Effect of natural starters used for sourdough bread in Morocco on phytate biodegradation

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تأثير مُبْدئات التخمر الطبيعية المستخدَمة في صناعة الخبز في المغرب على التدرُّك البيولوجي للفيتات

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الخلاصة: تمت دراسة نشاط إنزيم الفيتاز في مُبْدئات تخمر الخبز الطبيعية لتحديد الخصائص الفيزيائية الكيميائية للدقيق (مثل حَلّمهة حمض الفيتيك، والقدرة على انتفاخ العجين، ورقم الباهاء pH) في الدقيق وأثناء تخمر الخميرة. كما تمت دراسة خصائص مكروبات التخمر (الخمائر وجراثيم حمض اللاكتيك). ودلت النتائج على انخفاض حمض الفيتيك في العجين الذي استُخدمت فيه مُبْدئات التخمر التقليدية، كما دلت على تباين كبير في نشاط إنزيم الفيتاز. ولوحظ ارتفاع عدد المكروبات في نهاية التخمر، مما يشير إلى زيادة نشاط مُبْدئات التخمر. وأظهرت الخمائر أيضاً تبايناً كبيراً، وكان عدد جراثيم حمض اللاكتيك مرتفعاً في العجين المتخمر. وقد ثبت وجود نشاط لإنزيم الفيتاز في مُبْدئات التخمر المحتوية على جراثيم حمض اللاكتيك والخميرة، والتي كان أهمها السكيراء الجعوية *Saccharomyces cerevisae* والمبَّنة المسطَّحة *Lactobacillus plantarum* والسُّتْقة المسارِيقية *Leuconostoc mesenteroides*.

ABSTRACT Phytase activity was studied in natural sourdough bread starters to determine physicochemical characteristics (phytic acid hydrolysis, dough rising capacity and pH) in the flour and during sourdough fermentation. Fermentation microorganisms (yeasts and lactic acid bacteria) were also characterized. Results showed a decrease of phytic acid in sourdoughs started with traditional starters, and wide variation in phytase activity. Microorganism counts were high at the end of fermentation, indicating higher fermenting activity of the starters. Yeast populations showed wide variation and lactic acid bacteria had high counts in the fermentation. Phytase activity was demonstrated in starter cultures made of lactic acid bacteria and yeast isolates, the most interesting of which were *Saccharomyces cerevisiae* combined with *Lactobacillus plantarum* and *Leuconostoc mesenteroides*.

Effet des ferments naturels utilisés pour le pain au levain au Maroc sur la biodégradation des phytates

RESUME L'activité de la phytase a été étudiée dans les ferments naturels utilisés pour le pain au levain afin de déterminer les caractéristiques physico-chimiques (hydrolyse de l'acide phytique, capacité de la pâte à lever et pH) dans la farine et pendant la fermentation du levain. Les microorganismes responsables de la fermentation (levures et bactéries de l'acide lactique) ont également été caractérisés. Les résultats ont montré une réduction de l'acide phytique dans les levains traditionnels, et une grande variation de l'activité de la phytase. La numération des microorganismes à la fin de la fermentation était élevée, ce qui indique une activité de fermentation plus importante des ferments. Les populations de levures montraient des variations importantes et les bactéries de l'acide lactique étaient en nombre important dans la fermentation. L'activité de la phytase a été montrée dans les cultures de ferments composées de bactéries d'acide lactique et d'isolats de levures, la plus intéressante étant *Saccharomyces cerevisiae* associée à *Lactobacillus plantarum* et *Leuconostoc mesenteroides*.

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Introduction

Bread is a staple food in most developing countries. Bread consumption in Morocco is an estimated 210.44 kg/year in grain equivalent. Baker's yeast is now more frequently used than traditional starter, with a consequent loss of some of the nutritional properties of the bread. It is assumed that most of the minerals are bound by phytates in cereals due to the high chelating reaction of the myo-inositol hexakis dihydrogenphosphate (IP6) molecule.

The bioavailability of some important minerals is lowered by phytates [*1*] and the digestibility of proteins [*2*], starch [*3*] and lipids [*4*] is reduced. We have previously demonstrated that traditional sourdough starters in Morocco may contain two kinds of microorganisms, lactic acid bacteria and yeasts [*5*]. Faid et al. has demonstrated that controlled fermentation of sourdough bread inoculated with selected strains of lactic acid bacteria and yeasts has characteristics similar to bread made with traditional starters (the strains used were isolated from the traditional starters) [*6*].

There is a need to study the nutritional properties of natural sourdough starters and phytate destruction and the bioavailability of minerals to more clearly understand the role of mixed fermentation in bread-making by yeasts and lactic acid bacteria. In the present study we examined phytase activity in sourdough bread started with both traditional starters and selected isolates of lactic acid bacteria and yeasts.

Methods

Starter propagation

We propagated 16 samples of traditional sourdough bread starters on wheat flour in sterilized glass flasks. We mixed 10–20 g of each sample with 100 g of wheat flour and 35–40 mL of distilled water. The mixture was well-kneaded with a wooden spoon and the flasks were incubated at 30 °C for 6 hours.

Dough preparation

Doughs to be analysed were prepared by mixing 50 g of the starter with 200 g of wheat flour and 100 mL of water. The mixture was kneaded manually and the resultant dough introduced into a 500 mL graduated cylinder. Each assay was prepared in duplicate, one sample to determine the rising capacity of the dough and the second to determine pH, phytase activity and microbial count. The cylinders were placed in a water bath at 30 °C.

Chemical analysis

A pH-meter (Crison micro-pH 2000) was used to measure the pH of a 10 g sample mixed with 10 mL of distilled water. Dough rising capacity was determined according to the method described by Faid et al. [*6*].

Phytate determination

All glassware was rinsed with nitric acid (10%) and with distilled water prior to use. Precisely measured aliquots of the samples were introduced into 50 mL conical flasks. Nitric acid was added and the flasks were shaken for 4 hours. The mixture was centrifuged at 2000 g and the supernatant used for the determination of iron.

All reagents were analytical grade and all solutions were prepared just before use. The samples and the calibration curve were prepared as follows: to 0.5 mL of the supernatant, 1 mL of ammonium iron sulfate [$NH_4Fe(SO_4)$] (0.5 mmol) and 1.4 mL of distilled water were added in tubes. The mixture was heated in a boiling water bath for 20 minutes and allowed to cool to room temperature, after which 5 mL of amyl alcohol and 0.1 mL of ammonium thiocyan-

ate (10%) were added. The optical density of the red complex in the upper layer was checked at 465 nm in a spectrophotometer (C-Cil Instruments Type CE 303). The calibration curve was established using sodium phytate (2 mmol) (Merck, Germany) as a reference standard. The range was 0–320 μL.

Microbiological determinations

Yeasts and lactic acid bacteria were evaluated in the fermented sourdough to determine the origin of the phytases. Yeasts were counted on potato dextrose agar and the lactic acid bacteria counted on MRS (Merck, Germany) for lactobacilli. *Leuconostoc* were determined on the medium of Garvie (Merck, Germany). Pediococci were counted on APT agar (Merck, Germany), incubated at 40 °C.

Characterization of the lactic acid bacteria isolates

Isolates of lactic acid bacteria were collected from the samples, purified and studied for their morphological (Gram reaction and shape) and physiological characteristics (growth at 45 °C, 30 °C and 15 °C, arginine deaminase and carbon dioxide formation). Yeast isolates were also collected from the samples and broadly checked for their morphological and physiological characteristics. The isolates were first screened for their rising capacity.

Starter cultures

Isolates of each group, including *Lactobacillus* and *Leuconostoc*, were grown on MRS broth. The cultures were incubated 24 hours at 30 °C for *Lactobacillus* and 48 hours at 26 °C for *Leuconostoc*. Yeast isolates were grown on yeast extract glucose broth (yeast extract 5 g; glucose 20 g; distilled water 1 L). The biomass was harvested by centrifuging at 2800 g for 20 minutes and stored at 4 °C until use.

Dough inoculation

Soft wheat flour (200 g) was mixed with 100 mL distilled water in 500 mL wide-mouthed glass flasks and inoculated with a suspension of the isolates to be studied to obtain a final concentration of 10^6 cells/g in the dough. The flasks were incubated at 30 °C. The dough rising capacity, pH and phytates were determined as indicated above.

Results

Results for phytic acid reduction in the dough after a 10-hour fermentation showed a wide variation among the starters (Table 1). The highest values were observed in the assays commenced with starters L1 (84.7%), L2 (80%), L3 (82%), C1 (80.4%), C2 (80.5%) and C3 (79.6%). Other starters were also active on the phytates. Values ranged from 70.6% (A1) to 78.5% (L4). Lower phytase activity was observed in samples T2 (25.8%), A5 (42.7%) and T4 (23.2%) compared to those previously mentioned.

The physicochemical properties of the starters were also evaluated to study their characteristics in bread fermentation. It should be emphasized here that the technological factors are widely related to the nutritional characteristics. The pH was decreased in almost all the trials involving the traditional starters (Table 1). Values ranged between 3.02 (T2, A4) and 3.71 (L1). This was most probably due to the active microbiota (lactic acid bacteria). Low pH value in sourdough bread has become an area of increasing interest over the past few decades and a number of studies have been undertaken [7].

The dough rising capacity (DRC) was evaluated to provide accurate information about fermentation time at 30 °C and about the physical properties (rheology) of the

Table 1 **Phytate reduction levels and physicochemical properties of natural sourdough bread starters**

Starter code	pH	Dough rising capacity (mL)	Phytate reduction (%)
L1	3.71	65	84.7
L3	3.18	85	82.0
C2	3.15	90	80.5
C1	3.15	70	80.4
L2	3.07	110	80.0
C3	3.18	115	79.6
L4	3.11	130	78.5
L5	3.12	80	76.2
C5	3.07	110	74.8
C4	3.20	105	72.8
T1	3.20	90	71.8
A2	3.38	130	70.7
A1	3.35	40	70.6
A3	3.62	85	56.6
A4	3.02	20	53.9
A5	3.45	30	42.7
T5	3.30	40	40.4
T2	3.02	85	25.8
T4	3.56	125	23.2
Control	6.20	8	6.6

dough. DRC ranged from 20 mL (A4) to 130 mL (A2), indicating a wide variation among the samples (Table 1). The DRC was directly related to the nature of the fermenting yeasts derived from the starters.

Apart from phytate destruction activity in traditional starters, the physicochemical properties of the starters, including pH and DRC, are also of interest. Sourdough fermentation may take longer than baker's yeast fermentation, possibly because of the time required for growth of the lactic acid bacteria. Sourdough fermentation at home or in bakeries may involve a normal fermentation with active starters that can be maintained easily by regenerating them after each kneading operation.

We performed counts of the different microorganisms involved in sourdough fermentation in the samples that showed high phytate reduction activity. Table 2 shows the levels of the yeasts and lactic acid bacteria in the dough at the end of fermentation. Most of the samples showed the same profile for both microorganisms. Yeast populations ranged from 3.4×10^6 cfu/g (C1) to 2.6×10^8 cfu/g (L3). Counts of lactic acid bacteria were higher than those of yeasts. Cocci count reached 7.7×10^8 cfu/g, with one sample too numerous to count, and rods 5.8×10^9 cfu/g, with two samples too numerous to count. The high counts of lactic acid bacteria found in the samples may explain the low pH values of these samples and illuminate what typically occurs in sourdough fermentation. The

Table 2 **Yeast and lactic acid bacteria counts in fermenting sourdough bread**

No.	Yeasts ($\times 10^6$)	Lactic acid bacteria ($\times 10^6$)	
		Rods	Cocci
L1	15.0	480	770
L3	260.0	TNT	50
C2	150.0	580	240
C1	3.4	110	TNT
L2	230.0	450	260
C3	184.0	220	290
L4	8.5	560	310
L5	160.0	250	250
C5	140.0	240	40
C4	250.0	TNT	250
Control	0.4	0.01	0.02

*TNT = too numerous to count.

same suggestion can be applied to the results of the yeast counts and the DRC of the samples, i.e. high yeast counts are correlated to a high DRC.

It has previously been reported that lactic acid fermentation can reduce phytate levels in bread fermentation [*8,9*]. Khetarpaul and Chauhan have reported that mixed cultures of yeasts and lactobacilli may reduce the phytate content of bread [*10*].

The random combination of certain isolates of both lactic acid bacteria and yeasts showed high phytase activity (Table 3). The highest rate of phytate reduction was seen in the combination of strains of *Lactobacillus* with yeasts. The use of the lactic acid bacteria strains alone showed high phytate hydrolysis: 76.5% for *L. plantarum* and 67.0% for *Leu. mesenteroides*. The pH was decreased, but the dough rising capacity was low, so the bread could not be baked. The assay made with yeast strains alone showed a weak hydrolysis of phytates (28.0%), although the dough rising capacity was higher. A combination of yeast strains and lactic acid bacteria may overcome this situation. Our most interesting results were found by combining two strains of lactic acid bacteria with one strain of yeast (*L. plantarum* and *Leu. mesenteroides* with *S. cerevisiae*) because of the high DRC (120 mL) and the improvement in phytate reduction (85.4%).

The phytase activity found in the strains of lactic acid bacteria isolated from those starters showing high phytate reduction rates points to the existence of active strains of these microorganisms in traditional starters.

Discussion

The microbiota of traditional sourdough starters is complicated and in most cases not well understood. Control of fermentation may be achieved by using pure culture starters as a substitute for traditional starters to direct chemical reactions through a defined biotechnological process. Zyta [*11*] has reported on the use of mould phytases in the food industry and Wang et al. [*12*] have reported on their application in fermented oriental foods.

The use of sourdough starters with high phytase activities, high DRC and low pH is highly suited to the bread-making process. The reduction of phytic acid by the tradi-

Table 3 **Physico-chemical properties and phytate hydrolysis in starters made from lactic acid bacteria and yeasts strains**

Combinations	% phytate reduction	Dough rising capacity (mL)	pH
Lactobacillus plantarum SL50	76.5	16	3.4
Leuconostoc mesenteroides SC7	67.0	25	6.2
Saccharomyces cerevisiae SC20	28.0	100	5.6
Starter 1 (SL50/SC20)	88.8	100	3.4
Starter 2 (SC7/SC20)	86.7	45	3.5
Starter 3 (SL50/SC7/SC20)	85.4	120	3.8
Control (baker's yeast)	16.1	20	6.2

tional starter culture during fermentation is more practical and easier to maintain and to apply in bakeries than is the use of the more complicated two starters and enzymes.

Bread fermentation is better served by using species of lactic acid bacteria and yeasts not only for the organoleptic and gustatory benefits, but also for the enhanced nutritional properties such as phytate destruction and nutrient formation in the dough during the fermentation process.

Some inherent factors in fermentation, including pH and time of fermentation, may affect the destruction of phytates [*13,14*]. Some representative unidentified isolates of *Lactobacillus*, *Leuconostoc* and yeasts were inoculated in pure cultures separately and in combination with wheat flour doughs to study their hydrolyzing activity on phytic acid. In our results, phytic acid hydrolysis and dough characteristics showed evidence of net phytase activity in some combined cultures of the lactic acid bacteria and yeasts. The combination of *Lactobacillus* and *Leuconostoc* strains with the yeast strains showed a high phytate reduction (85.4%). This correlated with the traditional sourdough starters and is similar to the activity of these starters since the three microorganisms are combined in the pure culture starters to simulate the existing microorganisms in the traditional starter.

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FAO, WHO launch US$ 40 million trust fund to help poor countries participate in Codex Alimentarius

A US$ 40 million trust fund to help the world's least developed countries participate in Codex Alimentarius was launched in Geneva by the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO). Codex Alimentarius sets food standards that protect the health of consumers and ensure fair practices in food trade.

The Codex Alimentarius Commission (CAC) was established in 1962 by FAO and WHO and has 168 member countries today. Because the CAC establishes international food safety and trade standards, it is equally important to developed and developing countries. However, many developing countries, particularly the least developed ones, have not fully participated in the work of the CAC because of the cost involved in attending meetings and working groups.

The new fund will help some 120 developing countries and countries in transition increase their participation in the vital work of the Commission. The fund will also help regulators and food experts from all areas of the world to participate in setting international standards and enhance their capacity to develop effective food safety and quality standards, both within the framework of the Codex Alimentarius and national food safety systems in their own countries.

Source: WHO Press release, 14 February 2003