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Evaluación de los efectos de sustratos y tipos de harina de trigo sobre características microbiológicas, valores de pH, contenidos de compuestos fenólicos totales, capacidad antioxidante y capacidad fermentativa de levaduras naturales

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Abstract

Sourdough is a process that is expanding nowadays. However, the microorganisms present produce metabolites that can cause changes in the breads. Thus, the objective of this work was to evaluate the effects of substrates and types of wheat flour on microbiological characteristics, pH values, total phenolic compounds, antioxidant capacity and fermentative capacity of different types of sourdough, comparing them with dry yeast. In this way, six sourdough were elaborated, diversifying the type of wheat flour (whole or white) and the substrate (beer wort, pineapple juice or natural yogurt), being evaluated in the microbiological aspects (yeast count, bacteria under anaerobic conditions and under aerobic conditions), physicochemical (pH, phenolic compounds and antioxidant capacity (β-carotene/linoleic acid, ABTS and DPPH)) and fermentative capacity (only in sourdough). In view of the results obtained, it was observed that both white wheat flour and whole wheat flour, with the substrates yogurt, pineapple juice and brewer's wort, showed differences in microbiological characteristics, in pH values, in the content of compounds total phenolics, antioxidant capacity and fermentative capacity compared to dry yeast, with whole wheat flour, pineapple or beer wort providing microbial diversity, as well as lowering the pH. WY (white wheat flour and yogurt), WP (white wheat flour and pineapple) and IW (whole wheat flour and beer wort) sourdough were more resistant to temperatures (25 °C, 30 °C e 35 °C).

Keywords: Sourdough; Substrates; Fermentative capacity; Phenolic.

Resumo

O fermento natural é um processo que está em expansão nos dias atuais. No entanto, os microrganismos presente produzem metabólitos que podem causar alterações nos pães. Assim, o objetivo deste trabalho foi avaliar os efeitos dos substratos e dos tipos de farinha de trigo nas características microbiológicas, valores de pH, compostos fenólicos totais, capacidade antioxidante e capacidade fermentativa de diferentes tipos de fermentos naturais comparando-os com o fermento biológico liofilizado. Desta forma, foram elaborados seis fermentos naturais diversificando o tipo de farinha de trigo (integral ou branca) e o substrato (mosto de cerveja, suco de abacaxi ou iogurte natural), sendo avaliados nos aspectos microbiológicos (contagem de leveduras, bactérias em condições anaeróbicas e em condições aeróbicas), físico-químicos (pH, compostos fenólicos e capacidade antioxidante (β-caroteno/ácido linoleico, ABTS e DPPH)) e capacidade fermentativa (somente nos fermentos naturais). Diante dos resultados obtidos, observou-se que tanto a farinha de trigo branca como a farinha de trigo integral, com os substratos iogurte, suco de abacaxi e mosto cervejeiro, apresentaram diferenças quanto às características microbiológicas, nos valores de pH, no teor de compostos fenólicos totais, na capacidade antioxidante e na capacidade fermentativa em comparação com o fermento biológico liofilizado, sendo que a farinha de trigo integral, abacaxi ou mosto de cerveja proporcionaram diversidade microbiana, bem como o abaixamento do pH. Já os fermentos WY (farinha de trigo branca e iogurte), WP (farinha branca e abacaxi) e IW (farinha de trigo integral e mosto cervejeiro) foram mais resistentes às temperaturas (25 °C, 30 °C e 35 °C).

Palavras-chave: Fermento; Substratos; Capacidade fermentativa; Fenólico.

Resumen

La levadura natural es un proceso en expansión en la actualidad. Sin embargo, los microorganismos presentes producen metabolitos que pueden provocar cambios en los panes. Así, el objetivo de este trabajo fue evaluar los efectos de sustratos y tipos de harina de trigo sobre características microbiológicas, valores de pH, compuestos fenólicos totales, capacidad antioxidante y capacidad fermentativa de diferentes tipos de levaduras naturales, comparándolas con levaduras liofilizadas. De esta forma, se elaboraron seis levaduras naturales, diversificando el tipo de harina de trigo (integral o blanca) y el sustrato (mosto de cerveza, jugo de piña o yogur natural), siendo evaluadas en los aspectos microbiológicos (recuento de levaduras, bacterias en condiciones anaerobias) y en condiciones aeróbicas), fisicoquímicas (pH, compuestos fenólicos y capacidad antioxidante (β-caroteno/ácido linoleico, ABTS y DPPH)) y capacidad fermentativa (solo en levaduras naturales). A la vista de los resultados obtenidos, se observó que tanto la harina de trigo blanca como la harina de trigo integral, con los sustratos de yogur, jugo de piña e hierba de cerveza, presentaban diferencias en las características microbiológicas, en los valores de pH, en el contenido de compuestos fenólicos totales, antioxidantes capacidad y capacidad fermentativa en comparación con la levadura liofilizada, con harina de trigo integral, piña o mosto de cerveza proporcionando diversidad microbiana, además de reducir el pH. Los fermentos WY (harina de trigo blanca y yogur), WP (harina blanca y piña) e IW (harina de trigo integral y mosto de cerveza) fueron más resistentes a las temperaturas (25 °C, 30 °C y 35 °C).

Palabras clave: Levadura; Sustratos; Capacidad fermentativa; Fenólico.

1. Introduction

The increase demand for healthier, natural, and tasty foods has made the market return to traditional natural fermentation, which develops bread with the nutritional and sensory qualities of bread produced with sourdough (Silva & Fríscio, 2021).

The production of these sourdoughs occurs through the random proliferation of microorganisms (De Vuyst & Neysens, 2005; Silva & Fríscio, 2021). The bacteria that influence the characteristics of the doughs such as acidification, fermentation time, CO_2 production, flavor, enhancement in sensorial characteristics, alteration in texture and increases the shelf life of breads (Montemurro et al., 2019).

However, these random microorganisms from sourdoughs provide several species that result in varied metabolites influencing the softness, elasticity, and extensibility, which can be or not beneficial for the doughs (Gordún et al., 2015). And by using different substrates for yeast production, there is increase the variety of species and thus increases the diversity of metabolite levels (Gobbetti & Gänzle, 2013).

Some different ingredients, such as the types of flour, influence the species of bacteria and yeasts in the sourdough. Moreover, whole wheat flour has a higher microbial load than white wheat flour, since whole grains remain in contact with air and soil as this is where microorganisms are found (Neves et al., 2020).

Other ingredients, besides water and wheat flour, can be added as they help in the diversity of species in addition to providing enzymes, nutritional compounds, and preferential carbohydrates, such as beer must, yogurt and pineapple, thus being able to provide a better means of growth of the microbiota (Gordún et al., 2015; Zhao et al., 2015).

On the other hand, dry yeast is produced from selected yeasts and are able to fast production of carbon dioxide, resulting in a greater growth rate than sourdoughs; however, there is no production of acids in sufficient quantities to emphasize the flavor and aroma when compared to sourdoughs (Gélinas, 2019).

Therefore, the objective of this work was to evaluate the effects of substrates and types of wheat flour on microbiological characteristics, pH values, total phenolic compounds, antioxidant capacity and fermentative capacity of different types of sourdough, comparing them with dry yeast.

2. Methodology

2.1 Preparation of sourdough

For the elaboration of the different types of sourdough, beer wort (from an artisanal brewery), natural yogurt (Itambé®) and fresh pineapple juice were used as substrates. In addition, the yeasts were prepared with whole wheat flour (Vilma®) and white wheat flour (Boa Sorte®), according to Table 1.

Table 1. Sourdough formulation.

Types	Description
WY	White Wheat Flour + Natural Yogurt
IY	Whole Wheat Flour + Natural Yogurt
WP	White Wheat Flour + Pineapple Juice
IP	Whole Wheat Flour + Pineapple Juice
WW	White Wheat Flour + Beer Wort
IW	Whole Wheat Flour + Beer Wort

Source: Authors.

For the preparation of sourdough, the methodology proposed by Aplevicz (2013) was used, with modifications. In summary, at the beginning, each substrate was mixed with wheat flour (whole wheat flour or white wheat flour), this mixture remained for 24 h in a temperature-controlled chamber at 25 ºC, in the presence of oxygen, in glass jars. 30 g of wheat flour (whole or white) and 20 g of substrate were added to the mixtures, which remained for further 24 h in a temperature-controlled chamber at 25 ºC, in the presence of oxygen. Then, 50 g of wheat flour (whole or white) and 30 g of filtered water were added to each mixture, which remained for additional 24 h in the temperature-controlled chamber at 25 °C, in the presence of oxygen. Subsequently, 75 g of wheat flour (whole or white) and 30 g of filtered water were added to each mixture, remaining for 24 h in the temperature-controlled chamber at 25 °C, in the presence of oxygen. Finally, 300 g of wheat flour (whole or white) and 200 g of filtered water were added to the yeasts every 24 h twice every 24 h and stored under refrigeration (8 °C). The ready-made sourdoughs were stored in covered glass jars under refrigeration (8 ºC), and every 15 days the yeasts were fed in a 2:2:1 ratio (sourdough:wheat flour:water). In addition, dry yeast (Dr Oetker®) was used as a control. The experiment was carried out in three repetitions.

2.2 Microbiological evaluation of sourdough and dry yeast

The yeast and bacteria counting followed the methodology proposed by De Man et al. (1960). 10 g of each ferment (sourdough or dry yeast) were weighed using a sterile spatula and added to 90 mL of peptone water (Himedia®). Subsequently, manual homogenization was performed for 120 seconds, followed by serial dilutions and plating.

For bacterial counts, the samples were plated on MRS agar and subsequently incubated at 37 ºC for 48 h under aerobic and anaerobic conditions. Under anaerobic conditions, the plates were placed in anaerobic jars using an oxygen removal device. Results were expressed as log CFU/g. The total count of bacteria in the ferments was performed according to the methodology proposed by Garrote et al. (2001), with modifications.

The yeasts were plated on Potato Dextrose Agar (PDA) (Kasvi®) added 1% tartaric acid to 10% for yeast counting. The enumeration of yeasts was carried out according to the methodology proposed by Beuchat et al. (1990). The plates were incubated at 25 °C for 72 h under aerobic conditions. Results were presented in log CFU/g.

2.3 Evaluation of the hydrogenic potential (pH) of sourdough and dry yeast

The evaluation of the pH of sourdoughs and dry yeast was determined using a digital potentiometer, according to Instituto Adolf Lutz (2008), in triplicate.

2.4 Evaluation of total phenolic compounds and antioxidant capacity of sourdoughs and dry yeast

The evaluation of total phenolic compounds and antioxidant capacity using the DPPH, ABTS and β-carotene/linoleic acid methods present in sourdoughs and dry yeast was performed in triplicate.

2.4.1 Obtaining extracts from samples for analysis of total phenolic compounds and antioxidant capacity of sourdoughs and dry yeast

The procedure for obtaining the extracts from sourdoughs and dry yeast was adapted from Larrauri et al. (1997). Approximately 20 g of the samples were weighed in an erlenmeyer flask, with the exception of dry yeast, which was used 1 g. Samples were added by 40 mL of methanol/water solution (50:50 v/v), and kept under agitation (200 rpm) at 25 °C for 60 minutes. Subsequently, the solution was kept at rest in a refrigerated environment (8 °C) for 30 minutes. The supernatant was filtered, recovered, and transferred to a 100 mL flask. Then, 40 ml of acetone/water (70:30 v/v) were added to the residue and the mixture was stirred at 200 rpm, for at 25 °C for 60 minutes. The solution was also kept at rest in a refrigerated environment (8 °C) for 30 minutes. At the end of the period, the supernatant was transferred to the volumetric flask containing the first supernatant and the volume was made up to 100 mL with distilled water. The entire procedure was carried out protected from light, and the extract was stored at -18 ºC.

2.4.2 Evaluation of total phenolic compounds in sourdoughs and dry yeast

To determine the levels of total phenolic compounds in sourdoughs and dry yeast, used the adapted methodology of Folin-Ciocalteu (Waterhouse, 2002). The extract (0.5 mL) was pipetted and transferred to test tubes containing 2.5 mL of 10% (v/v) Folin-Ciocalteu reagent and 2.0 mL of 4% sodium carbonate solution (p/v). The contents of the tubes were homogenized and then kept for 120 minutes, protected from light, and the absorbance was determined at 750 nm. Absolute ethanol was used as a blank.

The content of total phenolic compounds was determined by interpolating the absorbance of the sample against the calibration curve constructed with gallic acid standards (5, 10, 15, 20, 30 and 40 μg/mL). Results were expressed as mg gallic acid equivalent (AGE)/g ferments.

2.4.3 Assessment of antioxidant capacity in sourdoughs and dry yeast

Antioxidant capacity was determined using the β-carotene/linoleic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis-(3-ethylbenzenethiazoline-6-sulfonic acid) methods. The β-carotene/linoleic acid method was determined following the procedure reported by Marco (1968), with some modifications. To prepare the solution of the β-carotene/linoleic acid system, 20 mg of β-carotene were used and to solubilize it was added 1 mL of chloroform, homogenized and, subsequently, the chloroform was evaporated with the aid of an oxygenator. Afterwards, 40 µL of linoleic acid and 530 µL of tween 40 were added. for 30 minutes) until absorbance between 0.6 nm and 0.7 nm at 470 nm was obtained. The system solution showed a yellow-orange color. Afterwards, 0.4 mL of each dilution of the extract was mixed with 5 mL of the system solution (β-carotene/linoleic acid system). It was used as a control 0.4 mL of the trolox solution with 5 mL of the β-carotene/linoleic acid system solution, the test tubes being homogenized in a shaker and kept in a water bath at 40 ºC. Then, the first reading (470 nm) was performed after 2 minutes of mixing and then at intervals of fifteen minutes up to 120 minutes. The spectrophotometer was calibrated with distilled water. Results were expressed as percent inhibition of oxidation.

The DPPH-free radical scavenging capacity was estimated using the method reported by Brand-Williams et al. (1995). The DPPH solution (600 µM) was diluted with ethanol to obtain an absorbance of 0.7±0.02 units at 517 nm. sourdoughs and dry yeast extracts (0.1 mL) were allowed to react with 3.9 mL of DPPH radical solution for 30 min in the dark, and the decrease in the absorbance of the resulting solution was monitored at 517 nm. Results were expressed as EC_{50} (g ferments/g DPPH).

The method of Re et al. (1999) was used for the ABTS assay, with minor modifications. The ABTS radical cation (ABTS+) was generated by the reaction of 5 mL of aqueous ABTS solution (7 mM) with 88 µL of 140 mM (2.45 mM final concentration) potassium persulfate. The mixture was kept in the dark for 16 h before use and then diluted with ethanol to obtain an absorbance of 0.7±0.05 units at 734 nm using a spectrophotometer. Sourdoughs and dry yeast extracts (30 µL), or a reference substance (trolox - 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), react with 3 mL of the resulting blue–green ABTS radical solution in the dark. The decrease in the absorbance at 734 nm was measured after 6 min. Ethanolic solutions of known trolox concentrations were used for calibration. Results were expressed as µM Trolox/g ferments.

2.5 Fermentative capacity of sourdoughs at different temperatures

Sourdoughs were estimated in terms of fermentative capacity, according to Aplevicz (2013) and Rouillé et al. (2000), with some modifications, in duplicate. 10 g of each sourdough were weighed, right after feeding, in a graduated test of 100 mL. Then, as tests were made at temperature in chambers with control of 25 °C, 30 °C and 35 °C, with the average volume every hour up to 13 °C. Using the fermentation x volume increase graph) the area under the greater curvature (fermentation time, corresponding to the fermentative capacity) was calculated.

2.6 Experimental design and evaluation of results

An experimental design with a completely random design (CRD) was used, and the microbiological were calculated through the results of calculating standards and standard deviations and, later, tested for descriptive data analysis. The data obtained from pH, total phenolic compounds, antioxidant capacity (DPPH, ABTS and β-carotene/linoleic acid methods) and fermentation capacity were obtained through analysis of variance (ANOVA) and Scott-Knott mean test at 5.0 % probability in Sisvar software (Ferreira, 2014).

3. Results and Discussion

3.1 Microbiological evaluation of sourdoughs and dry yeasts

Dry yeast (DY) had the highest yeast count (10.9 log CFU/g) (Table 2). This fact was expected since this dry yeast is composed of yeasts of the genus *Saccharomyces cerevisiae* (Castro & Marcelino, 2012). Among the sourdoughs, the highest value was obtained by WY yeast (whole wheat flour and yogurt) (8.58 log CFU/g), followed by IW yeast (whole wheat flour and beer wort) (8.48 log CFU/g).

Regarding sourdough from white wheat flour and yogurt, the symbiosis between lactic acid bacteria in yogurt and microorganisms in white wheat flour provided the maximum peak of yeast development in this analysis, as seen in the study by Gordún et al. (2015), who found that the mixture of white wheat flour with yogurt caused a slower growth of the yeast-forming units, but at the end of the seventh day there was an exacerbated growth. In the IW yeast result, the beer wort provided a better growth medium for the yeasts because it is rich in carbohydrates that are preferable to the yeasts, such as maltose and glucose, so the high levels of these carbohydrates help in the permanence of the high level of yeast colonies (Estracanholli, 2012).

In sourdoughs, aerobic bacteria (*Pseudomonas* sp.), facultative anaerobes (*Enterobacteria*) and predominantly lactic acid bacteria can be found (Nami et al., 2019). Most found are from the genera *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Weissella*, with *Lactobacillus* being the most found. For sourdoughs made with wheat flour, the dominant species is *Lactobacillus sanfranciscensis* (Corsetti & Settanni, 2007; Siragusa et al., 2008; Chavan & Chavan, 2011) and they are gram-positive, considered anaerobic or anaerobic optional (Saad, 2006; Nami et al., 2019). Viiard et al. (2013) analyzed lactic acid bacteria under aerobic and anaerobic conditions of sourdoughs and observed that some, such as *Lactobacillus helveticus*, showed favorable growth only in anaerobic conditions, and other lactic acid bacteria showed no difference between the anaerobiosis and aerobiosis treatments. of growth in treatments under anaerobic and aerobic conditions depends on the microbiota species.

Table 2. Values of yeast counts, and of bacteria cultivated under anaerobic and aerobic conditions in sourdoughs and dry yeast.

Types	Yeast (log UFC/g)	Bacteria cultured under anaerobic conditions (log CFU/g)	Bacteria grown under aerobic conditions (log CFU/g)
DY	10.9 ± 0.19	-	-
WY	8.58 ± 0.01	8.58 ± 0.03	8.66 ± 0.03
IY	7.60 ± 0.07	8.87 ± 0.00	8.59 ± 0.02
WP	8.18 ± 0.13	9.22 ± 0.12	9.00 ± 0.00
IP	7.90 ± 0.00	10.13 ± 0.07	10.28 ± 0.13
WW	7.70 ± 0.06	9.78 ± 0.04	9.35 ± 0.00
IW	8.48 ± 0.06	10.18 ± 0.00	10.40 ± 0.02

Note: n = 6. Means ± standard deviation. Dry Yeast (DY); Sourdoughs: white wheat flour and yogurt (WY), whole wheat flour and yogurt (IY), white wheat flour and pineapple (WP), whole wheat flour and pineapple (IP), white wheat flour and beer wort (WW), whole wheat flour and beer wort (IW). Source: Authors.

The highest bacterial counts, under both anaerobic and aerobic conditions, were observed for whole wheat flour and beer wort (IW) (10.18±0.00/10.40±0.02 log CFU/g) and whole wheat flour and pineapple (IP) (10.13±0.07/10.28±0.13 log CFU/g). According to Heiniö et al. (2016), the ingredients are responsible for providing the microbial load and substrates for the multiplication of microorganisms. Whole wheat flour naturally contains lactic acid bacteria in the bran from the environment where these cereals are grown (Neves et al., 2020). The same happens with beer wort, as it is a whole grain filtrate that, in addition to contributing to lactic acid bacteria, also has a high glycemic index as a substrate (Aliyu & Bala, 2011). Pineapple, on the other hand, also contains carbohydrates with a high glycemic index, in addition to providing lactic acid bacteria, which are naturally present in this fruit (Cordenunsi et al., 2010).

3.2 Evaluation of pH, contents of total phenolic compounds, and antioxidant capacity of sourdoughs and dry yeast

According to Clark et al. (2002), pH values promote characteristic flavors and aromas of bread, in addition to modifying proteinase enzymes, modifying the structure of the bread. It was observed that all the ferments were similar to the pH below 7, and sourdoughs made with white wheat flour obtained pH lower values when compared with sourdoughs with the same substrate but made with whole wheat flour (Table 3). This occurs because whole wheat present in this type of buffering present the mineral medium during fermentation (Gobbetti & Gänzle, 2013), in addition to the wheat bran, there is a symbiosis with the carbohydrates present in the pasta, causing a delay in the metabolism of the bacteria that decomposes the residues produced, being a beneficial effect, prolonging the effect of the enzymatic action (Sadeghi et al., 2019). Furthermore, dry yeast (DY) had the highest pH value (5.70) ($p \leq 0.05$).

In terms of phenolic compounds, it was observed that the sourdoughs had higher averages than dry yeast ($p \leq 0.05$) (Table 3), and they did not differ from each other ($p>0.05$). Phenolic compounds are hydroxylated substances naturally found in foods (Achkar et al., 2013). They are of plant origin (Tsamo et al., 2020), having a pigmentation function (Singh et al., 2019), free radical removal (Koley et al., 2014) and light absorption in plants (Naczk & Shahidi, 2004). The value of phenolic compounds for sourdoughs is related to larger wheat flour, being considered a source of phenolic compounds (Sivam et al., 2010). In wheat flour, the most abundant phenolic compounds are phenolic acids and lignans (Angelino et al., 2017). Wheat flour, (whole or white), has a fraction of non-bioavailable phenolics (Hung et al., 2009), but natural fermentation increases the bioavailability of these phenolics since enzymes produced by yeast microorganisms break down the acids insoluble phenolics increasing their bioavailability (Angelino et al., 2017).

Table 3. Average pH values, total phenolic compounds, and antioxidant capacity of sourdoughs and dry yeasts.

Types	**pH**	**Total Phenolic Compounds (mgAGE/g ferments)**	**Antioxidant Capacity**		
			β-carotene (% protection)	**DPPH (EC_{50} g ferments/g DPPH)**	**ABTS (µMtrolox/g ferments)**
DY	5.70 ± 0.04 a	0.004 ± 0.00 b	48.65 ± 2.31 b	1.36 ± 0.13 e	58.82 ± 0.44 a
WY	4.65 ± 0.04 e	0.19 ± 0.00 a	49.89 ± 3.65 b	151.36 ± 0.50 a	0.01 ± 0.01 e
IY	4.99 ± 0.01 b	0.23 ± 0.01 a	44.26 ± 4.02 b	27.38 ± 1.51 c	3.46 ± 0.01c
WP	4.70 ± 0.01 d	0.25 ± 0.06 a	52.08 ± 2.92 b	150.64 ± 0.51 a	0.47 ± 0.20 e
IP	4.78 ± 0.01 c	0.25 ± 0.02 a	50.77 ± 2.92 b	28.28 ± 0.01 c	3.07 ± 0.90 c
WW	4.52 ± 0.01 f	0.21 ± 0.03 a	57.57 ± 0.77 a	48.30 ± 0.35 b	1.83 ± 0.59 d
IW	4.74 ± 0.01 c	0.23 ± 0.01 a	55.96 ± 2.68 a	15.37 ± 0.22 d	4.58 ± 0.09 b

Note: n = 6. Means ± standard deviation followed by the same capital letter in the column and lower case in the row do not differ from each other by the Scott-Knott test at 5% probability. Dry Yeast (DY); Sourdoughs: white wheat flour and yogurt (WY), whole wheat flour and yogurt (IY), white wheat flour and pineapple (WP), whole wheat flour and pineapple (IP), white wheat flour and beer wort (WW), whole wheat flour and beer wort (IW). Source: Authors.

Antioxidants are important for health, they bind to the free radicals ones that are produced daily by human stress, therefore these radicals are harmful to health and other health hazards. They also protect foods by delaying rancid flavor and aroma and depigmentation (Gray et al., 1996).

By the methodology of inhibition of lipid peroxidation in the β-carotene/linoleic acid system, it was observed that beer wort sourdough, both white wheat flour and whole wheat flour, had a higher percentage of the others ($p \leq 0{,}05$). Beer wort is rich in antioxidant compounds such as B vitamins, vitamin C, melanoid (Yang & Gao, 2021). Jehle et al. (2011) mention that the highest antioxidant capacity in beers and beer wort, where he observed resistant antioxidants, thus being difficult to degrade over time.

Furthermore, it was observed that dry yeast (DY) showed higher antioxidant capacity by the DPPH and ABTS methods (Table 3). According to Hassan (2011), dry yeasts trigger the production of glutathione, produced from the carbon sources of glucose and alcohol during fermentation (Wen et al., 2005). In relation to sourdoughs, it was observed that the IW (whole wheat flour and beer wort) showed greater capacity by the antioxidant methods (DPPH and ABTS), inferring that of whole wheat flour and the beer wort may have the occasional interaction between the antioxidant compounds present in the two ingredients, thus increasing an antioxidant of this sourdough.

3.3 Evaluation of the fermentative capacity of sourdoughs at different temperatures

Fermentation capacity is the evaluation of fermentation development over time, which can be through the measurement of some parameters such as acid production, pH lowering, volume increase, alcohol and gas production, being important parameters to analyze the changes that occur during fermentation, from its beginning to the point of stability between the consumption of metabolites and the fermentation products (Qureshi et al., 2007; Samagaci et al., 2014).

Table 4. Average values of the evaluation of the fermentative capacity of sourdoughs at 25 °C, 30 °C and 35 °C.

Types	25 ºC (mL/h)	30 ºC (mL/h)	35 ºC (mL/h)
WY	124.5 ± 3.54 Ba	129.25 ± 3.24 Aa	116.00 ± 15.99 Aa
IY	36.75 ± 17.32 Cb	121.00 ± 14.14 Aa	97.50 ± 19.80 Aa
WP	87.50 ± 6.36 Ba	72.50 ± 19.09 Aa	92.00 ± 2.83 Aa
IP	203.50 ± 9.19 Aa	107.50 ± 23.13 Ab	86.05 ± 28.17 Ab
WW	173.50 ± 20.31 Aa	131.00 ± 29.70 Ab	101.00 ± 5.66 Ab
IW	118.50 ± 20.50 Ba	132.00 ± 31.11 Aa	152.50 ± 4.95 Aa

Note: n = 6. Means ± standard deviation followed by the same capital letter in the column and lower case in the row do not differ from each other by the Scott-Knott test at 5% probability. Sourdoughs: white wheat flour and yogurt (WY), whole wheat flour and yogurt (IY), white wheat flour and pineapple (WP), whole wheat flour and pineapple (IP), white wheat flour and beer wort (WW), whole wheat flour and beer wort (IW). Source: Authors.

The expansion power of the yeast dough is given by the production of carbon dioxide that expands the elastic matrix of gluten forming the dough structure (Castro & Marcelino, 2012). This expansion is facilitated by the maturation of the gluten network, as certain products, such as organic acids, improve the elasticity of this matrix by triggering the release of proteases (Pagani et al., 2014). It was observed that there were differences between the fermentative capacities of the sourdoughs only at the temperature of 25 °C (Table 4), and the IP (whole wheat flour and pineapple) and WW (white wheat flour and beer wort) sourdoughs showed higher mean values ($p \leq 0.05$), not differing from each other.

According to Chavan and Chavan (2011), a temperature between 30 °C and 35 °C improve the growth of lactic acid bacteria, which can raise the acidity to a point of expansive improvement, since whole wheat flour have difficulty in expanding and improving gas retention (Nami et al., 2019). As observed, the fermentative capacity of IW (whole wheat flour and yogurt) sourdough increased at higher temperatures (Table 4).

The ideal temperature for yeast multiplication is 25 °C (Chavan & Chavan, 2011) but Samagaci et al. (2014) reported that some yeasts develop a better resistance to the increase in temperature, due to a natural selection among them, improving the capacity to release CO_2. The fermentative capacity of WY (white wheat flour and yogurt), WP (white wheat flour and pineapple) and IW (whole wheat flour and beer wort) sourdoughs remained constant with increasing temperature (Table 4). These sourdoughs are among those with the highest amount of yeasts (Table 2); thus, it can be inferred that increasing the fermentation temperature does not reduce the multiplication of these microorganisms.

As for the IP (whole wheat flour and pineapple) and WW (white wheat flour and beer wort) sourdoughs, it was found that the increase in fermentation temperature caused a decrease in fermentative capacities. The ingredients pineapple and beer wort are rich in carbohydrates that are preferable for the microorganisms involved in fermentation, in addition to having enzymes that accelerate the fermentation process and, by increasing the temperature, the dough passed from tolerable fermentation, which made it reduce the volume (Estracanholli, 2012).

4. Conclusion

Sourdoughs is a favorable biotechnology to be used, because white wheat flour and whole wheat flour with yogurt or pineapple or beer wort substrates showed differences in relation to dry yeasts in relation to microbiological characteristics, pH values, content of total phenolic compounds, in the antioxidant capacity and in the fermentative capacity.

Whole wheat flour, pineapple or beer wort provided microbial diversity as well as carbon sources ideal for the multiplication of bacteria producing greater amount of organic acids, which left the pH lower. They promoted the bioavailability of phenolic compounds in sourdoughs in parallel with most of the protection principles and high protection by the β-carotene/chemical acid method, as it is rich in vitamins and mineral acids that are rich in vitamins and minerals. This ingredient also favors protection to DPPH and ABTS methods, but dry yeast was higher through its metabolites.

In the microbiological profile, as yeasts preferred ingredients with higher carbohydrate value, such as white wheat flour and beer wort. The WY (white wheat flour and yogurt), WP (white flour and pineapple) and IW (whole wheat flour and beer wort) sourdoughs were more resistant to temperatures.

An antioxidant between whole wheat flour with beer wort brought answers to the production of a bread with microbiological diversity, better resistance to temperature change in addition to the concentration of compounds. Thus, for a better understanding of the microbiota, it would be the count of bacteria and yeasts over time to trace the identification of the most resistant genera, analyzing an isolated development being studied a of them for biotechnology in bakery.

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