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Effect of the refreshment on the liquid sourdough preparation

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PAPER

Abstract

The aim of this work was to investigate the effect of refreshments on the growth of endogenous microorganisms during liquid sourdough preparation by using an Italian and Mexican wheat flours and its effects on the physicochemical properties (pH, total titratable acidity, water activity, moisture content and reducing sugars). The liquid sourdoughs were prepared (DY 200) and incubated for 6 days at 20°C. The sourdoughs were refreshed every day and compared with the not-refreshed ones. Preliminary results showed that in the early stages of the microbial growth process, their population was greater in the sourdough made from the Mexican wheat flour than that of the Italian one. However, after 6 days, the microbial population was not significantly different in refreshed or not-refreshed samples for both sourdoughs (Italian and Mexican). Similarly, physicochemical properties did not show significant differences.

Keywords: backslopping; leavening agent; sourdough; spontaneous fermentation

Introduction

The art of baking is a very ancient technology. The beer foam was initially used for leavening of bread by ancient Egyptians, which was then replaced by sourdough (Carnevali *et al.*, 2007); in fact the sourdough fermentation is one of the oldest cereal fermentations known by mankind. Sourdough is a mixture of wheat and/or rye flour and water, possibly with added salt, fermented by spontaneous lactic acid bacteria (LAB) and yeasts from the flour and environment. The microbial ecosystem varies from one sourdough to another depending on the geographical position, which determines its acidifying and leavening capability. The microbial community makes the dough metabolically active and can be reactivated and optimised in time through consecutive refreshments (or re-buildings, replenishments, backslopping) (Corsetti and Settanni, 2007). The term 'refreshment' deals with the technique by which a dough made of flour, water, and sometimes other ingredients ferments spontaneously, and it is subsequently added as an inoculum to start the fermentation of a new mixture of flour and water or other ingredients.

The sourdough fermentation is a process with very complex mechanisms (Hammes and Gänzle, 1998; Thiele *et al.*, 2002), and during fermentation carbohydrates and flour proteins undergo biochemical changes due to the action of microbial and indigenous enzymes (Spicher, 1983). The rate and magnitude of these changes greatly affect the sourdough properties and ultimately the quality of the final baked product (Arendt *et al.*, 2007). Many

intrinsic properties of sourdough depend on the metabolic activities of its resident LAB: lactic fermentation, proteolysis and synthesis of volatile compounds, production of anti-mold, and antiropiness are amongst the most important activities during the fermentation of sourdough (Gobbetti *et al*., 1999; Hammes and Gänzle, 1998). The fermentation of natural yeast consequently improves the dough properties, such as improving the volume, texture, flavour and nutritional value of bread; delaying the staling process of bread, and protecting bread from mold and bacterial spoilage (De Vuyst and Vancanneyt, 2007). In fact nowadays, its application is on the rise, and sourdough is used in the production of a variety of products such as bread, pizza, cakes and crackers, as the improved quality of sourdough bakery products became an important marketing tool (De Vuyst and Gänzle, 2005). Because fermentation can be performed as firm dough or as a liquid suspension of flour in water, sourdoughs can vary in its consistency. The ratio of flour and water is called the dough yield (DY) and is defined as: DY = (flour weight + water weight) × 100/flour weight. Following this approach, wheat sourdough with DY 160 is firm dough, while DY 200 is liquid sourdough (Decock and Cappelle, 2005). The liquid fermentation system is preferred by industries due to the following technological and analytical advantages: (1) ease of management and reproducibility under operating conditions; (2) easier control of fermentation parameters (e.g. temperature, pH, dough yield), and addition of nutrients (e.g. vitamins, peptides, carbohydrates) to condition microbial performance; (3) greater suitability to deal with microbial metabolism to obtain an optimal organoleptic profile; (4) greater suitability of application as natural starter without changes to the current bread formulations; and (5) increased suitability for use with different technologies to produce various baked goods (Carnevali *et al.*, 2007). This work was carried out to investigate the effect of refreshments on the growth of endogenous microorganisms during the preparation of liquid sourdough (DY 200) incubated for 6 days using wheat flours from two different geographical locations (Italian and Mexican flour), and their effects on physicochemical properties, such as pH, total titratable acidity (TTA), water activity (aw), moisture content and reducing sugars.

Materials and Methods

Materials

For liquid sourdough preparation, two types of commercial wheat soft flour '00' were used. The first flour type, Mexican flour, had a protein content of 11.1%, fat 2.2%, carbohydrates 71.6% and fibres 2.1% (San Antonio, Tres Estrellas, Toluca, México). The second one was the Italian flour, with a protein content of 11%, fat 2%, carbohydrates 72% and fibres 2% (La Molisana, Campobasso, Italy). The average moisture content of both flour types was 13%.

Chemicals

The following were used for the study: Plate count agar (PCA), potato dextrose agar (PDA) (BD, Franklin Lakes, NJ, USA), NaCl, NaOH, 3,5-dinitrosalicylic acid, sodium potassium tartrate, D-glucose. All chemicals used were of analytical grade, purchased from Sigma–Aldrich (St. Louis MO, USA).

Preparation of sourdoughs

Four types of liquid sourdough were prepared, two for each type of flour (Mexican and Italian flour). The liquid sourdough was prepared by mixing 500 g of flour with 500 mL of distilled water. The ingredients were mixed in a spiral mixer (Grilletta IM5, Famag s.r.l, Milano, Italy) for 10 min at speed 1, and the sourdoughs were fermented at 25°C ± 1 for 6 days. The samples were remixed every day for 5 min, and one sample for each type of flour was refreshed by removing 200 g of dough that was replaced with 100 g of flour plus 100 mL of distilled water. The aliquots of samples, taken each day before remixing, were used for the following experiments. Table 1 shows the different samples prepared.

Determination of microbial populations

Serial dilutions of liquid sourdough samples in 0.85 % NaCl solution were used for determining the microbial count using the following media: PCA for estimation of total aerobic mesophilic bacteria and PDA containing 14 mg/L of tartaric acid, 50 mg/L of chloramphenicol, and 50 mg/L of Rose Bengal for yeasts and other fungi. Exactly, 1 mL of appropriate dilutions was pour plated in triplicate. Counts of total aerobic mesophilic bacteria were obtained after 48 h of incubation at 37°C, while the count of yeast and other fungi were obtained after 5 days of incubation at 30°C (Ben Omar and Ampe, 2000). All values were performed by counting on a colony counter. Results were calculated as the means of three determinations ± standard deviation .

Table 1. Different samples of liquid sourdough.

DMNR	Sourdough not refreshed, prepared with Mexican flour
DMR	Sourdough refreshed, prepared with Mexican flour
DINR	Sourdough not refreshed, prepared with Italian flour
DIR	Sourdough refreshed prepared, with Italian flour

Determination of pH, titratable acidity, moisture content, water activity and reducing sugars

The values of pH were determined using a pH meter equipped with an immersion probe, calibrated using standard solutions at pH 7.00, 4.01 and 10.00. After calibration, the electrode was rinsed with distilled water, dried and immersed in the sample.

Total titratable acidity was measured in 10 g sample, which was homogenised with 90 mL of distilled water for 3 min in a Stomacher apparatus (Seward, London, UK) and expressed as the amount (mL) of 0.1 M NaOH needed to achieve a pH of 8.3 (Ercolini *et al.*, 2013).

The moisture content using the thermobalance (XM 50 Precisa, Biltek, Esenler, Istanbul, Turkey) was calculated using the following Equation 1:

$$\text{Moisture content (\%)} = \frac{(\text{Mi} - \text{Mf})}{(\text{Mi})} \times 100 \qquad (1)$$

Mi – fresh weight, g
Mf – dry weight, g

The values of water activity (aw) were determined by Aqua-Lab instrument (CX-2, Decagon Devices, Pullman, WA, USA), calibrated with saturated KCl (aw = 0.984) standard. The determination was carried out by preparing a homogeneous sample of the product. The value was detected in balanced conditions and read directly on the screen.

Reducing sugars were determined using DNS assay (Wood *et al.*, 2012). DNS reagent contain 3,5-dinitrosalicylicacid (10 g/L), sodium potassium tartrate (30 g/L) and NaOH (16 g/L) and is stored in darkness at room temperature. D-glucose calibration curves were created covering appropriate ranges as described in the relevant sections. Each reaction contained 50 µL of sample and 1 mL of DNS reagent (1:20, sample:DNS reagent). The resulting solutions were heated in a thermocycler (Biometra T-Gradient, Germany) at 100°C for 1 min, and held for 2 min at 20°C to cool, and analysed using a spectrophotometer (Genesys 10UV, Thermo Scientific, Waltham, MA, USA) at 540 nm.

Results and Discussion

The microbial population of the sourdoughs was enumerated using two different culture media: PCA for estimation of total aerobic mesophilic bacteria and PDA for yeasts and other fungi. Figure 1 shows the growth of aerobic mesophilic bacteria during the 6 days of incubation. The initial concentration of bacteria was higher in sourdoughs made with Mexican flour (4 Log UFC/g) than in sourdoughs made with Italian flour (3.2 Log UFC/g). In Mexican sourdoughs, refreshed or not, growth was intense and reached almost stationary phase in the first 3 days of fermentation; on the other hand, the Italian sourdoughs reached stationary phase after 5 days, probably due to lower initial population than Mexican sourdoughs.

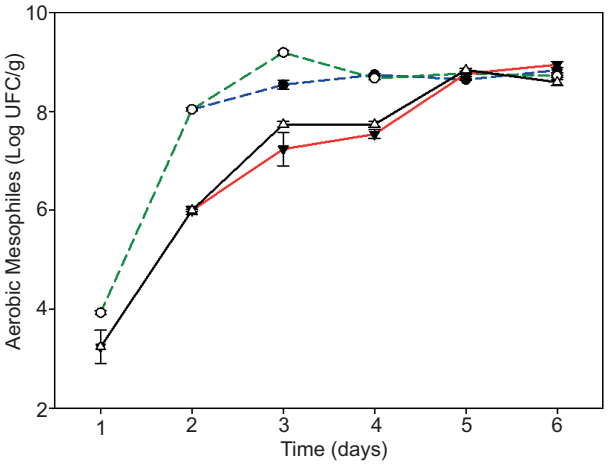

Figure 1. Growth of total aerobic mesophilic bacteria (Log UFC/g) of the different sourdoughs, with PCA method. (○): DMR, (●): DMNR, (△): DIR, (▲): DINR. Each value is represented as mean ± SD (n = 3).

The growth of yeasts during the 6 days of incubation (Figure 2) showed a growing trend similar to bacteria; in this case, the initial concentration of yeasts was higher in sourdoughs made with Mexican flour (4.2 Log UFC/g) than in sourdoughs made with Italian flour (3.8 Log UFC/g).

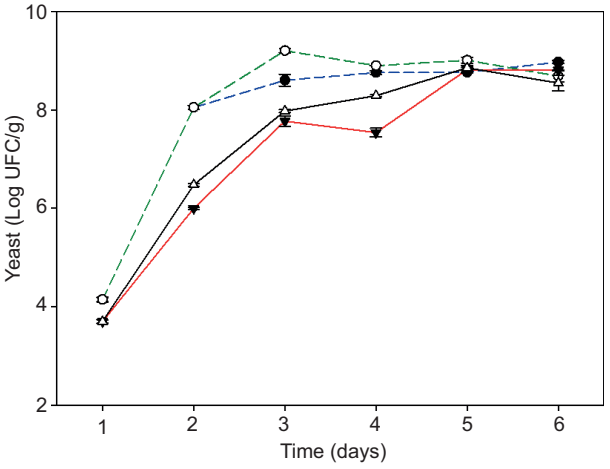

Figure 2. Growth of yeast and other fungi (Log UFC/g) of the different sourdoughs, with PDA method. (○): DMR, (●): DMNR, (△): DIR, (▲): DINR. Each value is represented as mean ± SD (n = 3).

Initially, the microbial population of the sourdough represents that of the flour. Each microbial group did not generally exceed 5 Log UFC/g. During the time, LAB and yeasts become more adapted to the environmental conditions of the sourdough, to the point of dominating the mature sourdough. Similar studies state that the population ranged from 6 to 9 Log UFC /g and 5 to 8 Log UFC /g, respectively (Minervini *et al.*, 2012).

Figures 3 and 4 show the results for pH and TTA. The initial pH values in Mexican and Italian sourdoughs were 5.9 and 5.6, respectively, while the TTA was 0.8 mL and 0.1M NaOH in each. During fermentation, the physicochemical parameters change, mainly due to the microbial metabolism (Paramithiotis *et al.*, 2014). The pH values decreased after 6 days of incubation to 3.7 both for Mexican and Italian sourdoughs. Similar pH values were also found by Vrancken *et al.*, (2011). Generally, the pH values between 3.5 and 4.3 are considered as an index of well-developed sourdough fermentation (Gobbetti and Gänzle, 2012). However, in the Mexican sourdoughs, the pH decreased quickly after 3 days of incubation with respect to the Italian sourdoughs that showed a gradual trend. No differences were observed between the pH values of refreshed or not-refreshed sourdoughs. These results are in accordance with the bacterial growth, and their produced metabolites such as lactic acid (Maifreni *et al.*, 2004). In fact, TTA values increased in both Mexican and Italian sourdoughs, with higher values in the Mexican one due to the higher bacterial population at the beginning. After 6 days of incubation the not-refreshed sourdoughs showed higher values of TTA than those refreshed for both flours. This behaviour can be related to the refreshment procedure that can act as a dilution factor on the sourdough.

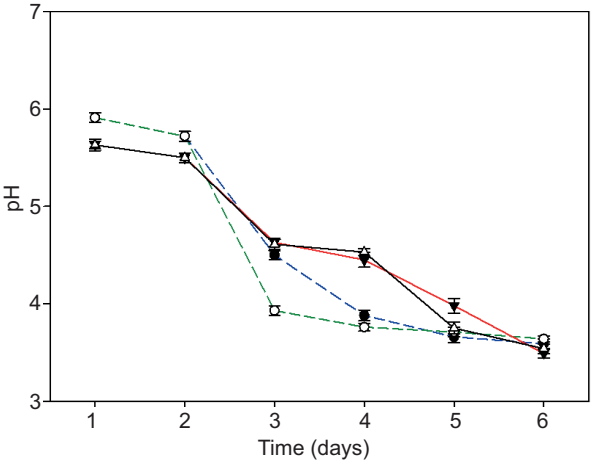

Figure 3. Evolution of pH of the different sourdoughs during 6 days of incubation. (○): DMR, (●): DMNR, (△): DIR, (▲): DINR. Each value is represented as mean ± SD (n = 3).

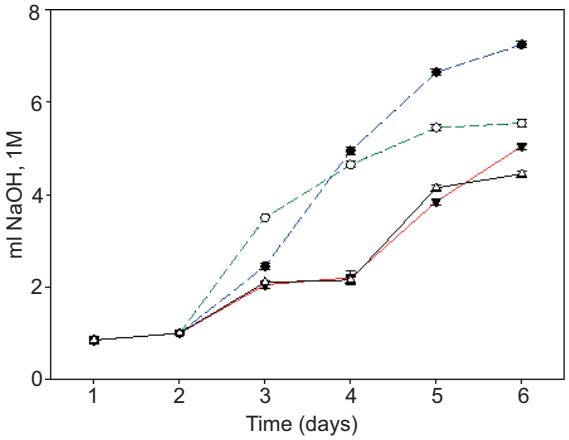

Figure 4. Evolution of TTA of the different sourdoughs during 6 days of incubation.(○): DMR, (●): DMNR, (△): DIR, (▲): DINR. Each value is represented as mean ± SD (n = 3).

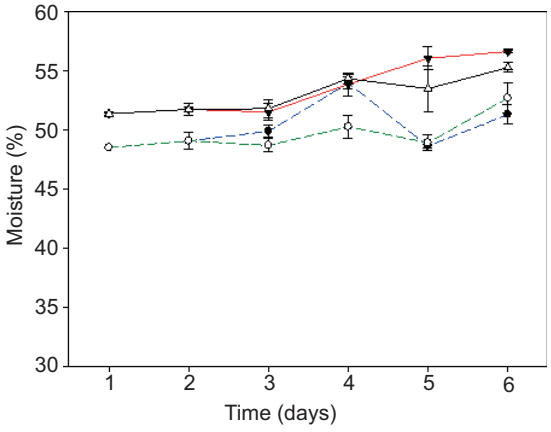

Figure 5. Evolution of Moisture content (%) of the different sourdoughs during 6 days of incubation (○): DMR, (●): DMNR, (△): DIR, (▲): DINR. Each value is represented as mean ± SD (n = 3).

Figures 5 and 6 show the moisture content (%) and aw values. In each sourdough, there are no significant differences in moisture content and aw values during the 6 days of incubation both in the refreshed and not-refreshed sourdoughs. These results confirm that both the incubation and refreshment did not affect the aqueous environment in the sourdoughs, preserving the favourable condition for microbial growth (Tecante, 2019). Minervini *et al.* (2014) stated that aw values between 0.96 and 0.98 do not limit the growth of most microorganisms.

Figure 7 shows the results of reducing sugar content during the fermentation. As shown during incubation, the reducing sugars increased linearly reaching its maximum concentration in each sourdough after 4 days, which can be related to the amylolytic activity of bacteria (Tecante, 2019). Also in this case, the values show

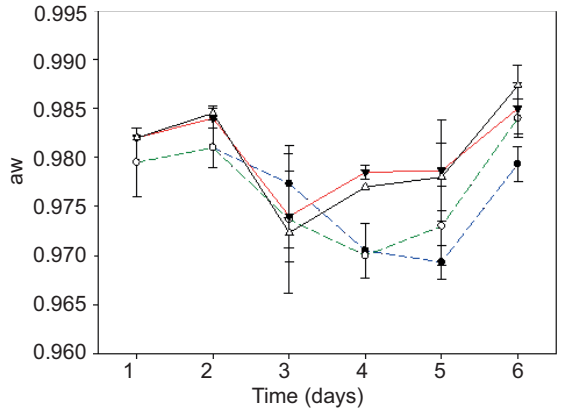

Figure 6. Evolution of water activity of the different sourdoughs during 6 days of incubation (○): DMR, (●): DMNR, (△): DIR, (▲): DINR. Each value is represented as mean ± SD (n = 3).

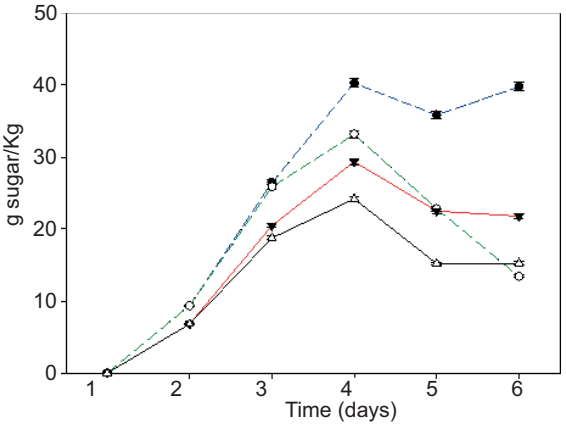

Figure 7. Evolution of reducing sugars (g/kg). (○): DMR, (●): DMNR, (△): DIR, (▲): DINR. Each value is represented as mean ± SD (n = 3).

greater reducing sugars in Mexican than in Italian sourdoughs, probably due to higher initial microbial population. Moreover, the differences in reducing sugar content observed in the refreshed and not-refreshed sourdoughs could be related to the sourdough refreshment, where there is increased polysaccharides concentration, due to fresh flour addition.

Conclusions

These results showed that in the early stages of microbial growth, the microbial population was greater in the sourdough made from the Mexican wheat flour than the Italian one, due to different geographic environments. However, after 6 days of incubation, the microbial populations were not significantly different in both types of sourdoughs, either refreshed or not refreshed. In addition, there were no significant differences in the physicochemical properties of refreshed or not-refreshed sourdoughs. In summary, daily refreshment is not necessary during the first 6 days of liquid sourdough preparation.

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