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Use of yeasts and Moulds in the manufacture of dairy products: A Review

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Abstract
Over the past years, the diversity of yeasts and mold in the environment has been remarkably understood. Due to the high adaptability of these fungal organs, they are ubiquitous in the environment. Yeasts and molds are widely used in the dairy industry due to their high capacity to ferment milk, as yeast commercial cultures are becoming increasingly popular for the production of fermented milk, cheese and ethanol from whey and lactose solutions. Many types of mold also find their use in the development of various dairy products. Different types of mold are used for ripening different types of cheese for example (Roquefort, Camembert), producing enzymes, and managing dairy waste.

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1. Introduction

In this review, we present data to show why we believe there is a need for a greater understanding of the key issues and for more substantive and directed research in the future. We have divided our discussion into three sections, dealing first with mycotoxigenic molds, which pose a potential hazard to the consumer but also tend to cause severe economic problems in various milk-producing countries. We then explore the possibilities presented by the desirable activity of yeasts or fungi in dairy fermentation, though the technological use of various strains is, as yet, basically untested. The third part is restricted to the problem exerted by sporadic spoilage of milk and dairy products due to the too often high levels of undesirable molds, which are not frequently reported but are of concern in cheese and dairy-based desserts. (Lima *et al*. 2021) [17] (Ayofemi 2020) [4].
Microorganisms have long been known to play an important role in the processing of a wide variety of dairy products. For instance, lactic acid bacteria have a key role in fermented milks and cheeses, and starter cultures are used to confer favorable texture and taste, as well as to act as preservatives. However, in addition to bacteria, molds and yeasts, either introduced or naturally present in milk, can have important technological and sensory consequences, particularly detrimental in dairy products. Yet relatively little research has previously addressed the possible economically important manipulation of yeasts and molds in milk and dairy products. Consequently, there is a general dearth of reliable data on the occurrence and type of yeast and mold species in dairy products as a whole and cheeses, in particular. (Carrillo-Lopez *et al*. 2021) [6] (Nikmaram & Keener, 2022) [26].

2. Yeasts and Moulds: Characteristics and Classification

For some yeasts and molds, rapid metabolism under aerobic conditions, at high osmotic pressure, low oxygen concentrations, and other unfavorable conditions is typical. They are less acid, temperature, salt, and zone-tolerant than lactic acid microorganisms considered in this review. Therefore, the established level of contamination and the presence of undesirable yeasts and molds in the end-products determine consumer characteristics. The current biotechnological approach is thus aimed at producing starter yeasts, including those with the ability to reduce pressure during maturation of the bottles with newly sterilized fermented milk, or a new mixture (reinoculating re-cultigated) with a desired resistance to ethanol toxicity. (Awasti & Anand, 2020) [3] (Yang *et al*., 2022) [41].

Desirable yeasts and molds must possess a number of biochemical and technological features. These include the ability to grow at low temperatures, aerobic and fermentative metabolism capabilities, the production of bio surfactants and peptides from milk proteins, softening, taste and texture improvement of cheese, the production of antimicrobial substances in fermentation, and a negative representation of opportunistic pathogens. In addition, the progression of technological features of starter and adjunct cultures for the manufacture of cheese, dry-sausages, and other fermented dairy products is associated with the understanding of their metabolic and biochemical properties. (Awasti & Anand, 2020) [3] (Zheng *et al*., 2021) [42].

2.1. Yeasts: Types and Properties

Yeast cells are elliptical in shape but can also be round, oval, or cylindrical. They can grow in an acidic, hyper- or hypoosmotic environment and in foods with low moisture or high-organic acid content. Yeasts tolerate pasteurization, refrigeration temperatures, high salt concentration, and desiccation. They may respond to antimicrobial compounds with an inhibition of growth and even induction of yeast cell death. At present, to use the potential of yeast in food products, they play an important role both as the product's primary and secondary indicators of harmful changes in the safety or quality of the products. Yeasts present in food products are inactivated by heat treatment, UV radiation, gamma-irradiation, freezing, or addition of chemical preservatives. Yeasts have reduced the epiphytic bacterium, fungi, and even pesticide resistance and are often used as a solvent for the extraction of essential oils. (Saldaña *et al*. 2021) [35] (Lucena *et al*. 2020) [20].

Yeasts are unicellular fungi of the family Saccharomycetaceae that are found naturally in the soil, air, plants, animals, and insects. They belong to both ascomycetes and basidiomycetes and occur predominantly on the surface of plants and fruits. The Ashbya gossypii is the yeast most extensively used in food and beverages, and among them, Saccharomyces cerevisiae is the largest member. But other yeasts such as Debaryomyces, Yarrowia, Kluyveromyces, and Candida are also used in food digestibility and for their other functional properties. Yeasts are devoid of a chlorophyll pigment, so they entirely rely on other organic materials for their nutrition. They degrade carbohydrates to alcohol and then to acetic acid and carbon dioxide. They are capable of reproducing sexually or asexually. Under adverse conditions, yeasts are capable of developing that make them survive by reverting to the resting stage or forming viscid or thick-walled cells. Yeasts asexually reproduce by budding. (Parapouli *et al*. 2020) [31] (Pereira *et al*. 2021) [32].

2.2. Moulds: Types and Properties

The filamentous fungi *Aspergillus clavatus*, *A. flavus*, *A. versicolor*, *Penicillium cyclopium*, *P. citrinum*, *P. commune*, *P. chrysogenum*, *P. corylophilum*, *P. digitatum*, *P. expansum*, *P. griseofulvum*, *P. minioluteum*, *P. nalgiovense*, *Mucor racemosus*, *Rhizopus nigricans*, and *Absidia spp*. have been isolated from a wide range of dairy products. The extent to which an inoculated mould that grows in parallel with the spontaneous microflora will affect the final characteristics of a cheese depends on a variety of intrinsic and technological parameters. The interactions and cooperation between the different communities that coexist can produce toxic/health hazard secondary metabolites from the action of their catabolic enzymes. These metabolites, generated by yeast or mould propagation, are responsible for spoilage of food products. (Souza *et al*. 2022) [38] (ALZAMILY, 2022) [1].

Moulds are eukaryotic microorganisms, characterized by thread-like structures called hyphae. The colonies present a vegetative structure, mycelium, and a reproductive one, by sexual and/or asexual reproduction. The colloidal state of organic matter in the substrate, the mechanism of degradation of insoluble substrates, and the extracellular accumulation of catabolic enzymes are the most important aspects of filamentous fungi in their ecological functions as saprophytes. (Mankar *et al*., 2022) [21].

3. Role of Yeasts and Moulds in Dairy Product Manufacturing

In specialty cheese production, contamination by molds may enhance the organoleptic and visual properties of certain cheeses, whereas, in an industry producing standardized cheeses, contamination events are not desirable. Generally, the growth of molds and yeasts on cheese can lead to problems such as the variation in taste and smell and in some cases lead to spoilage of the product, resulting from the production of extruded ascospores of the fungi or the biological processes involved. The major problem of the yeasts and molds present in cheeses arises from the production of off-flavors, and, in certain cases, mycotoxins. (Dolganova *et al*. 2020) [10] (Awasti & Anand, 2020) [3].

Just as the microorganisms present in raw milk can influence the quality and safety of the final dairy products, so too can the molds and yeasts that grow under particular conditions during the storage of fermented products. In this way, controlled spoilage by molds for enhanced cheese flavor is used, or in the case of early spoilage during refrigerated storage of yogurt, it constitutes a problem that is not related directly to food safety but rather to the preferences of the consumer. The conditioning of cheese is a process that commences with the selection of the initial starter cultures, and the characteristics of the final product can be manipulated to the liking of the consumer through the addition of flavor-producing adjunct cultures such as yeasts and molds. The knowledge of the cheese species and strains capable of growing during storage and effecting specific cheese ripening characteristics is applied for the selection of a combination of cultures or the use of appropriate packaging technologies. (Awasti & Anand, 2020) [3] (Zheng *et al*., 2021) [42].

4. Yeast and Mould Fermentation Processes in Dairy Products

We showed that the respective yeast and mold microbiomes of commercial starters were very similar to those of raw milk and this similarity was more closely related to the environmental parameters of the production of the milk itself. Hypothesizing that the microbiome of the starter propagated in a model farm could have an effect on the development of the final sensory profile of the product, we further pursued our study by comparing the yeasts and molds in the farm and the starter doughs to the development stages of the final product. Therefore, with the help of conventional plating, culture-independent techniques, and sensory analysis of drums disposed of at various positions within the growing rooms, we found that the microbiota of the farm and starter doughs had a less considerable effect on the development of the microbiota of blue cheese. (Vadopalas *et al*. 2020) [39] (Louw *et al*. 2023) [19].

Yeasts and molds have a generally beneficial role in the production of certain fermented dairy products, principally blue, Camembert, and Roquefort cheeses. Yeasts, or a mixture of yeasts and lactic acid bacteria, ferment lactose in cheese curd, producing carbon dioxide and ethanol that modify the curd texture and establish the internal atmosphere required for mold development. They can also make a limited contribution to the development of volatile flavors in blue and Camembert cheeses. In contrast, their presence in terms of the texture and the yeasty odor is undesirable in consumer-preferred cheeses such as Cheddar, Emmental, and Pecorino Romano. Their implication in the late blowing and late-release defects of soft cheese should also not be overlooked. This article displays the role of yeast strains in the physicochemical constitution of dairy products and their defects, focusing on the most prevalent strains isolated in a number of dairy farms. (Awasti & Anand, 2020) [3] (Yang *et al*., 2022) [41] (Shi & Maktabdar, 2022) [37].

4.1. Fermentation of Cheese

It has been reported that specific yeast species enhance the growth of some lactic acid bacteria and contribute to fundamental texturizing processes, namely the development of secondary aromas and the acceleration of ethanol conversion in lactate and of the second simultaneous elongation process of the precursor flavor compounds, obtaining new desirable effects of these strains, including enhanced intracellular amino acids and nucleotides. In spite of the extensive use of lactic acid, rheonomic investigations of specific strains, as well as their ability to lower acetaldehyde and diacetyl, cause them to lose their desirability and to generate enzyme inhibitors which can also reduce the milk's enzymatic coagulation capacity. Regarding the buttery taste, selected strains can produce a small number of putative potent aldehydes. (Yang & Yang, 2023) [40] (Gao *et al*.2020) [13] (Galli *et al*., 2022) [12].

Cheese manufacture represents one of the initial alternatives of biotechnological strain use, with domestication processes beginning 5,000 years ago. Modifications taken place by these microorganisms include lactic acid bacteria development, a parallel decrease in pH, and the synthesis of lactic and propionic acid, acetaldehyde, diacetyl, acetoin, and some enzymes that provide unique organoleptic qualities to the product. Usually, these microorganisms grow and cooperatively interact at optimized temperature and other fermentation conditions supplied by the cheesemaker. Their advantage includes the inhibition of some pathogenic microorganisms present initially in the milk or environment as well as the development of flavors, aromas, and nutritional values that are part of the final product. Using new methods, the balance of the microflora in the semi-ripened and ripened uncooked or thermally treated pasta cheeses is convenient to prevent some undesired metabolite adverse effects. (Oštarić *et al*. 2022) [28] (Danylenko *et al*. 2023) [9] (Rest, 2021) [33].

4.2. Fermentation of Yogurt

The choice and technology allow the recovery of larger accumulations of desired metabolites in a shorter period of time. So the use of proteolytic, peptidolytic soluble, user-friendly dry yeast preparations as starter cultures of dairy products of different fat content is a prerequisite factor for the accelerated production of nutritionally valuable functional food with a longer shelf life. Non-domesticated microorganisms isolated from dairy products play significant roles in the ripening and organoleptic qualities of the final fermented products. The yeast or mold fermentation products frequently introduce unique flavor attributes to food products, partly because of the goldilocks effect of swirling interactions among the chemical compounds produced including high-perceived and pleasant taste notes, and partly due to the many enzymes and other minor metabolites produced in the process.

Yogurt is a dairy product that is consumed worldwide. It is a nutritious and healthy food and more versatile in terms of their manufacturing and their consumption. It is an ancient fermentation product which possesses not only good nutritional properties but also a good medicinal activity. Microorganisms such as Lactobacillus acidophilus and L. bulgaricus, added deliberately, are widely used as starters, which are capable of fermenting the galactose of milk. Some yeasts and molds become residents of the microbial biotopes of various dairy products and offer strain character for industrially significant fermentation of dairy products. Furthermore, some yeasts and molds isolated from dairy products, particularly in fermented food matrices. Principles of selection of proteolytic, peptidolytic starter yeast cultures as a potential tool for developing high-quality fermented dairy products with bioactive properties, have been briefly summarized. (Farag *et al*. 2022) [11] (Montemurro *et al*., 2021) [25].

5. Biotechnological Applications of Yeasts and Moulds in Dairy Industry

Dairy industries are beginning to take advantage of the different metabolic pathways of yeasts which provide products that have unique nutritional and sensorial characteristics. With the increasing number of vegetarians and lactose-intolerant consumers around the world, activity of β-galactosidase-producing yeasts is attractive to dairy industries as these yeasts are suitable for the development of new lactose-free dairy products. Experimental whey and milk fermentation assays have indicated that several yeasts degrade all lactose available, and the short-chain oligosaccharides present at the end of the fermentation are prebiotic components with beneficial health effects for the host. For application in whey fermentation, yeasts require more specific characteristics than those applied in traditional alcoholic fermentations. Dairy yeasts need to be acid tolerant and able to grow at 4-5 °C, while at the same time being resistant to stress conditions imposed by the high maternal aspect of whey medium. Currently, it is possible to isolate yeasts with the unique combinations of metabolic, biotechnological, and industrial properties that the market requires for dairy products such as Priobacillus yogurt, from Arla Foods DK, which contains both Lactobacillus casei 431 and the lactose-free yeast Saccharomyces cerevisiae, and Danisco lactose-free starter cultures, KLDS 1.1014 from Yili, LCW-SC1 from Lick S.R.L. Twitchell S.C. from Chr. Hansen, and Codi lactobacilli-yeast mixyog from CSK Food Enrichment, which contain Saccharomyces cerevisiae var. boulardii, Weissella, Kluyveromyces burxanus, Lactococcus (LCW-ELC1, LCW-SC1, and Lactobacillus helveticus). (Awasti & Anand, 2020) [3] (Pang *et al*. 2022) [30] (Kumar *et al*. 2022) [16] (Ansari *et al*. 2023) [2] (Rocha & Guerra, 2020) [34].

The modern lifestyle has made consumers have an increasing demand not only for higher quality and safety of dairy products, but also for the appearance and presentation of

such. To meet such demands, the dairy industry has been working with modern biotechnological methods, focusing on obtaining more competitive starter cultures that provide a large number of characteristics, reducing the time of production, and ensuring more nutritional and sensorial characteristics such as taste and aroma, and producing new dairy derivatives with beneficial effects on human health. In this context, the implementation of yeasts and molds in the dairy industry deserves more attention from both industry and academia, as these microorganisms are still under-utilized for the biotechnological purposes they offer. (Jayasree *et al*. 2024) [14] (Campos-Vega *et al*. 2022) [5].

6. Health and Safety Considerations in the Use of Yeasts and Moulds

The possible transmission of yeasts and molds through dairy products is a potential food safety problem. Pathogens that can be associated with raw materials for the dairy industry, such as mastitis-causing yeast and molds, excrete them into milk. Post-process contamination or nisin-resistant strains from bacteria could get transmitted from the inoculum of cheese starter cultures. Because of the cheese's special treatment plan and specific approvals from the regulatory bodies for its use, GMOs are the phage lysin-encoding tests for water activity improvement. The use of approved antimicrobials to control Gram-positive bacteria avoids the isolation of the molds. Small quantities of mold inhibitors can be used to avoid toxin production. More research is needed on the routines of ineffective mold inhibitors. The proof of the pudding is that people consume it without health problems. Consumer health needs first and foremost to be properly handled, and dairy producers can benefit when the producers have developed the standards for cheese production. (Owusu-Kwarteng *et al*., 2020) [29] (Ntuli *et al*. 2023) [27] (Martin *et al*., 2021) [22].

Food safety is one of the high priorities for all dairy industries. The use of yeasts and molds in the dairy industry also appears to pose great safety risks that might be associated with the metabolic by-products of these microorganisms during fermentation. Nevertheless, when the fermentation is appropriately done and the "good" yeasts and molds are used, the cheese quality can be improved, and safety from putting pathogens can be improved as well. The manufacturers of yeast and mold strains have the responsibility to ensure that each of these microorganisms' strains is safe. Regulatory bodies require the substantiation of safety for the intended application in each animal and human food. This includes the evaluation of safe use through DNA subactivities of the antimicrobial, toxins, allergens, and candidate virulence genes, and other safety parameters. (Lindsay *et al*. 2021) [18] (Kapustian *et al*. 2021) [15] (Choi *et al*. 2024) [8].

7. Future Perspectives and Innovations in the Field

These valuable and attractive fungi, besides contributing to the relevant sensory, nutritional, and shelf-life attributes of the products, may offer several biotechnological alternatives and, in the future, bring direct benefit to producers, consumers, or the pharmaceutical industry. Unfortunately, the mycology of dairy products has been persistent in many urban legends, poor scientific knowledge, or confusion, and historical data are available. This review presents data that suggests areas where not only more research is needed but started a relationship with other specialists, such as biochemistry, and, in an approach more adequate to the questions posed, can be studied in order to disseminate knowledge or correct common preconceptions. (Money, 2022) [23] (Money, 2024) [24] (Seifert, 2022) [36].

Dairy products are highly diversified, and their production has become very innovative tough time. In addition to new products and changes in processing technologies, the requests of many people to arrive at new food formulations (lower calories, salt, sugar, free from lactose, cholesterol, animal protein) will need huge investments in research to find new alternatives to the traditional product formulations. The microflora of dairy products represents an area of study due to the wide diversity of dairy products. Many communities of microorganisms are explored, within which the diversity of yeasts and molds is still the most poorly studied. This review aims to discuss the dairy product research over the last 25 years, where the dairy mycoflora was one of the most important topics. (Awasti & Anand, 2020) [3] (Cenci-Goga *et al*. 2021) [7].

8. Conclusion

Yeasts and molds can act as spoilage agents in dairy products. However, the overall function of yeasts can be seen as more complex in that certain molds and yeasts are used in the production of many different fermented dairy products. The product is, in fact, their home, and the use by the dairy and milling industries still allows these species to survive. The problem is that this unwanted contamination of the raw material results in the need for continuous vigilance on the part of the industry by checking for contamination levels and conditions which are favorable to yeast and mold growth. Even though a greater understanding of the basic biology of yeasts and molds will ultimately help control these unwanted organisms, few studies on their morphology or interaction with their dairy environment at the gene level have been published. Initial microscopic examination, followed by molecular typing, can further our knowledge of the strain/species present and sometimes give information on the origin of the strain and its position in the ecosystem.

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