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The interaction between yeasts and bacteria in dairy environments

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Abstract

The general environment from which raw dairy products originate and the microbiological quality of the products in its processed state inevitably admit yeast growth and spoilage. Only part of the primary microflora survives under the selective pressures exerted by the intrinsic and extrinsic biotic factors present, processing procedures and preservatives. Yeasts that possess the proper physiological attributes to counteract the specific ecological determinants will be favored. Eventually, a particular yeast community will develop, and if the environmental factors permit, this characteristic yeast community will result in a specific association contributing positively or negatively to the final product.

The association that develops between yeasts and bacteria is governed by specific key properties selecting for a few predominant yeasts. These yeasts may either stimulate or inhibit normal bacterial growth. The extent to which interaction between yeasts and bacteria contribute to the final product is discussed.

Keywords: Yeasts; Lactic acid bacteria; Dairy products; Interaction

1. Introduction

Dairy products offer a special ecological niche that selects for the occurrence and activity of specific yeasts (Deàk and Beuchat, 1996). Despite the frequent occurrences of yeasts in many dairy products (Devoyod, 1990; Fleet, 1990; Fleet and Mian, 1987; Seiler and Busse, 1990), it is not generally accepted that these yeasts contribute significantly to the quality of the final product. Bacteria, especially the psychrotrophs causing spoilage, and bacterial starter cultures contribute most to the final product adding to the aroma and taste (Cousin, 1982). Yeasts, however, play an essential role in the preparation of certain fermented dairy products (Marshall, 1986; Marth, 1978; Gobbetti and Rossi, 1992) and in the ripening of certain cheeses (Devoyod and Desmazeaud, 1971; Seiler, 1991; Schlesser et al., 1992; Welthagen and Viljoen, 1998, 1999) and contribute substantially to the final product. These contributions are attributed to various interactions between the yeasts, starter cultures of lactic acid bacteria, and the secondary flora of bacteria and moulds (Welthagen and Viljoen, 1998, 1999).

The types of interaction found in the mixed populations of microorganisms are classified on the basis of effects, as direct or indirect interactions. Indirect interaction refers to competition, commensalism, mutualism, ammensalism or neutralism (Linton and Drozd, 1982), and direct interaction to predation and parasitism (Fredrickson, 1977; Bull and Slater, 1982). However, fermented products, such as dairy products, develop their nutritional and organoleptic quali-

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ties as a result of the metabolic activity of a succession of different microorganisms and it is unlikely that the interactions will separate into these discrete groups since more than one type of interaction may occur simultaneously (Verachtert and Dawoud, 1990).

The yeasts, as part of the interactions, either contribute to the fermentation by supporting the starter cultures (Jakobsen and Narvhus, 1996), inhibiting undesired microorganisms causing quality defects (Deiana et al., 1984; Siewert, 1986; Gedek, 1991), or adding to the final product by means of desirable biochemical changes like the production of aromatic compounds, proteolytic and lipolytic activities (Lubert and Frazier, 1955; Szumski and Cone, 1962; Nunez, 1978; Machota et al., 1987; Fernandez Del Poza et al., 1988a,b; Fleet, 1990; Besançon et al., 1992; Hostin and Palo, 1992). On the other hand, the interactions may be detrimental causing spoilage (Ingram, 1958; Walker and Ayres, 1970; Lenoir, 1984; Seiler and Busse, 1990; Viljoen and Greyling, 1995), by inhibiting the growth of starter cultures and producing excessive gas formation, off-flavors, slime formation or discoloration (Brocklehurst and Lund, 1985; Fleet, 1990; Rohm et al., 1992; Tudor and Board, 1993). The present review deals with the role of yeasts in interactions in dairy products with special emphasis on raw milk, fermented milk based beverages, and cheeses. The extent to which the interactions contribute to the final product, from where the yeasts originate, and the most prevalent yeasts involved in the interactions are also discussed.

2. Yeasts in the processing environment.

Since yeasts are not usually added as part of the starter culture during the processing of most dairy products, a normal question will be: from where do the yeasts evolve that play a role in the interactions? The immediate ecosystem of the dairy products itself, the environmental conditions prevailing, and the pasteurization of the raw milk contribute towards the selection of a uniform and well-defined yeast domain that initially originates as environmental contaminants (Baroiller and Schmidt, 1990; Jakobsen and Narvhus, 1996; Vadillo et al., 1987; Viljoen and Greyling, 1995). Only part of this primary microflora, however, will survive under the selective pressures exerted by the internal and external environments of dairy products and the presence of the rest is regarded as purely accidental (Deàk and Beuchat, 1996). Those strains capable to respond will develop in a dominant yeast community, and eventually play a major role in the interactions between the yeasts and the lactic acid starter cultures, and undesired bacterial cultures.

High numbers of yeasts are frequently observed on processing equipment, and in the air of the processing environment (Viljoen and Greyling, 1995; Welthagen and Viljoen, 1998, 1999). Normally, we may attribute the contamination of equipment to poor hygienic practices. Laubscher and Viljoen (1999), however, reported resistance of the dominant dairy associated yeasts to commercial sanitizers and cleaning compounds. Yeasts like *Debaryomyces hansenii*, *Candida versatilis*, *Torulaspora delbrueckii*, and others showed strong resistance, even after 60 min of exposure. None of the nine commercial cleaners and sanitizers examined sufficiently inhibited or killed the contaminating yeasts. Therefore, it is possible that the yeasts may colonize during cleaning and sanitation cycles (Laubscher and Viljoen, 1999). Consequently, the high numbers of yeasts in dairy products may be attributed to their ability to grow at low temperatures, assimilation/fermentation of lactose, assimilation of organic acids like succinic, lactic, and citric acid, lipolytic and proteolytic activities, low water activities, resistance against high salt concentrations and resistance against cleaning compounds and sanitizers (Fleet, 1990; Laubscher and Viljoen, 1999). All of these properties will determine their presence in dairy products, contributing towards the survival and progression of the yeasts.

The most prevalent yeast strains frequently isolated from dairy products are representatives of the genera *Kluyveromyces*, *Debaryomyces*, *Yarrowia* and *Candida Kluyveromyces* and *Debaryomyces* species are typically associated with cheeses and yoghurt (Tudor and Board, 1993), whereas *Candida* species (*C. lusitaniae, C. krusei*) are normally associated with yoghurt (Deàk and Beuchat, 1996). *Yarrowia* species are found in cheeses, milk and yoghurt samples and this can be attributed to their remarkable lipolytic and proteolytic activities

(Welthagen and Viljoen, 1998, 1999). Yoghurt, due to the addition of sugar and/or fruit, is particularly prone to spoilage caused by *Saccharomyces cerevisiae* (Fleet, 1990; Fleet and Mian, 1987).

3. Microbial interactions in dairy products

When the domains of individual microorganisms overlap, as observed in dairy products, it is likely that interactions will occur. The outcome of natural interactions in nature is evaluated based on the effect they have on population size (Steinkraus, 1982) regardless whether the interactions are detrimental, neutral or beneficial. When a food product is produced, however, the positive or negative aspects caused by interactions between microorganisms become very important.

Present understanding of the positive, negative or neutral role of interactions between yeasts, bacteria and moulds has its origins the first time fermentation has been employed. The fermentation of many dairy products includes the interaction of various microorganisms which physiological activity brings about desirable changes which decisively determine the character of a product and stabilize the population in a specific ecological niche (Deiana et al., 1984; Jakobsen and Narvhus, 1996). However, interaction does not necessarily only implies the positive or negative attributes within fermentation but also involves the antagonistic activity of yeasts against other microorganisms by means of the killer factor as observed in cheese brines (Seiler, 1991), secretion of antibacterial and antifungal compounds (Brugier and Patte, 1975; Lehmann et al., 1987; Polonelli and Morace, 1986), and binding of pathogenic bacteria (Gedek, 1991).

Microbial communities with their combined physiology, interactions and enzymatic activities are responsible for major biochemical and nutritional changes that occur in the substrates of fermented milk-based products (Steinkraus, 1982). Antimicrobial effects present in fermented products and beverages are attributed to organic acids, antibiotic factors, volatile acids, hydrogen peroxide and to a number of substrates excreted in the products (Borregaard and Arneborg, 1998; Bankole and Okagbue, 1992). These antimicrobial effects are the result of the presence of several kinds of microorganisms involved in the fermentation and putrefaction of products which inevitably lead to beneficial or detrimental interaction among the populations (Bull and Slater, 1982).

The metabolic interactions are governed by the yeasts inherent technological characteristics and biochemical activities providing essential growth metabolites, such as amino acids, vitamins, removing toxic end-products of metabolism, inhibit the growth of undesired microorganisms by lowering the pH, secretion of alcohol, CO_2 production, or encouraging the growth of the starter cultures by increasing the pH due to the utilization of organic acids (Devoyod, 1990; Kaminarides and Laskos, 1992; Seiler, 1991; Schlesser et al., 1992; Robinson and Tamine, 1990; Welthagen and Viljoen, 1999).

4. Microbial interaction in raw and pasteurized milk

The chemical composition of milk will support the growth of yeast species although they are usually out-competed by mainly the psychrotrophic bacteria (Cousin, 1982). Literature shows that yeasts occur in both raw (Engel, 1986; Randolph et al., 1973; Fleet and Mian, 1987; Roostita and Fleet, 1996), and pasteurized milk (Vadillo et al., 1987) at insignificant numbers. Yeasts in pasteurized milk, however, evolve as secondary contamination because they are killed during pasteurization. Populations less than 10^3 cfu/ml are generally reported although counts as high as 10^8 cfu/ml were reported when inoculated in UHT-treated milk (Roostita and Fleet, 1996). Again, the growth is attributed to the yeasts ability to utilize milk constituents. The high yeast counts observed when inoculated in UHT-treated milk, and the insignificant numbers observed under normal circumstances are an indication that definite interactions between the yeasts and bacteria exist. The low yeast numbers in raw milk, therefore, may be due to competitive utilization for the growth substrates, especially by the faster growing psychrotrophic bacteria or inhibition by metabolites excreted by bacteria.

When different dairy associated yeasts were grown at different temperatures, low counts were obtained at reduced temperatures, but if the milk was incubated at 25 °C, high yeast populations were evident (Roostita and Fleet, 1996; Loretan, 1999). Therefore, keeping milk at low temperatures is most likely to give psychrotropic bacteria a competitive advantage.

On the other hand, fermented milks, such as yoghurt, soft-cheeses as well as hard-cheeses, support the growth of both yeasts and bacteria which, consequently, lead to enhanced numbers of microorganisms (Loretan, 1999; Welthagen and Viljoen, 1998, 1999). Therefore, it may be an indication that yeasts survive and compete better in restricted environments when intrinsic properties like the high salt or lower pH, and reduced water activity play a substantial role.

5. Microbial interaction in fermented milk based products

The commensalistic interaction between *Lactobacillus acidophilus* and the lactose fermenting yeast, *Kluyveromyces fragilis,* in acidophilus-yeast milk (Subramanian and Shankar, 1983) relies on the coexistence of both organisms to secure a good product. Although the lactic acid fermentation originally relied on either the fermentation of *L. acidophilus* alone or in mixed cultures with other lactic acid bacteria, the overgrowth of these organisms resulted in lesser viable cells of *L. acidophilus* which consequently reduced the species contribution to gastro-intestinal disorders (Lang and Lang, 1975). The co-culture of *L. acidophilus* with lactose-fermenting yeasts reduces the time of coagulation of the milk due to acid production by the yeasts, elevates the number of viable lactic acid bacteria cells attributed to stimulating influences of yeasts, and inhibits the growth of *Escherichia coli* and *Bacillus cereus* (Subramanian and Shankar, 1983).

Mutualism (synergism) occurs between yeasts and lactic acid bacteria during the fermentation of milky kefir (Loretan, 1999; Rossi, 1978). The predominant species isolated from milky kefir are *S. kefir*, *C. kefyr*, *L. caucasicus*, *L. casei* and *Leuconostoc* spp. (Oberman, 1985). The yeasts provide growth factors like amino acids, vitamins and other compounds for bacterial growth which consequently lead to elevated acid production, while the bacterial end-products are used by the yeasts as an energy source (Loretan, 1999). This phenomenon creates stability in the product. However, a decrease in alcohol production by the yeasts might occur due to excessive lactic and acetic acids production by osmophilic lactic acid bacteria, competition for the carbon source or lysis of the yeast cell walls by bacterial enzymes (Loretan, 1999).

Similar symbiotic relationships based on acid- or alcohol fermentation occur when lactic acid bacteria are responsible for the lowering of the pH due to the secretion of organic acids allowing the yeast population to become competitive in the immediate environment, followed by yeast fermentation, like in various milk-based fermentations such as Leben, Dahi, Koumiss, etc. (Kosikowski, 1977; Vedamuthu, 1982; Steinkraus, 1982; Bankole and Okagbue, 1992). Oberman (1985) and Vedamuthu (1982) reviewed the fermented milks. The combination of conditions (acidic, saturated with carbon dioxide and alcohol) is inhibitory to many spoilage bacteria and filamentous fungi and thereby substantially increases the shelf-life and safety of the products (Wood, 1981, 1985).

Yoghurts exhibit a selective environment for the growth of yeasts due to their low pH (Suriyarachchi and Fleet, 1981). The yeasts are not involved in the fermentation process, mainly due to the high processing temperatures, but they appear as contaminants originating from the processing equipment and to a lesser effect from the fruit, honey, and sugar. Significant literature refers to the spoilage ability of yeasts, causing fermentative flavors and odours, and excessive gas production (Suriyarachchi and Fleet, 1981; Green and Ibe, 1987; Rohm et al., 1990; Jordano et al., 1991; McKay, 1992). The yeasts species, *K. marxianus*, *D. hansenii*, *Yarrowia lipolytica* and *Rhodotorula mucilaginosa* predominated in most studies. Average yeast counts of 10^4 cfu/g or more were frequently encountered, especially in the fruit-based and flavoured yoghurts where added sugar acts as fermentable growth substrate, and amplifies the risk of yeast spoilage (Deàk and Beuchat, 1996).

Although the populations of contaminating yeasts remain relatively stable at low temperatures, the numbers quickly increase when the yoghurts are exposed to higher temperatures. The shelf life of yoghurts, which is usually about 30 days, decreases substantially when the storage temperatures is abused. Despite the major increase in yeast numbers, the population of starter cultures of lactic acid bacteria remains constant or continues to increase probably due to a symbiotic effect whereby both populations benefit from the interaction. This mutualistic effect may be attributed to the yeasts providing the necessary growth factors or vice versa.

6. Microbial interaction in cheeses

The occurrence of yeasts in cheese is not unexpected because of the low pH, low moisture content, elevated salt concentration and low storage temperatures (Fleet, 1990). The significance of this presence depends on the type of cheese. Yeast populations exceeding 10^7 cfu/g were reported (Koburger, 1971; Nakase and Komagata, 1977; Jarvis and Shapton, 1986; Banks and Board, 1987) and counts of 10^4 to 10^6 cfu/g are frequently found (Nunez et al., 1981; Chavarri et al., 1985; Fleet and Mian, 1987; Welthagen and Viljoen, 1998, 1999).

The role of interactions between yeasts and the starter cultures is well documented for the soft cheeses and semi-soft cheeses (Devoyod and Desmazeaud, 1971; Lenoir, 1984; Kaminarides and Anifantakis, 1989; Hostin and Palo, 1992; Roostita and Fleet, 1996). The yeasts, originating as natural contaminants of the cheesemaking process, contribute to the ripening by metabolizing lactic acid (Lenoir, 1984), producing lipases and proteases, fermenting residual lactose, excreting growth factors and their autolysis (Schmidt and Lenoir, 1980; Fleet, 1990; Devoyod, 1990; Besançon et al., 1992; Roostita and Fleet, 1996). All of these characteristics contribute to the sensory quality of the cheese. The increase in pH due to lactic acid utilization will encourage the growth of bacteria which not only affect flavour and textural quality, but may pose a risk to public health. Yeasts also assist the development of fungi in blue-veined and Camembert cheeses (Kaminarides and Laskos, 1992; Schlesser et al., 1992) by gas production leading to curd openness (Coghill, 1979).

Despite the frequent references to interactions between the yeasts and the lactic acid starter cultures in soft and semi-soft cheeses, limited studies referred to interactions within the hard cheeses. Yeasts, however, are present during the maturation of Gruyere (Piton, 1988), Saint-Nectaire (Vergeade et al., 1976), Romano (Deiana et al., 1984), Parmesan (Romano et al., 1989), and Cheddar (Fleet and Mian, 1987; Welthagen and Viljoen, 1998, 1999) and therefore may contribute to the final product. Based on these studies, the inclusion of *D. hansenii* and *Y. lipolytica* (Guerzoni et al., 1996) as part of the starter culture was proposed to accelerate ripening based on their proteolytic and lipolytic abilities (Grieve et al., 1983; Deiana et al., 1984; El-Soda, 1986), the utilization of lactic and acetic acids, and the inhibition of *Clostridium* species. Studies on the interaction between yeasts and the starter cultures in Cheddar and Gouda cheeses by Welthagen and Viljoen (1998, 1999) and Laubscher (1999) indicated that yeasts also play a significant role during the ripening of these cheeses by supporting the growth of the starter cultures. The large number of yeasts present during the later stages of ripening, originating as contaminants from the immediate environment, is indicative of a possible mutualistic interaction between the microflora. During ripening, yeasts continue to increase at a faster rate than the starter cultures, but no inhibition of either of the populations was observed (Welthagen and Viljoen, 1998, 1999). Therefore, the mutualistic interaction may contribute to the final product due to the production of flavor compounds, supporting the growth and survival of starter cultures during maturation, excreting lipolytic or poteolytic enzymes, and metabolizing the organic acids.

7. Conclusion

Sufficient knowledge about the interactions between yeasts and bacteria in dairy products remains incomplete. More comprehensive studies are needed to determine the role of yeasts during interactions in dairy products and to assess its value in the outcome of the final product. Different interactions exist within dairy products, depending on the environmental stresses. In sterile milk, yeasts will grow and reach high numbers due to the absence of any competing microflora, but in raw milk a competitive interaction induced by the psychrotropic bacteria restricts yeast growth. The inability of the yeasts to compete under these conditions may also be incurred by inhibition. Mutualistic interaction or synergistic interaction, however, is evident in restricted environments like yoghurts and cheeses, whereby both populations will benefit. Based on the interactive associations, the deliberate use of yeast species in the maturation of all cheeses or the production of fermented dairy products requires intensive exploration.

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