Oleoresins effect on cooked poultry sausages microbiological stability

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Abstract

Introduction. The effect of coriander, mace and black pepper oleoresins on microbiological stability of a cooked poultry sausages during refrigeration storage was investigated.

Materials and methods. Cooked poultry sausages with different part non-meat raw material were examined. Microbiological attributes, such as Quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms (QMAFAnM), coliforms, *Salmonella*, Sulfite-reducing clostridia, *Proteus, Listeria monocytogenes*, *Staphylococcus aureus*, yeasts and molds were determined methods accepted in general lines.

Results and discussion. There was no significant difference between initial QMAFAnM for all samples after the thermal treatment (day 0). QMAFAnM for CO samples increase during entire research. At the end of storage, the count was increased to $1{,}1–8{,}5\times10^5$ cfu/g and was significantly higher than the other samples, except CO40. The QMAFAnM in MO samples was initially $1{,}0\times10^1–1{,}5\times10^2$ cfu/g and was maintained at this level until the seventh day of storage. However, after 13 days the increase of QMAFAnM was significant. Sausages with BPO showed stable meaning of QMAFAnM during storage time. After 13 days of storage, the BPO samples contributed to significantly lower QMAFAnM count than the CO and MO samples. The initial population of moulds was <10 cfu/g while on day 4 of storage a count of $2{,}5–7{,}0\times10^1$ cfu/g was recorded for treatments with CO. MO and BO samples demonstrated stable meaning of moulds during entire research, only on 13th day of storage BPO100 and MO60 samples showed $2{,}0\times10^1$ and $2{,}5\times10^1$ cfu/g respectively. Yeasts from MO and BPO samples did not differ after 7 d of storage, but were significantly lower than counts from CO samples. Yeasts from CO samples increased during entire storage. Sausages with MO showed stable meaning of yeasts during storage time. Initial meaning of BPO80 yeasts was $3{,}0\times10^1$ cfu/g, although on 7th day of storage inhibition of yeasts was observed. The samples with BPO and MO had lower yeasts counts than CO samples during the entire storage period.

Conclusions. Mace oleoresin and black pepper oleoresin have more antimicrobial activity than coriander oleoresin. Only black pepper oleoresin has shown antimicrobial effect during refrigeration storage more than 10 days. In processing meat containing products with oleoresin it's necessary to make accent on black pepper oleoresin addition.

Introduction

Qualitative characteristics of meat products preservation during storage is one of important task and is vital for meat industry.

The object of the present research was to study the coriander (CO), black pepper (BPO) and mace (MO) oleoresins effect on the microbiological stability and the shelf-life of a cooked chicken meat (more than 60% of meat in formulation) and meat containing (less than 60 % of meat in formulation) products.

Spice oleoresins application for food, in particular meat products analysis

In the last decade, chicken-based meat products have become increasingly popular worldwide due to their high nutritional quality and low cost and are available as either fresh or precooked (i.e. fried) chicken products, which after subsequent packaging are usually stored under refrigeration [1]. Additionally, frozen chicken-based meat products also available on the market include specialties such as: nuggets, meatballs, hamburgers, frankfurters, etc. Susceptibility of chicken meat and chicken-based meat products to microbial spoilage presents a potential health hazard, since poultry meat may harbor pathogenic microorganisms [2].

Poultry and poultry products are a highly perishable food and their shelf-life varies between 3 and 10 days under refrigeration. Deterioration depends mainly on the microbiological quality of the poultry carcasses, as poultry meat offers the perfect environment, pH, nutrients and humidity conditions for microorganism development.

The use of natural preservatives in foods has been widely accepted by consumers, who increasingly seek for natural and healthier products, free of synthetic additives [3, 4]. In addition, consumers are used to the presence of herbs and spices commonly added to provide flavor and aroma in meats.

Black pepper and coriander are the most widely spread in meat products processing, unlike mace.

The quality of black pepper depends on the contents of piperine and essential oil. Both pepper and piperine exert liver protective action. Kaul and Kapil found that piperine reduces *in vitro* and *in vivo* lipid peroxidation [5]. This is a very significant property, as lipid peroxidation causes free radical production that causes tissue damage. Pepper has antioxidant activity which is attributed to the tocopherol and polyphenol contents in pepper. Supercritical carbon dioxide extracts of ground black pepper have been found superior in reducing lipid oxidation of cooked ground pork [6]. The antioxidative activity of black pepper can, at least partially, be ascribed to the presence of glycosides of the flavonoids kaempherol, rhamnetin and quercetin [7], as well as to the phenolic amides. Nakatani *et al.* established that all the five phenolic amides present in pepper possess very good antioxidant property, which is even superior to that of the synthetic antioxidants like butylated hydroxy toluene and butylated hydroxy anisole [8]. Addition of pepper to foods increases their keeping quality and prevents their spoilage, due to the antimicrobial properties of pepper. The essential oil of pepper is found to be inhibitory to *Vibrio cholerae*, *Staphylococcus albus, Clostridium diphthereae, Shigella dysenteriae*, *Streptomyces faecalis*, *Bacillus* spp., *Pseudomanas* spp., etc. Pepper oil stopped the growth and aflatoxin production by *Aspergillus parasitics* at a concentration of 0,2–1%. Pepper leaf oil also exhibits antifungal activity.

The ethanol extract of *Coriandrum sativum* leaves is an excellent antioxidant, which is stable at high temperature and can serve as a substitute for synthetic antioxidants [9]. Further studies carried out by Melo *et al*. indicated that the four coriander extract fractions obtained from the crude extract using chromatography in silica gel possessed similar antioxidant activities, which can be measured by the β-carotene/linoleic acid system. The antioxidant activity was due to several phenolic acids and caffeic acid, which were present in all four fractions [10].

The greater antioxidant effect of a crude extract of coriander compared to its component fractions suggested a synergistic action between the carotenoids. Assessment of the total antioxidant activity of methanol and water extracts coriander leaves and stems using an iron-induced linoleic acid oxidation model system showed that the methanol-derived leaf extracts exhibited significantly greater radical-scavenging activity towards both lipid- and water-soluble radicals, which was attributed to the total phenolic content [11].

Coriander has strong antibacterial activity against *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* [12]. In 2002, a study carried out by Delaquis *et al*. reported that coriander oil strongly inhibited gram-positive bacteria (*Listeria monocytogenes* and *S. aureus*), but had little effect against gramnegative bacteria (*Pseudomonas fragi*, *E. coli*, *S. typhi*) [13].

Mace possess antioxidant properties. Checker *et al*. reported that the lignans present in the aqueous extract of fresh mace also possess antioxidant properties [14]. Acetone extract of mace-containing lignans inhibited lipid oxidation and prevented oxidative damage to cells [15]. Mace oil is inhibitory to the growth of *A. parasiticus* and *F. moniliforme* [16] and prevented the formation of aflatoxins.

Herbs and spices, and the oleoresins and essential oils extracted from them, are widely recognized as powerful agents for the preservation of food quality. Spices and herbs, in addition to contributing taste and aroma to foods, also contain a variety of bioactive substances which are of considerable use from the standpoint of food science and technology. These may be used singly or in combination, and some act synergistically to control spoilage of foods [17]. Their use has been well documented in terms of their ability to increase safety and shelf-life of pork, beef and poultry products through their antimicrobial [18, 19, 20] and antioxidant [21, 22, 23] capacities.

Therefore, oleoresins can be considered a good choice of natural preservatives for meat and meat products.

Oleoresin is a concentrated form of the spice containing the volatile essential oils as well as non-volatiles such as fixed oils, antioxidants, and pigments materials.

Oleoresins contain all of the volatile and nonvolatile flavor components and the natural antioxidants of the spices. In comparison to the ground spices, they are hygienic and can be standardized for acceptable flavour levels by blending. Unlike the essential oils, oleoresins contain natural antioxidants of the corresponding spices, which make them more stable. Oleoresin contains essential oils that make up the aroma, oleoresin also contains resins and compounds that did not volatile determine the characteristic flavor of spices. Moreover, the resin part in the oleoresins acts as natural fixatives to more volatile components. Oleoresins are quite concentrated and have good replacement value.

However, despite these advantages over ground spices, spice oleoresins exhibit sensitivity to light, heat and oxygen, and have short storage lives if not stored properly.

The process used for extraction depends on the nature of vegetable matter, and depending on its thermal instability, the operating temperature ranges from ambient to the boiling point. Oleoresin extraction is generally done with organic solvents, such as acetone, ethanol, methanol, hexane, ether and isopropyl alcohol. The choice of solvent affects the

quality and quantity of oleoresin obtained. Oleoresins were used in food processing safer extracted using ethanol solvent [24]. Extraction with polar solvents such as ethanol will be produced oleoresin with a low fat content.

In recent years, many researchers have evaluated the antioxidant properties of oleoresins from different spice and herbs [25, 26]. Oleoresin has been studied for its antimicrobial activity [27, 28]. Rosemary and onion oleoresins showed antioxidant effects in both raw and cooked irradiated pork loins. When these oleoresins were used in combination with α-tocopherol, the antioxidant activity was more distinct in irradiated cooked pork. Rosemary oleoresin–tocopherol had stronger antioxidant effect than onion–tocopherol [29]. The incorporation of nanoparticle paprika oleoresin in to meat using carrier system, demonstrated that the marinating performance and sensory acceptability of marinated meat products can be improved and optimized by the utilization of nanoparticle ingredients in marinating operations [30].

It appears a meat containing products with large part non-meat raw material problem shelf-life and ensuring microbiological deterioration.

Materials and methods

The present study focused on the monitoring of the following species of micro-organisms: Quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms (QMAFAnM), coliforms, *Salmonella*, Sulfite-reducing clostridia, *Proteus, Listeria monocytogenes*, *Staphylococcus aureus*, yeasts and molds.

After the preparation of each chicken batter, oleoresin was added according to the following formulations (Table 1).

Table 1

Formulation of poultry sausages with coriander, mace and black pepper oleoresins

	CO 100	**BPO 100**	**MO 100**	**CO 80**	**BPO 80**	**MO 80**	**CO 60**	**BPO 60**	**MO 60**	**CO 40**	**BPO 40**	**MO 40**
Poultry	100	100	100	80	80	80	60	60	60	40	40	40
Soyabean meal	–	–	–	10 (2%)	10 (2%)	10 (2%)	20 (4%)	20 (4%)	20 (4%)	30 (6%)	30 (6%)	30 (6%)
Protein	–	–	–	10	10	10	20	20	20	30	30	30
Water	30	30	30	30	30	30	30	30	30	30	30	30
Salt	2,2	2,2	2,2	2,2	2,2	2,2	2,2	2,2	2,2	2,2	2,2	2,2
Oleoresin	0,1	0,1	0,1	0,1	0,1	0,1	0,15	0,15	0,15	0,2	0,2	0,2
Smoke	–	–	–	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2

Minced poultry from each treatment was formed into sausages using a meat former. The sausages were boiled in a vessel at a water temperature of 75±1 ºC until a core temperature of 71±1 ºC was reached. After cooling in iced water, the sausages were stored at 5±1 ºC for 13 days.

Microbiological analysis of chicken sausages was carried out on days 0, 4, 7 and 13 of storage. At each sampling day, three independent samples from three different manufactured date for each treatment were analyzed.

10 g of each sample were aseptically placed into a stomacher bag. Afterward, 90 ml of Peptone Saline Solution (PSS) was added and homogenized using a stomacher for 60 s at

room temperature. Serial 10-fold dilutions were prepared by diluting 1 ml of homogenate in 9 ml of PSS.

Serial decimal dilutions were inoculated (1 ml) onto nutrient agar for QMAFAnM and onto Sabouraud agar for yeasts and molds. Plates were incubated at 30 ± 1 °C for 72 h for QMAFAnM and 24 ± 1 °C for 120 h for yeasts and molds.

Coliforms were determined on nutrient medium Kessler after incubation at 37±1ºC for 48 hours. After that, one loop of nutrient medium was streaked onto the surface of Endo agar and incubated at 37±1 ºC for 48 h.

Staphylococcus aureus was determined on Saline Solution after incubation at 37±1ºC for 48 hours. After that, was streaked on Baird Parker agar and incubated at 37±1 ºC for 48 h.

Sulfite-reducing clostridia were determined on Iron sulfite agar (Wilson-Blair) after incubation at 37±1ºC for 3 days.

Proteus was determined on nutrient broth. After incubation at 37ºC for 48 h the one loop was streaked on nutrient agar and incubated at 37 ºC for 48 h.

Presence of *Listeria monocytogenes* was determined by suspending 25 g of sausage into 225 ml enrichment medium with reduced concentration of selective agents (half-Fraser broth) with incubation at 30±1 ºC for 24 h. Then one loop was re-seeding in a selective liquid enrichment medium with full concentration of selective agents (Fraser broth) and streaked on PALCAM agar and incubated at 37±1 ºC for 48 h. After incubation Fraser broth one loop was streaked on PALCAM agar and incubated at 37±1 ºC for 48 h.

Presence of *Salmonella* was determined by suspending 25 g of sausage into 225 ml buffered peptone water followed by incubation at 37±1 ºC for 20 h. Then the culture was re-seeding on Rappaport-Vassiliadis medium and incubated at 37±1 ºC for 24 h. After that, one loop was streaked onto the surface of two selective solid media: Brilliant green agar and bismuth sulphite agar, both incubated at 37±1 ºC for 48 h.

After incubation, two plates with nutrient agar and Sabouraud agar for each sampling point were counted. Results were expressed as a number of colony forming units per gram (cfu/g). The article contains average meaning of three independent samples from three different manufactured date for each treatment. All plates were examined visually for typical colony types and morphological characteristics that were associated with each growth medium.

The lowest detection limit for QMAFAnM, yeasts and molds analysis was <10 cfu/g, for coliforms, *Staphylococcus aureus*; *Proteus*; Sulfite-reducing clostridia; *Salmonella* and *Listeria monocytogenes* the detection limit of which were absence in 1,0; 0,1; 0,01; 25 g, respectively.

Results and discussion

The changes in the QMAFAnM during storage are shown in Figs. 1, 2, 3 and 4.

There was no significant difference between initial QMAFAnM for all samples after the thermal treatment (day 0). It characterizes homogeneity of the initial condition.

QMAFAnM for CO samples increase during entire research. At the end of storage, the count was increased to 1,1-8,5×10^5 cfu/g and was significantly higher than the other samples, except CO40. It is attributable to low functional effect of this oleoresin component.

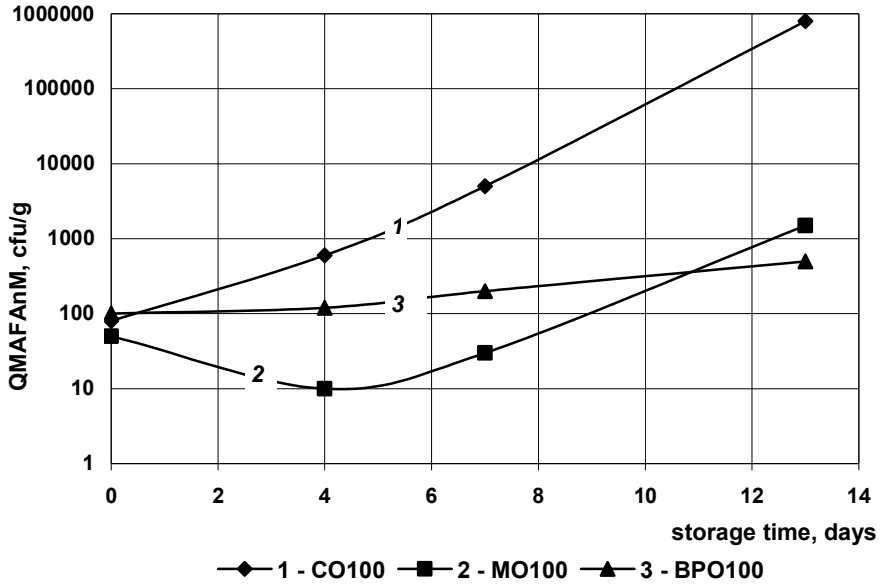

Fig. 1 Changes in QMAFAnM in cooked poultry sausage with 100% of meat stored under refrigeration (4±1 °C) during 13 days

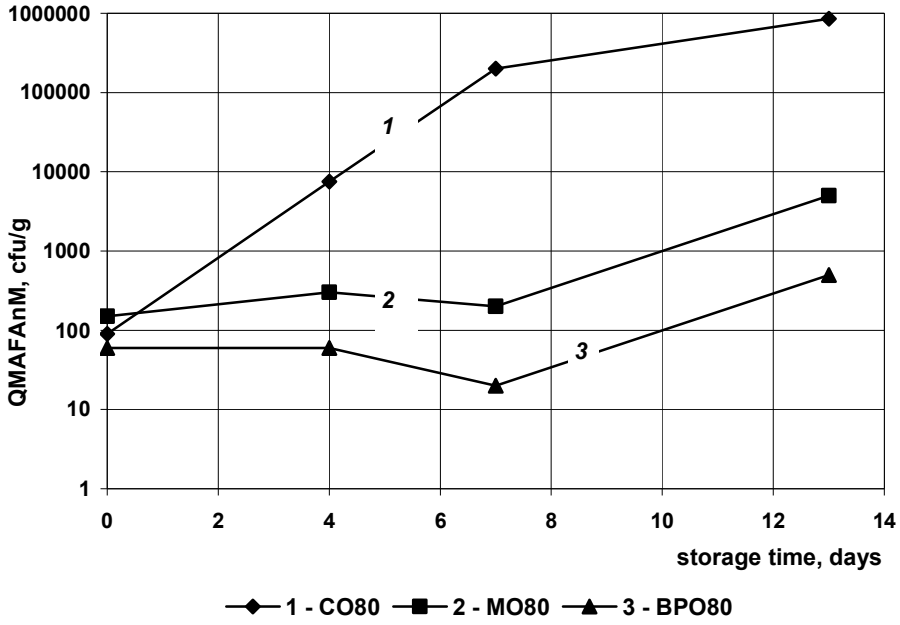

Fig. 2 Changes in QMAFAnM in cooked poultry sausage with 80% of meat stored under refrigeration (4±1 °C) during 13 days

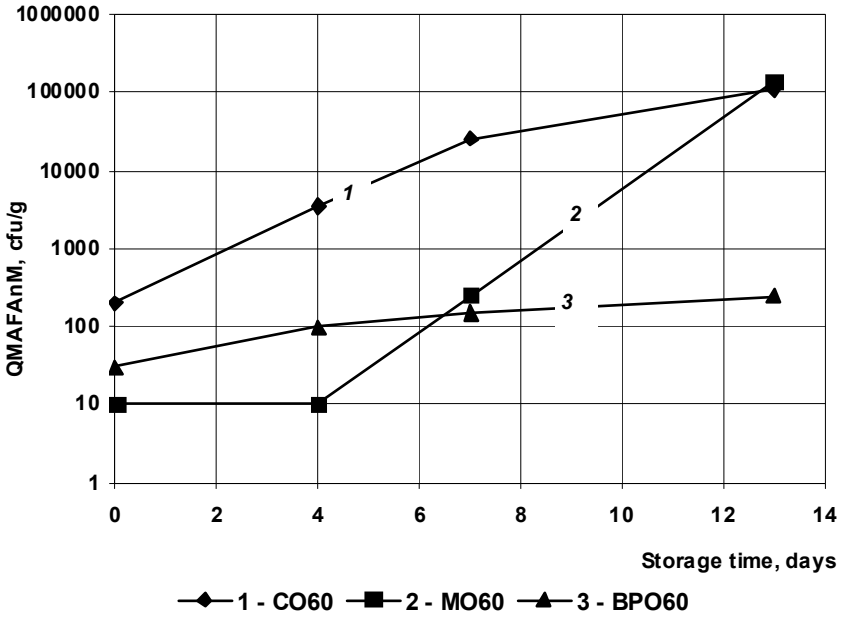

Fig. 3 Changes in QMAFAnM in cooked poultry sausage with 60% of meat stored under refrigeration (4±1 °C) during 13 days

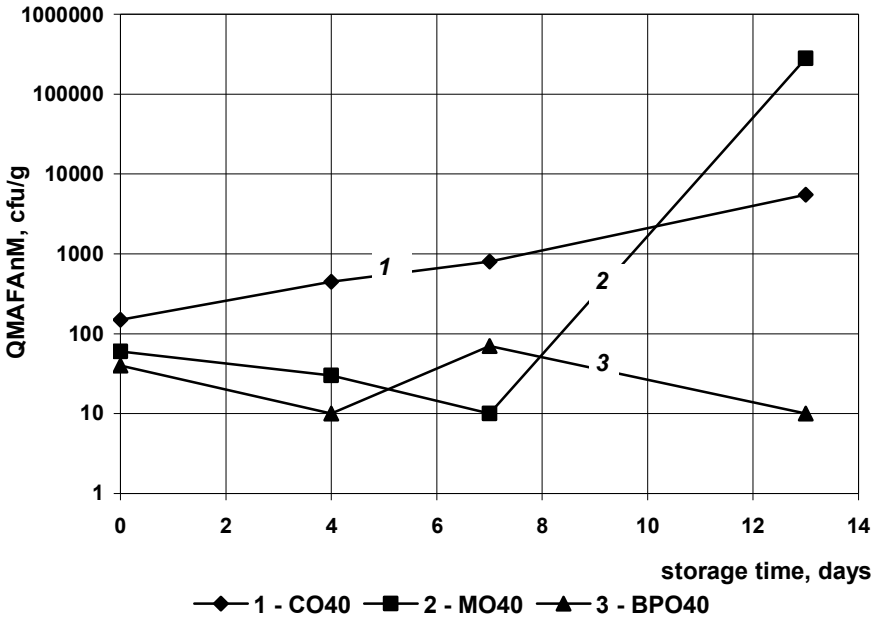

Fig. 4 Changes in QMAFAnM in cooked poultry sausage with 40% of meat stored under refrigeration (4±1 °C) during 13 days

The QMAFAnM in MO samples was initially $1,0\times10^1$-$1,5\times10^2$ cfu/g and was maintained at this level until the seventh day of storage. However, after 13 days the increase of QMAFAnM was significant and was more expressive in meat-containing samples than in the meat products. QMAFAnM shift character with MO addition shows this oleoresin as possible bacteriostatic agent.

Numbers QMAFAnM recovered from meat and meat-containing samples treated with MO and BPO were not significantly different from the numbers recovered from sample with 100% meat raw material.

Mace oleoresin and black pepper oleoresin were more effective than coriander oleoresin on reducing QMAFAnM.

After 13 days of storage, the BPO samples contributed to significantly lower QMAFAnM count than the CO and MO samples.

Sausages with BPO showed stable meaning of QMAFAnM during storage time, that pointed to BPO bactericidal effect and afford its recommendation as basic component for oleoresins mix, including meat containing products processing. It's approved by the fact that QMAFAnM was significantly lower for BPO40 samples as compared to all other treatments during storage.

Of the treatments examined in the present study, black pepper oleoresin was the most effective for the inhibition of QMAFAnM during storage.

The initial population of moulds (Table 2, 3) was <10 cfu/g while on day 4 of storage a count of $2,5$-$7,0\times10^1$ cfu/g was recorded for treatments with CO.

Table 2

Changes of moulds in meat products during refrigeration storage

Days of storage	Moulds, cfu/g					
	CO100	**BPO100**	**MO100**	**CO80**	**BPO80**	**MO80**
0	<10	<10	<10	<10	<10	<10
4	$2,5\times10^1$	<10	<10	$5,5\times10^1$	<10	<10
7	<10	<10	<10	$3,2\times10^2$	10	<10
13	<10	$2,0\times10^1$	<10	$1,0\times10^3$	<10	<10

Table 3

Changes of moulds in meat-containing products during refrigeration storage

Days of storage	Moulds, cfu/g					
	CO60	**BPO60**	**MO60**	**CO40**	**BPO40**	**MO40**
0	<10	<10	<10	<10	<10	<10
4	$5,0\times10^1$	<10	<10	$7,0\times10^1$	<10	<10
7	$3,0\times10^1$	<10	<10	<10	<10	<10
13	$3,0\times10^1$	<10	$2,5\times10^1$	$4,0\times10^1$	<10	<10

CO samples demonstrated moulds increase during storage, so coriander oleoresin did not inhibit moulds.

MO and BO samples demonstrated stable meaning of moulds during entire research, only on 13th day of storage BPO100 and MO60 samples showed $2,0\times10^1$ and $2,5\times10^1$ cfu/g respectively.

The development of the yeasts for the samples is shown in Table 4, 5.

Table 4

Changes of yeasts in meat products during refrigeration storage

Days of storage	Yeasts, cfu/g					
	CO100	BPO100	MO100	CO80	BPO80	MO80
0	<10	<10	<10	<10	$3,0\times10^1$	<10
4	$3,0\times10^1$	10	<10	$2,3\times10^2$	$2,5\times10^1$	10
7	$1,0\times10^2$	<10	<10	$1,5\times10^2$	<10	<10
13	$1,5\times10^3$	10	<10	$4,5\times10^3$	<10	10

Table 5

Changes of yeasts in meat-containing products during refrigeration storage

Days of storage	Yeasts, cfu/g					
	CO60	BPO60	MO60	CO40	BPO40	MO40
0	$2,0\times10^1$	<10	<10	<10	<10	<10
4	$9,0\times10^1$	<10	<10	$8,0\times10^1$	<10	<10
7	$5,5\times10^2$	<10	<10	<10	10	10
13	$5,5\times10^2$	$2,0\times10^1$	10	$3,5\times10^1$	$2,5\times10^1$	<10

Yeasts from MO and BPO samples did not differ after 7 d of storage, but were significantly lower than counts from CO samples.

Yeasts from CO samples increased during entire storage.

Sausages with MO showed stable meaning of yeasts during storage time.

Initial meaning of BPO80 yeasts was $3,0\times10^1$ cfu/g, although on 7th day of storage inhibition of yeasts was observed.

The samples with BPO and MO had lower yeasts counts than CO samples during the entire storage period.

Concerning yeasts and moulds, no significant differences between all treatments with BPO and MO were observed.

Mace oleoresin and black pepper oleoresin was a lot more effective in reducing yeast and moulds populations than coriander oleoresin.

The sample with CO showed a higher QMAFAnM, yeasts and molds count than the samples with MO and BPO. The mentioned microbial groups are considered as spoilage microorganism, and their presence in high amounts could affect the organoleptic properties of the samples. The relatively high population of yeasts and molds also may cause the formation of slime and greening on the sample surface. Therefore, it confirmed more powerful inhibitory action of oleoresins on QMAFAnM as well as yeasts and molds development.

In this work, the presence of coliforms, *Salmonella*, Sulfite-reducing clostridia, *Listeria monocytogenes*, *Proteus, Staphylococcus aureus* were not detected in any sausage samples, regardless of storage time (data not shown).

Conclusions

Meat and meat containing systems with coriander oleoresin demonstrated rapid increase of QMAFAnM, moulds and yeasts during entire research, so coriander oleoresin application in meat-containing products processing as microbial stabilizing agent is not recommended.

Meat systems with mace oleoresin kept microbiological stability during 10 days, meat containing products kept microbiological stability during 8 days. That's why for meat and meat containing systems with mace oleoresin could be recommended shelf life 7 and 5 days respectively.

Samples with black pepper oleoresin kept microbiological stability during all storage time. For meat and meat containing systems with black pepper oleoresin could be recommended shelf life 10 days.

Mace oleoresin and black pepper oleoresin have more antimicrobial activity than coriander oleoresin.

Only BPO has shown antimicrobial effect during refrigeration storage more than 10 days.

Offered shelf life for samples containing mace and black pepper oleoresins is in accord with trivial recommendation for cooked sausages.

In processing meat containing products with oleoresins mix inhibiting microorganisms growth it's necessary to make emphasis on black pepper oleoresin addition.

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