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Combined effect of alkali pretreatment and sodium chloride addition on the olive fermentation process

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Abstract

Green olives of the Tunisian variety "Meski" were treated according to a Spanish-style green olive preservation process by using an alkaline treatment (1.5, 2 and 2.5% (w/v) NaOH) to eliminate bitterness, combined with different brine concentrations (6, 9 and 12% (w/v) NaCl). A spontaneous fermentation by the environmental microflora took place. Results showed that 2% NaOH solution and 9% sodium chloride brine was an optimal combination inducing the best growth of *Lactobacillus* species (10^8 CFU/ml) and acidity of 0.726 g lactic acid/100 ml brine. In all trials and independently of the treatment, *Lb. plantarum* was the most dominant strain of *Lactobacillus*. Moreover, pretreatment with lye and lactic fermentation of olives contributed to coliform elimination.

Keywords: Olive fermentation; Alkali treatment; *Lactobacilli*; Free acidity; Coliforms

1. Introduction

Olive fermentation is considered to be one of the oldest processes of food preservation used in Mediterranean countries. Although traditional methods are still adopted in some regions, they are increasingly being replaced by new industrial processes with chemical pretreatment followed by fermentation.

Preparation of Spanish-style green olives includes an alkaline treatment aiming at an efficient hydrolysis of the bitter glycoside oleuropein, a washing step used to eliminate the excess of lye accumulated over the olives and on the inside walls of the PVC containers and finally a brining, where the typical lactic acid fermentation occurs (Férnández-Díez, 1985). This author suggested that the concentration of NaOH during the alkaline treatment, the period of immersion and the degree of penetration achieved depend on various factors such as the variety, the degree of maturity of the fruits and the temperature. Furthermore, the brining of olives takes place after washing them twice; this has an important influence on the pickling process (Le Guern, 1989). The natural selection of microbiological strains during fermentation depends on the initial concentration of brine. Adequate alkali pretreatment is also important because the use of low concentrations of lye leads to a bitterness in the fruit due to the weak hydrolysis of oleuropein,

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recognized as one of the fermentation inhibitors (Walter et al., 1973; Fleming et al., 1973; Brenes et al., 1992). Moreover, for each variety, the recommended degree of penetration of NaOH through the olive fruits varies from two-thirds (2/3) to three-quarters (3/4) of the distance between the skin and the stone of the fruit (Férnández-Díez, 1985). On the other hand, when the concentration of sodium chloride is too high, the olives become wrinkled and this phenomenon may be irreversible and detrimental to the final product quality.

Previous researchers have studied the effect of lye and sodium chloride treatments on the micro-flora and the quality of fermented olives. According to Bobillo and Marshall (1991) and Montano et al. (1993), the growth and the metabolism of *Lactobacilli* genera involved in food fermentation are influenced by the level of sodium chloride.

The present work deals with a comparative study of the effect of chemical pretreatments with lye and the addition of salt on the fermentation of Tunisian variety "Meski" green olives. The interaction of many physico-chemical parameters and the complexity of the microbiological flora were also examined.

2. Methods

2.1. Olive fruits

Experiments were carried out with green olive fruits of the "Meski" variety (*Olea europea sativa*) harvested in September from Borj Toumi, a region situated in the north of Tunisia. The olives were size graded to approximatively 220–240 fruits per kg and then washed simply with tap water to eliminate dirt and undesirable risks of spoilage.

2.2. Lye treatment, brining and fermentation

Olives were put in PVC containers (15 kg of olives and 8 l of liquid) and then submitted to a prior alkali treatment (1.5, 2 and 2.5% w/v) for 7–12 h until penetration of NaOH solution reached around 2/3 of the flesh thickness. Then, olives were washed by replacing lye with tap water and changing it twice at intervals of 3 and 12 h. After washing, fruits were brined and fermented at three concentrations of NaCl (6%, 9% and 12%). Therefore, nine PVC fermenters were prepared by combining these concentrations of NaOH and NaCl solutions. Experiments were carried out at ambient temperature (22–25 °C).

2.3. Sampling

Before any sampling, fermenter contents were homogenized by hand shaking. Samples of brine were taken under sterile conditions at the 1st and the 5th day of fermentation, each week for a further 21 days, then every two weeks for a month and finally each month until the 140th day of fermentation.

2.4. Microbiological analyses

Brine samples and appropriate decimal dilutions were plated. Gram-negative bacteria, represented essentially by the coliform group, were counted by adopting the Most Probable Number Method on Brilliant-Green Bile broth medium (Diagnostics Pasteur, France). Lactic acid bacteria were grown on Man Rogosa Sharpe agar (Pronadisa, Spain), yeasts on Sabouraud agar (Bio-Rad, France) and total flora on Plate Count Agar (Pronadisa, Spain).

2.5. Chemical analyses

Brine samples were routinely analyzed using a pH meter (Mettler Toledo, MP225) for pH and using a titration method with 0.22 N NaOH in the presence of an indicator (phenolphthalein) for free acidity.

2.6. Data analyses

The values of the different parameters from triplicate fermentations were calculated and graphed. The coefficients of variation were obtained for each experiment and average values plotted on graphs.

Table 1
Evolution of the pH average values (± standard deviation) during olive fermentation with different alkali treatments and sodium chloride concentrations

NaOH/NaCl (w/v)	1.5/6	1.5/9	1.5/12	2/6	2/9	2/12	2.5/6	2.5/9	2.5/12
Fermentation time (days)	pH value								
1	7.42 ± 0.15	7.1 ± 0.21	7.28 ± 0.26	7.72 ± 0.11	7.88 ± 0.21	7.61 ± 0.21	9.32 ± 0.42	9.36 ± 0.4	9.10 ± 0.15
5	5.58 ± 0.15	6.33 ± 0.11	6.82 ± 0.25	5.25 ± 0.26	5.35 ± 0.25	5.3 ± 0.2	5.29 ± 0.41	6.80 ± 0.15	6.81 ± 0.15
13	4.51 ± 0.41	4.75 ± 0.76	5.01 ± 0.21	4.39 ± 0.05	4.68 ± 0.05	4.66 ± 0.4	4.44 ± 0.26	5.12 ± 0.35	4.72 ± 0.35
19	4.42 ± 0.25	4.72 ± 0.35	5.00 ± 0.15	4.30 ± 0.15	4.56 ± 0. 4	4.73 ± 0.11	4.53 ± 0.42	5.22 ± 0.04	4.65 ± 0.15
26	4.43 ± 0.26	4.60 ± 0.15	4.76 ± 0.35	4.22 ± 0.15	4.50 ± 0.05	4.65 ± 0.35	4.48 ± 0.21	4.62 ± 0.11	4.46 ± 0.4
114	4.05 ± 0.41	4.25 ± 0.25	4.42 ± 0.25	3.96 ± 0.05	4.09 ± 0.70	4.19 ± 0.15	4.42 ± 0.25	4.49 ± 0.36	4.34 ± 0.21