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Effect of pomegranate based marinades on the microbiological, chemical and sensory quality of chicken meat: A metabolomics approach

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ABSTRACT

Pomegranate juice is a product with enhanced functional properties that could be used as an alternative to traditional marination ingredients and effectively retard microbial growth along with providing an improved sensory result. In this study, two pomegranate based marinades were prepared for the marination of chicken breast fillets and the marinated samples were aerobically stored at 4 and 10 °C for 9 days. Raw, non-marinated chicken samples were used as control. Levels of total viable counts (TVC), *Pseudomonas* spp., *Brochothrix thermosphacta*, *Enterobacteriaceae* and lactic acid bacteria (LAB) were determined together with sensory assessment to evaluate the evolution of spoilage. The profile of organic acids and volatile compounds was also analyzed during storage. The shelf life of marinated samples was significantly extended compared to control samples at both storage temperatures (e.g., up to 5 and 6 days for the pomegranate/lemon marinated samples stored at 4 and 10 °C, respectively) as evaluated by both microbiological and sensory analyses. The profile of the organic acids and the volatilome of marinated and control samples were remarkably differentiated according to storage time, microbial load and sensory score. The findings of this study suggest that pomegranate juice could be used as a novel ingredient in marinades to improve the sensory attributes, while prolonging the shelf life of chicken meat.

1. Introduction

In recent years, the chicken meat industry is steadily focusing on the development of new products that contribute significantly to the increasing global demand for poultry meat (FAO, 2016). These products, among other, involve the use of seasoned liquids to improve the flavor, tenderness, or texture of chicken meat. Various solutions can be added to poultries by several methods, such as injection, brining, or marinating (USDA, 2013). The marination process of chicken meat has become an important segment of the poultry industry because of the increased demand from consumers, institutional food services, and restaurants for ready-to-cook and convenience products. One issue that has already been reported regarding marinades and especially acidic ones, arises from the fact that a marinade with a relatively high pH (> 4.5), that may otherwise positively affect the overall sensory quality of the end product, is not efficient to adequately retard the growth of spoilage microorganisms, whereas a lower pH marinade that has a remarkable effect on the evolution of the microbial growth, could lead to degraded meat products from the sensory and nutritional viewpoint (Ke et al., 2009; Sharedeh et al., 2015; Yusop et al., 2010). Considering all the above, research should focus on the incorporation of novel ingredients that could conduce to the improvement of microbial, sensory and nutritional quality of meat products. The pomegranate juice could be a promising ingredient that covers the above mentioned criteria, since its content in organic acids, sugars and phenolic compounds renders a product of high nutritional value, with antimicrobial efficacy, antioxidant activity and desirable sensory attributes (Al-Zoreky, 2009; Maskan, 2006; Türkyılmaz et al., 2013). Moreover, the process of marination can cause alterations in the physicochemical profile of meat resulting in a shift in spoilage microorganisms that could possibly delay spoilage (Gram et al., 2002; Schirmer et al., 2009). This different profile can be determined by monitoring changes in metabolite composition occurring during processing and storage. Therefore, metabolite analysis - through the determination of organic acids and volatile organic compounds – plays a critical role in acquiring a detailed view of the whole physicochemical status, the evolution of the indigenous microbiota and the characterization of the type of spoilage in marinated and non-marinated meat. Thus, the objectives of this study were the evaluation of the effect of pomegranate juice based marinades on: (a) the microbial growth, (b) the sensory characteristics, and (c) the organic

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acids and volatile compounds profile of chicken breast fillets stored aerobically at 4 and 10 °C.

2. Materials and methods

2.1. Sample preparation

Fresh skinless chicken breast fillets were obtained from a local meat market and transferred to the laboratory under refrigeration within 30 min. They were subsequently cut into 10 g pieces (2 × 4 × 1 cm) with a sterilized knife and placed into polypropylene trays. For the marination of the samples two different marinades were prepared. The first contained commercially available 100% natural pomegranate juice as the main ingredient while for the second a combination of pomegranate and lemon juice was used. The composition of the marinade was (per 100 mL): i) pomegranate juice (70 mL), olive oil (30 mL), dried thyme (0.1 g), honey (2 g) (denoted PO marinade), and ii) pomegranate juice (35 mL), lemon juice (35 mL), olive oil (30 mL), dried thyme (0.1 g), honey (2 g) (denoted PL marinade). Marination was carried out in plastic trays at 4 °C for 3 h by adding 1200 mL of marinade in each tray containing 60 pieces of chicken meat (10 g each). This volume was sufficient for the complete immersion of the samples into the marinades. Following marination, samples were removed from the trays, allowed to drain off and placed into sterile Petri dishes. They were then wrapped with oxygen-permeable plastic film and stored at 4 and 10 °C for 9 days in high precision (± 0.5 °C) incubation chambers (MIR-153, Sanyo Electric Co., Osaka, Japan). Untreated (non-marinated) chicken samples were also prepared and used as control (denoted CO). At each sampling point, duplicate samples were subjected to microbiological, sensory and chemical analyses, while the experiment was conducted twice ($n = 4$) using different batches of chicken meat.

2.2. Microbiological analysis

Chicken samples (10 g) were transferred aseptically to a stomacher bag and diluted 10 times in sterile quarter-strength Ringer's solution (LAB 100Z, LAB M, Bury, UK). The mixture was homogenized in a stomacher (Lab Blender, Seward Medical, London, UK) for 60 s at room temperature. The resulting suspension was serially diluted in the same diluent and aliquots (0.1 or 1 mL) of the appropriate dilutions were spread or poured in duplicate in the following agar 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; de Man-Rogosa-Sharpe agar (401728, Biolife) (pH adjusted to 5.7) for LAB, overlaid with the same medium and incubated at 30 °C for 72 h; Rose-Bengal Chloramphenicol Agar Base (LAB036, supplemented with X009 chloramphenicol, LABM) for yeasts and molds, incubated at 25 °C for 72 h; STA Agar base (402079 supplemented with selective supplement 4240052, Biolife) for *Brochothrix thermosphacta*, incubated at 25 °C for 48 h; Violet Red Bile Glucose Agar (402188, Biolife) for *Enterobacteriaceae*, overlaid with the same medium and incubated at 37 °C for 24 h. After incubation, typical colonies for each microbial group were enumerated and colony counts were logarithmically transformed (log CFU/g). Results are presented as average values (± standard deviation) of the 4 samples analyzed at each sampling point. In parallel to microbiological analyses, the pH of chicken samples and marinades was recorded by means of a digital 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.

2.3. Sensory evaluation

Ten in-house trained assessors evaluated the odor and the overall appearance of the marinated and non-marinated samples using a 3-point hedonic scale (1-desirable; 2-acceptable; 3-unacceptable). The score of 2 was set as the threshold value for the acceptance of the samples. The assessors were not informed about the experimental procedure and the samples were blind-coded with random numbers. The samples were also subjected to thermal process (180 °C for 15 min in a preheated oven) and were assessed by the sensory panel for their flavor, odor and tenderness using the same scale as for the raw samples. The cooked marinated samples were presented warm to the panel along with water to clean their palates between each evaluation. The evaluation of the cooked samples was conducted for the marinated samples only, as the aim was to check the acceptability of the marinated chicken samples by the consumers with regard to sensory attributes of thermally processed marinated chicken. The sensory assessment of cooked samples was performed until the 7th day of storage.

2.4. Organic acids analysis

The extraction of organic acids was undertaken according to Argyri et al. (2011) with slight modifications. Specifically, 4 g of the sample were homogenized with a glass rod in 8 mL of HPLC grade water for 2 min and centrifuged (10 min at 4500 rpm 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 trifluoroacetic acid – TFA (for the precipitation of proteins) and 50 μL of 1% (v/v) sodium azide (as preservative) were added. Finally, the filtrate was stirred, centrifuged (10 min at 4500 rpm at 4 °C) and the supernatant filtrated through a 0.22 μm filter. HPLC analysis was carried out using 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. Optimum efficiency of separation was obtained by isocratic elution of the samples with a solution of 0.009 N H_2SO_4 through an Amminex 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 the collection and the processing of the spectra was the 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. Solutions of oxalic, citric, malic, lactic, acetic, pyruvic, tartaric, succinic, propionic and butyric acids were used as reference substances, analyzed using the same method and their spectra were compared with the spectra of the samples for the identification of the peaks. Determination of the concentration of organic acids was implemented using calibration curves for 6 different concentrations of a mixture of organic acids standard solution.

2.5. Volatile compounds analysis

The volatile compounds profile was determined by headspace SPME–GC/MS analysis. The volatile compounds of meat samples were isolated by the headspace solid phase micro-extraction method (HS–SPME). The fibre used for the absorption of volatiles was a DVB/CAR/PDMS — 50/30 μm (needle length 1 cm, needle size 24 ga) (Sigma Aldrich, Greece). For the preparation of the sample, 5 g were homogenized manually in 10 mL of 25% NaCl into a 20 mL glass vial. An amount of 10 μL of internal standard (4-methyl-1-pentanol, final concentration 1000 μg/L) was added, the vial was closed hermetically using a mininert valve (Sigma Aldrich, Greece) and the contents were magnetically stirred for 15 min at 40 °C. Then, the fibre was exposed to the headspace for another 30 min under the same conditions. The length of the fibre in the headspace was kept constant. Before each analysis, the fibre was exposed to the injection port for 7 min to remove any volatile contaminants. GC/MS analysis was performed on an Agilent 7890A gas chromatograph coupled to an Agilent 5973C mass