# ANTIMICROBIAL EFFECTS OF SELECTED PLANT EXTRACTS ON THE SHELF LIFE OF GOAT WHEY

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## ABSTRACT

One of the possibilities, how we can increase the shelf life of whey, as well as food in general, is using of essential oils and extracts. The aim of this paper was to evaluate the possibility of addition of an aqueous plant extracts to goat whey to ensure its microbiological stability and prolongation of its shelf-life. There were chosen these herbs: lemon balm (*Melissa officinalis* L.), wild garlic (*Allium ursinum* L.), rosemary (*Rosmarinus officinalis* L.) and Baikal skullcap (*Scutellaria baicalensis* L.). The aqueous extracts in the amount of 5% were added into goat whey. These samples were stored at 6 °C for four weeks. The microbiological analysis (determining the groups: total plate count, coli-form bacteria, enterococci, thermo-resistant microorganisms), measuring of pH and titratable acidity were performed during storage. The rosemary extract was the only one that significantly reduced the titratable acidity of goat whey. The other three extracts showed mild decrease of titratable acidity. The pH came into lower undesirable values in all prepared samples with the addition of aqueous plant extracts. The results of microbiological analyses suggested that the aqueous extract of rosemary was inappropriate. It contributed to an increase of the number of microorganisms in all the studied groups. A clear opinion cannot be expressed for the other three extracts. To increase the dose of aqueous extracts of selected plants in goat whey would be probably more appropriate performing the results of future experiments devoted to antimicrobial activity of plant extracts.

**Key words:** microorganisms, pH, titration acidity

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## INTRODUCTION

Whey or milk serum is liquid remaining after milk has been curdled by rennet or different coagulation preparation; it is a by-product of the manufacture of cheese or casein (Fox et al., 2000). Whey contains big amount of lactose (Panesar et al., 2007). For many years, this dairy product was neglected. In recent years, however, whey has experienced a renaissance, which is related to knowledge of nutrition, with the development of separation technologies and the ever-increasing volume of produced cheese products which causes higher production of whey (Suková, 2006).

Among the major groups of microorganisms affecting the quality of milk and thus subsequently the whey, we can include the total plate count (TPC), psychotropic microorganisms, coli-form bacteria, lactic acid bacteria, enterococci and sporulating anaerobic bacteria (Fernandes, 2009). It is necessary to count with a possible secondary contamination of the whey and the final product.

Whey cheeses and mainly whey beverages are more often offered to customers. Due to the fact that the un-modified whey experiences a low durability, one of the possible measures to extend the durability of the whey appears to be the use of vegetable oils, respectively their extracts.

Essential oils are volatile liquids possible to be gained from different parts of a plant such as straw, wood, leaves, fruit, rind, flowers or seeds. Essential oils are typical for their characteristic strong odor and they are formed in a form of secondary metabolites. They are also well known for their antiseptic medicinal properties such as bactericidal, virucidal and fungicidal characteristic. Essential oils are commonly used in food preservation (Bakkali, 2008) and they can be gained by different technologies such as pressing, fermentation, distillation or extraction (Burt, 2004).

The aim of this study was to evaluate whether the addition of an aqueous extract of selected drugs into goat whey can ensure microbiological stability and prolong its shelf life.

## MATERIAL AND METHODS

Goat whey was sampled at the goat farm Kozí farma Sedlák in Šošůvka, The Czech Republic. Following herbs were used for the testing of antimicrobial activities: lemon balm (*Melissa officinalis* L.), wild garlic (*Allium ursinum* L.), rosemary (*Rosmarinus officinalis* L.) and Baikal skullcap (*Scutellaria baicalensis* L.). Herbal drugs were purchased in the specialized store with medical plants Léčivé rostliny in Brno, The Czech Republic.

With use of these drugs, aqueous extracts were prepared. 10 grams of powdered drug were immersed into 100 ml of distilled water at 95 °C for 1 hour. The aqueous extracts in the amount of 5 % were added into goat whey. The prepared samples of the goat whey were stored in a refrigerator under the temperature of 6 °C during the whole time of 4 weeks.

Each sample was subjected to microbiological analysis with the use of pour plate method. Determined microorganisms, media and cultivation conditions are stated in the Tab. 1. The bacterial counts were expressed as CFU • $ml^{-1}$ and taken under a logarithm.

*Tab. 1 The culture conditions for the microorganisms*

| Microorganisms | Culture medium1 | Conditions of cultivation |
|---|---|---|
| **Total plate count** | **PCA with skimmed milk** | 30 °C; 72 hours |
| **Coli-form bacteria** | **VRBL** | 37 °C; 24 hours |
| **Enterococci** | **Compass Enterococcus Agar** | 44 °C; 24 hours |
| **Thermo resistant aerobic (TMRae) and anaerobic (TMRan) microorganisms** | **PCA with skimmed milk** | 30 °C; 48 hours, TMRan under anaerobic conditions |

[1] - producer: Biokar Diagnostics, France

pH and titratable acidity (SH) were chosen as the parameters to determine the shelf life of goat whey. The pH value is determined by hydrogen ions concentration and it was gauged with WTW pH 95 pH meter. Titration acidity is determined by titration process of 100 ml of sample with 0.25 mol.l-1 solution of sodium hydroxide with phenolphtalein indicator by Czech State Standard ISO 57 0530

Microbiological analyses, pH and titratable acidity were performed on the second day and then on the each following 7th day of analysis by pour plate method.

## RESULTS AND DISCUSSION

Zinoviadou et al. (2009) used oregano in their work. It studied properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. The results pointed to the effectiveness of oregano oil containing whey protein films to increase the shelf life of fresh beef. Gallego et al. (2013) studied antioxidant properties of three aromatic herbs (rosemary, thyme and lavender) in oil-in-water emulsions. In oil-in water emulsion, extracts from rosemary leaves and thyme leaves were most effective at retarding oxidation.

All prepared aqueous extracts were not tested on the same sample of whey. Therefore, it was necessary to provide values of whey without extracts and applicable values of samples of whey with aqueous extracts (samples with the addition of rosemary, Lemon balm, wild garlic, and Baikal skullcap). Different bacteria counts and begining values of pH and titratable acidity of the whey in both cases were the reason for this procedure.

pH and titratable acidity changed during 4 weeks of refrigerator storage of the whey with aqueous extracts are shown from the Fig. 1 to 4. The Fig. 1 and the Fig. 3 show there was no decline nor rise of pH values after the use of rosemary extract. Other aqueous extracts such as lemon balm, wild garlic, and Baikal skullcap showed sourer values than the control goat whey within the second week of the experiment. In the second week of analyse, the pH values of whey with the addition of aqueous extracts were similar with pH values of goat without the addition. Therefore, we can conclude the use of these aqueous extracts do not affect pH of goat whey.

The Fig. 2 shows the values of titratable acidity of whey sample with rosemary extract. They were lower than the control goat whey from the second week. During the first week, titratable acidity values of the goat whey with aqueous extracts such as Lemon balm, wild garlic, and Baikal skullcap were higher than the goat whey after cheese making. However, the control goat whey

values of these samples rose up during analysis but samples with aqueous extracts values were gradually decreasing.

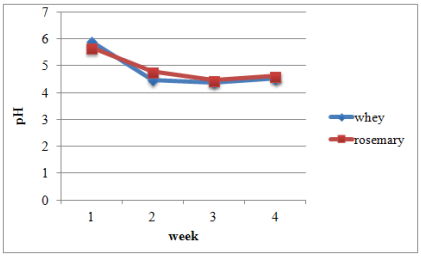


Fig. 1 Comparison of pH of goat whey and rosemary

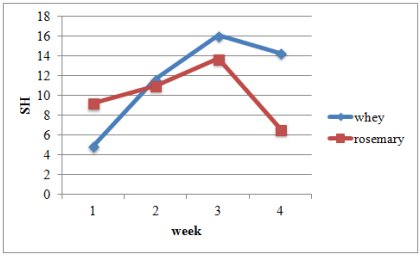


Fig. 2 Comparison of titratable acidity (SH) of goat whey and rosemary

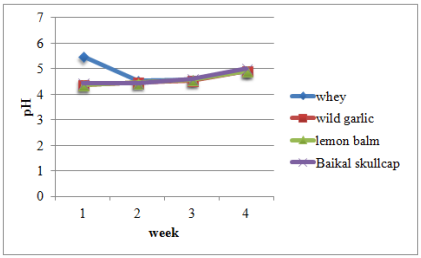


Fig. 3 Comparison of pH of goat whey and lemon balm, wild garlic and Baikal skullcap

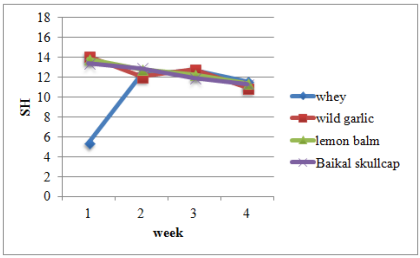


Fig. 4 Comparison of titratable acidity (SH) of goat whey and lemon balm, wild garlic and Baikal skullcap

In the Tab. 2 and 3, there are shown numbers of microorganisms in the goat whey and in the microorganisms with the addition of aqueous extracts. Development of microorganisms counts in the goat whey with aqueous extracts and goat whey without aqueous extracts is the main index of antimicrobial activities.

*Tab. 2: Counts of microorganisms after the addition of rosemary*

| | week | log N [log CFU·ml$^{-1}$] | | | | |
|---|---|---|---|---|---|---|
| | | **Coli** | **Ent** | **TMRan** | **TMRae** | **TPC** |
| **goat whey** | **1** | **5,05** | **1,67** | **1,34** | **0,44** | **6,54** |
| | **2** | **5,80** | **1,94** | **1,10** | **0,66** | **6,71** |
| | **3** | **5,94** | **1,23** | **1,88** | **1,92** | **6,85** |
| | **4** | **6,42** | **1,21** | **0,80** | **0,80** | **7,71** |
| **Whey with aqueous extract** | **week** | **log N [log CFU·ml-1]** | | | | |
| | | **Coli** | **Ent** | **TMRan** | **TMRae** | **TPC** |
| **Rosmarinus officinalis L.** | **1** | **5,20** | **1,91** | **1,62** | **1,69** | **6,56** |
| | **2** | **5,76** | **2,23** | **3,05** | **3,23** | **8,39** |
| | **3** | **7,82** | **2,09** | **3,24** | **3,26** | **8,10** |
| | **4** | **6,96** | **1,57** | **1,63** | **1,60** | **7,15** |

Coli – coli-form bacteria, Ent - enterococci, TMRan – thermo - resistant anaerobic microorganisms, TMRae – thermo - resistant aerobic microorganisms, TPC – total plate count

*Tab. 3 Counts of microorganisms after the addition of lemon balm, wild garlic and Baikal skullcap*

| | week | log N [log CFU ml$^{-1}$] | | | | |
|---|---|---|---|---|---|---|
| | | **Coli** | **Ent** | **TMRan** | **TMRae** | **TPC** |
| **goat whey** | **1** | **5,03** | **1,45** | **2,31** | **2,63** | **7,34** |
| | **2** | **4,65** | **1,34** | **1,74** | **0,69** | **6,78** |
| | **3** | **4,04** | **1,32** | **0,69** | **3,03** | **6,04** |
| | **4** | **4,39** | **1,16** | **0,69** | **0,69** | **5,56** |
| **Whey with aqueous extract** | **week** | **log N [log CFU·ml$^{-1}$]** | | | | |
| | | **Coli** | **Ent** | **TMRan** | **TMRae** | **TPC** |
| **Melissa officinalis L.** | **1** | **4,07** | **1,33** | **3,04** | **2,04** | **6,46** |
| | **2** | **4,67** | **0,61** | **2,32** | **0,26** | **6,88** |
| | **3** | **4,12** | **1,35** | **1,00** | **3,41** | **5,86** |
| | **4** | **4,01** | **1,21** | **0,91** | **ND** | **5,30** |
| **Allium ursinum L.** | **1** | **3,92** | **1,57** | **2,93** | **2,75** | **6,76** |
| | **2** | **4,61** | **0,94** | **1,54** | **2,18** | **6,39** |
| | **3** | **4,33** | **1,23** | **0,69** | **1,15** | **6,09** |
| | **4** | **4,15** | **1,39** | **0,61** | **0,50** | **5,56** |
| **Scutellaria baicalensis L.** | **1** | **4,08** | **1,74** | **2,85** | **2,69** | **5,91** |
| | **2** | **4,88** | **ND** | **1,54** | **0,44** | **7,06** |
| | **3** | **4,71** | **1,30** | **1,00** | **3,19** | **6,41** |
| | **4** | **3,94** | **1,25** | **0,66** | **0,36** | **5,36** |

ND – not detected, Coli – coli-form bacteria, Ent - enterococci, TMRan – thermo - resistant anaerobic microorganisms, TMRae – thermo - resistant aerobic microorganisms, TPC – total plate count

The microbiological results show that the addition of 5% aqueous rosemary extract conversely led to increase of the number of selected microorganisms groups. Therefore, the addition of rosemary extract in whey does not ensure the microbial stability of the goat whey. The results from other 3 extracts are not so conclusive. For example, during all 4 weeks of the analyses, the number of thermo-resistant microorganisms in the whey with lemon balm rose up. The total plate count has increased only in the second week of analysis. The coli-form bacteria number increased in the second and third week of analysis. Whey with rosemary and Baikal skullcap showed similar ambiguous results.

Efforts to extend the shelf life of whey can also be seen in other studies. Tomaino et al. (2004) demonstrated a longer shelf life of whey after the addition of the *Lactococcus lactis* in starter culture. The *Lactococcus lactis* affects taste and antioxidative stability and thus, it prolongs its applicability. Jasewicz et al (1959) studied addition of 0.02% $H_2O_2$ into the cheese whey. According to the results, $H_2O_2$ addition shortly after separation of the whey from curd is recommended.

## CONCLUSIONS

The use of activities of some plants seems to be an interesting alternative to decline the number of synthetic substances in food. The use of aqueous extracts in the conservation process of whey is one of the particularly interesting possibilities.

This work shows evaluation of aqueous extract addition of particular drugs such as: lemon balm (*Melissa officinalis* L.), wild garlic (*Allium ursinum* L.) rosemary (*Rosmarinus officinalis* L.) and Baikal skullcap (*Scutellaria baicalensis* L.). These drugs were added into goat whey to ensure its microbiological stability and to prolong its shelf life. pH and titratable acidity (SH) were chosen as parameters to determine the shelf life of the goat whey. The prepared samples of goat whey were stored in a refrigerator under the temperature of 6 ° C during the whole time of 4 weeks.

Only the rosemary extract shows significant decline of titratable acidity. Other aqueous extracts show only mild decline of titratable acidity. pH values continued to decline further even after aqueous extracts addition.

With the use of microbiological analysis, we were able to determine that rosemary aqueous extract does not decline the number of microorganisms not even in one of the particular groups. Therefore, we cannot recommend its use for this purpose. The results of lemon balm (*Melissa officinalis* L.), wild garlic (*Allium ursinum* L.) and Baikal skullcap (*Scutellaria baicalensis* L.) are not conclusive. Some of the numbers of these particular groups of microorganisms – even within one group –they rose up then decreased.

Due to obtained results, it is appropriate to follow up with other experiments and add other percentage of aqueous extracts of these particular plants, prepare the right recipe for a new whey beverage with optimal addition of aqueous extract due to sensory acceptability of this product for consumers.

## REFERENCES

BAKKALI, F., AVERBECK, S., AVERBECK, D. and IDAOMAR, M., 2008: Biological effects of Essentials oils – A review. *Food and Chemical Toxicology*, 46 (2).

BURT S., 2004: Essential oils: their antibacterial properties and potential applications in foods-a review, *International Journal of Food Microbiology*, 94 (3), pp 223-253, ISSN 0168-1605.

ČSN 57 0105-3, 2003: *Metody zkoušení mléka a tekutých mléčných výrobků*, Český normalizační institut, Praha.

FERNANDES R., 2009: *Microbiology handbook*. Cambridge: Leather head Pub., and Royal Society of Chemistry, xiii, 173 p. ISBN 19-052-2462-1.

FOX, P. F., GUINEE, T. P., COGAN, T. M., and MCSWEENEY, P. L. H., 2000: *Fundamentals of Cheese Science*. Gaithersburg: AspenPublishers.

GALLEGO, M. G., GORDON, M. H, SEGOVIA, F. J., SKOWYRA, M. and. ALMAJANO, M. P., 2013: Antioxidant Properties of Three Aromatic Herbs (Rosemary, Thyme and Lavender) in Oil-in-Water Emulsions. *Journal of the American Oil Chemists' Society*. 90 (10), pp. 1559-1568.

JASEWICZ, L. and PORGES, N., 1959: Whey Preservation by Hydrogen Peroxide. *Journal of Dairy Science*. 42 (7), pp. 1119-1125.

PANESAR, P. S., KENNEDY, J. F., GANDHI, D. N., and BUNKO, K., 2007: Bioutilisation of whey for lactic acid production. *Food Chemistry*, 105, pp. 1-14.

SUKOVÁ I., 2006: *Syrovátka v potravinářství.* Praha: Ústav zemědělských a potravinářských informací, 60 s., ISBN 80-7271-173-3.

TOMAINO, R. M., TURNER, L. G. and LARICK, D. K., 2004: The effect of Lactococcus lactis starter cultures on the oxidative stability of liquid whey. *Journal of Dairy Science*, 87 (2), pp. 300-307.

ZINOVIADOU, K. G., KOUTSOUMANIS, K. P. and BILIADERIS, C. G. 2009: Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. *Meat Science*, 82 (3), pp. 338-345.