

## Reducing the Bloater Spoilage During Lactic Fermentation of Moroccan Green Olives

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### Summary

The process of natural lactic fermentation of green olives is too long and usually associated with several types of olive deteriorations, mainly the »bloater spoilage«. The control of pH and salt level in brine, a practice mostly used in the olive industry, is not sufficient to avoid these problems. The main objective of this work is the control of the fermentation process in order to reduce the duration of the process and the olive spoilage incidence. Therefore, some Moroccan green olives were alkali-treated and brined at 5 % NaCl. The controlled fermentation was carried out by adjusting pH, by inoculation with *Lactobacillus plantarum* I159 and the addition of potassium sorbate (0.05 % brine). The results showed a rapid development of lactic fermentation with a remarkable reduction in »bloater spoilage«, without affecting the organoleptic properties, colour and texture of the final product.

*Key words:* green olives, fermentation, bloater spoilage, *Lactobacillus plantarum*, yeasts

### Introduction

Green table olives are prepared in Morocco according to the Spanish style. This process consists of sorting, grading, debittering of fruits in lye (1.5–2 % NaOH), washing and then brining them initially at 10–12 % NaCl to undergo a natural lactic fermentation. This high initial salt level, inhibiting the lactic acid bacteria activity (1), and the lack of control of the other environmental parameters (temperature, inoculation with starters) lead to a very slow (4–6 months) and complex process. In these conditions, the fermentation process is mostly associated with a high percentage of deteriorated fruits with several kinds of defects, such as softening, bloater deterioration and off-odour of fermentation (2).

The main defect encountered in Moroccan olives during fermentation is the bloater spoilage. This deterioration is caused by gas-producing microorganisms, such as coliforms, heterofermentative lactic acid bacteria, butyric acid bacteria and fermenting yeasts (2). Thus the suitable starters and good environmental conditions are important factors to optimise the process.

The control of pH and salt concentration in brine, mostly practiced in the olive industry, does not reduce the incidence of this spoilage in fruits, and it has an influence on the fermentation process. Hence, the high acidification to pH=3.5 and the addition of high amount of salt (10–12 %) in brine would have an inhibitory effect on the lactic flora, mainly involved in the fermentation process (3).

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Acidification and salt control of brine can inhibit the coliforms, the heterofermentative lactic acid bacteria and the butyric acid bacteria (2). However, the yeast population highly involved in the olive bloaters spoilage (4) and tolerating high salt levels and low pH cannot be controlled by these two factors. The use of antimicrobial compounds is necessary.

The essential oils of a seasoning plant showed a significant inhibitory effect of the yeast population in fermented green olives during storage, but with a high taste of garlic in the final product (5). The use of sorbates showed an important reduction of the yeast population in brines, with lower inhibitory effect on the lactic flora (6–8).

The improvement of the fermentation process depends on the optimisation of the main factors influencing the desirable action of the starters in olive brines, such as the brine strength, pH, sugar concentration, temperature, starter composition and the olive cultivar.

*Lactobacillus plantarum* is the most used species of lactic acid bacteria in controlled fermentation of green olives (2,9), because of its homofermentative metabolism and high acidification capacity. It enhances the speed of the process, but without affecting the yeast population involved in various defects, mainly the bloaters.

The aim of this work is to find the best combination of fixed process parameters involved in the lactic fermentation process of the olives in order to reduce the duration of the process and the olive spoilage level.

## Materials and Methods

### Preparation of olives

Green olives of the Moroccan »Picholine« cv. were harvested from the olive orchards in the Oujda area. The injured fruits were removed and the rest was treated by immersion in a 2 % NaOH solution for 6 h and then washed twice in tap water, for 3 h for the first time and for 14 h for the second time.

### Experimental design

The olives, debittered and washed, were then brined in one-litre bottles [600 g olives/400 mL of brine (5 % NaCl)]. This initial salt concentration was not kept constant during the fermentation process. By the osmosis phenomenon between fruits and brine, this concentration was reduced until the equilibrium was obtained, around 2 to 3 %. Except for the control, the other trials were acidified to pH=4 by lactic acid (0.1 %). After brining for two days, the trials were treated as follows: (i) trial 1 (E1): control (spontaneous fermentation); (ii) trial 2 (E2): acidified to pH=4; (iii) trial 3 (E3): acidified to pH=4 and inoculated with *L. plantarum* I159; (iv) trial 4 (E4): acidified to pH=4, inoculated with *L. plantarum* I159 and supplemented with potassium sorbate (0.05 % of brine).

Trials, made in duplicate, were incubated at 30 °C during fermentation.

### Starter preparation

The strain of *L. plantarum* I159, used for the inoculation of the olives, had been isolated from natural lactic fermentation of Moroccan green olives. The inoculation

was carried out with an overnight culture in MRS broth containing 5 % NaCl.

### Physicochemical analyses

All the analyses were made in brine solutions. A pH meter type Crison pH 2000 was used to determine the pH values. The reducing sugar contents were determined with the Nelson-Somogyi method, using copper and arsenomolybdate reagents (10,11). This method is based on the reduction of copper by sugars. The absorbance of the blue colour developed, measured at 520 nm, is proportional to the glucose taken.

### Microbiological analyses

From serial decimal dilutions, made with brine and sterile physiological water, each microbial group was inoculated, using pour plate method, in its specific medium. The Gram-negative bacteria, lactic acid bacteria, and yeasts and moulds were determined respectively on deoxycholate lactose agar (Biokar, France), de Man Rogosa Sharpe (MRS; Merck, Germany) containing pimaricin at 0.02 %, and potato dextrose agar (PDA; Biokar, France) acidified with lactic acid to pH=3.5. The Gram-negative bacteria were counted after 2 days of incubation at 30 °C. The lactic acid bacteria were counted after 3 days of incubation at 30 °C. The yeasts and moulds were counted after 3 to 4 days of incubation at 30 °C.

### Spoilage formation evaluation

At the end of the fermentation process, all the fruits of each trial were sorted by hand and analysed visually to evaluate the levels of deteriorations (in percentage) in terms of colour and texture.

## Results

The control (E1), without acidification nor inoculation, showed a drop of pH from 6.61 to 4.5 at the end of the fermentation process (42nd day), whereas the other trials (E2, E3 and E4), acidified to pH=4, showed a slight pH increase at the beginning of the process (during the first 7 days) to pH=(5.29–5.42), and a decrease followed by stabilisation at approximately pH=5 (Fig. 1).

At the beginning, reducing sugars showed a progressive increase in the brines of all trials, followed by a

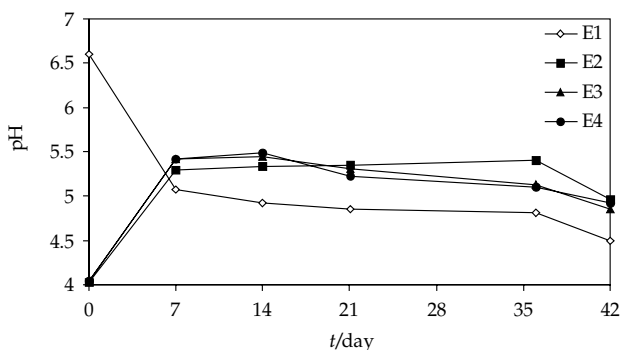


Fig 1. pH changes during lactic fermentation of green olives (E1: control, E2: pH=4, E3: pH=4 + *L. plantarum*, and E4: pH=4 + *L. plantarum* + sorbate)

reduction as a result of their consumption by microorganisms (Fig. 2). The acidified trials (E2, E3 and E4) showed a significant difference in the brine sugar contents and sugar concentration, which were approximately 1 g/L higher than in the non-acidified assay (E1: control).

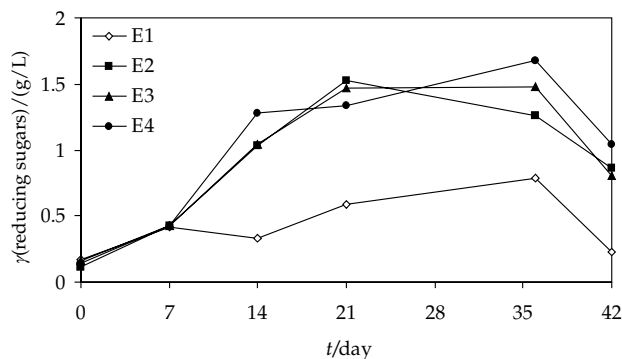


Fig 2. Contents of reducing sugars during lactic fermentation of green olives (E1: control, E2: pH=4, E3: pH=4 + *L. plantarum*, and E4: pH=4 + *L. plantarum* + sorbate)

A Gram-negative bacteria population can be observed between  $10^3$  and  $10^4$  CFU/mL in the trials E1, E2 and E3 during the fermentation (Fig. 3). However, the acidified and inoculated trials containing potassium sorbate (E4) showed a high reduction of this microbiota to the undetectable level ( $<10$  CFU/mL) at the 3rd week, probably due to the effect of potassium sorbate and the acidic conditions (12).

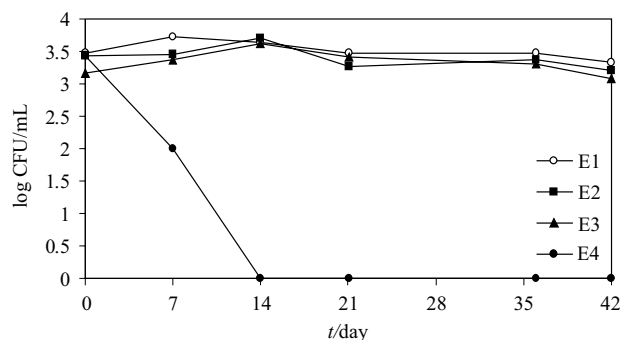


Fig 3. Gram-negative bacteria population changes during lactic fermentation of green olives (E1: control, E2: pH=4, E3: pH=4 + *L. plantarum*, and E4: pH=4 + *L. plantarum* + sorbate)

The trials inoculated with *L. plantarum* (E3 and E4) presented a lactic population of  $2\text{--}3 \cdot 10^4$  CFU/mL at the beginning of the process, where a slight decrease was followed by a slow development reaching  $10^6$  CFU/mL during 42 days (Fig. 4), whereas the non-inoculated trials (E1 and E2) indicate the presence of a natural lactic flora varying from  $50\text{--}80$  to  $10^4\text{--}10^5$  CFU/mL at the end of the fermentation process.

In the absence of potassium sorbate (E1, E2 and E3), the population of yeasts and moulds showed an important development from  $1.5\text{--}6 \cdot 10^3$  to  $2\text{--}5.7 \cdot 10^6$  CFU/mL at the end of the process (Fig. 5). However, in the presence

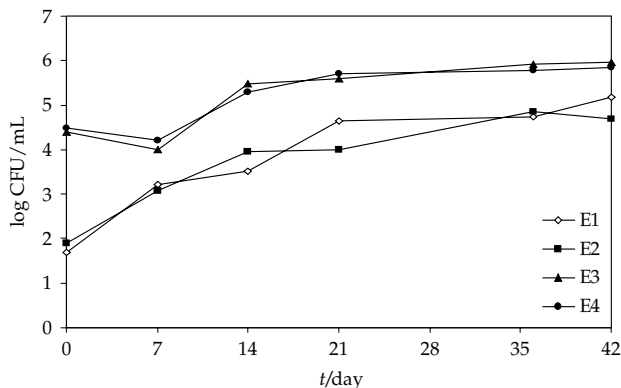


Fig 4. Lactic acid bacteria population changes during lactic fermentation of green olives (E1: control, E2: pH=4, E3: pH=4 + *L. plantarum*, and E4: pH=4 + *L. plantarum* + sorbate)

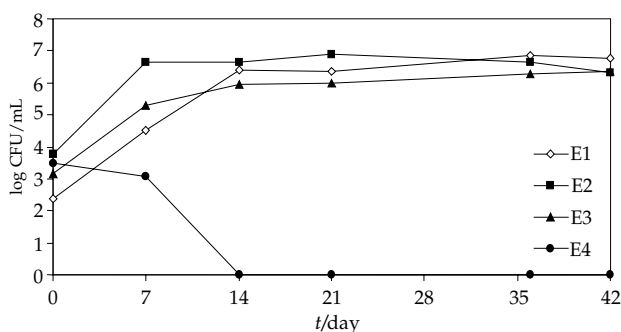


Fig 5. Population changes of yeasts and moulds during lactic fermentation of green olives (E1: control, E2: pH=4, E3: pH=4 + *L. plantarum*, and E4: pH=4 + *L. plantarum* + sorbate)

of potassium sorbate (E4) a high decrease of this population can be noticed from  $3 \cdot 10^3$  CFU/mL to the undetectable level ( $<10$  CFU/mL) at the second week.

After 42 days of fermentation, the main types of spoilage identified in fruits are the »bloaters spoilage« and the »lactic spot«. The bloater spoilage is identified as a gas pocket or a crack on the flesh. The lactic spot is identified as a white spot on the olive flesh, due to the development of microbial colonies in stoma apertures. The non-deteriorated fruits showed good organoleptic properties in terms of colour and texture.

The most important defect found in the olives concerns the bloater spoilage. The control (E1), having undergone natural fermentation, showed the highest level of spoiled fruits, 65.17 % (Table 1). The acidification to pH=4 (trial E2) reduced the Gram-negative and heterofermentative lactic populations, and consequently the bloaters caused by these microorganisms. Hence, the bloater spoilage level was reduced to 22.8 %. The acidification to pH=4 and the inoculation with *L. plantarum* I159 (trial E3) were more effective, so the level of the olives affected by bloater spoilage decreased to 15.88 %. The combination of the acidification, the inoculation with *L. plantarum* I159 and the addition of potassium sorbate 0.05 % (trial E4), inhibiting highly the yeast population, reduced drastically the incidence of the bloaters in olives to 3.68 %.

Table 1. Spoilage evaluation of the olives at the end of the lactic fermentation process (E1: control, E2: pH=4, E3: pH=4 + *L. plantarum* I159, and E4: pH=4 + *L. plantarum* I159 + potassium sorbate 0.05 % mass per volume ratio)

Trials	BLOATER SPOILAGE/%				LACTIC SPOT/%			
	Assay 1	Assay 2	m	s.d.	Assay 1	Assay 2	m	s.d.
E1	64.34	66	65.17	0.83	10.66	11.74	11.2	0.54
E2	22.26	23.34	22.8	0.54	0.7	0.94	0.82	0.12
E3	15.53	16.23	15.88	0.35	16.08	17.52	16.8	0.72
E4	3.26	4.1	3.68	0.42	16.46	17.74	17.1	0.64

m: mean, s.d.: standard deviation

## Discussion

BLOATER SPOILAGE is the main defect attacking the olives during fermentation and storage. The control of brine salt concentration and pH, practices widely used in the Moroccan table olive industry, are not sufficient to avoid or reduce the incidence of this defect (4). The control of other factors, such as the use of starters and antimicrobial compounds as well as the control of temperature, is necessary.

The pH levels found proved the development of lactic fermentation process in the assays, but with a slow rate. Hence, the debittering and washing operations cause a high degradation and elimination of bacterial nutrients and cells (13). Furthermore, slow acidification level observed in the acidified trials, compared to the control, is probably due to the residual sodium hydroxide in the olives, the lactic acid consumption by the yeast, and the inhibitory effect of the stressing factors on the growth and biochemical activity of lactic acid bacteria, including acidic pH and sorbate. The control of the incubation temperature (30 °C) improves the growth and the acidification rates of this microbiota. In fact, lactic acid bacteria are not acidophilic, but they support high acidities.

With natural lactic population of the inoculated trials (E3 and E4), the starter of *L. plantarum* I159 enhanced the dominance of the lactic microflora over the other groups of microorganisms, leading to a better development of the lactic fermentation process and good quality results in table olives, particularly the level of bloater spoilage in olives.

The potassium sorbate concentration used, which is lower than the level permitted in the olives (14), has the advantage of inhibiting high levels of yeast population without affecting the lactic population, which leads to the dominance of the biochemical activity of the lactic population over the yeasts, having as a result the reduction of bloater incidence in the olives. In fact, the balance between these two microbial groups is the most important factor for the success in table olive fermentation (2). The olives affected by bloater spoilage obtained in trial E4 (acidified to pH=4, inoculated by *L. plantarum* and supplemented with potassium sorbate) are probably the result of the gas-producing microorganisms before their inhibition, or of the indigenous fruit metabolism (15).

In comparison with our previous works (5), the control of the incubation temperature (30 °C), with low salt concentration in brines (5 %), improves highly the speed of the lactic fermentation process by assuring optimal growth and acidification rates of the lactic flora. However, these conditions lead to a high level of olives affected by bloater spoilage (E1: control), because of the optimisation of the metabolic activity of gas-producing microorganisms. Thus, the control of temperature can be used to improve the brine acidification rate, but not to reduce the incidence of the bloaters in olives.

The presence of the lactic population in the olive brine is important not only to assure continuous acidification of the medium, inhibiting the Gram-negative bacteria and some heterofermentative lactic bacteria, but also to inhibit the fermentative metabolism of yeasts (16) that produce bloaters in olives.

## Conclusions

The combination of the incubation at 30 °C, brine acidification to pH=4, inoculation with *L. plantarum* I159 and the addition of potassium sorbate (0.05 %) in low salted brine (5 %) showed a rapid lactic fermentation process with a remarkable reduction of bloaters in olives.

It is well known that the natural lactic fermentation requires a long period to complete the process and it is associated with a high level of olives affected by bloater spoilage, particularly in cold areas. Furthermore, the environmental temperature cannot be controlled in the table olives industry. Thus, this process may constitute an important alternative in temperate regions, such as Marrakesh in Morocco, leading to (a) promotion of the table olives industry by the reduction of the process duration and the level of economic losses (spoiled fruits), (b) protection of the consumer by the use of biological fermentation process and reducing the salt concentration in fruits, and (c) preservation of the environment by reducing the salt concentration of brine rejected in the environment.

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## Smanjivanje napuhavanja marokanskih zelenih maslina tijekom mliječno-kisele fermentacije

### Sažetak

Prirodni je proces mliječno-kisele fermentacije zelenih maslina predug i obično ga prate različiti tipovi kvarenja, najčešće pojava napuhavanja. Kontrola pH-vrijednosti i količine soli u rasolu, što se najčešće koristi u preradi maslina, nije dovoljna kako bi se izbjegli ti problemi. Svrha je ovoga rada kontrola fermentacijskoga procesa radi skraćivanja njegova trajanja i sprečavanja kvarenja maslina. Zbog toga su marokanske zelene masline obrađene lužinom i usoljene u 5 %-tnoj otopini NaCl. Fermentacija je kontrolirana reguliranjem pH, inokulacijom s *Lactobacillus plantarum* I159 i dodatkom kalijeva sorbata (0,05 % rasola). Rezultati pokazuju da je došlo do brze mliječno-kisele fermentacije uz značajno smanjenje napuhavanja, a pritom se nisu promijenila organoleptička svojstva, boja i tekstura gotovog proizvoda.