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EFFECT OF SOME ESSENTIAL OILS ON THE SHELF LIFE OF WHEY

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Abstract

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The aim of this work was to determine whether the addition of different herbal oils (anise, sweet basil, blue chamomile, clove, true cinnamon, common sage) into whey will extend its shelf life. Changes in pH, titration acidity and microbiological analysis of whey during storage life were chosen as parameters for monitoring of whey shelf life. Based on these analyses possibility of utilization of herbal oils has been confirmed for the purpose of extension of the lifetime of whey. The use of essential oils blue chamomile and common sage did not help to extend the shelf life of fresh whey. The treatment of whey by herbal essential oils anise, sweet basil, clove and cinnamon extend the shelf life up to 21 days without significant changes in pH and titratable acidity. The results of our study show that essential oils could be used to inhibit microbial spoilage whey and to prolong its shelf life.

Keywords: microorganisms, pH, titratable acidity, anise, sweet basil, blue chamomile, common sage, clove, cinnamon

INTRODUCTION

Value of whey has significantly increased in recent years. This is related to new knowledge about nutritional value, development of separation technologies and the necessity of utilization of large volumes of this by-product resulting from increasing volumes of cheese production (Suková, 2006).

Whey originates from production of cheese or casein. Whey composition varies considerably depending on composition and type of milk, but above all on process conditions used. It contains a considerable amount of water, 93%–94% on average. There is about 50% of milk dry matter in whey, the biggest part of which is lactose (above 70% of dry matter). Whey further contains proteins, forming about 10% of its dry matter, as well as mineral substances and non-protein nitrogen substances. About 40% of calcium and 43% of phosphorus passes from milk into rennet whey during cheese production. Besides these substances, whey contains special functional components enabling development of new food and pharmaceutical products (Jelen, 2011).

Whey is a suitable substrate for growth of a number of microorganisms, lowering its keeping quality. The high water content makes fresh whey very susceptible to microbial spoilage and therefore attempt to extend the shelf life of whey existed previously (Jeličić *et al*., 2008). An attempt to extend whey shelf life was made by Tomaino *et al.* (2004) by adding a starter culture containing *Lactococcus lactis* into whey. This research clearly proved the ability of *Lactococcus lactis* starter culture to extend whey shelf life for further processing. Roseiro *et al.* (1991) carried out an analysis of the influence of storage temperature upon whey quality and its subsequent usage for production of the Requeijao whey cheese.

Our work focused on biological means of whey preservation with utilization of herbal oils. Essential oils are volatile, high-scenting oil substances, formed in storage canals, glandules and glandular hairs of herbs. During evaporation they permeate through the upper surface of leaves and flowers, thereby effusing their typical scent. From the chemical point of view they are a complicated mixture of various organic compounds soluble in lipophilic solvents (ether, petrol). Prevailing composition includes hydrocarbons as alcohols, phenols, aldehydes, ketones, oxides, acids, esters etc. Furthermore, they may contain terpenes and their derivatives. Biological effects of essential oils are numerous, depending on their specific chemical composition. They are able to provide plant protection or to attract insects for pollination. Among the numerous of all pharmaco-therapeutic properties, the most characteristic are antiseptic and disinfecting effects (Pamukov, 1986; Seitz, 1999).

One of the possibility how we can use whey are beverages. Sweet or acid whey can be used for beverages. There are non alcoholic and alcoholic whey beverages (Holsinger *et al.*, 1974). Non alcoholic beverages are made with different additives like tropical fruits, apples, pears, strawberries or cranberries, crops and their products, isolates of vegetable proteins, chocolate, cocoa, vanilla extracts etc (Jeličić *et al.*, 2008). Whey beverages prepared by fermentation with probiotic bacteria are known, e.g. Kar and Misra (1999) studied wheyghurt drink, made with 4% yoghurt cultures inoculated in deproteinized whey. Alcoholic whey beverages comprise drinks with small amount of alcohol (to 1.5%), whey beer and whey wine (Jeličić *et al.*, 2008). The utilization of whey with essential oils (like natural preservative), we see in the beverage industry. The essential oils can usefully complement the taste of the drink and also extend their shelf-life. For example Baljeet *et al.* (2013) studied development and storage of whey-based pineapple and bottle gourd mixed herbal beverage. Whey beverage prepared from pineapple and bottle gourd juices in combination with extract of herbal like *Mentha arvensis* will not have only excellent nutritional properties but also possess therapeutic, prophylactic, antibacterial and organoleptic properties. There are also some researches of whey proteins film for food safety, e.g. Du *et al.* (2011) or Matan (2012).

There are publications dealing with the application of essential oils to some kinds of food materials and food. But so far none have dealt with the use of these oils in the whey in order to suppress microbial spoilage and extend the shelf life of whey. There are no many papers with this theme in dairy industry and with these herbal essential oils like compound with preservative effect. Let alone the use of these selected essential oils in the whey. For example Singh *et al.* (2011) studied addition of anise essential oil in yogurt. Yogurt from buffalo's milk was mixed with anise essential oil and its oleoresin (ethanol) at varying concentrations (0.1–1.0 g/L) and they were stored and analyzed. Mixing of essential oil and oleoresin of anise at 1.0 g/L concentration was quite effective in regulation the growth of spoilage microorganisms. Tsiraki *et al.* (2011) compared the effect of basil essential oil (in concentration 0.4% v/w)) and various packaging conditions (under aerobic, vacuum, modified atmosphere) on special Greek whey cheese and cheese was stored. The combined use of either vacuum packaging or modified atmosphere packaging, and basil essential oil, can prolong the shelf life of whey cheese. Menon *et al.* (2001) studied the efficiency activity of clove oil at concentrations of 0.5% and 1% against *Listeria monocytogenes*. The inhibitory activity of clove essential oil was better at a concentration of 1%. Their results showed the potential of clove oil as a natural preservative in meat and cheese.

The aim of this study was to find out whether the addition of different herbal oils into whey will extend its shelf life. Changes in pH, titration acidity (SH) and changes in whey microflora composition during its storage were chosen as parameters for monitoring of whey shelf life.

Whey of different weeks was used, but from the same production. The first samples were prepared from whey with sweet basil or clove or cinnamon. Anise, blue chamomile and common sage were added to the second whey.

MATERIALS AND METHODS

Sweet whey originating in the course of production of fresh cheeses had been used for analyses. Fresh cheeses were prepared by a standard procedure from cow milk (Necidová *et al.*, 2009). Various herbal oils (M + H, Míča a Harašta s. r. o., Czech republic) were added into whey samples: anise – 0.03 g (Elgayyar *et al.*, 2001); sweet basil – 1000 µL (Halley *et al.*, 2001); blue chamomile – 50 µL (Aggag *et al.*, 1972); common sage – 15 µL (Burt, 2004), clove – 200 µL and cinnamon – 50 µL (Oussalah *et al.*, 2007; Du *et al.*, 2009). The total volume was 100 mL. Prepared whey samples were subsequently preserved in a refrigerator at a temperature 9 °C and 78% humidity. There were 25 samples prepared in total, each was analyzed twice. Averages with standard deviations were used to evaluate the results.

Chemical analyses of whey samples were carried out always on the day of the whey production and subsequently on the first, seventh and fourteenth day of storage (anise oil, blue chamomile oil and common sage oil). For samples with sweet basil oil, clove oil and cinnamon oil, the storage period was extended to twenty one days. pH was measured by means of a potentiometer using a pH meter WTW pH 95 (Germany), titratable acidity (SH) has determined by titration of 50 mL of whey by NaOH solution 0.25 $mol \cdot L^{-1}$ concentration with addition of 2 mL of ethanol solution of phenolphthalein to obtain a lightly pink colouring.

In the microbiological analysis the following exquisite groups of microorganisms were also monitored: total plate count of microorganisms (TPC) on PCAM (Plate Count Agar with Skimmed Milk) cultivating medium (incubation at 30 °C for 72 hours); lactic acid bacteria (LAB) on MRS agar (incubation at 30 °C for 72 hours); thermo-resistant (aerobic and anaerobic) bacteria on PCAM cultivating medium (in case of spore-forming bacteria, inhibition of vegetative forms had been achieved by heating at 85 °C for 10 minutes. Incubation of spore-forming anaerobic bacteria took place in anaerobic environment secured by cultivation in containers with anaerocults at a temperature of 30 °C for 48 hours. Incubation of spore-forming anaerobic bacteria took place at a temperature of 30 °C for 48 hours. Psychrotrophic bacteria were determined on PCAM cultivating medium (incubation of psychrotrophic bacteria took place at a temperature of 6.5 °C for 10 days); Enterococci were enumerated on selective medium of Compass Enterococcus Agar (incubation at 45 °C/24 hours; coli-form bacteria on selective medium of VRBL (incubation at 37 °C/24 hours); molds and yeasts on agar with glucose and chloramphenicol (incubation at 25 °C/120 hours). Producer of all the cultivating media was Biokar Diagnostics, France. The bacterial counts were expressed as log CFU·mL^{-1}.

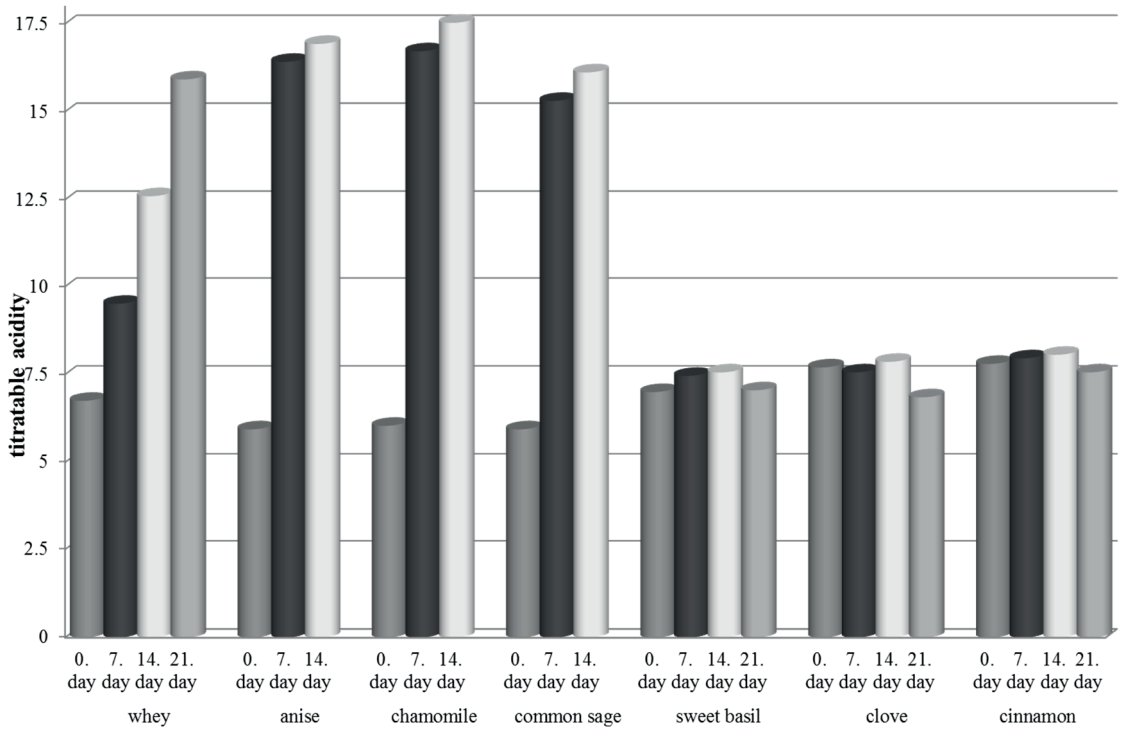
RESULTS AND DISCUSSION

Measurement of pH and Titratable Acidity

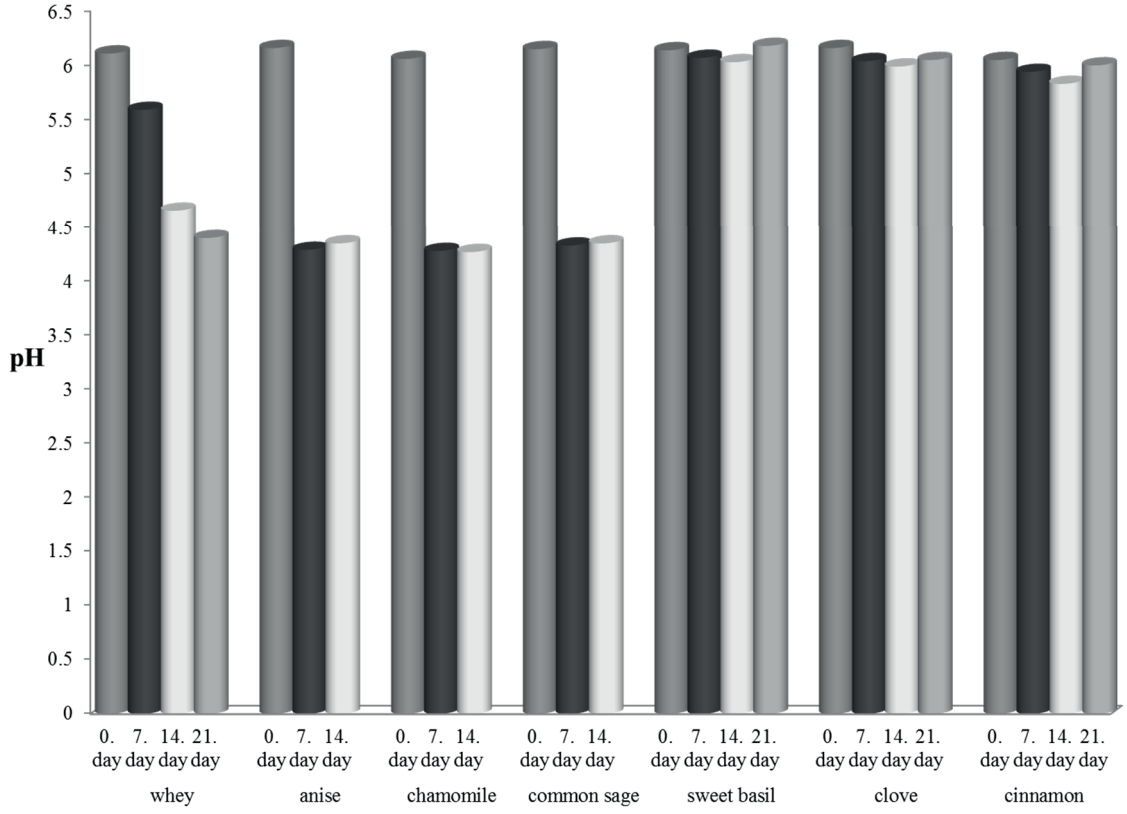
Addition of essential oils of anise, sweet basil, clove and cinnamon into whey was able to extend whey shelf life up to 21 days without material alterations of pH and titratable acidity as is evident from Fig. 1 and Fig. 2, where measured results of pH and SH changes during the storage time are summarized.

Whey treatment with blue chamomile and common sage oils led to increase in titratable acidity after a period of two weeks, which seems to show that application of these herbal oils into fresh whey did not help in extension of its lifetime. Tab. I shows a difference of measured pH and titratable acidity at the beginning and at the end of storage life of individual whey samples without treatment or with treatment using the relevant herbal oil.

These results confirm that pH and titratable acidity of samples without treatment changed markedly during the course of storage life. The same is true for samples with oil of anise, blue chamomile and common sage. However samples of whey with sweet basil oil, clove oil and cinnamon oil did not show any acidity increase during the course of the whole storage period in comparison to whey. The differences whey tested by means of Tukey – B test were not statistically significant. Statistical significance was determined at $P < 0.05$.



1: *Changes of titratable acidity of whey and whey with essential oils during storage after 7, 14 and 21 days*



2: *Changes of pH of whey and whey with essential oils during storage after 7, 14 and 21 days*

I: *The difference of measured pH and titration acidity of whey and whey with essential oils (EO) expressed at the beginning and at the end of storage*

	Difference of pH at the beginning and at the end of storage	Difference of titratable acidity at the beginning and at the end of storage
Whey	0.71	4.64
Whey with:		
anise EO	0.82	5.14
chamomile EO	0.89	5.59
common sage EO	0.84	4.89
sweet basil EO	0.02	0.04
clove EO	0.09	0.32
cinnamon EO	0.10	0.32

For each parameter, the values are not significantly different ($P < 0.05$)

Microbiological Analyses

In the course of storage in a sample of whey without treatment the TPC increased. After fourteen days storage the whey with common sage oil the biggest TPC development suppression was observed. In case of usage of sweet basil oil, clove oil and cinnamon oil, the results are in Tab. II and Tab. III. No microorganisms were deducted for samples with a description ND (= not detected in 1 mL of sample). The development of TPC was observed in samples treated with clove oil and cinnamon oil twenty-first day of storage. The results were statistically conclusive.

The oils of anise, blue chamomile and common sage in whey showed a marked ability to prevent also development of anaerobic thermo-resistant microorganisms. In whey samples with oils of sweet basil, increased counts of these bacteria were not observed for the whole storage period. A similar effect was observed in case of clove oil and cinnamon oil as well.

In the whey samples with sweet basil oil no presence of anaerobic thermo-resistant microorganisms were detected during the whole storage period. In the sample with cinnamon oil, growth of these microorganisms was observed after twenty one days of storage.

II: *The results of microbiological analysis (plate method) of whey and whey with anise, chamomile and sage essential oils during storage*

sample	day	TPC log CFU/mL	LAB log CFU/mL	TMRae log CFU/mL	TMRan log CFU/mL	Psychr. MO log CFU/mL	Micromycetes log CFU/mL
whey	0.	7.78	ND	1.93	ND	ND	0.30
	7.	8.08	3.90	1.40	0.48	1.65	ND
	14.	7.88	ND	1.74	ND	ND	ND
anise	0.	7.77	4.70	0.60	ND	ND	0.30
	7.	7.95	3.08	1.38	ND	2.85	ND
	14.	7.95	ND	1.83	ND	ND	ND
chamomile	0.	7.53	4.83	0.30	1.00	ND	ND
	7.	7.81	3.76	1.54	ND	2.50	ND
	14.	7.98	ND	1.08	ND	ND	ND
sage	0.	7.60	3.96	1.08	0.95	ND	ND
	7.	7.91	1.70	1.08	ND	4.33	ND
	14.	7.33	ND	1.45	ND	ND	0.30

ND = not detected in 1 mL of sample, TPC = total plate count, LAB = lactic acid bacteria, TMRan = thermo-resistant anaerobic microorganisms, TMRae = thermo-resistant aerobic microorganisms, Psychr. MO = psychrotrofic microorganisms, Micromycetes = yeasts and molds

III: *The results of microbiological analysis (plate method) of whey and whey with basil, clove and cinnamon essential oils during storage*

sample	day	TPC log CFU/mL	LAB log CFU/mL	TMRae log CFU/mL	TMRan log CFU/mL	Psychr. MO log CFU/mL	Micromycetes log CFU/mL
whey	0.	6.89	5.23	ND	ND	ND	0.30
	7.	ND	ND	ND	ND	3.71	0.30
	14.	4.94	0.95	2.22	1.11	4.31	1.23
	21.	6.80	3.52	ND	ND	0.70	0.60
basil	0.	6.50	5.32	ND	ND	ND	0.30
	7.	6.48	ND	ND	ND	0.70	ND
	14.	5.03	0.85	ND	ND	4.27	1.65
	21.	6.43	3.42	ND	ND	5.84	ND
clove	0.	7.74	5.48	0.48	0.78	ND	ND
	7.	6.13	ND	ND	ND	3.89	0.48
	14.	3.74	ND	0.60	0.60	1.81	0.85
	21.	5.35	ND	ND	ND	4.88	ND
cinnamon	0.	11.20	5.25	1.15	ND	ND	ND
	7.	7.60	ND	ND	ND	1.48	ND
	14.	5.22	ND	0.79	ND	3.77	1.36
	21.	5.53	3.30	ND	0.48	3.53	ND

ND = not detected in 1 mL of sample, TPC = total plate count, LAB = lactic acid bacteria, TMRan = thermo-resistant anaerobic microorganisms, TMRae = thermo-resistant aerobic microorganisms, Psychr. MO = psychrotrofic microorganisms, Micromycetes = yeasts and molds

After seven days of, micromycetes development was observed only in the sample with no herbal oil and with clove oil. Micromycetes development suppression by sweet basil, clove and cinnamon oils is apparent for the whole storage period. During the whole of twenty one days, micromycetes did not grow in any of these samples.

In all samples, development of LAB was observed already after one day of storage. After seven days of storage increase lower than 10^3 were observed. In the whey sample with no herbal oil and in the whey sample with sweet basil oil a slight LAB increase was observed after fourteen days, a higher LAB increase was observed in these samples after twenty one days. In samples with clove oil LAB development was observed after just one day of storage. Clove oil suppressed the LAB growth.

The biggest influence upon psychrotrophic bacteria development suppression was observed by the oils of sweet basil and cinnamon in working time of seven to fourteen days. On day 21, growth of these bacteria in whey samples with no oil was significantly lowered, in the whey sample with oils of sweet basil and clove it was on the contrary considerably increased in comparison with storage after 14 days.

Efforts to extend the shelf life of whey can also be seen in other papers or 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 so it prolongs its applicability.

CONCLUSION

The aim of the study was to determine whether the addition of different herbal oils to whey extend the shelf life. The growth of TPC, LAB thermo-resistant bacteria, psychrotrophic bacteria and yeasts and moulds was observed during 21 days in the whey after the production of fresh cheese and was measured titratable acidity and pH.

The oils of anise, blue chamomile, sweet basil, clove and common sage were added to the whey. The application of essential oils blue chamomile and common sage did not help to extend the shelf life of fresh whey. The treatment of whey by herbal essential oils scented anise, sweet basil, clove and cinnamon whey extend the shelf life up to 21 days without significant changes in pH and titratable acidity. The whey in combination with tested essential oils could be used for example in spa baths, because in this way would prolong shelf life and simultaneously improve her medical and cosmetic effects.

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