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Studies on Organic Acids and Minerals Content of Sourdough Naans Made from Different Extraction Rate Wheat Flours and Starter Cultures

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Abstract: Sourdough naans were prepared using different extraction rate wheat flours and sourdough starter cultures. Sourdough naans were analyzed to find out the effect of flour extraction rates on the production of organic acids and minerals content. It was concluded that organic acids (lactic, acetic and citric acid) increased with an increase in extraction rate and freeze dried cultures containing hetero-fermentative strains of LAB showed better performance than homo-fermentative in the production of organic acids. Similarly, minerals content were also increased with an increase in flour extraction and 100% extraction rate sourdough naan showed the highest minerals content.

Key words: Organic acids, minerals, sourdough naans, extraction rate

INTRODUCTION

Wheat (*Triticum aestivum*) is the most important crop for making bread, due to its absolute baking performance in comparison to all other cereals (Dewettinck *et al.,* 2008). In Pakistan, the annual wheat production stood at 21.74 million tones during the year 2007-08 (GOP, 2008). Almost 80% of this grain is consumed in the form of flat breads locally known as chapattis, rotis and naan (Anjum *et al.,* 1991). Naan is the usual commercial flat bread of Pakistan. It is mostly prepared from lower extraction rate wheat flour and dough is leavened either with yeast or sodium bicarbonate or both. The extraction rate of wheat flour has marked effect upon its nutritional content. As a result of milling process, nutrient level of high extraction rate flour increases directly with increase in flour ash values (Matthews and Workman, 1977).

In recent years, sourdough bread has enjoyed renowned success worldwide, due its natural taste and good health effects (Brummer and Lorenz, 1991). The sourdough bread is prepared from a mixture of flour and water that is fermented with (LAB), mainly hetero-fermentative strains, elaborating lactic acid and acetic acid in the mixture and hence, resulting a pleasant sour taste end product (De Vuyst and Neysens, 2004). The sourdough fermentation have a number of beneficial effects that include, a prolong shelf, accelerated volume, delayed staling, improved bread flavor and nutritional value (Thiele *et al*., 2002; Lavermicocca *et al*., 2000).

Mold growth is the most common cause of microbial spoilage in bread that results in huge economic losses. The sourdough inhibits the growth of the pathogens by synthesizing the antimicrobial compounds, like lactic acid, acetic acid, benzoic acid and hydrogen peroxide (Park *et al*., 2006). Whole meal cereals are an important source of minerals such as K, P, Mg, or Zn, but mineral utilization is limited by the presence of phytic acid (Lopez *et al*., 1998). The degradation of phytate, has repeatedly been reported in sourdough processes (Angelis *et al*., 2003). Use of sourdoughs or acidified sponges can be adjusted to improve mineral bioavailability by increasing phytic acid hydrolysis (Fretzdorff and Brummer, 1992).

Sourdough breads have been extensively investigated in the recent times but, no study has been made on the preparation of sourdough naans. The present study was designed to study the effect of flour extraction rates on the production of organic acids and mineral contents in sourdough naans.

MATERIALS AND METHODS

Procurement of wheat varieties: Different Wheat varieties were procured from Wheat Research Institute, Ayub Agriculture Research Institute, Faisalabad-Pakistan. After preliminary trials for making flat bread (naan), variety AS-2002 was selected for further studies.

Flour milling for different extraction rate flours: Selected wheat variety (AS-2002) was milled to get different extraction rate flours from 64% to 100%. Wheat tempered to 15% moisture was milled by using Quadrumate Senior Mill (C. W. Brabender, Duisburg, Germany), (AACC, 2000). Four milling fractions namely break flour, reduction flour, bran and shorts were obtained. Break and reduction flours were mixed to get straight grade flour. The by-products obtained during milling such as bran and shorts were milled in a hammer mill and blended with 64% extraction rate flour by passing through a 40-mesh sieve (610 µ). The 64%, 76%, 88% and 100% extraction rates flours were obtained by proportional mixing of the by-product of wheat with 64% extraction rate flour.

Sourdough cultures: Freeze dried cultures were procured from Lallemand Baking Solutions, Montreal, Canada. Freeze dried cultures namely, LA-1 containing homo-fermentative strain *Pediococcus acidilacti* along with yeast *Saccharomyces cerevisiae* and LA-5, mixed culture containing hetero-fermentative strain *L. brevis* and homo-fermentative strain *L. casei*, along with yeast *Saccharomyces cerevisiae*, was used.

Mother sponge: Mother Sponge was prepared from flour (200g), tap water (200ml at 30°C) and freeze dried starter culture was added to give colony-forming units (cfu) of 10^8 bacteria per gram and yeast count as 10^6 cfu per gram of sponge. This was mixed by hand with a spoon and incubated for 20 h at 30°C (Hansen and Hansen, 1994).

Sourdough preparation: Sourdough was prepared from flour with 64, 76, 88 and 100% extraction rates. The flour (200 g), water (100 ml) and fermented sponge (70 g) were mixed together into dough and incubated for 20 h at 30°C.

Preparation of sourdough naan: The sourdough naans were prepared by using wheat flours of different extraction rates. Dough was prepared by mixing flour (250 g), fermented sponge (100 g), sugar (10 g), salt (6 g), and water as determined by farinographic water absorption and incubated for 20 h at 30 °C. A dough ball of 100 g was taken and sheeted into a disk of 7-in. diameter with rolling pin. The disk was pressed with the fingertips in the center covering 5-in. diameter. Put it in proofing chamber at 37°C and 85% RH for 45 min. Finally it was baked in oven at 315ºC for 3 min. The sourdough breads were packed in a polyethylene bag and kept at -20°C for further analysis. Naan bread made from yeast *Saccharomyces cerevisiae* alone was kept as control treatment.

pH and acidity of sourdough naans: pH and total titratable acidity (TTA) of sourdough naans were determined according to the method given by Park *et al.* (2006), using the Inolab WTW Series 720 pH meter. A 10g sample was blended with 90 ml distill water and the suspension was then titrated with a 0.1 mol/L NaOH to a final pH of 8.5. The TTA was expressed as the amount (ml) of NaOH used.

Organic acids in Sourdough naan: Organic acids (lactic acid, acetic acid and citric acid) contents in sourdough naans were determined using High Performance Liquid Chromatography (HPLC) by following the method of Vernocchi *et al.* (2004).
HPLC (Model 1050, Hewlett-Packard, Waldbronn Germany) equipped with a Bio-Rad Aminex (Bio-Rad Laboratories, Hertfordshire, UK) HPX-87H column (300×7.8 mm) was used for organic acids determination. The following conditions were used: mobile phase, 0.08 M H_2SO_4; flow rate, 0.60 ml min^{-1}; temperature, 65°C; the column was connected with a UV detector (210 nm). For HPLC analysis, 100 ml of 0.1 N sulfuric solution was added to each 10 g samples, the mixture was homogenized for 5 min by an Omni Mixer Homogeniser (Omni International, Warrengton, VA), filtered through a 0.22-mm membrane filter and analyzed as described above.

Minerals content of sourdough naan: The minerals such as Fe, Ca, Zn and Cu were estimated according to the respective method as described in AOAC (2000) using Atomic Absorption Spectrophotometer (Varian, AA240, Victoria, Australia). A 0.5 g sample was digested separately by using wet digestion method. The sample was first digested with 10 ml HNO_3 at a temperature of 60-70°C for 20 min and then digested with $HClO_4$ at a temperature of 190°C till the solution become clear. The digested sample was transferred to 250 ml volumetric flask and volume was made with distilled water and then filtered. The solution was loaded into atomic absorption spectrophotometer apparatus. The standard curve was prepared by running samples of known strength through atomic absorption spectrophotometer. The mineral contents of unknown samples were estimated by using the respective standard curve prepared for each mineral.

Phosphorous content in sourdough naan: Ammonium vandate was used to determine phosphorus content by aminonaphthol sulphonic acid method as described by Kitson and Mellon (1992). In a test tube, add ammonium molybdate solution (1 ml), amminonaphthol sulphonic acid solutions (0.4 ml), and 0.1 ml of sample or standard at various dilutions. Distilled water was added to make up the volume (10 ml). Solution was shaken and allowed to stand for 5 min to develop color. Blank was used to set the zero absorbance at 720 nm wavelength.

Statistical analysis: Statistical analysis was carried out using Minitab (V.13.1, Minitab Inc., PA 16801-3008, USA). Duncan's Multiple Range Test was applied to calculate the level of significance (Steel *et al*., 1997).

RESULTS AND DISCUSSION

pH and acidity of sourdough naans: Statistical analysis (Table 1) exhibited that the treatments differed significantly ($P<0.01$) with respect to both pH and acidity of the sourdough naans. The comparison of yeast leavened naans with sourdough naans also resulted in significant differences. Two different LAB types varied significantly ($P<0.01$) with regards to acidity of sourdough naan but showed non-significant differences for pH. The mean values for the pH and acidity of naan

Table 1: Mean sum of squares for pH and acidity of sourdough naan prepared with different extraction rate flours and cultures

SOV	df	pH	Acidity (mL 0.1N NaOH)
Treatments	8	0.0858*	17.143**
Control V/S LAB fermented	1	0.5704**	31.970**
LAB types	1	0.0037[NS]	1.654**
Extraction rates	3	0.0337[NS]	31.914**
LAB types x Extraction rates	3	0.0037[NS]	2.594**
Error	18	0.0249	0.132
Total	26		

* = Significant ($p<0.05$); ** = Highly significant ($p<0.01$); NS = Non-significant

Table 2: Mean values for pH and acidity of sourdough naan prepared with different extraction rate flours and cultures

Starter cultures	Extraction rates	pH	Acidity (mL 0.1N NaOH)
Control		5.2±0.144[a]	3.5±0.046[f]
LA-1	64%	4.8±0.063[b]	4.7±0.092[e]
	76%	4.8±0.104[b]	5.7±0.196[d]
	88%	4.7±0.052[b]	6.9±0.069[c]
	100%	4.6±0.052[b]	9.5±0.387[a]
LA-5	64%	4.8±0.052[b]	3.5±0.087[f]
	76%	4.8±0.081[b]	7.2±0.248[c]
	88%	4.7±0.156[b]	8.5±0.179[b]
	100%	4.7±0.017[b]	9.7±0.300[a]

*Means with different letters have significant ($p<0.05$) and with no letter have non-significant variation ($p>0.05$), Control Naan bread made from *Saccharomyces cerevisiae* alone; LA-1: Starter culture containing *Pediococcus acidilacti* + *Saccharomyces cerevisiae*.
LA-5: Starter culture *L .brevis* + *L .casei* + *Saccharomyces cerevisiae*

given in Table 2 showed that yeast leavened naan showed the highest pH (5.2) and the lowest acidity (3.5) values. The sourdough naans prepared from different extraction rate flours using LA-1 starter culture showed acidity in the range of 3.5 to 9.5 ml, which increased with an increase in flour extraction rate. The sourdough naan with 100% extraction rate using LA-1 as starter culture showed the highest acidity (9.5 ml).

The results for pH and acidity are in accordance with findings of Park *et al.* (2007) who reported increased acidity and lower pH values of sourdough breads in contrast to yeast leavened bread which showed less acidity and higher pH values. Rouzaud and Martinez-Anaya (1997) also found that the addition of starter and strain significantly influence acidity related parameters.

Organic acids in Sourdough naan: The statistical analysis for organic acids produced by sourdough naans prepared from different extraction rate flours and cultures is given in Table 3. The treatments showed highly significant effect ($P<0.01$) on organic acids contents. Control bread showed the lowest organic acids production for all three organic acids. The sourdough naans prepared with different extraction rate flours and LA-1 starter culture produced lactic acid in the range of 7975 to 16800 ppm, acetic acid in the range of 515 to 1075 ppm and citric acid in the range of 450 to 950 ppm (Table 4), while lactic acid in the range of 8606 to 22600 ppm, acetic acid in the range of 1105 to 2050 ppm and citric acid in the range of 510 to 1360 ppm were produced by LA-5 starter culture.

The lactic acid production increased with an increase in flour extraction rates with highest amounts of lactic acid and citric acid found in 100% extraction rate sourdough naan. However, the acetic acid contents were extraction rate as well as starter culture dependant.

The results of present study are also supported by findings of Hansen *et al.* (1989) in which the production of acetic acid was much higher in the dough acidified with hetero-fermentative strains as compared to homo-fermentative strains which produced only lactic and citric acids. The increased production of acetic acid may improve the qualitative characteristics of sourdough naan. Hansen and Hansen (1994) which showed that acetic acid production is strain dependent and homo-fermentative strains did not produce acetic acid except for sourdough made from straight grade flour. The production of lactic acid and citric acids increased with an increase in flour extraction rate in the present study indicating that lactic acid and citric acids production is flour extraction rate dependent and their total contents were independent of starter cultures. The use of mixed culture resulted in higher lactic acid concentrations compared to yeast leavened bread (Plessas *et al.,* 2008).

Minerals content of sourdough naan: Statistical analysis for minerals content in sourdough naan prepared with different extraction rate flours showed highly significant effect ($P<0.01$) of treatments on minerals like Ca, Fe, Zn, Cu and P (Table 5). When sourdough naans were compared with control (yeast leavened naan), it resulted in highly significant ($P<0.01$) differences. The mean values for sourdough naan made from different extraction rate flours are given in Table 6. The control treatment showed lower mineral contents as compared to sourdough naan.

The flour extraction rate is the most important factor for controlling the amount of minerals (Pederson *et al.,* 1989).The results of the present study indicated that with the increase in flour extraction rate, minerals content of sourdough naans increased appreciably (Kent and Amos, 1967). The results are in accordance with the findings of Nilsson and Harkonen (1997) which showed high content of dietary fiber, Mg, P, Fe, Cu and Zn, in bread made from whole wheat flour.

With the increase in flour extraction rate minerals content increase, as does the buffering capacity of the flour due to the phytic acid from the aleurone layer of the cereal (Hansen, 2006). The utilization of minerals such as Fe, P, Cu and Zn is limited due to the presence of phytic acid. The phytic acid contents are decreased during sourdough bread making due to the presence of phytase enzyme in sourdough (Nilsson and Harkonen, 1997). From the health point of view sourdough

Table 3: Mean sum of squares for organic acids of sourdough naan prepared with different extraction rate flours and cultures

SOV	df	Lactic acid	Acetic acid	Citric acid
Treatments	8	86896899**	665421**	278738**
Control V/S LAB fermented	1	191032480**	319704**	349209**
LAB types	1	56742975**	3003338**	267759**
Extraction rates	3	142372240**	54113*	493134**
LAB types x Extraction rates	3	6761005**	612663**	44509**
Error	18	1087903	12797	1913
Total	26			

* = Significant (p<0.05); ** = Highly significant (p<0.01)

Table 4: Mean values for organic acids of sourdough naan prepared with different extraction rate flours and cultures

Starter cultures	Extraction rates	Lactic acid	Acetic acid	Citric acid
Control		5025±253.9^{e}	850±18.52^{e}	380±11.63^{e}
LA-1	64%	7975±181.1^{d}	1075±16.57^{d}	450±14.86^{de}
	76%	9820±474.3^{d}	920±29.48^{de}	520±8.52^{d}
	88%	13210±1133.8^{c}	860±85.99^{e}	670±23.84^{c}
	100%	16800±339.7^{b}	515±36.89^{f}	905±40.66^{b}
LA-5	64%	8606±133.8^{d}	1105±42.11^{d}	510±29.44^{d}
	76%	12960±138.4^{c}	1365±63.05^{c}	650±26.68^{c}
	88%	15940±108.4^{b}	1680±66.64^{b}	870±32.16^{b}
	100%	22600±1221.4^{a}	2050±134.02^{a}	1360±21.79^{a}

Table 5: Mean sum of squares for minerals content of sourdough naan prepared with different extraction rate flours and cultures

SOV	df	Ca	Fe	Zn	Cu	P
Treatments	8	0.020**	399.195**	225.936**	2.845**	3.729**
Control V/S LAB fermented	1	0.025**	430.954**	183.707**	2.802**	3.089**
LAB types	1	0.001^{NS}	0.004^{NS}	0.015^{NS}	0.001^{NS}	0.004^{NS}
Extraction rates	3	0.045**	920.414**	540.970**	6.645**	8.911**
LAB types x Extraction rates	3	0.001^{NS}	0.454^{NS}	0.285^{NS}	0.010^{NS}	0.002^{NS}
Error	18	0.001	4.924	1.295	0.067	0.038
Total	26					

* = Significant (p<0.05); ** = Highly significant (p<0.01); NS = Non-significant

Table 6: Mean values for minerals content of sourdough naan prepared with different extraction rate flours and cultures

Starter cultures	Extraction rates	Ca (mg/g)	Fe(ppm)	Zn (ppm)	Cu (ppm)	P (mg/g)
Control		0.21±0.006^{d}	19.5±0.13^{d}	8.3±0.18^{c}	1.4±0.01^{d}	1.48±0.06^{cd}
LA-1	64%	0.22±0.006^{d}	19.2±0.61^{d}	8.5±0.19^{c}	1.5±0.08^{d}	1.44±0.04^{d}
	76%	0.26±0.011^{c}	26.5±0.32^{c}	9.6±0.13^{c}	1.8±0.12^{cd}	1.82±0.06^{c}
	88%	0.33±0.012^{b}	34.8±1.49^{b}	19.4±1.06^{b}	2.7±0.17^{b}	2.76±0.12^{b}
	100%	0.42±0.006^{a}	48.4±1.35^{a}	28.8±0.96^{a}	3.8±0.17^{a}	4.15±0.16^{a}
LA-5	64%	0.22±0.005^{d}	19.7±0.38^{d}	8.2±0.12^{c}	1.4±0.03^{d}	1.46±0.02^{cd}
	76%	0.27±0.012^{c}	26.0±1.58^{c}	9.8±0.39^{c}	1.9±0.11^{c}	1.80±0.09^{cd}
	88%	0.32±0.011^{b}	35.2±0.22^{b}	20.0±0.12^{b}	2.6±0.01^{b}	2.82±0.16^{b}
	100%	0.42±0.029^{a}	47.9±2.75^{a}	28.5±1.25^{a}	3.8±0.33^{a}	4.20±0.17a

fermentation result in decrease of phytic acid by phytate hydrolysis, thus increase mineral bioavailability and would be beneficial and attractive in improving mineral status (Plessas *et al.,* 2008).

Conclusion: From the present research work it was concluded that, production of organic acids increased with an increase in extraction rate and freeze dried cultures containing hetero-fermentative strains of LAB showed better performance than homo-fermentative in the production of organic acids. The sourdough naans prepared with different extraction rates flour showed highly significant effect on minerals content and an increase in extraction rate resulted in increased minerals content in sourdough naans.

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