Food Microbiology 49 (2015) 56–64

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Fermentation profile of green Spanish-style Manzanilla olives according to NaCl content in brine

J. Bautista-Gallego*, F.N. Arroyo-López, V. Romero-Gil, F. Rodríguez-Gómez, P. García-García, A. Garrido-Fernández

Food Biotechnology Department, Instituto de la Grasa (CSIC), Avda. Padre García Tejero, n° 4, 41012, Seville, Spain

ARTICLE INFO

Article history:
Received 18 October 2014
Received in revised form
14 January 2015
Accepted 30 January 2015
Available online 10 February 2015

Keywords:
Calcium chloride
Green table olives
Mixture design
Potassium chloride
Sodium chloride

ABSTRACT

This work studies the effects of the partial substitution of NaCl with potassium and calcium chloride salts on the fermentation profile of Spanish-style green Manzanilla olives. For this purpose, response surface methodology based in an enlarged simplex centroid mixture design with constrain ($\sum$salts = 100 g/L) was used. Regarding to physicochemical characteristics, pH decreased when $CaCl_2$ increased, titratable acidity was lower in presence of KCl while combined acidity increased as the contents of KCl and $CaCl_2$ were close to the barycentre of the experiment (~33.33% each salt). Regarding to microbiological profile, *Enterobacteriaceae* growth was slight stimulated in presence of high $CaCl_2$ contents, yeast patterns were not linked to the initial brine compositions, while the maximum lactic acid bacteria population decreased slightly as KCl and $CaCl_2$ increased in the proportion 1:1, although a moderate (equilibrated) content of both may be stimulating. Results obtained in this work show that Spanish-style green Manzanilla cv. can be fermented in diverse mixtures of chloride salts, albeit the initial $CaCl_2$ should be limited to 20–30 g/L to prevent excessive *Enterobacteriaceae* growth; combining it with a similar proportion of KCl may also improve LAB predominance.

1. Introduction

Spanish-style green Manzanilla cv. is probably the most appreciated and marketable table olive product. The Spanish production was about 156,000 tons in the 2012 season (Agencia Española para el Aceite de Oliva, 2013) but it is also fairly popular in the rest of olive growing countries. Its processing is quite standard and is characterized by the use of an intermediate concentration of sodium hydroxide solution (20–25 g/L) during debittering. Then, the fruits are washed to remove the excess of alkali (18–24 h) and brined in a NaCl solution (110–120 g/L NaCl) where a spontaneous or inoculated lactic acid fermentation process is achieved (Garrido Fernández et al., 1997).

Fresh olive fruits (raw material) are low in Na content but on the contrary high in K and, in decreasing proportions, Ca, Mg, and P (Garrido Fernández et al., 1997). Most of the original elements in flesh, except Ca, are lost in great proportions during processing because the fruits' immersion in successive aqueous solutions. Hence, after the fermentation and conditioning, the final products are poor in K, Mg and P and other micronutrient minerals but rich in Na which, according to an industrial survey, has an average content of 17 g Na/kg olive flesh (equivalent to 42.5 g salt/kg flesh) (López et al., 2008). Therefore, in case of a 100 g olive flesh consumption, such level would supplied, approximately, 70% of the recommended daily intake for salt (WHO, 2003), established as 5 g/day (~2000 mg Na^+/day) (WHO, 2003; British Food Standard Agency (FSA), 2009).

The average intake of salt in Spain has been estimated in 9.8 g/day (Ortega et al., 2011); reduction of this level may, eventually, be achieved by limiting the salt content in the most contributing foods. In this survey, the impact of table olives on the overall salt intake was not considered of concern due to its low consumption (~4 kg olives (equivalent to 2.8 kg flesh)/year). In spite of this, a reduction of NaCl concentration in table olives would be favorable for consumers. Following the suggestions of the National Salt Initiative (Council, 2010), the EU Commission made a called to Member States to develop coordinate national nutritional policies to reduce salt intake. In this framework, the “Agencia Española de Seguridad Alimentaria y Nutrición” (AESAN) implemented in Spain the so called NAOS strategy, which includes an initiative to reduce salt consumption (AESAN, 2010).

* Corresponding author. Tel.: +34 954 692 516; fax: +34 954 691 262.
E-mail address: joaquinbg@ig.csic.es (J. Bautista-Gallego).

http://dx.doi.org/10.1016/j.fm.2015.01.012
0740-0020/

Different studies have been carried out to evaluate the reduction or substitution of NaCl in table olive and other vegetable fermentations (Bautista Gallego et al., 2013). They have shown that this possibility is becoming a reality but food reformulation must be checked product by product. Tassou et al. (2007) reported an increase in the cell wall breakage due to the presence of $CaCl_2$. Kanavouras et al. (2005) produced natural black olives using mixtures of NaCl (12.8%) and $CaCl_2$ (0.29%) without lost of acceptance by consumers. Di Silva (2000) also arrived to similar results after processing green olives in solutions containing KCl and $CaCl_2$. Bautista Gallego et al. (2010) established that NaCl could be substituted with KCl and/or $CaCl_2$ in diverse proportions in brined green olives. In addition, the composition of the initial brines caused a great impact on the Gordal cv. processing, decreasing sugar release and acidity production (Bautista Gallego et al., 2011). However, the fermentation of Manzanilla cv. as green Spanish-style in low NaCl brines, supplemented with other mineral nutrients, has not been investigated yet. However, these studies are necessary because the potential microbial risk inherent to any food reformulation (Sleator and Hill, 2007).

Experimental design and Response Surface Methodology (RSM) are powerful tools to study the simultaneous effects of several variables (Myers and Montgomery, 2002) and has been widely used in experimental studies related to salt substitution (Guillou and Floros, 1993; Tsapatsaris and Kotzekidou, 2004; Bautista Gallego et al., 2010, 2011).

In this context, the aim of this work was to study the effect of the partial replacement of sodium chloride by potassium and calcium chloride on the physicochemical and microbiological profile of green Spanish-style Manzanilla cv. fermentation, using RSM based on a simplex centroid mixture design (enlarged with central points).

2. Material and methods

2.1. Olives and experimental design

The experiments were carried out with Manzanilla olives (*Olea europaea pomiformis*), which is the most popular cultivar for preparing Spanish-style green table olives all over the world. Fruits were supplied by a local producer (JOLCA S.A., Huevar del Aljarafe, Seville, Spain). After a previous selection for removing deteriorated and low size fruits, the olives were introduced in the fermentation vessels (2.90 kg/container) and immersed in a 21 g/L sodium hydroxide solution (lye) (2.35 L solution/container). When the alkali had penetrated 2/3 of flesh, the lye was substituted with water. After washing for about 18 h, the liquid was removed and immediately substituted with different brine solutions. The fermentation vessels were let to equilibrate for 22 h and, then, carbon dioxide was bubbled until saturation (pH stabilization). The suspension was allowed to equilibrate for ~20 h and then all the fermentations vessels were inoculated with a 24 h *Lactobacillus* (*Lb.*) *pentosus* culture (strain IGLAC01) to reach an initial population of approximately 6 $\log_{10}$ CFU/mL. The growth of the rest of the microbiota (*Enterobacteriaceae* and yeasts) was left to be spontaneous.

The experimental design consisted of 15 runs composed of 10 independent treatments obtained from a simplex centroid mixture design, enlarged with some interior points and 5 replicates (Table 1). The composition of each brine was obtained using Design Expert v.6.06 software (StateEasy, INC., Minneapolis, USA). The total initial concentrations of the diverse salts was constrained to $[NaCl] + [KCl] + [CaCl_2] = 100$ g/L, with NaCl ranging from 40 to 100 g/L, KCl from 0 to 60 g/L and $CaCl_2$ from 0 to 60 g/L. The overall sum of mixture concentrations (100 g/L) mimicked that of brines containing only NaCl, commonly used in this process.

Table 1
Expanded simplex centroid mixture design used in the present study for the fermentation of Manzanilla olives. Constrains: $NaCl + KCl + CaCl_2 = 100$ g/L, with NaCl ranging from 40 to 100 g/L, KCl from 0 to 40 g/L and $CaCl_2$ from 0 to 60 g/L.

Treatments	NaCl (g/L)	KCl (g/L)	$CaCl_2$ (g/L)
1[a]	100	0	0
2	50	10	40
3[b]	40	30	30
4	70	30	0
5	80	10	10
6[c]	60	20	20
7	50	40	10
8[d]	40	60	0
9[a]	100	0	0
10[e]	40	0	60
11[b]	40	30	30
12[e]	40	0	60
13	70	0	30
14[d]	40	60	0
15[c]	60	20	20

Note: Runs with the same superscript correspond to duplicate experiments.

2.2. Physicochemical analyses

The analyses of olive brines for pH, titratable and combined acidity were carried out using the methods described by Garrido Fernández et al. (1997). The changes in pH were modelled using the following first order decay equation:

$$y = a + b\cdot\exp(-c\cdot x) \quad (1)$$

where *x* is the time (h), *a* is the lower asymptote, *b* the total change in pH and *c* the kinetic constant unit (h^{-1}).

The production of titratable acidity was modelled using a first order kinetic formation, according to the equation:

$$y = a\cdot(1 - \exp(-b\cdot x)) \quad (2)$$

where x is the time (h), *a* is the total final acid content expressed as g lactic acid (upper asymptote), and *b* is the specific formation rate unit (h^{-1}). In this case, the time to form half the amount of acid is given by $x_{50} = \ln 2/b$. The areas for the evolution of the physicochemical characteristics *versus* time were estimated by integration using Origin 7.5 software (OriginLab Corporation, Northampton, USA).

2.3. Microbiological analyses

Brine samples or their decimal dilutions were plated using a Spiral System model dwScientific (Don Whitley Scientific Limited, England) on the appropriate media. Subsequently, plates were counted, using a CounterMat v.3.10 (IUL, Barcelona, Spain) image analysis system, and results expressed as $\log_{10}$ CFU/mL. *Enterobacteriaceae* were counted on VRBD (Crystal-violet Neutral-Red bile glucose)-agar (Merck, Darmstadt, Germany), LAB on MRS (de Man, Rogosa and Sharpe)-agar (Oxoid) with 0.02% (wt/vol) sodium azide (Sigma, St. Luis, USA), and yeasts on YM (yeast–malt–peptone–glucose medium)-agar (Difco™, Becton and Dickinson Company, Sparks, MD, USA) supplemented with oxytetracycline and gentamicin sulphate as selective agents for yeasts. Plates were incubated at 30 °C for 48–72 h.

Changes in the *Enterobacteriaceae* populations *versus* time were modelled using the two-term Gompertz equation proposed by Bello and Sánchez Fuertes (1995), which has the following expression:

$$\log N_t = \log(N_0) + k_1 * \exp[-\exp(-k_2(t-k_3))] - k_4 * \exp[-\exp(-k_5(t-k_6))] \quad (3)$$