Fatty acid, sterol and triterpenic dialcohol compositions of 'Azeiteira' green table olives: the influence of starter utilization

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Abstract

Research has been carried out to ascertain the influence of *Lactobacillus pentosus* DSM 16366 as freeze-dried cells and culture in nutritive media on fatty acid, sterol and triterpenic dialcohol compositions of 'Azeiteira' Spanish style green table olives. Results showed that there were no relevant differences on spontaneously or induced fermented fruit when comparing those compositions.

INTRODUCTION

Table olive processing, like other natural vegetable fermentations, is spontaneous lactic acid fermentation. Recently, new starter cultures of lactic acid bacteria that can contribute to technological, microbial safety or offer sensorial or nutritional advantages are being developed (Leroy and De Vuyst, 2004). Nutritionally, the benefits of table olives are associated with major and minor constituents. Olive oil fatty acids and sterols play an important role in human nutrition as preventive agents against degenerative diseases (Awad et al., 2000; Escrich et al., 2006).

The purpose of the present study was to assess the influence of *Lactobacillus pentosus* DSM 16366, a strain originally isolated from green olives fermenting brines (Delgado et al., 2005), as freeze-dried cells and culture in nutritive media fatty acid, sterol and triterpenic dialcohol compositions of 'Azeiteira' Spanish style green table olives.

MATERIAL AND METHODS

Spanish style green olives preparation

Green olives of the Portuguese cultivar Azeiteira (*Olea europaea*) were harvested from an olive-grove in the Alentejo Northeast area in 29th September and 17th October of 2005. The steps of Spanish-style processing were as follows. Olives were submitted to an alkali treatment (15 g L^{-1}) until penetration of NaOH reached approximately 2/3 of the flesh thickness and then washed with tap water twice. Fruit were placed in brine (80 g L^{-1} NaCl) in 30 L plastic containers – fermentors. The fermentors were placed in a room maintained at 30 ºC.

Lactobacillus pentosus DSM 16366 was used as a starter suspending freeze-dried cells in brine from the corresponding fermentor and left for 4 h to rehydrate or with an overnight culture in Man Rogosa and Sharpe broth (Oxoid, Hampshire, England). Starter was added to brines giving a population in the vessels approximately 10^5-10^6 CFU mL^{-1}. Inoculation took place at day 5 of fermentation, when brine pH was c.a. 6 (Delgado et al., 2005). Spontaneous fermentation by the environmental microbiota represented the control treatment. Trials were prepared in duplicate for each harvesting date.

Olive oil extraction

After 50 days fermentation was completed. Samples of fruit (approximately 2 kg) were withdrawn from the centre of the each ferment. Oil extraction was performed using an Abencor laboratory oil mill system (Abengoa, Spain) equipped with a hammer mill, thermobeater, and centrifuge.

Analytical methods

The fatty acid methyl esters and sterolic fraction were prepared as described by Regulation (EC) No 1989/2003 (EUC, 2003). Both chromatographic separations were carried out using Hewlett-Packard (HP 5890 Series II) equipped with flame ionization detector. With respect to fatty acid methyl esters composition, a Supelco SP2380 column (60 m length x 0.2 mm internal diameter x 0.2 μm thickness) was used. The temperatures of the injector and detector were set at 240 ºC and 250 ºC; iniatially, oven temperature of 175 ºC was maintained for 25 minutes, and then increased to 220 ºC (at a rate of 5 ºC/min), which was kept for 10 minutes. Sterolic fraction was determined with a Permabond SE-52-DF (25 m length x 0.32 mm internal diameter x 0.25 μm thickness) and the temperatures of the injector and detector were set at 280 ºC and 290 ºC, respectively, with an oven temperature of 265 ºC.

Statistical analysis

Analysis of variance, followed by Scheffé's multiple range test with a significance level of $p<0.05$ were performed using the SPSS 11.5 statistical software.

RESULTS AND DISCUSSION

Results suggest that *Lactobacillus pentosus* DSM 16366 starter utilization, neither as freeze-dried cells nor culture in nutritive media, did not affect fatty acid, sterol and triterpenic dialcohol compositions, except for Δ-7-avenastenol, which was higher in spontaneously fermented fruit (Tables 1 and 2). The effects of processing on plant sterol had been extensively studied for oils but, to our knowledge, there are no data available on the variation of these compounds during olive fermentation. So, further studies are needed in order to explain the higher concentration of Δ-7-stigmastenol in spontaneous fermented fruit.

With regard to fatty acid composition, monounsaturated and polyunsaturated fatty acids represented 78.89 % and 4.78 %, respectively (Table 1). Total sterols content of 'Azeiteira' Spannish style table olives was 1054 mg kg^{-1}. Sterolic fraction is mainly composed by ß-sitosterol (94.00 %), (Δ-5-avenasterol (6.33 %), erytrodiol plus uvaol (3.39 %), campesterol (3.03 %) and stigmasterol (1.08 %) (Table 2).

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Tables

Table 1. Influence of *Lactobacillus pentosus* DSM 16366 as freeze-dried cells and culture in nutritive media on fatty acid composition of 'Azeiteira' table olives

Starter utilization	Fatty acids composition (%)							
	Saturated			Monounsaturated			Polyunsaturated	
	Palmitic	Stearic	Arachidic	Palmitoleic	Oleic	Eicosenoic	Linoleic	Linolenic
Fresh fruit	*14.05±0.65*	*1.71±0.07*	*0.34±0.03*	*1.23±0.02*	*77.17±0.08*	*0.37±0.02*	*4.05±0.53*	*0.78±0.03*
Table olives								
Spontaneous	13.75±0.37a	1.75±0.04a	0.37±0.02a	1.19±0.01a	77.43±0.56a	0.38±0.01a	4.02±0.44a	0.79±0.05a
Freeze-dried starter	14.16±0.30a	1.75±0.03a	0.35±0.03a	1.19±0.02a	77.06±0.73a	0.37±0.02a	4.02±0.47a	0.79±0.06a
Culture starter	13.77±0.29a	1.75±0.04a	0.38±0.01a	1.18±0.01a	77.48±0.38a	0.39±0.01a	3.95±0.47a	0.78±0.05a

Means and standard deviations. Means within a column followed by the same letters are not significantly different (p=0.05), according to Scheffé's multiple range test.

Table 2. Influence of *Lactobacillus pentosus* DSM 16366 as freeze-dried cells and culture in nutritive media on total sterols content and sterolic fraction of 'Azeiteira' table olives

Starter utilization	Total sterols ($mg\ kg^{-1}$)	Sterolic fraction (%)					
		Campesterol	Stigmasterol	ß-sitosterol	Δ-5-avenasterol	Δ-7-stigmastenol	Erythrodiol + uvaol
Fresh fruit	*1152±94*	*2.84±0.12*	*1.04±0.13*	*94.22±0.87*	*6.64±1.85*	*0.22±0.09*	*3.47±0.52*
Table olives							
Spontaneous	1087±51a	3.03±0.08a	1.07±0.08a	93.87±0.71a	6.40±1.62a	0.31±0.06b	3.31±0.67a
Freeze-dried starter	1043±23a	2.99±0.09a	1.07±0.06a	94.15±0.40a	6.45±1.80a	0.23±0.03a	3.30±0.98a
Culture starter	1042±34a	3.07±0.09a	1.10±0.12a	93.99±0.56a	6.44±1.65a	0.21±0.07a	3.55±0.63a

Means and standard deviations. Means within a column followed by the same letters are not significantly different (p=0.05), according to Scheffé's multiple range test.