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The Effect of Oatmeal And The Mucilage From The Nopal Cactus In The Production of Lactic Acid In Sourdough

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ABSTRACT
The use of sourdough process as a form of leavening is one of the oldest biotechnological processes in food production and has been used for thousands of years to improve flavor, texture and microbiological shelf life of bread. Its main function is to leaven the dough to produce a more gaseous dough piece and much more aerated bread. In Europe, cereal fermentations are mainly applied to the brewing industry, providing sourmashes, and to baking, in which sourdough plays an important role in the preparation of bread dough to improved dough machinability and bread crumb structure, keeping properties and flavor. The mixture modification has influence upon the production of lactic acid, in which the sourdough added with oat fiber to 24 h was of 634.59 mg•100 g^{-1} of dough (316.80 % in relation to control sample). On the other hand, nopal mucilage to 48 h, the lactate production went from 481.35 mg•100 g^{-1} of dough (414.20 % in relation to control sample), which depends on the kind of sold off mixture.

Keywords – dough, fermentation, lactobacilli, lactic acid, sourdough

I. INTRODUCTION

The use of sourdough is a form of unleavened bread and is one of oldest biotechnological processes that has been used, and considered safe, in the production of foods for hundreds of years. Traditionally, the acidification of the dough gave a signature flavor, texture, extended the shelf life of baked goods and was found to be used primarily in the Mediterranean region. In modern times, the baking of sourdough is an alternative to the use of additives [1]. The sourdough is a mixture of flour and water, of which is then fermented with lactic acid bacteria (LAB), primarily with heterofermented cultures that produce lactic and acetic acid in the mixture, from which the resulting sour taste originates in the final product [2]. introduction of the paper should explain the nature of the problem, previous work, purpose, and the contribution of the paper. The contents of each section may be provided to understand easily about the paper.

The sourdough was used in the baking process to achieve the unleavened bread effect (production of CO_2) and to have an impact upon the smell of the bread (organic acids and volatile compounds). Diverse studies have been made regarding the fermentation, microbiology, production of organic acids and CO_2, and some other compounds in the sourdough [3]. Many of these developments are related to the metabolic activity of the lactic microflora. The proteolytic system of the lactic acid bacteria is composed of systems that are associated with proteinases; peptides transport systems and peptidases intracellular. These can hydrolyze proteins, small peptides and amino acids, which are important for a rapid microbial growth and for the development of precursors of aroma in baked foods [4].

It is known that the soluble fiber is hypocholesterolemical, and it is known that soluble fiber reduces the risk of cancer in the colon. It is also known that the β-glucans also reduce the risk of cancer in the colon and also reduce the absorption of glucose in the digestive system. High concentrations of fibers exhibit many properties that affect the physiological functions of the food [5]. Cereals with β-glucans are considered unbranched, are linear polymers of β-D-glucopyranose, containing approximately 30% of links (1→3) and 70% of links (1→4) [6]. The β-glucans are presented in two forms, such as soluble and insoluble. The non-covalent interactions between β-glucans and cellulose or arabinoxylans makes possible their removal in aqueous media [7]. On the other hand, in some countries of the Mediterranean the fermentation of the chickpea is very useful as a leavening agent in a traditional manner in certain breads and biscuits. The addition of fermented chickpea flour, enhances the nutrient quality and shelf life of the bread [8], in addition, the increase of the polysaccharides qualities in the dough cause changes in the system, affecting the rheological behavior of the dough and improving the technological quality of the dough and the bread. The addition of hydrocolloids to the dough also

affects the retrogradation of the starches and delays the aging process of the bread [9].
The objective of this study is to determine the production of lactic acid in fermented dough with lactobacilli and yeasts, under varying conditions and mixture designs with different amounts of water, mother dough and fiber from oats and the mucilage from the nopal cactus.

II. MATERIALS AND METHODS

2.1 Preparation of Samples

The wheat flour and rye, chickpea and oats, were acquired in a commercial shop. The chickpea was ground and made into a fine powder. The oatmeal was then placed in a hammer mill and then were transferred to a sieve, to which he was appointed fiber oats (FO). In the case of the nopal cactus, the cladodes were purchased in a local market, and were compressed for 10 min, with what was extracted from the mucilage (FNM).

Table 1. Distribution of ingredients to the mixture of the sourdough.

Sample	Ingredients (g)		
	Water	Fiber	Mother dough
Fx_1	32.88	0.61	15.22
Fx_2	32.88	4.87	10.96
Fx_3	28.62	4.87	15.22
Fx_4	32.17	2.03	14.51
Fx_5	32.17	4.16	12.38
Fx_6	30.04	4.16	14.51
Fx_7	32.88	2.74	13.09
Fx_8	30.75	2.74	15.22
Fx_9	30.75	4.87	13.09
Fx_{10}	31.46	3.45	13.80

F: fiber; x: depends on the type of sample, Oats (FO); Mucilage of nopal (FNM)

2.2 Microorganisms

The microorganisms that were used was a mixture of dried *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium bifidus* contained in a commercial product, in addition to yeast for baking (*Saccharomyces cerevisiae*).

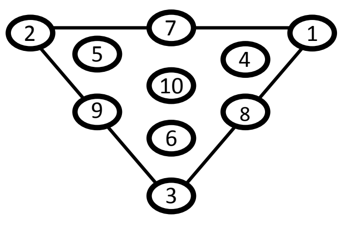

Figure 1.Location and design points of the mixtures

2.3 Mother dough

Mother dough was developed with 33 % of wheat flour, 2 % of dehydrated yeast, 1 % of sugar, 13 % of the produced yogurt and 12.72 % of the chickpea paste. This was fermented for 24 hours at 3± 1 °C.

2.4 Sourdough

The preparation for the base of the sourdough was: 24.58 g of wheat and rye flour, 0.35 g of dehydrated yeast, 1 g of starter cultures, 1.11 g of sucrose, 0.55 g of salt, 2.33 g of milk and 4.46 g of edible oil. For the mixing of the ingredients of water-fiber-mother dough in the base continued to be as shown in Table 1, and the accommodation is represented in Fig. 1. The fermentation was carried out in glass reactors 10 cm in height and 6 cm in diameter fitted with screw cap. Each reactor had 100 g of dough added and the reactors were allowed to cool in a refrigerated environment to 3± 2 °C for 48 hours producing a sample every 12 hours.

2.5 Analysis of pH and titratable acidity

The sourdough was macerated with distilled water (1:4) and the pH value of the suspension was obtained with a pH meter. The titratable acidity (TA) of the dough was determined with a pH meter and the addition of 0.01 N NaOH until a final pH of 8.1, and was expressed as ml of NaOH used.

2.6 Glucose

The sourdough was dried in a convection oven, for 24 hours and soaked to obtain a softened and homogeneous sample. A 100 mg sample was then placed with 300 µl of water in a microtube. The sample was then shaken vigorously and was left to stand for 15 min to then be centrifuged at 2500 rpm for 10 min. It took an aliquot of 30 µl and was added to 3 ml of the GOD PAD kit (Ehrlich Lab, Mexico). This was then incubated in a water bath at 37 °C for 10 min. The reading was performed with a UV/Vis spectrometer Lamda 25 Perkin Elmer (USA) to 505 nm.

2.7 Determination of lactic acid

5 g of sourdough was added to 20 ml of NaH_2PO_4 20 mM and shaken vigorously, leaving at rest for 10 minutes. It was then filtered with Whatman # 40 and centrifuged at 2500 rpm for 15 minutes, and filtered with a microfilter of 0.45 µm before being injected into the HPLC. The HPLC equipment was formed by a Perkin Elmer 200 series (USA), with a manual injector of 20 µl volume. The column was an Atlantis dc18 4.6 x 150 mm, 5 µ (Ireland), at 30 °C, with a UV detector Perkin-Elmer series 200 at 210 nm. The mobile phase used was a

solution of 20 mM NaH_2PO_4 adjusted to a pH of 2.7 with H_3PO_4. The flow was 0.5 ml min^{-1}.

2.8 Statistical analysis

The results were evaluated by Analysis of Mixture Design and Tukey´s tests using Statgraphics Centurion XV.

III. RESULTS AND DISCUSSION

The lactic acid bacteria (LAB) are not always the predominant organisms within the native flora of the meal, but its resistance to acids allows them to continue to grow during the fermentation of the sourdough. The LAB are capable of producing lactic acid (homofermented), some also produce acetic acid (heterofermented). This production of acid decreases the pH and increases the TA, with the passage of time. This behavior has been observed in the samples supplied with fiber from oats, it was found that all samples increased their TA above the control, but in the case of the samples supplied with fiber enriched oats with mucilage of nopal significant changes were not found ($p>0.01$)

Table 2. Physicochemical analysis of samples supplied with oats at 24 hour fermentation.

	Parameter			
Mixture	**pH**	**TA (ml NaOH)**	**Glucose (mg/100 g)**	**Lactate (mg/100 g)**
FO_1	4.87 ± 0.03^{a}	15.75 ± 3.88^{b}	24.32 ± 7.33^{b}	$190.33 \pm 5.73^{b,c}$
FO_2	4.81 ± 0.06^{a}	16.75 ± 0.35^{b}	$14.84 \pm 1.41^{b,c,d}$	$198.04 \pm 25.92^{b,c}$
FO_3	4.62 ± 0.62^{a}	26.50 ± 2.12^{a}	2.00 ± 0.30^{d}	87.46 ± 18.56^{c}
FO_4	4.74 ± 0.01^{a}	14.95 ± 0.07^{b}	$10.45 \pm 0.62^{c,d}$	$241.27 \pm 51.45^{b,c}$
FO_5	4.74 ± 0.01^{a}	$20.17 \pm 1.17^{a,b}$	$14.05 \pm 2.20^{b,c,d}$	101.82 ± 9.69^{c}
FO_6	4.75 ± 0.07^{a}	28.00 ± 1.97^{a}	$22.44 \pm 1.33^{b,c}$	$223.51 \pm 78.43^{b,c}$
FO_7	4.74 ± 0.04^{a}	16.00 ± 0.28^{b}	$9.63 \pm 1.22^{c,d}$	634.59 ± 151.10^{a}
FO_8	4.73 ± 0.04^{a}	14.80 ± 0.14^{b}	$14.55 \pm 2.62^{b,c,d}$	353.86 ± 10.23^{b}
FO_9	4.78 ± 0.37^{a}	14.63 ± 1.10^{b}	40.96 ± 2.93^{a}	$156.18 \pm 20.23^{b,c}$
FO_{10}	4.77 ± 0.02^{a}	13.23 ± 2.95^{b}	$19.61 \pm 3.52^{b,c}$	37.58 ± 1.32^{c}
Base	4.84 ± 0.02^{a}	11.50 ± 0.78^{c}	151.94 ± 12.13^{e}	$200.31 \pm 37.33^{b,c}$

± Standard Deviation; ^{a,b,c} Significant Difference by Tukey.

The control sample (base) was prepared without the addition of fiber (30.75 g of water and 13.09 g of sourdough). Giving a pH of 4.84 at 24 h of fermentation, which showed no significant difference ($p<0.01$) as compared to the rest of the mixtures made, this is to say that the variation within the ingredients does not affect the pH of the sourdough at 24 h of fermentation, both for oats (Table 2), as with the nopal cactus as well (Table 3).

Table 3. Physicochemical analysis of samples supplied with mucilage of nopal at 24 hour fermentation.

	Parameter			
Mixture	**pH**	**TA (ml NaOH)**	**Glucose (mg/100 g)**	**Lactate (mg/100 g)**
FNM_1	4.69 ± 0.63^{a}	$18.75 \pm 6.71^{b,c}$	$7.99 \pm 0.45^{d,c}$	$99.12 \pm 12.75^{b,c,d}$
FNM_2	4.42 ± 0.01^{a}	41.90 ± 2.40^{a}	$16.57 \pm 1.26^{c,d}$	$59.48 \pm 11.06^{c,d}$
FNM_3	4.36 ± 0.06^{a}	43.40 ± 3.46^{a}	10.13 ± 2.16^{d}	$155.19 \pm 1.18^{a,b}$
FNM_4	4.28 ± 0.01^{a}	35.00 ± 4.24^{a}	$17.06 \pm 0.46^{c,d}$	40.08 ± 8.50^{d}
FNM_5	4.45 ± 0.02^{a}	$31.60 \pm 3.17^{a,b}$	$19.75 \pm 1.37^{c,d}$	$81.59 \pm 9.62^{c,d}$
FNM_6	4.46 ± 0.00^{a}	$31.50 \pm 2.12^{a,b}$	$17.81 \pm 3.83^{c,d}$	$112.33 \pm 7.52^{b,c}$
FNM_7	4.53 ± 0.01^{a}	39.75 ± 0.35^{a}	45.40 ± 0.59^{b}	$97.89 \pm 17.99^{b,c,d}$
FNM_8	4.39 ± 0.00^{a}	33.50 ± 0.70^{a}	$18.08 \pm 2.71^{c,d}$	$80.27 \pm 0.02^{c,d}$
FNM_9	4.53 ± 0.79^{a}	$29.50 \pm 1.57^{a,b}$	$24.51 \pm 2.85^{c,d}$	$79.31 \pm 9.36^{c,d}$
FNM_{10}	4.52 ± 0.09^{a}	$30.02 \pm 4.28^{a,b}$	$33.43 \pm 4.32^{b,c}$	$50.73 \pm 7.38^{c,d}$
Base	4.84 ± 0.02^{a}	11.50 ± 0.78^{c}	151.94 ± 12.13^{a}	200.31 ± 37.33^{a}

± Standard Deviation; ^{a,b,c} Significant Difference by Tukey.

Normally the sourdoughs by the native microflora have a pH of 5.0 to 6.2 [2]. At 48 h of fermentation, the pH of the control drops to 4.71, but there have been no significant differences with the mixtures (Table 4). While this happened, the TA (Table 5) increased in comparison to the control sample at 24 h; however, the increase of the titrateable acidity at 48 h, showed no significant differences between them (oats and mucilage; $p<0.01$). It has been reported that when you combine the fermentation with culture of lactobacilli and yeasts the pH values decrease as, simultaneously the acidity increases, compared with the fermentation of a simple culture [10].

The concentration of lactic acid during the fermentation of the dough resulted an increase in the acidity, while the pH decreased [11]. Although the fermentation of dough is regarded as a traditional process, the acidic characteristics will depend on the flora present, or the type of mother dough used [12].

The analysis of samples fermented at 24 h, were found to have FO_7 pH from 4.74 to 4.76 (Fig. 2), constituting more uniform values in this parameter, where this behavior might be due to the fact that they lie close to the average values.

After 24 h of fermentation the growth of yeasts as monocultures, decrease the pH up to 6.8 and the TA rises to 2.4. When this is combined with LAB, it has been reported that the pH decreases below 3 [13].

Table 4. Physicochemical analysis of samples supplied with oats at 48 hour fermentation.

	Parameter			
Mixture	**pH**	**TA (ml NaOH)**	**Glucose (mg/100 g)**	**Lactate (mg/100 g)**
FO_1	4.80 ± 0.09^a	$20.00 \pm 4.24^{a,b,c}$	$10.25 \pm 0.46^{b,c}$	269.85 ± 42.64^a
FO_2	4.64 ± 0.16^a	$19.87 \pm 1.60^{c,d}$	$10.25 \pm 0.34^{b,c}$	178.28 ± 9.79^a
FO_3	4.56 ± 0.06^a	29.00 ± 1.41^a	0.00 ± 0.00^c	60.87 ± 1.24^a
FO_4	4.62 ± 0.06^a	16.66 ± 0.20^d	$4.79 \pm 0.67^{b,c}$	401.75 ± 25.33^a
FO_5	4.66 ± 0.04^a	$26.70 \pm 0.57^{a,b}$	$12.12 \pm 5.66^{b,c}$	42.42 ± 7.96^a
FO_6	4.56 ± 0.26^a	29.00 ± 1.13^a	$22.22 \pm 9.60^{b,c}$	79.98 ± 2.85^a
FO_7	4.65 ± 0.03^a	16.83 ± 0.03^d	0.00 ± 0.00^c	89.37 ± 1.97^a
FO_8	4.56 ± 0.02^a	16.60 ± 0.56^d	0.00 ± 0.00^c	106.79 ± 10.62^a
FO_9	4.62 ± 0.16^a	16.92 ± 3.15^d	$2.10 \pm 0.63^{b,c}$	160.14 ± 15.35^a
FO_{10}	4.57 ± 0.07^a	16.70 ± 1.5^d	$2.87 \pm 0.42^{b,c}$	175.37 ± 6.68^a
Base	4.71 ± 0.05^a	18.00 ± 2.82^d	116.21 ± 0.06^a	116.21 ± 0.06^a

± Standard Deviation; ^{a,b,c} Significant Difference by Tukey.

The values of TA are consistent with the results of the pH. The lower the water content, the greater is the value of TA. On the other hand, the decrease in the pH to the 48 h is shown in Fig. 2, where the samples show a pH in the range 4.5 to 4.8, and in the case of the nopal range between 4.3 and 4.6 (Fig. 3), these values are close to the middle area of the graph, which is inversely related to the amount of mother dough added.

Table 5. Physicochemical analysis of samples supplied with mucilage of nopal at 48 hour fermentation.

	Parameter			
Mixture	**pH**	**TA (ml NaOH)**	**Glucose (mg/100 g)**	**Lactate (mg/100 g)**
FNM_1	4.67 ± 0.07^a	19.34 ± 3.99^f	2.46 ± 0.64^d	27.15 ± 16.95^d
FNM_2	4.42 ± 0.02^a	52.30 ± 3.81^a	23.06 ± 1.88^b	4.33 ± 1.42^d
FNM_3	4.31 ± 0.05^a	$48.70 \pm 2.84^{a,b}$	$22.24 \pm 3.83^{b,c}$	$48.70 \pm 3.74^{c,d}$
FNM_4	4.34 ± 0.00^a	$38.05 \pm 1.48^{b,c,d,e}$	$21.08 \pm 1.13^{b,c}$	$27.70 \pm 8.63^{c,d}$
FNM_5	4.52 ± 0.05^a	$45.00 \pm 2.55^{a,b,c}$	$20.94 \pm 4.96^{b,c}$	7.45 ± 0.61^d
FNM_6	4.45 ± 0.03^a	$33.00 \pm 1.41^{d,e}$	$10.43 \pm 2.29^{c,d}$	$23.81 \pm 5.73^{c,d}$
FNM_7	4.40 ± 0.00^a	$43.50 \pm 2.12^{a,b,c,d}$	$20.99 \pm 2.44^{b,c}$	$48.72 \pm 7.84^{c,d}$
FNM_8	4.48 ± 0.01^a	$29.35 \pm 0.91^{e,f}$	$21.38 \pm 1.84^{b,c}$	481.35 ± 58.39^a
FNM_9	4.46 ± 0.06^a	$34.00 \pm 2.84^{c,d,e}$	$17.83 \pm 3.85^{b,c}$	227.67 ± 37.49^b
FNM_{10}	4.45 ± 0.40^a	$33.00 \pm 2.14^{d,e}$	24.31 ± 3.53^b	$46.65 \pm 11.4^{c,d}$
Base	4.71 ± 0.76^a	18.00 ± 2.82^f	116.21 ± 0.06^a	116.21 ± 0.06^c

± Standard Deviation; ^{a,b,c} Significant Difference by Tukey.

By increasing the amount of fiber, there is less variation of pH, observing that the pH of the samples with greater amount of fiber gradually decreases. The dough can reach acidic to have low pH in the range of 4.1-4.5 [14], considering the product bases, and the portions to make the fermentation.

The glucose release during the fermentation at 24 h was favored with the increase in the amount of fiber and water, and with an intermediate portion of mother dough (40 to 45 mg/100 g of dough) in the case of oats. Which is affected in the mixtures when there is less amount of water, giving lower yields of glucose (0 to 5 mg/100 g of dough), as shown in Fig. 4, while in the case of the nopal increases the concentration with an increase of water (40 to 45 mg/100 g of dough) and decreases with the decrease in concentration of fiber and water (5 to 10 mg/100 g dough) as seen in Fig. 5.

The addition of inoculum (mother dough) at low concentrations, as a result that the lactic acid bacteria begin to grow as monocultures, the source of carbon is not exhausted. But when you include yeast, the carbon source runs out before 12 h at 35 °C [13].

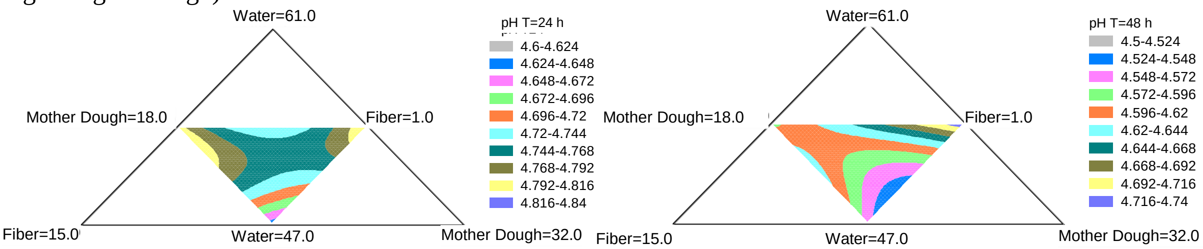

Figure 2. Movement of the pH during the fermentation of the sourdough with oatmeal added to 24 and 48 hours respectively.

Obtaining energy by microorganisms, is by the use of available sugars, when decrease in the concentration of these carbohydrates, are obtained from the starch and the fermentable fibers. This occurs with the release of amylolytic enzymes by the lactobacilli and yeasts, to release mono and disaccharides such as glucose, sucrose or maltose [15]. Another source for obtaining carbon source are the fibers of rye, oats, which contain β-glucans, to a lesser proportion in the rye, in addition to pectins in the case of the mucilage of nopal [16] to obtain glucose. In Fig. 4, it is shown that the concentration of glucose increases with decreasing of mother dough (16.0 to 18.0 mg/100 g of dough) in the fermentation of oatmeal, and in the case of the nopal glucose decreases (22.5 to 25.0 mg/100 g of dough) compared with the 24 h, when it is reduced the concentration of mother dough and water. This is due to the reduced microbial load, resulting in a lower competition between species. With the increase in the inoculum, there is competition for the substrate, which must occur in a higher concentration, which is consumed immediately.

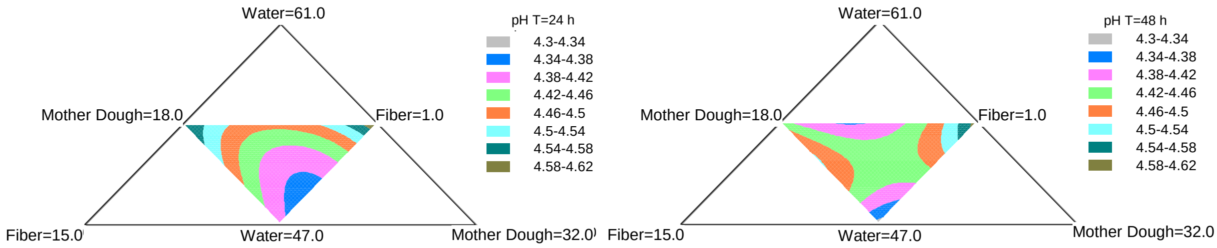

Figure 3. Movement of the pH during the fermentation of the dough mass with nopal added at 24 and 48 hours respectively.

The lactic acid, ethanol and CO_2, are the major products of fermentation processes. The homofermentatives obliged and facultatives microorganisms degrade the hexoses by Emden Meyerhof pathway, where the use maltose and fructose takes place only after having been consumed glucose by microorganisms [17]. The increased production of lactic acid at 24 h was introduced in the mixing FO_7, with 634.59 mg/ 100g of dough (Table 2), and the majority of the samples had values between 156 and 241 mg/100 g of dough (Table 4), while the 48 h the higher values were found in the FO_4 with 401.75 mg/100 g of dough, while to the mucilage of nopal samples FNM_8 and FNM_9 (481.35 and 227.67 mg/100 g of dough) showed the highest concentrations in a period of 48 h (Table 5) because at 24 h were obtained low values (Table 3). Lactic acid is the metabolite produced by the LAB, when placed only monocultures of LAB, the production of lactate is 810 mg/100 g of dough, but when combined with yeast, the production reaches up to 990.88 mg/100 g of dough [13]. In these point where mixtures are obtained the highest concentrations of lactic acid, is the best interaction between the yeast and lactobacilli, where the amylases formed to obtain simpler sugars act and are stable in pH of 5 to 7, and the increased production of lactic acid is found in a pH of 4.4 to 4.7 . It is possible that to being productive organisms of acids, enzymes produced have greater tolerance to pHs low. At a pH of 5 there is a good production of amylases of 8.1 U ml^{-1}, and pH of 4, there are 3.3 U ml^{-1}[18].

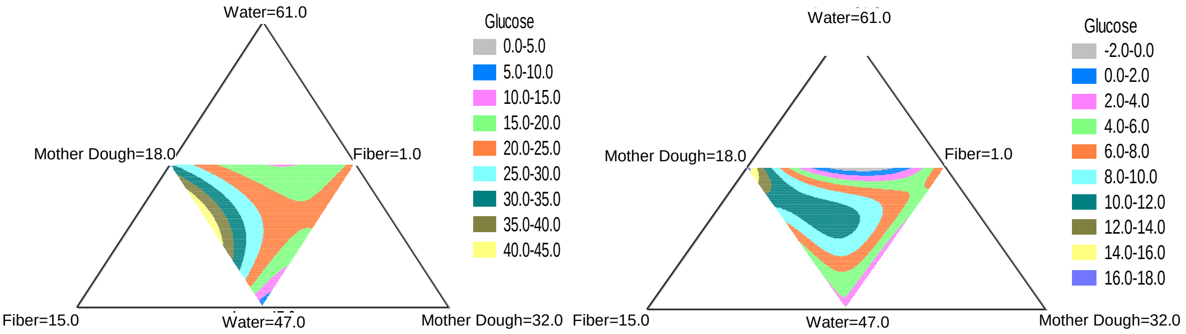

Figure 4. Movement of the glucose during the fermentation of the dough mass with oatmeal added at 24 and 48 hours respectively.

Glucose-1-phosphate is metabolized by pentose phosphate, where glucose is excreted as a result, it increases the concentration of external glucose, in addition to the consistent expression of the hexokinase activity [12]. The sucrose is metabolized to glucose and fructose and they are incorporated into the cycle of the hexose. It has been reported that the metabolic activity increases with the addition of yeast, because they produce greater amount of CO_2 to the leavened [19].

The incorporation of yeast to the dough, improve the fermentation process by the formation of the CO_2, improving the environmental conditions for the lactobacilli. However, when it reaches the 48 h regarding fermentation the dough

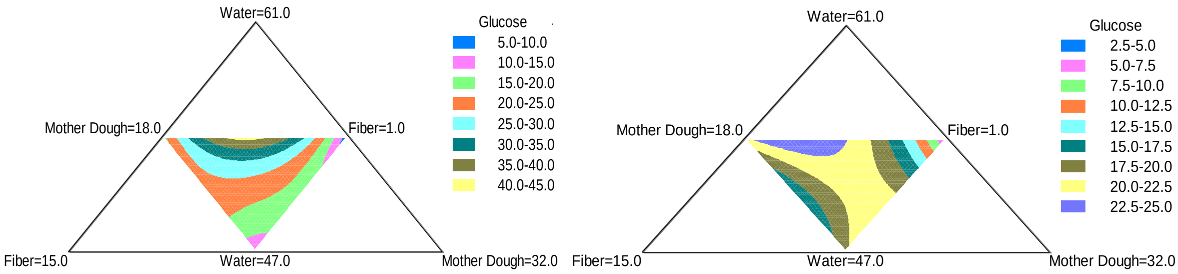

Figure 5. Movement of the glucose during the fermentation of the dough mass with nopal added at 24 and 48 hours respectively.

reaches the maximum concentration for the mixture FO_4 and FNM_8, which was formulated with fiber concentration close to average and greater amount of mother dough (Fig. 1). However, for FNM_9 the low production of lactic acid with the increase in the amount of fiber, while the 24 h for the FO_7 gives as a result the best fermentation, with the quantities of intermediate water, mother dough and the minimum water content. The production of organic acid is affected by the amount of water in the mixture. Since the higher content, favors the solubility of the nutrients, giving a better interaction with the microorganisms.

IV. CONCLUSION

The results obtained in this study, unveil a complexity of the interactions between *S. cereviseae* and the lactic acid bacteria, in which, which improves the lactic acid production by combining in a balanced manner the raw materials, where the best productions of lactate were found in the sample FO_7 at 24 h, and of FO_4, FNM_8 and FNM_9 at 48 h.

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