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Study of benzoate, propionate, and sorbate salts as mould spoilage inhibitors on intermediate moisture bakery products of low pH (4.5–5.5)

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Abstract

A hurdle technology approach has been applied to control common mold species causing spoilage of intermediate moisture bakery products (*Eurotium* spp., *Aspergillus* spp., and *Penicillium corylophilum*), growing on a fermented bakery product analogue (FBPA). The factors studied included a combination of different levels of weak acid preservatives (potassium sorbate, calcium propionate, and sodium benzoate; 0–0.3%), pH (4.5–5.5), and water activity (a_w; 0.80–0.90). Potassium sorbate was found to be the most effective in preventing fungal spoilage of this kind of products at the maximum concentration tested (0.3%) regardless of a_w. The same concentration of calcium propionate and sodium benzoate was effective only at low a_w levels. On the other hand, potassium sorbate activity was slightly reduced at pH 5.5, the 0.3% being only effective at 0.80 a_w. These findings indicate that potassium sorbate may be a suitable preserving agent to inhibit deterioration of a FBPA of slightly acidic pH (near 4.5) by xerophilic fungi. Further studies have to be done in order to adjust the minimal inhibitory concentration necessary to obtain a product with the required shelf life.

Keywords: Mould; Bakery product; Weak acid preservatives

1. Introduction

Bakery products are widely consumed and are becoming a major component of the international food market (Kotsianis et al., 2002). It is very difficult to assess the losses of baked goods attributable to moulds; however, mould growth is considered the most frequent cause of bakery product spoilage (Corsetti et al., 1998; Earle and Putt, 1984, Legan, 1993). Xerophilic fungi are the most common microbiota causing spoilage of baked goods, as they are intermediate moisture products, causing considerable economic losses (Pitt and Hocking, 1997). *Eurotium*, *Aspergillus*, and *Penicillum* are the main spoilage fungi in Spanish bakery products (Abellana et al., 1997b). The most important factors controlling the growth of undesirable

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fungi on foodstuffs are temperature, pH, and water activity (a_w; Gibson and Hocking, 1997). The a_w of Spanish bakery products varies between 0.70 and 0.88 approximately, and their pH goes from 5.5 (croissants) to 8.0 (sponge cakes) (Abellana et al., 1997a). Recently, in order to achieve longer shelf life for bakery products, refrigeration is employed for prebaked or not prebaked doughs (Kotsianis et al., 2002).

The major preservation technique currently employed to prevent or delay spoilage in food products is the application of a combination of parameters, which may act synergistically to inhibit or retard microbial growth. The most common parameters applied are slight reductions in a_w, lowered pH, addition of antimicrobial agents, moderate heat treatments, etc. (Chirife and Favetto, 1992; Leistner, 1992). Among the most extensively used combinations of treatments are those in which an antimicrobial acid is applied and its effectiveness is enhanced by lowering the pH (Gould, 1996).

Weak organic acids such as propionic, benzoic, and sorbic are used to suppress the growth of microorganisms and to lengthen usable life of bakery products (Legan, 1993; Gould, 1996). The antimicrobial activity of these weak acids is mainly dependent upon the undissociated molecules (Eklund, 1983, 1985; Pethybridge et al., 1983). Previous in vitro studies have shown that calcium propionate, sodium benzoate and potassium sorbate were effective to inhibit some isolates from bakery products at pH 4.5 when they where applied at a concentration of 0.3%. Potassium sorbate was also effective at a 0.03% concentration (Marín et al., 2002a). However, others studies showed that the addition of the same weak-organic acids salts in a sponge cake analogue of pH 6 appeared to be effective only at low a_w levels (0.80–0.85; Guynot et al., 2002, Marín et al., 2002b). These weak organic preservatives seem to have a poor efficacy in bakery products of high pH (like sponge cakes); however, their application could be useful to extend the shelf life of products of low pH (around 5; Marín et al., 2002b).

The aim of the present work was to study the suitability of using both standard and sub-optimal concentrations of sodium benzoate, calcium propionate, and potassium sorbate to prevent mould spoilage of intermediate moisture bakery products of relative low pH (4.5–5.5). For this purpose two studies were carried out using both: (i) fermented bakery product analogue (FBPA) at pH 4.5 and (ii) same analogue at pH 5.5.

2. Material and methods

2.1. Fungal isolates

A total of seven isolates from different bakery products were used. Five of them, *Eurotium amstelodami* (3.205), *Eurotium herbariorum* (3.209), *Eurotium rubrum* (3.228), *Aspergillus flavus* (3.226), and *Aspergillus niger* (3.227) were isolated by Abellana et al. (1997b) from Spanish bakery products. These isolates belong to the Food Technology Department microorganisms collection of the Lleida University. The other two isolates, *Eurotium repens* (IBT18000) and *Penicillium corylophilum* (IBT6978), were kindly provided by the Department of Biotechnology of the Technical University of Denmark and had been isolated from Danish bakery products.

2.2. Experimental design

2.2.1. FBPA at pH 4.5

The factors assayed were weak-acid preservatives: calcium propionate (Fluka, Switzerland), potassium sorbate (Panreac, Barcelona, Spain), and sodium benzoate (Probus, Badalona, Spain), a_w (0.80, 0.85, 0.90) and concentration of preservatives (0%, 0.03%, and 0.3%).

2.2.2. FBPA at pH 5.5

The factors assayed were different concentrations of potassium sorbate (0%, 0.03%, and 0.3%) and a_w levels (0.80, 0.85, 0.90).

In both studies, a full factorial design was used and all treatments were repeated twice. The response recorded was colony radius.

2.3. Preparation of the analogue

The composition of both doughs was similar, only the pH was modified by the addition of citric acid. Dough was composed of 220.0 g of wheat flour, 37.5 g of sucrose, 3.0 g of dehydrated yeast, 1.1 g of salt, 60.0 g of butter, 30.0 g of eggs, and 60.0 ml of warm water

(30 °C). It was prepared by first mixing 60.0 g of flour, with the yeast and the water. The mixture was kept covered with a wet cloth at 30 °C for 1.5 h to favour yeast rehydration and fermentation. After that, 60.0 g of flour, the total amount of sugar, salt, and eggs as well as citric acid and preservatives, if required by treatment, were added to the mix which was hand kneaded for 20 min. Butter and the remaining flour were added, and hand kneaded again until dough became uniform and smooth. Finally, the dough was kept at 30 °C for 30 min and then flat-shaped and placed on aluminium plates. Baking conditions: oven at 160 °C for 30 min. Cooking foil was also put in the oven for sterilisation. After baking, plates were covered with the sterile cooking foil and transferred to the laminar flow bench. The analogues were exposed to UV light for 10 min to eliminate surface contaminants.

2.4. Adjust of pH and water activity

The basic dough after baking had a pH of about 5.5 and a a_w level in the range 0.76–0.80. To reach the pH 4.5, 1.07 g of citric acid was added to the mixed solid ingredients. This concentration was assessed by interpolation in an experimental curve obtained by plotting added citric acid versus resulting pH of FBPA. Finally, a_w level was adjusted by placing the FBPA in Petri plates containing water–glycerol agar of a specific proportion depending on the desired a_w: 110.4, 77.6, and 47.0 g of glycerol in 100 ml of distilled water plus 1.5% of agar, to adjust a_w of FBPA to 0.80, 0.85, and 0.90, respectively. In addition, a calibration curve was made to determine the concentration of glycerol in the agar needed to increase and maintain the a_w of the FBPA during the incubation period (data not shown).

2.5. Inoculation, incubation, and measurement

After baking, analogues were aseptically cut into 5×5×0.5 squared pieces, placed into 9-cm sterile Petri plates containing water–glycerol agar and once sealed with parafilm, they were kept at 25 °C for a 48-h equilibration period.

Mould inoculum was prepared by growing each isolate in DG18 (glucose 10 g, peptone 5 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, glycerol 220 g, agar 15 g, dichloran 0.2 mg, chloramphenicol 100 mg, in 1000 ml of distilled water) for 14 days at 25 °C to obtain sporulating cultures. Then, a spore suspension of 10^6 spores/ml was prepared in a 0.005% Tween-80 solution. Squared pieces were needle-inoculated in the centre, sealed with parafilm, and incubated at 25 °C for 28 days. Growth observation was carried out daily (or less frequently if required, e.g. at low a_w values) using a binocular magnifier.

2.6. Statistical treatment of the results

Analysis of co-variance of colony radius measured during the storage period, with time as a co-variable, was carried out for each fungal species separately, in order to find significant differences between the levels of factors assayed and their interactions. For this purpose, Statistical Analysis System package (SAS®, version 8.02, SAS Institute, Cary, NC, USA) was used.

3. Results

3.1. Fungal growth inhibition on FBPA at pH 4.5

In general, according to the co-variance analysis, all single factors (preservative, concentration and a_w) and their interactions had a significant effect on the growth of all isolates tested. The behaviour of all species was quite similar and, as expected, the growth was favoured by increasing levels of water activity (Fig. 1).

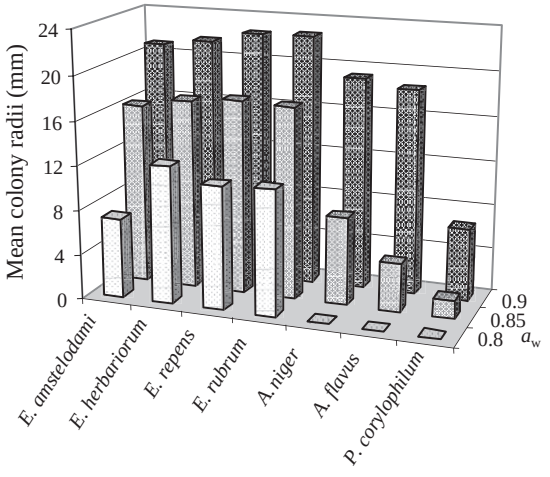

Fig. 1. Growth of all fungal species tested on a FBPA at pH 4.5 and at different a_w levels, after 28 days of incubation and 25 °C.

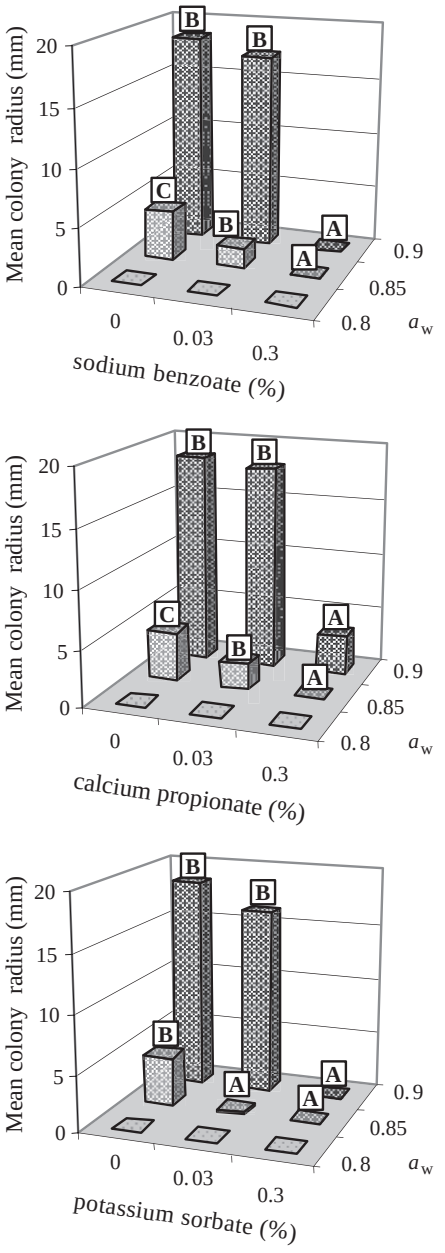

Fig. 2. Combined effect of preservative concentrations and a_w levels on *A. flavus* growth on a FBPA at pH 4.5, after 28 days of incubation at 25 °C. Bars with different letters within each preservative and within each a_w group are significantly different in LSMEANS test.

Further separate analyses were performed for each water activity level and the effect of each preservative concentration analysed by the LSMEANS test (represented by letters in Figs. 2 and 3).

Aspergillus spp. and *P. corylophilum* were more affected by some combinations of factors; they were unable to grow at 0.80 a_w. At 0.85 a_w, 0.03% of

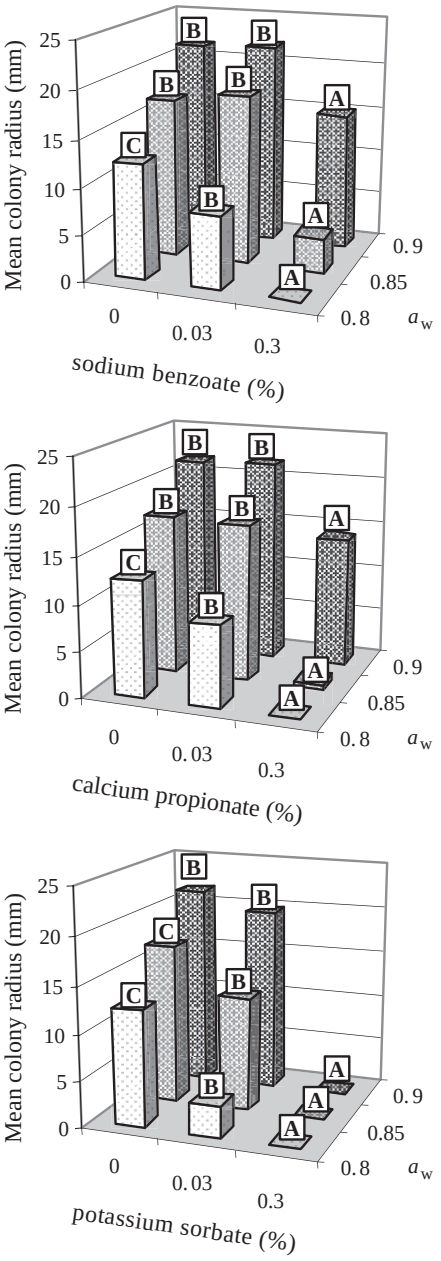

Fig. 3. Combined effect of preservative concentrations and a_w levels on *E. herbariorum* growth on a FBPA at pH 4.5, after 28 days of incubation at 25 °C. Bars with different letters within each preservative and within each a_w group are significantly different in LSMEANS test.

calcium propionate and sodium benzoate delayed their growth significantly; moreover, potassium sorbate completely prevented it (Fig. 2). In addition, a significant inhibitory effect was observed at the highest a_w tested (0.90), when assaying the higher concentration (0.3%).

Among the three preservatives, the most effective in fungal growth inhibition was potassium sorbate. All species growth was completely inhibited with 0.3% of potassium sorbate, regardless of a_w. Moreover, a 0.03% concentration was also effective at low a_w (0.80–0.85; Fig. 3).

Propionate and benzoate effectivity was restricted to the lower a_w levels and higher concentrations. In general no fungal growth was observed with 0.3% concentration of any of them at 0.80 a_w. At 0.85 a_w, the effect depended on the species, being *Aspergillus* spp. and *P. corylophilum* the more affected: 0.03% of benzoate or propionate only delayed *Eurotium* spp. growth at 0.80 a_w, and *Aspergillus* spp. and *P. corylophilum* at 0.85 a_w.

3.2. Fungal growth inhibition on FBPA at pH 5.5

According to the co-variance analysis, potassium sorbate concentration, a_w as well as their interaction had a significant effect on growth of all isolates. In general, analogues spoilage by all species was prevented by applying the highest concentration (0.3%) at the lowest a_w level (0.80). As water activity

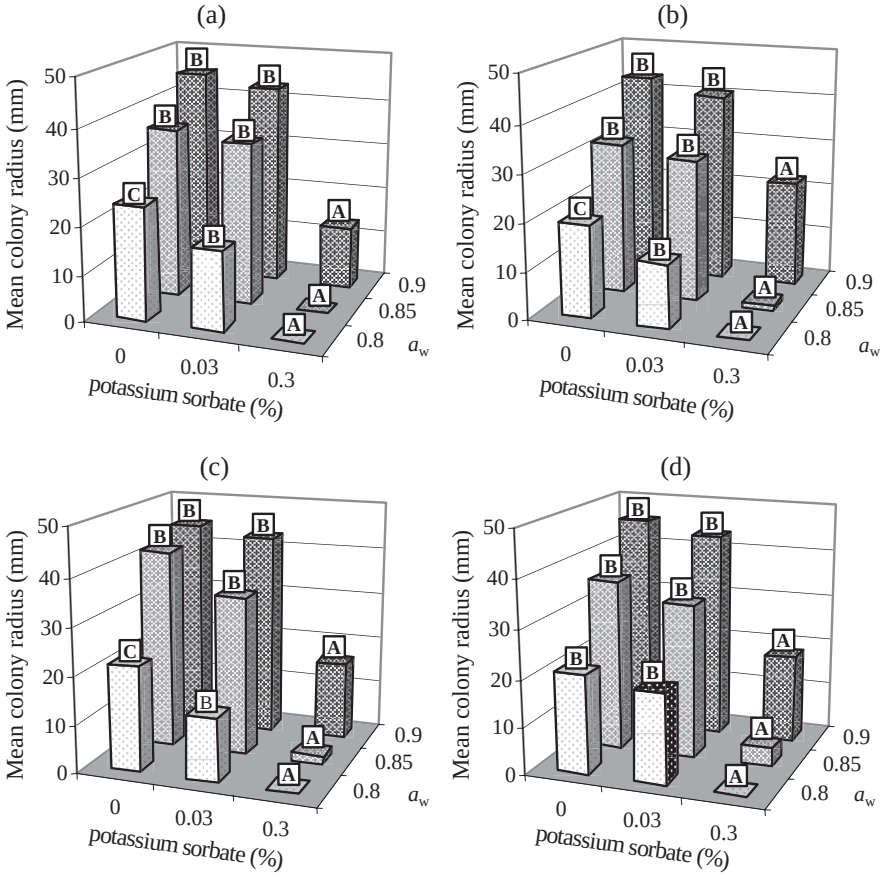

Fig. 4. Combined effect of potassium sorbate concentrations and a_w levels on *Eurotium* spp. growth, on a FBPA at pH 5.5, after 28 days of incubation at 25 °C: (a) *E. herbariorum*, (b) *E. amstelodami*, (c) *E. repens*, and (d) *E. rubrum*. Bars with different letters within each a_w group are significantly different in LSMEANS test.

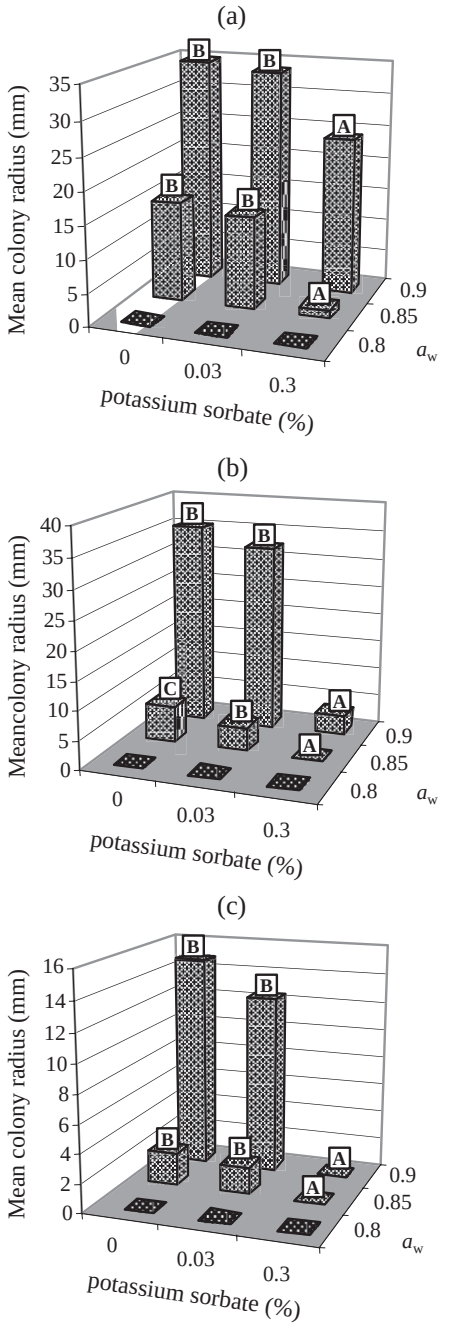

Fig. 5. Combined effect of potassium sorbate concentrations and a_w levels on *A. niger* (a), *A. flavus* (b), and *P. corylophilum* (c) growth on a FBPA at pH 5.5, after 28 days of incubation at 25 °C. Bars with different letters within each a_w group are significantly different in LSMEANS test.

increased, sorbate became less effective and the extent of the inhibition depended on the species. Among *Eurotium* species, *E. herbariorum* was the more sensitive as it was completely inhibited at 0.85 a_w, being other species growth only delayed (Fig. 4). At 0.90 a_w, growth of all fungi was significantly reduced by preservative addition, the inhibition being more important for *Aspergillus* spp. and *P. corylophilum* (Fig. 5).

On the other hand, a slight inhibition was observed with 0.03% of potassium sorbate at low a_w levels. *E. amstelodami*, *E. herbariorum*, and *E. repens* growth was significantly inhibited at 0.80 a_w, and that of *A. flavus* at 0.85 a_w.

4. Discussion

In this study, the combined impact of preservatives commonly used in bakery products and other important growth factors, such as a_w and pH on the prevention of fungal spoilage have been assayed. The range of microorganisms used in this study represents the typical mycobiota of Spanish bakery products consisting of xerophilic species of *Eurotium*, *Aspergillus*, and *Penicillium* (Abellana et al., 1997b). As bakery products are normally kept at room temperature, favouring xerophiles growth, studies were carried out at 25 °C.

In the present work, an analogue of fermented dough of acidic pH (4.5–5.5) was used. The two pH values assayed were chosen in the base of the well know dependence of weak acids activity with pH and also to keep the product near to its common organoleptic features.

Water activity is probably the most important environmental factor determining whether and at which rate a microorganism will grow on an intermediate moisture food (Seiler, 1988; Fustier et al., 1998; Membré et al., 1999). In general, a wide variety of Spanish bakery products have a_w values in the range 0.70–0.88 (Abellana et al., 1997a). However, water activity is not constant and undergoes changes during production and storage of foodstuffs (Vytrasová et al., 2002). One reason why water content or humidity in product surfaces becomes higher than expected, is the gradient of temperature between the product and the environment once

packed. Although common moulds present in raw materials and those that arise from the surfaces of the machinery are destroyed during baking, post contamination during cooling, finishing, or wrapping procedures is unavoidable (Seiler, 1988). No *Aspergillus* spp. either *P. corylophilum* growth was observed on a 0.80 a_w analogue free of preservative, at both pH assayed, like found before in sponge cake analogues (Marín et al., 2002b). *Eurotium* spp. were also more inhibited as water activity decreased; however, the addition of preservatives was always required to control their growth.

Our study confirms the dependence of such type of preservatives upon the pH of the product, as reported earlier by several studies (Earle and Putt, 1984; Seiler, 1988; Chirife and Favetto, 1992; Frías et al., 1996; Praphailong and Fleet, 1997; Fustier et al., 1998; Stratford and Anslow, 1998). Many investigators reported potassium sorbate as the most suitable preservative to be used in bakery products (Liewen and Marth, 1985; Thakur et al., 1994; Marín et al., 2002a; Guynot et al., 2002). Among the three preservatives tested in this study, potassium sorbate was found to be the most effective, as it was the only one that completely inhibited fungal growth at 0.90 a_w, at pH 4.5. The current accepted theory of week acid preservative action suggests an inhibition via internal pH depression by directly inhibiting glycolysis enzymes (Lück, 1981). The undissociated acid molecule, which is most lipophilic and therefore most ready to permeate the membrane, on entering the cytoplasm tends to dissociate (as normally internal pH is near 7), delivering hydrogen ions, along with particular anions (Gould, 1996). Stratford and Anslow (1998) suggest that sorbic acid have also an inhibitory role as a membrane-active compound, what makes its activity less linked to the pH medium as benzoic and propionic acid.

Potassium sorbate was found to be less effective in the FBPA at pH 5.5. At this pH, a 0.3% concentration was effective in controlling fungal growth only at 0.80 a_w; as water activity increased fungal growth was only delayed. Comparing with previous studies carried out in artificial agar medium by Marín et al. (2002a), in the present work less activity of potassium sorbate was observed against the same set of fungal species. This could be due to interactions of potassium sorbate with food components, such as proteins and lipids, decreasing their level (Ledward, 1990; Jideani and Wedzicha, 1994; Thakur et al., 1994).

Beside its less linked activity to pH, sorbic acid has others advantages that make it the most suitable preservative: it has not residual taste or at least less than other preservatives and has a lower price. On the other hand, its main drawback is that it has an adverse effect on yeast activity and thus on dough rheology, producing a serious reduction in loaf volume and making dough sticky and difficult to process (Legan, 1993). However, sorbates are widely used in German sourdough breads, where presumably the long fermentation times and mixed ferment of yeast and lactic acid bacteria make the inhibition of yeast activity less important (Legan, 1993). A good alternative could be the application of sorbic acid or sorbate immediately after baking by spraying (Legan, 1993; Earle and Putt, 1984).

This study indicates that potassium sorbate is a suitable preserving agent to inhibit growth of xerophilic fungi in bakery products of pH near 4.5 regardless a_w level. For products of slightly higher pH (5.5) the addition of this preservative must be combined with low water activity levels (0.80), otherwise other additional controlling factors must be applied. Further studies have to be done in order to adjust the minimal concentration of sorbate needed to preserve this kind of products.

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