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Effect of temperature on inactivation kinetics of three strains of *Penicillium paneum* and *P. roqueforti* during bread baking

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ABSTRACT

In this study, the inactivation kinetics of strains of *Penicillium paneum* and *P. roqueforti* were determined during bread baking. Three strains of *P. paneum* (PR03, PR04, and PR05) and *P. roqueforti* (PR06, PR11, and PR67) were used. Baking conditions were based on those used in baking industries (160 °C, 190 °C and 220 °C). The inactivation curves did not follow first-order kinetic, and as the primary model, the Weibull model was used with the fixed p-value. The $t4_D$ value was also determined, and the secondary model was built using the log δ as a function of the baking temperature. Lower values of δ and $t4_D$ were obtained at 220 °C, and the values of this parameter were different ($p < 0.05$) among the three *P. paneum* strains at 160 °C and 220 °C. Two strains of *P. roqueforti* (PR06 and PR11) showed the highest values of $t4_D$ at 190 °C and 220 °C (10.2 and 8.18 min. respectively). The results of this study demonstrate that the different baking temperatures of the bread making process may result in the survival of fungi in the product, which may be vital in limiting the shelf life of these products. The quantification of fungal inactivation during bread baking is critical for the design of thermal process aiming to balance quality and microbiological stability of industrialized loaves of bread.

1. Introduction

Fungi are the main microorganisms involved in the spoilage of bread, mainly due to their composition [moisture content (around 40%), water activity (a_w 0.94 to 0.98), intermediate acidity (pH = 5.5–6.0)] and the storage temperature these products are exposed during commercialization (20–35 °C). These factors will result in the shelf life of 3–7 days for most bread that does not contain preservatives (Legan, 1993; Saranraj & Geetha, 2012). However, losses in the bakery industry attributed to fungi may reach up to 10% in countries such as Brazil, depending on factors such as product formulation and storage method (Freire, 2011).

Flour and other ingredients used in the bakery industry are considered the primary sources of contamination due to the presence of fungal spores that are adhered and can be diffused through flour particles dispersed in the air of processing premises (Rogers & Hesseltine, 1978). The main fungal genera involved in the spoilage of bread are *Penicillium (P. roqueforti, P. brevicompactum, P. chrysogenum, P. paneum), Wallemia, Aspergillus [(*syn. *Eurotium), Chrysonilia, Rhizopus,* and *Mucor]* (Garcia, Bernardi, Parussolo, Stefanello, & Copetti, 2018; Legan, 1993; Magan & Aldred, 2006; Pitt & Hocking, 2009, p. 519; Santos et al., 2016).

While *P. roqueforti* is mostly considered beneficial due to its contribution to the taste and aroma of blue cheeses, it is also deemed a spoilage fungus able to grow rapidly at refrigeration temperatures (Pitt & Hocking, 2009, p. 519). *P. roqueforti* is also resistant to calcium propionate (Suhr & Nielsen, 2004), the mainly preservative used in the bakery industry. Furthermore, this species has been responsible for the spoilage of rye bread in Europe (Lund, Westall, & Frisvad, 1996) and several types of industrialized bread in Brazil (Garcia et al., 2018). *P. paneum* has also been reported as an important spoilage fungus of rye bread, silage, cassava (Frisvad & Samson, 2004), and multigrain bread (Santos et al., 2016).

Several physical, chemical and biochemical changes occur throughout bread processing steps, especially during baking (Cauvain & Young, 2007). In this step, the interior of bread reaches temperatures around 95 °C and higher values of a_w (> 0.90) than in the crust (< 0.50). Some studies report that most fungal spores are destroyed due to the temperature reached (Knight & Menlove, 1961). However, fungal survival after the processing depends on the initial fungal load

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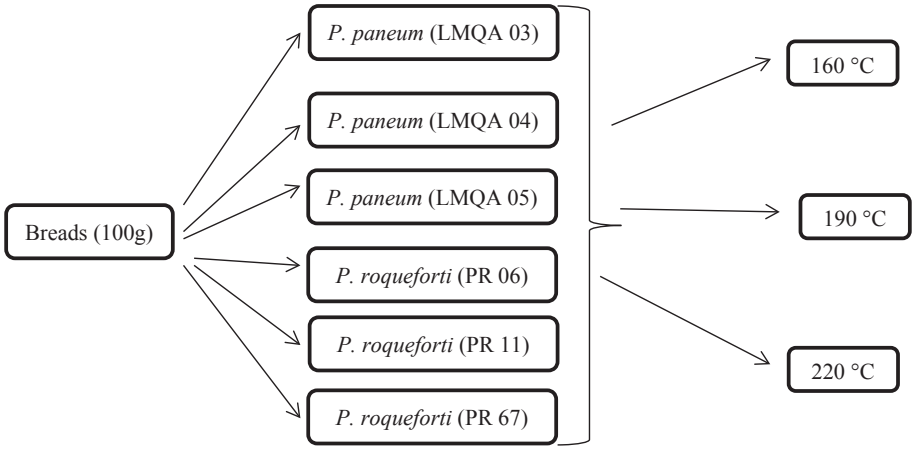

Fig. 1. Experimental design for assessment of *P. paneum* and *P. roqueforti* strains inactivation during bread baking.

present in the product, on bread's composition, and the storage conditions employed. Therefore, even though bread baking is applied for technological purposes, it plays a fundamental role in reducing the microbial contamination and contributing to the stability of the product during the shelf life. Despite its relevance of for bread's shelf life, there is a lack of studies describing the inactivation kinetic parameters of fungi and quantifying thermal inactivation of fungal spores during bread baking. The quantification of fungal inactivation during bread baking is key for the design of thermal process aiming to balance quality and microbiological stability of industrialized bread. Thus, the objective of this work was to model the inactivation kinetics of three different strains of *P. paneum* and *P. roqueforti* during the bread baking process.

2. Material and methods

2.1. Strains of fungi and preparation of the suspension of conidia

Three *P. paneum* strains (LMQA03, LMQA04, LMQA05) and three *P. roqueforti* (PR06, PR11, PR67) were used in the present study. These strains were isolated from different brands/lots of spoiled bread (Garcia et al., 2018; Santos et al., 2016).

For the preparation of conidial suspensions, the strains were individually inoculated in malt extract agar (MEA) (malt extract, 20 g (Neogen, Lansing, USA); glucose, 20 g (Labsynth, Diadema, BR); agar, 20 g (Kasvi, Curitiba, Brazil); casein peptone, 1 g (Kasvi, Curitiba, BR); distilled water, 1 L; 1 ml Trace Metal], and incubated at 25 °C for 7 days (Delgado, de Souza Sant'Ana, Granato, & Rodriguez de Massaguer, 2012b, 2012a; Pitt & Hocking, 2009, p. 519; Wigmann, Moreira, Alvarenga, Sant'Ana, & Copetti, 2016). The concentration of conidia in each fungal suspension was standardized in 10^8 conidia/mL using a Neubauer chamber (Sigma-Aldrich). The concentration of conidia was confirmed by plating the suspension in MEA (25 °C/5 days). The standardized suspensions were further stored at 2 °C for up to 3 months. Stored spore suspensions were periodically checked through microscopy and enumeration on MEA agar to ensure their concentration remained stable.

2.2. Bread making

Bread loaves (squared shape) of about 100 g were prepared [715 g of wheat flour, 14.3 g of sugar, 10 g of salt, 14.3 g of milk powder, 7.15 g of baking powder, 430 ml of distilled water per kg of bread]. The ingredients were weighed and homogenized in an electronic bread machine (Electronic Philco®, model 54-302-004, 110v, Manaus, Brazil), which was able to mix the ingredients to allow dough formation. The inoculum was added to the water before mixing the ingredients for better distribution in the dough, so it was possible to obtain the concentration of 10^6 conidia/g. Each bread (100 g) was molded and laid in individual baking trays of greased aluminum foil. Then, the filled baked trays were placed in an air-circulating incubator (45 °C) for 30 min for fermentation. Then, the bread was cooled down to 25 °C (around one h) inside an incubator regulated at 25 °C until the time of baking. This procedure was conducted in order to allow the bread loaves to reach the same initial temperature at the beginning of the baking process.

2.3. Baking of bread

After the fermentation, the loaves of bread were submitted to the following treatments: *i)* baking at 160 °C, *ii)* baking at 190 °C and *iii)* baking at 220 °C. An industrial oven (8–4000 W, Imequi, São Paulo, Brazil) was used. The temperatures chosen were based on those used for the production of form bread in bakery industries (personal data).

The temperature in the center of the oven was monitored with a thermocouple (flexible copper-constantan type T, RSA, Brazil) fixed immediately above the grid in which the bread was arranged to be baked. The thermocouple was coupled to a temperature recorder (Hydra Series II, Fluke, Everett, USA). After the oven reached the required temperature (160 °C, 190 °C or 220 °C), the loaves of bread were introduced in the oven for baking and the time registration was immediately initiated once the oven was closed. This procedure was repeated for all single bread subjected to different baking times. The total process time varied for each experimental condition, and it was adjusted to ensure an inactivation curve was obtained.

The internal temperature of each bread was monitored during the tests, also with the aid of a T-type thermocouple fixed in the center of the dough, and recorded as a function of the baking time. Two independent replicates were performed for each experimental condition. The experimental design is illustrated in Fig. 1.

2.4. Enumeration P. paneum and P. roqueforti conidia

At the pre-defined times, the loaves of bread were removed from the oven and immediately cooled, aiming to interrupt the thermal inactivation. For this, the loaves of bread were immediately packed in sterile stomacher bags (Nasco, Whirl-pak, USA) and submerged in a water-ice bath. After cooling, the samples were analyzed to quantify the population of surviving microorganisms in the dough. For this purpose, 25 g of sample was weighted (taking care to proportionately sample the crumbs and crust of bread and homogenized with 225 ml of 0.1% peptone water for 1 min (Stomacher 400, Seward Lab System, USA). Subsequently, the aliquots were subjected to serial decimal dilution and plated in MEA supplemented with chloramphenicol (50 mg/L, Inlab, São Paulo, Brazil) and incubated at 25 °C for five days. After the