

African Journal of Biotechnology Vol. 10(47), pp. 9668-9672, 24 August, 2011
Available online at <http://www.academicjournals.org/AJB>
DOI: 10.5897/AJB10.2428
ISSN 1684-5315 © 2011 Academic Journals

Full Length Research Paper

Investigating the sourdough potential for enhance microbiological shelf life and roasty aroma of traditional Lavash bread

Seyed Ali Mortazavi and Alireza Sadeghi*

Department of Food Science and Technology, Faculty of Agriculture, Ferdowsi University of Mashhad, P. O. Box: 91775-1163 Mashhad, Iran.

Accepted 25 April, 2011

In this study, potential effects of sourdough on microbiological shelf life and roasty aroma of traditional Lavash bread were studied. For investigating the biopreservation effect of sourdough, after intentional contamination of flour with hygiene indicator microorganisms that cause mouldiness and ropiness, survival curves of these microorganisms versus different sourdough fermentation times and temperatures, at finished-products were determined. Influence of sourdough fermentation conditions on crust roasty aroma was determined by gas chromatography and 2-acetyl 2-thiazoline was selected as standard compound for this aroma. The results showed that by increasing sourdough fermentation time and temperature, survival of indicator microorganisms in finished-products were decreased. But intensity of crust roasty aroma did not have the same profile. Therefore, process requirements for optimum microbiological shelf life and roasty aroma were different, which should be taken into account in designing sourdough baking processes.

Key words: Lavash bread, sourdough, microbiological shelf life, roasty aroma.

INTRODUCTION

Bread is one of the most popular foods consumed worldwide. With the world's population increasing, it is not only necessary to increase the supply of food but also to learn how to better preserve existing food supplies (Clarke and Arendt, 2005). Acidic sourdough is an ancient way to improve flavor, texture and microbiological shelf life of bread used in Mediterranean countries (Katina, 2005). Sourdough is capable to control and inhibition of spoilage organisms during fermentation is due to different factors especially low pH value and antimicrobial compounds produced by Lactic Acid Bacteria or LAB (Simsek et al., 2006). The influence of sourdough on bread flavor is based on three main factors: Formation of acidity, formation of flavor precursors such as amino acids and formation of volatile compounds or aroma. The generation of sufficient amounts of volatile compounds during fermentation

needs a multiple step process of about 12 to 24 h, while fermentation by baker's yeast alone is finished within a few hours (Ur-Rehman et al., 2006).

Traditional sourdough bread-making has been practiced for centuries in Iran. Although, rural bread making is still highly reliant on sourdough; urban bakeries usually use baker's yeast and also sodium bicarbonate instead (Fazeli et al., 2004). Microbiological shelf life in bread is limited by mouldiness and ropiness. Moulds (such as *Rhizopus stolonifer*, *Neurospora sitophila*) and *Bacillus* spp. (such as *Bacillus subtilis*, *Bacillus licheniformis*) are the key microorganisms in these spoilages, respectively (Clarke and Arendt, 2005). With respect to deterioration in the quality of bread during shelf life, mould growth is the most common cause of microbial spoilage. In addition to the economic yield losses associated with this kind of spoilage, another concern is the possibility that mycotoxins produced by the moulds that may cause public health problems (Fazeli et al., 2004). Rope spoilage is the most bacterial spoilage of bread and *Bacillus* spp. cause a potential risk to food borne illness when present at levels of 10^5 CFU/g in

*Corresponding author. E-mail: Sadeghi.Alireza@stu-mail.um.ac.ir Tel: 98-915-5080383.

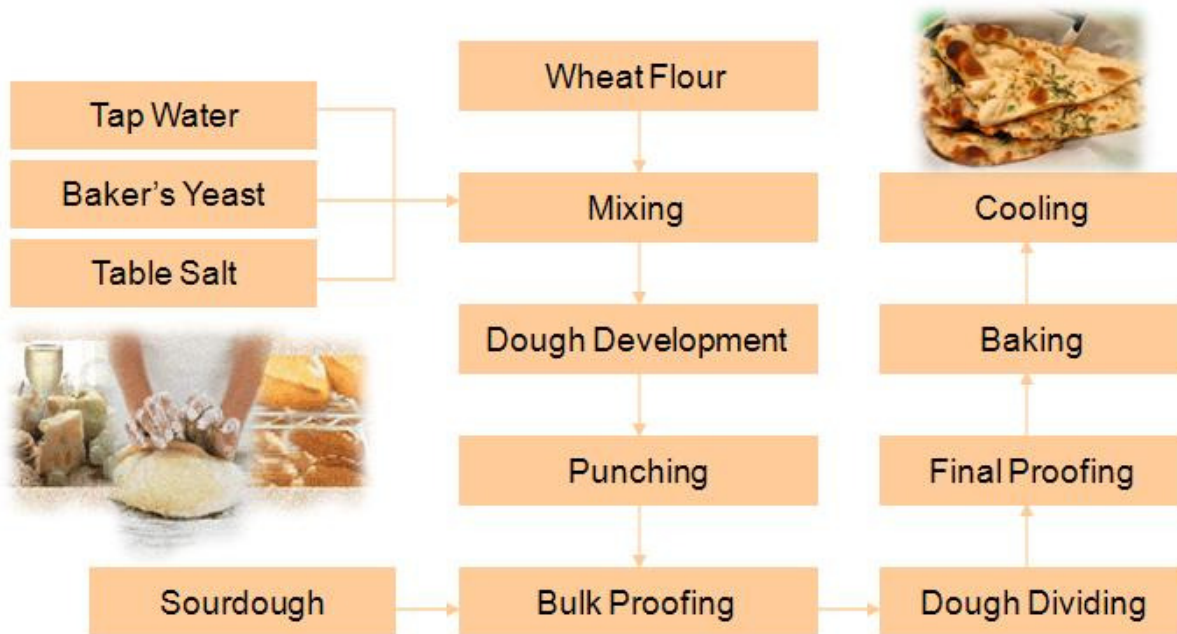


Figure 1. Scheme of the Lavash bread manufacturing process.

bread crumb (Katina, 2005). Impact of sourdough on bread shelf life has been well reported in several studies (Katina et al., 2002; Simsek et al., 2006; Sadeghi et al., 2008).

Bread aroma is one of the most important criteria of the quality of bakery product. Actually, it reflects the freshness of the product. Molecules exhibiting roasted notes largely contribute to this flavor and 2-acetyl 2-thiazoline (2-AT) was identified as one of the strongest (Fleury et al., 2002). The influence of sourdough fermentation on bread aroma was studied in several researches (Zehentbauer and Grosch, 1998; Fleury et al., 2002; Guerzoni et al., 2007). Recently, generation of aroma compounds during sourdough fermentation was also reviewed by Hansen and Schieberle (2005).

The aim of this case study was to investigate the sourdough potential in order to enhance microbiological shelf life and roasty aroma of traditional Lavash bread.

MATERIALS AND METHODS

The scheme of the Lavash bread manufacturing process is shown in Figure 1. Lavash is a traditional Iranian white wheat flatbread, can be used to make a sandwich or wrap. It is lightly baked bread that can be served with dips, as an appetizer or enjoyed as a healthy snack (ISIRI, 2002; Fazeli et al., 2004). In this study, control Lavash dough consisted of 59 g of wheat flour (the flour properties were based on ISIRI, 2002), 40 g of tap water, 0.5 g of baker's yeast and 0.5 g of table salt. The mixed ingredients were left at 30°C for a proof time of 1 h. Rural bread-making in Iran is reliant solely on sourdough, which is rich in lactobacilli as well as baker's yeast. For sourdough preparation, flour-water (4 g of wheat flour and 6 g of water) mix was fermented at temperatures of 28, 32 and 36°C. The mixture was fermented at three different times (8, 16 and

24 h) without agitation. Sample dough were prepared by adding 20% sourdough to the control Lavash dough following further incubation of the mixture for 10 min at 30°C. Loaves of 30 g each were baked on a rotary baking machine for 2 min and air dried for another 1 min. Baked breads were cut by sterile knives into small pieces (5×5×1 cm) before cooling and packaging into polyethylene bags (ISIRI, 2002; Fazeli et al., 2004; Sadeghi et al., 2008).

Microbiological shelf life of finished-products, were determined 1 h after baking by plating serial dilutions onto potato dextrose agar (Merck, Germany), supplemented with yeast extract, 24 h at 24°C for moulds detection and nutrient broth (Merck, Germany), 24 h at 30°C with shaking (shaker incubator at 30 rpm) for *Bacillus* strains detection, after intentional contamination (inoculums) of flour with these indicator microorganisms (10^5 CFU/g *B. subtilis*- PTCC 1023 and 10^4 CFU/g *Neurospora sitophila*- PTCC 5291 which prepared from Persian type culture collection). Before inoculums, mentioned strains were activated in specific media and incubation conditions, then biomass from actively growing microorganisms culture was collected with centrifugation (3000 g for *N. sitophila*, 5000 g for *B. subtilis*, 10 min and 4°C) based on McFarland method (specific CFU/ml) and resuspended in sterile tap water that was immediately mixed with flour until dough formation (Murray et al., 2007). Survival curves of these indicator microorganisms (*N. sitophila* and *B. subtilis*) versus different sourdough fermentation times and temperatures, as the main biopreservation step in Lavash processing (Fazeli et al., 2004; Sadeghi et al., 2008), at finished-products were also determined.

2-Acetyl 2-thiazoline as standard compound for roasty aroma was purchased from RT corp (manufacturer and distributor of certified reference materials- Wyoming, USA). For aroma extraction, the crust was separated from the crumb, frozen in liquid nitrogen and ground in a blender. The powder was transferred to a Soxhlet apparatus and soaked with dichloromethane and diethyl ether (Sigma-Aldrich), respectively. After storage of the suspension at 7°C for 15 h, the extraction was started and continued for 8 h at 50°C. The extract was concentrated, then distilled under high vacuum and the condensate obtained was separated in two fractions containing the neutral/basic and the acidic compounds.

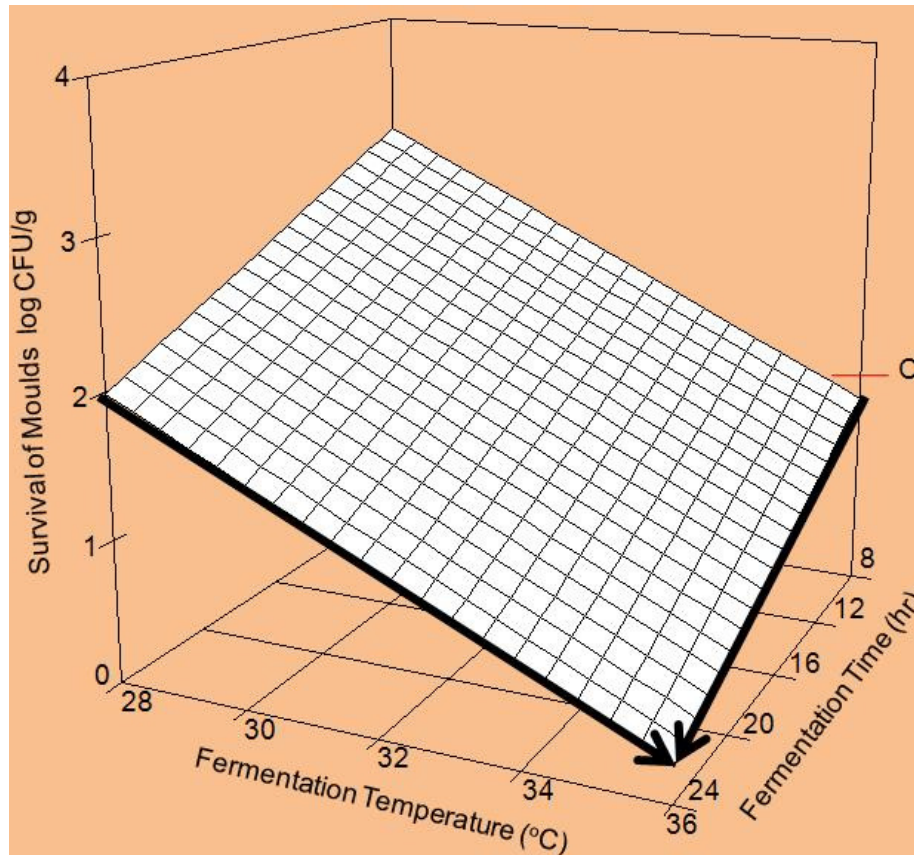


Figure 2. Survival curve of moulds, versus different sourdough fermentation times and temperatures, 1 h after baking in finished-products (C: control sample).

Finally, aroma detection was carried out by gas chromatography based on Zehentbauer and Grosch (1998) method.

For Statistical analysis, a completely randomized design with factorial arrangement by using statistical softwares including Microsoft Office Excel, CurveExpert 1.3 SlideWrite 2 and Sigma stat was employed.

RESULTS AND DISCUSSION

The survival curves of moulds and *Bacillus* spp., versus different sourdough fermentation times and temperatures, 1 h after baking, are shown in Figures 2 and 3, respectively. According to these figures, by increasing sourdough fermentation time and temperature, survival of moulds and *Bacillus* spp. in finished-products decreased. However, these changes were more regular for moulds than *Bacillus*. Sourdough significantly improved ($P \leq 0.05$) microbiological quality (reduction of moulds and *Bacillus*) of finished-products in comparison with control sample (without sourdough) and also the sourdough prepared at 24 h fermentation time and 36°C fermentation temperature had a significantly higher microbiological quality than the others.

The microbiological quality of Iranian breads in urban areas has been compromised by the expansion of semi-

automatic fast bakeries, which sometimes use sodium bicarbonate instead of sourdough. This has led to short-life bread and a huge waste of bread, equivalent to more than 1 million tons of wheat per year (Fazeli et al., 2004). Antifungal (Fazeli et al., 2004) and antibacterial (Sadeghi et al., 2008) activities of sourdough LAB on Iranian traditional breads have been studied previously. These results support the above idea about traditional sourdough bread making and the key role of lactobacilli sourdoughs as a potent antimicrobial agent.

In this study, our interest for aroma detection was focused on roasted notes. 2-Acetyl 1-pyrroline (2-AP) is the main key compound, but it is highly unstable and consequently very difficult to control. Then 2-acetyl 2-thiazoline (2-AT) was selected because it has a strong roasted and popcorn-like aroma and is more stable than 2-AP (Fleury et al., 2002; Hansen and Schieberle, 2005).

The results obtained from gas chromatography showed that the produced sourdough at 28°C fermentation temperature and 16 h fermentation time had the strongest effect on crust roasty aroma. Furthermore, the intensity of crust roasty aroma did not have an equal profile versus change of fermentation time and temperature (Figure 4).

The improved bread flavor required carefully optimized

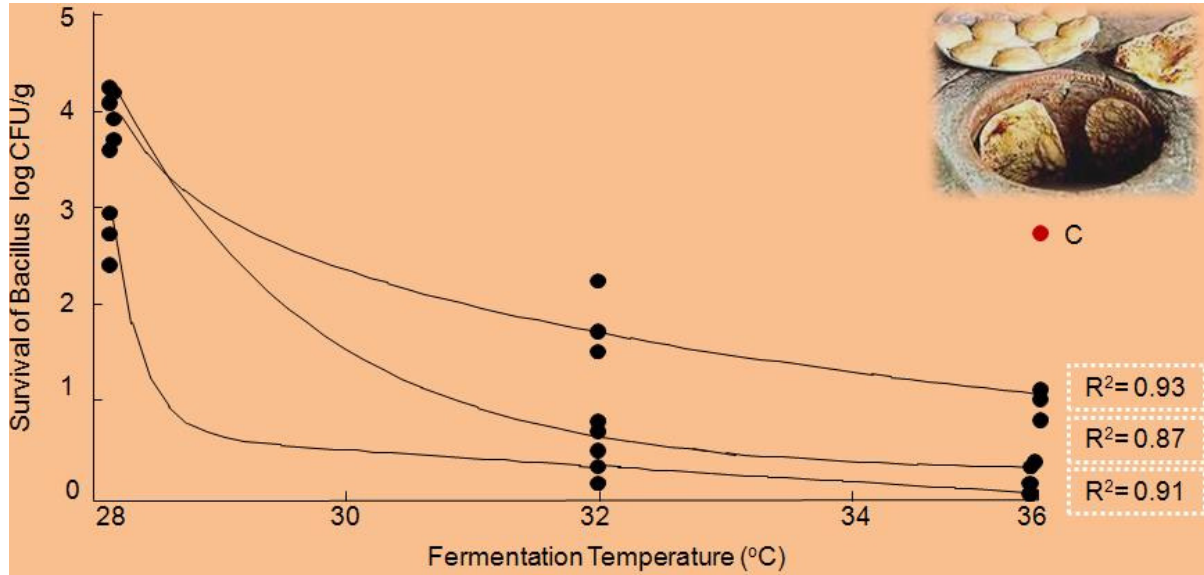


Figure 3. Survival curves of *Bacillus*, versus different sourdough fermentation temperatures, respectively in 8 ($R^2=0.93$), 16 ($R^2=0.87$) and 24 ($R^2=0.91$) h fermentation times, 1 h after baking in finished-products (C: control sample).

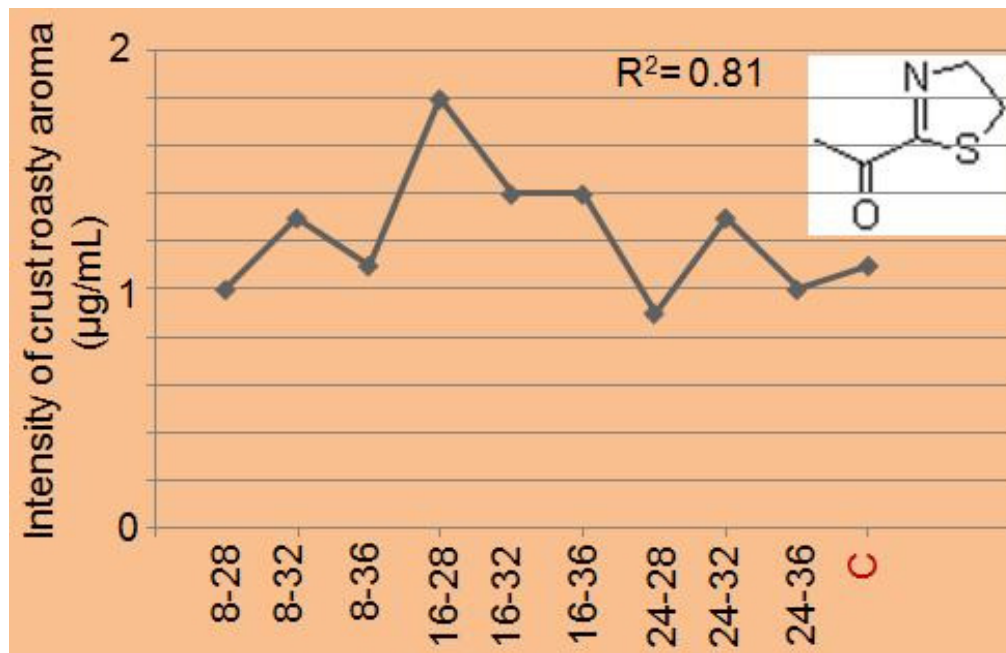


Figure 4. Intensity of crust roasty aroma versus different sourdough fermentation times (8, 16 and 24 h) and temperatures (28, 32 and 36°C), C is control sample.

fermentation time, temperature and ash content of flour in a strain-specific manner to achieve a balanced flavor profile between desired and undesired flavor attributes. The desired aroma development was related mainly to the moderate development of acidity and enhanced proteolysis during sourdough fermentation (Katina, 2005).

The influence of fermentation time on the sensory profile of subsequent bread is not well reported, but

longer fermentation times are generally assumed to create stronger flavors in comparison to no-time or short time processes in wheat baking (Guerzoni et al., 2007). Also, microbial metabolisms verify production of different volatile compounds for hetero- and homo-lactic LAB sourdough fermentations (Raccach et al., 2004).

The results obtained in this study suggest that the use of sourdough LAB as starter cultures for their anti-microbial

activities needs a controlled sourdough process. Most of the beneficial properties attributed to sourdough are determined by the acidification activity of lactic acid bacteria, however, a clear conclusion could not be made whether organic acids or other possible metabolites for example, bacteriocins, may be involved in the anti-microbial preservative effect of lactobacilli on bread. But process requirements for optimum microbiological shelf life and roasty aroma were different, which should be taken into account in designing sourdough baking processes. Therefore, it is noteworthy that the effective fermentation condition of enhanced microbial shelf-life does not meet the fermentation criteria for a good aroma of Lavash sourdough bread.

Conclusion

In this study, significant effect of sourdough process conditions (fermentation time and temperature) on Lavash bread microbiological quality was clarified. Based on these results by optimization of traditional sourdough process, control of indicator organisms in Lavash bread is possible. Furthermore, it is possible to control the formation of volatile compounds in sourdough fermentation, besides choosing the appropriate fermentation conditions such as time and temperature. Thus, it is noteworthy that the effective fermentation condition of enhanced microbial shelf-life does not meet the fermentation criteria for a good aroma of Lavash sourdough bread.

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