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Microbiological and biochemical profile of cv. Conservolea naturally black olives during controlled fermentation with selected strains of lactic acid bacteria

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Abstract

The effect of controlled fermentation processes on the microbial association and biochemical profile of cv. Conservolea naturally black olives processed by the traditional anaerobic method was studied. The different treatments included (a) inoculation with a commercial starter culture of *Lactobacillus pentosus*, (b) inoculation with a strain of *Lactobacillus plantarum* isolated from a fermented cassava product and (c) uninoculated spontaneous process. Microbial growth, pH, titratable acidity, organic acids and volatile compounds were monitored throughout the fermentation. The initial microbiota consisted of Gram-negative bacteria, lactic acid bacteria and yeasts. Inhibition of Gram-negative bacteria was evident in all processes. Both starter cultures were effective in establishing an accelerated fermentation process and reduced the survival period of Gram-negative bacteria by 5 days compared with the spontaneous process, minimizing thus the likelihood of spoilage. Higher acidification of the brines was observed in inoculated processes without any significant difference between the two selected starter cultures (113.5 and 117.6 mM for *L. plantarum* and *L. pentosus*, respectively). *L. pentosus* was also determined as the major species present during the whole process of spontaneous olive fermentation. It is characteristic that lactic acid fermentation was also initiated rapidly in the spontaneous process, as the conditions of fermentation, mainly the low salt level (6%, w/v) favored the dominance of lactic acid bacteria over yeasts. Lactic, acetic and propionic were the organic acids detected by HPLC in considerable amounts, whereas citric and malic acids were also present at low levels and degraded completely during the processes. Ethanol, methanol, acetaldehyde, ethyl acetate were the major volatile compounds identified by GC. Their concentrations varied among the different treatments, reflecting varying degrees of microbial activity in the brines. The results obtained from this study could help the Greek table olive industry to improve the existing processing schemes in order to increase product consistency and quality expanding the international market for naturally black olives.

Keywords: Conservolea; Fermentation; *Lactobacillus pentosus*; *Lactobacillus plantarum*; Naturally black olives; Organic acids; Volatile compounds

1. Introduction

Table olives are probably the most popular fermented vegetable in the Western world and a main part of the Mediterranean diet together with olive oil. Production for the 2004–05 season amounted to 1,465,500 ton, the majority of which (ca. 43.9%) was located in the European Union (EU). Spain is the leading producer with 467,600 ton (71.8%), followed by Greece 104,300 ton (16.0%), Italy 66,500 (10.2%), Portugal 11,100 ton (1.7%) and France 2000 ton (0.3%). Other significant non-EU producing countries include Turkey (10.5% of the world production), Egypt (10.6%), Morocco (5.6%), Syria (8.2%) and the United States (6.6%) (ICAP, 2005). Table olives are prepared from specifically cultivated fruit varieties harvested at the pre-determined stage of maturation. The most important industrial preparations are the Spanish

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preparation for green olives, the Californian preparation for black oxidized olives and the Greek preparation for naturally black olives (Garrido Fernández et al., 1997). The wide variety of table olives and the development of new commercial forms of presentation (whole, pitted, sliced, stuffed) has made product diversification possible and has contributed to the expansion of the table olive sector in the past years.

Naturally black olives in brine are obtained by directly brining olives without any prior debittering treatment. The final product is characterized by a fruity flavor and retains a slightly bitter taste. This kind of preparation is popular in Turkey, Greece and other northern African countries and although the production has been decreasing considerably since 1960, it still accounts for 30% of the world trade olive market (Piga et al., 2001). The cultivation of olive trees for table olive production is an economically important activity in Greece involving more than 50,000 farmers in the primary sector and more than 50 processing units throughout the country. On average, almost 90,000–100,000 ton of raw olives are subjected to processing every year to produce primarily naturally black olives in brine. Processing involves several steps, namely harvesting when the drups are fully ripe or slightly before full ripeness (3/4 of the mesocarp has attained black colour), transportation to the factory, sorting to exclude defective drups, washing to remove superficial dirt and finally brining in 8–10% salt concentration where olives undergo spontaneous fermentation by a mixed microbiota of Gram-negative bacteria, lactic acid bacteria and yeasts (Balatsouras, 1990; Brenes, 2004). The main driving forces of the process are the availability of fermentable substrates, salt content, pH, aerobic/anaerobic conditions and temperature control (Bobillo and Marshall 1991, 1992; García García et al., 1992; Fernández Gonzalez et al., 1993; Montaño et al., 1993; Durán Quintana et al., 1999; Spyropoulou et al., 2001; Castro et al., 2002; Tassou et al., 2002; Álvarez et al., 2003; Chorianopoulos et al., 2005). To improve fermentation and produce consistent and high quality final products, process control of these parameters is necessary. The primary purpose of table olive fermentation is to achieve a preservation effect and enhance the organoleptic attributes of the processed product. A simple criterion to determine the effectiveness of fermentation is the concentration of lactic acid produced. Lactic acid bacteria influence fermentation in a variety of ways, the most important being the production of lactic acid from fermentable substrates resulting in pH decrease (<4.0) with a concurrent increase in acidity, thus ensuring the microbiological stability of the product during storage.

In a normal fermentation, the prevailing microbial groups are lactic acid bacteria and yeasts, the relative population of which defines the characteristics of the final product. Thus, when lactic acid bacteria outgrow yeasts, lactic acid fermentation is favored rendering a more acidic product with a lower pH, which is greatly desirable in naturally black olive fermentation. The opposite happens when yeasts become the dominant group, resulting in a product with milder taste and less self-preservation characteristics. Brine inoculation with an appropriate starter culture of lactic acid bacteria helps to achieve an enhanced and more predictable fermentation process. In most cases, inoculation is carried out with wild strains of lactic acid bacteria isolated from previous fermentations, a process known as back-slopping. However, such lactic cultures exhibit a diversity of metabolic activities, which vary even among strains, including differences in growth rate, adaptation to a particular substrate, antimicrobial properties, flavor and quality attributes and competitive growth behavior in mixed cultures (Holzapfel, 1997), resulting in non-consistent and low quality final products. To avoid these drawbacks, modern table olive industry is directed towards the use of pure starter cultures to achieve an improved and more predictable fermentation process. The selection of starters is based on diverse criteria including homo- and hetero-fermentation, acid production, salt tolerance, flavor development, temperature range, oleuropein-splitting capability and bacteriocin production (Nout and Rombouts, 1992; Ciafardini et al., 1994; Ruiz-Barba et al., 1994; Durán Quintana et al., 1999; Delgado et al., 2005). The preparation of improved commercial starter cultures specifically for table olives has been reported previously (Roig and Hernández, 1991) with main focus on Spanish-style processing, whereas there is little information on the application of starter cultures on naturally black olive processing, which are prepared almost exclusively by natural spontaneous fermentation. One risk of such a practise is the increased likelihood of microbial spoilage, including the clearly differentiated and defined 'gas pocket' and 'zapatera' spoilage (Garrido Fernández et al., 1997). Today, the use of starter cultures is not very common in European vegetable fermentations, although commercial preparations are already available on the market. This is also the case with table olive processing in Greece, which remains mostly artisanal and empirical despite its economic importance. However, interest in the application of starter cultures in table olive fermentation is increasing steadily since industrial experience suggests that an appropriate inoculation helps to achieve a more controllable process.

The aim of the present work was (i) to study the growth profile of two starter cultures in the fermentation of naturally black olives of cv. Conservolea (*Olea europea media rotunda*) and (ii) to monitor the effects of inoculation on the biochemical profile in comparison with a spontaneous fermentation process.

2. Materials and methods

2.1. Olive samples

Natural black olives cv. Conservolea from the 2005/2006 crop were kindly provided by Konstantopoulos S.A. table