International Journal of Food Microbiology 239 (2016) 65–78

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Development of a method for the direct fermentation of semolina by selected sourdough lactic acid bacteria

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ARTICLE INFO

Article history:
Received 17 December 2015
Received in revised form 13 May 2016
Accepted 22 June 2016
Available online 25 June 2016

Keywords:
Fermentation
Lactic acid bacteria
Quality parameters
Starter culture
Sourdough
Triticum durum genotypes
Volatile organic compounds

ABSTRACT

Three obligately heterofermentative lactic acid bacteria (LAB) strains (*Lactobacillus sanfranciscensis* PON100336, *Leuconostoc citreum* PON10079 and *Weissella cibaria* PON10030) were used in this study as a multi-species starter culture for sourdough production. The starter inoculum was prepared and propagated in sterile semolina extract (SSE) broth. Acidification kinetics, microbiological counts detected on specific media for sourdough LAB, polymorphic profile comparison and species-specific PCRs evidenced a stability of the liquid inoculum over time determining its suitability for direct addition to semolina. In order to validate this innovative method for the production of durum wheat (*Triticum durum* Desf) sourdoughs, 15 semolinas (from ten old and five modern genotypes cultivated in Sicily, southern Italy) were used to prepare the SSEs and to produce sourdoughs and finally breads. Chemical and microbiological analyses of the sourdoughs and the evaluation of the quality parameters (weight loss, height, crumb and crust colour, image analysis and volatile organic compound generation) of the resulting breads indicated that the direct addition of the liquid inocula propagated in SSE is a valuable method to stabilise the production of sourdoughs. The differences registered during the technological characterisation of the breads were underlined by the sensory tests and the multivariate analysis and are mainly imputable to the type of semolina.

1. Introduction

Bread production can be assumed as a simple process. The ingredients, mainly flour, water, salt and a leavening agent are mixed together. However, the dough is left to ferment for a while when the leavening is carried out by biological agents, in order to develop the desired characteristics. Baker's yeast is the primary biological agent in dough formation, but typical breads are often produced with the sourdough technology (De Vuyst et al., 2009). Sourdough is an extremely complex ecosystem where several lactic acid bacteria (LAB) and yeasts cohabit (Corsetti and Settanni, 2007).

In general, the raw materials, the microbiota developing during the fermentation process and the technological parameters applied during bread making affect consistently the characteristics of the final products (Corsetti et al., 2000). In particular, the microbial composition of sourdough plays a major role during fermentation (De Vuyst and Neysens, 2005). However, a series of intrinsic and extrinsic factors may in turn influence the composition of the sourdough microbiota (De Vuyst et al., 2014).

The vast majority of bread is traditionally produced from wheat (Goesaert et al., 2005). Although bread is generally produced with the flour from common wheat (*Triticum aestivum* L), for this reason also called "bread wheat", the use of semolina from durum wheat (*Triticum durum* Desf) in bread production is quite common in southern Italy (Corsetti et al., 2001; Quaglia, 1988). Several typical breads produced in Sicily are made with semolina applying the sourdough technology (Ventimiglia et al., 2015).

The dominating LAB populations of a given type of sourdough are quite stable at species level (Meroth et al., 2003). Regarding the Italian sourdoughs, basically included in Type I sourdough produced with traditional techniques and characterised by continuous, daily refreshments and fermentation at ambient temperature (De Vuyst and Neysens, 2005), a few species are often found to dominate the lactic acid microbiota. The species most frequently found at the highest levels in Italian sourdoughs is undoubtedly *Lactobacillus sanfranciscensis* (Picozzi et al., 2010; Siragusa et al., 2009), but other obligately heterofermentative LAB, such as *Leuconostoc* and *Weissella* species, are often found to dominate this ecosystem (Coppola et al., 1996). However, during refreshments, the addition of flours stored in different conditions and obtained from wheat crops grown in different environmental conditions, cropping systems and genotypes might determine a variation of

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http://dx.doi.org/10.1016/j.ijfoodmicro.2016.06.027
0168-1605/

the microbial composition of sourdoughs over time (De Vuyst et al., 2014). Alfonzo et al. (2013) reported that flours and semolinas used to produce sourdough breads in a restricted area in Sicily (southern Italy) were characterised by different strains of the same species. Thus, a certain succession of the dominant strains during sourdough propagation for long times cannot be excluded.

The variability of the dominant strains is reflected in a limited reproducibility of the final characteristics of a given bread typology. When a starter culture is added to a sourdough ecosystem, it is important to ensure its stability over time, in order to warrant a certain reproducibility of the characteristics of the resulting bread. For this reason, a new method for the preparation of the starter culture for sourdough production has been developed in this study. The innovative method is based on the daily addition of a direct liquid inoculum to semolina. The methodology was validated with several semolinas from old and modern genotypes of durum wheat cultivated in Sicily.

2. Materials and methods

2.1. Starter strains

In this study, three obligately heterofermentative LAB strains (*Lb. sanfranciscensis* PON100336, *Leuconostoc citreum* PON10079 and *Weissella cibaria* PON10030) were used as a multi-species starter culture for sourdough production. The strains, belonging to the culture collection of the Department of Agricultural and Forest Sciences – University of Palermo (Italy), were previously isolated from wheat semolinas produced from durum wheats cultivated in Sicily (southern Italy) (Alfonzo et al., 2013) and selected for their potential during the production of experimental sourdough breads (Settanni et al., 2013). Recently, the performances of *Ln. citreum* and *W. cibaria* have been evaluated under industrial conditions without obligate heterofermentative *Lactobacillus* species, evidencing their specific abilities to carry out the sourdough fermentation (Corona et al., 2016). The strains *Ln. citreum* PON10079 and *W. cibaria* PON10030 were propagated overnight at 30 °C in modified-de Man-Rogosa-Sharpe (mMRS) broth, prepared from MRS (Oxoid, Milan, Italy) added with maltose and fresh yeast extract at the final concentration of 1% and 10%, respectively, and adjusted to pH 5.6 with 5 M lactic acid, while *Lb. sanfranciscensis* PON100336 was propagated overnight at 30 °C in Sour Dough Bacteria (SDB) broth prepared as described by Kline and Sugihara (1971).

2.2. Preparation and propagation of the liquid inoculum

Each broth culture for the preparation of the multi-species strain starter was grown overnight in the optimal conditions, centrifuged at 5000 × *g* for 5 min and washed twice in Ringer's solution (Sigma-Aldrich, Milan, Italy) before re-suspending to the value 1.00 of optical density at 600 nm using the 6400 Spectrophotometer (Jenway Ltd., Felsted, Dunmow, UK). The cell suspensions of each LAB strain included in this

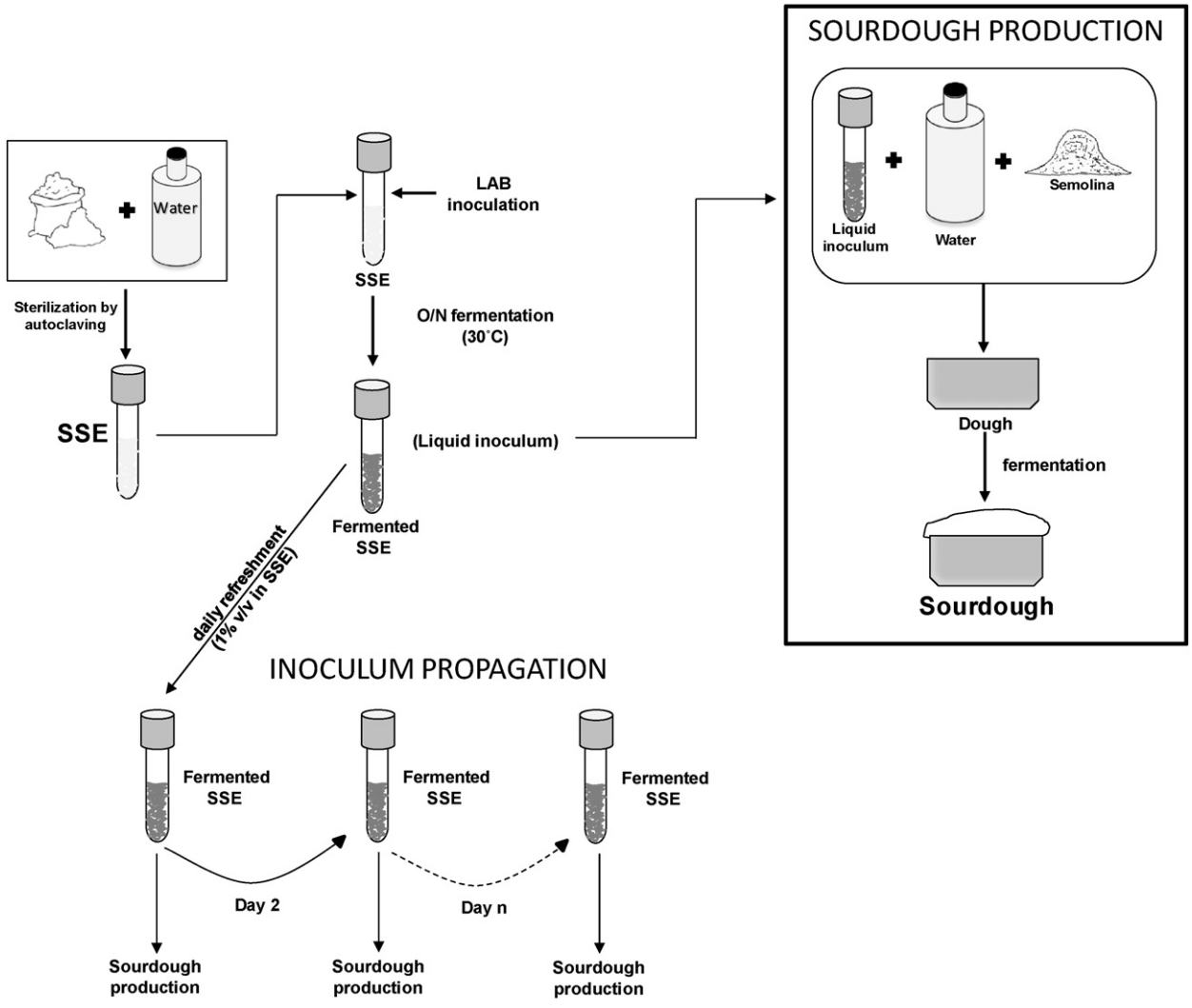

Fig. 1. Schematic representation of the innovative method for sourdough production.