The Processing of Turning Colour Olives of Oblica Cultivar

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SUMMARY

In this research the observation of the preservation process of turning colour olives of Oblica cultivar (an autochthonous Croatian cultivar) processed according to the Californian type of processing has been carried out.

Although the oblica cultivar is the most frequent cultivar in Croatian orchards, it is absolutely unexplored concerning the concentration of polyphenolic compounds (oleuropein and its derivatives) as well as concerning the composition of natural microflora in the fruit. It has been very important to determine to what extent the choice of preserving technology is optimal for the cultivar's particularities. The changes of fundamental physical and chemical features of brine (total acidity, pH value, the concentration of sodium chloride, the concentration of sugar, and brine temperature) have been screened during the process of preservation. The appeareance of the lactobacilli population has also been observed. The *Lactobacillus plantarum* species has been isolated and identified in this population on the seventh day after the fruit has been put in brine. The *Lactobacillus plantarum* species has been isolated and identified by means of the API 50 CHL ("bioMérieux", France) biochemical test and the APILAB PLUS ("bioMérieux", France) software. During further phases of the process, no appearance of the lactobacilli has been identified.

On the basis of our physical and chemical examinations, we have concluded that the type of processing of turning colour olives of Oblica cultivar (which was applied without a complete knowledge of the Oblica's particularities) did not give optimal and expected values (low brine temperature, exceptionally high acidity of the medium). We have also concluded that it is necessary for further research to include the examinations of the portion of polyphenolic compounds in the fruit, as well as the examinations of the composition of naturally present microflora. Both examinations represent the prerequisites for an optimal process of preservation and for reaching the best quality of the product.

KEY WORDS

lactic fermentation, *Lactobacillus plantarum*, Oblica cultivar, table olives

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INTRODUCTION

The tradition of olive growing in Croatia has been several centuries old. Considering its numerous nutritive values, the olive represents the basis of the so-called Mediterranean nutrition, which has often been identified with the term 'healthy nutrition' (Gracian Tous, 1968; Mataix Verdu, Munoz, 1988).

However, the production of table olives in Croatia has not reached a high level, so that the major part of annual crops is being used for the production of olive oil.

Preservation technology is the crucial factor for achieving the optimal quality of the product. Therefore, the main objective of this research has been the observation of changes of physical and chemical features of brine and the presence of the lactobacilli population during the process of preservation of turning colour olives of Oblica cultivar (an autochthonous Croatian cultivar) processed according to the Californian type of processing.

The Oblica cultivar has the characteristics suitable for the production of olive oil, and for the production of table olives. This cultivar is by far the most frequent olive cultivar in Croatia (making up over 75% of the total number of olive trees). One of the fundamental problems in the selection of optimal technological process and the determination of optimal parameters of such a process is complete ignorance as to the composition of oblica fruits, particularly of the portion of phenolic compounds (oleuropein and its derivatives) which have an impact on the development of the microbial population necessary for a normal course of the lactic fermentation process.

The Californian type of processing represents (Garrido Fernandez et al., 1997), together with the Spanish and Greek types, the third most important type of table olive processing in the world. It is the portion of phenolic compounds that is such an important factor for the Californian type of processing, because the phenolic compounds facilitate the browning of the fruit skin by their own oxidation. The lye treatment of the fruits and the oxidation process are the two most important steps in this type of processing with a significant influence on the quality of the final product (Fernandez Diez, 1969).

The fermentation process taking place during this type of processing is characterized by the appearance of lactic acid bacteria and by a permanent appearance of the yeasts population. Diffusion of fermentative substrates in brine is slower and, consequently, the fermentation process needs much more time than in the case of the preservation of green olives (Garrido Fernandez et al., 1997), because the lye treatment has not been carried out at the beginning of the process, which made the fruit skin much stronger (Brenes et al., 1996). During the processing of turning colour olives some authors notice the absence of the lactic acid bacteria due to the known inhibitory effect of oleuropein and some other phenolic compounds present in the olive fruit (Walter et al., 1973; Borbolla y Alcala, 1981; Ruiz-Barba et al., 1990; Ruiz Barba et al., 1993). However, some examinations have confirmed the possibility of a spontaneous lactic fermentation process in the case of the fruits which had been put in brine immediately after the harvesting (Garcia Garcia et al., 1992b). These facts have been proven by the results of the part of this research which is related to the identification of the *Lactobacillus plantarum* species on the seventh day after the beginning of brining.

MATERIALS AND METHODS

Olives

In the experimental part of this research the fruits of Oblica cultivar have been used. The fruits were harvested in the stage of their technological maturity (dark brown fruits with sporadic dark green spots) in the orchards in the area of Split (southern Croatia) during the period between mid-October and the end of October 1999.

Technological process

The harvested fruits were transported in crates and kept for a day or two before brining. The fruits were then put in brine with a concentration of 1.0% of acetic acid and the solution of 15% NaCl in plastic vessels of 200 kg each, (produced by Marvi plast Ltd., Greece). The solution of sodium chloride was being added in the following manner: 6% at the beginning, 3% after four days and 6% after the following four days. When the stability of brine was reached, the fruits were kept in it for a few months and then debittered and exposed to the air for the oxidation process. Before the process of debittering began, the fruits were carefully washed out and then treated with 1.5% NaOH solution and 1.5% NaCl solution for the period of 10 hours. The lye penetration was controlled by phenolphtalein. After the process of debittering, the fruits were exposed to the air for 8 to 12 hours. The oxidized fruits were also treated with 0.1% ferrous gluconate solution for the purpose of achieving colour stability. The coloured fruits were then washed out again and put in a 10% NaCl solution (6% of which was added at the beginning and the remaining 4% three days after). In such brine the fruits were pasteurized by water at a temperature of 85-90 ^{0}C for 45 minutes.

Physical and chemical analysis

– the brine pH (Mettler Toledo MP 230, Swiss) value and conductivity (Mettler Toledo MC 226, Swiss) were measured by potentiometric method

– the concentration of sodium chloride and the total acidity as well as the determination of reducing sugars and the total amount of sugar were carried out according to the methods as described in Analytical Chemistry of Foods, 1999
– determination of D-glucose concentration and lactose concentration was carried out by enzyme test combinations using the method of UV spectrophotometer*.
  The principle of the method is based on the hydrolysis of D-glucose and D-galactose by means of enzyme β-galactosidase in the presence of H2O. D-glucose is phosphorylated by enzyme hexokinase (HK) with a simultaneous formation of adenosine diphosphate (ADP). Glucose-6-phosphate is oxidized by glucose-6-phosphate dehydrogenase (G6P- DH) into gluconate-6-phosphate and nicotinamide adenine dinucleotide phosphate (NADP), present in this reaction, was transformed to reduce nicotinamide adenine dinucleotide phosphate (NADPH).
– determination of glucose/fructose/sucrose concentration was carried out by enzyme test combinations by method of UV spectrophotometer**
  The principle of the method is based on the reaction of glucose phosphorylation catalysed by enzyme hexokinase (HK) and with the presence of ATP. Thus formed glucose-6-phosphate is then oxidized into gluconate-6-phosphate in a reaction facilitated by nicotinamide-adenine-dinucleotide-phosphate ($NADP^+$) and enzyme glucose-6-phosphate dehydrogenase. $NADP^+$ is simultaneously reduced to NADPH. The concentration of the thus formed NADPH is equivalent to the concentration of glucose and it is measured at a wavelength of 340 nm. Enzyme invertase catalyses the hydrolysis of sucrose into glucose and fructose. After the determination of glucose concentration, according to the described principle, the concentration of sucrose is determined on the basis of the difference between the concentration of glucose before and after its inversion. Fructose also yields to the reaction of phosphorylation catalysed by hexokinase enzyme (HK) in the presence of ATP. Thus formed fructose-6-phosphate is then transformed into glucose-6-phosphate by phosphoglucose isomerase (PGI). The glucose-6-phosphate is oxidized into gluconate, as was the case with glucose.

Microbiological tests

These tests include:
– the isolation of the bacteria belonging to the *Lactobacillus* genus:
  It was carried out by the inoculation of 1 ml of decimal dilutions of the sample (10^{-1} to 10^{-6}) onto MRS agar ("Biolife", Italy). The incubation under anaerobic conditions ("bioMérieux", France) at a temperature of 30 ^{0}C lasted for 2–3 days. At the end of the incubation, grown colonies were coloured according to Gramm's method in order to determine whether they were Gram-positive. If the colouring turned out to be positive, the spore forming rods were inoculated onto MRS agar under the same conditions once again.
– the identification of the bacteria colonies belonging to the *Lactobacillus* genus:
  It was carried out by reading biochemical strips, API 50 CHL ("bioMérieux", France) and by using the APILAB PLUS software ("bioMérieux", France).

In this research the statistical analysis has been carried out by means of the SPSS 9.0 (Microsoft) software.

RESULTS AND DISCUSSION

Physical and chemical features

During the processing of turning colour olives of Oblica cultivar, the fundamental physical and chemical features of brine have been examined (brine temperature, pH value, total acidity, concentration of sodium chloride and the portion of natural invert).

During the first months of the processing, the examined values varied very little. Only the change of total acidity and, consequently, the change of the pH value were significant. These features are usually determined by the fermentation type. Some authors consider that the pH value always has to be lower than 4 in the case of the growth of the lactic acid bacteria, and approximately 4.4 in the case of the yeasts appearance (Garrido Fernandez et al., 1997).

A relatively low portion of fermentable substrates has been observed, which is a consequence of the cultivar's particularity and of its slow diffusion in brine.

Figures 1 and 2 show the curves of initial physical and chemical features of brine which indicate very low pH values (probably because of the presence of the acetic acid immediately at the beginning of brining), too high a value of total acidity and brine temperature below the lower limit.

Microbiological tests

The main purpose of microbiological tests has been to determine the beginning of the appearance of the lactobacilli population and the screening of their presence during the technological process.

It is not possible to distinguish clearly the fermentation phases in this type of processing as in the case of

* This method is described in the manual of "Boehringer" Mannheim, Germany, 1998
** The method is described in the manual of "Merck", Germany, 1998

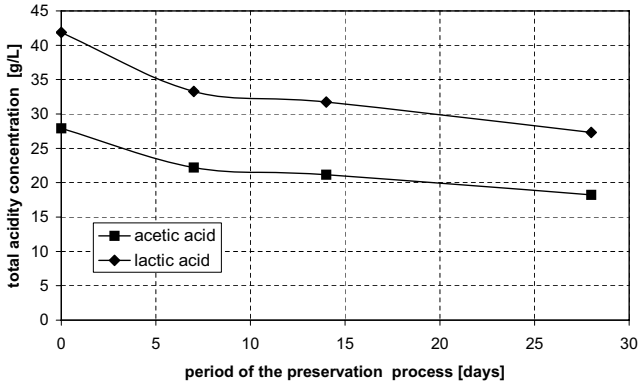

Figure 1.
Changes of total acidity value during the process of preservation Friedman'a analysis of variance: acetic acid - $x^2=9$, p=0.029

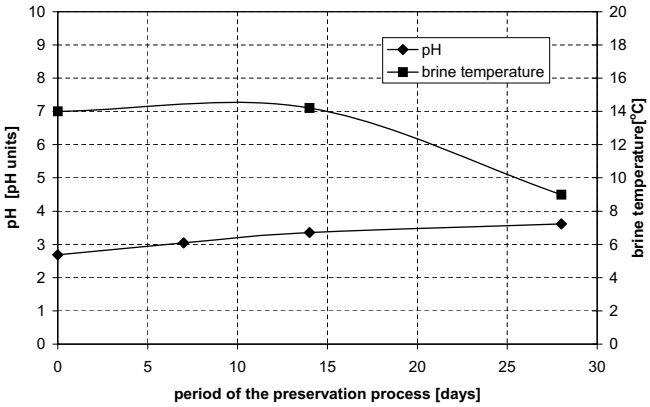

Figure 2.
Changes of brine pH value and temperature during the process of preservation Friedman's analysis of variance: pH - $x^2=9$, p= 0.029

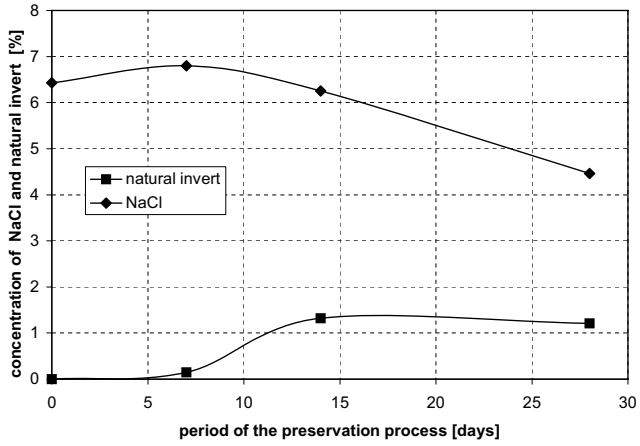

Figure 3.
Changes of concentration of sodium chloride and natural invert during the process of preservation Friedman'a analysis of variance: natural invert - $x^2=9$, p=0.029
concentration of sodium chloride - $x^2=6.6$, p=0.086

green olives processed according to the Spanish type of processing (Garrido Fernandez et al., 1997).

Table 1. The microbial population in brine during the processing of turning colour olives of Oblica cultivar

Beginning of fermentation	No microbial growth
7th day	*Lactobacillus plantarum*
14th day	Yeasts - Gram-positive diplococci
28th day	Yeasts - Gram-positive diplococci

Table 2. Results of the API 50 CHL bioMérieux's test 48 hours after the aerobic incubation at 30 ^{0}C for the *Lactobacillus plantarum* species.

0	control	–	25	esculin	–
1	glycerol	–	26	salicin	+
2	erythriol	–	27	celibiose	+
3	D arabinose	–	28	maltose	+
4	L arabinose	–	29	lactose	–
5	ribose	–	30	melibiose	+
6	D xylose	–	31	sucrose	–
7	L xylose	–	32	trehalose	+
8	adonitol	–	33	inulin	–
9	β-methyl-D xyloside	–	34	melezitose	+
10	galactose	+	35	raffinose	–
11	glucose	+	36	starch	–
12	fructose	+	37	glycogen	–
13	manose	+	38	xylitol	–
14	sorbose	–	39	gentibiose	–
15	rhamnose	–	40	D turanose	+
16	dulcitol	–	41	D lyxose	–
17	inositol	–	42	D tagatose	+
18	manitol	+	43	D fucose	–
19	sorbitol	–	44	L fucose	–
20	α-methyl-D mannoside	–	45	D arabitol	–
21	α-methyl-D glucoside	–	46	L arabitol	–
22	N-acetyl glucosamine	+	47	gluconate	–
23	amygdalin	–	48	2-keto-gluconate	–
24	arbutin	+	49	5-keto-gluconate	–

The results of the microbiological examinations are shown in Table 1. On the seventh day after the beginning of brining, only the species of *Lactobacillus plantarum* was isolated and identified by means of the API 50 CHL ("bioMérieux", France) biochemical test. The *Lactobacillus plantarum* is considered to be the most frequent and the most important microorganism in the process of lactic fermentation in olives (Ruiz-Barba et al., 1990; Duran et al., 1993; Ciafardini et al., 1994; Roses, Peres, 1996;). The fact that it appeared even in the very acid medium was explained by its own, relativly high, pH value (Kashket, 1987). However, the nature of its acidotolerance has not been completely examined so far (Brenes et al., 1996).

Table 2 shows the results of the biochemical testing carried out by means of the API 50 CHL biochemical

strips ("bioMérieux", France) for the *Lactobacillus plantarum* species in Oblica cultivar. By means of the APILAB PLUS software ("bioMérieux", France) a very high level of identification accuracy has been observed (99.3%). Three successive tests have been carried out.

Some authors also mention the apperance of the lactic acid bacteria in brine with a 5-9 % soduim chloride solution (Garcia Garcia et al., 1992). Meanwhile, some cultivars are able to have a good lactic fermentation process (Sevilliano cultivar has a high level of acidity), while others are not. It is observed that the fermentor materials can have some influence on the fermentation process (Borbola y Alcala et al., 1971). The reasons for this effect are still unkown, but it is certain that some attention should be paid to the type of plastic containers used in the technological process.

Table 1 shows a permanent presence of the yeasts population and gramm-positive bacteria which do not normally occur in brine with such a poor presence of the lactic acid bacteria.

CONCLUSIONS

On the basis of this research, we have concluded that the applied technological conditions have not provided optimal features of brine, the features that ensure a desirable lactic fermentation process which is the most natural way of reaching the best quality and prolonged shelf-life of the product.

The provision of optimal features during such a technological process necessarily requires that further research include the examinations of the particularities of this autochthonous cultivar concerning the portion of polyphenolic compounds in the fruit and, equally important, the composition of the naturally present microbial population of the Oblica cultivar.

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