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Green olives fermentation profile by using *Lactobacillus pentosus* DSM 16366 as a starter culture

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Abstract

Lactobacillus pentosus DSM 16366 was used as a starter culture for Azeiteira cultivar green olives Spanish-style preparation. Inoculum was added to the ecosystem suspended in nutritive media or as a lyophilized starter culture. Physico-chemical and microbiological fermentation profiles indicated that Lactobacillus pentosus DSM 16366 in nutritive media accelerated brine acidification, and reduced the survival period of Gram-negative bacteria, minimizing the danger of early stage spoilage.

Keywords: table olives, fermentation, *Lactobacillus pentosus*, physico-chemical, microbiological.

Introduction

Modern large-scale production of fermented foods is focused on the use of defined strain starter systems to ensure consistency and quality in the final product. In Spanish-style green olives process, initial conditions are usually somewhat restrictive for the growth of desirable microorganisms. The use of lactic acid bacteria starter cultures have the potential to improve the microbiological control of the process, increase the lactic acid yield and, accordingly, provide table olives of consistently high quality (de Castro *et al.* 2002; Leal-Sánchez *et al.* 2003; Sánchez *et al.* 2001).

In this regard, the bacteriocin producer *Lactobacillus pentosus* DSM 16366, a strain originally isolated from olive fermentation (Delgado et al. 2005), was used as a starter culture for Azeiteira cultivar Spanish-style green olives preparation. Physico-chemical and microbiological profiles of table olive fermentation using this lactobacillus strain as a lyophilized starter culture or suspended cells in nutritive media were studied.

Material and Methods

Experiments were carried out with Azeiteira cultivar. Olives exhibited a yellow-green to straw colour, but when squeezed between fingers they hardly released a creamy white juice – "early harvesting date".

Olives were treated with a NaOH solution (1.5% w v^{-1}) to reach 2/3 of the flesh, washed in tap water for 16 h, and then brined (8% NaCl, w v^{-1}), following the Spanish-style green olives preparation. For fermentation, plastic vessels of 30 l capacity were used. It was essential that all olives had been treated in exactly the same way, since alkaline treatment and washing are crucial for subsequent fermentation.

Two fermenters were subjected to spontaneous fermentation by the environmental microbiota (S); two fermenters were inoculated with lyophilized starter culture of *Lactobacillus pentosus* DSM 16366 (L); and other two were inoculated with starter cells suspended in MRS broth (Oxoid, Hampshire, England) after an overnight growing (M). Inoculation took place at day 5 of fermentation, when brine pH was c.a. 6 (Delgado *et al.* 2005), giving a population of approximately 10^5 CFU ml^{-1} in each vessel.

Brine samples were taken from the centre of each fermenter after 1, 5, 12, 19, 26, 33, 40, 47 and 54 days of fermentation to monitorize microbial growth, free acidity, and pH. For microbiological analyzes, brine samples and appropriate dilutions were plated on selective media. Gram-negative bacteria colonies were counted on crystal-violet neutral red bile glucose agar (Merck, Darmstad, Germany), lactic acid bacteria were enumerated on MRS agar (Oxoid, Hampshire, England) supplemented with sodium azide (Sigma-Aldrich, Madrid, Spain) and yeasts plus fungi were counted on DG18 agar base (Oxoid, Hampshire, England) with chloramphenicol selective supplement (Oxoid, Hampshire, England). For counting Gram-negative bacteria, lactic acid bacteria, and yeasts plus fungi, plates were incubated at 30 °C for 24 h, 30 °C for 48 h, and 28 °C for 72 h, respectively.

Results

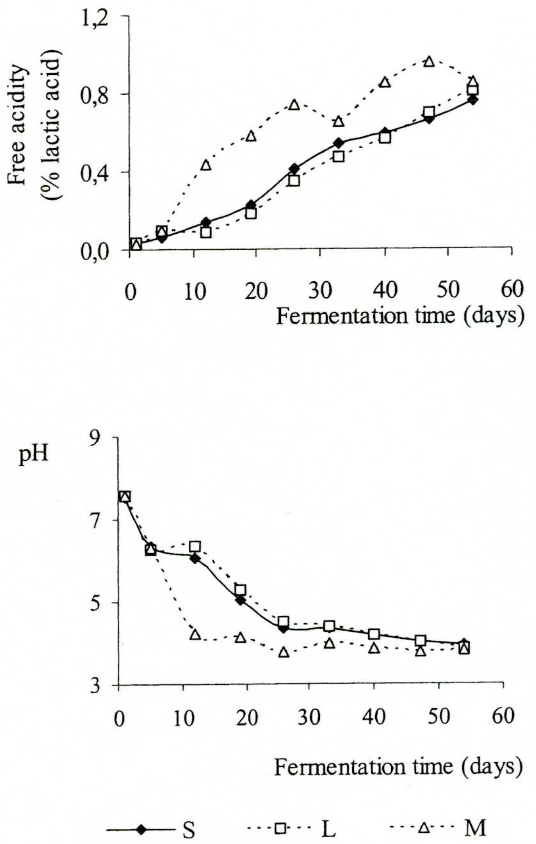

Figure 1. Free acidity and pH evolution in spontaneous (S) and induced fermentations by using *Lb. pentosus* DSM 16366 as a lyophilized (L) starter or suspended cells in nutritive media (M). Points are means of duplicate fermentations.

Production of acidity and decrease in pH occurred faster when starter cells were added to the fermenters suspended in nutritive media than in those inoculated with lyophilized starter or uninoculated. There were no substantial differences between spontaneous and lyophilized *Lb. pentosus* DSM 16366 starter culture application in brines free acidity and pH evolution (Figure 1).

Changes in free acidity were scarce during the first five days of fermentation and then raised rapidly. Starter cells suspended in nutritive media application resulted in highest free acidity levels. In parallel, M exhibited a pronounced decline of pH from 7.6 to 4.2 units during the first 12 days of fermentation, whereas the other trials showed similar pH levels after 40 days of fermentation. After 54 days of fermentation, all treatments showed similar free acidity and pH levels (approximately 0.8% and 3.8, respectively) (Figure 1).

Fermentation microbial profile for spontaneous and controlled fermentation is presented in Figure 2. Gram-negative bacteria showed an increase within the first 5 days in inoculated fermentors and within 12 days in spontaneous fermentation, which was followed by a strongly marked decrease; they "disappeared" by 26 days of fermentation in M and 33 days in S and L. The population of lactic acid bacteria in controlled fermentation exceeded that in the spontaneous fermentation during the first 26 days, especially when starter cells suspended in nutritive media were used. However, in the second month of fermentation, whether inoculated or not, all fermenters showed slight fluctuations in lactic acid bacteria (10^9 CFU ml^{-1}) and yeasts (10^4 CFU ml^{-1}) populations.

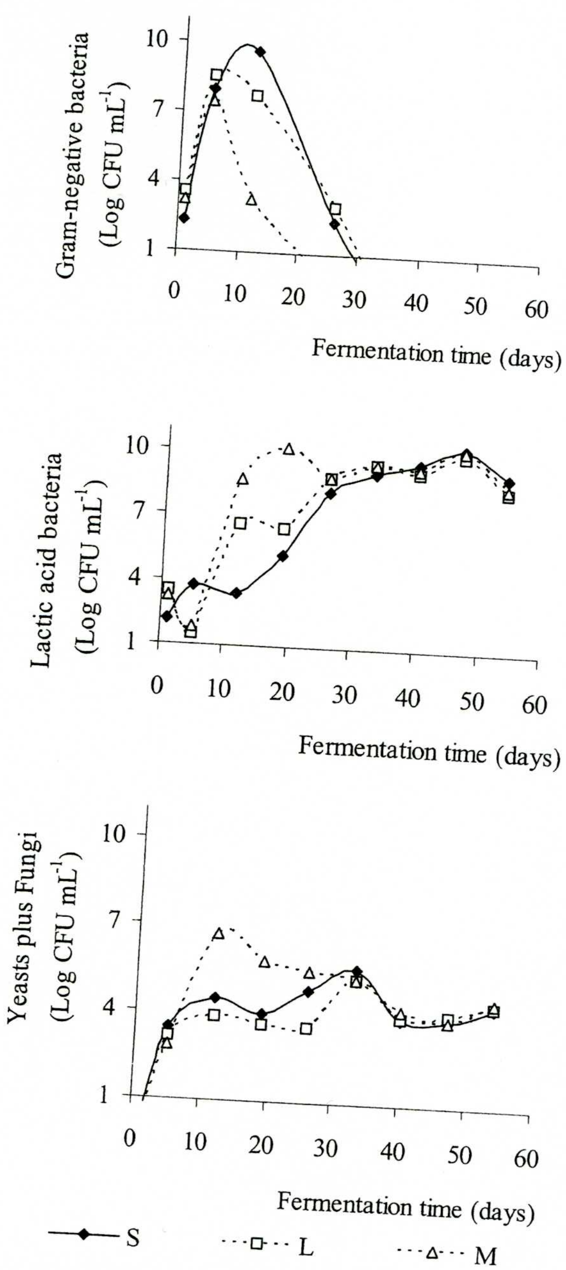

Figure 2. Microbiological profile of spontaneous (S) and induced fermentations by using *Lb. pentosus* DSM 16366 as a lyophilized (L) starter or suspended cells in nutritive media (M). Points are means of duplicate fermentations.

Discussion

The pH is a crucial indicator of fermentation progress and its drop occurs due to the metabolism of sugars by lactic acid bacteria, mainly to lactic acid. The evolution of free acidity and pH suggests a higher rate of fermentation when inoculation with *Lb. pentosus* DSM 16366 cells suspended in nutritive media took place. This delay on the onset of fermentation, when the risk of spoilage due to *Enterobacteriaceae* or butyric acid clostridia is highest, implies that the likelihood of spoilage is higher in spontaneous fermentation (de Castro *et al.* 2002; Leal-Sánchez *et al.* 2003; Sánchez *et al.* 2001). Gram-negative microflora dynamic confirms this assessment exhibiting higher population and lasting longer in spontaneous fermentation than in induced fermentation. In fact, the rapid decrease of pH brine is the determining factor for the success of fermentation and safety of the final product. Apparently, the microbiological control caused by the inoculation was completely effective during the first month of fermentation. Then spontaneous and inoculated fermentations exhibited similar microbiological profile.

Nutritive media MRS as starter carrier improved fermentation progress probably related with the presence of growth factors and/or protective effect on *Lactobacillus pentosus* DSM 16366, as previ ously commented for *Lactobacillus plantarum* starters (Durán Quintana *et al.* 1994; Ruiz-Barba and Jiménez-Díaz 1994).

In conclusion, 'Azeiteira' green olive Spanish-style preparation by using *Lactobacillus pentosus* DSM 16366 cells suspended in nutritive media as a starter culture conducted to a faster fermentation, decreasing the period during which growth of spoilage microorganisms could occur.

Acknowledgments

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