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Original article

Influence of microflora composition on safety and colour parameters of "kumpia wieprzowa" during ripening

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Abstract

The aim of this study was to define the influence of microbiological activity on the safety (microflora composition, biogenic amine amount) and colour of "kumpia wieprzowa" during the 3-month ripening period. The study included the amount of aerobic bacteria, yeast, lactobacilli rods, coagulase-negative cocci, pH and colour parameters as well as the content of nitrates (V) and (III), biogenic amines and amino acids. The lactobacilli and cocci constituted the predominant microflora of the ready-to-eat product (4.9-5.2 and 5.2-5.4 log cfu/g, respectively), although further mesophilic bacteria identification revealed the presence of numerous aerobic, aerotolerant and anaerobic species, mostly gram-positive, spore- and non-spore-forming. The absence of 2-phenylethylamine and putrescine and the low level of tryptamine (2.5 mg/kg) at the beginning of the ripening as well as the increase of tyramine and spermine amounts from 11.5 and 2.7 to 21.9 and 4.0 mg/kg, respectively during the treatment, denoted the good quality of raw meat used and dynamic growth of the desired acidifying and denitrifying microorganisms. The development of the coagulase-negative cocci population corresponded with the a* and C* colour parameters and the nitrate (III) content increase, the final result of which was 26.9, 27.5 as well as 19.4 mg/kg. The content of nitrates (V) and (III) was optimal to obtain a non-cured, safe and suitably coloured, long-term ripened meat product.

Key words: kumpia wieprzowa, microflora, colour, biogenic amines

Introduction

Traditional raw ripened meat products are an important part of the European Union meat market. Depending on the region, they differ with their recipes and technology as well as microflora composition. The interest in fermented meat products results from their characteristic aroma and their content of biologically active ingredients. "kumpia wieprzowa" is a unique pork product, entered on the "List of Polish

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traditional products" in 2005. Węsierska et al. (2012) reported the biochemical changes during the 3-month ripening period of "kumpia wieprzowa" (proteolysis, lipolysis, oxidation, release of volatile compounds). Up to the present time, knowledge of its properties and bacteria activity were not well reported. Nitrates (V) and (III) are not used in the production of "kumpia wieprzowa" but, as in other traditional meat products, both compounds may be present in low concentrations, without the use of any additives of this kind. Nitrates (V) are partly reduced to (III) by bacterial nitrate reductase. The presence of nitrate (III) in meat products is essential for the pink colour, characteristic meat flavor, enhancement of shelf life and protecting the consumer against bacterial spoilage and hazards caused by *Clostridium botulinum*. Literature on the effects of different food processing on the production of biogenic amines e.g. during long-term ripening is unfortunately scarce. During food spoilage, microorganisms can produce a high concentration of biogenic amines by decarboxylating the free amino acids. Their concentrations can indicate bacterial contamination of food. The aim of this study was to evaluate the influence of the microbiological activity on the safety (microflora composition, biogenic amine amount) and colour of "kumpia wieprzowa".

Materials and Methods

Processed meat product

"Kumpia wieprzowa" was manufactured in a small-scale plant using the traditional method of meat fermentation. The meat and spices were obtained from local producers (the Podlasie region in Poland). Smoking procedures and the composition of the spices were kept confidential. The product was processed as one full cut (meat with bones) from the pork shoulder of the Polish landrace pig breed (pbz) with external fat. The weight of the meat cut fluctuated between 4 and 7 kg, according to the age of the animal. The shoulder was dry salted with the use of non-iodized salt and seasoned for 3 weeks in cold storage at a temperature of 4-7°C. Nitrite curing was not practised. The excess salt was removed by drenching for 24 h (intended NaCl content: about 7% in the ready-to-eat product). After draining, it was smoked with cold smoke and ripened at a temperature of 12-15°C with a relative humidity of 85-90% for 3 months in a ripening room. Three batches were produced. Three products in each batch were wrapped in grease-proof paper, packed in thermo-isolated bags, stored at a temperature of 4-6°C and immediately distributed by courier to the laboratory for analysis for up to 24 h.

Sampling

Samples of raw meat of three processed cuts from each batch were collected, just before the smoking procedures, after dry salting (ripening period: 0). The cuts were also sampled at different times throughout the ripening process (after 1, 2 and 3 months of ripening). The microbial count, the content of nitrates (V) and (III), the biogenic amines and amino acids as well as the pH and colour parameters were determined in duplicate at each sampling point. Samples for microbiological analysis were taken from the surface layer (a depth of up to 2-3.5 cm) as well as from the inside of the meat products (internal layer). Initially, three 10g slices from each product, without casing, were weighed aseptically, cut into small pieces and placed in sterile stomacher bags. 90 ml of a sterile diluent (Peptone Water, Biocorp) was added and the mixture was homogenized using a laboratory blender (Stomacher 80, Seward). A series of decimal dilutions was prepared with the same diluent according to European standards (PN-EN ISO 6887-2:2005). Thereafter, three 20 g slices were taken for water activity, pH and colour measurements. After removing the casing, the remainder was comminuted using a kitchen blender (Multiquick Professional, Braun) to obtain a homogenous raw meat mixture for further investigation. The collected samples were analysed immediately according to relative standards.

Analysis

The bacteriological composition was determined according to the following methods: total plate count in mesophilic conditions by European standards (PN-EN ISO 4833:2004/Ap1:2005); yeasts by Polish standards (PN ISO 21527-1:2009); *Lactobacilli* rods by Polish standards (PN-ISO 15214:2002); *Micrococcus* sp. as well as a coagulase-negative and positive *Staphylococcus* sp. by European standards (EN ISO 6888:2001/A1:2004); *Enterobacteriaceae* by Polish standards (PN-ISO 21528-2:2005). Additional isolation and identification of the bacteria from the raw materials using the miniATB system, ID 32E, ID 32A and ID 32 Staph tests (bioMerieux), was conducted following the procedure of the producer. The nitrate (V) and (III) content was determined using the colorimetric method in accordance with Polish standards (PN-EN 12014-4:2006/Ap1:2007). The pH was measured with a pH-meter type CP-411 and electrode type PP-3 (Elmetron) in a water homogenate (meat:water 1:3). A Chroma Meter CR-400 with a C illuminant (Minolta) was used for measuring colour differences (ΔE^*). The analyser was calibrated

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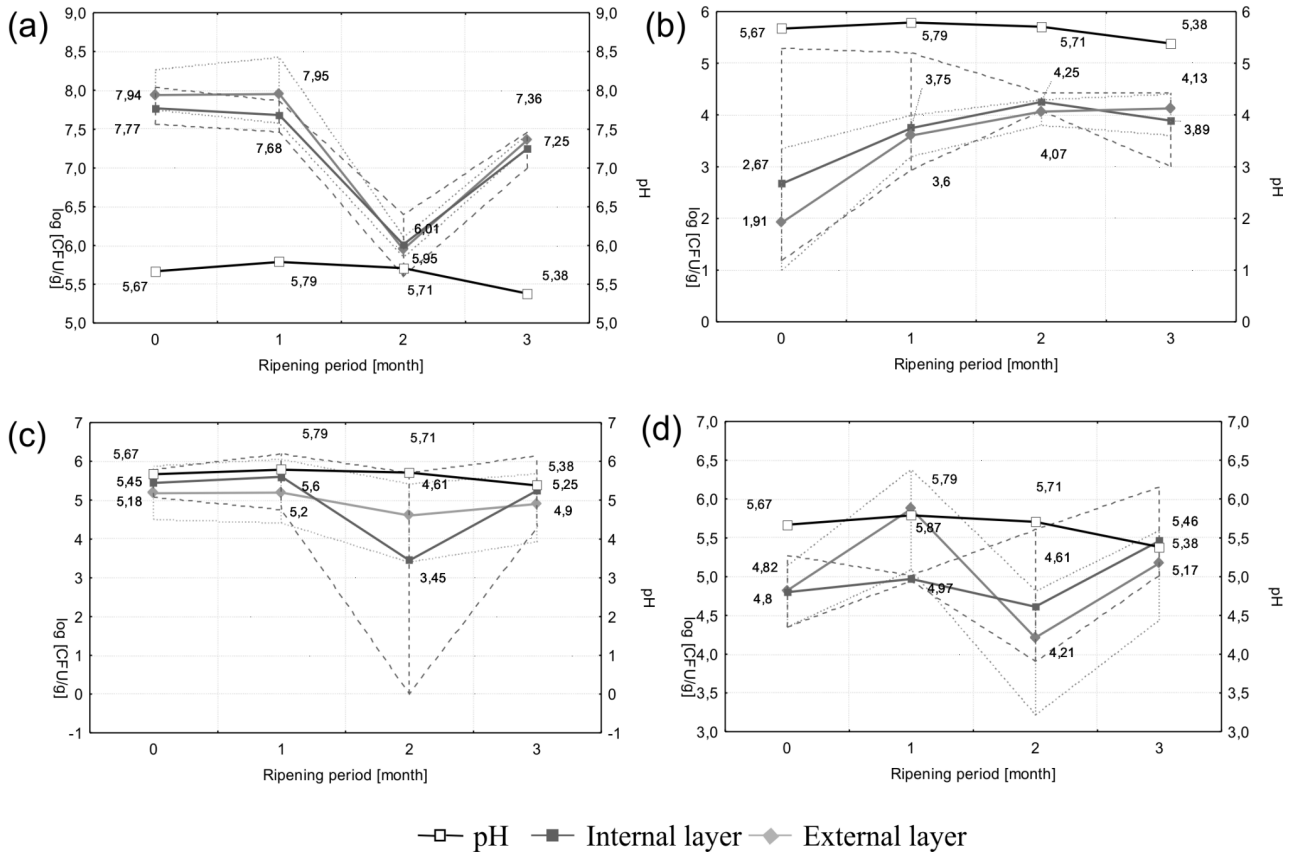

Fig. 1. Microflora growth dynamics in "kumpia wieprzowa" during ripening (0, 1, 2, 3 months): total count of aerobic bacteria (a), yeast (b), lactobacilli rods (c), coagulase-negative cocci (d). All values are mean with SD; data represent the average of three batches, each analysed in triplicate.

according to the white reference standard (L*=94.2; a*=0.3133; b*=0.320), and the values for coordinates L*, a* and b* were then determined. The hue angle (h^{o*}) and saturation (C*) were calculated on this basis. Biogenic amines were derivatised with dansylchloride, as was previously described by Paulsen and Bauer (2007). The dansylated amines were separated using the HPLC method (high performance liquid chromatograph, Hewlett Packard 1050 with UV/WIS detector, Rheodyne dispenser, LiChroCART HPLC 3 Purospher RP-18 5 µm 25 cm column and Hewlett Packard 3396 Series II Integrator). The amines were detected by UV-VIS absorption (Waters 996 at 254 nm). The liberated amino acid composition was analysed with ion-change chromatography with postcolumn derivatisation and the detection of a ninhydryn reaction with an automatic amino acid analyser (AAA 400, Ingos). The effect of time on the microflora composition, biogenic amine amount and colour of "kumpia wieprzowa" was tested using Analysis of Variance (ANOVA) with one factor: the ripening period (levels: months 0, 1, 2, 3). The Duncan post-hoc tests were used for the comparison of means (the significance of differences was investigated at P<0.05).

Results

Microbial composition

There were no significant differences in the count of the aerobic bacteria and lactobacilli rods in the external and internal layer of "kumpia wieprzowa" during the first month of ripening (Fig. 1a, c) nor in the count of coagulase-negative cocci in the internal layer (Fig. 1d). The final level of these bacteria was similar to the initial amount despite the cfu reduction between the 1st and the 2nd month (P>0.05) and changed respectively by about 0.5-0.6, 0.2-0.3 and 0.3-0.6 log cfu/g. In contrast, the yeast count increased significantly (P>0.05) during the first 2 months of ripening and changed insignificantly during the final month (Fig. 1b). *Enterobacteriaceae* and moulds were not found in "kumpia wieprzowa". *Staphylococcus aureus* was determined in one sample at the beginning of manufacture. It was not found in the samples from subsequent stages. Further mesophilic bacteria identification revealed the presence of aerobic, aerotolerant and anaerobic species. The following bacteria strains were identified: *Actinomyces naeslundii*, *Anaerococcus*

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Table 1. Overall effect of ripening on nitrate (V) and (III) content, pH and colour parameters of "kumpia wieprzowa" (mean, standard deviation, n = 3).

Ripening period [month]	0		1		2		3	
	M	SD	M	SD	M	SD	M	SD
Nitrates (V) and (III)								
$NaNO_2$ [mg/kg]	2.14[a]	0.76	5.06[b]	0.77	10.19[c]	2.91	19.36[d]	4.18
$NaNO_3$ [mg/kg]	22.54[a]	6.10	25.77[b]	2.75	35.42[c]	2.34	35.63[c]	2.82
pH	5.67[a]	0.05	5.79[a]	0.19	5.71[a]	0.52	5.39[b]	0.15
Color parameters								
L*	52.07[a]	0.15	51.53[b]	0.10	50.00[c]	0.15	48.27[d]	0.00
a*	26.23[a]	0.15	27.17[b]	0.11	26.86[c]	0.15	26.53[d]	0.00
b*	7.13[a]	0.06	6.23[b]	0.15	6.27[b]	0.06	5.96[c]	0.00
h^{o*}[1]	15.21[a]	0.04	12.92[b]	0.26	13.29[c]	0.07	12.51[d]	0.01
C*[2]	27.18[a]	0.16	27.87[b]	0.14	27.29[ac]	0.1	27.51[c]	0.01
ΔE^*_{ab}[3]								4.03

a, b, c Different letters in the same row indicate significant differences between means at P<0.05.
1 h^{o*} – hue angle defined as: arctgb*/a*
2 C* – saturation (chroma) defined as: $\sqrt{a^{*2} + b^{*2}}$
3 ΔE*ab – color difference during ripening defined as: $\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$

Table 2. Overall effect of ripening on biogenic amine amounts of "kumpia wieprzowa" (mean, standard deviation, n = 3).

Ripening period [month]	0		1		2		3	
	M	SD	M	SD	M	SD	M	SD
Biogenic amines [mg/kg]								
2-Phenylethylalanine	0.00[a]	0.00	0.00[a]	0.00	1.15[b]	0.15	1.95[c]	0.18
Putrescine	0.00[a]	0.00	3.41[b]	0.11	3.91[c]	0.09	3.90[c]	0.08
Cadaverine	2.47[a]	0.32	4.07[b]	0.16	5.29[c]	0.29	7.54[d]	0.15
Histamine	1.10[a]	0.20	2.69[b]	0.16	5.86[c]	0.05	6.52[d]	0.30
Tyramine	11.53[a]	0.50	11.98[a]	0.36	12.99[b]	0.17	21.90[c]	0.43
Spermine	2.71[a]	0.20	2.40[a]	0.24	3.82[b]	0.35	4.02[b]	0.10
Spermidine	6.13[a]	0.21	9.48[b]	0.24	12.57[c]	0.51	17.79[d]	0.33
Tryptamine	1.13[a]	0.15	3.05[b]	0.10	4.27[c]	0.14	4.78[d]	0.20

a, b, c Different letters in the same row indicate significant differences between means at P<0.05.

Table 3. Overall effect of ripening on aminoacid amounts in "kumpia wieprzowa" (mean, standard deviation, n = 3).

Ripening period [month]		0		1		2		3	
Amino acids composition [g/100 g]	The properties of residue	M	SD	M	SD	M	SD	M	SD
Proline	aliphatic	0.82[a]	0.05	1.00[b]	0.11	1.03[b]	0.02	1.30[c]	0.05
Glycine		1.00[a]	0.09	1.18[b]	0.19	1.28[b]	0.01	1.60[c]	0.04
Alanine		1.23[a]	0.04	1.40[b]	0.06	1.44[b]	0.07	1.83[c]	0.05
Valine		1.12[a]	0.02	1.23[b]	0.03	1.24[b]	0.03	1.60[c]	0.05
Isoleucine		1.01[a]	0.01	1.12[b]	0.04	1.12[b]	0.02	1.45[c]	0.05
Leucine		1.77[a]	0.02	1.95[b]	0.05	1.94[b]	0.03	2.52[c]	0.08
Threonine	with alcohol group	1.00[a]	0.01	1.12[b]	0.03	1.11[b]	0.02	1.42[c]	0.04
Serine	or sulphur	0.85[a]	0.02	0.93[b]	0.01	0.95[b]	0.02	1.21[c]	0.03
Cysteine		0.23[a]	0.02	0.23[a]	0.01	0.24[a]	0.01	0.28[b]	0.02
Methionine		0.49[a]	0.40	0.50[ab]	0.03	0.55[b]	0.01	0.61[c]	0.04
Tyrosine	with aromatic ring	0.86[a]	0.01	0.95[b]	0.02	0.98[b]	0.02	1.25[c]	0.04
Phenylalanine		0.90[a]	0.02	1.00[b]	0.02	1.01[b]	0.01	1.30[c]	0.04
Histidine	alkaline	0.71[a]	0.02	0.78[b]	0.01	0.86[c]	0.03	1.10[d]	0.04
Lysine		2.01[a]	0.03	2.27[b]	0.06	2.27[b]	0.05	2.97[c]	0.07
Arginine		1.67[a]	0.04	1.89[b]	0.04	1.94[b]	0.09	2.50[c]	0.10
Asparagine	acidic	2.02[a]	0.03	2.24[b]	0.05	2.26[b]	0.05	2.87[c]	0.07
Glutamine		3.04[a]	0.04	3.41[b]	0.08	3.43[b]	0.08	4.42[c]	0.13

a, b, c Different letters in the same row indicate significant differences between means at P<0.05.

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prevotti, *Bacillus cereus*, *B. subtilis*, *Clostridium bifermentans*, *C. clostridioforme*, *C. glycolicum*, *C. histolyticum*, *C. ramosum*, *C. sporogenes*, *C. subterminale*, *C. tyrobutyricum*, *Eggerthella lenta*, *Finegoldia magna*, *Gemella morbillorum*, *Lactococcus lactis subsp. lactis*, *Lactobacillus acidophilus*, *Propionibacterium acnes* and *P. granulosum* as well as *Staphylococcus xylosus.* They were mostly gram-positive, spore- or non-spore-forming, rod-shaped or coccoid bacteria.

Nitrate (V) and (III) amount, pH and colour parameters

A successive increase of nitrate (V) and (III) amounts, respectively of about 14 and 17 mg/kg ($P<0.05$) was observed during the ripening of "kumpia wieprzowa" (Table 1). A 2-fold increase of the nitrate (III) content was indicated every month, when the final product contained a similar quantity of nitrates (V) as a second month sample. The pH value stayed at about the same values during the first 2 months (5.7) and after the second month of ripening it decreased to 5.4 ($P<0.05$). "Kumpia wieprzowa" dried evenly and consequently, the L* parameter indicating the lightness of the meat, slightly decreased every month ($P<0.05$), although the surface was moist and strongly reflected the light during the entire ripening period (Table 1). The rise of the a* and C* parameters showed a red colour intensity increase during the first month ($P<0.05$) and significant decrease at the third month of processing ($P<0.05$). Between the first and second month, both parameters decreased from 27.2 and 27.9 to 26.5 and 27.3, respectively. The ΔE^* measurement, indicating the difference of the "kumpia wieprzowa" colour during ripening, was approximately 4.0.

Biogenic amine and amino acid amounts

The changes in the biogenic amine amounts are summarized in Table 2. Putrescine was not detected in the fresh meat and its content increased by about 3.4 mg/kg ($P<0.05$) during the first month and 0.5 mg/kg ($P<0.05$) during the second month of ripening. There were no significant differences between the second and third month in putrescine amount. 2-phenylethylamine was not found during the first month of ripening, although in the second- and third-month product, the content of 2-phenylethylamine was 1.1 and 1.9 mg/kg, respectively. Spermine, tryptamine, cadaverine and histamine content increased about 1.3, 3.6, 5.1 and 5.4 mg/kg, respectively during the processing. Greater differences between the initial and the final amount were noted for tyramine and spermidine, respectively about 10.4 and 15.7 mg/kg ($P<0.05$). The amino acid analysis (Table 3) confirmed the presence of biogenic amine precursors, among others: phenylalanine, lysine, histidine, tyrosine, arginine and methionine. The differences between the initial and the final amino acid contents were statistically significant and amounted from 0.05 (cysteine) to 0.96 g/100 g (lysine). In the ready-to-eat product, amino acids followed the following order: cysteine, methionine, histidine, serine, tyrozine, proline, phenylalanine, threonine, isoleucine, glycine, valine, alanine, arginine, leucine, asparagine as well as lysine and glutamine.

Discussion

According to Takahashi and Yamada (1999), Ezaki et al. (2001), Lavigne et al. (2003), Finegold et al. (2005), López-Enrtquez et al. (2007), Fiorentini et al. (2009), Labutti et al. (2009) and Liderot et al. (2010) the isolated microorganisms originated from animal/human skin, intestines, and tissues as well as from herbal additives, and the pigsty and manufacturing environment also constituted a specific part of the "kumpia wieprzowa house microflora". Their presence indicated the capacity for fresh meat contamination during the initial post-slaughter treatment (scalding, bleeding, skinning or evisceration as well as during technological treatment (trimming, salting, hanging, smoking, weighing). Nevertheless, the absence of *Enterobacteriaceae* and *Enterococcus* sp. excluded the contamination of the digestive track content with bacteria of the fresh shoulder. The low pH value, connected with the development of lactic acid bacteria, was the physico-chemical basis of the safety as well as the colour of "kumpia wieprzowa". The feed, water and nitrogenous substances, broken down during the ripening to oxide and ammonia definitively, could be the source of the nitrates (V) and (III) in the non-cured meat product. The conversion is pH-dependent and the value of pH>5.0 corresponds to high bacterial activity and high nitrite levels (Lorenzo et al. 2008). This was confirmed by the results of "kumpia wieprzowa" – the rise of the coagulase-negative cocci population conformed to the red colour intensity. The nitrate reductase is present in many bacteria normally occurring in the gastro-intestinal tract, among others *Clostridium clostridioforme* and *Actinomyces naeslundii* (WHO 1985) as well as nitrite reductase in *Lactobacillus acidophilus* (Hammens and Hertel 2006), also identified in "kumpia wieprzowa". The smoking procedure with nitrosylomyoglobin and carboxymyoglobin production, could be essential for the colour parameters of "kumpia wieprzowa" in the initial stage

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of the processing (Incze 1998). The absence or low level of 2-phenylethylamine, putrescine and tryptamine additionally confirmed the good quality of the raw meat used (Kalač 2006, Lorenzo et al. 2008, Stadnik and Dolatowski 2010). As reported by Suzzi and Gardini (2003), the dynamic growth of lactic acid bacteria and denitrifying microorganisms may result in the increase of tyramine and spermine amounts. Tyramine may also occur as a result of enterococci activity (Ansorena and Astiasarán 2000, Suzzi and Gardini 2003), whereas they were not found in "kumpia wieprzowa". The presence of spermidine, even in a large amount, was not alarming (Lorenzo et al. 2007, 2008, Stadnik and Dolatowski 2010). The rise of the biogenic amine and amino acid amounts was additionally associated with the phenomena of slight drying during the 3-month ripening of "kumpia wieprzowa" (Węsierska et al. 2012).

Conclusions

The composition of the aerobic and anaerobic microflora as well as the absence or low level of 2-phenylethylamine, putrescine and tryptamine at the beginning of "kumpia wieprzowa" production, denote the good quality of the raw meat used. The increase of tyramine and spermine amounts confirm the dynamic growth of lactic acid bacteria and denitrifying microorganisms. The development of the coagulase-negative cocci population corresponds with the nitrate (III) amount and the red colour intensity increase. The content of nitrates (V) and (III) is optimal to obtain a non-cured, safe and suitably coloured meat product during long-term ripening.

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