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Effects of Fortification of Beef Steaks with Krill Oil on Physical, Chemical, and Sensory Characteristics

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Abstract

Fortifying foods with oil from marine sources enhances the dietary availability of omega-3 fatty acids to consumers. Although the inclusion of omega-3s in foods has been studied for years, few research considered evaluating sensory attributes of beef enhanced with EPA (Eicosapentaenoic acid) and DHA (Docosahexanoic acid) from marine sources. Krill oil has recently emerged as a novel source of omega-3s, which manufacturers claim that it does not generate fishy aftertaste due to higher oxidative stability when compared to fish oils. This study tested the null hypothesis that enhancing nutritional values of beef by incorporating krill oil in the lean does not affect flavor when beef is stored under vacuum conditions, and color and lipid stability when steaks are displayed packaged in O2 permeable film. Strip loin steaks were enhanced with 47 mg (32 mg of EPA and 15 mg of DHA, E1), and with 94 mg (64 mg of EPA and 30 mg of an EPA and DHA from krill oil diluted in extra virgin olive oil. Steaks enhanced with the highest concentration showed improved tenderness when compared to control steaks (P = 0.01). Overall, enhanced steaks had higher off-flavor intensity when compared to control steaks whereas fishy and metallic were the most predominant off-flavor descriptors. As levels of enhancement increased, greater lipid oxidation on steaks was observed (P = 0.002). Enhancing beef with omega-3 fatty acids from krill oil led to detrimental effects on flavor and other quality attributes.

ABBREVIATIONS

EPA: Eicosapentaenoic Acid; DHA: Docosahexanoic Acid; ALA: α-linolenic Acid; LA: Linoleic Acid; TBA: Thiobarbituric Acid Assay; PUFA: Polyunsaturated Fatty Acids; USDA: United States Department of Agriculture; IMPS: Institutional Meat Purchase Specifications

INTRODUCTION

Consumer value judgment for beef products depends on intrinsic and extrinsic information cues [1]. Intrinsic attributes such as flavor, color, and nutritional values are important driving factors in a consumer decision's to purchase meat. Moreover, extrinsic factors, such as origin, finishing systems (grass vs grain fed), brand, and organic/natural claims have also increased in popularity, serving as an additional decision making factor, especially in local markets. Overall consumer's preference for foods is mainly dependent on flavor, color, and nutritional values [2-4]. Consumption of omega-3 fatty acids is usually associated to a general perception that it contributes to overall health [5]. Due to this, the food industry has been experiencing a substantial increase in the demand for supplements and enriched products containing omega-3s. Methods to fortify edible animal products such as meat and dairy include animal feeding and direct inclusion of omega-3 sources in final products [6,7]. However, these methodologies usually encounter obstacles such as limited levels of deposition in animal products and decrease in flavor desirability, respectively. Finishing cattle on grass or with ethanol production by-products increases the deposition of omega-3 fatty acid in the lean [7,8]. However, it is not biologically possible by feeding to increase values of omega-3s in beef at higher levels as found in seafood since absorption and deposition of those fatty acids in tissues are limited by the bio hydrogenation of α-linolenic acid (ALA, C18:3n3) during ruminant digestion [9].

Omega-3 fatty acids including ALA, Eicosapentaenoic Acid (EPA), and Docosahexanoic Acid (DHA) are essential for regulation of a variety of physiologic functions, nervous system development, and improvement of cognitive performance [10,11]. Although there is no official recommended daily allowance of omega-3s, the European Food Safety Authority and the United States Department of Health and Human Services agree that daily levels of 200-500 mg of combined EPA and DHA are

sufficient for adults to maintain overall health [12,13]. Benefits of having nutritional access to adequate levels of these fatty acids also include protection against cardiac arrhythmia, triglyceride-lowering, amelioration of inflammatory, and neurodegenerative disorders [14].

In humans, polyunsaturated fatty acids (PUFA) with more than 20 carbons are synthesized by fatty acid denaturizes, which are regulated by the ingestion of linoleic acid (LA, C18:2n6) and ALA [15]. Although these enzymes are present in different tissues, humans still have low ability of converting LA and ALA into 20 carbons PUFA [16]. Therefore, they must be acquired from different dietary sources [17]. Significant efforts have been made to incorporate omega-3 oils into foods, but inclusion of EPA and DHA usually leads to fishy aftertaste [18]. Recently, research suggested that krill oil benefits surpass fish oil due to better bioavailability of omega-3s [19]. Additionally, several supplement manufacturers (Potent Organics®, Schiff®, Down In The Park®, Member's Mark®, Nature's Reward®) claim that a unique advantage of krill oil is the absence of fishy aftertaste. However, there is minimal data that supports this claim [20,21]. Possibly, this claim is based on the biological availability of omega-3s (EPA and DHA) in krill oil, which are carried in a phospholipids form [22,23]. Nwosu, Boyd, and Sheldon [23] demonstrated that choline-containing phospholipid is better protected against oxidation and therefore, production of volatiles associated with fishy after taste may be inhibited. Winther, Hoem, and Berge [24] showed that the majority of omega-3s in krill oil is in choline-containing phospholipids.

The objective of this research was to evaluate the sensory attributes of striploin steaks fortified with krill oil and understand if enhancing beef with this novel marine oil source affects shelf life by compromising color and lipid stability.

MATERIALS AND METHODS

Raw meat and marine oil

In this study, a commercial red color food-graded krill oil solution containing 64 mg of EPA and 30 mg of DHA per gram diluted in purified water, ethyl vanillin, and sorbitol was used. In order to minimize any additional effects caused by different muscles or animals on palatability, a single United States Department of Agriculture (USDA) Choice-graded beef loin, strip loin, boneless (Institutional Meat Purchase Specifications-IMPS 180) weighing approximately 5 kg was procured from a commercial, USDA-inspected beef processing facility. After 14 d aging, the strip loin was fabricated into thirty (n=30) 2.54-cm steaks (150 g each).

EPA/DHA incorporation and sample assignment

A standard enhancing solution was formulated by diluting krill oil in extra virgin olive oil (50:50). Steaks were injected with a disposable syringe set in 8 different symmetric locations across the steak to ensure homogeneous distribution of the solution in the lean. The target green weights for treated samples were 100.7% (1.05 g of oil solution, final steak weight = 151.05 g - E1) and 101.3% (1.95 g of oil solution, final steak weight = 151.95 g - E2). Although concentrations of EPA and DHA in beef may vary based on animal feeding strategies, in our study, enhancement was performed to incorporate into beef approximately 47 mg and 94 mg per 150g of a combination of EPA and DHA from krill oil. Possible additional concentrations of both fatty acids from olive oil were not considered in this study. The scope of this work was not to precisely evaluate final levels of fatty acids in the lean, but effects of incorporating krill oil mainly on flavor and shelf life. Steaks from treatment E1 were enhanced with 32 mg of EPA and 15 mg of DHA, whereas steaks from treatment E2 were enhanced with 64 mg of EPA and 30 mg of DHA. Control (C) steaks were not injected (n=5 per treatment). Steaks used for sensory analysis were immediately vacuum packaged after being enhanced, and stored at -80°C to avoid oxidation and possible flavor deterioration before cooking. Steaks used for color and lipid oxidation analyses were displayed for 5 days in trays wrapped with permeable film.

Simulated retail display, instrumental color, and visual evaluation

Steaks were displayed in a refrigerated case maintained at 4°C ± 2°C (Master Bilt Refrigeration Solutions, model VOAM48-79, MS, USA). Samples were exposed to fluorescent lighting with an intensity of approximately 1614 lux. Objective color was measured for L* (psychometric lightness; black = 0, white = 100), a* (red = positive values; green = negative values) and b* (yellow = positive values; blue = negative values) using a Minolta Colorimeter (CR-400, Minolta Company, Osaka, Japan). Every day before measuring, the colorimeter was calibrated according to the manufacturer instructions. Area of measurement of the equipment was 8 mm diameter and the illuminant and standard observer were set at D65 and 10°, respectively. Visual evaluation was performed by scoring surface discoloration (from 0% to 100% discolored). Trained panelists (n = 4) scored discoloration by following a scale described by Senaratne [25]. Measures for all color attributes (L*, a*, and b*) and discoloration were taken during 5 consecutive days, whereas objective color readings were averaged from three different locations of each steak.

Lipid oxidation analysis

Lipid oxidation was evaluated after 5 days of display according to the protocol of Buege and Aust [26], modified by Ahn et al. [27]. Briefly, a Thiobarbituric Acid Assay (TBA) was performed by mixing 5 g of powdered sample with 14 ml of demineralized / deionizer water and 1 ml of Butylatedhydroxianisole (BHA). Subsequently, samples were homogenized for 15 s, centrifuged at 2000 rpm for 5 min, and 1 ml of the homogenate was transferred to a 15 ml conical tube. Tubes were vortexed after adding the 2-Thiobarbituric Acid / Trichloroacetic Acid (TBA/TCA - 15% TCA (w/v) and 20 mM TBA (molar weight= 144.5) reagent in ddH2O). Samples were incubated at 70°C for 30 min, and centrifuged at 2000 rpm for 15 min. Aliquots of 200 µl were transferred from tubes to a well plate, where absorbance was read at 540 nm (BioTek microplate reader - model Synergy HT, VT, USA). Lipid oxidation (TBA values) was expressed as malondialdehyde concentration (mg/kg) and quantization was realized by comparing samples to absorbance of standards.

Sensory evaluation

Sensory panels were approved by the University of Nevada,

Reno Institutional Review Board (IRB# 1076014-1). Panelists were screened and trained according to Meilgaard, Civille, and B.T. Carr [28]. A total of 6 out of 12 panelists were selected based on accuracy of detecting similar samples and off-flavor descriptors. Steaks were cooked on an electric grill (Hamilton Beach, USA) to an internal temperature of 71°C. During cooking, steaks were flipped after reached 35°C. Temperature was monitored using thermocouples attached to a temperature recorder data logger (RDXL 12SD, Omega, USA). Cooked steaks were subsample into 6 cubes of 1.3 x 1.3 cm x cooked steak thickness and randomly assigned to panelists. Cubes were served warm and panelists had approximately 3 minutes per sample to evaluate attributes. Panel sessions (n=3) were held in a sensory laboratory not attached to the preparation kitchen to limit panelist access to cooking aromas. Samples were served under a red fluorescent light to avoid visual differences. In order to eliminate residual flavor notes between the samples, double distilled, deionizer water and unsalted crackers were provided to panelists. Sensory attributes were rated in anchored intensity scales for tenderness (1 = extremely tough, 8 = extremely tender), connective tissue (1 = abundant amount, 8 = no connective tissue), juiciness (1 = extremely dry, 8 = extremely juicy), and off-flavor intensity (1 = extremely mild, 8 = extremely intense). If off-flavor was detected, panelists used lexicon descriptor for each sample. Descriptors included sweet, metallic, sour, oxidized, livery, bloody, bitter, and fishy [29,30] (Table 1).

Statistical analysis

Data of the experiment were analyzed using SAS® (SAS Inst., Inc. Cary, NC, USA) as a completely randomized design. Enhancement treatment was considered the main effect whereas day of display (color data) was analyzed as repeated measures using the best covariance matrix indicated by the smallest Akaike and Bayesian information criteria (AIC and BIC, respectively). Fixed effects of enhancement treatment and day of display were analyzed as 3x6 factorial (individual effects and interaction). Sensory and subjective color panelists were considered random effects. Experimental power was calculated by using PROC POWER to ensure at least 80% of power. Data sets were analyzed using PROC GLIMMIX and when significance ($P \leq 0.05$) was indicated by ANOVA, means were separated using the LSMEANS and DIFF functions. Chi-square, FREQ procedures were used to test the frequency distribution of off-flavor descriptors.

RESULTS AND DISCUSSION

Color

Effects of enhancement ($P = 0.03$) and day of display ($P = 0.01$) were observed for lightness (L*). Steaks were darker on day 3 and 5 when compared to days 1 and 2 (Figure 1). Steaks enhanced with E1were lighter ($P=0.003$) when compared to C and E2 steaks (Table 2). Enhancement levels did not affect redness (a*). However, a significant day of display effect was observed ($P < 0.0001$). Steaks gradually showed less redness as display time increased (Figure 1). As levels of enhancement increase, yellowness (b*) also increased ($P < 0.0001$) (Table 2). Interaction between enhancement and day of display effects was observed on surface discoloration (Figure 2, $P < 0.0001$). Initial signs of discoloration were observed only on day 3, whereas enhanced steaks had higher discoloration when compared to control steaks. When comparing enhancement treatments, E2 steaks had the highest discoloration, followed by E1, and control steaks. Fortification of steaks with the oils solution directly affected color attributes due to higher lipid oxidation found in enhanced samples (Table 2.).

Sensory attributes

Effects of enhancement treatments on sensory attributes are shown on Table 2. No effects were observed for connective tissue amount and juiciness. However, E2 samples were tenderer than control samples. In addition, off-flavor intensity was significantly higher in enhanced samples. Distribution of metallic, bloody, and fishy off-flavors was also different across treatments (Figure 3). Enhanced samples showed higher intensity of metallic off-flavor. Control samples had higher bloody flavor intensity when compared to enhanced samples. This was possibly due to intensity of other off-flavors detected in enhanced samples. Minimal fishy flavor intensity was observed on E1 samples (5.56%). However, a total of 41.67% of samples enhanced with the highest level of omega-3s (E2) were reported to have fishy off-flavor.

In this study, in order to minimize an additional random effect of muscle from different animals on all evaluated attributes, we used strip loin steaks from a single animal. This eliminated possible differences that could be led by individual physical and chemical characteristics of different muscles. Extra virgin olive oil was used as a lip soluble carrier because it may provide potential flavor perception benefits when incorporated to meats [31]. In addition, it contains highly valuable unsaponifiable

Table 1: Definitions and references for off-flavor *descriptor.*

Descriptor	Definition	Reference
Bitter	The fundamental taste factor associated with a caffeine solution [29].	0.02% caffeine solution
Bloody	The aromatics associated with blood on cooked beef.	Cooked beef blood
Fishy	The fundamental taste from fish products.	Sardine oil
Livery	The aromatics associated with cooked organ meat/liver [29].	Cooked beef liver
Metallic	The impression of slightly oxidized metal [29].	0.1% potassium chloride solution
Oxidized	The aromatics associated with oxidized oil.	Vegetal oil
Sour	The fundamental sour taste associated with animal foods.	Buttermilk
Sweet	The fundamental taste factor associated with sucrose [29]	2% sucrose solution

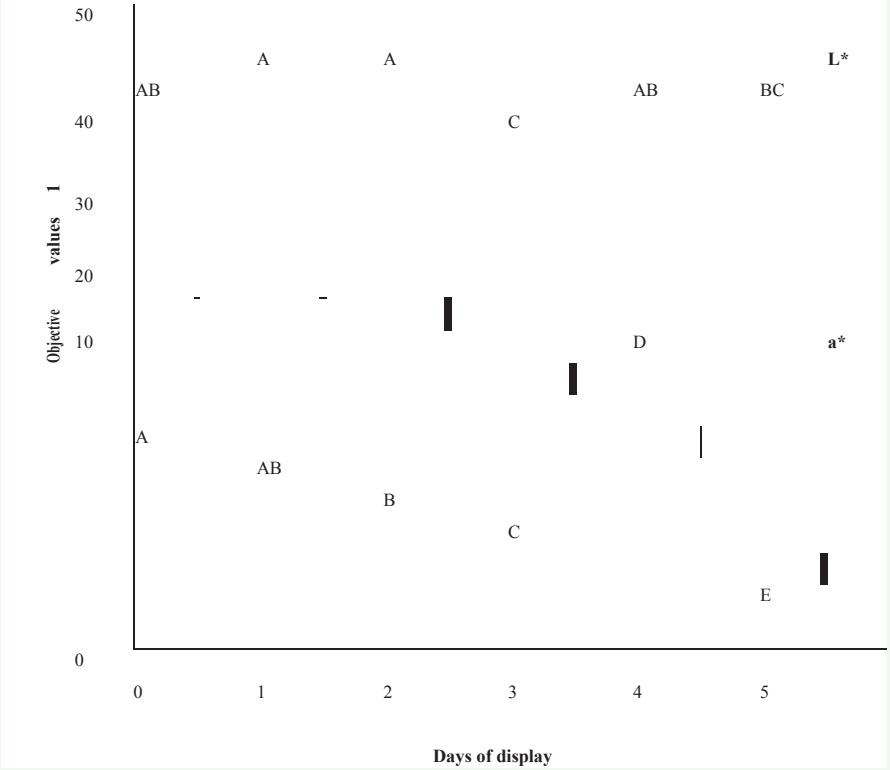

Figure 1 Day effect on lightness (L*) and redness (a*) of strip loin steaks during display.
1L*(psychometric lightness; black = 0, white = 100), a*(red = positive values; green = negative values)
A,B,C,D,E Means within objective color parameters having different letters are significant at P ≤ 0.05.

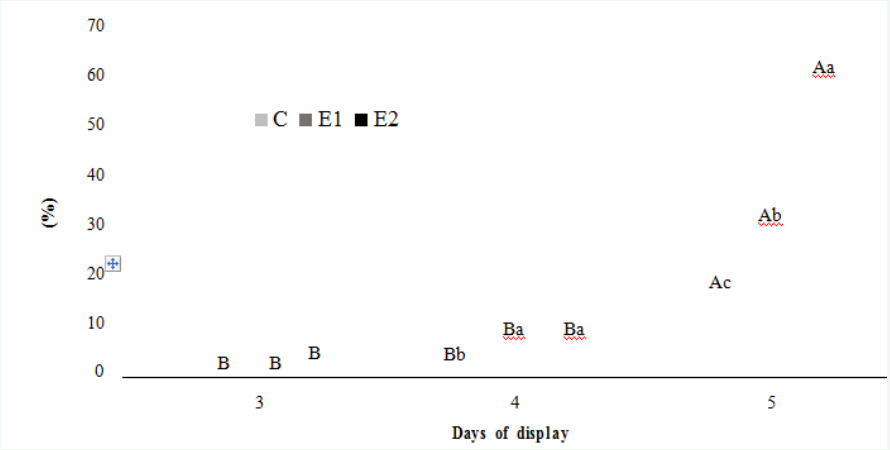

Figure 2 Surface discoloration (%) of strip loin steaks enhanced with krill oil (Control = not enhanced, E1 = enhanced with 32 mg of EPA and of 15 mg DHA, E2 = enhanced with 64 mg of EPA and 30 mg of DHA).
[A,B]Means within enhancement treatments having different letters are significant at P < 0.0001
[a,b,c]Means within days of display having different letters are significant at P < 0.0001.

ingredients with exceptional biological value [32]. As expected, treatments did not affect connective tissue amount since steaks were obtained from the same animal. Steaks from treatment E2 received higher tenderness scores, whereas E1 steaks had similar tenderness when compared to C and E2 steaks. While the exact reason for this is not known, it is possible that treatments with oils lubricated muscle fibers or fibrils [33], creating an improved perception of tenderness to panelists. Regarding juiciness, previous research associated greater levels of fat with higher juiciness perception by panelists [34,35]. In this study, although it was suggested that incorporation of oils in the lean improved tenderness perception, no effect on juiciness was observed.

Prior to conducting the aforementioned study, a pilot sensory testing of beef enhanced with marine oils using shellfish,

Table 2: Sensory attributes[1], instrumental color[2], and TBA[3] values of beef steaks enhanced with krill oil.

	Parameters		Treatments[4]			
Sensory Attributes		**Control**	**E1**	**E2**	**SEM[5]**	***P*-value**
	Tenderness	4.71[b]	5.26[ab]	5.75[a]	0.29	0.01
	Connective tissue amount	4.58	4.86	5.04	0.29	0.36
	Juiciness	4.92	4.5	5.17	0.24	0.11
	Off-flavor intensity	3.80[b]	5.42[a]	6.04[a]	0.42	<0.0001
Instrumental color						
	L*	44.47[b]	46.15[a]	45.13[b]	0.44	0.003
	a*	14.85	15.53	15.12	0.71	0.26
	b*	8.54[c]	10.39[b]	11.30[a]	0.37	<0.0001
Lipid Oxidation						
	TBA	0.85[c]	1.56[b]	2.32[a]	0.25	0.002

[1]Tenderness (1 = extremely tough to 8 = extremely tender), connective tissue (1 = abundant amount to 8 = no connective tissue), juiciness (1 = extremely dry to 8 = extremely juicy), and off-flavor intensity (1 = extremely mild to 8 = extremely intense).

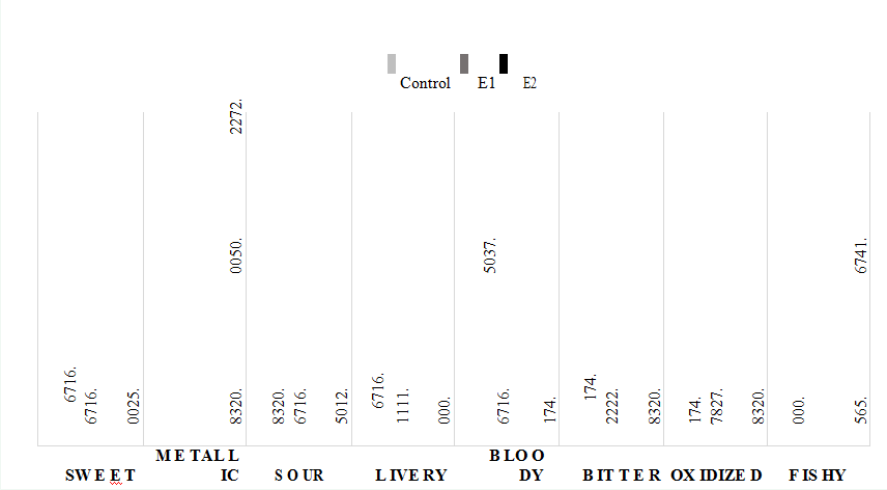

Figure 3 Sweet (P = 0.79), metallic (P = 0.003), sour (P = 0.91), livery (P = 0.12), bloody (P = 0.01), bitter (P =0.17), oxidized (P = 0.13), and fishy (P = 0.0001) off-flavor descriptor Chi-square frequency distribution (%). Off-flavors with significant P- values were not equally distributed across treatments (Control = not enhanced, E1 = enhanced with 32 mg of EPA and of 15 mg DHA from krill oil, E2 = enhanced with 64 mg of EPA and 30 mg of DHA from krill oil).

anchovy, sardine, salmon, and mackerel was conducted with two trained panelists to refine possible sources. All fish oils generated significant and undesirable fishy off-flavor, possibly due to the bioavailability of DHA and EPA in regular fish oils, which are usually susceptible to rapid oxidation [36]. This directly limits the incorporation of both fatty acids in foods since odorants generated by their auto oxidation lead to the development of fishy off-flavor [37]. Commonly, fortifying products with marine oil sources requires encapsulation and addition of antioxidants to protect omega-3s. However, oxidation of omega-3 fatty acids and generation of undesirable flavors still may occur when product is heated [37]. Previous research conducted by Martini, et al. [7], reported that fortification of Cheddar cheese with levels of 71 mg/28g of encapsulated fish oil led to the presence of fishy off-flavor during aging. This is due to the oxidation of omega-3s, which generates carbonyl compounds. In addition, the magnitude of off-flavor perception may also increase with cooking [38].

In our study, we hypothesized that fortifying fresh meats with krill oil would not lead to fishy off-flavor development due to two major reasons: 1. Krill oil contains intrinsic antioxidants including vitamins E, A, D, and canthaxathin, which makes this oil 48 times more resistant to oxidation when compared to fish oil on basis of oxygen radical absorption capacity [39]; and 2. Omega-3s (EPA and DHA) in krill oil are carried in a phospholipid form [21], which is generally stable to heating [40]. Conversely, enhancing beef with 47 (E1) and 94 mg/150g (E2) of EPA and DHA led to higher off-flavor intensity when compared to control samples. Fishy off-flavor was reported in 5.56% of samples from treatment E1 and 41.67% of samples from treatment E2. This suggests that the different chemical properties of EPA and DHA of krill oil when compared to regular fish oil are not able to inhibit fishy off-flavor development when the product is heated. In addition, a high

amount of enhanced samples was reported to have metallic off-flavor when compared to control steaks. This possibly happened due to the addition of olive oil in the enhancement solution. The second major fatty acid in olive oil is the Linoleic acid (C18:2n6) [41] and oxidation of this acid is directly correlated to metallic flavor development [42,43]. The lower perception of metallic off-flavor on E2 samples when compared to E1 was possibly due to the higher fishy intensity found on E2, which may have overwhelmed panelists leading to a lower perception of metallic.

Lipid oxidation

As levels of enhancement increased, higher oxidation was observed in steaks (Table 2). Lipid peroxidation is a primary cause of quality deterioration in meat products. Lipid peroxyl radical and alkoxyl radical generated in initial reactions abstract hydrogen atoms from lipids, initiating the chain reaction and propagating oxidation throughout the tissue [44]. In our study, as levels of oils increased, lipid content also increased and maximizing oxidation in the lean. Higher oxidation in fortified samples was directly responsible for high frequencies of metallic and fishy off-flavor detected by panelists, higher values of L* and a*, and greater discoloration of steaks. Samples enhanced with the highest concentration of oils showed the greatest discoloration. This was possibly due to increased levels of metmyoglobin in the lean since lipid and pigment oxidation is positively correlated [45].

CONCLUSION

Enhancing meat products with krill oil negatively impacts eating experience due to unacceptable flavor and shelf life due oxidation, and visual discoloration. Although EPA and DHA in krill oil possess unique biological characteristics that may improve lipid stability and delay oxidation even during heating, cooking temperature is still the factor with the largest influence on lipid oxidation. Heating fortified samples possibly generated volatiles related to fishy aftertaste. Therefore, it is not possible to decrease fishy off-flavor perception if the product is cooked. Future research must study novel methodologies to mask fishy-off flavor in fortified fresh foods that are intended to be cooked.

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