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Evaluation of the effects of selected plant-derived nutraceuticals on the quality and shelf-life stability of raw and cooked pork sausages

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ABSTRACT

The effect of lutein (200 μg/g meat), sesamol (250 μg/g meat), ellagic acid (300 μg/g meat) and olive leaf extract (200 μg/g meat) on total viable counts (TVC), pH, water holding capacity (WHC), cooking loss, lipid oxidation (thiobarbituric acid-reactive substances, TBARs), colour stability, texture and sensory evaluation of fresh and cooked pork sausages stored in aerobic or modified atmosphere packs (MAP) was investigated. Addition of sesamol, ellagic acid and olive leaf extract reduced ($P < 0.001$) lipid oxidation in all packaged raw and cooked pork sausages. Antioxidant potency followed the order: sesamol 250 > ellagic acid 300 > olive leaf extract 200 > lutein 200 for both raw and cooked pork sausages. Addition of sesamol increased ($P < 0.001$) WHC on days 2 and 12 of MAP storage. Meat addition of lutein, sesamol, ellagic acid and olive leaf extract had no detrimental effect on pH, cooking losses, TVCs, tenderness, juiciness, texture or product flavour. Lutein, sesamol, ellagic acid and olive leaf extract were effective as natural functional ingredients in suppressing lipid oxidation and have the potential to be incorporated into functional raw and cooked pork sausages.

1. Introduction

Meat is considered a vital component of a healthy diet, an excellent source of protein, essential minerals, trace elements and vitamins. Negative concerns regarding meat consumption and its impact on human health have prompted research into the development of novel functional meat products (Arihara, 2006). There is a high degree of certainty associating colorectal cancer with the consumption of processed meat products following several epidemiological studies in the US and the UK since the 1970s. In 2007, the World Cancer Research Fund (WCRF) as one of their universal guidelines recommended to limit intake of red meat and avoid processed meat (WCRF, 2007). In recent years, a greater emphasis has been placed on the link between diet and the prevention of chronic diseases. Plant biomass or its derived bioactive compounds have been considered as possible functional components in processed meat products for alleviation of the colorectal cancer risk associated with the consumption of processed meats (Demeyer, Honikel, & De Smet, 2008). The introduction of functional ingredients such as botanicals, plant extracts, seaweeds and whey proteins with probable biological activity into processed meat products is receiving abundant attention (Calvo, Garcia, & Selgas, 2008; Carpenter, O'Grady, O'Callaghan, O'Brien, & Kerry, 2007; Cofrades, López-López, Solas, Bravo, & Jiménez-Colmenero, 2008; Hayes, Desmond, Troy, Buckley, & Mehra, 2005; Hernández-Hernández, Ponce-Alquicira, Jaramillo-Flores, & Guerrero Legarreta, 2009; Ribnicky, Poulev, Schmidt, Cefalu, & Raskin, 2008; Valencia, O'Grady, Ansorena, Astiasarán, & Kerry, 2008). Meat is one of the most important and commonly-consumed foods and is an excellent way to promote intake of functional ingredients without any radical changes in eating habits.

Rancidity in processed meats products causes changes in odour, flavour, taste, colour, texture and appearance (Aguirrezábal, Mateo, Domínguez, & Zumalacárregui, 2000) but more importantly, linked to unhealthiness due to free-radical production in the body causing health problems. Therefore, delaying lipid oxidation and product enhancement are factors that can have a significant contribution towards the development of functional meat products with enhanced nutritional and health benefits, improved shelf-life and superior product quality.

Plant-derived ingredients possessing antioxidant and antimicrobial properties have the advantage of being readily accepted by consumers, as they are considered natural. Natural ingredients such as lutein, sesamol, ellagic acid and olive leaf extract are natural active compounds which in *in vitro* and animal studies have exhibited a variety of biological activities, including potent

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antioxidant effects (Ezdihar, Vodhanel, Holden, & Abushaban, 2006), anticancer effects (Edderkaoui et al., 2008), anti-inflammatory activity (Rogerio et al., 2008), blood pressure lowering effects (Khayyal et al., 2002), inhibition of lipid oxidation (Bouaziz, Fki, Jemai, Ayadi, & Sayadi, 2008), protection against age-related macular degeneration (O'Connell et al., 2008) and antimicrobial properties (Micol et al., 2005). For example, clinical evidence has proven the blood pressure lowering effects of carefully extracted olive leaf extracts (Perrinjaquet-Moccetti et al., 2008; Somova, Shode, Ramnanan, & Nadar, 2003). Daly et al. (2010) found that ellagic acid (600 μg/g muscle), lutein (200 μg/g muscle) and sesamol (500 μg/g muscle) exhibited cytoprotective and/or genoprotective effects as added ingredients in pork patties following cooking and digestion using an *in vitro* digestion and caco-2 cell model system, indicating the potential of lutein, ellagic acid and sesamol as functional ingredients.

The objective of this study was to investigate the effect of the addition of plant-based nutraceuticals, lutein, sesamol, ellagic acid and olive leaf extract on pH, water holding capacity, lipid oxidation, colour, texture and organoleptic properties of raw and cooked pork sausages.

2. Materials and methods

2.1. Preparation of samples, packaging and storage

All reagents and solvents used in this work were 'AnalaR' grade and were obtained from Sigma–Aldrich, Ireland and Lennox Laboratory Supplies, Dublin, Ireland. Lutein(4-[18-(4-Hydroxy-2,6,6-trimethyl-1-cyclohexenyl)-3,7,12,16-tetramethyloctadeca -1,3,5,7,9,11, 13,15,17-nonaenyl]-3,5,5-trimethyl-cyclohex-2-en-1-ol), sesamol (1,3-Benzodioxol-5-ol), ellagic acid (2,3,7,8-Tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione) and olive leaf extract were obtained from Guinness Chemical (Ireland) Ltd (Clonminam Industrial Estate, Portlaoise, Co. Laois, Ireland). White's sausage seasoning blend, fresh sausage casing 23/30 and rusk were obtained from McDonnells, Queen Street, Dublin, Ireland. The six main phenolic compounds present in olive leaf extract were oleuropein (1151.5 μg/ml), verbascoside (68.6 μg/ml), luteolin-7-O-glucoside (25.6 μg/ml), apigenin-7-O-glucoside (15.9 μg/ml), tyrosol (15.6 μg/ml) and hydroxytyrosol (10.2 μg/ml) (Hayes, 2009).

Fresh pork shoulder (95% visibly lean) and pork back fat were purchased from a local meat supplier (Olhausen Ltd, Dublin, Ireland). Pork shoulder was trimmed of visible fat and connective tissue. The raw materials were cut into cubes and minced twice separately through a 5 mm steel plate using meat a meat mincer (model PT-82/22 Mainca Barcelona, Spain). Following mincing, raw materials were assigned to one of five treatments: Control (no added functional ingredients); sausage formulation with added lutein at concentration 200 μg/g formulation (lutein 200); sesamol at concentrations of 250 μg/g formulation (sesamol 250); ellagic at concentrations of 300 μg/g formulation (ellagic 300); olive leaf extract at concentrations of 200 μg/g formulation (olive 200). The concentration of lutein, sesamol, ellagic acid and olive leaf extract used in this study was based on published data generated by Hayes et al. (2009) in porcine model muscle systems. All phytochemicals were dissolved or dispersed in cold water (5% w/v) prior to addition to sausage formulations. Throughout the sausage manufacture, the meat formulations were kept constant at 4 °C. All treatments contained pork shoulder (44.25%), pork fat (18.75%), white sausage seasoning (breadcrumbs, spices, pea protein, starch, phosphate E451i, sodium metabisulphite E221, ascorbic acid E300) (2.5%), rusk (11%) and iced water (23.5%).

The pork meat, pork fat were placed in a bowl chopper (CR.22 model, Mainca, Berkshire, England) for 2 min at bowl speed 508 g and knife speed of 508 g during which time half the iced was added to control any temperature rise in the batter. The seasoning, functional ingredient and remaining iced water were then added and the batter was mixed for a further 1 min at 0.14 g. The rusk was added and the batter mixed for 30 s at 0.04 g. Stuffing was carried out into 23 mm diameter cellulose casings (Viscofon, Food Process Technology) using a hydraulic piston-type sausage stuffer (Mainca, Berkshire, England) and hand linked at 12 cm intervals. The sausage batches were separated into raw (fresh) and cooked treatments. Sausages were grilled in a conventional oven, turning every 3 min until internal temperature reached 71 °C (temperature was monitored with a hand held thermocouple). Sausages were left to cool prior to packing. For cook loss measurements, the weights of the sausages before and after cooking, following cooling to 4 °C were recorded and the cook loss calculated for ten replicates per treatment.

Raw and cooked sausages were packed aerobically by overwrapping in oxygen permeable (8000 cm^3/m^2/24 h STP) cling film (Wrap Film Systems, Halesfield 14, Telford TF7 4QR, Shropshire, England). For MAP storage, sausages were placed in low oxygen permeable (<1 cm^3/m^2/24 h/atm) polystyrene/ethylvinylalcohol/polyethylene-based trays, and flushed with 80% O_2:20% CO_2 and 70% N_2:30% CO_2 for raw and cooked sausages, respectively (BOC Gases, Dublin, Ireland) (Ilpra Foodpack VG 400 packaging machine, Ilpra, Vigevano, Italy). Trays were covered and heat-sealed with a low oxygen permeable (3 cm^3/m^2/24 h) laminated polyethylene terephthalate (PET) barrier film. All treatments were placed in random order in an open front display cabinet for 21 days storage at 4 °C (Cronos fan assisted cabinet, Criosbanc, Padova, Italy). Lighting was approximately 600 lux with 58 W deluxe cool white bulbs at a temperature of 420 K (Phillips, Eastern Electric, Dublin, Ireland). The display temperature was monitored every 15 min using a temperature logger (TV-4076, Tinytag, Gemini Data Loggers (UK) Ltd, West Sussex, UK).

2.2. Determination of pH of raw pork sausages

The pH of the raw sausages was measured after homogenisation (Ultra-Turrax T25, Janke & Kunkel, Staufen, Germany) with distilled water at a ratio of 1:10 using pH meter model 420A (Orion, Germany). The pH was measured in triplicate on days 0, 14, and 21 for all aerobic and MAP stored samples.

2.3. Water holding capacity of raw pork sausages

On day 2, water holding capacity (WHC) was carried out on raw pork sausages stored in MAP. Ten samples per batch were analysed. Approximately 10 g per sample was weighed into a glass jar. The jars were placed into a water bath (Model No Y-38, Grant Instruments Ltd., Cambridge, UK) for 10 min at 90 °C. After heating, each sample was carefully removed from the jar using a forceps and wrapped in cheesecloth and placed into a 30 ml centrifuge tube (Model. 3118-0030, Nalgene Brand Products, NY, USA) each containing cotton wool in the bottom. The samples were centrifuged (Sigma SK 10, MSE Scientific Instruments, Sussex, England) for 10 min at 13,440 g at 4 °C. Samples were removed from the centrifuge, cheesecloth removed and reweighed. The percentage WHC was calculated using Equation (1) (Lianji & Chen, 1989). Measurements of moisture content (M_2) of samples were carried out on a CEM LabWave 9000 (Smart Trac 5 Model 907875, CEM Corporation, NC, USA).