Ciência e Tecnologia de Alimentos
ISSN: 0101-2061
revista@sbcta.org.br
Sociedade Brasileira de Ciência e Tecnologia de Alimentos
Brasil

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Ciência e Tecnologia de Alimentos, vol. 30, núm. 2, abril-junio, 2010, pp. 560-564
Sociedade Brasileira de Ciência e Tecnologia de Alimentos
Campinas, Brasil

Original

Influence of chitosan addition on quality properties of vacuum-packaged pork sausages

Influência da adição de quitosana sobre a qualidade de salsichas embaladas a vacuo

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Abstract

The aim of the study was to evaluate the influence of the chitosan addition on the quality of vacuum packaged pork sausages. A variant of the product was elaborated with 1% (w/w) of chitosan in lactic acid solution at 1% (v/v) and it was compared to a control. Sausages were mechanically stuffed and manually conformed and vacuum packaged. Sausages were stored at 4 °C and microbiological evaluations, pH measurements, texture profile analysis and sensorial evaluation were performed. The chitosan addition in the formulation of the sausages did not reduce the microbiological counts. The pH values obtained in all samples were similar, which suggests that the chitosan addition did not influence the pH values of sausages. The added chitosan did not affect significantly ($p \leq 0.05$) the results of the texture profile analysis and sensorial attributes and therefore, the overall acceptance of the sausages.

Keywords: *pork sausages; meat products; chitosan; microbiological quality; sensory quality; storage.*

Resumo

O objetivo do estudo foi avaliar a influência da adição de quitosana sobre a qualidade de salsichas embaladas a vácuo. Elaborou-se uma variante do produto com 1% (m/m) de quitosana adicionada em dissolução de ácido láctico a 1% (v/v) e manteve-se um lote controle sem adição de quitosana. Formaram-se peças mediante torção manual e envasadas a vácuo. Determinaram-se, durante o armazenamento a 4 °C, vários parâmetros de qualidade: avaliações microbiológicas, pH, análise do perfil de textura e avaliação sensorial mediante a análise descritiva quantitativa. A adição de quitosana na formulação das salsichas não reduziu os conteúdos microbiológicos e os valores de pH obtidos em todas as amostras foram similares, o que sugere que a adição de quitosana não influencia os valores de pH das salsichas. A adição de quitosana não afetou significativamente ($p \leq 0,05$) os valores de textura nem as análises sensoriais estudadas mantendo, portanto, a qualidade do embutido.

Palavras-chave: *salsichas; produtos cárneos; quitosana; qualidade microbiológica; qualidade sensorial; armazenamento.*

1 Introduction

Chitosan, a linear polymer of 2-amino-2-deoxy-β-D-glucan, is a deacetylated form of chitin, a naturally occurring cationic biopolymer (LIN; ZHAO, 2007; AIROLDI, 2008). It occurs as a shell component of crustaceans (crab and shrimp), as the skeletal substance of invertebrates, and as the cell wall constituent of fungi and insects. Applications of chitosan include as flocculating agent, clarifier, thickener, gas-selective membrane, coating material, promoter of plant disease resistance, wound-healing factor agent, and antimicrobial agent (DONG et al., 2000; GARCÍA, 2008; GARCÍA et al., 2008). Recently, use of chitosan was approved by European Pharmacopoeia (COUNCIL OF EUROPE EUROPEAN, 2002, 2005) for its application in pharmaceutical forms.

Chitosan exhibits in vitro antimicrobial activity against a range of foodborne microorganisms and consequently has attracted attention as a potential natural food preservative (DARMADJI; IZUMIMOTO, 1994; FANG; LI; SHIH, 1994; ROLLER; COVILL, 2000; ROLLER et al., 2002; SHAHIDI; ARACHCHI; JEON, 1999; SIMPSON et al., 1997; SUDARSHAN et al., 1992; WANG, 1992).

The objective of the study was to evaluate the influence of the chitosan addition on the quality of vacuum-packaged pork sausages.

2 Materials and methods

2.1 Sample preparation

Refrigerated lean pork and frozen pork backfat were obtained within 72 hours of slaughtering from a local meat packer and ground twice through a 9 and a 3-mm plate, respectively. An emulsion-type pork product was prepared using ground meat, NaCl, ice, pork backfat, trisodium phosphate, sugar, monosodium glutamate, sodium nitrite, and spicemix. Chitosan oligomer (1%, molecular weight = 1.23×10^6 and 85.33% degree of deacetylation, Biomaterials Center, University

Recebido para publicação em 11/3/2009
Aceito para publicação em 7/7/2009 (004081)
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of Havana, Cuba) was added to the treated products. Lean pork, salt and phosphate were placed in a silent cutter (MAINCA, Mod. 2130, Spain), mixed for about 1 minute after which 50% of the ice was added and mixed at high speed. When the temperature of mixture decreased around 1-2 °C, ground pork backfat was added and mixed until the temperature of mixture reached 10 °C. The remainder, 50% of the ice and other spices were added and mixed until the temperature of the mixture reached 13 °C. Total emulsification time was about 10 minutes and processing room temperature was 13 °C. The sausages were stuffed (MAINCA, Mod. 3763, Spain) into a fibrous casing (2.0 cm of diameter, NaloBar, Spain), dried (50 °C for 20 minutes), smoked (60 °C for 30 minutes) using sawdust, and cooked to 71 °C of internal temperature (about 1 hour) using a smokehouse. The cooked sausage was water-spray cooled for 5 minutes and dried at room temperature for 30 minutes. All samples (250 g in each bag) were vacuum-packaged in oxygen-impermeable polyester/polyethylene bags (30 × 40 cm) (Tables 1 and 2) using a vacuum packaging machine. The samples were stored for 26 days at 4 °C until analysis.

2.2 Microbiological evaluation and pH

A 10-g sample and 90 mL of sterilized distilled water were homogenized. The homogenate was serially diluted with sterilized distilled water and the dilutions were spread on the specific medium in aerobic conditions. Total aerobic bacteria (35 ± 1 °C, 48 hours), (NORMA CUBANA-INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, 2003), coliform bacteria counts (37 ± 1 °C, 48 hours), (NORMA CUBANA-INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, 2002a) and psychrophilic bacteria (4-6 °C, 10 d), (NORMA CUBANA, 1982a) were performed with plate count agar (PCA, Biocen, La Habana, Cuba). The violet red bile glucose agar (VRBGA, Biocen) and malt extract agar (MEA, Biocen) were used to grow *Enterobacteriaceae* (37 °C, 24 hours), (NORMA CUBANA, 1982b) and fungi and yeasts (30 °C, 48-72 hours), (NORMA CUBANA-INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, 2002b), respectively. Colony forming units (CFU) per gram in plates were counted, at a dilution giving 30–300 CFU per plate, with Micro Counter.

The pH was measured using a pH meter (Metrohm Herisau, Mod. E-510) according to ISO 2917 (INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, 1999).

Table 1. Base pack weight and thickness of complex film layers (n = 10).

Parameter	External layer	Intermediate layer	Internal layer
Weight/unit area ($g.m^{-2}$)	19.2 (1.2)	3.9 (0.5)	46.2 (2.8)
Thickness (μm)	22.4 (0.8)	11.0 (2.2)	77.3 (2.0)

Table 2. Water vapor and oxygen permeability, and thermal sealing resistance.

Parameter	Mean	Standard deviation
Water vapor permeability (g/m^2d) at 23 °C and 85% relative humidity	2.9	0.6
Gas permeability (cm^3/m^2d) a 25 °C	42.2	3.1
Heat seal resistance (N/15 mm)		
Heat seal of three sides bag	22.1	1.0
Heat seal of closing bag (vacuum-packaging manual machine)	17.9	0.9

2.3 Texture profile analysis

The sausage prepared from different treatment combinations was cut into 2-cm thick pieces, placed on the target plate and radially compressed at 25 °C. The two-bite compression test was conducted with a Universal Texture Analyzer (Instron, Model 1140, USA) as described by Bourne (1978). With a cylindrical probe, 75% compression was achieved and then, from the graphics, hardness, springiness, cohesiveness, gumminess and chewiness were calculated.

2.4 Sensory evaluation

Modified Quantitative Descriptive Analysis (STONE et al., 1974) was used to evaluate sensory qualities of the sausage. Five trained panelists evaluated products over a structured scale of 10 cm delimited at both ends. The sausage was reheated to a 70 °C internal temperature in an oven at 180 °C for about 15 minutes, sliced into 2-cm thick pieces, and served to the judges. The sensory attributes used in the current study were flavor, color, texture, and overall acceptance.

2.5 Statistical analysis

Experimental design used was 2 (chitosan treatment) × 1 (packaging) factorial and the whole experiment was replicated. Analyses of Variance were performed using STATISTICS software (STATSOFT, 1998) and the Duncan's multiple range tests were used to compare differences among mean values. Mean values were reported, and the significance was defined at $p \leq 0.05$.

3 Results and discussion

3.1 Microbiological results and pH measurements

The results of microbiological analyses of the sausages during the 26-days storage period are presented in Table 3. The addition of chitosan to the emulsion-type sausage did not reduce the number of microorganisms. The results of microbiological analyses of the sausages confirmed earlier results (YOUN et al., 1999; JO et al., 2001), showing that chitosan addition did not improve the microbiological quality of sausages.

After 14 days of storage at 4 °C the total viable counts were not significantly different, in the samples treated with 1% chitosan and the control samples, and total viable counts increased gradually in all samples during storage. An increase

Table 3. Microbial population changes (log CFU.g^{-1}) of pork sausage prepared with and without chitosan oligomer.

Treatments	Storage period (d)	Total aerobic bacteria	Coliform bacteria	Entero-bacteriaceae	Fungi	Yeast	Psychrophilic bacteria
Control	7	1.8	1	1	1	1	1.6
	14	2.8	1	1	1	1	1.6
	21	2.5	1	1	1	1	1
	26	2.3	1	1	1	2.0	2.1
Chitosan	7	1.5	1	1	1	1	1.8
	14	2.2	1	1	1	1	2.9
	21	3.0	1	1	1	1	1
	26	3.1	1	1.3	1	2.1	2.0

in total viable counts (approximately 1 log cycle, $p < 0.05$) was observed in the samples treated with 1% of chitosan after 21 days of storage. However, after storage for 26 days the total counts in the all samples had not already exceeded the maximum levels acceptable (10^7 CFU.g^{-1}) (INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY, 1999). By contrast, the total counts of sausages without chitosan were below the maximum acceptable levels for 21 days. These results partially agreed with those of Darmadji and Izumimoto (1994), who reported that *Staphylococci*, coliform, Gram-negative bacteria, *Micrococci*, and *Pseudomonas* in meat during storage at 30 °C for 48 hours and 4 °C for 10 days were inhibited by the presence of chitosan, and the results of Sagoo, Board and Roller (2002) and Georgantelis et al. (2007), who found that treatment with chitosan at a concentration of 0.3-0.6% and 1%, respectively, increased the shelf-life of fresh pork sausages stored at chill temperatures from 7 to 15 days.

Different mechanisms have been proposed for the antimicrobial effects of chitosan. Interaction between positively charged chitosan molecules and negatively charged microbial cell membranes leads to the leakage of proteinaceous and other intracellular constituents (CHEN; LIAU; TSAI, 1998; PAPINEAU et al., 1991; SUDHARSHAN; HOOVER; KNORR, 1992; YOUNG; KOHLE; KAUSS, 1982). Chitosan also acts as a chelating agent that selectively binds trace metals and thereby inhibits the production of toxins and microbial growth (CUERO; OSUJI; WASHINGTON, 1991). It also acts as a water binding agent and inhibits various enzymes (YOUNG; KOHLE; KAUSS, 1982). Chitosan also has bioabsorbant activity (KNORR, 1991) and can absorb nutrients of bacteria and may inhibit their growth. Binding of chitosan with DNA and inhibition of mRNA synthesis occurs via chitosan penetrating the nuclei of the microorganisms and interfering with the synthesis of mRNA and proteins (HADWIGER et al., 1985; SUDHARSHAN; HOOVER; KNORR, 1992).

The pH changes in fresh pork sausages during chill (4 °C) storage are shown in Table 4. On the day of preparation the pH of all batches ranged between 5.7 and 5.8. These values were similar to those reported in previous studies (CLAUS; HUNT, 1991; JO et al., 2001; SANTOS et al., 2003) for pork sausages. There was a gradual increase in pH in all samples during storage, probably due to the accumulation of basic compounds such as ammonia, derived from microbial action (NYCHAS; DROSINOS; BOARD, 1998). Throughout storage there was no significant difference ($p \leq 0.05$) in pH values between samples containing chitosan and control samples.

Table 4. Effect of chitosan on pH values of the experimental sausages during the 26-days storage period (n = 2).

Storage period (d)	Treatments	
	Control	Chitosan
0	5.70	5.80
7	6.21	6.10
14	6.26	6.34
21	5.82	5.75
26	5.85	5.90

3.2 *Texture and sensory analysis*

The sensory scores for sausages with vacuum packaging are shown in Table 5. Statistical significance was not found ($p \leq 0.05$) among all sensory parameters measured of sausages treated with chitosan and control samples. None of the mechanical texture profile analysis showed any difference either (Table 6). Therefore, the addition of chitosan oligomer in the emulsion-type pork sausage product did not change its color, flavor, texture and overall acceptance in agreement with Jo et al. (2001). The acid taste detected by judges from the twenty-first day onwards in samples with chitosan added were in line with the decrease in pH values (Table 4), and could be due to the growth of lactic acid bacteria, indicated by the increase in total aerobic bacteria counts (Table 3), what can be explained by the slight effectiveness of chitosan to inhibit this kind of bacteria.

Table 5 showed the results of the texture profile analysis of pork sausages during the storage period. The data showed that both samples prepared with chitosan and control samples had similar behavior ($p \leq 0.05$) during storage, which indicates that differences among the evaluated attributes were not due to the chitosan addition. Youn et al. (1999) reported,

Table 5. Sensory scores of pork sausage prepared with chitosan oligomer (n = 5).

Treatments	Storage period (d)	Attributes					
		Color	Typical odor	Typical taste	Acid taste	Texture	Overall acceptance
Control	0	5.5 (0.7)[a]	9.9 (0.2)[d]	9.7 (0.4)[c]	0.0 (0.0)[ab]	7.0 (1.0)[a]	9.3 (0.4)[b]
	7	5.0 (0.0)[a]	9.4 (0.6)[cd]	9.5 (0.3)[bc]	0.0 (0.0)[ab]	5.7 (1.1)[a]	8.5 (0.6)[a]
	14	5.1 (0.2)[a]	9.2 (0.5)[bc]	9.3 (0.5)[abc]	0.0 (0.0)[a]	6.8 (1.6)[a]	8.9 (0.2)[ab]
	21	5.6 (0.7)[a]	8.4 (0.6)[a]	9.2 (0.2)[abc]	0.2 (0.2)[abc]	6.3 (0.4)[a]	8.8 (0.2)[ab]
	26	5.7 (1.4)[a]	9.1 (0.2)[bc]	8.7 (0.2)[a]	0.4 (0.3)[c]	5.9 (0.9)[a]	8.4 (0.8)[a]
Chitosan	0	5.2 (0.4)[a]	9.7 (0.2)[cd]	9.5 (0.3)[bc]	0.0 (0.0)[a]	6.9 (1.1)[a]	9.3 (0.4)[b]
	7	4.7 (0.4)[a]	9.5 (0.4)[cd]	9.2 (0.4)[abc]	0.0 (0.0)[a]	5.7 (1.1)[a]	8.7 (0.5)[ab]
	14	5.4 (1.1)[a]	9.1 (0.3)[bc]	9.0 (0.6)[ab]	0.0 (0.0)[a]	7.1 (2.1)[a]	9.0 (0.3)[ab]
	21	5.6 (0.6)[a]	8.7 (0.4)[ab]	9.1 (0.6)[abc]	0.3 (0.4)[bc]	5.9 (0.4)[a]	9.2 (0.2)[b]
	26	5.6 (1.0)[a]	9.1 (0.3)[bc]	8.7 (0.4)[a]	0.7 (0.1)[d]	6.0 (0.3)[a]	8.7 (0.5)[ab]

Mean (Standard deviation). Different letters (a-d) within a same column differ significantly ($p \leq 0.05$).

Table 6. Texture profile analysis of pork sausage prepared with chitosan oligomer (n = 4).

Treatments	Storage period (d)	Cohesiveness	Gumminess (kgf)	Chewiness (kgf.mm)	Hardness (kgf)	Springiness (mm)
Control	0	0.28 (0.04)[abc]	4.0 (1.0)[bc]	34 (9)[bc]	14.2 (2.1)[bc]	8.4 (0.2)[b]
	7	0.31 (0.02)[bcd]	4.7 (0.3)[c]	39 (3)[bc]	14.9 (0.4)[c]	8.2 (0.2)[b]
	14	0.37 (0.03)[d]	4.1 (0.6)[bc]	39 (7)[bc]	11.2 (1.0)[a]	9.4 (0.4)[c]
	21	0.32 (0.01)[bcd]	4.1 (0.7)[bc]	40 (5)[bc]	12.8 (1.5)[b]	9.5 (0.4)[c]
	26	0.24 (0.05)[a]	3.2 (0.8)[ab]	19 (5)[a]	13.6 (0.7)[bc]	6.0 (0.0)[a]
Chitosan	0	0.31 (0.03)[bcd]	4.9 (0.8)[c]	42 (8)[c]	16.4 (1.3)[c]	8.5 (0.4)[b]
	7	0.33 (0.06)[cd]	4.8 (1.1)[c]	42 (10)[c]	14.2 (0.9)[bc]	8.9 (0.7)[bc]
	14	0.34 (0.01)[cd]	3.7 (0.2)[ab]	31 (1)[b]	11.3 (0.6)[a]	8.5 (0.7)[b]
	21	0.36 (0.01)[d]	3.6 (0.3)[ab]	35 (4)[bc]	10.4 (0.9)[a]	9.6 (0.2)[c]
	26	0.27 (0.02)[ab]	3.6 (0.5)[ab]	30 (5)[b]	14.1 (0.5)[bc]	8.2 (0.2)[b]

Mean (Standard deviation). Different letters (a-d) within a same column differ significantly ($p \leq 0.05$).

however, that the hardness of the sausage increased by an increase in molecular weight of chitosan, and, especially, at the highest molecular weight (120,000) increased viscosity which may cause adverse effect on processing. In our study, chitosan was of medium molecular weight, so it did not affect hardness.

4 Conclusions

The study showed that chitosan can be used in pork sausages without affecting texture and sensory attributes. Although the quality of the sausages with added chitosan was acceptable, the investigation of its activity in a range of food systems is still needed for successful application to meat products. Microbial growth was not inhibited by addition of chitosan to sausages and the pH values obtained in all samples were similar, which suggests that the chitosan addition did not influence these values.

Acknowledgements

The authors wish to thank the Food Industry Research Institute of the Ministry of Food Industry of Cuba.

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