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Storage of pork meat under modified atmospheres containing vapors from commercial alcoholic beverages

A.E. Kapetanakou, E.I. Agathaggelou, P.N. Skandamis *

Agricultural University of Athens, Department of Food Science and Human Nutrition, Laboratory of Food Quality Control & Hygiene, Iera Odos 75, GR-11855 Athens, Greece

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ABSTRACT

The present study aimed to evaluate the effect of AB vapors on microbial, physicochemical, and sensory profile of pork meat stored in different MAP conditions. Pork pieces (10 g) and cotton/cellulose absorbent cloths (2 × 2 cm) were placed into compartmentalized Petri-dishes in two sections. Aliquots (1 mL) of water (control), 30% v/v and 40% v/v ethanol, whisky, brandy, tsipouro, raki, and ouzo were added separately to the cotton/cellulose absorbent cloths. Each pork sample was placed in one compartment and cotton/cellulose absorbent cloths supplemented with different ABs were placed in a separate compartment of each Petri-dish. Samples were packaged in 40% CO_2: 30% O_2: 30% N_2 and 80% O_2: 20% CO_2 and stored at 4 and 10 °C. Total viable counts, *Pseudomonas* sp., *Brochothrix thermosphacta*, lactic acid bacteria, yeasts and molds, and Enterobacteriaceae, were enumerated during storage. Changes in pH, color (L^*, a^*, b^*), odor, taste, and overall appearance of pork meat were also evaluated along with changes in organic acid levels via HPLC. At 4 °C, lactic acid bacteria and *B. thermosphacta* were the dominant organisms under 40% CO_2: 30% N_2: 30% O_2 and 80% O_2: 20% CO_2, respectively, while at 10 °C, lactic acid bacteria dominated in both MAP conditions. All applied ABs were effective ($p < 0.05$) against lactic acid bacteria, pseudomonads, and *B. thermosphacta*. The inhibitory effect of ABs was also reflected through lower levels of glucose consumption or accumulation of lactic, acetic, succinic, and formic acid compared to controls. Moreover, packaged samples in 40% CO_2: 30% O_2: 30% N_2 exhibited a significant increase ($p < 0.05$) of acetic acid during storage at 4 °C, but the concentrations of acetic acid in samples exposed to AB vapors were lower than those in controls. Both antimicrobial active MAPs extended the shelf-life of pork meat by *ca.* 2-fold, while samples exposed to alcoholic beverages (especially ouzo) under 80% O_2: 20% CO_2 resulted in better ($p < 0.05$) sensory properties compared to the respective samples under 40% CO_2: 30% O_2: 30% N_2. Overall, vapor action of ABs in combination with MAP may constitute a promising, antimicrobial packaging technology for extending the shelf-life of pork meat.

1. Introduction

Pork meat is considered a popular food commodity worldwide with high exportation rates; however it is also perishable and the food industry is in need of applying modern preservation methods for the extension of its shelf-life. Nowadays at retail, the common packaging types of pork meat are air, modified atmosphere (MAP), or vacuum. The efficacy of MAP to maintain or increase the nutritional, quality, and safety attributes of fresh foods is already known. Inhibition of deterioration under MAP is achieved by adjusting the gas composition inside the packaging (Fraquez and Barreto, 2011; Lorenzo and Gómez, 2012). Indeed, the majority of meat products are packaged in high oxygen environment *ca.* 80% O_2 to reduce myoglobin oxidation and provide a stable, attractive, "bloomed" red meat color, in a proportion of at least 20% CO_2 to prevent growth of Gram-negative bacteria responsible for aerobic spoilage such as *Pseudomonas* sp. However, the high presence of O_2 in MAP may also have some drawbacks such as favor the dominance of a facultative anaerobic or microaerophilic microorganisms including *Brochotrix thermosphacta* and lactic acid bacteria, significant increase of lipid oxidation, high tenderness and juiciness scores, rancidity and off-flavor development, and premature browning (Cornforth and Hunt, 2008; Doulgeraki et al., 2012; Kim et al., 2010).

When MAP is combined with other preservation methods, its effectiveness may be highly enhanced (Arvanitoyannis and Stratakos, 2012; Mastromatteo et al., 2010; McMillin, 2008). In response to the dynamic changes in current consumer demands and market trends, the interest of using alternative and innovative methods is increased rapidly. Among them, is active packaging, which has been defined as a mode of packaging in which the package, the product, and the environment interact during storage to prolong shelf-life and/or enhance quality and safety of the product (Coma, 2008; Labuza and Breene, 1989). Different active packaging systems have been developed (e.g., oxygen scavengers, moisture absorbers, CO_2 controllers, ethanol emitters) with the version of antimicrobial active packaging to be of great importance (Coma, 2008; Suppakul et al., 2003). Antimicrobial agents used

* Corresponding author. Tel.: +30 210 5294684; fax: +30 210 529 4683.
E-mail address: pskan@aua.gr (P.N. Skandamis).

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through an active packaging may migrate into the food through diffusion and partitioning either by direct addition in the food matrix or by incorporation into edible carrier materials such as films or coatings (Emiroğlu et al., 2010; La Storia et al., 2012; Zinoviadou et al., 2009). Otherwise, antimicrobials may be released through evaporation in the headspace between food and packaging (Argyri et al., 2011; Laird and Phillips, 2011; Matan et al., 2006; Skandamis and Nychas, 2002). The latter application has both scientific and commercial interest, since its major advantage is being reflected to the lack of immediate contact between the food and antimicrobial agent, which may negatively impact the sensory attributes of the food product.

Research has been extensively focused on the application of alternative, natural antimicrobials in food e.g., essential oils, plant or fruit extracts, beverages, and food-grade ethanol through the aforementioned ways of action (evaporation or/diffusion) (Fratianni et al., 2010; Mexis et al., 2012; Oral et al., 2009; Suppakul et al., 2003; Ward et al., 1998). A literature and market research (patents) could provide many applications of ethanol, either generated by films or sachets (i.e., Ethicap™ sachet, Freund, Japan) or even adhesive-backed films that can be taped on the inside of a package to provide antimicrobial activity (Floros et al., 1997; Labuza and Breene, 1989; Smith et al., 1995; Suppakul et al., 2003). However, with regards to consumer demands for fresh products without the addition of preservatives, the application of pure ethanol, even if it is food-grade could raise suspicion (Zink, 1997). Thus, alcoholic beverages (ABs) could be potential adequate substitutes not only in food preservation level but also in terms of marketing, since they are used in many national cuisines e.g. Italian, Greek, (e.g., beer, wine), in order to give flavor to the food during cooking. With regards to the ethical issues that may arise when such antimicrobials (ethanol content) are used, their mild application through evaporation along with the facts that are added on non-ready-to-eat food products, is a major advantage, since it is expected that the levels of migrated ethanol will be dramatically reduced after cooking.

Considering the above, the objectives of the present study was to develop an active-like antimicrobial packaging system formed by the gradual release of vapors of Greek and international ABs and evaluate its effect on microbial, physicochemical, and sensory attributes of pork meat during storage at different storage temperatures and MAPs.

2. Materials and methods

2.1. Antimicrobials—Alcoholic beverages

Three commercial, traditionally Greek ("tsipouro", "raki" or "tsikoudia", and "ouzo") and two well-known internationally (whisky and brandy) ABs were used separately as volatile antimicrobials in the present study. Tsipouro and raki are produced by single and double distillation of grape pomace, respectively, sometimes including the stems and seeds. Ouzo, on the other hand, is not a distillation product but is being produced by infusing pure white alcohol, after double or triple distillation process, with various herbs such as aniseed (dominant), licorice, and mint. Ethanol content of whisky, brandy, tsipouro, raki, and ouzo was 40, 36, 41, 39.6, and 40% v/v, respectively.

2.2. Preparation and storage of pork samples

Fresh boneless rib eye pork steaks (*ca* 1–1.5 cm thickness; pH 5.54 ± 0.05), derived from different carcasses to increase sample variability, were purchased from a local butchery shop (Athens, Greece) and transported to the laboratory within 30 min, where they were stored at 1 °C for 1 h. The fat was removed from the steaks, which were cut into pieces of 10 g. Compartmentalized Petri-dishes in two sections (No 82.1195; ∅ 9.2 mm; Sarstedt AG & Co., Germany) were used in order to avoid any potential contact between the product and ABs during storage. In order to scale-up and commercialize the present product, further shelf-life experiments in commercial packaging including trays of two compartments and larger amounts of pork meat are required (Fig. 1). In laboratory scale, aliquots (1 mL) of water (control), 30% and 40% ethanol, whisky, brandy, tsipouro, raki, and ouzo were separately added to 2 × 2 cm absorbent cloths (35% cotton and 65% cellulose) (Wettex®, Vileda Professional, Germany). The soaked cloths served as vehicles ("carriers") of ABs, allowing the controlled release of AB vapors in the packaging atmosphere during storage. Cotton/cellulose absorbent cloths were selected for use in the present study, since they were capable to absorb 15 times its own weight, according to the manufacturer company. Each pork sample was placed in the first compartment and cotton/cellulose absorbent cloths supplemented with each AB in the second compartment of each Petri-dish. Following this, all Petri-dishes without their cap were placed in plastic bags with gas permeability *ca*. 25, 90, and 6 cm^3/m^2 per day/10^5 Pa for CO_2, O_2 and N_2, at 20 °C and 50% relative humidity (Flexo-Pack S.A., Athens, Greece). Different MAP compositions, 40% CO_2: 30% O_2: 30% N_2 and 80% O_2: 20% CO_2, were applied by using a gas flush, sealing packaging machine (Henko Vac 1900 Machine, Howden Food Equipment B.V., The Netherlands) and samples were stored at 4 and 10 °C in high precision (±0.5 °C) incubation chambers (MIR-153, Sanyo Electric Co., Osaka, Japan). Two independent storage experiments were performed and duplicate samples (from different steaks) were used for each trial ($n = 4$). Each sample derived from a different package and two independent packages were analyzed at each sampling point. Sampling was performed every 3–5 days at 4 °C and every 2 days at 10 °C.

The applied volume of 1 mL was decided after conducting preliminary experiments among 0.5, 1.0, and 3.0 mL per AB, which showed good antimicrobial activity against total viable counts and satisfactory sensory properties of pork samples packaged under aerobic conditions and stored at 4 and 10 °C. Growth inhibition of total viable counts in pork samples exposed to ABs vapors was ranged from 0.8 to 3.5 log CFU/g compared to controls, depending on the applied AB and storage temperature (data not shown).

Shelf-life of pork samples exposed or not to generated vapors of ABs and ethanol was estimated during storage at 4 and 10 °C as the time needed for TVC to reach the target level of 7.0 log CFU/g (Ercolini et al., 2011; Nychas et al., 2008; Tang et al., 2013).

2.3. Microbiological analysis

All packages were visually inspected for gas leaks prior to microbiological analysis. Pork samples (10 g) were aseptically removed from Petri-dishes, added to 90 mL of sterile ¼ strength Ringer's solution (Lab M, Lancashire, UK), and homogenized in a stomacher (Interscience, France) for 60 s. Following homogenization, decimal dilutions in ¼ strength Ringer's solution were prepared and 1 or 0.1 mL of the appropriate dilutions were poured or spread, respectively, on selective and non-selective culture media. Total Viable Counts (TVC) were enumerated on Plate Count Agar (PCA; Lab M, Lancashire, UK) and incubated at 30 °C for 48 h; Lactic Acid Bacteria (LAB) were determined on double

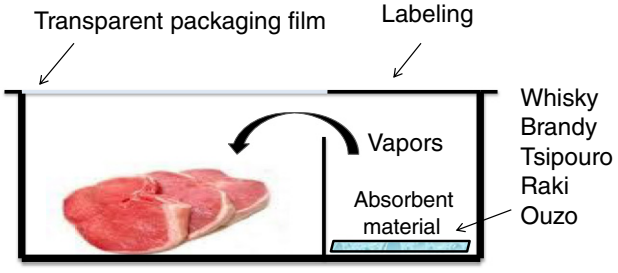

Fig. 1. Cross-section of the suggested, commercial antimicrobial MAP of pork steaks by using trays compartmentalized in two sections and expose the pork steaks (left section) in vapors produced by different ABs (right section).