An Approach to Manufacture of Fresh Chicken Sausages Incorporated with Black Cumin and Flaxseed Oil in Water Gelled Emulsion

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Running title: The use of gelled emulsion in fresh sausage formulation

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Abstract

In order to investigate the use of oil in water gelled emulsion (GE) prepared with healthier oil combinations as beef fat replacer in the fresh chicken sausage formulations, four batches of fresh sausages were produced. The first batch was control (C) sample formulated with %100 beef fat, other batches were codded as GE50, GE75, and GE100 respective to the percentage of beef fat replaced with GE. The addition of GE to sausage formulation resulted in an increment in moisture and protein contents while a decrement was observed in fat content ($p<0.05$). pH, cooking yield and water holding capacity values of GE added samples were found lower than C ($p<0.05$). GE addition caused lower CIE L* values in samples, however, this trend was not observed in CIE a* and CIE b* values. Initially, the lowest peroxide and the highest TBARS values were recorded in GE100 samples on the 0^{th} d ($p<0.05$). Peroxide and TBARS values were in the limits. The texture of samples was softened while total saturated fatty acid content reduced up to 52.61% with the incorporation of GE ($p<0.05$). Taken together, our results showed that gelled emulsions can be used as fat replacers in meat product formulations without causing undesirable quality changes.

Keywords: black cumin oil, flaxseed oil, fresh sausage, gelled emulsion, oxidation

Introduction

Recently poultry meat has become very popular owing to its high biological value, contains essential amino acids, high unsaturated fatty acid content, vitamins, and other nutrients as well as its price (Pereira and Vicente, 2013). Sausage is one of the most popular food products consumed worldwide. Fresh sausages are products that are not heat-treated or cured. Fresh sausages are formulated with variable meat species such as pork, beef, chicken, fish, and fat. They are more or less coarsely minced or emulsified and also contain additives, such as salt, flavoring agents, spices, coloring agents depending on local preparations (Pearson and Gillet, 2012). The sausage dough is stuffed into natural casings and due to the absence of heat and curing treatments in the production steps, products have limited shelf life and they are susceptible to oxidative changes thus, ready products should be kept under cold storage conditions until consumption. (Pereira et al.,2019).

Fat is considered a fundamental material in meat product formulations since it gives desirable textural and sensory properties to the products. However, consumption of saturated fat has been linked with serious health problems such as cholesterol, obesity, cardiovascular diseases and some types of cancer (Forouhi et al., 2018), thus developing a healthy lipid profile has become one of the most important attempts in meat industry. Omega-6 and omega-3 fatty acids (PUFAs) are essential fatty acids that are taken from the diet, due to the deficiency of enzymes for omega-3 desaturation (Bhardwaj, 2016).

Black cumin oil is the essential oil from the seeds of *Nigella sativa*, contains high amount of thymoquinone and its related compounds such as thymol and dithymoquinone, which have been utilized in the prevention of inflammation and oxidative changes (Lutterodt et al., 2010).

Dominating fatty acids of black cumin oil are 57% linoleic acid (C18:2), 23.9– 24.1% oleic acid (C18:1) (Ramadan and Mörsel, 2002).

Flaxseed oil is polyunsaturated oil extracted from the flax plant (*Linum usitatissimim*) rich in α-linolenic acid (n-3) which is 50-60% of its total fatty acids. The high content of n-3 fatty acid present means that the consumption of flaxseed oil may have health benefits, thus enrichment of products with flaxseed oil enables to produce functional food products (Singh et al., 2011).

Even though modification of the fatty acid composition of meat products could be accomplished by using oil sources rich in polyunsaturated fatty acids such as black cumin or linseed oil in formulation, one of the important problems regarding the use of oils rich in polyunsaturated fatty acids (PUFAs) is their high susceptibility towards oxidation and consequent generation of rancidity. Therefore, these oils should be protected in order to make them more stable against oxidative changes during processing and storage (Carneiro et al., 2013). In this respect, pre-emulsions create an opportunity to incorporate healthy oil mixtures to meat systems for increasing mono and polyunsaturated fatty acids content since adding healthy oil mixtures directly to product formulation can have technological problems and quality issues in meat products (Serdaroğlu et al., 2017).

It was shown that gelled emulsions have the potential to carry functional compounds and replace the beef fat in meat products (Pintado et al., 2015; Poyato et al., 2014; Serdaroğlu et al., 2016). Gelled emulsions prepared with olive, linseed, fish and sunflower seed oils have been used in different products such as frankfurters (Delgado-Pando et al., 2010; Pintado et al., 2016), pork meat system (Salcedo-Sandoval et al., 2015), fresh sausages (Pintado et al., 2018); meatballs (Serdaroğlu et al., 2017), patties (Alejandre et al., 2017; Alejandre et al, 2019), burgers (Poyato et al., 2015),

meat emulsions (de Souza et al., 2018; Serdaroğlu et al., 2016), dry fermented sausages (Alejandre et al., 2016; Glisic et al., 2019) and bologna (de Souza et al., 2019a; Poyato et al., 2014) to improve fatty acid composition.

In this study, it was aimed to design a technological strategy to produce functional fresh sausages by replacing beef fat in formulation with oil in water gelled emulsion prepared with flaxseed and black cumin oil mixture, inulin, sodium caseinate and gelatin. The technological and nutritional quality parameters, as well as susceptibility of sausages to oxidation, are examined throughout the short storage period.

Materials and methods

Materials

Chicken thighs were supplied from Lezita (Abalıoğlu, Izmir, Turkey), beef fat was purchased from a local butcher store. Black cumin oil was obtained from a local producer (Şifahane-i Kübra, Izmir, Turkey) according to the specifications of the supplier, fatty acid composition of black cumin oil as follows; 28.22% oleic acid (C18:1), 7.61% palmitic acid (C16:0), 58.85% linoleic acid (C18:2), 3.50% stearic acid (C18:0), and 0.72% linolenic acid (C18:3) and other fatty acids). Flaxseed oil (18.38 % oleic acid (C18:1), 5.53% palmitic acid (C16:0), 15.07% linoleic acid (C18:2), 3.88% stearic acid (C18:0), and 54.58% linolenic acid (C18:3) and other fatty acids) was supplied from Ege University Agriculture Faculty (Izmir, Turkey). All the other ingredients were obtained from local market. Sodium caseinate (SC) and gelatin were purchased from Sigma-Aldrich, Co. (St. Louis, MO, USA), polyglycerol polyricinoleate (PGPR) and inulin (Ash Content: 0.05-0.15% Glucose: 0-1.6% Saccharose: 1.05-3.05% Dry Matter Content: 93-97% Carbohydrates: 94.90%

Inulin: 88-92% Fructose: 1.2-3.2%) were supplied from Smart Chemical (İstanbul, Turkey) and BENEO-Orafti (Istanbul, Turkey) respectively.

Preparation of gelled emulsion

Oil in water (O/W) gelled emulsion (GE) was prepared according to the method described by Poyato et al. (2014) with some modifications. The oil phase (50%) was prepared with mixture of black cumin and flaxseed oil (1:1). Oil phase also contained PGPR. The water phase (50%) consisted 2% sodium caseinate (per 100 g emulsion), 3% gelatin (per 100 g emulsion) and 7% inulin (per 100 g emulsion). Both phases were heated to 55℃ on a water bath (121 rpm) after mixing them separately in homogenizer (WiseTis HG-15D, DAIHAN Scientific, Wertheim, Germany) at 6000 rpm. After the mixing and heating stage, the oil phase was added onto the water phase in a high-speed homogenizer with heating equipment (TM-31 Vorwerk, Wuppertal, Germany) at 275 rpm, and then further emulsified at 700 rpm. The prepared emulsion stored at 4℃ overnight.

Experimental design and preparation of chicken fresh sausage

Four batches (Table 1) were prepared for each treatment. In control samples (C) 10% beef fat was added, in other formulations beef fat was substituted with GE at levels of 50% (G50), 75% (G75), and 100% (G100). Chicken thigh meat and beef fat were minced through a 3 mm plate grinder (Promeat W2000 Grande, Arnica, İstanbul, Turkey). Ground meat, fat source (beef fat and/or GE), salt, half of the ice and other additives mixed in cutter (330S, Alpina, Schweiz, Switzerland) for 5 min. The remaining part of the ice was added to the mixture and the emulsification process was continued for 3 more min. Sausage doughs were stuffed into natural edible casings (sheep intestine) using a hydraulic sausage filling machine (SG-Alpina, Schweiz, Switzerland) and stored in sealed

polypropylene bags at 4°C for 5 d. Analyses were performed on 0^{th}, 3^{rd} and 5^{th} d of storage (Fig. 1).

Gelled emulsion stability

Gelled emulsion stability was determined after the application of centrifugal forces at 323xg, 3 min and heat treatment at 70 ºC, 30 min (Serdaroglu et al., 2016; Surh et al., 2007). Creaming stability of gelled emulsion was measured according to the method described by Gu et al. (2005) after 7 d of storage at 4°C, the separated layer was measured and compared to initial sample height. Syneresis (S) was analyzed according to Bot et al. (2014). All parameters related to the stability of GE were determined in triplicate.

Chemical composition

Moisture and ash contents of raw and cooked chicken fresh dough (uncooked) and sausages (cooked) were determined according to AOAC (2002a, 2002b). Protein content of the samples was determined using an automatic nitrogen analyzer (FP 528, LECO, Michigan, USA) based on the Dumas method. Fat content was analyzed according to Flynn and Bramblet. (1975).

pH

pH value of GE and chicken fresh sausages were measured in triplicate by using a pH-meter (pH 3110 set 2, WTW, Weilheim, Germany) equipped with a glass penetration probe.

Emulsion stability

Twenty-five gram of raw emulsion was centrifuged for 1 min at 2634xg. The samples were heated in a water bath (70°C, 30 min) than tubes were centrifuged for 3 min at 2634xg. The pellets were removed and weighed and the supernatants were separated into pre-weighed crucibles and dried at

100℃. The volumes of total expressible fluid (TEF) and the expressible fat (EFAT) were calculated according to Hughes et al. (1997).

Water holding capacity

The ability of the uncooked product to keep moisture was assessed by using the method stated by Hughes et al. (1997) with modifications. Ten g batter was placed into jars and heated in 90℃ water bath (10 min), cooled and then samples were wrapped in roll bandage. Wrapped samples were centrifuged at 323xg for 15 min and weighed again (W_2). Water-holding capacity (WHC) was calculated from the equation below:

$$WHC\ (\%) = (1 - \frac{W1 - W2}{M}) \times 100$$

Where M indicate total moisture content of the sample.

Jelly and fat separation

Jelly and fat separation (JFS) of chicken fresh sausages were measured (Bloukas and Honikel., 1992). Raw emulsion sample was placed in glass jars and heated in a boiling water bath for 35 min (core temperature about 90℃). After heat treatment, the jars were cooled to room temperature and stored at 4℃ for 24 h. Jars were then re-heated at 45℃ for 1 h. The fluid jelly and fat were drained in a volumetric cylinder and measured in mL, JFS was calculated as a percentage of the original weight of the emulsion.

Cooking yield

Cooking yields of samples were conducted according to the Fang et al. (2019) with some modifications. The weights of raw chicken fresh sausages were recorded, then sausages put into the boiling water until the core temperature is reached 70℃. Internal temperature of sausages

156 monitored by inserting a thermometer (Thermo TA-288, Teknogreen, Sakarya, Turkey) into the
157 sausages. After cooking, samples were cooled and weighed again. The cooking yield of samples
158 was determined by calculating weight differences for samples before and after cooking.

159 **Color**

160 Lightness (CIE L*), redness (CIE a*) and yellowness (CIE b*) parameters of GE and cooked
161 chicken fresh sausages were determined by using a portable colorimeter (Chromameter CR400,
162 Minolta, Tokio, Japan).

163 **Purge loss**

164 Three bags per formulation were used to determine purge loss (PL) during chilled storage. After
165 the chicken fresh sausages were removed from the package, the exudate was dried with paper
166 towels and weighed again. The purge loss was calculated by weight difference and expressed as a
167 percentage of the initial weight.

168 **Peroxide value**

169 The peroxide value (PV) content of the samples was analyzed by the method of Koniecko (1979).
170 10 g of sample was weighed and homogenized with 60 mL of chloroform for 2 min and filtered
171 with Whatman No. 1 and 25 mL of filtrate is added into 250 mL Erlenmeyer. Filtrates were treated
172 with 30 mL of glacial acetic acid and 2 mL of saturated potassium iodide solution. Then,
173 Erlenmeyers were stirred and kept closed for 5 min in the dark. The flask was then added with 100
174 mL of distilled water and 2 mL of 1% fresh starch solution. Titration was carried out with 0.1 N
175 sodium thiosulfate and results were expressed in terms of per $mEqO_2$ (milliequivalent peroxide
176 oxygen)/kg.

TBARS

The 2-thiobarbituric acid reactive substances (TBARS) value was measured using the method of Witte et al. (1970). Twenty g of sample was homogenized with 50 mL cold solution containing 20% trichloroacetic acid (TCA) in 2 M phosphoric acid for 2 min 50 mL distilled water was then added and homogenized again for 1 min. After that, the slurry was filtered through Whatman No.1 filter paper into a 100 mL flask. The volume was completed to 100 mL by 1:1 TCA: distilled water. 5 mL of the filtrate was then pipetted into a test tube while another 5 mL of fresh chilled TBA (0.02 M in distilled water) was added. The tubes were incubated at 80℃ for 35 min and cooled to room temperature. The absorbance of the solution was measured with a spectrophotometer (T-60, PG Instruments, Leicestershire, UK) at 532 nm against blind solution prepared with 1:1 TCA-distilled water. The results were expressed as TBARS values (mg malonaldehyde/kg sample), which was calculated by multiplying the absorbance by 5.2. Each sample was analyzed in triplicate at each storage time.

Fatty acid composition

Lipid extraction from samples was performed according to Flynn and Bramblet (1975) and methylated (IUPAC, 1992). Analyses of Fatty Acid Methyl Esters (FAME) were carried out on a gas chromatograph (HP5890 Series, Hewlett-Packard, Wilmington, USA). The fatty acids were identified by comparison of their retention times of the sample with those of standards. Three determinations were carried out per sample (silica capillary column: DB-23, 30 m × 0.25 mm id., 0.25 μm film thickness, 100°C to 220°C at 4℃/min and 15 min at 220℃., J.W. Scientific and injector and detector (FID) temperature were kept at 220℃, flow rate of hydrogen 1 mL/min).

Texture Profile Analysis

Texture profile analyze (TPA) of cooked sausage samples was performed using a texture analyzer (CT3-4500; Brookfield Engineering Laboratories, Middleborough, USA) with TA4/1000 probe. Samples were cut into cylinders (20 mm height ×19 mm diameter) and placed on the instrument's base (two compression cycles, 4500 g load cell, 40% compression, 1 mm/s crosshead speed and 1 s time interval). Texture Expert version 1.0 software (Stable Micro Systems, England) was used to collect and process the data.

Statistical Analysis

All analysis was carried out in triplicate and one-way analysis of variance (ANOVA) was applied in order to observe the statistical differences between the chicken fresh sausages. Significant differences that have an effect on analysis are further analyzed by Duncan multiple test at 95% confidence level by using SPSS for Windows statistical package program (version 21.0, IBM, USA).

Results and Discussion

pH, Color (CIE L*, CIE a*, CIE b*) and Stability of Gelled Emulsion

O/W gelled emulsions are promising fat replacers in meat products thus their characteristics play an important role in quality attributes associated with animal fat in final products. pH, CIE L*, CIE a*, CIE b* and syneresis values of gelled emulsion are measured as 6.35, 83.01, 3.88, 25.35 and 0.052% respectively. pH value of GE is in the range of previous studies recorded by Pintado et al. (2015) and de Souza Paglarini et al. (2018). Similar to our study, Verheyen et al. (2018)

found that pH value of gelled emulsions containing sunflower oil, calcium carbonate, and glucono delta-lactone as 6.34.

Serdaroğlu et al. (2016) reported that CIE L*, CIE a* and CIE b* values of gelled emulsion prepared with olive oil, inulin and gelatin were 81.43, 3.71 and 15.98 respectively. CIE L*, CIE a* and CIE b* values of gelled emulsion manufactured by using extra virgin olive oil and whey protein isolate were determined as 84.96, -0.51 and 12.91 (Freire et al., 2018).

GE showed high emulsion stability against the centrifugation force and heat treatment. Also, Pintado et al. (2015) reported that gelled emulsion prepared with olive oil and cold gelling agents showed no noticeable syneresis or release. Gelled emulsion prepared with olive oil, inulin and gelatin showed high thermal stability (93%) (Delgado-Pando et al., 2010) and using 3% carrageenan and 1% algae oil in gelled emulsion formulation induced 1.14% syneresis (Alejandre et al., 2017).

Chemical composition

Chemical compositions of uncooked and cooked sausages are shown in Table 2 and Table 3. Moisture content of uncooked sausages changed between 66.41-69.47%. Moisture content increased with the addition of GE ($p<0.05$), this result was due to the high water content of GE. Moisture content was significantly lower in the control than in the GE added treatments. Pintado et al. (2015) observed the same pattern in frankfurters added olive oil-in-water gelled emulsion. There are no significant differences in moisture content of cooked sausages except GE50. GE50 samples showed the highest moisture content ($p<0.05$), similar findings reported by Poyato et al. (2014) in Bologna type sausages where 50% animal fat was replaced with conventional O/W emulsion or O/W gelled emulsion.

241 Fat content was decreased with the GE addition both in raw and cooked samples ($p<0.05$). Fat
242 content of GE50 was found lower than other counterparts with respect to increment in moisture
243 content. GE addition affected protein content of raw samples, GE75 and GE100 samples had higher
244 protein content than control and GE50 ($p<0.05$), this finding could be explained by using sodium
245 caseinate as an emulsifying agent in the gelled emulsion. The protein content of the cooked
246 samples did not change significantly with the addition of GE. Serdaroğlu et al. (2017) also reported
247 no significant differences in protein content of raw chicken patties formulated with gelled
248 emulsions however in cooked samples except 100% replaced samples.

249 While GE75 had the highest ash content among the raw samples, no significant differences were
250 found in the ash content of cooked ones. Alejandre et al. (2016) also found that incorporating
251 linseed oil gelled emulsions as fat replacer up to 39.5% did not affect the ash contents of dry
252 fermented sausage samples.

253 pH values of GE added fresh sausages were found lower than C on final product ($p<0.05$), cooked
254 sausage samples present no significant differences. Opposite to our findings, pH increasing effect
255 of gelled emulsions is reported by Pintado et al. (2018) in fresh sausages.

256 **Water holding capacity (WHC), jelly and fat separation (JFS), cooking yield (CY) and**
257 **emulsion stability (TEF, EFAT)**

258 Technological properties of meat emulsions such as cooking yield, emulsion stability, and water
259 holding capacity are some of the most important factors for the food industry to predict the behavior
260 of products during cooking. The technological properties of fresh sausages could be seen in Table
261 3. It can be recognized that GE addition resulted in a decrement in WHC of samples when the

replacement level was more than 50% ($p<0.05$). This could be explained by the addition of more than 50% GE induces dilution of meat proteins which are capable of hold the water in meat system.

Jelly and fat separation is a parameter that shows the ability of meat products to keep its moisture and fat. Jelly and fat separation of fresh sausages were affected by the addition of GE ($p<0.05$). GE added samples had higher values than C sample ($p<0.05$). The highest levels of JFS recorded in GE75 samples, while GE50 and GE100 had similar values.

In proportion to JFS values, the highest TEF and EFAT were observed in GE75 samples ($p<0.05$). The lowest fluid release was obtained in C samples, however, fat releases of C, GE50, and GE100 were similar. It could be evaluated as GE75 samples had the lowest emulsion stability between the treatments while C and GE100 had the highest emulsion stability. The reason for decrement in emulsion stability could be explained by the type of fat or ratio of protein in sausage formulation. Due to the low melting point, utilizing unsaturated fatty acids in products can cause a decrease in emulsion stability values.

The highest cooking yields were observed in C samples, this finding could be explained by the low meat protein content of GE added samples, since cooking yield depends on the ability of the protein matrix to stabilize both fat and water molecules. GE75 samples had the lowest cooking yield ($p<0.05$), which would likely be the result of the blocking effect of GE on the water binding ability of meat proteins. When gelatin is used at an appropriate concentration in meat emulsions, it acts as a stabilizer; promotes cooking yield, reduces fat and water losses due to its gelling ability (Serdaroğlu et al., 2017). However, increasing gelatin concentration resulted in a decrement in cooking yield since gelatin might be melted out and could not interact with the protein in MSME treatments during cooking (Serdaroğlu et al., 2017). Similar to our results, Serdaroğlu et al. (2016),

indicated that replacing beef fat completely with GE can have negative impacts on the JFS, cooking yield and WHC. In contrast to our results, meat emulsion formulated with perilla-canola oil (O/W) gelled emulsion showed better emulsion stability, cooking yield than control samples (Utama et al., 2018).

Color

The color of the meat product is one of the important parameters that the consumer can predict the quality during the purchasing. The color parameters of the samples could be seen in Fig. 2. The addition of GE significantly affected Lightness (CIE L*), redness (CIE a*), and yellowness (CIE b*) values of samples due to the color of flaxseed and black cumin oils in formulation. On 0^{th} d and throughout the storage GE added samples showed darker color than C samples. Increasing GE levels more than 50% decreased CIE L* values of the final product ($p<0.05$). GE addition induced CIE L* values to decrease in all formulations during 5 d of storage ($p<0.05$).

CIE a* values were measured 3.19, 3.76, 3.28, 3.99, and CIE b* values were measured 18.60,18.84, 16.68 and 18.07 for C, GE50, GE75 and GE100 respectively. During the storage, CIE a* values of all samples were increased, while CIE b* values of all samples were decreased. These changes can be explained by the lipid oxidation during the storage. Besides, this noticeable decrement in CIE b* values can be attributed to the isomerization and potential degradation of carotenoids in black cumin oil (Zepka et al., 2009). Similar to our results, Pintado et al. (2016) reported that storage period had an increasing effect on CIE a* values while decreasing effect on CIE b* values.

Poyato et al. (2014), reported that CIE L*, CIE a*, and CIE b* were significantly higher in gelled emulsion prepared with linseed oil and carrageenan added products compared to control samples.

Gel emulsion containing microalgal oil and a branch extract did not influence the CIE L*, CIE a* and CIE b* parameters of beef patties (Alejandre et al., 2019).

Purge loss

Purge loss in packaged meat products affects the appearance of the product negatively and also limits the shelf life of the product by making it more vulnerable to microbiological deterioration (Lopez-Lopez et al., 2009). Purge losses of fresh sausages formulated with different levels of GE are presented in Fig. 3. GE addition did not alter the purge loss, during the storage purge loss of samples increased significantly except GE50 ($p<0.05$). Similar to our results Salcedo-Sandoval et al. (2015) reported that purge losses of frankfurters formulated with liquid fish oil, fish oil/water emulsion and fish oil filled hydrogel particles are ranged between 0.13-0.58% control and samples formulated with hydrogel particles had similar purge losses.

Peroxide value

Autoxidation is a reaction between unsaturated fatty acids, regardless of whether they are in their free state or esterified as a triglyceride molecule and oxygen. These reactions originate from hydroperoxides, which are rapidly turn to aldehydes, ketones, alcohols, hydrocarbons, esters, furans and lactones (Almeida et al., 2019). Peroxide values of fresh sausages could be seen in Fig. 4. Initial peroxide values were similar in C, GE50, GE75, however, GE100 samples had lower peroxide values than other experimental counterparts ($p<0.05$). Since black cumin and flaxseed are highly perishable oils, GE100 samples which have high amount of these oils also showed high initial TBARS values (Fig. 5). Peroxide values of final products were higher than the values on the 3rd d of storage except for GE100 ($p<0.05$). This decrement might be the result of transformation of lipid peroxides to further lipid or protein oxidation products (Aalhus and Dugan, 2004).

On 5th d only GE75 and GE100 samples were higher than the peroxide values of the 3rd d ($p<0.05$). The highest peroxide values were seen in GE75 at the end of the storage ($p<0.05$). Higher peroxide values than control samples also reported by Pelser et al. (2007) in Dutch style fermented sausages formulated with flaxseed oil or flaxseed oil pre-emulsified with sodium caseinate. However, Alejandre et al. (2016) indicated that replacing pork fat at a level of 39.5% with linseed gelled emulsion did not affect the peroxide value of dry fermented sausage samples.

TBARS

Meat products are exposed to oxidation by the action of metal ions, unsaturated fatty acids, and reactive oxygen species. The toxic compounds formed as a result of lipid oxidation induce discoloration, poor taste, loss of nutritional value and reduction of shelf life in meat products. Changes in TBARS values of fresh sausages during the storage are given in Fig. 5. Initial TBARS values of sausages were between 0.58-1.26 mg malonaldehyde/kg. The highest TBARS value was found in GE100 sample while GE50 had the lowest value at d 0 ($p<0.05$). These results could be associated with the highest unsaturated fatty acid contents of GE100 samples. TBARS increasing effect of vegetable oils also declared by Choi et al. (2010) in reduced fat frankfurters formulated with 10% pre-emulsified olive, grape seed, corn, canola and soybean oils in combination with 10% pork back fat and 2% rice bran.

TBARS values of fresh sausages changed between 0.29–1.43 mg malonaldehyde/kg. No significant changes were observed in TBARS values of treated counterparts until 5th d. Throughout the storage, when the peroxide values decreased, TBARS values were increased, however, no significant differences were obtained except GE50 sample. GE50 samples showed a decrement on the 5th d and also showed the lowest TBARS values at the end of the storage ($p<0.05$). These results are thought to be caused by antioxidant compounds found in black cumin and/or flaxseed oil.

Thymoquinone and flavonoids are the main antioxidants in black cumin and flaxseed oils respectively (Lutterodt et al., 2010; Wang et al., 2017). At the end of the storage TBARS values of all samples were lower than 2 mg malonaldehyde/kg which is a limit declared by Witte et al. (1997). It could be said that replacing beef fat with gelled emulsion is a suitable application in terms of oxidative quality of short period stored fresh chicken sausages.

Increases in TBARS values throughout the storage could be explained greater formation ratio of malonaldehyde than the disappearance, however after a point the ratio of disappearance pass the ratio of formation then TBARS values decrease (Delgado-Pando et al., 2011). Changes in TBARS values could also derive as a result of intermolecular reactions of malonaldehydes with amino acids or proteins. Therefore, the rate of malonaldehyde loss/disappearance during storage may have exceeded the rate of production through lipid oxidation (Jamora and Rhee, 2002).

Similar to our results, replacing 50% of pork back fat with avocado, sunflower and olive oils in pork patties resulted in lower TBARS values than patties added 100% of pork back fat due to antioxidant substances present in avocado, sunflower and olive oils (Rodriquez-Carpena et al., 2012). Dutch-style fermented sausages reformulated with encapsulated fish oil had lower quantity of lipid oxidation products than control samples and pure fish oil added samples (Josquin et al., 2012).

Fatty acid composition

While black cumin oil contains 58.5% linoleic acid, 23.8% oleic acid, total unsaturated fatty acid content of this oil is 82.9% (Gharby et al., 2015) and flaxseed oil is rich in linolenic acid 55% (Dubois et al., 2007). Fatty acid composition of samples was given in Table 4. The addition amount of GE affected the fatty acid composition of sausages. Increasing addition level of GE resulted and

increment in polyunsaturated fatty acid (PUFA) content of sausages ($p<0.05$). The same trend was also reported by Delgado-Pando et al. (2010) in low fat frankfurters added olive, linseed and fish oil mixture-in-water emulsions and de Souza Paglarini et al. (2019b) in frankfurters formulated with GE prepared with soybean oil, soy protein isolate, carrageenan and inulin. Major SFAs were C16:0 (palmitic acid) and C18:0 (stearic acid), the major unsaturated fatty acids were C18:1 (oleic acid), C18:2 (linoleic acid) in samples prepared with 100% of beef fat. Likewise, Asuming-Bediako et al. (2014) found that major SFAs were C16:0 (palmitic acid) and C18:0 (stearic acid) and major unsaturated fatty acids were C18:1 (oleic acid), C18:2 (linoleic acid) in UK-style sausages formulated with pre-emulsified pork fat.

Replacing beef fat with GE had a reducing effect on the saturated fatty acid content of fresh sausages ($p<0.05$). The reduction percentage of saturated fatty acid (SFA) was 18.80%, 28.94% and 52.61% for GE50, GE75, and GE100 respectively. The reduction in the saturated fat content in GE75 and GE100 samples can confers to the attribute of "reduced in saturated fat" product, as they reached a reduction of more than 25% for American rules and more than 30% for European Commission (de Souza Paglarini et al., 2019b). Decreases in saturated fat content are originated from the addition of healthier oil combination which contained high amount of unsaturated fatty acid instead of animal fat is in the formulation.

GE100 sample registered the highest C18:1 + C18:2 (oleic acid + linoleic acid) content while the lowest C18:1 + C18:2 (oleic acid + linoleic acid) content were observed in control samples ($p<0.05$). These findings could be supported by the fatty acid composition of black cumin and flaxseed oil mixture. Due to the fatty acid composition of both oils, healthier combination of these oils constituted high amount of oleic acid and linoleic acid together with linolenic acid. In general,

integrating GE prepared with these oils to formulation enabled healthier meat products with high percentage of unsaturated fatty acids.

Texture profile analysis

The results of texture profile analysis are given in Table 5. Using GE prepared with flaxseed and black cumin oils as beef fat replacer in fresh sausage formulation altered the texture profile properties ($p<0.05$). All reformulated fresh sausage samples showed a softer texture compared to control prepared with 100% of beef fat. GE75 and GE100 samples had similar hardness values, also both groups showed the lowest hardness values among the samples ($p<0.05$). The reason for the lowest hardness values could be related to fat reduction process. While the source of protein (chicken meat) kept constant, fat content of the system was decreased, and the amount of water increased along with GE addition thus, texture became less dense (Jimenez-Colmenero et al., 1996). Gumminess and chewiness results were in parallel with each other. C and GE50 samples had the highest gumminess and chewiness while lower values were found in GE75 and GE100 ($p<0.05$).

Similar to our results Andres et al. (2009) observed lower hardness and chewiness values in chicken sausages formulated with squid oil than sausages formulated with beef tallow. Also reformulating fresh sausages with chia added olive oil emulsion gel resulted softer products (Pintado et al., 2018), however, the different behavior has been reported in emulsion sausages formulated with 100% canola oil (Baek et al., 2016).

Conclusion

The result of this study demonstrated that incorporating GE into the fresh sausage formulation showed a decreasing effect on the fat content of sausages. Higher beef fat replacement ratio than 50% lowered the water holding capacity. Increasing the use of gelled emulsion resulted in darker sausages most probably as a result of pigments in black cumin oil. From the oxidation perspective, even sample formulated with 100% gelled emulsion was found acceptable. The use of GE leads to the way production of reduced fat meat products with more than 50% of reduction in saturated fatty acid content. Major unsaturated fatty acids of fresh chicken sausages were oleic acid+linoleic acids. Further studies can be done to determine the effects of GE on functional, technological and also sensory properties of different meat products such as emulsion type meatballs and nuggets.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author's Contributions

Conceptualization: Serdaroğlu M. Formal analysis: Nacak, B, Kavuşan H.S. Methodology: Serdaroğlu M. Software: Kavuşan H.S. Validation: Serdaroğlu M. Investigation: Serdaroğlu M. Writing-original draft: Nacak, B, Kavuşan H.S. Writing-review & editing: Kavuşan H.S.

Ethics Approval (IRB/IACUC)

This article does not require IRB/IACUC approval because there are no humans and animal participants.

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Figure legends

Fig. 1. Production flow chart of fresh chicken sausages

Fig. 2. Color parameters of fresh sausages formulated with different levels of gelled emulsion

Fig. 3. Purge loss of fresh sausages formulated with different levels of gelled emulsion

Peroxide Values

Fig. 4. Peroxide values of fresh sausages formulated with different levels of gelled emulsion

Fig. 5. TBARS values of fresh sausages formulated with different levels of gelled emulsion

611 **Table 1. Chicken fresh sausage formulation**

Samples	Chicken meat (g)	Fat (g)	GE (g)	Water (g)	Salt (g)	Spice mix and curing ingredients* (g)
C	75	10	0	10	2	3.36
GE50	75	5	5	10	2	3.36
GE75	75	2.5	7.5	10	2	3.36
GE100	75	0	10	10	2	3.36

612 **C:** fresh sausages formulated without GE; **GE50**: fresh sausages formulated with GE as 50% fat replacer; **GE75**: fresh
613 sausages formulated with GE as 75% fat replacer; **GE100**: fresh sausages formulated with GE as 100% fat replacer.
614 *0.25% sugar, 0.5% black pepper, 0.2% red pepper, 0.1% white pepper, 0.2% coriander, 0.1% ginger and 0.015%
615 sodium nitrite were added in all formulations.

616

Table 2. Chemical composition of fresh sausages formulated with different level of gelled emulsion

Chemical composition of uncooked fresh sausages (%) and pH					
Samples	Moisture	Fat	Protein	Ash	pH
C	66.41^{b}±0.50	15.51^{a}±0.64	15.26^{b}±0.34	2.82^{ab}±0.03	6.16^{a}±0.01
GE50	69.33^{a}±0.05	12.53^{b}±0.14	15.29^{b}±0.25	2.62^{c}±0.006	6.14^{b}±0.01
GE75	69.47^{a}±0.08	10.86^{c}±0.10	17.35^{a}±0.28	3.10^{a}±0.24	6.14^{b}±0.06
GE100	68.88^{a}±0.61	9.97^{d}±0.59	18.31^{a}±1.05	2.90^{ab}±0.02	6.14^{b}±0.03

Chemical composition of uncooked fresh sausages (%) and pH					
Samples	Moisture	Fat	Protein	Ash	pH
C	60.98^{b}±0.26	16.79^{a}±0.17	21.24±0.27	3.13±0.21	6.31±0.01
GE50	64.68^{a}±0.76	13.09^{c}±0.52	19.98±0.76	3.16±0.15	6.32±0.01
GE75	61.06^{b}±1.26	14.37^{b}±0.61	21.33±1.33	3.29±0.24	6.32±0.01
GE100	61.87^{b}±0.91	14.69^{b}±0.62	20.62±0.42	3.09±0.16	6.31±0.01

***C:** fresh sausages formulated without GE; **GE50**: fresh sausages formulated with GE as 50% fat replacer; **GE75**: fresh sausages formulated with GE as 75% fat replacer; **GE100**: fresh sausages formulated with GE as 100% fat replacer. Data are presented as the mean values of replications ± standard deviation. abc: Means with the different letter in the same column are significantly different ($p<0.05$).

Table 3. Water holding capacity, jelly and fat separation, cooking yield and emulsion stability of fresh sausages formulated with different levels of gelled emulsion

Samples	WHC (%)	JFS (%)	TEF (%)	EFAT (%)	CY (%)
C	73.20^{a}±1.02	13.57^{c}±1.49	5.92^{c}±1.76	15.44^{b}±2.88	89.37^{a} ±1.09
GE50	71.13^{ab}±3.11	17.19^{b}±1.14	13.27^{ab}±2.91	18.32^{b}±3.11	84.40^{b} ±4.62
GE75	67.57^{b}±1.34	25.40^{a}±1.01	17.53^{a}±1.67	25.45^{a}±5.33	75.90^{d} ±0.43
GE100	69.35^{b}±1.43	16.83^{b}±0.97	11.48^{b}±2.21	14.81^{b}±1.82	80.24^{c} ±0.67

***C:** fresh sausages formulated without GE; **GE50**: fresh sausages formulated with GE as 50% fat replacer; **GE75**: fresh sausages formulated with GE as 75% fat replacer; **GE100**: fresh sausages formulated with GE as 100% fat replacer. Data are presented as the mean values of replications ± standard deviation. abc: Means with the different letter in the same column are significantly different ($p<0.05$).

Table 4. Fatty acid composition of fresh sausages formulated with different levels of gelled emulsion

Fatty acids (%)		C	GE50	GE75	GE100
C14:0	Myristic acid	$2.89^{a}\pm0.07$	$1.63^{b}\pm0.01$	$1.36^{c}\pm0.02$	$0.38^{d}\pm0.00$
C14:1	Methyl myristoleate	$0.28^{a}\pm0.03$	$0.16^{b}\pm0.02$	$0.12^{c}\pm0.01$	$0.03^{d}\pm0.01$
C15:0	Pentadecanoic acid	$0.72^{a}\pm0.01$	$0.38^{b}\pm0.01$	$0.34^{c}\pm0.02$	$0.06^{d}\pm0.01$
C16:0	Palmitic acid	$21.82^{a}\pm0.18$	$18.89^{b}\pm0.21$	$17.43^{c}\pm0.28$	$14.08^{d}\pm0.09$
C16:1	Palmitoleic acid	$2.52^{a}\pm0.04$	$1.66^{c}\pm0.05$	$1.93^{b}\pm0.03$	$1.14^{d}\pm0.06$
C17:0	Heptadecanoic acid	$2.85^{a}\pm0.05$	$1.30^{c}\pm0.01$	$1.39^{b}\pm0.02$	$1.00^{d}\pm0.04$
C18:0	Stearic acid	$24.55^{a}\pm0.05$	$20.48^{b}\pm0.03$	$16.91^{c}\pm0.01$	$9.25^{d}\pm0.04$
C18:1+C18:2	Oleic acid + Linoleic acid	$41.56^{d}\pm0.05$	$53.46^{c}\pm0.11$	$55.75^{b}\pm0.04$	$69.78^{a}\pm0.13$
C20:0	Arachidic acid	$0.81^{b}\pm0.02$	$0.88^{a}\pm0.01$	$0.68^{c}\pm0.02$	$0.66^{c}\pm0.03$
C18:3n6	γ -Linolenic acid	$0.06^{b}\pm0.01$	$0.05^{bc}\pm0.01$	$0.08^{a}\pm0.01$	$0.05^{c}\pm0.01$
C18:3n3	Linolenic acid	$0.29^{c}\pm0.01$	$0.32^{b}\pm0.01$	$0.40^{a}\pm0.01$	$0.28^{c}\pm0.01$
C20:2	cis-11,14-Eicosanoic acid	$1.23^{c}\pm0.02$	$0.42^{d}\pm0.01$	$2.26^{b}\pm0.01$	$2.60^{a}\pm0.03$
C20:3n6	cis-8,11,14-Eicosadienoic acid	$0.30^{c}\pm0.01$	$0.35^{b}\pm0.02$	$0.39^{a}\pm0.01$	$0.28^{c}\pm0.02$
C20:4n6	Arachidonic acid	$0.32^{b}\pm0.06$	$0.47^{c}\pm0.01$	$0.76^{a}\pm0.01$	$0.56^{b}\pm0.01$
∑	Saturated (SFA)	$53.66^{a}\pm0.26$	$43.57^{b}\pm0.25$	$38.13^{c}\pm0.29$	$25.43^{d}\pm0.06$
	Polyunsaturated(PUFA)	$46.57^{d}\pm0.07$	$56.90^{c}\pm0.21$	$61.69^{b}\pm0.08$	$74.72^{a}\pm0.19$

***C:** fresh sausages formulated without GE; **GE50**: fresh sausages formulated with GE as 50% fat replacer; **GE75**: fresh sausages formulated with GE as 75% fat replacer; **GE100**: fresh sausages formulated with GE as 100% fat replacer. Data are presented as the mean values of replications ± standard deviation. abc: Means with the different letter in the same column are significantly different ($p<0.05$).

Table 5. Texture profile analysis of fresh sausages formulated with different level of gelled emulsion

Samples	Hardness(N)	Springness(mm)	Cohesiveness	Gumminess(N)	Chewiness (N .mm)
C	3.34^{a}±0.21	4.41^{ab}±0.59	0.37^{b}±0.06	1.22^{a}±0.15	5.41^{a}±1.36
GE50	2.16^{b}±0.35	5.14^{a}±0.52	0.46^{a}±0.03	0.98^{a}±0.20	5.12^{a}±1.53
GE75	1.55^{c}±0.06	4.36^{ab}±0.14	0.40^{ab}±0.01	0.62^{b}±0.01	2.72^{b}±0.05
GE100	1.36^{c}±0.09	4.03^{b}±0.06	0.39^{b}±0.01	0.53^{b}±0.05	2.15^{b}±0.18

***C:** fresh sausages formulated without GE; **GE50**: fresh sausages formulated with GE as 50% fat replacer; GE75: fresh sausages formulated with GE as 75% fat replacer; **GE100**: fresh sausages formulated with GE as 100% fat replacer. Data are presented as the mean values of replications ± standard deviation. abc: Means with the different letter in the same column are significantly different ($p<0.05$).

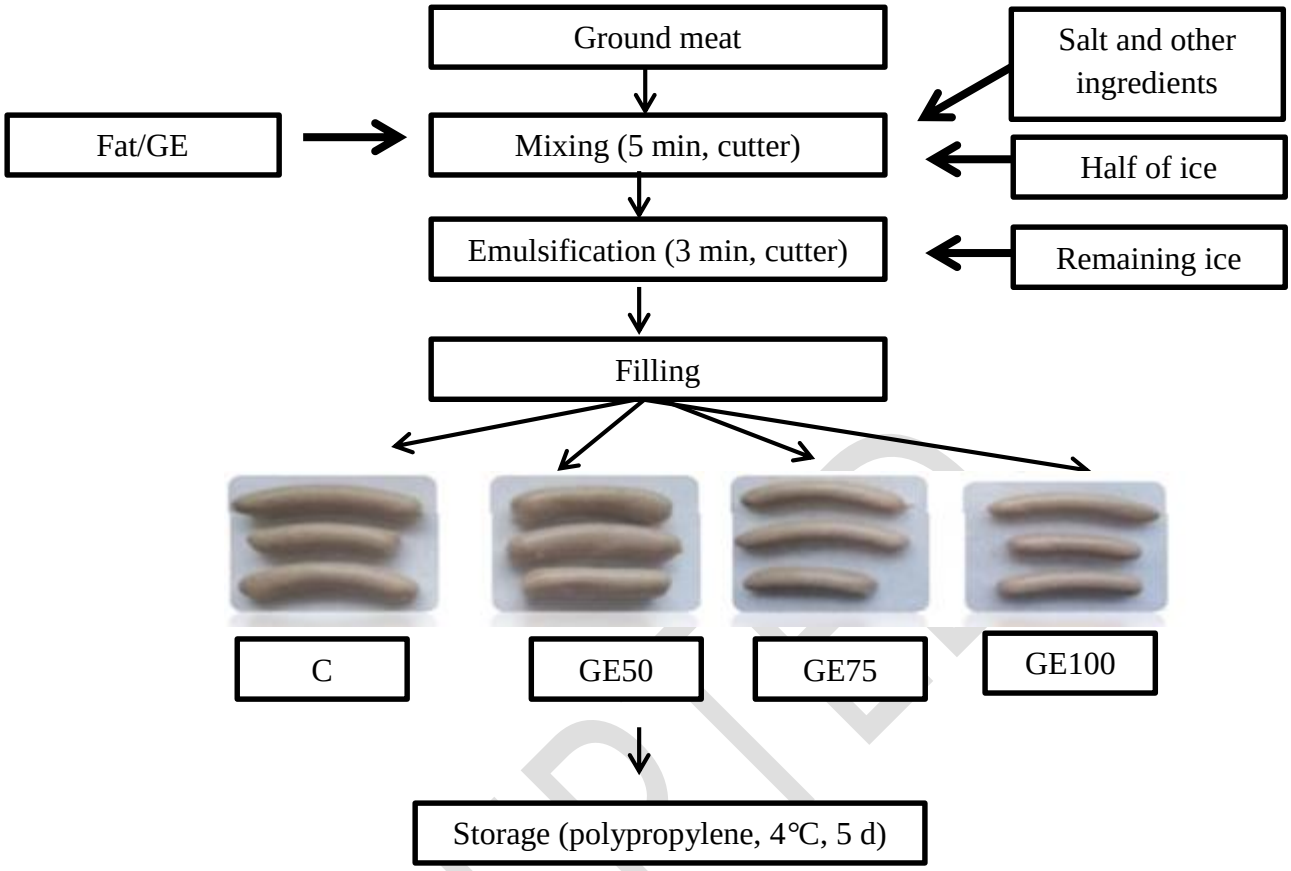
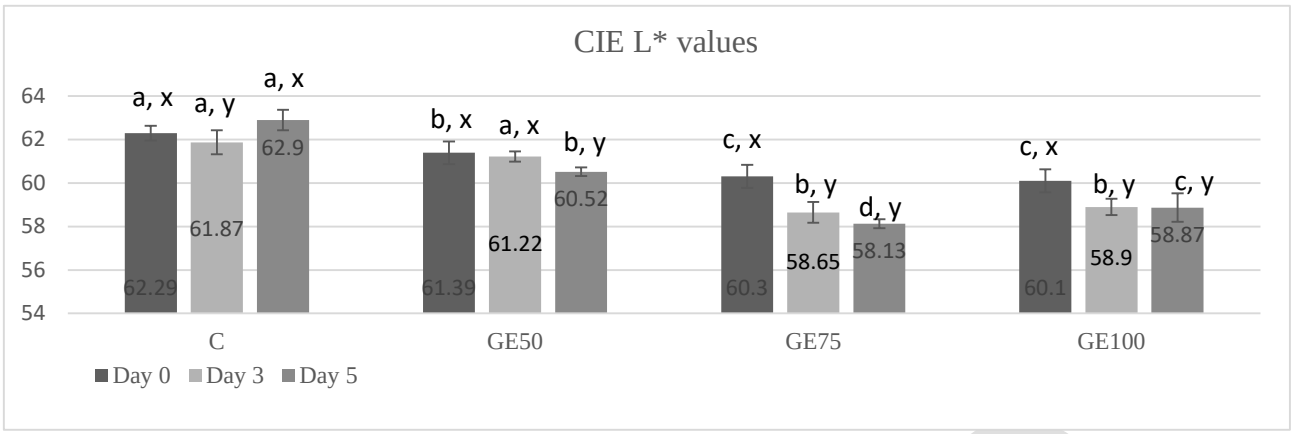
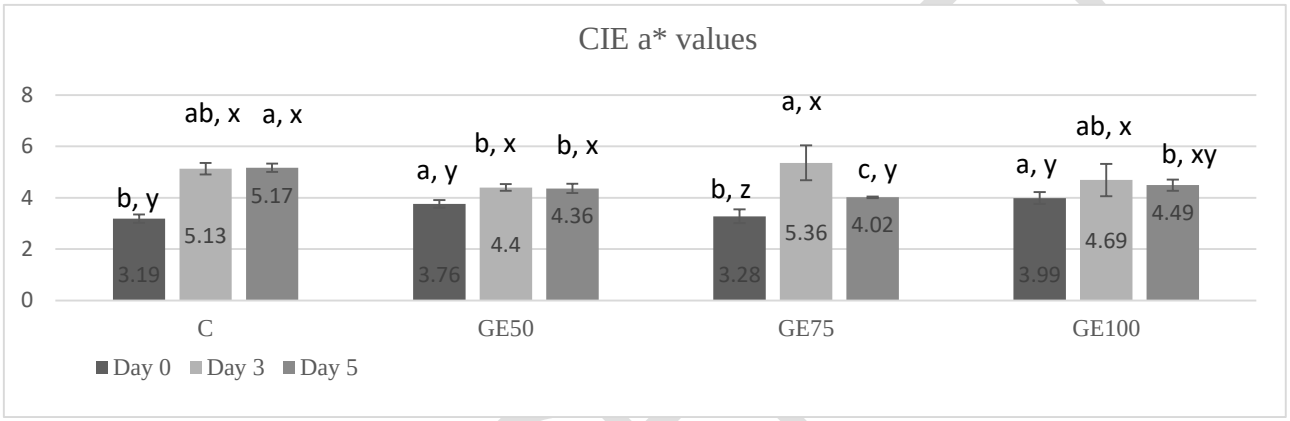


Fig. 1. Production flow chart of fresh chicken sausages

C: fresh sausages formulated without GE; **GE50**: fresh sausages formulated with GE as 50% fat replacer; **GE75**: fresh sausages formulated with GE as 75% fat replacer; **GE100**: fresh sausages formulated with GE as 100% fat replacer.





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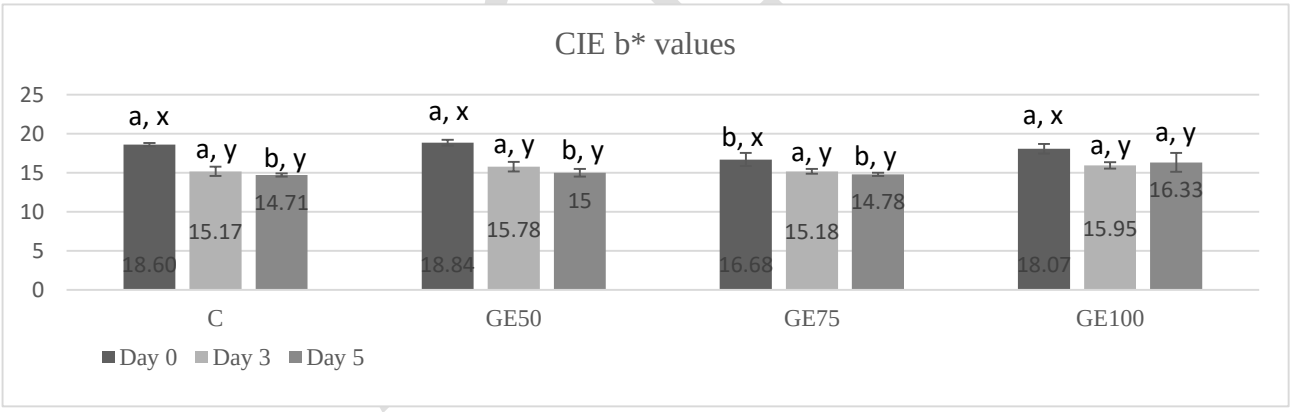

664

665 **Fig. 2. Color parameters of fresh sausages formulated with different levels of gelled emulsion**

666 abc: Means differences between groups, while, xyz means differences between storage time ($p<0.05$).

667 **C:** fresh sausages formulated without GE; **GE50**: fresh sausages formulated with GE as 50% fat
668 replacer; **GE75**: fresh sausages formulated with GE as 75% fat replacer; **GE100**: fresh sausages
669 formulated with GE as 100% fat replacer.

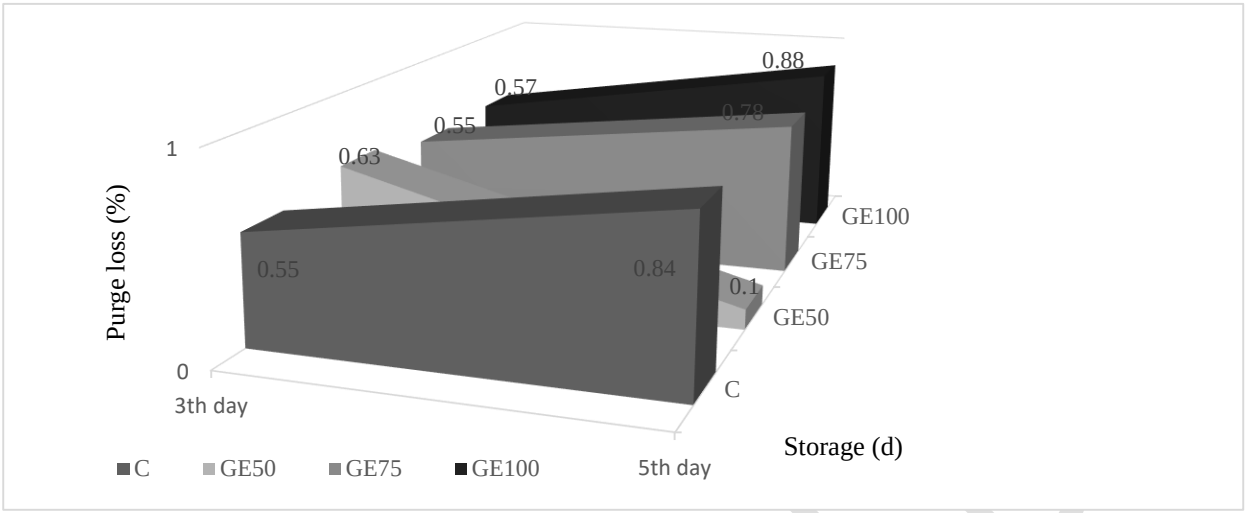

Fig. 3. Purge loss of fresh sausages formulated with different levels of gelled emulsion

C: fresh sausages formulated without GE; **GE50**: fresh sausages formulated with GE as 50% fat replacer; **GE75**: fresh sausages formulated with GE as 75% fat replacer; **GE100**: fresh sausages formulated with GE as 100% fat replacer.

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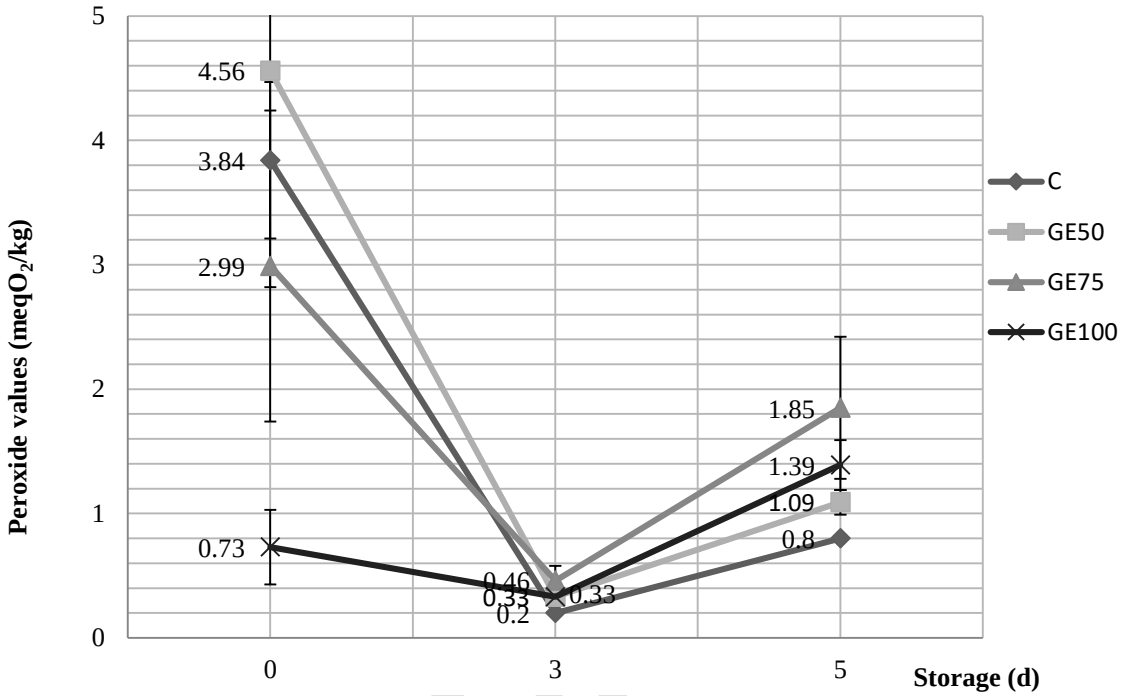

679

680 **Fig. 4**. **Peroxide values of fresh sausages formulated with different levels of gelled emulsion**

681 **C:** fresh sausages formulated without GE; **GE50**: fresh sausages formulated with GE as 50% fat
682 replacer; **GE75**: fresh sausages formulated with GE as 75% fat replacer; **GE100**: fresh sausages
683 formulated with GE as 100% fat replacer.

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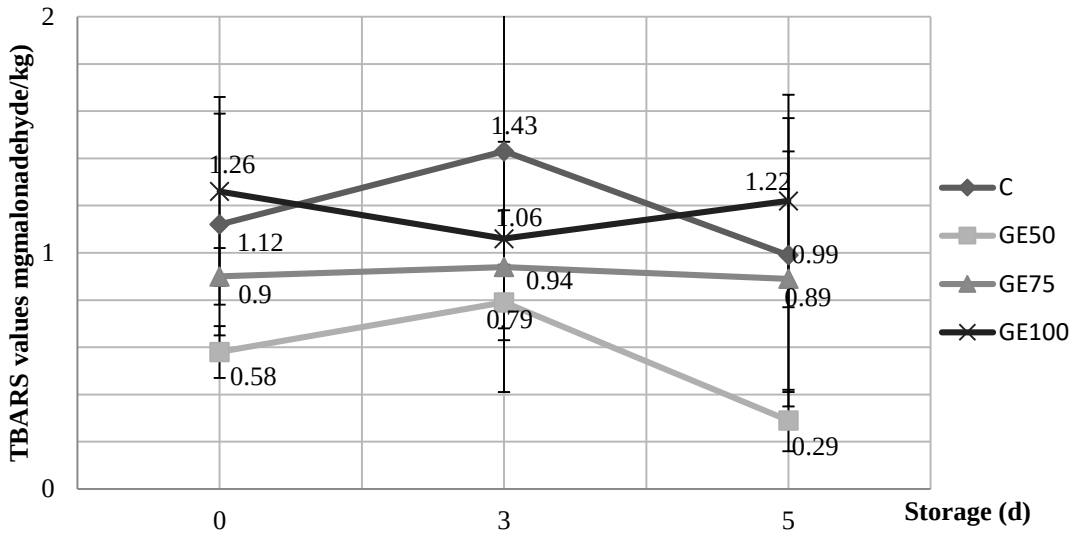

Fig. 5. TBARS values of fresh sausages formulated with different levels of gelled emulsion

C: fresh sausages formulated without GE; **GE50**: fresh sausages formulated with GE as 50% fat replacer; **GE75**: fresh sausages formulated with GE as 75% fat replacer; **GE100**: fresh sausages formulated with GE as 100% fat replacer.