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A study on the implications of NaCl reduction in the fermentation profile of Conservolea natural black olives

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ABSTRACT

This study examined the impact of different mixtures of NaCl, KCl, and $CaCl_2$ on the fermentation profiles of Conservolea natural black olives. Five different combinations of chloride salts were investigated, namely (i) 8% NaCl (control treatment), (ii) 4% NaCl and 4% KCl, (iii) 4% NaCl and 4% $CaCl_2$, (iv) 4% KCl and 4% $CaCl_2$, and (v) 2.6% NaCl–2.6% KCl–2.6% $CaCl_2$. The changes in the microbial association (lactic acid bacteria, yeasts, *Enterobacteriaceae*), pH, titratable acidity, organic acids, volatile compounds, and mineral content in olive flesh were analyzed. Results demonstrated that all salt combinations led to vigorous lactic acid processes based on the obtained values of pH (3.9–4.2) and titratable acidity (0.70–0.86 g lactic acid per 100 ml brine). Organoleptic evaluation was a critical factor in the acceptability of the final product. Increasing concentrations of $CaCl_2$ or a combination of KCl and $CaCl_2$ rendered the product bitter with low acceptability by the taste panel. Only one combination of chloride salts (4% NaCl and 4% KCl) could finally produce olives with lower sodium content and good organoleptic attributes. The results of this study could be employed by the Greek table olive industry in an attempt to produce natural black olives with less sodium without affecting the traditional taste of fermented olives in order to meet consumers' demand for low sodium dietary intake.

1. Introduction

Greece has a long tradition in producing natural black olives in brine, one of the three major commercial preparations of table olives in the international market. Every year, almost 50% of harvested olives in the country are processed as natural black according to well-established procedures (Sánchez Gómez et al., 2006). According to the Greek style process, olives are harvested when they are fully ripe or slightly before full ripeness and directly brined in 8–10% salt where they are subjected to the so-called 'spontaneous' fermentation by a mixed microflora of Gram negative bacteria, lactic acid bacteria and yeasts (Balatsouras, 1990; Brenes, 2004). The final product retains a fruity flavour and a slightly bitter taste due to the presence of residual polyphenols.

Sodium chloride (NaCl) has long been employed in the preservation of food commodities as well as a flavouring agent to enhance the organoleptic properties of food. The preparation of fermented vegetables in particular, relies on the use of common salt as the main ingredient of the brine because it reduces water activity, increases the ionic strength of the solution, reduces the solubility of oxygen in water, and inhibits undesirable spoilage and pathogenic microflora ensuring thus the microbiological safety of the final product during storage (Taormina, 2010; Albarracín et al., 2011). In recent years there has been a recommendation by public health and regulatory authorities to reduce dietary intake of sodium because of its association to hypertension (WHO, 2003, 2007). High blood pressure is an acknowledged risk factor for ischaemic heart disease, stroke, and renal disease which are major causes for morbidity and mortality in Europe (EFSA, 2005). Today, on average 75% of sodium intake comes from processed foods or restaurant foods, 10–12% is naturally occurring in food and the remaining comes from use of salt during home cooking (Dötsch et al., 2009). It is worth noting that the economic cost to treat hypertension is significant amounting to approximately € 169 billion per year in the EU only (Daniels, 2006). According to a recent report (Bibbins-Domingo et al., 2010) reducing daily salt intake by 3 g in the population of the USA would reduce health care costs from $ 24 billion to $ 10 billion on annual basis. The same authors concluded that it is more cost effective to reduce salt level than treat hypertension.

The negative effects of high sodium chloride (NaCl) intake could be possibly overcome by substituting, at least partially, this salt by other chloride salts with more favourable effect on human health such as potassium chloride (KCl), calcium chloride ($CaCl_2$), and magnesium chloride ($MgCl_2$) (Bautista-Gallego et al., 2008). This is especially important in Greek natural black olives which have been

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traditionally processed in high salt concentrations ranging from 8 to 14% or even higher depending on local processing methods (Balatsouras, 1990). So far, a number of studies have investigated NaCl reduction in vegetable fermentation including sauerkraut (Trail et al., 1996; Viander et al., 2003), kimchi (Choi et al., 1994), cucumber extracts (Naewbanij et al., 1990), cucumber (Guillou et al., 1992; Guillou and Floros, 1993), and turnips (Yamani et al., 1999). In the case of olives, the effect of chloride salts on the fermentation profile and physical attributes of cracked green table olives has been recently studied by Spanish researchers who reported effective substitution of NaCl by different proportions of KCl and $CaCl_2$ (Bautista-Gallego et al., 2010, 2011). The same chloride salts in directly brined green table olives resulted in the development of a final product with a lower sodium content and good sensory characteristics (Mulè et al., 2000). In natural black olives, the effect of calcium acetate, calcium lactate and the substitution of NaCl with 50% KCl on the growth kinetics of *Lactobacillus plantarum* and *Debaryomyces hansenii* in olive juice of Kalamon variety has been investigated (Tsapatsaris and Kotzekidou, 2004). The authors concluded that the replacement of NaCl by KCl resulted in a strong synergy between calcium lactate and calcium acetate with higher growth rates of single cultures of both microorganisms. In another study (Kanavouras et al., 2005) it has been reported that buffering the brine with acetic acid and calcium acetate resulted in reduction of NaCl, yielding a final product with low salt content, absence of spoilage microflora and better physicochemical and sensory attributes. However, information on the potential use of KCl and $CaCl_2$ in the fermentation of Conservolea black olives to produce a low sodium final product has not been reported so far.

The objective of the present study was to investigate the effect of selected concentrations of chloride salts (NaCl, KCl, $CaCl_2$) on the microbiological, physicochemical and organoleptic profile during the traditional fermentation process of Conservolea natural black olives. The results reported in this work could be adopted by the Greek table olive industry to modify the existing processing scheme to obtain a final product with lower sodium content while maintaining its traditional properties.

2. Materials and methods

2.1. Olive samples and fermentation procedures

Raw natural black Conservolea olives were obtained at their mature stage of ripening (in November) directly from the Union of Agricultural Cooperatives of Stylida in Central Greece and immediately transported to the laboratory to be processed according to the traditional anaerobic method. On arrival, olives were washed under tap water, hand selected to remove defective drups and immersed directly in plastic vessels containing 8 kg of olives and 6 L of brine. Sodium chloride (NaCl) which is normally employed in the preparation of the brine, was partially or totally substituted by potassium chloride (KCl) and calcium chloride ($CaCl_2$) in concentrations shown in Table 1. The initial concentrations of the chloride salts were constrained to NaCl + KCl + $CaCl_2$ = 8%, which is the usual level of salt applied by the Greek table olive industry today. All treatments were performed in duplicate. Fermentation vessels were kept at room temperature (ca. 20 °C) for a period of 55 days. The process was allowed to evolve spontaneously by the indigenous microbiota of olives based on the traditional anaerobic method as described elsewhere (Balatsouras, 1990).

2.2. Microbiological analysis

Brine samples (1 ml) or their decimal dilutions in sterile quarter strength Ringer's solution were analysed at different time periods to quantify the evolution of lactic acid bacteria (LAB), yeasts and *Enterobacteriaceae* in the brines using an Autoplate 4000 spiral plater system (Spiral Biotech Inc., Norwood, MA, USA). LAB were enumerated on de Man-Rogosa-Sharp medium (MRS; Merck 1.10660, Darmstadt, Germany) and incubated at 25 °C for 48–72 h. Yeasts were counted on Rose Bengal Chloramphenicol agar (RBC; Oxoid CM 549, Basingstoke, Hampshire, England, with selective supplement SR 78) and incubated at 25 °C for 48 h. Finally, *Enterobacteriaceae* were enumerated on Violet Red Bile Glucose agar (VRBGA; Oxoid CM 458, Basingstoke, Hampshire, England) incubated at 37 °C for 24 h.

Table 1
Experimental treatments of different concentrations of NaCl, KCl and $CaCl_2$ in the fermentation profiles of Conservolea natural black olives.

Run	NaCl		KCl		$CaCl_2$		Total IS	Initial a_w[b]
	(%)	IS[a]	(%)	IS	(%)	IS		
1	8	1.369	0	0.000	0	0.000	1.369	0.955 ± 0.004
2	4	0.684	4	0.537	0	0.000	1.221	0.951 ± 0.007
3	4	0.684	0	0.000	4	1.084	1.768	0.954 ± 0.002
4	0	0	4	0.537	4	1.081	1.618	0.961 ± 0.003
5	2.6	0.455	2.6	0.357	2.6	0.719	1.531	0.963 ± 0.002

[a] Ionic strength (IS) $= \frac{1}{2}\sum_{i=1}^{n} c_i z_i^2$, where c_i is the molality (mol/l) of ion i of the solute and z_i its charge.
[b] value ± standard deviation of duplicate analyses.

2.3. Physicochemical analysis and organoleptic evaluation

Fermentation of black olives was routinely analysed for pH using a digital pH meter (Metrohm AG, Herisau, Switzerland). Titratable acidity was also determined by titrating brine up to pH 8.2 with 0.2 N NaOH and expressed as percent of lactic acid (%, w/v) (Garrido-Fernández et al., 1997). Organic acids (lactic, acetic, succinic, propionic, citric, malic) were analysed by HPLC as previously described (Spyropoulou et al., 2001). The water activity (a_w) of the initial brines for each combination of chloride salts was measured with a Novasina Thermoconstander RTD 33 water activity meter (Novasina AG, Zürich, Switzerland) at 25 °C.

The volatile compounds of olive brine were isolated by the headspace solid phase micro-extraction (SPME) method. The fibre used for the absorption of volatiles was a DVB/CAR/PDMS - 50/30 μm (needle length 1 cm, needle size 24 ga) (Sigma Aldrich, Greece). The conditions of headspace SPME sampling used were as follows: 2 ml of diluted brine (1/10 in HPLC water, Merck) and 0.5 g NaCl were transferred in a 4 ml glass vial and spiked with 100 μl of a solution of 4-methyl-1-pentanol (final concentration 100 μg/l). A small magnetic bar was also added. The vial was tightly capped with a PTFE-faced silicone septum and placed in a thermostated block on a stirrer. The sample was equilibrated for 15 min at 35 °C, and after this, the fibre was exposed to the headspace for 30 min, under the same conditions. The length of the fibre in the headspace and the stirring were kept constant. Desorption of volatiles took place in the injector of the GC/MS for 5 min. Before the first daily analysis, the fibre was conditioned in the injector for 10 min at 250 °C to remove any volatile contaminants. For the following analyses, 5 min desorption after each extraction was used as conditioning time. Once a day, a blank test was performed to check the carry-over, which was negligible under the previous conditions. GC/MS analyses were performed on an Agilent 7890A gas chromatograph coupled to an Agilent 5973C mass spectrometer. Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The injection port was equipped with an SPME liner (0.75 mm × 6.35 mm × 78.5 mm). It was operated in split mode (1/5 split ratio) at 250 °C, because splitless desorption resulted in overloaded chromatograms with