



Fermentation in nutrient salt mixtures affects green Spanish-style Manzanilla table olive characteristics



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ABSTRACT

This work studies the effects of the substitution of NaCl with KCl and CaCl₂ on the physicochemical, mineral and sensory profile of fermented green Spanish-style Manzanilla olives, using an enlarged centroid mixture design. An increasing presence of CaCl₂ in the initial brines improved the colour index, *L**, *b** values, and firmness. The Na in the olives decreased (linearly) while the levels of K and Ca increased (quadratic) as a function of the KCl and CaCl₂ concentrations in the initial brines. CaCl₂ also improved the retention of Zn and P in the flesh. PLS showed a strong relationship between Ca and bitterness, hardness, fibrousness, crunchiness and saltiness (negative) and allowed for the prediction of sensory attributes (except acid) from the mineral contents in the flesh. Most of the treatments could lead to new green Spanish-style Manzanilla olive presentations with reduced Na and healthier characteristics.

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1. Introduction

The salt concentration in table olives depends on the processing system (Garrido-Fernández, Fernández-Díez, & Adams, 1997). In green olives, the NaCl concentration during the post-fermentation bulk storage may reach up to 90 g/L brine, although this level is usually reduced later during the packaging process to ~50 g/L brine a concentration in the final products (Garrido-Fernández et al., 1997). The substitution of NaCl by other mineral nutrient salts in fermentation would lead to healthier fermented products. The modifications might potentially affect around 1,500,000 t/year olive product (60% of the production) (IOC (International Olive Council), 2015). Currently, these olives are treated with a sodium hydroxide solution (20–30 g/L) until the alkali reaches 2/3 of the flesh. Then, they are washed and, afterward, immersed in a 100–110 g/L NaCl solution where they undergo a spontaneous lactic acid fermentation (Garrido-Fernández et al., 1997).

The addition of nutrients to foods, including minerals, is permitted by Regulation (EC) No 1926/2006, and Commission Implementing Regulations (EU) No 307/2012 and No 489/2012. They aim “to regulate the addition of different nutrients that are added to foods

under conditions that result in the ingestion of amounts greatly exceeding those reasonably expected to be ingested under normal conditions of consumption of a balanced and varied diet”. Among the minerals that can be added are calcium and potassium; however, the incorporation of magnesium to the fermentation brine is questionable due to the insignificant effect of this element, at the concentrations that could be added, on the common table olive microbial flora (Bautista-Gallego, Arroyo-López, Durán-Quintana, & Garrido-Fernández, 2008). The usual high proportion of sodium in table olives suggests the importance of developing products with reduced (healthier) levels of this element but fortified with K and Ca. Such innovation would improve the consumers’ opinion of this food and could contribute to reducing their daily Na intake, which has been established at 2000 mg sodium, or 5 g salt, by WHO/FAO (World Health Organization/Food (2003).

Preliminary results on the NaCl reduction in table olives have already been reported (Bautista-Gallego, Rantsiou, Garrido-Fernández, Cocolin, & Arroyo-López, 2013; Kanavouras, Gazouli, Tzouveleki, & Petrakis, 2005; Marsilio, Campestre, Lanza, de Angelis, & Russi, 2002). A detailed study of the NaCl replacement in brined cracked olives was published by Bautista-Gallego, Arroyo-López, Durán-Quintana, and Garrido-Fernández (2010). The substitution of NaCl with KCl and CaCl₂ during the fermentation of Gordal showed significant effects on both fermentation (Rodríguez Gómez, Bautista-Gallego, Romero Gil, Arroyo-López, & Garrido Fernández, 2012) and final product profiles

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(Moreno-Baquero, Bautista-Gallego, Garrido-Fernández, & López-López, 2012). However, the physicochemical and sensory profiles of fermented green Spanish-style Manzanilla olives have not yet been studied.

Multivariate analysis and quantitative descriptive analysis (QDA) have been useful for the development of new products (Leighton, Schönfeldt, & Kruger, 2010) and for describing the sensory profiles of diverse foods (Lee & Chambers, 2010). Usually, QDA is combined with experimental design (Myers & Montgomery, 2002) and multivariate (chemometric) analysis for describing relationships among variables and treatments (Tenenhaus, Pagés, Ambroisini, & Guinot, 2005).

This work studies the effects of fermenting green Spanish-style Manzanilla table olives in brines composed of mixtures of NaCl, KCl, and CaCl₂ on their chemical and sensory characteristics. Colour parameters, firmness, mineral content, and sensory characteristics were used to generate Response Surfaces (based on an enlarged centroid mixture design) as a function of the concentrations of the diverse salts. Multivariate analysis was used for revealing relationships among variables and between them and treatments (runs). The results can contribute to a better understanding of the role of the diverse mineral nutrients in table olive perception and the development of healthier presentations.

2. Materials and methods

2.1. Experimental design and samples

The experiment was carried out with Manzanilla fruits, supplied by JOLCA SA. (Seville, Spain). The design consisted of 15 independent runs from an enlarged simplex centroid mixture design with three replicates (Table 1). The total initial concentrations of the diverse salts were constrained to [NaCl] + [KCl] + [CaCl₂] = 100 g/L (the usual NaCl level utilized by the industry), with NaCl ranging from 40 to 100 g/L, KCl from 0 to 60 g/L and CaCl₂ from 0 to 60 g/L. Details of the debittering and fermentation processes are described elsewhere (Bautista-Gallego et al., 2015). After fermentation, olives from all runs were subjected to mineral content in flesh and sensory analyses.

2.2. Analyses of colour and firmness in olives

CIE coordinates *L** (lightness), *a** (negative values indicate green while positive values indicate magenta), and *b** (negative values indicate blue and positive values indicate yellow) of fermented

fruits were obtained using a BYK-Gardner Model 9000 Colour-view spectrophotometer (Columbia, USA) equipped with computer software to calculate the CIE coordinates. Samples were covered with a box with a matt black interior to prevent interference by stray light. Each determination was the average of 20 olive measurements. The colour index (*C_i*) was estimated using the formula:

$$C_i = \frac{-2R_{560} + R_{590} + 2R_{630} + 2R_{640}}{3} \quad (1)$$

where *R_s* stand for the reflectance at 560, 590 630, 640 nm (Sánchez, Rejano, & Montaña, 1985).

2.3. Sensory analysis

The sensory analysis was conducted by a panel of 9 experienced judges from the staff of the Food Biotechnology Department. All panelists have participated in previous table olive classification tests (Moreno-Baquero, Bautista-Gallego, Garrido-Fernández, & López-López, 2013; Moreno-Baquero et al., 2012), have between 4 and 15 years' experience and were familiar with sensory studies on table olive presentations. The tests were performed in individual booths under controlled conditions of light, temperature and humidity. For the analysis, only the descriptors included in the Sensory Analysis for Table Olives (IOC (International Olive Council), 2010a) sheet were used due to its familiarity to panelists and their clear meaning for consumers. Despite this background, the panelists were trained for the proper assessment of the descriptors and the objectives of the QDA in this experiment (Stone & Sidel, 2003). The samples were served in the cups recommend by the Method for Sensory Analysis of Table Olives (IOC, 2010b), coded with a 3-digit random number, and presented in a randomized balanced order to the panelists (Mailgaard, Cville, & Carr, 1991). Only four runs per session were tested. Samples were analysed in duplicate.

Olives were first tested for the perception of negative sensations (abnormal fermentation type and other defects: musty, rancid, cooking effect, soapy, metallic, earthy, winey-vinegary) and classified (IOC (International Olive Council), 2010a). Then, the analysis for descriptors corresponding to gustatory sensations (acidity, saltiness, bitterness) and kinaesthetic sensations (hardness, fibrousness, crunchiness) was carried out. The olives were scored according to a 10-cm unstructured scale. Anchor ratings were 1 (no perception) and 11 (extremely strong) for gustatory perceptions and low and high levels for kinaesthetic sensations (Lee & Chambers, 2010). The marks on the evaluation sheet were transformed into data by taking measurements (in 0.1 cm) from the left

Table 1
Spanish-style Manzanilla green olives fermented in salt mixtures. Expanded simplex centroid mixture design used in the experiment. Nutrient salt concentrations in the initial brine. Constrains were: NaCl + KCl + CaCl₂ = 100 g/L, with NaCl ranging from 40 to 100 g/L, KCl from 0 to 40 g/L, and CaCl₂ from 0 to 60 g/L. Colour and firmness characteristics, according to runs.

Treatments	NaCl (g/L)	KCl (g/L)	CaCl ₂ (g/L)	Colour index	<i>L*</i>	<i>a*</i>	<i>b*</i>	Firmness (kN/kg olive flesh)
1 ^a	100	0	0	26.37 (0.23)	52.10 (1.30)	3.20 (0.09)	37.38 (1.20)	22.1 (1.9)
2	50	10	40	28.99 (0.29)	55.19 (1.90)	3.05 (0.15)	39.55 (1.13)	30.0 (1.8)
3 ^b	40	30	30	28.64 (0.25)	54.90 (2.00)	2.50 (0.18)	39.91(0.18)	27.9 (2.6)
4	70	30	0	26.79 (0.30)	53.46 (1.93)	3.00 (0.25)	39.14 (0.91)	12.3 (2.0)
5	80	10	10	26.55 (0.23)	53.84 (2.10)	2.40 (0.31)	39.83 (0.87)	29.2 (1.5)
6 ^c	60	20	20	28.72 (0.27)	55.40 (0.90)	2.25 (0.15)	41.58 (0.37)	26.9 (1.6)
7	50	40	10	29.67 (0.36)	56.01 (2.15)	2.13 (0.17)	42.26 (0.89)	24.8 (2.3)
8	40	60	0	26.29 (0.31)	52.92 (2.45)	2.46 (0.21)	39.17 (0.74)	10.9 (1.0)
9 ^a	100	0	0	25.54 (0.24)	51.99 (2.35)	2.95 (0.13)	37.36 (0.69)	18.7 (1.2)
10 ^d	40	0	60	30.14 (0.32)	55.95 (1.95)	2.67 (0.19)	42.22 (0.59)	29.2 (1.5)
11 ^b	40	30	30	31.36 (0.41)	54.02 (1.92)	1.70 (0.13)	42.86 (0.76)	24.0 (1.9)
12 ^d	40	0	60	32.86 (0.29)	57.91(2.34)	2.37 (0.21)	43.58 (0.59)	32.7 (1.8)
13	70	0	30	31.99 (0.51)	58.25 (1.78)	2.00 (0.20)	39.79 (1.12)	27.1 (2.4)
14	40	60	0	25.82 (0.32)	52.99 (3.01)	2.44 (0.16)	40.03 (0.51)	13.0 (1.2)
15 ^c	60	20	20	30.68 (0.42)	57.25 (2.96)	2.07 (0.22)	40.90 (1.37)	28.2 (1.6)

Notes: Runs with the same superscript correspond to duplicate experiments; standard error in parenthesis.

anchor. The average scores (descriptors and runs) were standardized (Hibbert, 2009), and the values were treated as responses for the mixture design and PLS analyses.

2.4. Sodium, potassium, and calcium analysis in flesh

Mineral nutrients were analysed by atomic absorption spectrophotometry (López-López, García-García, & Garrido-Fernández, 2008), using a GBC model 932 AA (Victoria, Australia), atomic absorption spectrometer, equipped with three hollow multi-element cathode lamps, (Cu, and Mn) (GBC, Victoria, Australia), (Ca, Mg, and Zn) (Photron, Victoria, Australia), and (Na and K) (Photron, Victoria, Australia).

2.5. Effects of initial brine composition on the mineral content and on the sensory profile of fermented fruits

The Response Surface Methodology (RSM), based on an enlarged mixture design, was used to model each variable (Myers & Montgomery, 2002). In the canonical (Sheffé) form, the model took the expression:

$$R = \sum_{i=1}^3 \beta_i x_i + \sum_{i<j=2}^3 \beta_{ij} x_i x_j + \sum_{i<j<k=2}^3 \beta_{ijk} x_i x_j x_k + \varepsilon \quad (2)$$

where the variables x_i , x_j , and x_k stand for NaCl, CaCl₂, and KCl concentrations in the initial brines; R is the dependent variable/transformed value, the β s are the coefficients to be estimated and ε the error (Bautista-Gallego, Arroyo-López, López-López, & Garrido-Fernández, 2011).

2.6. Chemometric analysis

2.6.1. Auto-scale and sensory score centering

For the statistical analysis, mineral content data were auto-scaled (Kowalski & Bender, 1972) to prevent bias due to differences in scales while descriptor values were centered by assessors, a transformation that is particularly recommended when the analysis is focussed on the products and descriptors and not on panelists, as in this study (Hibbert, 2009).

2.6.2. Principal Component Analysis (PCA) and Partial Least Square analysis (PLS)

PCA is a procedure for condensing the maximum amount of variance with the minimum number of uncorrelated variables (usually called principal components or Factors). It is widely used to reduce the number of variables and avoid multicollinearity. PLS analysis is useful for studying the relationships between product characteristics and sensory judgments (Tenenhaus et al., 2005). Normally, the products (n) are described by a set (p) of characteristics (X table, dimensions n and p) and are the subject of q sensory judgments (Y table, dimensions n and q). For the representation, the principal components were replaced by the PLS components coming from the PLS regression of Y on X (Tenenhaus et al., 2005). The variables and runs are represented by coordinates equal to their correlations with the first two PLS components.

2.7. Statistical data analysis

Design Expert v6.06 (Stat-Easy Inc, Minneapolis, USA) was used for deducing the experimental design and its analysis. Modeling consisted of hypothesizing a model and checking its corresponding ANOVA for fit. The process was stopped when no further improvement in the variance explained was obtained. Coefficients were selected ($p \leq 0.05$) using the backward option. Nevertheless, those required to fulfill the hierarchical principle, necessary to transform

the equation back into actual percentages, were also retained (regardless of their significance). PCA and PLS analyses were performed with XLSTAT for Excel, v 2015 (Addinsoft, New York, USA).

3. Results and discussion

3.1. Effect of the initial composition of fermentation brines on colour

The average values of the colour parameters (responses) of the fermented olives (Table 1) were subjected to regression analysis as a function of the initial brine salt mixtures.

The models suggested for the colour parameters (except a^* , which was not related) were linear (C_i and b^*) and quadratic (L^*) (see Table 1 in López-López et al., submitted). The contour curves of C_i equation (see Fig. 1, panel A, in López-López et al., submitted) show an increase in C_i (high values are preferred) as the concentration of CaCl₂ is greater with the lines almost parallel to the NaCl-KCl base, regardless of the concentrations of these salts. Hence, only CaCl₂ affected and improved, the C_i .

L^* is a measurement of olive surface luminance. In Spanish-style olives, the higher the value of this parameter the better the colour. The equation corresponding to this parameter (see Table 1, Eq. (2), in López-López et al., submitted) reached its maximum in the NaCl-CaCl₂ base, at ~60 g/L NaCl and 40 g/L CaCl₂ (see Fig. 1, panel B, in López-López et al., submitted). The surface was convex with the minimum decreasing, along the line represented by the proportion of the two other salts, as the KCl content increased.

The contour lines of the equation corresponding to b^* (see Table 1, Eq. (3), in López-López et al., submitted) were perpendicular to the base NaCl-CaCl₂ (see Fig. 1, panel C, in López-López et al., submitted). Therefore, the changes in this parameter were affected by the NaCl:CaCl₂ ratio (the lower it was, the higher the values of b^*) but not by KCl. As high values of b^* are associated with yellowness, the presence of CaCl₂ improves b^* .

Hence, increasing proportions of CaCl₂ improves the C_i and L^* colour parameters of green Spanish-style Manzanilla olives (characterized by its yellowness). However, b^* reaches its maximum in the absence of KCl (NaCl-CaCl₂ base) and at 40 g/L CaCl₂.

3.2. Effect of the initial composition of the fermentation brines on instrumental firmness

The firmness was related to the initial salt concentrations by a cubic model (see Table 1, Eq. (4), in López-López et al., submitted). The firmness is represented as a hill with the lowest level on the NaCl-KCl base (see Fig. 1, panel D, in López-López et al., submitted). Then, firmness ascends towards the CaCl₂ vertex and has its steepest crest along the 50:30 (NaCl:KCl) ratio. Above ~20 g/L CaCl₂ concentration, the response reaches a plateau (possibly due to calcium saturation). This, approximately, includes the area within the polygon with vertexes in the barycenter (the central points of the CaCl₂-NaCl and CaCl₂-KCl axes, composed of 40 g CaCl₂ with 40 g/L of NaCl or KCl) and the CaCl₂ vertex. This saturation of the available bounding radicals in the flesh is in agreement with the results reported by Brenes, García, and Garrido (1994) for green (natural or lye treated) olives in an acidic medium. Therefore, concentrations of up to (or slightly above) 20 g/L CaCl₂ in the initial fermentation brines of green Spanish-style Manzanilla olives are convenient due to the favorable effect of calcium on firmness.

3.3. Effect of the initial composition of the fermentation brines on sodium, potassium and calcium contents in flesh

The use of salt mixtures in the initial fermentation brine led to a broad range of Na, K and Ca concentrations in the flesh (Table 2).

Table 2
Spanish-style Manzanilla green olives fermented in salt mixtures. Average content (\pm standard error) in mineral nutrients.

Treatments	[Na] _{pulpa} (mg/kg flesh)	[K] _{pulpa} (mg/kg flesh)	[Ca] _{pulpa} (mg/kg flesh)	[Mn] _{pulpa} (mg/kg flesh)	[Cu] _{pulpa} (mg/kg flesh)	[Zn] _{pulpa} (mg/kg flesh)	[Mg] _{pulpa} (mg/kg flesh)	[P] _{pulpa} (mg/kg flesh)
1	17,158 (139)	791 (10)	573 (7)	0.71 (0.01)	2.37 (0.02)	3.07 (0.04)	57.83 (3.09)	115.16 (0.89)
2	8985 (22)	2614 (14)	6009 (27)	0.34 (0.02)	2.33 (0.01)	3.30 (0.04)	74.36 (1.70)	124.04 (0.83)
3	6676 (42)	7444 (36)	4809 (54)	0.29 (<0.01)	1.94 (0.04)	3.04 (0.02)	54.55 (1.64)	123.84 (2.59)
4	11,634 (70)	7618 (94)	674 (12)	0.65 (0.01)	1.77 (0.02)	2.75 (0.08)	75.15 (3.95)	119.05 (1.22)
5	13,719 (231)	2676 (46)	2652 (35)	0.46 (0.01)	2.08 (0.22)	3.14 (0.04)	83.82 (2.39)	118.76 (2.14)
6	9555 (44)	4779 (18)	3464 (14)	0.48 (0.02)	2.19 (0.11)	2.91 (0.02)	69.59 (3.43)	125.57 (1.24)
7	9371 (28)	9727 (92)	2504 (16)	0.53 (0.02)	1.95 (0.02)	2.81 (0.02)	69.04 (1.22)	117.80 (0.39)
8	7567 (20)	17,802 (89)	684 (13)	0.54 (0.03)	2.02 (0.05)	2.62 (0.03)	63.49 (1.24)	113.79 (1.01)
9	19,412 (54)	682 (<1)	671 (13)	0.48 (0.01)	1.99 (0.16)	3.11 (0.01)	58.93 (2.78)	119.09 (1.62)
10	7111 (41)	956 (21)	8086 (42)	1.33 (0.01)	2.04 (0.09)	3.42 (0.13)	65.79 (1.42)	124.83 (1.44)
11	7174 (25)	7484 (17)	4791 (40)	0.61 (0.03)	1.67 (0.16)	3.58 (0.08)	65.82 (1.50)	116.81 (0.61)
12	6862 (46)	717 (7)	7942 (69)	0.58 (0.02)	1.96 (0.18)	3.57 (0.11)	71.74 (0.34)	123.31 (0.94)
13	11,851 (103)	817 (14)	4879 (17)	0.49 (0.01)	2.38 (0.02)	2.84 (0.02)	63.30 (3.57)	113.79 (0.79)
14	8342 (148)	17,392 (89)	522 (4)	0.34 (0.02)	1.77 (0.16)	3.04 (0.01)	47.46 (0.56)	109.93 (0.57)
15	10,563 (52)	5489 (180)	3488 (10)	0.41 (0.03)	1.92 (0.08)	3.11 (0.01)	60.47 (0.85)	123.07 (0.32)

Note: standard error in parenthesis.

The equations for Na and K as functions of the initial mixtures were linear and quadratic, respectively (see Table 1, Eqs. (5) and (6), in López-López et al., submitted). The equation for Ca was also significant, except for the lack of fit. Hence, in this case, only the trend observed is mentioned.

The contour lines from Na equation (see Table 1, Eq. (5), in López-López et al., submitted) show that the content of this mineral in flesh decreases as the proportions of CaCl₂ or/and KCl in the initial brines are higher (Fig. 1, panel A). At the maximum levels of these salts, the Na in the flesh may be reduced by about 50% (~8000 mg/kg). Owing to the inclination of the contour lines,

the presence of CaCl₂ produces a slight interference as NaCl decreases (Fig. 1, panel A). The Na reduction may decrease the contribution of these olives to the daily intake (DI) of Na from ~70% to ~40%, considering a serving size of 100 g. The potassium content in table olives is negatively affected by the debittering and washing processes that remove a sensible proportion of its original concentrations in the fresh fruits (Garrido-Fernández et al., 1997). Its addition to the fermentation brine might counterbalance such leakage. The graphical presentation of its equation (see Table 1, Eq. (6), in López-López et al., submitted) as a function of the salt mixtures (Fig. 1, panel B) shows that the contour lines were

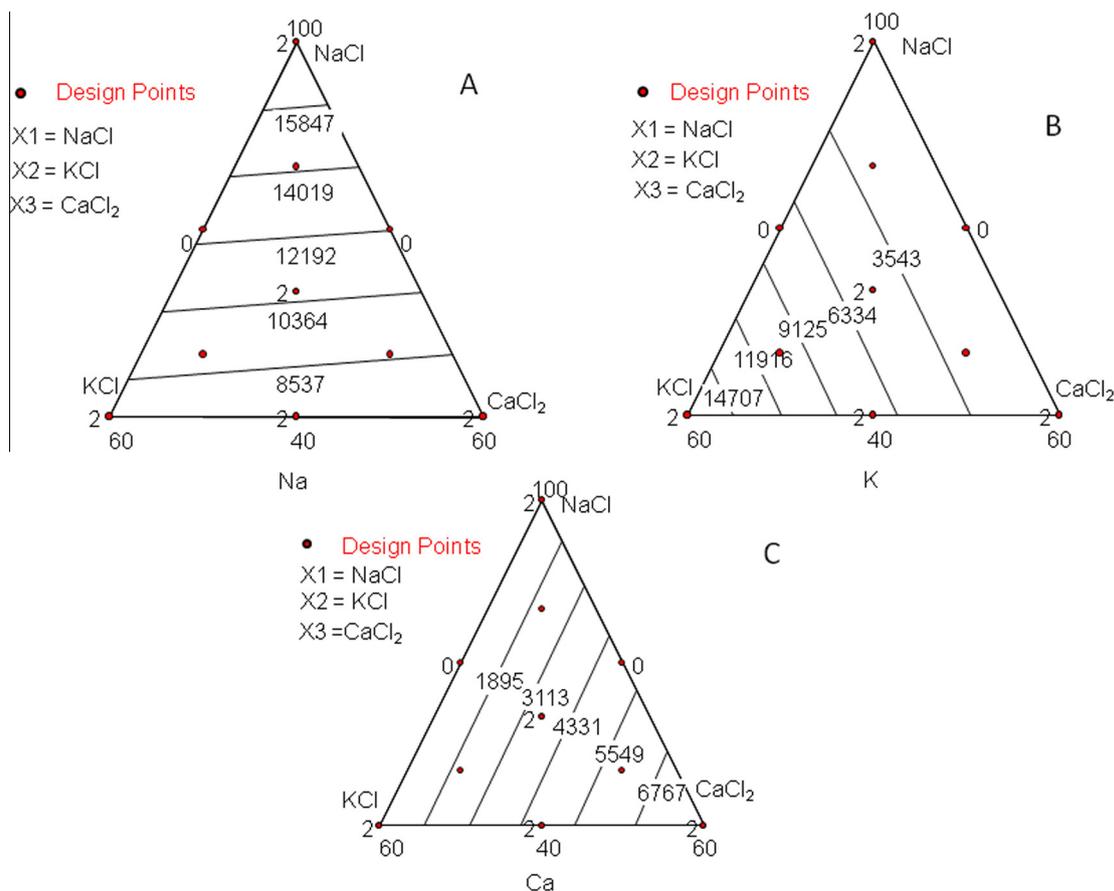


Fig. 1. Contour lines of A) sodium, B) potassium and C) calcium contents in the flesh as a function of the NaCl, KCl and CaCl₂ concentrations in the initial mixture brines (details of runs in Table 1). Duplicate design points are indicated by a two close to them.

parallel to the NaCl-CaCl₂ base. That is, the K content in flesh increases proportionally to the KCl content in the brining solution, without interference from NaCl or CaCl₂. The use of KCl in the fermentation brine can multiply the K content by up to five times the concentration expected in its absence. The use of salt mixtures would then increase the contribution of these olives to the DI of K from ~3% to ~50%, considering a serving size of 100 g flesh.

The model suggested for the Ca content in the flesh was cubic, had a significant fit ($p < 0.0001$), explained a high proportion of variance (99.91%) and had a good precision (65, far above 4, the lowest limit to be considered significant). However, it had significant ($p = 0.0083$) lack of fit. Its contour lines are then used as an approach for the trend observed in the Ca content according to the salt concentrations in the initial brines. The contour lines (Fig. 1, panel C) shows that the Ca content in flesh increases progressively as the proportion of CaCl₂ in the mixtures was higher, without any interference from KCl or NaCl (the contour lines are parallel to the base NaCl-KCl). The use of salt mixtures in the fermentation brine would then increase the contribution of these olives to the recommended DI of Ca from ~20% to ~90%, considering a 100 g flesh serving size.

3.4. Effect of the initial composition of the fermentation brines on mineral nutrients originally found in the flesh

The contents of other (non-added) mineral nutrients in the olives from the different runs (Table 2) ranged between the following values: Mn, 0.29–1.33 mg/kg; Cu, 1.67–2.38 mg/kg; Zn, 2.62–3.58 mg/kg; Mg, 47.46–83.82 mg/kg; and P, 109.93–125.57 mg/kg. Only the Zn and P contents in the flesh could be related to the diverse salt concentrations in the initial fermentation brines (see Table 1, Eqs. (7) and (8), in López-López et al., submitted).

For Zn (see Table 1, Eq. (7), in López-López et al., submitted), the contour lines showed that the concentration of Zn increased linearly (Fig. 2, panel A) as the CaCl₂ content in the initial fermentation brine was higher. Hence, the effect of Ca ions in the brine is not, apparently, due to Zn displacement from its complexes in the flesh but to retention. Possibly, Ca had caused a reinforcement of the flesh structure which prevented the Zn leakage. P profile in the flesh as a function of the initial brines (see Table 1, Eq. (8), in López-López et al., submitted) indicated an initial progressive increment in this element at low CaCl₂ to reach a plateau with a maximum concentration of P of about 122 mg/kg flesh (Fig. 2, panel B) at high CaCl₂. The content in P decreases at the sides of this plateau, for both low and high proportions of KCl. The changes in P may be related to the effect of Ca on the flesh (increasing the firmness and strengthening the structure that may prevent P leakage) but also with the lower P consumption by the microorganisms due to the microbial activity reduction caused by the Ca increment (Rodríguez Gómez et al., 2012).

3.5. The relationship between the initial chloride salt concentrations and sensory characteristics of olives

No abnormal fermentation or other defects were detected by more than 50% of panel tasters. Hence, the olives from all runs were considered *Extra* or *Fancy* commercial products.

The mean scores of all descriptors were subjected to PC analysis to check the correct panel scoring (Nyambaka & Ryley, 2004). Only one eigenvalue higher than 1 was found; which means that there were no panelists producing abnormal scores and the individual scores of all the panelists are properly expressed by their average (the first principal component). Furthermore, the contribution of each variable (panelist) to PC1 was similar in sign and magnitude, which indicates a homogeneous scoring. Therefore, the panelists showed a good general scoring ability and produced consistent

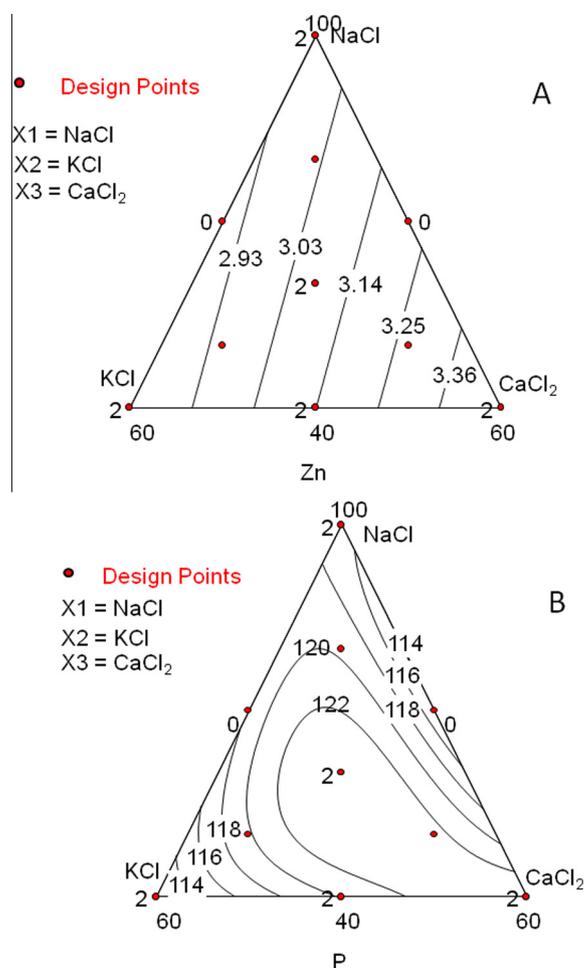


Fig. 2. Contour lines of A) zinc, and B) phosphorus contents in flesh as a function of the NaCl, KCl, and CaCl₂ concentrations in the initial mixture brines (details of runs in Table 1). Duplicate design points are indicated by a two close to them.

data. In this way, its performance was similar to the results found in previous studies (Bautista-Gallego, Moreno-Baquero, Garrido-Fernández, & López-López, 2013; Moreno-Baquero et al., 2012). Therefore, the average sensory values, centered by panelists (Table 3), were considered appropriate responses for the experimental design and analysed for their association with the initial salt contents in brines. All of them showed significant fit and insignificant lack of fit, except acidity, and are predicted as centered values:

The two dimension contour lines of the equation for saltiness (see Table 1, Eq. (9), in López-López et al., submitted) show that the scores of this attribute decreased as the concentrations of NaCl and KCl were lower (see Fig. 2, panel A, López-López et al., submitted). The lines were parallel to the base NaCl-KCl, indicating that the contribution of both salts to saltiness was approximately the same. On the contrary, saltiness decreased as the CaCl₂ concentration was higher; therefore, calcium does not contribute to this gustatory perception. A similar behavior was observed in seasoned cracked olives, but the contour lines were not parallel to the NaCl-KCl but perpendicular to the NaCl-CaCl₂ bases; so, in these olives, the saltiness scores depended more on the NaCl:CaCl₂ ratio than on the presence of KCl (Moreno-Baquero et al., 2013). In fermented green Spanish-style Gordal, the contour lines for saltiness were perpendicular to the base KCl-CaCl₂ and were then more dependent of the KCl:CaCl₂ ratio (Moreno-Baquero et al., 2012). Hence, in table olives, in general, the decrease in NaCl or KCl diminishes saltiness but their contributions depend on the process and/or

Table 3
Spanish-style Manzanilla green olives fermented in salt mixtures. Average centered sensory profile scores for green Spanish-style Manzanilla olives (gustative and kinaesthetic perceptions) according to runs. The concentrations of the diverse salts in the initial brines of runs are indicated in Table 1.

Treatments	Acidity	Saltiness	Bitterness	Hardness	Fibrousness	Crunchiness
1	0.17 (0.04)	0.49 (0.03)	-1.29 (0.03)	-0.48 (0.04)	-0.79 (0.03)	-0.41 (0.03)
2	0.96 (0.04)	0.53 (0.04)	-1.41 (0.04)	-3.87 (0.02)	-2.92 (0.04)	-3.53 (0.03)
3	0.79 (0.03)	0.38 (0.03)	0.80 (0.03)	1.23 (0.02)	0.96 (0.03)	1.20 (0.03)
4	0.41 (0.04)	0.94 (0.03)	-0.74 (0.04)	-0.20 (0.02)	-0.12 (0.02)	-0.69 (0.03)
5	0.19 (0.04)	0.71 (0.02)	-0.78 (0.03)	1.30 (0.02)	1.39 (0.02)	1.29 (0.03)
6	-0.41 (0.04)	0.48 (0.04)	-0.57 (0.03)	1.04 (0.02)	0.25 (0.03)	0.47 (0.03)
7	0.36 (0.03)	0.27 (0.02)	-0.27 (0.03)	1.52 (0.02)	1.08 (0.04)	1.93 (0.04)
8	-0.14 (0.04)	-1.24 (0.05)	-0.21 (0.05)	-4.18 (0.02)	-2.87 (0.03)	-3.58 (0.03)
9	0.37 (0.04)	0.23 (0.03)	-0.16 (0.02)	-2.45 (0.03)	-1.07 (0.03)	-2.36 (0.02)
10	-1.02 (0.05)	-1.64 (0.04)	3.32 (0.04)	2.54 (0.04)	1.08 (0.02)	1.22 (0.04)
11	0.01 (0.03)	0.22 (0.02)	0.04 (0.03)	1.79 (0.03)	1.39 (0.03)	2.06 (0.03)
12	-0.41 (0.04)	-0.70 (0.03)	3.07 (0.04)	2.57 (0.03)	1.64 (0.04)	2.11 (0.04)
13	-0.34 (0.03)	0.33 (0.03)	-0.13 (0.03)	1.31 (0.04)	0.77 (0.02)	1.16 (0.04)
14	-0.76 (0.04)	-1.81 (0.03)	-1.50 (0.03)	-3.38 (0.02)	-2.31 (0.03)	-2.82 (0.03)
15	-0.17 (0.04)	0.82 (0.02)	-0.17 (0.04)	1.26 (0.02)	1.52 (0.03)	1.95 (0.03)

Note: Standard errors in parenthesis.

cultivar. On the contrary, as CaCl_2 concentration increases there is always an apparent decrease in bitterness scores. Thus, calcium does not contribute to this perception nor can mask it. Marsilio, Russi, Iannucci, and Sabatini (2008) also reported that neutralization of the excess alkali with CO_2 after the debittering process may also significantly increase saltiness in Spanish-style olives. For bitterness (see Table 1, Eq. (10), in López-López et al., submitted), the two dimension contour lines show that its scores increased as the NaCl and KCl proportions decreased (see Fig. 2, panel B, in López-López et al., submitted), with similar contributions of both salts (contour lines almost parallel to the KCl-NaCl base) (see Fig. 2, panel A, López-López et al., submitted). The apparent increase in bitterness as CaCl_2 increases can be related to a decrease in the masking effect of the other two salts or to a direct effect of Ca presence. Therefore, the changes in saltiness and bitterness showed opposed trends. The average bitterness was observed for 40 g/L KCl and 20 g/L NaCl concentrations and around 20 g/L a CaCl_2 , while levels of KCl and NaCl below, or CaCl_2 above these contents markedly increased bitterness. This trend is in agreement with the behavior observed in green Gordal (Moreno-Baquero et al., 2012) and, in essence, similar to the results from seasoned cracked Aloreña olives (Moreno-Baquero et al., 2013). Hence, initial brines with moderate concentrations of CaCl_2 could be used for fermenting green Spanish-style table olives without effecting bitterness (scores below the average). This contrasts with previous reports that had attributed the bitter taste to potassium (Marsilio et al., 2002). The main contributor to the hardness perception (see Table 1, Eq. (11), in López-López et al., submitted) was CaCl_2 while both KCl and NaCl had negative effects. The contour lines (see Fig. 3, panel A, López-López et al., submitted) were almost perpendicular to the base KCl- CaCl_2 ; therefore hardness decreased from right to left as the proportion of KCl: CaCl_2 increased along this axis. The lowest hardness was appreciated in the KCl vertex and the maximum in the CaCl_2 vertex. In practice, NaCl had a very limited interference in the scores of this attribute. In seasoned cracked olives, the effect of CaCl_2 in the packaging brines was not significant (Moreno-Baquero et al., 2013), possibly due to the natural firmness of these non-lye-treated olives. On the contrary, the contour lines were perpendicular to the NaCl- CaCl_2 base while KCl played a reduced role in Gordal (Moreno-Baquero et al., 2012). The diverse trends in both cultivars can be due to their different olive sizes and the composition of the flesh. Due to the lower size of Manzanilla, the presence of Ca only scarcely interfere with sugar diffusion but in Gordal it caused a marked delay in the release of nutrients and acid formation. This slower acidification in Gordal might have caused a smaller degradative effect than on the

Manzanilla flesh. In any case, the role of Ca in hardness improvement is undeniable and has traditionally been accepted as convenient (Garrido-Fernández et al., 1997). Kanavouras et al. (2005) also noticed that Ca caused a significant hardness improvement in natural black olives. Jiménez, Heredia, Guillén, and Fernández Bolaños (1997) proposed two different mechanisms for cell wall stabilization and firmness improvement: i) the formation of complex coordinations (only by calcium), and ii) the formation of electrostatic bonds (by calcium and other mono or divalent cations).

Despite the similar structure of the equation for hardness and fibrousness (see Table 1, Eqs. (11) and (12), respectively, in López-López et al., submitted), the contour lines of the second (see Fig. 3, panel B, in López-López et al., submitted) were perpendicular to the KCl- CaCl_2 base, indicating that high scores of fibrousness were linked with high CaCl_2 concentrations. Hence, fibrousness is, in practice, a function of the KCl: CaCl_2 ratio; as the lower this ratio is, the lower the fibrousness score and vice versa. On the contrary, in Gordal, fibrousness was only slightly dependent on the KCl: CaCl_2 ratio and decreased as the concentration of NaCl was higher (Moreno-Baquero et al., 2012). In seasoned cracked olives, fibrousness increased as the NaCl content was lower; but, the maximum steepness was observed for an equilibrated KCl: CaCl_2 proportion (~1:1). Overall, fibrousness increases as the NaCl decreases, but the effects of other salts depend on cultivar and process. Crunchiness was mainly affected (see Table 1, Eq. (13), in López-López et al., submitted) by KCl and CaCl_2 concentrations in the fermentation brines while NaCl had only a slight, negative influence. Crunchiness contour lines (not shown) were similar to those of hardness (mainly) and fibrousness. Therefore, crunchiness scores were practically independent of the NaCl proportion in the initial brines but increased progressively as the ratio CaCl_2 :KCl was higher; the maximum and minimum scores were reached in the CaCl_2 and KCl vertexes, respectively. In Gordal, hardness and crunchiness followed similar trends, but the contour lines had different structures (Moreno-Baquero et al., 2012). In seasoned cracked Aloreña olives, crunchiness was not a function of the composition of the packaging brines (Moreno-Baquero et al., 2013) due to the marked natural crunchiness of these fruits (Jiménez Gómez, 2012). Differences in crunchiness between green olives fermented in brines with varying proportions of NaCl and KCl were also found by Marsilio et al. (2002).

3.6. Quantitative descriptive analysis

There were several significant ($p < 0.05$) correlations between the contents of Na, K, and P in the flesh at the end of the

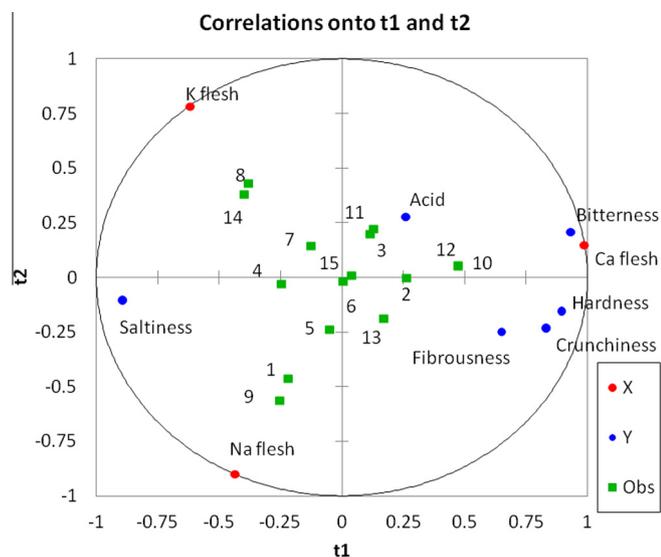


Fig. 3. Projection of variables (sensory and mineral content) and treatments onto the plane of the first two Principal Components. Details of runs in Table 1.

fermentation and the sensory scores that validate the data for multivariate analysis.

The PLS analysis showed that t_1 (which explained 48.05% variance) was positively linked to (correlation in parenthesis) calcium content (0.985), bitterness (0.931), hardness (0.894), fibrousness (0.650), and crunchiness (0.831) while was negatively associated to saltiness (-0.894); in other words, the t_1 component was related to the gustatory (except acid) and the kinaesthetic sensations. These relationships point out the relevant role that the presence of calcium may have on the sensory characteristics of green Manzanilla olives. In contrast, t_2 (31.13% variance) was only related to Na (-0.897) and K contents in the flesh (0.782) but not to any sensory attribute; then, t_2 could be associated with the other chloride salts. Hence, PLS has corroborated the limited influence of these elements on the sensory characteristics of table olives fermented in salt mixtures (within the ranges used in the experiment) and the competition between the elements for the active sites in the flesh.

The projections of the mineral contents and the sensory variables showed a strong relationship between the kinaesthetic sensations and bitterness, possibly due to their links with calcium, which is also in the proximity of these variables (Fig. 3). The saltiness was negatively associated with all of them, due to the Na masking effect on bitterness and its competition with Ca for the active sites (Fig. 3). The opposite relationship between Na and K contents in the flesh shows the mutual (opposed) dependence of the absorption of these elements that, in turn, compete with calcium in the flesh (opposed position on the graph). Saltiness has a position between Na and K which may reflect their balanced influence on saltiness (Fig. 3), within the ranges of salt mixtures used in the brine.

The relationships of the runs, mineral contents and sensory attributes (Fig. 3) show that runs 1 and 9 are characterized by their high NaCl content in the flesh. Run 8 and 14 by their contents in K. Run 10 and 12 are the closest treatments to Ca, indicating high proportions of this element in their olives. The rest of the treatments were situated around the origin, with scores close to the average; subsequently, they could be useful options for processing olives with rather plain profiles.

The PLS analysis also led to the equations that link the sensory scores (except acid, not related) with the mineral contents in flesh (see Table 1, EqS. (14)–(18), in López-López et al., submitted). The

quality parameters for the model (with three components) fit were $Q^2_{\text{accum}} = 0.539$, $Q^2_{\text{Yaccum}} = 0.668$, and $Q^2_{\text{Xaccum}} = 1.000$. Then, the model with three components explains the variance due to minerals (variable independent) completely and about 70% of the sensory scores (dependent). The confidence limits for the predictions of saltiness, bitterness, and hardness were reasonable (see Fig. 4, panels A, B and C, respectively, in López-López et al., submitted) while those for fibrousness and crunchiness were slightly wider but also acceptable.

4. Conclusions

The substitution of NaCl with other mineral nutrients during Manzanilla fermentation affected the characteristics of the products. The increasing presence of CaCl_2 in the initial brines led to olives with better C_i , L^* , b^* , and firmness. The modification also decreased the Na content in the flesh and increased those of K and Ca in proportion to their respective salt contents in the initial brines. Furthermore, CaCl_2 presence, due to its strengthening of flesh structures, also improved the retention of Zn and P. The increasing presence of Ca decreased saltiness scores but increased bitterness (an unfavourable effect) and the kinaesthetic sensations (hardness, fibrousness, and crunchiness); the effect increased as the CaCl_2 :KCl ratio was higher. In agreement with these results, the PLS analysis showed a strong relationship between Ca and bitterness, hardness, fibrousness, crunchiness and saltiness (negative) and led to equations for predicting the sensory attributes (except acidic) as a function of the Na, K, and Ca contents in the flesh, particularly saltiness, bitterness, and hardness. PLS projected most of the treatments of this experiment around the plot origin; they may constitute potential candidates for producing new (healthier) green Manzanilla table olives with average sensory characteristics, familiar to habitual consumers.

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