PAPER

RESPONSE OF THREE SARDINIAN OLIVE CULTIVARS TO GREEK- STYLE PROCESSING

ATTITUDINE DI TRE VARIETÀ DI OLIVE DELLA SARDEGNA ALLA TRASFORMAZIONE SECONDO IL METODO ALLA GRECA

A. PIGA*, F. GAMBELLA, V. VACCA and M. AGABBIO
Dipartimento di Scienze Ambientali Agrarie e Biotecnologie Agro-Alimentari - Università di Sassari - Viale Italia 39 - 07100 Sassari - Italy
* Corresponding author: Tel./Fax +39 079 229273 - e-mail: pigaa@ssmain.uniss.it

ABSTRACT

The response of three Sardinian olive cultivars to processing as table olives with the Greek-style was evaluated. "Bosana", "Manna" and "Sivigliana sarda" olives were characterised from the marketing, technological and chemical-physical points of view and brined with 8% NaCl. Fermentation was carried out according to the anaerobic method for 150 days. At fixed intervals the main brine and flesh parameters were monitored. Evolution of the chemical parameters of the brine showed yeast fermentation. Oleuropein decreased greatly in the flesh after 20 days

RIASSUNTO

In questo studio è stata valutata l'attitudine di tre varietà di olive della Sardegna alla trasformazione come olive da tavola secondo il metodo alla greca. A tal fine, dopo una caratterizzazione delle olive delle varietà "Bosana, "Manna" e "Sivigliana sarda" dal punto di vista merceologico, tecnologico e chimico-fisico, le olive sono state poste in salamoia alla concentrazione in NaCl dell'8%. La fermentazione è stata condotta secondo il metodo tradizionale anaerobico. Ad intervalli prefissati sono stati determinati i principali parametri delle salamoie e della polpa delle olive. L'evo-

- Key words: brining, fermentation, oleuropein, polyphenols, table olives -

and totally disappeared in "Bosana" after 90 days. Sensory determinations resulted in good taste and texture for the three cultivars, with "Bosana" being preferred. Gassy alterations were negligible, while shrivelling was not detected.

luzione dei parametri chimici delle salamoie suggerisce una fermentazione condotta fondamentalmente da lieviti. L'oleuropeina nella polpa delle olive ha subito una drastica diminuzione dopo appena 20 giorni di processo e nel caso della varietà "Bosana" è completamente scomparsa al novantesimo giorno. Le determinazioni sensoriali hanno evidenziato delle eccellenti caratteristiche di sapore e consistenza per tutte e tre le cultivar, con una certa preferenza per la "Bosana". L'incidenza delle alterazioni di natura gassosa è stata modesta, mentre non è apparso il fenomeno del raggrinzimento.

INTRODUCTION

Of pickled vegetables, table olive is an important fermented crop in the developed world. Total world production has increased linearly in the last 35 years at an average annual rate of 1.5 to 2%, reaching over 900,000 tonnes in 1994/1995 (GARRIDO FERNANDEZ *et al.*, 1997). Three table olive processing methods are primarily used: the Spanish, the Californian and the Greek-style, with olives being harvested, respectively, when they are green, turning and black. In the first two methods total removal of the bitter compound oleuropein occurs in a very short time (8-12 hours) by hydrolysis with NaOH (lye) (FERNANDEZ DIEZ, 1971; BORBOLLA Y ALCALA, 1981). In the latter case the olives are put in a NaCl brine without any other debittering treatment, so oleuropein is removed through diffusion from the flesh to the brine as well as by acid hydrolysis (BRENES BALBUENA *et al.*, 1992) in a period ranging from 6 months to one year. Fermentation as well is somewhat different, as a lactic acid microflora develops in the brines of Spanish processed olives, while mainly fermentative yeasts are found in the brines of Greek-style olives. All these variations lead to different kinds of products from the sensory point of view.

Production of black olives with the Greek-style has dropped considerably since 1960, but it still accounts for 30% of the world production. Italy, with about 40,000 tonnes produced in the south-central regions, is the third world producer. Sardinia is ranked fifth in the national production of table olives (SINI, 1993). Although the Sardinian production is mostly from green olives placed directly in the brine (natural-style), there is renewed interest among producers to process olives according to the Greek-style, due both to an increasing demand by the local market and to the possibility of reducing the debittering time by controlling fermentation with oleuropenolytic *Lactobacillus plantarum* strains (CIAFARDINI *et al.*, 1994).

Sardinian olive germplasm includes various cultivars that have performed well when processed according to the Spanish or the natural style. Reports on the response of the main local cultivars to the Greek-style, however, are rather incomplete. Marketing and sensory parameters have been thoroughly dis-

cussed, but the evolution of the main chemical parameters during fermentation has not been treated sufficiently (DENTI, 1982; SCHIRRA and AGABBIO, 1982; AGABBIO and MULAS, 1987). Moreover, the so called "afarolado", which is the formation of gas pockets in the fruit pulp and which is a serious defect of this processing method, has never been considered.

Hence, the aim of this study was to check the response of black ripe olives of three Sardinian olive cultivars to Greek-style processing, using the traditional anaerobic fermentation method. Special attention was given to some chemical parameters of the brine and fruit, including the evolution of the main bitter phenolic compound, oleuropein, in the flesh during 150 days of fermentation.

MATERIALS AND METHODS

Cultivar selection

Cultivars were selected according to DENTI (1982) and AGABBIO and MULAS (1987). Marketing (n. olives/kg, mean weight, % flesh and pit, flesh to pit mean ratio) and technological (percentage distribution in each calliper class) parameters were considered, as well as the aptitude of the cultivar to the Greek-style processing method. Based on these results the cultivars "Bosana", "Manna" and "Sivigliana sarda" were chosen for the investigation.

Harvesting and sampling

"Manna" and "Sivigliana sarda" were harvested during the first ten days of January and "Bosana" during the second ten days of February. Fruits of each cultivar were hand harvested at the black stage of ripeness (3/4 of the flesh was black) in an irrigated orchard in Sassari and immediately transported to the laboratory. Only olives free of blemishes, cuts and insect punctures were selected. The olives, calibrated with laboratory callipers, ranged from 16 to 21 mm in transversal diameter, and were equally divided into three 11 kg replications.

Processing

After washing with tap water to remove dust, the olives were put into 20 L sterilised plastic containers (two per cultivar) filled with a freshly prepared 8% (w/w) NaCl brine. The brine concentration was kept constant throughout the fermentation process. In particular, corrections were made at 5, 10, 15, 30 days and afterwards at monthly intervals. The olives were held submerged in the brine by a perforated cap. The lids of the containers were not totally screwed down during the first 10 days, in order to allow most of the CO_2 evolving from fermentation and fruit respiration to escape. After that, the containers were completely filled with fresh brine and closed tightly, so that air could not enter (to avoid growth of oxidative yeasts and moulds on the brine surface). The olives were kept in the fermentation brine for 150 days, then removed from the containers washed in tap water and placed in open air to oxidise and regain a uniform peel colour. After that they were packed with a freshly prepared 8% sodium chloride brine.

Assessment and determination

The following determinations were carried out at harvest on three replicates of 100 olives per cultivar: Marketing parameters: N. olives/kg, mean weight, relative percentage of flesh and pit, flesh to pit mean ratio; Technological parameters: percentage distribution in each calliper class; chemical parameters on homogenised flesh: pH, reducing sugars (Fehling method), protein content by the Kjeldahl method and percent dry matter and moisture in a vacuum oven (AOAC, 1990). These determinations were also

carried out at 150 days. Sodium chloride was analysed potentiometrically according to the method of HERRINGTON and KLEYN (1960). Phenols were extracted following AMIOT *et al.* (1986) with some modifications. Ten grams of olive pulp from ten olives were homogenised in a mill with 0.8 g of sodium metabisulphite (to stop enzymatic oxidation). The paste was then mixed with 100 mL of 80% methanol and homogenised for 2 min with an Ultraturrax on an ice bath. The resulting slurry was filtered under vacuum and the filtrate was saved. The operation was repeated twice. Solvent was evaporated in a rotary evaporator, and the resulting concentrate was collected and extracted four times in a separatory funnel with petrol ether to remove pigments and lipids. An equal volume of 20% ammonium sulphate was added to the residue and 20% metaphosphoric acid was used to adjust the pH to 2.6. The resulting volume was measured and 20% methanol was added. A final triple extraction in a separatory funnel with an equal volume of ethyl acetate was performed. The extracts were evaporated to dryness in a rotary evaporator, collected with 10 mL of methanol, filtered with a 0.45 μm filter and stored at -18°C until analysis. On this extract either total phenols were determined spectrophotometrically following SINGLETON and ROSSI (1965) or oleuropein was quantified by HPLC. For this purpose an HP 1050 chromatograph (Hewlett Packard, Palo Alto, California) equipped with a 1,050 pump and a 1,050 model diode array detector (DAD) were used. The column was a 250x4 mm i.d. Hibar RP 18 (Merck, Darmstadt, Germany) particle size 7 μm. Mobile phases were: eluent A: water plus 2.5% glacial acetic acid, eluent B: methanol:acetonitrile, 50:50. Eluent B was pumped as follows: 5% at 0 time to 15% at 20 min, then brought to 30% at 40 min, to 70% at 50 min, to 100% at 55 min, with a final hold of 5 min. Flow rate was 0.8 mL/min; column temperature was 30°C; injection volume was 5 μL (5 μL loop). Detection of phenols was carried out at 280 nm. Oleuropein was identified by comparison of both retention time and UV spectra (220-400 nm range) of the sample with that of an authentic standard (Extrasinthèse, Genay Cedex, France). Quantitative calculation of oleuropein was made using an HP Chemstation against the same standard (external). Two other compounds, verbascoside and hydroxytyrosol, were identified by comparison with spectra following AMIOT *et al.* (1986) and ESTI *et al.* (1998) and their chromatographic area recorded.

During processing at fixed times (5, 10, 20, 30, 60, 90, 120 and 150 days), the flesh of each olive cultivar was analysed for sodium chloride, total phenols and oleuropein (except at 5 and 10 days for oleuropein). The percent area of verbascoside and hydroxytyrosol was also determined, with respect to harvest time. The determinations were repeated in triplicate for each olive cultivar, (2 containers/cultivar), giving a total of six determinations.

At the above cited intervals, brines were also analysed for pH with a glass electrode. Free (g of lactic acid per 100 mL of brine), combined (mEq/L) and volatile acidity (g of lactic acid per 100 mL of brine), and reducing sugars (g of glucose/100 mL of brine) were determined according to GARRIDO FERNANDEZ *et al.* (1997).

Laboratory personnel (10 persons) performed an informal tasting at 150 days of brining. In particular, they were asked to detect off-flavours and to indicate the cultivar they preferred. Moreover, they judged consistency and crispness of the olives. Judgements of panelists were reported as a written comment and not as a rating.

Incidence of "alambrado" was calculated as the percentage of decayed fruits after taking 200 fruits from the top part of each container (affected olives tend to float) and checking for shrivelling and gas pockets both on the peel and in the flesh.

RESULTS AND DISCUSSION

Marketing, technological and chemical parameters at harvest

Data related to marketing and chemical (Table 1) and technological parameters (Table 2) show that the three olive cultivars can be considered as table olives. The flesh to pit ratio was always greater than 3, which is considered the threshold for table olive processing. Moreover, they were well distributed in the 15-20 mm calliper range and easily exceeded 70% of processable product, except for "Bosana", with more than 50% of the olives below 15 mm (data not shown). The main calliper classes were 17-18 for "Manna" and "Sivigliana sarda" and 15-16 for "Bosana". Percent dry matter and lipid content were quite similar in the three cultivars (the latter data not shown), while protein ranged from 0.92 to 1.44%. The pH value at harvest was close to 5 for all three cultivars.

Chemical parameters of brine

When olives are placed in brine drastic physical-chemical changes occur. Water soluble compounds diffuse into the brine, while salt goes from the brine into the flesh. Micro-organisms ferment some sugars to give other compounds (mainly lactic acid). The development of the different classes of micro-organisms (bacteria and yeasts), are influenced strictly by these variations and vice versa. Thus, by controlling the physical chemical parameters it is usually possible to predict which micro-organisms will grow and to avoid alterations or development of toxic microflora.

The pH and free acidity of the brine are of paramount importance from the technological and sanitary point of view when black olives are processed according to the Greek-style. The pH decreased sharply after only a few days in all three cultivars (Fig. 1). Values dropped from

Table 1 - Main marketing and chemical-physical parameters of "Bosana", "Manna" and "Sivigliana sarda" olives at harvest and after 150 days of processing with the Greek-style.

Cultivar	Sampling	N. olives/kg	Mean weight (g)	Flesh/pit	Main calliper (mm)	pH	Acidity (% citric acid)	Dry matter (%)	Moisture (%)	Protein (%)
Bosana	harvest	366	2.73	3.02	15-16	4.81	0.101	51.90	48.10	1.44
	150 days	-		-	-	5.11	-	46.93	53.07	1.79
Manna	harvest	216	4.62	3.65	17-18	5.25	0.091	50.60	49.40	0.92
	150 days	-		-	-	5.32	-	45.10	54.90	1.12
Sivigliana sarda	harvest	201	4.97	3.33	17-18	5.15	0.096	46.68	53.32	1.28
	150 days	-		-	-	5.23	-	42.65	57.35	1.40

Table 2 - Technological parameters of unprocessed "Bosana", "Manna" and "Sivigliana sarda" olives.

Technological parameters	Calliper classes[x] (mm of transversal diameter)					
Bosana	15-16	16-17	17-18	18-19	19-20	>20
Percent per class of calliper	65.63	27.87	6.28	0.22	-	-
N. olives/kg/class	376	366	>366	n.d.	-	-
Flesh/pit	2.76	2.89	3.1	3.2	-	-
Manna	15-16	16-17	17-18	18-19	19-20	>20
Percent per class of calliper	-	25.26	36.2	18.49	9.3	10.75
N. olives/kg/class	-	222	218	215	212	210
Flesh/pit	-	3.33	3.5	3.63	3.7	4.1
Sivigliana sarda	15-16	16-17	17-18	18-19	19-20	>20
Percent per class of calliper	-	35.15	43.43	13.22	4.83	3.37
N. olives/kg/class	-	245	219	186	165	187
Flesh/pit	-	2.73	3.2	3.33	3.6	3.8

[x] Distribution in each calliper class was calculated considering only processable olives and not fruit that had been classed under 15 mm of calliper.

7.5-7.7 at the start of the experiment to 4.65, 4.72 and 4.8 for "Bosana", "Sivigliana sarda" and "Manna", respectively, after 5 days. This pattern agrees with that observed by others during the first days of fermentation of both green or black olives processed according to the natural or Greek-style (DEIANA *et al.*, 1992; BORCACKLI *et al.*, 1993). After this drop the pH increased by 0.1 unit by the 10th day and then decreased again until reaching a steady-state at the beginning of the second month of brining, with values ranging from 4.65 to 4.70. This is in accordance with BRIGHIGNA *et al.*, (1978) for this kind of table olive processing and shows that fermentation is mainly due to yeasts. In fact, during fermentation in anaerobic conditions, apart from the first days when Gram-negative Coli-aerogenes bacteria develop with abundant gas production, different yeasts, but also some *Lactobacillus* species, are able to grow (BALATSOURAS, 1967). Anyway, BALATSOURAS (1990) reported that, when the final pH of the brine ranges from 4.5 to 4.8, fermentative yeasts are the main micro-organism that grow in the medium, as *Lactobacillus* development would have reduced the pH to 4.0.

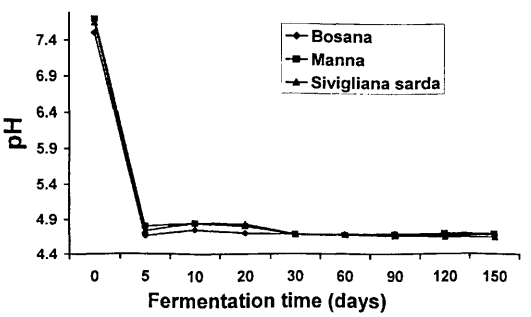

Fig. 1 - Trend of pH in brines of fermented "Bosana", "Manna" and "Sivigliana sarda" table olives during 150 days of brining. Each value is the mean of six determinations.

Free acidity values further confirm this hypothesis (Fig. 2). A weak acidity, below 0.6 g of lactic acid per 100 mL of brine, was attained in the brine of "Sivigliana sarda" and "Bosana" olives, while "Manna" cv reached a value of 0.45. According to some authors these levels of free acidity are evidence of fermentative yeast activity (BRENES BALBUENA *et al.*, 1986; FARRIS *et al.*, 1989; BALATSOURAS, 1990; MAR-

QUINA *et al.*, 1992; BORCACKLI *et al.*, 1993). In part the trend of free acidity mirrored the pH changes, i.e., the lower the pH, the higher the free acidity. Sometimes this pattern was not followed; probably because the three olive cultivars have different buffering capacity, which depends strictly on the acids arising from fermentation (BALATSOURAS *et al.*, 1983; GARRIDO FERNANDEZ *et al.*, 1997). In any case, the free acidity was quite low and stable during the first ten days of fermentation; then it increased and reached a steady state at the second month.

The high and constant sodium chloride concentration of the brines surely played an important role in driving the fermentation process. The decision to fix and maintain the sodium chloride concentration at 8% to prevent growth of putrefactive micro-organisms surely resulted in an inhibition in the growth of lactic acid bacteria (GONZALÉZ CANCHO, 1975), while yeasts tolerate higher salt concentrations.

Combined and volatile acidity also followed an expected trend. The former was in the range of 50-60 mEq/L at the end of the 150 days, while the latter was about 0.17 g lactic acid per 100 mL of brine for the three cultivars (data not shown). Combined acidity in this kind of product comes from organic acids, both from the olives (mainly polyphenolic) and from the fermentation process and reaches the values observed in this experiment. This leads to a maximum buffering capacity at pH 4.8 (BALATSOURAS, 1966; GARRIDO FERNANDEZ *et al.*, 1972), which is a value very close to what was attained and maintained starting from the thirtieth day of the process.

It is pointed out that either pH or free acidity reached the steady state starting form the 60th day of fermentation and after that very little variation occurred. Two hypotheses can be formulated to explain this: a) absence of oxidative or optional yeast growth such as *Candida* spp., *Debariomyces hansenii*, *Pichia membranefaciens*, *Torulopsis* spp., etc., that results in lactic acid uptake and pH increase and which is favoured by the presence of air on the container surface due to poor air-tight sealing (FERNANDEZ GONZALEZ *et al.*, 1993); b) fermentation has stopped from lack of sugar, thus lactic acid can not be produced and the pH does not decrease.

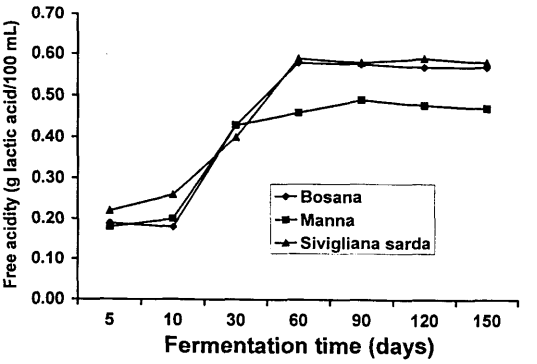

Fig. 2 - Changes in free acidity in brines during 150 days of fermentation of "Bosana", "Manna" and "Sivigliana sarda" table olives. Each value is the mean of six determinations.

The first situation can be easily detected, as growth of those micro-organisms causes the formation of a film on the brine surface; this was avoided by strictly monitoring and adjusting the salinity of the brine and controlling the air-tight closure of the containers. The lack of fermentable compounds is shown in Fig. 3, which indicates that sugars diffused rapidly into the brine reaching a maximum at the second month, followed by a sharp decrease, with small differences among cultivars; this resulted in similar pH and free acidity values, as previously reported.

Diffusion of sugars depends on different factors such skin permeability, fruit to brine ratio, salt concentration and temperature (GARRIDO-FERNANDEZ *et al.*, 1997); thus one of these surely affected the rate of the osmotic process. The residual 0.1–0.2% sugar content in the brine after 150 days can be due to the method of determination, which can be

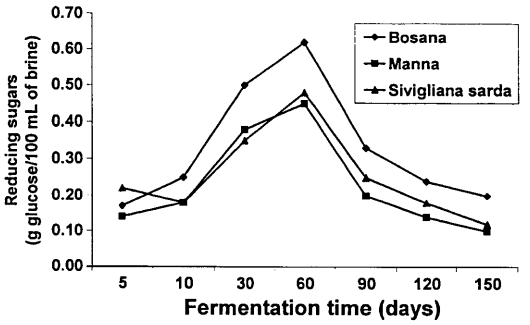

Fig. 3 - Reducing sugars in brines of "Bosana", "Manna" and "Sivigliana sarda" table olives during 150 days of brining. Each value is the mean of six determinations.

influenced by the interference of other organic compounds diffusing from olives to brine, such as polyphenols, which are easily oxidised by the Fehling reactive.

The fact that reducing sugars decreased after 60 days, while the pH did not change, could be due to the buffering capacity of the brines.

Chemical parameters of fruit flesh

The evolution of sodium chloride in the flesh is reported in Fig. 4. Total content in the fruit flesh ranged from 3.4 to 3.7 g per 100 g of flesh. Sodium chloride increased progressively in the flesh during processing, but a steady state was attained at different times, that is at 60, 90 and 120 days, for "Manna", "Bosana" and "Sivigliana sarda", respectively. The latter cultivar showed a slower rate of salt uptake, with respect to the other two. Diffusion of salt from brines to olives, due to the selective permeability of the fruits, is somewhat slow, as reported by GARCIA-GARCIA *et al.* (1982) and BORCACKLI *et al.* (1993).

Some others parameters such as moisture and protein content at the end of fermentation are reported in Table 1, which shows that both increased. The high pH at the end of the process may be of concern from the microbiological point of view, indicating that the product should be sterilised. Changes in the total phenol content of the flesh of the fruits during processing are reported in Fig. 5. Olives from "Bosana" cultivar had a very high content of phenols, compared to the other two cultivars and to reference data (GARRIDO-FERNANDEZ *et al.*, 1997). This is also confirmed by comparison of chromatograms (Fig. 6), in which the area of almost all peaks (Fig. 6C) was larger compared to the other two cultivars (Fig. 6A-6B). The amount of hydroxytyrosol, oleuropein and verbascoside from harvest to end of processing are

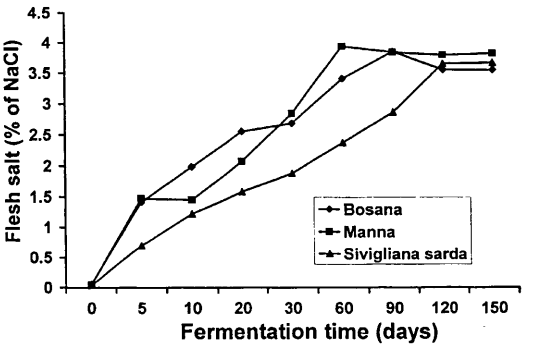

Fig. 4 - Total flesh salt content at harvest and during 150 days of fermentation with the Greek style of "Bosana", "Manna" and "Sivigliana sarda" table olives. Each value is the mean of six determinations.

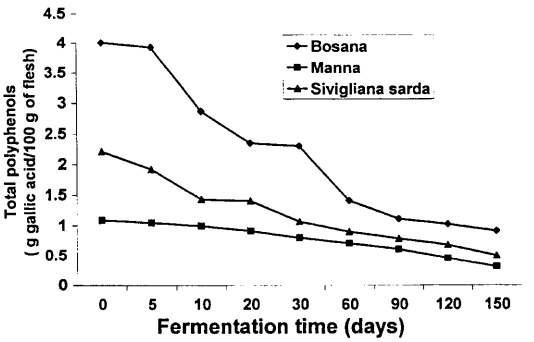

Fig. 5 -Total polyphenols in the flesh of "Bosana", "Manna" and "Sivigliana sarda" table olives during 150 days of brining. Each value is the mean of six determinations.

reported in Table 3. AMIOT *et al.* (1986) reported that the smaller the olive the higher or the lower the oleuropein or the verbascoside content, respectively. The data in Table 3 for harvest time show that "Bosana" olives, the smallest of the three cultivars, do not follow this rule. Moreover "Bosana" olives were harvested at the fully ripe stage, when verbascoside should have been very low, according to AMIOT *et al.* (1986). The evolution of oleuropein in the flesh showed a similar pattern for the three cultivars, with a consistent decrease after 20 days, accounting for 40, 63 and 80% with respect to that at harvest for "Sivigliana sarda", "Manna" and "Bosana", respectively. In general, hydroxytyrosol and verbascoside showed a similar pattern, with an initial rapid reduction in the first 30 days, followed by a slower one. Removal of the bitter taste from olives prepared with the Greek method is mainly attributed to diffusion of oleuropein from the flesh to the brine, even if BRENES-BALBUENA *et al.* (1992) report that acid hydrolysis of oleuropein may occur in acidic conditions. We can exclude this, since acid hydrolysis occurs only at a pH of 4.2-4.3, which was not reached in this experiment. Moreover, acid hydrolysis of oleuropein can be excluded, as it would have led to an increase of hydroxytyrosol content in the flesh. The UV spectra of oleuropein, hydroxytyrosol and verbascoside are reported in Fig. 7.

Sensory determinations

In general, the panelists did not indicate any off-flavours that could have derived from malodorous fermentations, such as butyric acid fermentation by *Clostridium* growth or the more frequent and deleterious "zapateria" by *Clostridium and Propionibacterium* development, which imparts a bad leather smell and taste (BALATSOURAS, 1990).

Although the pH and the free acidity were low enough to favour the spread of these micro-organisms, the continuous control of sodium chloride in the brines surely inhibited these fermentations, as reported by (GARRIDO-FERNANDEZ *et al.*,

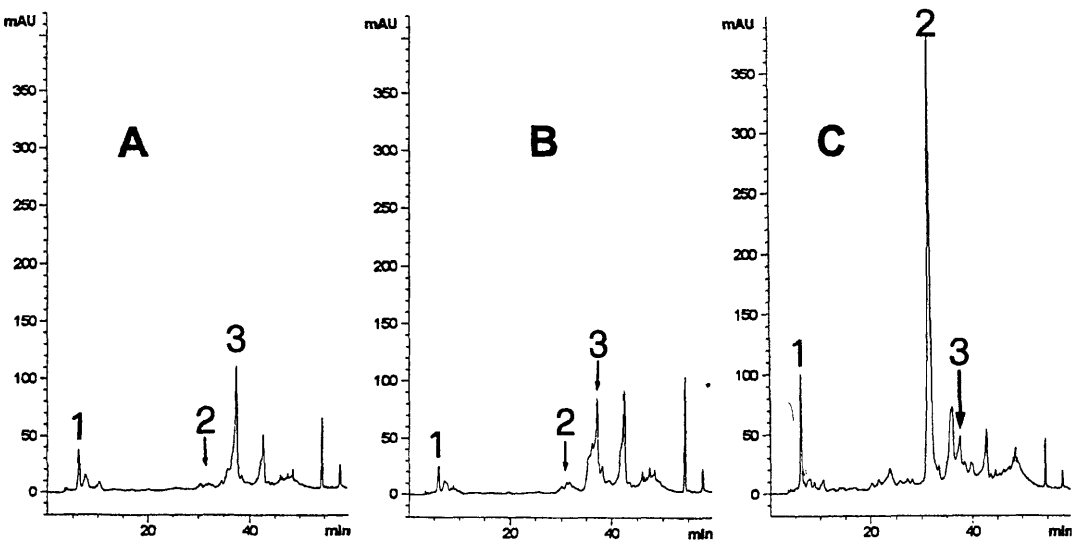

Fig. 6 - HPLC profiles of phenolic compounds extracted from fresh "Sivigliana sarda" (A), "Manna" (B) and "Bosana" (C) olives. Detection was carried out at 280 nm. Numbers on peaks identify hydroxytyrosol (1), verbascoside (2) and oleuropein (3).

1997). On the other hand, apart from the residual bitterness, which consumers like, and which totally disappeared in "Manna" olives, the fermented fruit had a well balanced taste and satisfactory consistency. Panelists considered the olives excellent and ready-to-eat after 150 days of brining. The fact that the

Table 3 - Oleuropein, hydroxytyrosol and verbascoside content in the flesh of "Bosana", "Manna" and "Sivigliana sarda" olives during 150 days of Greek-style fermentation.

Cultivar	Sampling (days)	Oleuropein (mg/g fw)	Hydroxytyrosol (% CA*)	Verbascoside (% CA)
Bosana	Harvest	1.401	100	100
	20	0.275	60.4	75.2
	30	0.256	53.7	33.8
	60	0.088	50.0	29.8
	90	0	46.8	24.1
	120	0	46.2	16.6
	150	0	42.5	12.4
Manna	Harvest	1.769	100	100
	20	0.676	74.1	46.4
	30	0.670	73.8	24.6
	60	0.353	51.7	19.3
	90	0.212	38.2	6.7
	120	0.102	21.1	5.2
	150	0.048	19.6	4.7
Sivigliana sarda	Harvest	2.469	100	100
	20	1.508	98.9	91.8
	30	1.461	74.7	87.6
	60	0.753	73.2	86.5
	90	0.660	76.2	85.7
	120	0.359	58.8	36.8
	150	0.184	52.2	32.5

* Percent of chromatographic area. Amounts were calculated as relative percentage area of peaks considering the harvest sampling as 100%.

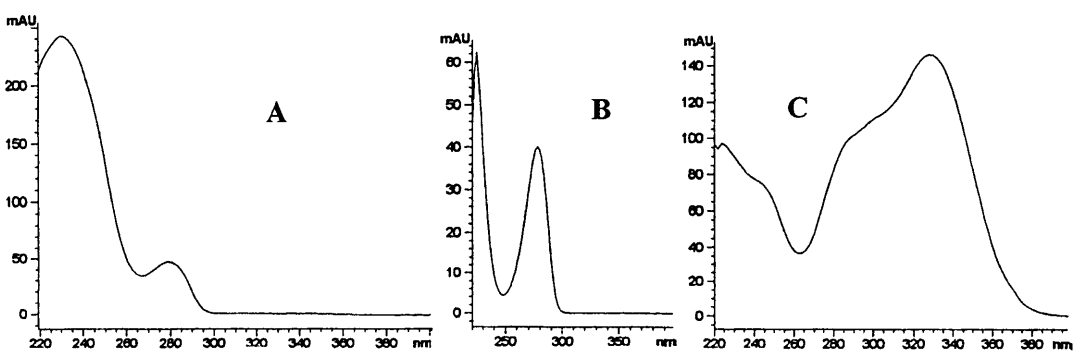

Fig. 7 - UV spectra of oleuropein (A), hydroxytyrosol (B) and verbascoside (C).

judges indicated that the "Bosana" olives had a bitter taste may be ascribed to the high amount of residual verbascoside, as oleuropein was not detected in these olives from the 90th day of processing. The panelists judged the "Bosana" olives as having better consistency and crispness (data not shown). The colour of the olives was good and uniform, but only after exposure to air for "Manna" and "Sivigliana sarda", while this step was not required for "Bosana" olives, that retained their original colour. Moreover, data from an unpublished preliminary trial showed that this cultivar can withstand pH values of 4.0 without irreversible discolouration, so initial pH acidification of the brine would be possible.

Product loss due to gaseous alterations was very low in the three cultivars and never exceeded 10%, with "Bosana" olives being rarely affected (data not shown). The main defect was what the Spanish call "afarolado", that occurs as pressurised gas between the peel and the flesh and that results in a transparent pocket. Shrivelling, another serious problem of this processing style, was not observed.

CONCLUSIONS

Data related to the chemical evolution of brines showed that the three Sardinian olive cultivars underwent fermentation mainly by yeasts. In particular pH and free acidity reached a steady state from the thirtieth to the sixtieth day, surely due to the process being strictly conducted in anaerobic conditions and to the low sugar content in the flesh. The latter parameter showed a rapid diffusion rate in the brine, as after sixty days of brining, reducing sugars were scarcely detectable. "Bosana" olives had a very high content of total phenols, which, contrary to expectations showed oleuropein and verbascoside content as the lowest and the highest, respectively; their pattern of disappearance from the flesh was similar. Hydroxytyrosol, on the other hand, did not increase in the fruits, suggesting that oleuropein removal was only through diffusion into the brine. Although oleuropein was not found in the "Bosana" cultivar after the third month of processing, the bitter taste was still detectable by the panelists, thus confirming the role of other phenols, such as verbascoside, giving a bitter taste to the olives. Even though all three cultivars were judged as excellent, "Bosana" was preferred by the panelists due to better texture. Incidence of alterations was very low, especially for the "Bosana" olives. Moreover, exposure to air was not necessary for this cultivar, which retained its original colour.

Taking these fact into account, it is concluded that the three olive cultivars are well adapted to table processing with the Greek style using traditional anaerobic fermentation. It is emphasized that good sanitary practice, strict control of both anaerobic conditions and concentration of sodium chloride allow the production of good black ripe table olives after five months of brining. The olives, anyway, due to their pH, should be stabilised from the microbiological point of view before marketing; pasteurisation or acidification with lactic acid are proposed for this, provided they are tested prior to sale. Despite its small size, the "Bosana" cultivar proved to be the best for this kind of speciality product, because, adjusting and keeping the pH value below the dangerous pH threshold of non acidic foods, fermentation may be carried out without risk of irreversible flesh discoloration.

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