EFFECT OF HIGH HYDROSTATIC PRESSURE TECHNOLOGY IN MICROBIOLOGICAL AND PHYSICAL PROPERTIES OF PORK NATURAL CASINGS

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Abstract – Natural casings are used in traditional sausage production and their sanitary hygienic quality is very important contributing to meat sausages safety. The effect of different high hydrostatic pressure (HHP) treatments (binomial pressure-time) on microbial load and physical resistance of casings was studied according to a response surface methodology (RSM-Response Surface Design), Microbial counts, tensile strength and elongation of treated casings were determined. Samples control had high *Enterobacteriaceae* (5.72 log cfu/g), *E. coli* (4.25 log cfu/g), *Pseudomonas* (2.37 log cfu/g), *Enterococci* (3.26 log cfu/g), coagulase negative *Staphylococcus* (3.38 log cfu/g) and lactic acid bacteria (4.48 log cfu/g) counts. The HHP treatments reduced the microbial groups studied particularly gram-negative bacteria (even in the treatments at lower pressures), being noticed that under pressure above 400 MPa the reduction was overall.

The maximum force values obtained in tensile radial width strength and perfuration tests suggested that HHP does not affect significantly the physical resistance of casings. This study suggests that HHP can be applied to natural casings in order to assure its microbial quality.

Key Words – Casings, Non-thermal treatment, Tensile strength

I. INTRODUCTION

The definition of natural casing includes intestine, stomachs, bladder and other membranes of food animals, principally, beef, pork or lamb. These natural casings play a significant role in today's sausage casing industry because it has interesting technological skills. Some producers of sausage consider that natural casing is the best choice due to their characteristics such as permeability to steam, fat and smoke, elasticity and tensile strength, allowing expansion during filling and giving the best flavor and appearance to the final product [1].

Microbial quality of natural casings depends on hygiene slaughter procedures, processing treatments on casing industry (selection, calibration, and packaging) and storage temperature [2]. Natural casings, by their nature, are contaminated with bacteria (10^4 to 10^7 cfu/g) [3, 4, 5]. Trigo and Fraqueza (1998) reported that the initial microbiota on small and large pork casings was dominated by fecal *Streptococci* (4.6 and 4.4 log cfu/g), *Enterobacteriaceae* (7.5 and 6.3 log cfu/g) and *Clostridium perfringens* (1.7 and 1.8 log ufc/g). This high microbial load increases the risk of meat products contamination with pathogenic microorganisms and it could pose health risk to the consumer [2, 6].

Casings are usually preserved by salting, curing and/or drying [7, 8, 9]. However, these methods are not effective to ensure the reduction of pathogens because, for example, in salted natural casings exist microorganisms extremely salt-tolerant or halophylic species [10].

Nowadays, consumers require minimally processed and microbiological safe products and as a consequence, industry search innovative technologies. High hydrostatic pressure (HHP) is a very promising non-thermal technology for food products submitted to pressure above 100 MPa [11]. This technology preserves the quality without significant modifications of food matrix (color, flavor, texture and nutritional values), with the advantage of efficiently eliminate microorganisms, providing microbiological safety and increased shelf life [12]. However, the effectiveness of treatments or the resistance of microorganisms can be extremely variable and depends on process parameters such as pressure (P), temperature (T) and exposure time (t).

The promising applicability of HHP in meat products is quite recent, but there are no studies concerning the application of this technology on natural casings.

The aim of this study was to evaluate the effect of high hydrostatic pressure, binomial impact of P and t on the initial microbial load and physical resistance of casings.

II. MATERIALS AND METHODS III.

The natural pork casings were delivered under refrigeration by a local slaughterhouse to the laboratory. These casings were divided in portions (twenty-five grams for microbiological analysis and fifty centimeters for physical tests), vacuum packaged in a EVT-7-CD machine (Tecnoprip, Barcelona, Spain) and immediately frozen (-20ºC) .

The casings vacuum packaged were submitted to high hydrostatic treatments in a High Pressure Food Processor (N.C. Hyperbaric, Wave 6000/135). The rates of compression and decompression were controlled automatically. The samples were submitted to isostatic pressure between 200 and 600 MPa at controlled temperature (10ºC) and 4 to 30 min according to a response surface methodology (RSM-Response Surface Design), in order to minimize the number of experiments needed but keeping statistical significance of the results [13].

For microbiological analysis were performed: *Enterobacteriaceae* count in Violet Red Bile Dextrose Agar (VRBD, Scharlau Chemie) incubated at 37ºC, 24 h; *E. coli* count in Gelose BCIG Tergitol (Biokar Diagnostic) at 44.5ºC, 24 h; *Pseudomonas* count in CFC medium(Oxoid) with supplements at 30ºC, 48 h; *Brochothrix thermosphacta* count in STAA (Oxoid) with supplements at 30ºC, 48 h; Enterococci count in Slanetz, Bartley agar (Scharlau Chemie) at 37ºC, 48 h; Coagulase negative Staphylococcus count in Mannitol Salt Agar (MSA – Scharlau Chemie) at 30ºC, 48 h; lactic acid bacteria (LAB) count in MRS Agar plates with supplement at 30ºC, 48 h in anaerobiosis; *Listeria monocytogenes* count in Agar Listeria Ottaviani Agosti (ALOA) plates at 37ºC, 24 h; *Salmonella* count in Cromo Agar plates at 37ºC, 24 h.

Two different physical tests were performed on a texturometer (Stable Micro System, England): tensile radial width strength and perforation. In first test was used tension gripping (A/TG) with an tool appendix attached and in the second was used one platform with central bore and needle as probe. For tensile strength width test, samples were cut with two centimeters wide. For perforation test, samples were prepared in one layer casing with approximately 16 cm^2.

III. RESULTS AND DISCUSSION

The enumeration of *Salmonella* and *Listeria monocytogenes* were for all tested samples <10ufc/g.

The microbial results of HHP effect on pork casings tested are presented in Table 1.

Table 1 Microbial results from treated HHP natural pork casings (expressed as log cfu/g)

P (MPa)	t (s)	*Enterobacteriaceae*	*E. coli*	*Pseudomonas*	*Brochothrix thermosplhacta*	*Enterococci*	*Coagulase negative Staphylococcus*	LAB
0.1	0	5.72	4.25	2.37	1.52	3.26	3.38	4.48
202	960	2.97	1.24	1.27	1.79	3.24	3.89	5.30
260	390	0	0	0	0.96	3.03	2.98	5.31
260	1530	0	0	0	1.16	2.51	2.35	3.92
400	154	0	0	0	0	2.94	2.65	4.32
400	960	0	0	0	0	1.3	0.38	3.45
400	1800	0	0	0	0	0	0	0
540	270	0	0	0	0	0	0	0
540	390	0	0	0	0	0	0	0
600	210	0	0	0	0	0	0	0

The spoilage microbiota represented by indicators *Pseudomonas* and *Enterobacteriaceae* shown a reduction of 1 to 3 log cfu/g with pressure ranges until 202 MPa and a complete reduction occurring from 260 MPa.

*T*he indicator of fecal contamination *E. coli* had a complete reduction with HHP treatments suggesting that if Salmonella was present probably would be completely eliminated.

At low pressure till 260 MPa, *Brochothrix thermosphacta* persists. HHP was efficient on microorganism elimination only at pressures over 400 MPa.

The reduction of technological microbiota (*Staplylococcus* and LAB) occurs at 400 MPa, being eliminated at pressures above 400 MPa.

In fact as pressure or time increases the counts of those bacteria decreased significantly to complete inactivation at higher pressures. This finding is in agreement with several studies about high hydrostatic pressure technology. In general, studies indicate that treatment at 600 MPa (5-10 min, 15-30ºC) is required to sufficiently reduce the level of pathogenic and to prevent their growth during storage [14, 15, 16]. Vercammen *et al.* [17] observed that meat treatment at 400 MPa causes inactivation of all bacteria studied and reduction levels were 3.2 log greater than with 300 MPa; at 500 MPa or more, the number of survivors was lower than detection limit.

The physical properties of natural casings evaluated in this study were essentially mechanical properties as elongation (ε) and tensile strength.

Maximum rupture force (Tensile strength and perforation force) was measured to indicate if casing´s capacity to resist the forces applied during stuffing was affected by the high-pressure treatments. These values are presented in Table 2.

Table 2 Maximum tensile strength (F_T), elongation (ε) and maximum perforation force (F_P) values of control and treated HHP natural pork casings

IV.

P (MPa)	t (s)	$F_T \pm \sigma$ (N)	$\varepsilon \pm \sigma$ (mm)	$F_P \pm \sigma$ (N)
0.1	0	14.633 ± 2.213	21.167 ± 0.704	2.570 ± 0.137
202	960	12.047 ± 1.236	20.619 ± 0.319	2.697 ± 0.216
260	390	11.818 ± 1.044	21.195 ± 0.146	2.905 ± 0.096
260	1530	12.628 ± 0.924	20.912 ± 0.518	2.811 ± 0.208
400	154	12.050 ± 0.579	17.832 ± 0.328	2.683 ± 0.200
400	960	14.323 ± 0.924	18.717 ± 0.518	2.710 ± 0.208
400	1800	14.563 ± 1.470	21.891 ± 2.029	2.595 ± 0.302
540	270	14.810 ± 0.243	23.494 ± 0.190	2.594 ± 0.032
540	390	12.396 ± 1.439	21.665 ± 0.459	2.449 ± 0.267
600	210	13.837 ± 0.620	24.522 ± 0.260	3.003 ± 0.166

Preliminary statistical data showed us that individually, both P and t, affected F_t significantly ($p<0.05$)

As can be seen on Figure 1., F_P needed to rupture casing increases with pressure and time. The same behavior was found for maximum perforation force (F_P).

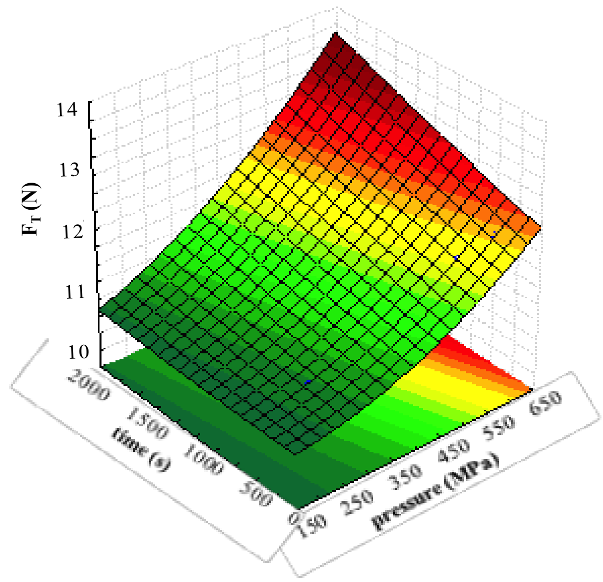

Figure 1. Response surface and respective contour plot, fitted to the experimental data points, corresponding to maximum tensile strength (F_T), as a function of time (s) and pressure (MPa).

Pressure and time did not affect significantly the elongation, ε, of the casings.

IV. CONCLUSION

The HHP treatments reduced the microbiota present on natural pork casings particularly gram-negative bacteria (even in the treatments at lower pressures), being noticed that under pressure above 400 MPa the reduction was overall for gram negative and positive bacterial groups under study.

The maximum force values obtained in tensile radial width strength and perforation tests suggested that HHP seems to affect positively the physical properties of casings.

This study also suggests that HHP can be applied on natural casings in order to assure its microbial quality.

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REFERENCES

1. Anonymous. The International Natural Sausage Casing Association (INSCA).
2. Trigo, M. J., & Fraqueza, M. J. (1998). Effect of gamma radiation on microbial population of natural casings. Radiation Physics and Chemistry 52: 125-128.
3. Bakker, W. A. M., Houben, J. H., Koolmees, P. A., Bindrich, U., & Sprehe, L. (1999). Effect of initial mild curing, with additives, of hog and sheep sausage casings on their microbial quality and mechanical properties after storage at difference temperatures. Meat Science, 51(2): 163-174.
4. Byun, M. W., Lee, J. W., Jo, C., & Yook, H. S. (2001). Quality properties of sausage made with gamma-irradiated natural pork and lamb casing. Meat Science, 59(3): 223-228.
5. Ockerman, H. W., & Hansen, C. L. (2000). Animal by-product processing and utilization. Lancaster, PA: Technomic Publishing Co.
6. Chawla, S. P., Chander, R., Sharma, A. (2006). Safe and shelf-stable natural casing using hurdle technology. Food control, 17: 127-131.
7. Fischer, A., Schweflinghaus, M., (1988). Naturdärme 1: anatomie und gewinnung. Fleischerei, 39: 10-14.
8. Fischer, A., Schweflinghaus, M., (1988). Naturdärme 2: Rechtliche Bestimmungen, Handelsbrauch, Verwendung, Darmfehler, Qualität und Qualitätsprüfung. Fleischerei, 39: 98-103.
9. Ockerman, H.W., Hansen, C.L. (1988). Animal by-product Processing. Ellis Horwood, Chichester, UK (pp. 202-231).
10. Houben, J. H. (2005). A survey of dry-salted natural casings for the presence of *Salmonella* spp., *Listeria monocytogenes* and sulphite-reducing *Clostridium* spores. Food Microbiology, 22: 221-225.
11. Cruz, A. G., Faria, J. A. F., Saad, S. M. I., Sant´Ana, A. S., & Cristianini, M. (2010). High Pressure Processing and Pulsed Electric Fields: Potential use in probiotic dairy foods processing. Trends in Food Science & Technology, 21:483-493.
12. Mathias, S. P., Rosenthal, A., Gaspar, A., Deliza, R., Slongo, A. P., Vicente, J., et al. (2010). Alterações oxidativas (cor e lipídios) em presunto de peru tratado por Alta Pressão Hidrostática (APH). Ciência e Tecnologia de Alimentos, 30(4): 852-857.
13. Ribeiro, M. H.L., Afonso, C., Vila-Real, H. J., Alfaia, A. J., Ferreira, L. (2010). Contribution of response surface methodology to the modeling of naringin hydrolysis by naringinase Ca-alginate beads under high pressure. LWT - Food Science and Technology, 43 (3): 482-487.
14. Aymerich, M. T., Jofré, A., Garriga, M., & Hugas, M. (2005). Inhibition of *Listeria monocytogenes* and Salmonella by natural antimicrobials and high hydrostatic pressure in sliced cooked ham. Journal of Food Protection, 68(1): 173–177.
15. Marcos, B., Jofré, A., Aymerich, T., Monfort, J.M., & Garriga, M. (2008). Combined effect of natural antimicrobials and high pressure processing to prevent *Listeria monocytogenes* growth after a cold chain break during storage of cooked ham. Food Control, 19(1): 76–81.
16. Jofré, A., Aymerich, T., Grèbol, N., & Garriga, M. (2009). Efficiency of high hydrostatic pressure at 600 MPa against food-borne microorganisms by challenge tests on convenience meat products. Lwt-Food Science and Technology, 42(5): 924-928.
17. Vercammen, A., Vanoirbeek, K. G. A., Luurquin, I., Steen, L., Goemaere, O., Szczepaniak, S., Paelink, H., Hendrickx, M. E. G., Michiels, C. W. (2011). Shelf-life extension of cooked ham model product by high hydrostatic pressure and natural preservatives. Innovative Food Science And Emerging Technologies, 12: 407-415.