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EVALUATION OF YOGHURTS WITH THYME, THYME ESSENTIAL OIL AND SALT

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Abstract

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The aim of this work was production and evaluation of yoghurts with different addition of thyme (*Thymus vulgaris*), thyme essential oil (EO) and salt. It was produced: control yoghurt, yoghurt with 0.45% of salt and 0.25% of thyme, yoghurt with 0.90% of salt and 0.50% of thyme, yoghurt with 0.45% of salt and 0.004% of thyme EO, yoghurt with 0.90% of salt and 0.008% of thyme EO. The time of yoghurt fermentation was extended from the previous 3.5 hours (control sample) to 5 hours for samples with thyme or even up to 7 hours for samples with thyme EO. The fermentation time also depended on the concentration of the used substances. The average titratable acidity of yoghurts fluctuated from 46.52 °SH to 49.51 °SH at day after the production and from 51.57 °SH to 55.75 °SH after 7 days of storage. Average pH values of yoghurts fluctuated from pH 4.77 to pH 4.81 at day after production and from pH 4.48 to pH 4.63 after storage. In all samples of yoghurts were not detected coliform bacteria (< 10 CFU.g^{-1}). The yeasts were appeared rarely (10^{1} CFU.g^{-1}), and their numbers were increased during cold storage (10^{2} up to 10^{3}CFU.g^{-1}). Moulds appeared rarely (10^{1} CFU.g^{-1}). The number of lactic acid bacteria reached required minimum value of 10^{7} CFU.g^{-1}. Samples of yoghurts reached characteristic yoghurt and thyme aroma. The intensity of sour, salty and thyme taste, determined by sensory evaluation was in experimental samples of yoghurts at different level. A higher concentration of thyme and thyme EO gave to yoghurts a bitter taste. The dense consistency of the yoghurts was decreased with the increased addition of thyme and thyme EO. According to results, we recommend the recipe of yogurt with 0.45% of salt and 0.25% of thyme or 0.004% of thyme EO.

Keywords: yoghurt, essential oil, thyme

INTRODUCTION

Milk and dairy products are consumed all over the world. Of these, fermented dairy products, especially yoghurts are the most popular and unique, which is being enjoyed for their refreshing taste and beneficial properties.

Ayran is fermented milk beverage traditionally manufactured by mixing yoghurt with water and salt. It has been one of the popular drinks in Turkey and is drunk as a refreshing (Simsek *et al.*, 2007). Labneh, a traditional fermented milk product is consumed in the Middle Eastern countries. It is obtained from yoghurt after removal of part of its whey. It is produced with salt addition (Otaibi and Demerdash, 2008). Salted yoghurt or winter yoghurt is traditional fermented milk manufactured using goat's milk or the mixture of cow's and goat's milk in the south of Turkey, in Syria or Lebanon. The unique production process of salted yoghurt is concentration and conservation of yoghurt by heating and addition of salt, respectively (Hayaloglu and Karagul-Yuceer, 2011).

Herbs and spices are used as culinary ingredients for taste and tang. The antibacterial activities of spices and herbs, their extracts and essential oils have been known for a long time, and a numbers of researchers on antimicrobials effects e.g. mint, thyme, garlic etc. essential oils were reported (Burt,

2004; Holley and Patel, 2005; Tajkarimi *et al.*, 2010; Carvalho *et al.*, 2015; Shahbazi, 2015).

The numbers of essential oil components have been identified as effective antimicrobials, e.g. carvacrol, thymol, eugenol, perillaldehyde, cinnamaldehyde and cinamic acid. *In vitro* studies have demonstrated antibacterial activity of essential oils against *Listeria monocytogenes, Salmonella typhimurium, Escherichia coli* O157 : H7, *Shigella dysenteria, Bacillus cereus* or *Staphylococcus aureus* (Burt, 2004). Results from studies regarding the effect of different concentrations of essential oils on different microorganisms present in food have been varied, ranging from partial to complete inhibition (Otaibi and Demerdash, 2008). By Burt (2004) higher concentration of essential oils is needed to achieve the same effect in food in compared to *in vitro* studies.

Essential oils that are considered Generally Recognized as Safe (GRAS) at doses typically used in foods and have been approved by the Food and Drug Administration (FDA) for use in foods and drinks include the essential oil from *Thymus vulgaris* L. (Carvalho *et al.*, 2015).

The antimicrobial activity of essential oils and spices are considered to result in extending the shelf life of a wide variety of foods (Burt, 2004).

Although there have been reports regarding the antimicrobial activity of spices on viability of lactic acid bacteria, most of them suggest, that lactic acid bacteria are relatively resistant to the inhibitory effect of spices and essential oil. Moreover, the viability of lactic acid increased at adjusted concentrations of essential oil (Ismail *et al.*, 2006; Otaibi and Demerdash, 2008).

Markets in Slovakia offer unflavored yoghurts or yoghurts with sweet flavor (e.g. with addition of chocolate or fruit compounds). In compare with other countries in our market are missing yoghurts or fermented dairy products with salty taste. The aim of this work was the production and the evaluation of new types of yoghurts – with different addition of thyme, thyme essential oil and salt.

MATERIALS AND METHODS

Manufacturing of yoghurts

Sixty grams of skimmed dried milk (fat 1.5%) was added per 1 liter of homogenized, semi-skimmed milk (fat 1.5%). The mixture was pasteurized at 85 °C for 10 minutes, cooled to 43 °C and then inoculated with yoghurt starter culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus)* (*Lactoflora,* Czech Republic) – 1 pack per 1 liter of milk. Inoculated milk was agitated and dispensed into glass containers and then was mixed thoroughly with NaCl, thyme and thyme essential oils.

Fresh thyme was purchased in local market in Nitra (Slovakia) and then for yoghurts production was frozen and stored at –18 °C.

Thyme essential oil – "White Thyme (100% Pure) – Now Food Essential Oil" was purchased from WFmed Lorton (Canada).

Together 5 types of yoghurt samples were prepared from these ingredients:

- control sample of yoghurt,
- yoghurt with addition of 0.25% thyme and 0.45% of salt,
- yoghurt with addition of 0.50% thyme and 0.90% of salt,
- yoghurt with addition of 0.004% thyme essential oil and 0.45% of salt,
- yoghurt with addition of 0.008% thyme essential oil and 0.90% of salt.

Samples of prepared mixtures were incubated at 43 °C until they were completely coagulated. After coagulation were yoghurt samples cooled to 6 °C and stored at this temperature for 7 days.

Samples were taken for analysis at 1 day after production and after 7 days of storage.

Experiment was done in four replicates.

Chemical analysis

The pH of yoghurts was measured with Gryf 209 L pH meter (*Gryf HB*, Czech Republic).

The titratable acidity was determined in °SH after titration 100 g of yoghurt with 0.25 N NaOH (Cvak *et al.*, 1992).

Microbiological analysis

For microbiological analysis 10 g of yoghurt was homogenized with 90 mL of saline and then decimal dilutions were prepared.

The determination of:

- coliform bacteria were cultivated on VRB agar (*HiMedia*, India), at 30 °C for 24 hours, (SÚTN, 2009),
- yeasts and moulds were cultivated on DRBC (*HiMedia*, India), at 25 °C for 5 days, (SÚTN, 2010),
- lactic acid bacteria were cultivated on MRS (*HiMedia*, India), at 37 °C for 72 hours, under anaerobic conditions (SÚTN, 2002).

Sensory analysis

Sensory properties – intensity of yoghurt aroma, taste and consistency were evaluated. Sensory analysis was performed by five-member committee of assessors who evaluated selected parameters by five point scale.

Statistical analyses

Obtained results were processed by variation-statistical methods in ANOVA. The differences between groups were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The average titratable acidity of yoghurts ranged from 46.52 °SH to 49.51 °SH at day after the production and from 51.57 °SH to 55.75 °SH after 7 days of storage. Average pH values of the yoghurts fluctuated from pH 4.77 to pH 4.81 day after production and from pH 4.48 to pH 4.63

after storage. No significant differences ($P > 0.05$) in titratable acidity and pH values were detected among the yoghurt samples at day after their production or 7 days after storage. However, it is important to note that addition of salt, thyme or thyme essential oil significantly affected fermentation time of yoghurts. The yoghurts fermentation time was extended from the previous 3.5 hours (control sample) to 5 hours for samples with thyme or even up to 7 hours for samples with thyme essential oil. The fermentation time depended also on the concentration of the added substances.

Also no significant effect on pH values were found in samples of yoghurts after addition of cinnamon, licorice (Behrad *et al.*, 2012), mint or eucalyptus essential oil (Shahdadi *et al.*, 2015).

Mahmoudi *et al.* (2014) studied effect of *Teucrium polium* essential oil (40, 60, 80 ppm) on the physicochemical properties of probiotic yoghurt with *Lactobacillus casei* (10^8–10^9 cfu.mL^{-1}). They reported initial pH value for different yoghurt types in the range from 4.45 to 4.60. They also found out gradual and consistent decrease of pH during 28 days of storage. In pH values no significant differences were observed. However, they conclude that additives have significantly ($P < 0.05$) affected titratable acidity treated samples on later days. They suggested that used essential oil had a stimulatory effect on the starter culture and *Lactobacillus casei*.

Similarly Otaibi and Demerdash (2008) suggest that thyme essential oil significantly increased acidity values in labneh (the highest values with 0.2 ppm) during 21 days of storage. However, mentioned authors in contrast to our manufacturing added thyme essential oil and 0.5 % of salt to product when was completely coagulated.

In all samples of yoghurts were not detected coliform bacteria (< 10 CFU.g^{-1}) at day after yoghurt production or after 7 days of cold storage. Otaibi and Demerdash (2008) also reported that coliform bacteria and spore-forming bacteria were not detected in any of labneh samples prepared by addition of thyme, marjoram or sage essential oil. Simsek *et al.* (2007) investigated the behaviour of *Escherichia coli* O157 : H7 during storage of Ayran produced with mint, thyme, garlic and salt. *Escherichia coli* O157 : H7 (initial count 6.40 log CFU.g^{-1}) was not present at the end of 14th day of storage. The inhibitory effect of spiced Ayran on *Escherichia coli* O157 : H7 were similar to the control sample without spice.

The yeasts and moulds are considered as indicator of the hygienic quality and shelf life of fermented dairy products. The yeasts appeared rarely (10^1 CFU.g^{-1}) at one day after yoghurts production. Their numbers increased during cold storage (Tab. I).

Significant differences were not determined among samples of yoghurts ($P > 0.05$). Moulds appeared rarely (10^1 CFU.g^{-1}). Otaibi and Demerdash (2008) not detected occurrence of yeasts and moulds in labneh treated with thyme, marjoram and sage essential oil during the storage period, but this group of microorganisms detected in the untreated control sample after 14 and 21 days of storage.

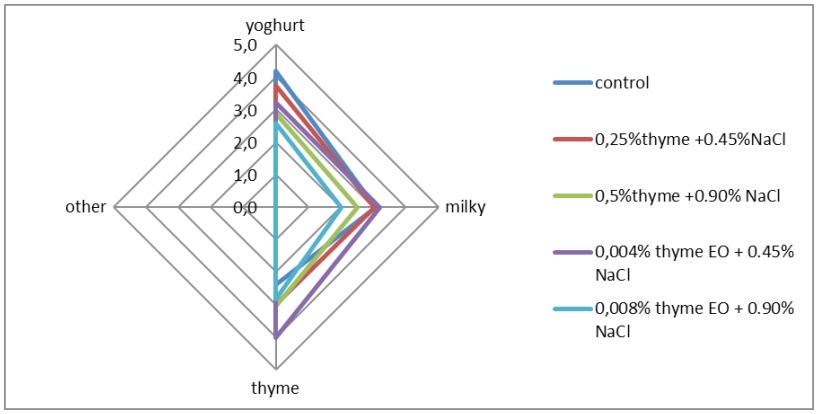
The numbers of lactic acid bacteria in our samples, reached required minimum value of 10^7 CFU.g^{-1} at day after production and also after storage. However, the counts of lactic acid bacteria were decreased during storage. Significant differences ($P > 0.05$) were not found among samples of yoghurts. The counts of lactobacilli and streptococci in study of Simsek *et al.* (2007), in all samples of ayran with and without addition of spice, significantly decreased ($P < 0.001$) during the storage time and were approximately 4–5 log CFU.g^{-1} at 21st day. Authors of this study concluded that effect of spices on lactic acid bacteria were not different from control sample. Ersöz *et al.* (2011) investigated effect of phenolic compounds extracted grape seed and pomegranate seed on counts of lactobacilli and streptococci in yoghurts. In their study, bacterial population were not supressed by the phenolic compounds during fermentation and at the first day, whereas the storage period led to the decrease in bacterial counts. Contrary to these results, El-Nawawy *et al.* (1998) reported that presence of some herbs, including thyme, in the manufacture of yoghurt increased the counts of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* compared to untreated control during storage.

Sensory properties of yoghurt samples (fat 1.5 %) were also evaluated and the results are presented in Fig. 1 and 2.

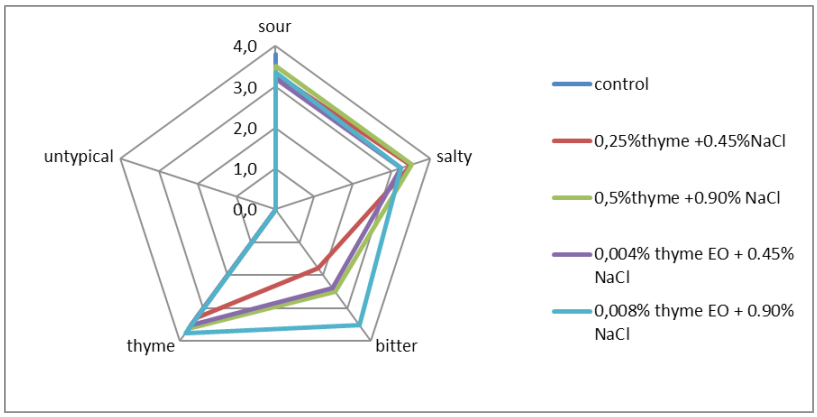
Samples of yoghurts had characteristic yoghurt and thyme aroma. The taste was sour, salty and with the taste of thyme. A higher concentration of thyme and thyme EO gave the yoghurts a bitter taste. The dense consistency of the yoghurts was decreased with the increased addition of thyme and thyme essential oil. The best score of sensory analyse

I: *Counts of yeasts in yoghurts after 7 days of storage at 6 °C*

	Counts of yeasts in CFU.g^{-1}				
	control sample	**0.25 % thyme 0.45 % NaCl**	**0.50 % thyme 0.90 % NaCl**	**0.004 % EO 0.45 % NaCl**	**0.008 %EO 0.90 % NaCl**
1	2.3×10^3	3.1×10^3	4.0×10^3	1.0×10^3	1.2×10^3
2	2.3×10^2	8.0×10^2	< 10	1.2×10^2	< 10
3	2.0×10^3	1.2×10^3	2.7×10^3	2.6×10^3	1.0×10^3
4	< 10	< 10	< 10	< 10	< 10



1: *Evaluation of yoghurt aroma after 7 days of storage*



2: *Evaluation of yoghurt taste after 7 days of storage*

had samples of yoghurts with lower concentrations of thyme or thyme essential oil and salt. According to sensory results, we recommend the recipe of yoghurt with 0.45% of salt and 0.25% of thyme or 0.004% of thyme EO.

Otaibi and Demerdash (2008) reported that total scores of sensory analyses of labneh containing essential oils decreased with an increase in the concentration of essential oil. They concluded that the lowest tested addition – 0.2 ppm of thyme can be used for manufacture of labneh. Ersöz *et al.* (2011) found out that while addition of phenolic compounds (from grape and pomegranate seeds) affected chemical and microbiological properties positively, sensory quality was affected negatively.

CONCLUSION

In this research, the yoghurts with different addition of thyme, thyme essential oil and salt were produced and evaluated. It is generally known, that essential oil of thyme have potential to inhibit some types of microorganisms. Tested addition of thyme, thyme essential oil and salt led to prolongation of yoghurt fermentation process. However counts of lactic acid bacteria reached required minimum value for dairy fermented products. Spoilage bacteria – coliform bacteria, yeasts and moulds were not detected or were appeared rarely. According to sensory results, lower addition of salt (0.45%) and thyme (0.25%) or thyme EO (0.004%) is preferable. This type salted flavored yoghurts can enrich the assortment of yoghurt products on our market and also represent healthier alternative e.g. to mayonnaise dips.

REFERENCES

BEHRAD, S., YUSOF, M. Y., GOH, K. L. and BABA, A. S. 2012. Manipulation of Probiotics Fermentation of Yogurt by Cinnamon and Licorice: Effects on Yogurt Formation and Inhibition of Helicobacter Pylori Growth in vitro. *International Journal of Biological and Life Sciences*, 8(3): 135–139.

BURT, S. 2004. Essential oils: their antibacterial properties and potential aplications in food – review. *Journal of Food Microbiology*, 94(3): 223–253.

CARVALHO, R. J., SOUZA, G. T., HONÓRIO, V. G., SOUSA, J. P, CONCEICAO, M. L. and MAGANANI, M. 2015. Comparative inhibitory effect of Thymus vulgaris L. essential oil against Staphylococcus aureus, Listeria monocytogenes and mesophilic starter co-culture in cheese-mimicking models. *Food Microbiology,* 52: 59–65.

CVAK, Z., PETERKOVÁ, Ľ. and ČERNÁ, E. 1992. *Chemické a fyzikálně-chemické metódy v kontrole jakosti mléka a mlékarenských výrobků*. Praha: VÚPP.

EL-NAWAWY, M. A., EL-KENANY, Y. M. and ABD EL-GHAFFAR, E. A. 1998. Effect of some herb plants on the use of yoghurt culture. In: *Annals of agriculture Sci. 7th Conf. Agric. De. Res. Fac. Agric*. Ain Shams University of Cairo, Egypt, 15–17 December.

ERSÖZ, E., KINIK, O., YERLIKAYA, O. and MERVE, A. 2011. Effect of phenolic compounds on characteristics of strained yoghurts produced from sheep milk. *African Journal of Agricultural Research*, 6(23): 5351–5359.

HAYALOGLU, A. A. and KARAGUL-YUCEER, Y. 2011. Utilization and characterization of small ruminants´milk and milk products in Turkey: Current status and new perspectives. *Small Ruminant Research,* 101(1-3): 73–83.

HOLLEY, R. A. and PATEL, D. 2005. Improvement in shelf-life and safety of perishable foods by plants essential oils and smoke antimicrobials. *Food Microbiology,* 22(4): 273–292.

ISMAIL, A. M., HARBY, S. and SALEM, A. S. 2006. Production of flavored labneh with extended shelf life. *Egyptian Journal of Dairy Science,* 34: 59–68.

MAHMOUDI, R., ZARE, P., HASSANZADEH, P. and NOSRATPOUR, S. 2014. Effect of Teucrium polium essential oil on the physicochemical and sensory properties of probiotic yoghurt. *Journal of Food Processing and Preservation*, 38(3): 880–888.

OTAIBI, M. A. and DEMERDASH, H. E. 2008. Improvement of the quality and shelf life of concentrated yoghurt (labneh) by the adittion of some essential oils. *African Journal of Microbiology Research,* 2: 156–161.

SHAHBAZI, Y. 2015. Ziziphora clinopodioides Essential Oil and Nisin as Pontential Antimicrobial Agents against Esherichia coli O157:H7 in Doogh (Iranian Yoghurt Drink). *Journal of Pathogen,* 2015: 176024.

SHAHDADI, F., MIRZAIE, H., KASHANINEJAD, M., MORTEZA, K. M., ZIAIIFAR, A. M. and AKBARIAN, A. 2015. Effects of Various Essential Oils on Chemical and Sensory Characteristics and Activity of Probiotic Bacteria in Drinking Yoghurt. *Agricultural Communications*, 3(1): 16–21.

SIMSEK, B., SAGDIC, O. and OZCELIK, S. 2007. Survival of Escherichia coli O 157:H7 during the storage of Ayran produced with different spices. *Journal of Food Engineering,* 78(2): 676–680.

SÚTN. 2010. *Mikrobiológia potravín a krmív. Horizontálna metóda na stanovenie počtu kvasiniek a plesní. Časť 1: Metóda počítania kolónií vo výrobkoch s aktivitou vody väčšou ako 0,95*. STN ISO 21527-1. Bratislava: Slovenský ústav technickej normalizácie.

SÚTN. 2002. *Mikrobiológia potravín a krmív. Horizontálna metóda na stanovenie počtu mezofilných kyslomliečných baktérií. Metóda počítania kolónií kultivovaných pri 30 °C*. STN ISO 15214. Bratislava: Slovenský ústav technickej normalizácie.

SÚTN. 2009. *Mikrobiológia potravín a krmív. Horizontálna metóda na stanovenie počtu koliformných baktérií. Metóda počítania kolónií*. STN ISO 4832: 2009 (56 0085). Bratislava: Slovenský ústav technickej normalizácie.

TAJKARIMI, M. M., IBRAHIM, S. A. and CLIVER, D. O. 2010. Antimicrobial herb and spice compounds in food. *Food Control,* 21(9): 1199–1218.

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