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# Rosemary and oxygen scavenger in active packaging for prevention of high-pressure induced lipid oxidation in pork patties

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ABSTRACT

Three different packaging systems: vacuum packaging, rosemary active packaging, and oxygen scavenger packaging were compared for their ability to counteract lipid oxidation in pork patties upon storage at 5 °C for 60 days following high pressure processing (HPP) (700 MPa, 10 min, 5 °C). Lipid oxidation was studied at the surface and the inner part by measuring secondary lipid oxidation products (TBARs) and the tendency to form radicals by electron spin resonance (ESR) spectroscopy. Lipid oxidation was lower in the inner part than at the surface for all three packaging systems. Rosemary active packaging was the most effective method to protect pork patties from the HPP-induced lipid oxidation, while oxygen scavenger packaging was not effective since residual oxygen remained in the package in the initial period of storage. The kinetics of the oxygen trapping by oxygen scavengers appears to be a crucial factor for this application.



## 1. Introduction

High pressure processing (HPP) is the most successful non-thermal food preservation technology so far and is becoming increasingly important in the production of minimally processed foods for which shelf life may be extended primarily through inactivation of bacteria (Hendrickx & Knorr, 2002). HPP preserves food with minimal losses of nutritional and sensory value and is very useful to keep food fresh without chemical additives (Bolumar, Georget, & Mathys, 2014; Hendrickx, & Knorr 2002; Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007). HPP relies on the application of hydrostatic pressures between 100 and 1000 MPa (1000–10,000 atmospheres), and due to the isostatic principle, the material experiences the same pressure instantaneously throughout the entire product (Hendrickx & Knorr, 2002). Therefore, regardless of size and composition of the product the result is a uniform treatment without gradients, and prepared, and processed meats, and cured meat products are already on the market. HPP permits the reduction of microbial load resulting in extension of shelf life (Bajovic, Bolumar, & Heinz, 2012; Garriga, Grèbol, Aymerich, Monfort, & Hugas, 2004). However, HP treatment also induces lipid oxidation especially in meat products with no added sulphites or nitrites, compounds that otherwise confer both antimicrobial and antioxidant protection (Bañón, Díaz, Rodríguez, Garrido, & Price, 2007; Kumar, Yadav, Ahmad, & Narsaiah, 2015; Shah, Bosco, & Mir, 2014). HPP can be useful to extend the shelf life of such additive-free meat products, and thus ensure food safety, ease logistic opportunities by allowing long-distance distribution in the global market. However, appropriate methods are still required to control lipid oxidation.

HP-induced lipid oxidation in meat has been related to increased accessibility of iron from haemoproteins, membrane disruption and radical formation under high pressure (Beltran, Pla, Yuste, & Mor-Mur, 2003; Bolumar, Skibsted, & Orlien, 2012; Orlien, Hansen, & Skibsted, 2000). It has been concluded from several studies, that ethylene diamine tetra-acetic acid (EDTA), which can chelate metal ions like iron, can reduce lipid oxidation in HP-treated meat (Beltran, Pla, Yuste, & Mor-Mur, 2004; Cheah & Ledward, 1996, 1997; Ma, Ledward, Zamri, Frazier, & Zhou, 2007). This indicates that transition metal ion catalysis is a major mechanism behind initiation of lipid oxidation in HP-treated meat. Furthermore, formation of radicals has been found to occur during HP-treatment of meat with a pressure threshold of 400 MPa at 25 °C and 500 MPa at 5 °C as initiation of lipid oxidation (Bolumar et al., 2012). More recently, it was described that the presence of iron and different antioxidants (rosemary, ascorbic acid, caffeic acid) in meat during HPP resulted in an increased formation of

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radicals, while EDTA limited radical formation (Bolumar, Andersen, & Orlien, 2014). This suggested that $Fe^{2+}$ is directly involved as a catalyst leading to radical formation when meat systems are submitted to high pressure. However, a combination of antioxidants were found to promote radical formation most likely through reduction of $Fe^{3+}$ to $Fe^{2+}$ in effect regenerating the active catalyst in a reaction similar to the Fenton reaction (Bolumar et al., 2014a). Nevertheless, HP-induced lipid oxidation can be restrained by the addition of a variety of antioxidants (Beltran et al., 2004; Bragagnolo, Danielsen, & Skibsted, 2005; Bragagnolo, Danielsen, & Skibsted, 2007; Bolumar, Andersen, & Orlien, 2011).

Several critical control points throughout processing, packaging and storage of meat may be identified including mincing, addition of antioxidants, exposure to light, increasing temperature, and high oxygen availability, which each can affect the oxidative stability of meat and meat products negatively (Skibsted, Mikkelsen & Bertelsen, 1998; Skibsted, 2010). Lipid oxidation can be limited either by scavenging radicals using antioxidants as hydrogen or electron donors. Another strategy to prevent oxidation is to limit oxygen access to the product otherwise oxygen will react with initially formed lipid radical producing peroxide radicals as precursors to lipid hydroperoxides (Min & Ahn, 2005).

Active packaging is a novel technology designed to incorporate components in the packaging material that release substances to the food or to the environment surrounding the food in order to extend shelf-life. In active packaging, compounds responsible for off-flavours may also be absorbed by packaging material. The technology provides several advantages compared to direct addition of antioxidants to the food as a smaller amount is required and the antioxidant activity may be centred at the more sensitive product surface through migration from packaging film to the food matrix resulting in longer storage times and elimination of additional processing steps for incorporation such as mixing, immersion, or spraying. In recent years, the development of active packaging for meat packaging has been successful for antimicrobials (Coma, 2008; Chen & Brody, 2013; Jofré, Aymerich, & Garriga, 2008; Kerry, O'Grady, & Hogan, 2006; Quintavalla & Vicini, 2002) and for antioxidants especially for fresh meat in modified atmosphere packaging (Barbosa-Pereira, Aurrekoetxea, Angulo, Paseiro-Losada, & Cruz, 2014; Camo, Beltrán, & Roncalés, 2008; Camo, Lorés, Djenane, Beltrán, & Roncalés, 2011; Nerín et al., 2006; Walsh, 2012). The use of antioxidants for active packaging for food protection seems very promising as active packaging based on rosemary has been proved to be an efficient method to prevent lipid oxidation in HP-processed chicken patties (Bolumar et al., 2011). Depletion of oxygen in the packaging atmosphere is another active packaging method in practical use for prevention of lipid oxidation in meat products (Limbo et al., 2013; Walsh, 2012), although it has not found use for prevention of HP-induced lipid oxidation.

The aim of the present work was to compare standard vacuum packaging (as a control method) with rosemary active packaging and oxygen scavenger packaging for their efficiency to prevent HP-induced lipid oxidation in order to contribute to the improvement of new preservation methods combining antimicrobial (HPP treatment) and antioxidant (antioxidant active packaging) protection. The progression of lipid oxidation was studied at the surface and in the inner parts of pork patties for the three packaging systems by measuring secondary lipid oxidation products by thiobarbituric acid reactive substances (TBARS) and by determining the tendency to form radicals by spin-trapping and electron spin resonance (ESR) spectroscopy in order to gain insights into the mechanisms for most effective antioxidant protection.

## 2. Materials and methods

### 2.1. Preparation of rosemary active packaging

Domestic household plastic for wrapping foods, low density polyethylene of 12 μm thickness, was used to prepare the antioxidant active packaging in $30 \times 22$ cm = 660 $cm^2$ pieces (Bolumar et al., 2011). A 10% solution of commercial food grade lipid soluble rosemary extract (Kemin Food Ingredients BVBA, Herentals, Belgium) containing 4.5% of carnosic acid in ethanol was used to prepare the active films. The active packaging film was prepared by spreading the required ethanol solution of rosemary extract on the plastic foil in order to reach a final concentration of 1000 ppm (mg rosemary extract/kg meat). The solution was spread uniformly over the surface of the film by using a brush followed by overnight ethanol evaporation. The antioxidant active film had 0.45 mg rosemary extract/$cm^2$.

### 2.2. Oxygen scavenger

Freshcare® oxygen absorbers (Mitsubishi Gas Chemical, Tokio, Japan) were used. Scavenger sachets of different capacity of oxygen absorption, ST50 (50 ml), ST100 (100 ml) and ST150 (150 ml), were employed for continuous absorption of oxygen in the packaging headspace. Preliminary tests were carried out to ensure that oxygen scavenger had the capacity to bring the oxygen level below 0.1% before HP treatment. An incubation period of 24 h with the oxygen scavengers (one ST50 plus one ST150) was found to reduce oxygen concentration in the small head-space around the meat patties (see Section 2.3) below 0.1%.

### 2.3. Preparation of pork patties and packaging

The pork patties consisted of 80% pork lean meat from the topside part of the ham and 20% pork fat from adipose tissue of the back. The pork used was commercial Danish pork meat cut in small pieces with a knife and minced using an electric mincer for approximately 20 s. The minced meat was mixed thoroughly to obtain a homogeneous batter prior to the preparation of the patties. The pork patties were prepared with the help of a plastic box of the size ($11.5 \times 7.5 \times 4.0$ cm, a total surface of 345 $cm^2$). A plastic foil was carefully placed within the box and the compartment filled up with minced meat to reach 300 g. The pork patties were packaged in three different systems: standard vacuum packaging (C), rosemary active packaging (R), and oxygen scavenger packaging (OS). In the case of R, a plastic foil containing rosemary extract as described above (Section 2.1) was used. Subsequently, all pork patties were vacuum-packed in plastic bags (PA/PE 20/70, 32 oxygen $cm^3/m^2$ d bar at 23 °C and 75% RH, SFK, Hvidovre, Denmark). The level of vacuum was adjusted to 100% and the vacuum time was 10 s for the C and R samples (Electronic VacuMIT PM Pack Service Pakkemaskiner, Horsens, Denmark). In the case of OS, two oxygen scavenger patches were placed inside the bag (one ST50 and one ST150) prior to vacuum-packaging. For the OS samples, the level of vacuum was adjusted to 75% in order to have a small head-space, or circulation channels, around the pork patty and the plastic bag which allows the oxygen to be trapped by the scavenger sachet, and the vacuum time was set to 10 s. All samples were kept for 24 h before HP treatment in order to ensure that oxygen concentration was below 0.1% in the OS sample.

### 2.4. High pressure processing (HPP) treatment

The packed pork patties were submitted to HPP treatment in a Food Processing Cold Isostaic Press QFP-6 (Avure Technologies AB, Västerås, Sweden) with a pressure chamber of 0.9 l and a