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Isolation and Identification of Yeasts Associated with Intermediate Moisture Meats

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Summary

Biltong, cabanossi, dry sausage, salami are typical intermediate moisture meats produced and consumed to a large extent in South Africa. A study was undertaken with the objective of isolating and identifying the dominant yeasts associated with these meat products. Microbiological analyses were performed by the enumeration of all relevant microbial organisms on selective agar, and the isolation and identification of all visually distinct yeast colonies by means of conventional methods. A total of 11 different yeast species, representing nine genera, were present in the samples analysed. Although a broad spectrum of yeasts were found in the meats, *Debaryomyces hansenii* was the most abundant yeast isolated. Other species encountered, were *Cryptococcus laurentii, Cryptococcus hungaricus, Torulaspora delbrueckii, Rhodotorula mucilaginosa, Sporobolomyces roseus, Debaryomyces vanriji, Trichosporon beigelii, Yarrowia lipolytica, Saccharomyces cerevisiae* and *Candida zeylanoides.*

The chemical and physical composition of these intermediate moisture meat products were also established. Representative samples of biltong, cabanossi, dry sausage and salami were evaluated and the water activity, pH, moisture fraction (%) and salt fraction (%) were measured.

Key words: isolation, identification, yeast, intermediate moisture meats

Introduction

From an ecological point of view, food is considered to be a habitat for microbial growth (*1*). The survival and growth of microorganisms in food are determined, apart from the availability of nutrients, by general factors such as temperature, moisture availability (generally expressed in terms of water activity, a_w), pH, the oxidation-reduction potential and the nature of the gaseous environment to which the organisms are exposed (*2*). The removal of available moisture is an effective strategy for inhibiting microbial growth, used in the processes of dehydration during curing, resulting in products with high salt contents. This, combined with the difficulty of rehydration of dehydrated products, led to the development of the so-called intermediate moisture technology (*3*). According to the a_w parameter, foodstuffs can be classified as intermediate moisture foods (IMF) having a_w values ranging from 0.90 to 0.60 (*4*).

The principle of reducing the a_w of food to prolong its shelf life is not new, although the origin remains unknown (*5*). The production of intermediate moisture meats (IMM), a heterogeneous group of foods, as well as other IMF, have great applications in space travels and other circumstances where stability, palatability and convenience is essential (*2*). IMM products such as biltong in South Africa (*6*), charqui in South America (*7,8*), sa-

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lami and rohwurst (*9,10*), speck of Switzerland (*7*) and dried and other fermented sausages (*2,11*) all fall into this category, and have been manufactured for centuries (*7,8*). Most of these traditional IMM products are prepared from comminuted, of non-comminuted, semi--dried and uncooked meats, involving processes of fermentation and chemical curing. Meat is salted with sodium chloride, nitrate or nitrite. Spices and seasonings are added. Bacteria of the *Micrococcus* or *Staphylococcus* spp. reduce nitrate to nitrite, whilst the lactic acid bacteria (LAB) are involved in the reduction of pH (*12*). Yeasts, often *Debaryomyces* spp., may be involved in flavour development (*11*).

The development of microbiology in recent years has led to an understanding of how the availability of moisture controls the growth of microorganisms (*4,9*). The availability of water is complementary to osmotic pressure, whereby the lowering of a_w, by increasing the salt concentrations, as performed in the curing processes, effectively prevents growth of many microorganisms, but selects growth of salt-tolerant (halophilic) bacteria (*13*).

Various microbial groups are involved in the manufacture and ripening processes of dry, fermented sausages (*14–16*), using the traditional »back-sloping« methods (*17*) or a more modern approach of starter cultures (*10*). Lactic acid bacteria, staphylococci and micrococci are the predominant microorganisms used in the production of fermented meats. However, since certain moulds and yeasts are considered as common contaminants of cured and fermented meats, they have also been included in some starter preparations (*17,18*). Moulds and yeasts have rarely been associated with the spoilage micro-flora of IMM, largely because they are out-grown by the faster growing bacteria. This unique combination of microorganisms present in this specific environment also suppress the growth of pathogenic organisms by their enzymatic activities, induction of organoleptic changes in the meat (*19*), as well as result of the low a_w of IMM (*2*). Generally, bacteria are able to grow at an a_w of just under 1.0 to 0.75. Yeasts and moulds grow slowly at an a_w of 0.62.

Like moulds, yeasts are usually present in low numbers on fresh meat, but counts may increase during low temperature storage and eventually dominate the microflora (*16*). Species of *Candida*, *Debaryomyces* and *Torulopsis* are the most frequently isolated genera from meats (*20,21*). Other genera include *Bullera*, *Cryptococcus*, *Pichia*, *Saccharomyces*, *Schizosaccharomyces*, *Torulaspora*, *Trichosporon* and *Williopsis* (*16*). It has been suggested that yeasts play a dual role in the production and ripening of IMM, either by contributing positively during development of the typical »red colour« of ripened sausages and an acceptable aroma of the final product (*22–24*), or being detrimental in causing spoilage (*25,26*).

The present study was undertaken with the objective of isolating and identifying the principal yeast contaminants, as well as determining mould and bacterial population counts for four traditional IMM products, *i.e.* biltong, cabanossi, dry sausage and salami. The chemical and physical characteristics of these products were also determined.

Materials and Methods

Media

The following media and incubation conditions were used during the enumeration of specific classes of organisms: Plate count agar (PCA, Biolab C6) for total counts (48 h at 25 °C), M17 agar (M17, Oxoid CM785) for the lactococci counts (24 h at 30 °C), MRS agar (MRS, Oxoid CM361) for the lactobacilli counts (48–72 h at 37 °C), yeast glucose chloramphenicol agar (YGC, Merck) for total yeast counts (5 days at 25 °C), potato dextrose agar (PDA, Oxoid CM139) for mould counts (48–72 h at 25 °C), violet red bile agar (VRB, Oxoid CM107) for the enumeration of *E. coli* (48 h at 37 °C) and Baird Parker agar (BPA, Oxoid CM275) for the enumeration of staphylococci (48 h at 37 °C). Yeast isolates were maintained on YM (yeast extract malt extract) agar (containing per litre: 10 g glucose, 3 g yeast extract, 3 g malt extract, 5 g peptone, 2 % agar).

Sampling

Before analysis, 10 g portions of random samples of biltong, cabanossi, dry sausage and salami were cut and grated under sterile conditions in the laboratory. These 10 g portions were homogenised in 90 mL sterile peptone water in a Colworth 400 stomacher (London, U.K.) for 2 min. Appropriate decimal dilutions of the liquid portions were performed. Aliquots (0.1 mL) of the dilutions were spread-plate inoculated, in duplicate, onto the surface of solidified media and incubated at the specific temperatures for the appropriate time. Yeast colonies were isolated from the highest dilutions on plates containing YGC agar. The yeast isolates were sub-cultured on YM agar and incubated at 25 °C for 72 h for control of purity by colony morphology and microscopy. The pure cultures were stored on YM slants at 4 °C during the period of investigation, until characterisation.

Chemical and physical analysis

The a_w, pH, fraction of moisture and salt for the biltong, cabanossi, dry sausage and salami samples were determined according to standard procedures. The pH of the homogenised biltong, cabanossi, dry sausage and salami samples was measured at 24 °C with a HI 9321 microprocessor pH meter (Hanna Instruments), calibrated with saturated salt solutions. The a_w was determined on a Thermoconstanter Navasina TH 200. The fraction of moisture was determined according to Thomas Scientific, Philadelphia, P.A. The fraction of salt of the samples was determined on the principle of the AOAC (*27*).

Identification

Individual yeast isolates were identified with the aid of conventional identification methods (*28*).

Results and Discussion

Physical and chemical composition

Moisture fraction is a useful means of predicting the shelf life of food, but a_w will give a more precise indica-

Table 1. Number and identification of yeast isolates obtained during the sampling of biltong, salami, dry sausage and cabanossi

ISOLATES	BILTONG	SALAMI	DRY SAUSAGE	CABANOSSI	TOTAL
Candida zeylanoides	0	1	0	1	2
Cryptococcus hungaricus	0	0	1	0	1
Cryptococcus laurentii	1	1	2	3	7
Debaryomyces hansenii	4	8	4	9	25
Debaryomyces vanriji	0	0	1	4	5
Rhodotorula mucilaginosa	1	1	2	0	4
Saccharomyces cerevisiae	1	1	0	0	2
Sporobolomyces roseus	1	1	1	0	3
Torulaspora delbrueckii	1	0	2	0	3
Trichosporon beigelii	1	1	2	4	8
Yarrowia lipolytica	2	0	0	0	2
T o t a l	**12**	**14**	**15**	**21**	**62**

tion of its stability. It is one of the most important environmental factors affecting the growth and metabolic activity of microorganisms (*29*). Water activity can be regarded as a measure of the availability of water for microbial growth. It gives some measure of the effective concentration of the water in the substrate (*7*) and depends largely on the concentration of solutes either naturally present or added to foods (*30,31*). The a_w is expressed in the range from 0 to 1 and can be regarded of as equal to the relative humidity (divided by 100) of the atmosphere that would be in equilibrium with the food. Bacteria grow from an a_w of just under 1.0 to 0.75, yeasts and moulds grow slowly at an a_w of 0.62 (*2*). *Salmonella*, for instance, was unable to survive in salami when the a_w was <0.96 and pH<4.84 (*32*). Thus, most yeasts are more tolerant to reduced a_w than most bacteria are, with the yeast *D. hansenii* being very resistant to low levels of a_w, being capable of growth at levels as low as 0.88 (*33*). The mechanism of sugar tolerance, and more precise to the present study, salt tolerance in yeasts, still needs to be completely elucidated (*34,35*). However, the ability to accumulate high concentrations of polyols appears to be the most important criterium in the yeast's capability to adapt to reduced a_w (*33*).

Shelf-stable IMF is usually defined as food having a_w between 0.65 and 0.90 although it seems that this classification is not absolute. Different threshold levels of a_w in combination with pH, have been suggested in order to secure the safety of the product (*36*). The a_w determined during this study ranged from 0.62 to 0.86 for the biltong samples, from 0.73 to 0.88 for the dry sausage samples, from 0.81 to 0.91 for the salami samples and from 0.83 to 0.94 for the cabanossi samples (Figs. 1–4). As far as the moisture and a_w values are concerned, these were lowered during processing by the addition of salt, fat and partial dehydration (*37*). According to a_w values reported in Figs. 1–4, the biltong samples had the lowest average a_w (0.72), followed by the dry sausage (0.80), salami (0.87) and cabanossi samples (0.88). The low a_w of the biltong samples is mainly due to the curing period (3 to 4 days) and concentration of the salt solution in which these meats are soaked (*38*). Fat in raw meats and its influence on palatability has received much attention in the past. The addition of fat to fermented or dried sausages may increase the yield and reduce a_w of the final product due to the lower water content of fat, in comparison to meat (*39*).

During the ripening process of IMM, weight loss is reflected as a function of the decline in the moisture content, an acceptable criterium to determine the stage of ripening of dried sausage (*40*). In the present study, the lowering of the a_w of the biltong samples was directly related to the drying period of 3 to 4 weeks, with a final moisture content of 19.68 %. The average moisture fraction of the dry sausage (34.76 %), the salami (45,98 %) and cabanossi samples (35.76 %) were much higher than that of the biltong samples (19.68 %). The higher percentage moisture of the dry sausage, salami and cabanossi samples, in comparison to the biltong samples, can be attributed to the influence of the sausage-skin of these samples, as well as the addition of fat during the processing of these IMM. According to Mellett (*39*), the sausage-skin plays an important role in the inhibition of moisture loss during the drying process of these products.

Low pH levels enhance the shelf life of the product. Thus, pH directly effects microbial growth. According to Demeyer *et al.* (*41*), interaction of lactate, ammonia and water content with proteins principally determines the pH of dry sausage. The pH of the IMM in the present study was measured immediately after the 10 g portions were homogenised in 90 mL sterile peptone water. According to the results, the average pH of biltong was 5.84, of the dry sausage 5.56, of the salami 5.12 and of the cabanossi samples 5.83 (Figs. 1–4). In the study by Marchesini *et al.* (*42*), the average pH of the different salami samples were between 5.0 and 5.8, which was in agreement with the average pH value of 5.12 determined for the salami samples in the present study. The decline in pH that is experienced during manufacturing can be attributed to utilisation of sugars by LAB, resulting in the production of organic acids. High yeast counts can actually lead to an increase of pH by the production of amines and ammonia (*43*), and thereby favour the growth of spoilage bacteria (*44*).

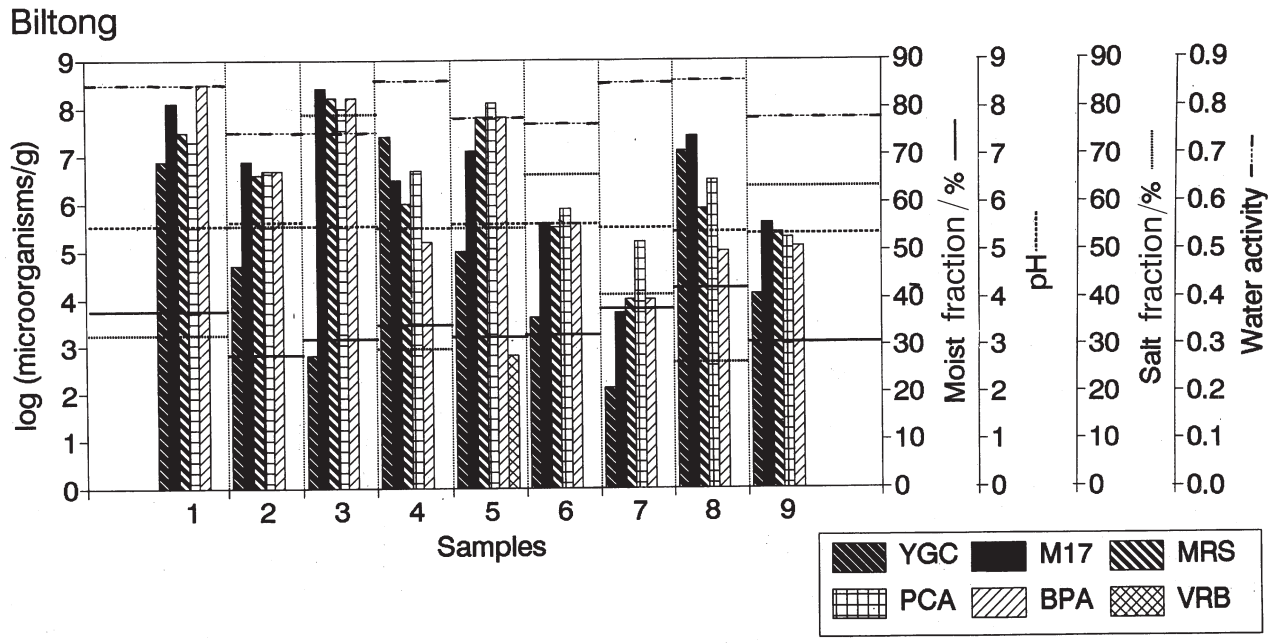

Fig. 1. Microbial enumeration, expressed as log(microorganisms/g sample) and chemical and physical analysis of biltong samples; microbial counts are bar-coded according to the media used. Chemical and physical parameters are distinguishable by different lines

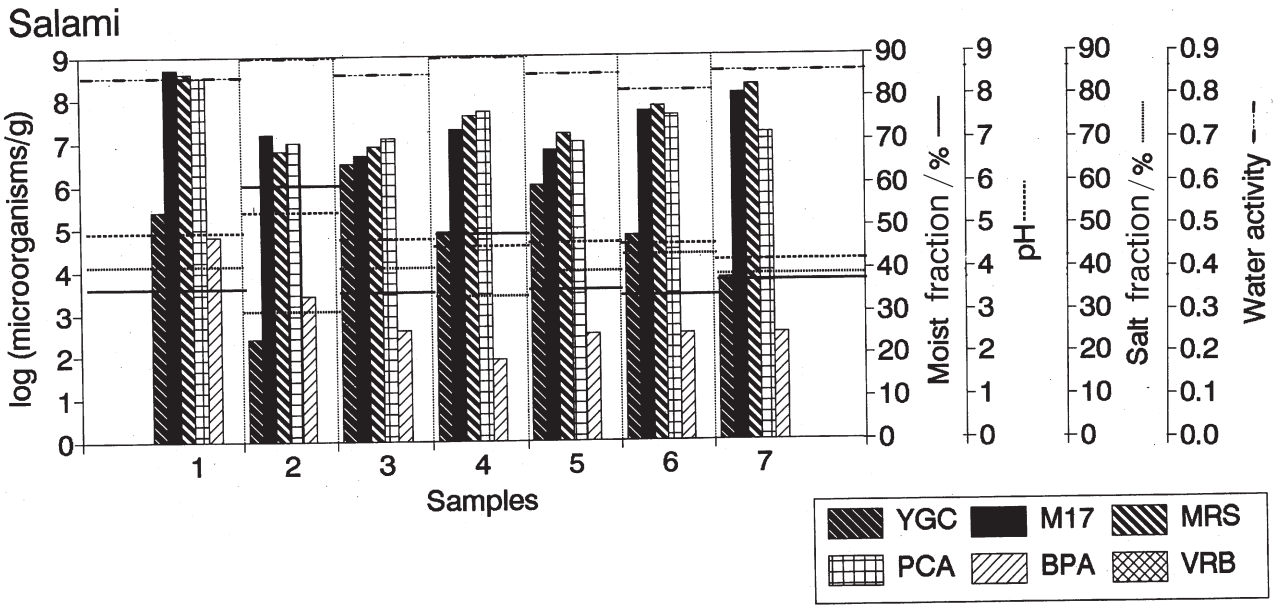

Fig. 2. Microbial enumeration, expressed as log(microorganisms/g sample) and chemical and physical analysis of salami samples; microbial counts are bar-coded according to the media used. Chemical and physical parameters are distinguishable by different lines

The addition of salt reduced the a_w, which caused the reduction of microbial growth. The fraction of salt for the biltong samples was 3.77 %, for the dry sausage samples 5.03 %, for the salami samples 3.53 % and for the cabanossi samples 3.25 %. Drying of meat products, for example, biltong, salami, cabanossi, raw ham, saveloys and dry sausage is always combined with other techniques, such as salting and/or smoking (*42*).

Microbial enumeration

The unique balance of microorganisms, *i.e.* the LAB, Micrococcaceae, yeasts and fungi (*14,15*), involved in the manufacture and ripening of IMM, as well as the principle of reduction of the a_w of the foodstuff (*4*), perform a dual role. Firstly, they suppress the growth of pathogenic organisms by their enzymatic activities, and secondly, they contribute to the final flavour of the product

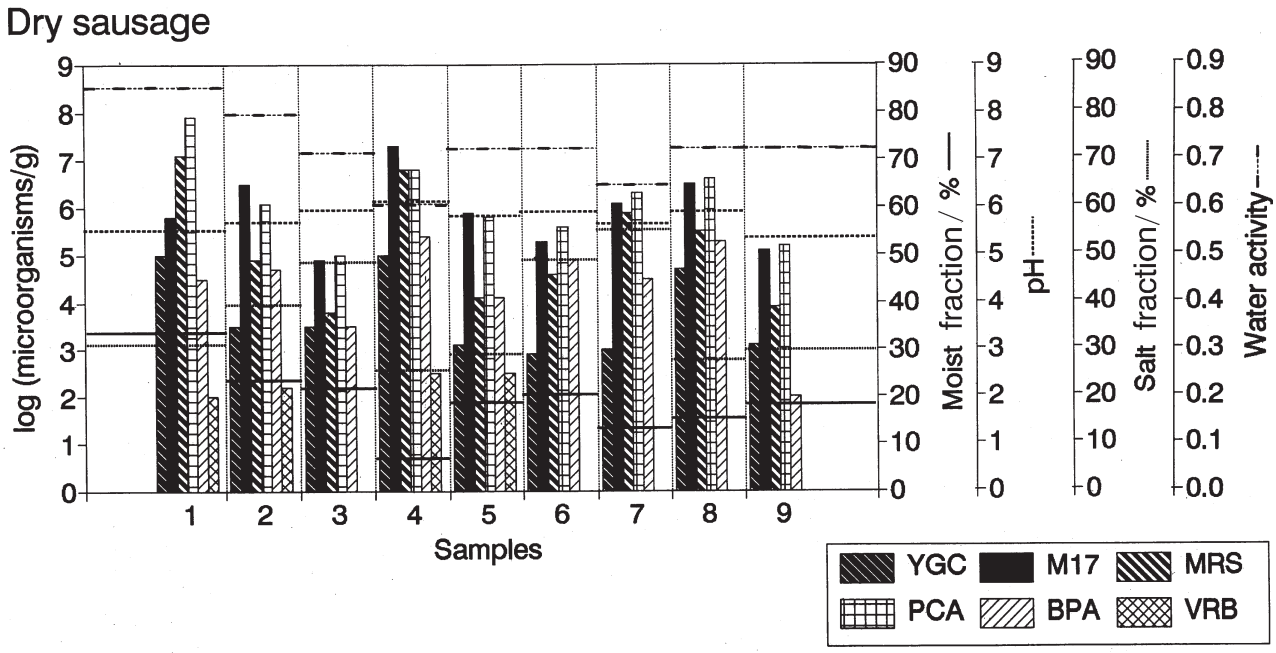

Fig. 3. Microbial enumeration, expressed as log(microorganisms/g sample) and chemical and physical analysis of dry sausage samples; microbial counts are bar-coded according to the media used. Chemical and physical parameters are distinguishable by different lines

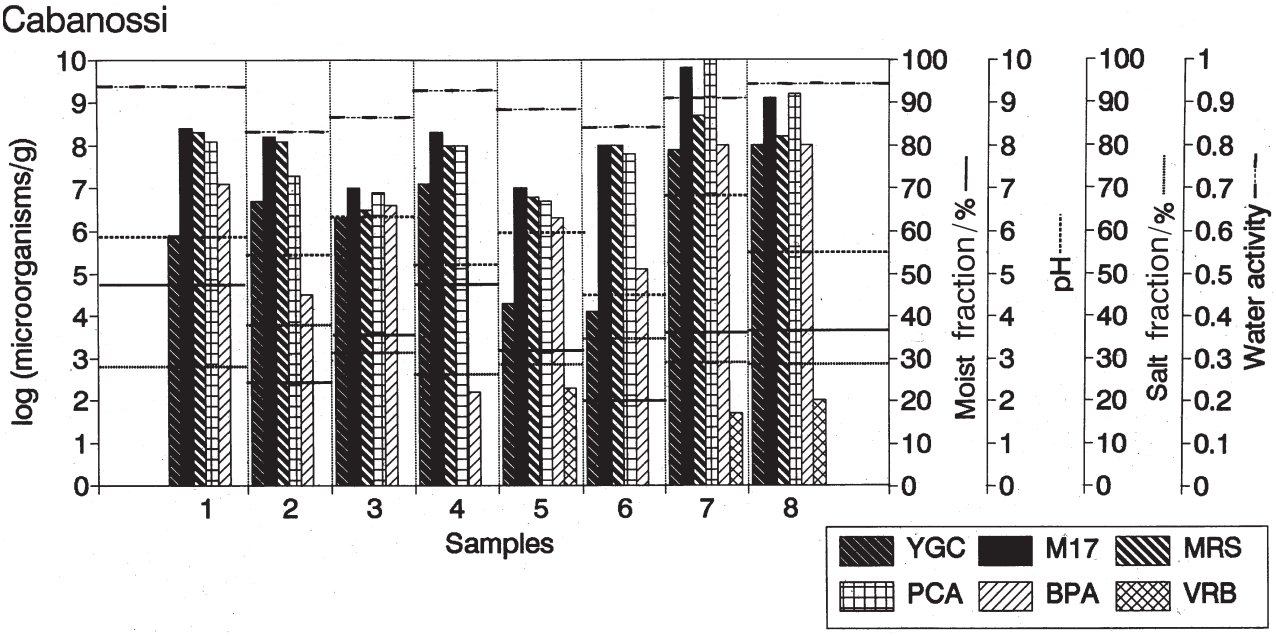

Fig. 4. Microbial enumeration, expressed as log(microorganisms/g sample) and chemical and physical analysis of cabanossi samples; microbial counts are bar-coded according to the media used. Chemical and physical parameters are distinguishable by different lines

by bio-converting lipids and proteins, and exertion of certain anti-oxidant effects by destroying peroxidase and depletion of oxygen from the surface of the product (*45*). All the IMM samples were tested for the presence of yeasts, moulds, lactococci, lactobacilli, *E. coli* and staphylococci. According to the results (Figs. 1–4), the LAB were the major bacterial component of the biltong, cabanossi, dry sausage and salami samples. These results concur with previous reports concerning the dominance of LAB in fermented dry sausages, whether or not LAB starter cultures were used (*46,47*). LAB form lactic acid that, apart from contributing to the taste of the sausage, also has the essential role in food technology to inhibit food spoilage bacteria such as *Salmonella*, *Listeria* and

certain staphylococci, by making the substrate unfavourable for proliferation of these organisms (*18*). The general a_w levels of the IMM, analysed in this study, were unfavourable for growth of certain bacterial species, with the exception of the LAB, explaining the very low counts of *E. coli*, and is in concurrence with previous reports (*48*). Higher counts were encountered for the staphylococci, which is also considered as an important group of bacteria contributing to sausage ripening (*18*). Micrococcaceae are added, together with LAB, as starter cultures to raw sausage meat (*49*). In addition to the inhibitory effect of lowered a_w, antimicrobial activity resulted from an interaction of low pH, added preservatives, the competitive microflora, generally low storage temperatures and the pasteurisation or other heat processes applied during processing.

Yeast, like moulds, contributes a small, but definite part of the natural microflora of meat. Although dry-cured meat products are frequently contaminated with yeast, they have been less intensively studied, with counts usually low in comparison to those of bacteria (*16*). These results are confirmed in the present study, with the total yeast counts of all four IMM samples examined, being lower than that of the LAB and staphylococcal counts (Figs. 1–4). Off-flavour of cold-stored meat spoiled by yeast is mainly the cause of lipolytic activity by yeast (*25,26*).

Yeast identification

Although a_w levels of IMF (0.9–0.6) generally inhibit the growth of bacteria, it permits the growth of xerophilic fungi (*50*). This group also includes the so-called osmo-tolerant yeasts. The most frequently isolated yeasts from meats include species of the genera *Candida*, *Debaryomyces* and *Torulopsis* (*20,21*). Since yeasts are ubiquitous in agricultural environments, a broad spectrum of yeasts was, as expected, found in the IMM analysed. A total of 11 species, representing nine genera, were present in the biltong, cabanossi, dry sausage and salami samples (Table 1). *D. hansenii* (25 isolates) was the most abundant yeast isolated. Other species encountered were *Cryptococcus laurentii* (7 isolates), *Cryptococcus hungaricus* (1 isolate), *Torulaspora delbrueckii* (3 isolates), *Rhodotorula mucilaginosa* (4 isolates), *Sporobolomyces roseus* (3 isolates), *D. vanriji* (5 isolates), *Trichosporon beigelii* (8 isolates), *Yarrowia lipolytica* (2 isolates), *Saccharomyces cerevisiae* (2 isolates) and *Candida zeylanoides* (2 isolates) (Table 1). Yeasts occurring naturally, or their deliberate addition as part of starter cultures, apparently contribute to the aroma of the fermented meat product (*i.e.* sausages) by breaking down of lipids and proteins, by forming specific metabolic products (*24*), as well as the depletion of oxygen. This causes the sausage to turn red rapidly (*18*), being an indication that the product is fully cured. By the formation of catalase, the onset of rancidity is also delayed. *D. hansenii* predomination in the present study concurs with results of Leistner and Bem (*51*) and can be related to the species' tolerance of high salt concentrations, together with the ability to grow at low a_w and the utilisation of organic acids (*33,52,53*). Apparent co-existence of the isolated yeast species with the relatively high LAB counts may be indicative of an definite synergistic relationship between these microorganisms (*11,12*).

Conclusions

Based on the results obtained during the present study, it would appear that yeasts are substantially represented in the total ecology of the IMM industry. A total of 11 different yeast species, representing nine different genera, were present in the biltong, cabanossi, dry sausage and salami samples. Although a broad spectrum of yeasts was found in the IMM analysed, *D. hansenii* was the most abundant yeast isolated. Bacterial counts obtained for lactococci, lactobacilli, *E. coli* and staphylococci in each of the samples analysed, contributed to the final taste, flavour and stability of the product. The physical and chemical characteristics of each product, combined with the specific balance between bacteria and yeasts, also contributed to the inhibitory growth of undesirable spoilage organisms. This resulted that neither pathogenic bacteria, nor pathogenic yeasts were detected in the IMM samples analysed, making these products suitable for human consumption.

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Izolacija i identifikacija kvasaca u mesu srednje vlažnosti

Sažetak

»Biltong«, »cabanossi«, suha kobasica i salama, koji se proizvode i koriste u Južnoj Africi, sadržavaju meso srednje vlažnosti. U radu su opisani izolacija i identifikacija dominantih kvasaca povezanih s tim mesnim proizvodima. Provedene su mikrobiološke analize, kojima je utvrđen broj svih relevantnih mikroorganizama na selektivnim agarnim podlogama, a izolacija i identifikacija svih okom vidljivih kolonija kvasaca provedene su uobičajenim postupcima. U analiziranim uzorcima nađeno je 11 različitih vrsta kvasaca, koje predstavljaju 9 rodova. Iako je u uzorcima mesa nađen širok spektar kvasaca, najraširenija je bio soj *Debaryomyces hansenii*. Ostale su vrste *Cryptococcus laurentii, Cryptococcus hungaricus, Torulaspora debrueckii, Rhodotorula mucilaginosa, Sporobolomyces roseus, Debaryomyces vanriji, Trichosporon beigelii, Yarrowia lipolytica, Saccharomyces cerevisiae* i *Candida zeylanoides*.

Utvrđen je kemijski i fizikalni sastav proizvoda od mesa srednje vlažnosti. Reprezentativnim uzorcima »biltong«, »cabanossi«, suhe kobasice i salame utvrđena je aktivnost vode, pH, postotak vlage i udjel soli.