CHEMICAL AND MICROBIOLOGICAL ANALYSIS OF FRESH, FROZEN AND EMULSIONS OF PORK SKINS.

D. Andronikov[1], D.Naseva[1], N. Taskov[2], D. Saneva[2], A. Kuzelov[1]

[1]Department of Food Technology and processing of animal products, Faculty of Agriculture, University Goce Delcev Stip, Krste Misirkov bb, Stip, R. Macedonia
[2] Faculty of Tourism and Business Logistics, University Goce Delcev Stip, Krste Misirkov bb, Stip, R. Macedonia
Corresponding author: darko.andronikov@ugd.edu.mk; tel: +389 32 550 111.

Original scientific paper

Abstract: The objective of our survey was to determine the content of proteins in fresh and frozen pork skins, as well as in the emulsion of skins and the influence of different acids on their microbiology before and after the cuttering. Altogether 120 kg pork skins (selected by random choice) split in 2 groups per 60 kg fresh and 60 kg frozen skins and immersed in three different combinations of acid solutions (acetic, lactic, wine, lemon) and water. 6 groups have been obtained for further analysis: fresh and frozen skins immersed in A, B, C solutions. Before and after the immersing, samples were taken from all groups for chemical and microbiological analysis. Previously grinded and well homogenized, the following parameters were analyzed: water, fats, ash and proteins. Pork skins were immersed for 72 in total in a solution, then washed with water and well drained. Prepared in that manner - 20 kg skins + 20 kg water (ice) + 200 g soy proteins. The so prepared emulsion of skins undergoes chemical and microbiological analysis. The content of proteins in emulsion of fresh skin is bigger when compared to frozen pork skins. The microbiological analysis showed us that in pork skins immersed in different solutions of acids and water, as well as in the emulsion of skins, no bacteria were found of the following types *clostridium, staphylococcus, proteus,* escherichia, and the total number of bacteria (bacillus) showed decrease of bacteria in all groups of fresh and frozen pork skins. The biggest decrease in the total number of bacteria before and after the immersed skins, as well as on the emulsion of skins, was found in the A solution.

Key words: Pork skins, protein, emulsion of skins, chemical and micro-biological analysis.

Introduction

The skin of pigs is in direct contact with polluting sources and represents excellent place for developing numerous surface microbiological pollutions *(Kim et*

*al. 199*6). The pork skin represents a great source of proteins, and the emulsion of skins (water, fats, proteins) serves as a supplement to many boiled products *(Osburn and Mandigo 1998; Radetic 2000).* Due to the joint tissues (collagen, elastin, retikulin) and the proteins are very hard and that is why they are treated by means of different combinations of acids (lactic, acetic, wine, lemon) and water for the purpose of softening *(Andronikov et al. 2013).* At the same time, the immersing in such solutions serves as a good bacteriological protection. Different types of bacteria (*clostridium, staphylococcus, proteus, escherichia, salmonella, listeria*) were tested on fresh pork and chicken skins by many authors *(Pipek et al. 2006; Trivedi et al. 2008; Lecompte et al. 2009; Rikke et al. 2011; Chaine et al. 2013).*
The objective of this survey was to determine the content of proteins and fats in fresh and frozen skins, as well as in the emulsion of skins before and after the immersing in different solutions of acids and water. Moreover, the aim was to witness the microbiological difference between fresh and frozen pork skins before and after the immersing in different solutions of acids and water and the microbiological difference between the emulsion of fresh and frozen pork skins.

Material and methods

A total of 120 kg pork skins were taken (selected randomly), divided in two groups - 60 kg fresh and 60 kg frozen, immersed in three different combinations of acid solution (acetic, lactatic, tartaric, citric) and water. Six groups were obtained - fresh (3 x 20 kg) and frozen (3 x 20 kg) skins immersed in:

- A - solution (15 g acetic acid, 200 g lactatic acid, 100 g tartaric acid, 100 g citric acid, 18,5 l water),
- B - solution (200 g lactatic acid, 100 g tartaric acid, 19,7 l water),
- C - solution (50 g acetic acid, 200 g lactatic acid, 19,7 l water).

Pork skins are separated from the bacon using a special machine called DERINDER. This machine is composed of a base, electric motor, grinning roller and sharp knife. The bacon and the skin are putted on the drum DERINDER - with the skin downward, the roller takes the pieces and move them right to the blade which peels off the skin of bacon. Samples from all groups were taken before and after immersion, for chemical and microbiological analysis. Earlier samples were cutted ground and well homogenized in a small mixer for home use. Pork skins were putted in solution for 72 hours, then washed and well drained. Then every group of skins (6 x 20 kg) is separately placed in a cutter and mixed with 20 kg water + 200 g soy proteins, whereby 6 groups of skin emulsions are prepared. Chemical and bacteriological analyses have been done on all groups.

Chemical analyses

Total nitrogen (TN) was determined according to the Kjeldahl method. Moisture content was determined by drying at (103 ± 2) °C to constant mass. The

intramuscular fat content was determined according to AOAC International method, with petroleum ether as solvent. Minerals were determined by burning and combustion (4 – 5 h) at 525 – 550 °C.

Microbial analysis

Following bacteria were determined: Total bacteria (*Bacillus*) number - ISO 4833 / 2003. Sown on nutrient agar to 37 °C during 24 hours. *Staphylococcus* ISO 6888 - 1 / 1999. Sown on ETGP agar (barit parker agar) after termostating on 37 °C during 24 hours. *Enterobacteriaceae* ISO 21528 - 1 / 2004; ISO 21528 - 2 / 2009. *Escherichia coli* are sown on lactoza bujon and brilian green, thermostated on 37 °C during 24 - 48 hours. *Clostridium* sown on sulfiten agar, thermostated on 37 °C during 24 - 48 hours

Data were transformed into $\log_{10}$ CFU/g before comparison of means.

The results were statistically processed using mathematical program Microsoft EXEL ANOVA (single factor) 2009 / 2013.

Results and discussion

Chemical composition

The results of the chemical analysis of fresh skin before and after the immersing in solutions of acids and water are presented in Table No. 1. In fresh skin before immersing in solution, we established significant differences ($p < 0.05$) in the content of water. After immersing the frozen skins, a significant difference was noticed ($p < 0.05$) in the content of water and fats between Frozen skin+ A sol. and Frozen skin+ C sol. The content of proteins at frozen skin has also shown significant difference ($p < 0.05$) between Frozen skin+ B sol. and Frozen skin+ C sol. Our results were simillar to the results obtained by *Radetic 2000; Andronikov et al. 2013*.

Table 1. Results of chemical composition of fresh and frozen pork skins before and after immersion in different acids solutions.

Before immersion			
Parameters	Fresh skin $\bar{X}$ ± Sd	Fresh skin $\bar{X}$ ± Sd	Fresh skin $\bar{X}$ ± Sd
Water	57.08 ± 0.28A	58.85 ± 1.37	52.36 ± 1.67C
Fats	29.06 ± 1.04	27.90 ± 1.27	35.77 ± 0.48
Proteins	13.10 ± 0.16	12.90 ± 0.10	11.52 ± 0.70
Minerals	0.26 ± 0.04	0.29 ± 0.05	0.47 ± 0.02
After immersion			
Parameters	Fresh skin + A	Fresh skin + B	Fresh skin + C sol.

	sol. $\bar{X}$ ± Sd	sol. $\bar{X}$ ± Sd	$\bar{X}$ ± Sd
Water	61.04 ± 1.15	56.28 ± 0.31	54.59 ± 0.79
Fats	24.27 ± 1.43	28.96 ± 0.22	34.94 ± 0.20
Proteins	12.62 ± 0.38	14.35 ± 0.06	11.51 ± 0.09
Minerals	0.27 ± 0.02	0.19 ± 0.01	0.53 ± 0.39
Before immersion			
Parameters	Frozen skin $\bar{X}$ ± Sd	Frozen skin $\bar{X}$ ± Sd	Frozen skin $\bar{X}$ ± Sd
Water	57.66 ± 0.31	52.40 ± 0.49	54.60 ± 0.88
Fats	28.99 ± 0.15	35.92 ± 0.95	31.56 ± 0.6
Proteins	13.17 ± 0.07	11.25 ± 0.65	12.76 ± 0.13
Minerals	0.23 ± 0.03	0.19 ± 0.01	0.53 ± 0.39
After immersion			
Parameters	Frozen skin+ A sol. $\bar{X}$ ± Sd	Frozen skin+ B sol. $\bar{X}$ ± Sd	Frozen skin+ C sol. $\bar{X}$ ± Sd
Water	63.44 ± 1.63A	63.82 ± 0.38	59.58 ± 0.27C
Fats	24.22 ± 1.50A	34.47 ± 0.49	31.95 ± 0.51C
Proteins	12.95 ± 0.04	11.49 ± 0.05B	13.18 ± 0.31C
Minerals	0.26 ± 0.02	0.21 ± 0.04	0.29 ± 0.03

$\bar{X}$ = mean, Sd = standard deviation; mean values with different capital letters (A, B, C) between each row differ significantly ($p < 0.05$).

The chemical composition of fresh and frozen skins emulsion is presented in Table 2.

The differences between the different parameters are not significantly important ($p < 0.05$). In general, the results as regards the content of proteins and water at fresh skin are bigger than at frozen skin. This means the fresh skin when compared to frozen skin better joints the added proteins and water.

Table 2. Results of chemical composition of emulsion of fresh and frozen pork skins in different acids solutions.

Fresh skin			
Parameters	Emulsion of skin + A sol. $\bar{X}$ ± Sd	Emulsion of skin + B sol. $\bar{X}$ ± Sd	Emulsion of skin + C sol. $\bar{X}$ ± Sd
Water	63.40 ± 1.1	58.50 ± 1.13	56.20 ± 0.54
Fats	23.18 ± 1.49	25.80 ± 0.52	31.28 ± 0.65
Proteins	13.17 ± 0.02	15.50 ± 0.45	12.10 ± 0.72
Minerals	0.25 ± 0.03	0.20 ± 0.28	0.44 ± 0.45
Frozen skin			

Parameters	Emulsion of skin + A sol. $\overline{X}$ ± Sd	Emulsion of skin + B sol. $\overline{X}$ ± Sd	Emulsion of skin + C sol. $\overline{X}$ ± Sd
Water	66.50 ± 0.25	55.05 ± 0.42	55.50 ± 0.22
Fats	21.15± 0.52	33.50 ± 0.48	32.30 ± 0.35
Proteins	12.12 ± 0.48	11.78 ± 0.32	12.05 ± 0.48
Minerals	0.28 ± 0.22	0.20 ± 0.55	0.15 ± 0.25

$\overline{X}$ = mean, Sd = standard deviation;

Microbiological analysis

Table 3. Results of microbiological analysis of fresh and frozen pork skins before and after immersion in different solutions of acids and water.

Bacteriology **Before immersion**	Bacteriology **Before immersion**	Bacteriology **Before immersion**
Fresh skin A Total bacteria number	Fresh skin B Total bacteria number	Fresh skin C Total bacteria number
2.50 log CFU/g	2.57 log CFU/g	2.65 log CFU/g
Frozen skin A Total bacteria number	Frozen skin B Total bacteria number	Frozen skin C Total bacteria number
2.44 log CFU/g	2.47 log CFU/g	2.54 log CFU/g
Bacteriology **After immersion**	Bacteriology **After immersion**	Bacteriology **After immersion**
Fresh skin + A sol. Total bacteria number	Fresh skin + B sol. Total bacteria number	Fresh skin + C sol. Total bacteria number
/	1.30 log CFU/g	1.69 log CFU/g
Frozen skin + A sol. Total bacteria number	Frozen skin + B sol. Total bacteria number	Frozen skin + C sol. Total bacteria number
/	1.30 log CFU/g	1.84 log CFU/g

Results of microbiological analysis of fresh and frozen pork skins before and after immersion in different solutions of acids and water are given in Table No. 3. We found no statistical significant difference ($p < 0.05$) between the fresh and frozen skin. It is obvious that different solutions of acids and water affected the decrease of the total number of bacteria (bacillus) of fresh and frozen skin. This is especially noted with solutions A and B, but those differences are not statistically significant ($p > 0.05$).

Results of microbiological analysis of emulsion of fresh and frozen pork skins in different acids solutions are given in Table No. 4. The total number of bacteria in the process of cuttering is increased in all groups of emulsion of fresh and frozen skin in A, B, C solution. The results obtained for the emulsion of fresh skin in all solutions of acids and water are lower than the emulsion of frozen skin. The results we received, have no statistical significant differences ($p > 0.05$).

Table 4. Results of microbiological analysis of emulsion of fresh and frozen pork skins in different acids solutions.

Bacteriology	Bacteriology	Bacteriology
Emulsion of fresh skin + A sol. Total bacteria number	Emulsion of fresh skin + B sol. Total bacteria number	Emulsion of fresh skin + C sol. Total bacteria number
2.74 log CFU/g	2.85 log CFU/g	2.87 log CFU/g
Emulsion of frozen skin + A sol. Total bacteria number	Emulsion of frozen skin + B sol. Total bacteria number	Emulsion of frozen skin + C sol. Total bacteria number
2.85 log CFU/g	2.87 log CFU/g	2.90 log CFU/g

Conclusion

Our survey has shown us the current chemical composition, including the content of proteins, water and fats in fresh and frozen pork skins on one part of the territory of the Republic of Macedonia. We found that in the emulsion of skins, fresh pork skins joint better proteins and water. In pork skins immersed in different solutions of acids and water, as well as in the emulsion of skins, no bacteria were found of the types *clostridium, staphylococcus, proteus, escherichia*, and the total number of bacteria (bacillus) showed decrease of bacteria in all groups of fresh and frozen pork skins. The best results and the biggest decrease in the total number of bacteria before and after the immersion of skins, as well as the emulsion of skins, were noted in solution A.

Hemiska i mikrobiološka analiza svežih, zamrznutih i emulzija svinjskih kožica.

D. Andronikov[1], D.Naseva[1], N. Taskov[2], D. Saneva[2], A. Kuzelov[1]

Rezime

Cilj našeg istraživanja bio je da se utvrdi sadržaj proteina u sveže i zamrznute svinjske kože i kože od emulzije kao i uticaj različitih kiselina na njihovu mikrobiologiju pre i posle kuterovanja. Uzeta su ukupno 120 кg svinjskih kožica (odabrani su slučajnim izborom) podeljene u 2 grupe po 60 кg svežih i 60 кg zamrznutih kožica i potopljene u tri različite kombinacije rastvora kiselina (sirћetna, mlečna, vinska, limunska) i vode. Dobijene su 6 grupe za dalju analizu: sveže i zamrznute kože potopljene u A, B, C rastvora. Pre i nakon potapljanja su uzorci uzeti iz svih grupa za hemiske i mikrobiološke analize. Ranije samljeveni i dobro homogenizovani, analizirani su sledeће hemiske paramtre: voda, pepel, masti i proteina. Svinske kožice su bile potopljene 72 sata u rastvoru, a zatim su oprani vodom i dobro su iscedene. Tako pripremljene stavljene su u kuter u odnosu 20 кg kožica + 20 кg vode (leda) + 200 g sojinih proteina. Na ovaj način je pripremljena emulzija od kože a potom i izvrsena je hemiska i mikrobiloška analiza. Sadržaj proteina u emulziju od svežih kožica je veћi od emulzije zamrznute svinjske kože. Mikrobiloška analiza nam je pokazala da u svinjskih kožica potopljene u različitim rastvorima kiselina i vode kao i u emulzijom od kože nisu pronaћeni bakterije iz vrste *clostridium, staphylococcus, proteus, escherichia*, a ukupan broj bakterija (bacila) pokazao je smanjenje bakterija u svim grupama svežih i zamrznutih svinjskih kožica. Najveће smanjenje u ukupnom broju bakterija pre i posle potapljanja kože i emulzije od kože utvrћeno je u rastvoru A.

Kljucne reci: Svinjske kože, proteini, emulzija od kože, hemiske i mikrobiološke analize.

References

ANDRONIKOV D., NASEVA D., DIMITROVSKI Z., SANEVA D., KUZELOV A. (2013): The amount of protein and influence of various acid solution and water in microbiology of fresh and frozen pork skins, 23rd International Scientific Conference, Stara Zagora, x-x.

Ash of Meat, AOAC Official Method No. 920.153, (1997): AOAC International, Gaithersburg, MD, USA.

CHAIN, A., ARNAUD, E., KONDJOYAN, A., COLLIGNAN, A.,., SARTER., S. (2013): Effect of steam and lactic acid treatments on the survival of Salmonella Enteritidis and Campylobacter jejuni inoculated on chicken skin, International journal of food microbiology, 162, 3, 276 – 82.

Enterobacteriaceae, ISO 21528 - 1 / 2004; ISO 21528 - 2, 2009.

Fat (Crude) in Meat and Meat Products, AOAC Official Method No. 991.36 (1997): AOAC International, Gaithersburg, MD, USA.

KIM, K. Y., FRANK, J. F., S., CRAVEN E. (1996): Three-dimensional visualization of *Salmonella* attachment to poultry skin using confocal scanning laser microscopy. Letters Applied Microbiology 22, 4, 280 – 282.

LECOMPTE, J. Y., COLLIGNAN, A., SARTER, S., CARDINALE, E., KONDJOYAN A.: (2009) Decontamination of chicken skin surfaces inoculated with Listeria innocua, Salmonella enteritidis and Campylobacter jejuni by contact with a concentrated lactic acid solution, British Poultry Science, 50, 3, 307 – 317.

Moisture in Meat, AOAC Official Method No. 950.46 (1997): AOAC International, Gaithersburg, MD, USA.

MORILD, R. K., OLSEN, J. E., AABO, S. (2011): Change in attachment of Salmonella Typhimurium, Yersinia enterocolitica, and Listeria monocytogenes to pork skin and muscle after hot water and lactic acid decontamination, International Journal of Food Microbiology, 145, 1, 31, 353 – 358.

Nitrogen in Meat, AOAC Official Method No. 928.08 (1997): AOAC International, Gaithersburg, MD, USA.

OSBURN W. N., AND MANDIGO R. W. (1998): Reduced-Fat Bologna Manufactured with Poultry Skin Connective Tissue Gel1. Poultry Science 77 : 1574 – 1584.

PIPEK, P., HOUŠKA, M., HOKE, K., JELENÍKOVÁ, J., KÝHOS, K., M., ŠIKULOVÁ. (2006): Decontamination of pork carcasses by steam and lactic acid, Journal of Food Engineering, 74, 2, 224 – 231.

RADETIC P. (2000): Barene kobasice. Tehnologija hrane, 34 - 39.

Staphylococcus, Official Method ISO 6888 - 1, (1999).

Total bacteria (Bacillus) number, Official Method ISO 4833, (2003).

TRIVEDI S., REYNOLDS A. E., CHEN J. (2009): Effectiveness of commercial household steam cleaning systems in reducing the populations of Listeria monocytogenes and spoilage bacteria on inoculated pork skin surfaces, Food