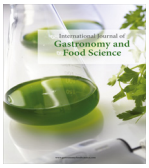

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Scientific Paper

Microbiota distribution in sourdough: Influence of high sucrose resistant strains

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Abstract

Microorganism distribution found in wild pre-fermentations in bakery (sourdough) may influence final properties of produced bread. The intent is to study the general microbiological distribution by executing four different cultures: fungus, yeasts, total counting and sucrose resistant, in the same sourdough's samples.

The effect of fruit addition into the mixture between flour and water determine significant changes in the final sourdough stability and strength. This addition increases the possibility of obtaining a proper environment for the controlling microorganisms. Sucrose presence, allows a natural selection of white-cream sucrose-resistant colonies. Yeast cells are a collection of single-cell fungi that will rapidly reproduce in the right conditions. Yeast requires a form of sugar or starch as food and a moist environment to grow in. The best temperature for growth is in the range of 43 to 46 °C, but the best products to make bread are formed from 27 to 35 °C although the growth rate achieved is smaller. If bread dough is kept colder than this temperature range, the yeast will not grow sufficiently. On the other hand, if water that is too hot is added to yeast, the yeast cells will not grow since the high temperatures can kill them. A temperature of 60 °C will kill most yeast cells. Obviously, the temperature at which bread dough rises is important to the overall results. Presence of dextran, increase the quality of formed bread. This study pretends an approach to microbiology of baking fermentation cell density and influence of sucrose resistant strains.

Keywords: Sourdough; Micro-biota distribution; Quorum sensing; *Leuconostoc mesenteroides*; Zoogleal

Introduction

When we use the term sourdough we are speaking about a wide range of things. Natural bread fermentations, are different between spontaneous sourdough (as in this study) and continuously backs lopped sourdoughs (were *L. sanfranciscensis* is dominant) (Mine and Sugihara, 1971). Also different biota are reported in different world populations (Galal et al., 1977; Scheirlinck et al., 2007) but in general it is able to maintain a established biota for long time in all of them and use it as baking CO_2 starters.

The presence of *Leuconostoc mesenteroides* strains in pre-fermentations can be crucial for the microbial habitat and culinary properties of sourdough. Besides, the kind of the additive used is critical for the success of the experiment. The bread flavour is implemented in their organoleptic characteristics with an intense biological culture; those microorganisms contribute to develop acid-lactic, fruity or roasted hints.

Fermented foods produced from cereals, for example beer, spirits, sake, porridge and baked goods, have a very long history, and nutritional and economic importance (Bottéro., 2005). One of the ancient means of cereal fermentation is the traditional sourdough process (Vogel et al., 1999). Sourdough is a mixture of flour and water that is fermented with lactic acid bacteria (LAB) and yeasts (Gänzle et al., 1998; Vogel et al., 1999). Nevertheless, there are few studies related to bakery

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microbacterial activity (De Vuyst et al., 2009). The interest was in the general bacterial microbiota distribution, but when the study began, the initial focus of the work moved on to a specific microbe, which would have a proper growth in a sugary environment like sourdough. The predominant lactic acid bacterium in type I sourdoughs are *Lactobacillus sanfranciscensis* (Mine and Sugihara, 1971), but other species of the genera *Weissella*, *Lactococcus*, *Leuconostoc* and *Enterococcus* are also found (Rocha and Malcata, 1999; Corsetti et al., 2001, 2003; De Vuyst et al., 2002; Ehrmann et al., 2003; Meroth et al., 2003; De Vuyst and Neysens, 2005). The initial idea, was that this key microbe could grow up easier if the progress was carried out in a sourdough, where sucrose or any kind of fruit addition was added, and control the development of micro flora distribution (Quorum sensing). The percentage and the fruit type had to be controlled, because each additive has different levels of sucrose and affect in a different way. In any case sugar excess in the ambient wouldn't be positive, for sucrose resistant microorganisms, and that situation would change the microbiological development and stabilization of sourdough. Use of selective culture media is the most usual way to establish micro-biota distribution but, this approach has been repeatedly criticized because only easily cultivable microorganisms can be detected, while members that need selective enrichments or require particular physiological conditions (in a sub lethal or injured state) are lost, (Iacumin et al., 2009). Here we also use high sucrose media in order to determine presence of sucrose-resistant strains.

Material and methods

Different culture media types have been used in order to determine the microbiological growth in seven different sourdoughs. Sourdoughs for testing have been done using traditional techniques, described by (Marqués et al., 2007), but different additives were added to obtain different microbiological populations in sourdough: Apricot, Spelt, Strong wheat flour, White cider, Rye cider, Blueberries and Rye. The sourdough has been stabilized according with (Tejero, 2006; Barber et al., 1989) and (Martínez-Anaya et al., 1990). The sourdough was ready for use when it had reached three times its initial volume. This should be achieved at temperatures near 25 °C. Once it has reached primary part of fermentation, it is possible to allow cold storage to keep dough cold. This method lets the dough at room temperature (25 °C) and once reached twice its initial volume, about 6 h, place it in the cold chamber at 10 °C. This will have a wider range of time the mass in good condition. All the old sourdough was used in every refreshing step (increasing the volume) and fruit and additives are added initially not in refreshments.

Six different samples for every experiment were taken aseptically, and analysed immediately. Five different experiments were performed (35 total samples for each data). In order to get the exact dilution we need to achieve countable colonies in all samples simultaneously, been made in previous experiments. Sourdough samples were dissolved (1:500, w/v) and serially diluted in sterile peptone-physiological solution [0.1% (w/v) bacteriological peptone (Oxoid) and 0.85% (w/v) NaCl between −4 and −5 decimal dilutions.

One ml from diluted samples was inoculated in pure agar plaques with 15% sucrose according with the reference for high sucrose media (Bravo, 2010). In order to favour different bacteria's development they were used four different standard culture media: Total counting: APHA (Cultimed), Glucose-Potato Agar Cultimed), Saboreau-Chloramphenicol, (Yeast and mould Cultimed) and agar with 15% sucrose according with (Bravo, 2010). As well as *Leuconostoc mesentoroides* grows selectively in a sucrose media a high sucrose environment has been used.

Cream coloured single colonies from high concentration sucrose cultures, were analysed through the RapID STR System test (Remel Microbiology), in order to determine the bacterial enzymatic spectre of isolated colonies.

Results

Different types of agars have been used, in order to determine the microbiological growth in several sourdoughs simultaneously (Fig. 1). Previous other authors (De Vuyst et al., 2009; Ravyts and Vuyst, 2011) situate the microbiological total count level in sourdough in a wide range between 2×105 and 7×107 cfu/ml. In the study, with a dilution of 1/50,000 (5×104 cfu/ml), it can be appreciated by using different culture media that microorganism distribution is extended in a wide range. It's important to consider the use of this specific dilution, because of the high variability of sourdough samples. In some cases (dry apricots, rye cider and spelt) the total count is up 1.5×107 but in some others (strong wheat flour, blueberries and rye) are less than 5×106 cfu/ml (Fig. 1).

Is also relevant the different response of classical yeast culture media, like yeast and mould or potatoes-sucrose agar. In dependence of additives, colonies grow in yeast culture

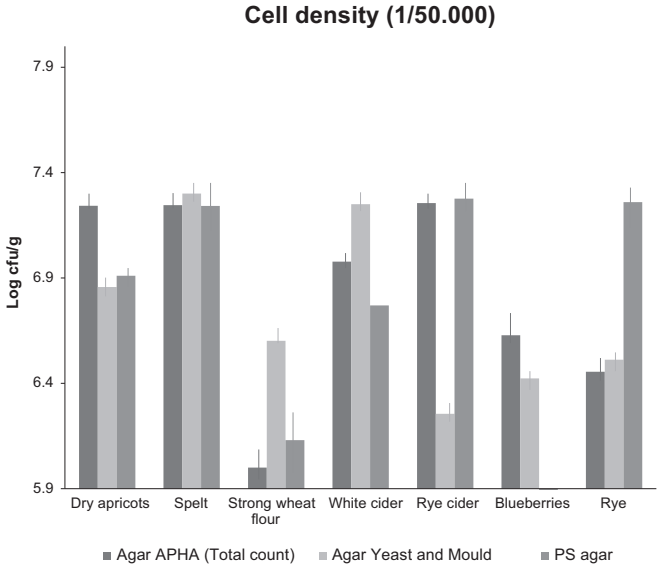

Fig. 1. Cell density from different sourdough in specific culture media.