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Effect of donkey milk lactoferrin and lysozyme on yoghurt properties

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H. Ceren Akal[1], Sebnem Ozturkoglu-Budak[1], Nilay Bereli[2], Duygu Cimen[2], Semra Akgonullu[2]*

[1]Ankara University, Faculty of Agriculture, Department of Dairy Technology, Turkey
[2]Hacettepe University, Faculty of Science, Department of Chemistry, Turkey

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*Corresponding author: budak@ankara.edu.tr

Abstract

Food industry has mostly focused on natural preservatives due to the undesirable effects of chemical additives on the human health. Among milk proteins, lactoferrin and lysozyme are the best-known for their antimicrobial. In this study, lactoferrin and lysozyme were extracted from donkey milk and applied on the yoghurt surface by spraying. The obtained yoghurt samples enriched with antimicrobial proteins were compared with the control sample produced without the addition of any preservatives as well as the samples treated with natamycin, a commercial preservative used in dairy products. Thereby physicochemical, microbiological and textural properties of the samples were investigated during the 30 days of storage. Yoghurt samples treated with antimicrobial agents had lower microbial load than control samples, which indicated that the donkey milk lactoferrin and lysozyme inhibit microbial activity in yoghurts. However, the addition of the mentioned preservatives did not change the gross composition and the textural properties of the yoghurt samples. Most importantly, the incorporation of lactoferrin or lysozyme did not adversely affect the sensory properties of yoghurt samples, but achieved higher appreciation points than the control sample on the 30th day of storage. In brief, lactoferrin and lysozyme extracted from donkey milk could be used to control the undesirable microbial growth, hence extending the shelf life of yoghurt.

Key words: lysozyme; lactoferrin; yoghurt; donkey milk

Introduction

Recently, many studies have focused on donkey milk due to its similar composition to human milk (Altomonte et al., 2019). Additionally, unlike bovine milk, donkey milk proteins are non-allergenic (Cunsolo et al., 2017). Therefore, donkey milk and donkey milk proteins are important for human health. Studies demonstrated that donkey milk proteins/peptides have antidiabetic (Li et al., 2020), antioxidant (Akan, 2020), antimicrobial (Massouras et al., 2020; Spada et al., 2021), bioactive (Vincenzetti et al., 2017), antiviral (Brumini et al., 2013) properties.

Lactoferrin and lysozyme are the two important components which have antimicrobial properties. However, the antimicrobial action mechanism of both proteins are different from each other. Lactoferrin not only limits bacterial growth by binding iron, but also changes the permeability of bacterial cells by attaching to the lipopolysaccharides of bacterial cell walls (Brumini et al., 2016). The availability of lysozyme in higher amounts in donkey milk than other kinds of milk could catalyze the hydrolysis of the glycoside 1-4 bond of peptidoglycans in bacterial wall and chitin in fungi walls (Derdak et al., 2020).

Yoghurt is a fermented dairy product consumed worldwide because of its functional properties and beneficial health effects. It has a relatively short shelf-life compared to other dairy products such as cheese or butter. Yoghurt spoilage is mainly caused by microbial contamination with yeasts and molds. (Mataragas et al., 2011). Several methods are applied to prevent microbial spoilage of yoghurt such as the use of commercial bioprotectant agents (Serna-Jimenez et al., 2020) or chemical additives (Ribes et al., 2018). Recently, the increasing interest of consumers in natural products has led to a focus on preserving foods via natural methods. Therefore, biopreservatives (Buehler et al., 2018), essential oils (Milanovic et al., 2021) or fruits which have antimicrobial components (Mataragas et al., 2011) could be added to yoghurt to control the microbial spoilage.

The aim of this study is to examine the effects of applying lactoferrin and lysozyme derived from donkey milk to prevent microbial contamination of yoghurt. Natamycin was also used individually in another batch of yoghurt production in order to compare its effect with commercial preservatives. That way, the effects of the used additives on the chemical, textural, and sensory properties as well as the microbial composition of yoghurt were determined.

Materials and methods

Material

Donkey milk was provided by the Korukoy Donkey Farm (Kirklareli, Turkey) while cow milk was obtained from the Dairy Factory of Ankara University (Ankara, Turkey). Commercial natamycin was obtained from Delvocid (İstanbul, Turkey). Starter culture including *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus,* was acquired from Chr Hansen (Hoersholm, Denmark). Each analytical standard was obtained from Sigma-Aldrich (St. Louis, MO, USA).

Extraction and purification of lysozyme and lactoferrin

Extraction of antimicrobial substances were performed by FPLC according to Billakanti et al. (2010) and chromatographic separation was applied for purification as stated in Ozturkoglu-Budak et al. (2021). By these procedures, spraying solutions of lysozyme and lactoferrin were obtained and diluted to 0.25% with distilled water for yogurt applications.

Yoghurt production

The raw cow milk (with 13.2 %±1.14 total solid, 3.45 %±0.05 fat, 3.32 %±0.16 protein and 6.82±0.01 pH value) was homogenized by Ultraturrax at 10000 rpm for 3 min. After homogenization, milk was pasteurized at 90 °C for 5 min, then rapidly cooled down to 45 °C and inoculated with starter culture (*Lactobacillus bulgaricus*:*Streptococcus thermophilus*; 1:1) at a rate of 2 % (v/v). Afterwards, this bulk mixture was distributed into 200 mL cups and incubated at 43 °C until the pH value dropped to 4.6-4.7. At this point, samples were rapidly cooled to 4 °C and kept at refrigeration temperature for 24 h before treatments. The next day, all sample cups were divided equally for 4 different treatment groups which are control, treatment with lactoferrin, treatment with lysozyme and treatment with natamycin. Except control samples, each cup belongs to other three groups was treated by spraying with the corresponding antimicrobial agents, individually. The spraying process was done on a surface area of 10 cm^2 with 1.5 mL of each type of liquid antimicrobial solution with 0.25 % concentration, which was determined by preliminary experiments. All yoghurt samples were stored at 4 °C until the analyses performed on days 1, 15 and 30.

Sample codings were as follows:
Y-C: Control sample without antimicrobial agents
Y-La: Yoghurt sample with lactoferrin
Y-Li: Yoghurt sample with lysozyme
Y-N: Yoghurt sample with natamycin

Physicochemical analyses

The pH was obtained by a pH-meter (MP 225 Mettler Toledo, Columbus, OH, USA) and titration acidity (lactic acid %) was determined by Bradley et al. (1993) method. The dry matter content was determined by oven drying method at 100 °C (AOAC, 1997). The fat content was determined by the method of Gerber-Van Gulik method (AOAC, 1997).

The total nitrogen (TN) contents were done by Kjeldahl method as given in IDF (1993). All measurements were carried out in technical duplicate.

Microbiological analyses

10 g of yoghurt sample were weighed and homogenised in a Stomacher (Bag Mixer 400 VW; Interscience, St Nom, France) with 90 mL Ringer solution for 2 min. Ten-fold dilutions were plated on the Plate Count Agar (PCA) with 1 % skimmed milk and incubated at 35 °C for 48 h for total aerobic mesophilic bacteria (TAMB) count. Yeast and mold count were determined on the Malt Extract Agar (MEA) acidified with 10% tartaric acid, following the incubation period at 28 °C for 7 days.

Physical properties

Water holding capacity (WHC) of the yoghurt samples were measured by centrifugation (Sigma 3-18K, Germany) (Isanga Zhang, 2009), as 25 g yoghurt were weighed and then centrifuged at 4500 x *g* for 15 min at 4 °C. Finally, the water holding capacity was calculated by following the equation below:

WHC (%) = $(1-W_1/W_2) \times 100$ (1)

where WHC: water holding capacity, W_1: weight of whey after centrifugation, W_2: weight of yoghurt.

Textural properties of yoghurt samples were determined using a TA.XT Plus Texture analyzer (Stable Micro Systems, Surrey, UK) according to the parameters of do Espírito Santo et al. (2012) and Brennan and Tudorica (2008) with some modifications. A 40 mm back-extrusion rig (A/BE-d40) and a 30 kg force load cell were used at compression test mode. Probe was moved at a pre-test speed of 5 mm/s and test speed was 1 mm/s through 25 mm within the yoghurt sample in the 70 mm sample container. End of the analysis firmness (maximum positive force), consistency (area of positive region), cohesiveness (maximum negative force), and index of viscosity (area of negative region) values were considered.

Determination of antimicrobial agent content in yoghurt

Lysozyme and lactoferrin content

Lysozyme and lactoferrin content in yoghurt samples were chromatographically determined (Billakanti et al., 2010). The same procedure was applied for the quantification of the both antimicrobial proteins. 10 g of the sample were heated to 45 °C for 20 min and acidified to pH 4.6 with 1 M HCl and then centrifuged at 18500 x g (Sigma K 3-18 Centrifuge, Sartorius AG, Gottingen, Germany, UK) for 20 min to remove caseins. Supernatant was seperated and adjusted to the pH 7.0 with 1 N NaOH. The solution was filtered through a 0.22-µm cellulose-acetate filter (Milex, Millipore Millex, Bedford, MA, USA) and 50 µL of the sample was injected into a high pressure liquid chromatography (HPLC) system (1100 series; Agilent Technology, Santa Clara, California, USA) with a flow rate of 0.8 mL/min. HPLC was equipped with a UV-Detector at 214 nm and a C18 column (4.6 cm x 250 mm x 5 µm) (ACE, Advanced Chromatography Technologies Ltd, UK) at 40 °C. Gradient elution was applied with mobile phases of (A) deionised water with 0.1% TFA, (B), deionised water with 95 % acetonitrile and 0.1 % TFA. The program was as below:

First 5 min: 100 % solvent A, 15 min: 50 % A and 50 % B, 5 min: 40 % A and 60 % B and running for 10 min with 60 % B.

The lysozyme standard solutions from the chicken egg white and lactoferrin from human milk were used to prepare standard curves. Curves were created at the concentrations of 10, 25, 50, 75, 150 µg/mL in NaCl solutions. Peak areas from HPLC analyses were used to quantify the lysozyme and lactoferrin concentrations.

Natamycin content

Natamycin was analysed according to the method of IDF (2007) and Alkaya and Karalomlu (2016). 5 g of the yoghurt sample were mixed with 50 mL MeOH and the mixture was stirred for 90 min. The flasks were put in a freezer for 1 h before the filtration of the solution inside through glass wool subsequently passed through a 0.22 µm PTFE membrane filter. Finally 40 µL sample were injected into the RP-HPLC system equipped with UV detector. Measurements were performed at 303 nm, using a C18 column (4.6 cm x 250 mm x 5 µm) (ACE, Advanced Chromatography Technologies Ltd, UK). Mobile phase was Methanol:Water:Acetonitrile (60:40:5) at a flow rate of 1.0 mL/min and a column temperature of 20 °C. Quantification was done by external calibration using natamycin solutions at 2, 5, 10, 20, and 50 µg/mL concentrations. Natamycin content of the samples were calculated by the equation given below:

Cs (mg/kg) = (V/mt) x Cm (2)
Where Cs: Natamycin content of sample (mg/kg),
V: Total volume of sample (mL),
mt: Sample content (g),
Cm: Measured sample concentration.

Sensory analysis

The yoghurt samples were evaluated by 7 panelists trained in yoghurt aroma and taste (Ankara University, Department of Dairy Technology academic staff) using the method described by Clark and Costello (2016) on the 1st, 15th and 30th day of storage. Each panelist evaluated the products on three sensory criteria: colour-appearance; body & texture; and taste & flavour.

Statistical analysis

Statistical analyses were performed by MINITAB program (version Minitab ®16.1.1, Minitab Inc., State College, PA, USA). Analysis of variance (ANOVA) was

carried out to identify statistical differences among treatments. Finally, Tukey's Multiple Range Test was applied for the determination of statistical significant differences (P<0.05).

Results and discussion

Physicochemical properties of yoghurt

Physicochemical properties of yoghurt samples treated with lactoferrin, lysozyme and natamycin are shown in Table 1.

There were no differences among the total solid content of the yoghurt samples (P>0.05). In addition, the total solid content of samples among the storage days were close to each other (P>0.05). There were no differences between fat and protein contents of samples as well as the total solid values during storage (P>0.05). The similarity of gross composition parameters of all yoghurt samples with or without antimicrobial agents demonstrated that the addition of donkey milk protein or natamycin did not affect these values. The main reason for this result could be little amount of agents applied to the yoghurt.

The pH values of yoghurt samples were similar (P>0.05) but there was a variation during storage (P<0.05). The pH values of the samples slightly decreased until day 15 (P>0.05), then sharply increased on day 30 (P<0.05). Depending on the run out of carbohydrate source, lactic acid bacteria begins to breakdown proteins and can produce some metabolites having alkali properties (Costa et al., 2015a). These metabolites can increase the pH value of yoghurt during the long term storage (Costa et al., 2015b). In our study, this may be the reason for the increase in the pH value of yoghurts during the 30-day storage period.

On the other hand, according to results presented in Table 1 lactic acid content was similar during storage (P>0.05) in all samples, but significant difference (P<0.05). was determined between the control sample (Y-C) and sample treated with Lysozime (Y-Li). The lactic acid content of the control sample remained at the highest levels among all samples during the storage period. Lactic acid values of all the other samples were similar to each other (P>0.05). However, the control sample had the lowest lactic acid value on the 1st day of the storage and it reached to the highest acidity level at the end of the storage. The acidity of the fermented products increased during the storage depending on the production of lactic acid from lactose via bacterial activity (Gaspar et al., 2013). It is noted that there is a greater microbial activity in samples having higher acidity. However, our results showed that both, the antimicrobial proteins and natamycin reduced the bacterial growth. The yoghurt samples treated with lysozyme had the lowest average lactic acid values among all the storage days.

Table 1. Physicochemical properties of yoghurt samples

		Storage days		
	Samples	**Day 1**	**Day 15**	**Day 30**
Total solid (%)	Y-C	13.11±0.27	12.63±0.07	13.19±0.16
	Y-La	12.80±0.06	13.20±0.19	12.70±0.09
	Y-Li	12.72±0.25	12.60±0.16	12.86±0.08
	Y-N	12.78±0.08	13.02±0.08	13.10±0.15
Fat (%)	Y-C	3.00±0.20	3.10±0.30	2.90±0.10
	Y-La	2.90±0.10	3.30±0.30	2.80±0.00
	Y-Li	2.80±0.00	3.00±0.20	2.90±0.10
	Y-N	2.90±0.10	3.20±0.20	2.90±0.10
Protein (%)	Y-C	4.64±0.175	4.33±0.057	4.06±0.019
	Y-La	4.05±0.089	4.26±0.112	4.36±0.067
	Y-Li	4.40±0.063	4.31±0.039	4.25±0.096
	Y-N	4.31±0.067	4.32±0.065	4.06±0.118
pH value	Y-C	$3.94±0.05^{b}$	$3.82±0.04^{b}$	$4.33±0.20^{a}$
	Y-La	3.97±0.01	3.95±0.07	4.33±0.17
	Y-Li	4.03±0.02	4.02±0.11	4.35±0.18
	Y-N	3.99±0.03	4.10±0.08	4.30±0.25
Lactic acid (%)	Y-C	$0.80±0.02^{A}$	0.85±0.01	0.84±0.02
	Y-La	$0.84±0.01^{AB}$	0.80±0.02	0.73±0.00
	Y-Li	$0.81±0.04^{B}$	0.76±0.01	0.75±0.03
	Y-N	$0.84±0.03^{AB}$	0.79±0.00	0.80±0.03

Y-C: Control Yoghurt, Y-La: Yoghurt treated with lactoferrin, Y-Li: Yoghurt treated with lysozyme, Y-N: Yoghurt treated with natamycin. The different lower case letters in the same row indicate the significant differences during storage period (P<0.05). The different uppercase letters in the same column indicate significant differences among the samples (P<0.05).

Microbiological analysis

Total aerobic mesophilic bacteria (TAMB) and yeast-mold counts for each yoghurt sample during the storage period is presented in the Table 2, respectively.

Table 2 shows that the control sample had the highest total mesophilic aerobic bacterial count in all storage days.

Table 2. Total aerobic mesophilic bacteria and yeast-mold counts of yoghurt samples

		Storage days		
	Samples	**Day 1**	**Day 15**	**Day 30**
TAMB count (log CFU/g)	Y-C	$2.54±0.22^{A}$	1.15±0.20	3.65±0.35
	Y-La	$1.07±0.09^{AB}$	0.00±0.00	1.15±0.08
	Y-Li	$0.00±0.00^{B}$	0.00±0.00	1.50±0.06
	Y-N	$2.10±0.20^{B}$	0.85±0.02	0.00±0.00
Yeast and mold counts (log CFU/g)	Y-C	$0.00±0.00^{Ab}$	$1.24±0.08^{b}$	$4.85±0.15^{a}$
	Y-La	$0.00±0.00^{B}$	0.00±0.00	0.00±0.00
	Y-Li	$0.00±0.00^{B}$	0.00±0.00	0.00±0.00
	Y-N	$0.65±0.05^{AB}$	0.00±0.00	1.00±0.01

Y-C: Control Yoghurt, Y-La: Yoghurt treated with lactoferrin, Y-Li: Yoghurt treated with lysozyme, Y-N: Yoghurt treated with natamycin. The different lowercase letters in the same row indicate significant differences during storage period (P<0.05). The different uppercase letters in the same column indicate significant differences among the samples (P<0.05)

A slight variation was observed during storage (P>0.05), but the TAMB counts of the samples differed from each other (P<0.05). Natamycin and lysozyme treated yoghurt samples had significantly lower bacterial load than the control samples (P<0.05), similar to lactoferrin treated samples having lower TAMB count. However, the difference was insignificant (P>0.05). The antimicrobial effect of lactoferrin is based on iron binding principle for both bacteria and yeast-molds (Steijns and van Hooijdonk, 2000).

Natamycin is known primarily as an effective antifungal agent towards yeasts and molds. However, some more recent studies have shown that natamycin also has an antimicrobial effect on some bacteria (Shah et al., 2020) such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Serafini et al., 2020). Accordingly, natamycin treated yoghurt samples in our study have an inhibitive effect on TAMB. Lysozyme has also antimicrobial effect on bacteria especially on the gram positives and a large spectrum of pathogens (Cosentino et al., 2016; Silvetti et al., 2017; Zhang et al., 2008). The action mechanism of lysozyme is based on splitting the ß-1,4-glycosidic linkages between N-acetylglucosamine and N-acetylmuramic acid in the peptidoglycan layer of bacterial cell wall (Arabski et al., 2015). Since lysozyme affects the peptidoglycan structure, it is more effective on the gram positive bacteria. Besides, the inhibition effect of lysozyme on yeast and mold could be due to the chitinase activity of lysozyme. The cell wall of yeast and molds contain chitin, which has a similar structure to bacterial peptidoglycan. Lysozyme shows antimicrobial effect by binding chitin that is an important component of cell walls in yeast and molds (Benkerroum, 2008).

The control sample had the highest yeast and mold count, similar to that of the TAMB count. As expected, the number of yeast and mold increased during storage (P<0.05). The application of controlled conditions during production led to no yeast and mold detection in the samples on day 1, except for Y-N in which 0.65 log CFU/g yeast and mold number was obtained. It could also remarkably observed that all the applied antimicrobials prevented the growth of yeasts and molds. Higher counts of yeasts and molds were detected only in the control sample at the end of storage.

Physical properties of yoghurt samples

The back-extrusion method measures firmness which is the necessary force to attain a given deformation and consistency, a force needed to attain a given deformation during the compression cycle of the plunger and cohesiveness, a force required to overcome the attractive forces between the surface of the sample, the probe, and the index of viscosity hence a necessary force to pull the plunger up through the sample (De Vuyst et al., 2003). These physical properties of yoghurt samples during storage are given in Table 3.

The WHC shows the physical stability of yoghurt and usually increases during storage. The WHC indicates the ability of proteins to retain water in the structure of yoghurt (Wu et al., 2001). The was no difference obtained

Table 3. Physical properties of yoghurt samples

		Storage Days		
	Samples	**Day 1**	**Day 15**	**Day 30**
Water holding capacity (%)	Y-C	39.19±3.08	39.35±0.13	41.80±1.64
	Y-La	39.25±1.88	40.08±1.22	43.00±1.31
	Y-Li	38.82±2.47	39.62±0.36	42.20±1.64
	Y-N	38.98±2.61	41.07±0.63	43.00±1.60
Firmness (g)	Y-C	333.82±23.90	384.24±6.37	397.15±5.62
	Y-La	373.32±27.70	390.24±8.55	388.35±11.95
	Y-Li	360.72±5.180	400.69±5.32	394.64±16.91
	Y-N	328.20±12.82	379.98±7.08	413.25±5.180
Consistency (g.s)	Y-C	8214.4 ± 293.20^{DE}	9199.1 ± 42.89^{BC}	9093.4 ± 166.54^{BC}
	Y-La	8061.9 ± 236.06^{E}	9408.5 ± 63.40^{BCD}	10074.3 ± 165.84^{A}
	Y-Li	8599.7 ± 209.09^{CDE}	9408.1 ± 57.53^{ABC}	9708.8 ± 158.36^{AB}
	Y-N	7769.5 ± 59.850^{E}	8978.4 ± 51.44^{BCD}	9702.0 ± 43.630^{AB}
Cohesiveness (-g)	Y-C	120.45±3.94	101.03±1.15	101.16±7.29
	Y-La	98.03±6.40	97.300±1.91	109.98±4.40
	Y-Li	97.48±9.07	110.39±2.93	110.46±1.95
	Y-N	110.0±5.25	103.52±7.89	111.53±4.76
Index of viscosity (-g.s)	Y-C	3.25±0.39	2.72±0.14	3.52±0.17
	Y-La	3.38±0.22	3.13±0.13	2.76±0.23
	Y-Li	2.51±0.46	4.93±0.54	2.71±0.45
	Y-N	4.20±0.32	3.92±0.94	2.80±0.82

Y-C: Control Yoghurt, Y-La: Yoghurt treated with lactoferrin, Y-Li: Yoghurt treated with lysozyme, Y-N: Yoghurt treated with natamycin. The differences in values with detected interactions are shown with capital letters (P < 0.05)

among the samples in terms of water holding capacity (P>0.05). This indicates that the addition of the donkey milk protein or natamycin had no effect on WHC of yoghurt samples. However, the storage period changed the water holding capacity. The WHC value on the 1st day of storage increased through the 30th day of storage (P<0.05).

Similar to these results, firmness values showed an increasing trend during storage and no significant difference was determined among samples (P>0.05). The firmness of yoghurts based on the strength of the three-dimensional protein network may have been altered by the total solids content, acidity and proteolytic activity of lactic acid bacteria (Lee and Lucey, 2010; Liu et al., 2014). The composition and acidity of all yoghurt samples in this study were similar. The firmness values were higher in whey protein added yoghurt samples (Bierzunska et al., 2019; Brodziak et al., 2020), but opposite to that, the addition of lactoferrin or lysozyme did not affect the textural parameters in this study probably due to the incorporation in little amounts.

The firmness values showed an increase during the storage period (P<0.05). The increase of firmness during storage is usually due to the shrinkage of the protein gel which occurs due to the pH change (Sah et al., 2016). Similarly, different studies on textural properties of yoghurt have also reported an increase in firmness values during storage (Gürbüz et al., 2021; SalvadorFiszman, 2004; Vieira et al., 2019). There was an interaction between samples and storage days in terms of consistency and the differences, which were presented in Table 3. Similar to firmness data, consistency values of all yoghurt samples also increased during the storage period.

As a result of the textural profiling, similar results were observed among yogurt samples in terms of values related to the negative region (below zero on the vertical axis) of the graphics (cohesiveness and index of viscosity) (P>0.05) and storage did not affect these values (P>0.05).

Table 4. Lactoferrin, lysozyme and natamycin content of yoghurt samples (μg/mL)

		Storage Days		
	Samples	Day 1	Day 15	Day 30
Lactoferrin	Y-La	22.76±1.02^{c}	43.29±0.24^{b}	60.15±3.83^{a}
Lysozyme	Y-Li	14.66±0.68^{b}	21.22±0.69^{a}	24.61±1.09^{a}
Natamycin	Y-N	27.79±0.72^{b}	32.18±2.74^{b}	50.22±1.15^{a}

Y-C: Control Yoghurt, Y-La: Yoghurt treated with lactoferrin, Y-Li: Yoghurt treated with lysozyme, Y-N: Yoghurt treated with natamycin. The different lowercase letters in the same row indicate significant differences during ripening period (P<0.05)

Lactoferrin, lysozyme and natamycin content

Table 4 shows the amount of preservatives in yoghurt samples except the control which had negligible values. Both, the preservatives derived from donkey milk and commercial natamycin amounts of yoghurt samples showed an increase during storage (P<0.05) since the protein network responsible for yoghurt texture changes during storage due to the dissolution. Protein degradation also plays an important role in the formation and release of peptides and free amino acids (Amani et al., 2017). For this reason, the amount of lactoferrin and lysozyme is thought to increase during storage. It was also stated in another report that the proteolysis level of yoghurt samples with different starter cultures increased during 28 days of storage (Amani et al., 2017). An increase was also observed in the amount of natamycin during storage (P<0.05), which is thought to be the reason of the slight increase in the total solid of yoghurt samples treated with natamycin. Furthermore, the slight increase in the total

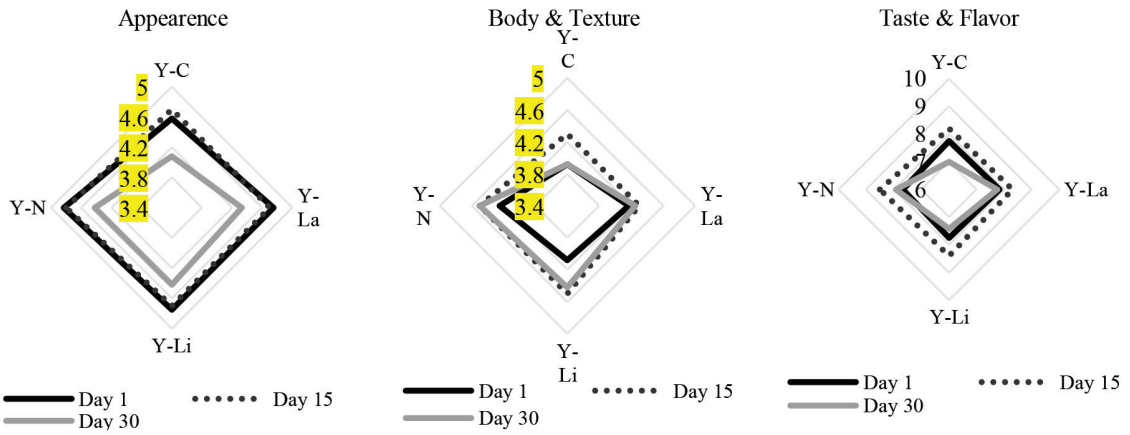

Figure 1. Sensory evaluation of yoghurt samples

Y-C: Control Yoghurt, Y-La: Yoghurt treated with lactoferrin, Y-Li: Yoghurt treated with lysozyme, Y-N: Yoghurt treated with natamycin

solid value of the control sample could be due to the loss of moisture during storage.

Sensory evaluation

The sensory attributes of cheeses treated with lysozyme, lactoferrin and natamycin are given in Figure 1.

There were no considerable differences between the evaluated sensory parameters (appearance, body and texture, and taste and flavour) of yoghurt samples ($P>0.05$) examend in this study. However, the lowest scores in terms of all the three parameters were obtained in the control sample, which might indicate positive effects of the applied preservatives on the sensory properties of yoghurt. The scores of appearance, taste and flavour of yoghurt samples showed a decrease on the 30th day of storage ($P<0.05$), but no change was observed in the body and texture scores ($P>0.05$). Moreover, with regard to the evaluated sensory parameters, yoghurt samples on day 15 were evaluated by the highest scores.

Similar studies performed on different dairy products also reported that the addition of lactoferrin (Zakaria et al., 2020), lysozyme (Saad et al., 2019), and natamycin (Nottagh et al., 2020) had no adverse effects on the sensory properties of products.

Conclusion

The treatment of yoghurt with lactoferrin, lysozyme and natamycin as antimicrobial agents did not affect the physicochemical (gross composition, pH, titratable acidity) and physical (water holding capacity, textural characteristics) properties. However, the TAMB and yeast-mold counts were significantly higher in the control sample than in the samples treated with lactoferrin, lysozyme and natamycin. This study showed that the addition of donkey milk proteins to yoghurt prevented the microbial growth without changing other quality properties. Besides, in terms of sensory evaluation, scores of yoghurt samples treated with all three preservatives were higher showing an increased variation between the control and the treated samples at the end of the storage period. Such results reveal that the applied preservatives most probably prevented the growth of undesirable microorganisms, hence extending the shelf life of yoghurt as well as maintaining desirable sensory properties.

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Utjecaj dodatka laktoferina i lizozima izoliranih iz mlijeka magarice na svojstva jogurta

Sažetak

Zbog neželjenih učinaka kemijskih konzervansa na zdravlje ljudi, prehrambena se industrija uglavnom usredotočila na primjenu prirodnih konzervansa. Laktoferin i lizozim ubrajaju se u mliječne proteine najjačeg antimikrobnog djelovanja. U ovom su istraživanju ti proteini izolirani iz mlijeka magarice te prskanjem naneseni na površinu jogurta. Tako dobiveni uzorci jogurta obogaćeni proteinima s antimikrobnim djelovanjem uspoređivani su s kontrolnim uzorkom proizvedenim bez dodatka ikakvih konzervansa, kao s i uzorcima tretiranim natamicinom kao komercijalnim konzervansom, a koji se često koristi u mliječnim proizvodima. Svim uzorcima su ispitivana fizikalno-kemijska, mikrobiološka i teksturalna svojstva tijekom 30 dana skladištenja. Prema dobivenim rezutatima, uzorci jogurta tretirani antimikrobnim sredstvima imali su manje mikroorganizama od kontrolnog uzorka, što ukazuje da laktoferin i lizozim izolirani iz mlijeka magarice djeluju inhibitorno na mikrobnu aktivnost u uzorcima jogurta. S druge strane, dodatak spomenutih konzervansa nije promijenio sastav ni teksturalna svojstva ispitivanih uzoraka jogurta. Međutim, najvažniji rezultati odnose se na senzorska svojstva jogurta koja su ostala nepromijenjena odnosno dodatak laktoferina ili lizozima nije negativno utjecao na njih. Naprotiv, jogurti s dodatkom laktoferina ili lizozima su nakon 30 dana skladištenja bolje ocijenjeni od kontrolnog uzorka. Uzimajući sve rezulatte u obzir, laktoferin i lizozim izolirani iz mlijeka magarice mogli bi se koristiti za kontrolu rasta neželjenih mikroorganizama te za produljenje roka trajanja jogurta.

Ključne riječi: lizozim; laktoferin; jogurt; mlijeko magarice

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