UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE CIÊNCIA E TECNOLOGIA DE ALIMENTOS

CURSO DE ENGENHARIA DE ALIMENTOS

Influência de diferentes concentrações de células viáveis de *Lactobacillus plantarum* e *Saccharomyces cerevisiae* como culturas *starters* em pães *sourdough*

Renata Ferreira Ferraz

Porto Alegre

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Trabalho de Conclusão de Curso, apresentado ao Instituto de Ciência e Tecnologia de Alimentos, da Universidade Federal do Rio Grande do Sul, para obtenção do Título de Engenheiro de Alimentos.

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“É exatamente disso que a vida é feita: de momentos. Momentos que temos que passar, sendo bons ou ruins, para o nosso próprio aprendizado. Nunca esquecendo o mais importante: Nada nessa vida é por acaso. Absolutamente nada. Por isso, temos que nos preocupar em fazer a nossa parte, da melhor forma possível. A vida nem sempre segue a nossa vontade, mas ela é perfeita naquilo que tem que ser”.

Chico Xavier

ACKNOWLEDGEMENT

To my family, for the education, the support, the love and especially all the patience dedicated to me, not only during college, but also throughout my entire life. I dedicate this work to my father Sergio, and my mother, Marta.

To my boyfriend Rafael who was present during all phases of this work, always encouraging and helping me when needed! Thank you so much for handling my college complaints and stresses! Your affection and patience were very important!

To my friends Bruna, Dani, Natasha and Stefani who were with me during these long and difficult years of Engineering! It was great spend this last year of college even more united! You sure have given me the best moments of UFRGS! Without you, none of this would have been so graceful! Thank you so much for all the happy moments, all the times you have help me to do the endless exercise lists, for all the conversations we had, everything! I wish you all a lot of success in the future!

To my friend Laura that is present in my life since the time we used to count "4 years to 20" and we thought we were old! Thank you for waking me up and letting me know that I had passed the entrance exam! Maybe I did not know until today! Thank you for the friendship and all the moments that we spent together! Thank God, you moved to Porto Alegre and we stayed even closer!

To my professors Jeverson and Roberta! Thank you for all the valuable advice and teachings, not only for this work but also for my life! Thank you for all the time dedicated to me and for always being willing to help me! I loved having you as supervisors! Our passion for breads has united us!

Finally, to all the people who helped me during all this work and who were part of my academic life, but were not mentioned here! You are also part of this achievement!

Influência de diferentes concentrações de células viáveis de *Lactobacillus plantarum* e *Saccharomyces cerevisiae* como culturas *starters* em pães *sourdough*

RESUMO

O uso da fermentação tipo *sourdough* para a produção de pães contribui para a melhoria de muitos atributos de qualidade nos pães. A utilização de culturas *starters* pré-selecionadas surge como uma alternativa para controlar o processo e otimizar a fermentação e a qualidade final do pão. O objetivo deste estudo foi avaliar a influência de diferentes concentrações de células viáveis de *Lactobacillus plantarum* e *Saccharomyces cerevisiae*, quando utilizados como culturas *starters* para a fabricação de pães tipo *sourdough*. Para avaliar a influência da cultura *starter* nas propriedades dos pães foi utilizado o método de superfície de resposta. Através de um planejamento fatorial 2^2, sete pães foram estudados no que diz respeito atributos sensoriais, características de acidificação, volume específico, cor da crosta e firmeza do pão. Para fins de comparação, foram analisados alguns atributos de um pão *sourdough* comercial adquirido de uma padaria local. A quantidade de células viáveis de *L. plantarum* adicionadas ao *sourdough* influenciaram diretamente nas características de acidificação do miolo do pão, em que a concentração máxima de células resultou em um valor de pH do miolo mais baixo. Os resultados de sabor e sabor residual revelaram uma correlação entre o pH e aceitabilidade do pão, indicando uma resistência por parte dos consumidores para pães com elevada acidez. No que diz respeito a firmeza, uma redução da dureza do miolo foi observada em pães com quantidades mais elevadas de ambos microrganismos. Não foram observados efeitos significativos no volume dos pães. *Sourdoughs* sem adição de *S. cerevisiae* resultaram em pães com uma coloração mais intensa de crosta, indicando uma maior quantidade de açúcar residual na massa de pão**.** Este resultado indica que a levedura adicionada como cultura *starter* não foi afetada pela presença de LAB e agiu no processo fermentativo. O melhor resultado para a textura do pão foi alcançado em concentrações de *Lactobacillus plantarum* e *Saccharomyces cerevisiae* diferentes daquelas encontradas para o caso de maior aceitação sensorial. Isto implica na existência de uma relação entre a característica que se deseja alcançar no pão e as concentrações combinadas destes microrganismos quando utilizados como *staters*.

Palavras-chave: *Sourdough*; *Lactobacillus plantarum*; *Saccharomyces cerevisiae*; superfície de resposta

ABSTRACT

The use of sourdough in breadmaking contributes to the improvement of many quality attributes of breads. The use pre-selected starter cultures is an alternative to control the process and optimize the sourdough fermentation and the final bread quality. The aim of this study was to assess the influence of different viable cell concentrations of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* when used as culture starters in sourdough breads. The influence of the starter culture on the characteristics of wheat sourdough bread was established by using response surface methodology. Seven experimental breads resulting from a central composite design were analyzed with regards sensory attributes, acidification characteristics, specific volume, crust color, and bread hardness. For comparative purposes, some attributes of a commercial sourdough bread from a local bakery was also analyzed. The amount of *L. plantarum* inoculated had direct effect on acidification characteristics of the bread crumb, indicating that the maximum cell concentration lead to bread crumb with lower pH. Results from taste and aftertaste reveled a correlation between pH and acceptability of the bread, indicating a resistance by consumers to breads with high acidity. With regards firmness, decrease in crumb hardness was observed in breads with higher amounts of lactobacilli and yeast. No significant effects were observed in specific volume. Sourdoughs with no addition of *S. cerevisiae* resulted in breads with a more intense crust color, indicating a higher amount of residual sugar in dough. This finding shows that yeast added as starter culture was not affected by the presence of LAB and did acted in the fermentation process. The best result for the bread texture was achieved using concentrations of *Lactobacillus plantarum* and *Saccharomyces* cerevisiae different from those found in breads with higher sensory acceptance. This finding implies that the desirable characteristics of the final bread are affected by the combined concentrations of these microorganisms when used as starters.

Keywords: Sourdough; *Lactobacillus plantarum*; *Saccharomyces cerevisiae*; Response surface methodology

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1 INTRODUCTION

The use of the sourdough process as a form of leavening is one of the oldest biotechnological processes in food production (RÖOCKEN; VOYSEY, 1995). In recent years, the traditional sourdough bread production has passed through renewed success with the growing demand by the consumer for more natural, tasty, and healthy foods (BRUMMER; LORENZ, 1991). Sourdough is an intermediate product that contains metabolically active yeast and lactic acid bacteria (LAB) strains.

The composition of the sourdough microbiota plays an important role for bread taste and quality. For this reason, scientific research focuses mainly on determination of the microbiota of sourdoughs fermented under different conditions; on the search for appropriate starter cultures for artisanal and industrial use; and on metabolic properties of the sourdough microflora. Even with intense research, it is still uncertain to which extent the fermentation microbiota is affected and selected by the kind of substrate and the process parameters like temperature, dough yield, redox potential, refreshment time, and number of propagation steps (HAMMES; GÄNZLE, 1998).

There is a growing interest in using pre-selected starter cultures in order to control the process and optimize the sourdough fermentation and the final bread quality. Starters composed of specific individual LAB, or mixed LAB and yeasts, became available a few years ago allowing the production of a full sourdough in a one-stage process (ROBERT et al., 2006). The choice of microorganisms used is of great importance, not only to ensure reliable sourdough quality, but also for easier control and economic viability of the sourdough making process. The combination of starter cultures requires prior knowledge of the biochemical characteristics and baking potential of the microorganisms (PLESSAS et al., 2008).

Growth of lactic acid bacteria is believed to be stimulated when co-cultured with yeasts, mostly due to the excretion of specific amino acids and small peptides by yeasts during growth (GOBBETTI et al., 1997). As presented by Gül et al. (2005), individual strains and combinations strongly affect the final bread texture. Differences have been reported in parameters such as specific volume (CROWLEY et al., 2002), crumb (CLARKE et al., 2003) and crust hardness

(CHIAVARO et al., 2008). However, the influence of cell concentration of yeast and LAB as inoculum in sourdough has not been reported before.

The present study was designed: (i) to determine the effect of different cell concentrations of a mixed starter cultures *Lactobacillus plantarum* and *Saccharomyces cerevisiae* on sensory attributes, acidification, volume, texture and color of wheat sourdough breads; and (ii) to improve the sensory profile and texture of wheat bread by using an optimized sourdough process through the surface response methodology. Furthermore, a commercial sourdough from a local bakery was analyzed in order to correlate and compare some attributes with the experimental breads.

2 OBJECTIVES

2.1 General objectives

The aim of this study was to evaluate the effect on bread properties of different cell concentrations of a mixed starter cultures containing *Lactobacillus plantarum* and *Saccharomyces cerevisiae* on sourdough wheat bread. In addition, to write a literature review was another objective.

2.2 Specific objectives

More precisely, the focus of this study is to evaluate the sensory attributes, acidification, volume, texture and crust color of wheat sourdough breads. Moreover, the experiments performed were designed to find an optimal concentration to improve the sensory profile and texture of wheat bread using the response surface methodology.

3 BIBLIOGRAPHIC DEVELOPMENT

3.1 Definition and origin of sourdough

Sourdough is an intermediate product between dough and traditional bread preparation, containing flour, water, and metabolically active microorganisms, mainly lactic acid bacteria (LAB) and yeast (HAMMES; GANZLE, 1998). Traditional sourdough was simply a piece of dough from the previous baking, which was mixed with flour, salt and water to make the bread dough (JACOB, 1997). While this piece of dough was stored, lactic acid fermentation took place caused by the metabolic activity of those lactic acid bacteria originally present in the flour or in the ambient. Additionally, selection and multiplication of yeasts from the flour occur in the dough. Due to their ability to produce carbon dioxide, LAB and yeasts in the sourdough are responsible for the leavening capacity of bread dough (HANSEN; SCHIEBERLE, 2005). This fermentation event may have been one of the first microbial processes employed by man (HAMMES; GANZLE, 1998).

The ancient Egyptians knew both the brewing of beer and the process of baking leavened bread with use of sourdough, as proved by wall paintings and analyses of desiccated bread loves and beer remains. The first documented production and consumption of sourdough bread can be traced back to the second millennium B.C. Egyptians saw that a mixture of flour and water, after left for a time to ferment, expanded and, after baking along with other fresh dough, it produced soft and light breads. The sourdough method remained the traditional form of leavening down into the European Middle ages until being substituted by barm (the foam, or scum, formed on the top of the liquor-fermented alcoholic beverages such as beer or wine from the brewing process), and after that by purpose-cultured yeast (KARRAR, 2016).

The tradition of making sourdough wheat bread is widely used in the Mediterranean and the Middle East countries and in the San Francisco bay in United States since 1849. During the 1849 Gold Rush, San Francisco was invaded by thousands of men and women in the grip of gold fever. Following the gold rush, sourdough bread remained an element that distinguishes the local tradition until today. Some bakeries in San Francisco claim to use sourdough that has been propagated for over 150 years (CAPPELLE et al. 2013).

Due to the superior sensory quality and the prolonged shelf life of the resulting baked products, sourdoughs are still important to the modern baking technology (HAMMES; GANZLE, 1998). In fact, sourdough improves dough properties and bread texture and flavor, retards staling process and protects baked products against spoilage (CORSETTI et al., 1998).

3.2 Classification of sourdoughs

Sourdough bread making is an ancient biotechnological process and various protocols for its use are applied in many countries. On the basis of the technology applied, sourdoughs have been grouped into three types (VUYST, DE; NEYSENS, 2005), to which a fourth type, named sponge-dough, can be added. This classification is based on the kind of technology applied for their production.

3.2.1 Type I

Traditional sourdoughs whose microorganisms are kept metabolically active through daily refreshments are included in this group. The process is performed at ambient temperature (20–30ºC) and the pH is about 4.0 (DE VUYST; NEYSENS, 2005). Type I sourdoughs are generally suitable for achieving dough leavening without addition of baker's yeast. Generally, traditional three-stage fermentation process is used, which relies on three refreshments over 24h in order to obtain the leavened dough to bake. Each step is characterized by a given DY (Dough Yield) which is defined as DY = (amount of flour + amount of water) x 100/amount of flour, as well as fermentation temperature and time. At the end of the last step of fermentation, the sourdough is used as the leavening agent; thus, it can be considered as a natural starter culture containing many microbial strains (HAMMES, 1991). In wheat and/or rye flour sourdoughs, dominating strains belong to the species *L. sanfranciscensis* which can co-exist with other obligately heterofermentative lactic acid bacteria such as *L. pontis , L. brevis , L. fermentum , L. fructivorans* and with the yeasts *Candida milleri, C. holmii, Saccharomyces cerevisiae and S. exiguus* (recently renamed *Kazachstania exigua*) (CORSETTI, 2013; HAMMES; GANZLE, 1998)

3.2.2 Type II

The development of type II sourdoughs came up due to industrial demand for faster, more efficient, controllable, large-scale sourdough fermentation processes. Typical type II processes last for 2–5 days without feedings and are often carried out at increased fermentation temperature

(usually greater than 30ºC) to speed up the process. Those sourdoughs exhibit a high acid content at a pH of < 3.5 after 24 h of fermentation. The microorganisms are commonly in the late stationary phase and therefore possess restricted metabolic activity. (HAMMES; GÄNZLE, 1998).

Industrially, it can be produced in large volumes, stored up to one week in silos, and are taken in working-day portions for the production of sourdough breads. These sourdoughs are generally fluid (DY of ca. 200) so it is easy pumpable in an industrial bakery. (CORSETTI et al., 2013; DE VUYST; NEYSENS, 2005). They are also applied for the production of dried sourdough products as well (HAMMES; GANZLE, 1998).

Sourdough type II serves to provide acidification to doughs. Leavening is performed by baker's yeast that has to be added to the dough, differently to traditional three-stage fermentation processes performed with type I doughs. Because of the long fermentation time, high DY, and temperature of fermentation, lactobacilli such as *L. panis, L. reuteri, L. johnsonii,* and *L. pontis*, which are resistant to low pH, dominate these sourdoughs (HAMMES; GANZLE, 1998).

3.2.3 Type III

Type III sourdoughs are dried doughs in powder form, which are initiated by defined starter cultures. They are used as acidifier supplements and aroma carries during breadmaking (DECOCK; CAPPELLE, 2005). They mostly contain LAB that are resistant to drying and are able to survive in that form. Bocker et al. (1995) have identified in type III doughs *Pediococcus pentosaceus*, *L. plantarum* and *L. brevis* at numbers ranging from 10^7 to 10^9 cfu/g.

Different drying protocols can be applied, for example freeze-drying, spray granulation, fluidized bed drying, and the two most common forms for type III sourdough: spray drying and drum drying (BRANDT, 2007). Different type III sourdoughs are obtained based on the various combinations of time and temperature of the drying process and on the extent of the Maillard reaction. These different degrees of caramelization and toasting bring as a consequence different aroma and flavor results in final bread. However, many volatile compounds (especially acetic acid) are lost due to the evaporation, even if to a different extent depending on the drying technique (DECOCK; CAPPELLE, 2005).

There are other ways to stabilize the sourdough as pasteurization, cooling or salting, resulting in a liquid or pasty sourdough. With the exception of cooling, all the other stabilization systems

stop gas and acid production, giving a sourdough, which can be categorized as type III. The use and storage of a stabilized sourdough is simple. It can be stored at room temperature for 30 to 60 days and directly added to the final dough at a proportion of 5 to 10%. To keep the sourdough liquid prevents the loss of volatile flavor compounds. In the same way of type II, baker's yeast needs to be added to leaven the dough (BRANDT, 2007; DECOCK; CAPPELLE, 2005; CORSETTI et al, 2013).

3.2.4 Sponge Dough

Sponge dough can be incorporated in the category of sourdoughs. The sponge dough is envisioned for increasing dough extensibility, bread volume, taste, and flavor of bread and its shelf life. It is an indirect process that may be considered as an intermediate procedure between straight-mass (or a straightforward process, where only baker's yeast is used to start the fermentation) and sourdough. (CORSETTI et al, 2013). Sponge dough is obtained in two steps: in the first dough (pre-dough) the baker's yeast is mixed with a part of the flour and water of the recipe, while the second dough is obtained by adding the rest of the others ingredients to the fermented pre-dough. The sponge is then, placed to rest and ferment for a period in an environment of a desired temperature and humidity. Lactic acid bacteria present as contaminants from either baker's yeast or flour grow in the dough mass reaching typically greater than 10^8 cfu/g (HAMMES; GANZLE, 1998).

3.3 Sourdough Applications

Artisan bread production, that often employs sourdough processes or the use of pre-ferments, provides a wide, regional variety of breads and specialty bakery products. In fact, many wheat breads and cakes came from Mediterranean countries, the San Francisco bay, and Southern America. On the other hand, numerous bakery preparations made with rye, wheat, barley, or mixed flours are typical for Germany, Central and Eastern Europe, and Scandinavia. In Italy, sourdough is used in more than 30% of bakery products, which include numerous different types of sourdough breads (OTTOGALLI; GALLI; FOSCHINO, 1996). Most of these products originate from very old traditions and differ in the type of flour, other ingredients, type of sourdough, technology, and shelf-life. The procedure for obtaining Italian Sourdough Bread, French Bread, Rye sourdough, Panettone Cake and San Francisco Sourdough are shown below.

3.3.1 Traditional Italian Sourdough Bread: The Altamura Bread

The Altamura bread is the first European bread that received the PDO (Protected Denomination of Origin) status. The technology is based on type I sourdough and it is manufactured in the Apulia region in Italy using specific cultivars of durum wheat flour. The final sourdough is obtained by a three-stage procedure in order to gradually increase the amount of leavened dough. At each step, water and durum wheat flour are mixed with previously fermented dough, which is added at the proportion of 20% based on flour weight. The time and temperature used at each stage are not defined but determined based on the bread-makers experience, as in traditional recipes. (CHIAVARO et al., 2008)

3.3.2 French Bread (*Pain au levain*)

A three-stage system is usually applied to prepare the traditional wheat flour sourdough French bread, according to the type I procedure. The first step consists of the mixing of a part of the mother sponge (*levain chef*) with flour and water to achieve around four times the initial mass. This dough (DY 160) ferments for 1.5–2h at 25°C and is named *levain de première* (fresh sour). It is used as a starter to obtain a dough with a DY of 185, which ferments for 7–8h at a temperature slightly higher than the previous one. In order to stimulate the growth of yeasts and the leavening capability of the *levain de seconde* (basic sour) the procedure requires a long mixing time to oxygenate the dough. By using this dough as the starter for the third refreshment, the last dough or *levain tout point* (full sour) is obtained after a short fermentation time (around 2h) with the aim of controlling the hydrolytic activities of the dough and of preserving the gas-retaining and bread-making capabilities. The *levain tout point* is added in a proportion of 25% with respect to the mass of the final dough and is ready for baking after 30min of leavening. (ONNO B., ROUSSEL P, 1994).

3.3.3 Rye sourdough bread

In rye bread making, the use of sourdough is also essential to achieve the desired texture. Dough acidification is a prerequisite for rye baking to inhibit the flour α-amylase. Further, sourdough fermentation promotes a solubilisation of rye pentosans at the dough stage and thus enhances water binding of the dough, since gluten are lacking in rye (MARTINEZ-ANAYA; DEVESA, 2000). Rye sourdoughs are predominantly used in the rye-growing North, Central and Eastern European countries (HANSEN; SCHIEBERLE, 2005). The “three-stage sourdough process, basic-sour overnight” requires about 15–20h and, as described by Spicher and Pomeranz

(1985), represents a good reference model for type I sourdough application in rye-flour-based bread making. The first stage leads to the fresh sour, which, in turn, is started by the mother sponge, a commercial starter or a part of a full sour from a previous bread making. The fresh sour has a DY of 200–250 and ferments for 6 h at 25–26°C. It contains a high number of yeasts and it is used to start a new dough based on rye flour and water (DY of about 160–180). After 5–8 h of overnight fermentation at 26–30°C, lactic acid bacteria grow and the basic sour is obtained. A further refreshment (3 h at 30–33°C), using the basic sour as the starter for a mix of rye flour and water (DY 180–200), leads to the full sour, which has values of pH and TTA of 4.0 and 10.5ml NaOH 0.1 N/10 g of dough, respectively. A part (40%) of this sourdough represents the natural starter for the final dough (DY 170), which contains rye flour and an equivalent amount of wheat flour in the case of a rye/wheat flour bread preparation. (GOBBETTI; GÄNZLE, 2012)

3.3.4 Panettone Cake

Panettone is a traditional Italian cake consumed over Christmas and famous throughout the world. Panettone has a soft structure, with regular holes, and characteristic flavour, which is derived from dough ingredients (water, flour, butter, sugar, eggs, salt, and others) and processing. Both at artisan and industrial levels, the sourdough biotechnology is traditionally based on many refreshments (type I sourdough) and an increased concentration of sugar is added during the last steps (OTTOGALLI; GALLI; FOSCHINO, 1996). Comparable to type I sourdoughs, sourdough microbiota are predominantly composed of *L. sanfranciscensis*. Time and temperature of fermentation are strictly controlled. The dough temperature does not exceed 30°C and the temperature of water and other ingredients has to be not lower than 20–22°C (CORSETTI et al, 2013).

A lot of studies have attributed the prolonged softness and shelf life of Panettone cake to the presence of dextran produced by *Leuconostoc mesenteroides* in the sourdough, which uses sucrose as an exo-polysaccharide (EPS) precursor (DECOCK; CAPPELLE, 2005). A sourdough containing 25% (on dry matter) of dextran, which is stabilized by refrigeration, pasteurization or drying, has been used to shorten the time to obtaining a product with similar characteristics to the traditional Panettone cake (CORSETTI et al, 2013).

3.3.5 San Francisco Bread

San Francisco bread is manufactured using the famous San Francisco sourdough, which possesses a typical microbial community, mainly consisting of *L. sanfranciscensis* and *S. exiguus* (renamed to *K. exigua*). In order to increase the concentration of acetic acid synthesized by the obligately heterofermentative *L. sanfranciscensis*, several refreshments and long fermentation times at low temperature are used. Recently, a liquid San Francisco sourdough, which is stabilized through pasteurization, has been introduced to the market (CORSETTI et al., 2013). Type III sourdough is being used as an alternative to acid and flavoring agent for the manufacture of the San Francisco bread in about 3 h (DECOCK; CAPPELLE, 2005).

3.4 Microflora of sourdough

In order to develop the microflora naturally presented in the raw material, spontaneous fermentation is used in the traditional production of sourdough. The microbial ecology of the sourdough fermentation is dependent on both endogenous and exogenous factors (HAMMES; GÄNZLE, 1998; HAMMES; STOLZ, GÄNZLE, 1996). Endogenous factors are determined by the chemical and microbiological composition of the dough. Exogenous factors are related mainly by temperature and redox potential. In practice, some process parameters such as dough yield (water activity), addition of salt, number of propagation steps, amount and composition of the starter, and fermentation time strongly affects the characteristics of sourdough. The impact of these parameters during continuous propagation of sourdough causes the selection of the characteristic LAB and yeast microflora, and meanwhile prevents the growth of other microorganisms originating from contamination of the raw materials or the bakery environment. In some industrial sourdough processes, such microbial associations may endure for years due to the selective pressure exerted by the environmental conditions, although the fermentation process is performed under non-aseptic conditions (VUYST, DE; NEYSENS, 2005).

Microbiological studies have revealed that many species, mostly of the genus *Lactobacillus*, and several yeast species, especially species of the genera *Saccharomyces* and *Candida*, occur in this ecological niche. The LAB:yeast ratio in sourdoughs is generally 100:1 (OTTOGALLI; GALLI; FOSCHINO, 1996). Although in the majority of fermented foods homofermentative LAB are the main microorganisms involved, heterofermentative LAB are dominating in sourdough, especially when traditionally prepared and maintained with propagations. Indeed,

acetic acid, an important end product of heterofermentation, plays a main role in the flavor of sourdough (CORSETTI et al., 2003).

Lactobacillus strains are more frequent than *Leuconostoc*, *Weissella*, and *Pediococcus* species. Lacto-cocci, enterococci, and streptococci are found with less frequency. The domination of heterofermentative lactobacilli in sourdoughs can be explained essentially by their competitiveness and adaptation to this particular environment (VUYST, DE; NEYSENS, 2005). In mature sourdoughs LAB reaches typically greater than 10^8 CFU per gram sourdough, and yeasts from 1×10^6 to 5×10^7 CFU per gram sourdough (HAMMES *et al.*, 2005).

The sourdough microflora is usually composed of stable associations of lactobacilli and yeasts, because of their growth requirements with respect to pH, temperature, organic acids, as well as metabolic interactions between them. However, in some sourdoughs, LAB and yeasts compete for the available substrates, resulting in heterogeneous populations. The antagonistic and synergistic interactions between lactobacilli and yeasts are related to the metabolism of carbohydrates, amino acids and the production of carbon dioxide (VUYST, DE; NEYSENS, 2005).

Typical mutual associations involve *L. sanfranciscensis* and either *S. exiguus* or *C. humilis* (GOBBETTI; CORSETTI, 1997). This association is explained because of the lack of competition between *L. sanfranciscensis* and *S. exiguus* for maltose. The sourdough yeasts do not affect the cell yield of *L. sanfranciscensis*, because pH is the limiting factor for growth of the lactobacilli. The maltose, amino acid, and peptide concentrations are not depleted during wheat or rye sourdough fermentations. The growth of maltose-negative yeasts is inhibited by the accumulation of metabolic end products. However, the glucose concentration in rye flours and whole-wheat flours remains high enough to support yeast growth throughout the fermentation. But in fermentations that takes white wheat flours are characterized by low concentrations of glucose, then small amounts of lactic acid are produced because of the low buffering capacity. In these doughs, depletion of glucose and fructose may occur and limit the growth of the yeasts (GOBBETTI; CORSETTI, 1997; VUYST, DE; NEYSENS, 2005).

3.4.1 Lactic Acid Bacteria

LAB comprise a varied group of nonsporulating, Gram-positive, strictly fermentative lactic acid-producing bacteria that plays a significant role in the organoleptic, health-promoting,

technological, and safety aspects of fermented food products. As a result of natural contamination through the flour or the environment or by deliberate addition via dough ingredients, an extensive taxonomic variety of LAB has also been found in sourdoughs. In sourdough environments, LAB contribute most to the process of dough acidification, whereas yeasts are mainly responsible for the leavening (CORSETTI; SETTANNI, 2007; HUYS; DANIEL; DE VUYST, 2013).

Sourdough has an initial pH of 5.0–6.2, which is quite low. It allows the spontaneous development of only selected acid-tolerant microorganisms, i.e. lactic acid bacteria, from the cereals or flours, depending on the flour preparation and the technology applied to sourdough production. Traditional sourdough processes, however, do not usually rely on the casual flora, but on the use of mother doughs that are incessantly propagated over long periods of time according to a defined cycle of preparation. In this case, the mother dough represents the natural microbial inoculum for the following doughs (DE VUYST; NEYSENS, 2005).

Homofermentative LAB are dominant in spontaneous fermentation processes and heterofermentative lactobacilli dominate sourdough fermentation processes with propagation. Still, this does not eliminate the presence of homofermentative LAB in the latter sourdoughs. More than 55 Lactobacillus species have been identified, of which the great majority are obligately heterofermentative. Many researchers still report on the existence of non-identifiable and perhaps new sourdough LAB species and/or strains (VUYST, DE *et al.,* 2002). An updated overview of the LAB species most commonly found in fermented sourdough is compiled in Table 1.

Table 1: LAB species generally associated with sourdough fermentation or found in fermented sourdoughs

Obligately heterofermentative	Facultatively heterofermentative	Obligately homofermentative
Lactobacillus acidifarinae	*Lactobacillus alimentarius*	*Enterococcus casseliflavus*
Lactobacillus brevis	*Lactobacillus casei/paracasei*	*Enterococcus durans*
Lactobacillus buchneri	*Lactobacillus coleohominis*	*Enterococcus faecalis*
Lactobacillus cellobiosus	*Lactobacillus kimchi*	*Enterococcus faecium*
Lactobacillus collinoides	*Lactobacillus paralimentarius*	*Lactobacillus acidophilus*
Lactobacillus crustorum	*Lactobacillus pentosus*	*Lactobacillus amylolyticus*
Lactobacillus curvatus	*Lactobacillus perolens*	*Lactobacillus amylovorus*
Lactobacillus fermentum	*Lactobacillus plantarum*	*Lactobacillus crispatus*
Lactobacillus fructivorans	*Lactobacillus sakei*	*Lactobacillus delbrueckii subsp. delbrueckii*
Lactobacillus frumenti	*Pediococcus acidilactici*	*Lactobacillus farciminis*
Lactobacillus hammesii	*Pediococcus dextrinicus*	*Lactobacillus gallinarum*
Lactobacillus hilgardii	*Pediococcus pentosaceus*	*Lactobacillus gasseri*
Lactobacillus homohiochii		*Lactobacillus helveticus*
Lactobacillus kefiri		*Lactobacillus johnsonii*
Lactobacillus kunkeei		*Lactobacillus mindensis*
Lactobacillus lindneri		*Lactobacillus nagelii*
Lactobacillus mucosae		*Lactobacillus salivarius*
Lactobacillus namurensis		*Lactococcus lactis subsp. lactis*
Lactobacillus nantensis		*Streptococcus constellatus*
Lactobacillus nodensis		*Streptococcus equinus*
Lactobacillus oris		*Streptococcus suis*
Lactobacillus panis		
Lactobacillus parabuchneri		
Lactobacillus pontis		
Lactobacillus reuteri		
Lactobacillus rossiae		
Lactobacillus sanfranciscensis		
Lactobacillus secaliphilus		
Lactobacillus siliginis		
Lactobacillus spicheri		
Lactobacillus vaginalis		
Lactobacillus zymae		
Leuconostoc citreum		
Leuconostoc Gelidum		

Table 2: LAB species generally associated with sourdough fermentation or found in fermented sourdoughs (continuation)

Obligately heterofermentative	Facultatively heterofermentative	Obligately homofermentative
Leuconostoc mesenteroides subsp. Cremoris		
Leuconostoc mesenteroides subsp. Dextranicum		
Leuconostoc mesenteroides subsp. Mesenteroides		
Weissella confusa		
Weissella hellenica		
Weissella kandleri		
Weissella paramesenteroides		
Weissella viridescens		

Source: HUYS; DANIEL; DE VUYST, 2013

The established LAB associations commonly reflect the media resources like carbohydrates, amino acids, and vitamins. They also reflects environmental conditions such as temperature, pH and redox potential. Further, process parameters and the use of a starter or baker's yeast influences a lot their microbiological composition (VUYST, DE; NEYSENS, 2005).

Even though the LAB microbiota of sourdoughs is evidently dominated by the genus lactobacilli, other less predominant LAB species may also be found, including members of the genera *Weissella*, *Pediococcus*, *Leuconostoc*, *Lactococcus*, *Enterococcus* and *Streptococcus* (Table 1). Of these, specific species of *Weissella*, *Pediococcus*, and *Leuconostoc* are particularly well adapted to survive and grow in flours for example and their presence in sourdoughs is restricted to only a few species (CORSETTI et al., 2008).

3.4.2 Yeasts

Yeasts are members of the fungus kingdom that grow as single cells. They produce daughter cells by budding (the budding yeasts) or by binary fission (the fission yeasts). They differ from most fungi, which grow as thread-like hyphae. From the agro-alimentary and scientific point of view, yeasts are one of the most important eukaryotes. Species found in sourdough microbial associations share an adaptation to the specific environment formed mainly by a low pH, high carbohydrate concentrations and high cell densities of lactic acid bacteria (LAB) (HUYS; DANIEL; DE VUYST, 2013).

Several yeasts are found in sourdoughs but *Saccharomyces cerevisiae* is considered the prevailing organism for leavening of bread (CORSETTI *et al.*, 2001). Important yeasts in sourdough starters include *Saccharomyces exiguus* (physiologically similar to *Candida milleri*), *Candida krusei*, *Pichia norvegensis* and *Hansenula anomala*. Other yeasts present in sourdough sponges include *Saccharomyces delbrueckii, Torulopsis holmii* and *Torulopsis unisporus* (GÜL; ÖZÇELIK; SA, 2005).

The study of Vernocchi et al. (2004) shows an example of how this association of LAB and yeasts results in a cooperation with important technological and sensory consequences. According to their work, *Candida milleri* and *S. cerevisiae* supply LAB population present in Colomba Cake with an electron source, fructose, that contribute to their growth and acetic acid production. In return, LAB such as *Lactobacillus sanfranciscensis* does not compete with maltose negative yeasts (*S. exiguus, C. humilis and C. milleri*). This fact emphasize the importance of antagonistic and synergistic interactions between yeast and LAB.

As said before, numerous yeasts have been isolated from sourdoughs (see Table 2) but only part of them play a substantial role in fermentation processes. For example, non-fermenting species may be just ubiquitous contaminants and they may affect only the flavor of the baked goods when present at high numbers. Well adapted and frequently isolated sourdough species are printed in bold in Table 2 (HAMMES *et al.*, 2005).

Table 3: Yeasts found in sourdoughs

Species	Synonyms
Candida boidinii	
Candida glabrata	***Torulopsis glabrata***
Candida humilis	***Candida milleri***
Candida parapsilosis	
Candida stellate	*Torulopsis stellata*
Dekkera bruxellensis	***Brettanomyces custersii***
Debaryomyces hansenii	***Torulopsis candida, Candida famata***
Galactomyces geotrichum	*Geotrichum candidum*
Issatchenkia orientalis	***Candida krusei***
Kluyveromyces marxianus	
Pichia anomala	*Candida pelliculosa, Hansenula anomala*
Pichia fermentans	*Candida lambica*
Pichia ohmeri	
Pichia subpelliculosa	*Hansenula subpelliculosa*
Pichia minuta var. minuta	*Hansenula minuta*
Saccharomyces bayanus	***Saccharomyces inusitatus***
Saccharomyces cerevisiae	***Saccharomyces fructuum***
Saccharomyces exiguus	***Torulopsis holmii, Candida holmii, Saccharomyces minor***
Saccharomyces kluyveri	
Saccharomyces servazzi	
Saccharomycopsis fibuligera	*Endomyces fibuliger*
Saturnispora saitoi	*Pichia saitoi*
Torulaspora delbrueckii	***Torulopsis colliculosa, Candida colliculosa, Saccharomyces rosei, Saccharomyces delbrueckii, Saccharomyces inconspicuous***
Torulaspora pretoriensis	***Saccharomyces pretoriensis***

Source: HAMMES et al., 2005

3.5 Process parameters that influence the sourdough

3.5.1 Consistency

The consistency of the sourdough can be a variable. The sourdough fermentation can be performed as a firm dough or as a liquid suspension of flour in water. This proportion between flour and water is called the dough yield (DY). The firmer the sourdough (lower DY value) the more acetic acid is produced and the less lactic acid. The flavor of lactic acid is rather mild acid and slow acting, where acetic acid has a sharp acidic taste, which is instantly perceived. The DY of a sourdough also influences the acidification rate. In higher DY value, i.e. soft dough, acidification will occur faster, most probably due to the better diffusion of the produced organic acids into the dough (DECOCK; CAPPELLE, 2005).

3.5.2 Substrate

The substrate, mainly flour, used for sourdough fermentation is a significant parameter influencing the final flavor and acidity of the sourdough.

3.5.2.1 The ash content in flour

The ash content in flours varies according to the extraction rate; the higher the extraction, the higher the ash content will be. The bran fraction contains more minerals and micronutrients which are important for the growth of LAB. The ash will also influence the buffering capacity of the sourdough system, resulting in a higher total titratable activity (TTA). TTA is a major quality criterion of sourdoughs and is determined by titration of 10g dough to pH 8.5 with 0.1M NaOH. The consumption of NaOH in milliliters is defined as TTA. This TTA value expresses the total amount of organic acids produced during the sourdough fermentation (BRANDT, 2007).

3.5.2.2 The falling number of the flour

The falling number of the flour is an indicator for the enzymatic activity of the flour. The lower the value the more amylase activity present in the flour and consequently more free sugars will be available for microflora to consume it, leading to faster metabolic activity in LAB and yeast (CHAVAN, R. S.; CHAVAN, S. R., 2011).

3.5.3 Temperature

The temperature during the fermentation will influence, similar to the DY, the acidification rate. The temperature has also an influence on the microbial composition of the sourdough. The temperature is even more important if the successive propagation method is used because part of

the microflora can be lost over the different sourdough refreshments if it is not controlled (VUYST, DE; NEYSENS, 2005).

According to Gobbetti et al. (1995), the production of acids in sourdoughs increased with increasing temperature due to a higher production of lactic acid, whereas the production of acetic acid was not influenced. Ideal temperatures for the growth of Lactobacilli are 30 to 40ºC depending on strain and for yeasts, 25 to 27ºC (CHAVAN, R. S.; CHAVAN, S. R., 2011).

On the basis of microbial adaptation to the various environmental factors, the combination of DY and temperature during refreshments markedly influences sourdough microbiota and its performance. According to Decock & Cappelle (2005) more acetic acid is present in firm dough fermented at 25–30°C, while more lactic acid is found in higher DY fermented at 35–37°C.

3.5.3.1 Stater cultures

A 4th parameter is the amount and composition of the microflora used for the fermentation. As said before, there are two main families: the heterofermentative and the homofermentative LAB. Currently, various commercial preparations of stabilized sourdough are available on the market and it consists mixtures of different LAB groups to assure good acidification and aromatization (DECOCK; CAPPELLE, 2005).

In case of sourdough made by continuous propagation method, parameters like dough yield, temperature, number of propagation steps and fermentation time causes the selection of a characteristic microbiota. This selected microbiota will determine the quality and handling properties of the sourdough (ARENDT; RYAN; DAL BELLO, 2007).

3.6 Characteristics of sourdough bread

Sourdough have earned special interest of researchers due to the benefits achieved by their use in breadmaking industry. It has been reported that sourdough contribute to the improvement of the volume, texture and sensory quality of the bread, as well as to the increase of desirable bread acidity and to the improvement of bread physical and microbiological shelf-life (PARAMITHIOTIS *et al.*, 2005).

3.6.1 Acidification

The pH of a ripe sourdough varies with the nature of the process and starter culture used but wheat sourdoughs there is a variation between 3.5 to 4.3 (THIELE; GÄNZLE; VOGEL, 2002).

Depending on the amount of full sour that is used as the inoculum, the pH of the sourdough affects the values of pH of the final dough and bread. In the case of a standard inoculum of 20% (of the dough weight), values of pH that range from 4.7 to 5.4 are usually found in the final dough. The acidification of the sourdough and the partial acidification of the bread dough will influence on structure-forming components like gluten, starch and arabinoxylans (ARENDT; RYAN; DAL BELLO, 2007).

The primary effect of acids on the protein fraction of the dough is the increased swelling and solubility of gluten proteins. This happens due to the positive net charge of the proteins in an acidic environment. Increased intramolecular electrostatic repulsion makes gluten proteins to unfold so the hydrophobic groups are more exposed. Because of the presence of strong intermolecular electrostatic repulsive forces the formation of new bonds are inhibit. The consequences of this is a softer dough with less stability and shorter mixing time. Also, softness of the gluten favors swelling and increase water absorption (TAKEDA; MATSUMURA; SHIMIZU, 2001).

The acidification of the dough is due to the production of lactic and acetic acids. The FQ value indicates the molar ratio between lactic and acetic acids during sourdough fermentation. The determination of the concentration of lactic and acetic acids is made by enzymatic or chromatographic methods and the FQ is calculated according Equation 1:

$$FQ = \frac{\text{g of lactic acid in 100 g of dough/molecular weight of lactic acid}}{\text{g of acetic acid in 100 g of dough/molecular weight of acetic acid}} \quad (1)$$

This parameter depends on the type of lactic acid bacteria dominating the fermentation and the balance between homo and heterofermentative lactobacilli. In turn, it depends on exogenous and endogenous factors during fermentation (e.g. fermentable sugar and oxygen concentration, DY, time and temperature, etc.) (CORSETTI et al., 2013).

3.6.2 Shelf life

The most common cause of microbial spoilage in bread is mould growth. Common spoilage fungi from bakery products belong to the genera *Penicillium, Aspergillus, Monilia, Mucor, Endomyces, Cladosporium, Fusarium* and *Rhizopus*. A number of methods are applied to prevent or reduce microbial spoilage of bread, such as addition of propionic acid and its salts, modified

atmosphere packaging or irradiation (LEGAN, 1993). Recently there has been an increasing interest in the application of biopreservation in the food industry. LAB and, in particular lactobacilli, are of special interest, since they have an extensive use in food and are generally regarded as safe (GRAS). Beside the weak organic acids, i.e. lactic and acetic acids LAB produce a wide range of low molecular weight compounds, peptides and proteins with antifungal activity (MAGNUSSON et al., 2003).

Numerous studies have been published describing the isolation and characterization of antifungal components from LAB (DAL BELLO et al., 2007; LAVERMICOCCA et al., 2000). LAB produce a diversity of antimicrobial compounds, as hydrogen peroxide, formic acid, propionic acid, and diacetyl. The precise mechanism of antimicrobial action is difficult to elucidate due to complex and normally synergistic interactions between different compounds. Research has mostly been directed towards identifying different antimicrobial substances, primarily antibacterial, using in vitro systems, but little is known about the general mechanisms of complex preservation systems within food environments (SCHNU, 2005).

3.6.3 Texture

Acids produced during fermentation strongly influence the texture of a sourdough bread. The acidification of wheat sourdough results in a large reduction of elasticity and firmness of the dough. Due to acidification of the dough by LAB growth, proteolysis occurs and alters the gluten network. The rheological consequence of gluten degradation is a significant reduction of elasticity and firmness of the sourdough and subsequent bread dough (ARENDT; RYAN; DAL BELLO, 2007).

Some strains of LAB synthesize exopolysaccharides (EPS) from sucrose. EPS exhibit a positive effect on the texture, mouthfeel, taste perception, and stability of fermented food. Also, prebiotic effects have been described for specific exopolysaccharides. EPS produced by LAB during fermentation is one of the aspects of sourdough technology with the potential to replace or reduce the addition of hydrocolloids used to improve bread texture (KORAKLI *et al.*, 2003).

3.6.4 Volatile compounds

Taste and smell of bread or any other baked product are the main characteristics taken into account by consumers to determine its quality. The flavor of wheat bread is influenced by the nature of the raw materials as well as the conditions of the fermentation and baking process. The

odor of bread crumb is mainly determined by microbial fermentation products, whereas the flavor products originating from thermal reactions dominate in the crust (PIGGOTT, 2006). Compounds strongly related to bread flavor are mainly organic acids, alcohols, esters and carbonyls (HANSEN; LUND; LEWIS, 1989). The process of generation of volatile compounds in sufficient amounts needs multiple steps of about 12–24h, but when baker's yeast is used the fermentation is completed within a few hours (HANSEN; SCHIEBERLE, 2005).

Bacterial proteolysis during sourdough fermentation was shown to contribute to the development of typical sourdough flavors of baked breads. According to Gänzle et al. (2007) there is an important relation between amino acid conversion to flavor volatile compounds. Amino acids are substrates for microbial conversions or are converted to flavor compounds during baking. Proteolytic strains of LAB in sourdough may affect amino acids level in doughs, but there is evidence that the activation of cereal proteases is a major driving force for protein degradation in sourdoughs (THIELE; GANZLE; VOGEL, 2002).

In sourdoughs, LAB and yeasts produce flavor compounds independently and through their interactions. Heterofermentative LAB mostly produce ethyl acetate and certain alcohols and aldehydes, whereas homofermentative LAB synthesize diacetyl and other carbonyls (PIGGOTT, 2006). Sourdoughs made with both LAB and yeasts resulted in more aroma compounds as compared to sourdoughs made using single starter based either on LAB (e.g. *Lb. brevis*) or yeast (e.g. *S. cerevisiae*) (MEIGNEN *et al.*, 2001).

4 ARTICLE

Article to be submitted to the Journal of Food Research International, previously formatted in the standards for publication.

https://www.elsevier.com/journals/food-research-international/0963-9969/guide-for-authors

The influence of different viable cell concentrations of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* as starter cultures on sourdough breadmaking

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Abstract

The aim of this study was to assess the influence of different cell concentrations of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* when used as culture starters in sourdough breads. The influence of the starter culture on the characteristics of wheat sourdough bread was established by using the response surface methodology. Seven experimental breads resulting from a central composite design were analyzed with regards of sensory attributes, acidification characteristics, specific volume, crust color and bread hardness. For comparative purpose, some attributes of a commercial sourdough bread from a local bakery was also analyzed. The amount of *L. plantarum* inoculated had direct effect on acidification characteristics (pH and total titratable acidity) of sourdough and final bread crumb, indicating that the maximum cell concentration lead to bread crumb with lower pH. Results from taste and aftertaste reveled a correlation between pH and acceptability of the bread, indicating a resistance by consumers to breads with high acidity. A decrease in crumb hardness was found with higher amounts of lactobacilli and yeast, but regarding specific volume, no significant difference was observed between breads. Sourdoughs with no addition of *S. cerevisiae* resulted in breads with a more intense crust color, indicating a higher amount of residual sugar in dough. Textural improvement and sensory acceptance are achieved using different optimal concentrations, which should be considered when using these microorganisms in future sourdough breads.

KEYWORDS: Sourdough; *Lactobacillus plantarum*; *Saccharomyces cerevisiae*; Response surface methodology

1 INTRODUCTION

The mixture of flour and water results in the formation of a dough, which, after some time, will turn into a sourdough characterized by acid taste, aroma and increased volume due to gas formation. Sourdough is an intermediate product that contains metabolically active yeast and lactic acid bacteria (LAB) strains. The LAB developed in the dough may be originated from selected natural contaminants in the flour or from a starter culture containing one or more known species of LAB (Vogel, Müller, Stolz, & Ehrmann, 1996). The use of sourdough has become popular over the last decades due to the superior quality of the sourdough baking products. The benefits of sourdough over yeasted breads can be related by its influence on the following features: (i) technological properties including improved dough machinability, (ii) nutritional properties, such as phytate hydrolysis, (iii) organoleptic properties like improved bread volume, crumb texture and flavor and (iv) improvement of bread physical and microbiological shelf life (De Vuyst & Neysens, 2005; Collar Esteve, Benedito de Barber, & Martínez-Anaya, 1994). The properties of sourdoughs and its effects on final bread rely on several factors, such as the sourdough making process and the microorganisms involved (Chavan & Chavan, 2011). Sourdough microflora generally contains a complex mixture of yeasts (mainly *Saccharomyces cerevisiae*) and hetero and homofermentative LAB. A great number of *Lactobacillus* species have been isolated including *Lactobacillus sanfranciscensis*, *L. pontis*, *L. brevis* and *L. plantarum* as the strains most commonly found (Ottogali, Galli, & Foschino, 1996; De Vuyst et al., 2002). In mature sourdoughs, LAB reaches typically greater than 10^8 CFU/g, and yeasts range from 1×10^6 to 5×10^7 CFU/g (Hammes et al., 2005).

Sourdough fermentation has been widely studied, however, due to its complicated biological system and the many factors affecting its final characteristics, it is still not a well-understood process (Plessas et al., 2008a). Hammes and Gänzle (1998) described the ecological factors affecting the microbiota of sourdough fermentations. They differentiate between endogenous and exogenous factors. Endogenous factors are the composition of the cereal substrate, as fermentable carbohydrates, N-sources, minerals, vitamins, lipids, enzyme activities, growth inhibitors, and the original microbiota of the grains. Exogenous factors are the applied process

parameters like temperature, oxygen, dough yield, amount of backslopping dough, addition of sodium chloride, fermentation time, number of propagation steps and the used starter cultures.

Sourdough microorganisms are traditionally allowed to develop naturally. However, in order to control the process and optimize the sourdough fermentation and the final bread quality, there is a growing interest in using pre-selected starter cultures. Starters composed of specific individual LAB, or mixed LAB and yeasts, became available a few years ago allowing the production of a full sourdough in a one-stage process (Robert et al., 2006). Currently, traditional and industrial processes have specific needs such as constant rheology and flavor properties of the bread, thus, daily propagation of sourdoughs must be reduced to lower time-consuming processes and risks, without losing the major properties of lactic acid bacteria (Gaggiano et al., 2007). The choice of microorganisms used is of great importance, not only to ensure reliable sourdough quality, but also for easier control and economic viability of the sourdough making process. The combination of starter cultures requires prior knowledge of the biochemical characteristics and baking potential of the microorganisms (Plessas et al., 2008). In industrial applications, it is common to add some amount of baker's yeast to bread dough as leavening agent (Hammes & Gänzle, 1998).

Growth of lactic acid bacteria is believed to be stimulated when co-cultured with yeasts, mostly due to the excretion of specific amino acids and small peptides by yeasts during growth (Gobbetti, & Corsetti, 1997). However, the influence of cell concentration of yeast and LAB as inoculum in sourdough has not been reported before. Performance of LAB has mainly been studied by characterization of the acidification properties such as pH, total titratable acidity (TTA) and lactic and acetic acids production during sourdough fermentation (Corsetti et al., 1997; Hammes & Gänzle, 1998). As presented by Gül et al. (2005), individual strains and combinations strongly affect the final bread texture. Differences have been reported in parameters such as specific volume (Crowley, Schober, Clarke, & Arendt, 2002), crumb (Clarke, Schober, Angst, & Arendt, 2003) and crust hardness (Chiavaro, Vittadini, Musci, Bianchi, & Curti, 2008).

In order to use wheat sourdough efficiently, the influence and interactions of sourdough process conditions on biochemical activity of sourdough and subsequent bread quality have to be understood and optimized accordingly. The present study was designed: (i) to determine the

effect of different cell concentrations of a mixed starter cultures *L. plantarum* and *S. cerevisiae* on sensory attributes, acidification, volume, texture and color of wheat sourdough breads; and (ii) to improve the sensory profile and texture of wheat bread by using an optimized sourdough process. Furthermore, a commercial sourdough from a local bakery was analyzed in order to correlate and compare some attributes with the experimental breads.

2 MATERIALS AND METHODS

2.1 Strains and growth conditions

Lactobacillus plantarum ATCC 8014 was kindly provided by FIOCRUZ Institute (Rio de Janeiro, Brazil) in lyophilized form and grown in de Man-Rogosa-Sharpe (MRS) broth (Merck, Darmstadt, Germany). Streak plate method was used to get pure culture of lactic acid bacteria. Then a single colony was transferred to 5mL MRS incubated under aerobic conditions at 37°C (24h) and then sub-cultured into 10mL MRS and incubated in the same conditions until the late exponential growth phase was reached (ca. 12h). Twelve-hour-old LAB cells were harvested by centrifugation at 5000 × g for 10 min at 4ºC, washed twice with saline solution (0.9% NaCl), and suspended in the same buffer. Serial dilutions in saline solution plated on MRS agar were made, resulting in a concentration of about 10^8 CFU/ml.

Saccharomyces cerevisiae strain was obtained from commercial baker's yeast manufactured by Fleischmann. Ten grams of the yeast were suspended using 90ml of peptone water for 30min. In order to determine cell viability the suspended cells were seeded on Petri dishes containing solid Yeast malt broth (YM) (Sigma-Aldrich, Germany) medium and incubated for 48 hours at 28°C, resulting in a final concentration of 10^9 CFU/ml. For each experiment, serially dilution was made to achieve the required final cell concentration.

2.2 Experimental design

An experimental design 2^2 with triplicate at the central point was designed to determine the relative contributions of two predictor variables: the cell concentration of *L. plantarum* and the presence of *S. cerevisiae* in the sourdough preparation. The results were analyzed using response surface methodology (RSM). The respective design is describe in Table 1. In order to minimize systematic errors, the experiments were conducted in a random order.

Table 1: Coded and uncoded levels of variables established according to the experimental design

Test	*Lactobacillus plantarum* (CFU/ml)		*Saccharomyces cerevisiae* (CFU/ml)	
	Coded	Uncoded	Coded	Uncoded
1	-1	10^2	-1	0
2	1	10^8	-1	0
3	-1	10^2	1	10^6
4	1	10^8	1	10^6
5	0	10^5	0	10^3
6	0	10^5	0	10^3
7	0	10^5	0	10^3

2.2.1 Statistical analysis

Data were subjected to one-way ANOVA; pair-comparison of treatment means was obtained by Tukey's procedure at $P < 0.05$, using the statistical software Statistica 12.0 (StatSoft Inc., Tulsa, USA).

2.3 Sourdough preparation

Each dough of 405g was produced with a dough yield (weight of the dough/weight of the flour×100) of 162 adding 55mL of tap water and 100ml containing the cell suspension of *L. plantarum* and *S. cerevisiae* to 250g of commercial flour (Tondo S.A., Forqueta, RS). The main specifications of the flour were moisture 13.3%; ash dry weight 0.48% and Falling Number 420s, gluten dry weight 8.5%; gluten index 98.80. The farinograph and alveograph parameters were water absorption (WA) 57.1%; development time (DT) 12.1min; stability time (ST) 15.2min; deformation energy (W) 221 × 10−4 J and curve configuration (P/L ratio) 1.31. For experiments made with absence of *S. cerevisiae*, only 50ml of *L. plantarum* cell suspension and 105 ml of tap water was added to the flour. For each experiment, the specific cell concentration was achieved by serial dilution. The doughs were mixed for 5–10min manually until the correct consistency was obtained, poured into a large beaker, covered, and placed in an incubator (Venâncio, AC40T) at 30 °C and 85% relative humidity (RH), for 20h. A reference (control) dough without starter was also produced.

2.4 Bread making

For bread making, 400g of flour, 215ml tap water, 200g sourdough prepared as described above, 4g of compressed baker's yeast and 8g salt were mixed for 19min with an automatic bread machine Britânia (Multipane). After a resting period of 15min, dough was divided into 100g dough ball, mechanically molded and placed in a French bread pan and kept in a proofer for 2.5h and 85% RH at 30ºC. Baking was carried out at 220°C for 9min in a convection oven (Tedesco, FTT150E). Bread products were cooled at room temperature (20°C) for 45min.

2.5 pH and total titratable acidity (TTA)

Sourdough samples collected before and after fermentation and were immediately frozen at -22ºC for further analyses. The same was made with final bread crumb sample. The pH and TTA values were measured in a suspension of 10g of each sample of dough or bread crumb blended with 90ml of distilled water according to Spicher e Stephan (1993). The TTA value was expressed as the amount (ml) of 0.1mol/L NaOH needed to achieve a final pH of 8.5. The pH was determined using a pHMeter (Quimis Q400A). Only the crumb of commercial sourdough bread was analyzed.

2.6 Specific volume and color analysis

The bread quality attributes were evaluated after cooling at ambient temperature. The loaves were weighed and loaf volume was measured by the rapeseed displacement method (AACC, 1988). Each loaf was put in a container and covered with rapeseed to fill the container totally. After the removal of the loaf, the volume of the rapeseed was noted. Loaf volumes were calculated by subtracting the rapeseed volume from the container volume. Specific loaf volume was calculated as ml/g.

Color was measured on two points of the crust of the central slices by means of a colorimeter (Chroma Meter CR-400C, Minolta, Osaka, Japan). The Hunter's scale parameters were determined: L*, a* and b*.

2.7 Texture analysis

Instrumental texture evaluation was performed 1h after baking to evaluate crumb and crust texture using a universal testing machine TA-XT2i (Stable Micro Systems, Surrey, UK) equipped with a 25kg load cell.

Texture profile analysis (TPA) was carried out to evaluate crumb texture using a 35mm aluminum cylindrical probe. Analyses were conducted according to the AACC method 74-09 (1986). A test speed of 1.7mm/s was used to compress the middle of the breadcrumb to 40% of its original height. Two bread slices of 25mm thickness taken from the center of each loaf were evaluated in this manner. The textural parameter considered was hardness (peak force of the first compression cycle in N). All measurements obtained with the two loaves from one batch were averaged into one value i.e. one replicate.

Crust penetration test was carried out on 10 mm thick and 25 mm wide crust pieces from bread top using a 2 mm stainless steel probe and a test speed of 60 mm min^{-1} (Bourne, 1978). Maximum peak force (N) was measured from the penetration curve and taken as crust hardness. Measurements were taken on the crust of two loaves at preselected locations.

2.8 Sensory evaluation

A panel of 50 non-trained testers was used to evaluate the sensory characteristics of the sourdough breads produced. They also evaluated a commercial sourdough bread from a bakery in Porto Alegre, RS and the control bread, in which no microorganisms where added on sourdough fermentation. Participants were asked to evaluate the acceptance of each loaf concerning appearance, color, aroma, texture, taste, after taste and overall acceptance. The sensory evaluation of samples was performed by the hedonic scale of 9 points (1 = disliked extremely, 9 = liked extremely).

3 RESULTS AND DISCUSSION

3.1 Full experimental design

The regression coefficients from the second order equations for each dependent variable can be observed in Table 2, along with their corresponding p, F (calculated and tabulated) and R^2 values. The analysis of variance (ANOVA) at the 95% confidence level implied that the majority

of the attributes evaluated resulted in F values lower than the tabulated values, as well as a low R^2 value, meaning that, for the studied range, the model was not able to explain the linear data behavior. However, pH and TTA values of fermented sourdough, aftertaste sensory score and crumb firmness were statistically significant, with high coefficient of determination ($F_c > F_t$; $R^2 \geq 0.94$; $p < 0.05$). A reduced model was used to investigate the effects of the significant factors for bread crumb pH ($F_c > F_t$; $R^2 \geq 0.80$; $p < 0.05$) and TTA ($F_c > F_t$; $R^2 \geq 0.75$; $p < 0.05$) values. The sensory attribute "taste" fitted well to the model with a confidence level of 90% ($F_c > F_t$; $R^2 \geq 0.86$; $p < 0.10$).

The one-way analysis of variance was made for an over-all comparison of the attributes that did not fit the linear model proposed in this study, as well as to compare the results of the control and commercial sourdough bread with experimental breads.

Table 2: Regression coefficients and analysis of variance (ANOVA) for pH and TTA of sourdough after 20h fermentation, pH and TTA of bread crumb, crumb firmness and sensory attributes taste and aftertaste.

		β_0	β_1	β_2	β_{12}	F_c	F_t	R^2
pH 20h	Cf	4.27	-0.58	-0.03	0.03	14.78	9.28	0.94
	pv	< 0.05	< 0.05	0.77	0.79			
TTA 20h	Cf	10.39	3.75	0.30	0.35	67.80	9.28	0.99
	pv	< 0.05	< 0.05	0.34	0.28			
pH crumb	Cf	4.51	-0.31	-	0.07	8.20	6.94	0.80
	pv	< 0.05	< 0.05	-	0.42			
TTA crumb	Cf	6.84	0.95	-	-	15.14	6.61	0.75
	pv	< 0.05	< 0.05	-	-			
Crumb firmness	Cf	23.55	-2.52	-3.78	0.80	34.05	9.28	0.94
	pv	< 0.05	< 0.05	< 0.05	0.18			
Taste	Cf	7.07	-0.07	-0.05	0.05	6.38	5.39	0.86
	pv	< 0.05	0.05	0.12	0.12			
Aftertaste	Cf	6.63	-0.29	0.14	0.18	14.78	9.28	0.94
	pv	< 0.05	< 0.05	0.09	< 0.05			

Cf, regression coefficient; pv, p value; β_0, constant; β_1, *Lactobacillus plantarum* cell concentration; β_2, *Saccharomyces cerevisiae* cell concentration; β_{12}, interaction between cell concentrations of *L. plantarum* and *S. cerevisiae*; Fc, F calculated; Ft , F tabulated; R^2, coefficient of determination.

3.2 Acidification characteristics

Initial sourdough pH value was quite similar (~5) in all experiments. At the end of the sourdough fermentation, pH value ranged between 4.9 to 3.7, similar range found in previous studies with different lactic acid bacteria (Collar Esteve, Benedito de Barber, & Martínez-Anaya, 1994; Thiele, Gänzle, & Vogel, 2002). The pH of fermented sourdough decreased significantly ($p \leq 0.007$) on experiments 2 and 4, both with higher amount of *L. plantarum* viable cells. In addition, experiments 1 and 3 resulted in higher sourdough pH values showing that the lowest inoculum level of bacteria has less effect on sourdough acidification. Response surface is shown in Fig. 1a. Such pH changes confirms that lactic acid production depends on the amount of viable LAB cells inoculated and was unaffected by the presence of the yeast. Paramithiotis et al. (2005) also reported no effect on lactic acid production when LAB strains (*Lactobacillus brevis, Lactobacillus sanfranciscensis, Lactobacillus brevis, Weissella cibaria, Lactobacillus paralimentarius, Pediococcus pentosaceus, Enterococcus faecium*) were co-cultured with *S. cerevisiae*.

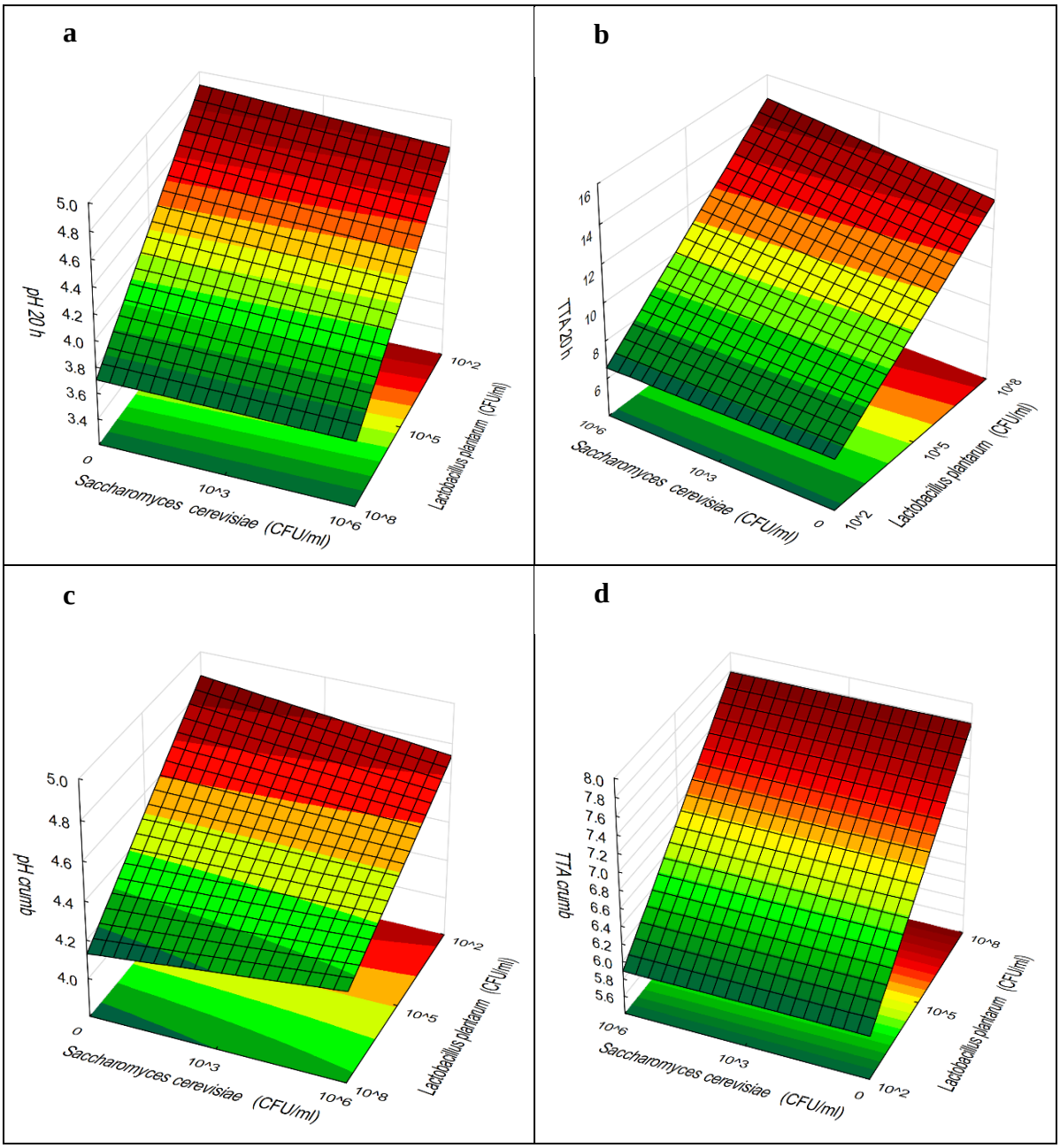

Figure 1: Response surfaces showing the effect of viable cell concentration of *Saccharomyces cerevisiae* and *Lactobacillus plantarum* on (a) sourdough pH after 20 h fermentation (Y1), (b) sourdough TTA after 20 h fermentation (Y2), (c) bread crumb pH (Y3), (d) bread crumb TTA (Y4)

Initial sourdough TTA, ranging from 3.75 to 3.00, did not showed significant differences between the experiments. After 20 h fermentation, TTA sourdough value was found to increase with an increase in LAB inoculum level ($p \leq 0.001$) as shown in Fig 1b. In addition, yeast had not a significantly association with TTA values, as organic acids are not principle products of alcoholic fermentation (Clarke, Schober, Angst, & Arendt, 2002).

The bread crumb pH value also exhibited correlation with LAB cell concentration, indicating that after bread baking, pH remains significantly ($p \leq 0.05$) lower in breads made with higher viable cells inoculated on sourdough (Fig. 1c). Increases in bread crumb TTA values continue to correlate with an increase in LAB cell concentration ($p \leq 0.05$) as shown in Fig. 1d. Once again, adding yeast did not significantly influenced the pH and the TTA of bread crumb. For both attributes, only terms statistically significant were included in the model instead of all possible interaction effects. With regards the control bread, a significant higher pH and lower TTA crumb values were observed. The commercial sourdough showed the opposite result, with significant lower pH and higher TTA values for crumb as shown in Table 3.

Lower pH is associated with positive effects on bread staling. However, it has been observed that the anti-staling effect depends on the specific strain performing the fermentation. This consequence involves dynamics other than those associated with the degree of acidification. Starch molecules, for instance, can be affected by enzymes produced by LAB, modifying the retrogradation properties of the starch (ARENDT; RYAN; DAL BELLO, 2007). In addition, LAB produces a wide range of low molecular weight compounds, peptides, and proteins with antifungal activity (Niku-Paavola, Laitila, Mattila-Sandholm, & Haikara, 1999).

Table 3: pH and TTA values of initial and fermented sourdough and bread crumb.

Test	pH	pH 20h	TTA	TTA 20h	Crumb pH	Crumb TTA
1	5.07 ± 0.03a	5 ± 0.02a	3.75 ± 0.35a	6.75 ± 0.35c	4.95 ± 0.01a	6.35 ± 0.21d
2	4.98 ± 0.01a	3.8 ± 0.01d	3.1 ± 0.28a	13.55 ± 1.2ab	4.2 ± 0.14cd	7.70 ± 0b
3	5.04 ± 0.05a	4.89 ± 0.01b	3.65 ± 0.64a	6.65 ± 0.35c	4.87 ± 0.02ab	5.60 ± 0.14e
4	4.98 ± 0.01a	3.79 ± 0.01d	3.3 ± 0.14a	14.85 ± 0.21a	4.4 ± 0.01bc	6.65 ± 0.21cd
5	5.05 ± 0.35a	4.13 ± 0.11c	3.2 ± 0.14a	11.05 ± 1.77b	4.4 ± 0.14bc	7.05 ± 0.07bcd
6	4.95 ± 0.07a	4.1 ± 0.57bc	3 ± 0.14a	10 ± 1.41b	4.4 ± 0.28bc	7.30 ± 0.28bc
7	5.05 ± 0.07a	4.23 ± 0.25c	3.35 ± 0.21a	9.9 ± 0.42b	4.33 ± 0.04c	7.35 ± 0.07bc
Control*	5.26 ± 0.01a	4.82 ± 0.03b	2.7 ± 0.14a	6.4 ± 0.14c	4.97 ± 0.1a	5.45 ± 0.07e
Commercial**	-	-	-	-	3.76 ± 0.01d	10.1 ± 0.28a

Results indicate mean values ± SD of one measurement.
Means with the same letter are not significantly different (Tukey, $p < 0.05$).
*Control bread produced with sourdough without starter, in the same conditions
**Commercial sourdough bread obtained from a bakery in Porto Alegre, RS.

3.3 Loaf specific volume, texture and color

Regarding to loaf specific volume, no noticeable differences were observed between the experimental breads neither between the control bread (Table 4). At first, this contradicts the study performed by Hansen and Hansen (1996) in which the addition of yeasts in sourdoughs affect positively the breads volume. At first, the result suggest that the leavening process was mainly performed by baker's yeast added to bread dough, independently of the amount of yeast added to sourdough. Additionally, the amount of LAB cell inoculated in sourdough did not affect volume. This finding was expected since *L. plantarum* is a homofermentative bacteria and do not contribute to the leavening process (Gobbetti et al., 1995).

Table 4: Differences in crumb and crust texture, specific volume of bread and color determination results (L*, a*, b*) on crust

Test	Crumb firmness (N)	Crust hardness (N)	Specific volume (ml/g)	L*	a*	b*
1	30.85 ± 0.46a	2.29 ± 0.32b	2.54 ± 0.41a	63.53 ± 3.46a	8.31 ± 1.95b	35.97 ± 0.73a
2	24.21 ± 0.21b	2.82 ± 1.15b	2.52 ± 0.10a	63.82 ± 2.43a	7.74 ± 1.5b	34.06 ± 0.47a
3	21.68 ± 0.1b	2.81 ± 0.51b	2.37 ± 0.21a	56.46 ± 2.62b	12.05 ± 0.96a	30.33 ± 0.33a
4	18.23 ± 0.77c	3.63 ± 0.67b	2.71 ± 0.21a	56.71 ± 0.14b	7.84 ± 0.87b	34.47 ± 0.53a
5	24.27 ± 0.9b	3.1 ± 0.24b	3.11 ± 0.05a	53.53 ± 0.87b	13.43 ± 0.96a	30.78 ± 6.02a
6	23.52 ± 0.27b	2.65 ± 0.61b	2.73 ± 0.27a	53.37 ± 5.24b	15.42 ± 0.12a	33.11 ± 3.21a
7	22.19 ± 0.76b	2.36 ± 0.26b	2.38 ± 0.32a	51.5 ± 5.27b	13.32 ± 0.9a	30.24 ± 3.68a
Control*	27.28 ± 2.95a	3.57 ± 0.44b	2.65 ± 0.09a	54.46 ± 4b	14.03 ± 0.99a	35.52 ± 1.89a
Commercial**	17.56 ± 0.09c	6.4 ± 0.32a	-	-	-	-

Results indicate mean values ± SD of one measurement.
Means with the same letter are not significantly different (Tukey, $P < 0.05$).
*Control bread produced with sourdough without starter, in the same conditions
**Commercial sourdough bread obtained from a bakery in Porto Alegre, RS.

Inoculation of higher amounts of viable cell counts of LAB and yeast led to significant lower crumb firmness and showed high values of coefficient of determination ($R^2 \geq 0.97$), suggesting that the model fitted well with the experimental data (Fig. 2). Crumb firmness is in general inversely related to bread volume (Clarke, Schober, & Arendt, 2002). Although breads in this study had not significant differences in specific volume, the decrease in crumb hardness may be explained by the influence of lower pH on cereal enzymes, which consequently affects the dough properties (GOCMEN *et al.*, 2007). This elucidates the fact that control bread (crumb pH of 4.97) had a firmer crumb and commercial sourdough (pH of 3.76) had the softer one.

Sourdough has been related to its positive effects in gas holding capacity of gluten in acidic dough (Gobbetti, Corsetti, Rossi, 1995), as well as solubilisation of pentosans (Corsetti et al., 2000) and modification in endogenous enzymes activities (Clarke et al., 2003). However, if fermentation is allowed to continue long enough to obtain intensive acidification, a stepwise degradation of gluten proteins can occur (Thiele, Gänzle, & Vogel, 2002) and may result in less elastic dough with poorer gas holding properties (Clarke et al., 2003). From these findings, another reason to lower firmness values of crumb might be the higher starch consumption due to the presence of higher concentration of *S. cerevisiae* inoculated on sourdough. Again, it would be expected that these breads would have higher volume due to the higher gas production by yeast. However, it did not occur because the gluten network was weakened and not able to hold the formed gas. In the study of Katina, Heiniö, Autio and Poutanen (2005), it was observed a significant decrease in bread volume when sourdough fermentation exceeded 14h using *L. brevis* as a starter.

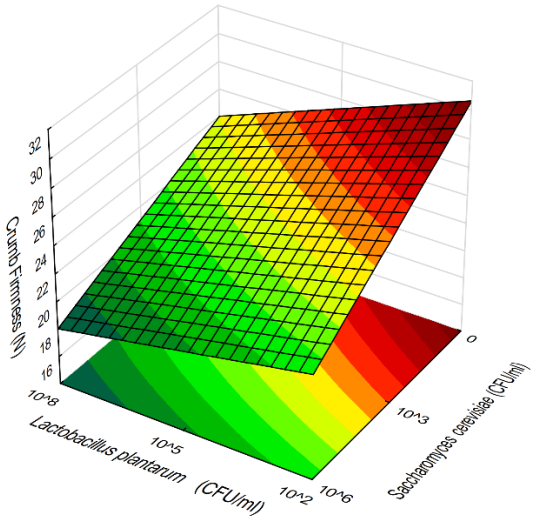

Figure 2: Response surface showing the effect of viable cell concentration of *Saccharomyces cerevisiae* and *Lactobacillus plantarum* on crumb firmness

Finally, there were no significant differences between experimental loafs with regard to crust firmness, with exception to the commercial sourdough that showed a higher crust hardness value. A possible explanation may be that the long fermentation time (about 6h) that the commercial sourdough bread goes through, favors the formation of a thicker crust. Moreover, the replacement level of the commercial dough with the sourdough is 20%, differently of the experimental breads in this study (50%). The study of Crowley, Schober, Clarke and Arendt (2002) identified that breads containing 20% sourdough had harder crusts in comparison to the bread containing higher amount (40%) of sourdough.

The color of the final breads obtained with different cell density of starters in sourdough registered small differences for the parameter a*. The parameter L* is the most interesting to be observed in order to correlate some difference in crust color hue (darker or lighter) with the levels of residual sugar due to lack or excess of fermentative activity from yeast. Sourdoughs with no addition of *S. cerevisiae* resulted in breads with a higher L* value, indicating a higher amount of residual sugar in doughs. This result supports the fact that the yeast added in sourdough did act in the fermentation process and consumed the hydrolyzed starch, resulting in a dough with less residual sugar and lighter crusts.

3.4 Sensory analysis

The results of the sensory evaluation of the appearance, color, aroma, texture, taste, after taste and overall acceptance of the experimental breads are shown in Table 5. Most of the sensory attributes did not differ between experimental breads. This low level of detectable difference was expected, due to the high number of consumers and their varying perceptions of sourdough bread. Several reasons for low variation have been attributed to consumers not being able to differentiate among products and scoring products randomly due to low motivation (Lawless & Heymann, 1999).

Table 5: Sensory analysis of bread produced with different viable cell counts of yeast and lactic acid bacteria

Test	Appearance	Color	Aroma	Texture	Taste	Aftertaste	Overall acceptance
1	7.36 ± 1.01ab	7.49 ± 1.12a	7.43 ± 1.06a	6.81 ± 1.48ab	7.26 ± 1.24a	6.91 ± 1.33a	7.32 ± 1.14a
2	7.57 ± 1.06a	7.53 ± 1.08a	6.86 ± 1.50a	6.96 ± 1.29ab	7.02 ± 1.30a	5.96 ± 1.57b	6.92 ± 1.19a
3	7.57 ± 0.96a	7.57 ± 1.00a	7.16 ± 0.96a	7.2 ± 1.27a	7.06 ± 1.43a	6.82 ± 1.55ab	7.12 ± 1.20a
4	7.44 ± 1.07a	7.54 ± 1.13a	7.08 ± 1.31a	7.06 ± 1.32a	7.02 ± 1.49a	6.6 ± 1.40ab	7.14 ± 1.28a
5	7.62 ± 0.82a	7.45 ± 1.04a	7.00 ± 1.08a	6.98 ± 1.03ab	7.11 ± 1.17a	6.68 ± 1.32ab	7.17 ± 1.01a
6	7.55 ± 0.95a	7.59 ± 0.97a	7.2 ± 0.86a	7.06 ± 1.00a	7.04 ± 1.18a	6.78 ± 1.37ab	7.02 ± 0.82a
7	7.69 ± 1.06a	7.55 ± 1.17a	6.98 ± 1.16a	7.18 ± 1.35a	7.04 ± 1.22a	6.65 ± 1.47ab	7.16 ± 1.12a
Control*	7.68 ± 0.98a	7.73 ± 0.85a	7.16 ± 1.33a	7.32 ± 1.22a	7.39 ± 1.26a	6.95 ± 1.51a	7.36 ± 1.24a
Commercial**	6.75 ± 1.26b	6.25 ± 1.38b	6.02 ± 1.45b	6.16 ± 1.41b	5.23 ± 1.79b	4.84 ± 1.83c	5.32 ± 1.65b

Results indicate mean values ± SD of one measurement.
Means with the same letter are not significantly different (Tukey, $P < 0.05$).
* Control bread produced with sourdough without starter, in the same conditions
** Commercial sourdough bread obtained from a bakery in Porto Alegre, RS.

Although sourdoughs made with co-cultures of lactic acid bacteria and yeasts may result in increased production of aroma compounds compared to monocultures (Hansen & Hansen, 1994), no significant difference was detected by the panel in sensory aroma scores. Considering a significance level of 90%, taste scores indicate that experiments with lower viable cells of LAB and yeast seemed to be more acceptable, as shown in Fig. 3a. According to Katina et al. (2005), the inability of sourdough containing yeast to enhance desired flavor is because in yeast fermentation, accumulation of amino acids is limited, because of a high demand for amino acids by yeast metabolism. This may explain why breads without yeast inoculation in sourdough received the highest scores for taste. However, incorporation of yeast was only significant in combination with minimum amount of LAB. Regarding to aftertaste, the model fitted well with the experimental data (Fig. 3b) and indicated low acceptance to breads with maximum LAB cell density ($p \leq 0.05$). The model also suggests that the presence of yeast ($p \leq 0.10$) is related to better acceptance of aftertaste. Control bread scores showed almost no significant difference between experimental breads. However, it is interesting to note that control bread (experiment 8) received the higher scores in almost all the attributes.

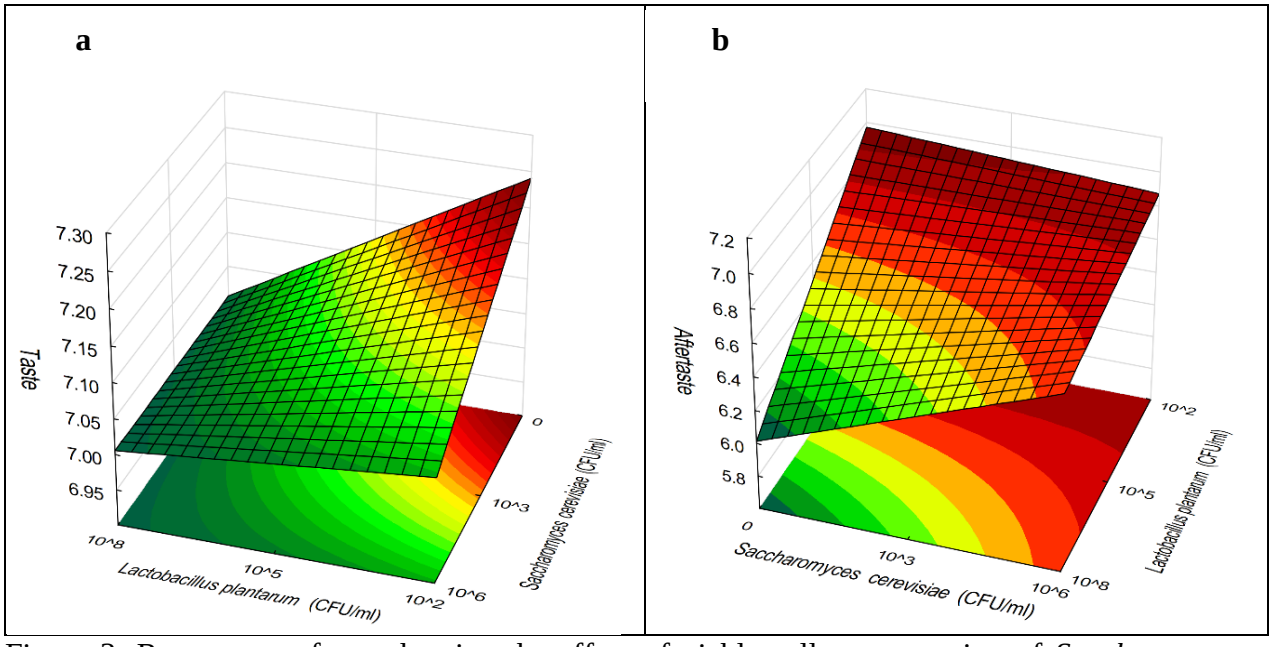

Figure 3: Response surfaces showing the effect of viable cell concentration of *Saccharomyces cerevisiae* and *Lactobacillus plantarum* on the following sensory attributes (a) taste (Y1), (b) aftertaste (Y2)

Commercial sourdough bread presented significant difference between the experimental breads in all attributes. The fact that commercial sourdough had a significant lower crumb pH value indicates that the panel´s preference was associated with breads with minor striking characteristics of a sourdough bread (high acidic).

According to Katina et al. (2005), the sensory analysis made by a trained panel showed that the desired flavor attributes of sourdough bread (intensity of overall flavor, roasted flavor and intensity of aftertaste) are strongly associated with undesired flavor attributes such as pungent flavor and reduced fresh flavor. This can be explained by the fact that acidification, and specifically formation of acetic acid, is the main factor enhancing pungent flavor. In their study, the undesirable intensity of pungent flavor had the highest scores when sourdough made with *L. plantarum* was fermented in 20 h. This result may explain why bread crumbs with lower pH values (higher formation of acetic acid) resulted in the lowest scores in taste and aftertaste.

In addition, a sensory evaluation of wheat sourdough bread crumb performed by Hansen and Hansen (1996) showed that bread made from sourdough fermented with the heterofermentative *L. sanfranciscensis* had a pleasant, mild, sour odor and taste, whereas sourdough bread fermented with the homofermentative *L. plantarum* had an unpleasant metallic sour taste. This finding supports the sensory scores obtained in this study, indicating that *L. plantarum* may not be the preeminent microorganism to achieve the desired sensory characteristics.

4 CONCLUSION

Significant influences of different cell concentrations of *L. plantarum* and *S. cerevisiae* starters was detected and able to fit in an experimental design model in some attributes evaluated such as bread taste, aftertaste, crumb texture and acidity. The optimal concentration of both microorganisms were specific and different for textural improvement and for sensory acceptance, which should be taken into account in future sourdough baking processes using these microorganisms. Results from taste and aftertaste reveled a correlation between pH and acceptability of the bread, indicating a resistance by consumers to breads with high acidity. However, softer breads were found in low pH values, when the maximum amount of yeast and LAB viable cells were inoculated.

The insignificant difference in specific volume and the significant differences in crumb firmness between breads revealed that incorporation of maximum cell concentration of yeast leads to a significant contribution in starch consumption, resulting in a softer bread. However, the gluten network presented a poor capacity of holding gas formed by yeast, thus, increase in bread volume was not possible. These findings correlate with the crust color results, which show that breads with no inoculation of *S. cerevisiae* present an intense coloration, since more residual sugar was present. No correlation could be made between the crust hardness and the different cell concentration of mixed starters, which indicates that, for this attribute, the final proof and baking procedures should be considered more relevant than the incorporation of sourdough.

The findings of this study confirm that expectations with regards the effect of sourdough on wheat bread quality must be analyzed with precaution and the used starter culture type must be carefully selected in order to achieve the desired quality. Although *L. plantarum* is one of the most common LABs found in sourdough, the relatively low sensory acceptance of final breads may suggest that this strain is not recommended to use as the only LAB in a sourdough starter. A possibility would be to mix a strain with different carbohydrate metabolism, such as a heterofermentative LAB, since they have better capacity to enhance sensory properties (Gobbetti, 1998). Additionally, further researches should be conducted in order to determine the influence of other parameters such as fermentation time, properties of the flour and amount of sourdough added in bread dough.

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5 CONCLUSION

At present, not much is known about the interactions among sourdough microorganisms and between environmental factors, hence, additional investigations of microbial dynamics, such as lactobacilli and their interactions with subdominant LAB species and yeasts, are needed in order to describe the real microflora of these ecosystems. The actual trend of using defined starter cultures to initiate sourdough fermentation has stimulated several studies of sourdough microorganism properties and their capability to enhance final bread quality.

Significant effects of different cell concentrations of *L. plantarum* and *S. cerevisiae* starters was detected and able to fit in an experimental design model in some attributes evaluated such as bread taste, aftertaste, crumb texture and acidity. The optimal concentration of both microorganisms for textural improvement and sensory acceptance were different, and it should be taken into account when preparing future sourdough baking processes with these microorganisms.

The principal purpose in using pre-selected starter cultures is to provide acidification and flavor to breads. However, depending on the process parameters, the selected microorganisms and the cell density of each one, sensory characteristics change and can result in final breads with low acceptance. Results from taste and aftertaste reveled a correlation between pH and acceptability of the bread, indicating a low acceptance by consumers to breads with high acidity. However, it is important considering that a non-trained panel, which was not used to consume sourdough breads, made the analysis. Thus, different results could be obtained if a trained panel did this evaluation.

The insignificant difference in specific volume and the significant differences in crumb firmness between breads revealed that incorporation of maximum cell concentration of yeast lead to a significant contribution in starch consumption, resulting in a softer bread, but the gluten network presented a poor capacity of holding gas formed by yeast, thus, increase in bread volume was not possible. These findings correlate with the crust color results, which intense coloration was detected in breads with no inoculation of *S. cerevisiae*, since more residual sugar was present.

No correlation could be made between the crust hardness and the different cell concentration of mixed starters, which indicates that, for this attribute, the final proof and baking procedures might be more determinant to results than the incorporation of sourdough in bread dough.

The results of this study confirm that expectations with regards the effect of sourdough on wheat bread quality must be analyzed with precaution and the starter culture type used must be carefully selected in order to attain the desired quality. Although *L. plantarum* is one of the most common LABs found in sourdough, the relatively low sensory acceptance of final breads may suggest that this strain is not recommended to use as the only LAB in a sourdough starter. A possibility would be to mix a strain with different carbohydrate metabolism, such as a heterofermentative LAB, since they have better capacity to enhance sensory properties (GOBBETTI, 1998). Additionally, further researches should be conducted in order to determine the influence of other parameters such as fermentation time, properties of the flour and amount of sourdough added in bread dough.

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APENDICE A – Sensory Analysis Form

Análise Sensorial de pães de fermentação natural

Nome: Data:

Idade:

Você está recebendo 2 amostras de pães de fermentação natural (*sourdough* ou *levain*). Prove as amostras, ingerindo água entre elas, e avalie os parâmetros de acordo com a escala abaixo.

1- Desgostei muitíssimo
2- Desgostei muito
3- Desgostei moderadamente
4- Desgostei levemente
5- Nem gostei nem desgostei
6- Gostei levemente
7- Gostei moderadamente
8- Gostei muito
9- Gostei muitíssimo

Amostra	**145**	**719**
Aparência		
Cor		
Aroma		
Textura		
Sabor		
Sabor residual		
Aceitação global		

Comentários: _____

_____