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Research Note

Characterisation of the dry salted process for the production of the msayer, a traditional lemon aromatising condiment

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ABSTRACT

Msayer is a dry salted lemon used as an aromatising condiment in many North-African food recipes. This preparation is obtained by adding coarse salt (10 g/100 g fresh lemons) to fresh cut lemons in containers kept under dark conditions away from air for around a month and a half. A survey of 52 days showed low initial uptake of citric acid and sugars from the formed liquid phase. Nevertheless, there were still sugars detected even at the end of the survey attesting for the low microbial activity as also shown by carbon dioxide production and microbial viable counts. Indeed, the microbial counts showed a steady decrease reaching 2 log units at the 52nd day of curing. Moulds were detected only at the beginning but at low numbers. No viable counts of lactic acid bacteria were observed on MRS medium despite detection of lactic acid (8 g/l). In contrast, yeasts seem to dominate in the msayer preparation. They count for almost all the microbial population especially after the first week. Six of these acid and halotolerant yeast isolates were identified, by sequence analysis of the amplified 5.8S rRNA and the two internal transcribed spacer regions, as *Candida parapsislosis* (2 isolates) and unclassified Saccharomycetales (4 isolates).
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1. Introduction

The salted fermented lemon called 'msayer' in Morocco is used as an aromatising condiment in many North-African food recipes. The pulp of msayer is added to many food dishes as a salt replacement but also bringing special aromas. The skin characterized by low or no bitterness is also consumed usually added as laces once the cooking is finished for food decoration. The most traditional process of msayer preparation used in most households is a dry salted process. The lemons are cut in half or forth until the middle of the fruit to allow addition of coarse salt at a rate of 10 g/100 g of fresh weight of the lemons. The fruits are subsequently stacked in glass jars until no head space is left. Sometimes a layer of olive oil is added on top of the fruits. The filled jars are tightly closed and left at room temperature for a month or two. At the end of this period, there is a slight browning of the lemon skins that is taken as an indication of the end of msayer maturation. The shrunk fruits are then completely submerged in a very thick salty liquid resulting of osmotic exchanges due to the salt addition.

Dry salting is also used in the Mediterranean region for the production of preserved vegetables or fruits such as naturally black olives prepared in Greece and some North-African countries (Panagou, 2006). Like olives lemons also present some bitterness but they are mainly due to naranjins in the lemon skin (Puri & Banerjee, 2000). The bitterness of naranjins is at least masked by the salty taste if not lowered by degradation (Puri & Banerjee, 2000) in the msayer.

Compared to some fermented acidic fruits known to the scientific community, the preparation of msayer presents many original aspects in terms of processing, food biochemistry and microbiology because of the low pH (pH around 2.5), the high concentration of citric acid (around 40 g/kg lemon) and salt (10 g/100 g fresh lemons). The lemons are also rich in glucose, fructose and sucrose (Souci, Fachmann, & Kraut, 2000). This chemical composition is close to cocoa beans (Ardhana & Fleet, 2003) but the citric acid concentration is lower, the pH is higher (around pH 4) and the sugar concentration is also higher in cocoa beans. Furthermore, no salt is added in the case of chocolate fermentation (Ardhana & Fleet, 2003). Tempoyak is also a traditional food of Malaysia that uses the

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lightly acidic fruit *Durio zibethenus* fermented with salt addition. Durio fruits contain low concentration of acetic and lactic acid with a pH of 6.7 (Leisner et al., 2001).

In this work we present results of 52 days survey of some changes in biochemical and microbiological parameters during lemon curing including citric acid, sugars, lactic acid, pH and also yeast, moulds and bacterial population concentrations. We also identified the dominant microbial population represented by halotolerant yeasts.

2. Materials and methods

2.1. Msayer preparation

Lemon fruits were bought from a local market in Marrakech. They belong to the species *Citrus limon limon*. The fruits presenting cuts or microbial growth were discarded. Fruits of close size were then selected and washed with distilled water. Ten fruits were then weighted, cut open and salted using coarse marine salt. The salt was adjusted to 10 g/100 g total fresh weight of the fruits. The fruits were placed in 745 ml container made of Polypropylene. The containers were tightly closed with plastic lids presenting a rubber stopper at their centre for gas and liquid sampling. The containers were then placed at room temperature (22–28 °C) in a dark box. Ten containers were thus prepared with an average lemon fruit weight of 595 g ± 24,5 (wet weight ± 95% confidence interval).

2.2. Sampling

Three out of the ten containers were drawn using random numbers on MS Excel to give triplicates for each measurement. Samples of gas were taken using gas tight Hamilton syringes of 5 ml volume. Liquid samples were taken using sterile 10 ml disposable syringes. Samples were taken at day 1, 2, 3, 5, 7, 10, 13, 17, 24, 31, 38 and 52 after salt addition.

2.3. Microbiological analyses

Liquid samples of 5 ml each were drawn aseptically from the containers using disposable sterile syringes. Decimal dilutions in sterile distilled water containing 9 g/l NaCl were prepared. Three appropriate dilutions for each sample were spread on Nutrient Agar (Biokar Diagnostics) for total viable counts at 25 °C for 6 days; de Man–Rogosa–Sharp medium (MRS Biokar Diagnostics) supplemented with 40 mg/l cycloheximide (Sigma) for lactic acid bacteria, at 30 °C for 48 h; YM agar (3 g/l malt extract (Difco), 3 g/l yeast extract (Biokar Diagnostics), 5 g/l peptone (Biokar Diagnostics), 10 g/l glucose (Labosi), 12 g/l agar (Biokar Diagnostics) at pH 6) containing 20 μg/l tetracycline (SIGMA) for yeasts at 35 °C for 48 h and YM agar containing 35 mg/l Rose Bengal (SIGMA) and 20 μg/l tetracycline (SIGMA) for moulds.

2.4. Yeast identification

Yeast identification was carried out by PCR amplification of the ribosomal RNA 5.8 S using the specific primers of the preserved areas ITS1 (ITS-1: 5′ TCCGTAGGTGAACCTGCGG 3′) and ITS4 (5′ TCCTCCGCTTATTGATATGC 3′) according to Las Heras-Vazquez, Mingorance-Cazorla, Clemente-Jimenez, and Rodriguez-Vico (2003). PCR products were purified using the Magnesil yellow solution (Promega) to eliminate the primers used for PCR reactions. The sequencing reaction was performed in a total volume of 20 μl containing 20 pmol of each primer (ITS-1 or IST-4), 3 μl of Big Dye (version 1.1) and 2 μl of purified PCR product. Twenty five cycles were performed: denaturation at 96 °C for 10 s, primer annealing at 55 °C for 10 s, and extension at 60 °C for 4 min. In order to eliminate the excess of labeled ddNTPs, sequencing reaction products were purified using the above mentioned Magnesil green solution. Direct sequencing of amplified PCR products for the ITS fragment of the ribosomal RNA 5,8S gene was performed on a sequencing apparatus (3130 Genetic Analyser, Applied Biosystem) and data analysis was done by sequencing analysis software. The sequencing was carried out for both strands.

2.5. Biochemical analyses

Organic acids (lactic, malic, citric acid and galacturonic acids) and sugars (glucose, fructose and sucrose) were silylated according to Molnar-Perl (1999) and then separated and detected using FID (Flame ionisation Detector) on a 6820 Agilent GC. A volume of 0.5 ml sample was freeze dried then mixed with 100 μl of 20 g/l hydroxylamine in pyridine and heated to a temperature of 80 °C for 30 min. The reaction was completed with 900 μl of HMDS (HydroxyMethylDisilazane) and 100 μl of trifluoracetic acid at a temperature of 100 °C for 1 hour. The organic compounds were separated using a DB5 (J&W Scientific) column of 50 m (0,20 mm I.D., 0, 33 μm film thickness) using the following temperature program: 60 °C for 2 mn and then 150 °C at a rate of 20 °C/mn and then 260° at a rate of 60 °C/mn. The injector temperature was 250 °C while the flame ionisation detector (FID) temperature was 280 °C. A volume of 1 μl of the sample was injected.

Oxygen and carbon dioxide in the containers head space were separated using a Supelco Carboxen™ 1010 Plot column (30 m × 0.32 mm I.D.) and detected using a Thermo-Conductivity Detector on 6820 Agilent GC.

Dry weight was estimated by freeze drying. The pH was measured using a calibrated (JENCO Electronics LTD) pHmeter.

3. Results and discussion

At the beginning of the preparation there is almost no liquid phase visible. As time progresses liquid starts to form between the fruits until it completely immerses all the fruits by the 6th day. The concentration of almost all solutes including glucose, fructose, citric and malic acids progressively increased from the first up to the fifth day (Fig. 1). Then, the concentrations of all these components progressively decreased until day ten and remained almost constant for the rest of the process. This evolution seems to be confirmed by the dry matter content (Fig. 1) of the liquid phase that showed an increase up to the fifth day then a drop thereafter with almost no changes after the tenth day. The differences observed after the tenth day were probably due to the random variation between the containers.

The kinetics of these solutes concentration in the liquid phase is probably related to the diffusion of solutes out of the fruit into the liquid phase and their subsequent degradation by the microbial population. Maldonado, Zuritz, and Assof (2008) have shown a gradual increase of reducing sugars in the liquid phase until the 15th and 16th day of green olives curing. The phenomenon was clearly observed because the authors blocked microbial sugar uptake using nisin and chloramphenicol. A similar, but less pronounced, increase of sugars in the liquid phase of the Greek naturally black dry salted olives was also reported up to the 25th day of the process (Panagou, 2006). Furthermore, Panagou and Tassou (2006) have reported a gradual drop of reducing sugar concentration starting at the 5th day of Conserveola green olives fermentation.

However, based on the initial concentration of solutes in the lemon juice (citric acid 39.7 g/l; malic acid 1.9 g/l; glucose 5.7 g/l; fructose 5.1 g/l and sucrose 6.8 g/l) the decrease of citric acid

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