PE8.09 Antioxidant and n-3 fatty acid emulsion effects on color and lipid oxidation in raw and cooked beef patties during chilled storage 63.00

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***Abstract*- This study was carried out to evaluate the effect of n-3 polyunsaturated fatty acid (PUFA) emulsion, with or without antioxidants, on lipid oxidation and color of raw and cooked patties during chilled storage. An emulsion of n-3 PUFAs (25% algal oil) was added to obtain 500 mg n-3/110g product. The effect of a mixture of antioxidants (citrate, erythorbate and rosemary) was also studied. The treatments were: control (C), n-3 emulsion (E), antioxidant (A) and n-3 emulsion + antioxidant (EA). Patties with emulsion (E, EA) presented greater (P<0.05) lightness (L*) in raw patties. In general, E samples demonstrated greater (P<0.05) lipid oxidation than other treatments. Samples treated with the antioxidant mixture had greater (P<0.05) red color (a*) than the other treatments; hue angle was lower (P<0.05) than in C and E. Antioxidant treatment delayed lipid oxidation ($TBARS_{532}$) in raw and cooked beef patties with emulsion (EA). The antioxidant mixture had lower $TBARS_{450}$ values for raw but not cooked beef patties. These results support the incorporation of a stabilized n-3 emulsion with a mixture of antioxidants for delaying oxidation in raw and cooked beef patties.**

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I. INTRODUCTION

Recently, there has been an increased demand for 'healthier' meat products. Formulation-based processing is a promising approach for development of meat-based functional foods. Functional foods containing n-3 lipids are one of the fastest growing food product categories in the US and Europe [1]. The potential health benefits for increasing consumption of dietary n-3 polyunsaturated fatty acids (PUFAs) have been well documented [2, 3].

Algal oil (DHA, docosahexaenoic acid-rich) has been incorporated in pork frankfurters, ground turkey patties and restructured ham products [4, 5]. A reasonable estimate for optimal intake would be 0.8-1.4 g for EPA (eicosapentaenoic acid) and DHA, or 3.0-5.5 g for total n-3 PUFA per day [6].

A prerequisite for successful development of n-3 PUFA-enriched foods is to evaluate the oxidation potential of these lipids, because of the potential to affect flavour and color of the products. One promising approach involves the inclusion of stabilized fatty acid emulsions which are made prior to meat product manufacture and added as a fat ingredient to meat products. Oil pre-emulsion technology with a non-meat protein improves the system's fat binding ability, since the oils can be stabilized or immobilized in a protein matrix [7]. DHA-enriched emulsions have been previously incorporated in meat products [5, 8].

One of the most common methods to evaluate lipid oxidation in meat products is the TBARS (thiobarbituric acid-reactive substances) procedure which is used to quantify secondary products of polyunsaturated fatty acids oxidation. TBARS reactions typically produce a stable pink chromophore with maximal absorbance at 532 nm ($TBARS_{532}$) [9]. It is also possible for some aldehyde products to react with TBA to produce yellow pigments with maximal absorbance at 450 nm ($TBARS_{450}$) [10].

The objective of the present study was to (1) manufacture beef patties to meet a target of 500mg DHA/110 g product, and (2) evaluate the effect of DHA (incorporated in an emulsion) and an antioxidant mixture on lipid oxidation and color in raw and cooked beef patties during chilled storage.

II. MATHERIALS AND METHODS

A. Preparation of emulsion

An oil-in-water emulsion containing oil-soluble and water-soluble components was prepared [11]. Algal oil (25% w/w) (Martek Biosci. Co., Columbia, MD) containing approximately 40-42% DHA, was used to obtain the stable n-3 emulsion [8].

B. Preparation of antioxidant mixture

The antioxidant combination was prepared according to the procedure of Lee et al. [12]. Sodium erythorbate (1g/1kg product; reductant) and sodium citrate (0.5% w/w; secuestrant) were dissolved in distilled water (1.5% w/w), and rosemary extract (0.2% w/w) in ethanol. Samples without added antioxidants contained an equal volume of distilled water or ethanol.

C. Preparation of beef patties

Coarse ground beef (10% fat) was obtained locally. The meat from 8 different chubs (n=8) was used to prepare 4 treatments/chub and ground fine (5mm) (Oster Food Grinder, Sunbeam-Oster Co., Inc., USA). The different treatments are presented in Table 1. The n-3 emulsion was added to obtain a concentration of 500 mg DHA/110g product. Patties were formed (5 cm dia., 1.5 cm thick, 25g) and wrapped with oxygen-permeable PVC film (15,500-16,275 cm^3 O_2/ m^2). For the study of raw chilled beef, patties were stored at 4 ºC in darkness and sampled on days 0, 2, 4 and 6 of storage. For the cooked patty study, 3 raw patties were frozen at -18 ºC for 3 weeks after product manufacture after which time they were thawed for 3 hrs at 4°C and then cooked to an internal temperature of 75 °C. Cooked patties were then wrapped with PVC film and stored at 4 °C in darkness for 0, 2 and 4 days.

D. Colorimetric analysis

Surface color of raw and cooked samples was measured in duplicate with a Minolta Chromameter (Model CR-200 b, Minolta Co., Osaka, Japan). L*, a* b* parameters were obtained and hue angle was calculated as $\tan^{-1}(b^*/a^*) \times 360°/2$ [13].

E. Lipid oxidation analysis

The thiobarbituric reactive substances (TBARS) procedure was used according to Yin et al. [14]. Each sample was measured in duplicate and absorbance values recorded using a Shimadzu 2101PC spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD). Results were expressed as absorbance of the solution at 532nm ($TBARS_{532}$) and 450 nm ($TBARS_{450}$).

F. Statistical analysis

Data were analysed using Statgraphics Plus 5.1 (STSC Inc. Rockville, MD, USA) for two-way ANOVA. Least squares differences were used for comparison of mean values among treatments and the Tukey HSD test to identify significant differences ($P<0.05$) between treatments and storage time. The analysis was made for raw and cooked experiment for each one separately.

III. RESULTS AND DISCUSSION

A. Color of raw and cooked patties during chilled storage

Color parameters for raw (Table 2) and cooked (Table 3) patties demonstrated a significant effect of treatment, storage and treatment x storage ($P<0.05$).

In general, the effect of treatments on color were more often significant for raw than for cooked samples (Tables 2 and 3). In raw patties, the main effect of added emulsion (E, EA) yielded products with greater ($P<0.05$) lightness (L*) (Table 2). These results were attributed to the milky appearance of the emulsion [5] and might be due to light scattering [11]. There was no clear effect of treatment for L* values in cooked patties (Table 3). For raw samples, the emulsion (E, EA) produced darker ($P<0.05$) patties than C and A at day 0 (Table 2). From day 2 forward, antioxidant samples (A, EA) had similar ($P>0.05$) a* values and were redder ($P<0.05$) than C and E treatments (Table 2).

Heat processing induces oxidation, and in cooked samples at day 0 patties with antioxidants were redder ($P<0.05$) than C and E (Table 3). Similar results were found in n-3 enriched fresh pork sausages with antioxidants [8]. Hue angle value represents the degree of change from redness to yellowness and this correlates well with subjective assessment of discoloration [13, 15]. At day 0 all samples (raw and cooked) presented the same ($P>0.05$) hue angle value. Beginning on day 2, samples with antioxidants (A, EA) presented lower ($P<0.05$) values than samples without antioxidants. This can be explained by the effectiveness of the antioxidant mixture to maintain the color during chilled storage. Similar results were reported for ground turkey patties [8].

In general, storage affected color parameters more in raw than cooked samples with the main changes in a* and hue angle values. In raw patties, a* values decreased ($P<0.05$) with storage. The decline was less in antioxidant-containing samples than in their non-antioxidant counterparts (Table 2). No significant changes were observed in redness of cooked samples as a function of time (Table 3). In general, hue angle for all samples increased ($P<0.05$) in raw patties with time (Table 2), especially for C and E; cooked patties with antioxidant presented a lower hue angle values than C and E ($P<0.05$) at the end of storage (Table 3). Therefore the antioxidant mixture appeared to delay discoloration in raw and cooked beef patties.

B. Lipid oxidation in raw and cooked patties during chilled storage

TBARS values for raw patties are presented in Figures 1a and 1b. On day 0, all samples presented similar ($P>0.05$) $TBARS_{532}$ values (Figure 1a); from day 2 forward, E samples had the greatest ($P<0.05$) values and increased with storage. Similar results were reported for ground turkey patties with n-3 emulsion [5]. It is important to note that C samples were stable throughout storage suggesting that lipid oxidation was

due to the emulsion PUFAs. The $TBARS_{450}$ values (Figure 1b) showed a normal oxidation pattern such that there was a plateau or decrease presumably due to secondary products reacting with other meat components. Lipid oxidation in C samples reached maximum $TBARS_{450}$ values on day 4 (Figure 1b) and then decreased (day 6) until they approximated the values of antioxidant-containing samples (A and EA). The E samples demonstrated the greatest ($P<0.05$) values ($P<0.05$) until day 4. Samples with antioxidants (A and EA) presented similar low values ($P>0.05$) throughout storage.

Lipid oxidation behavior over time for the cooked patties (Figures 2a and 2b) was different from that of raw samples. Only E samples demonstrated increased ($P<0.05$) $TBARS_{532}$ values (Figure 2a) with storage time (similar to raw samples); the other sample treatments showed increased values until day 2 and then decreased ($P<0.05$). This tendency is similar to that reported for cooked patties stored in vacuum conditions [16]. Antioxidant-containing patties (A and EA) had similar $TBARS_{532}$ values ($P>0.05$), and were lower ($P<0.05$) than samples without antioxidants. A similar antioxidant effect on lipid oxidation was reported in cooked ground turkey patties with n-3 emulsion [5]. At day 2, C patties had greater ($P<0.05$) $TBARS_{532}$ values than emulsion patties, an observation also noted by Lee et al. [12]. This could be due to the presence of mixed tocopherols used in the preparation of the n-3 emulsion, although we cannot readily explain why this observation differs from results observed in raw samples. $TBARS_{450}$ values (Figure 2b) showed an unexpected behavior because, in general, antioxidant samples (A, EA) presented greater ($P<0.05$) values than the other treatments which was opposite to the result observed in raw patties. It's important to note that Maillard reaction products (e.g., HMF) react with TBA to yield a yellow chromophore and so it is possible that values in cooked products reflected this reaction more than that of lipid oxidation [17,18].

IV. CONCLUSION

The development of potentially healthier beef patties rich in n-3 fatty acids is possible from a technological point of view when an effective antioxidant mixture containing a radical quencher (rosemary extract), sequestrant (sodium citrate) and reductant (sodium erythorbate) is used. This mixture protected redness in raw and cooked patties during storage, while n-3 emulsion increased the lightness in raw products. An important effect of the antioxidant cocktail is that it decreased product discoloration (Hue angle) and lipid oxidation ($TBARS_{532}$) for raw and cooked patties during storage.

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Table 1. Formulation (%, w/w) of raw and cooked beef patties*.

Samples	Beef	*n*-3 emulsion	Sodium erythorbate	Sodium citrate	Rosemary extract	Water	Ethanol
C	97	0	0	0	0	1,5	1,5
E	92,5	4,5	0	0	0	1,5	1,5
A	96,2	0	0,1	0,5	0,2	1,5	1,5
EA	91,7	4,5	0,1	0,5	0,2	1,5	1,5

*C, control patties; E, n-3 emulsion patties; A, antioxidant patties; EA, n-3 emulsion+antioxidant patties.

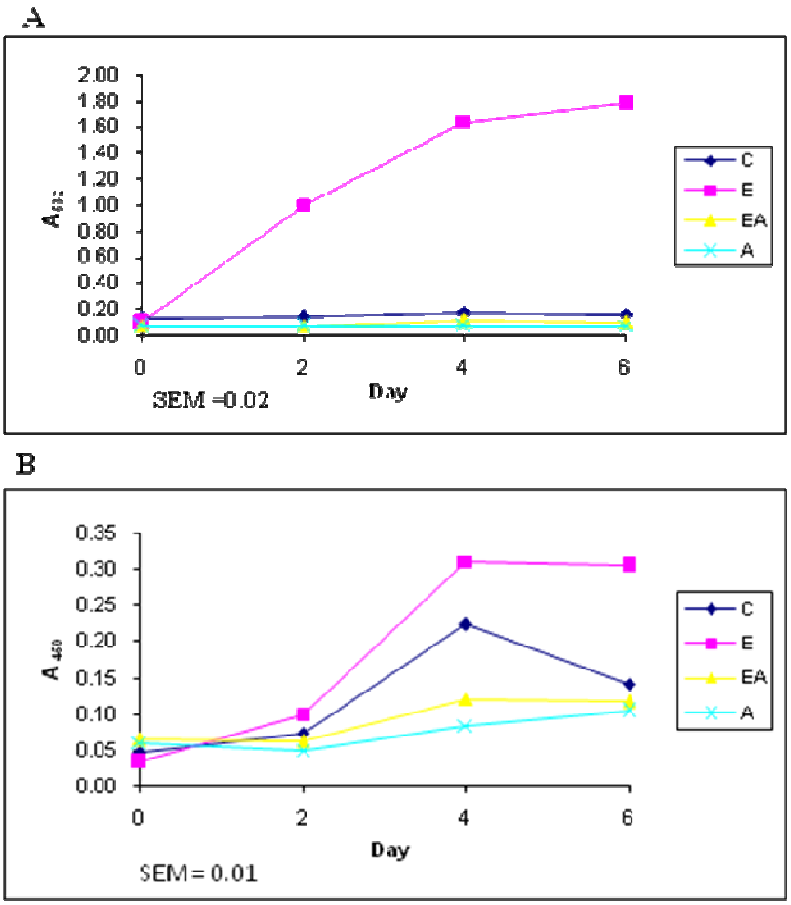

Figure 1 Lipid oxidation of raw beef patties during chilled storage: $TBARS_{532}$ (A) and $TBARS_{450}$ (B)- C, control patties; E, n-3 emulsion patties; A, antioxidant patties; EA, n-3 emulsion+antioxidant patties. SEM= Standar error of the mean(n=8).

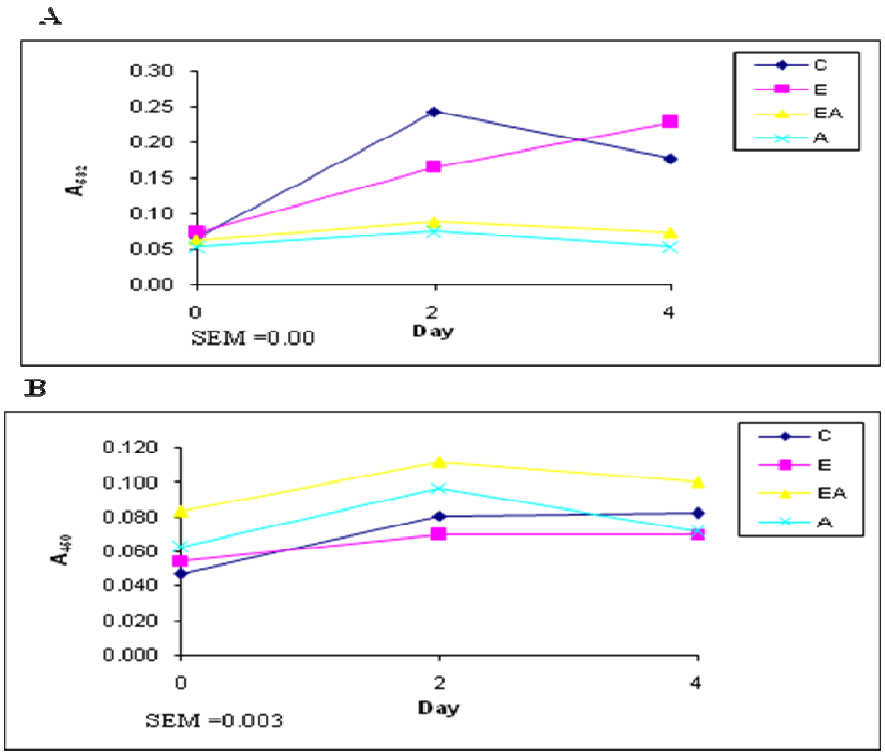

Figure 2. Lipid oxidation of cooked beef patties during chiled storage: $TBARS_{532}$ (A) and $TBARS_{450}$ (B)- C, control patties; E, n-3 emulsion patties; A, antioxidant patties; EA, n-3 emulsion+antioxidant patties.SEM= Standar error of the mean(n=8).