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Chemical profile of industrially fermented green olives of different varieties

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Abstract

Over 160 fermented brines, from green olives of Manzanilla, Hojiblanca, and Gordal varieties processed in five companies in two consecutive seasons, were analysed for physicochemical characteristics, organic acids, sugars, and volatile components. The composition of the brine following fermentation was assumed to be identical to that of the aqueous phase of the olives. Olive variety and processor were found to have a greater influence than season on both physicochemical characteristics and chemical composition. Hojiblanca olives presented values of pH, combined acidity, and volatile acidity significantly ($P<0.05$) higher than those of Manzanilla and Gordal, reflecting different processing conditions. The volatile/total acidity ratio, which did not differ between varieties or seasons, appeared to correlate with development of the "fourth stage" of fermentation. The major compounds were lactic, acetic, succinic and formic acids, ethanol, and methanol, with the contents of ethanol and formic acid being significantly different in all three varieties. Residual fermentation substrates, such as mannitol, glucose, sucrose, and citric acid, in addition to propanol, propionic acid, 2-butanol, and acetaldehyde, were found in low concentrations.

Keywords: Chemical; Table olive; Olive variety; Processing; Fermented olive brine

1. Introduction

Spanish-style green olive production in the EU is about 290 000 t/year; Spain is the main producer, with some 250 000 t/year (IOOC, 1999). The procedure for preparing this product consists of treating the fruits with dilute NaOH solution, followed by one or two water washes to remove the excess alkali. Subsequently, a 10–13% (w/v) NaCl solution is added to the fruits, in which they undergo a spontaneous lactic acid fermentation (Fernández-Díez et al., 1985). Microbial growth during fermentation can be considered in four, extensively studied, stages (Borbolla y Alcalá & Rejano, 1979, 1981; Vaughn, 1985). Free acidity, combined acidity, pH, and salt are the main chemical characteristics analysed in brine during fermentation. Under normal conditions, the product in bulk should have a pH value of 3.8–4.2, a free acidity (expressed as lactic acid) of 0.8–1.2%, a combined acidity of 0.09–0.11 N, and a salt concentration of 5–6% (Fernández-Díez, 1983). Another chemical characteristic is volatile acidity. Its normal range has not been indicated, but values may vary from 0.4–0.7%, expressed as lactic acid (Ramos, Vega, & Ladrón, 1979). A more detailed study of this characteristic could yield useful information about development of the "fourth stage" of fermentation, characterised by growth of species of *Propionibacterium,* with formation of propionic and acetic acids at the expense of lactic acid, and concomitant increase in both volatile acidity and pH (Borbolla y Alcalá, Rejano, & Vega, 1975; Cancho, Rejano, & Borbolla y Alcalá, 1980; Rejano, Cancho, & Borbolla y Alcalá, 1978).

Evolution of the main fermentation substrates and end-products during both spontaneous and controlled green olive fermentation has been recently studied (Sánchez, de Castro, Rejano, & Montaño, 2000). Until now, however, the concentration ranges of these compounds in the industrially processed fermented product have not been reported. Such information would supply a more complete chemical profile for this product, which would be useful for detecting cases outside the

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''normal'' range, and help decide corrective methods for improving the processing or preventing the appearance of microbial spoilage. Among other lactic acid-fermented vegetables, chemical characterisation of the commercial product has been reported for sauerkraut (Trail, Fleming, Young & McFeeters, 1996).

The chemical profile of Spanish-style green olives may vary from season to season, or as a consequence of the different way of processings, from one company to another. It is also known that, for each processor, the initial treatments (lye treatment, washing) are olive variety-dependent, due to the different physical properties (texture, size) of each variety, but it is not clear whether this affects the chemical profile of the final product.

The objectives of this work were to characterise Spanish-style green table olives in bulk using chemical methods (titrimetric and chromatographic analyses) and to study the effect of olive variety, processor, and season on mean data of both physicochemical characteristics and chemical composition. The composition of the aqueous phase of the olives was assumed to be identical to that of the brine following fermentation.

2. Materials and methods

2.1. Samples

Brine samples from Spanish-style green olives of Manzanilla, Hojiblanca, and Gordal varieties were collected in five companies (A–E) during April–early May of two consecutive seasons. In our lab, these samples were kept frozen until their analysis for physicochemical characteristics (pH, salt, free acidity, combined acidity, volatile acidity) and chemical components (sugars, organic acids, and volatile compounds). The number of samples included was 69 (27, first season; 42, second season), 68 (23; 45) and 30 (13; 17) for Manzanilla, Hojiblanca, and Gordal varieties, respectively.

2.2. Analysis of physicochemical characteristics

Analyses of olive brine for pH, free acidity, combined acidity, volatile acidity, and salt (%, w/v, NaCl) were carried out by the routine methods (Fernández-Díez et al., 1985). Values of the different acidity parameters were expressed as % (w/v) lactic acid.

2.3. Analysis of organic acids

The HPLC methods described by Sánchez et al. (2000) were used. Malic, citric, lactic, acetic, succinic, and propionic acids were analysed using a Spherisorb ODS-2 (5μm, 25 cm × 4 mm i.d., Teknokroma, Barcelona, Spain) column with deionised water (pH adjusted to 2.2 using concentrated H_3PO_4) as mobile phase at a flow rate of 1.0 ml/min. Formic acid was analysed using an Aminex HPX-87H (300 × 7.8 mm i.d., Bio Rad Labs, Richmond, CA) column, held at 65 °C, with 0.005 M H_2SO_4 as mobile phase at a flow rate of 0.7 ml/min.

2.4. Analysis of sugars

Sucrose, glucose, fructose, and mannitol were analysed by HPLC using an Aminex HPX-87C column (300×7.8 mm i.d., Bio Rad Labs) held at 70 °C and deionised water as eluent at 0.7 ml/min according to the method described by Sánchez et al. (2000).

2.5. Analysis of volatile components

Acetaldehyde, ethanol, methanol, diacetyl, 2-butanol, and *n*-propanol were analysed by GC using a Supelcowax 10 fused-silica capillary column (30 m×0.53 mm i.d. 1.0μm film thickness, Supelco, Bellefonte, PA) following the headspace method described by Montaño, Sánchez, and Rejano (1990), except that the sample was heated at 80 °C. Acetoin and 2,3-butanediol were analysed by GC using a PorapaK Q (mesh 80–100, 2 m × 0.25 in o.d.) column, as described by Sánchez et al. (2000).

2.6. Statistical analysis

Statistica software version 5.5 (StatSoft Inc., Tulsa, OK) was used for data processing. One-way analysis of variance (ANOVA) was used to test the effects of variety, season, and processor factors. Duncan's multiple range test was used to compare means when a significant variation was highlighted by ANOVA. Significance was defined at $P < 0.05$.

3. Results and discussion

3.1. General

Since all samples were collected in April–May, after 6–7 months of brining, we assumed that fermentable material had already been exhausted, and equilibrium between olive juice and the surrounding brine had been reached for physicochemical characteristics, as well as for individual compounds. Sánchez et al. (2000) found that, once fermentation substrates were exhausted, equilibrium between brine and olive juice for metabolic products was reached in a few days at 25 °C, although it appeared to be reached more slowly for physicochemical parameters, such as free acidity or combined acidity. Under equilibrium conditions, chemical analysis of olive juice should not significantly differ from that of its corresponding brine. For this reason, and because brine samples are easier to handle than olive juice, brine samples were used in the present study.

3.2. Physicochemical characteristics

Mean, range, and coefficient of variation for physicochemical characteristics of brines— that is, salt, pH, free acidity (FA), combined acidity (CA), and volatile acidity (VA)—were the following: pH, 4.04, 3.65–4.40, and 3.6%; salt, 6.3%, 4.0–9.9%, and 14.7%; FA, 0.93%, 0.35–1.41%, and 18.2%; CA, 1.16%, 0.54–2.32%, and 23.8%; VA, 0.60%, 0.27–1.06%, and 20.7%. For selected ratios, namely, FA/VA, CA/VA, CA/FA, and VA/TA, where TA is the total acidity (FA + CA), the corresponding values were: FA/VA, 1.57, 1.02–2.72, and 19.8%; CA/VA, 1.94, 1.31–3.14, and 15.9%; CA/FA, 1.27, 0.58-2.15, and 22.2%; and VA/TA, 0.29, 0.19–0.37, and 12.5%. Since large numbers of samples were used, the present survey gives a complete and true picture of physicochemical characteristics found today in industry. Ranges in pH, free acidity, and combined acidity were higher than the normal ranges cited by Fernández-Díez (1983). In addition, the range in volatile acidity was higher than that found by Ramos et al. (1979) who analysed only a few samples for each olive variety.

The patterns of physicochemical profiles between processors, seasons, and olive varieties were quite similar (graphs not shown). Moreover, with the exception of salt content, no significant ($P < 0.05$) differences were found between seasons for each physicochemical characteristic (Table 1). However, physicochemical characteristics were generally affected by processor (free acidity was the only exception) and olive variety. The mean value of free acidity for Manzanilla olives was slightly lower than those for Hojiblanca and Gordal. Greater differences were found for combined acidity, with Hojiblanca olives presenting the highest values. This result appears to be in connection with either the higher concentration of NaOH or longer duration of lye treatment generally used for Hojiblanca olives because of the higher texture of this variety in comparison with that of Manzanilla and Gordal (Borbolla y Alcalá, 1981; Fernández-Díez et al., 1985). The higher volatile acidity for Hojiblanca olives can be similarly explained. Formation of volatile acidity during lye treatment in green olives was demonstrated by Borbolla y Alcalá et al. (1956). For the FA/VA ratios, variations in volatile acidity appear to explain the observed differences between olive varieties. Variations in combined acidity values appear to be responsible for the varying of both CA/VA and CA/PA ratios. For all the experimental data, the pH values were directly related to both the CA/FA ratio, as expected according to the Henderson–Hasselbach equation, and VA values. The equation showing the best fit was the following:

$$\text{pH} = 3.40 + 0.43\ (\text{CA/FA}) + 0.15\ \text{VA} \qquad r = 0.874$$

For individual olive varieties, the following equations were obtained:

Manzanilla

$$\text{pH} = 3.31 + 0.45\ (\text{CA/FA}) + 0.31\ \text{VA} \qquad r = 0.839$$

Hojiblanca

$$\text{pH} = 3.63 + 0.33(\text{CA/FA}) \qquad r = 0.803$$

Gordal

$$\text{pH} = 3.23 + 0.45\ (\text{CA/FA}) + 0.32\ \text{VA} \qquad r = 0.952$$

In the case of Hojiblanca olive brines, the factor multiplying volatile acidity was not significant at the 5% level.

The VA/TA ratio did not differ between varieties or seasons. In 15 industrially fermented green olives of

Table 1
Main effects of olive variety, season and processor on physicochemical characteristics of Spanish-style green olives[a]

Physicochemical characteristic[b]	Variety			Season		Processor				
	Hojiblanca	Manzanilla	Gordal	First	Second	A	B	C	D	E
Salt (%)	5.9 a	6.7 b	6.1 a	6.7 b	6.0 a	6.3 c	5.9 c	6.0 c	7.7 a	6.9 b
pH	4.12 b	4.00 a	3.96 a	4.04 a	4.04 a	4.03 b	4.08 ab	4.05 ab	4.13 a	3.90 c
CA(%)	1.34c	1.00a	1.12b	1.13a	1.18a	1.04b	1.28a	1.22a	1.23a	0.98b
FA (%)	0.96 b	0.89 a	0.97 b	0.91 a	0.95 a	0.91 a	0.94 a	0.95 a	0.90 a	0.95 a
VA (%)	0.66 b	0.56 a	0.59 a	0.58 a	0.62 a	0.52 c	0.64 ab	0.63 ab	0.67 a	0.58 bc
FA/VA	1.48 a	1.61 b	1.71 b	1.59 a	1.56 a	1.75 a	1.48 bc	1.54b	1.34 c	1.63 ab
CA/VA	2.07 b	1.82 a	1.90 a	1.97 a	1.91 a	1.98 a	1.99 a	1.95 a	1.95 a	1.68 b
CA/FA	1.41b	1.16a	1.19a	1.29a	1.26a	1.18cd	1.37ab	1.30bc	1.46a	1.04d
VA/TA	0.29 a	0.29 a	0.29 a	0.29 a	0.29 a	0.27 b	0.29 ab	0.29 ab	0.31 a	0.30 a

[a] Means with different letters within a row for each effect are significantly different ($P < 0.05$).

[b] FA, free acidity; CA, combined acidity; VA, volatile acidity; TA, total acidity (= FA + CA). All of these characteristics are expressed as % lactic acid.

different olive varieties (all with clear evidence of the presence of the "fourth stage") analysed in previous studies (unpublished data), a mean±SD value of 0.42±0.05 was obtained for the VA/TA ratio (range: 0.35–0.52). That mean value was significantly ($P<0.05$) higher than the mean value (0.29±0.04, $n=167$) obtained in the present study, where presence of the "fourth stage" was negligible (see later). It is concluded that the VA/TA ratio could be used as an index to know the degree of development of the "fourth stage" of fermentation in Spanish-style green olives. Determination of this ratio, requiring only routine analysis of physicochemical characteristics, would be particularly interesting for labs of small olive-processing companies, which in general suffer from shortage of both equipment and skilled personnel.

3.3. Chemical composition

Fig. 1 shows the mean profile of the major compounds in brine after lactic acid fermentation for all samples. Lactic and acetic acids, along with ethanol and methanol, were present in all cases. Their means, ranges, and coefficients of variation were the following: lactic acid, 138 mM, 54–245 mM, 23%; acetic acid, 42 mM, 16–83 mM, 27%; ethanol, 21 mM, 4.6–53 mM, 49%; and methanol, 19 mM, 0.4–36 mM, 35%. Formic acid (12 mM, 0–28 mM, 54%) was present in all but six samples, and succinic acid (4 mM, 0–9.5 mM, 49%) in all but 13. Other compounds detected in most samples, but in small amounts (mean concentration <1 mM), were n-propanol, 2-butanol, and acetaldehyde. Residual fermentation substrates, namely, glucose, sucrose, mannitol, and citric acid, were detected in a limited number ($n<40$) of samples, where their mean concentrations were 0.8, 0.7, 8.4, and 1.3 mM, respectively (data not shown). Propionic acid was also detected in a limited number ($n=48$) of samples, in a concentration range of 0.4–11 mM. This represented between 1 and 22% of this acid relative to the total amount of acetic and propionic acids (data not shown). Based on the scale proposed by Rejano et al. (1978), these relative amounts of propionic acid can be classified as negligible (<10%) or scarce (11–22%), indicating that growth of *Propionibacterium* species, characteristic of the "fourth stage" of fermentation, was insignificant. Montaño, de Castro, Rejano, and Brenes (1996) found concentrations of propionic acid between 22 and 42 mM in olive samples with abundant "fourth stage" development (> 33% propionic acid). Fructose, diacetyl, acetoin, and 2,3-butanediol were not detected in any of the samples of the present study. This contrasts with the findings of Sánchez et al. (2000), who found relatively large amounts (11–13 mM) of 2,3-butanediol immediately after spontaneous green olive fermentation. In the present study, this compound may have been metabolised by some species of yeast during the olives' prolonged brining (6–7 months) following lactic acid fermentation. This assumption warrants confirmation by further investigation. The enzyme 2,3-butanediol dehydrogenase has been found in *Saccharomyces cerevisiae* (Heidlas & Tressl, 1990), a species of yeast frequently found in green olive fermentation (Fernández-Díez et al., 1985). The chemical profile of another typical fermented vegetable—sauerkraut—has been found to have noticeable differences from that of Spanish-style green olives; in particular, it has higher contents of residual glucose, mannitol, and ethanol, confirming a greater involvement of heterofermentative lactic acid bacteria in sauerkraut fermentation (Fleming, Kyung & Breidt, 1995; Trail et al., 1996).

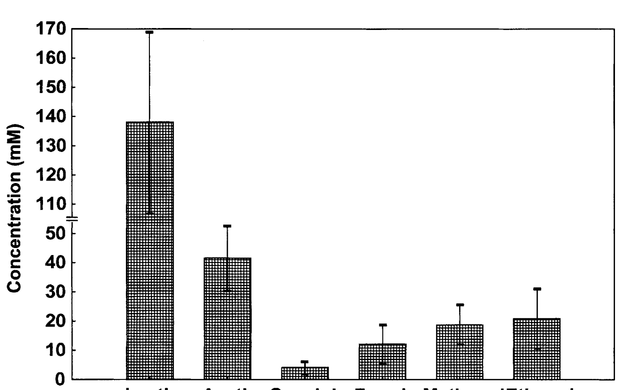

Fig. 1. Major compounds profile of industrially fermented green olives. Error bars represent ±1 standard deviation, $n=167$.

As with physicochemical characteristics, the patterns of chemical composition profiles were similar between olive varieties, seasons, and processors (graphs not shown), but the mean concentrations of the major compounds significantly ($P<0.05$) differed between olive varieties and processors; methanol and succinic acid were also affected by season (Table 2). Lactic acid in Manzanilla variety was significantly lower than in Hojiblanca and Gordal, consistent with values of free acidity. Acetic acid was significantly higher in Hojiblanca than in Manzanilla and Gordal, consistent with values of volatile acidity. Methanol in Gordal was lower than in Hojiblanca and Manzanilla. The contents of formic acid and ethanol were significantly different in all three varieties, both contents following the order Gordal > Hojiblanca > Manzanilla.

Of the minor compounds, mannitol was higher in Hojiblanca than in Manzanilla and Gordal. Since initial quantities of mannitol appear to be similar in all three varieties (Fernández-Bolaños, Fernández-Díez, Moreno, Serrano, & Romero, 1983; Marsilio, Campestre, Lanza, & De Angelis, 2001), this result could be explained by a lower rate of fermentation in the case of Hojiblanca, due mainly to a lower ambient temperature (harvest time and subsequent processing for Gordal and

Table 2
Main effects of olive variety, season and processor on chemical composition of Spanish-style green olives[a]

Compound (mM)	Variety			Season		Processor				
	Hojiblanca	Manzanilla	Gordal	First	Second	A	B	C	D	E
Lactic acid	152b	118a	152b	136a	139a	122c	155a	143ab	138bc	122c
Acetic acid	47.5 b	37.1 a	39.0 a	43.3 a	40.7 a	38.9 bc	44.1 b	41.0 bc	51.5 a	36.9 c
Ethanol	21.9b	17.4 a	26.5 c	22.7 a	19.8 a	24.1 a	23.2 a	18.7 a	20.2 a	12.5 b
Methanol	20.2 b	18.9 b	15.7 a	22.7 b	16.5 a	17.3 c	18.5 c	21.9 b	25.4 a	15.0 c
Formic acid	12.1 b	9.4 a	18.3 c	12.0 a	12.2 a	7.7 b	13.9 a	14.3 a	10.1 b	14.1 a
Succinic acid	4.2 b	3.7 a	4.7 ab	5.1 b	3.5 a	3.5 b	4.3 ab	4.9 a	4.6 ab	3.4 b
Mannitol	4.0b	0.6a	0.6a	0.4a	3.0b	1.8ab	4.1 a	0.6b	0.0b	0.2b
Propanol	0.9 a	0.5 a	2.3 b	1.4 a	0.8 a	1.1 ab	1.4 a	0.4 b	1.5 a	0.4 b
Propionic acid	0.6 a	0.4 a	0.9 a	1.5 b	0.0a	0.5 b	0.5 b	0.6 b	2.1 a	0.0 b
2-butanol	0.3 b	0.1 a	0.4 b	0.3 a	0.3 a	0.2b c	0.4 a	0.2 bc	0.3 ab	0.1 c
Glucose	0.2 a	0.2 a	0.1 a	0.0 a	0.3 b	0.4 a	0.1 a	0.1 a	0.0 a	0.4 a
Acetaldehyde	0.1 a	0.1 a	0.1 a	0.1 a	0.1 a	0.2 a	0.1 a	0.1 a	0.1 a	0.1 a
Sucrose	0.0 a	0.0 a	0.1 a	0.1 a	0.0 a	0.1 a	0.0 a	0.1 a	0.1 a	0.0 a

[a] Means with different letters within a row for each effect are significantly different ($P < 0.05$).

Manzanilla is normally between mid-September and early October, whereas the season for Hojiblanca commences about one month later). Propanol was higher in Gordal than in Manzanilla and Hojiblanca, and 2-butanol was lower in Manzanilla than in Hojiblanca and Gordal. The other compounds (acetaldehyde, propionic acid, sucrose, glucose) did not differ between varieties.

4. Conclusions

This is the first time that a survey of physicochemical characteristics and chemical composition in industrially fermented green olives of different varieties has been carried out. Lactic, acetic, formic and succinic acids, ethanol, and methanol were found as the major compounds. Residual fermentation substrates and other compounds such as propanol, 2-butanol, acetaldehyde, and propionic acid, which are not characteristic of lactic acid fermentations, were also detected in small amounts. The chemical profile patterns were similar between olive varieties, seasons, and processors, but mean values of both the physicochemical characteristics and the major compounds were significantly ($P < 0.05$) affected (mainly by olive variety and processor). Hojiblanca variety showed the highest values of pH, combined acidity, and volatile acidity, while the physicochemical characteristics of Manzanilla variety hardly differed from those of Gordal. The volatile/total acidity ratio could be used as an index showing the degree of development of the "fourth stage" of fermentation. The contents of volatile compounds (ethanol, propanol, formic acid) were highest for Gordal variety, which could be a reflection of a more intense aroma in this variety. However, in the present study only the major volatile components, partially responsible for aroma, were analysed. Further research, including a correlation between chemical composition of the aroma and sensory analyses, is needed to characterise the quality of Spanish-style green olives from a flavour standpoint.

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