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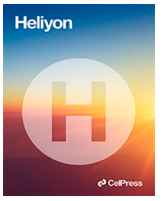

Research article

Innovative salt replacement for green Spanish-style olives using potassium, calcium, and magnesium chlorides during packaging

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ABSTRACT

This work aimed to enhance green Spanish-style Manzanilla table olives by replacing salt with K, Ca, and Mg chlorides in innovative packaging, utilising Response Surface Methodology (RSM). Both the added replacers and naturally occurring minerals were considered. RSM allowed the development of predictive models for K, Ca, Mg, and Mn (initially present) in olive flesh and their contributions to Reference Daily Intakes (RDI) based on the added salts. The sodium content in the new products decreased from 1.4 g/100 g flesh to 0.68 g/100 g flesh, while K, Ca, and Mg concentrations could increase up to 0.50, 0.45 and 0.15 g/100 g flesh, respectively. Added salt contributions to RDI could reach 25, 60, and 44 % for K, Ca, and Mg. Minimal differences between analytical data-derived minerals and predicted values were minimal, suggesting reliable model performance for nutrition labelling. Results assist the industry in creating nutritionally enhanced table olive products.

1. Introduction

Table olives are a widely consumed fermented product worldwide, with approximately 3.1×10^6 tons produced in the 2021/2022 season [1]. Olive processing typically involves using brine in critical steps such as fermentation, storage, or packaging [2]. To ensure the physicochemical stabilisation in packaging, minimum levels of NaCl at equilibrium are fixed at 5 %, 6 % and 10 % for lye-treated, natural, and dehydrated olives. However, the salt content in pasteurised or sterilised products depends on Good Manufacturing Practices (GMP) [3]. It is worth noting that table olives can significantly contribute to consumers' daily sodium intake [4].

Excessive intake of NaCl increases the risk of cardiovascular diseases [5,6], and there is a trend to reduce food content. Substitution of salt with other salts has been common in food products [7–9]. Regarding table olives, Kanavouras et al. [10] evaluated the effect of using brines buffered with acetic acid (0.05M) and 1.85 % $Ca(OH)_2$ with 12.8 % NaCl or without salt against the traditional process (14 % NaCl) while Erdogan et al. [11] investigated the quality of black table olives (Gemlik cv) by partially replacing NaCl (10 %) with KCl and $CaCl_2$ salt mixtures. Similarly, Panagou et al. [12] studied the fermentation profile of *Conservolea* natural olives using mixtures of NaCl, KCl, and $CaCl_2$, while Bautista-Gallego et al. [13] successfully assayed several mixtures of NaCl, KCl, and $CaCl_2$ in the fermentation/storage of *Aloreña de Málaga*. Furthermore, Bautista-Gallego et al. [14] did not observe significant changes in the shelf life and product quality when packaging cracked *Aloreña* olives using Na, K, and Ca chloride mixtures. Studies have also shown that it is possible to reuse brines in successive processes without compromising food safety. Zinno et al. [15] reported that reusing 25 %, 50 %,

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and 75 % brines in successive processes of Nocellara del Belice Spanish style (9 % NaCl) and Castelvetrano (7 % NaCl) types did not lead to an increase in contamination, overgrowth of pathogens, or spoilers. Despite the success of previous studies on olives, few have devoted themselves to reducing the salt level in the final products, which is the primary concern for consumers. Moreover, previous studies have primarily focused on investigating the changes of the mineral added as replacers in the olive flesh but not the effects on the other elements also present in the fermented products.

Besides, $MgCl_2$ has been rarely used as a NaCl replacer in table olives because of its low inhibitory effect on the spoiling microflora [16]. However, it is essential in numerous human enzymatic reactions such as energy production, protein synthesis, and DNA/RNA synthesis [17]. A balanced diet should provide enough Mg, but the excessive consumption of refined foods and animal proteins has reduced its intake [18]. Recent studies suggest that Mg supplementation can decrease the risk of hospitalisation in pregnant women, reduce the frequency and intensity of migraine, and lower the risk of type 2 diabetes and stroke [19,20]. Therefore, exploring the potential benefits of Mg incorporation in the packaging of table olives could be a valuable avenue for research. The effect of magnesium salts as NaCl replacers on the mineral content of olive products has never been assayed before.

This study introduces a novel approach by hypothesising that partially replacing salt during the packaging of green Spanish-style table olives offers distinct advantages over the salt substitution during fermentation. These benefits include lower production costs by avoiding mineral losses during olive conditioning before packaging and the ability to produce batches with diverse characteristics, such as varied concentrations or components. This innovative strategy aims to create healthier products without compromising safety, leveraging pasteurisation in the final preservation process a significant departure from conventional practices. This research explores replacing 50 % NaCl with KCl, $CaCl_2$, and $MgCl_2$ in packaged whole (plain) green Spanish-style Manzanilla table olives. It assesses the distribution of new and existing minerals between olive flesh and brine; additionally, it examines the changes in olive flesh to the added salts and considers the impact on existing minerals in the fermented olives and the implications for nutritional labelling. The incorporation of magnesium is also expected to enhance the nutritional value and improve the healthier profile of the new products.

2. Materials and Methods

2.1. Olives

The whole green Manzanilla olives (240 fruits/kg) were provided by JOLCA SCA (Huevar, Sevilla, Spain). They underwent the traditional green Spanish-style processing (2.4 g NaOH/100 mL lye treatment for 7 h, soaking in tap water for 18 h, brining in a 10 g NaCl/100 mL solution, and fermenting for six months). Before packaging, the olives were stored in a cold room and desalted to reduce the NaCl content in the flesh moisture to 2.5 g NaCl/100 mL [21]. For clarification, it is convenient to notice that the term "olive juice" used in the Trade Standards of Table Olives [3] refers just to a part of the flesh moisture, extracted by physical procedures, for characterizing some of its physicochemical properties, while in this work, the expression 'flesh moisture' is used to describe the total moisture content in olives.

Table 1
Replacing salt (50 %) with potassium, calcium, and magnesium chloride in plain green Spanish-style Manzanilla table olives. Experimental design. Expected concentrations in the equilibrium (levels in design) and in the cover brine, considering the composition of the different salts and the proportion of olive/brine in the glass bottle packaging.

Design point	Proportions of the diverse salts (g/100 mL)					
	KCl	KCl	$CaCl_2$	$CaCl_2{\cdot}2H_2O$	$MgCl_2$	$MgCl_2{\cdot}6H_2O$
	Levels in design	Initial cover brine	Levels in design	Initial cover brine	Levels in design	Initial cover brine
1	0.5	0.875	1	2.24	1	3.7375
2	1.5	2.6255	1	2.24	0	0
3	1.5	0.875	0	0	1	3.7375
4	0.5	0.875	1	2.24	1	3.7375
5	1	1.7505	0.5	1.12	1	3.7375
6	1.5	2.6255	0.5	1.12	0.5	1.8685
7	0.833	1.458	0.833	1.866	0.833	3.1135
8	1.5	2.6255	0	0	1	3.7375
9	1.5	2.6255	1	2.24	0	0
10	1.333	2.333	0.333	0.746	0.833	3.1135
11	1.333	2.333	0.833	1.866	0.333	1.2445
12	1.167	2.0425	0.667	1.494	0.667	2.493
13	1	1.7505	0.5	1.12	1	3.7375
14	1	1.7505	1	2.24	0.5	1.8685

Notes: KCl was anhydrous. Brines also contained enough NaCl and 90 % commercial lactic acid to reach 2.5 % NaCl and 0.5 % acid in equilibrium, respectively. In addition to the experimental design, another treatment (15, "Current") was also prepared, employing the usual industrial packaging conditions (5 % NaCl and 0.5 % lactic acid in equilibrium). Levels in design refer to salt concentrations of the anhydrous salt. Initial cover brine refers to salt concentrations actually used in the experiment.

2.2. Packaging

The characteristics of the olives before packaging were as follows: NaCl in flesh moisture, 2.5 % (w/v); lactic acid in flesh moisture, 0.39 % (w/v); flesh moisture, 67.84 % (w/w); and flesh proportion, 84.58 % (w/w). The glass containers used for packaging contained 170 g of olives and 130 mL of brine. The characteristics of the cover brine were set to reach, after equilibrium, 0.5 % (w/v) titratable acidity and 5 % (w/v) salts (2.5 % (w/v) NaCl (sodium chloride PA-ACS-ISO) + 2.5 % (w/v) of a mixture of KCl (potassium chloride PA-ACS-ISO, Panreac), $CaCl_2$ (calcium chloride 2-hydrate powder PA-ACS, Panreac) and $MgCl_2$ (magnesium chloride 6-hydrate PRS-CODEX, Panreac) in the solution).

The range of added salt proportions expected in the equilibrium (g/100 mL) were 0.5–1.5 % KCl, 0–1% $CaCl_2$, 0–1% $MgCl_2$, with the constrain that KCl (%) + $CaCl_2$ (%) + $MgCl_2$ (%) = 2.5 % (w/v). The combinations of salt proportions were designed using a D-optimum (simplex lattice) experimental design (Table 1, expected levels), expanded with several interior points to reduce the standard error. It was generated by Design Expert v.13.0 (Stat-Easy Inc. Minneapolis, USA).

The actual levels in the initial cover brine used in packaging were adjusted according to the composition of the salts (hydration degree) and the proportion of olives and brine in the glass bottles (Table 1, initial cover brine). Brines were prepared with tap water at 55 °C to facilitate the solubilisation of salts and induce a vacuum in the headspace of the containers. After adding the brine, the bottles were closed, pasteurised (autoclave Stériflow, BARRIQUAND) at 85 °C for 8.5 min ($PU^{5.25}_{62.4°C} \geq 15$), and stored at room temperature (20 ± 2 °C) in the pilot plant facilities of Instituto de la Grasa (Sevilla, Spain) for 30 days. Subsequently, the olive flesh and brines were subjected to mineral analysis.

2.3. Physicochemical analysis of brines

The brine pH, titratable acidity, and combined acidity were measured according to Garrido Fernández et al. [2]. Moisture was estimated by drying an aliquot of the olive flesh samples on stainless plates till constant weight, using a Selecta electric oven (Dry-Big 2002970, Abrera, Barcelona, Spain) set at 106 °C.

2.4. Mineral analysis in the flesh and brines

All glassware was immersed in 6N HCl overnight and then rinsed several times with distilled deionised water. The 6N HCl solution was obtained by diluting hydrochloric acid 37 % PA-ACS-ISO, Panreac, Barcelona, Spain. Two methods were checked for mineral analysis. For dry-mineralisation, 5 g of homogenised olive flesh was weighed exactly into a quartz capsule and was introduced at 550 °C for ≈8–10 h in the oven (L-9/11-B-180, Nabertherm GmbH, Lilienthal, Germany). The resulting ashes were dissolved (slightly heating) with 2 mL of 6N ultra-pure hydrochloric acid and filtered through filter paper into a 25 mL volumetric flask. The filter was washed with 3 mL of deionised water, and the resulting solution was added to the volumetric flask. This operation was repeated twice, and the volumetric flask was finally filled with deionised water to level. The mineral analysis of the brine was done directly. In this case, the brines were filtered through a 0.2 μ pore size membrane and an aliquot was analysed. Simultaneously, a blank was prepared with only the reagents. The analyses for each run (olive and brine) were carried out in triplicate.

For the wet-mineralisation [22], 2.5 g of flesh was introduced into a 50 mL screw cap Pyrex cylindrical bottle, which was heated at 100 °C until constant weight to remove moisture. For liquid samples such as brines, 20–25 mL was introduced in the same bottles, and the volume was reduced to 10–15 mL. Both sample bottles were digested by adding 5 mL of HNO_3 65 % PA-ISO, Panreac, Barcelona, Spain. The solution was heated at 180–220 °C in a sand heater (Selecta Combiplac-Sand 2 6000709, Barcelona, Spain) until grey fumes disappeared and the solution became transparent. Next, 5 mL of a mixture composed of HNO_3 65 % PA-ISO, Panreac:$HClO_4$ 60 % PA-ACS-ISO, Panreac (1:4) was added, and the tubes were heated until the brown/black fumes disappeared, and the solution became transparent and colourless. The bottles were then removed from the sand bath to cool, and their solutions were transferred to a 25 mL volumetric flask; the bottles were washed with double-deionised water, which was also incorporated into the flask, and this filled to the appropriate volume with double-deionised water (Sigma, St. Louis, Mo. USA).

Regardless of the sample preparation procedure, the mineral nutrients were determined by Atomic Absorption (AA) using an air-acetylene flame in a GBC model 932 AA (GBC, Braeside, Victoria, Australia) atomic absorption spectrometer, equipped with three two hollow multi-element cathode lamps Cu, Cr, Co Fe, Mn, and Ni (GBC, Braeside, Victoria, Australia), Na and K (Photron, Narre Warren, Victoria, Australia) and Ca and Mg (Photron, Narre Warren, Victoria, Australia) for major elements. For Ca and Mg determinations, 2000–5000 μg/mL de lantan (La^{+3}) ($LaCl_3.7H_2O$ PA, Panreac, Barcelona, Spain) was added to the final solution to prevent interferences. For Na (or K) analysis, 2000 μg/mL (1000 μg/mL) of K (or Na) was added to avoid sodium and potassium ionisation.

For P analysis, we followed the spectra-colourimetric method described in AOAC nº 970.39 [23]. This procedure involves the formation of a yellow complex from a mixture of phosphate, vanadate, and molybdate in a strongly acidic medium [24].

The Ca, Na, and K stock solution was obtained from Sigma Aldrich (St. Louis, USA) and PACISA (Madrid, Spain). All other reagents were of analytical purity. Further details can be found elsewhere [4].

2.5. Modeling the effect of the chloride salt concentrations on selected packaged product characteristics

The concentrations of KCl, $CaCl_2$, and $MgCl_2$ salts used in the experimental design were related to various parameters of the final products, such as the distribution coefficients (K_d), mineral content in the flesh (both added and existing the fermented product), and

contributions to the daily reference intake. This was analysed using a multiple special cubic regression model which, expressed in the canonical (Sheffé) form, took the expression:

$$R = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{1 \le i < j}^{n} \beta_{ij} x_i x_j + \sum_{1 \le i < j < k}^{n} \beta_{ijk} x_i x_j x_k + \in \quad \text{Eq. 1}$$

The response variable, denoted by R, is the abovementioned parameters or their transformation. The βs represent the coefficients that must be estimated, and the error term is represented by $\in$ [25]. A sequential sum of square ANOVA was conducted for each response variable to test whether a more complex model could fit the data better. The background option was used for variable selection, with $p \le 0.01$ for entering and $p \le 0.1$ for removing (attending its previous relevance at choice). The models were considered significant if the fit was significant $p < 0.05$ and the lack of fit was insignificant ($p > 0.05$).

In the case of significant interactions, linear or lower terms interactions were also retained because only hierarchical models are scale-independent and can be expressed into actual units. Three replicates in the experiment allowed for the estimation of pure error and lack of fit. The results are presented using ternary plots in the simplex, with contour lines for diverse levels of R.

Data on overall mineral content in olive flesh were also subjected to multivariate analysis to find similar profiles that may help producers select specific treatments according to consumer demand.

2.6. Statistical software

Design Expert v.13.0 (Stat-Easy Inc. Minneapolis, USA) was used to design the experiment and analyse responses. XLSTAT v. 2017 (Addisonsoft, Paris, France) was used for K_d estimations and the multivariate analysis.

3. Results and discussion

3.1. Selection of a method for mineral analysis

Previous analyses of minerals in the olive flesh used protocols developed for other products [4,26]. However, several mineralisation options are now available. Therefore, as a first step, this study compared wet mineralisation (for flesh and brines) with dry mineralisation (ashing for flesh and direct analysis for brines). In both cases, the element contents in the resulting solutions were determined by AA. The main nutritional elements in the flesh were statistically the same regardless of the mineralisation method (Table 1S). Similarly, levels found in brine by wet mineralisation and direct analysis (except for P, which was affected by brine colour) were identical (Table 1S). Therefore, this study adopted wet mineralisation for flesh and brines. Consequently, only data obtained through wet mineralisation are presented. Moreno-Torres et al. [27] did not find statistically significant differences in Ca and P contents in milk when comparing wet mineralisation using an $HNO_3/HClO_4$ (4:1) mixture and dry mineralisation in a furnace at 550 °C.

3.2. Mineral concentrations in olive flesh and brine according to treatments and their relationships

Analysis of olive flesh and brines from the experimental design and "Current" treatments after equilibrium (Tables 2 and 3) showed that, as intended, the Na content in the experimental runs was reduced by approximately 50 %, from 1380 mg/kg flesh in the "Current" treatment to 673 mg/kg flesh. A similar reduction was reported by Maestralexi et al. [28] through partial substituting NaCl with various combinations of salts in the fermentation brines. Mantzouridou et al. [29] achieved a reduction in NaCl during Chalkidiki Spanish-style fermentation to 4 %, with the concentrations of the other salts being 2.8 % for KCl, 1.0 % for $CaCl_2$, and 0.12 % for $MgCl_2$. It is important to notice that the concentrations of salt substitutes would diminish during olive conditioning, necessitating re-addition in packaging. However, substituting salt at the packaging stage does not result in salt loss, and the process is more effective regarding salt use and production costs. Concerning the minerals in the flesh, besides the higher contents of the added elements in the experimental runs, P and Fe had higher values in the "Current" packaging (Table 2). Meanwhile, Cu, Zn, and Mn showed minimal differences, suggesting higher leaching for the first minerals than the latter during desalting. Overall, the mineral nutrient levels in the "Current" packaging were comparable to those in a commercial survey for this style reported by López et al. [4], making it a good reference for the typical whole (plain) green Spanish-style table olives. Na, K, and Mg concentrations in brine (Table 3) were consistently higher than in the flesh due to the below-commented reasons. Conversely, Ca, Fe, Cu, Zn, and Mn were present at lower levels in brine, indicating a strong (especially in the case of Ca) association with flesh organic components. P levels were similar in both flesh and brine. Adding K, Ca, and Mg chloride salts caused fluctuations in added and non-added mineral levels.

This first approximation suggests replacing salt in plain green Manzanilla Spanish-style table olives is feasible after proper desalting. However, this substitution may alter the product's traditional mineral profiles, necessitating further study of these new relationships.

3.3. Effect of the mineral fortification on the distribution coefficient

The first approach to quantitatively evaluate the effect of salt substitution on the minerals in the flesh and brines was through the (pseudo) distribution coefficient, K_d, recently developed for table olives [30]. Originally, K_d described the distribution of organic compounds between two phases in equilibrium or predicted the mobility of species [31]. The EPA (Environmental Protection Agency)

Table 2
Replacing salt (50 %) with potassium, calcium, and magnesium chloride in plain green Spanish-style Manzanilla table olives. Concentration (mg/kg) and standard errors (SE) of mineral nutrients in the olive flesh according to the experimental design treatments. The table also includes data from the olive flesh employing the usual packaging conditions ("Current").

Treatment	Na		K		Ca		Mg		Fe		Cu		Zn		Mn		P	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	6734	14	1631	21	4686	16	1586.3	18.8	3.40	0.10	1.52	0.03	1.56	0.01	0.300	0.005	59.25	0.62
2	6886	14	4963	10	4736	14	43.3	0.4	3.35	0.13	1.39	0.02	1.64	0.02	0.238	0.013	65.12	0.52
3	6734	4	5140	6	603	13	1564.0	24.5	3.26	0.06	1.58	0.02	1.52	0.01	0.258	0.005	62.17	0.76
4	6836	9	1644	14	4750	6	1550.5	24.8	3.62	0.08	1.45	0.01	1.71	0.03	0.324	0.010	60.40	0.35
5	6796	12	3573	32	2733	9	1632.4	7.0	3.76	0.15	1.33	0.02	1.68	0.04	0.270	0.030	67.30	0.21
6	6745	19	5022	1	2692	3	799.2	9.2	3.64	0.07	1.31	0.03	1.65	0.01	0.298	0.010	58.76	0.34
7	6843	21	2656	26	4035	34	1368.1	3.7	3.31	0.08	1.50	0.04	1.48	0.02	0.267	0.009	65.38	0.68
8	6812	3	5147	13	657	8	1584.0	27.1	3.35	0.03	1.31	0.03	1.61	0.01	0.258	0.007	58.53	0.63
9	6665	6	5151	5	4760	21	57.5	2.1	3.21	0.05	1.53	0.08	1.55	0.01	0.283	0.018	60.02	1.17
10	6692	23	4480	19	2035	11	1300.3	4.3	3.77	0.04	1.59	0.04	1.63	0.04	0.234	0.013	60.70	0.05
11	6736	23	4473	9	3989	24	598.8	7.9	3.25	0.05	1.43	0.03	1.55	0.02	0.233	0.008	64.11	1.75
12	6783	14	4009	29	3349	11	1138.0	15.4	3.76	0.03	1.63	0.03	1.63	0.01	0.213	0.012	63.02	1.58
13	6785	9	3352	22	2742	24	1651.5	11.2	3.64	0.03	1.53	0.06	1.64	0.06	0.328	0.015	65.10	1.22
14	6840	21	3337	19	4747	10	890.6	7.3	3.41	0.02	1.53	0.11	1.69	0.03	0.267	0.012	64.73	0.96
15("Current")	13795	39	99	1	542	16	50.8	0.8	4.02	0.03	1.58	0.05	1.54	0.01	0.265	0.006	68.35	0.89

Table 3
Replacing salt (50 %) with potassium, calcium, and magnesium chloride in plain green Spanish-style Manzanilla table olives. Concentration (mg/kg) and standard errors (SE) of mineral nutrients in the brines according to the experimental design treatments. The table also includes data from brines employing the usual packaging conditions ("Current").

Treatment	Na		K		Ca		Mg		Fe		Cu		Zn		Mn		P	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	10043	10	2287	1	3268.7	8.1	2085.4	10.9	1.043	0.029	0.154	0.003	0.561	0.016	0.139	0.003	61.6	2.8
2	10120	9	7221	6	3276.6	3.0	41.0	0.2	1.033	0.041	0.126	0.004	0.622	0.006	0.152	0.003	59.3	0.7
3	9931	18	7269	19	213.1	0.5	2077.9	1.3	0.967	0.032	0.135	0.008	0.562	0.007	0.173	0.005	58.7	0.8
4	10201	41	2148	6	3357.6	6.6	2081.6	8.3	0.940	0.025	0.131	0.015	0.611	0.007	0.196	0.004	62.9	1.7
5	10096	154	4900	32	1734.1	15.2	2112.7	3.0	0.996	0.018	0.142	0.010	0.650	0.009	0.165	0.007	60.6	2.4
6	10145	28	7346	26	1751.6	4.5	1129.9	3.4	0.923	0.014	0.147	0.003	0.572	0.030	0.195	0.006	63.3	1.2
7	10010	104	4176	21	2946.1	0.9	1747.8	4.7	0.858	0.008	0.162	0.003	0.609	0.010	0.159	0.005	58.9	0.6
8	10013	121	7143	25	229.0	1.3	2120.8	2.4	0.921	0.027	0.166	0.006	0.581	0.008	0.155	0.007	61.1	1.8
9	10100	61	7309	7	3276.4	4.8	66.3	0.1	1.042	0.029	0.145	0.006	0.607	0.007	0.176	0.004	60.7	2.5
10	10271	32	6330	27	1226.2	1.2	1770.5	9.2	0.994	0.033	0.153	0.008	0.597	0.018	0.198	0.003	58.5	1.7
11	10187	16	6709	49	2885.2	12.2	771.5	2.2	0.846	0.028	0.157	0.012	0.620	0.011	0.196	0.010	63.0	1.1
12	10048	8	5734	32	2264.7	5.8	1366.4	5.0	0.910	0.032	0.137	0.009	0.667	0.007	0.140	0.002	62.3	1.5
13	9917	74	4865	26	1786.7	4.0	2026.2	4.9	0.919	0.073	0.156	0.004	0.544	0.040	0.201	0.009	62.9	1.3
14	9903	130	4841	5	3212.5	5.2	1011.4	4.6	0.873	0.016	0.132	0.003	0.569	0.005	0.167	0.005	58.5	0.7
15 ("Current")	19078	32	134	3	211.4	0.2	45.2	0.1	0.907	0.008	0.161	0.003	0.647	0.033	0.169	0.009	59.6	1.7

[32] defines K_d as partitioning species between a solid and an aqueous matrix after equilibrium [33,34]. Given that mineral nutrients in table olives are distributed between the olive flesh and brine, an "empirical" (or pseudo) distribution coefficient, K_d, was calculated, according to López-López et al. [30], as the ratio of each element concentration in the flesh to that in the brine at equilibrium. K_d is a simple parameter for describing the relationship between the mineral nutrient contents in the fruits or olive moisture and the brine in table olives.

Chloride salt mixtures in packaging resulted in K_d values below 1 for Na, K and Mg, indicating higher concentrations in brine than in the flesh. Conversely, K_d values for Ca, Fe, Cu, Zn, and Mn were above 1, implying a solid association with olive flesh components. P was the only element that showed a moderate proportion and apparent equilibrium between the olive flesh and brine, suggesting a partial link to the olive flesh (Table 2S). The differences in K_d values among the various treatments indicate that packaging green Spanish-style olives in chloride salt mixtures can affect the distribution of added and naturally occurring minerals in the fruits.

When K_d was estimated under the assumption that minerals were exclusively solubilised in the flesh moisture (Table 3S), the values for Na, K (primarily), and Mg were around 1, indicating similar concentrations in flesh moisture and brine (equilibrium). However, the K_d values for Ca, Fe, Cu, Zn, Mn, and P increased markedly compared to those based on olive flesh, suggesting that it is unrealistic to consider them exclusively present in the flesh moisture; they are primarily associated with the olive flesh (still showing high K_d values, Table 2S). The highest K_d value was for Cu, which reacts with the olive chlorophyll and its derivatives, causing the "green spot" effect [35]. The interaction of Ca with the olive flesh is well-known for improving texture [36]. This effect is crucial in processing ripe olives to prevent softening during storage in the acidic medium, during the darkening phases (lye treatment, washing, and storage in iron solution), or warm brine packaging and product sterilisation [37]. Additionally, calcium (4 %) in the *Conservolea* cultivar natural black olives led to the strongest and firmest fruits [38]. In green Spanish-style table olives, the firming effect of calcium was more potent at higher pH values, although its influence diminished as the cation concentration increased [39]. The effect of magnesium on olives is often associated with chlorophylls and Ca. A study on *Conservolea* olives showed that treating them with $CaCl_2$ before harvesting increased the Mg content without affecting chlorophyll levels and photosynthesis rate [40].

The observed fluctuations in the distribution coefficient (K_d) may be related to the initial concentrations of salt mixtures used in packaging. The model was selected based on the partial sum of squares method, which involves selecting the equation with the highest-order term that significantly increases the explained variance and has a non-significant lack of fit. This approach was feasible due to

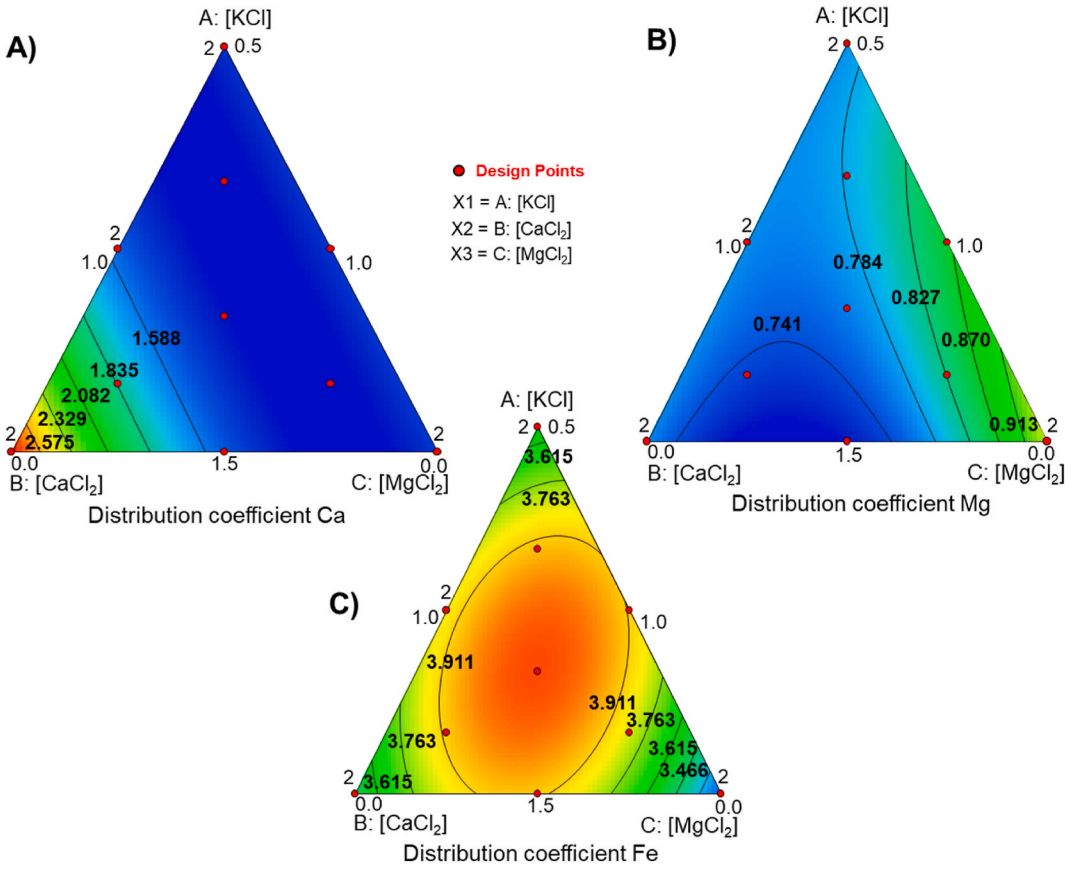

Fig. 1. Replacing salt (50 %) with potassium, calcium, and magnesium chloride in plain green Spanish-style Manzanilla table olives. Changes in the pseudo distribution coefficient K_d as a function of the KCl, $CaCl_2$, and $MgCl_2$ concentrations in the packaging brine. A), $K_{d\ Ca}$; B), $K_{d\ Mg}$; and C), $K_{d\ Fe}$. Number 2, close to design points, indicates a replicated treatment.

duplicated points in the design. Only Mg and Fe showed a significant regression model among the minerals studied. Although Ca exhibited a significant lack of fit (Table 4S), its model could help explain its trend at high concentrations. The ternary plot of the K_{dCa} in the simplex, considering the sum constraint of the initially added salts that provide a clear matrix of compositional data, revealed that high K_{dCa} values corresponded to low added Ca (Fig. 1A). This was due to the high content of Ca in the olives before packaging. As the concentration of $CaCl_2$ in the initial brine increases, the K_{dCa} decreases until stabilisation, likely when the flesh reaches saturation, and then remains similar up to the maximum levels of addition (Fig. 1A). This saturation likely caused the model's lack of fit. Above saturation concentration, Ca could follow a trend similar to Na, K or Mg, reflecting normal equilibrium in flesh moisture. The presence of Ca in olives is convenient due to the favourable effect of this element on health maintenance [41,42].

The models for Mg and Fe, which were significant with a non-significant lack of fit, explained a relatively high proportion of the variance (71.85–63.41 % and 79.28–66.32 % for Mg and Fe). Interpretation using a ternary plot shows that K_{dMg} increases as concentrations of $MgCl_2$ decrease (Fig. 1B), reaching their maximum at the highest levels of KCl and $CaCl_2$ ($MgCl_2$ vertex). The minimum K_{dMg} is observed at maximum KCl levels on the $CaCl_2$-$MgCl_2$ border at about 1/3 and 2/3 of the $CaCl_2$ and $MgCl_2$ ranges (Fig. 1B). For K_{dFe}, the highest values are reached around the barycenter region, with similar concentrations (33.33 %) of the three added salts (Fig. 1C). The presence at moderate concentrations of the added salts prevented the leaching of Fe already in the flesh. Therefore, the initial concentrations of the salts used in the packaging can be responsible for the oscillations observed in the added Mg and non-added Fe distribution coefficients.

3.4. Insights on the distribution of the mineral nutrients in the olive flesh

López-López et al. [30] suggested evaluating the relationship between nutrient minerals in olive flesh (or moisture) and brine in fermented green Spanish-style olives by regression. Since this approach has not yet been tested in packaged olives using salt mixtures, applying it to final products of this style is challenging. Additionally, the analysis provides parameters such as intercept and slope, whose values could help interpret the relationships between mineral nutrients and olive flesh. Moreover, no data regarding Mg distribution in table olives is currently available.

If the model is significant, the β-value indicates the element's brine contribution to the prediction, the intercept measures the initial excess or deficiency of each mineral in the flesh (or moisture) compared to brine, and the slope shows the relationship between the concentration in both substrates. In this context, the study complements K_d analysis by providing insights into the causes behind the mineral behaviours observed in the previous section. It helps determine whether the levels of each element in the flesh are due to a particular interaction with olive components or if their presence is merely due to solubilisation in the flesh's moisture.

Among the various regression models comparing the concentrations of minerals in the flesh and brine (Table 5S, upper half), only the models for Na (which was not included in the design but added at 2.5 % in all treatments), K, Ca, and Mg were significant. In these models, the β values were consistently high (ranging from 0.9952 to 0.9980), indicating that their presence in brine significantly contributed to the concentrations in the flesh. However, the regression parameter differed among the elements.

Regarding the intercept, only Na showed negative values, suggesting that part of the Na released during desalting was not recovered after packaging despite the NaCl presence in the initial brine, possibly due to its substitution by other added elements. K, Ca, and Mg had positive intercepts, with Ca being exceptionally high, indicating a considerable presence in the flesh. Na, K, and Mg slopes were lower than 1, implying lower *apparent* concentrations in the flesh compared to the brine. In contrast, the slope for Ca was higher than 1, meaning its *apparent* predominance in the flesh over the brine.

When mineral values were regressed, considering they were exclusively present in the flesh moisture (Table 5S, bottom half), significant models were also obtained for Na, K, Ca and Mg. The intercepts were similar to those in the previous models, referring to flesh as a whole. However, the slopes changed drastically. For Na, K, and Mg, the slopes were around 1, indicating an equilibrium between their concentrations in flesh moisture and brine. These minerals are distributed solely by solubilisation and do not interact (i.e., have no affinity) with the rest of the flesh (solid) components. In contrast, when considering only flesh moisture, the regression for Ca resulted in a high slope (Table 5S, bottom half), implying an unrealistic overconcentration of this element in flesh moisture. This suggests that attributing the high proportion of Ca bound to the flesh to the moisture has caused an abnormal and unrealistic overconcentration in this substrate. Most Ca is not solubilised in flesh moisture but is bound to flesh components.

The comparison between both regressions demonstrated that desalting reduces the Na in flesh moisture and removes some Na initially linked to flesh organic components (possibly during the lye treatment) that is not recovered after packaging. Subsequently, during packaging, the Na only equilibrate with flesh moisture. K and Mg follow a similar trend. However, Ca is already initially present and associated with olive flesh components. Further addition of calcium leads to greater solubilisation in the moisture but primarily results in binding to reactive sites in the olive flesh until saturation is reached. According to Del Pino et al. [43], cell plants are abundant in cytosolic-Ca^{2+}, suggesting that the natural Ca in table olives may partially be associated with the cytosolic-Ca^{2+}. However, the added Ca is hardly unlikely to penetrate the olive cell significantly, at least not in the proportion used in this experiment. It is more plausible that the added Ca primarily reacts with cell wall components since pectin fractions and hemicelluloses are abundant in olive pulp, as found by Jimenez et al. [36]. Supporting this hypothesis, Cardoso et al. [44] found that pectic fractions of olive flesh can form stable gels with Ca^{2+}.

This information extends the hypothesis of Na equilibrium between flesh moisture and brine, studied by López-López et al. [30] in fermented green Spanish-style olives, to their packaged products and K and Mg minerals. It reveals that a proportion of the Na leached during desalting is not recovered later and that a high proportion of Ca is strongly associated with flesh components. However, it may also be present in flesh moisture after the active sites are saturated.

Regarding mineral micronutrients, no significant models were detected for the regression between content in flesh and brine. In the

regression between flesh moisture vs brine, only the model for Mn was significant, but its contribution was weak (about 0.31). The intercepts were always positive (Table 5S) because their source was the initial presence in the olive flesh. These values can be interpreted as residual micronutrients remaining in the flesh after processing due to their strong bond to flesh components, which restricts their leakage even when packaged. This explanation is supported by the observation that intercepts increase when the regression is based on the concentration of flesh moisture.

3.5. Predictions of mineral concentrations in olive flesh after equilibrium as a function of their experimental design levels

The design did not include sodium (Na). Its concentration was regulated during desalting and by adding 2.5 % NaCl to the packaging brine. The concentrations in the experimental treatments were around 6778 mg/kg flesh, whereas in the current packaging practices ("Current"), it was 13,976 mg/kg, approximately double that in the fortified treatments. Therefore, the objective of reducing NaCl in the proposed new packaging conditions was adequately fulfilled.

Regarding the models for estimating the concentrations of other minerals based on the initial KCl, $CaCl_2$, and $MgCl_2$ in the packaging brine, the equations were deduced as explained in the Materials and Methods section. Only the elements of the salts used in the packaging brines and Mn were significant and could be used for predictions. Significant models mean a good fit (p-value <0.05), non-significant lack of fit (p-value >0.05), precision (signal-to-noise ratio) > 4, and high explained variance (R^2 and adjusted R^2 > 0.995, except for Mn that were about 0.79, 0.60, respectively) (Table 6S). The specific characteristics of each model will be described case by case. The equations were always deduced to express the concentrations in actual units (e.g., mg/kg flesh); to obtain values as mg/100 g, the coefficients for the linear terms and two-way interactions should be divided by 10 and 100, respectively.

Potassium. Only the linear terms of the model were significant for this element (Table 6S), and their coefficients had standard errors of around 0.24. Transposing the coded coefficients to actual units, the equation for the estimation of the expected K content in the flesh as a function of the KCl, $CaCl_2$ and $MgCl_2$ concentrations (percentages, w/v) in the packaging brine took the form:

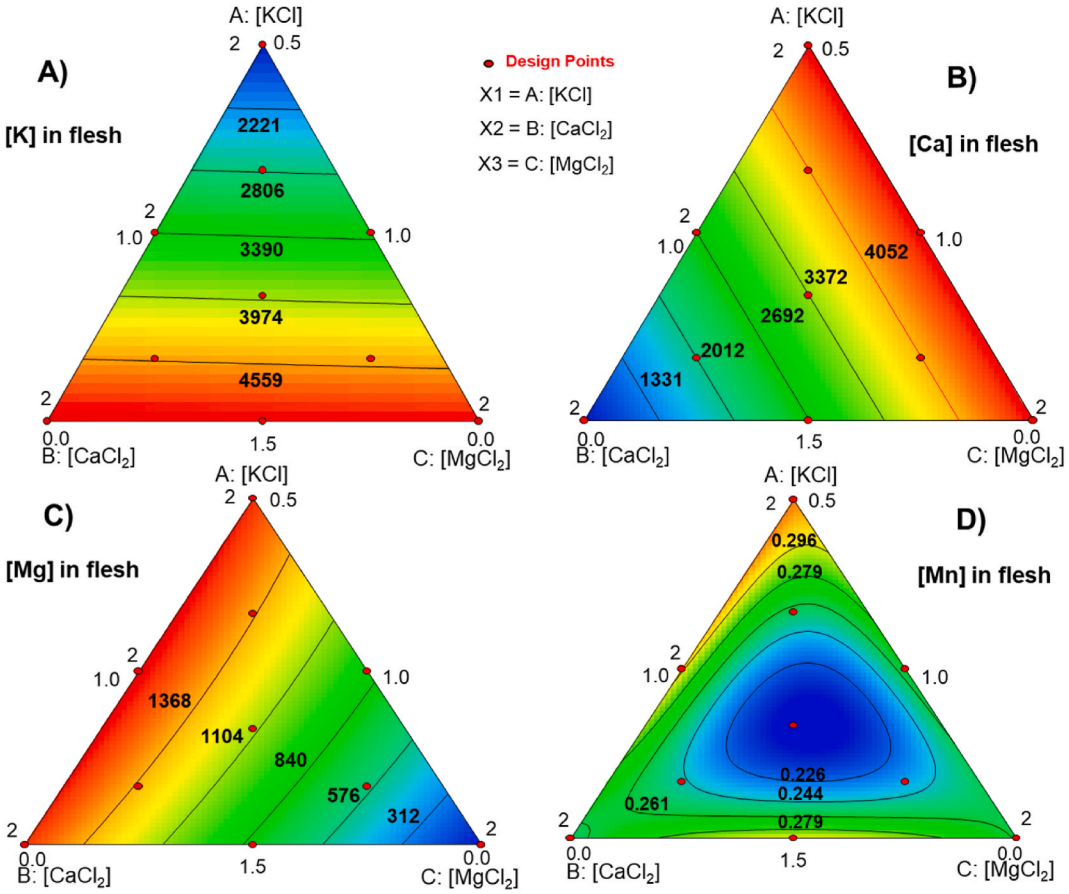

Fig. 2. Replacing salt (50 %) with potassium, calcium, and magnesium chloride in plain green Spanish-style Manzanilla table olives. The relationship between the concentrations of fortifying KCl, $CaCl_2$, and $MgCl_2$ salts in the packaging brines and their contents in the flesh of the corresponding final products are presented as follows: A), potassium (K); B), calcium (Ca); C), magnesium (Mg); D), manganese (Mn). Number 2, close to design points, indicates a replicated treatment.

$$\text{K in flesh (mg/kg)} = +3415.842 \cdot [\text{KCl}] - 90.682 \cdot [\text{CaCl}_2] + 14.865 \cdot [\text{MgCl}_2] \quad \text{Eq 2}$$

The function indicates that the proportion of K in the flesh mainly depends on the initial KCl level in the packaging brine. At the same time, the presence of calcium and magnesium chlorides reduces (primarily) and increases, respectively, the K absorption. These effects may be explained by the rise of firmness caused by Ca (resulting in more difficult diffusion of K) and the interference of Mg with Ca absorption due to the similar charges of both cations to form complexes with olive flesh pectic extracts as found by Cardoso et al. [44]. The behaviour is well interpreted in the ternary plot (Fig. 2A). K levels increase as the KCl concentrations are higher (direction opposed to KCl vertex), with the contour lines of the evolution almost parallel to the base. The slight inclination towards the Mg vertex reflects the slightly higher $CaCl_2$ than $MgCl_2$ interference in the K absorption in the flesh. However, when fermenting *Aloreña de Málaga* olives with a mixture of NaCl, KCl and $CaCl_2$, the model used to estimate K content in the flesh indicated that, in addition to linear terms, there were negative interactions between KCl and NaCl or $CaCl_2$. These interactions suggest a reduction in the K content in the flesh, possibly due to the common ion effect in the first case or an increase in texture due to the Ca, which may have reduced K accessibility to the flesh [14].

Replacing NaCl with selected combinations of KCl and $CaCl_2$ increased the presence of K in *Aloreña de Málaga* olive flesh from 1 to 16 g/kg flesh, according to the levels in the initial brines, without affecting the sensory attributes [14]. In *Gordal* fermentations, the K content ranged from 0.7 g/kg flesh to 7.5 g/kg flesh, with high contents related to saltiness [26].

Calcium. The model suggested was also linear (Table 6S), with the standard error of coefficients approx. 0.26. The equation for the prediction of Ca resulted in the following:

$$\text{Ca in flesh (mg / kg)} = +259.040 \cdot [\text{K Cl}] + 4340.554 \cdot [\text{CaCl}_2] + 262.533 \cdot [\text{MgCl}_2] \quad \text{Eq 3}$$

The high coefficient value for $CaCl_2$ reflects that Ca in the flesh strongly depends on its percentage in the packaging brine. At the same time, the (positive) influence of K and Mg chloride salts is somewhat similar and means a specific contribution to Ca diffusion into the flesh. As a visual confirmation, the ternary plot in the simplex (Fig. 2 B) shows that the Ca concentration in the flesh increases as the added Ca salt rises while maintaining parallel to the KCl-$MgCl_2$ borders, indicating no interference from any of them (i.e., no competition for the flesh active sites). The greatest concentration of Ca in fresh is obtained at the border $MgCl_2$-KCl, regardless of their proportions. These concentrations may convert olives into an exciting source of calcium since concentrations may reach sensibly high levels.

In *Aloreña de Málaga* cracked olives, adding KCl and $CaCl_2$ salts during the fermentation/storage (with NaCl in reduced proportions) resulted in a more complex model for estimating Ca content in the flesh. This model included several interactions between $CaCl_2$ and the other salts, suggesting that the different salt composition or the unique structure of these olive untreated olives leads to behaviour distinct from that of Spanish-style Manzanilla olives [14]. The presence of $CaCl_2$ in the fermentation brine significantly markedly increased Ca contents in the flesh, reaching up to 11 g/kg flesh [14]. Similarly, the models for K and Ca contents in the flesh of Spanish-style Gordal olives, fermented under conditions similar to those used in *Aloreña de Málaga*, also included interactions resembling those of the last cultivar. This supports the idea that the composition of the salt mixture can influence the absorption of elements into the flesh [26]. However, the process scarcely affected the actual concentration of Ca in the flesh, which was also relatively high and reached above 9 g/kg flesh [26].

Magnesium. The model for Mg had significant two-way interaction (Table 6S). In actual units, the function took the expression:

$$\text{Mg in flesh (mg / kg)} = -215.51772 \cdot [\text{KCl}] - 24.57867 \cdot [\text{CaCl}_2] + 1411.22392 \cdot [\text{MgCl}_2] + 263.3741 \cdot [\text{KCl}][\text{CaCl}_2] + 316.54281 \cdot [\text{KCl}][\text{MgCl}_2] \quad \text{Eq 4}$$

The interpretation of this complex equation (linear and quadratic terms for KCl and $MgCl_2$) requires plotting in a ternary plot (Fig. 2C). The Mg contents increase almost linearly as the design concentrations increase. The effect of interactions is reflected as a slight curvature, progressively more observable as the $MgCl_2$ concentration is higher. Its effect is more pronounced on the border $CaCl_2$-KCl and rate 80/20, respectively. No previous studies are available to compare this mineral model with others related to the packaging brine of green Spanish-style Manzanilla olives.

Manganese. This element was the only non-added microelement showing a significant relationship with the KCl, $CaCl_2$, and $MgCl_2$ concentrations in the experimental design (Table 6S). The three-way interaction in the model was significant (Table 6S), and subsequently, to preserve the hierarchical property, the corresponding linear and two-way interactions were also retained. The VIF values for coefficients indicate a slight increase in collinearity, and the explained variance was moderate (r-squared 0.78 and adj. R-squared 0.600). The equation took the form:

$$\text{Mn in flesh (mg / kg)} = +1.02005 \cdot [\text{KCl}] + 1.93841 \cdot [\text{CaCl}_2] + 2.12079 \cdot [\text{MgCl}_2] - 2.13895 \cdot [\text{KCl}] \cdot [\text{CaCl}_2] - 2.26320 \cdot [\text{KCl}] \cdot [\text{MgCl}_2] - 3.15201 \cdot [\text{CaCl}_2] \cdot [\text{MgCl}_2] + 2.19515 \cdot [\text{KCl}] \cdot [\text{CaCl}_2] \cdot [\text{MgCl}_2] \quad \text{Eq 5}$$

In the ternary plot (Fig. 2D), the lowest Mn in the flesh (indicating the highest Mn leaching) was observed at the barycenter of the plot, where the concentrations of the three salts are at 33.33 % of their ranges. Then, KCl, $CaCl_2$, and $MgCl_2$ contribute similarly to Mn release from the olive flesh. No previous studies are available to compare this mineral model in the packaging brine of green Spanish-style Manzanilla olives.

Regarding other non-added mineral elements, the "Control" had, in general, slightly higher levels than the design treatments; therefore, packaging with salt mixtures scarcely affected their contents due to their strong linkage to flesh components. In the

experimental packaging, Fe contents ranged from 3.21 to 3.77 mg/kg (mean, 3.49 mg/kg), Cu was in the range 1.31–1.63 mg/kg flesh (mean, 1.48 mg/kg), Zn ranged from 1.48 to 1.69 mg/kg (mean, 1.61 mg/kg), and P presence was the highest, ranging from 58.76 to 68.34 mg/kg (mean, 62.47 mg/kg).

3.6. Multivariate analysis of the mineral composition of treatments

From an industrial perspective, comparing the mineral profiles of various treatments helps select conditions such as type of salt, cost efficiency, or sensory profiles among treatments with similar mineral profiles. This comparison was performed using clustering (Fig. 3). Fig. 3A shows that the current packaging (cluster C4, treatment 15) is markedly different from the others, presenting an entirely different profile compared to packages using salt mixtures (Fig. 3B). This "Current" mineral profile is characterised by the high Na content and very low concentrations of the other macro and microminerals (Fig. 3B, cluster C4). Conversely, the experimental treatments present relatively low dissimilarity among themselves. Treatments 1, 4, 7, and 14 (cluster C1) are the most dissimilar within the experimental group but still differ notably from other treatments with added salt mixtures (Fig. 3A). These treatments are characterised by high initial proportions of Ca and Mg (Table 1) and show relevant content in Ca (mainly) and Mg (Fig. 3B, cluster C1). The third cluster includes treatments 3 and 8, which exhibit high contents of K and Mg (Fig. 3B). Finally, cluster C2, the larger group, includes treatments 2, 9, 5, 13, 11, 12, 6, and 10. This cluster is characterised by intermediate yet relatively high K, Ca, and Mg (Fig. 3B). It offers diverse conditions options, though it has the most varied mineral profiles compared to the "Current" product.

Overall, the multivariate study represents an important tool for the industry. Producers can choose from initial salt mixtures to achieve similar mineral profiles. This allows for selecting mixtures that best adapt products to consumer demand, processing conditions, or specific product characteristics.

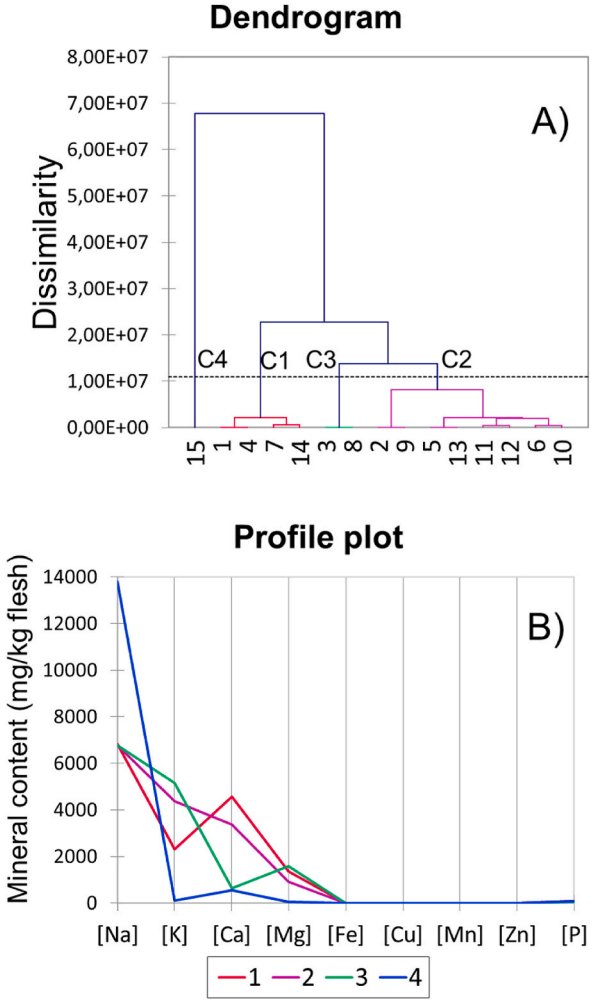

Fig. 3. Replacing salt (50 %) with potassium, calcium, and magnesium chloride in plain green Spanish-style Manzanilla table olives. Grouping the experimental treatments based on their nutrient mineral profiles, shown as follows: A), Clustering treatments based on dissimilarities; B), Mineral profiles of the clusters identified in A).

3.7. Effect of the experimental design salt mixture on the contribution to the Reference Daily Intake (RDI) of mineral nutrients

This approach is based on the following Reference Daily Intakes (RDIs), taken from Annex XIII of Regulation (EU) 1169/2011 [45]: 6 g salt or 2300 mg Na/day; 200 mg K/day; 800 mg Ca/day; and 375 mg Mg/day. Substituting 2.5 % salt in the packaging reduced Na contribution from these table olives to the RDI by half compared to the "Current" product, decreasing it from 58.50 % to approximately 28.72 %. This substitution only slightly reduced the levels of other non-added mineral nutrients. Among microelements, only Cu had a RDI contribution of around 15 %, sufficient to recognise the product as a potential source of this element (Table 7S).

Since RDI proportions are linear combinations of the mineral concentrations in the flesh, they can be related to the levels of fortifying elements in the design. Therefore, their model characteristics and ANOVA are similar and are omitted from the discussion. The model for Mn is also not discussed, as the Mn level was below the threshold of nutritional labelling interest. The functions and plots in the simplex for K, Ca, and Mg will be discussed because of the interest in the contributions of the added salts to the RDI for producers. Proportions above the established levels could allow specific claims.

Potassium. The model predicting the contribution of the salt mixtures used in packaging plain green Spanish-style Manzanilla table olives to the RDI of this element is:

$$\%\text{RDI (K)} = +17.07921 \cdot [\text{KCl}] - 0.45341 \cdot [\text{CaCl}_2] + 0.097434 \cdot [\text{MgCl}_2] \quad \text{Eq 6}$$

Its ternary plot (Fig. 4A) shows that the range of K concentrations used in the design includes contributions that reach RDI proportions up to approximately 25 % (border $CaCl_2$-$MgCl_2$), which could support, aside from other considerations, health claims recognised for K.

Calcium. The model equation for predicting the contribution to Ca is:

$$\%\text{RDI (Ca)} = +3.23800 \cdot [\text{KCl}] + 54.25693 \cdot [\text{CaCl}_2] + 3.28166 \cdot [\text{MgCl}_2] \quad \text{Eq 7}$$

The ternary plot (Fig. 4B) predicts a range of RDI percentages, up to 60 % (at the $MgCl_2$-KCl border). Due to olives' usual high Ca

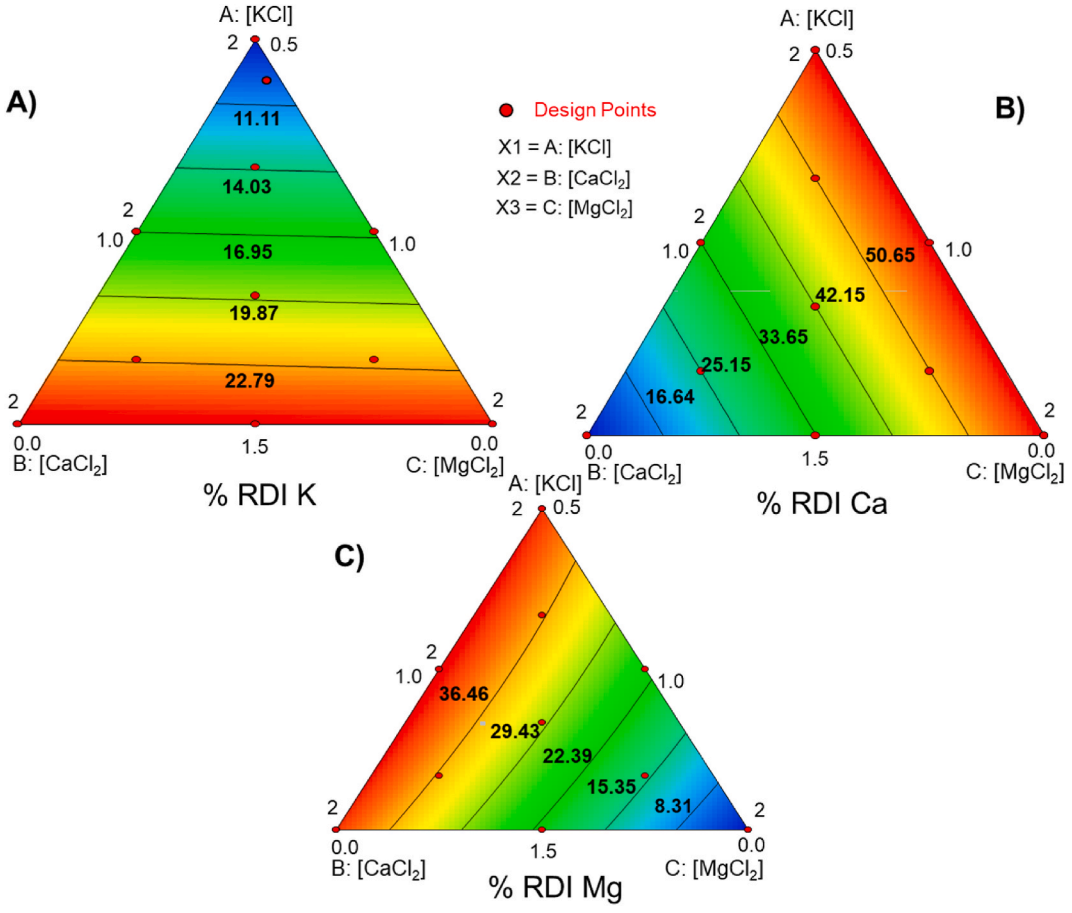

Fig. 4. Replacing salt (50 %) with potassium, calcium, and magnesium chloride in plain green Spanish-style Manzanilla table olives. Estimated Reference Daily Intake of selected mineral nutrients as a function of the KCl, $CaCl_2$, and $MgCl_2$ concentrations in the packaging brines are shown as follows: A), potassium (K); B), calcium (Ca); and C), magnesium (Mg). Number 2, close to design points, indicates a replicated treatment.

content, a relatively low addition of $CaCl_2$ will be sufficient to exceed the 15 % threshold necessary to consider plain green Spanish-style Manzanilla table olives a source of Ca. Higher concentrations is also possible but may affect the sensory characteristics [26].

Magnesium. The equation for predicting the contribution of Mg was:

$$\%RDI\ (Mg) = -5.74713 \cdot [KCl] - 6.5541 \cdot [CaCl_2] + 37.63263 \cdot [MgCl_2] + 7.03298 \cdot [KCl] \cdot [CaCl_2] + 8.44113 \cdot [KCl] \cdot [MgCl_2] \quad \text{Eq 8}$$

The ternary plot (Fig. 4C, values at the $CaCl_2$-KCl border) predicts RDI values up to around 44 % at the highest concentration of $MgCl_2$. However, contributions higher than 15 % can be obtained with moderate concentrations.

Several reports have estimated the concentrations of Na, K and Ca in the olive flesh after fermentation in salt mixtures, but references for Mg are scarce. Panagou et al. [12] reported a sodium concentration of around 1000 mg/100 g flesh potassium in *Conservolea* natural black olive processed through the conventional process. In olives prepared with various NaCl, KCl, and $CaCl_2$ proportions, sodium ranged from 16.4 mg/100 g flesh (without NaCl) to 446 mg/100 g flesh (with 4%NaCl). For K, the conventional process yielded 136.7 mg/100 g flesh, while in those using salt mixtures, potassium levels ranged from 119.7 (in the absence of KCl) to 453.6 mg/100 g flesh (with 4 % KCl). Calcium concentrations were 180 mg/100 g flesh for the conventional process, ranging between 176.3 (in the absence of $CaCl_2$) to 245 mg/100 g flesh (with 4 % $CaCl_2$). However, no data was provided for Mg.

In the fermentation of Spanish-style *Gordal* olives with NaCl, KCl, and $CaCl_2$, Moreno-Baquero et al. [26] estimated sodium levels ranging from about 700 mg/100 g flesh to above 1500 mg/100 g flesh (with a intended NaCl concentration of 4%–10 %), potassium levels from approximately 170 mg/100 g flesh to 860 mg/100 g flesh (with an intended KCl concentration of 4%–10 %) and Ca levels fluctuating between 170 mg/100 g flesh and 760 mg/100 g flesh (with an intended $CaCl_2$ concentration of 0%–6%). However, as noted in several occasions, the mineral concentrations after fermentation not necessarily reflects what reaches the consumer, as conditioning processes (such as size grading or pitting) can reduce these levels. In addition to incorporating Mg, this study has the advantage of maintaining the mineral concentrations until the product reaches the consumer.

3.8. Comparing mineral nutrient contents and proportions of RDI in partially replaced treatments estimated from flesh analysis and the proposed functions

To assess the accuracy of the data derived from the above relationships for nutritional labelling, the estimations were compared with those previously obtained by direct analysis of the olive flesh [46]. The mineral concentrations and the %RDI obtained from both procedures (regardless of rounding rules) are presented in Table 8S. The median deviation (due to work in percentages) for K concentration was 0.85 %, and for %RDI was 0.17 %, while for Ca, the deviations were −0.26 % and −0.14 %, and for Mg, they were 0.23 % and 0.29 %. The disparities between concentrations and %RDI were minimal, particularly about the latter, which, after rounding, would remain practically the same regardless of the data source.

However, it is important to note that these equations would not be universally applied. Tailored functions specific to particular fermentations, packaging conditions, other cultivars, or types of containers need to be developed and rigorously verified. Additionally, such functions will only be applicable within the concentration range of potassium (K), calcium (Ca), and magnesium (Mg) chloride salts considered in the experimental design.

Therefore, the provided information strongly supports the initial hypothesis favouring salt substitution during packaging over fermentation. Moreover, the experimental design allowed for the creation of products with varied mineral contents and enabled the derivations of functions for precise prediction of the added element concentrations in flesh and their contributions to the dietary reference intakes, compatible with their use in nutritional labelling. Notably, all products exhibited reduced sodium and enhanced nutritional value compared to traditional presentations, particularly in terms of calcium, potassium and magnesium, without compromising stability due to the routinary stabilisation of these products by pasteurisation. So, this study unveils a research avenue with considerable potential.

4. Conclusions

This work introduced an innovative strategy to reduce sodium (Na) content in green Spanish-style Manzanilla olives while enhancing levels of potassium (K), calcium (Ca), and magnesium (Mg). The study's results offer detailed insights into how the addition of these minerals affects the equilibrium between both added and naturally occurring minerals, as well as their interactions with olive components. Evidence supports the presence of strong bonds between various minerals and olive pulp components using the newly proposed mineral distribution coefficient. Furthermore, the study developed predictive models to estimate expected concentrations of the added minerals in olive pulp based on added salt concentrations in brine and their impact on the nutritional labels. These models offer a range of options for the industry, helping it select the best choices to meet consumers' demands. Below, specific details regarding these aspects are enumerated.

Mineral distribution. The fortifying salts altered the distribution of minerals between the olives and brine. The *Kd* for Ca, Mg, and Fe was significantly influenced by the initial concentrations of the three salts in brine. Regression analysis showed that Na, K, and Mg are primarily distributed between flesh moisture and brine. In contrast, Ca and initially present Fe, Cu, Zn, and Mn strongly associate with the flesh components, with P in an intermediate position in this distribution.

Development of predictive models. Applying RSM, predictive models for the added minerals and initialy present in olives Mn were successfully developed as a function of KCl, $CaCl_2$ and $MgCl_2$ levels in the initial brine. All models included linear terms for the three

added salts, with only the Mg model including two-way interactions. The contour lines in the simplex showed increased mineral content in the flesh with higher concentrations of their respective salt in brine. The influence of the other salts was relevant only in the Mg model. For Mn, the model included three-way interactions. The minimum Mn content was observed around the barycenter (33.33 % of each salt range in the initial brines), while the highest retention (about 0.30 mg/kg flesh) occurred at the KCl vertex.

Impact of the substitution proposal on sodium reduction and mineral enhancement. The proposed methods greatly reduced the Na content in green Spanish-style Manzanilla olives from 1.40 g/100 g flesh in the current product to 0.68 g/100 g flesh in the experimental treatments. In contrast, the concentrations of K, Ca, and Mg could increase up to 0.5, 0.45, and 0.15 g/100 g flesh, respectively. This resulted in a markedly different mineral profile in the fortified products compared to the current product. The fortified products could be further categorized into three distinct groups of several treatments, offering diverse options for the industry even within these clusters.

Model development for mineral contribution to RDI. After transforming mineral contents into RDIs, models were developed to predict the mineral's contribution to the RDI based on the initial brine salt contents. The models showed that the contribution to the RDI could reach about 25 % for K, 60 % for Ca, and 44 % for Mg.

Model accuracy. The predictive models demonstrated high accuracy, with minimal median deviations for mineral contents and their respective RDI, which were: potassium (K), 0.85 % for content and 0.17 % for RDI contribution; calcium (Ca), −0.26 % for content and −0.14 % for RDI contribution; and magnesium (Mg), 0.23 % for content and 0.29 % for RDI contribution. These models could then generate data for nutrition labelling, assuming that the packaging characteristics remain consistent with those used in the model development.

Given the varied requirements of different companies, this work did not aim to optimise a single solution. Instead, we provide derived equations and ternary plots in the simplex that can assist each industry in designing its own new products with enhanced nutritional mineral profiles. This approach facilitates the development of olives with customised mineral content tailored to specific nutritional goals.

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Antonio López-López: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **José María Moreno-Baquero:** Investigation. **Antonio Garrido-Fernández:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix. ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37901.

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