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EVALUATION OF SELECTED PARAMETERS OF EDAM TYPE CHEESE PACKED UNDER FOIL WITH NATURAL ANTIMICROBIAL AGENTS

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ABSTRACT
The aim of this study was to evaluate the properties of essential oils packed in foils derived from different plant sources used in Edam type cheese on selected parameters (total viable count of microorganism, coliform bacteria, micromycetes, sensory parameters and instrumental colour). Essential oils have antibacterial and antifungal activities against microorganisms. However, the concentration of these substances applied in cheeses should be considered carefully because of their possible negative influences on sensory parameters. Mixture of the essential oils (clove/cinnamon/thymol in a 1:2:1 ratio), three concentrations (3.9 %, 6.6 %, 9.0 %), respectively mixture of the essential oils (eugenol/thymol/cinnamon in a 1:1:1 ratio), three concentrations (0.10 %, 0.19 %, 0.24 % as a 5% solution in limonene in a dry coating) were used. Samples wrapped in polystyrene dishes were stored in the refrigerator at 3 – 6 °C. Analyses were made after 48 h, 168 h (144 h), 216 h (240 h) respectively. Taste is the most affected by presence of essential oils. The effectiveness of the film with the mixture A seems to be more effective in eliminating microorganisms. Negative sensory changes were observed at higher concentration. Based on the results, the tested foils seem to be promising materials suitable for packaging of cheese.

Keywords: packaging; sensory evaluation; colour; microbiological quality

INTRODUCTION
Active packaging becomes more and more important, rapid progress and growth of applications have been observed because of consumers food preferences **(Khaneghah, Hashemi and Limbo, 2018)**.

Active packaging has appeared as strategy for the control of microbial growth, thereby increasing food quality and safety of the packaged foodstuffs **(Wen et al., 2016)**.

Plants contain many substances that show antimicrobial effects. The most well-known and long-term studied are the essential oils that are carriers of the aromatic properties of many spices and medicinal plants such as cumin, anise, fennel, cinnamon or cloves. Substances as carvone, eugenol, thymol, anethol, phenchon and others are often occurring **(Conner and Beuchat, 1984; Doyle, Beuchat and Montville, 2001)**.

The application of essential oils in the film has been proven to be advantageous for avoiding direct application on the food, thus reducing undesirable interference in sensory parameters. It also allows for a gradual release of antimicrobial compounds on the surface of the packed food, prolonging the time of action/protection, and acting exactly where the risk of contamination is greatest **(Coma, 2008)**.

Active compounds from plants show a different effect on microorganisms. Gram-negative bacteria are more resistant to antimicrobial agents than gram-positive bacteria due to their cellular structure walls, as reported by **Gyawali and Ibrahim (2012)**. Their effectiveness depends on several factors, as pH, temperature, oxygen, concentration and variability of the components **(Tajkarimi, Ibrahim and Cliver, 2010; Burt, 2004)**.

Antimicrobial agents derived from spices are used to reduce the incidence of pathogenic bacteria and improve overall quality, can also be used to prolong the shelf life of food products **(Tajkarimi, Ibrahim and Cliver 2010; Jay, Loessner and Golden, 2005)**.

Milk and dairy products are an important part of the diet. Dairy products cover from 20 to 30% of protein, 15% of lipids and about 80% of calcium from food and positive aspects of milk are enhanced by the used cultural microflora **(Vorlová et al., 2017)**.

Scientific hypothesis
The aim of this work was to test the impact of the spray film mixtures of plant extracts without direct contact with the packaged food on microbiological parameters (Total viable count of microorganism, coliform bacteria, micromycetes), sensory characteristics (colour, odour, consistency, taste) and instrumental colours (L* lightness, a* redness, b* yellowness) of packaged Edam type cheese during storage.

MATERIAL AND METHODOLOGY

Experimental design

Two mixtures of natural antimicrobial agents were prepared for spreyed on foils.

Mixture A: a mixture of the essential oils (clove/cinnamon/thymol in a 1:2:1 ratio), three concentrations (3.9%; 6.6%; 9.0%).

Mixture B: a mixture of the essential oils (eugenol/thymol/cinnamon in a 1:1:1 ratio), three concentrations (0.10%; 0.19%; 0.24% as a 5% solution in limonene in a dry coating).

A layer of 27.5 x 17 cm lacquer coating, corresponding to the area of disposable polystyrene plates for food packaging in which antimicrobial tests were performed, was applied to the film. To the cleaned dishes (alcohol 60%) were added 100 g Edam type cheese, the active foil was glued to the dishes by means of a hot-pistol, the plates were incubated in the refrigerator at 3 – 6 °C for 48 h, 168 h (144 h) and 216 h (240 h).

Microbiological analysis

Microbiological analysis was performed by smear of the surface area of 5 x 5 cm. The following groups of microorganisms were determined for control and specimens taken at the time of establishment of the experiment. Total viable count (TVC) on PCA agar with skimmed milk on cheese samples (Biokar Diagnostics, France) at 30 °C for 72 hours, coliform bacteria on VRBL (Biokar Diagnostics, France) at 37 °C for 24 hours, micromycetes (mould and yeast) to Chloramphenicol glucose agar ((Biokar Diagnostics, France) at 25 °C in 120 hours. The results are shown in CFU x 25 cm^{-2}.

Sensory analysis

Sensory evaluation of cheeses packaged in active foil was performed in the tests of mixtures A and B. The sensory parameters evaluated colour on cut, odour, consistency and taste during the storage time. A sensory evaluation was carried out by 8 evaluators (3 men, 5 women) in special room under ISO 6658:2010 condition in the sensory laboratory of the Department of Food Technology, Mendel university in Brno, Czech Republic. All sensory evaluators buy and consume cheeses regularly. 100 mm line scale ranging from 0 at the left to 100 at the right were used. Descriptors expressed as the hedonic scores, where 0 is unpleasant and 100 is pleasant.

Instrumental colour analysis

The surface colour of cheese was measured on the Konica Minolta Spectrophotometer CM-3500d (Konica Minolta, Japan). The SCE (specular component excluded) mode and 8 mm slot were used. The L* (lightness), a* (redness) and b* (yellowness) were evaluated. Colour variation was determined as total colour difference ΔE^*_{ab} **(Saláková, 2012)**.

Statistic analysis

Data collected from experiments were statistically rated nonparametric Kruskal-Wallis multi-choice test for comparing the treatment by programme STATISTICA 12.

RESULTS AND DISCUSSION

Two mixtures od natural anitimicrobial agents (essential oils) were used in these experiments.

Total viable count of microorganisms

The results of microbiological analysis of mixture A (clove/cinnamon/thymol in a 1:2:1 ratio) in three concentrations (3.9%; 6.6%; 9.0%) are shown in Table 1. The data was compared with control packaging without essential oils.

The microbiological analysis was done during storage time 48 h, 168 h and 216 h. There were found no statistically significant differences (p >0.05) among treatment with different concentration of essential oils in mixture A. The TVC of fresh cheese (before packaging) was 2.1 x 10^4 CFU.cm^{-2}. After packaging, the TVC decreased in all concentration of essential oils.

The result of microbioogical analysis of mixture B (eugenol/thymol/cinnamon in a 1:1:1 ratio) in three concentrations (0.10%; 0.19%; 0.24% as a 5% solution in limonene in a dry coating) are shown in Table 2. The microbiological analysis was done during storage time 44 h, 144 h and 240 h. The TVC of fresh cheese (before packaging) was 4.2 x 10^4 CFU.cm^{-2}. There were found no statistically significant differences (p >0.05) among treatment with different concentration of essential oils in mixture B.

Coliform bacteria

Coliform bacteria were not detected or were detected in minimal CFU in both mixtures. Essential oils containing carvacrol and thymol can decompose the membrane of *E. coli*, facilitating the entrance of strong essential oils components such as eugenol into the *E. coli* and allowing them to interact with the relevant proteins **(Pei et al., 2009)**. **Khaneghah, Hashemi and Limbo (2018)** reported, that antimicrobial packaging technology reduces health risks and improves the safety and quality of food products by reducing or inhibiting microbial growth.

Micromycetes

There were found minimum CFU of micromycetes in mixture A with different concentrations of essential oils during storage time. Higher incidents of micromycetes was detected in cheese packed under foils with mixture B in all concetrations of essential oils. It seems that mixture B is less effective against growth of yeast and moulds. There were not found statistically significant differences (p >0.05) among concentrations or days of storage, respectively. According **Garnier, Valence and Mounier (2017)** more than 60 species of yeast have been identified as spoilage agents of dairy products and about 100 mould species have been identified so far as being responsible for dairy product spoilage. Mould spoilage can also lead to the formation of off-flavors and can also contaminate heat-treated dairy product.

Table 1 Microbiological analysis of Edam type cheese – mixture A (clove/cinnamon/thymol in a 1:2:1 ratio).

		Mixture A			
parameters	**time**	**0% A**	**3.9% A**	**6.6% A**	**9.0% A**
TVC CFU x 25 cm^{-2}	48 h	1.3 x 10^4	8.7 x 10^2	1.3 x 10^4	1.6 x 10^4
	168 h	8.5 x 10^3	1.2 x 10^4	9.3 x 10^3	4.0 x 10^3
	216 h	6.6 x 10^3	5.6 x 10^3	4.6 x 10^3	1.5 x 10^4
coliform bacteria CFU x 25 cm^{-2}	48 h	ND	ND	ND	ND
	168 h	ND	ND	ND	ND
	216 h	ND	ND	ND	ND
micromycetes CFU x 25 cm^{-2}	48 h	3	ND	ND	ND
	168 h	5	ND	1.7 x 10^2	2.8 x 10^3
	216 h	3	1.1 x 10^2	7.3 x 10^2	3

Note: TVC – total viable counts of microorganism, CFU – colony forming units, ND – not detected.

Table 2 Microbiological analysis of Edam type cheese – mixture B (eugenol/thymol/cinnamon in a 1:1:1 ratio).

		Mixture B			
parameters	**time**	**0% B**	**0.11% B**	**0.19% B**	**0.24% B**
TVC CFU x 25 cm^{-2}	48 h	2.1 x 10^5	3.6 x 10^5	1.8 x 10^5	2.1 x 10^5
	144 h	7.7 x 10^4	5.8 x 10^4	1.7 x 10^5	1.5 x 10^5
	240 h	1.5 x 10^6	6.4 x 10^5	9.5 x 10^5	7.9 x 10^5
coliform bacteria CFU x 25 cm^{-2}	48 h	ND	4.5 x 10^1	ND	ND
	144 h	ND	ND	ND	18
	240 h	3	3	ND	ND
micromycetes CFU x 25 cm^{-2}	48 h	1.9 x 10^3	1.1 x 10^2	1.1 x 10^3	6.3 x 10^2
	144 h	6.9 x 10^3	9.2 x 10^3	5.8 x 10^4	3.6 x 10^4
	240 h	6.4 x 10^5	8.5 x 10^5	6.2 x 10^5	1.5 x 10^6

Note: TVC – total viable counts of microorganism, CFU – colony forming units, ND – not detected).

Table 3 Sensory evaluation of Edam type cheese – mixture A (clove/cinnamon/thymol in a 1:2:1 ratio).

		Mixture A			
parameters	**time**	**0% A**	**3.9% A**	**6.6% A**	**9.0% A**
colour	48 h	54 ±13	57 ±10	53 ±15	55 ±14
	168 h	74 ±22	69 ±20	81 ±20	70 ±18
	216 h	81 ±15	71 ±20	85 ±17	75 ±21
odour	48 h	33 ±22	31 ±15	19 ±22	26 ±17
	168 h	81 ±19	30 ±18	47 ±18	21 ±16
	216 h	78 ±16	46 ±16	45 ±11	28 ±11
consistency	48 h	60 ±17	66 ±14	58 ±17	63 ±12
	168 h	66 ±17	46 ±22	65 ±13	61 ±20
	216 h	61 ±15	63 ±9	67 ±8	39 ±20
taste	48 h	35 ±18	41 ±21	21 ±25	30 ±21
	168 h	69 ±22	25 ±26	33 ±19	12 ±14
	216 h	71 ±11	56 ±12	64 ±15	35 ±12

Note: mean ± S. D.

Table 4 Sensory evaluation of Edam type cheese – mixture B (eugenol/thymol/cinnamon in a 1:1:1 ratio).

		Mixture B			
parameters	**time**	**0% B**	**0.11% B**	**0.19% B**	**0.24% B**
colour	48 h	81 ±14	81 ±14	80 ±14	80 ±14
	144 h	79 ±19	75 ±20	78 ±18	75 ±21
odour	48 h	83 ±16	65 ±+á	65 ±18	51 ±25
	144 h	76 ±21	68 ±23	65 ±19	61 ±17
consistency	48 h	81 ±13	79 ±14	76 ±14	66 ±15
	144 h	80 ±17	77 ±16	78 ±15	80 ±12
taste	48 h	84 ±11	70 ±10	53 ±18	29 ±23
	144 h	74 ±16	69 ±13	70 ±14	59 ±15

Note: mean ± S. D.

Table 5 Instrumental colour evaluation of Edam type cheese – mixture A (clove/cinnamon/thymol in a 1:2:1 ratio).

		Mixture A			
parameters	**time**	**0% A**	**3.9% A**	**6.6% A**	**9.0% A**
L*	48 h	83.60 ±0.13^{a}	82.76 ±0.36^{a}	80.38 ±1.18^{a}	81.37 ±1.29^{a}
	168 h	76.86 ±1.83^{b}	79.00 ±1.41^{b,c}	79.05 ±0.93^{a}	79.62 ±0.73^{a}
	216 h	80.56 ±0.48^{c}	80.00 ±0.89^{c}	80.23 ±0.30^{a}	79.08 ±0.62^{a}
a*	48 h	2.16 ±0.40^{a}	1.86 ±0.31^{a}	1.46 ±0.50^{a}	1.26 ±0.43^{a}
	168 h	0.62 ±0.48^{b,c}	1.56 ±0.31^{a}	1.67 ±0.15^{a}	1.05 ±0.10^{a}
	216 h	1.32 ±0.22^{c}	1.49 ±0.24^{a}	1.91 ±0.04^{a}	1.98 ±0.19^{a}
b*	48 h	28.06 ±0.41^{a}	28.09 ±0.80^{a}	29.17 ±1.02^{a}	28.86 ±0.59^{a}
	168 h	29.90 ±0.62^{b}	31.35 ±1.23^{b}	31.17 ±0.84^{b}	29.01 ±0.45^{a}
	216 h	29.42 ±0.24^{a}	29.75 ±0.96^{a}	31.09 ±0.31^{a}	33.19 ±0.64^{b}

Note: L* – lightness, a* – redness, b* – yellowness, mean ± S.D., different superscripts in the same columns show significant differences (p <0.05).

Table 6 Instrumental colour evaluation of Edam type cheese – mixture B (eugenol/thymol/cinnamon in a 1:1:1 ratio).

		Mixture B			
parameters	**time**	**0% B**	**0.11% B**	**0.19% B**	**0.24% B**
L*	48 h	82.67 ±0.17^{a}	82.13 ±0.41^{a}	83.97 ±0.04^{a}	81.96 ±0.15^{a}
	144 h	83.04 ±0.30^{b}	82.50 ±0.79^{b}	83.57 ±0.54^{a}	83.97 ±0.23^{b,c}
	240 h	83.46 ±0.47^{b}	83.99 ±0.77^{c}	83.43 ±0.27^{a}	83.69 ±0.44^{c}
a*	48 h	2.58 ±0.46^{a}	3.03 ±0.06^{a}	2.56 ±0.11^{a}	2.58 ±0.35^{a}
	144 h	3.24 ±0.27^{a}	3.23 ±0.23^{a}	2.74 ±0.16^{a}	2.76 ±0.41^{a}
	240 h	2.75 ±0.12^{a}	2.74 ±0.35^{a}	2.67 ±0.35^{a}	2.69 ±0.14^{a}
b*	48 h	29.92 ±0.42^{a}	29.87 ±0.38^{a}	29.25 ±0.40^{a}	30.24 ±0.36^{a}
	144 h	30.64 ±0.37^{a,b}	30.53 ±1.05^{a,b}	28.79 ±0.68^{a}	29.77 ±0.85^{a}
	240 h	28.55 ±0.04^{a,c}	28.78 ±0.57^{a,c}	28.79 ±0.26^{a}	28.48 ±1.00^{a}

Note: L* – lightness, a* – redness, b* – yellowness, mean±S.D., different superscripts in the same columns show significant differences (p <0.05).

Sensory analysis

The data collected during sensory evaluation of cheese packed under foil with mixture A of essential oils are shown in Table 3.

Colour on cut of cheese before packaging was according sensory evaluators 64 ±15. Sensory assessors judged cheese colour after 48 h worse than cheese before packaging in all essential oil concentration. The best colour evaluation had cheese with concentration 6.6% of essential oils during time of storage. Odour of cheese before packaging was 77 ±14. The best odour evaluation was for cheese packed without essential oils and the worst wit concentration of essential oils 9.9%. The use of essential oils had negative effect on odour, it corresponded with **Garnier, Valence and Mounier (2017)**.

No statistically significant effect (p >0.05) on consistency was found for all concentratin of essential oil. Consistency of cheese before packaging was 67 ±17, after 216 h of storage time the worst evaluation had 9.9% of essential oils on foil. Taste is the most affected by presence of essential oils. Before packaging was 70 ±13 and the worst evaluation was for concentration 9.9% of essential oils during storage time. Essential oils have intensive aroma, application at high concentrations could result in sensory defects **(Khorshidian et al., 2018)**.

The data collected during sensory evaluation of cheese packed under foil with mixture B of essential oils are shown in Table 4. Sensory analysis was performed only after 48 h and 144 h due to deteriorative microbiological parameters.

No statistically significant effect (p >0.05) on colour and consistency was found for every concentration of essential oils. The worst evaluation was for concentration 0.24% of essential oils during storage time.

Characteristic of sensory properties of cheese, i.e. appearance, colour, taste and aroma and texture, are the result of running biochemical process primarily during ripening of cheeses **(Vítová et al., 2011)** and during packaging.

Instrumental colour measurement

The results of instrumental colour are presented in Table 5 and Table 6. Colours of cheese may scope from pale yellow to deep red-orange, depending upon the application and consumer preference **(El-Nimr et al., 2010)**.

There were found statistically significant differences (p >0.05) during storage time. Minor differences were noted in use mixture B. Colour at the beginning of experiment range from L* 81.69, a* 2.13, b* 28.98 (mixture A) to L* 83.53, a* 2.70, b* 27.50 (mixture B). After 168 h were found colour difference ΔE^*_{ab} from 2.33 to 5.14, the highest colour difference was found in concentration 9.9% (mixture A) after 216 hours of storage. Statistically significant differences (p <0.05) in L* value between concentrations of mixture A at time of storage 168 h. After 144 h were found colour differences ΔE^*_{ab} from 0.55 to 1.36.

It is clear that the lightness decreased during storage and the yellowness b* increased during storage for use of mixture A. When using mixture B, the opposite trend was found.

CONCLUSION

These films appear to be promising materials for packaging cheese. However, it is necessary to confirm this other test focusing primarily on the choice of the appropriate concentration of the active substance and possible negative effects on the sensory properties of the food. The effectiveness of the film with the mixture A (clove/cinnamon/thymol in a 1:2:1 ratio), seems to be more effective in eliminating microorganisms. Negative sensory changes were observed at higher concentration.

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