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Microbiological and Sensory Aspects of Kojic Acid Added to Smoked Meat Products

Mikrobiološki i senzorski aspekti dodatka kojične kiseline dimljenim mesnim proizvodima

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Summary

In this work, the additive effect of two different kojic acid mass fractions (100 and 500 ppm) on selected groups of microorganisms (total number, coliforms, lactic acid bacteria, aerobic sporeforming bacteria, yeasts and moulds) in the smoked meat product called »Bratislavská« sausage during its fermentation and storage (50 days) has been studied. The results have shown that the applied kojic acid mass fractions had no significant influence on the microorganisms investigated with regard to their contents in the control sample (except for the partial effect on moulds). The positive effect of this additive manifested itself by stabilizing the investigated meat product colour.

Sažetak

U radu je opisan utjecaj dodavanja dvaju različitih masenih udjela kojične kiseline (100 i 500 ppm) na odabrane skupine mikroorganizama (ukupne mikroorganizme, koliformne bakterije, bakterije mliječno-kiselog vrenja, aerobne sporulirajuće bakterije, kvasce i plijesni) u dimljenom mesnom proizvodu »Bratislavskoj« kobasici tijekom zrenja i čuvanja (50 dana). Rezultati pokazuju da, u usporedbi s kontrolnim uzorkom, primijenjeni maseni udjeli kojične kiseline nisu imali značajnog utjecaja na mikroorganizme (osim djelomičnog utjecaja na plijesni). Pozitivan učinak dodavanja kojične kiseline iskazao se u stabiliziranju boje proučavanog mesnog proizvoda.

Introduction

In recent years, an increased interest in biological substances involving also kojic acid and its derivatives has come to the fore. Kojic acid (KA) is the secondary metabolite of some types of fungi of the genera *Aspergillus* and *Penicillium*. It is produced in the aerobic fermentation process on various carbon sources (pentoses, hexoses, polysaccharides, etc.) *(1)*. The acid is found in the natural form in oriental fermented foods (meat, saké, soya sauce, etc.) having its characteristic taste and flavour *(2–4)*. Both the kojic acid and its halogenderivatives (chloro-, bromo-, iodo-) have a wide spectrum of action (antibacterial, antifungal, antiprotozoal, insecticidal, antioxidative), which offers a great possibility for various applications (agriculture, human medicine, cosmetics, food industry) *(1)*.

The utilization of kojic acid and its derivatives, the biodegradability without undesirable effects on living organisms and on the ecological system, and the low or no mutagenicity *(1,6)* have initiated the more profound study of this problem in order to implement, on an industrial scale, the before – mentioned applications in our conditions as well. Till now, in our country careful attention has been paid to this problem only in the agricultural field, namely to the development and applications of halogenderivatives of KA in the form of antifungal agent *(5)*, or to the stabilization of wine in the viniculture industry *(6)*.

Antioxidative properties of kojic acid are attributed to its ability to form metal complexes, thereby preventing the change in colour of foods. In the presence of Fe^{3+} and oxygen the formation of an expressively red chelate takes place. This fact is the fundamental principle for determination of the KA *(7)*.

Since the fermented meat products are very often contamined by undesirable microorganisms (mainly by yeasts and moulds of *Candida, Penicillium, Mucor, Aspergillus, Rhizopus, Trichoderma*, etc.), we have studied the extent of the inhibition effect of addition of both KA mass

fractions on the selected groups of microorganisms contained in the product »Bratislavská« sausage. Besides microbiological parameters the influence on colour stability and organoleptic properties was also examined.

Material and Methods

The durable meat product »Bratislavská« sausage with addition of KA was prepared according to the standardized technological procedure *(8)* as follows: frozen pork was ground with a meat cutter having the size of particles approximately 3 mm. After adding the prescribed ingredients (nitrite salting mixture, spices, garlic, caraway-seeds, red pepper, sugar) the meat mixture was divided into 3 parts. Then the KA of both mass fraction was added to two of them in the amounts of 100 ppm and 500 ppm of the meat mixture (KA approved by the hygienist, 1986, content of aflatoxins – negative, mutagenic effect – negative, LD_{50} – 0.27 %; in Slovakia, the strain *Aspergillus tamarii* is used for its production) *(1)*. The third part was prepared without KA and served as control. The meat mixture was stuffed into intestines 1.10 m long. The filled meat product was then bound with a piece of string at its ends and in the centre, then hung up in a climatized drying room and smoked for 3 days at RH of 85–90 % and at temperature < 20 °C. After smoking, the product was dried again for 16 days at the temperature of 15–18 °C, RH of 75–85 % and then it was sensorically analyzed. For microbiological tests the samples were taken at weekly intervals (excluding the determination of lactic acid bacteria) at the 1st, 8th, 15th, 22nd, 30th, 36th, 42nd and 50th day of the ripening and storage period; the sensory evaluation was done at weekly intervals after the 15th day from the sausage production.

1. Determination of mesophilic and aerobic sporeforming bacteria by the plate count method, on the tryptone glucose extract agar (Difco) *(9)*,

2. Determination of coliform bacteria by the plate count method, on the Mc Conkey agar (Šarišské Michal'any) *(10)*,

3. Determination of yeasts and moulds by the plate count method, on the chloramphenicol glucose extract agar (Šarišské Michal'any) *(11)*,

4. Determination of lactic acid bacteria by the plate count method, on the Tomato Juice Agar (Oxoid) *(12)*,

5. Sensory evaluation: point-scale evaluation and profiling of tastiness (of 8–18 members) according to the 4–6 points of hedonic scale *(13)*,

6. Water activity (a_w) and pH values were measured in weekly intervals (a_w-Metem, Rotronic Ag-Hydroskop, Switzerland; pH-Meter, OP-109, Hungary).

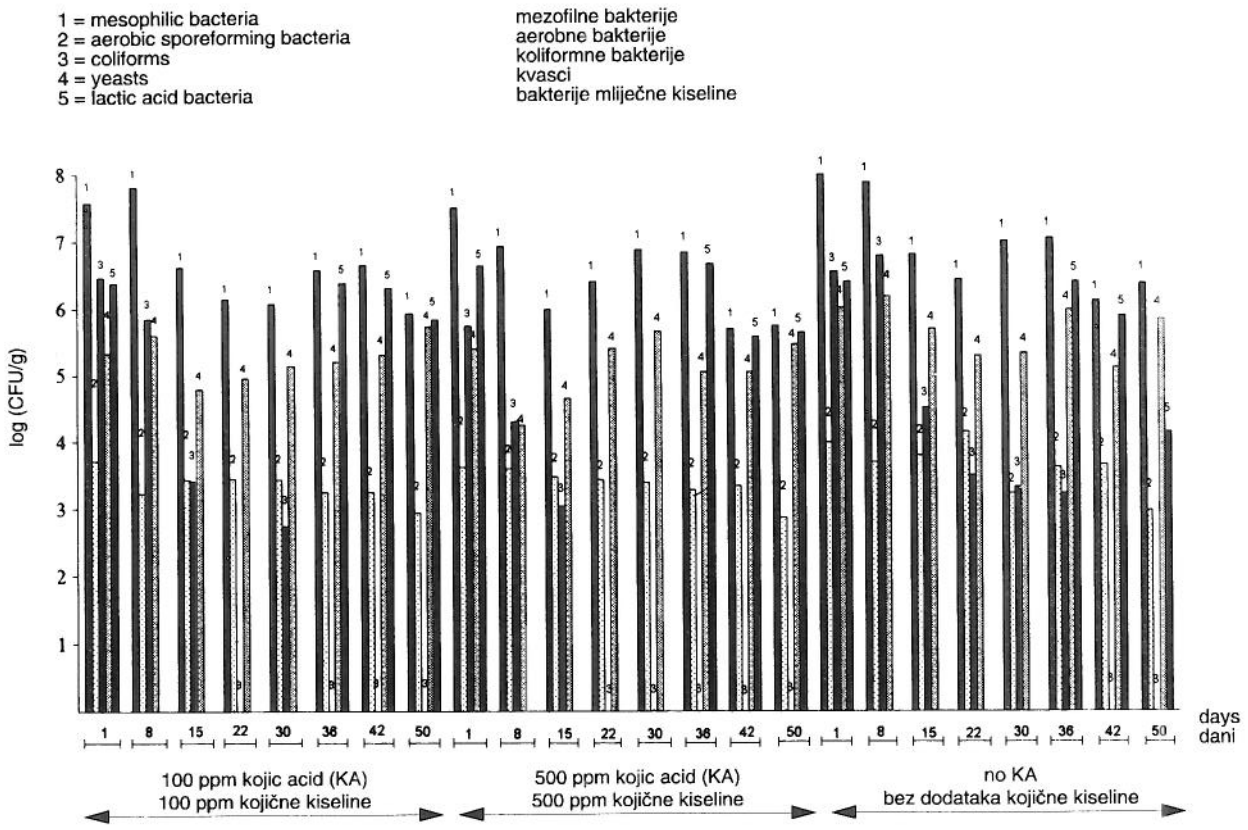

Fig. 1. Changes of the microorganisms count during ripening and storage of »Bratislavská« sausage with addition of KA

Slika 1. Promjene broja mikroorganizama tijekom zrenja i skladištenja »Bratislavske« kobasice s dodatkom kojične kiseline i u kontrolnom uzorku

Table 1. Organoleptic properties of "Bratislavská" sausage with addition of KA mass ratio 100 and 500 ppm
Tablica 1. Ocjena organoleptičkih svojstava "Bratislavske" kobasice s dodatkom kojične kiseline masenog udjela 100 i 500 ppm

Sensoric parameter Organoleptičko svojstvo	Ripening and storage / days Zrenje i skladištenje / dani														
	15			22			29			36			50		
	0	100	500	0	100	500	0	100	500	0	100	500	0	100	500
Consistency Konzistencija	2.6	2.7	3.2	3.8	3.4	3.3	3.9	3.0	3.2	3.7	3.5	3.3	3.6	3.5	3.8
Colour and appearance Boja i izgled	3.1	3.1	3.5	3.6	3.5	3.5	3.7	3.2	3.3	3.4	3.6	3.4	3.5	3.5	3.5
Odour Miris	4.8	4.9	5.1	5.5	5.1	5.4	5.6	5.4	5.4	5.3	5.6	5.6	5.3	5.5	5.5
Taste Okus	4.5	4.5	4.6	5.2	4.8	5.2	5.3	5.2	5.3	5.4	5.2	5.2	5.2	5.5	5.3
In total Ukupno	15.0	15.2	16.4	18.1	16.8	17.4	18.5	16.8	17.2	17.8	17.9	17.5	17.6	18.0	18.1
Total tastiness Cjelovitost okusa	2.3	2.3	2.3	2.8	2.3	2.6	2.5	2.3	2.3	2.6	2.7	2.6	2.5	2.6	2.6

Table 2. Profiling of tastiness of "Bratislavská" sausage with addition of KA mass ratio 100 and 500 ppm
Tablica 2. Ocjena okusa "Bratislavske" kobasice s dodatkom kojične kiseline masenog udjela 100 i 500 ppm

Descriptor Opis okusa	Ripening and storage / days Zrenje i skladištenje / dani														
	15			22			29			36			50		
	0	100	500	0	100	500	0	100	500	0	100	500	0	100	500
Meaty Mesni	3.4	3.4	3.4	3.1	3.4	3.4	3.5	3.5	3.4	3.3	3.4	3.4	3.7	3.4	3.5
Salty Slan	2.8	3.1	3.1	2.6	3.0	3.1	3.0	3.1	3.2	3.1	3.2	3.1	3.2	3.2	3.1
Spicy Pikantan	2.6	3.0	2.6	2.8	3.2	3.0	3.1	3.0	2.8	2.7	2.8	2.8	3.3	3.0	3.1
Sourish Kiselkast	2.0	2.2	2.4	2.2	2.9	3.0	2.4	2.9	2.8	2.2	2.5	2.4	2.8	2.6	2.5
Smoky Dimljen	2.8	2.7	2.8	2.4	2.6	2.5	2.5	2.5	2.4	2.3	2.3	2.2	2.8	2.6	2.7
Rawish Sirov	2.1	1.8	1.7	1.2	0.6	0.6	0.3	0.6	0.5	0.2	0.2	0.2	0.1	0.2	0.2

Table 3. Mean pH and a_w values of "Bratislavská" sausage during ripening and storage
Tablica 3. Prosječne vrijednosti pH i a_w "Bratislavske" kobasice tijekom zrenja i skladištenja

"Bratislavská" sausage "Bratislavska" kobasica		Ripening and storage / days Zrenje i skladištenje / dani					
w(KA)/ppm	Values Vrijednosti	1	8	22	29	36	50
0	pH	5.6	5.3	5.3	5.1	5.1	5.1
	a_w	0.97	0.95	0.93	0.89	0.85	0.82
100	pH	5.9	5.2	5.1	5.2	5.3	5.3
	a_w	0.97	0.95	0.93	0.88	0.88	0.85
500	pH	5.9	5.2	5.0	5.2	5.2	5.2
	a_w	0.97	0.94	0.93	0.93	0.88	0.85

Results and Discussion

Fig. 1 shows the results of the microbiological analysis; Tables 1–3 present the results of sensory evaluation and the pH and a_w measurements.

Mesophilic bacteria

As shown in Fig. 1, no significant microbiological effect could be attributed to addition of KA, versus control. On the 1st day after preparation the meat product contained 10^7 CFU g^{-1}; within a 50-day storage this count decreased by one (control) or two (100 and 500 ppm) logarithmic cycle. The products contained neither pathogenic (*Salmonella*) nor facultatively pathogenic (*Pseudomonas*) microorganisms.

Aerobic sporeforming bacteria

The average initial content of all samples ranged between 10^3 and 10^4 CFU g^{-1}. In the control samples it was diminished by one logarithmic cycle (9.3×10^2) at the end of storage; in the samples with addition of 100 ppm KA it was reduced to 8.7×10^2, and with addition of 500 ppm to 7.3×10^2 CFU.

Coliform bacteria

The starting content of coliforms ranged between 10^5 and 10^6 CFU g^{-1} (without the presence of Proteus and *Escherichia coli*). During the storage characterized by decreasing the pH and a_w, the number of these bacteria was falling and after the 36th day of storage it reached the value of 1.7×10^3 CFU g^{-1} (a connexion with the growing number of lactic acid bacteria). Our results correspond to the data found in literature on the course of natural fermentation *(14)*. It is interesting that with a lower additon of KA the coliforms were present even after the 30th day of storage (5.5×10^2) and with a higher addition of KA they were undetectable already after the 15th storage day. This fact points to the higher sensitivity of coliforms.

Yeasts and moulds

After the 1st day from the preparation of »Bratislavská« sausage samples the number of yeasts corresponded to 10^4 to 10^5 for all samples examined (supposed contamination by ingredients). During the whole time of storage no significant decrease or increase in the initial number of yeasts was noted either in the samples with addition of KA or in the control samples. The moulds occurred only sporadically (below 10) in the control samples, and in the samples with addition of KA they were unnoticed over the whole time of storage.

Lactic acid bacteria

For technical reasons (lack of culture medium) the lactic acid bacteria were examined after the preparation (on 1st day) only – at the 36th, 42nd and 50th day. The addition of KA did not have any influence on the growth of these microorganisms, the number of which varied between 10^5 to 10^6, that is from the beginning up to the end of storage. This fact is positive also from the point of view of the retention of starting cultures inoculated into meat products.

Sensory evaluation

a. *Point-scale evaluation*. This involved the evaluation of colour, appearance, odour, taste and consistency on a scale of 0–6 points for particular sensory characteristics (consistency 0–4, colour and appearance 0–4, taste 0–6, total tastiness 0–3). The points 6 and 5 refer to a very good and good quality, the points 0 and 1 refer to a bad or insufficient quality. For the total tastiness 0 was inacceptable, 3 was excellent. Simultaneously, the taste descriptors intensity was evaluated from the unperceivable feeling (0) to the hardly perceivable (1), weak (2), medium – expressive (3) and expressive (4) ones.

During ripening and storage the organoleptic properties of model samples were improved. As it can be seen from Table 1, the best quality (colour, consistency, appearance) was observed in the sample with addition of 500 ppm KA after the 15th day (16.4 points of the maximum 20 points) as well as after the 50th day of storage (consistency).

b. *Profiling of tastiness*. The profiles of tastiness were evaluated by intensity of particular distinguishing characteristics (descriptors) according to a scale ranging from 0–4 in comparison to a control sample prepared without KA. The evaluated samples were characterized by following descriptors: meaty, salty, spicy, sourish, smoky, rawish.

From the results of profiling of tastiness in Table 2 it followed that no significant differences among taste descriptors were found, except for the descriptor of »rawish« which was progressively reduced during ripening and storage.

Water activity and pH

The starting a_w (water activity) of products was 0.97 and the pH ranged between 5.6 and 5.9. During ripening and storage both parameters decreased gradually so that a_w and pH values after 50 days were the following: $a_w = 0.85 - 0.82$ and pH = 5.1 – 5.3, which was in agreement with the development of the microbiological status (Table 3).

Conclusion

From the microbiological analysis it follows that our results correspond with the literature data on antifungal properties of KA, since, in comparison to the control, no moulds occurred in the whole storage period. The other microorganisms were affected by KA insignificantly. The sensory analysis indicated a favourable effect of KA on the colur, consistency and appearance of smoked meat products.

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