THE ROLE OF YEASTS DURING THE RIPENING OF SALAMI

by

Akua Abrafi Osei-Abunyewa

Submitted in fulfilment of the requirements
for the degree

MAGISTER SCIENTIAE

in the

Department of Microbiology and Biochemistry, Faculty of Science,
University of the Free State, Bloemfontein 9300, South Africa

Promotor: Prof. B.C. Viljoen
Co-promotor: Mr. Arno Hugo

March 1999

CONTENTS

Not unto us, O Lord, not unto us, but unto thy name give I glory, for thy mercy, and for thy truth's sake. Wherefore should the heathen say, where is now thy God? But my God is in the heavens: He hath done whatsoever He hath pleased.(Ps 115,1-3).. I will therefore sing unto my Lord, I will make a joyful noise to the rock of my salvation. I will go before His presence with thanksgiving and make a joyful noise unto him with psalms. The Lord is a great God, and the greatest king above all gods.

For his pleasure he created all things - therefore I dedicate this work to him.

Receive thy praise O God

Acknowledgements

I wish to thank:

Mr Osei Kwadwo Abunyewa, who encouraged and supported me,

Prof Bennie C Viljoen, whose constructive criticism and contribution made this work a success,

Mr Arno Hugo, who despite a tight schedule helped with the manufacture of the salami,

Ms Eileen Roodt and Mr Piet Botes, for helping with the chemical analysis,

Mrs Carol Viljoen, whose typing skills helped in producing good prints

CHAPTER I

LITERATURE REVIEW

1.1 Introduction

Preservation by means of fermentation is one of the oldest food technologies, yet it continues to play an important role in meat preservation in many parts of the world. These processes can be simple, with minimal microbial involvement, or more complex, involving defined ingredients and starter cultures with controlled environmental conditions. Most meat fermentations rely mainly on the use of salt and spices as ingredients, and if permitted, the addition of nitrate and nitrite. The fermentation of meats furthermore, depends on the interaction of various intrinsic and extrinsic environmental as well as microbiological factors including the pH, water activity, redox potential and the presence of preservatives and naturally competitive microflora. The final fermented product, however, granted a long and safe shelf-life of high nutritious quality and wholesomeness.

The microbiological attributes of bacterial starter cultures have been studied in detail. However, despite frequent occurrences of yeasts in fermented products, little consideration has been given to the ability of yeasts to grow in salami, its contribution to the final product or the application as possible starter cultures. Therefore, this study reports the cell numbers and species of yeasts found in commercial salami and examines some properties that govern the ability of yeasts to grow in salami. In addition, selected yeast species, are incorporated individually and in association with conventional lactic acid bacteria as starter cultures during the processing of salami.

1.2. Meat

Meat is defined as 'the flesh of animals used as food, now chiefly butcher's meat, excluding fish and poultry'. Since the predominant portion of the edible flesh of animal carcasses consists of muscular tissue, meat can be conveniently regarded as the post mortem aspects of muscles (Lawrie, 1995). The principle attributes of eating quality in meat thus depend upon the structure and chemistry of muscle.

1.2.1. Chemical composition of meat

The proximate composition of a typical, adult mammalian muscle post mortem, after the onset of rigor mortis, but before degradative changes commence, is 75% water, 19% protein, 2.5 % lipids, 1.2% carbohydrate and 2.3% of miscellaneous non-protein substances (Lawrie, 1995). These findings apply generally, since the basic structure of muscles is similar between species and classes of animals. A number of factors, however, impose variation on the relative quantities of the components of muscles, including species, breed, sex, age, anatomical location, nutrition, and exercise. The approximate composition of lean muscle tissues is presented in Table 1.

Table 1

Approximate composition of lean muscle tissues of meat animals (%)

Species	Water	Protein	Fat	Ash
Beef	70-73	20-22	4.8	1.0
Pork	68-70	19-20	9-11	1.4
Lamb	73	20	5-6	1.4
Chicken	73-70	20-23	4.7	1.0

Data from Fennema, O. R. (1985).

Meat is a nutritious protein-rich food, which is highly perishable with a short shelf-life unless preservation methods are incorporated (Campbell-Platt, 1995). Meat product thus provides an excellent growth media for micro-organisms.

The slaughter of the live animal destroys the inherent defense mechanism in the meat tissue that resist microbial invasions and growth. The nature of the slaughtering process and further handling of raw product also allow spoilage and pathogenic micro-organisms from fleece, hide (Dillon and Board, 1991) and gut to readily contaminate the raw meat (Niven, 1961). The combination of a high water activity with a moderate pH and the availability of a range of nutrients further encourage the rapid growth of bacteria.

1.2.2. Microbial spoilage of meat

Raw meat may generally contain lactobacilli, micrococci, enterococci, *Pseudomonas, Escherichia*, anaerobic bacteria, yeasts and moulds contaminated either endogenously in the living animal or by subsequent post mortem contamination. Overt disease caused by *Bacillus anthracis, Mycobacterium tuberculosis* or *Brucella abortis* would lead to condemnation by veterinary inspectors and the meat would not reach the consumers. However, several conditions are not readily detectable and the composition of meat renders a good substrate for growth of spoilage bacteria such as *Pseudomonas* or food poisoning bacteria including *Salmonella, Listeria, Campylobacter* and *Clostridium* (Leistner *et al.,* 1989; Katsaras *and Leistner,* 1985; Hechelmann *et al.,* 1988) Post mortem contamination of meat is the major cause both of organoleptic spoilage and of food poisoning. Apart from the availability of nutrients, the survival and growth of these micro-organisms in meat is determined by factors like temperature, moisture availability, pH, and the gaseous environment to which the organisms are exposed.

1.2.3. Modes of meat preservation

It is evident that the requirements of micro-organisms for survival and growth afford the possibility of their control by storing meat under conditions which fail to provide these requirements, or under conditions which directly inhibit the growth and progression of the micro-organisms. Accordingly, temperature control, moisture control, or direct microbial inhibition by means of ionizing radiation, antibiotics or various chemicals are implemented. Fermentation as a mode of preservation, depending on the breakdown of carbohydrates to yield various alcohols and organic acids, resulting in the inhibition of growth of undesirable micro-organisms, was inadvertently exploited by humans many years ago. In respect of meat, fermentation has been employed to preserve or enhance the organoleptic attributes of comminuted products such as salami's (Zottola, 1992).

1.2.3.1. Fermentation

Meat product safety and shelf-life are dependent upon rapid preservation techniques instituted after-slaughter or freezing (Zottola, 1972). In general, preservation is accomplished by synergistic effects of several methods rather than the use of a single procedure (Zottola, 1972). Fermentation is an important preservation method, which has evolved for meat but is rarely used alone. Preservation is usually achieved by a combination of fermentation with the use of water activity lowering techniques including dehydration and the addition of salt (Campbell-Platt, 1995). These techniques have been the basis of traditional technologies used before the scientific basis of their action was understood (Campbell-Platt, 1995).

Historically, man made the observation that the addition of salt and sugar to ground meat followed by a holding period was conducive to preservation and resulted in an acceptable product. As time passed various geographical areas developed unique varieties of preserved meat products that varied in size, shape, texture and flavor. The

flavor and texture difference were mainly attributed to variations in spicing, sugar and salt content, meat formulation and processing characteristics. However, the stability of these products as well as their consistency, was primarily dependent upon the controlled conversion of sugar to lactic acid by bacteria (Deibel, 1974).

As with dead organic matter, muscles from slaughtered animal as a whole or in a particular form, may be modified by micro-organisms during prolonged storage. Environmental conditions and storage time greatly influence the sort and extent of modification. With food, there can be desirable and less desirable micro-organisms, which bring about desirable and less desirable changes. Desirable modifications are improvement in flavor aroma, palatability, appearance and storage characteristics. Food is fermented if micro-organisms, or enzymes contribute to its final characteristics (Campbell-Platt, 1987).

The main principle for fermented sausage manufacture is the function of lactic acid-producing bacteria (Diebel *et al.,* 1961), which reduces the pH of the meat and provides stability against the proliferation of food pathogens and other undesirable micro-organisms such as proteolytic and lipolytic organisms (Nurmi, 1966; Bacus and Brown 1981). Therefore, these bacteria control the ripening process and develop the required characteristics for the manufacture of fermented meat products (Inal, 1969; Diebel et at., 1961; Coretti, 1977). The lactic acid development inhibits undesirable micro-organisms and allows efficient dehydration. Specific micrococci cultures also enhance cure-meat color stability and prevent rancidity development by means of the reduction of peroxide formation via a catalase system (Andres, 1977). Certain yeast cultures of the *Debaryomyces* family have also been shown to accelerate and stabilize the color development at the surface of dry sausages (Coretti, 1977).

Although micro-organisms have been used to enhance and preserve fermented meat products for centuries, the respective manufactures were unaware of the technical aspect of the process (Leistner and Rodel, 1975).. As early as 1921 researchers

recognized the contribution of micro-organisms to meat processing. Later studies in the 1940s and 1950s further documented the role of micro-organisms and these led to the suggested usage of microbial cultures to achieve greater consistency. Fermented meat products have traditionally demonstrated an extended shelf-life through a combination of reduced moisture content and pH.

The USDA recognizes sausage having a moisture/protein ratio of 3.1 or less and a pH 5.0 or less as not requiring refrigeration (USDA, 1977). Moreover shelf-stable meat products are classified as having a pH at or below 5.2 and water activity at or below 0.95 or a pH at or below 5.0 and water activity at or below 0.91 (Leistner and Rodel, 1975). Microbial cultures have proven effective as "acidulation agents" for meat, since the relatively slow, consistent and uniform acid release, via metabolism does not prohibit the extraction and binding of the soluble meat proteins (Bacus, 1984). The use of microbial cultures as a "natural preservative" is therefore appealing. Fermented dry sausage in federally inspected meat plants is increasing by 10% per year (AMI, 1979). Attempts to duplicate microbial action with chemical acidulants added directly to the meat has been unsuccessful since direct rapid acidulation prohibits "binding formation" yielding an unacceptable product texture. In addition, the organic acids, primarily lactic acid produced by the micro-organism, are relatively "mild" and acceptable to the palate.

Fermentation as a means of meat preservation is becoming more significant with increasing energy cost for refrigeration, freezing and/or dehydration. Lowering meat pH provides an economic method to enhance product stability while preserving the nutritive and quality characteristics (Bacus, 1984). Although the direct contributions of micro-organisms to the nutritive value of meat products have not been studied extensively, the prolonged stability of fermented meats allows for greater consumption of perishable raw material. This natural preservation system also precludes alternative means of preservations such as extreme heat and chemicals that may reduce nutritive value (Bacus and Brown, 1981). The high protein value of most fermented meats (Kiernat *et al.,* 1964) generally results from the drying process that is consistently

achieved through controlled fermentation. Fermented products will certainly play a key role in the increasing meat product market development since these products have good stability without refrigeration and the initial nutritive value is maintained. According to the FAO the production and consumption of meat products will increase in future. Growth is expected in industrialized and especially in developing countries (Bacus, 1984).

Over the years, producers of fermented meats and other fermented foods have been bothered by the long production time involved with the consequent relatively high prices of the finished products. Therefore, developing work has been aimed at reducing the time of manufacture. As far as fermented meat products are concerned we are most likely at the beginning of a new era of class of foods and food ingredients.

1.3. Historical background of salami production

1.3.1. Origin of salami

A significant advance in man's history was the transition from food gathering to food production. Man learned that the proper handling and storage of many perishable food stuffs brought about changes in their physical, chemical, and organoleptic characteristics that proved desirable and yielded greater product stability. Meats could be grounded, mixed with salt and spices and held at cool temperatures to provide a wide variety of sausage products that were both safe for consumption and were acceptable (Bacus, 1984).

Sausage is one of the oldest forms of processed food and was consumed by the ancient Babylonians, Romans and Greeks during their military campaigns. Preserved sausages, as a meat supply, were credited as one of the main factors in the success of Caesar's legions (Pederson, 1979). The origin of meat processing probably occurred when primitive man first realized that he must either rapidly consume the fresh meat after

slaughter, or it would spoil and be unfit for consumption. Egyptians recorded the preservation of meat by salting and sun drying. The early Romans are credited with first using ice and snow to preserve food. The preparation of sausage by cutting or grinding the meat, seasoning it with salt and spices, and drying it in rolls became an effective means to preserve fresh meat (Bacus, 1984).

Man's oldest method of cooking was the open fire. Therefore the use of heat and smoke would have been recognized early as useful methods for preparing and preserving meat. The use of drying, whether by air, sun or fire was also known long before recorded history (Smith, 1987). Most reviews on fermented meats pointed out that drying and fermentation are probably the oldest form of preservation (Bacus, 1984; Smith, 1987; Roca and Incze, 1990). These authors claimed that these preservation methods are several thousands of years old. Smith (1987) made reference to Homer's Odyssey, ca 900 BC and sausages of the old Roman empire. Leistner (1986a) citing Lissner (1939), mentioned that "sausage" as such is an ancient word in many languages. Thus Wurst is an Indo- German word meaning "to turn" or "to twist" probably derived from Latin. Sausage is also well known as *kolbasa* in Slavic, derived from Hebrew, meaning "all kinds of meat". The origin of the name "salami" seem uncertain. Most authors such as Leistner (1986a) reported that it is derived from Latin, simply meaning "salt", whereas Bacus (1984) claimed that it is derived from the name of the city Salamis on Cyprus. Adams (1986) claimed that the production of fermented sausages is thought to have originated in the countries surrounding the Mediterranean Sea (Zeuthen, 1995).

It seems the art of sausage production, in most cases can be traced back to southern Europe, from where it spread to other European countries. Leistner (1986a) thus mentioned that the most well known cured and fermented German sausage probably first produced by Italians, are only ca.250 years old and the Hungarian salami is not more than 150 years old. Adams (1986) wrote that the European emigrants established production both in the USA, South America and Australia and that knowledge about

fermented sausages in the Seychelles, the Philippines and Papua New Guinea is largely a result of European influence. Climatic variation during the year has influenced the names of fermented sausages in some countries. Hungarian salami, the "winter salami" according to Incze (1986) originated in Italy where the climate in northern Italy was far better suited for the production of fermented sausages. Although the conditions were less optimal in Hungary, it turned out to be possible to dry fermented sausages in Hungary during winter months without difficulty hence the name "winter salami". Similarly the name "summer sausage" was given because the sausage was manufactured mainly during the summer, where for safety and shelf-life reasons microbial growth was stopped by heating the sausage as the last processing step (Zeuthen, 1995).

With a less lavish supply of red meat throughout Europe and the Mediterranean area through recorded history, evolved the need and opportunity for people of these regions to combine all potentially edible by-products with muscle meats and spices. Consequently, a significant proportion of traditional European sausages consist basically of a cured (i.e. nitrite-containing) red meat emulsion of widely varying texture and moderately varying fat content in which is suspended fat and/or one or more edible offals in some preferred form (e.g. chopped, diced, minced or strips). A very large proportion of the meat consumed in European countries is in the form of sausage products most of which are seen as high quality items equivalent in value to fresh meat (Smith, 1987). The drying of meat became very common along the shores of the Mediterranean when artificial refrigeration was not yet available as a means of food preservation. The early Roman butchers cut beef and pork in small pieces, added salt and spices, stuffed them into skins, or washed animal intestine and placed them in special rooms to dry.

Preparations and spicing of various sausages became a culinary art in these Mediterranean countries and later in upper Europe. These meat-processing operations grew rapidly and have lead to the development of our current dry and semi-dry

sausages. The manufacturing practices are still considered to be more of an art than a science.

The dry and semi-dry sausages were developed to maintain stability under the prevailing conditions of each area. The basis for processing the meat was preservation by inhibiting or deterring microbial decomposition (Bacus, 1984). To direct the sausage fermentation, "back slopping" was used originally, in which some meat from a previous fermentation was added to encourage establishment of desired microflora. Presently manufacturers use starter cultures (Smith and Palumbo, 1973). Fermentation as an effective method of both preservation and flavor development appears to date from at least several centuries BC (Smith, 1987). Coretti, (1971) defined fermentation as the production of lactic acid, but he used the term "ripening" to refer to all the chemical, physical, microbiological and enzymatic changes taking place in the sausage and which are temperature and humidity controlled.

Whilst the use of fermentation was fortuitous, and not properly understood until recent times it has been used for well over 2000 years thereby establishing a distinctive category of sausage products. The process of salting, curing, smoking, drying and fermenting all contribute greatly to the early development of sausage through their preservation effects on the meat products. The addition of salt reduces the availability of water to inhibit bacterial growth, whilst the addition of nitrite prevents the growth of *Clostridium* organisms. Many of the phenolic compounds deposited by smoking makes the surface of sausage much less suitable for microbial activity. Drying, like salting, also reduces the availability of water whereas fermentation increases the population of desirable bacteria, thereby making it difficult for undesirable bacteria to become established (Smith,1987).

The efficacy of the process could only have been established by trial and error over a long period of time. This gives rise to a deal of mythology surrounding traditional sausage products to this day. In this, only back slopping is stuck to. The fact that

without elaborate microbiological testing, one cannot be sure early enough that fermentation in that preceding batch was complete, is ignored by the traditionalist. (Smith, 1987).

The traditional process in the pre-1940 period relied on "natural fermentation" which was governed by the controls inherent in the process. Some manufacturers observed that, better consistency and stability were achieved by "back inoculating" portions of the recently fermented meat into a freshly prepared batch. Both this "back slopping" technique and the traditional process of relying on the natural fermentation by the indigenous bacteria are still commonly practiced in modern times (Daly *et al.,* 1973; USDA, 1977).

1.3.2. Current practices

1.3.2.1. The use of starter cultures

It is now known that the processing of sausages involves a biological fermentation process whereby specific bacteria and/or yeast, transform sugars to a variety of acids and/or alcohol that inhibit undesirable micro-organisms including food pathogens. These fermented meat owe their production, flavor, texture, nutritional, stability and/or other characteristics to the activities of the beneficial micro-organisms (Bacus, 1984).

According to Lucke (1985), intensive research in sausage fermentation was only initiated when the traditional empirical methods of manufactures no longer met the requirement of large-scale, low-cost industrial production with short ripening and highly standardized products (in the USA) in the 1930s. In Europe it was in the 1950s when the first systematic studies on the microbiology and production of fermented sausage were first published (Zeuthen, 1995). In 1940, following the successful use of starter cultures in cheese fermentation, attempts were made to develop prepared starter cultures for meat fermentation (Bacus and Brown, 1981). The concept of using an

inoculum of a defined microbial culture originated in the United States with a series of patents issued in the period 1920-1940 (Everson *et al.,* 1970; Kurk,U.S. Patent 1921).

However, the majority of these bacteria strains were of dairy origin and did not proliferate in meat mixtures, presumably because the lack of tolerance to salt and/or nitrite (Jensen and Paddock, 1940). Subsequent attempts utilized lactobacilli, the predominant microflora of the marketed meat products, but these strains did not readily survive lyophilization, which was the main method of distributing dairy starters. *Pediococcus cerevisiae* was introduced as the first commercially available meat starter culture, since it survived lyophilization (Deibel *et al* 1961).

The desirable micro-organisms have been isolated, identified, propagated separately in the laboratory, and subsequently reintroduced as starter cultures. The starter cultures are added to achieve a controlled fermentation that ensures and enhances the production of the desired end products (Bacus, 1984). Presently, the primary bacterial genera which are successfully utilized as meat starter cultures, are *Micrococcus* (Niinivaara, 1955; Nurmi, 1966), *Lactobacillus* (Nurmi 1966; Everson *et al.,* 1970), and *Pediococcus* (Deibel and Niven, 1957).

In Europe, meat starter cultures consisting of specific strains of molds and yeasts are also utilized for unique flavor development and prolonged shelf-life (Eilberg and Liepe, 1977; Coretti, 1977). New cultures are designed to yield unique flavor attributes, function as more effective preservatives of color and flavor, and/or to retard rancidity development and proliferation of undesirable micro-organisms. Consistent acid production will be maintained through the utilization of culture blends, whereby the specialized cultures will be combined with proficient acid producers (Bacus, 1984). The emergence of genetic manipulating techniques probably will contribute to a greater degree of control of microbial characteristics and improve production yields (Lee *et al.,* 1971).

Based on the large number of different sausage varieties recognized, it is not difficult to account for this phenomenon. There are many possible variations associated with the formulation, flavoring and processing of sausages. It requires only a relatively small change in any one of these, to make a substantial difference to the final product. (Smith, 1987).

1.4. Bacterial fermentation

1.4.1. Lactic acid bacteria (LAB)

Micro-organisms important for the normal raw sausage aging/curing belong to the lactic acid bacteria *Lactobacillus*, *Pediococcus* and Micrococcaceae (Leistner, 1991). Lactic acid bacteria as starter culture, cause the decline in pH by the fermentation of sugars under which nitrite degradation is supported, which improves colour formation. They also produce bacteriocin which in combination with reduced pH are capable of inhibiting growth of undesirable micro-organisms. Reduced pH consequently produces a firm product.

In meat fermentation two important types of microbial contaminants are required. One is needed to reduce added nitrate to nitrite thus producing cured meat color in sausage. The second is needed to ferment the added sugar and produce the tangy flavor, which characterizes these sausages (Deibel *et al.,* 1961; Nurmi, 1966; Coretti, 1977). Lactic acid bacteria and micrococcaceae starter cultures in perfect conditions are added to sausage emulsion at levels of 10^6-10^7 org/g of meat. Yeasts are added at levels of about 10^6 (Lucke and Hechelmann, 1987), the starter cultures grow to a high number of about 10^8 and then stabilize during late ripening. Samelis *et al.* (1994), yeast numbers were lower not exceeding 10^6cfu/g throughout processing. Lactic acid bacteria produce lactic acid from the fermentation of sugar thereby decreasing the pH and producing a sour or tangy taste.

1.4.2. Micrococcaceae

Unlike lactic acid bacteria members of the family Micrococaceae are acid-sensitive. Strain of the genera *Micrococcus* and *Staphylococci* by enzymatic actions reduce nitrate to nitrite, and produce calatase capable of breaking down peroxides (Leisner, 1991; Lucke and Hechelmann, 1987).

Micrococci capable of anaerobic growth ferment glucose (Jessen, 1995) Catalase formation by *Micrococcaceae* and yeasts reduce peroxides consequently delaying the onset of rancidity and giving flavour to the meat. (Leistner, 1991). Coagulase negative nonpathogenic strains of *Staphylococcus carnosus* and *Staphylococcus xylosus* are often used as starter cultures in dry sausage fermentation. Gokalp and Okerman (1985) and Sanz *et al.* (1988) noticed a high numbers (10^8cfu/g) of lactic acid bacteria and 10^7cfu/g of Micrococcaceae after four days fermentation.

1.5. Yeasts associated with meat

Like molds, yeasts are usually present in low numbers on fresh meat but can compete with bacteria if the surface of the meat becomes dry or competition with bacteria is reduce due to the presence of sulphite (Dillon and Board, 1991). Jay and Margitic (1981) reported yeast counts of 200 to 6.2 x 10^4/g on fresh ground beef. During low temperature storage of meat, yeast counts may increase and eventually dominate the microflora. Lowry and Gill (1984) observed that yeast counts on the loins of lamb packaged in gas-permeable plastic film increased from 10/cm^2 to 10^6/cm^2 after storage for 20 weeks at -5°C suggesting successful competition with psychrotrophic bacteria flora (Cook, 1995).

In review by Jay (1978) species of *Candida, Debaryomyces*, and *Torulopsis* were listed as the most frequently isolated genera from meats. Other genera associated with fresh

meat include *Bullera, Cryptococcus, Pichia, Saccharomyces, Schizosaccharomyces, Torulaspora, Trichosporon* and *Williopsis*. On processed meat such as sausages, burgers, luncheon meat and smoked ham, *Debaryomyces hansenii, Trichosporon spp.* and Candida spp. such as *Candida zeylanoides* may form a significant component (Jay, 1978; McCarthy and Damoglou, 1993; Viljoen *et al.,* 1993). Although numbers of yeasts on meat are generally lower than the numbers of spoilage bacteria, they can occasionally proliferate to high numbers forming a visible surface slime, particularly on some types of sausages. Dry cured meat products such as sausage and ham are frequently contaminated with yeasts (Leistner and Bem, 1970) being more typical on the surface than inside the product. The most common genera found included *Debaryomyces* and *Candida*. On Spanish dry ham yeasts have been demonstrated in high numbers throughout the ripening period (Casado *et al.,* 1991).

1.5.1. Yeasts associated with fermented meats

Most studies on yeast in fermented meats have been focused on fermented sausages rather than hams (Deak and Beuchat, 1987). Work performed by Leistner and Bem (1970) and Monte *et al.* (1986) showed *Debaryomyces hansenii* as the most frequent yeast on fermented meats despite the occurrence of *Candida rugosa, Candida cutenula* and *Yarrowia lipolytica*. Species of *Candida, Cryptococcus, Debaryomyces, Pichia, Rhodotorula* and *Trichosporon* have been isolated by Hadlock *et al.* (1976). Studies on yeasts microflora of dry-cured Spanish hams showed numbers of 10^3/g after the addition of salt rising to to 10^6/g in the middle of fermentation and stabilizing at 10^4at the end of the curing process. (Huerta *et al.,* 1988).

1.5.2. Yeasts as starter cultures

The intrinsic factors of sausage mix possess a natural selectivity for promoting the development of the desired microflora. A traditional means of ensuring that the proper microbial flora were present, was to add up to 5% of a previous mix to a fresh batch,

the so called "back slopping" method, which has been known for centuries in bread manufacturing. However, due to the increasing use of starter cultures, which are applied to great effect in the dairy industries, attempts were made to develop starter cultures for other foods such as meats (Jessen, 1995).

The starter cultures provide the sausage maker an additional control mechanism to ensure that the product is what is desired. Micro-organisms are considered desirable as natural food preservatives which have an extensive record of effectiveness and safety in a wide range of food systems. The use of cultures can often preclude the necessity for other food preservatives (Bacus, 1984). Although lactic acid bacteria and micrococci are the predominant micro-organisms associated with fermented meats, yeasts have also been incorporated. The yeasts used, have often been isolated from cured fermented meats (Zeuthen, 1995). When added directly to meat mix at an inoculation level of 10^6-10^7/g, the activity of the yeasts is mainly observed in the periphery, and the oxygen consumption accelerates the exterior color formation. The addition of yeasts result in a characteristic flavor, particularly desirable for Italian types of sausages (Leistner and Bem, 1970; Rossmanith *et al.,* 1972; Coretti, 1977).

Although little is known about the growth and kinetics of yeasts in fermented meats, the yeasts can tolerate the reduced water activity, high salt concentrations, low pH, and may contribute to the organoleptic properties of salami. *Debaryomyces* and *Candida* spp. growing on the surface, consume oxygen, degrade peroxide, show lipolytic and proteolytic activity, reduce moisture loss during curing and protect the meat from light. Both flavor and colour, have been reported to be improved by the addition of *Debaryomyces hansenii* (Rossmanith *et al.,* 1972; Coretti, 1977).

Some starter cultures contain yeasts. Early workers such as Rossmanith *et al* (1972) and Lucke and Hechelmann (1987) reported that *Debaryomyces hansenii,* identified as *D. kloeckeri,* was found to give the best performance in dry sausage ripening. Coretti (1973) in his review on the microbiology of fermented sausages, reported that rapid

and stable development of red color and acceptable aroma could be obtained with *D. kloeckeri, D. canterelli* or *D. pfaffi* as well as with a mixture of micrococci, lactic acid bacteria and *D. hansenii*.

Yeasts have also been used as starter cultures by Miteva *et al.* (1986), who reported on the use of *Candida utilis.* Similarly, Gehlen *et al.* (1991) reported on the influence of *Debaryomyces hansenii* in association with lactic acid bacteria and micrococci. In two reports on soujouk, a fermented Turkish sausage made from beef and mutton, Gokalp (1985, 1986) stressed how the use of various mixtures of cultures can be improved by adding D. hansenii.

1.5.3. Importance of yeasts in fermented meat

In commercial starter culture preparations, a yeast strain is offered for exterior and interior use (Rudolf Muller; Rhone-Poulenc Texel; Laboratories Roger). The strain used is classified as *Debaryomyces hansenii.* The species is characterized by high salt tolerance, no nitrate decomposition and high oxygen demands (Jensen, 1995). When added to the raw sausage mix as a starter culture (*D. hansenii*), it utilizes oxygen, causing the sausage to turn red rapidly.

By breaking down fat and protein, forming specific metallic products, yeasts can improve the aroma of fermented sausages (Metiva *et al.,* 1986) and, by the formation of catalase delay the onset of rancidity. In France consumers prefer sausages with a fine white coating (sausage bloom), and accordingly yeasts are used to inoculate the surface (Leisner, 1995).

In yeast and mold-ripened products, free fatty acids react with air oxygen, producing first hydroperoxides (Cerise *et al,* 1973) and then aldehydes and ketones and volatile fatty acids. These substances have a very strong aroma and are to be found especially in high quality dry sausages that have been ripen for a very long time (Langner, 1972).

CHAPTER II

THE GROWTH AND SURVIVAL OF YEASTS IN COMMERCIAL SALAMI

A.A. Osei Abunyewa; B.C. Viljoen; *A. Hugo.
Department of Microbiology and Biochemistry; *Department of Food Science, U.O.F.S., P.O. Box 399, Bloemfontein 9300, South Africa.

Abstract

The survival of yeast during the production of commercial salami was investigated. 108 distinctive yeast strains were isolated and identified during the processing of the salami. Initially, the number of yeasts remained below 10^3 cfu/g, but their numbers increased after the 12^{th} day of maturation reaching a maximum of 2.0x 10^5cfu/g at day 20. During maturation, the pH declined from 5.72 to 4.36, water content from 58% to 43% while the salt content increased by 1%. The number of lactic acid bacteria remained above 10^5 cfu/g throughout processing and maturation. Of the 108 yeast strains isolated, 22 strains were identified as members of the species *Debaryomyces hansenii* being present in all samples taken. *Rhodotorula mucilaginosa*, *Bullera variabilis* and *Cryptococcus albidus*, in that order, were also frequently isolated during processing and maturation.

2.1. Introduction

Life is characterized by constant interactions between organisms; this obviously applies to plants and animals but it certainly also applies to microbes. The functional ecological co-existence of microbes within a limited biotope is referred to as bioecoenosis. The species involved may either exert a positive effect on each other (synergism) or may suppress each others growth and development (antagonism) (Liepe, 1981). With the application of synergism and antagonism, starter cultures have been developed. The use of a starter culture provides sufficient microbial numbers to ensure the numerical dominance over the natural contaminating flora (as meat is an excellent growth medium for the growth of microorganisms) which include pathogens. The use of starter cultures in combination with the proper processing controls, therefore guarantee the safety and quality of the final product (Bacus and Brown, 1981).

Systematic inoculation with microorganisms, as it has been in practice in other nutritional sectors such as the brewing and dairy industries, was introduced to meat technology only recently (as compared to when it was applied to nutritional sectors mentioned). Nevertheless, it has been several decades that the fermentation technology for meat products has been available for use of microorganisms as starter cultures. The starter culture consists of single or mixed cultures of assorted non-hazardous strains of microorganisms. The selected strains may induce specific enzymatic activities to yield specific modification of the substrate under controlled conditions (Liepe, 1978d). Under controlled conditions, it was possible to eliminate potentially harmful salmonellae, staphylococci and clostridia. Consequently quality requirements in food sanitation could be met (Barber and Diebel 1972; Haines and Harmon, 1973; Liebetrau and Grossmann, 1976; Niskanen and Nurmi, 1976; Sirvio *et al.*,1977; Masters, 1979; Tanaka *et al.*,1980). Medical research on substitution therapy and biotechnological investigations on starter cultures used in food production have revealed a multitude of bacterial interactions in such biotopes as the intestinal tract

(Reuter, 1965; Tramer, 1966; Dahiya and Speck, 1967; Bungay and Bungay, 1968; Reuter, 1969; Daly *et al.,* 1971; Reuter, 1972a-c and Rantala and Nurmi, 1973). In this context, fermented sausages may act similarly as the intestines.

The inclusion of bacteria in the development of starter cultures has been frequently investigated, while little attention is given to the role of yeasts in the fermentation of sausages. Pioneer work on the yeast flora present in fermented sausages was conducted by Cesari (1919) and Cesari and Guilliermond (1920) who established the importance of the "fleur du saucisson" and recommended the use of pure yeast cultures for flavouring in fermented sausages (Liepe, 1981). Work performed by Jay and Margitic (1981) showed that on untreated meat like fresh ground beef, low counts of yeasts ($2x10^1$-$6.2x10^4$ cfu/g) existed, which corresponded with similar findings of Dowell and Board (1968) and Dyett and Shelley (1966) who stated that yeasts are common contaminants of sausages. Several studies have since dealt with the yeasts supposed to participate in the maturing of various dried meat products, contributing to the organoleptic characteristics of the products (Arnau *et al.,* 1987; Comi and Cantoni, 1983; Inigo *et al.,* 1970; Smith and Palumbo, 1973). Strains of the genus *Debaryomyces* predominated on dried sausages due to their exceptional high tolerance of salt (Leistner and Bem 1970; Comi and Cantoni, 1980).

Rossmanith *et al.* (1972) reported that curing color and flavour of sausages could be improved by the addition of selected *Debaryomyces* strains as part of the starter culture. Correti (1977) supported the findings and stressed that a combination of *D. hansenii,* lactobacilii and micrococci resulted in better flavour and taste development of sausages. It is evident from literature that yeasts are widely distributed on/in plants, air, water, soil and animals (Walker, 1977). The live animal and particular its hide, hair or fleece, obviously contribute substantially to the microbial contamination in the abattoir (Empey and Scott, 1939; Ayres, 1955) and since meat is an ideal growth medium for many microorganisms, yeast contamination may cause spoilage especially when bacterial loads are inhibited.

This study was therefore conducted to determine the growth and survival of the natural contaminating yeasts present during the manufacture of commercial salami.

2.2. Material and Methods

2.2.1 Commercial salami manufacture

Three 10kg batches of salami were prepared. From each batch a total of 25 individual salamis weighing 350g-450g were made according to the protocol indicated in Table 2.1. All meat portions were frozen at a temperature of -15°C to avoid "smearing" of the fat on the lean meat surface, which may cause problems during sausage dehydration. The fat used was from good quality pork lard. Meat portions and the ingredients as given in Table 2.1 were mixed sequentially in a bowl cutter. Beef was chopped into 10mm particle size. Starter culture and spices were evenly sprinkled on the beef and chopped to fine particles. Pork was then added and chopped to 20mm particle size followed by the addition of lard and curing salt. Finally, all were chopped to particle size of 4.5mm making sure that the entire batch was thoroughly mixed. The mixture was carefully packed into Calpak Fibrous Bak 65/50 to avoid trapping air and both ends were tied with strings. Three samples from each batch were weighed and marked for weight loss determination. On sampling occasions, they were weighed to determine the weight loss of the salami. The prepared salami samples were hanged on racks in a fermentation chamber at a temperature of 22°C and relative humidity of 90% for 2days. After 2days of fermentation, a 10 min smoke treatment at 18°C was carried out. Finally the sausages were transferred to a drying room at a temperature of 12°C and 75-80% RH until the product lost 20% and more weight.

Table 2.1: Composition of commercial salami

Ingredients	Percentage by weight (%)
Beef	40.000
Pork	34.730
Pork back fat	20.000
Spices	2.176
Curing salt	3.046
Starter culture (freeze dried)	0 11/26/98.500
Staphylococcus carnosus & Lactobacillus penttosus	2:1

2.2.2 Sampling methods and selection of isolates

Samples of the salami were taken from each batch on a 6hr basis during fermentation for a 2 day period. After 2 days, sampling was performed consecutively every 48hrs. One salami from every batch was transferred to the laboratory in a cooler box and analyzed in duplicate on every sampling occasion. Samples of the fresh meat and frozen meat were also taken.

2.2.3 Sampling during manufacture of salami

Salami samples were prepared for microbiological analysis by cleaning the surface of the salami with 70% ethanol. A cut was made through the salami using a sterile knife and the casing removed aseptically. 10g sample was cut using a sterile knife and transferred into a Mason jar (Metaxopoulos *et al.* 1981b) containing 90ml of sterile peptone water and blended for 3minutes Appropriate serial dilutions were made from the slurry and plated on MRS, PCA, and YGC agar by the spread plate method and incubated at temperatures as indicated in Table 2.2

2.2.4 Physical and Chemical Analysis

On every sampling occasion the a_w, pH, % fat content, % protein content % salt content and % moisture content were measured. The a_w of the salami was determined on a TH 200 Novasina Thermoconstanter. With a Janke and Kunkel Ultra Turrax T.25, 10g of the samples were homogenized in 100ml of distilled water and the pH was measured at 24°C with a HI 9321 Microprocessor pH meter (Hanna instruments). Salt, protein, fat and moisture contents were determined by the methods proposed by the Association of Official Analytical Chemist (AOAC) (1990).

Table 2.2 : Culture media, temperature and times of incubation used for microbial analysis of commercial salami

	Incubation time (h)	Growth medium
Yeast count, YGC	96	Yeast extract; glucose; chloramphenicol agar (Oxoid CM 139)
Yeast count, YM	96	Yeast extract malt extract agar (pH 3.5)
Total count, PCA	48	Standard plate count agar (Oxoid CM 361)
Lactobacilli, MRS	48	De Man Rosoga and Sharpe (MRS) (Oxoid CM 361)

2.2.5 Yeast Identification

Representative yeast isolates obtained from the highest dilution on YGC plates were purified by streaking on Yeast extract malt extract agar (YM) (Wickerham, 1951) and maintained at 4°C on the same media. Strains were identified to the species level according to the conventional methods of Barnett *et al.,* (1983) and Kreger-van Rij (1984).

2.3 Results and discussion

2.3.1 Physical and Chemical analysis

The pH in dry sausages as indicated by Demeyer *et al.* (1981) is principally determined by lactate, ammonia and water content interacting with proteins resulting in the variation of pH. The pH of the salami (Table 2.3) during the fermentation period of 48hrs increased from 5.72 to 5.88 within the first 18hrs ending at 5.78 after 48hrs. During the ripening period, pH decreased gradually reaching a lowest value of 4.36 at the end after 696hrs. The decrease in pH is attributed to the utilization of available sugars by the lactic acid bacteria resulting in the production of organic acids. There have been many debates regarding the precise pH and /or a_w levels require for a shelf-stable meat product that has not undergone heat processing. Many published criteria suggested different threshold levels of pH and/or a_w to secure the safety of the product. (Lee and Styliadis 1996). Ledward (1985) suggested an a_w < or = 0.85 or pH < 5 as the criteria, while Canadian Federal Guideline suggested an a_w< 0.92 and pH < 5.3. Meisel et al (1989) studied the survival of salmonella in salami and indicated that salmonella did not survive when the a_w was < 0.96 and pH< 4.84.

According to Roca and Incze (1990) to secure the expected stability and safety of meat that are not heated during processing and are consumed raw, the a_w has to be in the range of 0.80-0.94 depending on the drying process used and the actual pH value. The

pH and a_w values reported in this study were less than the ranges given (Table 2.3). A pH level less than 5.30 was obtained after 288 hrs while a a_w level less than 0.92 was reached after 432hrs. At the same time, the salami had lost 21.41% of its initial weight (Table 2.3). Spoilage organisms and pathogens are not resistant to low moisture content which corresponded with low a_w values and furthermore the low pH enhances the product shelf life. The final weight loss of the salami, which is a function of moisture loss, was 26.66% when the water activity reached a lowest value of 0.909. The moisture content decreased from 58% to 43.22% (Table 2.3) during the total time of processing. The gradual moisture loss of the salami reflected the rate and extent of increase in protein content of the salami from 16.834% to 22.711%, fat content from 19.659% to 26.487% and salt content from 3.070% to 4.131% during the processing period (Table 2.3).

2.3.2 Microbial Enumeration

Table 2.4 shows the comparative analysis of microbial growth. Lactic acid bacteria counts were obtained on De Man, Rogosa and Sharpe agar (MRS), total bacteria counts were determined using standard plate count agar (PCA) and yeast counts on yeast glucose chloramphenicol agar (YGC). Lactic acid bacteria numbers increased gradually from log 5.36/g from the beginning of processing and reached a maximum of log 6.52/g after 240 hrs during the ripening stage. The total bacteria and lactic acid bacteria counts were similar during fermentation and maturation with the exception of the time lapse between 42 and 96 hrs. The similar counts clearly indicated that the processing of salami is mainly governed by the added starter culture and the possible inhibition of normal contaminating bacteria, which corresponded with the decline in pH as a result of the production of lactic acid bacteria. The total bacteria numbers increased from log 4.99/g to log 6.89/g during the fermentation stage. The high bacterial numbers during the fermentation stage may contribute to the reduction of nitrate to nitrite thereby influencing the appearance, odour, flavour and safety of the product (Adams, 1986). Bacterial numbers continued to increase (Coretti, 1956) during

Table 2.3: The changes in chemical and physical analysis during the fermentation of salami over a period of 700hrs

Time (h)	Protein content (%)	Moisture content (%)	Fat content (%)	Salt content (%)	pH	Wt Loss (%)	a_w
Fermentation							
0	16.834	57.986	19.659	3.0707	5.72	0	0.950
6	16.964	57.608	19.832	3.0983	5.72	0.89	0.949
12	17.255	56.943	20.135	3.1474	5.80	2.42	0.909
18	17.427	56.504	20.337	3.179	5.88	3.39	0.947
24	17.649	55.945	20.812	3.2196	5.83	4.60	0.947
30	17.838	55.475	20.812	3.2541	5.80	5.60	0.947
36	18.057	54.918	21.070	3.2943	5.00	6.91	0.946
42	18.282	54.325	21.342	3.3272	5.79	7.91	0.944
48	18.484	53.848	21.563	3.3373	5.78	8.87	0.944

Wt loss = Weight loss a_w = Water activity

Table 2.3 continued

Time (h)	Protein content (%)	Moisture content (%)	Fat content (%)	Salt content (%)	pH	Wt Loss (%)	a_w
Ripening							
96	18.940	52.644	22.084	3.4551	5.64	11.05	0.936
144	19.270	51.917	22.467	3.5149	5.58	12.58	0.938
192	19.558	51.200	22.806	3.5675	5.55	13.80	0.937
240	19.864	50.436	23.168	3.6233	5.45	15.23	0.930
288	20.263	49.441	23.640	3.6960	5.31	16.90	0.929
336	20.636	48.511	24.079	3.7640	4.96	18.41	0.923
384	21.035	47.523	24.546	3.8367	4.60	19.95	0.922
432	21.229	46.908	24.809	3.8893	4.88	21.41	0.922
480	21484	46.401	25.072	3.9185	4.77	22.21	0.919
528	21.746	45.735	25.393	3.9666	4.72	23.09	0.919
576	22.010	45.058	25.719	4.0149	4.53	24.09	0.921
624	22.326	44.251	26.104	4.1063	4.52	25.35	0.917
696	22.711	43.220	26.488	4.1312	4.36	26.66	0.909

Wt loss = Weight loss a_w = Water activity

the first part of the maturation stage probably due to the higher pH values and water content present at the time. After 240hrs, however, the bacterial numbers remained constant.

Although no yeasts were added to the salami formulation an increase in yeast numbers from log 3.66/g to Log 5.36/g during ripening was noticed. The high yeast numbers is attributed to natural contaminating yeast present in the meat (Dillon and Board, 1991), processing equipment, and workers' hands and aprons. Yeast counts remained low during the fermentation stages when bacterial numbers progressed but their numbers rapidly increased during the maturation stage. A significant increase (log 2/g) in yeast numbers, was observed during the maturation stages when the bacterial numbers stabilized (Table 2.4). The highest yeast count of log 5.36/g was observed after 528 hrs. The high number of yeasts observed during the later stages of maturation suggests that the yeasts may have played an important role in the ripening of the salami.

The progressive growth of lactic acid bacteria during the fermentation stages, and yeasts during the ripening stages may indicate a competition between the microorganisms for available substrates. However, the interaction between the yeasts and lactic acid bacteria at the later stages appears to be synergistic since both populations continued to survive at high numbers with none being inhibited by the other.

2.3.3 Yeast enumeration

Of the yeasts isolated, 12 species from 7 different genera were isolated from the raw meat (Table 2.5), *Candida, Bullera, Trichosporon, Cryptococcus, Rhodotorula, Debaryomyces and Lipomyces.* Dillon and Board (1991) have confirmed most of these yeasts as natural flora present in the hide, fleece, and carcasses of field animals. In addition to the above genera, six other genera were isolated from the salami during processing, (*Schwanniomyces, Galactomyces, Sterigmatomyces, Pichia, Torulaspora* and

Table 2.4: Log counts g^{-1} during the production of salami over a period of 700hrs

	log counts g-1		
Time (hrs)	PCA	MRS	YGC
Fermentation			
0	4.99	5.36	3.90
6	5.36	5.88	3.51
12	5.61	5.71	3.64
18	5.45	5.63	3.99
24	5.45	5.41	3.91
30	5.43	5.78	3.90
36	5.64	5.91	3.72
42	6.63	6.03	3.49
48	6.89	6.08	3.57
Ripening			
96	6.27	6.11	3.66
144	6.26	6.21	4.24
192	6.47	6.39	4.39
240	6.58	6.52	4.52
288	6.48	6.33	4.43
336	6.49	6.42	4.24
384	6.42	6.34	5.08
432	6.33	6.31	5.31
480	6.43	6.43	5.25
528	6.57	6.14	5.36
576	6.53	6.48	5.17
624	6.23	6.14	5.24
696	6.35	6.39	5.18

PCA= Total bacteria counts MRS= Lactic acid bacteria counts YGC= Yeast counts

Table 2.5: Yeasts associated with salami processing

	Source of contamination					
	Fresh meat			Frozen meat		
Isolates	**Beef**	**Pork**	**Lard**	**Beef**	**Pork**	**Lard**
Bullera variabilis	+	+	+			
Candida haemulonii			+			
Candida vinaria	+					
Candida zeylanoides	+	+		+	+	
Cryptococcus albidus	+				+	
Cryptococccus hungaricus	+					
Cryptococcus laurentii					+	
Debaryomycse hansenii		+	+	+	+	+
Lipomyces tetrasporus						+
Rhodotorula minuta	+	+		+		
Rhodotorula mucilaginosa		+	+		+	
Trichosporon beigelii	+	+	+	+	+	

Sporobolomyces). *Candida, Cryptococcus, Debaryomyces, Galactomyces, Rhodotorula, Pichia, Trichosporon* and *Torulaspora* were frequently isolated from raw and fermented meats (Leistner and Ayres, 1968; Hadlock *et al*, 1976; Smith and Hadlock, 1976; Jay, 1978; Comi and Cantoni, 1983; Williams, 1990; McCarthy and Damoglou, 1993). The other yeast species being present might have originated from the meat, hands of those who prepared the salami, processing equipment or the air.

Although the initial yeast flora present in the sausage emulsion and raw meat was extremely variable, *D. hansenii* strains were isolated frequently during the fermentation and ripening stages. The species appeared to be the most abundant yeast species associated with the processing of salami (Table 2.6) representing 20.37% of the total number of yeast strains isolated. The frequent presence of the species corresponded with results obtained by various authors (Hammes *et al.,* 1985; Lucke and Hechelmann, 1987; Samelis *et al.,* 1994). *Rhodotorula mucilaginosa,* a typical air contaminant strain, described by Deak (1993) as a frequent food isolate followed with 14.81%. *Bullera variabilis, Cryptococcus albidus, and Trichosporon beigelii* were isolated at percentages of 13.89%, 10.18% and 9.26% respectively while the same number of *Candida zeylanoides* and *Schwanniomyces occidentalis* strains were isolated, 5.5%. Each of the remaining species represented less than 5% of the total number of yeast isolated. *Torulaspora delbruecki, Rhodotorula mucilagenosa, R. minuta, Cryptococcus albidus, C. laurentii, Candida zeylanoides and Galactomyces geotrichum* are reported to be frequently isolated from meats and meat products. Deak and Beauchat (1996)

The incidence of yeast isolated throughout the salami processing is shown in Table 2.7. *C. gropengiesseri, P. philogaea, P. farinosa, R. minuta, S. halophilus* and *Sp roseus* were isolated only during the fermentation stage which may be an indication that these yeast were inhibited by the reducing water activity. Despite the frequent occurrence of *T. beigelii* strains during the fermentation stage, no strains appeared during the ripening stages. *T. delbruecki, D. polymorphous, S. occidentalis* and *C. haemulonii* were not frequently isolated during processing which may be an indication that these yeast

Table 2.5: Yeasts associated with salami processing

	Source of contamination					
	Fresh meat			Frozen meat		
Isolates	Beef	Pork	Lard	Beef	Pork	Lard
Bullera variabilis	+	+	+			
Candida haemulonii			+			
Candida vinaria	+					
Candida zeylanoides	+	+		+	+	
Cryptococcus albidus	+				+	
Cryptococccus hungaricus	+					
Cryptococcus laurentii					+	
Debaryomycse hansenii		+	+	+	+	+
Lipomyces tetrasporus						+
Rhodotorula minuta	+	+		+		
Rhodotorula mucilaginosa		+	+		+	
Trichosporon beigelii	+	+	+	+	+	

Table 2.6: **Frequency of occurrence of yeast isolates during salami processing over a period of 696 hrs**

Isolates	Number of Strains	%
Bullera variabilis	15	13.89
Candida haemulonii	5	4.29
Candida gropengiesseri	1	0.93
Candida zeylanoides	6	5.55
Cryptococcus albidus	11	10.18
Debaryomyces hansenii	22	20.37
Debaryomyces polymorphus	1	0.93
Debaryomyces vanrijiae	5	4.29
Galactomyces geotrichum	1	0.93
Pichia farinosa	1	0.93
Pichia philogaea	1	0.93
Rhodotorula minuta	2	1.85
Rhodotorula mucilaginosa	16	14.81
Sterigmatomyces halophilus	1	0.93
Schwanniomyces occidentalis	6	5.55
Sporobolomyces roseus	1	0.93
Trichosporonn beigelii	10	9.26
Tolulopsis delbruecki	1	0.93

Table 2.7: The incidence of yeast during salami processing over a period of 696 hrs

	Time (hrs)										
	Fermentation									Maturation/Ripening	
Yeast Strain	0	6	12	18	24	30	36	42	48	96	144
B. variabilis	+	+	+	+	+	+	+			+	
C. gropengiensseri			+								
C. haemulonii								+		+	
C. zeylanoides					+	+	+		+		
Cry. albidus						+			+		
D hansenii	+	+	+	+	+	+	+	+	+	+	+
D. vanrijiae				+				+			
G. geotrichum	+										
P. farinosa				+							
P. philogaea						+					
R. minuta							+		+		
R. mucilaginosa	+	+	+	+	+			+	+		
S. halopilus				+					+		
S. occidentalis	+			+				+			
Sp. roseus									+		
T. beigelli	+	+	+	+	+	+	+			+	+

Table 2.7: continued

	Time (hrs)										
	Maturation/Ripening										
Yeast strain	**192**	**240**	**288**	**336**	**384**	**432**	**480**	**528**	**576**	**624**	**696**
B. variabilis	+	+	+	+	+			+	+		
C. haemulonii		+	+								
C. zeylanoides				+		+					
Cry. albidus	+	+	+	+	+	+	+	+	+		
D. hansenii	+	+	+	+	+	+	+	+	+	+	+
D. polymorphus											+
D. vanrijiae							+	+		+	
G. geotrichum							+				
R. mucilaginosa	+	+	+	+	+	+	+	+	+		
Sw. occidentalis									+	+	+
T. beigelii	+										
To. delbruecki	+										

B.= Bullera C.= Candida Cry.= Cryptococcus D.= Debaryomyces G.= Galactomyces P.= Pichia R.= Rhodotorula S.= Sporoblomyces St.= Sterigmatomyces Sw.= Schwanniomyces T.= Trichosporon To.= Torulaspora

strains are not part of the yeast community of the salami that developed during salami processing. Since small portions (10g) of the salami were taken for microbial analysis they might have been missed out during sampling. Deak and Beuchat (1996) indicated that micro-organisms favoured in foods are those that posses the necessary physiological attributes to respond to ecological determinants. The frequent occurrences of *C. albidus, B. variabilis, R. mucilaginosa, C. zeylanoides and D. hansenii* can therefore be attributed to their tolerance of low temperatures, high salt concentrations low pH levels and resistance against their environment. *C. albidus* an anamorphic yeast with basidiomycetous affinity exhibits proteolytic activity (Huerta *et al.,* 1988) and may cause spoilage by hydrolysation of the proteins in the salami. *D. hansenii* and *C. zeylanoides* are able to reduce the fat rancidity of the salami by hydrolyzing lipids through lipolytic activity (Metiva *et al.,* 1986). Furthermore, most of these species have the ability to utilize organic acids produced by the lactic acid bacteria (Fleet, 1990; Besancon *et al.* 1992; Roostita and Fleet, 1996) which result in an increase in pH. An increase in pH due to excessive growth of contaminating yeasts, might result in a decline in its preservation action (Fleet, 1992) making the salami susceptible to microbial spoilage of pathogens and undesired bacteria. Strong growth in the presence of salt, growth at low temperatures and the ability to utilize organic acids are considered as key determinant that encouraged the presence of *Debaryomyces hansenii* (Guerzoni, 1993b; Van Eck *et al.* 1993). Considering that this species was the most resistant and proliferating yeast found in this study, further research on its effect on organoleptic characteristics of salami and interaction with the lactic acid bacteria seems promising.

CHAPTER III

KEY PROPERTIES FOR THE SELECTION OF YEASTS AS POSSIBLE STARTER CULTURES IN THE MAKING OF SALAMI

Abstract

Practices in the manufacture of raw, dry sausage have been of much public concern since the finished products are not cooked before eating. The use of starter cultures assures better quality products with shorter production time, and a longer shelf-life. Care must be taken, however, not to put the consumers' health at risk since raw meat is an ideal habitat for the growth of pathogenic micro-organisms. Nineteen yeast species isolated from the natural microflora of commercial salami during production were examined based on relevant key properties proposed for selecting good starter cultures. All the yeasts survived NaCl concentrations at 4-8%, 80-240ppm nitrite concentrations and lacked proteolytic activity except for *Trichosporon beigelii.* Lipolytic activity proved to be variable. Selected lipolytic positive *Debaryomyces hansenii* and *D. polymorpus* strains were inactivated at temperatures above 50°C.

3.1. Introduction

The application of starter cultures in the fermentation of food products is derived from the isolation and identification of micro-organisms responsible for the desired effects and its addition to fermented food at the appropriate stage of processing (Bacus,

1984). This resulted in the selection of lactic acid bacteria, *Micrococcaceae* (Jensen and Paddock, 1940), and yeast strains (Coretti, 1977) for use as starter culture in sausage fermentation.

Flavour development in raw sausages is attributed to the action of microbial enzymes (Coretti, 1965) while lowering the pH and, the excretion of anti-microbial compounds ensure a safe and long shelf-life stable product (Lucke and Hechelmann, 1987). The first starter cultures were commercially available in the 60's, although suggestions and patent registration had long been made in 1919, for the use of various micro-organisms in the preparation of dry sausages (Coretti, 1977; Bacus and Brown, 1981; Liepe 1983). In the USA, Deibel (1956), Deibel and Niven (1957) and Deibel *et al.* (1961) recommended *Pediococcuss cerevisiae* to be used in meat fermentation as starter culture. Yet the first starter culture used on a large scale consisted of *Pediococcus acidulatici,* whereas *Micrococcus* ("M53") proposed by Niinivaara (1955), was used in Europe.

Many patent publications proposed the use of *Pediococcus* and *Lactobacillus plantarum* (Everson *et al.,* 1974), as well as mixed cultures (Gryczka, 1977; Gryczka and Shah, 1979). Bacterial starter cultures in Europe therefore, comprised of staphylococci in combination with lactobacilii or pediococci (Coretti, 1977; Liepe, 1978d; Bacus and Brown, 1981). A changeover to mixed cultures consisting of lactic acid bacteria used in combination with *Micrococcaceae* was established a few years later in Central Europe (Lucke and Hechelmann, 1987). Although mould ripened sausages are not so common, patented procedures for the covering of fermented sausages with mycelia were established in the sixties. Racovita and Racovita (1968) later described the method of spraying sausages with spores of certain species of *Penicillium.* Research on the improvement of fermented sausages resulted in the inclusion of yeasts as potential starter cultures for meat fermentation as eventually applied in the fermentation of Italian salami (Liestner and Bem, 1970; Coretti, 1977).

To be accepted as starter culture, it is expected that the micro-organisms used be more reliable and faster growing than the natural microflora, without endangering the quality of the product or health of the consumers (Lucke and Hechelmann, 1987). Lactic acid bacteria involved in meat fermentation produce organic acids, which inhibit the growth of undesirable micro-organisms and contribute to the development and stability of colour and aroma. *Micrococcaceae* strains reduce nitrate to nitrite and form catalase which breaks down peroxide (Lucke, 1985). Moulds protect the surface of the dry sausage from sunlight and oxygen, and prevent colonization of other pathogenic moulds. Stiebing and Rhodel (1988) reported that *Penicillium nalgiovence* contributed to better aroma. Yeasts contribute to the safety by breaking down peroxides within the sausage and utilize oxygen, which might be available for the growth of undesirable micro-organisms.

During the production of commercial salami, *Debaryomyces hansenii* strains were most frequently isolated. Coretti (1973) reported rapid and stable red meat colour development and acceptable aroma with the application of *Debaryomyces kloeckeri*, *D. cantarelli* or *D. pfaffi* as well as with a mixture of micrococci, lactic acid bacteria and *D. hansenii*. Huerta *et al.* (1988) found *Debaryomyces spp.* to be the dominant yeast in ham. *Debaryomyces hansenii* has been indicated by Leistner and Bem (1970), Jay (1978), Monte *et al.* (1986), McCarthy and Damouglou (1993) and Viljoen *et al.* (1993) as the most significant yeast in processed meat. Good performance of *D. hansenii* in sausage ripening was reported by Rossmanith *et al.,* (1972) and Gokalp (1986). *D. hansenii*, a psychrotrophic yeast species can tolerate low pH values, low water activity and high salt content, (Besancon *et al.,* 1992; Roostita and Fleet, 1996) Besancon *et al.* (1992) demonstrated that some strains of *D. hansenii* could tolerate up to 20% NaCl. Based on these properties, Vayssier (1979) suggested the use of *D. hansenii* as starter culture alone or in combination with *Penicillium nalgeovense.*

In a previous study, two *Debaryomyces* species identified as *Debaryomyces hansenii* and *Debaryomyces polymorphus* were frequently isolated during the processing and ripening of commercial salami indicating that they were able to survive at low pH levels,

water activity and high salt concentrations. The two species are therefore selected for further examination and compared with other yeasts naturally isolated during salami making. The yeasts are selected based on some of the key properties (Deibel, 1974) proposed to select a good starter culture.

Characteristics of a good starter culture as suggested by (Deibel, 1974) are the following:

1. Should be salt tolerant
2. Have the ability to grow well in the presence of 80-100ppm nitrite
3. Have an optimum growth at 32.2°C with series of 26.6-43°C
4. Be non-proteolytic
5. Be non-lipolytic
6. Must not produce off flavors as by-product of fermentation
7. Be non pathogenic
8. Be inactive at temperatures above 50°C
9. Have a rapid growth in 6% pickling solution.

3.2. Materials and methods

Yeasts isolated and identified according to the conventional methods of Barnett *et al.* (1983) from a previous study (Table 3.1), were maintained on YM (yeast extract malt extract, Wickerham, 1951) agar stored at 4°C.

3.2.1. Salt tolerance

The isolated yeasts were inoculated into sterile test tubes containing YM broth medium (Table 3.2) containing 4%, 6%, and 8% NaCl and incubated at 25°C for 5 days. Media appeared cloudy with positive results and remained clear with negative results.

Table 3.1 List of yeast isolates identified previously

1. *Bullera variabilis*
2. *Candida haemulonii*
3. *Candida gropengiesseri*
4. *Cryptococcus albidus*
5. *Cryptococcus laurentii*
6. *Candida zeylanoides*
7. *Debaryomyces hansenii*
8. *Debaryomyces polymorphus*
9. *Debaryomyces vanrijiae*
10. *Galactomyces geotrichum*
11. *Pichia farinosa*
12. *Pichia philogaea*
13. *Rhodotorula minuta*
14. *Rhodotorula mucilaginosa*
15. *Sporobolomyces halophilus*
16. *Sterigmatomyces roseus*
17. *Schwanniomyces occidentalis*
18. *Trichosporon beigelii*
19. *Torulaspora delbruecki*

Table 3.2: Media formulation, sterilizing temperature and time

Media	Ingredients	Quantity(g/ml)	Sterilizing temperature (°C)	Time (min)
YM broth	Yeast extract	3g	121	15
	Malt extract	3g		
	Peptone	5g		
	Glucose	10g		
	Distilled water	1000ml		
Casein Digestion	Skim milk powder	50g	121	15
	Bacteriological agar	25g		
	Distilled water	1500ml		
Tributyrin	Peptone	5g	121	15
	Yeast extract	3g		
	Glycerol tributyrate	10g		
	Bacteriological agar	20g		
	Distilled water	1000ml		
Rhodamine B	Nutrient broth	8g	121	15
	NaCl	4g		
	Bacteriologocal agar	16g		
	Olive oil	32.25ml		
	Distilled water	1000 ml		

3.2.2. Nitrite tolerance

Yeast isolates (Table 3.1) were streaked on sterile Petri dishes with YM agar containing 80ppm, 160ppm and 240ppm nitrite and incubated at 25°C for 5days (Table 3.2).

3.2.3. Proteolytic activity

Yeast isolates (Table 3.1) were streaked on Petri dishes with casein digestion agar (Ahearn *et al.*, 1968), and incubated at 25°C for 5 days (Table 3.2). Clear transparent zone around the streaked area indicated a positive result

3.2.4. Lipolytic activity

Isolated yeasts (Table 3.1) were streaked on sterile petri dishes with olive oil and Rhodamine B agar (Kouker and Jaeger, 1987) and Tributyrin (glycerol tirbutyrate) (Fryer *et al.,* 1966) agar, and the plates were incubated at 30°C for 3 to 6 days.

Rhodamine B (Table 3.2) was emulsified with a Brason Sonifer Cell Disrupter B-30 and the final pH of the solution adjusted to 7.0 before sterilizing. Media were cooled to 70°C and 10ml of filter sterilized Rhodamine B solution [(0.1% w/v) final concentration] added. The mixture was shaken and allowed standing for 10min to reduce foaming before pouring into sterile Petri dishes. Flourescence of the streaked area under a flourescent light in the dark gave positive results

3.2.5. Rate of inactivation at temperatures above 50°C

Yeast isolates (Table 3.1) were seeded in triplicate in YM broth (Table 3.2) (200ml) in 1000ml Elernmeyer flasks and incubated at 50°C, 55°C and 60°C with vigorous agitation on rotary a shaker (180rpm) through 150min. From each flask 10ml was taken for microbial enumeration at consecutive intervals of 6 hrs for a period of 60hrs. Serial

dilutions were prepared in buffered peptone water and spread plated on YGC (yeast glucose chloramphenicol) agar. Highest serial dilutions which represented colonies between 30-300 colonies per plate were counted.

3.3. Results and discussion

Results obtained are indicated in Table 3.3 indicating the ability of all the isolated yeasts to grow in the presence of 4-8% NaCl and 80-240ppm nitrite, proteolytic and lipolytic activities are also represented in Table 3.3. Several reports referred to the isolation of yeasts from dry cured meats with reduced product a_w by the addition of salts (Comi *et al.,* 1982; Monte *et al.,* 1986; Huerta *et al.,* 1988; Molina *et al.,* 1990; Gimenez, 1992). In a review by Jay (1978), *Candida, Debaryomyces* and *Torulopsis* (now regarded as *Candida*) were listed the most frequently isolated genera from meats. Other genera associated with fresh meat include *Bullera, Cryptococcus, Pichia, Saccharomyces, Schizosaccharomyces, Torulaspora, Trichosporon* and *Williopsis*. McCarthy and Damouglou (1993) and Viljoen *et al.* (1993) reported on the predominance of *Candida spp., Trichosporon spp.* and *Debaryomyces hansenii* in sausage, luncheon meat and smoked ham and considered these species as part of the established yeast community. Work performed by Saldanha-da Gama *et al.* (1997) showed the predominating yeasts in Portuguese pork based products to be *Debaryomyces hansenii, Cryptococcus laurentii, Cryptocuccus humicolus, D. polymorphus* and *Pichia spp.* These authors noted that all yeast isolated from ham were able to grow in the presence of 5-8% NaCl. Some *D. hansenii* strains have been demonstrated to tolerate up to 20% NaCl (Besancon *et al.,* 1992).

Yeasts grow at lower a_w levels in a sugar medium than compared to growth in a salt medium (Van Eck et al., 1993). Tokuaka et al. (1985) listed 30 yeast strains isolated from high sugar foods, which showed exceptional tolerance against high sugar concentrations capable of growth at a a_w range of 0.912-0.876. *Torulaspora delbrueckii* and *D. hansenii* are typical osmotolerant species isolated from high sugar foods.

Table 3.3: Key properties of a good starter culture

	Nitrite tolerance			Salt tolerance			Lipolytic acivity		Casein digestion
	Concentration (ppm)			Concentration (%)					
Isolates	80	160	240	2	4	6	Tributyrin	Rhodamine B	
1. Bullera variabilis	+	+	+	+	+	+	+	+	-
2. C. haemulonii	+	+	+	+	+	+	+	+	-
3. C. gropengiesseri	+	+	+	+	+	+	+	+	-
4. Cry. albidus	+	+	+	+	+	+	+	+	-
5. Cry. laurenti	+	+	+	+	+	+	+	-	-
6. Cry. zeylanoides	+	+	+	+	+	+	+	+	-
7. D. hansenii	+	+	+	+	+	+	+	+	-
7. D. hansenii	+	+	+	+	+	+	+	-	-
8. D. polymorphus	+	+	+	+	+	+	+	+	-
9. D. vanrijiae	+	+	+	+	+	+	+	-	-
9. D. vanrijiae	+	+	+	+	+	+	+	+	-
10. G. geotrichum	+	+	+	+	+	+	+	+	-
11. P. farinosa	+	+	+	+	+	+	+	+	-

C.= Candida Cry.= Cryptococcus D.= Debaryomyces G.= Galactomyces P.= Pichia

ppm = Parts per million % = percentage

Table 3.3: continued

Isolates	Nitrite tolerance			Salt tolerance			Lipolytic acivity		Casein digestion
	Concentration (ppm)			Concentration (%)					
	80	160	240	2	4	6	Tributyrin	Rhodamine B	
12. P. philogaea	+	+	+	+	+	+	+	+	-
13. R. minuta	+	+	+	+	+	+	+	-	-
14. R. mucilagenosa	+	+	+	+	+	+	+	-	-
15. S. halophilus	+	+	+	+	+	+	+	+	-
16. St. roseus	+	+	+	+	+	+	+	+	-
17. Sw. occidentalis	+	+	+	+	+	+	+	+	-
18. T. beigelii	+	+	+	+	+	+	+	+	+
19. To. delbrueckii	+	+	+	+	+	+	+	+	-

P.= Pichia R.= Rhodotorula S.= Sporoblomyces St.= Sterigmatomyces Sw.= Schwanniomyces T.= Trichosporon To.= Torulaspora

ppm =Parts per million %= percentage

Touaka *et al.* (1991) indicated the minimum a_w for growth in NaCl for *D. hansenii, T. delbruecki* and *Rodotorula mucilaginosa* are 0.84, 0.90 and 0.90 respectively. According to Van Eck *et al.* (1993) the minimum a_w for growth in NaCl is 0.93 for *Schwanniomyces occidentalis,* 0.90 for *Pichia farinosa* and 0.88 for *D. hansenii.* The spoilage of yeasts in meat products due to tolerance of low a_w, values, however, is not considered to be of any great importance (Deak, 1991), the spoilage appears to be related to the yeasts' proteolytic and lipolytic activity which also include desirable effects.

Table 3.3 shows the lipolytic and proteolytic activities of the yeasts. All the species exhibited no proteolytic activity, except *Trichosporon beigelii.* Huerta *et al.* (1988) found that *Trichosporon spp.* were the only yeast species with proteolytic activity in ham. All the yeast isolates included in this study proved to be lipolytically positive with the exception of *Rhodotorula* species and *Cryptococcus laurentii. Debaryomyces spp.* showed variable lipolytic results. The high frequency of yeasts presenting lipolytic active results is in agreement with report by of Saldanha-da-Gama *et al.,* (1997).

Despite several good characteristics presented by most of the isolated yeasts, two strains from the genus *Debaryomyces* were selected for use as starter cultures in the production of commercial salami as they appeared to remain stable in meat related environments, reaching high population cells when grown in meat (Dowell and Board, 1968; Samelis *et al.,* 1994). Work done on yeasts in meats and meat products depicted the promising use of *Debaryomyces,* especially *D. hansenii* as a good starter culture for sausage production. (Coretti, 1977; Hammes *et al.,* 1985; Lucke and Hechelmann, 1987; Gehlen *et al.,* 1991). Yeasts are generally not considered to be of any importance in the spoilage of refrigerated, cured and vacuum packed meat, and related meat products (Jay, 1987; Dillon and Board, 1991).

The positive contributions of yeasts to humankind are known and their public health significance in foods and beverages has been considered negligible. *Debaryomyces*

hansenii is regarded generally as safe and obtained GRAS status (Fleet, 1992) Lipolytically active *Debaryomyces hansenii* and *D polymorphus* strains were selected and their survival rate at high temperatures determined. Both species were inactivated within 60 hrs at temperatures above 50°C (Figs 1, 2 and 3). Yeasts are generally killed within minutes at temperatures between 55°C and 65°C however, the composition of food affects the rate of in-activation (Lund, 1951; Put and De Jong, 1982b; Su and Beauchat 1984; Engel *et al.*1994). *Debaryomyces spp.* are capable of surviving a wide range of temperatures with an optimum growth temperature of 32-37°C (Vidal-Liera *et al.,* 1979).

However, species were isolated from sugar cane mill capable of growth at 40°C (Stokes, 1971). The minimum growth temperature for most yeast is 0°C, although obligate pychrophlic strains can grow at –7°C. *D. hansenii* is frequently isolated from chilled foods (Guerzoni *et al.,* 1993b).

Despite the less obvious but detrimental effects, including production of off-flavours and taints, the production of metabolites such as acetaldehyde that can neutralize the preservative effects of sulphur dioxide and the utilization of sodium nitrite added as curing agent and preservative, (Dalton *et al.,* 1984; Dillon and Board, 1990), *D. hansenii* contributes positively to product quality in Italian salamis (Grazia *et al.,* 1989). Based on the results obtained in this study, and the proposed key characteristics to assure a stable starter culture (Deibel, 1974). *Debaryomyces spp* proved to be accepted as a potential starter culture in salami processing.

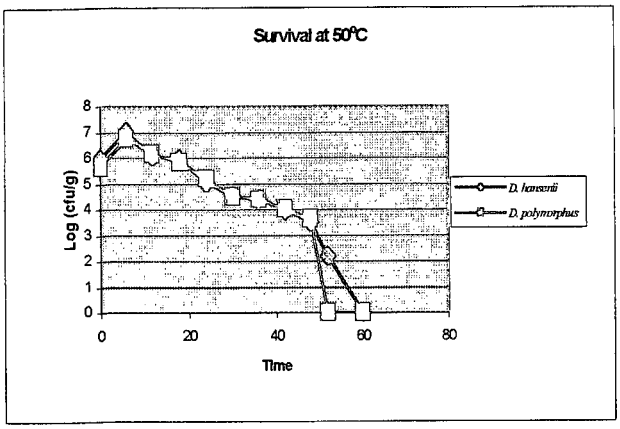

Fig. 1 The survival of *D. hansenii and D. polymorphus* at 50°C

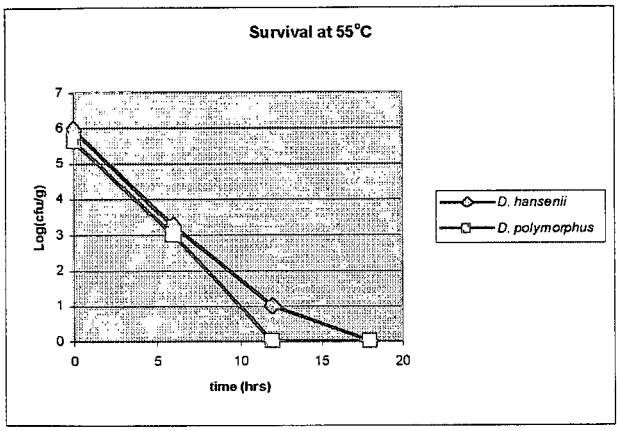

Fig. 2 The survival of *D. hansenii and D. polymorphus* at 55°C

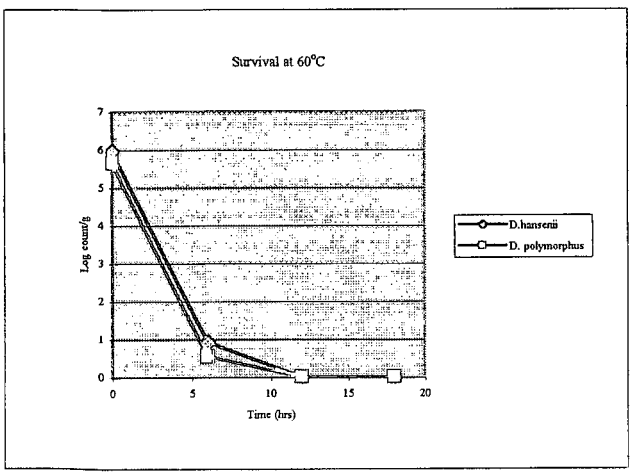

Fig. 3 The survival of *D. hansenii and D. polymorphus* at 60°C

CHAPTER IV

THE APPLICATION OF YEAST STRAINS AS STARTER CULTURES IN TRADITIONAL FERMENTED SAUSAGE

A. A. Osei-Abunyewa, B. C. Viljoen, *A Hugo.

Department of Microbiology and Biochemistry, *Department of Food Science U.F.S., Bloemfontein, 9300,South Africa

Abstract

Natural yeast strains isolated from traditional salami identified as *Debaryomyces hansenii* and *Debaryomyces polymorphus* were incorporated as starter cultures in the production of salami. *Debaryomyces hansenii* in combination with lactic acid bacteria were used as starter culture in the first batch (DhS). In the second batch (Dh), *Debaryomyces hansenii* as a single strain was used. The third batch (DhDp) contained *Debaryomyces hansenii* in combination with *Debaryomyces polymorphous* as starter culture, a fourth batch (S) serving as control contained lactic acid bacteria as starter culture. After a maturation time of 624 hours, all the batches attained a 20% moisture loss require for maturity with the exception of batch 4 which had obtained a moisture loss of 19.05%. DhDp obtained 21.0659% moisture loss, which was an equivalent of the weight loss; DhS attained 20.7834% and Dh, 20.3484%. The results obtained for physical and chemical analysis between the different batches were similar. The salamis appeared reddish brown after 48hrs but the batch containing yeast and lactic acid

bacteria as starter cultures was slightly firmer than the others were. The pH values of the salamis at the end of maturation were higher for batch 2 using a yeast isolate as a single starter culture, while the highest pH values were obtained when two yeast strains were incorporated.

4.1. Introduction

Ready-to-eat dried meat products, which may or may not be fermented, present a unique concern because they are prepared from raw meat which has not been cooked (Lee and Styliads., 1996). No matter how hygienic raw meat may appear, it contains numerous micro-organisms, which may potentially be harmful. Jay (1996) established that the typical contaminating microflora in fresh meat and meat products predominantly consist of bacteria. Yeast, however, despite limited references, also play a substantial role in the spoilage of meat and meat products (Deak 1991, Fleet 1992). Nevertheless, occasionally the role of yeasts in meat has been described (Dalton and Board, 1984; Bank and Board, 1987; Viljoen *et al.,* 1993).

Bacus (1986) and Lucke (1988) reported that various microbial groups are involved in the ripening process of dry fermented sausage ie. lactic acid bacteria, micrococcaceae, yeasts and fungi. According to Metaxopoulos *et al.* (1996) the microorganisms suppress the growth of pathogenic organisms due to their enzymatic activities while yeast play a dual role by either contributing to the final product or being detrimental in causing spoilage. Most sausage industries ignore the importance of yeast strains as starter cultures (Lucke and Hechel-mann, 1987) which may be a preventing strategy since improper usage of yeast strains as starter culture may lead to severe defects (Meisel *et al.,* 1989). However, the meat industry remains interested in the addition of yeasts as starter culture mainly because of their safety record (Nagodawithama, 1992). Yeasts furthermore contribute to sausage flavour by attacking lipids and proteins and

exert certain anti-oxidant effects by destroying peroxide and depleting oxygen from the surface of the product (Nagodawithama, 1992).

Gehlen *et al.* (1991) reported strains of *Debaryomyces hansenii* have a strong influence on the microbiology and chemical composition of dry sausages due to its reducing ability of fortuitous staphylococci and their contribution to the improvement of taste and surface color in dried sausages. Meisel *et al.* (1989) however, warned that care must be taken to control the reduction of anti-microbial properties of organic acids and to limit high residual values of nitrate in fermented sausages by the addition of yeast cultures. Consequently the meat industry continues to seek a proper yeast species that has the ability to impart the expected rich meaty flavor.

Debaryomyces hansenii, the proven predominant yeast species in traditional salami, requires oxygen for reproduction (Metaxopoulos *et al.,* 1996), destroys peroxides (Coretti 1973), is salt tolerant, does not reduce nitrate, and occurs frequently in cured meat products (Leistner and Bem, 1970). Even though *Debaryomyces hansenii* strains do not reduce nitrate, some starter cultures on the German market contain *Debaryomyces hansenii* as a single strain or in combination with lactic acid bacteria and staphylococci (Hammes *et al.,* 1985). Therefore, in this study we endeavoured to determine the performance of *Debaryomyces hansenii* as a single starter culture, in combination with another *Debaryomyces* species, and when used in combination with traditional lactic acid bacterial starter culture in the manufacture of salami.

4.2. Materials and methods

4.2.1. Yeast preparation for use as starter culture

Debaryomyces hansenii and *Debaryomyces polymorphous* isolated during a previous study were selected as yeast representatives for usage as starter cultures. *Debaryomyces hansenii* proved to be the predominant yeast isolate during processing

of salami while *Debaryomyces polymorphous* was isolated during the period of maturation. The yeast cells were prepared by seeding in glucose–yeast nitrogen base (YNB, Difco, Detroit, Michigan, U.S.A.) medium (400ml) in 1000ml Elernmeyer flask and cultured with vigorous agitation on a rotary shaker (180rpm) at 30°C until late exponential phase. The cells were harvested by centrifugation for 5 minutes using a Beckman J2-21 centrifuge and a JA-14 rotor spinning at 11000rpm.

4.2.2. Manufacture of salami

Four batches of 5kg of salami were prepared. Individual salamis weighing 250g-300g were prepared from the different batches. The formulation for salami processing is according to the protocol indicated in Table 4.1. For Batch 1 (DhS), the starter composition used was FloraCarn (Chr. Hansen, Denmark) consisting of *Staphylococcus carnosus* and *Lactobacillus pentosus* in the ratio of 2:1 and *Debaryomyces hansenii*. The ratio of FloraCarn to *Debaryomyces hansenii* was 1:1. For Batch 2 (Dh), *Debaryomyces hansenii* was used as the single starter organism and for Batch 3 (DhDp) *Debaryomyces hansenii* and *Debaryomyces polymorphous* were mixed in the ratio of 1:1. Batch 4 (S), served as a control used for comparable reasons to establish the maturation time. The only analysis which was done on batch 4 was the rate of weight loss. Batch 4 comprised of only FloraCarn as starter culture.

All meat portions were frozen at a temperature of -15°C to avoid "smearing" of the fat on the lean meat surface, which may cause problems during sausage dehydration. The fat used was from good quality pork lard. Meat portions and the ingredients were mixed sequentially in a bowl cutter. Beef was chopped into 10mm particle size. Starter culture and spices were evenly sprinkled on the beef and chopped to fine particles. Pork was then added and chopped to 20mm particle size after the lard and curing salt were added. Finally, the mixture was chopped to a particle size of 4.5mm making sure that the entire batch was thoroughly mixed. The mixture was carefully packed into Calpak Fibrous Bak 65/50 to avoid trapping air and both ends were tied with strings. Three

Table 4.1: Composition of commercial salami

Ingredients	Percentage by weight (%)
Beef	40.000
Pork	34.730
Pork back fat	20.000
Spices	2.176
Curing salt	3.046
Starter culture (freeze dried)	0 11/26/98.500
Staphylococcus carnosus & Lactobacillus penttosus	2:1

samples from each batch were weighed and marked for weight loss determination. On sampling occasions, the marked samples were weighed to determine the weight loss of the salami. The prepared salami samples were hanged on racks and placed in a fermentation chamber with a temperature of 22°C and relative humidity of 90% for 48hrs. After 48hrs of fermentation, a 10 min smoke treatment at 18°C was carried out. Finally the sausages were transferred to a drying chamber with a temperature of 12°C and 75-80% RH until the product lost 20% and more weight.

4.2.3. Sampling

Sampling was done 6 hourly for the first 2 days of fermentation starting directly after processing. Consecutive sampling was performed every 4 days. On every sampling occasion, one salami from each batch was transferred to the lab, in a cooler box for microbial and chemical analysis.

4.2.4. Chemical and Physical Analysis

On every sampling occasion a 10g sample from each batch of salami was homogenized using a Janke & Kunkel Ultra-Turrax T 25 for 60 s in 20ml of distilled water and made up to 100ml with distilled water. The pH was measured with a HI 9321 Microprocessor pH meter (Hanna Instrument). Another 10g sample from each salami was taken, homogenized with 12ml distilled water and centrifuged for 5min at 11000rpm with a Beckman J2-21centrifuge. The supernatant was extracted and used for lactic acid determination by liquid chromatography (Waters Lambda-Max 480) with a high performance (Bio-Rad. Aninex APX-87H) exclusion column and 0.01N sulphuric acid as eluent. The flow rate was 0.5ml/min and the detection wavelength 210nm. Samples from the same salami were also taken for water activity determination using the Novasina Thermoconstanter TH200. Salt, protein, fat and moisture were determined by the methods proposed by the Association of Official Analytical Chemist (AOAC, 1990).

4.2.5 Microbiological analysis

The salami samples were prepared for microbial analysis by cleaning the salami surface with 70% ethanol. A cut was made through the salami casing with a sterile knife and the casing removed aseptically. For each sample, 10g portions of the meat were taken with a sterile knife and homogenized in a stomacher (Janke & Kunkel Ultra T25) for 60 s in 90ml sterile bactopeptone. Further decimal dilutions of the suspensions were carried out as required for microbiological assay in 9ml sterile bactopeptone. Plating was done in duplicate by the spread plate method. The growth media used and incubation periods are indicated in Table 4.2.

4.3. Results and discussion

4.3.1. Chemical and physical composition

The changes in chemical and physical analysis of the three batches of salami are indicated in Tables 4.3-4.6. As frozen raw material was the main ingredient for processing, the temperature of the sausage after filling was about 0°C. When sausages at this temperature were transferred immediately into an air-conditioned chamber at a temperature of 22°C and 90% humidity, a microclimate with a temperature approximately that of the sausage was established. The saturation vapour pressure of the chamber declined, leading to a less water vapour absorption by the air. Surplus humidity was deposited on the surface of the salami as condensate (Stiebing and Rhodel, 1988) resulting in an increase in the initial weight of all the salamis with the exception of batch 1 (DhS, containing bacteria mixed with yeast as starters). Dehydration rate for all the salamis was therefore generally poor during fermentation, which resulted in an extended maturation period. Moulds grew on the surface of all the salamis sporadically during fermentation and maturation. Frequent cleaning with 5% vinegar solved this problem.

Table 4.2 : Culture media, temperature and times of incubation used for microbial analysis of commercial salami

	Incubation time (h)	Growth medium
Yeast count, YGC	96	Yeast extract; glucose; chloramphenicol agar (Oxoid CM 139)
Yeast count, YM	96	Yeast extract malt extract agar (pH 3.5)
Total count, PCA	48	Standard plate count agar (Oxoid CM 361)
Lactobacilli, MRS	48	De Man Rosoga and Sharpe (MRS) (Oxoid CM 361)

Table 4.3: Comparative chemical and physical changes during the processing of salami over a period of 624 hours

	Weight loss (%)				Moisture content (%)		
Time	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 4**	**Batch 1**	**Batch 2**	**Batch 3**
Fermentation							
0	0	0	0	0	57.0714	56.7393	56.4988
6	0.1467	-0.1215	-0.5129	-0.2501	57.0083	56.7913	56.7208
18	1.4559	1.1991	1.4065	0.8531	56.4372	56.2143	55.8782
24	1.2914	0.8791	0.6852	0.5219	56.5098	56.3556	56.1987
30	1.3772	0.8143	0.6051	0.3161	56.4719	56.3842	56.2340
42	0.8621	0.2107	-0.1563	-0.1630	56.6981	56.6480	56.5667
48	0.8013	0.1215	-0.3045	-0.3745			
Ripening							
144	3.799	3.6014	3.4783	1.85 00	55.3762	55.1231	54.9312
240	7.3761	7.2271	7.8141	5.2112	53.6528	53.3693	52.8115
336	9.2899	8.9569	9.1324	7.4133	52.6749	52.4833	52.1268
432	13.7028	13.3482	13.6905	10.6411	50.2549	50.0753	49.5986
528	15.3139	15.0334	15.3997	15.0251	49.3086	49.0850	48.5803
624	20.7834	20.3484	21.0659	19.0509			

Batch 1= *Debaryomyces hansenii* and conventional starters; Batch 2= *D. hansenii* as starter culture; Batch 3= *D. hansenii* and *D. polymorphus* strains as starter cultures; Batch 4= conventional starter culture.

Table 4.4: Comparative chemical and physical analysis during the processing of salami over a period of 624 hours

	Aw			Lactic acid content (mg/g)		
Time	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**
Fermentation						
0	0.950	0.947	0.947	0.169487	0.128554	0.103405
6	0.946	0.944	0.946	0.374401	0.142380	0.118556
18	0.944	0.948	0.950	0.163500	0.132979	0.135425
24	0.946	0.947	0.948	0.174230	0.142737	0.150471
30	0.945	0.946	0.948	0.244217	0.140796	0.158247
42	0.945	0.949	0.948	0.158174	0.184229	0.149979
48	0.947	0.947	0.947	0.149227	0.177125	
Ripening						
144	0.945	0.944	0.945	0.241101	0.215974	0.222855
240	0.943	0.942	0.941	0.343361	0.250833	0.265239
336	0.942	0.941	0.942	0.329300	0.180192	0.294203
432	0.937	0.937	0.937	0.343087	0.313001	0.219847
528	0.936	0.935	0.936	0.358796	0.309592	0.335808
624	0.930	0.928	0.929	0.409496	0.346934	0.304665

Batch 1= *Debaryomyces hansenii* and conventional starters; Batch 2= *D. hansenii* as starter culture; Batch 3 = *D. hansenii* and *D. polymorphus* stains as starter cultures

Table 4.5: Comparative chemical and physical analysis during the processing of salami over a period of 624 hrs

	Salt content (%)			pH		
Time	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3
Fermentation						
0	3.0250	3.0250	3.0250	5.26	5.16	5.15
6	3.0294	3.0213	3.0096	5.46	4.96	5.10
18	3.0697	3.0617	3.0682	5.33	5.20	5.23
24	3.0646	3.0518	3.0549	5.26	5.24	5.22
30	3.0672	3.0498	3.0434	5.36	5.24	5.24
42	3.0513	3.0314	3.0203	5.39	5.27	5.43
48	3.0494	3.0287	3.0158	5.45	5.30	5.40
Ripening						
144	3.1445	3.1380	3.0340	5.10	5.14	5.29
240	3.2659	3.2606	3.2814	4.82	4.92	5.04
336	3.3348	3.3226	3.3290	4.75	4.72	4.83
432	3.5053	3.4910	3.5048	4.92	4.86	5.00
528	3.5720	3.5602	3.5756	4.85	5.04	4.94
624	3.8186	3.7978	3.8328	4.66	4.87	4.85

Batch 1= *Debaryomyces hansenii* and conventional starters; Batch 2= *D. hansenii* as starter culture; Batch 3= *D. hansenii* and *D. polymorphus* strains as starter cultures

Table 4.6: Comparative chemical and physical analysis during the processing of salami over a period of 624 hrs

	Fat content (%)			Protein content (%)		
Time	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**
Fermentation						
0	20.3152	20.9494	20.3230	16.5251	16.6339	15.5188
6	20.345	20.9240	20.9250	16.5494	16.6137	15.4396
18	20.6153	21.2037	21.3323	16.7692	16.8358	15.7402
24	20.5810	21.1352	21.1774	16.7414	16.7814	15.6295
30	20.5989	21.1214	21.1666	16.7559	16.7709	15.6133
42	20.4919	20.9936	20.9995	16.6688	16.6690	15.4946
48	20.4793	20.9749	20.9684	16.6586	16.6541	15.4717
Ripening						
144	21.1174	21.7321	21.7902	17.1777	17.2553	16.0780
240	21.9330	22.5814	22.8151	17.8411	17.9299	16.8342
336	22.3957	23.0104	23.1461	18.2175	18.2703	17.0780
432	23.5410	24.1765	24.3685	19.1962	19.1962	17.9804
528	23.9888	24.6560	24.8608	19.5770	19.5770	18.3437
624	25.6451	26.3013	26.6454	20.8833	20.8833	19.6605

Batch 1= *Debaryomyces hansenii* and conventional starters; Batch 2= *D. hansenii* as starter culture; Batch 3= *D. hansenii* and *D. polymorphus* strains as starter cultures

Within 6hrs of fermentation the prepared salamis from batch 2 (Dh, with *Debaryomyces hansenii* as starter) exhibited an increase in weight of 0.1215%, batch 3 (with a mixture of two strains of yeast added as starter culture) 0.5129% and batch 4 (control) 0.2501% (Table 4.3). Weight loss determination is accepted as a method to determine the ripening pattern in dry sausages (Stiebing and Rhodel, 1988). The weight loss increased during ripening when the R.H. was reduced from 90 to about 75%. After 624hrs, all the salamis containing yeast starters attained a percentage weight loss above 20% (DhS, 20.78%; Dh, 20.35%; DhDp, 21.07). Batch 4 that comprised of the conventional bacteria starter cultures had lost only 19.05%. The weight loss which, is a function of the weight loss reflected a decline in the moisture content. The trend of moisture loss for all three batches was similar. The initial moisture contents of batches 1, 2 and 3 were 57.0714%, 56.7393% and 56.4988% respectively but at the end of ripening, batch 1 had lost 11.2628%, batch 2, 11.0517% and batch 3, 11.6096%.

The water activity of foods influences the growth and metabolic activity (including toxin production) of micro-organisms as well as their survival and resistance (Troller and Christian, 1978). *Debaryomyces hansenii* is very resistant to low levels of water activity, capable of growth in water activity levels as low as 0.88 (Van Eck *et al.,* 1993). The water activity and lactic acid content of the three batches are presented in Table 4.4. From initial water activity levels of 0.950, 0.947 and 0.947 for batches 1, 2 and 3 the water activity levels gradually declined to 0.930, 0.928 and 0.929 respectively after 624 hrs. Despite continued disagreements on the water activity requirements of all micro-organisms (Leistner *et al.,* 1976), it is accepted that enteropathogenic *Escherichia coli* strains are inhibited at water activity levels below 0.96 (Tomtov *et al.,* 1974).

During fermentation, the lactic acid content for batch 1 which contained lactic acid bacteria and yeast as starter cultures, increased rapidly within six hrs followed by a rapid decline in the next 12 hrs. Thereafter, a gradual increase was recorded until the end of maturation when a maximum of 0.409mg/g, which was the highest of the three batches, was obtained. Lactic acid content for batches without lactic acid bacteria

starter cultures (batches 2 and 3) fluctuated but both showed a gradual increase from the beginning to the end of production. Batch 2 exhibited an initial lactic acid content of 0.128mg/g and batch 3, 0.103mg/g. At the end of ripening, lactic acid content for batch 2, was 0.347mg/g and batch 3 a content of 0.304mg/g. An interesting feature is the similar increases in lactic acid content in batches 2 and 3 compared to batch 1, although lower final contents after 624hrs were obtained, despite the absence of lactic acid bacteria as part of the starter culture. However, high numbers of contaminating lactic acid bacteria originating from processing equipment, hands and aprons of workers or the meat might have contributed to the high lactic acid content.

Salt content and pH levels for all three batches are shown in Table 4.5. Salt content remained similar for all three batches exhibiting an increase less than 1% (DhS, 3.025-3.8186%; Dh, 3.025-3.7978% and DhDp, 3.025%-3.8328%). The total decline in pH during processing was minimal (less than 1 pH unit) as compared to Belgian, German and Spanish dry salamis which obtained pH values of 4.6-5 after fermentation and continued to decline during ripening (De Ketelaere *et al.,* 1974; Palumbo *et al.,* 1976; Lucke, 1985 and Sanz *et al.,* 1988). The slight decrease in pH in this study correspond with results obtained with Italian and Hungarian salamis which exhibit a pH decrease of about 0.5 units (Komendy and Gantner, 1962; Baldini *et al.,* 1983). The slight pH increase observed after 432hrs might be due to the utilization of lactic acid (Raimahone *et al.,* 1988) and/or the production of amine and ammonia by the high number of yeast present at the time (Lucke, 1988) which could favour the growth of spoilage bacteria (Walker, 1977). Despite the detrimental effect of pH increase caused by yeasts, the strong synergistic inhibitory effect on fortuitous staphylococci by *Debaryomyces hansenii* and *Lactobacillus curvatus* has been demonstrated (Sorrels and Speck, 1970; Meisel et al. 1989). The individual inhibitory effect is attributed to nitrite production by *L. curvatus* and oxygen depletion by *D. hansenii.* Fat content represented in Table 4.6 shows an increase of 5.6%, 5.4% and 5.3% fat content for batches 3, 2 and 1 respectively; an increase of approximately 5.6% protein content for all the batches is

indicated in Table 6. Batch 2 and 1 had the highest protein contents (20.8833%) after maturation.

4.3.2 Microbiological enumeration

The progression of the lactic acid bacteria present similar growth patterns for all three batches, the highest microbial counts recorded were obtained during ripening after 144hrs for batches 1 and 3 (Log 6.717/g and Log 6.647/g respectively) and after 240hrs for batch 2 [(Log 6.723/g) (Table 4.7)]. Similar growth patterns were reported by Lucke (1985).

The processing of the meat into raw sausages that reduced the a_w and rapidly consumed the oxygen present within the mixture (Lucke, 1985) might have eliminated pseudomonas (Hechelmannet al. 1977) and *Enterobacteriaceae* (Barth, 1960; Grau, 1981; Gill. 1982). Consequently, a shift in predominating microflora composition present in the salami towards the lactic acid bacteria, micrococci (Niinivaara and Pohja, 1956; Ten Cate, 1960; Incze, 1965; Reuter, 1967; Ayroulet and Fournaud, 1976; Hofmann and Scharner, 1980) and yeasts resulted. The availability of sugars, low acid production and relatively high water activity further enhanced the rapid growth of lactic acid bacteria during fermentation. The rapid growth and progression during fermentation corresponded with results reported by Lucke (1985), indicating that the activity of *Micrococcaceae* and Gram-negative bacteria under these conditions outgrow competing micro- organisms. Lactic acid bacteria proved to be the major microbial component present in the meat and during salami processing, confirming previous reports on the predominance of lactic acid bacteria in dry fermented sausages whether or not lactic acid bacteria starter cultures were added (Smith and Palumbo, 1973; Metaxopoulos *et al.,* 1981a,b; Gokalp and Okerman, 1985; Cahalan and Genigeorgis, 1986). Samelis et al. (1996) reported on the elimination of streptobacteria II, betabacteria and streptococci in Greek dry salami during fermentation even if significant numbers were present during early fermentation.

Table 4.7: Comparative microbial counts during the processing of salami over a period of 624 hrs

	Log counts g^{-1}					
	MRS			YGC		
Time	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**
Fermentation						
0	5 155	5.037	5.418	4.771	5.152	5.415
6	5.037	4.929	5.143	4.832	5.037	5.190
18	5.497	5.301	5.324	5.223	5.283	5.152
24	5.924	5.021	5.398	5.243	5.471	5.356
30	6.041	5.881	5.875	5.021	5.486	5.326
42	6.324	6.149	6.093	5.272	5.885	5.179
48	6.576	6.134	6.310	5.093	5.378	
Ripening						
144	6.717	6.620	6.647	5.545	5.535	5.354
240	6.556	6.723	6.635	6.057	5.939	5.505
336	6.577	6.560	6.536	4.732	5.143	4.903
432	6.600	6.577	6.468	4.531	5.093	4.845
528	6.522	6.375	6.635	4.544	4.785	4.991
624	6.539	6.240	6.417	4.505	5.090	4.653

MRS =Lactic acid bacteria count YGC = yeast counts

Batch 1= *Debaryomyces hansenii* and conventional starters; Batch 2= *D. hansenii* as starter culture; Batch 3= *D. hansenii* and *D. polymorphus* strains as starter cultures

The bacteria being not capable of resisting the low water activity, low pH and high salt content were inhibited leaving the competing lactic acid bacteria to establish itself in the stressful environment which consequently resulted in the stabilization of bacterial numbers. According to Reuter (1972), *Micrococcaceae* strains showed little or no growth during ripening. Hammes *et al.* (1985) confirmed the poor growth and survival of Micrococcaceae during sausage ripening despite being present. Yeasts, however, competed very well under the environmental stresses exerted (high salt, low water activity and low pH value) (Leistner and Bem, 1970; Jay, 1979; Beuchat, 1983). During fermentation, the bacterial numbers increased rapidly while yeast numbers remained stable due to the inability of the yeasts to proliferate when bacterial numbers competing for the same nutrients are high (Walker and Ayres, 1970; Walker, 1977). In all batches, highest counts of yeast numbers were recorded after 240hrs during ripening. At that time bacterial numbers remained relatively high but yeast competed better probably due to the availability of higher amounts of organic acids. Surprisingly, batch 1 with a mixture of bacteria and yeast as starter cultures, exhibited the highest yeast count (Log 6.057). The higher proportions of organic acids produced by the lactic acid bacteria starter cultures resulting in the progression of *D. hansenii* strains has been reported (Raimihone *et al.* 1988). The lower numbers of lactic acid bacteria present during the early stages of maturation also enhanced the proliferation of the yeasts (Miller 1979, Walker and Aryes, 1970; Walker, 1977).

The decline in yeasts' numbers during ripening corresponded with result by Gehlen *et al.* (1991) who indicated on the inhibition of yeast species by bacteria *Lactobacillus curvatus* and *Micrococcus varians* in the later stages of ripening.

In this study the production time for batch 3 with yeasts as starter culture was reduced by 48hrs as compared to batch 4, which contained the standard starter cluture. All the salamis exhibited a substantial red surface colour which had already occurred after 48hrs of fermentation, a situation noticed by Gehlen *et al.* (1991) in sausage fermented with and without conventional starter culture indicating all batches were fully cured.

The batches prepared without lactic acid bacteria starter cultures were judged better with smooth "sweet" taste according to an unprofessional taste panel. The taste panel all agreed on a less firm structure for the yeast fermented salamis compared to the salamis with lactic acid bacteria starter culture, an observation confirmed by Lucke and Hechelmann (1987).

CHAPTER V

GENERAL DISCUSSION AND CONCLUSIONS

The traditional approach to salami fermentation has been well documented. Although there have been numerous reports on the role of lactic acid starter bacteria in salami, little consideration has been given to the role of yeasts in fermented meats. Reports usually referred to the presence of yeasts in meat, but few have attempted to quantify the yeasts present in fermented meat, to examine the ability of yeasts to grow in fermented meats or to seek biochemical explanations of such growth. The concept that fermented meats, as a group, may represent a specialized ecological environment for the selective occurrence and growth of certain yeast species, is also neglected.

5.1 The growth and survival of yeasts in commercial salami

The results obtained in this study, obtained from South African commercial salami, were in agreement with results reported in literature. A decrease in the pH values, was observed as expected, since the fermentation process was mainly governed by the activities of lactic acid bacteria, which ferment simple sugars forming organic acids. The decrease in a_w levels was due to high salt concentrations, which consequently resulted in reduced weight of the salami's as a function of moisture loss. The moisture loss corresponded with an increase in salt, fat and protein contents.

An increase in both yeasts, and bacterial numbers confirmed the synergistic relationship that exists between yeasts and bacteria in restricted environments. Lactic acid bacteria

predominated in all salami's processed, despite the rapid progression of yeasts during ripening. This situation controlled the proliferation of yeasts by preventing the progression of numbers high enough to cause spoilage.

Based on the results, *Bullera variabilis, Candida haemulonii, Candida gropengiesseri, Crytopcoccus albidus, Cryptococcus laurentii, Candida zeylanoides, Debaryomyces hansenii, Debaryomyces polymorphus, Debaryomyces vanrijiae, Galactomyces geotrichum, Pichia farinosa, Pichia philogaea, Rhodotorula minuta, Rhodotorula, mucilaginosa, Sporobolomyces haophilus, Sterigmatomyces roseus, Schwanniomyces occidentalis, Torulaspora delbruecki and Trichosporon beigelii* were established as typical contaminants associated with meat and salami. Except for *Bullera, Schwanniomyces, Sporobolomyces and Sterigmatomyces,* all the above mentioned genera were previously isolated from meats and meat products. The sources of contamination mainly included the raw meat, air equipment and from animals' skin and hide.

D. hansenii, R. mucilaginosa, B. variabilis, C. albidus and *C. zeylanoides* were considered the dominant yeast community associated with commercial salami, being present at frequent intervals. *D. hansenii* strains were the most frequently isolated, and clearly predominated in salami processing. The substantial role played by *D. hansenii* during the ripening stages of the salami, can therefore not be ignored.

5.2 Key properties for the selection of yeasts as possible starter cultures in salami production.

Results obtained, indicated that all the yeast species isolated from commercial salami are tolerant to 4-8% NaCl and 80-240ppm nitrite. Of the nineteen species investigated, only *Trichosporon beigelii* exhibited proteolytic activity. *Debaryomyces hansenii* and *D. vanrijiae* showed variable lipolytic activity. All *Rhodotorula* species showed no lipolytic activity, whilst the rest were all lipolytically active.

According to literature, *Debaryomyces hanseniii* has attained GRAS status. The species, furthermore, is capable of growth at low temperatures, low a_w in a salty medium, low pH and generally is frequently associated with fermented meat products. Based on these properties, *D. hansenii* and *D. polymorphus* were selected, both of which were isolated on the last sampling occasion of commercial salami production. The two species, on examination for inactivation temperatures, proved to be inactivated at temperatures above 50°C within a maximum of 60hrs.

5.3 The application of yeast strains as starter culture in traditional fermented sausage

Despite the use of *Debaryomyces hansenii* as a single strain, or mixed with *Debaryomyces polymorphus* or conventional starter cultures as starter cultures in the formulation of traditional salami, there was a decline in pH values at the end of production. This is confirmed in literature indicating that the process of fermentation in salami making is governed by the activities of lactic acid bacteria. The decline in pH was restricted due to the activities of yeasts in the utilization of lactic acid and the production of amine and ammonia.

Water activity values declined in correspondence with decreases in the weight, moisture, fat, protein and salt contents. The implementation of yeasts as starter culture did not inhibit the reddish-brown colour formation in the meat. Lactic acid bacteria in this study were the most predominant microflora found in the salami. The proliferation of the yeasts to numbers higher than the bacterial numbers would have left the salami susceptible to spoilage by undesirable bacteria.

Debaryomyces hansenii and *Debaryomyces polymorphus* added to the salami formulation without the conventional starter culture resulted in a less sour and tastier,

product. Moreover, ripening time was reduced but the consistency was less firm. The softness of the salami obtained with the inclusion of only yeasts as starter cultures, was a result of the higher pH according to literature. The application of yeasts as starter cultures in this study, has been successful, resulting in a product comparable with products prepared with the conventional starter culture and without any major defect.

The positive results obtained in this study is a clear indication that *Debaryomyces hansenii* and *Debaryomyces polymorphus* could be used in the future as starter culture in the formulation of salami leading to a better taste and shorter ripening time than salami produced with the present traditional bacteria starter cultures.

Future Research: Recommendation is therefore made for future research to include,

A. The survival levels of *Debaryomyces hansenii* and *Debaryomyces polymorphus* after lyophilization.
B. The contribution of strains to the organoleptic characteristics of salami.
C. The type of possible antibiotics excreted by the isolated yeast strains.
D. The possible antagonistic effect of the isolated strains against fungi and bacterial contamination.

CHAPTER VI

SUMMARY

Yeasts play a substantial role in the processing of salami, being present at high numbers during the ripening stages contributing to aroma and flavour development. Accordingly, the yeasts occurring as natural microflora in commercial salami were quantified, isolated and identified according to conventional identification and enumeration techniques. Depending on conditions, the yeasts grew to maximum populations of 10^7cfu/g during the ripening stages.

Characterization of the naturally contaminating yeasts of commercial salami revealed 108 yeast species, belonging to 12 genera. The yeasts most frequently isolated were from the genera *Debaryomyces, Rhodotorula, Bullera, Candida, Cryptococcus, Trichosporon* and *Schwanniomyces* in that order. Low appearances of *Pichia, Galactomyces, Sporobolomyces, Sterigmatomyces and Torulaspora* were observed. *Debaryomyces hansenii, Rhodotorula mucilaginosa, Bullera variabilis* and *Cryptococcus albidus* were established as representing the typical yeast community associated with salami. These species were frequently isolated from the meat, during processing and maturation. The most frequently isolated yeast species, was *D. hansenii,* isolated on every sampling occasion.

The isolated yeasts were examined based on relevant key properties that governed their growth and survival in fermented meat, and proposed as representative selective characteristics to determine a good starter culture. All the yeasts were tolerant to 4-8%

NaCl and 80-240ppm nitrite. All the yeasts proved to be non-proteolyic with the exception of *Trichosporon beigelii,* whereas lipolitic activity appeared variable.

Two predominating yeast isolates, *Debaryomyces hansenii* and *Debaryomyces polymorphus,* exhibited relevant qualities regarding fermentation of meat. Consequently, they were applied in the formulation as starter cultures in an attempt to prepare a salami based on yeast fermentation. The incorporation of *D. hansenii,* individually, or in association with conventional starter cultures, resulted in a product with improved taste and reduced maturation time.

REFERENCES

Adams, M.R., (1986) Fermented flesh foods. Progr. Indust. Microbiol., 23, 159-198.

Ahearn, D. G., Meyers, S.P. and Nichois, R.A. (1968) Extracellular proteinase of yeasts-like and fungi. Appli. Microbiol. 16, 1370-1374.

AMI, (1979) "Meat Facts: A Statistical Summary about America's Largest Food Industry Am. Meat Inst., Washington, D.C.

Anderson, P.J., MacNeil, K. and Watson, K.A., (1986) High-efficiency carbohydrate fermentation to ethalate temperatures above 40°C by *Kluyveromyces marxianus* var *marxianus* isolated from sugar mills Appli. Environ. Bacteriol. 51, 1314-1320.

Andres, C., (1977) Starter cultures for sausages has two micro-organisms for better performance. Food Proc., 38(1), 132.

AOAC., (1990). Association of Official Analytical Chemist

Arnau, J., Maneja, E., yMontfort, J. M., (1987). Factores que influyen en la apricon de precipitados de tirosina. Carnica 2000, 48. 106-110.

Ayres, J.C., (1955). Microbiological implications in the handling slaughtering and dressing of meat animals. Advances in Food Research 6,106-161.

Ayroulet, M., and Fournaud, J. (1976) Mikrobiolgische Wechselwirkungen bei der Rohwurstherstellung. Fleischwirtschaft 56, 1331-4.

Bacus, J., (1984) Utilization of micro-organisms in meat processing. A handbook for meat. Plant operators. John Wiley, New York.

Bacus, J. N. (1986). Fermented meat and poultry products. In Advances in Meat Research: "Meat and Poultry Microbiology" Pearson, A. M. and Dutson, T.R. (Eds) V. Z. P 123. Macmillan.

Bacus, J.N. and Brown, W.L., (1981) Use of microbial cultures: meat products. Food Technol., 35, 74-78.

Baldini, P., Mamia, F. and Raczynski, R. G. (1983) Influence of the chemical and physical-chemical properties of the formulations on pH lowering and weight loss in the ripening of Italian salami. Proceedings of the 29th European Congress of Meat Research Workers Salsomaggiore, pp 189-97.

Banks, J.G. &Board R.G. (1987) Some factors influencing the recovery of yeast and mould from chilled foods. Int. J. Food Microbiol. 4. 197-206.

Barber, L. E. and Diebel, R. H. (1972). The effect of pH and oxygen tension on staphylococcal growth and enterotoxin production in fermented sausage. Appli. Microbiol. 24,891.

Barnett, J.A., Payne, R.W., and Yarrow, D., (1983) Yeast: Characteristics and identification of yeasts. Cambridge. Cambridge University Press.

Barth, W. (1960) Welchen Einfluss haben pH-Wert, Kochsalz und Nitrit auf die Bakterienflora des Rohwurstbrates/ Dissertation, Veterinarmedizinische Fakultat, Freie Universitat Berlin, 50 pp.

Besancon, X., Smet, C., Chabalier, C., Rivemale, M., Reverbel, J.P., Ratomahenina, R., and Galzy P. (1992) Study of surface yeast flora of Roquefort cheese. Int. J. Food microbiol. 17, 9-18.

Beuchat, L. R. (1982) Thermal inactivation of yeasts in fruit juices supplemented with food preservatives and sucrose Journal of food science 46, 771-777.

Bungay,H.R and, Bungay, M.L., (1968). "Microbial interactions in continuous culture. Adv. Appl. Microbiol. 10, 269-290.

Cahalan, D. L. and Genigeorgis, C. (1986) Study of the microflora during the natural commercial fermentation of Italian-dry ansalami and identification of species suitable as starters. In Proceedings of the 32nd European meeting of meat research workers 5,263-266.

Campbell-Platt, G., (1987). Fermented foods of the world : A dictionary and guide. 314pp. Butterworths, London.

Campbell-Platt, G., (1995) Fermented Meats -A world perspective. Blackie Academic . and Professional. London. pp 39-51.

Casado, M.-J.M, Borras, M.-A D. and Agnilar, R.V., (1991) Fungal flora present on the surface of cured Spanish ham. Fleischwirtschaft, 71(11). 1300-1302.

Cerise, L., Bracco, U., Horman, I., Sozzi, T. and Wuhrmann, T. J. (1973) Veranderung des Lipidanteils wahrend des Reifungsprozesses von salami aus reinem Scheweinefleisch. Fleischwirtschaft, 53, 223-225.

Cesari, E.-P., (1919). "La maturation du saucisson" Academie des Sciences Paris. 168, 802-803 .

Cesari, E.P. and Guiliermond, A., (1920). Les levures de saucissons" Ann Inst. Pasteur 34, 229-248.

Comi, G. and Cantoni, C., (1980). Yeast in matured raw sausage. Industrie Alimentari 19, 850-60.

Comi, G., Cantoni, C., and Traldi, C., (1982) Conservazione di sciruppi con aife Conzentrzioni di succheri. Ind. Bevande 11(59), 169-171.

Comi, G. and Cantoni, C., (1983). Presenza di lieviti nei pros ciulti crudi stagionati. Ind. Alimentar, 22, 102-104.

Cook, P.E., (1995) Fungal ripen meats and meat products. In Fermented meats.eds G. Campbell-Platt, P.E. Cook, Blackie Academic and Professional. London. pp 110-125

Coretti, (1956). Fleischwirtschaft, 8, 197.

Coretti, K., (1965) Vorkommen und Bedeutung lipolytischer Mikroorganismen in Dauerwursten. Fleischwirtschaft, 45 21-23.

Coretti, K., (1971) Rhowurstreifung und Fehlerzeugnisse bei der Rhowurstherstelliung.Verlag Rheinhessische Druckwerkstatte, Alzey (fed. Rep. Germany.

Coretti, K. (1973) Murum interessiert den Praktiker die Microbiologie der Rohwurstrei Feischwirtschaft 53, 907-903.

Coretti, K., (1977) Starterkulturen in der Fleischwirtschaft. Fleischwirtschaft, 57,386-394.

Dahiya, R. S. and Speck, M.L., (1967). Hydrogen peroxide formation by lactobacilli and its effect on *Staphylococcus aureus*. J. Dairy Sci. 51, 1568

Dalton, H.K. and Board, R.G., (1984) The yeast of fresh British sausage and minced Beef. J. Microbiol. Serl. Antonie van Leeuwenhoek, 50, 227.

Daly, C., Sandine, W.E. and Elliker, P.R., (1971). "Interactions of food-starter cultures and food-borne pathogens" J. Milk Food Technol. 35, (6) 349-357.

Daly, C., Chance, N., Sandine, W.E. and Elliker, P.R., (1973) Control of *Staphylococcus aureus* in sausage by starter culture and chemical acidulation. J. Food Sci. 38: 426

Deak, T., (1991) Food borne yeast. Adv. Appli. Microbiol. 36, 179-279.

Deak, T., (1993) Simplified techniques for identifying food borne yeasts. International Journal of Food Microbiology 19 (1) 15-26

Deak, T. and Beuchat, L. R. (1987) Identification of food borne yeasts J. Food Protect 50, 243-264.

Deak, T. and Beuchat. L.R., (1996) Handbook of Food Spoilage. Yeasts. CRC press, New York.

Deibel, R.H., (1956) Bacteriological aspects of pure starter culture in the manufacture of sausage. American meat Institute Foundation, Circular No. 20, p. 14.

Deibel, R.H., (1974). Technology of fermented semi-dried and dried sausage. In Proc. Meat Ind. Res. Conf., P.57. Am Meat Inst. Found., Washington D.C.

Deibel, R.H. and Niven, C.F., (1957) *Pediococcus cerevisiae*: A starter culture for summer sausage. Bacteriol. Proc. 1957: 14.

Deibel, R.H., Niven, C.F. and Wilson, G.D., (1961a) Appl. Micobiol. 9, 156-161.

Deibel, R.H., Wilson, G.A., and Niven Jr., C.F., (1961b) Microbiology of meat curing. IV. A lyophilyzed *Pediococcus cerevisiae* starter culture for fermented sausage. Appli. Microbil. 9, 239-243.

De Ketelaere, A.,Demeyer D., Vandekerckhove, P. and Vervaeke, J. (1974) Food Sci., 39, 297

Demeyer, D. I.,Vandekerckove, L. and Moerman, R., (1979). Meat Sci. 161

Demeyer, D. I., Vandekerckove, L., Vermeulen, L.and Moerman R., (1981) Compounds determining pH in dry sausages, Europ. Meet. Of Meat Res. Worker, Kulmbach, C.4.

Dillon, V.M. and Board, R.G., (1990) A study of sulfite tolerant yeast from comminuted lamb products Biotech. Appli. Biochem., 12, 99.

Dillon, V.M. and Board,R.G., (1991). Yeast associated with red meats (A review), Journal of Applied Bacteriology, 71, 93-108.

Diara, H. A., (1992) The indigenous fermented foods of Sudan. In: Applications of Biotechnology in food processing in Africa. Pp. 23-40. UNDO Veinna.

Dowdell, M.J. and Board, R.G., (1968). A microbiological survey of British fresh sausage. Journal of applied bacteriology 31, 378-396.

Dyett, E.J.and Shelley, D., (1962). The microiology of British fermented sausage. 1st Int. Cong. Food Sci. and technol. London.

Dyett, E.J. and Shelley, D., (1966). The effect of sulphite preservative in British J. Appl. Bact. 29, 439. Fresh sausage

Eilberg, B.L. and Lieip, H., (1977) Possible improvement in dry sausage technology by adding *Streptomyces* as a starter culture. Die Fleischwirtschaft 9 1698.

Empey, W.A. and Scott, W.J., (1939). Investigations on chilled beef. Part1 Microbial contamination acquired in the meatworks. Australian Council for Scientific and Industrial Research Bulletin.

Engel, G., Rosch, N.and Heller, K.J., (1994) Heat tolerance of heat of yeasts. Kieler Milchwietsch. Forschungsber, 46, (1), 81-90.

Everson C.W. Danner, W.E. and Hammes, P.A. (1970) J. Agr. Food Chem., 18 (4) pp 570-571.

Everson, C.W., Danner, WE. and Hammes, P.A., (1974) Process for curing dry and semi-dry sausages. U.S. Pat. 3814817.

Fennema, O.R. (1985) Food chemistry 2nd ed. Rev and expanded, N.Y., Dekkar.

Fleet, G.H. (1990) Cell walls, In The Yeast. Vol. 4, 2nd ed. Rose A. H. and Harisson J. S. Eds. Acadamic Press, London, 200.

Fleet, G. (1992) Spoilage Yeast. Crit Rev. Biotechnol. 12, 1-14.

Fryer, T. F., Lawrence, R. C. and Reiter, B., (1966). Methods for the isolation and enumeration of lipolytic oranisms. J. Dairy Sci. 50, 477-484.

Galzy, P., (1992) Study of yeast flora of roquefort cheese. Int. J. Food Microbiol. 17, 9-18.

Gehlen, K-H.; Meisel C.;Fisher, A; Hammes W.P (1991) Influences of the yeast *Debaryomyces hansenii* on dry sausage fermentation. Proc. 37th int. Congr. Meat Sci. Technol. 2 pp871-876.

Genigeorgis, C. A. (1976) Quality control of fermented meats. Javma 169,1220-1228.

Gill, C. O. (1982) Microbial interaction with meats. In meae Microbiology, M. H. Brown, Ed. Applied Science Publishers, London, pp. 225-64.

Gimenez, E.H., (1992) Evolution of the microbiological parameters in the production of Dry–cured hams (in Spanish) Proc. of the 8th Scientific Meeting of the Food Microbiol. Group.

Gokalp, H. Y. and Ockerman, H. W. (1985) Turkish-styled fermented sausages (soudjouk) manufactured by adding different starter cultures and using different ripening temperatures. Fleishwirtsch. 65, 1235-1239

Gokalp, H.Y., (1986) Residual nitrate,nitrite, carbonyl and TBA values of Turkish soujouk manufactured by adding different starter culture and using different ripening temperature. J. Food Technol.21, 615-625.

Grau, F. H. (1981) Role of pH, lactate, and aerobosis in controlling the growth of some fermentative gram negative bacteria on beef. Appl. Environ. Microbiol. 42, 1034-50.

Grazia, I., Suzzi, G., Romanio, P. and Giudici, P., (1989) The yeasts in meat products, 5, s495.

Gryczka, A.J., (1977) Bacterial composition and their use in fermented meats. U.S. Pat. 4013797

Gryczka, A.J. and Shah, R.B., (1979) Process for the treatment of meat with composition including *Micrococcus* varians and lactic acid producing bacteria. U.S. Pat. 4147807.

Guerzoni, M.E., Sinigaglia, M. and Gardini, F., (1993) Interaction between isolation source, cellular fatty acid composition and stress tolerant *Saccharomyces cerevisiae* and its subspecies. J. Appli. Bacteriol. 75: 588-294.

Hadlock, D., Samson R.A. and Schinorr, B., (1975). Moulds and meat: The genus *Penicillium*. Feischwirtschaft, 55, 969-983.

Hadlock D, Samsom R.A. and Schipper M.A.A. (1976). Schimmelpilze und Fleish Kontaminationflora. Fleischwirtschaft 56, 372-376.

Haines, N.C. and Harmon L.G., (1973). Effect of lactic acid bacteria on growth of *Staphylococcus aureus* and production of enterotoxin. Appli. Microbiol. 25, 436.

Hammes, W.P., Rolz, I. and Blantleon, A., (1985). Microbiologische Untersuchung der auf dem deutschen Markt vorhandenen Starterkulturpraparate fur die Rohwurstbereitung. Fleischwirtsch. 65, 1235-1239.

Hechelmann, H., Holzapfel, W. and Bem, Z. (1977) Vorkmmen und Bedeutung von Pseudomonadaceae bei der Rohwurstreifung Mitteilungsblatt der Bundesanstalt fur Fleischforschung Kulmbach Paryt 55. 2963-5.

Hechelmann, H., Lucke, F.-K. and Schillinger, U., (1988) Ursachen und Vermeidung von *Staphylococcus aureus*-Intoxikationen nach Verzehr von Rohwurst und Rohschinken. Mitteilungsbl. Bundesanstalt Fleischforsch. N. 100, 7956-7964.

Hofmann, H-B. and Scharner, E. (1980) Microbiologische und sensorische Untersuchungen zur Reifungadynamik von Rohwursten, ausgereift. I. Mitt.: Veranderung der Mikrflora wahrend der Reifung Nahrung 24,285-93.

Huerta, T., Querol, A. and Hernandez-Haba, J., (1988). Yeast of dry cured hams . Qualitative and quantitative aspects. Microbiologie, aliments, nutrition 5,247-252.

Inal, T., (1969) Fleicherei 1, 6-8.

Incze, K. (1965) Die mikrobiologische Veranderungen des Reifungsprozesses. 1. Gyulaer wurst. Husipar 14, 208-12 (In Hungarian)

Incze, K., (1986) Technologie und Mikrobiologie der ungarischen Salami. Fleischwirtschaft, 66, 1305-1311.

Inigo, B., Arroyo, V. and Somavilla, J., (1970). Estudio microbiologico del proceso de fabricacion de embutidos. Identificacion de lavaduras. Rev. Agroquim. Technol. Aliment. 10, 406-410.

Jay, J.M., (1978a). Meat and seafood. In: Food and Beverages Mycology. (ed. L.R. Beuchat) pp. 155-173 Avi Publishing.

Jay, J.M., (1978b) Meats, poultry and seafoods. In: Foods and Beverage Mycology (ed. Beuchat, L.R.) AVI Publishing Westport, Ct. pp 129-144.

Jay, J.M., (1987) Meats , poultry and seafoods. In: Foods and Beverage Mycology, 2nd ed. (ed. Beucht, L. R.) Van Nostrand Reinhold, New York.

Jay, J. M. (1979) Occurrence and significance of specific yeasts and moulds in meats, poultry and seafoods. In food Mycology ed. Rhodes, M. E. pp. 44-55. Boston: G. K. Hall.

Jay, J. M. (1996) Modern Food Mirobiology, 5th ed Chapman and Hall, New York

Jay, J.M. and Margitic S., (1981) Incidence of yeast in fresh ground beef and their ratios to bacteria Journal of food Science 46, 648-649.

Jensen, LB., (1935) 'Meat treating method' U.S. Pat. 2002146

Jensen, L.B., Paddock, L.S., (1940) US Patent 2, 225-783

Jessen, B. (1995) Starter culture for meat fermentation In Fermented Meats eds G. Campbell-Platt and P.E. Cook, Blackie Academic and Professional. London. pp 130-154

Katsaras, K. and Leistner L. (1985) *Staphylococcus aureus* und *Clostridium botulinum*. Bedeutung bel Rohwurst und Rohschinken. In Mikrobiologie und Qualtat von Rohwurst und Rohschinken. Herausgegeben vom Institut fur Mikrobiologie Toxikologie und Histologie der Bundesanstalt fur Fleischforschung,Kulmbach, pp. 152-172.

Kiernat, B.H., Johnson, J. and Siedler, A., (1964) A summary of the nutrient content of meat. Bull 57. Am. Meat Inst. Found. Chicago III

Kormendy, L. and Gantner G. (1962) Uber freie Aminosauren bei der Rohwurstreifung Fleischwirtschaft 12, 774-80.

Kouker, G. and Jaeger, K-K., (1987). Specific and sensitive plate assay for bacterial lipases. Appli. Environ. Microbiol. 53,211-213.

Kreger-Van Rij, N. J. W. (ed) (1984). The yeast – a taxanomic study. Amsterdam; Elsevier sceince.

Kurk, U.S. Patent 1,380,070, (1921)

Langner, H.J., (1972) Aromastoffe in der Rohwurst. Fleischwirtschaft 52, 1299-1306.

Lawrie, R. (1995) The structure , composition and preservation of meat In Fermented meats eds G. Campbell-Platt and P. E. Cook Blackie Academic and Professional. London. pp 1-33.

Lee, W.H., Reimann, H.P. and Al-Mashat, A.I., (1971) Controlled fermentation and prevention of undesirable bacteria growth in food. U.S. Patent 3,794,739.

Lee M. B. and Styliadis S. (1996) A survey of pH and water activity levels in processed salami and sausage in Metro Toronto.

Ledward, D.A. (1985). Novel intermediate moisture meat products pp. 447-450. In

Leisner, L., (1991) Fleischerei 42, (3) pp1-5; (4)3-5

Leistner, L., (1995) Stable and safe fermented sausages world-wide. In Fremented Meats. (Ed Campbell-Platt)

Leistner L. and Ayres J.C. (1968). Moulds in Meat. Fleishwirtschaft 48, 62-65.

Leistner I. und Bem, Z.(1970) Vorkommen und Bedeutung von Helfen bei Pokeelfleischwaren Fleischwirtschaft 50, 350-351.

Leistner, L.and Rodel, W., (1975) The significant of water activity for micro-organisms in meats. In water relation of foods (Edited by R.B.Duckworth); P.309. Academic Press, New York.

Leistner, L. and Rhodel, W. (1976) Inhibition and Inactivation of Vegetative Microbes . (F. A. Skinner and W. B. Hugo, eds.) Academic Press, New York.

Leistner, L., (1986a) Allgemeines uber Rohwurst. Fleischwirtschaft, 66 290-300.

Leistner, L., Schmidt, U. and Kaya, M., (1989) Bedeutung des Vorkommens von Listerien bei Fleisch und Fleischerzeugnissen. Mitteilungsbl. Bundesanstalt Fleischforschung, 28, 440-445.

Lerche, M., (1957). Bert. Munch. Tierarztl. Wschr. 70, 13.
a. Fleischwirtschaft 56(2), 178, 180-183.

Liebetrau, B. and Grossman, G. (1976). Die bedeutung von Starterkulturen zur Rohwurstreifung m Rahmen der Lebensmittel-und Ernahrungshygiene. Nature 20(5), 489-493.

Liepe, H.-U., (1978a) In La Charcuterie Crue et Les Produits Saumures. (Migaud-Frentz, ed.) Editions, Soussana, S.A., Orly (France).

Liepe, H-U., (1978b) Herstelung eines Inokulationmaterials zur Erzeugung einse dichten Schimmelrasens. German Pat. 2614147.

Liepe, H.-U., (1978c) L'utilisation des bacteries dan la technologie du saucisson cru. RTVA (Revue Technique Veterinaire de I'Alimentation) 138, 3, 5-15.

Liepe,H-U (1978d). Starterkulturen fur die Lebensmitteltechnik 4, 26-30.

Liepe, H-U (1981) . Starter culture in meat production, Biotechnology. 5, 399-424.

Liepe, H-U., (1983) Daten-Taschenbuch fur die Rohpokelwaren-Technologie. Rudolf Muller and Co., Pohlheim 1. Druckerei Gebhard, Offenbach (Fed. Rep. Germany).

Lissner, E., (1939) Wurstologia oder es geht um die Wurst. Hauserpresse, Hans, Schaefer, Frankfurt am Main.

Lowry, P.D. and Gill, C.O., (1984) Temperature and water activity minima for growth of spoilage mold from meat. J. Appli. Bact. 56 193-199.

Lucke, F-K, (1985) Fermented sausages. In Microbiology of Fermented foods. Vol 2. (Ed. B.J.B. Wood), pp. 41-83. Elsevier Applied Science. London.

Lucke, F-K., (1985) Mikrobiologische Vorgange bei der Herstellung von Rohwurst und Rohschinken. In: Mikrobiologie und Qualitat von Rohwurst und Rohschinken. Her ausgegeben vom Institut fur Mikrobiloge, Toxikologie und Histokologie der Bundesanstalt fur Fleischforschung, Kulmbach. 85-102.

Lucke F-K. (1988) Microbiological processes in the manufacture of dry sausages and raw ham. Fleischwirtsc. Inter . 2: 3.

Lucke, F-K. and Hechelmann, H., (1987) Starter cultures for dry suasage and raw ham. Composition and effect. Fleischwirtschaft. Composition and effect. Fleischwirtschaft. 67, 307-314.

Lund, A., (1951) Some beer spoilage yeast and their heat resistance. J. Inst. Brew. 57, 36-41.

Masters, B.D. (1979). Fate of *Salmonella* inoculated into fermented sausage. M.S. Thesis, University of Florida, Gainsville.

McCarthy, J.A. and Damoglou, A.P., (1993) The effect of low-dose gamma irradiation on yeast of British fresh sausage. Food Mocrobiol. 10, 439-446.

Meisel, C., Gehlen, K.H. ,Fischer A. and Hammes W.P. (1989) Inhibition of the growth of *Staphylococcus aureus* in dry sausage by *Lactobacillus curvatus*, *Micrococcus varians* and *Debaryomyces hansenii*. Food biotechnol 3:145

Metaxopoulos, J.,Genigeorgis, C., Fanelli, M.. J., Franti, C. and Cosma, E. (1981a) Production of Italian dry salami. I Initiation of staphylococcal growth in salami under commercial manufacturing conditions. J. Food Protect. 44, 347-352.

Metaxopoulos, J., Genigeorgis, C., Fanelli, M. J. Franti, C. and Cosma, E. (1981b) Production of Italian dry salami. Effect of starter culture and chemical acidulation on *Staphylococcus aureus* growth in salami under commercial manufacturing conditions Appli. Environ. Microbiol. 42, 863-871.

Metaxopoulos, J.; Stavropoulos, S.;Kakouri, A. and Samelis J. (1996) Yeast isolated from traditional Greek dry salami. J. Food Sci. 8 (1) pg 25-32.

Metiva, E., Kirova, E.,Gadjeva, D. and Radeva, M., (1986) Sensory aroma and taste profiles of raw dried sausages manufactured with a lipolytically active yeast culture. Nahurung 829-832.

Miller, M. W. (1979) Yeasts in food spoilage. An update, Food Technol., 33, 76.

Molina, I., Silla, H., and Flores, J., (1990) Study of the microbial flora in dry ham 4. Yeasts. Fleischwirtschaft 70, 74-76.

Monte, E., Villanueva, J.R., and Dominguez, A., (1986) Fungal profiles of Spanish country cured hams. Int. J. Food Microbiol.3, 355-359.

Nagodawithama, T. (1992) Yeast derived flavours and flavour enhancers and their probable mode of action. Food Technology 46 (11) pgs 138, 140-142. 144.

Niinivaara, F. P. and Pohja. M. S. (1956) Erfahrungen uber die Herstellung von Rohwurst mittels einer Bakterienreinkultur. Fleischwirtschaft, 9, 264-286.

Niinivaara, F.P., (1955) Uber den Einfluss von Bacterienkulturen auf die Reifung und . Umrotung der Rohwurst. Acta Agralia Fennica, 85 1-128.

Niinivaara, F.P., (1955). The influence of pure bacteria cultures on ageing and changes of the red color of dry sausage. Suomen Maataloustieteellisen seuran Julkaisuja No 84, Acta Agralis Fennica (Helsinki).

Niskanen, A. and Nurmi, E, . (1976). Effects of starter culture on staphylococcal enterotoxin and thermonuclease production in dry sausage. Appl. Micobol. 34, (1), 11.

Niven, C.F., (1957) Lactobacillus viridescens nov. spec., a heterofermentative species that produces a green discolouration of cured meat pigments. J. Bact., 73, 758-59

Niven, C.F., (1961). Microbiol. of meats. Circular 68. Am Meat Inst. Found. Washington D.C.

Nurmi, E., (1966) Acta. agr. Fenn. No 8.1-77

Palumbo, S. A., Zaika, L. L., Kissinger, J. C. and Smith, J. L. (1976) Microbiology and Technology of the pepperoni process. J. Food Sci. 41, 1270- 1272.

Pederson, G.S., (1979). Microbiology of food fermentations (2nd ed.). AVI Publishing Co., Westport, Conn.

Put, H.M.C. and De Jong, J., (1982b) The heat resistance of ascospore of four *Saccharomyces* spp isolated from spoiled heat processed soft drinks and fruit products. J. Appli. Bacteriol. 40,135-152.

Racovita, M., and Racovita, A., (1968) Die Reimpfung von Dauerwurst mit Schimmelpilzen.. Fleischwittschaft 48(7), 893-987.

Ramihone, M., Sirami, J., Larpent, J. P. and Girard, J. P. (1988) Gout acide des saucissons secs. Viandes et Produits Carnes, 9 (6), 291-298.

Rantala, M. and Nurmi, E (1973) Prevention of growth *of Salmonella infantis* in chicks by the flora of the alimentary tract of chicken. Br. Poult. Sci. 14, 624-630.

Reuter, G., (1965). "Untersuchungen uber die Zusammensetzung und die BeeinfluBbarkeit der menschichen Magen-undd Damflora unter besonderer Berucksichtigung der Laktobazillen. Ernanrungsforschung X, 429-435.

Reuter, G. (1967) Atypische Steptobakterien als dominierende Flora in reifer und gelagerter Rowurst. Fleischwitschaft 47, 397-402.

Reuter, G. (1969) . Zusammensetzung und Anwendung von Bakterienkulturen fur therapeutische Zwecke. Arzneimittelforschung 19 (1), 103-109

Reuter, G., (1972a). Reifung von Rohwursten vom Typ der Deutschen Salami mit Laktobazillen- und Mikrokokken-Starterkulturen. Proc. 18th Meeting Meat Research Workers, Guelph (Canada), pp. 666-669

Reuter, G. (1972b). Versuche zur Rohwurstreifung mit Laktobazillen- und Mikrokokken-Starterkulturen. Fleischwirtschaft 52 (4), 465-473.

Reuter, G. (1972c). Untersuchungen zur antagonistischen Wirkung von Milchsaurebakterien auf andere Keimgruppen der Lebensmittelflora. Zentralbl. Vet. Med. B. 19, 320-334.

Reuter, G. (1972). Versuche zur Rohwurstreifung mit Laktobazillen- und Mikrokokken-Starterkulturen. Fleischwirtschaft 52, 465-468, 471-473.

Roca, M. and Incze, K. (1990) Fermented sausages. Food Reviews International, 6(1), 91-118. Rudolf Muller data sheets, Giessener Strasse 94, D-35415, Pohleim. Germany.

Roostita, R., Fleet G. H. (1996) The occurrence and growth of yeast in camembert and blue-veined cheeses International Journal of Food Microbiology 28 (3) 393-404

Rossmanith, E., Mantzlaff, H-j., Streng, B., Christ, W and Leistner, L., (1972) Hefen als Starterkulturen fur Rohwurste. Jahresbericht der BAFF, Kulmbach, I, 47-48.

Saldanha-da-Gamma, A., Malfeito-Ferreira, M., and Loureiro, V., (1997) Characterization of yeasts associated with Portuguese pork-based products. Int. J. of Food Microbiol., 37, 201-207.

Samelis, J., Stavropoulos, Kakouri, A., Metaxopoulos (1994). J. Food Microbiology, 11,447-460.

Samelis, J., Tsakalidou, E., Metaxopoulos, I. and Kalantzopoulos, G. (1996). Differentiation of *Lactobacillus sake* and *Lactobacillus curvalons* isolated from

naturally fermented Greek dry salami by SDS-PAGE of whole cell proteins. J. Applied Bacteriology, 78 (2), 157-163.

Sanz, B., Selgas, D.,Parejo, I. and Ordonez, J. (1988) Characteristics of lactobacilli isolated from dry fermented sausages. Int. J. Food Microbiol. 6,199-205.

Simatos, D. and Multon, J.L., (ed) Properties of water in foods. Martinus Nijhoff Publishers, Dordrecht, Netherland.

Sirvio, P., Nurmi, E., Poulanne, E. and Niinivaara, F. P., (1977) Der EinfluB uon Starterkulturen und verschiedenen Zusatzstoffen auf das Wachstum von Salmonella Senftenberg in Rohwurst Fleischwirtschaft 56 (5), 1007-1012.

Smith, D.R., (1987) Sausage a food of myth, mystery and marvel. CSIRO. Food Res Quart., 47, 1-8.

Smith, G. and Palumbo, S., Microbiology of Lebanon Bolonia. Appli. Microbiol.. (1973) 26, 489-496.

Smith, M. TH. and Hadlok, R., (1976) Hefen und feisch: vorkommer, systematik und differenzierung. Feischwirtschaft 56, 379-384

Smith, J.L. and Palumbo, S.A., (1983) Use of starter cultures in meats. J. Food Protect; 46, 997-1006.

Sorrels, K. M and Speck, (1970) Diary Sci., 53, 239.

Stiebing, A. and Rhodel, W., (1988) Influence of relative humidity on the ripening of dry sausage. Fleischwirtschaft 68 (10), 1287-1291.

Stokes, J.L., (1971) Influence of temperature on the growth and metabolism of yeasts. In The Yeast. Vol. 2 (ed Rose, A. H. and Harrison, J. S.) Academic Press New York.

Su, W. and Beuchat, L.R., (1984) Combined effect of growth medium, age and phase of sporulation on heat resistance and recovery of *Hansenula anomala.* ycopathlogia 87, 129-134.

Tanaka, N., Traisman, E., Lee, M.H., Casseus, R.G.and Foster, E.M., (1980) "Inhibition of botulinum toxin formation in bacon"

Ten Cate, L., (1960) Fleischwirtschaft 40.

Tomtov, D., Bem, Z. and Leistner, L. (1974) RIM, 6, 3.

Tokuaka, K., Ishitani, T., Goto, S., and Komagata, K., (1985) Identification of yeast isolated from high sugar foods. J. Gen. Appli. Microbiol. 31, 411-427.

Touaka, K., and Ishitani, T., (1991) The minimum water activity for the growth of yeast isolated from high sugar foods. J. Gen. Appli. Microbil. 37, 111-119.

Tramer, J. (1966) Inhibitory effect of Lactobacillus acidophiluus. Nature (London) 211, 204-205.

Troller, J. A. and Christian, J. H. B. (1978) Water Activity and Food. New York: Academic Press.

USDA (1977f) The staphylococcal enterotoxin problem in fermented sausage. Task Force Report. F.S.Q.S., Washington D.C.

Van Eck, J. H., Prior, B.A. and Brandt, E. V. (1993) Journal of General Microbiology 139, 1047-1054.

Vayssier , Y. (1979) New mould culture for surface ripening of dry sausage. RTVA, 18, 50.

Vidal-Leira, M., Buckley, H. and Uden, N., (1979) Distribution of the maximum temperature for growth among yeasts. Micologia 71, 493-501.

Viljoen, B.C., Dykes G.A., Callis M., von Holy, A., (1993) Yeast associated with vienna sausage packaging. Int. J. Food Microbiol. 37, 201-207.

Walker, H.W., (1977) Spoilage of food by yeast. Food Technology 31, 57-61, 65.

Walker, H. W. and Ayres, J. C. (1970) Yeasts as spoilage organisms. In Yeasts. Yeast Technology, Vol. 3 (eds Rose, A. H. and Harrison, J. S.) Academic Press, London. pp. 463-527.

Wickerham, L. J., (1951) Taxanomy of Yeast. Technical Bulletin 1029, US Department of Agriculture, Washington, DC.

Williams, W. P (1990) *Penicillium* and *Aspergillus* in the food microbiology laboratory. In Modern Concepts in *Penicillium* and *Aspergillus* Classification. Eds R. A. Sampson and J. J.I. Pitt, pp. 121-137, Plenum Press, New York.

Zeuthen, P., (1995) Historical aspect of meat fermentation. In Fermented Meats (Ed Campbell-Platt)

Zottola, E.A., (1972) Introduction to meat microbiology, AMI. Center for continuing education, Am. Meat Inst., Washington, DC.