MICROBIAL QUALITY OF LOW-FAT BOLOGNA SAUSAGES DURING PROCESSING AND CHILLING STORAGE

Alfonso V. Carrascosa[1],Francisco Jiménez-Colmenero[2], Paloma Fernández[2] and José Carballo[2].

[1] Instituto de Fermentaciones Industriales (C.S.I.C.). C/ Juan de la Cierva 3. 28006 Madrid (Spain).
[2] Instituto del Frío (C.S.I.C.). Ciudad Universitaria s/n. 28040 Madrid (Spain).

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BACKGROUND AND OBJECTIVES

Microbiological aspects of pork bologna sausages has been studied in several research works (Locatelli *et al.*, 1988; Zanoni *et al.*,1993). While limited information is available on the refrigerated storage characteristics of low-fat pork bologna, microbial quality has not been reported. The aim of this work was study the evolution during processing and chilled storage of microbial risk groups of low-fat pork bologna as influenced by fat level, and its implication on Hazard Analysis and Critical Control Point (HACCP) of the manufacture process.

MATERIALS AND METHODS

The raw materials (pork meat and fat) and the bologna sausage preparation used in these experiments was the reported by Cavestany *et al.* (1994), except that the batters were chopped for a period till to give final temperature of 15±1°C. Pork meat, back fat and water were combined in the requesite proportions to give products that would yield the following approximate composition on analysis: low-fat samples (LF) with protein 13.5%, moisture 75% and fat 8%; high-fat samples (HF) with protein 13.5%, moisture 60% and fat 22%. All samples further contained 2.5% NaCl, 0.18% phosphate, 0.012% $NaNO_2$ and 0.4% of a commercial flavouring mixture (Gewurzmüller,Germany).

The batter was stuffed into 11 cm of diameter fibrous casings (Viscora, Beauvais, France) and cooked to an internal temperature of 70°C in a forced-air oven (Rational CM6, GroBküchentechnik GmbH, Landsberg a. Lech, Germany) set at 90°C. After sitting for 3 hr at ambient temperature (20-22°C), the sausages were cooled to 2°C in a chill room and kept there until used. Cooking loss was estimated as (%) weight loss occuring during the cooking process.

Moisture, protein, fat, ash and pH (in triplicate) of bologna sausage were determined as described by Carballo *et al.* (1995). Emulsion stability was determined on four replicates of meat batter as described by Jiménez-Colmenero *et al.*(1995). Total cooked-out fluid (TC, %), water released (WR, %) and fat release (FR, %) were obtained.

Inmediatly after fabrication bolognas were sliced and vacuum packed in plastic bags (Cryovac BB4L) (approx. 17g/slice, 5 slices/package) and stored at 2±1°C until use, for the study of microbial quality during chilled storage. Microbial analysis were made before and after cooking, after slicing and vacuum packing, and after one week, two weeks and one month of chilled storage. 10g of each sample (at least by triplicate) were weighed in sterile plastic bags in a laminar air flow cabinet (Gelaire,Italy), and stomached in 90ml of peptone water (0.1%) for two minutes in a commercial homogenizer (MASTICATOR, IUL, Spain). Appropriate dilutions were spread plated on Plate Count Agar (OXOID) and incubated during 48hr at 30°C to obtain Total Aerobic Count, and during 10 days at 7°C to obtain Total Psycrotrophic Count; on Malt Agar (DIFCO) to obtain Yeasts and Moulds count after incubation at 25°C during 5 days; on Mannitol Salt Agar (DIFCO) to obtain *Micrococcaceae* counts; on Baird Parker Agar (OXOID) to obtain presumptive *Staphylococcus aureus* counts after incubation during 48hr at 37°C; on Violet Red Bile Glucose agar to obtain *Enterobacteriaceae* counts after incubation in two layer plates during 48hr at 37°C; on MRS Agar (OXOID) to obtain Lactic Acid Bacteria counts after incubation in two layer plates during 48hr at 30°C; on Slanetz-Bartley Agar (OXOID) to obtain Enterococci counts after incubation of two layer plates during 48hr at 37°C; on Tripticase Soy Agar (BBL), after pasteurizing appropiate dilutions during 10 minutes at 80°C, and after incubation during 48hr at 30°C to obtain Sporulated Acrobes (*Bacillus*) counts and on SPS agar (DIFCO) tubes to obtain sluphite reducing Clostridia after incubation in anaerobiosis at 37°C during 48hr. Counts were expressed as $\log_{10}$ c.f.u./g.

One- and two-way analysis of variance using and F test and least-squares difference in means between pairs was utilize to obtain confidence intervals, using STATGRAPHICS package.

RESULTS AND DISCUSSION

Bologna mean values of moisture content were: 73.0% for LF and 58.7% for HF bolognas. Protein mean values were: 14.5% for LF and 14.2% for HF. Mean fat was: 8.6% for LF and 22.8% for HF. The composition of LF and HF bologna sausages (close to the target levels), indicated that modifications to fat content was largely at the expense of moisture level. The small variations found in actual as opposed to target proximate composition may also be ascribed partly to two causes: slight errors in the actual make-up of target formulations, and alterations to the composition of the sausages due the variation in the behaviour of meat batters as a function of the variables studied (Carballo *et al.*,1995). Added water (Added water= % moisture- 4 x % protein) was 1.9% for HF and 15% for LF samples. Values of pH in all samples were in the range of 6.36-6.51 and did not vary throughout storage.

The TC was noticeably greater ($p<0.05$) in the samples with the LF content (higher percentage of moisture)(Table 1) and could be explained to a higher water released. Others authors have reported similar results (Claus *et al.*,1989; Jiménez-Colmenero *et al.*, 1995). As expected, cooking losses were higher the lower the percentage of fat (Table 1), due to an increase in added water (Ahmed *et al.*,1990; Gregg *et al.*,1993; Cavestany *et al.*, 1994).

Microbiological counts before cooking were greater ($p<0.05$) on HF bolognas (Table 2). Cooking had a letal effect on all microbial risk flora, thus it microbial counts over 10^2 c.f.u./g were are not detected, except for the Total Aerobic Counts (TAC) and Sporulated Aerobes counts (TSC) and Yeasts and Moulds Counts (YMC) on HF samples. These levels could be explained considering the thermotolerant microorganisms.

During chilling storage, microbial counts were greater ($p<0.05$) on LF samples (Table 2), probably as a consequence of the higher moisture content of these bolognas. Whereas chill temperatures avoid the recovering of microbial risk groups of pathogens (e.g *Enterobacteriaceae*) before one month in HF bolognas, in LF bolognas the recovering was observed after one week. LF bolognas seems to have a more reduced shelf-life than HF bolognas. *S. aureus* and Enterococci were not recovered neither HF nor LF samples.

Although no spoilage was observed, considering 10^7 c.f.u./g of total aerobic count as a generally-accepted level for the onset of spoilage in bologna sausage (Mussato *et al.*,1984), LF levels reduces the shelf-life of bolognas at least at two weeks.

CONCLUSIONS

- As in the case of HF bologna, cooking of LF bologna must be considered Critical Control Point 1 (CCP1) and chill storage Critical Control Point 2 (CCP2).
- Shelf life of bologna LF is reduced.

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Table 1. Batter stability and cooking loss of meat batters as influenced by fat content[1].

Samples	Total cooked-out fluid (%)	Fat released (%)	Water released (%)	Cooking loss (%)
LF	2.29^a	0.11^a	2.18^a	6.56^a
HF	1.05^b	0.06^b	0.99^b	5.25^b
SEM	0.08	0.003	0.079	0.2

[1]Differents letters in the same column indicate significant differences ($p<0.05$). LF: Low Fat; HF: High Fat. SEM = Standard error of the mean.

Table 2. Microbiological analysis of bolognas during processing and chilled storage as influenced by fat content[1].

	TAC		TPC		YMC		MC		SC		EC		LABC		ECC		TSC	
	HF	LF	HF	LF	HF	LF	HF	LF	HF	LF	HF	LF	HF	LF	HF	LF	HF	LF
1	6.30^a	5.46^b	6.22^a	5.23^b	6.21^a	5.61^b	4.84^a	4.55^b	3.64^a	3.67^a	5.16^a	4.86^b	5.07^a	4.23^b	4.58^a	4.11^b	3.35^a	3.25^b
2	2.41^a	2.26^a	<2	<2	2.96	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	2.62^a	2.32^a
3	2.30^a	3.25^a	<2	2.53	<2	2.70	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
4	4.00^a	4.82^a	4.08^a	4.92^a	3.28^a	3.82^a	<2	<2	<2	<2	<2	3.25	2.73^a	3.82^a	<2	<2	<2	<2
5	5.04^a	7.49^b	5.11^a	7.08^b	3.56^a	4.91^b	4.19^a	4.97^a	<2	<2	<2	3.19	4.09^a	4.75^a	<2	<2	2.26^a	3.82^b
6	8.05^a	8.69^a	8.24^a	8.58^b	6.94^a	7.32^a	5.98^a	4.77^b	<2	<2	3.56^a	5.73^b	6.81^a	7.65^a	<2	<2	3.41^a	4.32^a

[1]TAC: Total aerobic count; TPC: Total psycrotrophic count; YMC: Yeasts and moulds count; MC: *Micrococcaceae* count; SC: *S. aureus* count; EC: *Enterobacteriaceae* count; LABC: Lactic Acid Bacteria count; ECC: Enterococci count; TSC: Total sporulated count; 1. Meat batter; 2. After cooking; 3. After slicing and vacuum-packing; 4. After one week at 2±1°C; 5. After two weeks at 2±1°C; 6. After one month at 2±1°C. Different superscript beetwen HF and LF counts indicates significant differences ($p<0.05$).