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Natural conservation attached to the use of microorganisms

Conservação natural atribuída ao uso dos micro-organismos

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Abstract

Breads are part of our basic food, with rare exceptions. We are currently involved in such a wide variety of brands and products that we end up opting for the item that matches our immediate desire for consumption. Consumers, on the other hand, are more concerned about their food and are looking for healthier foods with as few preservatives as possible. In this way, industries need to innovate and launch more natural options to meet these current changes. However, it is still necessary to combine research with technology to produce products that meet this new reality. This review includes the objective of reporting on bioconservation using micro-organisms that produce antimicrobial substances in order to sensitize industries and potential consumers about some studies on products made with bioconservants.
Keywords: Bioconservation, Lactic Acid Bacteria, Fermentation, Sourdough.

Resumo

Os pães fazem parte da nossa alimentação básica, salvo raras exceções. Atualmente estamos envolvidos por uma variedade tão grande de marcas e produtos que acabamos optando pelo item que corresponde ao nosso desejo imediato de consumo. Os consumidores, por sua vez, estão mais preocupados com sua alimentação e procuram alimentos mais saudáveis e com o mínimo de conservantes possível. Dessa forma as indústrias necessitam inovar e lançar opções mais naturais para satisfazer essas atuais mudanças. Contudo, ainda se faz necessário aliar a pesquisa com a tecnologia para elaborar produtos que atendam essa nova realidade. Essa revisão traz consigo o objetivo de informar sobre a bioconservação utilizando micro-organismos produtores de substâncias antimicrobianas a fim de sensibilizar as indústrias e os potenciais consumidores a respeito de alguns estudos sobre produtos elaborados com bioconservantes.
Palavras-chave: Bioconservação. Bactérias Ácido Láticas. Fermentação. Sourdough.

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Introduction

Fungi are versatile organisms capable of growing in various types of foods, including cereals, meats and fruits. They are important as deteriorators of different foods and cause significant economic losses in the food industry. Several strategies have been used to extend product shelf life, such as heat treatments, irradiation of infrared or microwave products, use of modified atmosphere in the package and addition of chemical preservatives (such as sorbic acid, benzoic and propionic), as well as prevention through the application of hygienic practices that reduce the microbiological contamination of the products.

In bakery products, the adoption of good manufacturing practices reduces fungus contamination, especially from ambient air, and the use of chemical preservatives makes fungus germination and growth difficult (LEGAN, 1993). A solution without the use of chemical preservatives would be the use of microorganisms during the elaboration of the products, which through their metabolites would offer a bioconservation to the products for the production of natural antimicrobial compounds (SCHNÜRER, MAGNUSSON, 2005). Food preservation based on the use of natural compounds or microorganisms has been highlighted as an alternative to reduce economic losses due to microbiological contamination of raw materials and food products in order to reduce the incidence of foodborne diseases (GALVEZ et al., 2008), as well as the occurrence of fungal deterioration.

Baked goods are easily subject to deterioration caused by molds and yeasts, especially when moisture is quite high, as is the case of sweet breads and breads (Smith et al., 2004). The development of molds leads to economic losses that are relevant to the baking industry, reducing sales, causing the formation of unpleasant odors, known as "off-flavors", and also promote the formation of substances harmful to health, such as mycotoxins (SMITH et al., 2004). Among the protective microorganisms, lactic acid bacteria (BAL) stand out due to their ability to produce compounds with fungicidal or fungicidal effects (DALIÉ; DESCHAMPS; RICHARD-FORGET, 2010). Some studies have demonstrated the antifungal activity of BAL in laboratory conditions (HASSAN; ZHOU; BULLERMAN, 2015; LAVERMICOCCA; VALERIO; VISCONTI, 2003), while other investigations have sought to highlight the ability of some antifungal BAL strains to effectively prevent fungal baked bakery products (CODA et al., 2011; RIZZELLO et al., 2011; RYAN; DAL BELLO; ARENDT, 2008; STRAIN et al., 2000; ZHANG et al., 2010).

Quality of baked products

Breads, like other baked goods, are perishable foods that undergo physical, chemical, sensory and microbiological changes during storage. The decrease in the quality and / or freshness of these products is closely related to storage time and is known as staling or staling. Over time, the firmness increases significantly, the crunchiness of the bread crust decreases, the bread loses its fragrance, assuming a rancid flavor. These complex phenomena are a consequence of the retrogradation of the gelatinized starch granules during cooking, the exchange of moisture between the starch and the constituent proteins of the bread, an increase in the interaction between the protein and starch fraction, a redistribution of water in bread and a removal of aromatic molecules. The occurrence of mold in food is potentially dangerous to public health and is also an important economic problem. In Western Europe, economic losses related to the presence of fungi on bread were estimated at more than 200 million euros per year (LEGAN, 1993), leading to losses of 1% and 5% of bakery products, depending on the season, type of product and mode of production (VEGA et al., 1998).

Among the most isolated fungi and related to the deterioration of baked goods, we can mention those of the genus *Penicillium, Aspergillus, Fusarium* and *Rhizopus* (LEGAN, 1993), while deteriorating yeasts of bakery products and ingredients belong to the genera *Zygosaccharomyces, Saccharomycopsis, Pichia, Candida* and *Debaryomyces* (LEGAN, VOYSEY, 1991). Baked products can be protected against deterioration caused by fungi and yeasts and by the spores that have contaminated the products using BAL in the preparation of fermented pasta since the use of these products provides certain antimicrobial activity (LAVERMICOCCA et al., 2000).

Some fungi are able to resist the use of certain preservatives. For example, some species of the genus *Penicillium* can grow in the presence of potassium sorbate (DAVIDSON, 2001) and other fungi have the ability to degrade the sorbate, including by stimulating the production of ochratoxin A (NIELSEN; RIOS, 2000). Bacterial breakdown of bread is mainly caused by a microorganism called *Bacillus subtilis* (COLLIN et al., 1991), derived from raw materials, bakery atmosphere and equipment surfaces (BAILEY, VON HOLY, 1993), becoming a major economic concern in the bakery industry (SUHR; NIELSEN, 2004).

Chemical preservatives

The most commonly used chemical preservatives are weak organic acids, such as propionic, benzoic and sorbic

acid; whose efficiency is higher at low pH because of the dissociation of their molecules (LEGAN, 1993). Previous studies have shown that calcium propionate, sodium benzoate and potassium sorbate were effective in inhibiting some isolates from bakery products at pH 4.5 when a concentration of 0.3% was applied. Potassium sorbate was also effective at a concentration of 0.03% (MARIN et al., 2002).

Bioconservation

Several reasons have led to the search for new alternatives to minimize the risks associated with presence or deterioration by fungi in food, which include consumer demands for food quality and safety, and increased government concern about environmental and safety issues. The term "bioconservation" refers to the prolonged shelf life and safety of foods that use microorganisms or their metabolites in their manufacture (ROSS et al., 2002). Thus, this concept occupies a prominent place, exploring the use of microorganisms and/ or their metabolites in different food processes.

Lactic Acid Bacteria (LAB) as Bioconservants

LABs are food grade microorganisms whose diverse metabolism and the ability to produce natural antifungal compounds become a versatile, technically feasible and cost-effective biological alternative for fungus control (SCHWENNINGER et al., 2005). Due to their nutritional needs, LABs are generally grown in enriched media and are found in dairy products, meats, meat products and cereal products (CARR et al., 2002). These bacteria are mainly divided into four genera: *Lactococcus, Lactobacillus, Leuconostoc and Pediococcus*. They are traditionally used as preservatives to prevent spoilage and to extend the shelf life of many foods.

LABs play a key role in the fermentation of foods, contributing not only to the development of desired sensory properties in the final product, but also to their microbiological safety (SMAOUI et al., 2010). According to Magnusson et al. (2003), three mechanisms may explain the antimicrobial efficacy of these microorganisms: the production of organic acids, competition for nutrients and the production of antagonistic compounds. The antimicrobial effect of LAB is mainly related to the production of lactic, acetic, propionic, sorbic, benzoic, hydrogen peroxide, diacetyl, ethanol, phenols and other protein compounds. Some strains are capable of synthesizing antimicrobial substances such as bacteriocins (DALIÉ; DESCHAMPS; RICHARD-FORGET, 2010).

However, the use of LAB requires an in-depth study on the parameters that modulate its antifungal properties. Several are the parameters, including temperature, incubation time, growth medium, pH and nutritional factors (BATISH et al., 1997). The studies performed by Sathe et al. (2007) demonstrated that the antifungal activity of Lactobacillus plantarum CUK501 was maximal (1,280 AU/ ml) at 30°C when the culture was at the end of its log phase. These results were consistent with those obtained previously by Batish et al. (1990), who observed that the antifungal activity of a strain of Lactobacillus acidophilus was maximal at 30°C after 48 hours of incubation, while increasing the incubation period resulted in a lower antifungal activity. This decrease was related to a metabolism of active compounds or to their enzymatic degradation.

The culture medium in which the microorganism will be inoculated can significantly modulate the metabolism of the antifungal compounds generated by LABs (BATISH et al., 1990; ROY et al., 1996). From some studies (BATISH et al., 1990) the Elliker broth was presented as the best medium for the production of antifungal compounds of *Lactococcus lactis* subsp. *lactis* CHD 28.3 and *L. lactis* subsp. *lactis biovar. diacetylactis* against fungi, compared to media M17 and MRS. The antifungal effectiveness of LAB can be enhanced with a slight increase in NaCl concentration (from 0.5% to 3%) in the growth medium for *Lactobacillus rhamnosus* (EFFAT et al., 2001) which may be related to a synergistic effect between NaCl with organic acids and antifungal substances metabolised by LAB (EFFAT et al., 2001). The pH also exerts a strong influence on the production of antifungal compounds by LAB (BATISH et al., 1997; SATHE et al., 2007). Studies have reported (CORSETTI; GOBBETTI; ROSSI, 1998) that the optimum pH range for the production of these compounds is between 5.0 and 6.0.

Many are the compounds released by the BAL. As its name suggests, lactic acid and acetic acid are the main products of the fermentation of carbohydrates by LAB. These acids generally recognized as safe agents for preserving foods, diffuse through the target microorganism membrane (PIARD, DESMAZEAUD, 1991) and then reducing the pH to inactivate cytoplasmic most metabolic activities of micro-bodies of interest. The acetic acid, by having a high dissociation constant and not being assimilated by the energy metabolism has been described as being more effective than lactic acid and is by far the best mold growth inhibitor (BATISH, et al., 1997). However, some authors (GAGIU, et al, 2013; GEREZ et al, 2013; SCHNÜRER; MAGNUSSON 2005), which describe antifungal activity of another organic acid from the LAB, as phenylaactic

acid that was able to inhibit the growth *Penicillium expansum* of MPI/FS2, and Aspergillus niger FTDC3227 iDM1, *Aspergillus flavus and Fusarium graminearum* FTDC3226 IDM623 at a concentration of about 50 mg/ml (LAVERMICOCCA et al., 2000).

Applications of Bioconservation

The use of natural ferments as a form of initiation and propagation of a fermented mass in brewing processes already have a long tradition and still plays a very important role in improving the quality and prolongation of the useful life of these fermented products (ARENDT; 2007, KATINA et al., 2006). The use of yeast has been intensely studied in recent times, due to consumer demand for foods containing a lower amount of chemical preservatives. Various yeasts have been developed to increase shelf life and improve sensory characteristics of bread. Natural yeast is a complex ecosystem in which microorganisms, exerting a symbiosis between yeasts with lactic acid bacteria, are highly adapted to environmental conditions (temperature, pH, acidity, maltose as the most abundant fermentable carbohydrate and fructose as a potential electrons), for the production of antimicrobials (VERA et al., 2009).

In a study by (CIZEIKIENE et al., 2013) strains producing organic acids and bacteriocins (*Lactobacillus sakei* KTU05-6, *Pediococcus acidilactici* KTU05-7, Pediococcus pentosaceus KTU05-8, KTU05-9 and KTU05-10) were used in preparation of fermented bread dough showing fungistatic and fungistatic activity against *Fusarium culmorum, Penicillium chrysogenum, Aspergillus fumigatus, Aspergillus versicolor, Penicillium expansum and Aspergillus niger*. This work indicates that these bacteria can be widely used in the food industry as bioconservants because of their broad spectrum of inhibition.

The use of L. *plantarum*, *Lactobacillus brevis* CR772 and CRL 796 in the formulation of starter cultures together with *Saccharomyces cerevisiae* was studied to significantly improve bread quality and prevent fungal decay (GEREZ et al., 2013). The viability of NaCl reduction in wheat bread focusing on shelf life and compensation using natural yeast in association with a chemical preservative has been studied (BELZ et al., 2012). The authors performed challenge tests using fungi commonly found in bakeries such as *Penicillium expansum, Fusarium culmorum and Aspergillus niger*. In this study, the NaCl reduction reduced the shelf life by 1-2 days. The addition of fermented pasta with antifungal activity prolonged the shelf life of 12-14 days, while the addition of 0.3% of calcium propionate provided a shelf life of only 10-12 days. The results of this study indicate that the addition of natural yeast prepared with a specific antifungal strain (Lactobacillus amylovorus DSM 19280) can replace the addition of chemical preservatives and compensate the reduced level of NaCl, guaranteeing product safety (BELZ et al., 2012).

Conclusion

From this analysis of data available in the literature on the antifungal activity of LAB, the ability of some strains to reduce or even suppress fungal growth through the production of several low molecular weight antifungal metabolites was highlighted. The control of the optimal conditions responsible for the higher production of in vitro and food matrix antifungal metabolites could increase the potential offered by BAL as natural food-grade bioconservatives. Thus, the use of BAL and its metabolites to control mold development seems to be a promising strategy for bioconservation in perishable foods or frequently contaminated by toxigenic fungi strains, especially bakery products. In view of the growing concern for food safety, microorganisms that are used because of their antifungal potential need careful food safety assessment in order to ensure a safe product for the consumer.

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