Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 107 (2008) 883–889
Food Chemistry
www.elsevier.com/locate/foodchem

Evolution of aroma volatiles during storage of sourdough breads made by mixed cultures of *Kluyveromyces marxianus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* or *Lactobacillus helveticus*

S. Plessas [a,*], A. Bekatorou [a], J. Gallanagh [b], P. Nigam [b], A.A. Koutinas [a], C. Psarianos [a]

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

Received 29 December 2006; received in revised form 4 August 2007; accepted 6 September 2007

Abstract

Two mixed starter cultures were used for sourdough bread making to evaluate their ability to improve quality and increase bread shelf-life: *Lactobacillus delbrueckii* ssp. *bulgaricus* or *Lactobacillus helveticus* mixed with the lactose fermenting yeast *Kluyveromyces marxianus* as alternative baker's yeast. Control sourdough breads (*K. marxianus*) without the addition of bacteria, were also prepared. The changes on the headspace aroma volatiles during storage were assessed using solid-phase microextraction (SPME) GC–MS analysis. The effect of these changes on bread flavour was evaluated by consumer preference evaluations and the results were co-evaluated with those from the GC–MS analysis. The obtained results showed differences in the volatile composition of the different types of breads examined, as well as dramatic decreases of the number and the amount of volatiles after five days of storage. The sourdough breads made with *K. marxianus* and *L. bulgaricus*, had a more complex aroma profile, longer shelf-life and achieved the highest scores in the sensory tests.

Keywords: Bread; Sourdough; *Lactobacillus helveticus*; *Lactobacillus delbrueckii* ssp. *bulgaricus*; *Kluyveromyces marxianus*; Volatiles; Shelf-life

1. Introduction

The extension of shelf-life is one of the biggest challenges for the baking industry today. The shelf-life of bread and other baking products is small, mainly as a result of staling, which is a number of physicochemical alterations that occur after baking and during storage. Staling is characterised by crumb firming mainly due to retrogradation of the starch polymers and interactions between starch and proteins, crust softening due to transfer of moisture from the crumb to crust and finally flavour changes. Consequently, these changes are responsible for the disposal of large quantities of bread (8–10%), therefore resulting in economical losses (Corsetti et al., 2000; Guarda, Rosell, Benedito, & Galotto, 2004; Inagaki & Seib, 1992; Katina, Salmenkallio-Marttila, Partanen, Forssell, & Autio, 2006; Lorenz & Maga, 1972). The main ways for delaying staling and extending shelf-life are the use of hydrocolloids, emulsifiers, exogenous enzymes, etc. (Corsetti et al., 2000; Guarda et al., 2004; Katina et al., 2006). Recently, return to traditional processes like sourdough bread making, employing pure cultures of lactic acid bacteria (LAB), has been proposed as a significant means for improving bread quality in terms of delaying staling, improving taste, texture, aroma and generally increasing shelf-life (Gobbetti, 1998; Hansen & Schieberle, 2005; Messens & De Vuyst, 2002).

Defined mixed starter cultures containing both LAB and yeasts, in free cell suspensions, or even immobilised in suitable matrices, such as kefir (Plessas, Pherson, Bekatorou, Nigam, & Koutinas, 2005), baker's yeast, kefir and *Lactobacillus casei* (Plessas et al., 2007), baker's yeast and

* Corresponding author. Tel.: +30 2610 997104; fax: +30 2610 997105.
E-mail address: splessas@upatras.gr (S. Plessas).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.09.010

milk LAB immobilised on a flour/milk matrix or kefir immobilised on orange peel (Plessas et al., 2007, 2008b), *Kluyveromyces marxianus, Lactobacillus delbrueckii* ssp. *bulgaricus* and *Lactobacillus helveticus* (Plessas et al., 2008a), etc., was proposed for the production of sourdough bread making. The LAB and the lactose fermenting yeasts employed in these studies, can be grown on whey, therefore their use in baking applications was proposed as an alternative for the utilisation of this seriously polluting waste. These starter cultures proved efficient as leavening agents, and lead to products of longer shelf-life (delayed staling and resistance to mould spoilage), as well as improved organoleptic properties, which was justified by GC–MS assays of volatiles revealing more complex aroma profiles. The effect of sourdough on the aroma profile of breads has been widely studied and reviewed (Ganzle, Vermeulen, & Vogel, 2007; Gobbetti, 1998; Hansen & Hansen, 1994; Hansen & Schieberle, 2005; Kirchhoff & Schieberle, 2001, 2002; Maga, 1974; Rehman, Paterson, & Piggott, 2006).

Even though extensive research has been carried out regarding bread shelf-life, proximate analysis of quality features and aroma composition after baking, there are few studies examining the changes of the aromatic composition of bread, during storage, which may reflect the effects of staling, evaporation and microbial activities in the stored product. Therefore, the aim of this study was to monitor the quality degradation during storage of breads produced using mixed starter cultures of yeast and LAB, through the qualitative and quantitative determinations of aroma volatiles, combined with respective consumer evaluations.

2. Materials and methods

2.1. Microorganisms and media

The homofermentative LAB *L. delbruekii* ssp. *bulgaricus* (*L. bulgaricus*) (ATCC 11842) isolated from Bulgarian yoghurt and *L. helveticus* (ATCC 15009) isolated from cheese, were obtained from Deutsche-Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Germany. The cultures were grown and maintained at MRS broth (De Man, Rogosa and Sharpe), they were incubated at 40 °C for 24 h and then stored at 4 °C. The broth was refreshed regularly during the course of the experiment. *K. marxianus* (IFO 0288) was also obtained from DSMZ. The culture was grown and maintained on nutrient broth consisting of 3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone and 10 g/L glucose. It was incubated at 30 °C for 48 h and then the produced culture was stored at 4 °C. For further production, an initial amount of 4 g of each microorganism was added to 2 L of cheese whey and incubated at 30 °C for about 24 h. In all cases cells were harvested by centrifugation (Sigma 3K12, Rotor No. 11133, 5000*g*, 10 min). All media were sterilised by autoclaving at 120 °C for 15 min.

Commercial hard type wheat flour manufactured by Allatini S.A. (Thessaloniki, Greece), containing 13% protein, 69% carbohydrates, 1.5% fat and 22% dietary fibre was used for bread making. Cheese whey was prepared from cow's milk, after rennin coagulation, filtration to separate casein proteins and subsequent heat treatment at 90 °C for 15 min for removal of whey proteins.

2.2. Preparation of sourdough

For preparation of the sourdoughs, 400 g of flour and various amounts of starter cultures were mixed with 200 mL tap water. The doughs were mixed manually for 5–10 min until the correct consistency was obtained and then sourdough fermentation was allowed to occur at 30 and 40 °C, for 16 h. The amounts of microorganisms examined (% w/w on flour basis) were 1% of *K. marxianus* mixed with 4% of either *L. bulgaricus* or *L. helveticus*. Control breads were also made using sourdoughs prepared with the addition of 1% *K. marxianus* as the leavening agent and no addition of pure LAB culture (Plessas et al., 2007, 2008a).

2.3. Bread making

Sourdough breads were prepared according to the sourdough method. The general process involved mixing 400 g flour, 200 g (50% w/w on flour basis) of sourdoughs prepared as describe above, addition of 1.5% salt (on flour basis) and 200 mL tap water. The doughs were kneaded mechanically for 5 min (BBA 2866 Automatic Bead Machine Clatronic International GmbH, Germany) and then allowed to ferment at 37 °C for 2 h. Baking was carried out at 200–210 °C for 1 h. The process was repeated three times.

2.4. Determination of volatiles

Headspace analysis of headspace aroma volatiles was carried out by gas chromatography mass spectrometry (GC–MS) using the solid-phase microextraction technique (SPME). For each SPME analysis, 2 g of bread sample were introduced into a 20 mL vial and the SPME needle was introduced through the vial septum. The vial was then immersed in a water bath at 60 °C and the SPME fibre (2 cm–50/30 mm DVD/Carboxen/PDMS Stable Flex Supelco, Bellefonte, PA, USA) was exposed to the headspace for 60 min. When the extraction process was completed, the fibre was inserted into the injector port (set at 280 °C) of the gas chromatograph (GC) for thermal desorption of volatiles for 5 min in splitless mode. The GC–MS instrumentation included a Shimadzu model GC-17A gas chromatograph coupled to a GC–MS-QP5050A mass spectrometer. A Supelcowax-10 column (60 m, 0.32 mm i.d., 0.25 μm film thickness) was used. The GC temperature program was set as follows: 35 °C for 5 min, increased by 5 °C/min to 50 °C (held for 5 min), increased by 5.5 °C/min to 230 °C (held for