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Antioxidant activity of different cheese-honey combinations before and after *in vitro* gastrointestinal digestion

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ABSTRACT

This study aimed to evaluate the effect of different cheese-honey combinations on antioxidant activity before and after *in vitro* gastrointestinal digestion, using the 2,2′-azino-bis-3-ethylbenzothiazoline- 6-sulfonic acid (ABTS), and ferric-reducing antioxidant power (FRAP) assays. Honey addition significantly enhanced the antioxidant activity of cheese samples ($P < 0.05$). The antioxidant activity of *in vitro* digested samples was significantly lower than the undigested ones. However, digested cheese-honey combination showed a percentage decrease of antioxidant activity lower than plain cheese. The cheese and honey types affected the percentage decrease of antioxidant activity in the digested samples, this is mainly due to the bioaccessibility of the honey polyphenols after digestion. Sensory analysis showed that all cheese-honey combinations were well accepted by the consumers, the health information on higher antioxidant capacity of dark honeys has not influenced the degree of acceptability of consumers, who preferred the cheese - clear honey combinations.

1. Introduction

Food combinations have always been used in gastronomy to enhance the sensory characteristics of food products. Among these the honey addition in dairy products is now widely used in order to improve the sensory properties and at the same time the nutraceutical characteristics of these foods (Perna, Simonetti, & Gambacorta, 2019); just think that today the cheese-honey combination, used since ancient times, represents an important Italian culinary tradition. Moreover, cheese and honey are both considered functional foods (Luchese, Ribeiro Prudêncio, & Fioravante Guerra, 2017; Summer et al., 2017). The cheese is a rich source of proteins, vitamins, minerals (in particular calcium in a highly bioavailable form), and fatty acids important in a healthy diet; in addition, during cheese ripening the caseins are hydrolyzed by different enzymatic systems into a large variety of peptides that influence several biological functions (Donkor, Henriksson, Singh, Vasiljevic, & Shah, 2007; Perna, Simonetti, Intaglietta, & Gambacorta, 2015); finally is know that during gastrointestinal digestion several bioactive peptides are released from milk casein mostly by gastric and pancreatic enzymes (Kanwar et al., 2009). However, the biological capacity of cheese depends on the used milk type, cheese-making process, and cheese ripening (Lucas et al., 2006). Honey's functional importance due to its antioxidant, anti-inflammatory, and antitumoral properties is amply demonstrated (Orsolic, Terzic, Sver, & Basic, 2005). The biological activity of honey depends on the floral and geographical origin (Perna, Simonetti, Intaglietta, Sofo, & Gambacorta, 2012) which markedly influence its functional composition, and thus the quality and the quantity of important components such as flavonoids, phenols, enzymes, ascorbic acid, Maillard reaction products, organic acids, amino acids, and proteins (Al-Mamary, Al-Meeri, & Al-Habori, 2002; Aljadi & Kamaruddin, 2004). In light of the above, the study from the nutraceutical point of view of cheese-honey combination can provide new information to consumers increasingly oriented towards foods that exert a positive effect in the body. However, to evaluate the beneficial effects of peptides and polyphenols it is necessary to consider their bioavailability and metabolic fate. In fact, it is know that the compounds bioavailability depends on its: bio-accessibility (understood as release from food matrix); digestive stability; and transepithelial passage efficiency (Manach, Williamson, Morand, Scalbert, & Rémésy, 2005). Thus, the aim of this study was to evaluate the effect of different cheese-honey combinations on antioxidant activity before and after *in vitro* gastrointestinal digestion using ABTS and FRAP assays.

2. Materials and methods

2.1. Samples

A total of 38 cheeses and 9 honeys, randomly selected according to

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consumers' preferences, were purchased from Italian stores in April 2019. The cheeses were all Italian Protected Designation of Origin (PDO) cheese, consequently their production geographical area, livestock system (breed, diet, and farming conditions), and manufacturing technologies are reported in public guidelines which are approved and regulated by the European Union and local authorities. Cheese varieties included 2 long-aged pressed cheeses: Parmigiano Reggiano cheese (PaRe; also known as Parmesan cheese, 24 months of ripening), and Pecorino Romano cheese (PeRo; 8 months of ripening) made from cow and sheep milk, respectively; and 2 long-aged stretched-curd cheese: Caciocavallo Silano cheese (CS; 6 months of ripening) and Provolone Valpadana cheese (PV; 2 months of ripening) both made from cow milk. All cheese samples were stored at 4 °C in the dark until analyzed. Honeys collected for this study were: sulla, chestnut and eucalyptus. For cheese-honey (C–H) combinations, on each cheese sample (1.5 × 2.0 cm side and 7 cm in length) was added 10% (w/w) of each honey before analysis.

2.2. In vitro gastrointestinal digestions

The *in vitro* gastrointestinal digestion of honey, cheese, and C–H samples was simulated using pepsin and pancreatin according to the method reported by Simonetti, Gambacorta, and Perna (2016), with some modifications. Fifteen grams of each sample were mixed with 40 mL of bidistilled water and homogenized in a Stomacher (Steward Stomacher 400 Lab Blender, London, UK) for 1 min for simulate the human chewing. Then HCl (3 mol/L; Sigma-Aldrich, Milan, Italy) was used to bring the pH of the solution to 2 (model PHM 92, Radiometer, Copenhagen, Denmark), and stomach phase was simulated by adding pepsin (Sigma-Aldrich P6887, Milan, Italy) at a 1:200 (enzyme:substrate) ratio. After 2 h of digestion at 37 °C, the pepsin was inactivated by adjusting the pH to 7.2 with 1 mol/L $NaHCO_3$ and the pancreatin (Sigma-Aldrich P3292, Milan, Italy) was added at a 1:25 (enzyme:substrate) ratio to simulated intestinal phase. After 4 h of digestion at 37 °C, pancreatin activity in the solution was terminated by heating for 10 min at 95 °C. Samples were collected before adding the enzymes (undigested sample), and after *in vitro* digestion (digested sample). Each sample was centrifuged at 5,000 × *g* for 20 min at 4 °C to remove large particles, the supernatant was filtered through a 0.2 μm cellulose acetate membrane filter (Sigma-Aldrich), and it was frozen and kept a −55 °C until analysis.

2.3. Antioxidant activity

The ABTS radical cation and FRAP assays were carried out according to the methodology described by Perna, Intaglietta, Simonetti, and Gambacorta (2014). Results were expressed as milligrams of Trolox equivalents (TE) per gram of sample.

2.4. Consumer acceptability

An sensory method to evaluate consumer acceptability of different C–H combinations was used. The test consisted of 270 untrained consumers who had been selected based on their regular consumption of ripened cheese (at least once per week) as well as their sex (132 females and 138 males) and age (25 ÷ 65 years of age), attempting to represent the distribution of the population as closely as possible. The test was conducted on 45 d with one session per day carried out between 9:30 and 12:30. Each consumer participated in three sessions and tasted four C–H samples. C–H samples (1.5 × 2.0 cm side and 7 cm in length) were presented to consumer at room temperature in glass tumblers covered with clock glasses, coded with 3 digit numbers and order of tasting was balanced to account for first order and carry over effects. Consumers were asked to evaluate the C–H samples, visually (appearance, and color) and then organoleptically (taste and odor), finally expressing a judgment on overall acceptability. Each consumer was isolated in individual booths and was asked to express their acceptability on a nine point hedonic scale, between 1 (dislike extremely) and 9 (like extremely) (Peryam & Pilgrim, 1957). Consumers were provided with oligomineral water and asked to thoroughly rinse the oral cavity after each sample. To examine the influence of potential health message, the same consumers in the fourth session (test with the information) received four C–H combinations: PaRe-chestnut honey, and CS-chestnut honey (cheese with dark honey), PaRe-sulla honey, and CS-sulla honey (cheese with clear honey). Before starting the analysis, to consumers was provided the following health information: "dark honeys are rich in antioxidant components". The sensory test was conducted as the previous one: briefly, to informed consumers was asked to evaluate the samples for appearance, color, taste, odor, and overall acceptability, and to express their acceptability on a nine point hedonic scale. All assessments were carried out in a sensory laboratory equipped according to UNI–ISO 8589 recommendations (International Organization.for International Organization for Standardization, 1988)

2.5. Statistical analysis

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

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where y_{ijk} is the *k*th observation at the *i*th level of factor α and the *j*th level of factor β; μ is the overall mean; α_i is the fixed effect of the *i*th cheese type ($i = 1, 2, 3, 4$); β_j is the fixed effect of the *j*th honey type ($j = 1, 2, 3$); $(\alpha\beta)_{ij}$ is the interaction of cheese type x honey; and ε_{ijk} 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. Antioxidant activity of undigested samples

The antioxidant activity of the honey samples, evaluated by ABTS and FRAP assays, is reported in Table 1. Overall, the average antioxidant activity of the honeys was 27.7 and 10.72 mg TE/g honey for ABTS and FRAP assay, respectively. In particular, the antioxidant activity varied in a wide range: eucalyptus honey showed the lowest ABTS and FRAP values (23.38 and 8.29 mg TE/g honey, respectively) than other tested honeys ($P < 0.05$); on the contrary, chestnut honey showed the highest values (34.09 and 13.35 mg TE/g honey for ABTS and FRAP assay, respectively; $P < 0.05$), confirming what was reported in our previous study (Perna et al., 2012). The differences found

Table 1
Antioxidant activity[1] (mg of Trolox equivalents/g of honey) of both honey samples from different botanical origin and different cheese samples, before *in vitro* gastrointestinal digestion.

Honey	ABTS		FRAP	
	Mean	SD	Mean	SD
Chestnut	34.09^{c}	0.18	13.35^{c}	0.04
Eucalyptus	23.38^{a}	0.06	8.29^{a}	0.03
Sulla	25.58^{b}	0.81	10.53^{b}	0.04
Cheese				
Parmigiano Reggiano	20.43^{d}	0.10	3.79^{b}	0.19
Pecorino Romano	17.99^{c}	0.07	3.13^{a}	0.11
Caciocavallo Silano	17.15^{b}	0.13	3.87^{b}	0.18
Provolone Valpadana	16.26^{a}	0.07	3.75^{b}	0.03

$^{a–d}$ For each food, means within a column with different superscripts differ ($P < 0.05$).
[1]ABTS = 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical cation. assay; FRAP = ferric-reducing antioxidant power assay.

among the studied honeys highlighted that the antioxidant activity of honey, closely related to quality and quantity of polyphenols, varies considerably depending on the floral origin (Gomez-Caravaca, Segura-Carretero, & Fernandez-Gutierrez, 2006; Perna, Intaglietta, Simonetti, & Gambacorta, 2013). In line with this results, in our previous study (Perna et al., 2013) we found a higher phenolic and flavonoid content in chestnut honey than eucalyptus and sulla ones. In particular, in the study mentioned above, we found that chestnut honey is characterized by a higher content of both phenolic acids (such as caffeic acid, p-coumaric acid, ferulic acid, benzoic acid), and flavonoids (such as catechin, epicatechin, gallocatechin, rutin, myricetin, quercetin) compared to eucalyptus and sulla honeys. Cheese contains numerous bioactive components with a strong protective activity against oxidative damage (Alberti-Fidanza, Burini, & Perriello, 2002). The ABTS and FRAP values of studied cheeses are reported in Table 1. The PaRe cheese showed the highest ABTS value ($P < 0.05$) probably linked to the greater ripening time of this cheese compared to other analyzed ones. Several authors (Elias et al., 2006; Phelan, Aherne-Bruce, O'Sullivan, Fitzgerald, & O'Brien, 2009) reported that the antioxidant activity of cheese is largely due to the concentration of macro and micronutrients, to enzymatic systems, to hydrolyzed proteins, and to free amino acids; however, the ripening time markedly influences the antioxidant activity of cheese (Gupta, Mann, Kumar, & Sangwan, 2009; Perna et al., 2015) since many bioactive peptides, such as antioxidant ones, are released from precursor protein, where they are encrypted, by the proteolytic system during ripening (Donkor et al., 2007). The PeRo cheese instead showed the lowest FRAP value ($P < 0.05$). It is known that the manufacturing technology influences the presence of bioactive components in the cheese: Gagnaire et al. (2011) reported that the stretching at 70–80 °C in Ragusano stretched-curd cheese favored the plasmin action with consequent casein degradation; however, the heat treatment exposes reactive thiol groups which can form disulfide links with consequent decrease of antioxidant activity (Perna et al., 2015). Overall, all studied C–H combinations showed a significant increase in antioxidant activity compared to the plain cheese ($P < 0.01$; Table 2); this is due to honey polyphenols, the main compounds responsible of the biological activities of honey (Al-Mamary et al., 2002; Aljadi & Kamaruddin, 2004). Alonso, Guillèn, Barroso, Puertas, and Garcìa (2002) reported a positive correlation between antioxidant activity and phenols content in food. A support, Lucera et al. (2018) in spreadable cheese enriched with different by-products flours such as red and white grape pomace, tomato peel, broccoli, corn bran, and artichokes, detected a significant increase of phenols and flavonoids content, and antioxidant activity compared to the control cheese. The different antioxidant activity detected among the studied C–H combinations could be due to both antioxidant activity of cheese and honey type and interactions between the constituents of dairy products and the polyphenols of honey which interact with milk proteins by both hydrophilic and hydrophobic interactions (Lamothe, Azimy, Bazinet, Couillard, & Britten, 2014). Considering the antioxidant activity of the C–H combinations, for each studied cheese, the findings showed that the increase or decrease in antioxidant capacity is strongly correlated to the type of added honey (Table 2). In particular, for the PaRe cheese the addition of different honey varieties did not result in significant differences in terms of ABTS assay, whose average value was 33.16 mg TE/g. For the PeRo cheese instead the combination with sulla honey showed the highest ABTS value ($P < 0.05$), whereas the one with chestnut honey was the worst one ($P < 0.05$). On the contrary, for the CS cheese the combination with chestnut honey showed the highest ABTS value ($P < 0.05$), whereas the one with sulla honey the lowest ($P < 0.05$). Finally, the addition of sulla honey on PV cheese showed the lowest ABTS value, whereas PV-eucalyptus honey combination showed the highest value ($P < 0.05$). The FRAP assay did not confirm what was observed with the ABTS assay, and for all C–H combinations the findings showed a high variability (Table 2). However, the cheese

Table 2
Antioxidant activity[1] (mg of Trolox equivalents/g of sample) of the cheese-honey combinations before *in vitro* gastrointestinal digestion.

Cheese	Honey					
	+ Chestnut		+ Eucalyptus		+ Sulla	
	ABTS					
	Mean	SD	Mean	SD	Mean	SD
Parmigiano Reggiano	$32.99^{a,D}$	0.80	$33.09^{a,D}$	1.16	$33.40^{a,D}$	0.68
Pecorino Romano	$19.07^{a,A}$	0.86	$22.66^{b,A}$	0.18	$31.22^{c,C}$	0.43
Caciocavallo Silano	$28.60^{c,C}$	0.55	$25.60^{b,B}$	0.12	$25.09^{a,B}$	0.01
Provolone Valpadana	$24.88^{b,B}$	0.12	$27.37^{c,C}$	0.48	$16.74^{a,A}$	0.25
FRAP						
	Mean	SD	Mean	SD	Mean	SD
Parmigiano Reggiano	$7.24^{b,C}$	0.06	$5.41^{a,B}$	0.35	4.87^{aA}	0.12
Pecorino Romano	$5.23^{b,A}$	0.01	$4.45^{a,A}$	0.06	$5.13^{a,b,A}$	0.48
Caciocavallo Silano	$5.43^{b,B}$	0.12	$4.91^{a,b,A}$	0.91	$4.61^{a,A}$	0.15
Provolone Valpadana	$5.35^{b,B}$	0.08	$5.40^{b,B}$	0.16	$4.66^{a,A}$	0.32

[a–c]Means within a row with different superscripts differ ($P < 0.05$).
[A–D] For each antioxidant assay, means within a column with different superscripts differ ($P < 0.05$).
[1]ABTS = 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical scavenging assay; FRAP = ferric-reducing antioxidant power assay.

Table 3
Antioxidant activity[1] (mg of Trolox equivalents/g of honey) of both honey samples from different botanical origin and different cheese samples, before (undigested), and after (digested) *in vitro* gastrointestinal digestion.

Honey	Undigested		Digested		Loss (%)[2]
	Mean	SD	Mean	SD	
	ABTS				
Chestnut	$34.09^{b,C}$	0.18	$24.60^{a,C}$	0.54	27.84
Eucalyptus	$23.38^{b,A}$	0.16	$18.25^{a,A}$	0.34	21.94
Sulla	$25.58^{b,B}$	0.81	$18.12^{a,A}$	0.21	29.16
Overall	27.68	4.82	20.32	3.74	26.59
FRAP					
Chestnut	$13.35^{b,C}$	0.14	$9.43^{a,C}$	0.12	29.36
Eucalyptus	$8.29^{b,A}$	0.13	$6.35^{a,A}$	0.09	23.40
Sulla	$10.53^{b,B}$	0.11	$7.54^{a,B}$	0.07	28.39
Overall	10.72	2.41	7.77	1.37	27.51
Cheese	**ABTS**				
Parmigiano Reggiano	$20.43^{b,D}$	0.10	$15.84^{a,D}$	0.36	22.47
Pecorino Romano	$17.99^{b,C}$	0.07	$14.27^{a,C}$	0.20	20.68
Caciocavallo Silano	$17.15^{b,B}$	0.13	$13.72^{a,B}$	0.22	20.00
Provolone Valpadana	$16.26^{b,A}$	0.09	$12.60^{a,A}$	0.27	22.51
Overall	17.96	1.61	14.11	1.37	21.44
FRAP					
Parmigiano Reggiano	$3.79^{b,B}$	0.19	$2.90^{a,B}$	0.03	23.48
Pecorino Romano	$3.13^{b,A}$	0.11	$2.51^{a,A}$	0.08	19.81
Caciocavallo Silano	$3.87^{b,B}$	0.18	$2.61^{a,A}$	0.01	32.56
Provolone Valpadana	$3.75^{b,B}$	0.03	$2.50^{a,A}$	0.05	33.33
Overall	3.64	0.35	2.63	0.23	27.65

[a–c] Means within a row with different superscripts differ ($P < 0.05$).
[A–D] For each antioxidant assay, means within a column with different superscripts differ ($P < 0.05$).
[1]ABTS = 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical scavenging assay; FRAP = ferric-reducing antioxidant power assay.
[2]Calculated as percentage ratio: 100 x (antioxidant value of undigested sample - antioxidant value of digested sample)/antioxidant value of undigested sample).

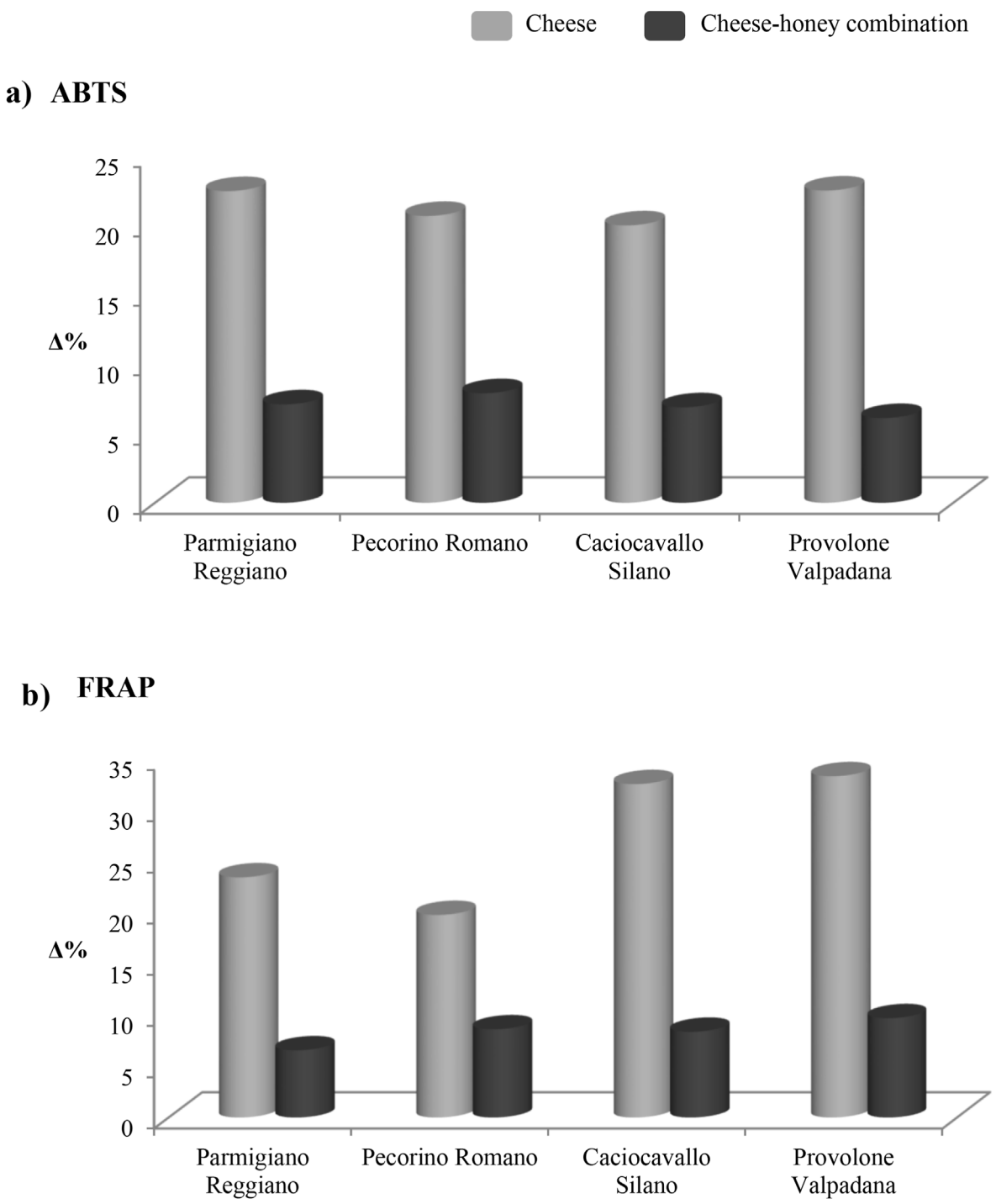

Fig. 1. Percentage decrease[1] (Δ%) of antioxidant activity after *in vitro* gastrointestinal digestion in cheese and cheese-honey combination measured by ABTS (a) and FRAP (b) assays, separately for studied cheese and independently of the used honey.

combination with chestnut honey showed the highest values ($P < 0.05$). Among studied cheeses, PaRe cheese with chestnut and eucalyptus honeys showed the highest antioxidant values, while PeRo cheese with these honeys showed the lowest values, for both ABTS and FRAP assays. Moreover, the PaRe cheese with sulla honey showed the highest ABTS value, whereas PV-sulla honey combination the lowest value ($P < 0.05$). Considering the FRAP assay, no significant difference was found among studied cheeses and sulla honey addition. This wide variability of results could be due to the formation of different protein-polyphenol complexes that could influence the polyphenols bioavailability, and consequently the antioxidant activity of dairy products (Arts et al., 2002; Perna, Simonetti, Grassi, & Gambacorta, 2018). Authors (Hasni et al., 2011; Kanakis, Hasni, Bourassa, Tarantilis, Polissiou, & Tajmir-Riahi, 2011) detected that the formation of protein-polyphenol complexes decreases the electron donation capacity of catechins by reducing the number of hydroxyl groups available in the solution.

3.2. Effect of in vitro gastrointestinal digestion on antioxidant activity

In vitro digestion model provides an indication of bioaccessibility of antioxidants in a biological system. In this study, all samples were

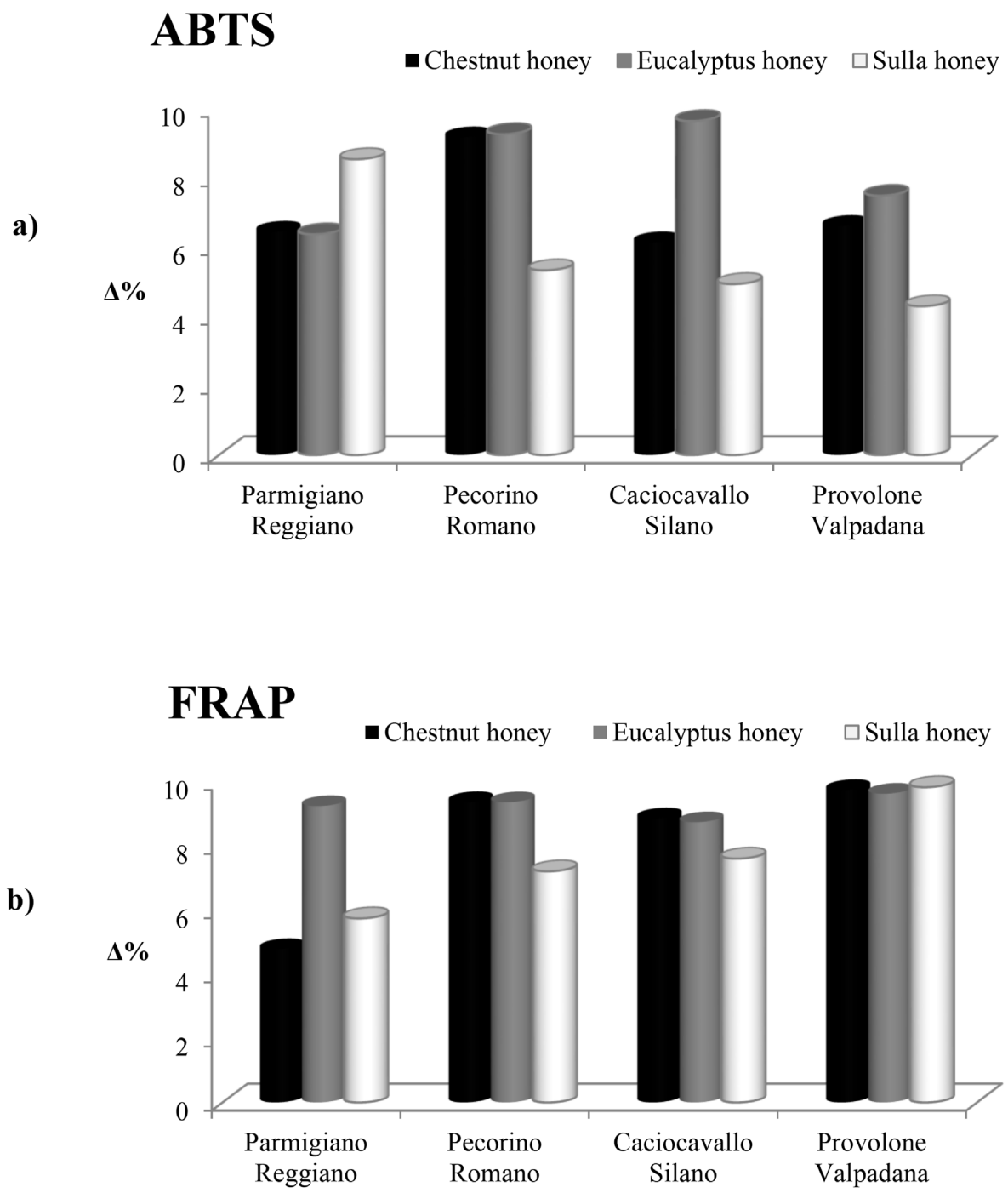

Fig. 2. Percentage decrease[1] (Δ%) of antioxidant activity in cheese-honey combination after *in vitro* gastrointestinal digestion, measured by ABTS (a) and FRAP (b) assays.

subjected to *in vitro* gastrointestinal digestion, and on each hydrolyzed sample was determined the antioxidant activity by ABTS and FRAP assays. Overall, antioxidant activity of all studied samples (honey, cheese, and C–H combinations) decreased after *in vitro* gastrointestinal hydrolysis: in honey, it reached values of 19.98 and 7.77 mg TE/g for ABTS and FRAP assay, respectively, with a decrease compared to the undigested, of about 26.5 and 27.5%, respectively (Table 3). These findings are in agreement with O'Sullivan, O'Callaghan, O'Connor, and O'Brien (2013) who reported that hydrolytic enzymes and pH changes during digestion can lead to the degradation of some antioxidant compounds in honey, thus reducing their bioavailability. Neilson, Bomser, and Ferruzzi (2007) reported that the catechins showed gastric stability because they are stable at pH values below 6, while they were largely decomposed at neutral or slightly alkaline pH similar to that found in the small intestine. A support of this, *in vivo* studies in humans showed that, in spite high concentration of polyphenols in the diet, their plasma concentration ranged from 0 to 4 μmol/L, and they had a small effect on the total antioxidant capacity of blood plasma (Henning et al., 2004; Lotito & Frei, 2006). Moreover, it is known that many polyphenols remain in the gastrointestinal tract where they may exert their biological activity (Cheynier, 2012). In the cheese samples, the digestion has led to a decrease in antioxidant capacity of about 21.44 and 27.65% for ABTS and FRAP assays, respectively (Table 3). This could be due to the fact that the studied cheeses are long-aged cheeses,

Table 4
Sensory acceptability[1] of the studied cheese-honey combinations.

Descriptor											
Cheese[2]	**Honey**	**Appearance**		**Color**		**Odor**		**Taste**		**Overall acceptability**	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
		Without the health-related information									
PaRe	+ Chestnut	6.84^{a}	0.15	6.69^{a}	0.21	7.21^{a}	0.47	6.85^{a}	0.31	6.91^{a}	0.25
	+ Eucalyptus	7.27^{b}	0.25	7.58^{c}	0.15	7.35^{a}	0.36	7.43^{c,d}	0.22	7.37^{c,d}	0.20
	+ Sulla	7.32^{b}	0.12	7.29^{b}	0.15	7.24^{a}	0.29	7.16^{b}	0.17	7.18^{a,b}	0.25
PeRo	+ Chestnut	6.80^{a}	0.15	6.76^{a}	0.18	6.95^{a}	0.26	6.85^{a}	0.16	7.02^{a,b}	0.15
	+ Eucalyptus	7.31^{b}	0.08	7.45^{b,c}	0.12	7.15^{a}	0.37	7.35^{b,c,d}	0.19	7.23^{b,c,d}	0.21
	+ Sulla	7.46^{b}	0.10	7.71^{c}	0.25	7.55^{a}	0.28	7.79^{e}	0.22	7.82^{e}	0.15
CS	+ Chestnut	6.78^{a}	0.17	6.79^{a}	0.23	7.62^{a}	0.35	7.45^{d}	0.29	7.51^{d}	0.36
	+ Eucalyptus	7.30^{b}	0.13	7.59^{c}	0.26	7.38^{a}	0.29	7.12^{a,b}	0.14	7.01^{a,b}	0.29
	+ Sulla	7.32^{b}	0.20	7.43^{b,c}	0.27	7.20^{a}	0.25	7.10^{a,b}	0.18	7.22^{b,c}	0.10
PV	+ Chestnut	6.95^{a}	0.12	6.74^{a}	0.33	6.99^{a}	0.29	7.40^{c,d}	0.19	7.46^{c}	0.17
	+ Eucalyptus	7.35^{b}	0.14	7.38^{b,c}	0.12	7.43^{a}	0.34	7.17^{b,c}	0.18	7.28^{b,c,d}	0.15
	+ Sulla	7.43^{b}	0.15	7.51^{b,c}	0.20	7.41^{a}	0.34	7.15^{b}	0.24	7.17^{a,b,c}	0.23
With the health-related information[3]											
PaRe	+ Chestnut	6.95^{a}	0.17	6.73^{a}	0.18	7.33^{a}	0.26	6.91^{a}	0.11	6.95^{a}	0.24
	+ Sulla	7.24^{b}	0.11	7.38^{b,c}	0.13	7.54^{a}	0.38	7.13^{a,b}	0.21	7.17^{a,b}	0.14
CS	+ Chestnut	6.59^{a}	0.21	6.72^{a}	0.28	7.47^{a}	0.29	7.51^{d}	0.19	7.62^{d}	0.13
	+ Sulla	7.37^{b}	0.18	7.39^{b,c}	0.17	7.18^{a}	0.35	7.16^{b}	0.17	7.18^{a,b}	0.21

[1]Each attribute was evaluated on a hedonic scale from 1 (dislike extremely) to 9 (like extremely).
[2]PaRe = Parmigiano Reggiano, PeRo = Pecorino Romano, CS = Caciocavallo Silano, PV = Provola Valdostana
[3] Health-related information was: "dark honeys are rich in antioxidant components".
[a-e]Means within a column with different superscripts differ ($P < 0.05$).

so they have already undergone a high hydrolysis during ripening, consequently many ingested peptides might be hydrolyzed by digestive enzymes during digestion, reducing their bioavailability and thus their antioxidant capacity. Bottesini et al. (2013), in Parmigiano Reggiano cheese, hypothesized that the decrease in antioxidant activity after *in vitro* digestion could be due to degradation of antioxidant amino acids, as well as tryptophan, in gastric environment. Antioxidant activity of different C–H combinations after *in vitro* gastrointestinal digestion were also evaluated, and in Fig. 1 is reported the percentage decrease (Δ%) of antioxidant activity of both plain cheese and C–H combination, separately for considered cheese and independently of the used honey. After *in vitro* digestion, antioxidant activity of the C–H combinations showed a lower decrease compared to the plain cheese. This could be due to the protein-polyphenol complex that physically trapping the polyphenols exerted a protective effect protecting them from the attack of pepsin during gastric digestion (Helal & Tagliazucchi, 2018), and increase their stability during this digestion phase (Hasni et al., 2011); thereafter, during *in vitro* gastro-pancreatic digestion, the proteins and peptides were hydrolyzed and the polyphenols are released with a consequent increase of their bioaccessibility (Helal & Tagliazucchi, 2018). Considering the percentage decrease of individual C–H combinations (Fig. 2), the combination of sulla honey with PeRO, CS, and PV cheeses showed the lower decrease of ABTS value after *in vitro* gastrointestinal digestion; whereas, the PaRe-sulla honey combination showed the higher decrease. FRAP assay instead showed a high individuality; however, the combination of all the studied cheeses with eucalyptus honey showed a high decrease. Moreover, among studied cheeses, PV-honey combinations showed the higher decrease of FRAP value.

3.3. Sensory analysis

The aim of this sensory analysis was twofold: to investigate the degree of acceptance of the different C–H combinations, and to examine the influence of health-related message on the sensory acceptability of C–H combinations. Today the consumers are more health conscious and to perceive a food as healthy can increase the intake and acceptability of that food, therefore several health claims which state, suggest or imply that a relationship exists between food and health are used by food companies. (Schouteten et al., 2015). Overall, it was observed that the addition of honey to cheese showed a good acceptability score for all assessed descriptors (Table 4TtTtttt), this underlines the fact that the Italian consumers are used to eating C–H combinations. However, the addition of honey from different botanical origin to different cheese types showed a significant effect on all sensory properties ($P < 0.05$), except for odor, where no difference among C–H combinations was detected. In particular, the cheese-chestnut honey combinations showed the lowest appearance and color scores ($P < 0.05$). These descriptors are very important because could be affect the consumer acceptance, changing their flavor perception with negatively impacting on marketing (Spence, 2015). The lowest appearance and color score for cheese-chestnut honey combinations can be explained by the fact that consumers are used to eating honey with a colour slightly straw yellow, while chestnut honey, due to the high content of polyphenols, is a dark honey. The PeRo-sulla honey combination showed the highest taste and overall acceptability scores ($P < 0.05$) compared to the other C–H combinations. Considering the studied cheeses, PaRe cheese showed higher taste and overall acceptability scores when eucalyptus honey was added, while for CS and PV cheeses higher taste and overall acceptability scores were detected when chestnut honey was added. Since the findings showed that the cheese-chestnut honey combinations were less accepted by consumers for color and appearance scores, another sensory test with the same consumers, but with a health-related information was conducted. The health-related massage was: "dark honeys are rich in antioxidant components", the samples evaluated were four: two cheeses with sulla honey (straw yellow honey), and two cheeses with chestnut honey (dark honey; Table 4). Our findings showed that the health-related information did not have an effect on the sensory acceptability, in contrast to what has been reported by Schouteten et al. (2015) who showed that health claim may affect cheese flavor perception and overlap the sensory characteristics.

4. Conclusions

This study highlighted the complex dynamic actions that occur when foods with different biochemical characteristics interact. Honey addition significantly enhanced the antioxidant activity of cheese samples. The lower antioxidant activity of digested samples than the undigested ones was probably due to the further hydrolytic degradation of the samples during *in vitro* digestion. However, after *in vitro* digestion, C–H combinations showed a percentage decrease of antioxidant activity lower than plain cheese; this could be due to the polyphenol-protein complexes that improve the stability of polyphenols in a gastrointestinal environment, with an additional advantage for consumer health. The polyphenols, in fact, can reduce oxidative damage in the gastrointestinal tract and are also involved in many complex mechanisms such as modulation of cell survival signalling pathways and modification of gut microbiota composition. The cheese and honey type affected the percentage decrease of antioxidant activity in digested samples, this is mainly due to the bioaccessibility of the honey polyphenols after digestion. The PeRo-sulla honey combination showed a higher consumer acceptability, indicating advantages not only on antioxidant capacity but also in sensory properties compared to the other C–H combinations. The health information on higher antioxidant capacity of dark honeys has not influenced the degree of acceptability of consumers, who preferred the cheese - clear honey combinations. Certainly more in-depth knowledge is needed to identify the best cheese-honey combinations, in terms of the availability of bioactive components useful for consumer health. Moreover, further *in vivo* studies evaluating both the action of digestive enzymes and the action of microbiota metabolism should be performed.

CRediT authorship contribution statement

Amalia Simonetti: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Annamaria Perna:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing - original draft, Writing - review & editing. **Giulia Grassi:** Formal analysis, Investigation, Methodology. **Emilio Gambacorta:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Resources, Software, Supervision, Validation, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financialinterestsor personal relationships that could have appeared to influence the work reported in this paper.

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