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Antioxidant and polyphenol content of different milk and dairy products

Running title: Antioxidants in milk and dairy products

Abstract

The aim of the present study was to determine the antioxidant capacity and total polyphenol content of raw milk, dairy products (ricotta and cottage cheese), and by-products (sweet and acid whey) from different animal breeds (cow, goat).

Overall, the total polyphenol content of raw milk ranged from 420.34 to 490.72 mg GAE/100mL, while the total antioxidant content changed between 8.95 and 28.72 mg AAE/100mL. These values in the case of cottage cheeses were 32.29-124.29 mg GAE/100mL for polyphenols and 14.12-16.38 mg AAE/100g for antioxidants. Significant differences were observed between the total polyphenol content and antioxidant properties of sweet- (10.85-197.55 mg AAE/100g antioxidant; 32.29-124.29 mg GAE/100g polyphenol) and acid whey (13.28-158.69 mg AAE/100g antioxidant; 43.50-98.03 mg GAE/100g polyphenol). In addition, slight differences in total polyphenol content (10.55-19.01 mg GAE/100g) and antioxidant capacity (10.84-15.93 mg AAE/100g) were observed for ricotta cheeses made from milk of different animal breeds. The results show that milk and dairy products are excellent sources of antioxidants and polyphenols.

Keywords: milk, whey, ricotta, antioxidants, polyphenols, spectroscopy

1. Introduction

According to the latest forecast, world milk production will reach 937 million tonnes in 2022, an increase of 1.0 percent compared to 2021. These predictions also confirm that Asia will continue to produce the most cow's milk in the world due to the increase in the number of dairy cattle. Milk production may increase moderately in North and Central America, as well as in the Caribbean region, mainly due to improved yields. In contrast, milk production is expected to decline in Europe, South America, and Oceania because of the decrease in the number of dairy cattle, and the increase in feed costs, due to the increasing shortage of skilled labor and the deteriorating pasture quality (FAO, 2022). The planet's population is growing, and the industries dealing with agriculture, animal husbandry, and food processing began to develop rapidly. The dairy industry needs to meet the growing needs of the world's population for milk, cheese, butter, yogurt, milk powder, and other dairy products (Jaganmai and Jinka, 2017).

The main components of milk are water, fat, lactose, and protein (casein and whey protein), while other minor components include minerals, specific blood proteins, vitamins, and enzymes. The proportion of ingredients from the milk of different mammalian species may vary widely. For this reason, it is obvious that the processing methods for different types of milk may also differ (Robinson, 2002). Lipophilic (phospholipids, α-tocopherol, β-carotene, conjugated linoleic acid, vitamins D3 and A, coenzyme Q10) and hydrophilic (proteins, peptides, vitamins, minerals, and trace elements) milk antioxidants play a significant role in the balance of prooxidant- antioxidants homeostasis in the human body (Baldi and Pinotti, 2008). Lipophilic antioxidants have excellent thermal stability, so they are present in the active form in all dairy products. Milk antioxidants interact and deactivate reactive oxygen species (ROS) and the final products of lipid peroxidation, these facts confirm that dairy product consumption has health benefits (Cichosz et al., 2017). Many dairy products and milk fractions have antioxidant effects (milk caseins, whey, lactoferrin), which contribute to the value of milk, and their consumption may even have an anticarcinogenic effect on the human body cells (Tong et al., 2000).

One of the main problems of the dairy industry is the large amount of by-products produced. For example, during the production of 1 kg of cheese produces 9 liters of whey. (Parashar et al., 2016). The whey is a yellowish-green liquid caused by the riboflavin content. Whey makes up 85-95% of the milk and contains 65g of dry matter per liter. After cheese production, 55% of the whole milk's nutrients and 20% of the total protein content remains in the whey (Ryan and Walsh 2016). The composition of whey may affect many factors such as milk origin or type (acid or rennet coagulated) of the produced cheese. According to the coagulation method, two types of whey can be distinguished, namely sweet and sour whey. In addition, the quality of raw milk can be affected by breeding, circadian rhythm, feeding or lactation phase (Székelyhidi, 2017; Usmani et al., 2022). Several studies have shown that whey products are an excellent source of antioxidants (Iskandar et al., 2015; Mohammadian et al., 2016; Power-Grant et al., 2016), making them potential raw materials for functional foods. Nowadays, functional foods are very popular because they support consumer health and may reduce the amount of by-products generated by the food industry. This study examined the total antioxidant and polyphenol content of raw milk, cottage cheese, ricotta, furthermore sweet, and sour whey which was left behind in the milk processing. Our aim was to prove that the consumption of milk, dairy products, and dairy by-products has beneficial physiological effects. In this study, we also offer a possible solution for utilizing whey in the production of a traditional Northern European confectionery (mysost or other name whey caramel).

The purpose of the study was to determine the general chemical composition, antioxidant, and polyphenol content of the milk of different dairy cow breeds (Jersey, Simmental, Holstein-Friesian) and the Saanen goat breed. The dairy products and by-products made from different kinds of milk were also examined for the parameters mentioned above, and to what extent the processing methods affect them.

2. Materials and Methods

2.1. Chemicals

Chemicals for the determination of polyphenol, and antioxidant content were 99% methanol (Reanal, Hungary), anhydrous sodium carbonate (Riedel-de Haen, Germany), Folin-Ciocalteu reagent (Merck, Germany), 2-4-6-tripyridyl-s-triazine (TPTZ) (Sigma-Aldrich, USA), acetic acid (Reanal, Hungary), anhydrous iron chloride (Merck, Germany), gallic acid (Sigma-Aldrich, USA), ascorbic acid (Sigma-Aldrich, USA), and citric acid from trade.

2.2. Milk samples

The Saanen goat's milk originated from the Kránicz farm (Győr, Hungary). Holstein milk was purchased from the farm of Attila Berebora (Darnózseli, Hungary). Jersey cattle milk was received from Milán Meiszner (Levél, Hungary). Simmental milk was obtained from the farm of Miklós Varga (Dunakiliti, Hungary).

2.3. Manufacture of cottage cheese

5-5 L of different kinds of raw milk (Jersey, Simmental, Holsten-Friesian, and Saanen) were weighed into a pot. Each type of milk was heated to 65 ° C and kept at this temperature for 30 min for pasteurization. Then, it was cooled to 36 °C. When the right temperature was reached, 5mL of rennet, which was previously diluted in 10 mL of ultra-high purity water, was added. The inoculated milk was rested for 45 minutes. The clot was cut both vertically and horizontally using a cheese harp. It was then left to rest for 10 minutes to release the whey. After 10 minutes, the larger clot clumps were crushed with stirring and left to rest for 15 minutes. The resulting curd was placed in cheesecloth and hung to allow the remaining whey to drip out.

2.4. Ricotta production

To make the ricotta, the leftover sweet whey was heated to 89-92 ° C. Then, 5 g of citric acid was added and mixed thoroughly. The stove was turned off, and after 30 minutes, the whey proteins precipitated. The whey proteins were put into a cheesecloth and hung up to drip the remaining sour whey.

2.5. Mysost production

Mysost was made from sour whey. Briefly, 700 g of sugar is added to the uncooled whey per liter. Then, it was cooked for 5-8 hours with constant stirring and poured into molds, left to cool down.

2.6. Determination of constituents of milk and milk products

All sample constituents were tested according to the standards of the Hungarian Standards Institution. These standards for dry matter content: MSZ 3744:1981, chapter 1, for fat content: MSZ 3703:2018, chapter 5/ MSZ EN ISO 1211:2010, for whey content: MSZ EN ISO 8968-1:2014/MSZ EN ISO 8968-3:2004, for lactose content: AM 18:2016/AM 19:2016/ ISO 5548:2004. The ash content is determined by Methodenbunch Band VI (C 10.2).

2.7. Determination of total antioxidant and polyphenol content

2.7.1. Sample preparation

Raw milk, sour whey, and sweet whey samples did not require sample preparation. The total antioxidant and polyphenol content of the samples could be determined directly. 5 g from the previously prepared cottage cheese and ricotta samples were weighed into 250 mL Erlenmeyer flasks. The samples were extracted for two hours after adding of 20 mL extractant (70:30 V/V% methanol: ultra-high purity water). The samples were then centrifuged (Hermle Z206A Germany) at 6000 RPM for 20 min and filtered.

2.7.2. FRAP assay

The antioxidant content of the samples was estimated according to the method described by Benzie, and Strain (1996) as modified by Amamcharla and Metzger (2014) with minor modifications. 300 μL of the extracted sample and 4.5mL of FRAP solution were pipetted into a test tube. The finished solutions were placed in a dark place for 5 min and then their absorbance was measured with a Spectroquant Pharo 100 spectrophotometer (Merck, Germany) at a wavelength of 593 nm against the blank which contained only the FRAP solution. Ascorbic acid (40-500 mg/L) was used as a standard and the results were expressed as mg ascorbic acid equivalent capacity (AAE)/ g dry matter.

2.7.3. Folin-Ciocalteu assay

The determination of total polyphenol content based on the Folin–Ciocalteau method was described by Singleton et al. (1999) with some modifications (Barba et al., 2013). To 300 μL of milk, whey, and milk product extract, 1.5 mL of ultra-high purity water was pipetted, and the reagents were added. First 2.5 mL of Folin-Ciocalteu reagent, then 2 mL of Na_2CO_3. The tubes with the solutions were placed in a dark place for 90 minutes, and then the absorbance was measured at 725 nm versus the blank which was similar to the test solution except for the sample extract. Gallic acid solutions were used as standards (25- 500 mg/L).

2.8. Data analysis

The total antioxidant and polyphenol contents of milk and dairy products were determined in Microsoft Office Excel from the absorbance values measured for raw milk and milk products using the equation of the second-order least squares analytical curve fitted to the measurement solutions using the nonlinear least-squares method. All the results are expressed as means (n=3) + / - standard deviation.

3. Results and discussion

3.1. Constituents of milk and milk products

The composition of milk and milk products of different dairy species is shown in Table 1. Results are expressed in g/100g. The dry matter content of the tested raw milk varied between 12.23 and, 16.11 g/100. The fat content ranged from 3.25 to 6.20 g/100g, while the protein contents changed between 3.40 and 4.44 g/100g. Holstein Friesian raw milk had the lowest, and Jersey milk had the highest ash content. The lactose content was the lowest in the milk of Saanen goats, and the highest in Simmental raw milk. The received values approach the data in the literature for both bovine milk (Stocco et al., 2017) and goat milk (Chavez and Gonzáles, 2010). Khastayeva et al. (2021) determined the biological value of Simmental and Holstein cow milk, and they found similar results. Sanjayaranj et al. (2023) studied Holstein Friesian, and Jersey milk composition. In the case of Jersey samples, they determined similar results. However, in the case of Holstein Friesian milk, they found higher values.

Jersey cottage cheeses were outstanding in terms of dry matter and fat, but the lactose content of these products was the lowest. Cottage cheese made from Simmental milk had the lowest fat content and the highest protein and ash content. Goat cottage cheese had the lowest dry matter and protein content. In terms of fat content, cottage cheese made from Holstein Friesian milk was outstanding. Ali et al. (2022) examined the chemical composition of cottage cheeses, they reported fat and protein content similar to our values.

The chemical composition of the leftover sweet whey was also examined. Based on the results, the lowest values were obtained for Simmental sweet whey for all parameters except lactose. In contrast, the highest values were measured from Saanen sweet whey in all cases except protein content. Jersey whey had the highest protein content.

In the case of ricotta products, Holstein Friesian ricotta had the lowest fat and ash content, but the protein content was the highest among the studied varieties. Simmental ricotta was outstanding in dry matter and fat content, but the lactose content was negligible compared to other ricotta samples. Goat ricotta had the lowest dry matter, and protein content, while lactose content was the highest. In terms of ash content, the Jersey ricotta showed outstanding results. Semeniuc et al. (2015) analysed the physicochemical properties of whey cheeses and recorded similar results.

The by-product of ricotta production experiments is sour whey. Holstein Friesian whey contained the least dry matter, protein, and fat, while Jersey whey contained most of these components. The sour whey from the goat's milk had the lowest ash and the highest lactose content. In contrast, Jersey whey had the highest ash content, while Simmental whey had the lowest lactose content. Tsakali et al. (2010) reported lower chemical composition values in the case of sweet and acid whey samples.

3.2. Antioxidant content of milk and milk products

The antioxidant content of raw milk samples, dairy products, and whey was determined (Figure 1). Based on the results, Jersey milk had the highest antioxidant content (28.2 mg AAE/100g), followed by Holstein Friesian milk (26.24 mg AAE/100g). Simmental milk contains only half of this amount with a 13.22 mg AAE/100g value. The raw milk with the lowest total antioxidant content was Saanen goat milk, which contained only 8.95 mg AAE/100g antioxidant. Sreeramulu and Raghunath (2011) also obtained similar results when examining the antioxidant content of milk and dairy products.

There were significant differences in the antioxidant content of cottage cheeses. Jersey cottage cheese had the highest antioxidant content with 16.38 mg AAE/100g value. Holstein Friesian and Simmental cheeses contained 14.12 and 14.13 mg AAE/100g antioxidants, and the cottage cheese made from the Saanen goat milk contained 15.01 mg AAE/100g antioxidants. Ogunlade et al. (2019) obtained similar results when examining cheeses made from goat milk. Jersey sweet whey samples which remained after cottage cheese preparation contained 66.87 mg AAE/100g antioxidants. The Holstein Friesian sweet whey's antioxidant value was only 10.85 mg AAE/100g. In the case of Simmental whey, this data was 47.49 mg AAE/100g. From the tested four sweet whey samples, Sanental Goat whey contained an exceptionally high amount of antioxidants (197.55 mg AAE/100g). Significant differences were observed in the antioxidant content of sweet whey samples, just like in the case of raw milk.

There were few differences in the ricotta samples total antioxidant content. In the case of Jersey (14.21 mg AAE/100g) and Simmental (13.71 mg AAE/100g) ricottas the antioxidant values were statistically the same. The most antioxidants can be found in Holstein Friesian ricotta with a 15.93 mg AAE/100g value. In contrast, Saanen goat ricotta contained the least total antioxidants (10.84 mg AAE/100g). The antioxidant values of ricotta and whey cannot be compared with the data in the literature, as to the best of our knowledge, no article is available that gives the results by a similar method and in terms of ascorbic acid units.

3.3. Polyphenol content of milk and milk products

In the analysis of the total polyphenol content of raw milk samples, it was found that the highest polyphenol content (490.72 mg GAE / 100mL) was in the milk of the Saanen goat. The total polyphenol content of Jersey milk was 483.53 mg GAE/ 100mL, and this value in Holstein milk was 478.79mg GAE/ 100mL, there were no significant differences compared to the milk of the Saanen goat. The raw milk of Simmental cows contained 420.34 mg GAE/100 mL polyphenol. Alyaqoubi et al. (2014) reported similar results when examining goat milk.

There were significant differences in the polyphenol content of the cottage cheeses. Jersey cottage cheese contained 32.58 mg GAE/100g, while Holstein Friesian cottage cheese contained 56.70 mg GAE /100g polyphenols. In Simmental cottage cheese was detected an exceptionally high polyphenol content with 124.29 mg GAE/100 g value. The least phenolic components were goat cottage cheese, which contained only 32.29 mg GAE/100g. Similar results were obtained by Farrag *et al*. (2020) when examining soft cheeses.

In all cases, there were significant differences in the amount of phenolic components of sweet whey samples. The total polyphenol content of Jersey sweet whey was 196.76 mg GAE/ 100mL, in Holstein Friesian sweet whey, this value was 313.33 mg GAE/ 100 mL. The total polyphenol of Simmental cottage cheese was 265.85 mg GAE/ 100 mL, while in Saanen goat whey, this value was 347.34 mg GAE/ 100 mL.

In Jersey ricotta, 19.01 mg GAE/100g total polyphenol content was detected, while Holstein ricotta had the lowest total polyphenol content (10.55 mg GAE/ 100g). In the case of Simmental and Saanen goat ricotta samples, the total polyphenol content was the same (15.42-15.42 mg GAE/ 100g).

Finally, the total polyphenol content of sour whey samples was also shown significant differences. Jersey sour whey had 98.03 mg GAE/ 100mL polyphenol content, and in the case of Holstein Friesian sour whey, this value was 43.50 mg GAE/ 100 mL, while in the Simmental whey 93.22 mg GAE/ 100 mL polyphenol content was determined. In contrast, the total polyphenol content of Saanen goat whey was 67.35 mg GAE/ 100 ml. To the best of our knowledge, there is no article available on the total polyphenol content of whey and ricotta which the similar in terms of sample preparation and test method, so we cannot compare our results with literature data. We found a single article (Bennato et al. 2022) that examined the total polyphenol content of ricotta and whey, however, these compounds were detected in significantly lower amounts than we did. This may have been due to the extremely short (30s) extraction time.

Mysost (whey caramel) made from sour whey makes milk processing completely by-product-free.

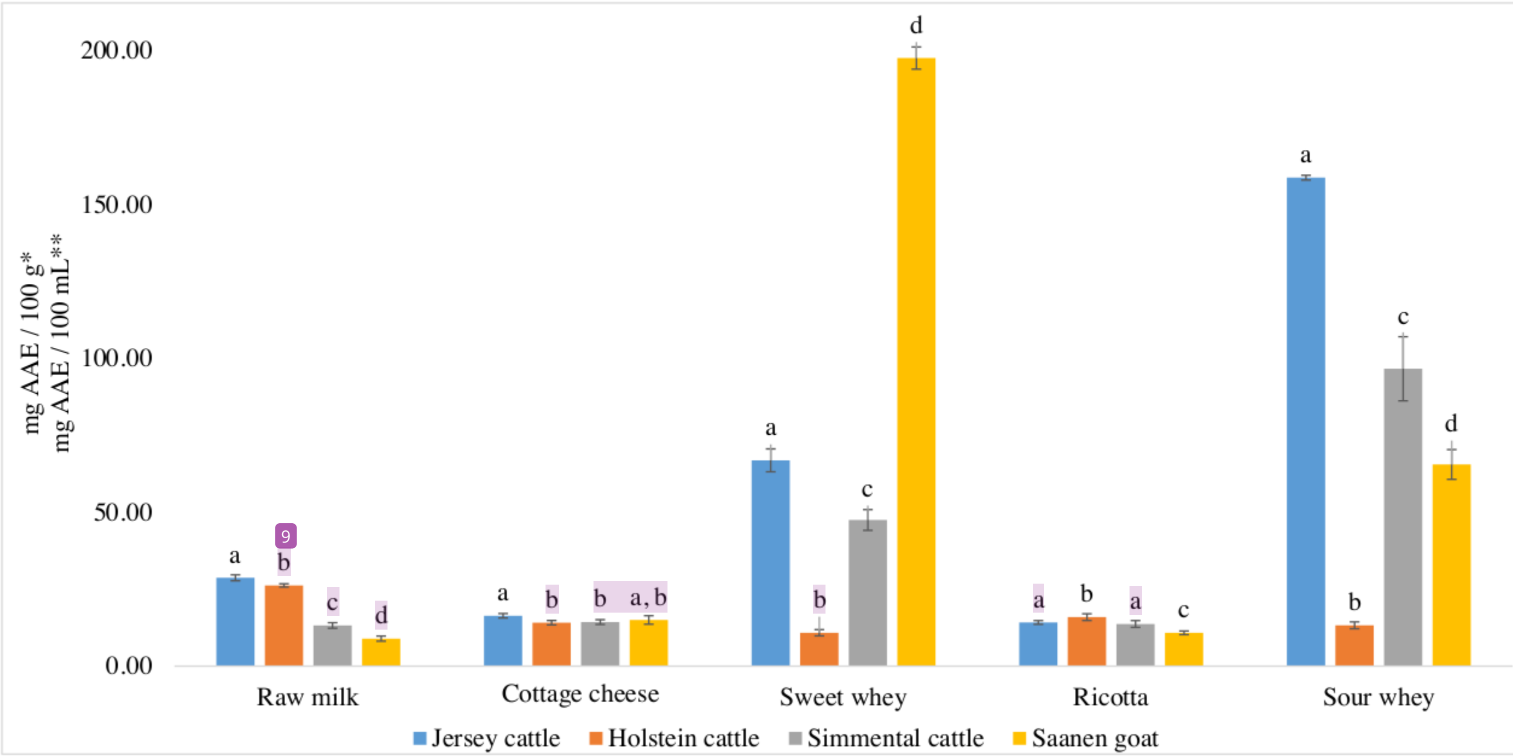
4. Conclusion

All in all, our study clearly supported that raw milk and dairy products are excellent sources of antioxidants and polyphenols. The total polyphenol content in different kinds of raw milk ranged from 420.34 to 490.72 mg GAE/ 100mL, while the total antioxidant content changed between 8.95 and 28.72 mg AAE/ 100mL. The measured values in the case of different kinds of cottage cheese samples were 32.29-124.29 mg GAE/ 100mL for polyphenols and 14.12-16.38 mg AAE/ 100g for antioxidants. There was a significant difference between the measured antioxidant and polyphenol content of sweet (10.85-197.55 mg AAE/ 100g antioxidant; 32.29-124.29 mg GAE/ 100g polyphenol) and sour whey (13.28-158.69 mg AAE/ 100g antioxidant; 43.50-98.03 mg GAE/ 100g polyphenol). Slight differences were observed between the ricotta cheeses made from different types of c ow milk. Specifically, antioxidant contents ranged from 10.84-15.93 mg AAE/ 100g, and polyphenol contents changed between 10.55-19.01 mg GAE/ 100g.

The production of cottage cheese and ricotta removes small amounts of antioxidant compounds from the liquid phases. Furthermore, significant differences were observed in the antioxidant and polyphenol contents of each product when comparing the tested dairy breeds and species. The health-protective effect of whey is highlighted, due to the large amount of free radical scavengers it contains. This study proves that the processing of dairy products, especially the whey produced as a by-product of the dairy industry, enables the production of dairy products that significantly contribute to preserving the health of consumers. The results also show that full processability is possible in the dairy industry, and all of the by-products have usable and valuable properties. The sour whey remaining after acid coagulation of the whey proteins can also be used to produce sweets, making milk processing loss-free.

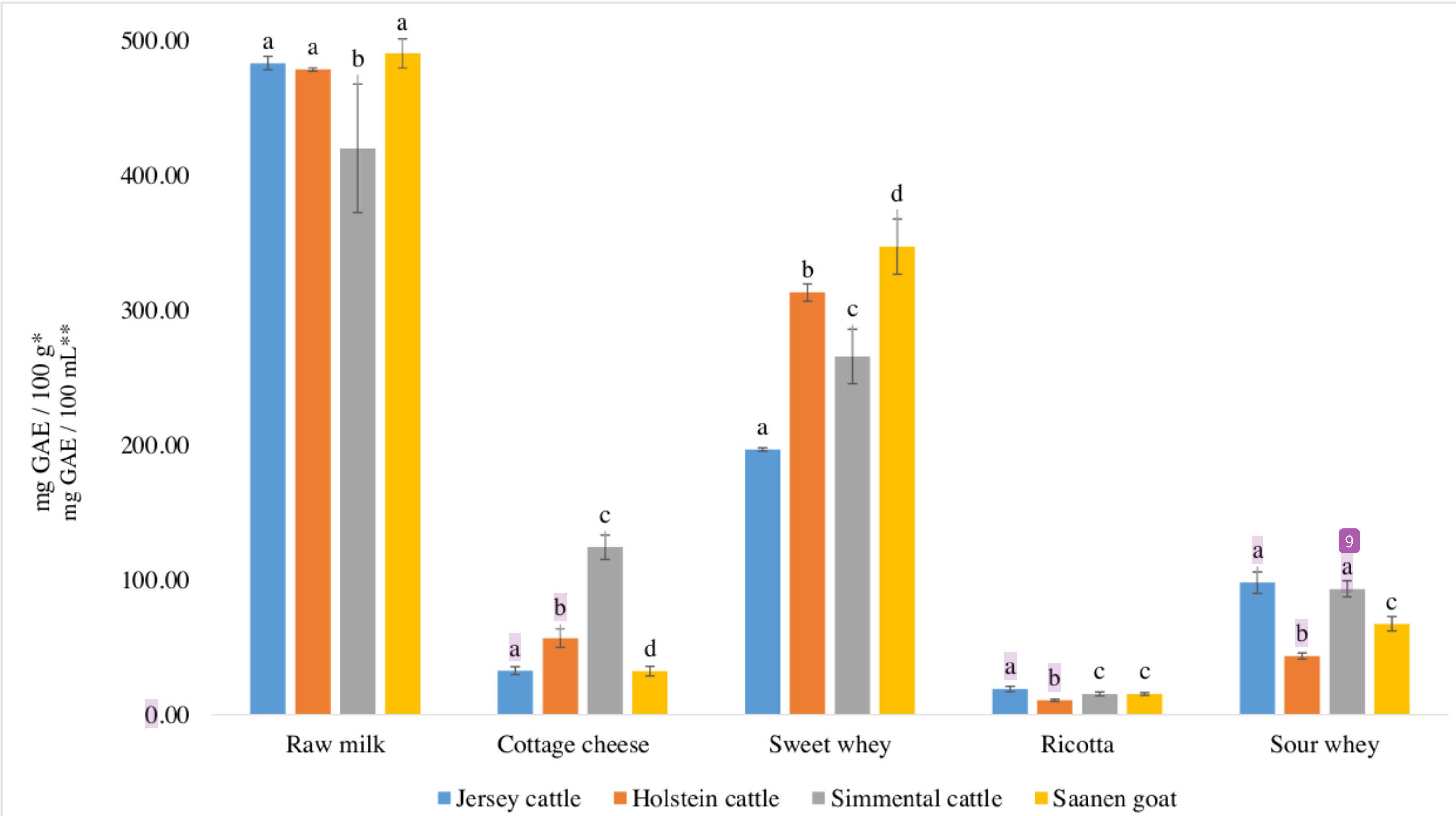
Table 1. Composition of milk and milk products of different dairy species

Breed	Product	Dry matter	Fat	Protein	Ash	Lactose
				g/100g		
Simmental cattle	Raw milk	12,79	4,00	3,40	0,72	4,67
	Cottage cheese	41,13	14,80	22,02	2,60	1,71
	Sweet whey	7,15	0,65	1,04	0,55	4,91
	Ricotta	43,15	31,50	10,06	0,57	1,02
	Sour whey	7,61	1,04	0,57	0,56	5,44
Jersey cattle	Raw milk	16,11	6,20	4,44	0,85	4,62
	Cottage cheese	46,44	26,50	16,84	1,93	1,17
	Sweet whey	7,79	0,83	1,29	0,59	5,08
	Ricotta	39,15	25,26	9,42	0,72	3,75
	Sour whey	8,78	1,37	1,29	0,63	5,49
Holstein Friesian cattle	Raw milk	12,23	3,25	3,44	0,70	4,84
	Cottage cheese	41,31	18,40	18,09	2,14	2,68
	Sweet whey	7,18	0,72	1,19	0,56	4,71
	Ricotta	29,03	13,20	11,42	0,55	3,86
	Sour whey	6,98	0,20	0,47	0,58	5,73
Saanen goat	Raw milk	13,21	4,30	3,61	0,79	4,51
	Cottage cheese	38,18	19,00	15,22	2,00	1,96
	Sweet whey	8,22	1,35	1,20	0,59	5,08
	Ricotta	28,99	16,00	8,00	0,64	4,35
	Sour whey	7,62	0,31	0,86	0,45	6,00



*: In the case of cottage cheese and ricotta. **: In the case of raw milk, sweet and sour whey.
Different letters (a, b, c, d) denote significant differences ($p \leq 0.05$)

Figure 1. Antioxidant content of different raw milk, milk products, and by-products



In the case of cottage cheese and ricotta. **: In the case of raw milk, sweet and sour whey.
Different letters (a, b, c, d) denote significant differences ($p \leq 0.05$)

Figure 2. Polyphenol content of different raw milk, milk products, and by-products

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