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Effect of combined treatment with supercritical CO_2 and rosemary on microbiological and physicochemical properties of ground pork stored at 4 °C

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A B S T R A C T

The effect of combined treatment with supercritical CO_2 (2000 psi, 35 °C for 2 h) and rosemary powder (2.5% and 5.0% (w/w)) on microbiological and physicochemical properties of ground pork stored at 4 °C was investigated. The changes in total viable count, pH, total volatile base nitrogen (TVB-N), lipid oxidation and instrumental color (CIE L^*, a^*, b^*) were analyzed during a week period of refrigerated storage. It was found that microbial populations were reduced by supercritical CO_2 treatment, with the more pronounced effect being achieved by combined treatment with supercritical CO_2 and 5.0 g rosemary powder/100 g meat. Supercritical CO_2 treatment for 2 h could accelerate lipid oxidation of ground pork during refrigerated storage, whereas combination with rosemary can significantly slow down the increase of oxidation rate. Combined treatment of supercritical CO_2 and rosemary significantly increased L^* and b^* values of the ground pork, while the a^*, pH and TVB-N value were not affected as compared to the treatment with supercritical CO_2 alone. The results of this study indicate that combined treatment of supercritical CO_2 and rosemary may be useful in the meat industry to enhance the storage stability of ground pork treated with long time exposure of supercritical CO_2 during refrigerated storage.

1. Introduction

Raw meat is a rich nutrient matrix and is highly perishable. Spoilage of raw meat may occur in two ways during storage: microbial growth and oxidative rancidity (Sebranek, Sewalt, Robbins, & Houser, 2005). This is especially the case for ground meat, since ground meat is more sensitive to oxidation because of its porous structure and it has more susceptibility to microbial spoilage due to the grounding process (Esmer, Irkin, Degirmencioglu, & Degirmencioglu, 2011).

Spoilage of ground meat could bring about heavy losses to the producer. Therefore, adequate preservation technologies must be used to preserve its safety and quality which is the major problem faced by meat processing industry.

Recently, supercritical CO_2 ($scCO_2$) treatment has been considered by the food industry as a viable alternative to traditional pasteurization/sterilization processes in the preservation of natural flavors and nutrients of foods (Ferrentino, Balzan, & Spilimbergo, 2012). It has been used to inactivate microorganisms in various food products, including meat and meat products (Bae, Choi, Kim, Kim, Kim, & Rhee, 2011; Bae, Kim, Kim, Kim, & Rhee, 2011; Choi, Bae, Kim, Kim, & Rhee, 2009; Choi, Kim, Kim, Kim, & Rhee, 2009; Ferrentino, Balzan, & Spilimbergo, 2013). It is reported that $scCO_2$ processing of meat has a favorable bacterial disinfection effect to the produced meat, while keeping its texture and taste, which is not possible using other sterilization techniques (Taher, Al-Zuhair, AlMarzouqui, & Hashim, 2011). Moreover, $scCO_2$ processing can be an effective processing technique for protein-based foods since the covalent bonds of food components including free amino acids, proteins, lipids, vitamins and polysaccharides are able to resist the effects of high pressures (Taher et al., 2011). Finally, $scCO_2$ processes can protect the treated materials from oxidation and contamination with organic solvents and prevent bio-burden increase (Perrut, 2012).

Extracts from herbs and spices have also been used to extend the shelf life of food products (Chouliara, Karatapanis, Savvaidis, & Kontominas, 2007; Li et al., 2012; Michalczyk, Macura, Tesarowicz, & Banaś, 2012). Many research showed that rosemary extracts possess antioxidant and antibacterial activity (Bubonja-Sonje, Giacometti, & Abram, 2011; Georgantelis, Ambrosiadis, Katikou, Blekas, & Georgakis, 2007; Ozogul et al., 2010; Zhang, Kong, Xiong, & Sun, 2009) and could be used as natural agents for food preservation. Hernandez, Ponce-Alquicira, Jaramillo-Flores, and Legarreta (2009) studied antioxidant effects of rosemary in model raw pork batters. Georgantelis et al. (2007) investigated the effect of rosemary extract on lipid oxidation of fresh pork sausages stored at 4 °C. The antioxidant effects of rosemary in

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frozen vacuum packaged beef and pork was also evaluated by Rojas and Brewer (2008). Numerous studies have showed that compounds responsible for antioxidant and antimicrobial activity of rosemary are phenolic diterpenes such as carnosol and carnosic acid, royleanonic acid and 7-methoxyrosmanol, rosmadial, rosmanol, epirosmanol and isorosmanol, methyl carnosate, rosmanol-9-ethyl ether (Babovic et al., 2010). These phenolic compounds can delay or inhibit the oxidation of lipids or other molecules by the following mechanisms: quenching free radicals, acting as reducing agents, and chelating ferrous ions. These compounds can also degrade the cell wall, disrupt the cytoplasmic membrane, cause leakage of cellular components, change fatty acid and phospholipid constituents, influence the synthesis of DNA and RNA and destroy protein translocation, thus inhibiting bacteria (Shan, Cai, Brooks, & Corke, 2007).

Although many references can be found on the separate application of $scCO_2$ and rosemary to different food products, little is known about their combined effects. Thus, the objective of this study was to investigate the effect of combined application of $scCO_2$ and rosemary on physicochemical and microbial qualities of ground pork.

2. Materials and methods

2.1. Materials

Dried rosemary (*Rosemarinus officinalis*) was obtained from Hangzhou Efuton Tea Co., LTD (Hangzhou, China). It was ground in a FW100 universal high-speed smashing machine (Tianjin Taisite Instrument Co., LTD., Tianjin, China). The ground powder was passed through a 1.0 mm sieve and kept in a desiccator at room temperature until further use.

Meat from *longissimus lumborum* muscle of *porcine* carcasses at 24 h postslaughter was purchased from a local wholesale supplier and then transported to the laboratory within 1 h. The meat was trimmed to remove all subcutaneous and visible connective tissues. About 2.0 kg of the obtained lean meat was passed through a sterilized meat grinder with 6 mm holes. Then the meat samples were divided into four different batches of 200 g each and randomly assigned to one of the following four treatments: control (meat without any treatment), treatment with $scCO_2$ at 2000 psi (13.8 MPa) and 35 °C for 2 h only (SCO_2), combined treatment of $scCO_2$ and 2.5 g (SCO_2 + R 2.5%) or 5 g (SCO_2 + R 5%) dried rosemary powder/100 g meat. Each treatment was prepared in duplicate. The levels of rosemary were selected on the basis of the extraction yield (2.09 ± 0.16%) of rosemary extracted by $scCO_2$ under the treatment conditions and experimental information available in the literature for the addition of rosemary extract into meat and meat products (Yin, Zhang, & Zhou, 2014). Therefore ground pork contained ~0.05% and 0.1% rosemary extract following $scCO_2$ treatment of pork with rosemary (R 2.5% and 5%).

2.2. Supercritical CO_2 treatment and packaging of ground pork

Fig. 1 schematically shows the supercritical experimental setup. Fig. 2 depicts a schematic diagram of meat samples treatment by supercritical CO_2. One batch of meat (ca. 200 g) was placed in a charging barrel. Desired amount of dried rosemary powder was weighed and wrapped by filter paper to form a sachet. The sachet was also put into the charging barrel and separated from the meat by a plate. The plate is round (Φ60), and there are many small through holes (Φ3) uniformly distributed in it, which can make carbon dioxide flow through the ground pork and the rosemary sachet freely. Then the charging barrel was sealed into the high-pressure stainless steel vessel (500 mL) that was pre-heated to the desired temperature (35 °C). After sealing, CO_2 gas was introduced into the vessel to purge it for 2 min. Subsequently, the vessel was pressurized with CO_2 using a constant flow/constant pressure dual piston pump (SFT-10, Supercritical Fluid Technologies, INC., USA). When the desired pressure (2000 psi) was reached, the system was kept at that pressure and temperature for 2 h. During the holding time, the rosemary powder was subjected to supercritical CO_2 extraction and the resulting extracts were subsequently impregnated in ground pork by $scCO_2$. At the end of this period, the vessel was depressurized and venting the CO_2 in <10 min. During the decompression, solvent power of the $scCO_2$ can be decreased, causing the precipitation of rosemary extract onto the surface of ground pork. After that, the sample was removed from the vessel, subdivided into four groups (each treatment × 4 storage times) and then aerobically packaged in low density polyethylene bags. Ground pork was stored for up to 7 days at 4 °C (± 1 °C) and experimental measurements were recorded at 2 day intervals. Each treatment repeated the same procedure once.

2.3. Microbiological analysis

The total viable count (TVC) was measured with standard plate count method according to the Chinese national standard GB 4789.2 (2010) with slight modification. In brief, at each selected time, each sample (10 g), in duplicate, was taken aseptically and homogenized in 90 mL of sterile physiological saline. Then the resulting suspension was serially diluted (1:10) in sterile physiological saline and in duplicate 1 mL samples of appropriate dilutions were poured onto total count agar plates. After incubation for 48 h at 30 °C, colonies on the plates were counted and expressed as $\log_{10}$ cfu (colony forming units)/g pork meat.

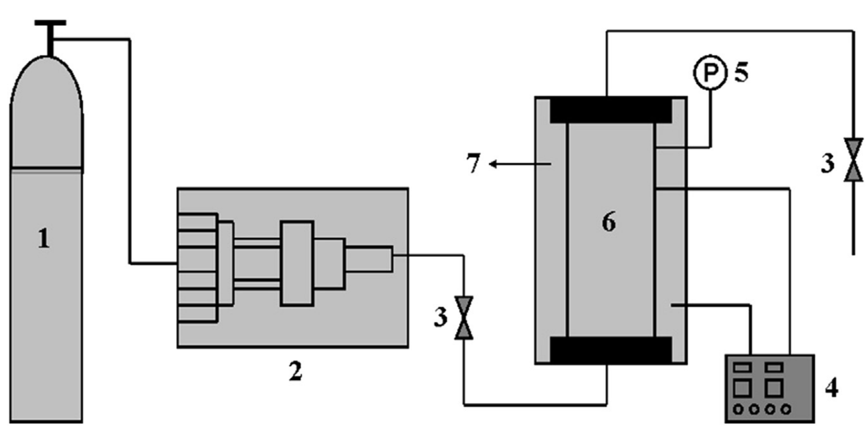

Fig. 1. Schematic illustration of the supercritical CO_2 setup employed: 1, CO_2 gas cylinder; 2, pump (equipped with a pre-cooler); 3, valve; 4, temperature controller; 5, pressure gauge; 6, pressure vessel; 7, heating jacket.