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OXIDATIVE STABILITY OF CHICKEN MEAT EMULSION SYSTEMS: THE EFFECTS OF GELLED EMULSION AND USE OF ASCORBIC ACID AND ROSEMARY EXTRACT IN DIFFERENT PHASES

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ABSTRACT

In order to examine the effects of using ascorbic acid, rosemary extract, or ascorbic acid-rosemary extract combination in different phases of flaxseed oil gelled emulsion (GE) formulation on oxidative stability, model chicken meat emulsions (CMEs) formulated with as follows; beef fat-no antioxidant (C), gelled emulsion-no antioxidant (GE-No), GE containing 100 ppm ascorbic acid in the water phase (GE-A), GE containing 100 ppm rosemary extract in the oil phase (GE-R) and GE containing 100 ppm ascorbic acid in water phase and 100 ppm rosemary extract in oil phase (GE-A/R). Protein content of samples increased from 12.99% to 14.58% with the addition of GE ($P<0.05$). Water holding capacity of reformulated CMEs increased up to 66.01%. At the end of the storage using ascorbic acid and rosemary extract individually or combined in GE formulation was effective to delay the primary lipid oxidation of samples, while ascorbic acid and ascorbic acid+ rosemary extracts retarded the formation of malonaldehyde. Initial free fatty acid values ranged between 0.34%- 1.07% and the initial trend was proportional to TBARS values. Reformulated samples were lighter than the control group. a* value of control was higher while b* values were lower than reformulated CMEs throughout the storage.

1.Introduction

Increasing trends in consumer's demand for healthy foods have led to meat industry to focus on research and development studies to improve healthier meat product formulations. In the application of producing healthier meat products, using vegetable and seed oils in liquid form yields technological problems, undesired organoleptic properties, and increase oxidative changes. To prevent these undesirable changes in quality, liquid oils can be modified by using various methods such as structured emulsions which involve gel and double emulsions (Alejandre *et al.*, 2016; Öztürk *et al.*, 2017), interesterification (Kılıç and Özer, 2017), and organogelation (Barbut *et al.*, 2016) have been researched previously. Gelled emulsions (GE) are considered to be one of the effective ways to solidify liquid oils by entrapping the liquid oil in gel network which is created by polysaccharides and/or enzymes (Serdaroğlu *et al.*, 2016; Serdaroğlu *et al.*, 2017).

One of the main problems when improving the fatty acid composition of meat products by the addition of oils that contains high amounts of unsaturated fatty acids is their oxidative susceptibility which results in deterioration of the nutritional and sensorial perspective of meat products (Kumar *et al.*, 2015). Therefore, these oils should be protected to make them more stable against oxidative changes during processing and storage (Carneiro *et al.*, 2013). In this respect gel networks which are created by polysaccharides, enzymes, and heat treatment

provide an opportunity to protect unsaturated fatty acids against oxidation (Wang *et al.*,2018; Kavuşan *et al.*,2020). In some cases, using highly oxidative oils in gelled emulsion formulations remains incapable in terms of inhibition of oxidative changes due to high perishability, therefore antioxidant incorporation to gelled emulsion formulation may retard the oxidation reactions better than gelled emulsion itself (Alejandre *et al.,* 2019). For this purpose, *Murraya koenigii* berries extract (Kumar and Kumar, 2020) and olive leaves extract (Robert *et al.,* 2019), rosemary extract (Erdmann, *et al.,* 2017) loaded O/W emulsions or double emulsions were used successfully in meat products. Also, lyophilized *Melissa* extract (Poyato *et al.,* 2013) and curcumin, quercetin, rutin hydrate, and ascorbic acid (Noon *et al.,* 2020) were used in gelled emulsion formulations for the prevention of sunflower, olive, and linseed oils. Up to now, there is only one research regarding the use of green tea extract in the formulation of fish oil gelled emulsion (Pourashouri *et al.,* 2020). The findings of this research presented green tea extract loaded gelled emulsion did not improve oxidative stability. Instead of using antioxidant in the water phase, Mosca *et al.* (2013) stated that the best strategy to preserve the emulsions is the combined use of antioxidants both in water and oil phases, to promote a synergistic effect and the regeneration of antioxidants mediated by the interfacial layer.

Lipid oxidation could be retarded by sequestration of free radicals from the medium, chelation of metallic ions, inhibition of free radical producing enzymes, activation of endogenous antioxidant enzymes, and prevention of lipid peroxidation (Carocho *et al.*, 2018). Using synthetic antioxidants such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) has been related health problems and their application in the food industry has limited (Rojas and Brewer, 2008). Thus, the use of natural antioxidants such as fruit or vegetable extracts and essential oils in meat product formulations have been subjected to researches in recent years (Oswell *et al.*,2018; Pateiro *et al.,* 2018).

Water-insoluble rosemary (*Rosmarinus officinalis* L.) extracts have strong antioxidant effects depending on their phenolic compounds such as rosmanol, rosmariquinone, rosmaridiphenol, carnosic acid, and carnosol. The antioxidant mechanism of rosemary extract is based on its breaking free radical chain as a result of hydrogen donation provided by phenolic compounds (Haile, 2015). Ascorbic acid is water-soluble organic compound that one of the most important properties of ascorbic acid is its ability to remove environmental oxygen (Varvara *et al.*, 2016).

"Polar paradox" is explained by Porter (1993) stated that polar antioxidants are more effective than apolar antioxidants in bulk oils while apolar antioxidants were more efficient in O/W emulsions due to the localization of antioxidants in different phases where oxidation occurs (Shahidi and Zhong, 2011; Sørensen *et al.*, 2011). Although this phenomenon has been predominantly approved, new pieces of evidence from elaborative studies have found that not all antioxidant compounds in O/W emulsions behave according to this phenomenon (Shahidi and Zhong, 2011; Poyato *et al.*, 2013).

To the best of our knowledge, there is limited search focused on the combined use of different antioxidants in gelled emulsion formulations (Noon *et al.,* 2020) or their use in meat products. Therefore, the objective of this study was to examine the effects of the combined use of ascorbic acid and rosemary extract in different phases of GE formulation on oxidative stability of model chicken meat emulsion where beef fat is replaced by GE prepared with flaxseed oil.

2. Materials and methods (TNR 12 Bold, No ident)

2.1. Materials

Chicken breast meat (74.72% moisture, 2.43% fat, 20.77% protein, 1.67% ash), skinless and boneless was kindly donated by a local processor (Abalıoğlu, Manisa, Turkey). Flaxseed oil (17% oleic acid, 15% linoleic acid,

and 59% linolenic acid) was taken from Ege University Agriculture Faculty (Izmir, Turkey). Polyglyserol polyricinoleate (PGPR) was obtained from Çağdaş Chemicals Co. (Turkey). Gelatin was purchased from Sigma-Aldrich (USA) and egg white powder was purchased from Dr. Gusto (Turkey). Inulin powder (Inulin: 88-92%) was obtained from BENEO-Orafti. Ascorbic acid and rosemary extracts were supplied from Kimbiotek (Turkey).

2.2.Preparation of emulsion gels formulated with different antioxidant

Gelled emulsions were prepared with flaxseed oil, egg white powder, inulin, and gelatin according to the method implemented by our research group (Kavuşan *et al.*, 2020). The formulation of gelled emulsions is given in Table 1. Four different batches of gelled emulsions are formulated as follows: (No) no antioxidant added to GE; (A) 100 ppm ascorbic acid added to the water phase of GE; (R) 100 ppm rosemary extract added to the oil phase of GE, and (A/R) 100 ppm ascorbic acid added to the water phase and 100 ppm rosemary extract added to the oil phase of GE respectively. Antioxidant addition was performed after the heating procedure when the temperature of emulsions reached room temperature. Prepared emulsions were stored at 4 ºC overnight to use in the model chicken meat emulsion formulation.

Table 1. Formulation of gelled emulsions used in model chicken meat emulsion

	100 g emulsion							
	Water phase (g)					**Oil phase (g)**		
	Egg white powder	**Inulin**	**Gelatin**	**Water**	**Ascorbic acid**	**PGPR**	**Flaxseed oil**	**Rosemary extract**
No	3	8	2	37	-	3.2	46.8	-
A	3	8	2	37	0.01	3.2	46.8	-
R	3	8	2	37	-	3.2	46.8	0.01
A/R	3	8	2	37	0.01	3.2	46.8	0.01

*Rosemary extract added into oil phase, while ascorbic acid added into water phase. Addition level of antioxidants was 100 ppm.

No= no antioxidant added to gelled emulsion formulation

A= 100 ppm ascorbic acid added to water phase of gelled emulsion formulation

R= 100 ppm rosemary extract added to oil phase of gelled emulsion formulation

A/R=100 ppm ascorbic acid added to the water phase and 100 ppm rosemary extract added to the oil phase of GE respectively

Table 2. The formulation of model chicken meat emulsion

Samples	**Meat**	**Beef fat/GE**	**Water**	**NaCl**	**STTP**	**$NaNO_2$**
C	63.41	9.75	24.39	1.95	0.49	0.01
GE	63.41	20.83	13.31	1.95	0.49	0.01
GE-R	63.41	20.83	13.31	1.95	0.49	0.01
GE-A	63.41	20.83	13.31	1.95	0.49	0.01
GE-A/R	63.41	20.83	13.31	1.95	0.49	0.01

C: only beef fat added chicken meat emulsions

GE: only GE added chicken meat emulsions

GE-R : CME prepared with GE incorporated with rosemary extract in oil phase

GE-A: CME prepared with GE incorporated with ascorbic acid in water phase

GE-A/R: CME prepared with GE incorporated with rosemary extract in oil phase and ascorbic acid in water phase.

2.3.Processing of model chicken meat emulsion

The preparation of the chicken meat emulsion method of Cofrades *et al.* (2008) was used with some modifications. After trimming visible fat and connective tissue, chicken breast and fat are minced through a grinder with a 3 mm plate. Minced meat was mixed for 60 s. in Thermomix (Vorwerk, Wuppertal, Germany), fat or GE, fifty percent of the ice, and curing ingredients (NaCl, STTP, $NaNO_2$) were added and homogenized at 2500 rpm for 60 s. more. Control sample was formulated with 9.75% beef fat, while 20.83 % gelled emulsion was added to all other CME samples to reach the same fat content as the control samples. The formulation of the model chicken meat emulsion is given in Table 2. The final temperature of emulsions was lower than 12 ºC for all batches. Twenty-five g of samples were filled in 50 ml tubes, then tubes were centrifuged at 2500 rpm for 60 s. Samples were then heated for 30 min. in a 70 ºC water bath, after the heating process, all heat-treated samples were cooled (approximately 25 ºC) and afterward stored for 7 days at 4 ºC.

2.4.Experimental design

Five different batches of chicken meat emulsions (CMEs) were produced. Control (C) samples formulated with beef fat and no antioxidant, four other CMEs were prepared with gelled emulsions without antioxidant (GE-No), gelled emulsion added rosemary extract to oil phase (GE-R), gelled emulsion added ascorbic acid to water phase (GE-A) and gelled emulsion added rosemary extract to the oil phase and ascorbic acid to the water phase (GE-A/R).

2.5.Chemical composition

Moisture and ash contents of samples were determined following AOAC (2012) procedures. Fat content was analyzed according to Flynn and Bramblet (1975). Protein content of samples was determined by using DUMAS method with LECO nitrogen analyzer (FP-528, USA).

2.6.Water holding capacity (WHC)

The ability of CMEs to keep water determined as described by Hughes *et al.* (1997). 10 g CME samples heated in a water bath (90 ºC, 10 min) and centrifuged for 10 min. at 4000 rpm. Final weight of samples recorded and WHC was found as the percentage of retained water related to moisture content.

2.7. pH

pH values were measured with a pH-meter (WTW pH 330i/SET, Germany) on different points of CME samples for every individual group.

2.8.Oxidation analysis

Peroxide and TBARS analyses were performed to observe oxidative changes in CME samples on the 0, 3^{rd,} and 7th days of storage. In the CME samples, the peroxide value was determined after the fat/oil in the sample was extracted (chloroform). The potassium iodide was oxidized with the peroxide oxygen in the fat/oil afterward the iodine was released, and this free iodine was titrated with thiosulfate (Koniecko, 1979).

TBARS analysis was based on the measurement of the intensity of the formed pink color (malonaldehyde) as a result of oxidation at a wavelength of 532 nm. The obtained absorbance value was multiplied by 5.2 and the malonaldehyde concentration of the product was determined as mg MA/ kg (Witte *et al.*, 1970).

2.9.Free fatty acid (FFA)

Free fatty acid value was analyzed according to AOAC (2012) procedure.

2.10 Color

Color parameters of chicken meat emulsions were measured quadruple for each group during refrigerated storage by using a portable colorimeter (Chromameter CR400, Minolta, Japan) and parameters were phrased as lightness (L*), redness (a*), and yellowness (b*).

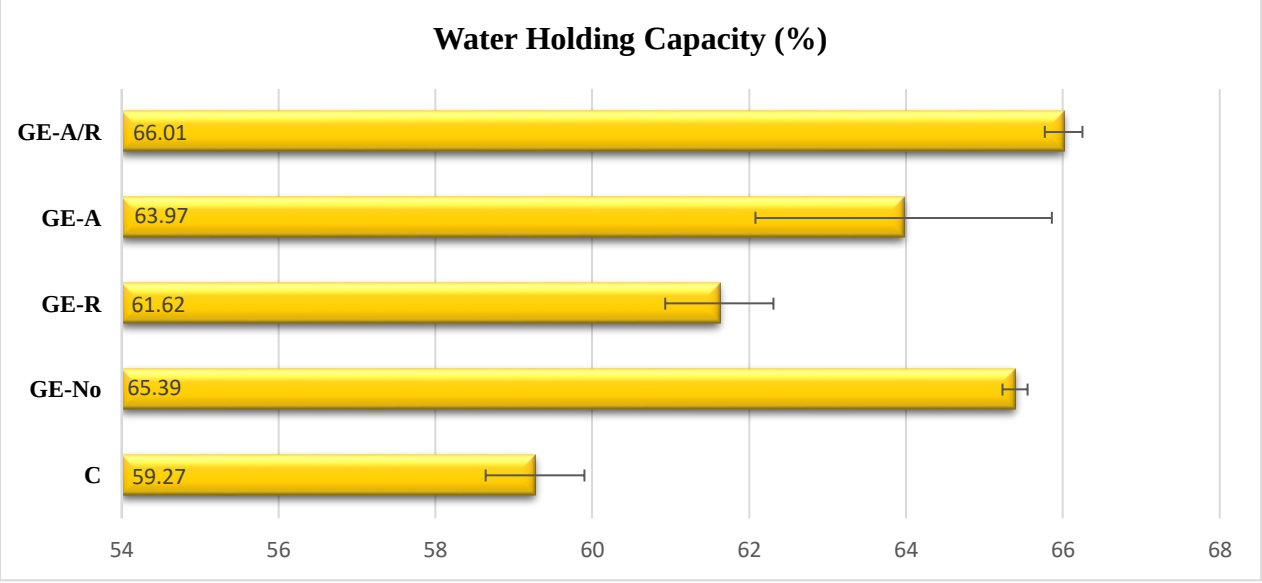
2.11.Statistical analysis

Statistical analyses were carried out using SPSS program (IBM, version 21.0, USA). A one-way ANOVA was applied to examine the effects of using gelled emulsions loaded with antioxidants to different phases on the chemical composition and water holding capacity. A model that included a two-way analysis of variance (ANOVA) was carried out to analyze the effect of treatment (C, GE-No, GE-R, GE-A, GE-A/R) and storage day (days 0, 3, and 7) on the pH, color, peroxide and TBARS value analyses. For these analyses, treatments and storage days were considered as fixed effects, while trial was included in the model as a random effect. Significant differences that affect analysis are examined by Duncan multiple tests at a 95% confidence level. The results of this study were reported as the mean values and standard deviation of the mean.

3.Results and discussions

3.1. Chemical composition and water holding capacity

Table 3 presents the chemical composition of heat-treated chicken meat emulsions. All chemical composition parameters except fat content are affected by the fat source ($P<0.05$). Moisture, protein, fat, and ash contents of samples varied between 69.46-71.71%, 12.99-14.58%, 12.36-12.76%, and 2.94-3.14% respectively. It is observed that samples incorporated with GE had lower moisture content compared to control sample prepared with beef fat, however, the adverse trend has been observed in protein and ash contents ($P<0.05$). These findings could be associated with egg white powder added to GE formulation. Lower moisture content of samples could be the result of the calculated equal fat contents of CME samples. In model meat emulsions when fat is completely replaced with gelled emulsion, lower moisture content was reported by Serdaroğlu *et al.* (2016). In a study where pork back fat in frankfurter formulation replaced by unripe banana by-products and pre-emulsified sunflower oil, the addition of pre-emulsion had a moisture increasing effect was reported (Pereira *et al.*, 2020).



ab: means with the different letter in the same column are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates.

C: only beef fat added chicken meat emulsions, GE: only GE added chicken meat emulsions, GE-R : CME prepared with GE incorporated with rosemary extract in oil phase, GE-A: CME prepared with GE incorporated with ascorbic acid in water phase, GE-A/R: CME prepared with GE incorporated with rosemary extract in oil phase and ascorbic acid in water phase.

Figure 1. Water holding capacity of chicken meat emulsions

Table 3. Chemical composition and of chicken meat emulsions

Sample	Moisture (%)	Protein(%)	Fat(%)	Ash(%)
C	71.71±0.72^{a}	12.99±0.64^{b}	12.36±0.05	2.94±0.12^{b}
GE	69.46±0.67^{b}	14.19±0.09^{a}	12.98±0.42	3.08±0.01^{a}
GE-R	70.14±0.28^{b}	14.13±0.32^{a}	12.67±0.04	3.06±0.00^{a}
GE-A	69.81±0.14^{b}	14.58±0.29^{a}	12.57±0.47	3.04±0.04^{ab}
GE-A/R	69.88±0.67^{b}	14.22±0.41^{a}	12.76±0.32	3.14±0.06^{a}

ab: means with the different letter in the same column are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates.

C: only beef fat added chicken meat emulsions, GE: only GE added chicken meat emulsions, GE-R : CME prepared with GE incorporated with rosemary extract in oil phase, GE-A: CME prepared with GE incorporated with ascorbic acid in water phase, GE-A/R: CME prepared with GE incorporated with rosemary extract in oil phase and ascorbic acid in water phase.

WHC values of CME samples are shown in Figure 1. All GE added samples were more successful in terms of keeping water compared to C treatment (P<0.05). The increment in WHC could be explained by the water absorption ability of inulin in GE formulation. Inulin also reduced the water release in CME probably due to the strong electrostatic interactions and hydrogen bonds between meat proteins. The same phenomena are explained by covalent bonds of gelatin which is included in the aqueous phase of gel emulsion, which may also increase the water-holding of the system (Serdaroğlu *et al.*,2016). Negative effects on the liquid release have been reported in a study where gelled emulsion containing olive oil, chia mucilage + carboxymethylcellulose, or sodium alginate was added to model meat emulsions (Câmara *et al.*,2020). GE-No samples had good water retention ability than GE-R sample (P<0.05), however, they had similar WHC values with GE-A and GE-A/R. The lowest WHC values (61.62%) were found in samples incorporated with GE loaded with rosemary extract (P<0.05). WHC of samples formulated with GE is in line with pH results, samples with higher pH had high WHC (Figure 1). Additives might affect the space between the filaments and net charges of protein molecules thus help to keep moisture in the structure (Yogesh *et al.*, 2012). Another reason for alterations in WHC could be the possible effect of protein oxidation that yielded conformational changes in proteins. Also, similarly, Serdaroğlu *et al.* (2017) reported an increment in WHC in samples prepared with a gelled emulsion containing pumpkin seed oil in model meat emulsions.

3.2. pH

Table 4 shows the pH values of CME samples throughout the 7 days of storage at 4 °C. Initial pH values ranged from 6.11-6.16. Antioxidant addition to GE affected the pH values (P<0.05). Control samples prepared with beef fat, GE-No, and GE-A/R samples had similar pH values which were higher than GE-R and GE-A (P<0.05). Also, meat emulsions formulated with a gelled emulsion containing olive oil, chia mucilage + whey resulted in lower pH values (Câmara *et al.*, 2020). Pourashouri *et al.*, (2020) reported that fish sausages formulated with fish oil gelled emulsion had higher pH values than control treatments, nevertheless, samples that contain green tea extract in gelled emulsion formulation had lower pH values than gelled emulsion added samples. At the end of the storage pH values were 6.23, 6.18, 6.22, 6.22, and 6.19 for C, GE-No, GE-R, GE-A, and GE-A/R respectively. The highest pH value belonged to C sample (P<0.05). Relatively lower pH values could be attributed to the usage of flaxseed oil or gelatin. Similarly, Pintado, Herrero, Jimenez-Colmenero and Ruiz-Capillas (2016) reported that frankfurters formulated with gelled emulsion consisting of 2 % gelatin showed the lowest pH.

Table 4. pH values of chicken meat emulsions throughout 7 days of storage

pH	0th day	3rd day	7th day
C	$6.16\pm0.02^{a,y}$	$6.22\pm0.02^{a,x}$	$6.23\pm0.01^{a,x}$
GE	$6.15\pm0.01^{a,z}$	$6.23\pm0.01^{a,x}$	$6.18\pm0.01^{c,y}$
GE-R	$6.11\pm0.01^{b,z}$	$6.20\pm0.01^{b,y}$	$6.22\pm0.01^{b,x}$
GE-A	$6.12\pm0.01^{b,y}$	$6.22\pm0.01^{a,x}$	$6.22\pm0.01^{b,x}$
GE-A/R	$6.15\pm0.00^{a,z}$	$6.22\pm0.01^{a,x}$	$6.19\pm0.00^{c,y}$

abc: means with the different letter in the same column are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates. xyz: means with the different letter in the same row are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates.

C: only beef fat added chicken meat emulsions, GE: only GE added chicken meat emulsions, GE-R : CME prepared with GE incorporated with rosemary extract in oil phase, GE-A: CME prepared with GE incorporated with ascorbic acid in water phase, GE-A/R: CME prepared with GE incorporated with rosemary extract in oil phase and ascorbic acid in water phase.

3.3. Peroxide value

Peroxide values of samples are displayed in Table 5. Initial peroxide values were affected by fat replacement (P<0.05). All peroxide values were lower than 25 meqO_2/kg which is a limit for fatty food products (Evranuz, 1993). At the beginning of the storage, even samples formulated with GE had higher PV than the control treatment, combined use of antioxidants lowered the PVs amongst the GE added treatments. Peroxide values of all samples increased (P<0.05) during the storage except GE-A, the addition of ascorbic acid maintained the oxidative stability along with the storage. Freire *et al.* (2017) reported that when animal fat in pork patties was replaced with perilla oil gelled emulsion at levels of 66 and 100%, modified samples had higher hydroperoxide concentration at the end of the storage. The highest PVs were obtained in C (7.98 meqO_2/kg) and GE-No (7.96 sample meqO_2/kg) groups (P<0.05). In a study conducted by Pelser *et al.* (2007) using pre-emulsified flaxseed oil resulted in higher peroxide values in fermented sausages than control groups at the end of the storage. At the end of the storage replacing beef fat by flaxseed oil had neither lowering nor increasing effect on PV, however, the use of ascorbic acid and rosemary alone or combined in GE are found to be effective to delay primary lipid oxidation by locating oil-water interphase where hydroperoxides exist. Similar to our results, incorporation of blackthorn branch extract through gelled emulsion prepared with microalgal oil in reduced-fat beef patties resulted in lower peroxide values (Alejandre *et al.*, 2019).

Table 5. Peroxide values of chicken meat emulsions throughout 7 days of storage

Peroxide (meqO_2/kg)	0th day	3rd day	7th day
C	$2.95\pm1.01^{b,z}$	$5.96\pm0.05^{a,y}$	$7.98\pm0.01^{a,x}$
GE	$6.96\pm0.96^{a,x}$	$4.98\pm1.00^{a,y}$	$7.96\pm0.02^{a,x}$
GE-R	$6.91\pm1.01^{a,x}$	$4.44\pm0.51^{a,y}$	$5.63\pm0.56^{b,xy}$
GE-A	$5.95\pm0.05^{a,x}$	$4.97\pm0.98^{a,x}$	$5.99\pm0.02^{b,x}$
GE-A/R	$3.39\pm0.48^{b,z}$	$4.61\pm1.17^{a,xy}$	$6.00\pm0.00^{b,x}$

ab: means with the different letter in the same column are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates. xyz: means with the different letter in the same row are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates.

C: only beef fat added chicken meat emulsions, GE: only GE added chicken meat emulsions, GE-R : CME prepared with GE incorporated with rosemary extract in oil phase, GE-A: CME prepared with GE incorporated with ascorbic acid in water phase, GE-A/R: CME prepared with GE incorporated with rosemary extract in oil phase and ascorbic acid in water phase.

3.4. TBARS values

Replacement of beef fat and storage period were found effective on TBARS values ($P<0.05$). Initially, C sample had the lowest (0.25 mg MA/kg) TBARS value. All modified treatments had higher TBARS values due to the presence of flaxseed oil in formulation, however, induvial use of rosemary (GE-R samples) or ascorbic acid (GE-A samples) resulted in lower TBARS values. The mean TBARS values of CME samples which ranged from 0.25- 1.04 mg MA/ kg are displayed in Table 6. TBARS values of all samples were lower than limiting threshold value (2 mg MA/kg) (Witte *et al.*,1970). During the storage decrements and followed by increments have been observed in TBARS values of all antioxidants added counterparts ($P<0.05$). Increments recorded in TBARS values during storage possibly be explained by a higher formation ratio of malonaldehydes than the disappearance, nonetheless, after a while, the disappearance ratio surpasses the formation ratio, thus, TBARS values tend to decrease (Delgado-Pando *et al.*,2011). The rate of malonaldehyde disappearance throughout the storage may have exceeded the rate of production as a consequence of intermolecular reactions between malonaldehydes and amino acids or proteins (Jamora and Rhee, 2002). Throughout the 3 days of storage, the C sample had the lowest value while GE-A/R had the highest ($P<0.05$). Up to 3-day TBARS values of antioxidant added samples raised, GE-A/R showed the highest value amongst all samples and storage days, however, on day 7 rosemary and ascorbic acid showed an antioxidant effect. Similar to our findings, W/O nanoemulsion formulated with orange essential oil and cactus fruit acid was used in emulsified meat products to substitute pork fat up to 5%, resulted in lower TBARS values in a dosed manner at the end of the storage (Almaráz-Buendia *et al.,* 2019). Oxidative reactions could be retarded better in salami formulated with O/W emulsion (caprylic, capric, and lauric triglyceride mixture) loaded with 100 g/kg rosemary than sample formulated with emulsion without rosemary (Erdmann *et al.,* 2017). Even so, the amount of GE is the same as beef fat added to control group, TBARS values were lower in samples formulated with gelled emulsion containing flaxseed oil after 7 days of storage. Lower TBARS values are probably linked to the preservation of easily perishable flaxseed oil against oxidation by the surrounding gel network which is created by inulin and gelatin. Also, it could be said that using GE incorporated with ascorbic acid or combined with rosemary was found as a successful strategy to control the oxidation reactions at the end of 7-days of storage. Also, some studies reported that the combined use of various antioxidants with ascorbic acid decreased the TBARS values (Kim *et al.,* 2013; Hwang *et al.,* 2017).

It is thought that well protection of highly perishable flaxseed oil by the gelled emulsion which is formed with inulin and gelatin could be the reason for lower TBARS values. Similarly, Robert *et al.* (2019) reported that in a meat system double emulsion added with olive leaves extract exhibited a successful barrier against oxidation for linseed, fish, and olive oils mixture.

In our study, we found that ascorbic acid with a hydrophilic character was found more effective than lipophilic rosemary extract in model chicken meat emulsions. This opposite 'polar paradox' behavior could be explained using egg white powder and inulin may enhance the antioxidant activity of ascorbic acid through interactions or the level of rosemary extract could be insufficient. Also, oxidation occurs in oil-water interphase, so nonpolar antioxidants above critical concentration goes into the oil phase instead of collecting at the oil-water interphase. Another probable reason for this particular

result is that pro-oxidants in the aqueous phase could be inactivated by the effect of ascorbic acid. Opposite to our results, in fish sausages, hydrophilic polar green tea extract in fish oil gelled emulsion formulation did not show an antioxidant effect compared to the samples formulated with gelled emulsion alone due to soy protein aggregates placed in the interphase (Pourashouri *et al.*, 2020). In a study reported by Noon *et al.* (2020) low concentration (0.1 and 0.4 mg) of ascorbic acid was found more effective than non-polar curcumin in terms of delaying the oxidation in sunflower oil emulsions where polysorbate 20 is used as surfactant throughout the 7 days of storage.

Table 6. TBARS values of chicken meat emulsions throughout 7 days of storage

TBARS (mgMA/kg)	0th day	3rd day	7th day
C	$0.25\pm0.01^{e,y}$	$0.25\pm0.03^{c,y}$	$0.94\pm0.03^{a,x}$
GE	$0.71\pm0.03^{b,y}$	$0.71\pm0.02^{b,y}$	$0.83\pm0.03^{b,x}$
GE-R	$0.62\pm0.02^{c,z}$	$0.97\pm0.00^{a,x}$	$0.86\pm0.03^{b,y}$
GE-A	$0.57\pm0.01^{d,y}$	$0.72\pm0.08^{b,x}$	$0.48\pm0.02^{c,y}$
GE-A/R	$0.87\pm0.01^{a,y}$	$1.04\pm0.13^{a,x}$	$0.53\pm0.01^{c,z}$

abcd: means with the different letter in the same column are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates. xyz: means with the different letter in the same row are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates

C: only beef fat added chicken meat emulsions, GE: only GE added chicken meat emulsions, GE-R : CME prepared with GE incorporated with rosemary extract in oil phase, GE-A: CME prepared with GE incorporated with ascorbic acid in water phase, GE-A/R: CME prepared with GE incorporated with rosemary extract in oil phase and ascorbic acid in water phase.

Table 7. Free fatty acid values of chicken meat emulsions throughout 7 days of storage

FFA (%oleic acid)	0th day	3rd day	7th day
C	$0.34^{c,z}\pm0.07$	$0.68^{d,y}\pm0.13$	$1.68^{b,x}\pm0.07$
GE	$1.01^{a,y}\pm0.08$	$0.83^{cd,y}\pm0.01$	$1.45^{b,x}\pm0.33$
GE-R	$0.36^{c,y}\pm0.12$	$1.15^{ab,xy}\pm0.15$	$1.69^{b,x}\pm0.28$
GE-A	$0.64^{b,y}\pm0.13$	$0.97^{bc,y}\pm0.13$	$2.79^{a,x}\pm0.55$
GE-A/R	$1.07^{a,y}\pm0.08$	$1.23^{a,y}\pm0.12$	$2.40^{a,x}\pm0.14$

abc: means with the different letter in the same column are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates. xyz: means with the different letter in the same row are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates.

C: only beef fat added chicken meat emulsions, GE: only GE added chicken meat emulsions, GE-R : CME prepared with GE incorporated with rosemary extract in oil phase, GE-A: CME prepared with GE incorporated with ascorbic acid in water phase, GE-A/R: CME prepared with GE incorporated with rosemary extract in oil phase and ascorbic acid in water phase.

3.5. Free fatty acid

The initial FFA values were between 0.34 and 1.07% (oleic acid) and increased to 1.45–2.79% (oleic acid) after 7 days of storage (Table 7). At each period of storage treatment formulated with beef fat had lower FFA value (0.34%). Microbial and muscle lipases attack mainly unsaturated fatty acids for hydrolysis. Since flaxseed oil contains high amounts of unsaturated fatty acids it was expected that lipolysis was more rapid in all modified treatments. It could be seen that, just after the production (day 0), FFA value of GE-No and GE-A/R showed similar trends

with TBARS values (Table 6). Samples with rosemary extract and control samples had similar FFA values at the beginning of storage. No significant differences were observed in the FFA values on the 3rd day of storage compared to day 0 except C samples. Ascorbic acid addition was ineffective to block the generation of FFA ($P<0.05$). FFA values of GE-No and GE-R were similar to C at the end of storage. However, on the 7th day, FFA values of all samples showed an increase, as a result of the hydrolysis of phospholipids which is in an agreement with other studies (Geçgel *et al.,* 2015; Reddy *et al.*, 2017).

3.6. Color

Table 8 shows the L* values of treatments formulated with GE and/or GE combined with antioxidants. L* values varied between 76.33-82.94, and the incorporation of GE resulted in higher L* values ($P<0.05$). Same findings also reported for model meat emulsions formulated with GE consisted of gelatin and inulin (Serdaroğlu *et al.,* 2016; Serdaroğlu and Öztürk, 2017).

Table 8. L*, a* and b* values of chicken meat emulsions throughout 7 days of storage

L*	**0th day**	**3rd day**	**7th day**
C	$76.33\pm0.49^{c,y}$	$78.69\pm0.13^{d,x}$	$78.06\pm0.50^{e,x}$
GE	$82.94\pm0.40^{a,x}$	$82.67\pm0.93^{a,x}$	$82.49\pm0.47^{a,x}$
GE-R	$82.30\pm0.39^{a,x}$	$82.33\pm0.16^{a,x}$	$81.76\pm0.32^{b,x}$
GE-A	$82.32\pm0.32^{a,x}$	$81.42\pm0.13^{b,y}$	$81.10\pm0.10^{c,y}$
GE-A/R	$78.75\pm0.50^{b,y}$	$80.11\pm0.37^{c,x}$	$80.33\pm0.26^{d,x}$
a*	**0th day**	**3rd day**	**7th day**
C	$0.65\pm0.16^{b,y}$	$2.07\pm0.17^{a,x}$	$1.98\pm0.13^{a,x}$
GE	$-0.20\pm0.11^{c,z}$	$0.77\pm0.15^{c,x}$	$0.51\pm0.02^{d,y}$
GE-R	$0.36\pm0.21^{b,y}$	$0.83\pm0.15^{c,x}$	$0.76\pm0.16^{c,x}$
GE-A	$0.51\pm0.25^{b,y}$	$1.31\pm0.13^{b,x}$	$0.99\pm0.03^{b,x}$
GE-A/R	$1.14\pm0.07^{a,x}$	$0.83\pm0.07^{c,y}$	$0.53\pm0.04^{d,z}$
b*	**0th day**	**3rd day**	**7th day**
C	$8.46\pm0.36^{c,x}$	$7.42\pm0.25^{c,y}$	$6.81\pm0.16^{c,z}$
GE	$14.24\pm0.06^{ab,x}$	$12.67\pm0.15^{b,z}$	$13.05\pm0.25^{b,y}$
GE-R	$14.48\pm0.22^{ab,x}$	$13.57\pm0.25^{a,y}$	$13.73\pm0.59^{a,xy}$
GE-A	$13.94\pm0.45^{b,x}$	$12.86\pm0.55^{b,y}$	$13.01\pm0.13^{b,y}$
GE-A/R	$14.70\pm0.22^{a,x}$	$13.84\pm0.21^{a,y}$	$13.89\pm0.36^{a,y}$

abc: means with the different letter in the same column are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates. xy: means with the different letter in the same row are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates.

C: only beef fat added chicken meat emulsions, GE: only GE added chicken meat emulsions, GE-R : CME prepared with GE incorporated with rosemary extract in oil phase, GE-A: CME prepared with GE incorporated with ascorbic acid in water phase, GE-A/R: CME prepared with GE incorporated with rosemary extract in oil phase and ascorbic acid in water phase.

Gelled emulsions have abundantly smaller droplet sizes than beef fat globules therefore the reflection of light on the surface of these treatments was more than in control groups. Increased L* values were also reported by Poyato *et al.* (2014). Using rosemary or ascorbic acid separately in GE did not affect initial L* values. L* values of GE-No and GE-R groups remained stable throughout the storage, while L* values of C and GE-A/R

samples increased. The results obtained on day 7 showed that C samples had the lowest L* values (P<0.05). Similar to our results, Barros *et al.* (2020) reported that tiger nut oil emulsion increased the L* values of beef burgers.

a* values of samples are presented in Table 8. Concerning a* values (red color), there was a significant increase from day 0 to 3rd day for all treatments (P<0.05). This increment indicates the conversion of meat pigment to nitroso pigments (Sakata, 2000). On day 0, GE treatment showed the lowest a* value, while GE-A/R had the highest (P<0.05). a* values were affected by the storage time (P<0.05). An increment followed by a decrement was seen in a* values of all treatments throughout the storage, C treatment showed higher a* values compared to other counterparts. After 3rd day of storage using GE and incorporating the antioxidants reduced the * values. Green tea extract-loaded fish oil gelled emulsion also had the same reducing effect on the fish sausages (Pourashouri *et al.,* 2020).

Yellowness (b*) does not greatly impact the appearance of meat color, however, this value is negatively correlated with the oxidation processes (Luciano *et al.,* 2009) while as the lipid oxidation proceeds a* values of meat decrease (Zhang *et al.,* 2013). The lowest b*values of control samples are related to the oxidation process. As can be seen in Table 5, on the last day of storage higher TBARS values were found for C samples, therefore the color of C treatment may have been negatively influenced.

b* values were changed between 6.81-14.70 during 7 days of storage (Table 8). At all storage periods, C treatment formulated with beef fat exhibited the lowest b* values (P<0.05). Similar findings also reported by Wang *et al.* (2018) in Harbin sausages formulated with camellia oil gelled emulsion at different levels. Also, an increase in b* values reported in hamburgers with animal fat is replaced with hydrogelled emulsion formulated with linseed oil and chia (Heck *et al.,* 2019). There was a general decline in all samples throughout the storage most probably due to the lipid oxidation reactions (P<0.05).

4. Conclusions

According to the results found in this study, it was possible to produce stable gelled emulsions formulated with rosemary extract and/or ascorbic acid. Oxidation reactions of model chicken meat emulsions were affected by both GE addition and antioxidant incorporation. Lipid oxidations at the end of the storage were lower in samples formulated with a gelled emulsion containing flaxseed oil by the reason of the preservation of easily perishable flaxseed oil against oxidation by surrounding with gel network which is created by inulin and gelatin. Gelled emulsion is a good source for highly perishable oils; however, inhibition of oxidation is more pronounced when ascorbic acid or ascorbic acid+rosemary extract loaded to the gelled emulsion formulation which was used as a fat source. Polar ascorbic acid was found the most effective antioxidant unlike 'polar paradox'. However further researches should be implemented with different emulsion formulations and antioxidants or different meat products

5.References

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