Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 93 (2005) 585–589
Food Chemistry
www.elsevier.com/locate/foodchem

Bread making using kefir grains as baker's yeast

S. Plessas [a], L. Pherson [b], A. Bekatorou [a,*], P. Nigam [b], A.A. Koutinas [a]

[a] Department of Chemistry, Food Biotechnology Group, University of Patras, GR-26500 Patras, Greece
[b] School of Biomedical Sciences, University of Ulster, BT52 1SA Coleraine, Northern Ireland, UK

Received 1 March 2004; received in revised form 4 October 2004; accepted 4 October 2004

Abstract

The leavening activity of kefir grains in lean dough and their efficiency in producing bread of good quality, in terms of loaf volume, flavour, texture and shelf life, were evaluated. Although the leavening rate of commercial baker's yeast was higher (30 ml/h), kefir performed well (24 ml/h), and produced bread of good quality, resembling traditional sourdough bread. Rising was satisfactory (specific loaf volume 3.0–3.3 ml/g) and breads produced with kefir retained more moisture, had a firmer texture, lower acidity (pH 4.9–5.5) and retained their freshness for longer compared to baker's yeast bread. Consumers showed a preference for kefir-leavened bread, although a preliminary headspace GC–MS analysis of volatiles did not reveal significant differences between the two types of bread.

Keywords: Bread; Kefir; Baker's yeast; Leavening activity; Shelf life; Volatiles

1. Introduction

Bread is a major nutritional component and bread making is one of the oldest processes, known and practised for thousands of years, worldwide. Yet, the mechanisms associated with bread staling have not been fully understood. Bread staling is a result of changes in the starch and protein microstructures and moisture migration and redistribution (Shewry & Tatham, 1996; Hallberg & Chinachoti, 2002). The economical problems arising from staling and microbial spoilage have recently triggered extensive research. Another important issue in the developed countries is the degraded organoleptic quality of industrial bread and the consequent preference of consumers for other types of food. In the developing countries, on the other hand, longer preservation and higher nutritional value are issues of major importance. To achieve better mechanical properties of flour, extend preservation time, improve organoleptic and nutritional quality of bread, and satisfy the recent demand of consumers for "*natural*" technologies, research has been focussing on the use of enzymes (Bombara, Anon, & Pilosof, 1997; Dunnewind, Van Vliet, & Orsel, 2002; Van der Maarel, Van der Veen, Uitdehaag, Leemhuis, & Dijkhuizen, 2002), natural preservatives, genetic engineering of yeast and plants (Randez-Gil, Sanz, & Prieto, 1999; Linko, Javanainen, & Linko, 1997) and sourdough bread production, using well-defined mixed starter cultures containing lactic acid bacteria (LAB) and yeasts (Gobbetti et al., 1995; Meignen et al., 2001; Caballero et al., 1995; Linko et al., 1997). The use of mixed cultures has a number of important advantages over baker's yeast, such as improved flavour and texture (Meignen et al., 2001), as well as extension of preservation time through the in situ production of antimicrobial compounds (organic acids and bacteriocins) (Katina, Sauri, Alakomi, & Mattila-Sandholm, 2002; Messens & De Vuyst, 2002). In such mixed-cultures, yeasts act mainly as leavening agents, while LAB contribute mainly to the sensory quality and longer shelf life

* Corresponding author. Tel.: +30 2610 997 104; fax: +30 2610 997 105.
E-mail address: abecatorou@in.gr (A. Bekatorou).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.10.034

of bread. Kefir is a natural mixed culture used for centuries in the Caucasus area for the production of the traditional milk drink through lactic acid and alcoholic fermentations. The kefir grains are clusters of microorganisms with a cauliflower-like structure held together by a polysaccharide matrix. Many microorganisms have been isolated from kefir microflora, sharing symbiotic relationships, including yeasts (*Kluyveromyces, Candida, Torulopsis* and *Sacharo myces* sp), lactobacilli (*L. brevis, L. acidophilus, L. casei, L. helveticus, L. delbruecki*), streptococci (*Streptococcus salivarius*), lactococci (*Lc. Lactis* ssp. *thermophilus*, *Leuconostoc mesenteroides* and *L. cremoris*) and occasionally acetic acid bacteria (Simova et al., 2002). The use of kefir as baker's yeast for sourdough bread production has not been reported and it is an interesting perspective, expected to increase resistance to spoilage and produce bread of improved aroma and taste. The aim of this study was the evaluation of kefir grains as baker's yeast in terms of leavening activity, flavour formation and preservation time.

2. Materials and methods

2.1. Microorganisms and growth media

Kefir grains, isolated from Russian kefir drink (Athanasiadis, Boskou, Kanellaki, & Koutinas, 2001), were obtained by aerobic fermentation (3 l/min air supply), at 30 °C, in liquid nutrient media containing 20 g/l glucose, 20 g/l lactose, 1 g/l $(NH_4)_2SO_4$, 1 g/l KH_2PO_4, 10.2 g/l, $MgSO_4 \cdot 7H_2O$ and 4 g/l yeast extract. All media were sterilized at 120 °C for 15 min. The produced cells (small grains of approximately 0.5 mm diameter) were harvested by centrifugation at 5000 rpm for 10 min to yield pressed kefir biomass with 70% moisture, like pressed baker's yeast, which was a commercial product used in the Greek baking industry.

2.2. Evaluation of leavening activity

To evaluate and compare the leavening activity of kefir grains and baker's yeast in lean dough (without addition of sugars), dough samples were prepared by mixing 20 g flour, 15 ml water and 0.7 g of kefir or baker's yeast (on a dry weight basis) in 100 ml volumetric cylinders. Maximum leavening rate (ml/h) at 30 °C, was calculated from the highest volume reached in 120 min, divided by the time at which that volume was first recorded (Caballero et al., 1995).

2.3. Bread making

Bread making was carried out in a Greek bakery according to the traditional method for baking white, yeast-leavened bread. Dough samples were prepared by mixing 500 g flour with 300 ml water and with 15 g of pressed baker's yeast or either 5, 10 or 15 g of pressed kefir biomass. Salt and sugar were not added (lean dough). Samples were kneaded for 10 min and then they were incubated for 30–60 min at 30 °C to allow initial rising. Proofing was carried out at 46 °C for 45 min. The loaves were then baked at 190 °C for 60 min. The process was repeated five times.

2.4. Sensory evaluation

Finished breads were allowed to cool at room temperature and a preliminary sensory evaluation was carried out by a number of non-trained testers (consumers) and bakers (totally 20 people), who were asked to evaluate the breads, using a taste-test protocol based on a 1–10 preference scale. Testers were asked to evaluate (separately) the aroma and taste of breads as well as the overall quality on the basis of loaf volume, texture, colour and flavour. The results are given in Table 3 as average scores plus standard deviations.

2.5. Assays

An amount of 15 g of breadcrumb and 100 ml of distilled water were placed in a clean dry container, which was sealed and stirred until the bread dispersed into a semi-liquid mixture. The pH was recorded using a Cyberscan 10 pH-meter. Then, an amount of 0.11 N NaOH solution was added until the pH was fixed at 6.6. The total titratable acidity (TTA) was determined by the consumed ml of NaOH, as mg of lactic acid per g of sample (Gélinas, McKinnon, & Pelletier, 1999). The loaf volumes, after cooling for 15 min, were measured using the rapeseed displacement method (Hallén, Ibanoglu, & Ainsworth, 2004). Each loaf was put in a container and covered with rapeseed to totally fill the container. Then the loaf was removed and the volume of the rapeseed noted. Loaf volumes were calculated by deducting the rapeseed volume from the container volume. Moisture loss was measured by deducting the weight of bread from the initial weight of the dough before baking. Moisture contents of breadcrumb samples were determined according to the AOAC (1990) Method 925.09, involving heating at 98–100 °C in a partial vacuum, and reporting loss in weight as moisture (indirect method). The results are presented in Table 1 as average values of three repetitions. The standard deviations were: pH ± 0.3, TTA ± 0.4, loaf weight ± 23, loaf volume ± 85, moisture loss ± 12, breadcrumb moisture ± 0.09.

Headspace analysis of volatiles in breadcrumb samples was carried out by GC–MS analysis. A solid phase micro-extraction (SPME) sampling method was used (DVB/CAR/PDMS fibre) (Mallouchos, Komaitis, Koutinas, & Kanellaki, 2002; Ruiz, Quilez, Mestres, & Guash, 2003). Specifically, 0.25 g of sample and 10 ml

Table 1
Characteristics and shelf life of bread made with kefir and baker's yeast

Bread sample	pH	TTA (mg lactic acid/g)	Loaf weight (g)	Loaf volume (ml)	Moisture loss (g)	Breadcrumb moisture ($g\ H_2O/g$)			Specific loaf volume (ml/g)	Mould spoilage (days)
						Day 1	Day 3	Day 5		
Baker's yeast	5.7	2.1	649	2356	166	0.20	0.17	0.13	3.6	3–4
Kefir (1%)	5.5	2.8	665	2214	140	0.24	0.20	0.16	3.3	4–5
Kefir (2%)	5.2	3.6	675	2123	135	0.25	0.22	0.19	3.1	5–6
Kefir (3%)	4.9	5.0	683	2058	132	0.27	0.24	0.20	3.0	5–6

of a 20% w/v NaCl solution were transferred into a 20 ml sealed glass vial and the content was stirred for 5.5 min at 50 °C. The fibre was then exposed to the headspace for 60 min. Desorption of volatiles took place in the injector port of the GC in splitless mode at 230 °C for 70 min. Separation of volatiles was performed on a Shimadzu GC-17A Gas Chromatograph (SUPELCO WAX-10 column), connected with a GCMS-QP505A Mass Spectrometer (70 eV ionisation energy; 29–400 *m/z* mass range). Helium was used as carrier gas (2 ml/min). Oven temperature was programmed at 35 °C for 5 min, and then it was raised to 60, 200, and 250 °C at rates of 2.0, 5.0 and 25.0 °C/min, respectively. Identification was carried out by comparison of retention times and MS data with those of standard compounds and data obtained from NIST libraries.

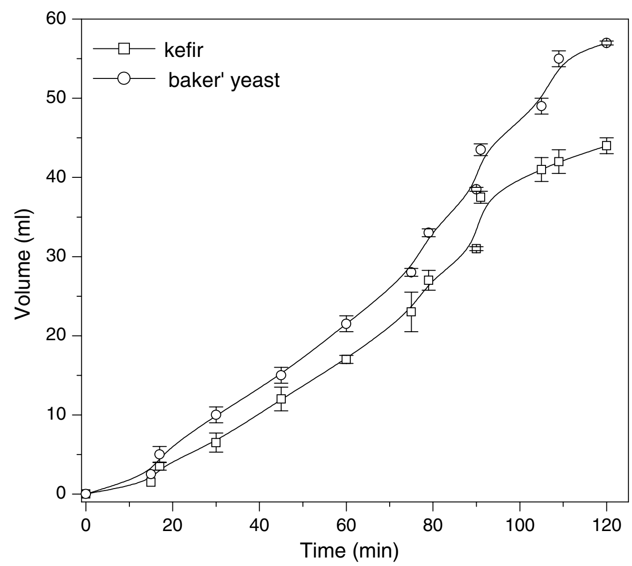

Fig. 1. Leavening activity of kefir and baker's yeast in lean dough.

3. Results and discussion

The leavening activity of kefir grains in lean dough was compared to traditional baker's yeast, and their efficiency in producing bread of good quality, in terms of loaf volume, flavour, texture and shelf life, were evaluated. Kefir exhibited lower leavening rate (24 ml/h) and lower maximum rising (44 ml) compared to baker's yeast (30 ml/h and 57 ml, respectively) (Fig. 1). Despite this observation, bread was produced successfully using kefir grains. Bread samples were prepared from lean dough with either 1%, 2% or 3% kefir grains and were compared with bread made with 2% of commercial pressed baker's yeast. Rising was satisfactory in all cases. The specific loaf volumes were 3.6 ml/g for baker's yeast and 3.0–3.3 ml/g for kefir bread (Table 1). Additionally, the breads produced with kefir retained more moisture after baking (Table 1) and had a firmer texture than bread made with baker's yeast. The acidity of bread made with kefir (pH 4.9–5.5 and TTA 2.8–5.0 mg lactic acid/g, respectively) was higher than baker's yeast bread (pH 5.7; TTA 2.1 mg lactic acid/g) (Table 1). This suggested that it would probably have a positive effect on the preservation of kefir bread and contribute to longer microbiological shelf life, therefore avoiding the addition of preservatives to bread. Therefore, the bread samples were kept at 25 °C to estimate preservation time as far as staling and appearance of mould spoilage are concerned. The baker's yeast-leavened bread retained its freshness for only 1 day and then became dry and stale. Breadcrumb moisture was reduced by 15% in 3 days and 35% in 5 days (Table 1). Mould spoilage (green hyphae) appeared after 3 days. Kefir-leavened breads remained fresher for longer. Breadcrumb moisture was reduced by an average 13% in 3 days and 28% in 5 days (Table 1). Staling was obvious after 3 days and mould spoilage after 5 days.

The 20 random customers and bakers, who were asked to evaluate the quality of the bread samples at the first day of baking showed a bigger preference for bread made with kefir as far as flavour and overall quality were concerned (Table 2). Nevertheless, they did not observe significant differences between the two types of bread and therefore, it was concluded that bread made with kefir could reach the same standards as normal

Table 2
Sensory evaluation of the breads produced by baker's yeast or kefir, as average scores plus standard deviations for aroma, taste and overall quality (volume, texture, colour and flavour)

	Baker's yeast	Kefir (1%)	Kefir (2%)	Kefir (3%)
Aroma	7.5 ± 0.98	7.5 ± 0.92	7.9 ± 1.00	8.1 ± 0.95
Taste	8.0 ± 0.88	8.2 ± 0.96	8.4 ± 1.02	8.8 ± 1.05
Overall quality	8.2 ± 0.79	8.4 ± 0.86	8.6 ± 0.94	8.8 ± 0.83

Table 3
Volatile compounds detected in breadcrumb samples of breads made with kefir and baker's yeast

Retention time (min)	Compound	Reliability of identification	Baker's yeast	Kefir
4.425	Acetaldehyde	a	+	+
5.356	Ammonia	b	+	+
5.793	Ethanol	a	+	+
7.394	Ethyl propanoate	a	+	+
20.663	Amyl alcohols	a	+	+
10.833	2-Hydroxy butanoic acid	b	+	+
12.633	Ethanodioic acid dibutyl ester	a	+	+
13.450	Propanoic acid	b	+	+
13.558	1-Pentanal	a	–	+
17.908	Acetic acid	a	+	+
21.317	1-Butene	b	+	+
22.633	1,3-Dioxolane-2-one	a	+	+
33.550	1,5-Diisopropyl-2,3-dimethyl-cyclohexane	a	–	+
43.442	1-Hexanal	a	–	+
45.083	1-Heptanal	a	+	+
45.558	Tetrahydro-4H-pyran-4-ol	a	+	+
48.200	Ethanol 2-(ethenyloxy)	b	+	+
49.417	1(Methoxymethoxy)-3-methyl-3-hydroxybutane	b	–	+
49.683	2-Hydroxy-3-pentanone	b	–	+

a: Identification by comparison of gas chromatographic retention times and MS data with those of available pure compounds, b: Mass spectrum in agreement with spectra in the literature.

loaves. Generally, bread baked with 3% kefir had better flavour and remained fresh for longer. Although, loaf volume was lower, its texture was firmer, holding more moisture and resembling traditional sourdough bread. The increased shelf life of bread produced with kefir could be attributed to its higher acidity and probably to bacteriocin formation by kefir microflora. The preliminary headspace GC–MS analysis of volatiles did not reveal significant differences between the two types of bread (Table 3). In total, 19 compounds were identified, 5 of which were identified only in bread produced by kefir (1-hexanal, 1-pentanal, 1,5-diisopropyl-2,3-dimethyl-cyclohexane, 1-(methoxymethoxy)-3-methyl-3-hydroxybutene, 2-hydroxy-3-pentanone). Nevertheless, the method used needs to be optimised and an extraction technique should be more appropriate for analysis of breadcrumb aroma.

The above results suggest that kefir grains can be used successfully for sourdough type bread making, producing bread with improved flavour and extended shelf life. Also, as kefir microflora can ferment lactose, and milk whey, a liquid waste of the dairy industry, could be used as a low cost raw material for the production of kefir biomass (Koutinas, 2003). Additionally, this would offer an interesting alternative to the utilization of whey, whose disposal causes serious environmental problems due to its high organic load. In the future, further investigation is needed on the contribution of kefir to flavour formation through optimised GC–MS analysis of aroma and analysis of amino acids and free fatty acid profile. Also, the extension of shelf life (reduction of staling rate and increase of microbial stability), and potential probiotic properties of bread made with kefir, should be investigated through microbiological examinations, analysis of organic acid formation and detection of bacteriocins.

Acknowledgement

We thank European Social Fund (ESF), Operational Program for Educational and Vocational Training II (EPEAEK II) and particularly the Program IRAKLEITOS for funding the above work. We also thank Mr T. Fotopoulos for the kind use of his bakery.

References

AOAC (1990). In: P. Cunniff (Ed.), *Official methods of analysis of AOAC International*. (16th ed., Vol. II, pp. 32–1). Arlington, Virgini DC: The Association of Official Analytical Chemists.

Athanasiadis, I., Boskou, D., Kanellaki, M., & Koutinas, A. A. (2001). Effect of carbohydrate substrate on fermentation by kefir yeast supported on delignified cellulosic materials. *Journal of Agricultural and Food Chemistry, 49*, 658–663.

Bombara, N., Anon, M. C., & Pilosof, A. M. R. (1997). Functional properties of protease modified wheat flours. *Lebensmittel – Wissenschaft und – Technologie, 30*, 441–447.

Caballero, R., Olguin, P., Cruz-Guerrero, A., Gallardo, F., Garcia-Garibay, M., & Gomez-Ruiz, L. (1995). Evaluation of *Kluyveromyces marxianus* as baker's yeast. *Food Research International, 28*(1), 37–41.

Dunnewind, B., Van Vliet, T., & Orsel, R. (2002). Effect of oxidative enzymes on bulk rheological properties of wheat flour doughs. *Journal of Cereal Science, 36*, 357–366.

Gélinas, P., McKinnon, C. M., & Pelletier, M. (1999). Sourdough-type bread from waste bread crumb. *Food Microbiology, 16*(1), 37–43.

Gobbetti, M., Simonetti, M. S., Corsetti, A., Santinelli, F., Rossi, J., & Damiani, P. (1995). Volatile compound and organic acid productions by mixed wheat sour dough starters: influence of fermentation parameters and dynamics during baking. *Food Microbiology, 12*, 497–507.

Hallberg, L. M., & Chinachoti, P. (2002). A fresh perspective on staling: the significance of starch recrystallization on the firming of bread. *Journal of Food Science, 67*, 1092–1096.

Hallén, E., Ibanoglu, S., & Ainsworth, P. (2004). Effect of fermented/germinated cowpea flour addition on the rheological and baking properties of wheat flour. *Journal of Food Engineering, 63*, 177–184.

Katina, K., Sauri, M., Alakomi, H. L., & Mattila-Sandholm, T. (2002). Potential of lactic acid bacteria to inhibit rope spoilage in wheat sourdough bread. *Lebensmittel – Wissenschaft und – Technologie, 35*(1), 38–45.

Koutinas, A. A. (2003). Kefir yeast technology. In S. Roussos, C. R. Soccol, A. Pandey, & C. Augur (Eds.), *New horizons in biotechnology* (pp. 297–310). Dordrecht: Kluwer Academic.

Linko, Y., Javanainen, P., & Linko, S. (1997). Biotechnology of bread baking. *Trends in Food Science and Technology, 8*, 339–344.

Mallouchos, A., Komaitis, M., Koutinas, A., & Kanellaki, M. (2002). Investigation of volatiles evolution during the alcoholic fermentation of grape must using free and immobilized cells with the help of solid phase microextraction (SPME) headspace sampling. *Journal of Agricultural and Food Chemistry, 50*, 3840–3848.

Meignen, B., Onno, B., Gélinas, P., Infantes, M., Guilois, S., & Cahagnier, B. (2001). Optimisation of sour dough fermentation with *Lactobacillus brevis* and bakers yeast. *Food Microbiology, 18*, 239–245.

Messens, W., & De Vuyst, L. (2002). Inhibitory substances produced by Lactobacilli isolated from sourdoughs-a review. *International Journal of Food Microbiology, 72*, 31–43.

Randez-Gil, F., Sanz, P., & Prieto, J. A. (1999). Engineering baker's yeast: room for improvement. *Trends in Biotechnology, 17*, 237–244.

Ruiz, J. A., Quilez, J., Mestres, M., & Guash, J. (2003). Solid-phase microextraction method for headspace analysis of volatile compounds in bread crumb. *Cereal Chemistry, 80*(3), 255–259.

Shewry, P. R., & Tatham, A. S. (1996). Disulphide bonds in wheat gluten proteins. *Journal of Cereal Science, 25*, 207–227.

Simova, E., Beshkova, D., Angelov, A., Hristova, T., Frengova, G., & Spasov, Z. (2002). Lactic acid bacteria and yeasts in kefir grains and Kefir made from them. *Journal of Industrial and Microbiological Biotechnology, 28*(1), 1–6.

Van der Maarel, M. J. E. C., Van der Veen, B., Uitdehaag, J. C. M., Leemhuis, H., & Dijkhuizen, L. (2002). Properties and applications of the starch-converting enzymes of the α-amylase family. *Journal of Biotechnology, 94*, 137–155.