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Review

Alternative methods for mould spoilage control in bread and bakery products

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Abstract

Mould contamination of bread and bakery products is a cause for concern, and generates economic losses and consumers' dissatisfaction. The addition of organic acid salts to the process, as a preservative, is the main method to prevent this problem. However, other methodologies can be used to extend the product shelf life. Physical procedures, such as the modified atmospheres and the application of gamma irradiation, provide both advantages and disadvantages. On the other hand, processes including biopreservation and the action of antimicrobial compounds extracted from plants have been highlighted in the literature because of their considerable efficiency in retarding fungal growth. Therefore, the present review shows that, in general, different unit operations, natural preservatives, and predictive methods are effective tools to increase the shelf life of bread and bakery products. However, the large-scale use of these methods still relies on factors related to economic practicality, consumers' acceptance, as well as further studies in real food matrices for the validation of their effectiveness.

Introduction

Bread is one of the most important basic foods consumed by people around the world. It is an important part of the daily diet and, therefore, finding ways to improve its quality and increase the shelf life of bakery products is of utmost importance (Chaudhary, 1991). Wheat bread aging can occur through several factors, including microbial deterioration, mainly fungal, which is a common cause (Ryan *et al*., 2008).

Fungal deterioration implicates significant economic losses to the baking industry; affect the market, increase the disposal of products, cause distasteful odours known as "off-flavours", and even form harmful substances, such as mycotoxins (Legan, 1993; Smith *et al*., 2004; Dalié *et al*., 2010). Fungi are microorganisms capable of growing in all types of foods, including cereals, meats and fruits. These microorganisms are capable of spoiling numerous kinds of foods, especially when the intrinsic factors restrict bacterial growth (Pitt and Hocking, 2009). Fungal deterioration of wheat bread is mainly caused by *Penicillium* spp., which is responsible for about 90% of the spoilage of wheat products (Legan and Voysey, 1991). Other common fungi that deteriorate bakery products belong to the genus *Aspergillus*, *Wallemia*, *Mucor*, *Endomyces*, *Cladosporium*, *Fusarium*, *Rhizopus*, *Hyphopichia*, and *Chrysonilia* (Legan, 1993; Pitt and Hocking, 2009).

Chemical preservatives, such as propionic, sorbic, and acetic acids, as well as their salts, can retard fungal deterioration of bread. Although these compounds are classified as GRAS (generally recognized as safe), their use generates dissatisfaction due to resistance developed by fungal strains, solubility, toxicity, and potency (Dengate and Ruben, 2002; Suhr and Nielsen, 2004; Jing *et al*., 2014). In this context, studies have demonstrated the ability of alternative compounds and their antimicrobial effectiveness in food, including enzymes, bacteriocins, and essential oils (Suhr and Nielsen, 2004; Cagri *et al*., 2004; Avila-Sosa *et al*., 2012; Samapundo *et al*., 2017; Piwowarek *et al*., 2018). Moreover, other known methods include heat etreatments, infrared ray or microwave irradiation, and modified atmosphere packaging (MAP) (Gould, 1996).

Over the years, researches have focused on increasing the shelf life of various types of foods. Knowledge on deterioration by fungi is the main problem reported in bakery products; therefore, the objective of the present review was to describe the main alternative methods of fungal deterioration control in bread and bakery products.

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Factors influencing fungal contamination of foods

The growth of unwanted microorganisms, such as bacteria and fungi in foodstuffs, in addition to being responsible for deteriorating products, may offer risks to consumers' health and generate considerable economic losses (Filtenborg *et al*., 1996, Pitt and Hocking, 2009). Fungal spores are the main culprits of bread and bakery product deterioration. This process takes place after the appearance of visible mycelium from spores that develop on the product surface, which may happen after germination and before the end of the product's life, resulting in consumers' rejection (Baert *et al*., 2007; Dagnas and Membré, 2013). In addition to the unwanted appearance, fungi are also responsible for changes in the product sensory characteristics, such as taste and odour (Nielsen and Rios, 2000), due to the production of exoenzymes such as lipases, proteases, and carbohydrases (Filtenborg *et al*., 1996). Studies have aimed to determine the factors that lead to the contamination by unwanted moulds (dos Santos *et al*., 2016; Garcia *et al*., 2019). In bakery products, the air is described as one of the principal sources of contamination. Therefore, the spores present in the industrial processing environment may re-contaminate the food after baking, which happens mainly in the slicing and packaging steps (Andrade and Salustiano, 2008; Freire, 2011; dos Santos *et al*., 2016). The raw materials are the main source of fungal spore dissemination. The hygienic-sanitary conditions of the production environment and time that the bread is exposed to environmental air after their removal from the oven are also relevant factors that influence fungal load (Figure 1).

Other factors, such as temperature and relative humidity of the environment and product water activity (a_w), have also been reported as important factors for fungal growth in bakery products. This is because some raw materials, including barley, wheat flour, linseed, and maize have high risks of being contaminated by fungi, which may deteriorate the final product if found in the propitious conditions (Cauvain, 2003; Pitt and Hocking, 2009; dos Santos *et al*., 2016).

Increasing the shelf life of bread and bakery products

The baking industry has employed different methods to achieve significant microbiological stability of bakery products regarding shelf life. Among the available methods are techniques that reduce the contamination of freshly processed products (raw material quality, hygiene conditions of the production environment, factory layout), control of spore germination after mycelium growth (product formulation, packaging and storage conditions) and techniques for contaminant inactivation during processing (Dagnas and Membré, 2013). Cauvain (2003) emphasized that control of fungal deterioration in bakery products can be accomplished in several ways. The basic principles are based on:

(A) Access restriction of deteriorating fungi to products;

(B) Inactivation of deteriorating fungi or;

(C) Growth inhibition of deteriorating fungi.

The most common method for controlling fungal growth in foods is the use of antifungal agents. These are chemical substances that, when added to food, tend to prevent or retard fungal deterioration. In practice, most of these agents have fungistatic activity and are not fungicides. In other words, they stop germination when present, although growth may still occur in some cases. Fungicidal agents are more effective because they destroy microorganisms responsible for deterioration (Cauvain, 2003).

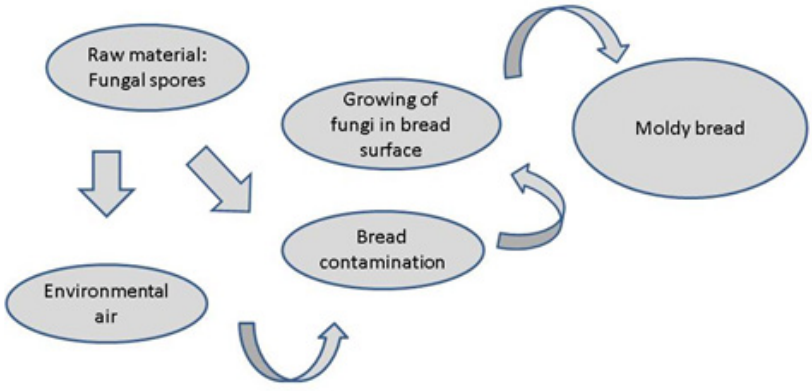

Figure 1. Processes involved in fungal contamination of breads in industry.

Table 1. Alternative methods to improve the shelf life of bread and bakery products.

Methods	Bakery product	Microorganism, technology, compound or predictive parameters	Shelf life	Action against fungi or mycotoxins	Reference
Modified atmosphere packaging	Sliced bread	50% CO_2 and 50% N_2	10 days	General fungi	Rodríguez *et al*. (2000)
	Soy bread	50% CO_2 and 50% N_2 or 80% CO_2 and 80% N_2	8 days	General fungi	Fernandez *et al.* (2006)
	Gluten-free fresh filled pasta	70% N_2 and 30% CO_2 and refrigeration	42 days	General fungi	Sanguinetti *et al*. (2016)
	Wheat and rye bread	CO_2 + N_2 + mustard essential oil	30 days	*Penicillium commune, P. solitum, Aspergillus flavus, Endomyces fibuliger, P. roqueforti, P. corylophilum, A. pseudoglaucus*	Suhr and Nielsen (2005)
	Prebaked pizza dough	100% CO_2	13 days	General fungi	Rodríguez *et al*. (2003)
	Sponge cake	CO_2 + a_w + pH	28 days	*Aspergillus montevidensis, A. glaucus, A. pseudoglaucus, A. ruber, A. niger, A. flavus, and Penicillium corylophilum*	Guynot *et al*. (2003a)
	Part baked bread	40% CO_2 + 60% N_2 + storage at 4°C	91 days	General fungi	Leuschner *et al.* (1999)
	Cakes	30% CO_2 or 100 CO_2 + a_w + pH	> 28 days	*Aspergillus montevidensis, A. glaucus* and *A. ruber*	Guynot *et al*. (2003b)
	Part-baked flat bread	100% CO_2	21 days	General fungi	Khoshakhlagh *et al.* (2014)
	Calcium-enriched wholemeal bread	60% CO_2 + 40% N_2	27 days	General fungi	Fik *et al*. (2012)
	Pita bread	100% CO_2	28 days	General fungi	Hasan *et al*. (2014)
	Rye bread	1% ethanol emitter	26-27 days	General fungi	Salminen *et al.* (1996)
Irradiation	Chiffon cake	Gamma irradiation: 4 kGy	90 days	General fungi	Sirisoontaralak *et al*. (2017)
	Bread and flour	Gamma irradiation: 6 kGy	-	A sharp drop in Fusarium toxins less than 5 µg/kg occurred in bread with flour irradiated	Aziz *et al*. (1997)
	White sandwich bread	Gamma irradiation: 0.2 to 0.5 kGy	5 days	General fungi	Hamza *et al.* (2016)
	Mexican wheat flour	Gamma irradiation: 1 kGy	Decrease in 75% of moulds	General fungi	Agúndez-Arvizu *et al*. (2006)
Biopreservation	Packed bread	*Lactobacillus plantarum*	2 days	*Penicillium sp.*	Gerez *et al*. (2010)
	Wheat flour hydrolysate	*Lactobacillus plantarum*	2 days	*Fusarium sp., Aspergillus sp.* and *Penicillium sp.*	Lavermicocca *et al.* (2003)
	Wheat germ	*Lactobacillus plantarum*, *Lactobacillus rossiae*	28 days	*Penicillium roqueforti*	Rizzello *et al*. (2011)
	Flour-based medium	*Weissel lacibaria*, *Leuconostoc citreum*, *Leuconostoc mesenteroides*, *Lactococcus lactis*, *L. rossiae* and *L. plantarum*	-	*Aspergillus niger, Penicillium roqueforti, Endomyces fibuliger*	Valerio *et al*. (2009)

Table 1. (Cont.)

	Wheat bread	*Lactobacillus plantarum, Wickerhamomyces anomalus*	28 days	*Penicillium roqueforti*	Coda *et al*. (2011)
	Bread	*Lactobacillus hammesii*	6 days	*Aspergillus niger, Mucor plumbeus, Penicillium roqueforti*	Black *et al*. (2013)
	Quinoa and rice bread	*Lactobacillus reuteri, Lactobacillus brevis*	2 days	General fungi	Axel *et al*. (2016)
	Bread	*Lactobacillus plantarum*	> 14 days	General fungi	Gerez *et al*. (2015)
	Bread	*Lactobacillus sakei, Pediococcus acidilactici, Pediococcus pentosaceus*	8 days	*Rhizopus stolonifer, Penicillium expansum, Aspergillus niger, A. versicolor, A. fumigatus, P. chrysogenum*	Cizeikiene *et al*. (2013)
	Gluten-free breads	*Lactobacillus amylovorus*	4 days	General fungi	Axel *et al*. (2015)
	Wheat bread	*Lactobacillus plantarum*	7 days	*Fusarium culmorum, F. graminearum*	Dal Bello *et al*. (2007)
	Pound cake and milk bread	*Leuconostoc citreum, Lactobacillus sakei, Lactobacillus plantarum, Lactobacillus spicheri, Lactobacillus reuteri, Lactobacillus brevis*	Data not shown	*Penicillium corylophilum, Aspergillus niger* and *A. pseudoglaucus*	Le Lay *et al*. (2016a; 2016b)
	Wheat bread	*Lactobacillus amylovorus*	2 days	*Fusarium culmorum, Aspergillus niger, Penicillium expansum, P. roqueforti*	Ryan *et al*. (2011)
	Dough	*Lactobacillus plantarum, Lactobacillus reuteri* and *Lactobacillus brevis*	5 days	Aspergillus niger	Gerez *et al*. (2009)
Essential oils	Edible films	Chitosan films	Increase in lag phase in culture media	*Aspergillus niger, Penicillium digitatum*	Avila-Sosa *et al*. (2012)
	Bread	Citrus peel (Orange) essential oil	4 days	General fungi	Rehman *et al*. (2007)
	Cakes	Encapsulated thyme (*Thymus vulgaris)* essential oil	30 days	*Penicillium raistrickii, Aspergillus fumigatus*	Gonçalves *et al*. (2017)
	Sliced bread and hot-dog bread	Mustard essential oil + MAP	-	*Penicillium commune, P. roqueforti, Aspergillus flavus, Endomyces fibuliger*	Nielsen and Rios (2000)
	Bread	Lemongrass essential oil	21 days	*Penicillium expansum*	Mani López *et al*. (2018)
Active packaging	Bread	Gliadin films	10 days	*Penicillium expansum, Aspergillus niger*	Balaguer *et al*. (2013)
	Sliced bread	Shelf-adhesive with polypropylene and Cinnamomum zeylanicum essential oil	90 days	General fungi	Gutiérrez *et al*. (2011)
	Sliced bread	Cellulose acetate films with sodium propionate	3 days	General fungi	Soares *et al*. (2002)

Table 1 (Cont.)

	Sliced wheat bread	Nanocomposite film and coating based on chitosan-carboxymethyl cellulose-oleic acid with zinc oxide nano-particles	3 to 35 days	General fungi	Noshirvani *et al*. (2017)
	Slice wheat bread	Packaging with ethanol emitter or ethanol emitter combined with and oxygen absorber	24 and 30 days	General fungi	Latou *et al*. (2010)
Predictive microbiology	Madeline cake	Growth: a_w and temperature	14 days	*Aspergillus candidus*	Huchet *et al*. (2013)
	Sliced bread	Growth: Ethanol	21 days	*Chrysonilia sytophila, Hyphopichia burtonii*	Berni and Scaramuzza (2013)
Emerging alternatives	-	Monocaprin	3 to 9 days	*Aspergillus niger, Penicillium citrinum*	Ma *et al*. (2018)
	Bread	β-defensin-3	> 13 days	*Fusarium culmorum, Penicillium expansum, Aspergillus niger*	Terry *et al*. (2016)
	Bread	Fermentates	20 days	*Penicillium paneum, P. chrysogenum*	Samapundo *et al*. (2017)
	Pound cake	Fermentates	46 days	*A. tritici, A. amstelodami*	Samapundo *et al*. (2016)
	Wheat bread	Hydrolysate from a mixture of legume flours	14 days	*Penicillium roqueforti, Aspergillus parasiticus, P. carneum, P. paneum, P. polonicum*	Rizzello *et al*. (2017)
	White bread	$CaCl_2$, $MgCl_2$, KCl, $MgSO_4$	No differences in lag phase	*P. roqueforti*	Samapundo *et al*. (2010)
	Gluten-free and wheat bread	Water-soluble extract from Amaranthus spp.	28 days	*Penicillium roqueforti*	Giuseppe Rizzello *et al*. (2009)
	Loaf bread	Allyl isothiocyanate	3 to 4 days	*Aspergillus parasiticus* and *aflatoxins*	Saladino *et al*. (2016)

Several reasons led to the search for alternatives that minimise the hazards associated with the presence of fungal spoilage in food, which include consumers' demands regarding the quality and safety of food, and increased government concern about environmental and safety issues. Therefore, the next topics will expose the main alternative and emerging methodologies being used to increase the shelf life of bread and related products. Some studies using alternatives and emerging methods to increase the shelf life of bread and bakery products are shown in Table 1.

Alternative methods

Modified atmosphere packaging

The purpose of food packaging is to minimise the changes that may occur in products. Therefore, modified atmosphere packaging (MAP) may be a useful method. This process consists of modifying the atmosphere where the food packaging is inserted with gases, such as nitrogen (N_2) and carbon dioxide (CO_2). These gases are used in bakery products mainly to increase shelf life by inhibiting fungal multiplication (Galić *et al*., 2009). The fungistatic action of CO_2 occurs by inhibiting metabolism and interrupting enzymatic activity. In addition, CO_2 can react with proteins and affect dissolution rates in water. However, the composition of the packaging materials and their permeability may interfere with the action of these gases (Ooraikul, 1991). Rodríguez *et al*. (2000) evaluated the shelf life of processed and preserved bread stored under different concentrations of gases, and observed that 50% CO_2 and 50% N_2, with and without calcium propionate, were the most effective in controlling fungal and yeast growths. Combined storage also helps increase the shelf life of bakery products. Ooraikul (1991) observed an increase of 10% in CO_2 concentration in the atmosphere inside the package and storage temperature reduction of 5.5°C, which doubled the shelf life of bread and cake.

Gamma and ultraviolet irradiation (UV)

Food irradiation consists of exposing a given material to ionising radiation coming from an electron machine or radioactive sources. Only the sources of ^{60}Co and ^{137}Cs are considered for commercial use due to the production of gamma rays of adequate energies, availability, and cost. Furthermore, the source of ^{60}Co is generally more accepted because it is used in the metallic form, which is insoluble in water and, thus, provides greater environmental safety (Silva and Roza, 2010).

The advantages of using gamma-irradiation in bakery products, such as wheat flour include food spoilage prevention through the reduction in insect infestation and microbial load (Agúndez-Arvizu *et al*., 2006). The disadvantage of the method is the high cost of irradiation chambers and equipment maintenance since no changes were observed in moisture, protein, and ashes in gamma-irradiated samples as compared to non-irradiated samples (Agúndez-Arvizu *et al*., 2006). Hamza *et al*. (2016) evaluated the effect of gamma irradiation on the reduction of microbiological contamination of white bread, and observed that loaves exposed to concentrations of 0.2 to 0.5 kGy showed decreased microbial counts.

Ultraviolet light (UV) is recommended to control the occurrence of fungal spores on bread, and the wavelength of 256 nm is known to have the best germicidal action spectrum. The fact that this technology does not emit heat or cause condensation on the packaging is a positive aspect; although its low penetrability into the food is a limitation. To mitigate this, it is also used on packaged bakery products during the slicing step (Cauvain, 2015). The disadvantages are related to the difficulty of radiating a multi-superficial product (considering the sliced surface), which makes penetrating the spores present on the bread surface difficult (Seiler, 1988; Cauvain, 2015).

Biopreservation

The increased interest in the biopreservation of food systems led to the use of new natural antimicrobial compounds from different origins. These include systems derived from animals (lysozyme, lactoferrin, magainin, etc.), plants (phytoalexins, herbs, spices, etc.), and microbial (bacteriocins, hydrogen peroxide, organic acids, etc.) metabolites. In addition, the biopreservation of food has been an alternative in the maintenance of foods that are considered healthy. The method consists of applying and/or producing *in situ* natural antimicrobials obtained usually by microbial fermentation and capable of inhibiting the proliferation of other microorganisms (Ross *et al*., 2002).

Among the microorganisms used in the biopreservation of bakery products are lactic acid bacteria because of its ability to produce organic acids with fungistatic or fungicidal effects. The organic acids generally produced are lactic, acetic, formic, phenylacetic, and citric acids (Valerio *et al*., 2009; Dalié *et al*., 2010; Rizzello *et al*., 2011; Magnusson *et al*., 2013; Axel *et al*., 2015). Samapundo *et al*. (2017) evaluated the substitution of calcium propionate by fermentates, which are products obtained from the

fermentation of raw food by microorganisms, such as lactic acid and propionic bacteria. These fermentates are declared as "clean label" and allowed for use in bakery products (Elsser-Gravesen and Elsser-Gravesen, 2014). The authors used the fermentate solids from corn syrup, citric acid, wheat, and dextrose solids, and observed that the fermentates FA (cultured with syrup and acetic acid) and FC (cultured dextrose) showed significant inhibitory activity ($p < 0.05$) against *Penicillium chrysogenum* and *P. paneum* and, as expected, the inhibitory activity of calcium propionate and fermentates increased and pH decreased.

Axel *et al*. (2015) observed that the application of *Lactobacillus amylovorus* as an antifungal compound producing agent (carboxylic acids) obtained from quinoa sourdough extended the shelf life of gluten-free bread by four days when compared with non-acidified control. Lavermicocca *et al*. (2003) evaluated the production and effect of the phenyllactic acid obtained from *L. plantarum*, and observed that concentrations up to 7.5 mg/L were able to inhibit 90% of fungal strains derived from bakery products, which included *Aspergillus ochraceus*, *A. flavus*, *Penicillium roqueforti*, *P. chrysogenum*, *P. solitum, P. commune, P. polonicum*, and *Fusarium* sp. Gerez *et al*. (2009) demonstrated that *Lactobacillus brevis*, *L. plantarum* and *L. reuteri* strains tested for bread preservation were able to inhibit the *Penicillium* sp. growth and extend the shelf life by two days when compared with bread prepared only with Saccharomyces cerevisiae. Valerio *et al*. (2009) reported that *Leuconostoc citreum*, *L. rossiae* and *Weisella cibaria* were able to inhibit in equal or higher concentrations the growth of *Aspergillus niger*, *Penicillium roqueforti* and *Endomyces fibuliger* when compared with the use of calcium propionate (0.3 w/v).

Essential oils

Extensive researches have been carried out to elucidate the chemical structures and activities of natural antimicrobials of fruits, vegetables, grains, herbs and spices (Fogliata *et al*., 2000; Kalemba and Kunicka, 2003; Suhr and Nielsen, 2003; da Cruz Cabral *et al*., 2013). For this, efforts were focused on the use of extracts, the extraction methods and verification of the antifungal actions of their essential oils (EOs).

Essential oils are liquid aromatic oils obtained from plant materials which generally consist of complex mixtures of various substances. The inherent flavour and antimicrobial activity of the EOs are commonly associated with the chemical structure of these components, the concentration present, and the interactions between them can affect the bioactive properties (Avila-Sosa *et al*., 2012). López *et al*. (2007) observed the fungicidal effect of the EO of cinnamon and oregano against *Penicillium islandicum* and *Aspergillus flavus* with concentrations near 0.5%. Another study showed that the inclusion of cinnamon and lemongrass EOs (2%) in active polypropylene films for packaging bakery products inhibited *Aspergillus niger* and *Penicillium commune* growth and increased the shelf life of these products by more than three times.

It is important to mention that the extraction methodology of EOs can interfere with their effect. Suhr and Nielsen (2003) reported that thyme EO (major chemical component thymol) had higher inhibition values when added to a culture medium made with rye bread, while the mustard (allyl isothiocyanate) and citrus EO's (citral b) showed the highest inhibition when applying the volatile exposure method against *Penicillium roqueforti*, *P. corylophilum*, *Aspergillus flavus* and *Endomyces fibuliger* strains.

Rehman *et al*. (2007) observed technological and sensorial modifications when using orange peel EO, and observed significant differences among the symmetry, crust, colour, taste, texture, and aroma of the formulated breads. However, it was possible to obtain the highest inhibitory action of microorganisms by spraying the orange extract on the slices of bread, which caused lower counts of fungi and bacteria. In addition, Krisch *et al*. (2013) reported that bread treated with EO vapour of marjoram and clary sage from the closed system exhibited strong odour, and had almost the same intensity after staying one hour at room temperature on a plate. The panellists reported that the taste and odour of the EO steam treated bread were unacceptable and strange.

Active packaging

The basic function of packaging is delaying product deterioration, quality maintenance, and food safety. In this case, packaging technologies with active antimicrobial properties prolong shelf life and control the quality of food products, reduce microbial action, and biochemical and enzymatic reactions through different strategies such using chemical additives/preservatives, oxygen, humidity and temperature control or a combination of these methods (Russo *et al*., 2017).

Antimicrobial packaging can be divided into two groups. The first one consists of the antimicrobial agent migrating from the package to the surface of the product. In the second one, the agents are effective

against surface microbial multiplication without needing to migrate into the product. In other words, the requirement for the operation of the antimicrobial package is intense contact with the food. Nevertheless, it is necessary to restrict the number of compounds in prepare the antimicrobial films for use in the food industry as they cannot contaminate or leave waste in the food (ANVISA, 2010).

Inedible films, for example, may reduce antimicrobial diffusion in the product because the essential oil is part of the chemical structure of the film and interacts with the polymer and plasticiser. Additionally, the release of antimicrobial compounds from the edible film depends on many factors, including electrostatic interactions between the antimicrobial agent and the polymer chains, osmosis, and antimicrobial and environmentally induced changes in structure. When compared with the direct application, smaller amounts of antimicrobial agents are necessary when edible films are used to achieve a specific shelf life due to gradual release on food surfaces (Sebti *et al*., 2005; Ponce *et al*., 2008).

Balaguer *et al*. (2013) evaluated the use of gliadin films containing 1.5, 3, and 5% of cinnamaldehyde, and observed that 3% concentration was able to increase the shelf life of bread and cheese by up to 10 days after inhibiting *Penicillium expansum* and *Aspergillus niger* growth. Kechichian *et al*. (2010) evaluated the effect of adding cinnamon and clove powder to edible films. The authors did not notice any difference between fungal growth when compared with the control. On the other hand, Otoni *et al*. (2014) noted a decrease in microbial counts when nano-emulsions of clove were added to the bread package and stored up to 15 days.

Predictive microbiology

Microbial modelling or predictive microbiology is the use of mathematical models or equations to predict the growth and/or activity of a microorganism in a food system over time (Jay, 2005). In the past, all existing models were used to describe bacterial behaviour, although not necessarily for the same purpose (Dantigny *et al*., 2011). With the development of predictive mycology, models have been developed to describe, mainly, fungal germination (Marín *et al*., 1996; Dantigny *et al*., 2005; 2011) and the inactivation of these microorganisms (Sant'ana *et al*., 2009; Dao and Dantigy, 2011; Garcia *et al*., 2019).

Dantigny *et al*. (2011) described germination models for fungal species know as food spoilers, such as *Aspergillus carbonarius*, *A. ochraceus*, *Fusarium verticillioides, F. proliferatum*, *Gibberella zeae*, *Mucor racemosus*, *Penicillium chrysogenum* and *P. verrucosum*. The authors suggested that the observed growth model was effective in predicting the germination rate of these fungal isolates when compared with the Gompertz model and the logistic equation that was previously used to describe the process. Huchet *et al*. (2013) developed a predictive model for *Aspergillus candidus*, which is the main spoilage agent for grains and flour products. The results showed a satisfactory fit for the model to the t_v (mycelium onset time), both *in vitro* and when tested in Madeline cake matrices. Dagnas *et al*. (2014) evaluated the influence of temperature, pH, and a_w in the growth rate of bakery product spoiling fungi such as *Aspergillus pseudoglaucus* (*Eurotium repens*), A. niger and *Penicillium corylophilum.* The authors observed that the same model can be applied to describe the effect of temperature on fungal growth for all species tested. However, the effect of a_w on the growth of *A. pseudoglaucus* mycelium differed from the other two species, concluding that another model would be necessary.

A growth/no growth model to verify the interference of pH, a_w, and ethanol concentration on the growth of *Wallemia sebi* and *Aspergillus glaucus* (*Eurotium herbariorum*) was developed by Deschuyffeleer *et al*. (2015). These types of growth models were designed to predict the probability of growth of a microorganism under a specific set of environmental conditions. The authors observed that the growth of the fungi tested was inhibited (>three months) at 5% ethanol concentration in the aqueous phase in a food matrix, regardless of the a_w values (between 0.75 and 0.89). The authors emphasized that, although the models were not fully validated in a real food matrix, the results indicated that the models were able to provide reliable predictions.

Kalai *et al*. (2017) modelled the effect of temperature, pH, organic acids and a_w in the germination time of *Penicillium camemberti* and *P. roqueforti*, which are the main species responsible for bakery products deterioration (Lund *et al*., 1996; Garcia *et al*., 2019). They observed a dependence of optimum pH for delaying the germination of conidia, and *P. camemberti* was more sensitive to propionic acid (Suhr and Nielsen, 2004).

Few studies have reported the thermal resistance of fungal conidia (Sant'ana *et al*., 2009). However, it is known that the cooking operation is a basic unit in the processing of bakery products and, assuming that raw materials can be contaminated with fungi that may damage the final product, Garcia *et al*. (2019) evaluated the kinetics of inactivating *Penicillium paneum* and *P. roqueforti* conidia during bread baking. The results demonstrated that, at 220°C, a smaller

delta value (time for the first decimal reduction) and t4D (time to four decimal reductions from the initial population) were required to inactivate the conidia of both fungi. However, *P. roqueforti* showed more resistance to higher temperatures than *P. paneum*.

Emerging alternatives

Several studies have demonstrated the potential for using new technologies and new chemical compounds that may increase the shelf life of bread and bakery products. Dao and Dantigny (2011) observed that the use of ethanol vapour has proven to be an efficient methodology for eliminating fungal conidia, and that it may be used to reduce contamination by toxigenic species in grains and stored cereals. In addition, fumigation with ethanol vapour in bakery chambers was effective in reducing fungal counts. However, this method required further optimisation.

Nano-particles of some compounds, such as titanium dioxide (TiO_2), zinc oxide (ZnO), and magnesium oxide (MgO), have been used in food packaging materials because of their antifungal capacity (Van Long *et al*., 2016). The action of these compounds is through the disruption of the fungal cell wall, which causes cytoplasm leakage and cell death (Pinto *et al*., 2013). Despite the efficacy, there is concern about the toxicological effects on consumers due to the residue presence of these compounds (Rhim *et al*., 2013).

Thery *et al*. (2016) evaluated the antifungal action of the synthetic peptide β-defensin-3 (HBD-3) and concluded that the compound had a deleterious effect on *Fusarium culmorum*, *Penicillium expansum* and *Aspergillus niger* growth. This reinforces the potential of these peptides to minimise the microbial deterioration of cereal products, mainly because this peptide is a heat resistant compound.

Ma *et al*. (2018) evaluated the effect of monocaprin, which is a compound obtained from capric acid and possesses antifungal abilities. The results showed that the compound efficiently inhibited Saccharomyces cerevisiae, *Aspergillus niger* and *Penicillium citrinum* growth over a broad pH range in addition to being more durable than potassium and sodium benzoate sorbate.

Conclusion

In general, the use of different unit operations, natural preservatives, and predictive methods are important tools to extend the shelf life of bread and bakery products. However, the large-scale use of these tools depends on economic practicality and consumers' acceptance.

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