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Effects of innovative and conventional sanitizing treatments on the reduction of *Saccharomycopsis fibuligera* defects on industrial durum wheat bread

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ABSTRACT

Wickerhamomyces anomalus, *Hyphopichia burtonii* and *Saccharomycopsis fibuligera* are spoilage yeasts causing chalk mold defects on sliced bread packaged under modified atmosphere. The first objective of this study, carried out in a bread-making company for two consecutive years, was to genetically identify yeasts isolated from spoiled sliced bread in Modified Atmosphere Packaging (MAP) and to determine the dominant species among identified strains. The second objective was to evaluate the effects of hydrogen peroxide and silver solution 12% (HPS) treatment in the leavening cells and cooling chambers, in comparison with the conventional Ortho-Phenylphenol (OPP) fumigating treatment, on the incidence of chalk defects of the commercialized products. One-hundred percent of the isolated yeasts were identified as *S. fibuligera*, while *H. burtonii* and *W. anomalus* were not detected. Concerning mean water activity (a_w) and moisture content values, packaged bread samples were, respectively, included in the range 0.922–0.940 and 33.40–35.39%. *S. fibuligera* was able to grow in a wide range of temperature (11.5 to 28.5 °C) and relative humidity (70.00 to 80.17%) in the processing environments, and product $a_w < 0.94$. Compared to OPP, the combined treatment with hydrogen peroxide and silver solution, in association with MAP, reduced to a negligible level yeast contamination of industrial sliced bread. The identification of the spoilage organisms and a comprehensive understanding of the combined effects of a_w, pO_2/pCO_2 inside the packages, environmental conditions and sanitizing treatment on the growth behaviour is essential for future development of adequate preventive process strategies against chalk mold defects.

1. Introduction

Consumer preferences push towards alternative techniques that maintain the fresh and natural character of baked goods without the use of traditional chemical preservatives. Along with the increased interest towards health benefits, this trend is also known as green consumerism (Corbo et al., 2009).

Bakery products are intermediate to high moisture products and by consequence highly susceptible to spoilage by fungi. To control this loss, manufacturers of bakery products use traditional chemical preservatives (i.e. sorbic, benzoic and propionic acid and their corresponding salts at ambient storage temperatures) or Modified Atmosphere Packaging (MAP). MAP is now commonly used as an alternative preservation technique for a specific type of bakery product, namely baked goods packed in protective atmosphere. The utilization of MAP meets consumer demands since only a gas mixture of nitrogen (N_2) and carbon dioxide (CO_2), generally applied at levels of 40 and 60% respectively (Galic et al., 2009), is used to prevent spoilage caused by aerobic microorganisms. CO_2 is the most important gas in the mixture since it is both fungistatic and bacteriostatic (Galic et al., 2009). In addition, the low levels of O_2 (<1%) ensure the inhibition of molds and other aerobic spoilage organisms.

However, if under these strict conditions of MAP, baked goods are well protected against aerobic spoilage organisms, the growth of anaerobic microorganisms causing early spoilage of bread may occur.

Among spoilage organisms, yeasts *Wickerhamomyces anomalus* (formerly *Pichia anomala*), *Hyphopichia burtonii* and *Saccharomycopsis fibuligera*, usually resulting from post-baking contamination, cause visually spoiled breads by growing in low, white, spreading colonies that sometimes look like sprinkling of chalk dust on the product surface (Legan and Voysey, 1991). Two of these yeast species, *H. burtonii* and *S. fibuligera*, even show structures resembling molds as they have the

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tendency to grow as hyphae and form mycelium. These hypha-like structures are called pseudo hypha and are actually chains of budded yeast cells that did not separate after duplication. Because of this close resemblance to molds and the white, powdery colonies they produce, *H. burtonii* and *S. fibuligera*, are also referred to as chalk molds (Legan and Voysey, 1991). With reference to the latter species, Suhr and Nielsen (2005) demonstrated that it is the least affected by the different O_2 residual levels, as it is not inhibited by any MAP treatments.

Research on spoilage of bakery products has to date mainly focused on molds. Less attention has been paid to yeasts, probably because their public health significance is negligible, since human consumption of viable yeast cells present in fermented foods and beverages had no adverse effects. However, the early bread spoilage caused by yeasts represents an actual problem for the bakery industry in terms of production losses. The majority of these microorganisms is carried by dust particles and water droplets suspended in the air, which constitute the so-called "bio aerosol." The formation of bio aerosol is influenced by different factors, like the use of high-pressure water for surface cleaning (Braymen, 1969; Spurlock and Zottola, 1991), air currents, relative humidity, and temperature (Stetzenbach, 2007).

To contribute to the reduction of the bakery industry losses due to yeast spoilage, without affecting the additive-free character of MAP packaged bread, the main objectives of this study, carried out in an industrial bread-making company for two consecutive years, were to identify the spoilage yeasts of sliced bread and to evaluate the influence of environmental conditions, moisture content and water activity of bread, MAP packaging and two different sanitizing treatments applied to leavening and cooling chambers on the spoilage incidence.

2. Materials and methods

2.1. Study design

The study was performed in a bread-making company located in Sicily (37°33′52.15″N 14°27′44.74″E), from August 2013 to December 2014.

2.2. Plant environmental conditions and sanitizing treatments

The following processing areas of a bakery plant, as two of the main critical points in which microbiological contamination may occur, were considered: leavening cells and cooling cells (Fig. 1). Antimicrobial treatment, used from August to December 2013 (season 2013), consisted in 20% OrthoPhenylPhenol (Fumispore OPP, International PBI, Milano, Italy) conventionally used as fungicide of facilities and equipment used for the storage and production of foodstuffs intended for human consumption. A new generation commercial sanitizing product, based on 120 mL/L hydrogen peroxide stabilized by 30 mg/kg colloidal silver complex (Nocolyse®, OXYPHARM, Champigny-Sur-Mame, France), manufactured according to ISO 9001 and EN 13485, was used from August to December 2014 (season 2014).

Environmental temperature and relative humidity were measured by using agro-meteorological bulletin Decade of Agira (EN), agro-meteorological information service of Sicily Region. Weather parameters monitored included maximum and minimum daily air temperature, daily total precipitation, and daily relative humidity.

2.3. Bread sample production and packaging

A base dough formulation, consisting of durum wheat semolina, water (66% semolina basis), compressed yeast (0.47% semolina basis), salt (2.2% semolina basis), anti-staling enzyme (0.05% semolina basis) and mono and diglycerides of fatty acids (E 471, 0.22% semolina basis), was used to produce the bread. Dough was mixed for 17 min in high-speed mixer (Pietro Berto, Marano Vicentino, VI, Italy). Final dough temperature was 26 ± 1 °C. The dough was rested in bulk for 15 min, scaled into 1160 ± 20 g portions, placed into the proofer set and leavened at 32 ± 1 °C and 66 ± 2% relative humidity (RH) for 150 min. The baking was carried out at 240 °C for 60 min, in industrial tunnel oven measuring 33 × 3 m (Pavailler Engineering, Galliate, NO, Italy). The baked loaves, weighting approximately 1.040 kg each, were automatically transported to the cooling chambers (S.L.C, Copit, Trecate (NO), Italy; Tecnopool, S. Giorgio in Bosco, PD, Italy) set at 20 ± 1 °C for

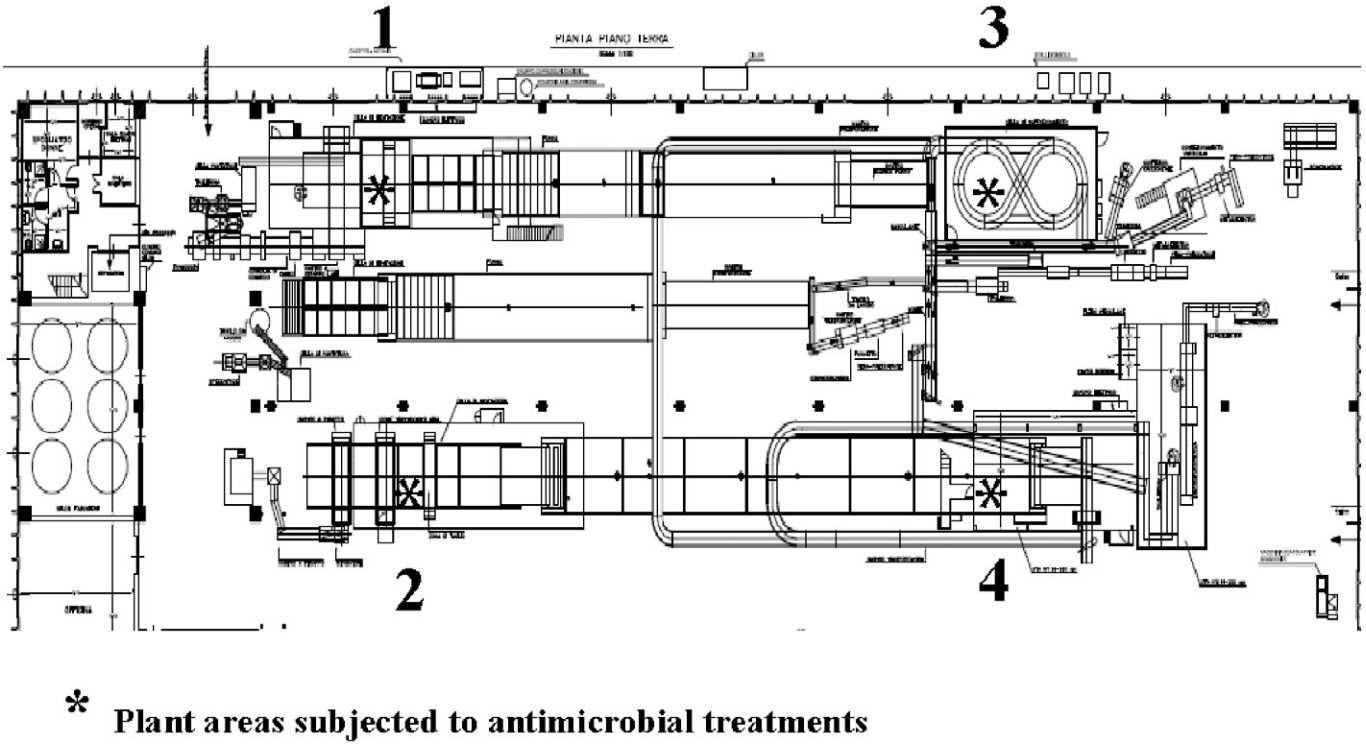

Fig. 1. Production site map and sanitizing treatment points. 1, leavening cell (30 ± 2 °C, 60 ± 0.5% RH); 2, leavening cell (32 ± 2 °C, 64 ± 0.5% RH); 3, cooling cell 320 m^3 (20 ± 1 °C); 4, cooling cell 375 m^3 (20 ± 1 °C).