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STARTER CULTURE FOR USE IN SOURDOUGH BREAD MAKING: A REVIEW

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Abstract: Fermentation has been used by humans since 6000 b. C. and in recent years, natural fermentation products, such as bread, preserves, *kombucha* (fermented tea) and *kimchi* (fermented napa cabbage) have gained notoriety for their probiotic effects on people's health, as well as for providing different flavors, tastes and aromas. Currently, the yeast available for bakeries and bread making presents only one species of yeast: *Saccharomyces cerevisiae*. The fermentation process is important for the modifications on the texture and flavor of the final product. Therefore, if it is used only one species of microorganism, the final product will have the same result regarding taste and texture. The sourdough, a wild culture of bacteria and yeast has been used for homemade bread making mainly because of the final flavor that this fermentative culture provides to bread and baking products. The problem this fermentative culture poses for the general public is that it is not available in the market, therefore, resulting difficult for home and professional bakers to have access to it, unless a starter culture is developed by them, a process that can take as long as seven days. Sourdough bread and baking goods present a unique set of aromas, flavors and texture not found in products resulting from the use of *Saccharomyces cerevisiae*. This article is a review approaching several aspects of sourdough, such as how it can be established, kept and the types of it. The elements that constitute bread and the most common microorganisms found in sourdough, as well as possible techniques used to lyophilize starter cultures. Lyophilization method requires the use of a cryoprotectant indispensable for the later viability of samples.

Keywords: Fermentation, Yeast, Levain, *Saccharomyces cerevisiae*.

BREAD

Bread is undoubtedly one of the main goods consumed around the world. It is a constant presence in almost every household in several different ways, varying according to country, region, ingredients available and culture. Bread, amongst other baking goods, are the base of the population food pyramid.

Bread is all products obtained from wheat flour and other flours with the addition of a liquid, resulted from fermentation process or not and cooked or baked. Flours that may be used for the production and propagation of sourdough are quinoa (Rizello et al., 2017), amaranth (Alencar et al., 2015), semolinas (*Triticum durum*) (Alfonzo et al., 2016) and chia (*Salvia hispanica*) (Constantini et al., 2014). Bread has several forms and shapes, may contain other ingredients as long as they do not change its main characteristics and present fillings and toppings in its final product.

The process of making bread can be divided into three steps: the first, is the one in which the main ingredients are combined and mixed and the dough is formed. The second, involves the dough fermentation and the third and final is baking. During the third step, the starch is gelatinized, the protein is denatured, and the raw dough is baked into a porous, digestible caramel-color product. The gluten protein controls the viscoelastic properties of the dough. During the mixing process, glutenins align and form cross-links between glutenin molecules, which increases the dough strength (Gupta, Batey and MacRitchie, 1992). According to McCann and Day (2013), the hydrated gluten protein network provides a structure which holds starch granules this way trapping air bubbles. On the fermentation processes, which uses *Saccharomyces cerevisiae*, sugar is converted in carbon dioxide, water and ethanol. When exposed to higher temperatures while baking,

carbon dioxide is expanded as well as water, therefore creating a natural isolation that prevents the humidity to escape (Mondal and Datta, 2008).

The main ingredients for making bread are flour and its types, i.e. whole wheat, rye, and spelt; water, salt and yeast (Camargo, 2013). Other non-essential ingredients may be added, such as fats (butter, olive oil, lard). Water hydrates the flour dissolving part of the proteins and starches binding them together. Other ingredients may include water, milk, yeast, sugar and some types of fat, such as butter and vegetable oil.

Wheat flour is composed by starch (70 – 75%), water (12 – 14%), protein (8 – 16%) as well as other components, such as non-starch polysaccharide (2 – 3%), lipids (2%) and ashes (1%). These values may vary accordingly to weather and soil conditions. The quality of the gluten and starch play an important role in bread making, regarding texture. Fibers and lipids also contribute to bread quality (Morita et al., 2002).

During the baking process, the dough receives high temperature (180°C to 230°C), therefore changing its original characteristics. The main chemical reactions are the protein denaturation, starch gelatinization, enzymatic activation or deactivation, production of color and aroma and caramelization and Maillard reaction (Germani, 2018). Different types of flour, additional ingredients and the bread making process technology may influence the microbiological composition of the sourdough, therefore, resulting in different products regarding flavor, aroma and texture.

SOURDOUGH

Sourdough is the process of combining flour and water and have it fermented with microbes originated from preceding sourdough, starter culture (commercial or non-commercial) and other agents (Katina, 2005). According

to Katina (2005), the use of sourdough in bread making is an alternative to the use of preservatives and additives. Some studies demonstrate the effectiveness of sourdough fermentation in the improvements of aspects, such as nutritional value (Liukkonen et al., 2003; Kariluoto et al., 2004).

Most bakeries and the bread production industry in Brazil adopt rapid fermentation processes using commercial yeasts, such as *Saccharomyces cerevisiae*. Traditional and artisanal bakeries commonly use sourdough, in some cases propagated over centuries. *Saccharomyces cerevisiae* is the most common commercial yeast used in bread making around the world. Under anaerobic conditions, yeast cells metabolize fermentable sugars, i.e. fructose, glucose, maltose and sucrose, producing carbon dioxide, enhancing the dough volume (Chavan and Chavan, 2011). Commercial yeast derives from selected stocks of industrial cultures and may be lyophilized or not. The lyophilized version is frequently called “instant”. Commercial yeast using *Saccharomyces cerevisiae* presents standardized quality, and breads using it present low acidity levels.

A commercial lyophilized ready-to-use version of sourdough can be found but its composition is not specified. They are quite popular in France, Germany, Italy and even the USA. The most common yeast used in bakeries and households is *Saccharomyces cerevisiae*, which presents only one type of product regarding texture, flavor and aroma. It is widely used and easily found in any supermarket or supplier for baking goods, therefore, it simplifies the bread making process and provides a standardization of production. Most of the sourdough properties are the outcome of lactic acid bacteria's (LAB) metabolic activity present in the process: lactic fermentation, proteolysis, volatile compounds synthesis and anti-fungal activity (Stefanello,

2014).

The advantages of sourdough in bread making over the baker's yeast (*Saccharomyces cerevisiae*) as a leavening agent relies on a number of aspects: it develops a characteristic flavor of the bread, resulting in a high sensory quality product through more elasticity (Takeda et al. 2001; Clarke et al. 2004), internal humidity (Corsetti and Setanni 2007), an improved nutritional value due to the reduction of phytase, an anti-nutritional enzyme (Leenhardt et al. 2005; Gänzle 2014), antifungal production, resulting in a longer shelf life (Hammes and Gänzle, 1998; Manini et al., 2016), production of bacteriocins and higher acidity (Messens and De Vuyst, 2002; Gänzle, 2004; Poutanen et al., 2009).

Acidification of the dough is mainly influenced by temperature, type of flour and starter culture used. The acidic dough has an intricate biologic system in terms of microorganisms' composition and the influence of several aspects upon them. Fermentation time and temperature both impact on the result acidity formation. It is assumed that higher levels of acidity in sourdough may require higher temperatures for fermentation as well as longer fermentation time (Katina, 2005).

Chavan and Chavan (2011), as well as Katina (2005) and Reddy et al. (2009) have reported the benefits of the biological acidification on the bread quality due to the metabolites formed during fermentation.

COMMON MICROORGANISM FOUND IN SOURDOUGH

The microflora of spontaneous dough fermentation depends on the microflora of the ingredients used to make the dough, as well as the hygienic conditions, origin of products, kind and flour storage, and the technological aspects involved in the fermentation process (Katina, 2005). The majority of microorganisms

in spontaneous fermented dough are homofermentative lactobacilli and pediococci (Katina, 2005). Typical homofermentative lactic acid bacteria found in sourdough are *Lactobacillus casei*, *Lactobacillus delbrueckii*, *Lactobacillus farciminis*, *Lactobacillus phantarum* and *Pediococcus pentosaceus*. Typical heterofermentative lactic acid bacteria include *Lactobacillus brevis*, *Lactobacillus buchneri*, and *Lactobacillus fermentum* (Stolz et al., 1995).

Microbiologic studies presented more than fifty species of LAB, most of them *Lactobacillus*, and more than 20 species of yeast, mostly *Saccharomyces* and *Candida* (Aplevicz, 2013). Many factors influence the quality of the sourdough, i.e. temperature, type of starter culture and acidification (Chavan and Chavan, 2011).

Lactic acid bacteria cause the acidification of the dough by producing organic acids, mostly lactic acid. Besides organic acids, LAB produces ethanol, aromatic compounds, bacteriocins and a number of enzymes (Aplevicz, 2013). According to Aplevicz (2013), Corsetti and Setanni (2007), the ratio between lactic acid and acetic acid affect immensely the final product aroma. Lactic bacteria produce a wide array of compounds from fermented foods, providing them with the characteristic natural fermentation flavor, as well as food safety and rheology (Arendt et al., 2007). *Lactobacillus* (Lb.) can be divided into three groups accordingly to the final product resulting from their fermentation: obligate thermophilic homofermentative *Lactobacillus*; facultative heterofermentative mesophilic *Lactobacillus*, and obligate heterofermentative mesophilic *Lactobacillus* (Aplevicz 2013).

Besides the ones mentioned, the undesired *Staphylococcus aureus* and *Bacillus cereus* can be found. *Staphylococcus aureus* is a Gram-positive pathogen and can cause harm

not only to people but also to animal's health. Sometimes *S aureus* colonies persist in some fermented food causing food poisoning. In an environment dominated by LAB, *Staphylococcus aureus* may have inhibited them. *Bacillus cereus* is also a Gram-positive bacteria and some strains can cause foodborne illnesses to humans whereas other strains may be beneficial to animals as probiotics.

Yeast can be spherical, oval shaped or elliptical. They do not possess flagellum and reproduce through vegetative reproduction known as sprouting or gemmulation (Aplevicz, 2013). In order to reproduce, yeasts need certain elements, such as water, nitrogen and carbon sources, oxygen, and minerals (Tortora, Funke and Case, 2002). Common yeasts found in sourdough are *Kazachstania exigua*, *Candida milleri* and *Candida humilis*.

PRESERVING METHODS

LYOPHILIZATION/FREEZE DRYING

Lyophilization is a method that removes water through the sublimation of ice crystals from frozen materials. Sublimation is a phenomenon in which water passes directly from solid state (ice) to a vapor state without passing through a liquid state. There are parameters and settings to obtain the best results from different materials. Lyophilization is a rather costly process due to the time and energy spent to achieve the desired results (Gaidhani et al., 2015).

Lyophilization is one of the most useful methods of preserving food. It is widely applied in several fields. This method is the most satisfactory for long-term LAB preservation and stabilization until use (Kearney et al., 1990).

Microbial cell surviving after the lyophilization process depends on the protective medium (cryoprotectant) used for the protection of the cells during the freeze procedure and the rehydration method used

for the cells reactivation. The protective medium prevents cell damage and improve stability during storage time. The medium used to maintain the cultures viable are, in general, skimmed milk with an addition of yeast and glucose extract (Badis et al., 2004), BHI broth (Gálvez et al., 2007), MRS broth and M17 broth (Marino et al., 2003).

There are several ways to dry sourdough, but among useful methods, freeze drying is very popular and useful for food preservation (Rhodes, 1993). The drying method to be chosen depends on the Lyophilization method may at first seem costly but in comparison with freezing rates, it is still a very good manner to storage starter cultures. Once the water is evaporated through sublimation, the weight of the final product reduces considerably, therefore reducing transportation costs.

CRYOPROTECTANTS

In addition to the method chosen for cryopreservation, a cryoprotective agent is also made necessary for an efficient process. Cryoprotectants preserve the microorganisms' cells when they are stressed during the dry-freezing process. Reddy et al. (2009) used different cryoprotectants, such as Trehalose, Sucrose, Lactose, Skimmed Milk, Maltodextrin, on *Pediococcus acidilactici*, *Lactobacillus plantarum*, and *Lactobacillus salivarius* to evaluate the retention of probiotic properties and viability. Also, lactose, skimmed milk and maltodextrin were used as cryoprotectants and the retention of probiotics properties was verified.

When lactobacilli were freeze dried with no addition of a cryoprotectant agent, a severe decrease in viability of 50% was perceived (Reddy et al., 2009)

Several carbohydrates have been proposed as cryoprotectants for freeze drying lactic acid bacteria and subsequent storage. Amongst them are trealose, sucrose, lactose, skimmed

milk and maltodextrin. Sucrose was used as a cryoprotectant for lactic acid bacteria in freeze dried powdered kimchi which improved the survival rate of LAB (Song et al., 2015).

Cold shock has also been applied in some cases, which resulted in an increase of 6-8% of both, probiotic properties and viability compared to the samples freeze dried without the cold shock. Samples of *Lactobacillus* which were freeze dried with sucrose presented more than 92% retention in viability compared to initial numbers.

THE IMPORTANCE OF THE CHOICE OF INGREDIENTS TO FORM SOURDOUGH

The choice of ingredients to produce a starter culture, especially the liquids, will determine the strains of microorganisms that will feed from the carbon sources and multiply. The microorganisms' metabolism present different aromas, texture and flavor to the final product. This is the main reason there are differences between the starter cultures made from pineapple juice and sugar cane juice. The baking products made out of them present different organoleptic characteristics.

Sugarcane is composed mainly of sucrose, whether pineapple is composed mainly by fructose. According to a study carried out by Rehman, Paterson and Piggott (2006), the addition of sucrose in wheat flour to produce starter cultures is favorable for both yeasts and LAB. It also stimulates the bacterial production of acetic and lactic acid. Bread made from those two starter cultures present different characteristics regarding aroma and texture, resulting from the metabolism of these microorganisms.

They also present different strains of bacteria and microorganisms which also affects the characteristics of the final product, presenting different aromas, acidity and possibly an increase in shelf life.

Lactobacillus plantarum is commonly found starter cultures made from cereal flours. *Lactobacillus plantarum*'s metabolism plays a very important role in the process of bread fermentation and proving due to its proteolysis activity and acidification properties (Rizzello et al., 2017).

Yeasts tend to thrive quite well at temperatures ranging between 20°C and 30°C. *Saccharomyces* can develop at low and high temperatures. Yeasts are more tolerant to the absence of water since they are facultative anaerobes. Bacteria on the other hand, prefer an acidic environment and need water activity to thrive (Fleet, 2011).

Fleet (2011) and Weusthuis et al. (1994) both state yeast cells do not thrive well at low temperatures, low pH and high levels of salt and sugar. The presence of excess sugars can also inhibit the fermentative metabolism of yeasts (Weusthuis et al., 1994). Not only the low pH can harm yeasts, but the variation of pH and other factors may difficult or prevent the survival of microorganisms.

BENEFITS OF SOURDOUGH

The digestibility of bread produced from sourdough is higher than of the ones using commercial yeast (*Saccharomyces cerevisiae*) probably due to the longer period of fermentation and the type of microorganisms present in the starter culture. A high quantity of starch is metabolized in ethanol, CO₂, lactic and organic acids and protein is broken down into amino acids and peptides, whereas complex carbohydrates are broken down into and digestible simple sugars. Since the amount of resulting starch is reduced, the glycemic index is of the bread or baking good is affected (Liljeberg et al., 1995).

The application of sourdough to wholegrain bread reduces the content of FODMAPs (fermentable, oligosaccharides, disaccharides, monosaccharides and

polyols) in baking products. FODMAPs are significantly reduced during the propagation of microorganisms in sourdough and sucrose and glucose are degraded in the first step of fermentation. Sourdough also presents a lower concentration of carbohydrates and fructants, glucose, fructose and sucrose, as well as a higher content of organic acids and polyols (Menezes, 2019).

Sourdough bread, as well as other fermented products, present different aromas and flavors, better food safety due to a more acidic pH, which prevents certain strains of bacteria to develop, and also, it modulates nutritional properties of sourdough bread in a number of ways, such as increasing the levels or bioavailability of bioactive compounds in addition to retarding the digestibility of starches (Stefanello, 2014).

AROMA

One of the factors that makes the sourdough bread more attractive is undoubtedly the aroma it presents. It affects the acceptance of it by consumers immensely. The aromas present in the sourdough bread comes from the volatile compounds generated in the proteolysis of gluten, processing phases and the heat applied during the baking process (Heiniö et al., 2003).

Compounds of chemical classes are aldehyde, acids and alcohol. The three of them provide sourdough bread its distinctive aroma. The sourdough microbiota produces compounds that improve the sensory qualities of sourdough bread (Ripari et al., 2016a; Ripari et al., 2016b).

Kim et al. (2009) performed a characterization of aromas, producing bread with 20% sourdough in 2009. Amongst the 53 compounds identified, ethanol and 3-methyl-1-butanol were found in higher concentrations. Ethanol and 3-methyl-1-butanol are commonly found in yeast

fermentations. In another study, volatile compounds found in samples were 16 alcohols, 13 esters, 8 aldehydes and 6 ketones. The others were acids, furans, pyrazines, phenol, terpenes, lactones and alkanes (Aponte et al., 2013). Whenever sourdough is used in bread making, aromas different from the ones presented by *Saccharomyces cerevisiae* may be expected.

TEXTURE

Bread texture is defined by microbial acidification and rate of substrate breakdown and the microorganisms involved in the process. Individual strains of LAB and perhaps a combination of several present a positive effect on the final sourdough texture. Expolysaccharides produced by LAB can replace expensive hydrocolloids commonly used as bread improvers (Arendt et al., 2007).

A study carried out about the influence of the lactate and NaCl on gluten showed that low pH has great influence on firmness and elasticity of wheat bread (Schober et al., 2003).

The longer the time between production and consumption, the harder the crumb will be. As shelf time increases, there is a decrease in bread quality and freshness, as well as consumer acceptance known as staleness (Hebeda et al., 1990).

SHELF LIFE

Bakery products using *Saccharomyces cerevisiae* have a short shelf life if antifungal additives and enzymes are not added to it during the bread making process. The quality of the bread depends on the time period between bread production and consumption. Once ready for consumption, bread can take some time stored until it is purchased and consumed. And as time passes, there is a decrease in bread freshness and an increase in crumb hardness. During this time, several changes occur in the quality of

the bread. Amongst them, the crust becomes harder and difficult to chew, and the texture becomes crumbly and the exterior harder. As bread ages, several rearrangements in the starch fraction lead to several changes in bread structure, such as crystallization and gelatinization (Arendt et al., 2007). All these factors lead to a staling effect in the bread, this process is called retrogradation.

What determines the staling rate are changes in the aroma, hardness, opacity, crumbliness, absorptive capacity, starch crystallinity, susceptibility to alpha amylase and soluble starch content.

Sourdough bread presents a longer shelf life and a reduction in the staling rate. The decrease in the staling rate can be measured by differential scanning calorimetry.

According to Mohsen et al. (2016), sourdough bread containing 2 or 3% of *Lactobacillus plantarum* had an increase in shelf life up until 8 days, whereas the control sample only presented 3 days. Sourdough bread containing 20% sourdough with at least 3% of LAB had a staling rate retarded by 19.98% and 19.30% after 3 days, compared to the control sample, which was 42.84% staling rate (Mohsen et al., 2016).

Mould growth is the most common cause of bread spoilage and mycotoxins produced by them may present public health problems. Fungi may remain static due to the effect of sourdough's LAB production of organic acids. Therefore, sourdough is the most promising procedure to extend the bread shelf life (Sadeghi, 2008).

It has also been noticed that the anti-staling effect is due not only to the acidification of the bread, but also due to some strains of bacteria found in it during the fermentation process. LAB produces enzymes which affect starch molecules, which cause a variation in the retrogradation properties of the starch, reducing, therefore, the rate of staling (Corsetti

et al., 1998).

CONCLUSION

Natural, or spontaneous fermentation has never been extensively studied and tested as it has been in recent years. People are curious and stepping into the kitchen to produce their own food in search for better quality of life through food. A lyophilized starter culture would benefit that industry immensely, since it would turn a rather complicated and long process into a more accessible and easier one. The microorganisms present in the starter culture used in bread making are responsible for several qualities which improve significantly bread quality. Due to the metabolism of bacteria, an acidification can be perceived as well as an improvement in texture, flavor and aroma, not to mention a longer shelf life. The obstacles faced by bakeries and the bread industry lie in the fact that the starter culture needs constant care and attention through weekly additions (refreshments or backslopping) of flour and water and quality in the final product may vary. A lyophilized ready-to-use starter culture represents economy and better quality bread in all senses. If added to regular bread, besides flavor and aroma it would represent a longer shelf life and thus, a lower spoilage rate, which would be economically beneficial to the industry.

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