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Combined effects of ozone and freeze-drying on the shelf-life of *Broiler* chicken meat

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ABSTRACT

The effects of ozone on the shelf-life extension of freeze-dried chicken meat fillets stored at 21 ± 1 °C were investigated. The samples were exposed to gaseous ozone with three ozone concentrations of 0.4, 0.6 and 0.72 ppm during 10, 30, 60 and 120 min. The shelf-life of the ozonated freeze-dried chicken meat samples was determined using both microbiological and sensory analyses during 8 months of storage. Ozone and lyophilisation (Combined treatments) were shown to be valid in retarding the growth of most microbial groups from the first month of storage. High microbial counts were found in frozen meat from month 6 onwards, with total aerobic mesophilic bacteria (TAMB) counts above 7 log cfu/g and lactic acid bacteria (LAB) counts above 5 log cfu/g, whereas decreases of 6.8 and 4.77 log cfu/g for TAMB and LAB counts were reported in combined treated samples when compared with untreated meat (frozen meat). Also, 3.26 and 1.41-log reductions were observed with respect to non-ozonated freeze-dried meat (trt-0). Regarding sensory characteristics, the combined use of ozone and lyophilisation would be useful in extending the shelf-life of raw chicken breast meat up to 8 months, whilst the samples treated only with lyophilisation showed a 4 month shelf-life.

1. Introduction

Chicken meat is one of the most popular food commodities in Europe and the second most preferred meat by European Union consumers after pork meat (FAO, 2014). Some of the reasons for the popularity of this kind of meat are the relatively low price, low fat content and the high nutritional value. Generally, poultry meats are highly perishable to bacterial contaminants due to large amounts of variable nutrients, a high water activity (a_w) and a higher final pH limiting the shelf-life of the product (Lawrie, 1998). In the case of meat and meat products, enzymatic and chemical reactions are responsible for the initial loss of freshness, while microbial activity is responsible for subsequent spoilage. The contamination by several pathogenic microorganisms can cause severe foodborne diseases in consumers (Jayasena et al., 2015).

However, the manufacturing of meat products is constantly challenged to meet rapid changes in consumer tastes and demands for healthier food products, safe, natural, free of conventional chemical preservatives with an extended shelf-life. Consumer acceptance is the key success factor for the development of successful meat products (De Barcellos et al., 2010) and meat safety is considered to be a prerequisite by consumers (Van Wezemael, Verbeke, Kügler, de Barcellos, & Grunert, 2010). For this purpose, the multiple hurdle concept is an integrated basic approach in food preservation and the hurdle technology is generally defined as using the simultaneous or the sequential application of factors and/or treatments affecting microbial growth (Turantaş, Kılıç, & Kılıç, 2015). The principle of this concept can be explained as two or more inhibition and inactivation methods (hurdles) at suboptimal levels are more effective than one (Leistner, 1992). This method is becoming attractive, because several hurdles are used to obtain the optimum combinations which do not affect the sensory quality, while maintaining the microbial stability and safety of the food (Alzamora, Tapia, Argaíz, & Welli, 1993; Leistner, 1992). In fact, ozonation and freeze-drying were employed as hurdles in the present study to develop a new raw meat product from *Broiler* chicken breasts. Ozone is a powerful antimicrobial agent very effective in destroying a wide range of microorganisms including viruses, bacteria, fungi, protozoa, and bacterial and fungal spores (Khadre & Yousef, 2001). This agent inactivates bacteria by disrupting the cell membrane and cell wall, leading to cell lysis (Muhlisin, Cho, Choi, Hahn, & Lee, 2015). Ozone is used in an

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extensive range of agricultural products, such as vegetables, fruits, fish (Manousaridis et al., 2005) and meat products (Muhlisin et al., 2015; Sekhon et al., 2010; Stivarius, Pohlman, McElyea, & Apple, 2002). The bactericidal effect of ozone depends on several factors, such as temperature, relative humidity, pH and the presence of organic matter (Kim, Yousef, & Chism, 1999).

Freeze-drying is the most common form of food preservation to improve the long-term stability of food because the percentage of humidity and the water activity can be reduced, if the product is well lyophilized, which retards the growth of microorganisms for a long period. This process applies only for high added-value products (Abdelwahed, Degobert, Stainmesse, & Fessi, 2006). Freeze-drying has many applications on food products, such as chicken meat, raw beef, mushrooms, fruits, carrots, tomato, eggs, etc. (Babić, Cantalejo, & Arroqui, 2009; Chang, Lin, Chang, & Liu, 2006; Hammami & René, 1997; Litvin, Mannheim, & Miltz, 1998). The many advantages of lyophilisation make it one of the technologies attracting the attention of the food industry, including: (i) the conservation of the primary physical and chemical characteristics of the product, (ii) a low residual humidity (<10%) providing easy handling during shipping and storage of the lyophilized product and, (iii) long-term stability.

The aim of this research was to study the combined effects of ozone and lyophilisation on the shelf-life extension of *Broiler* chicken meat fillets, stored at room temperature by evaluating microbiological load and sensory characteristics, in order to develop new high-quality raw meat products from fresh chicken meat, safe, with a high nutritional value, with no additives added and long-lasting at room temperature. Therefore, these meat products can be preserved and transported with no refrigeration, due to the relative reduction of moisture content and water activity (energy saving, as no freezing is required). Furthermore, this type of food product would allow a long shelf-life in the case of natural catastrophes (earthquakes, floods, …), export to third countries, military campaigns, mountain climbers and scarcity in electricity supply.

2. Materials and methods

2.1. Raw matter and sample preparation

Broiler chicken breast meat was obtained from U.V.E., S.A. (Tudela, Navarre, Spain). Chickens were 42 days old before slaughtering with approximately 2 kg of weight. All breasts were stored in a refrigerated room (2–4 °C) for the time of reception until used. The samples were trimmed of visible fat and nerves. They were cut into pieces (approximately 3 × 3 cm^2 of section and of 0.7 cm in thickness), before the analyses. Then, they were divided into three batches. The first batch was vacuum-packed, refrigerated and stored at 4 ± 0.5 °C (P Selecta, Pharmalow, Tarre, Navarra, Spain). To characterize the fresh meat, physical-chemical measurements (pH, colour, water activity, humidity and texture) were performed. After characterization, the same batch was vacuum-packed, deep-frozen, and stored at −40 ± 1 °C (Climas, Barcelona, Spain) and used as an external reference of raw meat for sensory and microbiological analyses. The second batch of meat samples was subjected to freeze-drying only, and vacuum packed and stored in a dark place at room temperature (21 ± 1 °C) and used as an internal control. The third batch of meat samples was treated with ozone, freeze-dried, vacuum-packed and stored in a dark place at 21 ± 1 °C.

2.2. Ozone treatments

Ozonation assays were carried out in a 3 m^3 volume refrigerated chamber (Eurozon, Ecologyc 2000, Sestao, Vizcaya, Spain) to a continuous flow of ozone gas at 4 ± 0.5 °C and 90 ± 1% relative humidity. These conditions are important for the efficiency of the bactericidal effect of ozone (Kim et al., 1999). Ozone was generated *in situ*, utilizing a UV radiation using an ozone generator (Rilize, model 3060 Eurozon, Sestao, Spain). Ozone concentrations inside the chamber were monitored continuously by circulating air from the chamber through an ultraviolet absorption ozone gas analyzer (Ozomat MP, Anseros, Germany). The different treatments are shown in Table 1. Treatment combinations for this study included three ozone concentrations (0.72, 0.6 and 0.4 ppm) and four exposure times (120, 60, 30 and 10 min).

2.3. Freeze-drying process and packaging of samples

After ozone treatments, samples were dehydrated in a pilot scale freeze-dryer (Model Lyobeta 25, Telstar Industrial, S.L., Barcelona, Spain). The different parameters of the freeze-drying process assayed in this study were the same in all treatments and were the best conditions described in the research work of Babić et al. (2009). Briefly, slow freezing, 20.5 h of primary drying (12 h at 0 °C and 8.5 h at 10 °C) at 30 Pa.

All the samples were vacuum-packed, using a vacuum packaging machine (Model SAMMIC V-640, Gipuzkoa, Spain), in impermeable plastic trays type polyamide/polyethylene PA/PE 20/70 200 × 300 (Ilpra, Barcelona, Spain). The double-layer of the trays resulted in a strong and relatively impenetrable bag for both air and moisture and had an oxygen transfer rate of less than 50 cm^3 $m^{-2}d^{-1}bar^{-1}$, permeability to CO_2 less than 150 $cm^3m^{-2}d^{-1}bar^{-1}$ and a water vapour permeability of less than 2.8 g m^{-2} d^{-1}.

Two meat controls were used in this study: (1) Lyophilized chicken samples (trt-0), that were not exposed to ozone treatment and were used as an internal control in order to analyse the efficacy of the combination of ozone and lyophilisation on the self-life of meat. (2) Frozen meat used as an external reference of raw meat (due to the similarity of those samples with the ozonated freeze-dried samples) for sensory and microbiological analyses.

2.4. Analyses of samples

2.4.1. Physical and chemical analyses

Physical and chemical analyses (pH, water activity (a_w), humidity, percentage of rehydration, colour and the texture) were carried out during the first day of storage for characterising the fresh meat and all treated samples.

The pH was measured using a pH-meter (Crison PH 25, S.A, Barcelona, Spain) with a combined electrode, which penetrates the meat samples. Water activity (a_w) was measured by means of a hygrometer (Novasina RS-232, LabMaster, Switzerland). Humidity of fresh meat was determined in a stove (P Selecta, Digitronic, Barcelona, Spain) at 102 ± 2 °C until constant weight, according to the ISO R-1442 regulation (ISO, 1973) and the Spanish Official Method for the Analysis of Meat Products (B.O.E., 29/8/79). Humidity of dried meat was determined following the ISO R-1442 method (AOAC, 1975), by using a gravimetric infrared stove (Gram, ST-H 50, Barcelona, Spain).

In order to know how much water was absorbed by freeze-dried chicken meat and their fully rehydration characteristics, the samples were rehydrated in trays filled with distilled water at 21–22 °C. The change in mass of freeze-dried chicken meat was measured each half an hour, when all meat samples were taken out and dried with a blotting paper, then each sample was weighed. This procedure was repeated until obtaining constant weight of the samples. The percentage of rehydration was calculated using the following