PHYSICO-CHEMICAL CHARACTERIZATION OF FERMENTED ORANGE PEEL: A NOVEL PRODUCT MADE FROM ORANGE JUICE MAKING BYPRODUCTS

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KEYWORDS

Food waste, msayer, texture, volatile composition, consumer study

HIGHLIGHTS

- *Msayer*'s traditional recipe was used to develop a product using orange peel
- Yeasts seemed to drive the whole fermentation process
- Results showed that the best product was the one with 5% salt content

ABSTRACT

A traditional fermentative process typical of North-African countries has been used to develop a new product using orange peels from orange juice making. The impact of different NaCl, sucrose and time conditions on the physicochemical characteristics of the final product (instrumental texture, color, volatile composition) and consumers' perception, as well as the making process (pH, total soluble solids, sugars, and Plate Count Agar method - PCA for aerobic and anaerobic microorganisms) has been studied. Results showed that NaCl was a significant effect, and the interaction 'NaCl*time' was significant in some parameters, suggesting that the appropriate NaCl content was 5%. Although PCA was not significantly different among samples, pH decrease, sugars contents, instrumental texture, and volatile composition of the samples fermented in a 5% brine were significantly different than those of the samples fermented in the 10 or 15% NaCl brines. Slightly significant differences were detected along time for some of the physico-chemical parameters, but none of the results suggested that continuing the fermentative process over 40 days would results in better product. Consumer study results confirmed the physico-chemical ones, indicating that the sample made using a 5% NaCl content could be the one with the best potential success.

1. INTRODUCTION

Global orange production (*Citrus sinensis*) amounted to about 53.8 mill. t during the marketing year 2018-2019 (Shahbandeh, 2020), and its main derivative, orange juice, is maybe the most important fruit juice consumed worldwide. Industrial, HORECA (HOtels REstaurants CAtering sector) or household production of orange juice is similar, generating the same byproduct, orange peels, which represent a waste of approximately 50% weight of the raw material. Food loss and waste, differentiated by the Food and Agriculture Organization (FAO) depending on the food supply chain moment in which the loss or waste is generated (early on *vs* later and as a result of users/consumers' decisions, respectively), have become important worldwide problems, meaning over 1.3 billion tons of edible material per year. Most of these food losses/wastes have a plant origin, coming from fruits, vegetables, roots, and tubers (FAO, 2011). As well as food industry, the HORECA segment has a significant effect on producing food waste, but the main reasons for this waste generation are shared with the household sector: portion sizes, staff's awareness, and some logistics difficulties such as planning based on reservations (Chalak, Abou-Daher, & Abiad. 2018; Monier et al., 2010).

Different studies have been published including relevant information for valorizing orange peels coming from orange juice industry (Rezzadori, Benedetti, & Amante, 2012). Most of the proposed solutions included a complex treatment of the peels to extract specific fractions (e.g.: essential oils), in turn causing new byproducts, and no solutions have been studied for HORECA's or household stakeholders, whose interest in food waste management is increasing. Some recipes and formulas have been published recommending fermentation as a friendly technique to transform vegetable wastes into new products, reproducing traditional processes of specific cultures (e.g.: sauerkraut making), but using different raw materials (e,g.: del Noval & Prado, 2019; Katz, 2012; Redzepi & Zilber, 2018). In most of these cases, the byproduct is transformed into an edible ingredient avoiding the generation of new by-products. Following this trend, orange peels discarded from orange juice making could be used as raw material in a fermentation process, obtaining an orange *msayer*. Fermented or "pickled" lemons, also called *msayer,* are typical products of the Moroccan and other North-African countries culinary tradition. The process of making *msayer*, described in popular recipes, is the following: to add cut lemons to a brine (with a variable concentration of NaCl), sometimes using a starter (e.g.: kombucha, or brine

obtained from a previous production of *msayer*), and to let the fermentation occur during approximately 30 days, generally at room temperature. Then, the pulp of *msayer* is frequently used as a salt substitute which also adds citric aromas, and the peel is directly consumed, generally served cut in strips or cords. The fermented peel is characterized by its low bitterness if compared with the original lemon peel (Aayah et al., 2010). Although the production process could be used as an example for valorizing orange peel, *msayer* and its making process has been barely investigated.

Aayah et al. (2010) detailed *msayer* making as a dry salting process in which deep incisions are made into whole lemon cut, and covered with a salt layer in a ratio of 10:100 g of salt:weight of fresh fruit respectively. After the salt addition, the lemons are stacked in glass jars until there is no free space. Sometimes a layer of oil is added to avoid the presence of air in the jar, which is tightly closed and remains at room temperature for 1-2 months. After this period, the lemon skin has browned, and the fruits have shrunk, remaining in a brine which results from changes in the osmotic pressure between the lemon and the medium. After a sampling process that reached 52 days, these authors described the presence of some microorganisms, mainly yeasts of the genera *Candida* and *Saccharomyces*, and the composition dynamics of the medium with a low pH (approximately 2.5) and a high NaCl content: a highly restrictive medium for the vast majority of microorganisms. On the other hand, Bousmaha, Ouhssine, & ElYachioui (2006) used 2 microorganisms previously isolated from a spontaneous fermentation of a *msayer*'s brine to select and inoculate new batches of *msayer*. These authors reported using a 15% NaCl brine enriched with glucose (3 g L^{-1} addition) and proposed changing the traditional spontaneous fermentation process by inoculating a mixed and selected culture, in which a yeast and a lactic acid bacterium were chosen by its high acidifying capacity.

The objective of the present study was to characterize and optimize the production of fermented orange peels coming from orange juice making, reproducing the aforementioned *msayer* making process. A Box-Behnken design was used to determine the optimal sucrose and NaCl concentration, as well as time, to develop a highly accepted product, conducing a consumer study to determine liking of the different samples. Total soluble solids (TSS), pH, and sugars concentrations (High Performance Liquid Chromatography) were determined to study the process, as well as the presence of different microbial populations using traditional microbiology

methods (total plating counts). In addition, color (CIE *L*a*b**), instrumental texture (cutting force), and volatile composition (Gas Chromatography-Mass Spectrometry) were determined in the final *msayer* samples, with the aim of characterizing the developed product.

2. MATERIAL AND METHODS

2.1. Reagents and culture media

Standards of inositol, erythritol, sorbitol, xylitol, glucose, lactitol, fructose, lactose, sucrose, and maltose, analytical quality, were supplied by Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Peptone saline solution, Rose Bengal agar and Nutrient Agar were from VWR, (VWR Inc, Germany). WLD agar, Wort agar, and tetracycline solution were purchased at Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). The alkane standard mixture for Gas Chromatography-Mass Spectrometry compounds identification was purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).

2.2. Samples: *msayer* making

Approximately 25 kg of orange peels discarded from making orange (*Citrus sinensis,* Navel lane late cultivar) juice in the restaurant of Basque Culinary Center were used for the present study. The juice was made in a Zumex V machine (Zumex®, Valencia, Spain). Each *msayer* sample was prepared as indicated below: approximately 300 g of peels were weighed and placed in 750 mL glass jar, then, the peels were fully covered with the corresponding brine-type (500 ml approximately, brines details described below) and a top layer of olive oil to ensure avoiding air-contact. The filled jars were tightly closed with lids, which had a rubber septum stopper for sampling, and placed at room temperature (21-24 °C) for the duration of the experiment.

An experimental design was carried out to study the optimization of some of the independent variables that could affect the characteristics of *msayer:* 'NaCl content', 'sucrose content', and 'time'. The design consisted of 3 factors and 3 levels: a) a brine with a NaCl concentration of 5, 10 and 15% w/v, b) a brine with a sucrose concentration of 30, 40, and 50 g L^{-1}, and c) 40, 65, or 90 days of making. These conditions were decided based on the NaCl content reported in 2 studies which treated *msayer* making (Aayah et al., 2010; Bousmaha et al., 2006) and to increase sugars content, without reaching the reported concentration of sugars in orange juice (Pepin, Stanhope, & Imbeault, 2019). The combination of variables resulted in a total of 27 different samples (named T1-T9 as indicated in Table 1) which were made in triplicate. The dependent

variables under study were cutting force (instrumental texture analysis), volatile composition, and liking.

2.3. Brine sampling

With the aim of monitoring the fermentation process, brine samples were taken using sterile 10 ml disposable syringes at days 0, 1, 4, 7, 40, 65, and 90, and characterized using different physico-chemical and microbiological analyses.

2.3.1. Determination of pH and Total Soluble Solids (TSS)

The pH of the original brine and the brine samples was measured with an electronic pH meter Crison Basic 20 (Crison Instruments SA, Spain). The total soluble solids of the brines were measured at 20 ± 0.5 °C using a refractometer Digital Handheld Refractometer VWR® (VWR Inc, Germany), results were obtained in ºBrix.

2.3.2. Sugars and polyalcohols determinations using High Performance Liquid Chromatography (HPLC-PAD)

Brine samples were diluted 1:50 (v/v) with ultrapure water at 60 °C, then shaken for 30 minutes and centrifugated at 4200 rpm for 10 minutes. The supernatant was filtered using a 25 mm, 0.45 µm nylon VWR® Syrenge Filter (VWR Inc, Germany). Fifteen µL of each sample were injected in a Knauer HPLC system (Knauer Scientific Devices GmbH, Germany) equipped with a low-pressure gradient pump (Knauer Azura® P 6.1L, Knauer Scientific Devices GmbH, Germany), using an automatic injector (Knauer Azura ® AS 6.1L) with a partial loop (100 µL loop). A Metrosep Carb 2 150 / 4.0 column (15 cm long and 4 mm internal diameter), placed in a CT 2.1 column thermostat programmed at 35 ± 0.1°C, was used for the separation of compounds. An isocratic mobile phase of 300 mmol L^{-1} NaOH and 1.0 mmol L^{-1} NaAc, at a flow rate of 0.5 ml min^{-1} and 80 bar backpressure, was used. The equipment was coupled to an amperometric detector 945 Professional Detector Vario (Metrohm AG, Herisau, Suiza) in pulsed amperometric mode. Standards for inositol, erythritol, sorbitol, xylitol, glucose, lactitol, fructose, lactose, sucrose, and maltose were used to quantify the presence of the different compounds.

2.3.3. Microbiological analyses

The brine samples were diluted in tenfold series in saline peptone solution (0.85% NaCl and 1% peptone) and plated onto different culture media to quantify the presence of different microorganisms using a traditional Plate Count Agar (PCA) method: Nutrient Agar for total viable

colonies in aerobic conditions (30 °C for 48 h), WLD agar for lactic acid bacteria (30 °C for 72 h, in anaerobic conditions), Wort agar supplemented with 20 mg L^{-1} tetracycline for yeast and mold count (30 °C for 48 h), and 35 mg L^{-1} Rose Bengal for molds (30 °C for 72 hours) (Aayah et al., 2010).

2.3. *Msayer* characterization

All physico-chemical analyses mentioned below were conducted to characterize the original orange peel samples and the final *msayer* samples (after 40, 65, and 90 days of fermentation).

2.3.1. *Instrumental texture: cutting force*

The conditions reported in Singh and Reddy (2006) for orange peels hardness determination were used to determine the original orange-peel and *msayer* hardness. Pieces of 20 x 40 mm were placed in a Texture Analyzer Aname (Microstable system, UK) with a HDP/BSK blade set probe for determining cutting force. The load cell was calibrated to 295 N and the cutting was done at a speed of 1 mm s^{-1}. Peak cutting force was considered as the first peak force in g. A total of 10 replications were done for each sample.

2.3.2. *Instrumental color*

The color of both, orange and *msayer* peels, was measured using Chroma Meter CR 400 (Konica Minolta, Inc, Japan) using the CIE *L*a*b** color space. L* indicates lightness (range 0–100), and a* and b* are the chromatic coordinates; a* takes positives values for reddish colors and negatives for the greenish ones, whereas b* takes positive values for yellowish colors and negative values for bluish ones (Minolta, 1994). Data were expressed with the *L*, a*, b** values and then, chroma [$C^* = (a^{*2} + b^{*2})^{1/2}$] and Hue angle [$H = \tan^{-1}(b^*/a^*)$], were calculated. Thirty different marked sections of each sample were measured.

2.3.3. *Volatile composition (solid phase microextraction / gas chromatography-mass spectrometry)*

The volatile composition of the samples was determined using headspace solid phase micro-extraction (HS-SPME). A total of 0.01 g of milled and lyophilized sample (Telstar lyophilizer, LyoQuest, España) was weighted, and ultrapure water (10 mL), NaCl (1.0 g) and benzyl acetate (5 µL of 1000 mg L^{-1}, internal standard for semi-quantification of compounds) were added into a 40 mL vial with polypropylene caps and PTFE/silicone septa. The vial was placed in a AS-10 autosampler (Shimadzu Corporation, Kyoto, Japan), after 5 min of equilibration time, a 50/30 µm

DVB/CAR/PDMS fiber was exposed to the sample headspace for 50 min at 40 °C. The identification of compounds was conducted by GC (Shimadzu ICP), with a Sapiens X5MS (Teknokroma, Barcelona, Spain) column of 30 m × 0.25 mm i.d., 0.25 µm film thickness, and coupled with a Mass Spectrometer detector (Mass Spectrometer ICPMS-2030; Shimadzu Corporation, Kyoto, Japan) in split mode 1/35 to allow limonene quantification, the main volatile compound of orange peels. Retention indexes of a commercial alkane standard mixture were used to identify the compounds, as well as the NIST library indexes.

2.3.5. *Sensory analysis: consumer study*

The protocol for the consumer study was approved by the Basque Culinary Center scientific committee, which stated a waiver consent. All articles from the Declaration of Helsinki and the 2016/679 EU Regulation on the protection of natural persons regarding the processing of personal data and on the free movement of such data were met. The experimental procedure was explained and a written consent indicating voluntary participation was obtained from each participant prior to beginning the study.

Approximately 250 g of each *msayer* sample, drained from the brine, and cut into roughly equal pieces, were ground using a Thermomix TM6 (full speed, 3 seconds) (Thermomix, Germany). Then, 75% of water (w/w) was added and mixed with the ground peels (full speed for 1 min; the sides and lid of the Thermomix were scraped down with a silicone spatula, and the mix was processed for 1 minute more on full speed; this process was repeated twice) to turn the *msayer* peels into a finely chopped homogeneous *msayer* cream.

A total of 60 consumers participated in the consumer test, tasting each sample and rating liking using a 9-points hedonic scale (1 = extremely dislike, 9 = extremely like) and 9-points Just About Right (JAR) questions (1 = too much light, 5 = Just About Right, 9 = too much intense) about salty taste, bitter taste, and intensity of global flavor. Consumers attended 3 times, tasting 9 different samples on each occasion. Samples were coded with 3-digit numbers randomly assigned and served to each consumer with the questionnaire to complete. Consumers were free to choose bread, or no carrier, to taste the different orange creams but, once selected, the use of the same vehicle was mandatory for tasting all samples.

2.4. Data analysis

A three-ways ANOVA test was conducted using 'sucrose content', 'NaCl content', and 'time' as factors. Post-hoc test was conducted using Tukey's HSD. All data analyses were conducted using the statistical package XLSTAT Version 2009.6.03 (Addinsoft, USA) (Addinsoft, 2019). Results were considered significant when $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Brines characterization

The pH of the original 9 brine samples, before adding them to the orange peels and start the *msayer* making process, varied between 6.55 and 7.30. Table 2 shows the latter pH decrease during the fermentation process, reaching values below 3.5 after the 7th day. TSS decreased over time, but significant differences were only detected between samples of day 0 and all other samples. Three-ways ANOVA results indicated a significant effect 'NaCl content*time', with a faster pH decrease when the NaCl concentration was lower; a pH = 2.95 was reached at day 65 in the samples with a 5% of NaCl (Figure 1). The effect 'sucrose content*time' was not significant. pH and TSS content increased from samples day-60 to samples belonging to day-90, maybe because of a higher degradation of the orange peel matrix with a variety of soluble compounds release. Previous studies in which the *msayer* making process has been characterized reported pH values below 3 (Aayah et al., 2010; Bousmaha et al., 2006). Although the pH of the *msayer* made with peels was not as low as the one reported by the traditional product, it could be considered low enough to ensure no pathogen bacteria growth because of being under 4 (Rahman & Rahman, 2020).

Microbiological analyses showed a great variability among total aerobic counts (TAC) of the samples, with no significant differences among samples, and independently of the brine used in the treatment. Significant differences ($p < 0.05$) were found along time, with a marked increment in the UFC ml^{-1} during the first week of fermentation (from a mean of 164 UFC ml^{-1} in day 0 up to over 157,000 UFC ml^{-1} in day 7). After the first week, a plateau period was reached, and a decrease in the TAC seemed to start between days 40 and 65, reaching a mean of approximately 95,000 UFC ml^{-1} at day 90. Some references had suggested that *msayer* making could be an acid-lactic fermentation process, similar to kimchi or sauerkraut making, in which yeast are inhibited by adding salt (Katz, 2012). Results of the present study showed that, after day 4, no anaerobic bacteria were present in the different brines of the samples, because no acid-producing

252 bacteria growth was detected in the WLD media in anaerobic conditions. Yeast populations
253 seemed to be the predominant ones from the beginning of the microbiological sampling, and only
254 yeast colonies were detected after day 4, which corroborated the results of Aayah et al. (2010).
255 These authors reported that yeasts dominated, and were the only microbial representatives, after
256 the second week of *msayer* making, identifying *Candida* and *Saccharomycetales* species as
257 potential drivers of the transformation. Previously, Arias, Burns, Friedrich, Goodrich, & Parish
258 (2002) had identified some *Candida* species as predominant microorganisms in orange juice,
259 using different identification methods, and supporting the idea that these might be the main
260 microorganisms present in orange derivatives.
261 Results of the sugars determination by HPLC showed the expected detriment in sucrose from
262 day 0 until day 40, 65 or 90. Significant differences ($p < 0.05$) were detected among samples for
263 sucrose, glucose and fructose, being time and NaCl concentration the main factors driving the
264 differences among treatments (Figure 2). No significant differences were identified when studying
265 the effect 'sucrose content*time'. Figure 2 shows the dynamics of sugars concentrations, as well
266 as polyalcohols. As seen in this figure, sucrose concentration decreased in all samples, while
267 glucose and fructose levels increased over time in the samples with a 10 and 15% of added NaCl,
268 probably due to the sucrose hydrolyzation in the brine, but not in the samples with the 5% NaCl-
269 brine. Slight increases of glucose and fructose concentrations were observed during the first 4
270 days of fermentation in samples T1, T2 and T3 (5% NaCl), from 2591 up to 6592 mg L^{-1}, and
271 2837 to 6340 mg L^{-1} respectively, but then a plateau period was observed, and the concentration
272 was kept similar until the end of the process. The different microorganisms present in the media
273 could metabolize these sugars, producing fermentation-derived metabolites as carbon dioxide,
274 ethanol, or organic acids, and resulting in a decrease of the pH of the brine and hindering the
275 development of potential pathogen microorganisms. Xiong et al. (2016) showed that using a 2%
276 NaCl w/v brine accelerated the maturation of sauerkraut samples but did not effectively inhibited
277 the growth of harmful microorganisms; using a 5% NaCl brine successfully difficulted the growth
278 of fungi and *E. coli*, and using a brine with an 8% NaCl w/v content excessively delayed the
279 maturation of sauerkraut and slowed down the metabolism of lactic acid bacteria. Therefore, these
280 authors recommended conducting fermentations with a brine containing a 5% NaCl to improve
281 the quality of sauerkraut in traditional fermentations. During the present research, a significant

282 'NaCl content*time' effect was observed when studying the evolution of sugars content, and the
283 samples with the lower added NaCl were the ones in which sucrose, glucose, and fructose
284 concentrations were the lower during the whole fermentation time (Table 3). Therefore, these
285 results suggested a higher microbiological activity in those samples in which the NaCl content
286 was lower, similarly as proposed by Xiong et al. (2016), although the microbiological analyses
287 (PCA) of the present study had not shown significant differences among treatments.
288 Results of HPLC analysis also showed an increment of some polyalcohols, highlighting the
289 appearance of erythritol in the final samples (day 40, 65, and 90) with a significantly different
290 content when studying the 'NaCl content*time' effect (Figure 2 and Table 3). Samples which
291 fermented in a brine with a 5% NaCl content had a significantly higher content on this polyalcohol,
292 a sugar alcohol produced by osmophilic yeasts as osmoprotectant, and which can be used as a
293 natural sweetener (Carly & Fickers, 2018).
294 3.2. Orange peel and orange *msayer* characterization
295 Significant differences were determined between the original orange peel and the fermented
296 *msayer* samples texture, increasing from 6,374 g of hardness (cutting force) in the original peel
297 sample up to different hardness values, depending on the treatment (Table 4 and Figure 3). As
298 shown in Table 4, significant differences were detected among fermentation treatments (T1-T9),
299 and also due to fermentation time, although the main results showed that hardness was lower in
300 the samples with the lower NaCl content. In concordance with the results of the process
301 characterization through the brines analyses, no differences were found in the texture of the
302 samples due to the 'sucrose content' or the interaction 'sucrose content*time' factors.
303 Color of the original orange peel significantly changed over fermentation time, with a decrease of
304 *L** and Chroma values from 64.3 to 56.9 and 71.7 to 58.8 respectively, and the increment of Hue
305 from 71.0 up to 78.6 at day 90. As reported by the previously mentioned parameters, the 'sucrose
306 content*time' interaction was not significant, while 'NaCl content*time' were identified as
307 significant factors and interaction. The samples with a lower content of NaCl (5%) had significantly
308 higher values ($p < 0.05$) for all color parameters (*L*, a*, b*,* Chroma, and Hue) than the samples
309 made using the 10 or 15% NaCl-brine, which were similar between them, and had a less luminous
310 and less orange color (considering orange a mixture of red and yellow colors) than the samples
311 with less salt. No color data has been reported on *msayer* samples during previous research but

312 mentioning that lemons became browner and the change in the skin color is used as indicator of
313 the end of the fermentation process (Aayah et al. 2010).
314 The volatile composition of the original and *msayer* samples, determined by SPME-GCMS, is
315 shown in Table 5. Some of the compounds were significantly different depending on the
316 treatment, being 'NaCl content' the main effect, and not 'sucrose content', 'time', or any of the
317 possible interactions. *Msayer* samples and the original orange peel had significantly different
318 amounts of many volatile compounds ($p < 0.05$), highlighting the decrease of practically all
319 monoterpenes during the fermentation process. Monoterpenes and its derivatives (e.g.:
320 monoterpenes with hydroxy groups such as linalool) are common volatile compounds found in
321 fruits, vegetables, and byproducts such as wine, and are considered pleasant aromas with
322 descriptors associated to floral, herbaceous, and fruity odors (Belitz, Grosch, & Schieberle, 2009).
323 Limonene was the main compound in all samples, and its concentration significantly decreased
324 during the fermentation process, decreasing up to a third from the orange peel to the samples
325 made with the brines containing 10% and 15% of NaCl. Alcohols and esters significantly
326 increased during the *msayer* making, highlighting the increment in the samples made with a 5%
327 NaCl brine, which seemed to have a more complex volatile profile. Mantzouridou,
328 Paraskevopoulou, & Lalou (2015) used solid state fermentation of sterilized orange peel waste to
329 produce volatile esters with fruity descriptors using a fermentation process driven by
330 *Saccharomyces cerevisiae*. During that study, the orange peels were autoclaved to decrease
331 limonene content down to a 38% of the initial, trying to eliminate this compound with a significant
332 antimicrobial activity (Pourbafrani, Talebnia & Niklasson, 2007). Results of the present study
333 showed that NaCl content seemed to be more limiting for the fermentative process than the
334 limonene content, and that the content of limonene decreased during the fermentation. Samples
335 made in the 5% NaCl-brine had a higher limonene concentration, as well as a higher
336 concentration of esters developed during the *msayer* making. Bevilacqua, Corbo & Sinigaglia
337 (2010) showed that limonene and citrus extract had antimicrobial effect, being some yeasts
338 species more susceptible to its presence than bacteria typical of juice microflora, and being citrus
339 extract more inhibitory than the isolated limonene. During the present study, similarly as reported
340 by Aayah et al. (2010), yeasts drove the fermentation process of the orange peels into *msayer*,

and bacteria seemed to be more sensitive to the inhibitory compounds present in the media, either NaCl or the essential oil components.

3.3. Consumer study results

All samples were assessed by a consumer panel (n = 60), rating liking and the appropriateness (Just About Right - JAR questions) of the saltiness, bitterness, and overall flavor intensity of each sample served as an orange *msayer* cream. Liking was similar for all samples, receiving all of them scores close to the "neither like-nor dislike" value, and indicating that some corrections could be made on the samples to increase liking. Differences were found for all JAR responses with significant 'NaCl content' effect, but no significant 'sucrose content', 'time' effects, or interactions were identified. The samples made with a 5% NaCl-brine were closely perceived as just-about-right (values close to 5) for saltiness and overall intensity, but were the samples more penalized by bitterness, opposite to the samples made with a 15% NaCl-brine (Figure 4). It is important to note that *msayer* is usually consumed as a seasoning or ingredient for salads and, during the present research, the developed cream was tasted directly. Although previous research reported making *msayer* using a 10-15% NaCl content, (Aayah et al., 2010 and Bousmaha et al., 2006, respectively) results of the present research suggested that a product with a lower salt content could be more appreciated by consumers, although bitterness was perceived significantly higher than samples made with a 15% NaCl-brine. To optimize the consumer study and conclude about liking of the developed product, a consumer study with a higher number of participants should be conducted, as well as using the orange *msayer* as it is usually consumed (e.g.: seasoning).

4. CONCLUSIONS

Results of the present research provided useful information to better understand the *msayer* making process, using orange peels coming from orange juice production to develop a new product. The different physico-chemical and sensory analyses developed during the study suggested that using a 5% NaCl brine for 40 days was the most suitable combination of variables to develop an orange peel *msayer*. Sucrose addition at different concentrations was not a significant effect, therefore using a 30 g L^{-1} seemed to be enough to favor the fermentative process. Microbiological analyses were difficult to interpret due to the high variability among samples, and probably because of being a spontaneous process, although the development of yeast species was evident along time, and the absence of LAB was clear from day 4^{th} of

371 fermentation. A deeper study is necessary to better understand the whole process and the
372 potential of the orange peel *msayer*, a product which would allow revalorizing orange peels as a
373 whole and making a new ingredient from what nowadays is considered a byproduct.

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437 **Table 1.** Samples codification. Legend: three sets of T1-T9 samples were analyzed each final
438 day (40, 65, 90).

Sample code	NaCl% w/v (added brine)	Sucrose% w/v (added brine)
T1(40)	5	3
T2(40)	5	4
T3(40)	5	5
T4(40)	10	3
T5(40)	10	4
T6(40)	10	5
T7(40)	15	3
T8(40)	15	4
T9(40)	15	5
T1(65)	5	3
T2(65)	5	4
T3(65)	5	5
T4(65)	10	3
T5(65)	10	4
T6(65)	10	5
T7(65)	15	3
T8(65)	15	4
T9(65)	15	5
T1(90)	5	3
T2(90)	5	4
T3(90)	5	5
T4(90)	10	3
T5(90)	10	4
T6(90)	10	5
T7(90)	15	3
T8(90)	15	4
T9(90)	15	5

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441 **Table 2.** Results of pH and TSS during the fermentation process (average value of all samples
442 for each day). Legend: Different letters within the same column to indicate significant differences
443 among samples.

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Time (days)	pH	TSS (°Brix)
0	3.83 a	13.65 a
1	3.63 b	12.79 b
4	3.54 c	12.82 b
7	3.46 d	12.77 b
40	3.27 e	12.79 b
65	3.12 f	12.41 b
90	3.45 d	12.97 b
p-value	<0.0001	<0.0001

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447 **Table 3.** Results of ANOVA and post-hoc analysis (Tukey HSD) for sugars and polyalcohols
448 content grouped by the significant NaCl factor at t(0) and t(90). Legeng: “n.d. = not determined”
449 and “n.s. = not statistically significant”.

NaCl%	Sucrose (mg L^{-1})	Glucose (mg L^{-1})	Fructose (mg L^{-1})	Inositol (mg L^{-1})	Lactitol (mg L^{-1})	Erythritol (mg L^{-1})
5 (t0)	32972 ± 9883	2590 ± 626	2837 ± 788	242 ± 66	80.8 ± 27	n.d.
10 (t0)	33683 ± 9875	2174 ± 582	2432 ± 781	206 ± 58	69.4 ± 26	n.d.
15 (t0)	33546 ± 9110	1990 ± 448	2262 ± 628	205 ± 59	65.0 ± 18	n.d.
	n.s.	n.s.	n.s.	n.s.	n.s.	
5 (t90)	2297 ± 612 c	5699 ± 1210 c	8596 ± 1531 b	896 ± 63 ab	497 ± 184	2223 ± 615 a
10 (t90)	11484 ± 3104 b	14473 ± 4111 ab	20498 ± 6812 a	860 ± 121 ab	538 ± 105	950 ± 302 b
15 (t90)	16599 ± 3478 a	18525 ± 5102 a	21141 ± 6422 a	827 ± 105 b	507 ± 165	483 ± 150 c
p-value	<0.0001	<0.0001	<0.0001	0.025	0.091	<0.0001

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Table 4. Results of one-way ANOVA and post-hoc analysis (Tukey HSD) of the orange *msayer* texture depending on time and NaCl content.

NaCl %	Cutting force (g)	Time (days)	Cutting force (g)
5	8102 b	40	11565 ab
10	11618 a	65	10223 b
15	10524 a	90	12164 a
p-value	<0.0001	p-value	<0.0001

460 **Table 5**. Volatile composition of samples grouped by 'NaCl content'. Legend: values followed by
461 different letters were significantly different *msayer* samples (Tukey HSD), also indicated in bold.
462 ANOVA results including orange peel were not included in the table to highlight the *msayer*
463 samples differences. Retention index of literature from NIST (2021).

Compounds	Ret. time	RI (EXP)	RI(LIT)	Orange peel	*Msayer samples* (mg kg^{-1})			p-value
					NaCl (5%)	NaCl (10%)	NaCl (15%)	
Ethanol	2.17	508	493	0.60	0.68	0.83	0.85	0.384
1-Octanol	19.72	1070	1070	1.19	3.67	5.17	4.11	0.514
1-Nonanol	26.59	1172	1172	2.38	2.05	2.12	1.58	0.678
1-Decanol	32.75	1273	1273	1.54	**35.47 a**	**12.61 b**	**8.72 b**	**<0.0001**
Alcohols total				5.72	41.87	20.74	15.26	
Ethyl acetate	2.81	616	616	1.34	0.68	0.79	0.74	0.786
Isoamyl acetate	8.58	873	874	1.28	0.89	0.66	0.38	0.064
Ethyl hexanoate	15.03	999	999	1.35	**5.76 a**	**0.53 b**	**0.34 b**	**<0.0001**
Methyl octanoate	23.29	1124	1123	1.62	**2.87 a**	**0.62 b**	**0.47 b**	**<0.0001**
Ethyl octanoate	28.23	1196	1196	1.41	**83.5 a**	**8.13 b**	**2.36 b**	**<0.0001**
Ethyl decanoate	39.07	1394	1394	0.33	**30.3 a**	**3.15 b**	**1.24 b**	**<0.0001**
Octyl acetate	29.11	1211	1211	7.25	**16.4 a**	**4.66 b**	**6.38 b**	**0.001**
Ethyl nonanoate	34.03	1295	1294	1.92	**1.76 a**	**0.54 b**	**0.46 b**	**<0.0001**
Esters total				16.49	142.1	19.08	12.37	
p-Cymene	16.62	1023	1023	39.65	**26.04 a**	**15.32 b**	**14.55 b**	**<0.0001**
Benzene derivatives total				39.65	26.04	15.32	14.55	
α-Pinene	11.35	932	932	119.4	38.63	27.41	32.42	0.578
Sabinene	13.50	971	971	12.07	1.97	1.43	4.47	0.272
β-Myrcene	14.49	989	989	1271	484.6	328.7	339.6	0.192
α-Phellandrene	15.47	1005	1005	25.40	**9.30 a**	**5.80 b**	**5.18 b**	**0.010**
α-Terpinene	16.17	1016	1016	32.66	**12.16 a**	**5.44 b**	**5.58 b**	**0.002**
Limonene	16.99	1029	1029	56977	25370	17978	18689	0.122
trans-b-Ocymene	18.06	1045	1045	16.11	9.38	4.26	4.71	0.075
γ-Terpinene	18.82	1056	1056	75.05	**29.71 a**	**13.89 b**	**14.86 b**	**0.017**
Terpinolene	20.64	1084	1084	123.5	**50.51 a**	**26.14 b**	**25.77 b**	**<0.0001**
Linalool	21.62	1099	1099	111.9	44.20	52.39	45.47	0.795
d-3-Carene	15.64	1008	1008	47.05	15.76	9.37	9.88	0.147
4-Terpineol	27.05	1179	1179	27.94	**20.94 a**	**10.65 b**	**8.62 b**	**0.002**
α-Terpineol	28.01	1193	1192	35.59	**15.37 a**	**7.24 b**	**5.08 b**	**<0.0001**
Citronellol	30.04	1226	1226	1.79	**7.77 a**	**5.20 ab**	**3.69 b**	**0.018**
Carvone	30.99	1243	1243	6.18	3.57	1.23	2.13	0.284
Citronellyl acetate	36.82	1349	1348	0.34	**4.97 a**	**0.91 b**	**0.71 b**	**<0.0001**
Neryl acetate	37.24	1357	1357	2.70	**3.91 a**	**0.98 b**	**1.05 b**	**<0.0001**
Monoterpenes total				58885	26122	18479	19198	

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466 **Table 5. Continuation.**

Compounds	Ret. time	RI (EXP)	RI(LIT)	Orange peel	*Msayer* samples (mg kg^{-1})			p-value
					NaCl (5%)	NaCl (10%)	NaCl (15%)	
trans-Caryophyllene	40.26	1419	1419	1.97	5.02 a	1.68 b	2.46 ab	0.029
Germacrene D	40.71	1429	1451	1.43	3.29	1.01	1.90	0.185
trans-β-Farnesene	41.76	1452	1452	0.29	4.97	0.71	0.95	0.055
Valencene	43.54	1492	1492	22.42	**66.3 a**	**31.7 a**	**35.9 a**	**0.041**
α-Selinene	43.63	1494	1494	0.61	**2.68 a**	**0.77 b**	**0.87 b**	**0.017**
δ-Cadinene	44.65	1518	1518	3.43	**9.22 a**	**2.58 b**	**3.35 b**	**0.002**
Sesquiterpenes total				30.26	91.49	38.49	45.46	
Nonanal	21.95	1104	1104	31.98	6.53 b	10.10 ab	11.03 a	**0.031**
Decanal	28.81	1205	1205	283.3	125.8	120.4	114.5	0.887
Undecanal	34.66	1306	1306	2.23	**3.31 a**	**1.48 a**	**1.69 a**	**0.040**
Aldehydes total				317.5	135.7	132.0	127.3	
Hexane	2.72	602	600	1.09	1.33	3.47	4.62	0.715
Tetradecane	39.74	1408	1400	3.73	**18.17 a**	**6.23 b**	**8.05 b**	**0.005**
Hexadecane	48.11	1601	1600	1.75	2.86	0.54	1.62	0.060
Heptadecane	52.00	1703	1700	1.70	**5.21 a**	**0.56 b**	**1.44 b**	**0.001**
Liner hydrocarbons total				8.26	27.58	10.81	15.74	
TOTAL				59264	26587	18716	19429	

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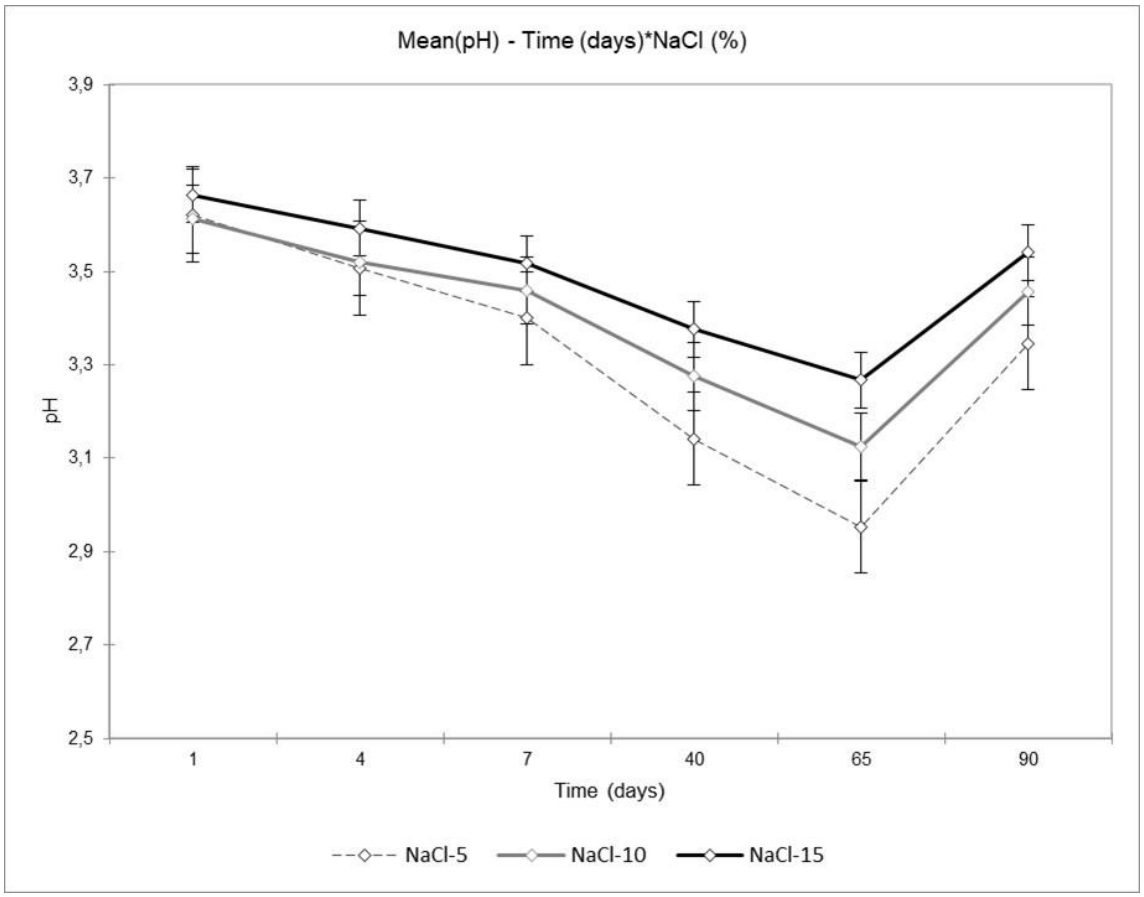
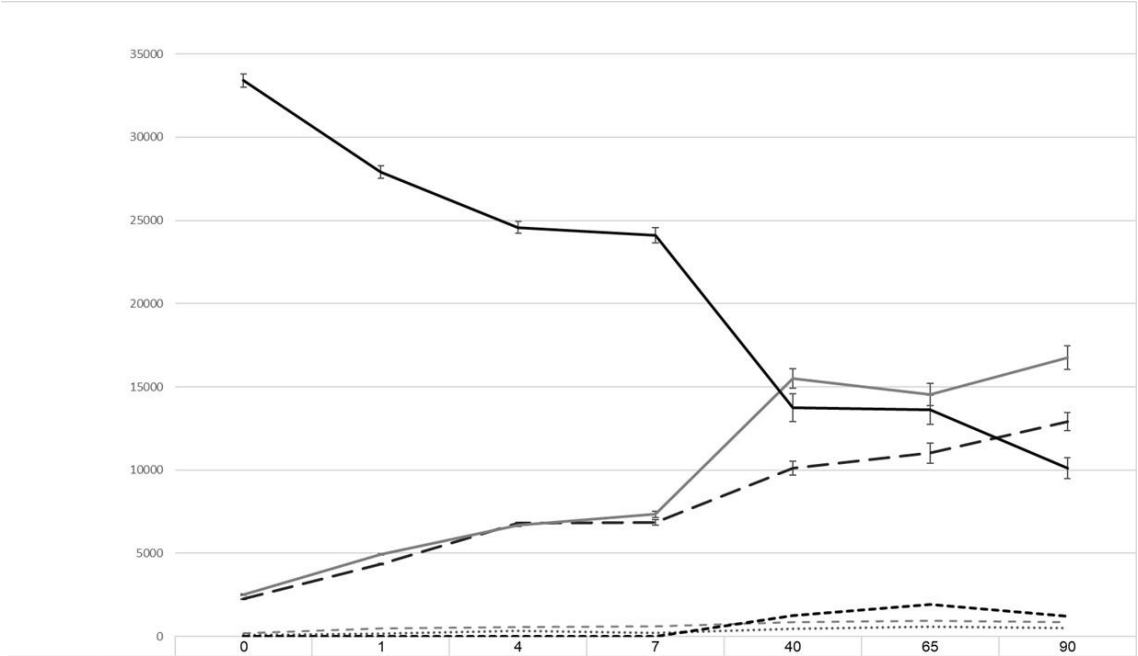


Figure 1. Results of pH (a) and TSS (b) for the 'NaCl*time' effect, shown as significant interaction in the three-way ANOVA analysis.



	0	1	4	7	40	65	90
Inositol (mg L-1)	218 e	479 d	578 c	614 c	878 ab	928 a	861 b
Glucose (mg L-1)	2251 e	4369 d	6807 c	6844 c	10104 b	11014 ab	12899 a
Lactitol (mg L-1)	72 e	171 d	328 c	232 d	476 b	592 a	514 b
Fructose (mg L-1)	2510 c	4940 bc	6710 b	7357 b	15494 a	14534 a	16745 a
Sucrose (mg L-1)	33401 a	27906 b	24576 bc	24106 c	13748 d	13620 d	10127 d
Erythritol (mg L-1)	0,0 c	0,0 c	0,0 c	0,0 c	1244 b	1933 a	1219 b

Figure 2. Sugars and polyalcohols concentration over time (x-axis expressed in days). Legend: mean of all treatments each sampling day; different letters within the same row to indicate significant differences among samples ($p < 0.001$).

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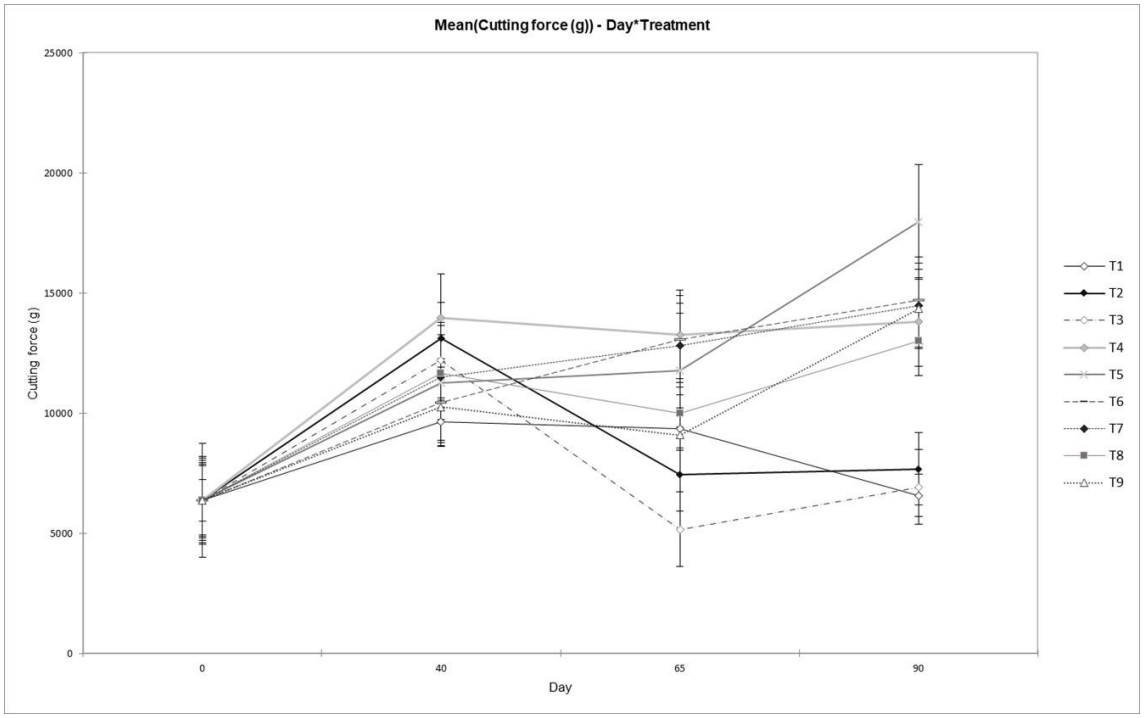

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487 **Figure 3.** Cutting force, expressed in (g), of *msayer* samples after 40, 65 and 90 days of

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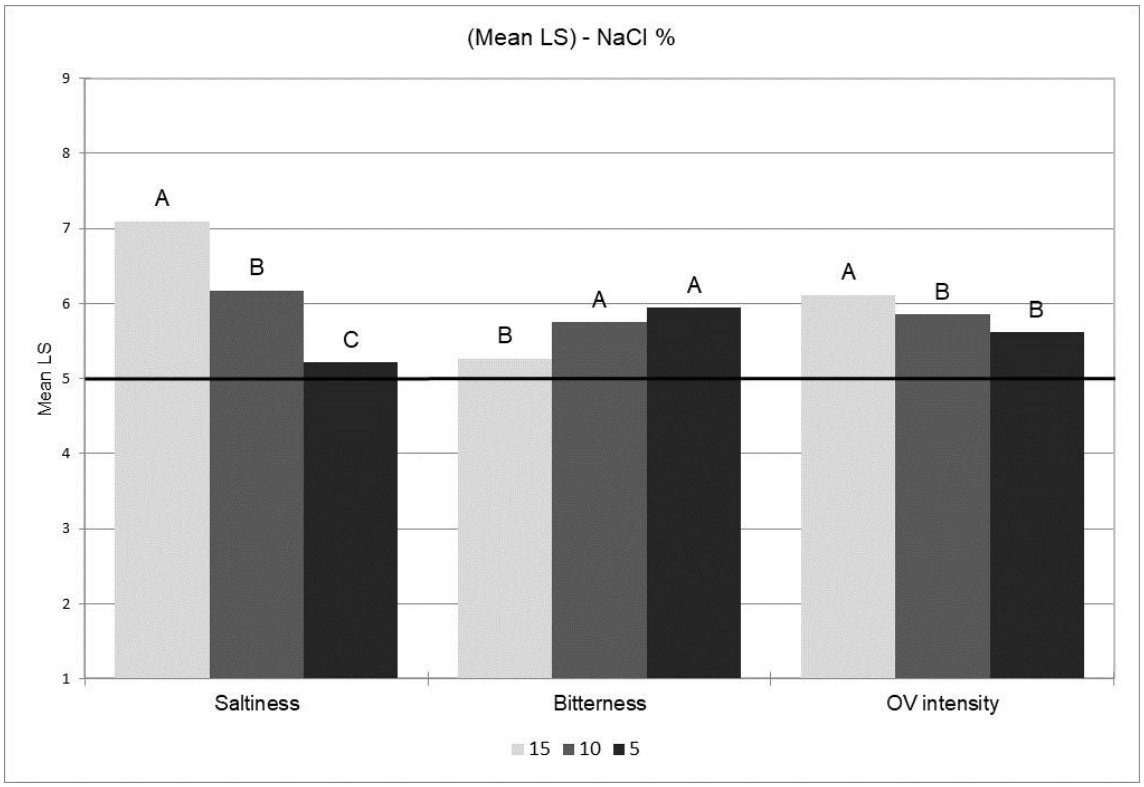

Figure 4. Results of JAR questions of the consumer study. Mean of samples by NaCl content, detected as significantly different. Legend: black line indicating the Just About Right score in the scale. Different letters to indicate significant differences among samples among attributes ($p < 0.05$)