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ABSTRACT

The bacteria Total Plate Count (TPC), *Lactobacilli*, pH and water activity influencing the shelf life of Greek vacuum-packed, heat-treated sausages kept at 7 °C were investigated. Samples of six types of whole and sliced, vacuum packed sausages were tested at weekly intervals and until sensory defects occurred. The TPC and *Lactobacilli* reached counts of about or over 10⁸ cfu/g at the end of the products' shelf life and were followed by a drop in pH to about or below 5.7. The findings showed that the shelf life of all the products falls short of the producer's recommended sell-by-date, due to higher counts of bacteria and to a faster pH fall of the products.

Key words: heat treated sausages, total plate count, pH, shelf life

Introduction

Vacuum-packed, heat-treated red meat sausages become a source of microorganisms important for public health either by contamination during slicing and packaging or due to the survival of pre-existing heat resistant bacteria and spores (GARDNER, 1982; FÄHNLE et al., 1985). The latter could contaminate the production premises through contaminated carcasses of carrier animals. Potential human pathogens could multiply even without visible changes in the colour, taste and odour of the product if the initial contaminating dose is high, the storing temperature is not the recommended one (HECHELMANN et al.,

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1974; SILLA and SIMONSEN, 1985; MARRIOT, 1992) and the product lacks the beneficial inhibitory effect of *Lactobacilli* (KAYA and SCHMIDT, 1989; SCHMIDT, 1989). In addition, the shelf life of a product depends, excluding the composition of the initial microflora and storing temperature, on the product's pH, water activity (a_w), and concentration of added NaCl, nitrites and other additives (LEISTNER, 1987; REICHERT, 1985; SHAY et al., 1978).

Increases in the number of microorganisms during storage are proportional to the initial bacteria Total Plate Counts (TPC), storage temperature (ALLEN and FOSTER, 1960; MISKIMIN et al., 1976; SILLA and SIMONSEN, 1985) and the a_w value. The optimum a_w values for bacteria growth are between 0.98 to 0.99, with the exception of halophilic, osmophilic and xerophilic ones (HANSEN and RIEMANN, 1962; SCOTT, 1957). Various recipes, processing technologies and additives used in heat-treated meat products reduce the a_w value of the product, thus reducing microbial multiplication (LEISTNER, 1970; TÄNDLER, 1985). In heat-treated products with added water both a_w and pH values are high (0.97-0.98 and 6.0-6.4, respectively), resulting in a restricted preservation and the need for proper product refrigeration (TÄNDLER, 1985).

With regard to the knowledge accumulated about heat-treated meat sausage products, the purpose of the present initial investigation was the study of the influence of TPC, lactic acid bacteria, pH and a_w values on the shelf life and sensory characteristics of Greek vacuum-packed, heat-treated sausages, produced by the same manufacturer, and stored at 7 °C, which is the usual storage temperature in many stores in Greece.

Materials and methods

Sausage samples. The study was planned to last 12 weeks, a period exceeding the manufacturer's recommended sell-by-date of each product. First testing was performed on the day of production. Six types of commercially heat-treated and vacuum-packed sausages were selected for the study. They were all products of the same production plant and packaging line: Parisaki, Country, Frankfurter, Parisa, Mortadella and Pick-Nick type sausages. Parisaki was individually vacuum-packed as a whole, while Country-and Frankfurter-type sausages were packed into packs of five pieces. Parisa, Mortadella and Pick-Nick sausage types were initially produced in loaves and vacuum-packed after slicing. Twelve randomly selected packages of each product and the same product lot weighing about 360 g each were brought to the laboratory. They were transported packed in ice at a maintained temperature of about 7 °C. Once at the laboratory they were refrigerated at 7 °C for the duration of the experiment. Each product was examined weekly, starting from the date of vacuum packing.

Microbiological examination, pH and water activity. Five samples of 25 g each from each vacuum-packed product were aseptically removed on each testing date and

individually placed in individual sterile plastic bags containing 225 mL of sterile 0.1% buffered peptone water (BPW). After homogenation for two min. using a masticator microprocessor (IUL Instruments, Athens, GR), the homogenates were tested for the TPC according to ISO 4833 (ANON., 1991) and the colony forming units (cfu) of *Lactobacilli* according to ROGOSA et al. (1951). The pH of each product was measured using a Smart Chem Lab, (TP-126124, Sper Scientific Ltd, USA). The initial a_w value was determined with a NOVASINA electric hygrometer (HANNA, Italy) at a temperature of 20 °C.

Sensory defects. Sensory defects were clear deviations of odour, mainly acidic smell, and visible manifestations of changes indicating spoilage of the product. For securing an objective evaluation of each product's odour, the same person evaluated sensory defects until the end of the experiment, immediately after the opening of the vacuumed pack, and two hours after storage at room temperature, left opened. Visible manifestations of spoilage were considered as changes in colour, flavour or texture (gummy slime development).

Statistical analysis. 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

For an immediate comparison of important changes Table 1 details only the median TPC, lactic acid bacteria (Lactobacilli) cfu and pH at initial testing, and again on the day sensory defects were observed. Intermediate measurements have been omitted as having minimal importance. Testing ended when sensory defects indicative of spoilage were detected. Table 1 shows that none of the examined products lasted to the end of the set sell-by-date. Preservation time ranged between 49 days for all three sliced products and up to 84 days for Parisaki. The first evidence of sensory defects was an acidic odour, which occurred at a TPC of about 107 cfu/g, signs of atypical flavours and/or the appearance of gummy slime at a TPC equal or above 108 cfu/g. Increases in TPC and numbers of Lactobacilli showed a similar pattern in the increasing values of cfu. The product's pH values at first testing ranged between 5.8 and 6.4 and a_w values between 0.95 and 0. 97. The correlations between TPC, Lactobacilli and pH values were significant, with P values equal to 0.006, 0.016, 0.002 and 0.002, respectively, for Frankfurter, Country, Parisaki and Parisa type vacuum-packed, heat-treated products. The various sensory defects were associated with the increases of TPC counts of about 10⁸ cfu/g and above and to a drop of pH to about or below 5.7.

Type of Sausage							
Whole	Initial TPC./g	T.P.C/g at sensory defects	Initial lactic acid bacteria/g	Lactic acid bacteria/g at sensory defects	Initial pH	PH at sensory defects	End of shelf life in days
Frankfurter $a_w = 0.950$	1.9×10^{2}	9.3 × 10 ⁸	<10	4.8×10^{9}	6.21	5.75	63
Country $a_w = 0.965$	9.8 × 10 ²	9.7 × 10 ⁸	2.1 × 10	5.1 × 10 ⁹	6.19	5.55	49
Parisaki $a_w = 0.960$	7.0×10^{2}	9.7 × 10 ⁷	<10	$8.5 imes 10^8$	6.35	5.68	84
Sliced							
Pick-Nick $a_w = 0.970$	5.5×10^{4}	9.8 × 10 ⁸	9.8 × 10 ³	6.7 × 10 ⁹	6.36	5.67	49
Mortadella $a_w = 0.963$	3.5×10^{4}	7.4×10^{8}	9.1 × 10 ³	5.6 × 10 ⁹	6.40	5.79	49
Parisa $a_w = 0.960$	2.5×10^{3}	9.8 × 10 ⁹	9.5 × 10 ⁴	3.3×10^{10}	5.80	4.90	49

Table 1. TPC, *Lactic Acid Bacterial* cfu/g, pH and a_w values in whole and sliced heat- treated Greek sausages

Discussion

As expected, the starting counts of bacteria TPC were higher in the sliced sausages than in the whole piece ones, and appeared to be composed mainly of *Lactobacilli*. As time proceeded, both TPC and the number of *Lactobacilli* increased in a similar way. TPC and *Lactobacilli* were in similar numbers when sensory defects were first detected. The increases in the cfu of *Lactobacilli* appeared to be the reason of the pH lowering, and in four products (Frankfurter, Country, Parisaki and Parisa) this correlation was statistically significant. It is generally accepted that the most important determinant for the quality and length of shelf life of a product's pH and a_w values, the various food additives and the type of microflora acquired during handling. It has been reported that the pH and a_w values of heat-treated, well-preserved sausages are between 6.0 to 6.4, and from 0.97 to 0.98, respectively (MATTILA et al., 2003; SAMELIS et al., 1998; TEUFEL, 1985). However, the a_w value is mainly dependent on the recipe and the processing technology. For example, the a_w value of long life heat-treated products is reported to be between 0.91 and 0.95 (HECHELMANN and LEISTNER, 1984; TÄNDLER, 1985). These values are

closer to the a, values observed here. Although KORKEALA et al. (1987) suggested that the pH and CO₂ of vacuum-packed ring sausages could be used as indicators of freshness, STOJANOVIC and FLEMMIG (1988) believe that the pH value is not a safe indicator of freshness, at least for some types of product. The latter appears to be true in the present study, in which the pH value first dropped below 6.0 at about or immediately after bacteria TPC reached numbers of 10⁶ or above. In addition, one of the freshly prepared products (Parisa), had a pH value of below 6 at the time of production, but its shelf life was 49 days. However, the sensory defects justifying the end of the product's shelf life were observed two or more weeks after this drop in pH values. These observations indicate, as previously observed (STOJANOVIC and FLEMMIG, 1988), that pH values could not always be indicative of a product's eatable state. The latter appears to depend on the TPC and its quality. Thus, also bacteria species forming the microflora. Lactobacilli in particular affect the product's pH values and appear in this study to be the determining factor for the presence of sensory defects being prominent at a count of about or above 10^8 . These counts were observed earlier in the sliced products due to a higher initial count affected, apparently, by the additional processing (EGAN et al., 1980; ANONYM., 1980; SAMELIS et al., 1998; TEUFEL, 1985).

In conclusion, increased protective handling measures must be taken for Greek heat-treated sausages, especially sliced ones, in order to ensure longer shelf life. That the shelf life of all the products examined here falls much shorter than the sell-by-date recommended by the manufacturer (90 days), indicates the empirical setting of the dates on products. This situation must change since such products, although spoiled, could be sold. The widening of research encompassing similar types of sausage produced by other manufacturers is recommended as a follow up.

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SAŽETAK

Istraživanje je provedeno da bi se odredio utjecaj ukupnog broja bakterija, laktobacila, pH i djelovanje vode na trajnost grčkih kobasica pakiranih u vakuumu pohranjenih pri temperaturi od 7 °C. Uzorci šest vrsta cijelih ili narezanih kobasica bili su pretraživani u tjednim razmacima sve dok se nisu promijenila njihova organoleptička svojstva. Ukupan broj bakterija i laktobacila dosegao je ili je bio veći od 108 kolonija/g pri kraju roka upotrebe što je pratio i pad pH vrijednosti na oko 5,7 ili niže. Rezultati su pokazali da bi, zbog većeg broja bakterija i bržeg pada pH vrijednosti proizvoda, rok upotrebe svih testiranih proizvoda morao biti kraći od onoga koji preporučuje proizvođač.

Ključne riječi: toplinski obrađene kobasice, ukupni broj bakterija, pH, rok trajanja