COMPARISON OF ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS AND AQUEOUS EXTRACTS

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ABSTRACT

In this study antimicrobial activity of essential oils and aqueous extracts on microorganisms contained in goat whey was researched. The analyses were realized in four weeks. Tested groups of microorganisms were total plate count (TPC), coliform bacteria, enterococci and aerobic and anaerobic thermo-resistant microorganisms. Essential oils and two types of aqueous extracts (seven-day extract and hour extract) from three herbs were used for analyses – thyme (Thymus vulgaris, L.), peppermint (Mentha piperita, L.) and fennel (Foeniculum vulgare, Mill). Thyme essential oil was the most effective and peppermint essential oil was the least effective. Hour extracts were more effective than seven-day extracts.

Key words: goat whey, bioactive agents, antimicrobial activity, essential oil, aqueous extract

Acknowledgments: This work was supported by the project IGA MENDELU FA TP 9/2013.

INTRODUCTION

Small farms use produced milk mainly to manufacture their own cheese, yoghurt and other dairy products. Whey arises from the cheese production. Whey was considered as a waste product, but now, it is used to a wide range of whey drinks and whey cheese production (Suková I. 2006). Whey without treatment is subjected to microbial spoilage primarily due to high lactose content (Jeličić I. 2008). Therefore, it might be interesting to develop a new food product from whey with the addition of bioactive agents from plants to extend its shelf-life. This product should have been positively perceived by the consumer.

Whey is a by-product obtained during the production of cheese and casein. Therefore, it contains microorganisms originally contained in milk. Total plate count, coliform bacteria, enterococci, aerobic and anaerobic thermo-resistant microorganisms are ranked among important groups of microorganisms, which have an impact on quality of milk and subsequently on whey quality (Fernandes R. 2009). Total plate count is determined by legislation. Exceeding this limit informs us about the lack of hygiene in obtaining milk, insufficient cooling, and errors in storage or possible secondary contamination (Görner F., Valík Ľ. 2004). The group of coliform bacteria include genus *Escherichia*, *Enterobacter*, *Citrobacter* and *Klebsiella* (Marth E., Steele J. 2001). Coliform bacteria can be used as an indicator of pollution. The presence of enterococci in dairy products signifies a lack of sanitary conditions during the acquisition and processing of milk. Deciding enterococci contamination of the milk comes from milking equipment and plant feed (Greifová M. et al. 2003).

Plants are rich in some functional compounds which include phytochemicals, phenols, polyphenols, essential oils (EO) and micronutrients (Tajkarimi M. et al. 2010). For these compounds antimicrobial and antioxidant activity was demonstrated. Phenols influence low concentrations of enzyme activity associated with the energy production. In high concentrations, they cause denaturation of proteins. This means that the antimicrobial activity of phenols contained in EO may be affected by their concentration (Gyawali R., Ibrahim A. 2012). Bajpai et al. (2008) found that antimicrobial activity is caused by phenols ability to modify cell wall permeability causing the loss of macromolecules as well as disruption of cell wall functionality. Previous studies have shown that gram positive (G^+) bacteria are more susceptible to the effects of antimicrobial agents in comparison with gram negative (G^-) bacteria, which is influenced by the outer lipopolysaccharide membrane that is relatively impermeable to the phenolic compounds (Smith-Palmer A. et al. 2001). In conclusion, the phenolic compounds can increase the sensitivity of the phospholipid bilayer cytoplasmatic membrane resulting in increase of its permeability, the unavailability of the necessary intracellular components and in damage of the bacterial enzyme system (Gyawali R., Ibrahim A. 2012).

MATERIAL AND METHODS

Three kinds of herbs were selected for the purposes of this study. They have been tested for their antimicrobial activity – fennel (*Foeniculum vulgare*, Mill.), peppermint (*Mentha piperita*, L.) and thyme (*Thymus vulgaris*, L.). Antimicrobial effects were tested on goat whey, which was obtained from Kozí farma Sedlák in Šošůvka, The Czech Republic.

Observations took place over a period of four weeks. From herbs two types of aqueous extracts – seven-day and hour extract were prepared. Extracts were prepared from powdered herbs, which were bought in specialized shop Léčivé rostliny in Brno, The Czech Republic. Preparation of 7-day extract: 10 g of powdered herbs were added into 100 ml distilled water at room temperature and left to infuse for seven days. Preparation of hour extract: 10 g of powdered herbs were added into 100 ml distilled water at 95 °C and left to infuse for one hour. Infusions were subsequently filtered. Essential oils were bought from two manufacturer – Míča a Harašta and Manipura, The Czech

Republic. Essential oils and extracts were added first day to whey in different concentrations (Tab. 1). Treated whey was stored at 6°C. Microbiological analyses were performed on the second day and then on the each following 7th day of analysis by pour plate method. Overview of determined microorganisms is shown in the Tab. 2 together with the conditions of cultivation. The bacterial counts were expressed as colony forming units (CFU) in 1 ml and logarithm.

Tab. 1: Addition of essential oils and extracts to goat whey

Essential oil/extract	Added volume [µl/100ml]
Fennel	100
Peppermint	2000
Thyme	600
Extracts	5000

Tab. 2: Conditions of cultivation

Microorganisms (MO)	Culture medium	Conditions of cultivation
Total plate count (TPC)	PCA with skimmed milk	30°C, 72 hours
Coliform bacteria (Coli)	VRBL	37°C, 24 hours
Enterococci (Ent)	Compass Enterococcus Agar	44°C, 24 hours
Aerobic (TMRae) and anaerobic (TMRan) thermo resistant MO	PCA with skimmed milk	30°C, 48 hours, TMRan under anaerobic conditions

The manufacturer of the culture media is Biokar diagnostics, France

RESULTS AND DISCUSSION

Counts of microorganisms in whey control samples are shown in the Tab. 3. All essential oils and extracts were not tested on the same sample of whey. Therefore, account must be taken on the fact that each of the tested whey contained different initial numbers of microorganisms, from which microbial counts subsequently developed during testing. Efficacy can-not be compared on the basis of values given in the Tab. 4 and the Tab. 5.

Tab. 3: Counts of microorganisms (MO) in whey without added essential oil/extract [log CFU·ml^{-1}]

Repetition of whey	Day of observation	log N [log CFU·ml^{-1}]				
		Coli	Ent	TMRan	TMRae	TPC
I.	2	3,94	0,96	0,91	0,83	4,28
May	8	3,99	ND	1,41	0,69	6,58
	15	2,56	ND	ND	0,80	6,24
	22	2,44	ND	0,44	1,09	6,03
II.	2	5,05	1,67	1,34	0,44	6,54
June	8	5,80	1,94	1,10	0,66	6,71
	15	5,94	1,23	1,88	1,92	6,85
	22	6,42	1,21	0,80	0,80	7,71

ND = not detected, Coli = coliform bacteria, Ent = enterococci, TMRan = thermo resistant anaerobic MO, TMRae = thermo resistant aerobic MO, TPC = total plate count of microorganisms

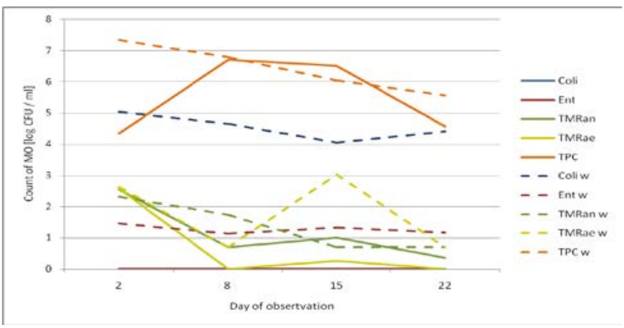
Tab. 4: Counts of microorganisms after the fennel addition [log CFU·ml^{-1}]

Essential oil/extract	Day of observation	log N [log CFU·ml^{-1}]				
		Coli	Ent	TMRan	TMRae	TPC
Essential oil (II)	2	5,22	1,66	1,30	0,44	5,84
	8	5,72	1,58	0,26	0,61	6,49
	15	5,56	1,54	0,56	2,59	6,28
	22	6,33	0,26	0,69	2,77	7,05
7-day extract (II)	2	5,26	2,20	1,38	0,94	6,09
	8	7,00	1,57	1,13	1,51	7,69
	15	6,54	1,49	2,43	2,94	6,99
	22	7,08	1,16	2,61	3,72	7,26
Hour extract (II)	2	4,98	2,20	1,12	1,65	5,94
	8	6,44	2,16	1,99	2,48	7,20
	15	6,51	1,86	1,71	2,48	6,45
	22	7,13	1,91	0,94	0,86	7,49

Tab. 5: Counts of microorganisms after the peppermint addition [log CFU·ml^{-1}]

Essential oil/extract	Day of observation	log N [log CFU·ml^{-1}]				
Essential oil (I)	2	3,27	ND	0,77	1,84	4,56
	8	5,94	ND	ND	ND	7,05
	15	4,59	ND	0,80	0,36	6,38
	22	3,75	ND	3,75	3,92	6,77
7-day extract (II)	2	5,76	1,89	0,66	2,12	6,53
	8	5,93	1,72	1,07	1,31	6,69
	15	8,03	1,26	2,26	3,00	8,93
	22	5,94	ND	2,23	2,20	8,22
Hour extract (II)	2	5,09	2,08	1,67	1,75	6,13
	8	6,22	1,95	1,37	1,50	7,04
	15	6,52	1,33	1,60	1,00	6,54
	22	6,78	1,74	1,67	1,71	7,08

Results of microbiological analyses are summarized in the Tabs. 3, 4 and 5 and in the Figs. 1, 2 and 3. An important indicator of antimicrobial activity is the development of microorganisms and the difference in their numbers towards samples of whey without the addition of essential oils and extracts. Under the title of each plant, from which extract/EO was obtained, there is stated a number of analysis providing particular results. The dynamic of micro-flora in whey with the addition of thyme essential oil or extracts is given in the Figs. 1, 2 and 3.



Coli w = coliform bacteria in control sample of whey, Ent w = enterococci in control sample of whey, TMRan w/TMRae w = = thermo-resistant aerobic/anaerobic MO in control sample of whey, TPC w = total plate count in control sample of whey

Fig. 1: The dynamics of the count of MO in the whey with added thyme essential oil

After the addition of thyme essential oil, the growth of coli-form bacteria and enterococci was significantly inhibited. Although, control samples contained coliform bacteria, samples with the addition of thyme essential oil did not show any of them. The fact proves antimicrobial effect of thyme EO.

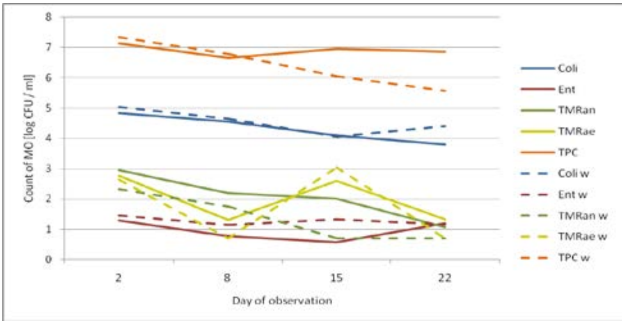


Fig. 2: The dynamics of the count of MO in the whey with added 7-day thyme extract

Microorganisms were not visibly inhibited after addition of 7-day extract; their counts were very similar like in a control sample. Number of some groups of microorganisms has even increased.

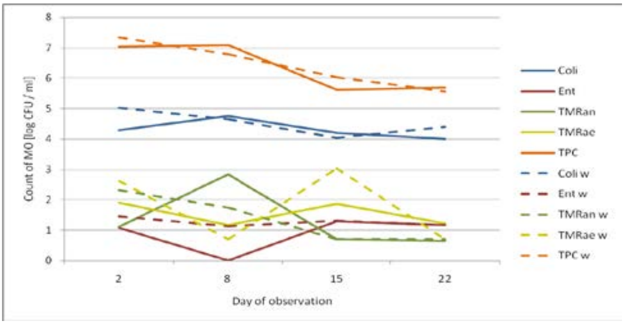


Fig. 3: The dynamics of the count of MO in the whey with added thyme hour extract

Antimicrobial activity of hour extract was apparent in the case of enterococci and thermo-resistant aerobic microorganisms. Other groups of microorganisms were not visibly inhibited, in some groups there was even a short-term increase of their number.

These results show that the best inhibitory effects were reached by essential oils addition, due to the highest concentration of active compounds. Hour extract has been proven to be more effective than 7-day extract. Hour extract inhibited the growth of microorganisms less than EO, but after addition of 7-day extracts, there was an apparent increase of the number of microorganisms compared to the control sample of whey.

The best antimicrobial effects showed thyme, in the case of EOs and both types of extracts as well. These results are confirmed by many studies where antimicrobial activity of thyme is demonstrated. Bouzidi et al. (2012) recommended the possibility of use thyme EO as natural food preservative. Gyawali and Ibrahim (2012) demonstrated colistatic and colicid properties of thyme EO – it irreversibly damages the cells of *E. coli*. Fennel EO decreased the number of microorganisms less significant, the extracts showed no visible antimicrobial effects while the numbers of certain microorganisms were higher than in the control sample of whey. Roby et al. (2012) showed that fennel EO is more effective against G^+ bacteria. The lowest antimicrobial effect was showed by peppermint oil. Better results than the EO of peppermint were reached even both aqueous extracts of thyme. The addition of peppermint EO significantly reduced better *Staphylococcus aureus* numbers than the numbers of *S. enteritidis* (Tassou C. et al. 2000). This is consistent with the fact that G^+ bacteria are more sensitive to the effects of EOs than G^- bacteria, which is probably due to the protective role of the outer membrane of G^- bacteria (Govaris A. et al. 2010).

CONCLUSION

In this work, antimicrobial activity of EOs and aqueous extracts of fennel, peppermint and thyme was evaluated. The most significant inhibitory effects was showed by used concentrations thyme, EO and both extracts. Fennel EO inhibited the growth of microorganisms less than thyme. Fennel extracts showed relatively low antimicrobial effect and the lowest antimicrobial activity was shown by peppermint on observed microorganisms, however, the concentration of peppermint EO was more than three times higher in comparison with thyme EO. These results are one of the first outcomes of a larger experiment devoted to the influence of EOs on the growth of selected microorganisms. It is important to continue in solution of whey conservation due to its considerable production and also due to the increasing trend in food preservation by bioactive substances.

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