological properties due to its richness in polyphenols (Kosar et al., 2007). In the last decade, several studies have been published on the biological properties of sumac and its use as a functional ingredient in foods (Bozan et al., 2003; Kosar et al., 2007) such as fermented dairy foods (Perna et al., 2018). Goat yogurt contains high amounts of proteins, lipids,

and carbohydrates which form reversibly or irreversibly complexes with polyphenols, depending on factors such as the molecule type, phenols content, pH, and temperature (Roura et al., 2008). In support of this, several studies highlighted the high binding affinity between phenols and milk proteins (Helal & Tagliazucchi, 2018; Kanakis et al., 2011). In particular, authors (Helal & Tagliazucchi, 2018; Perna et al., 2018) showed that the interaction between proteins and/or peptides and polyphenols depends on both amino acid composition of protein and type of phenol. These complexes interfere with polyphenols bioaccessibility, that is, the phenols fraction released from yogurt matrix following digestion, solubilized into small intestine, and therefore available for subsequent absorption (Tagliazucchi et al., 2010). This last point is very important since only the compounds released from the yogurt and stable in the digestive environment are potentially able to exert their biological effects. Therefore, in fortified foods the evaluation of phenols bioaccessibility is more important than the content of these compounds in the undigested food. In this regard, *in vitro* gastrointestinal digestion system is considered a useful tool to address these aspects (Bohn et al., 2018). However, in our knowledge few studies were reported in literature on polyphenols bioaccessibility from fortified yogurts, and no reports are available on the antioxidant capacity and recovery of phenolic compounds from *R. coriaria*-fortified yogurts after *in vitro* gastrointestinal digestion. Thus, the aim of the present study was to evaluate the phenolic content, antioxidant activity (AA), and bioaccessibility of phenolic compounds in goat milk yogurt fortified with *R. coriaria* leaf powder after simulated gastro-pancreatic digestion.

2 | MATERIALS AND METHODS

2.1 | Preparation of *R. coriaria*-fortified yogurts

Goat milk (Derivata di Siria breed) used in this study was provided by a farm situated in the Basilicata region (Southern Italy). The milk showed the following composition (g 100/g): 12.02 dry matter, 3.59 fat, 3.19 total protein, 0.16 non-nitrogen protein, 0.75 ash, and 4.48 lactose. The pH of the milk was 6.68. Turkish *R. coriaria* leaf powder was purchased from Terza Luna (http://www.terzaluna.com/prodotto/sumach-o-sommaco/). *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* were purchased from Insao s.r.l. (Liscate, Milan, Italy). The preparation of yogurt samples with 20% (w/v) *R. coriaria* leaf powder was carried out as described by Perna et al. (2018). After that yogurt samples were subjected to *in vitro* gastrointestinal digestion.

2.2 | *In vitro* gastrointestinal digestions

The *in vitro* gastrointestinal digestion of fortified yogurts was simulated according to the method reported by Simonetti et al. (2016), with some modifications. Briefly, 8 g of each sample were mixed with 20 mL of bidistilled water and homogenized in a Stomacher (Steward Stomacher 400 Lab Blender, London, UK) for 1 min to simulate the human chewing. Then 3 M HCl was used to bring the solution pH to 2 (model PHM 92, Radiometer, Copenhagen, Denmark), and stomach phase was simulated by adding 20 mL gastric juice (1.25 mg pepsin per mL of 0.1 M HCl; Sigma-Aldrich P6887, Milan, Ialy). After 2 hr of digestion at 37 °C, the pepsin was inactivated by adjusting the pH to 7.2 with 1 M $NaHCO_3$ and 40 mL pancreatin juice (4.50 mg pancreatin per mL of 20 mM phosphate buffer, pH 7.2; Sigma-Aldrich P3292) was added to simulate intestinal phase. After 4 hr of digestion, pancreatin activity was terminated by heating for 10 min at 95 °C. Aliquots of the samples were collected before adding the enzymes (undigested), after peptic (gastric) and pancreatic (intestinal) digestion. The digestion was carried out in triplicate.

2.3 | Samples preparation for analysis

Each sample was centrifuged at 5,000 x g for 20 min at 4 °C to remove insoluble material, the supernatant was filtered through a 0.2 μm cellulose acetate membrane filter (Sigma-Aldrich), and it was frozen and kept a −20 °C until analysis.

2.4 | Determination of total phenolic content (TPC)

TPC in *R. coriaria*-fortified yogurts before and after *in vitro* peptic and pancreatic digestion was determined using Folin–Ciocalteu reagent (Carlo Erba, Milan, Italy), as described by Perna et al. (2018). Each sample was tested in triplicate and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of yogurt.

2.5 | Analysis of AA

AA in *R. coriaria*-fortified yogurts before and after *in vitro* peptic and pancreatic digestion was determined by the 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS; Sigma-Aldrich) radical scavenging and ferric-reducing antioxidant power (FRAP; Sigma-Aldrich) assays, in according to the methodology described by Perna et al. (2014). Each determination was carried out in

triplicate and the results were expressed as milligrams of trolox equivalents (TE) per gram of yogurt.

2.6 | HPLC-UV analysis of phenolic compounds

Extraction procedure of phenolic compounds was carried out as described by Perna et al. (2018). The phenolic profile analysis was carried out in liquid chromatography (Varian Inc., Walnut Creek, CA, USA) as described by Perna et al. (2013). The identification and quantification of phenolic compounds at 280 nm was performed by comparison of their retention times and spectral characteristics with those of commercial standards (Sigma-Aldrich) analyzed under the same conditions, and the results were expressed as micrograms of phenolic compound per gram of yogurt.

2.7 | Bioaccessibility index (BI)

BI was calculated using the following equation:

$$\mathrm{BI}\,(\%) = \left(\mathrm{PC}_{\mathrm{digested}}/\mathrm{PC}_{\mathrm{undigested}}\right) \times 100 \qquad (1)$$

where $\mathrm{PC}_{\mathrm{digested}}$ is the phenol content in post-pancreatic digested sample and $\mathrm{PC}_{\mathrm{undigested}}$ is the content of the same phenol in undigested sample.

2.8 | Statistical analysis

Data were analyzed according to the following linear model (SAS Institute, 1996):

$$y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where y_{ij} is the observation; μ is the overall mean; α_i is the fixed effect of the ith treatment ($i = 1, 2, 3$); and ε_{ij} is the random error. Student's t-test was used for all variable comparisons and differences between means at the 95% ($P < 0.05$) confidence level were considered statistically significant.

3 | RESULTS AND DISCUSSION

3.1 | Effect of *in vitro* digestion on TPC and AA of *R. coriaria*-fortified yogurt

In this study *R. coriaria* polyphenols are consumed with yogurt, so the effects of the polyphenol-milk complex on the biological capacity of the digested were examined. Thus, all samples were subjected to *in vitro* digestion, and on each hydrolyzed sample was determined the TPC, AA, and phenolic profile. Overall, both TPC and AA of studied samples increased after *in vitro* digestion ($P < 0.001$; Table 1). In particular, the TPC varied from 12.09 (undigested yogurt) to 25.14 mg GAE/g of yogurt (digested yogurt), increasing about twice. Folin-Ciocalteau method detected the free polyphenols from *R. coriaria*, the endogenous milk phenols deriving from the animal feeding, and the milk nonphenolic compounds such as free amino acids and peptides that interfere with the Folin-Ciocalteu reagent (Helal & Tagliazucchi, 2018); whereas, the polyphenols fraction that remains linked to milk components such as protein, lipids, and carbohydrates was not detected. In fact, it is known that polyphenols have a high binding affinity with proteins and/or peptides which depends on the amino-acid composition of the proteins and the type of phenols (Helal & Tagliazucchi, 2018). Numerous noncovalent interactions such as hydrophobic interactions, hydrogen bonds, and van der Waals attractions, and some covalent bonds due to specific enzymatic, thermal or oxidative conditions were reported in literature between proteins and polyphenols (O'connell, & Fox, 2001; Yuksel et al., 2010). Richard et al. (2006) reported that numerous hydrophobic interactions occur between hydroxyl (−OH) groups of polyphenols and proline-rich proteins, such as caseins and/or derived peptides. Also numerous noncovalent interactions were detected between whey proteins (such as β-lactoglobulin, α-lactalbumin, serum albumin) and polyphenols (Mohammadi & Moeeni, 2015; Papadopoulou et al., 2005; Stojadinovic et al., 2013). The pH changes influenced the conformation of the protein molecules with effects on the protein–polyphenol interactions (Yildirim-Elikoglu & Erdem, 2018); in particular, authors (Naczk et al., 2006; Rawel et al., 2005) reported that the polyphenols showed a higher affinity to proteins when pH was close to its isoelectric point.

In support of this, Helal and Tagliazucchi (2018) found that at yogurt pH (4.5) the binding affinity between polyphenols and milk proteins enhance, consequently this leads to a decrease of health benefits of yogurt. However, during *in vitro* digestion, hydrolytic enzymes and pH changes lead to the hydrolysis of proteins and/or peptides, resulting in the release of polyphenols and therefore an increase in their bioaccessibility (De Carvalho et al., 2019; Helal & Tagliazucchi, 2018). In this study employed both pepsin and pancreatin: the pepsin treatment at low pH digests majority of the proteins thus interrupting the protein–polyphenol interaction, resulting in the release of more phenols into the digestive fluid; whereas, the pancreatin treatment at pH around neutrality completes the proteins hydrolysis (Cutrim & Cortez, 2018). In line with this, the TPC after *in vitro* gastric digestion was 21.76 mg

TABLE 1 Total phenolic content (TPC; mg of gallic acid equivalents/g of yogurt) and antioxidant activity[1] (mg of trolox equivalents/g of yogurt) of *R. coriaria*-fortified yogurts, before (undigested), and after *in vitro* peptic (gastric) and pancreatic (intestinal) digestion

	***Rhus coriaria*-fortified yogurt**					
	Undigested		**Gastric digestion**		**Intestinal digestion**	
	Mean	SD	Mean	SD	Mean	SD
TPC	12.09[a]	1.14	21.76[b]	1.37	25.14[c]	2.02
ABTS	7.88[a]	1.24	53.97[b]	2.59	86.12[c]	8.34
FRAP	1.11[a]	0.25	4.08[b]	0.82	6.69[c]	0.86

[a–c]Means within a row with different superscripts differ ($P < 0.001$).

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

of GAE/g of yogurt, that represents about 75% of the TPC released after complete *in vitro* digestion. A similar trend was observed by De Carvalho et al. (2019) in stevia-fortified yogurt, and by Helal and Tagliazucchi (2018) in cinnamon-fortified yogurt. Saura-Calixto et al. (2007) reported that during gastric digestion a high percentage of phenols bound to high molecular weight compounds (such as proteins) were released. After digestion, the BI of TPC, calculated as percentage ratio between TPC in yogurt sample after digestion and TPC in the same yogurt sample before digestion, was 208%. Authors (Green et al. 2007; Perez-Vicente et al., 2002) in fruit juices, and green tea, after simulated gastric digestion, detected a BI of almost 100%. Milk matrix enhances the polyphenols recovery and thus their bioaccessibility in a simulate digestive system (Granato et al., 2018; Green et al., 2007). In fact initially the polyphenols are physically trapped in protein–polyphenol complexes that protect them from possible degradation; however, as the digestion proceeds, the milk proteins hydrolysis allowed the release of the bound compounds, with a consequent increase of their bioaccessibility (Hasni et al., 2011). Moreover, after yogurt digestion, an increase of TPC is due to the release of nonphenolic substances, such as amino acids, aromatic amines, and peptides which are reduced by the Folin-Ciocalteau reagent (Prior et al., 2005).

ABTS and FRAP assays were used to determine the AA of *R. coriaria*-fortified yogurts before and after *in vitro* digestion, and the results are reported in Table 1. The AA is closely related with polyphenols content (Perna et al., 2019) and as expected after *in vitro* digestion it increased significantly ($P < 0.001$). Rashidinejad et al. (2016) reported that the AA in vegetable-fortified dairy products increased during digestion as a result of peptic and pancreatic enzymes which lead to the release of both polyphenols encrypted in protein–polyphenol complex and bioactive peptides and amino acids encrypted in milk protein sequences. Moreover, Wootton-Beard et al. (2011) detected that the AA of vegetable juices is relatively stable after *in vitro* digestion, highlighting that the majority of free polyphenols are resistant or not degraded to derivatives not-antioxidant during digestion. In this study, after *in vitro* gastric digestion, AA values was 53.97 and 4.08 mg TE/g of yogurt for ABTS and FRAP assays, respectively (Table 1), which corresponded to an increase of about 70% compared to AA values detected after complete *in vitro* digestion. This highlighted that the acid pH together with enzymes action led to both higher polyphenols extractability from protein–polyphenol complexes and release of bioactive peptides. After *in vitro* pancreatic digestion, the AA reached values of 86.12 and 6.69 mg TE/g of yogurt for ABTS and FRAP assays, respectively, with an increase compared to the undigested yogurt of about 10 and 6 times, respectively. Wootton-Beard et al. (2011) reported that during digestion the pH changes affected the molecules racemization creating enantiomers with different biological reactivity. The same AA trend was observed by Oliveira and Pintado (2015) in strawberry and peach enriched yogurts. However, De Carvalho et al. (2019), in stevia-fortified yogurt, and Helal and Tagliazucchi (2018), in cinnamon-fortified yogurt, detected a low or nonsignificant increase in AA from undigested to postgastric digested; whereas, the significant increase of AA after intestinal digestion detected by the aforementioned authors was attributed to the transition from acidic to alkaline environment that led to the deprotonation of the hydroxyl radicals of the aromatic rings.

3.2 | Effect of *in vitro* digestion on polyphenols bioaccessibility of *R. coriaria*-fortified yogurt

After ingestion, the phenols undergo a biotransformation and a digestion process in the gut and it is not said that the obtained products have the same biological activities, therefore it is important to detected which polyphenols are bioaccessible, and thus potentially able to exert their bioactivity after digestion. In this study, the amount of monomeric phenolic compounds in *R. coriaria*-fortified yogurt before and after *in vitro* digestion was determinate by HPLC (Figure 1), and the results are summarized in Table 2. In the undigested *R. coriaria*-fortified yogurt

TABLE 2 Phenolic compounds (μg of individual compound/g of yogurt) and bioaccessibility index[c] of goat milk yogurt fortified with *Rhus coriaria* leaf powder before (undigested), and after (digested) *in vitro* gastrointestinal digestion

	R. coriaria-fortified yogurt				Bioaccessibility Index (%)
	Undigested		Digested		
	Mean	SD	Mean	SD	
Phenolic acids					
Gallic acid	286.08[a]	26.95	15.52[b]	1.50	5.42
Rosmarinic acid	11.41[a]	1.62	20.61[b]	2.44	180.63
Chlorogenic acid	5.22[a]	0.65	18.85[b]	3.30	361.11
Vanillic acid	7.00[a]	1.07	19.96[b]	2.14	285.14
Caffeic acid	1.54[a]	0.17	6.00[b]	0.51	389.61
p-Coumaric acid	2.97[a]	0.41	5.46[b]	0.62	183.84
Syringic acid	2.17[a]	0.25	5.44[b]	0.44	204.61
Sum Phenolic acids	316.39		91.84		29.03
Flavonoids					
Epicatechin	35.75[a]	3.24	29.33[b]	3.52	82.04
Catechin	24.39[a]	3.57	85.11[b]	16.37	348.95
Rutin	28.42[a]	4.04	14.64[b]	2.12	51.51
Narirutin	10.86[a]	1.21	20.84[b]	2.58	191.89
Sum Flavonoids	99.42		149.92		150.79
Total	415.81		241.76		58.14

[a–b]Means within a row with different superscripts differ ($P < 0.001$).

[c]Calculated as percentage ratio between phenol content in post-pancreatic digested and the content of the same phenol in undigested sample.

the gallic acid was the most present phenolic compound in line with what detected in our previous study (Perna et al., 2018). After *in vitro* digestion, the amount of phenolic compounds has changed considerably, and a different behavior of these phenols was observed. Overall, the content of identified polyphenols varied from 415.81 (undigested yogurt) to 241.76 μg/g of yogurt (digested yogurt; Table 2), showing a low BI (58.14%). This decrease is largely due to the loss of gallic acid after digestion, as reported below. It is known that polyphenols can inhibit digestive enzymes (He et al., 2007) resulting in loss of both catalytic activity of enzyme and phenols bioaccessibility. Moreover, in alkaline environment, polyphenols may be oxidized to their corresponding quinone which can undergo attack by nucleophiles such as cysteine, lysine, methionine, with changes in their structural and biological properties (Kroll et al., 2003). Furthermore, oxidation and polymerization of phenolic compounds can lead to the formation of derivatives, such as chalcones, which are not bioaccessible due to their high molecular weight and low solubility (Kroll et al., 2003). Finally, under simulated digestive conditions, polyphenols can be degraded to other derivatives which may or may not show AA but are not detected as original compound by HPLC. Regarding to phenols class, the phenolic acids content varied from 316.39 (undigested yogurt) to 91.84 μg/g of yogurt (digested yogurt), showing a BI of 29.03%; whereas, the flavonoids content varied from 99.42 (undigested yogurt) to 149.92 μg/g of yogurt (digested yogurt), with a BI of 150.79% (Table 2). Gallic acid, active principle of the sumac extract, is the primarily responsible for its beneficial effects (Kosar et al., 2007). After *in vitro* digestion, the gallic acid resulted the most degraded, with a BI of 5.42%. Liu et al. (2013) reported that free phenol showed a high instability under alkaline conditions. In line with our findings, Desseva & Mihaylova (2020), after *in vitro* digestion of pomegranate, reported a loss of gallic acid of 93.5%. He et al. (2007) reported that different interactions occur between gallic acid and digestive enzymes such as pepsin which could cause both a change in enzyme molecular configuration and the masking of phenol, no longer detectable by HPLC analysis. An interesting result after *in vitro* digestion was instead the increase of other identified phenolic acids which showed a BI well above 100% (Table 2). The high recovery of these phenolic acids after simulated digestion highlighted both the high breakdown of protein–phenolic acid complexes by digestive enzymes and high stability of these phenolic acids in the digestive system. These findings also underlined that the BI of phenolic compounds depends on the physicochemical conditions of the digestive environment (pH, temperature, and enzymatic activity) as well as the type of the food matrix (Bouayed et al., 2011). In this study, caffeic acid and chlorogenic acid, powerful antioxidants (Rice-Evans et al., 1996), showed the highest BI (389.61% and 361.11%,

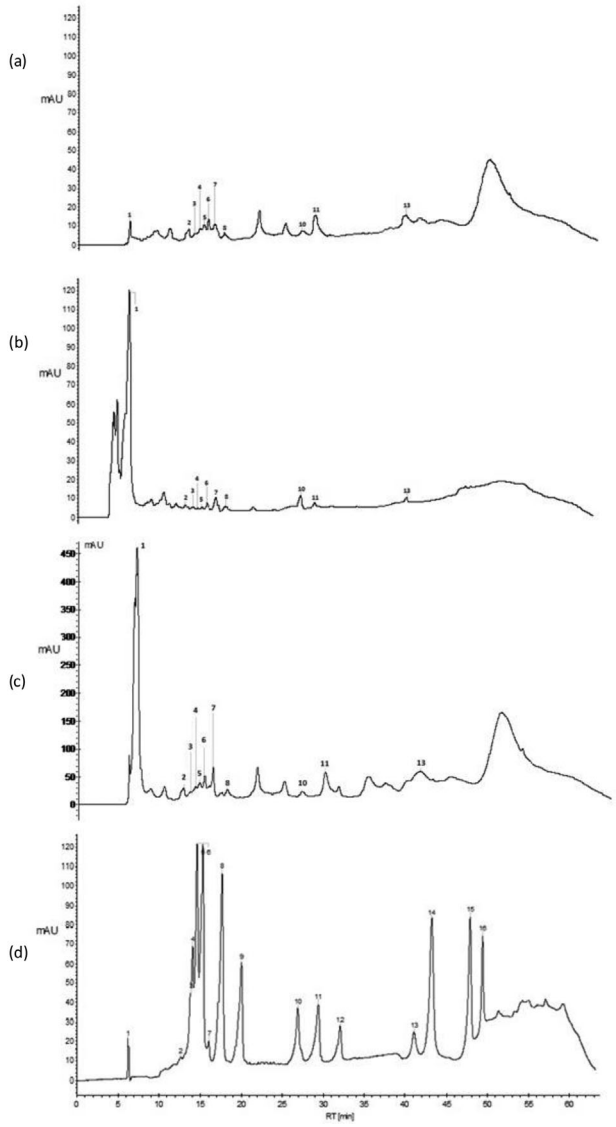

FIGURE 1 High-performance liquid chromatograms (detected at 280 nm) of undigested *Rhus coriaria*-fortified yogurt (a), digested *Rhus coriaria*-fortified yogurt (b), *Rhus coriaria* water extract (c), and standard mixture of polyphenols (d). Peaks: 1, gallic acid; 2, (+)-catechin; 3, vanillic acid; 4, chlorogenic acid; 5, caffeic acid; 6, syringic 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

respectively; Table 2). Tagliazucchi et al. (2012) detected that milk proteins have a protective action on caffeic acid or its derivatives under intestinal conditions. A higher BI of caffeic acid was observed in oat phenolic extract when mixed with 50% of milk. Dupas et al. (2006) found that the chlorogenic acid in coffee readily forms stable milk casein complexes under simulated gastrointestinal conditions. The high stability of these phenolic acids under digestive conditions was also demonstrated by Olthof et al. (2001) who detected that chlorogenic acid and caffeic acid were recovered almost completely after *in vitro* digestion. Vanillic acid showed a high BI (285.14%), following by rosmarinic, p-coumaric, and syringic acids which showed a similar BI (180.63%, 183.84%, and 204.61%, respectively). These phenolic acids have several biological properties (Kiliç & Yeşiloğlu, 2013; Kumar et al., 2011; Moreno et al., 2006). In agreement with our findings, Chen et al. (2019) detected a higher BI of vanillic acid in oat phenolic extract when mixed with 50% of milk. Zorić et al. (2016) detected a high gastrointestinal stability of pure rosmarinic acid. Moreover, a high BI for vanillic, p-coumaric, and syringic acids (about 68%, 60%, and 53%, respectively) was detected by Freitas et al. (2019) in soybean meal.

Catechins are a class of flavonols with demonstrated antiallergic, antimicrobial and anticarcinogenic properties (Higdon & Frei, 2003). On the basis of a daily consumption of 125 g of yogurt, after digestion, *R. coriaria*-fortified yogurt provides 10.64 mg of catechin which represents 21.3% of recommended mean allowance in Dutch diet (50 mg/die; Arts et al., 2001). After digestion (+)-catechin content increased about 3.5 times, showing the highest BI (Table 2). In support of this, Su et al. (2003) reported that the low yogurt pH leads to the formation of stable catechin-protein complexes during storage, and consequently a greater catechin recovery in intestinal system. Arts et al. (2002) reported that the catechins were able to form stable complexes with proline-rich proteins, such as β-casein. Moreover, a high catechins recovery from milk-tea beverages after simulate digestion was detected by Green et al. (2007) who attributed this to the breakdown of protein–catechin bound by digestive enzymes. In particular, Ferruzzi and Green (2006) reported that during digestion the pepsin can sufficiently disrupt the proteins-catechin bound. In disagreement with our study, Oliveira and Pintado (2015) after *in vitro* digestion of strawberry and peach enriched yogurt detected a low BI (53% and 20%, respectively); they attributed this to the masking of the catechin due to catechin-protein interactions that make it undetectable with HPLC analysis. After *in vitro* digestion, the (-)-epicatechin content decreased, showing a BI of 82.04% (Table 2). Hasni et al. (2011) reported that epicatechin compared to catechin has lower interactions with α- and β-casein, both in terms of lower binding constant value and number of polyphenol bound for casein molecule; this result in less protection of flavonol and, therefore, a greater susceptibility to the enzymes action. Rashidinejad et al. (2016), in cheese fortified with green tea catechins, reported a lower retention coefficient in curd and a lower recovery after *in vitro* digestion of epicatechin compared to catechin. Moreover, catechins contain a ring structure that facilitates their solubility in milk fat globule membrane (MFGM; Sirk et al., 2009), with consequent lower release of these molecules during digestion.

A strong affinity to the lipid bilayer surface was detected for epicatechin (Sirk et al., 2009); whereas, a lower affinity to the MFGM was detected for catechin when compared to (-)-epigallocatechingallate and green tea extract (Rashidinejad et al., 2016). After simulate digestion, the rutin showed a recovery of 51.51% (Table 2). Oliveira and Pintado (2015) after *in vitro* digestion of strawberry and peach enriched yogurt observed a rutin BI slightly higher than our own (about 60% and 67%, respectively). Rutin is a flavones with a wide range of biological properties such as antibacterial, anti-oxidant, and antiproliferative activities (Kessler et al., 2003); however, it is known that rutin, begin slightly soluble in both water and oil, has low dissolution rate and bioavailability (Mauludin et al., 2009). Moreover, in literature is reported that, among milk proteins, the rutin forms complexes only with the bovine serum albumin and the β-lactoglobulin (Sahihi et al., 2012), whereas, the free rutin can undergo several chemical and enzymatic degradation in the gastrointestinal environment (Baldisserotto et al., 2015). Narirutin is a flavanone with wide range of therapeutic properties (Funaguchi et al., 2007). Hou et al. (2019) after *in vitro* gastrointestinal digestion of orange juice detected a high narirutin recovery (about 56%). In this study, after digestion, the narirutin content increased about two times, showing a BI of 191.89%. These findings allow us to hypothesize the formation of lactoprotein–narirutin complexes that are broken up following digestion, making this flavanone bioaccessible in intestinal tract, even if to our knowledge no information is reported in literature on recovery of narirutin ingested with dairy products. Several clinical trials have examined the effects of polyphenol administration in humans. Among these, Jensen et al. (2008), in a randomized, double-blind, placebo-controlled, crossover trial with healthy subjects, after consumption of antioxidant-rich fruit and berry juice blend observed a decrease of lipid peroxidation and an increase of antioxidants in serum. Georgakouli et al. (2016) showed that body weight, body mass index, hip circumference and systolic blood pressure diminished considerably after consumption of olive polyphenol-enriched yogurt for 2 weeks; these authors also observed a tendency to reduce the low-density lipoprotein cholesterol level and thiobarbituric acid-reactive substances.

4 | CONCLUSIONS

R. coriaria leaf powder was successfully employed to produce *R. coriaria*-fortified yogurts characterized by higher nutraceutical quality. Nevertheless, the nature of the food matrix in which bioactive molecules are contained affected both their antioxidant capacity and bioaccessibility. In particular, this study demonstrated that *in vitro* gastrointestinal digestion significantly influenced the TPC and AA of *R. coriaria*-fortified yogurt. Moreover, the polyphenols bioaccessibility was modulated by both the food matrix and the phenol stability under digestion system. Thus, digested *R. coriaria*-fortified yogurt is a model for design of dairy products with enhanced nutritional and functional quality. However, to obtain a better understanding on polyphenols bioaccessibility from *R. coriaria*-fortified yogurt further *in vivo* studies evaluating both the action of digestive enzymes and the action of microbiota metabolism should be performed.

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AUTHOR CONTRIBUTIONS

Amalia Simonetti and Annamaria Perna conceived the research, supervised the research, and drafted the manuscript. Amalia Simonetti and Giulia Grassi collected samples and carried out the chemical analysis. Emilio Gambacorta carried out the chemical and statistical analyses and collected all the data. Annamaria Perna and Amalia Simonetti jointly analyzed, drafted, and revised the manuscript. All authors read and approved the final article.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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