Shelf Life Extension of Vacuum Packaged Vienna Sausages by In-package Pasteurization

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SUMMARY: The effects of in-package pasteurization and subsequent storage temperature variation on the microbiological shelf life and predominant spoilage populations of vacuum packaged vienna sausages were examined. Pasteurization in 80°C water for 20 minutes was followed by constant temperature storage at 7°C and 25°C (accelerated test). Post-pasteurization storage at 7°C led to a ca. four-fold shelf life increase, but did not eliminate spoilage. Although lactic acid bacteria, specifically homofermentative lactobacilli and leuconostocs, dominated the spoilage ecology in pasteurized and control samples at both storage temperatures, pasteurization and storage at 25°C led to a diversification of the lactic spoilage populations.

INTRODUCTION: Despite chilling, the growth of psychrotrophic lactic acid bacteria (LAB) is favoured in vacuum packaged processed meats due to their tolerance of refrigeration temperatures, microaerophilic conditions, low pH values and curing salts (SHARPE, 1962). They can reach numbers of 10^8 g^{-1} or more after relatively short times, depending on the storage temperature and most studies accord the highest predominance to lactobacilli and leuconostocs in varying percentages (REUTER, 1969; HOLZAPFEL and GERBER, 1986; SCHILLINGER and LÜCKE, 1988; BORCH and MOLIN, 1988). Spoilage of vacuum packaged processed meats by LAB leads to undesirable souring of the product, the development of off odours and slime and gas formation in the packs.The resultant reduction of refrigerated shelf life causes product returns to the manufacturers from the marketplace which result in large scale economic losses (SCHILLINGER and LÜCKE, 1988; KORKEALA et al., 1988). Similar, severe problems are experienced by local manufacturers of vacuum packaged processed meats and specifically vienna sausages (VON HOLY and HOLZAPFEL, 1989).

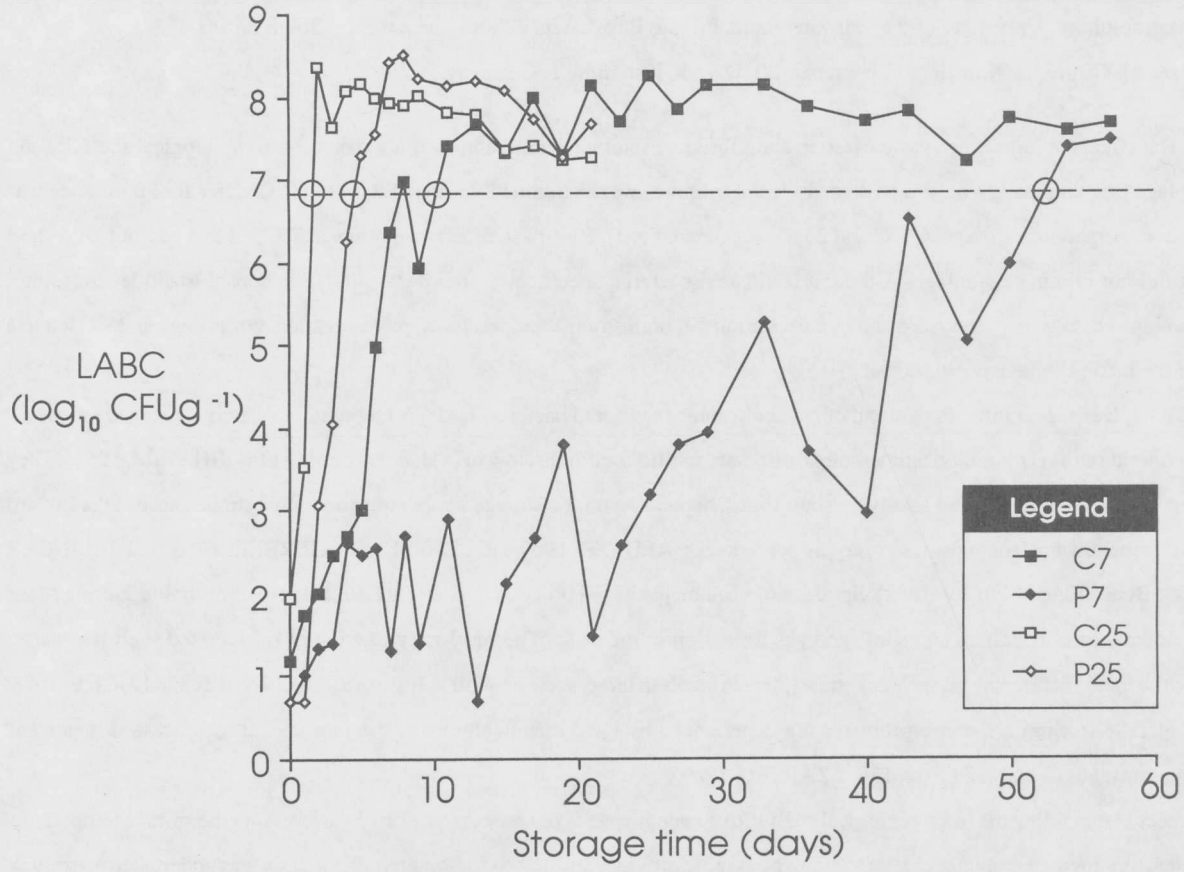
Since the prospect of extending the microbiological shelf life of vacuum packaged processed meats by decreasing the storage temperature alone is limited, heat treatment of retail packs after vacuum packaging provides an attractive alternative. This study was undertaken to evaluate a moderate, post-packaging pasteurization step for increasing the shelf life of vacuum packaged, smoked vienna sausages.

MATERIALS and METHODS: Vienna type emulsion sausages were manufactured at a meat processing plant according to a standardized commercial recipe and vacuum packaged into 500g packages by routine procedures. The first group of samples (C) was stored on ice without further treatment. The second group (P) was pasteurized in a water cooker held at 80°C ± 1°C for 20 minutes. After heating, packages were cooled rapidly to below 4°C in crushed ice. Core temperatures of randomly chosen packs on removal from the cooker ranged from 52 to 57°C, and peripheral temperatures from 58 to 59°C. Samples were stored at controlled temperatures of 7°C and 25°C and analysed by standard methods (VON HOLY and HOLZAPFEL, 1989) for numbers of total aerobic bacteria (TAPC) and lactic acid bacteria (LABC) by duplicate spread plating. Microbiological shelf life was taken as storage time needed to reach a microbiological count of 5×10^6 colony forming units (CFU) g^{-1}(FRUIN et al., 1978).

Four colonies of predominant spoilage bacteria per sample were randomly selected in equal proportions from TAPC and LABC plates of the highest dilutions showing growth and characterized. Three hundred and sixty eight LAB from TAPC (174 isolates) and LABC plates (194 isolates) were assigned to six biogroups (VON HOLY and HOLZAPFEL, 1989), based on key physiological and biochemical tests, viz.: heterofermentative lactobacilli (group I); leuconostocs (group II); homofermentative lactobacilli (group III); pediococci (group IVA); streptococci/enterococci (group IVB); carnobacteria (group V) and thermobacteria (group VI).

RESULTS and DISCUSSION: The TAPC and LABC (Fig. 1) increased with increasing storage time to numbers above the cut-off point for microbiological shelf life ($\log_{10}$6, 70). It was evident that microbial numbers of the TAPC were closely approximated by those of the LABC (VON HOLY and HOLZAPFEL, 1989) and that lactic acid bacteria therefore dominated the TAPC. Final population densities in the pasteurized samples were equal to those found in the control samples and did thus not depend on the size of the initial inoculum (REUTER, 1969). Based on microbial numbers, however, pasteurization led to increased shelf life, and this effect was synergistically enhanced by low temperature (i.e. 7°C) storage (Fig. 1). At 25°C storage, only a marginal gain in shelf life of 2,5 to three days was observed in the pasteurized samples compared to the controls (Fig. 1). At 7°C storage, this gain increased to 38 to 40 days. The obvious importance of constant

Figure 1: Lactic acid bacteria counts (LABC) of control (C) and pasteurized (P) vacuum packaged, smoked vienna sausages stored at 25°C and 7°C indicating intersection of counts (○) with the microbiological shelf life limit (---) of $5x10^6$ CFU g^{-1} (log_{10} 6,70).



maintenance of this temperature in the extension of microbiological shelf life emphasized the central role of the cold chain from producer to consumer. The considerable heat resistance documented for psychrotrophic meat spoilage LAB, however, (BORCH et al., 1988) could possibly explain the apparent failure of the pasteurization process to curtail product spoilage.

The shelf life figures obtained in this study at 7°C for pasteurized samples correlated with findings of HILL et al. (1979) on vacuum packaged luncheon meats, while a study on a variety of sliced vacuum packaged smallgoods stored at 5°C suggested a shelf life of two weeks (SHAY et al., 1978). This finding correlated well with the shelf life established for control samples stored at 7°C in this study (Fig. 1). REUTER (1969) reported a shelf life of three days and 12-17 days at 20°C and 4-6°C storage, respectively, for sliced, pre-packaged, cooked emulsion sausage products, which again was in agreement with our findings for control samples stored at 7°C and 25°C.

Lactic acid bacteria isolates predominated during the entire storage period of both pasteurized and control samples stored at 7°C and 25°C which was in agreement with microbial counts. Their distribution over pasteurized and control samples at the two storage temperatures is shown in Table 1. At both storage temperatures it was evident that pasteurization led to a diversification of the lactic spoilage populations

Table 1: Composition of predominant lactic acid bacteria populations from pasteurized (P) and control (C) vacuum packaged, smoked vienna sausages stored at 7°C and 25°C.

Sample treatment	Percentage isolates per biogroup[a]							Number of isolates
	I	II	III	IV A	IV B	V	VI	
25C	6,7	13,3	58,3	5,0	13,4	-	3,3	60
25P	21,8	9,1	21,8	18,2	25,5	-	3,6	55
7C	4,9	34,0	50,0	6,2	4,9	-	-	144
7P	10,0	32,1	39,4	9,3	9,2	-	-	109

[a] Biogroup identification: I-heterofermentative lactobacilli; II – leuconostocs; III – homofermentative lactobacilli; IV A – pediococci; IV B streptococci/enterococci; V – carnobacteria; VI – thermobacteria.

At 25°C storage, this was particularly noticeable for the heterofermentative lactobacilli (group I), and the pediococci and streptococci/enterococci (groups IVA and B, respectively), which all showed marked proportional increases in the pasteurized samples. Conversely, the proportion of leuconostocs (group II) and homofermentative lactobacilli (group III) were decreased in the pasteurized samples stored at 25°C. In samples stored at 7°C similar trends were observed although these were not as marked. Of particular note, however, were the greatly increased leuconostoc proportions observed in both pasteurized and control samples stored at 7°C when compared to the corresponding samples stored at 25°C.

However, in view of the synergistic increase in product shelf life by pasteurization and 7°C storage, changes in the proportions of predominant spoilage populations with increasing storage time were examined in detail over two time intervals, viz. 0 to 14 days' and 15 to 115 days' storage at 7°C (Table 2).

Table 2: Changes in predominant lactic acid bacteria populations from pasteurized (P) and control (C) vacuum packaged, smoked vienna sausages over storage time at 7°C.

Treatment and storage time (days)	Percentage isolates per biogroup[a] I	II	III	IV A	IV B	Number of isolates
7C (0-14)	2,2	32,6	47,8	8,7	8,7	46
7P (0-14)	18,1	4,5	68,3	-	9,1	22
7C (15-115)	6,1	34,7	51,0	5,1	3,1	98
7P (15-115)	9,2	39,1	32,2	10,3	9,2	87

[a] For definition of biogroups, see Table 1; no representatives of biogroups V and VI were found.

From these results it was evident that the lactic spoilage populations in the control samples were virtually mature after 14 days' storage, with a clear predominance of leuconostocs (group II – 32,6%) and homofermentative lactobacilli (group III – 47,8%). This result agreed with previous findings on the strong predominance of these groups in terminally spoiled vienna sausage samples (VON HOLY and HOLZAPFEL, 1989), which also highlighted low proportions of heterofermentative lactobacilli and pediococci, streptococci and enterococci. Only slight compositional changes occurred in the lactic spoilage populations of control samples stored at 7°C during 15 to 115 days' storage compared to the 0 to 14 day storage period (Table 2). It was noted that microbial numbers in the control samples stored 7°C had exceeded the microbiological shelf life threshold of 5 x 10^6 CFU g^{-1} ($\log_{10}$ 6,70) after 14 days' storage at 7°C (Fig. 1).

Conversely, major changes in the composition of lactic spoilage populations occurred in pasteurized samples stored at 7°C between the two time intervals. While the proportions of heterofermentative (group I) and homofermentative (group III) lactobacilli each showed a ca. 50% decrease with increasing storage time, the proportions of leuconostocs (group II) and pediococci (group IVA) showed marked increases of 4,5 to 39,1% and 0 to 10,3% respectively. By comparison, microbial numbers in the pasteurized samples stored at 7°C only exceeded the microbiological shelf life threshold approximately halfway during the 15 to 115 day storage period (Fig. 1). Although the proportions of leuconostocs were comparable between control and pasteurized samples after 115 days' storage at 7°C, the proportions of homofermentative lactobacilli decreased to 32,2% in the pasteurized samples compared to the 51,0% of the control samples. Pasteurization followed by 7°C storage thus resulted in a diversification of spoilage populations. In addition, it resulted in an initial (0 – 14 days) inhibitory effect on leuconostoc predominance, while the opposite response was observed for the homofermentative lactobacilli with increasing storage time (Table 2). Nevertheless, the leuconostocs and homofermentative lactobacilli individually and collectively comprised the largest proportions of the predominant lactic spoilage populations after 115 days' storage at 7°C in both pasteurized and control samples. The high proportions of leuconostocs recovered here (Table 2) were of special note since a comparable study on spoiled, South African processed meats identified only 2,8% (HOLZAPFEL and GERBER, 1986) and a study on prepacked, Swedish meat products (BORCH and MOLIN, 1988) identified 18% of predominant isolates as leuconostocs. The high percentages of leuconostocs in spoilage populations of vacuum packaged, smoked vienna sausages found in this and related studies (VON HOLY and HOLZAPFEL, 1989) suggests that the composition and status of this group in the spoilage process should be re-evaluated .

CONCLUSIONS: Although the in-package pasteurization treatment did not eliminate microbiological spoilage, ca. four-fold microbiological shelf life increases compared to untreated controls were achieved, when the product was stored at 7°C. Nevertheless, final

population densities in pasteurized samples equalled those of control samples and LAB still predominated. Homofermentative lactobacilli and leuconostocs jointly comprised the largest proportion of spoilage isolates in both pasteurized and control samples, although a ca. 20% reduction in the proportion of homofermentative lactobacilli was achieved in pasteurized samples stored at 7°C. These were replaced by heterofermentative lactobacilli and pediococci. We concluded that this ecological diversification did not represent an advantage with respect to microbiological spoilage potential. Consequently, approaches to increase product shelf life by additional "hurdles" (LEISTNER, 1987) should be investigated.

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