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Comparison of buffalo's, sheep's and goat's yoghurts in terms of their antioxidant activity, angiotensin-converting enzyme (ACE) inhibitory activity, volatile compound content and 5-hydroxymethylfurfural (HMF) content

MURAT EMRE TERZİOĞLU, İHSAN BAKIRCI

Department of Food Engineering, Faculty of Agriculture, Atatürk University, 25240, Erzurum, Turkey

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Terzioğlu M. E., Bakırcı İ.

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Summary

This study examined yoghurt samples produced using classical yoghurt culture from buffalo's, goat's and sheep's milks in terms of their physicochemical characteristics, DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity, angiotensin-converting enzyme (ACE) inhibitory activity, and contents of total phenolic compounds (TPC), volatile compounds and 5-hydroxymethylfurfural (HMF). It was determined that the use of different types of milk in yoghurt production had a statistically very significant effect ($p < 0.01$) on all physicochemical and biochemical properties analyzed, whereas the storage period had a very significant effect ($p < 0.01$) on pH and titratable acidity. Compared to other types of milk, sheep's milk was found to be superior in terms of antioxidant activity and ACE inhibitory activity, whereas buffalo's milk was found to be superior in its content of volatile compounds. The highest HMF formation was observed in yoghurt samples produced from goat's milk. It is therefore suggested that the consumption of yoghurts produced from different types of milk should be expanded because of their nutritional value and bioactivity.

Keywords: antioxidant activity, ACE inhibitory activity, volatile compounds, HMF

In recent years, it has been increasingly popular to take advantage of antioxidant, antihypertensive, antimicrobial and anticancer properties of food proteins. The fact that food proteins have these activities is due to bioactive peptides they contain. Bioactive peptides are formed during enzymatic hydrolysis, digestion or fermentation (8). Among these activities, antihypertensive activity plays an important role in the prevention of cardiovascular diseases, which are currently the main cause of death. Bioactive peptides in foods also have ACE inhibitory activity, which is the mechanism of action of drugs used in the treatment of hypertension (10, 24). Antioxidant activity, on the other hand, has a key role in preventing oxidative damage caused by free radicals in the body. It is known that free radicals are involved in the development of many diseases, especially cancers. The consumption of foods with antioxidant properties is a primary method of preventing oxidation reactions and oxidative damage (19). In this context, milk and dairy products, which are rich in bioactive peptides, come to the fore both in the treatment of hypertension and as a source of antioxidants (9, 24). On the other hand, HMF, a compound belonging to the group of furfurals which is formed as a result of the Maillard reaction during storage or heat treatment of milk, has been proven to produce tumoral, genotoxic and cytotoxic effects. Therefore, HMF formed in the advanced stages of the Maillard reaction in milk and dairy products is regarded as an important quality parameter (13, 23).

Although cow's milk is the first one that comes to mind when we think about milk and dairy products, it is known that buffalo's, goat's and sheep's milks are richer in nutrients (3). It is therefore desirable that these milk types, which are consumed less often, are transformed into dairy products so that their consumption becomes widespread and consumers can benefit from their rich nutritional contents (9, 20). To the best

of our knowledge, there has been no study in which buffalo's, goat's and sheep's milks were used simultaneously as raw materials in yoghurt production, and the parameters we examined have not hitherto been evaluated comparatively. Our research is original in this respect. In this study, experimental yoghurts were produced from three types of milk and stored at 4°C for 14 days, and the analyses detailed in the Material and methods section were performed at different storage times.

Material and methods

Material. Buffalo's, goat's and sheep's milks used as raw material in the present study were obtained from local farms in Erzurum/Turkey, whereas cultures of *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Streptococcus salivarius* subsp. *Thermophilus* were obtained from Süt-Sa Süt Sanayii İht Malz Tic, distributor of CHR Hansen in Adapazarı/Turkey. The starter culture used in this research was a lyophilized (YC-350) culture (Direct Vat Set type).

Experimental yoghurt production. To obtain the experimental yoghurt samples, buffalo's, goat's and sheep's milks were pre-treated and then heat treated in stainless steel containers at 90°C for 10-15 minutes. Then, the milks were cooled to 43-45°C and left for incubation (for approximately 3-4 h) in sterile glass jars after culture addition. The incubation was terminated after the pH value, measured with a pH meter (Mettler Toledo), reached 4.6 ± 0.1, and the yoghurt samples were stored at 4°C ± 1.

Physicochemical analyses of raw milk. In the present study, physicochemical analyses (total solids (%), fat (%), protein (%), ash (%), pH (pH meter/Mettler Toledo 420, MA 02129, USA) and titratable acidity (lactic acid%)) of different types of milk used as raw material were carried out according to Kavaz (11).

Physicochemical analyses of yoghurt samples. Physicochemical analyses of the experimental yoghurt samples produced from buffalo's, goat's and sheep's milks, comprising measurements of total solids (%), fat (%), protein (%), ash (%), pH (pH meter/Mettler Toledo 420, MA 02129, USA) and titratable acidity (lactic acid%), were performed by methods described by Kavaz (11).

Determination of DPPH free radical scavenging activity. To measure DPPH free radical scavenging activity, which is used to determine antioxidant activity, 5 mL of methanolic DPPH solution (0.004%) was added to 50 µL of the yoghurt sample and mixed. After the mixture had been kept at room temperature for 30 minutes, the absorbance was measured on a spectrophotometer (Optizen POP) at a wavelength of 517 nm. Trolox was used as a reference for the determination of DPPH free radical scavenging activity, and the results were calculated as mg TE/100 g (2).

Determination of total phenolic compound amount. In order to determine the total amount of phenolic compounds, 0.1 mL of the yoghurt sample, 1 mL of Folin-Ciocalteu reagent, and 46 mL of water were mixed, 3 mL of Na_2CO_3 (2%) was added to the mixture, and the mixture was left for 2 hours. The same procedures were applied to gallic acid solution, and the absorbances of all samples were measured on a spectrophotometer (Optizen POP) at a wavelength of 760 nm (2).

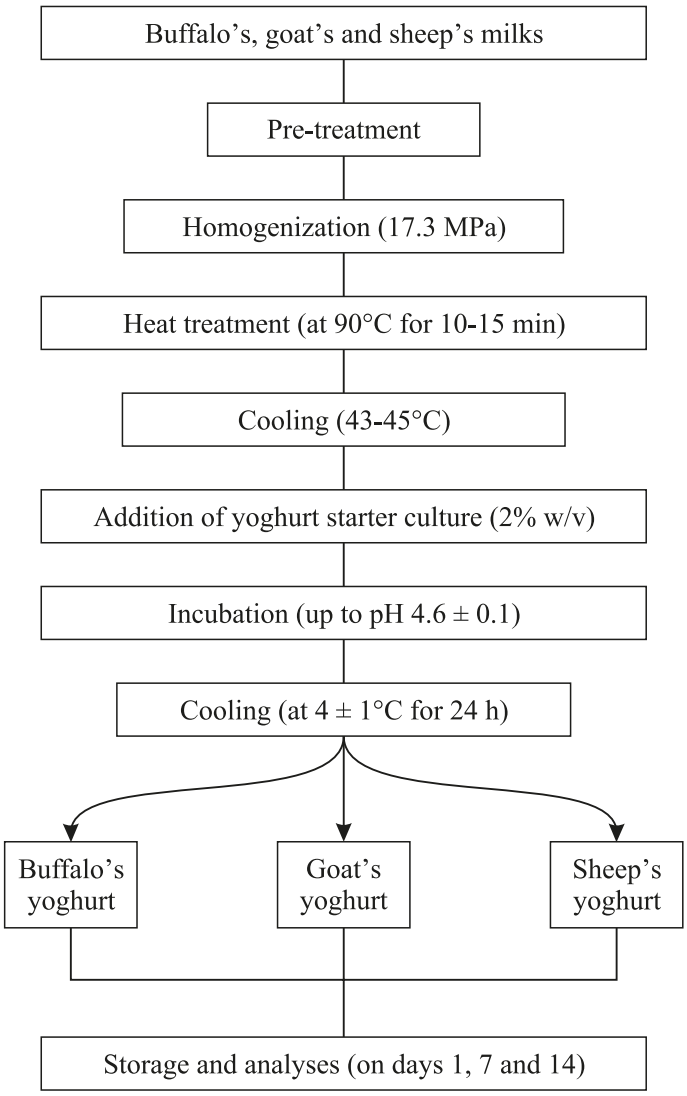

Fig. 1. Flow chart of the production of the experimental yoghurt samples

Determination of ACE inhibitory activity. The ACE inhibitory activities of yoghurt samples were determined according to Cushman and Cheung (5) and Nakamura et al. (16) with some modifications. For this purpose, 180 µL of HHL solution and 20 µL of ACE solution were added to 50 µL of the yoghurt sample, the mixture was kept at 37°C for 90 minutes, and then 250 µL of 1 M HCl was added. The extraction was performed by adding 1.7 mL of ethyl acetate to the mixture obtained, and the evaporation process was applied at 100°C for 15 minutes. Then 1 mL of distilled water was added to the hippuric acid solution obtained, and absorbance was measured on a UV spectrophotometer (Optizen POP) at a wavelength of 228 nm.

Determination of volatile compound content. GC-MS (Shimadzu, GC-MS-QP2010) and DB-WAX Ultra Inert (Agilent, 30 m, 0.25 mm, 0.25 µm) columns were used to determine the amount of volatile compounds in the experimental yoghurt samples produced from different milk types, and the analysis was carried out in the headspace injection mode. To collect the volatile compounds, 5 g of the yoghurt

sample weighed into the headspace vial was shaken for 30 minutes and then incubated at 80°C (11).

Determination of HMF content. The HMF content of the experimental yoghurt samples produced from different milk types was carried out according to Urguet al. (22) with some modifications. For this purpose, after adding 5 mL of oxalic acid to the yoghurt sample, it was mixed and kept in a water bath for 1 hour. After the mixture had been cooled to the room temperature and filtered, 1 mL of thiobarbituric acid solution (0.05 M) was added to 4 mL of the filtrate and placed in a water bath again. Then, absorbance was measured on a spectrophotometer (Optizen POP) at a wavelength of 443 nm.

Statistical analysis. The analysis of variance (ANOVA) of the yoghurt samples produced from buffalo's, goat's and sheep's milk was performed using the SPSS 20 software package (SPSS Inc., Chicago, IL) according to the randomized blocks experimental design. The experiment was carried out in 2 replications. Differences between samples (buffalo's, goat's and sheep's yoghurts) and storage periods (days 1, 7 and 14) were analysed by Duncan's Multiple Range Test.

Results and discussion

Results of the physicochemical analysis of raw milk. The results of the physicochemical analysis the raw buffalo's, goat's, and sheep's milks used in the experimental yoghurt production are given in Table 1.

Although the animal type, breed, feeding and milking method affect the composition of milk, it was determined that the composition of different milk types was compatible with the literature data. Erkaya and Şengül (7) and Boukria et al. (3) reported that buffalo's, goat's and sheep's milks had total solid contents of 16.56%, 14.14% and 17.40%, fat contents of 7.40%, 4.70% and 5.90%, protein contents of 4.60%, 3.83% and 6.21%, and ash contents of 0.77%, 0.78%, 0.91%, respectively.

Results of the physicochemical analysis of yoghurt samples. The results of the physicochemical analysis of the experimental buffalo's, goat's and sheep's yoghurt samples are given in Table 2.

It was determined that the use of different types of milk in the experimental yoghurt production had a statistically very significant effect ($p < 0.01$) on the results of all physicochemical analyses. The storage period, on the other hand, had a statistically very significant effect ($p < 0.01$) on pH and titratable acidity values, and a statistically significant effect ($p < 0.05$) on the protein content. The sheep's yoghurt samples had the highest contents of total solids, fat, protein and ash. As a matter of fact, it was also determined that the sheep's milk used in yoghurt production was richer in total solids, fat, protein and ash than other milk types. It was observed that the pH value decreased, and the titratable acidity value increased during the storage period, as expected. Erkayaand Şengül (7) reported that the total solids, protein and ash content of sheep's yoghurt was higher than that of buffalo's yoghurt or goat's yoghurt. In addition, the results of Boukria et al. (3) and Shalabi (18) on the composition of buffalo's, goat's and sheep's yoghurts are in line with the results of our study.

DPPH free radical scavenging activity and the amount of TPC. It is known that components of milk, such as α-tocopherols, conjugated linoleic acid, β-carotene, vitamins (A and D_3), phospholipids and mineral substances, contribute to its antioxidant activity. In addition, antioxidant peptides formed during fermentation in milk and dairy products have a significant effect on antioxidant activity (19). The results concerning DPPH free radical scavenging activity and the amount

Tab. 1. Physicochemical analysis of the buffalo's, goat's and sheep's milk

Milk type	Total solids (%)	Fat (%)	Protein (%)	Ash (%)	pH	Titratable acidity (Lactic acid%)
Buffalo's milk	17.72 ± 0.10	7.20 ± 0.12	5.46 ± 0.03	0.85 ± 0.04	6.63 ± 0.01	0.20 ± 0.01
Goat's milk	12.99 ± 0.14	4.22 ± 0.11	3.73 ± 0.06	0.83 ± 0.04	6.68 ± 0.01	0.19 ± 0.01
Sheep's milk	18.57 ± 0.09	7.62 ± 0.13	5.73 ± 0.06	0.95 ± 0.01	6.65 ± 0.01	0.20 ± 0.00

Tab. 2. Physicochemical analysis of the experimental yoghurts

		Total solids (%)	Fat (%)	Protein (%)	Ash (%)	pH	Titratable acidity (Lactic acid %)
Experimental yoghurt samples	BY	18.39 ± 0.13^{b}	7.51 ± 0.02^{b}	5.67 ± 0.02^{b}	0.92 ± 0.01^{b}	4.36 ± 0.17^{ab}	0.97 ± 0.12^{b}
	GY	15.55 ± 0.09^{c}	4.43 ± 0.03^{c}	5.10 ± 0.06^{c}	0.93 ± 0.02^{b}	4.42 ± 0.18^{a}	0.93 ± 0.12^{b}
	SY	19.33 ± 0.10^{a}	7.78 ± 0.05^{a}	6.00 ± 0.06^{a}	1.03 ± 0.02^{a}	4.34 ± 0.20^{b}	1.01 ± 0.14^{a}
	Sig.	**	**	**	**	**	**
Storage period	Day 1	17.74 ± 1.79a	6.58 ± 1.68^{a}	5.56 ± 0.39^{b}	0.96 ± 0.06^{a}	4.58 ± 0.07^{a}	0.84 ± 0.03^{c}
	Day 7	17.79 ± 1.72a	6.58 ± 1.67^{a}	5.59 ± 0.45^{ab}	0.97 ± 0.07^{a}	4.35 ± 0.05^{b}	0.95 ± 0.06^{b}
	Day 14	17.74 ± 1.79a	6.56 ± 1.65^{a}	5.62 ± 0.38^{a}	0.95 ± 0.05^{a}	4.18 ± 0.06^{c}	1.12 ± 0.04^{a}
	Sig.	Ns	ns	*	ns	**	**

Explanations: a-c – Different letters indicate significant differences in columns; BY – buffalo's yoghurt; GY – goat's yoghurt; SY – sheep's yoghurt; ** – $p < 0.01$; * – $p < 0.05$; ns – $p > 0.05$

Tab. 3. Biochemical analysis of the experimental yoghurts

Experimental yoghurt samples	DPPH (mg TE/100 g)	TPC (mg GAE/100 g)	ACE inhibitory activity (%)	Acetaldehyde (µg/mL)	Diacetyl (µg/mL)	Acetoin (µg/mL)	HMF content (µmol/L)
BY	7.06 ± 0.04^{c}	5.98 ± 0.01^{c}	28.82 ± 0.04^{c}	28.64 ± 0.02^{a}	1.37 ± 0.01^{c}	43.17 ± 0.03^{a}	16.33 ± 0.16^{c}
GY	8.18 ± 0.05^{b}	6.61 ± 0.05^{b}	34.69 ± 0.04^{b}	8.26 ± 0.01^{c}	1.56 ± 0.03^{a}	36.87 ± 0.11^{b}	21.59 ± 0.11^{a}
SY	9.34 ± 0.02^{a}	8.24 ± 0.04^{a}	38.51 ± 0.08^{a}	9.36 ± 0.03^{b}	1.43 ± 0.01^{b}	36.86 ± 0.04^{b}	17.81 ± 0.12^{b}
Sig.	**	**	**	**	**	**	**

Explanations: a-c – different letters indicate significant differences in columns; BY – buffalo's yoghurt; GY – goat's yoghurt; SY – sheep's yoghurt; ** – $p < 0.01$

of TPC for the experimental buffalo's, goat's and sheep's yoghurt samples are given in Table 3.

It was determined that the use of different types of milk in the experimental yoghurt production had a statistically very significant effect ($p < 0.01$) on DPPH free radical scavenging activity and the amount of TPC. While the sheep's yoghurt samples had the highest DPPH free radical scavenging activity, the lowest value was found in the buffalo's yoghurt. It is thought that the difference in antioxidant activity in the yoghurts produced from different types of milk may be due to different amounts of proteins and degrees of protein hydrolysis (14). According to Tami et al. (21), two possible causative factors of this situation are polyphenols transferred from animal feed to milk and the interaction of a specific non-reactive Folin-Ciocalteau reagent with some food compounds.

ACE inhibitory activity. It has been reported that starter cultures used in yoghurt production produce peptides with ACE inhibitory activity through proteolytic activity during fermentation. This also allows yoghurt to be used as a carrier of bioactive compounds to the body (1). ACE inhibitory activity results for the experimental buffalo's, goat's and sheep's yoghurt samples are given in Table 3. It was determined that the use of different types of milk in the experimental yoghurt production had a statistically very significant effect ($p < 0.01$) on ACE inhibitory activity. The sheep's yoghurt samples had the highest ACE inhibitory activity (38.51%), while the lowest value was found in the buffalo's yoghurt (28.82%). It has been noted that differences in ACE inhibitory activity in yoghurt samples may be due to many factors, such as the use of different analytical methods, differences in the amino acid profile of milk, the degree of proteolysis, fermentation conditions and storage conditions (15).

Volatile compounds. It has been reported that starter cultures in yoghurt produce volatile compounds in different ways by using milk components, such as protein, carbohydrate, fat and nucleic acid. The amount of volatile compounds depends on various factors, such as the type of milk, enzyme activities of starter cultures and the production method (12). The volatile compound (acetaldehyde, diacetyl, and acetoin) results for the experimental buffalo's, goat's and sheep's yoghurt samples are given in Table 3. The use of different types of milk in the experimental yoghurt production had a statistically very significant effect ($p < 0.01$) on acetaldehyde, diacetyl and acetoin contents. It was established that the amount of acetaldehyde was highest in the buffalo's yoghurt (28.64 µg/mL), followed by the sheep's yoghurt (9.36 µg/mL) and the goat's yoghurt (8.26 µg/mL); the diacetyl content was highest in the goat's yoghurt (1.56 µg/mL), followed by the sheep's yoghurt (1.43 µg/mL) mL) and the buffalo's yoghurt (1.37 µg/mL), whereas the acetoin content was highest in the buffalo's yoghurt (43.17 µg/mL), followed by the goat's yoghurt (36.87 µg/mL) and the sheep's yoghurt (36.86 µg/mL). Acetaldehyde, diacetyl and acetoin are produced in the process of fermentation by *S. thermophilus* and *L. bulgaricus* using precursors, such as lactose, threonine, methionine and pyruvate. The amount of these volatile compounds produced is affected by factors such as milk type, culture rate and production process (4, 27). The amounts of volatile compounds in yoghurts reported by the literature are similar (17).

HMF content. It is known that the heat treatment of milk before it is processed into products results in the formation of certain harmful compounds, such as HMF, due to high temperature and pressure. HMF can be produced by the Maillard reaction or by heating hexoses under acidic conditions (6, 26). The HMF results for the experimental buffalo's, goat's and sheep's yoghurt samples are given in Table 3. It was determined that the use of different types of milk in the experimental yoghurt production had a statistically very significant effect ($p < 0.01$) on the HMF content. The HMF content was highest in the goat's yoghurt (21.59 µmol/L), followed by the sheep's yoghurt (17.81 µmol/L) and the buffalo's yoghurt (16.33 µmol/L). It has been reported that heat treatment temperature, heat treatment time, milk composition and pH affect the HMF content of foods (25).

Various bioactive compounds produced by the proteolytic activity of starter cultures during fermentation make it possible to use yoghurt as a functional food or a nutraceutical. In the current research, it was determined that the use of different types of milk in yoghurt production resulted in different physicochemical properties, aroma and bioactivity due to composition differences. Among the yoghurt samples produced

from different types of milk, the sheep's yoghurt stood out in terms of its high DPPH free radical scavenging activity, TPC content, and ACE inhibitory activity. In terms of the characteristic volatile compounds found in yoghurt, the buffalo's yoghurt was superior to the others. On the other hand, the amount of HMF, which has various harmful effects, was highest in the goat's yoghurt.

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Corresponding author: Murat Emre Terzioğlu, Research Assistant, Atatürk University, Faculty of Agriculture, Department of Food Engineering, 25240, Erzurum, Turkey; e-mail: murat.terzioglu@atauni.edu.tr