Bulgarian Journal of Veterinary Medicine (2005), **8**, No 4, 245–254

SAFETY AND SHELF-LIFE OF WIDELY DISTRIBUTED VACUUM PACKED, HEAT TREATED SAUSAGES

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Summary

Mantis, F. N., I. Tsachev, O. Sabatakou, A. R. Burriel, A. Vacalopoulos & S. B. Ramantanis, 2005. Safety and shelf-life of widely distributed vacuum packed, heat treated sausages. *Bulg. J. Vet. Med.*, **8**, No 4, 245–254.

The hygienic status of Greek vacuum-packed, heat-treated sausages kept at 7 ^{0}C was evaluated. Six types of widely consumed products (whole and sliced) were investigated from the date of production to the time of sensory defects development. Shelf life was shorter than the recommended sell-by-date for all products examined. The starting pH values and water activity were between 5.8 to 6.4 and 0.95 to 0.97 respectively. The initial total plate counts and lactic acid bacteria colony forming unites were higher for the sliced products than the whole piece ones and they were correlated positively with the end of shelf life. Potential human pathogens such as *Salmonella* spp., *Listeria monocytogenes* and sulfite reducing clostridia *(Clostridium perfringens)* were not isolated from any of the products, but *Enterobacteriaceae* and *Staphylococcus* spp**.,** probable contaminants during handling, were present. The evidence supports the shortening of the recommended sell-by-date and stringent HACCP measures after the heat treatment of the sausage mass.

Key words: bacteria, sausage, shelf life, total counts

INTRODUCTION

Quality of starter raw materials is one of the most important factors in producing safe and high quality meat products (Holley *et al*., 1988) with a reasonably long shelf life. The shelf life of vacuum packed, heat-treated meat products depends on the composition and increase of the initial microflora. Microflora increases depend on the storing temperature, pH values, water activity (a_w), concentration of added *NaCl*, nitrites and other additives (Korkeala *et al*., 1990; Buncic *et al*., 1997; Aymerich *et al*., 2000). Since microorganisms need water for their multiplication, the initial value of a_w in particular is very important. Optimum a_w values for bacteria growth range between 0.98 and 0.99, with the exception of halophilic, osmophilic and xerophilic bacteria (Scott, 1957; Hansen & Riemann, 1962). In heat-

treated products with added water both the a_w and pH values are high (0.97–0.98 and 6.0–6.4, respectively). In addition to a_w value, the pH is closer to the optimum for bacteria multiplication (Tändler, 1985). Processing technology and various food additives affect both the product's pH and a_w values, thus they are used to regulate them. Food additives are added either as beneficial bacterial products or inorganic and organic ingredients (Raevuori, 1975; Korkeala *et al*., 1992; Aymerich *et al*., 2000). Greek Food Code has adopted European food directives and codes on the manufacturing process including additives of vacuum packed, heat-treated sausages. It is evident, that a product's safety and shelf life depend on the increases in the number of microorganisms of the microflora. Total bacteria counts and eventual composition of a product's microflora is dependent on the microflora of the sausage mass and the handling during manufacturing. Its later composition is dependent both on the storing temperature during selling or consumption and the effectiveness of heat treatment (Raevuori, 1975; Miskimin *et al*., 1976; Holley *et al*., 1988; Nychas *et al*., 1988; Korkeala *et al*., 1992; Samelis *et al*., 1998a). Vacuum-packed, heat-treated red meat sausages become a source of microorganisms important for public health either by contamination during slicing and packing or the survival of pre-existing heat resistant bacteria and spores (Gardner, 1982; Samelis *et al*., 1998a). The latter could become contaminants for other products during manufacturing if animal infections are contaminating carcasses at slaughter and, through it the production line of a sausage making facility (Gardner, 1982; Holley *et al*., 1988; Bender *et al*., 2004). Potential human pathogens, such as species of the families Enterobacteriaceae and Staphylococcaceae, and specifically *Staphylococcus* spp. or *E. coli,* could multiply and cause consumer infections or food poisoning. These are possible without visible changes in the color, taste and odor of the product, if the initial microbial contaminating dose is high. The problem is worsening when the storing temperature is not the recommended (Stiles & Ng, 1981; Gardner, 1982; Samelis *et al*., 1998a; Bover-Cid *et al*., 2001). Among the species of microorganisms having increased public health importance are *Listeria monocytogenes,* different species of the genus *Salmonella* and *Clostridium perfringens,* belonging to the sulfite reducing clostridia. *Cl. perfringens* survives heat treatment if meat contamination is very high (Ladiges *et al*., 1974; Samelis *et al*., 1998a; Bender *et al*., 2004). *Salmonella* spp., either surviving ineffective heat treatment or contaminating a product during manufacturing, will increase in numbers at storing temperatures above 10 ^{0}C. Storing temperatures between 4 ^{0}C and 10 ^{0}C inhibit *Salmonella* spp. multiplication (Stiles *et al*., 1979; Buncic *et al*., 1997). *L. monocytogenes* grows well to an infectious dose at temperatures below 7^0 C if the product is lacking of the beneficial inhibitory effect of lactobacilli (Aymerich *et al*., 2000; Tantillo *et. al*, 2002). Lactobacilli are either acquired during manufacturing (Holley *et al*., 1988; Bover-Cid *et al*., 2001) or are added as starter culture (Buncic *et al*., 1997; Aymerich *et al*., 2000).

With regard to the knowledge accumulated about heat treated meat sausage products, the purpose of the present study was to investigate selected parameters affecting the safety, the shelf life and the sensory characteristics of some Greek vacuum-packed, heat-treated sausages stored at 7^0 C and produced by the same

large-scale manufacturer. The study widened previously accumulated knowledge (unpublished).

MATERIALS AND METHODS

Sausage samples

Six types of sausages from comminuted meat, commercially vacuum-packed were selected for the study. They were all products of the same packing plant and production line. They were Parisaki, Country, Frankfurter, Parisa, Mortadella and Pick-Nick type red meat sausages. Parisaki was individually vacuum-packed as a whole, while Country and Frankfurter type sausages were packaged into packs of five pieces. Parisa, Mortadella and Pick-Nick sausage types were vacuum-packed after slicing. The study was planned to last 13 weeks overlapping the manufacturer's recommended sell-by-date of each product. Thirteen randomly selected packages of each product and the same product lot were brought to the laboratory within one hour from collection. Once at the laboratory they were refrigerated at 7 ^{0}C, a temperature that is the maximum possible for reasonable handling, and for the duration of the experiment. Examination of each product was in weekly intervals starting at the date of vacuum packing.

Microbiological examination

Isolation of microorganisms aimed in determining: 1. The total plate counts (TPC) of microorganisms, and 2. The presence of *Staphylococcus spp., L. monocytogenes,* sulfite reducing clostridia, Enterobacteriaceae, among which are *Salmonella* spp. and lactobacilli. At each testing date 25 g samples from each vacuum-packaged product and from the mass of the product (surface and center) were aseptically homogenized in 225 mL of sterile 0.1% buffered peptone water (BPW). A recommended quantity of the liquefied sample was tested using the working protocols of the ISO 6888 (1983), 4833 (1991), 7402 (1993), 6579 (1993), 11290 (1995). In addition, lactobacilli were isolated according to Rogosa *et al*., (1951), and sulfite-reducing clostridia were investigated by culturing duplicate tissue dilutions on Sulfite-Polymyxin-Sulfadiazine (SPS) agar (Becton Dickinson Hellas S.A, GR). Specifically, one mL of homogenate was mixed by rotation with 15 to 20 mL of SPS agar kept in liquid state at 45 ^{0}C. Inoculated plates were left covered to solidify at room temperature and layered with an additional 5 to 8 mL of the same SPS agar at 45 ^{0}C. Layered plates were incubated in anaerobic conditions for 24 hours at 37 ^{0}C.

Measurement of pH and water activity (a_w)

All samples subjected to microbiological examinations were also tested for their pH and a_w. The value of pH was determined by a Smart Chem Lab, TP-126124 (Sper Scientific Ltd, USA). The a_w value was determined with a Novasina electric hygrometer (Hanna, Italy) and was the mean value of three measurements of each sample at 20^{0}C. pH values were taking at each testing date.

Histological examination of products

Ten pieces free of lipoid tissue 1–3 cm in length and 50 mm in thickness were removed from each examined product. They were immediately placed in 10% formalin and fixed for 24 hours. After fixing they were dehydrated and embedded in paramat. The paramat blocks were cut into sections of 4–5 μm, stained by haematoxylin-eosin and examined under light microscopy.

Sensory defects

They were clear deviations of odor, smell, flavor and visible manifestations indicating spoilage of the product. The same examiner evaluated the odor at each sampling date immediately after the opening of the vacuumed pack, and two hours after storage at room temperature. Changes in color, flavor or texture (gummy slime development) were considered as evidence of spoilage.

Statistical analysis

The data were statistically analyzed using the SPSS computer program version 8.0 for Windows. Correlations were made between pH, a_w, TPC and lactobacilli.

RESULTS

The first observation was that none of the examined products lasted up to the end of the recommended sell-by-date. Maximum shelf life was 11 weeks for Parisaki and six weeks for all three sliced products. The first evidence of sensory defects was an acidic odor occurring at a TPC of about 10^7 cfu/g (*lg*=7), but the product remained in the experiment until atypical flavors and gummy slime were developed. The end of the shelf-life coincided with TPC and lactobacilli counts over 10^8 cfu/g (*lg*=8), and pH values below 5.6 (Table 1). Sulfite reducing clostridia, *Salmonella* spp. and *L. monocytogenes* were absent from all the products examined. Other Enterobacteriaceae were isolated from only Pick-Nick and Mortadella sausage. *Staphylococcus* spp. were not isolated from Frankfurter and Parisaki type sausages. Their cfu in the other products gradually increased and either stabilized or decreased close to the development of sensory changes. The initial pH and a_w values varied among the products and were ranging between 5.8 to 6.4 and 0.95 to 0.97 respectively. Only in Frankfurter and Pick-Nick type sausages was not found tissue not permitted by the Greek Food Code. The other four types of sausages showed evidence of two or more non-permitted tissue types (tongue, glandular tissue and cartilage). The correlations between TPC, lactobacilli, pH and a_w values were positively significant (P equal to 0.006, 0.016, 0.002 and 0. 002 for Frankfurter, Country, Parisaki and Parisa type, vacuum-packed, heat-treated products, respectively).

DISCUSSION

The absence of *Salmonella* spp., sulfite reducing clostridia and *L. monocytogenes* indicates either absence of these microorganisms from the meat mass used for this lot of products or their presence in very low numbers, thus effectively destroyed during heat treatment. In the present study handling appears to be the most probable factor of contamination of sausages with *Staphylococcus* spp., a genus usually found on the skin of product handlers (Lee *et al*., 2001). The same is true for Enterobacteriaceae. Enterobacteriaceae are usually present in much higher numbers in the meat than *Staphylococcus* spp. (Heredia *et al*., 2001; Bender *et al*., 2004) because they are present in the gut of healthy animals whose carcasses are contaminated at slaughter (Bender *et al*., 2004; Gill, 2004). Members of the family Enterobacteriaceae and *Staphylococcus* spp. are important human pathogens causing either clinical disease (Baillargeon *et al*., 2004; Bender *et al*., 2004) or food poisoning (Atanassova *et al*., 2001; Lee *et al*., 2001;

Table 1. Lg of bacterial counts and pH values of whole piece sausages

Testing time	Frankfurter sausages $a_w = 0.950$			Parisaki $a_w = 0.960$			Country type sausages $a_w = 0.965$			
	TPC/g (lg)	Lactic acid bacteria/g (lg)	pH	TPC/g (lg)	Lactic acid bacteria/g (lg)	pH	TPC/g (lg)	Lactic acid bacteria/g (lg)	*Staphylococcus* spp./g (lg)	pH
1st Day	2.2	-	6.2	2.8	-	6.3	2.9	1.3	1.9	6.2
1st Week	2.9	1.4	6.2	3	-	6.3	4.8	3.9	2.9	6.1
2nd Week	3.5	3.4	6.1	3.7	1.9	6.3	5	4.6	2.9	6.1
3rd Week	4.8	4.8	6.1	4.6	2.1	6.2	5.1	4.1	3.8	6.1
4th Week	4.9	5	6.1	4.7	2.8	6.2	5.8	7	3.9	6.0
5th Week	5.8	5.9	6.1	4.9	2.9	6.2	6.1	7.5	3.9	5.9
6th Week	5.9	6.7	6.0	5.5	3.2	6.2	7.9	8.9	3.9	5.6
7th Week	7.8	7.9	5.9	5.8	4.9	6.1	8.9	9.7	3.6	5.5
8th Week	8.9	9.6	5.7	6	5.6	6.1				
9th Week				6.8	6.4	6				
10th Week				7.9	7.9	5.9				
11th Week				7.9	8.9	5.6				

Table 2. Bacterial growth and changes of pH and a_w values in sliced sausages

Testing time	Pick-Nick $a_w = 0.970$					Mortadella $a_w = 0.963$					Parisa $a_w = 0.960$				
	TPC/g (lg)	Lactic acid bact./g (lg)	*Staph.* spp./g (lg)	*E. coli*/g (lg)	pH	TPC/g (lg)	Lactic acid bact./g (lg)	*Staph.* spp./g (lg)	*E. coli*/g (lg)	pH	TPC/g (lg)	Lactic acid bact./g (lg)	*Staph.* spp./g (lg)	pH
1st Day	4.7	3.9	2.4	2.9	6.4	4.5	3.9	2.7	2.6	6.4	3.4	4.	2.8	5.8
1st Week	4.9	4.7	2.9	2.9	6.2	4.9	4.	2.8	2.	6.2	4.9	5.5	3.1	5.8
2nd Week	6	5	3.8	3.2	6.2	5.4	4.9	3.4	2.9	6.1	5.8	6.2	4.9	5.7
3rd Week	6.9	6.7	4.8	3.9	6.1	5.7	5.4	3.9	3.9	6.0	6.9	7.4	3.9	5.6
4th Week	7	7.9	4.7	4.9	5.8	6.9	6.7	4.4	4.8	5.9	7.5	8.2	2.8	5.6
5th Week	7.8	8.9	3.4	5	5.7	7.6	7.9	4.2	4.9	5.9	7.9	8.8	1.6	5.5
6th Week	9	9.8	3.2	4.9	5.6	8.8	9.7	3.8	4.6	5.7	9.9	10.5	-	4.9

Chen *et al*., 2004). Of particular concern should be the presence of *Staphylococcus* spp. because their multiplication to high numbers occurs without sensory defects (Johnston & Tompkin, 1992; Snyder, 1995; Chen *et al*., 2004). Thus, they could cause food poisoning without the beneficial warning effect of sensory defects.

As expected, handling increased the TPC and the cfu/g of lactobacilli, which increased in a similar way and numbers, as time proceeded indicating that the TPC were mainly made up from lactobacilli. These microorganisms could exert an inhibitory effect on pathogenic microorganisms (Buncic *et al*., 1997; Tantillo *et al*., 2002). Nevertheless they were ineffective in reducing the cfu of Enterobacteriaceae and *Staphylococcus* spp. in some of the products examined here. Lactobacilli are mentioned as inhibitory for specific species or strains of certain pathogens (Tantillo *et al.*, 2002). However, they are, perhaps, effective in higher initial cfu than the observed here or in a lower cfu/gr of contaminating pathogenic bacteria (Buncic *et al*., 1997; Cavadini *et al*., 1998). The significant correlation (P equal to 0.006, 0.016, 0.002 and 0.002 respectively) observed between the cfu of lactobacilli and the pH values for four of the products (Frankfurter, Country, Parisaki and Parisa) indicate that the pH lowering resulted from the increases of lactic acid bacteria. It is generally accepted that the most important determinant for the quality and length of shell life of a product is the processing technology, and specifically initial pH, a_w, and various food additives or the quality of the microflora acquired during handling (Egan *et al*., 1980; Korkeala *et al*., 1990). The pH and a_w values of heat-treated, well-preserved sausages are reported to be from 6.0 to 6.4 and from 0.97 to 0.98 respectively (Samelis *et al*., 1998b). However, the a_w value is mainly depended on the recipe and the processing technology, as observed in long life heat-treated products. This type of product has an a_w value between 0.91 and 0.95 (Tändler, 1985). The a_w values observed here are mostly closer to those recommended for the latter products (Holley *et al*., 1988) and could have been the most beneficial factor for the shelf life of the examined products in light of the high TPC, Enterobacteriaceae and *Staphylococcus* spp. Thus, the evidence of this study indicates that the values of a_w are more important than the values of pH. Although some authors (Korkeala *et al*., 1987; Buncic *et al*., 1997) suggest that the pH values can be used as indicators of freshness, others (Stojanovic & Flemmig, 1988) think that the pH value isn't a safe indicator, at least for some products, something appearing to be true here. The pH of the examined products dropped below the possible critical value of 6.0 at about or immediately after bacteria TPC reached numbers of 10^6 (*lg*=6) or over. However, the sensory defects justifying the end of the products' shelf life were detected two or more weeks later. In addition, Parisa sausage had a pH below 6.0 from the first date of its production, although its shelf life lasted for six weeks. The same product had a value of a_w closer to that of long life heat-treated products (Tändler, 1985). Lactobacilli in particular affect the product's pH values (Korkeala *et al*., 1990). Meanwhile sensory defects were being prominent at a count of lactobacilli of about or over 10^8 (*lg*=8). These counts were observed earlier in sliced products due to a higher initial count. Increased handling could also be the factor influencing the presence and the

counts of Enterobacteriaceae and *Staphylococcus* spp. in vacuum packaged, heat-treated Greek sausages. The same could have shorten considerably the shelf life of the products.

In summary, increased protective handling measures must be taken for Greek vacuum packed, heat-treated sausages, especially the sliced ones, to ensure longer shelf life and better consumer protection. That the real shelf life of these products falls much shorter than the recommended by the manufacturer sell-by-date reveals the empirical setting of dates on products. This situation must change since such products, although spoiled, could be sold. The widening of research encompassing similar types of sausages produced by other manufacturers is recommended.

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Paper received 24.01.2005; accepted for publication 15.11.2005

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