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Effect of different marinating conditions on the evolution of spoilage microbiota and metabolomic profile of chicken breast fillets

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ABSTRACT

Five different marinades were prepared containing lemon juice, apple cider vinegar, pomegranate juice and combinations of them. Three different temperatures (4, 10, and 20 °C) and five marinating time intervals (1, 3, 6, and 9 h) were tested. Microbial, physicochemical as well as sensory analyses were performed to assess marination. Noticeable microbial reductions and satisfactory sensory results were observed only in samples treated for short time (1 and 3 h). The marinade in which pomegranate and lemon juices were combined caused a decrease in microbial counts and led to desirable sensory attributes. Each of the marinades was characterized by a distinguishable organic acid profile, while the discrimination of the samples, based on organic acid concentration, between low (1 and 3) and high (6 and 9) marinating time was feasible. It can be concluded that marinating time affected the indigenous microbiota and the sensory characteristics of chicken meat while pomegranate could be a promising marinating ingredient from a microbiological and physicochemical perspective.

1. Introduction

Poultry meat production and consumption have been rapidly increased in many parts of the world in the last years, whereas per capita consumption is expected to grow continuously (FAO, 2014) probably due to its low price, nutritional characteristics (e.g., low fat content), and organoleptic attributes (Anang et al., 2010; Barbut, 2002; Radha Krishnan et al., 2014). Another reason for the high demand of poultry meat is the increased availability of further processed products (Fletcher, 2002). From the gastronomic point of view, ingredients such as lemon, wine or vinegar are traditionally used in marination, while new trends propose various alcoholic beverages such as whiskey, brandy and also fruit juices like pomegranate, pineapple or kiwi. So far, studies related to marination focus on the effect of marinades on flavor improvement, tenderness, water-holding capacity, yield, plus the reduction of off flavors (Goli et al., 2014; Smith and Acton, 2010; Yusop et al., 2009, 2010), while in fewer works the effect of marinades on the improvement of microbiological quality and safety of poultry meat has been reported (Kargiotou et al., 2011; Nisiotou et al., 2013; Pathania et al., 2010). It should be noted that limited information is available on (i) marinated chicken meat and marinades containing organic acids as active ingredients against microorganisms, (ii) the shelf life of marinated poultry meat, given that it is a highly perishable foodstuff (Fernández-López et al., 2005; Jimenez et al., 1997; Wang et al., 2004) stored under refrigerated conditions (Al-Nehlawi et al., 2014; Morshedy and Sallam, 2009), and (iii) the effect of marinating conditions (marinating temperature and time) on the microbial succession and physicochemical profile of chicken meat. In the latter issue, the information provided concerns pH, moisture or mechanical texture analysis (Burke and Monahan, 2003; Yusop et al., 2010, 2012) which are features that only partially contribute in the holistic view of quality. To our knowledge, the potential of using metabolomics in the food sector is rather limited since this approach has been predominantly used so far in clinical and pharmaceutical research areas (Shulaev, 2006; Trivedi and Iles, 2015). However, its versatility was found to be crucial since this "*comprehensive analysis of the whole metabolome, which refers to the full complement of small molecule metabolites in a tissue (such as poultry tissue) under a given set of conditions*" could be used to determine the quality and safety of these products (Argyri et al., 2011).

The purpose of this study was to investigate the effect of five different home-made marinades, for several time intervals at 3

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different temperatures (4, 10, and 20 °C) on the (a) spoilage microbiota of chicken breast fillets, and (b) quality parameters based on both sensory and metabolomics analysis.

2. Materials and methods

2.1. Sample preparation

Fresh chicken breast fillets were obtained from a local meat market and transported under refrigeration to the laboratory within 20 min. Ten (10) gram portions were cut aseptically and placed onto sterile trays until marination. Five home-made marinades differentiated by the main commercially available ingredient were prepared, namely (i) lemon juice (LE), (ii) apple cider vinegar (VI), (iii) pomegranate juice (PO), (iv) combination of pomegranate and lemon juices (PL), and (v) combination of pomegranate juice and apple cider vinegar (PV). The additional ingredients added in all marinades to provide flavor balance were: olive oil, dried thyme and honey. The exact composition of the marinades is presented in Table 1. Marination was performed at three different temperatures (4, 10, and 20 °C) and four time intervals (1, 3, 6, and 9 h). A total volume of 100 mL of each marinade was prepared in which seven chicken samples were completely immersed throughout marination. Untreated (non-marinated) samples were used as control treatment and kept in Petri dishes under the same conditions.

2.2. Microbiological analysis

Prior to marination, microbiological analysis was undertaken in chicken breast fillets for the determination of the indigenous microbiota. After each marination process, the microbiota of marinated and non-marinated samples (control) was also determined. Chicken samples (10 g) were added aseptically to 90 mL of sterile quarter-strength Ringer's solution (LAB 100Z, LAB M, Bury, UK) and homogenized in a stomacher (Lab Blender, Seward Medical, London, UK) for 60 s at room temperature. Serial decimal dilutions in the same medium were prepared and 0.1 mL aliquots of the appropriate dilutions were spread in duplicates on the following growth media: Tryptic Glucose Yeast Agar (402145, Biolife, Milan, Italy) for total viable counts (TVC), incubated at 30 °C for 48 h; Pseudomonas agar base (LAB 108, supplemented with selective supplement X108, LAB M) for *Pseudomonas* spp., incubated at 25 °C for 48 h; STA Agar Base (402079, supplemented with selective supplement 4240052, Biolife) for *Brochothrix thermosphacta*, incubated at 25 °C for 72 h, while 1.0 mL aliquots were poured in duplicates in the following media: Violet Red Bile Glucose Agar (402188, Biolife) for *Enterobacteriaceae* counts, overlaid with the same medium and incubated at 37 °C for 24 h; de Man-Rogosa-Sharpe agar (401728, Biolife) (pH adjusted to 5.7) for lactic acid bacteria (LAB), overlaid with the same medium and incubated at 30 °C for 72 h. In order to reduce the detection threshold of *B. thermosphacta* and *Pseudomonas* spp. using the spread-plating method, 1 mL from the first dilution of chicken sample homogenate was spread equally on 3 STA and Pseudomonas agar plates, respectively. Duplicate samples were analyzed at each temperature and time interval and the experiment was carried out twice (n = 4).

2.3. Sensory evaluation

Sensory evaluation was undertaken by 10 in-house trained assessors who were all staff of the laboratory. They evaluated the attributes of flavor, odor and tenderness of thermally processed (180 °C for 15 min in preheated oven) marinated samples that were presented in random order using a 5-point hedonic scale for assessment (1-undesirable; 2- moderately undesirable 3- good; 4-very good; 5- excellent).

2.4. pH measurement

The pH of the chicken samples and the marinades was routinely recorded by a pH meter (Metrohm 691 pH meter, Ion Analysis, Switzerland) after the end of microbiological analysis with direct immersion of the glass electrode in the marinade solution or in the sample homogenate (1:10).

2.5. Organic acids determination

Organic acids were extracted according to Argyri et al. (2011) with slight modifications. Specifically, four (4) grams of chicken breast fillet were homogenized manually with a glass rod in 8 mL of HPLC grade water for 2 min and the homogenate was centrifuged at 4500 g for 10 min at 4 °C. The supernatant was then filtered through filter paper and 5 mL of the filtrate was transferred in Eppendorf tubes where 50 μL of trifluroacetic acid − TFA (for the precipitation of proteins) and 50 μL of 1.0% sodium azide (as preservative) were added. Finally, the supernatant was stirred, centrifuged (4500 g for 10 min at 4 °C) and filtered through 0.22 μm filter. The HPLC system consisted of a JASCO LC-Net II/ADC system controller, a JASCO AS-2055 Plus Autosampler with a Model PU-980 Intelligent pump, a Model LG-980-02 ternary gradient unit pump and a MD-910 multiwavelength detector. The operating conditions resulting in optimum separation of the acids are reported elsewhere (Argyri et al., 2011; Skandamis and Nychas, 2001). The samples were isocratically eluted with a solution of 0.009N H_2SO_4 through an Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad Laboratories, Richmond, CA) at a rate of 0.7 mL/min and oven temperature at 65 °C while the injection volume was 20 μL. The software used for spectra collection and processing was Jasco Chrompass Chromatography Data system v1.7.403.1. Although spectral data were collected from 200 to 600 nm, the optimum wavelength for determination was 210 nm. The purity of peaks was examined using all spectral ranges. The coefficient of variation (CV %) of the results was always lower than 5%. The organic acids selected for the analysis, their retention times and concentrations in the marinades are presented in Table 2. The selection of organic

Table 1
Marinades composition employed in the present study.

Main ingredients	Volume (mL)	Common ingredients
	per 100 mL of marinade	
Lemon juice (LE)	70	30 mL Olive oil
Apple cider vinegar (VI)	70	0.1 g Dried thyme
Pomegranate juice (PO)	70	2.0 g Honey
Pomegranate + Lemon juices (PL)	35 + 35	
Pomegranate juice + Apple cider vinegar (PV)	35 + 35	