PE4.102 Effect of Lutein, Sesamol, Ellagic acid and Olive leaf extract on the Quality and Shelf-life Stability of Packaged Raw Minced Beef Stored Under MAP 374.00

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Abstract— The effects of lutein (100 and 200 µg/g muscle), sesamol (250 and 500 µg/g muscle), ellagic acid (300 and 600 µg/g muscle) and olive leaf extract (100 and 200 g muscle) on total viable counts (TVC), lipid oxidation (TBARS, mg malondialdehyde (MDA)/kg muscle), colour (CIE a* value), oxymyoglobin oxidation of raw minced beef (*M. longissimus thoracis et lumborum*) stored under modified atmosphere (MAP) at 4 °C for up to 12 days was examined. The addition of lutein, sesamol, ellagic acid and olive leaf extract reduced TVCs (P < 0.001) relative to controls. Lipid oxidation was reduced (P < 0.001) in raw beef patties following the addition of lutein, sesamol, ellagic acid and olive leaf extract. Sesamol addition to beef resulted in lower a* redness values and increased oxymyoglobin oxidation (P < 0.01). Conversely, lutein and olive leaf extract reduced oxymyoglobin oxidation (P < 0.001) relative to the control. pH was unaffected following the addition of lutein, sesamol, ellagic acid and olive leaf extract. The results indicate that lutein, sesamol, ellagic acid and olive leaf extract have the potential for use as health promoting functional ingredients in minced beef products.

Keywords: lipid oxidation, natural antioxidant, lutein, sesamol, ellagic acid, olive leaf extract, beef, MAP

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I. INTRODUCTION

Lipid oxidation, colour and microbial growth, are factors important to shelf-life and consequently to consumer acceptance of fresh meat [1]. Lipid oxidation is one of the main factors limiting the quality and acceptability of lipid containing foods as it affects the sensory quality, due to off-flavour and off-odour development, and the production of potentially toxic compounds [2]. Colour tends to be used as an indicator of perceived quality and freshness of meat. It is regarded as the first limiting factor in beef shelf-life [3] and is also an important factor in the marketing of meat.

Muscle foods are also susceptible to microbial contamination leading to food borne illnesses hence, the control of microbial contamination is a major concern for the meat industry. In recent years there has been much interest in adding naturally occurring antimicrobial and antioxidant compounds derived from plant sources to meat products because of their potential health benefits and safety compared with synthetic preservatives, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary-butyl hydroquinone (TBHQ). The use of natural compounds to increase the shelf life of meat products is a promising technology since many plant derived substances have antioxidant and antimicrobial properties.

Lutein, an oxygenated carotenoid, significantly reduces the risk of age-related macular degeneration [4]. Ellagic acid and sesamol are polyphenol antioxidants found in numerous fruits and vegetables and have been found to exhibit anticarcinogenic activity and inhibit atherosclerosis [5]. Sesamol is a natural phenolic antioxidant and has been shown to significantly reduce lipid oxidation in raw and cooked pork patties [6], salami [7] and turkey breast [8].Olive leaf extract, a phenolic compounds derived from olive leaves, is known to have anti-oxidative properties.

Incorporation of phytochemicals/nutraceuticals into 'functional' meat products offers considerable health benefit potential to consumers and may afford the meat industry an opportunity to develop novel meat products with enhanced nutritional and health benefits, improved shelf-life and quality. The scientific literature contains little information regarding functional ingredients such as lutein, sesamol, ellagic acid and olive leaf extract in meat products. Therefore, the influence of these ingredients on meat quality parameters merits investigation. The objective was to determine the effect of lutein, sesamol, ellagic acid and olive leave extract on the microbial status, lipid oxidation, colour stability and pH of minced beef stored under MAP at 4^{o}C.

II. MATERIALS AND METHODS

Materials and chemicals: 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, sesamol (98%), ellagic acid (90%) and olive leaf extract were obtained from Guinness Chemicals (Ireland) Ltd, Clonminam Industrial Estate, Portlaoise, Co. Laois, Ireland.

Antioxidant addition and sample preparation: Fresh beef (*M. longissimus thoracis et lumborum*) was obtained from Kepak Group, Co. Meath, Ireland. The muscles were trimmed to remove connective tissue and surface fat and minced twice through a 5-mm plate using a Mainca mincer (model PT-82/22 Mainca, Barcelona, Spain). Minced beef was assigned to one of the following nine treatments: control (no added nutraceuticals); lutein added at concentrations of 100 (L100) and 200 μg/g muscle (L200); sesamol added at concentrations of 250 (S250) and 500 μg/g muscle (S500); ellagic acid added at concentrations of 300 (EA300) and 600 μg/g muscle (EA600); olive leaf extract added at concentrations of 100 (OLE100) and 200 μg/g muscle (OLE200). Lutein, sesamol ellagic acid and olive leaf extract were dissolved in distilled water (5% v/w), added to the raw minced beef and mixed thoroughly. The same volume of distilled water (with no functional ingredients) was added to the control minced beef treatment.

Minced beef from each treatment was formed into patties (80 g portions) using a meat former and placed in laminated low oxygen permeable polystyrene retail trays. The minced beef patties were separated into cooked and raw meat batches. The raw patties were gas flushed (Ilpra Foodpack VG 400 packaging machine, Ilpra, Vigevano, Italy) using a modified atmosphere (MAP) of 80% O_2: 20% CO_2 (BOC Ltd., Dublin Ireland) and sealed with a low oxygen permeable barrier film (3cm^3/m^2/24h at STP). All samples were placed in random order in an open front display cabinet (Cronos fan-assisted cabinet, Criosbanc, Padova, Italy; lighting: 58W, deluxe cool white bulbs, colour temperature: 420^{o}K, Phillips, Eastern Electric, Dublin, Ireland) for the required storage period. The display temperature was monitored every 15 minutes using a temperature logger.

pH analysis: The pH of the raw beef patties was measured after homogenisation (Ultra-Turrax T25, Janke & Kunkel, Staufen, Germany) with distilled water at a ratio of 1:10 using a pH meter model 420A (Orion, Germany). Beef pH was measured on days 0, 8 and 12.

Microbiological analysis: The total viable counts (TVC) of raw and cooked beef patties (10 g) were determined (ISO, 2003) using plate count agar (tryptone glucose yeast agar) (Oxoid Ltd. CM0325, Basingstoke, Hampshire, United Kingdom). Plates were incubated at 30^{o}C ± 1 ^{o}C for 72 hr on days 0, 3, 6, 9 and 12. Results were expressed as $\log_{10}$CFU (colony forming units)/g muscle.

Lipid oxidation measurement: Thiobarbituric acid reactive substances (TBARS) were determined by the distillation method [9]. The malondialdehyde content of the sample was calculated using an extinction coefficient of 1.56 x 10^5 M^{-1}cm^{-1}. Results were expressed as TBARS in mg malondialdehyde (MDA)/kg product. TBARS were determined in raw beef patties on days 0, 3, 6, 9 and 12. *Colour evaluation:* Surface colour measurements of raw and cooked beef patties were determined using the CIE L*a*b* system with a dual beam xenon flash spectrophotometer (UltraScan XE, Hunter lab, Reston, Virginia, U.S.A). Colour measurements of raw beef patties were determined on days 0, 3, 6, 9 and 12.

Oxymyoglobin oxidation: Absorbance spectra were recorded on days 0, 3, 6, 9 and 12 for raw beef patties. Absorbance measurements were taken at 525, 545, 565, 572 and 730nm. From these the relative proportions of oxymyoglobin were calculated [10].

Sensory evaluation of cooked beef patties: Samples of freshly cooked beef patties were evaluated by a semi-trained panel of laboratory co-workers (n = 52). Beef patties were cooked by grilling with turning every 3 minutes until cooked to an internal temperature of 71^{o}C. Samples were labelled with 3

digit random numbers and served within 2 min of cooking in random order to panellists in individual booths. Panellists were instructed to cleanse their palates with water between samples. Panellists were asked to evaluate overall appearance, overall texture, overall flavour and overall acceptability on a six point hedonic scale where 1 and 6 were the extremes of each characteristic and to evaluate tenderness and juiciness on an 8 point hedonic scale ranging from extremely juicy/tender to extremely dry/tough.

Statistical analysis: The significance of differences among samples at each day of storage was determined by analysis of variance (ANOVA) using the Least Square Difference method of GenStat (Release 10.1 Copyright 2007, Lawes Agricultural Trust, Rothamsted Experimental Station). Differences were considered significant at the $P < 0.05$ level. The entire experiment was replicated three times.

III. RESULTS AND DISCUSSION

The pH of raw beef patties decreased from 5.7 to 5.5 over the 12 day storage period. The addition of lutein, sesamol, ellagic acid and sesamol had no influence on pH ($P > 0.05$) relative to the control.

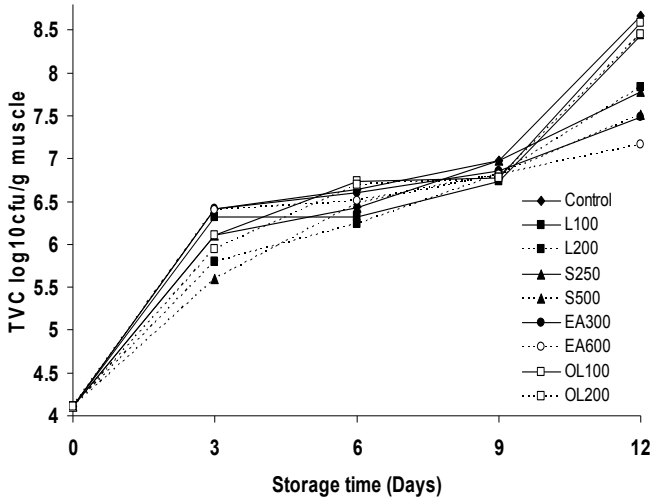
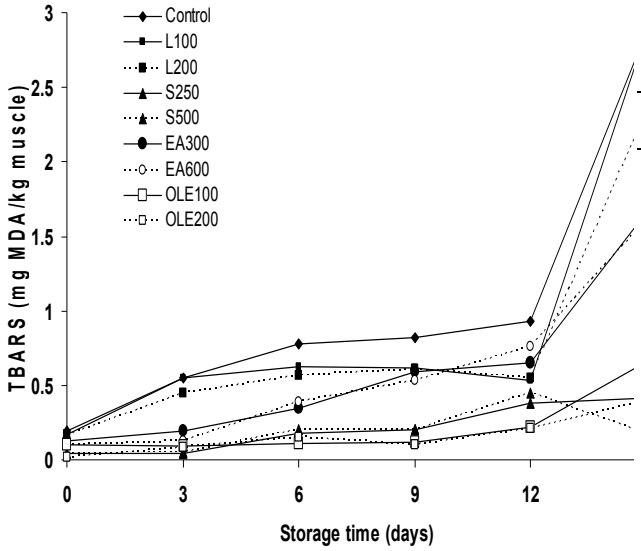


Fig. 1: Mean TVC in raw beef patties stored in MAP conditions for 0, 3, 6, 9, and 12 days at 4°C.

The addition of lutein, sesamol, ellagic acid and olive leaf extract reduced TVCs ($P < 0.001$) in raw beef patties relative to the control ($P > 0.05$) on certain storage days (Fig.1).

Antioxidant potency in raw beef patty samples was determined to be in the order: sesamol > olive leaf extract > ellagic acid > lutein following storage under MAP conditions (Fig. 2). Lipid oxidation increased ($P < 0.001$) in control beef patties between days 0 and 12. All compounds exhibited antioxidant activity over the 12 day storage period. Lipid oxidation was reduced ($P < 0.05$) in the presence of lutein at a concentration of 100 µg/g muscle on days 9 and 12 and at 200 µg/g muscle on days 3, 6, 9 and 12 relative to the control. Sesamol at concentrations of 250 and 500 µg/g muscle reduced lipid oxidation by an average of 80%. Ellagic acid reduced lipid oxidation ($P < 0.05$) on days 3, 6, 9 and 12 with a concentration effect ($P < 0.01$) on days 3, 9 and 12. Olive leaf extract at concentrations of 100 and 200 µg/g muscle had consistently lower ($P < 0.05$) TBARS values compared to the control. Olive leaf extract at concentrations of 100 and 200 µg/g muscle reduced lipid oxidation by an average 76 and 84% respectively over the 12 day storage period.

Fig 2: Effect of lutein, sesamol, ellagic acid and olive leaf extract on lipid oxidation in raw beef patties



Redness (a*) was increased ($P < 0.05$) following the addition of lutein at both levels on days 0, 3 and 6 of MAP storage relative to the controls (Table 1). The addition of sesamol at 250 and 500 μg/g muscle to raw beef patties had a negative effect on the colour stability as the a* value was lower than that of the control ($P < 0.05$) over the 12 day storage period. Several researchers have reported that sesamol had a very strong antioxidant effect when used in meat but showed negative effects on the colour, resulting in reduced redness of ground beef [11] [12]. There was no effect ($P > 0.05$) of ellagic acid on the redness of raw beef patties from day 3 to 12 of storage relative to the control. Olive leave extract at concentrations of 100 and 200 μg/g muscle reduced redness at day 0 but enhanced colour stability ($P > 0.05$) by increasing the a* value compared to the control on days 3, 9 and 12.

[abc]Within each day (each treatment type is compared separately to the control) mean values in the same column bearing different superscripts are significantly different, $P <$ 0.05

Treatment	Day 0	Day 3	Day 6	Day 9	Day 12
Control	23.6^{aA}	18.1^{aB}	16.9^{aC}	12.9^{aD}	7.2^{aE}
L100	27.4^{bA}	21.4^{bB}	19.0^{bC}	16.0^{aD}	7.3^{aE}
L200	26.9^{bA}	22.7^{bB}	18.9^{bC}	11.5^{aD}	8.9^{aE}
S250	14.9^{bA}	10.2^{bB}	7.5^{bC}	7.0^{bC}	5.9^{bC}
S500	14.9^{bA}	8.2^{cB}	6.2^{bC}	5.7^{bC}	5.7^{bC}
EA300	24.46^{bA}	15.9^{aB}	13.3^{aC}	8.0^{aD}	7.2^{aD}
EA600	24.4^{bA}	16.6^{aB}	13.4^{bC}	8.3^{aD}	7.9^{aD}
OLE100	19.8^{bA}	20.4^{bA}	16.7^{aB}	15.8^{bB}	15.7^{bB}
OLE200	16.0^{cA}	18.8^{cB}	17.2^{aBC}	16.2^{bC}	16.5^{bC}

[ABCDE]Mean values in the same row bearing different superscripts are significantly different, $P < 0.05$.

The addition of lutein at both concentrations reduced oxymyoglobin oxidation in raw beef patties up to day 9 of MAP storage ($P < 0.001$) (Table 2). Oxymyoglobin oxidation, indicated by the decrease in percentage oxymyoglobin in raw beef patties stored under MAP occurred to the greatest extent following the addition of sesamol ($P < 0.001$).

Table 1: Effect of lutein, sesamol, ellagic acid and olive leaf extract on surface redness (a* value) of raw beef patties

Table 2: Effect of lutein, sesamol, ellagic acid and olive leaf extract on oxymyoglobin levels in raw beef patties

Treatment	Day 0	Day 3	Day 6	Day 9	Day 12
Control	70.4^{aA}	66.6^{aB}	63.4^{aB}	52.2^{aC}	45.2^{aD}
L100	73.9^{bA}	73.5^{bA}	74.2^{bA}	65.3^{bB}	45.2^{aC}
L200	75.8^{cA}	71.2^{bA}	70.8^{bA}	58.8^{bB}	45.1^{aC}
S250	20.3^{bA}	55.4^{bB}	47.0^{bC}	43.6^{bC}	37.5^{b}
S500	18.1^{bA}	48.8^{cB}	39.7^{cC}	38.1^{bC}	36.0^{bC}
EA300	74.4^{bA}	70.7^{bA}	68.7^{bA}	59.5^{bB}	44.5^{aC}
EA600	75.0^{bA}	70.9^{bA}	69.5^{bA}	58.6^{bB}	46.8^{aC}
OLE200	74.2^{bA}	69.5^{bA}	66.9^{bA}	64.2^{bA}	63.0^{bA}
OLE200	72.2^{bA}	69.9^{bA}	69.6^{cA}	67.1^{cA}	65.2^{cA}

^{abc}Within each day (each treatment type is compared separately to the control) mean values in the same column bearing different superscripts are significantly different, $P < 0.05$

^{ABCDE}Mean values in the same row bearing different superscripts are significantly different, $P < 0.05$.

These results suggests that lipid oxidation and oxymyoglobin oxidation may not always be positively correlated as often reported since the addition of sesamol resulted in a decrease in lipid oxidation and a reduction in a* redness ($P < 0.001$). Beef patties containing added sesamol exhibited increased ($P < 0.001$) oxymyoglobin oxidation up to day 12 of MAP storage. Sesamol had a negative influence on the oxymyoglobin levels of raw beef patties in agreement with the lower redness values. Hence, the use of sesamol would not be recommended in raw beef products.

The addition of ellagic acid reduced oxymyoglobin oxidation up to day 9 in comparison to the control. Olive leaf extract reduced oxymyoglobin oxidation with graded addition of the extract resulting in lower oxymyoglobin oxidation on days 6 to 12 of MAP storage relative to the control. Previous research confirmed that there is a close link between colour preference and the decision to purchase [13]. The results show that lutein, olive leaf extract and ellagic acid extend the red colour shelf-life of minced beef patties and demonstrated that lutein, ellagic acid and olive leaf extract were effective in improving the oxidative and microbial stability of fresh minced beef patties held under MAP conditions.

IV. CONCLUSION

Oxymyoglobin and lipid oxidation as well as microbial contamination are serious concerns for meat producers and consumers. The exogenous application of functional ingredients such as lutein, ellagic acid, sesamol and olive leaf extract not only enhanced product colour and minimized lipid oxidation but also enhanced microbial safety of the minced beef product. Due to concerns regarding the safety and toxicity of synthetic antioxidants, lutein, sesamol, ellagic acid and olive leaf extract may prove useful as safe, natural, health promoting functional ingredients to the meat industry.

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