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Oxidative Effects of Raw Chickpea in Reformulated Pork Patties: Level of Chickpea, Temperature, and Use of Selected Natural Antioxidants

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Abstract: Raw pulses as extenders in meat preparations result in oxidative processes. The oxidative effects of using a raw chickpea paste (CP; 1/2; chickpea/water) in pork patties were evaluated. In a first experiment, patties were prepared with increasing levels of CP (0 to 25%); in a second experiment, patties with CP (25%) and without CP (controls) were kept at 4 °C or 22 °C for 18 h before patty production; in a third experiment, chitosan, garlic, and cumin (from 0.5 to 2%) were added in patties with CP (25%) and controls, and their antioxidant effects were evaluated. Patties were analysed for pH, colour, and thiobarbituric acid reactive substances (TBARS) on days 1, 3, and 7 of refrigerated aerobic storage. Discoloration on day 1 and TBARS levels on days 1 to 7 of storage increased with the CP used. Higher batter temperature after mixing did not activate oxidative processes in the CP patties. Garlic showed pro-oxidant effects in controls and no effects in the CP patties. Chitosan and cumin did not reduce CP patties oxidation on the first day of storage, but they controlled oxidation during subsequent storage. More research is needed to prevent oxidation caused by using raw chickpeas in meat preparations.

Keywords: meat replacer; lipoxygenase; meat discoloration; meat oxidation; natural antioxidant

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1. Introduction

Partial replacement of meat with meat extenders, such as non-meat proteins, i.e., soy isolates, caseinates, or gluten, or fillers, such as starch or carrageenan, is a common practice in the meat industry, mainly aimed at reducing raw material costs and increasing water retention [1]. In recent decades, however, there has been growing interest in reducing the amount of meat in meat products for purposes beyond cost reduction and improved cooking yield [2,3]. The aim would be not only to produce meat products with a lower meat content, but also with a high nutritional value, not inferior to that of conventional products, a good sensory quality comparable to that of conventional meat products and a reduced environmental impact. The extenders proposed to replace meat in these novel reformulated meat products are dry grain legumes (pulses), cereals, oilseeds, or mushrooms. Pulses are a good and cheap source of dietary protein, with levels usually above 20%, fibre, minerals, and vitamins, and at the same time, their components have technological functionalities

necessary for their use in processing food applications, such as water retention, emulsification, and gelling [4]. Furthermore, their consumption plays an important role in the health of the world's population, as their regular consumption is associated with numerous health benefits, such as reduced risk of cardiovascular disease, cancer, diabetes, and hypertension, as well as reduced low-density lipoprotein cholesterol, among others [5,6].

In this context, there is a growing interest in the study of meat reformulated by the inclusion of pulses to produce so-called hybrid meat products. This research has mainly focused on fresh meat preparations such as hamburgers, meatballs, and fresh sausages [7–11]. In these studies, quantities of between 5% and 75% of pulses were tested in the formulations. Overall, the results show that the use of pulses in low–medium amounts (<15%) increases the cooking yield and firmness of meat preparations, without negatively affecting their sensory evaluation. However, the effects of reformulation depend not only on the amount of meat substituted, but also on the type of pulses [12] and their pre-treatment prior to use [13]. In this respect, studies do not show uniformity in the way pulses should be pre-treated before use (grinding into flour, soaking and grinding in water, heating, etc.).

Interestingly, the use of raw pulses, such as raw chickpeas, in meat preparations has been associated with oxidative changes, leading to lipid oxidation and resulting in off-flavours, described as "beany" or "hay", and discolouration, which compromises consumer acceptance [13–16]. These effects, which are eliminated when pulses or their flours are sufficiently heated before being added to meat, have been attributed to enzymatic oxidation of lipids presumably caused by the enzyme lipoxygenase contained in pulses. Lipoxygenases, non-heme iron dioxygenases, are natural enzymes that catalyse the deoxygenation of polyunsaturated fatty acids with a cis,cis-1,4-pentadiene moiety, thereby producing hydroperoxides and the subsequent formation of volatile lipid-derived compounds, mainly carbonyls. These can be responsible for desirable and undesirable flavours in pulses and processed food products containing them [17,18]. Lipoxygenase-mediated oxidation is induced by several factors, such as light, metal ions, and tissue disruption [19].

Different antioxidant families, such as competitive reaction substrates, e.g., ascorbic acid; Fe^{2+} chelating agents, e.g., catechins; reducing agents, e.g., selenide; or free radical inhibitors, e.g., tocopherols and some polyphenols, have been suggested to at least partially prevent lipoxygenase-associated oxidation of pulses in food applications [17]. However, few studies have been conducted to assess the efficacy of those antioxidants in preventing oxidation derived from pulses in meat matrices. Studies found in the literature on this topic report that an antioxidant composed of α-tocopherol plus and ascorbyl palmitate used at a concentration of 1% on fat content was able to partially inhibit the oxidative effect of raw chickpea in a fresh sausage [14], while the use of sodium ascorbate (0.5 g/kg) or sodium metabisulphite (0.45 g/kg) showed no antioxidant effect in a sausage batter [16].

Numerous natural ingredients or their extracts, containing high levels of phenolics or other active ingredients, have been proposed for use in meat products as an alternative to synthetic antioxidants [20]. Some of these natural ingredients may also increase the nutritional value of meat products or possibly contribute antimicrobial agents and functional components. Three natural ingredients recognised as having potential for use in meat preparations are chitosan, fresh garlic (*Allium sativum* L.), and cumin (*Cuminum cyminum*). Chitosan is a polysaccharide of animal origin whose use in fresh meat products has been shown to have a lipid oxidation stabilising effect, also contributing to retarding microbial growth and improving technological quality, i.e., texture and cooking performance [21–24]. Garlic is commonly used as a seasoning in fresh meat preparations, where its use at levels between 0.5 and 5% has been shown to improve lipid oxidation stability [25–27]. In terms of antioxidant effect, the most relevant active agents are specific organosulphur compounds and polyphenols. Cumin is a commonly used spice with a strong antioxidant potency attributed to a high phenolic content and specific terpenoids, such as cuminaldehyde [28,29], which shows competitive inhibition of lipoxygenase binding at the active site [30].

The aim of this study was to increase the knowledge on the effects of the use of raw chickpeas in reformulated fresh meat products on the discoloration and lipid oxidation

processes occurring during mixing and storage of these meat products. More specifically, the study had a threefold objective: (i) to assess the effects of the amount of chickpeas used on the oxidative processes triggered, (ii) to understand whether the temperature of the meat–chickpea mixture affects the degree of oxidation, and (iii) to evaluate the performance of a selection of natural ingredients or their extracts with proven antioxidant capacity, namely, chitosan, garlic, and cumin, on the oxidation generated by chickpeas.

2. Materials and Methods

2.1. Experiment Plan

The research consisted of three experiments to characterise the effects of using a raw chickpea paste (chickpea–water mixture, 1/2 *w*/*w*) as an ingredient (up to 25%) in reformulated pork patties (60% pork) on adverse changes in pH, discolouration, and lipid oxidation during refrigerated aerobic storage (up to 7 days). Three experiments were conducted, all including a set of control patties without chickpea paste and patties with chickpea paste. The control and chickpea-containing patties had always the same target amount of fat (15%). In addition, to obtain similar levels of starch, protein, and water (4, 16, and 68%, respectively) in the control and reformulated patties, adequate amounts of sodium caseinate and potato starch were included in the patty formulation where necessary. The experiments, summarised in Figure 1, and their purpose were as follows: experiment one, to evaluate the effect of the level of raw chickpea paste (four levels; from 0 to 25%) on the quality changes (pH, discoloration and oxidative stability of lipids) of patties during aerobic storage; experiment two, to evaluate the effect of temperature in the patty batter before forming (4 °C and 15 °C for 18 h) on these changes in patties with chickpea paste (25%); experiment three, to evaluate the antioxidant effect of selected natural ingredients used at two levels (chitosan, garlic, and cumin) on these changes in patties with chickpea paste (25%).

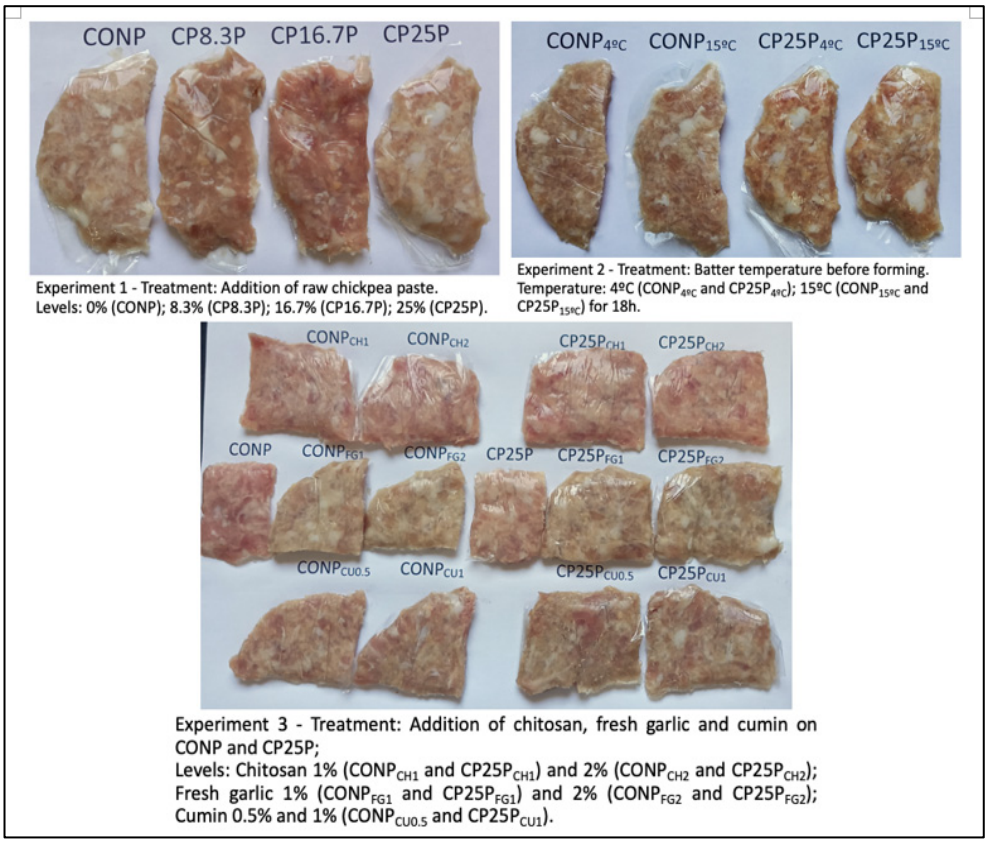

Figure 1. Treatments included in each of the three experiments carried out and the corresponding patty portions.

The patties were prepared at the Food Processing Pilot Plant of the Faculty of Veterinary Medicine (University of León, León, Spain). Once the patties were formed, they were packaged in trays covered with cling film and stored under aerobic conditions at 4 ± 1 °C for up to seven days. For each experiment, a randomised complete block design was followed by two replicates (two batches of patties, each prepared on a different day). Each batch contained from four to six treatments (formulations): four for experiments one and two, and six for experiment three. For each batch, three patties from each treatment were sampled on days 1 (24 h after patty packaging), 4, and 7 of storage for further analysis, i.e., three patties were sampled for each formulation x storage time combination for analysis. The sampled patties were analysed in duplicate for pH, instrumental colour, and thiobarbituric acid reactive substances (TBARS).

2.2. Ingredients Used in Patty Formulations

The ingredients used to make the patties were lean pork shoulder, pork back fat, sodium caseinate, sodium chloride, potato starch, cream-coloured chickpea (*Cicer arietinum* L. variety Pedrosillano; La Asturiana, Vidanes, León, Spain), and water. The natural ingredients used as antioxidants in experiment three were chitosan (C3646, deacetylation degree ≥75%, average molecular weight 1260 kDa; Sigma-Aldrich, St. Louis, MI, USA), fresh garlic, and cumin seed powder (*Cuminum cyminum*) purchased locally. The pork and pork fat were minced with a meat mincer through an 8 mm diameter plate just before preparing the patty mixes.

The chickpea paste was prepared in duplicate from 1 kg of chickpeas and 2 kg of water as follows: chickpeas were soaked in 3 L of distilled water for 24 h at room temperature (22 °C); the excess water was removed, the chickpeas were washed with tap water and then drained and weighed, and distilled water was added to the soaked chickpeas until a total weight of 3 kg was reached. The soaked chickpeas and added water were mixed using a Stephan UMC5 (Saint-Cannat, France) operating at 4 °C, 2400 r.p.m., and 0.5 atm for 8 min to form a paste. A 100 g sample of the paste was used for characterisation, which consisted of triplicate analysis of pH, composition, colour, TBARS (determined as indicated for patties), and lipoxygenase activity. Lipoxygenase activity was determined from a subsample of 2 g of paste following the methodology of [31]. The remaining chickpea paste was divided into three similar portions (approximately 1 kg each), vacuum packed, and frozen until further use.

2.3. Preparation of Patties and Sampling

2.3.1. Effects of the Amount of Raw Chickpea Paste on the Quality of Reformulated Patties

Four types of patty mixes (four treatments; 2 kg each) were prepared in duplicate (two batches) using 60% lean pork, 15% fat, and increasing levels of chickpea paste from 0 to 25% (Table 1): control patty, no chickpea paste, CONP; patty with 8.3% chickpea paste, CP8.3P; patty with 16.7% chickpea paste, CP16.7P; and patty with 25.0% chickpea paste, CP25P. Starch, caseinate, and water were added at different levels in order to obtain patties with similar target concentrations of protein, starch, and water, regardless of treatment. Next, 20 g of sodium chloride per kg was added to all mixtures. The meat and all ingredients were then mixed in a meat mixer for 6 min until a homogenous mass was obtained. Patties were then formed into 80 g units (10 cm in diameter and 1 cm thick) using a patty press, covered with food grade cellophane patty discs, and placed on a plastic tray. The trays with the patties were wrapped with transparent polyvinyl chloride film and stored at 4 ± 1 °C for up to seven days. On day 1, three patties CONP and CP25P were sampled for chemical composition analysis, pH, colour, and TBARS. In addition, on days 3 and 7, three other patties per treatment and per day of storage were sampled for pH, colour, and TBARS analysis.

Table 1. Formulations, expressed as percentage, for the patties [&] in experiment one.

	CONP	CP8.3P	CP16.7P	CP25P
Lean pork	60	60	60	60
Pork back fat	15	15	15	15
Water	19	12.7	6.4	-
Potato starch	4	2.7	1.3	-
Sodium caseinate	2	1.3	0.6	-
Chickpea paste [#]	-	8.3	16.7	25

CONP: control patty; CP8.3P, CP16.7P, and CP25P: 8.3%, 16.7%, and 25% chickpea-paste-containing patties, respectively. [&] The four patty mixes were also added with 2 g of common salt per 100 g of mix. [#] Prepared from one part of raw chickpea and two parts of water (weight/weight).

2.3.2. Effects of Temperature on the Quality of Reformulated Patties with Raw Chickpea Paste

A batter of a control patty and a patty containing 25% chickpea paste (two batches; 2 kg per batch) were prepared. The batter composition of both types of patties and the patty preparation process were the same as described in the previous experiment for CONP and CP25P (Table 1). The batters for each formulation were divided into two similar portions and wrapped in cling film. One of these portions was stored at 4 ± 1 °C for 18 h, and the other portion was subjected to a temperature abuse of 15 ± 1 °C for 18 h. Thus, the experiment had four treatments, two batter compositions x two temperatures (4 °C or 15 °C) during the 18 h storage period prior to patty formation: $CONP_{4°C}$, $CONP_{15°C}$, $CP25P_{4°C}$, and $CP25P_{15°C}$. Patties were then prepared from each treatment, placed in trays covered with cling film, and stored under refrigeration, as described in experiment two. The pH, colour, and TBARS of three patties from each batch and treatment were analysed on days 1, 3, and 7 of storage.

2.3.3. Effect of the Use of Selected Natural Ingredients on the Quality of Reformulated Patties with Raw Chickpea Paste

In this experiment, two batches of eighteen types of patties were prepared, nine of which used the same formulation and procedure as described in the previous experiments for CONP (without chickpea), while the other nine were for CP25P (Table 1), except that selected natural antioxidant ingredients (no antioxidant, chitosan, garlic, and cumin) were added to the formulations at two levels (low and high). Thus, the following treatments were obtained: two without antioxidants (CONP and CP25P), two with 1 g chitosan/100 g of batter ($CONP_{CH1}$ and $CP25P_{CH1}$), two with 2 g chitosan/100 g ($CONP_{CH2}$ and $CP25P_{CH2}$), two with 1 g fresh garlic/100 g ($CONP_{FG1}$ and $CP25P_{FG1}$), two with 2 g garlic/100 g ($CONP_{FG2}$ and $CP25P_{FG2}$), two with 0.5 g cumin/100 g ($CONP_{CU0·5}$ and $CPP25_{CU0·5}$), and two with 1 g cumin/100 g ($CONP_{CU1}$ and $CP25P_{CU1}$). For the CONP treatments, the antioxidant ingredients were added before the mixing of the ingredients, and for the CP25P treatments, the antioxidant ingredients were added to and mixed with the chickpea paste. After the mixing, patties were then formed, refrigerated, stored, and sampled for analysis following the abovementioned conditions in the previous experiments.

2.4. Quality Analysis in Patties

The chemical composition (moisture, protein, and ash content) of the raw CONP and CP25P from experiment one and of the chickpea paste (the same chickpea paste was used for all experiments) was determined in triplicate following the procedures recommended by [32], i.e., Official Methods 950.46, 991.36, 981.10, and 920.153, respectively. The pH of all patties was measured using a pH meter equipped with a penetration electrode calibrated with buffer solutions of pH 4 and 7. Instrumental colour was measured at three different points on the surface using a portable colorimeter (Konica Minolta CM-600d, Osaka, Japan) operating in SCI mode, with D65 illumination, 11 mm aperture, and 10° viewing angle. Colour was described in terms of lightness (L*), redness (a*), and yellowness (b*). Lipid

oxidation was estimated as TBARS value, which was measured according to [33] with modifications described by [16] and expressed as mg malondialdehyde equivalents/kg patty sample. Briefly, a 2 g aliquot of patty was homogenised at 9500 rpm for 60 s with 20 mL of distilled water using an IKA T-18 basic Ultra Turrax (Staufen, Germany). The mixture was then passed through a wire mesh filter and 1 mL of filtrate, 50 µL of 7.2% butylated hydroxytoluene ethanolic solution, and 1 mL of thiobarbituric acid 20 mM in 15% trichloroacetic acid were placed in a test tube, shaken, heated in a water bath at 80 °C for 20 min and cooled in cold water for 10 min, centrifuged at 4000× *g* for 20 min, and filtered through a 0.45 µm syringe filter. Afterwards, the absorbance was read at 531 nm, and the TBARS were quantified using 1,1,3,3-tetra-ethoxypropane standard solutions.

2.5. Statistical Analysis

The data were analysed by one-way analysis of variance (ANOVA; general linear model) using the software SPSS v.26 (IBM, Somers, NY, USA). For the chemical composition, the mean values obtained from each of the two batches of experiment one was compared by ANOVA. For the pH, colour, and TBARS values, two ANOVA were carried out. In the first one, the effect of treatment within each time was assessed using replication as a random factor. In the second, the effect of time within treatment was used as the fixed factor, and replication was also used as a random factor. When the analyses proved to be significant ($p < 0.05$), they were followed by the Tukey test, considering statistical significance for $p \leq 0.05$. Pearson correlations were calculated between the amount of chickpea paste added in experiment one and the corresponding dependent variables (pH, colour values, and TBARS).

3. Results and Discussion

3.1. Chickpea Paste and Patty Chemical Characteristics

The pH value, chemical composition (expressed as a percentage), colour (L*, a*, and b*), and TBARS values of the chickpea paste were as follows (mean ± standard deviation; $n = 2$): pH, 6.24 ± 0.02; moisture, 69.5 ± 0. 1; protein, 6.8 ± 0.2; lipid, 2.1 ± 0.2; ash, 0.86 ± 0.01; carbohydrate by difference, 20.7; L*, 77.7 ± 0.3; a*, 2.65; b*, 21.0 ± 0.1; and TBARS, 1.2 ± 0.1 mg malondialdehyde equivalents/kg. The amount of fibre in chickpea paste, according to the information provided on the chickpea package label (14.4 g dietary fibre/100 g chickpea), was 4.8%. The lipoxygenase activity of the chickpea paste was 20,036 ± 2217 units/g dry matter. The results of the compositional analysis and lipoxygenase activity of chickpea paste are consistent with those found in other studies on chickpea [31,34]. The composition of the control patty and the one reformulated with 25% chickpea paste is shown in Table 2. No significant differences in moisture, lipids, and protein were detected, as the formulation was designed to obtain similar levels between treatments. The use of chickpea paste increased ash and carbohydrate levels. The higher carbohydrate content in CP25P can be explained by the fibre (and starch) provided by chickpea paste, as starch and caseinate, added to CONP, do not contain fibre. CP25P was expected to have more ash, as the levels of minerals contributed by the chickpea paste (0.9% ash) to the patties would have been higher than those contributed by the sodium caseinate and starch added to the patties (according to the supplier's analytical data, the sodium caseinate and starch contained 3.5% and 0.8% ash, respectively).

Table 2. Chemical composition (%) of control and reformulated patties.

	CONP (n = 2)	CP25P (n = 2)	p-Level
Moisture	70.13 ± 0.53	67.01 ± 1.80	0.14
Lipids	8.01 ± 1.40	8.76 ± 1.38	0.64
Protein	15.83 ± 0.77	15.68 ± 0.15	0.81
Ash	2.72 ± 0.05	2.90 ± 0.02	0.042
Total carbohydrate [#]	3.31 ± 0.05	5.69 ± 0.55	0.026

CONP: control patties; CP25P patties with 25% of chickpea paste. [#] Obtained by difference: 100 − (% moisture + % fat + % protein + % ash).

3.2. Effects of the Level of Raw Chickpea Paste on the Quality of Reformulated Patties

Increasing levels of chickpea paste were used up to 25% (8.3% chickpea) in the patty formulation. The maximum value was within the range used in previous studies on fresh minced meat products reformulated with pulses and was below the levels found in those studies to negatively affect sensory acceptability [10]. The results obtained for pH, colour, and TBARS of patties with different levels of chickpea paste are shown in Table 3. The amount of chickpea paste did not affect the pH on the first day of storage. This is consistent with the pH value found in the chickpea paste (6.2), which was close to the normal values expected for pork. During storage under aerobic conditions, from day 4 to day 7, the pH of patties with higher amounts of chickpea paste increased significantly. This could have been the result of chickpea paste providing favourable conditions for the growth of aerobic psychrotrophic bacteria that produce metabolites related to proteolysis that contribute to the increase in pH. The growth of psychrotrophic bacteria can be used as an index of spoilage in minced meat stored under aerobic conditions [35]. The observed increase in pH is consistent with a study by [14], where the use of raw chickpea flour in a fresh sausage decreased the microbial quality of sausages stored under refrigeration.

The colour of raw patties was affected by the use of raw chickpea, with redness (a*) being the most affected coordinate. The a* values are shown in Table 3 and Figure 2. On the first day of patty storage, redness (a*) was lower in patties with chickpea. The decrease in a*, which is the consequence of myoglobin oxidation and result in patty discoloration, can negatively influence consumer intention to purchase meat preparations with pulses [9]. Raw chickpeas when mixed with minced meat would generate peroxides from polyunsaturated fatty acids, presumably via lipoxygenase activity, thus promoting pigment oxidation together with lipid oxidation [13,14]. Consistent with this study, Verma et al. [14] found that the mixing raw chickpea flour with meat in a reformulated sausage oxidised myoglobin to metmyoglobin (up to 80%), and that most of the formation of metmyoglobin took place during the first few minutes of mixing the batter. In this study, in addition to the previously described effect of raw chickpeas on colour oxidation [14], we found a relationship between patty discoloration and the level of chickpea in the formulation. Pearson's correlation analysis between the level of chickpea paste used (from 8.3 to 25%) and the a* values of patties with chickpea on day one of storage showed significance ($r = -0.618$, $p = 0.006$), indicating that the concentration of chickpea paste explained 0.38% of the variation in discoloration.

As for the other colour coordinates, on day 1, lightness (L*) increased with the amount of chickpea, and significant differences were found between CONP and CP25P. The effect of chickpea paste on L* was also observed on days 4 and 7 of storage and could have been related to the high L* value of chickpea paste (L* = 78), which was greater than that of meat and control patties. In contrast, the yellowness (b*) coordinate was not affected by reformulation.

Table 3. Effects of partial pork lean meat replacement with different levels of chickpea paste on the colour, pH, and lipid oxidation (TBARS) of the patties during refrigerated aerobic storage.

	CONP	CP8.3P	CP16.7P	CP25P	*p*-Level
pH					
Day 1	5.98 ± 0.02	6.00 ± 0.01	6.00 ± 0.20 ^{1}	5.99 ± 0.02 ^{2}	0.283
Day 4	5.95 ± 0.03 ^{c}	5.99 ± 0.01 ^{b}	6.01 ± 0.01 ^{ab,2}	6.02 ± 0.01 ^{a,2}	<0.001
Day 7	5.99 ± 0.03 ^{b}	6.00 ± 0.01 ^{b}	6.06 ± 0.02 ^{a,2}	6.08 ± 0.02 ^{a,1}	<0.001
p-level	0.060	0.090	0.003	0.003	
Color					
L*					
Day 1	53.62 ± 2.47 ^{b}	54.84 ± 1.20 ^{ab}	55.58 ± 1.84 ^{ab}	57.74 ± 0.66 ^{a}	<0.001
Day 4	52.42 ± 1.79 ^{b}	54.66 ± 0.64 ^{ab}	56.50 ± 1.80 ^{a}	56.89 ± 0.90 ^{a}	<0.001
Day 7	52.61 ± 3.17 ^{b}	55.90 ± 2.61 ^{ab}	56.28 ± 2.05 ^{ab}	57.90 ± 1.92 ^{a}	<0.001
p-level	0.093	0.260	0.088	0.100	
a*					
Day 1	8.97 ± 0.87 ^{a,1}	7.61 ± 0.96 ^{b,1}	7.49 ± 0.67 ^{b,1}	6.32 ± 0.19 ^{b,1}	0.002
Day 4	4.94 ± 0.23 ^{2}	5.03 ± 0.83 ^{2}	5.50 ± 0.46 ^{2}	5.31 ± 0.33 ^{2}	0.221
Day 7	5.16 ± 0.87 ^{2}	4.59 ± 0.63 ^{2}	4.76 ± 0.59 ^{2}	4.66 ± 0.35 ^{2}	0.102
p-level	<0.001	0.005	<0.001	0.002	
b*					
Day 1	17.95 ± 0.91 ^{1}	17.37 ± 1.12 ^{1}	17.82 ± 1.07 ^{1}	17.93 ± 0.69	0.620
Day 4	15.94 ± 0.67 ^{b,12}	16.98 ± 0.79 ^{a,1}	17.69 ± 0.68 ^{a,1}	17.32 ± 0.79 ^{a}	0.048
Day 7	14.58 ± 1.94 ^{b,2}	15.15 ± 0.1 ^{b,2}	16.69 ± 0.33 ^{ab,2}	17.80 ± 0.78 ^{a}	<0.001
p-level	0.003	0.026	0.016	0.346	
TBARS					
Day 1	0.28 ± 0.09 ^{d,2}	2.11 ± 0.36 ^{c,2}	2.65 ± 0.42 ^{b,2}	3.83 ± 0.34 ^{a,3}	<0.001
Day 4	0.67 ± 0.18 ^{c,12}	3.01 ± 0.22 ^{b,1}	3.52 ± 0.61 ^{b,1}	5.18 ± 0.33 ^{a,2}	<0.001
Day 7	0.75 ± 0.24 ^{c,1}	3.34 ± 0.24 ^{b,1}	3.90 ± 0.26 ^{b,1}	6.06 ± 0.51 ^{a,1}	<0.001
p-level	<0.001	0.004	0.023	<0.001	

TBARS: thiobarbituric reactive substances expressed as mg malondialdehyde equivalents/kg of patty. CONP: control patty; CP8.3P, CP16.7P, and CP25P: patties with 8.3%, 16.7%, and 25% of chickpea paste, respectively. ^{abc} Means within the same row showing any common letter superscript are significantly different ($p \leq 0.05$; Tukey post-hoc test). ^{123} Means within the same column showing any common number superscript are significantly different ($p < 0.05$; Tukey post-hoc test).

Storage time significantly reduced redness (a*) of patties from day 1 to 4 in all treatments. No decrease in a*, however, was observed from day 4 to 7. The reduction in a* during the first four days of storage could be attributed to oxidation of myoglobin. It was more intense in CONP and less intense in CP25P, with the reductions shown by CP8.3P and CP16.7P being in an intermediate position. As a result, from day 4 onwards, the value of a* became similar between treatments, irrespective of whether chickpea was used or not and the amount used. Similarly, Verma et al. [14] found that unoxidised myoglobin remaining in fresh sausages with and without raw chickpea at the time of packaging became progressively oxidised over a one-week storage period until it was completely oxidised in both the control sausage and the one containing chickpea. In this study, yellowness (b*) showed a significant and steady decrease during storage in all sausages except CP25P.

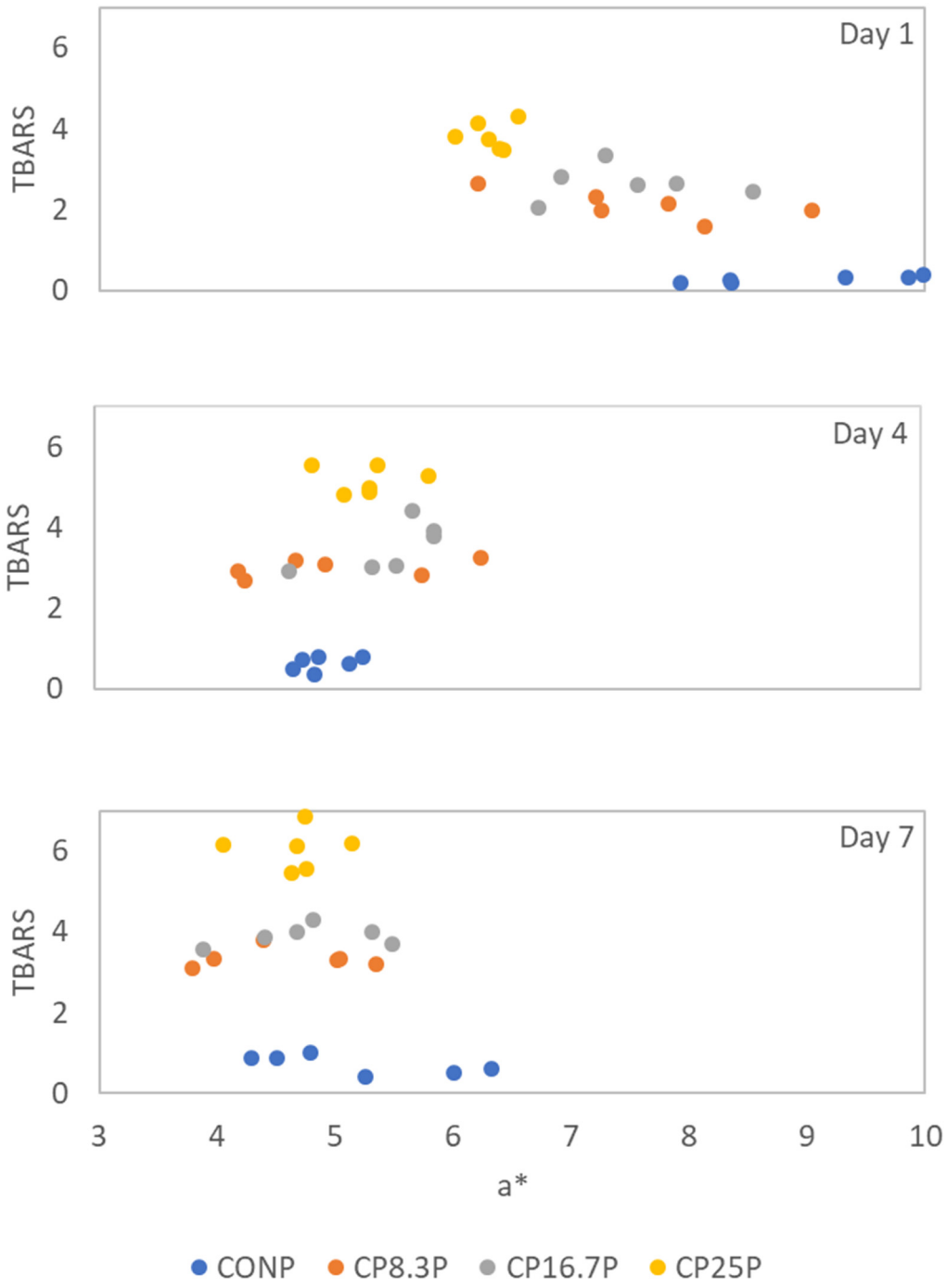

Figure 2. Values of thiobarbituric acid reactive substances (TBARS; expressed as mg of malondialdehyde equivalents/kg) vs. redness (a*) of the patties on each day of storage (n = 24). CONP: control patty; CP8.3P, CP16.7P, and CP25P: patties with 8.3%, 16.7%, and 25% of chickpea paste, respecitvely.

The TBARS assay was used to monitor lipid oxidation during patty storage. This is a method widely used in meat research and industry to assess the oxidative status a of meat or fresh meat products during short storage times, and TBARS levels have been correlated with the consumer perception of rancid flavour [36]. The TBARS values of the patties were significantly increased by the use of raw chickpea on day 1 of storage, and the increasing effect was also observed on days 4 and 7 (Table 3, Figure 2). A similar pattern was previously reported in fresh sausages [14]. Furthermore, the results of this study showed that lipid oxidation was related to the level of chickpea used, and the relationship was stronger than that found for discolouration versus chickpea level. On day 1 of storage,

the Pearson correlation between the level of chickpea paste (from 0 to 25%) and TBARS values was 0.952 ($p < 0.001$). Thus, accepting that oxidation is produced by chickpea lipoxygenase, these results are in line with previous studies in various non-meat food applications showing that the enzyme concentration determines the rate and extent of lipid oxidation [37]. Previous studies on meat products have found that lipid oxidation by raw chickpea has resulted in the formation of a number of volatile compounds that could negatively affect the acceptability of its aroma and flavour. [13,16].

As suggested by [14], a relationship between lipid oxidation and discoloration of patties was observed. The variation of TBARS in patties due to the use of chickpea showed a high correlation with the variation of a* (discoloration) on each of the three days of storage (Figure 2), i.e., the correlations between TBARS and a* were r = −0.775 ($p < 0.001$; $n = 24$) on day 1, r = −0.512 ($p = 0.011$; $n = 24$) on day 4, and r = −0.694 on day 7 ($p < 0.001$; $n = 24$).

Lipid oxidation in patties increased with storage time, and the increase was the lowest in CONP and the highest in CP25P. Verma et al. [14] also found that the lipid oxidation rate of a control fresh sausage (with no chickpea) during storage was lower than that of a sausage containing raw chickpea. This means that the oxidative effect mediated by the raw chickpea continues after the patty making during aerobic refrigerated storage. Results in this study also show that the rate of oxidation during storage was positively related to the level of chickpea used. In contrast, a previous study with reformulated cooked sausages containing raw chickpea showed no increase in TBARS during a similar period of aerobic storage [16]. The discrepancy could be attributed to the inactivation effect of sausage cooking on lipoxygenase activity.

3.3. Effects of Temperature of the Patty Batter on the Quality of Reformulated Patties with Raw Chickpea Paste

The oxidation of chickpea-containing patties, presumably mediated by lipoxygenase [13,17], could be affected by temperature, as the catalytic activity of lipoxygenases is temperature dependent, with the optimum being between 30 and 40 °C [38]. To determine the possible effect of temperature, a patty batter without chickpea (control) and one with 25% chickpea paste were prepared and kept in the dark for 18 h at two different temperatures: refrigerated (4 °C) and at abuse temperature (15 °C). The patties were then formed, packaged, stored, and refrigerated for up to 7 days and then analysed. Table 4 shows the effect of temperature on the patty quality traits. The temperature at which the patty batter was held before forming did not affect the pH on any of the storage days, except for a lower pH in $CONP_{15°C}$ compared to $CP25P_{4°C}$ on day 7. No increase in pH was observed in the chickpea patties during storage, indicating a possible lower growth of aerobic psychrophilic bacteria in CP25P in this experiment than in the previous one. The use of chickpeas in the patties, as seen in experiment one, tended to increase L* and decrease a*, further reducing a* during storage. In addition, lipid oxidation on day 1 increased with the use of chickpeas, and the oxidative effect of raw chickpeas continued for longer storage periods.

No effect of temperature on discoloration or lipid oxidation of patties containing chickpeas was detected at any of the storage periods. Therefore, contrary to our hypothesis, a higher temperature of the patty batter after mixing during the hours following mixing did not promote the oxidative processes triggered by the raw chickpeas. The lack of response of lipid oxidation to temperature suggests that lipoxygenase would have lost activity just after mixing. Therefore, the oxidation in CP25P observed on day 1 must have developed mainly during the mixing of chickpea paste and minced meat. In agreement, discoloration of a sausage batter containing raw chickpea flour was observed to occur immediately after mixing [14]. The probable loss of oxidative activity after mixing could have been the result of an irreversible change of the iron contained in the enzyme from the ferric to the ferrous form, as the active state corresponds to the high-spin oxidised Fe^{3+}, or inhibition of enzyme active sites by the secondary oxidation products formed [17,39].

Table 4. Effects of the temperature abuse in patty batter and formulation (control and chickpea-containing patties) on patty colour, pH, and lipid oxidation (TBARS) during refrigerated aerobic storage.

	CONP$_{4°C}$	**CONP$_{15°C}$**	**CP25P$_{4°C}$**	**CP25P$_{15°C}$**	***p*-Level**
pH					
Day 1	5.90 ± 0.01	5.86 ± 0.01	5.94 ± 0.04	5.94 ± 0.02	0.051
Day	5.87 ± 0.02	5.88 ± 0.05	5.95 ± 0.02	5.93 ± 0.02	0.149
Day 7	5.93 ± 0.03 ^{ab}	5.84 ± 0.04 ^{b}	6.00 ± 0.01 ^{a}	5.93 ± 0.01 ^{ab}	0.018
p-level	0.114	0.684	0.162	0.821	
Color					
L*					
Day 1	53.76 ± 0.45 ^{b}	53.96 ± 0.74 ^{b}	57.98 ± 0.85 ^{a}	58.17 ± 0.22 ^{a}	0.003
Day 4	53.09 ± 0.35 ^{b}	52.74 ± 0.88 ^{b}	58.72 ± 1.02 ^{a}	58.99 ± 0.83 ^{a}	0.004
Day 7	55.47 ± 0.93 ^{b}	54.57 ± 0.40 ^{b}	59.64 ± 0.34 ^{a}	59.03 ± 0.58 ^{a}	0.003
p-level	0.071	0.163	0.256	0.387	
a*					
Day 1	7.72 ± 0.39 ^{a,1}	6.20 ± 0.26 ^{b,1}	6.30 ± 0.35 ^{b,1}	6.13 ± 0.12 ^{b,1}	0.050
Day 4	4.69 ± 0.31 ^{2}	5.78 ± 0.28 ^{12}	5.06 ± 0.21 ^{2}	4.97 ± 0.33 ^{2}	0.070
Day 7	5.35 ± 0.58 ^{12}	5.23 ± 0.18 ^{2}	4.34 ± 0.13 ^{2}	4.96 ± 0.54 ^{2}	0.153
p-level	0.043	0.048	0.009	0.050	
b*					
Day 1	16.80 ± 0.96	16.03 ± 0.10	18.02 ± 0.49	18.36 ± 0.91	0.089
Day 4	16.13 ± 0.21 ^{b}	16.07 ± 0.47 ^{b}	18.44 ± 0.45 ^{a}	17.57 ± 1.01 ^{ab}	0.043
Day 7	15.27 ± 1.06	15.22 ± 0.11	17.36 ± 0.26	16.71 ± 0.93	0.094
p-level	0.323	0.094	0.165	0.352	
TBARS					
Day 1	0.29 ± 0.39 ^{b}	0.48 ± 0.41 ^{b}	4.47 ± 0.60 ^{a,2}	3.82 ± 0.82 ^{a,2}	0.019
Day 4	0.56 ± 0.06 ^{b}	0.75 ± 0.10 ^{b}	5.15 ± 0.59 ^{a,12}	5.02 ± 0.12 ^{a,12}	<0.001
Day 7	0. 86 ± 0.29 ^{b}	1.10 ± 0.52 ^{b}	6.35 ± 0.30 ^{a,1}	6.84 ± 0.23 ^{a,1}	<0.001
p-level	0.275	0.396	0.048	0.040	

TBARS: thiobarbituric reactive substances expressed as mg malondialdehyde equivalents/kg of patty. CONP: control patty; CP25P: patty with 25% of chickpea paste; $_{4°C}$ and $_{15°C}$: the patty batter was stored at 4 ± 1 °C or 15 ± 1 °C (temperature abuse), respectively, for 18 h before patty formation. ^{ab} Means within the same row showing any common letter superscript are significantly different ($p \leq 0.05$; Tukey post-hoc test). ^{12} Means within the same column showing any common number superscript are significantly different ($p \leq 0.05$; Tukey post-hoc test).

The subsequent increase in TBARS observed in the chickpea patties during storage from day 1 to 7 was also unaffected by the above temperature conditions. The progressive oxidation, not significant in the control patties, could have been the result of the gradual decomposition of residual hydroperoxides together with peroxy and alkoxy radicals [37] previously formed by the lipoxygenase enzyme during the mixing process, or by residual enzymatic activity. In contrast, in a previous study, Kasaiyan et al. [16] did not find that the use of raw chickpea increased lipid oxidation during aerobic storage of an emulsion-type cooked sausage with olive oil. The reason for this discrepancy could be the denaturation of the lipoxygenase enzyme during heating of the sausage together with the protective effect of olive oil against oxidation.

3.4. Effect of the Use of Selected Natural Ingredients on the Quality of Reformulated Patties with Raw Chickpea Paste

Lipid oxidation (and discoloration) in reformulated meat products promoted by the use of raw chickpea can be avoided by sufficiently heating the chickpea or chickpea flour (at least above 100 °C) before use [13,16]. If raw chickpeas are to be used, antioxidants capable of controlling the oxidation produced by the chickpea should be used. Three natural ingredients with proven antioxidant effect in meat systems were added to both control patties and patties with 25% chickpea paste. Tables 5–7 show the effects of chitosan, garlic,

and cumin, respectively, on pH, colour, and lipid oxidation of the control and chickpea paste patties stored under refrigeration.

The most obvious effect of using chitosan in patties was an increase in pH of approximately 0.5 pH units per g of chitosan added to 100 g of patty batter (Table 5). This effect was expected [22,40], as chitosan is a basic polysaccharide [23]. During storage, pH decreased significantly in $CONP_{CH1}$ and $CONP_{CH2}$, while no significant changes in pH were observed in the other patties. In contrast, a pH increase in patties containing chitosan (up to 2%) during patty storage was observed in other studies [22,40], although the increase was lower than that observed in patties without chitosan. The antimicrobial effect of chitosan [22] could have slowed bacterial growth in the patties in this study. However, the efficacy of chitosan as an antimicrobial agent would not have been equal in all patties, as its effect depends on the components of the meat matrix and the type of bacteria considered [41]. The decrease in pH observed for $CONP_{CH1}$ and $CONP_{CH2}$ suggests that chitosan may have selectively promoted the growth of acidifying microorganisms, such as lactic acid bacteria, in these patties during storage, and that the use of chickpea would have interfered with this effect.

Chitosan did not affect the L* values of the patties, irrespective of treatment and day of storage. However, it did affect a* and b* values. In the control patties, the use of chitosan reduced the a* and b* values on day 1 of storage, while no effect was observed on days 4 and 7. In patties with chickpea paste, chitosan did not affect a* and b* values. In patties with chickpea paste, chitosan did not cause any colour change during storage. Previous studies on the effect of chitosan on the colour of fresh patties have been inconclusive, as this effect was dependent on the molecular weight of the chitosan. An increase in L*, a*, and b* was observed in fresh patties added with a low molecular weight chitosan [22,40]. However, when a high molecular weight chitosan was used, as in this study, no effect on colour was observed [22]. The discrepancies between the studies could be at least partially explained by light scattering. Chitosan, and eventually other polysaccharides such as starch, could affect the light scattering properties of the patty surface in a variable and complex way, thus affecting colour values along with myoglobin concentration and chemical state [42].

During storage, redness decreased significantly in all control patties and in the patty containing chickpeas without chitosan (CP25P), but not in those containing chickpeas and chitosan. Previous studies have shown that the use of chitosan in patties at levels of 1–2% can exert a positive effect on red colour retention during frozen storage [22,43]. In this study, however, a protective effect of chitosan on colour was observed in patties containing chickpea paste, but not in patties without chickpea paste. In the three patties without chickpea and CP25P, the a* value on day 7 reached values indicating that most of the myoglobin had been oxidised to metmyoglobin [14], while in $CP25P_{CH1}$ and $CP25P_{CH2}$, the a* values on day 7 were higher, suggesting that some of the myoglobin remained unoxidised.

A lipid antioxidant effect of chitosan has been described in reformulated fresh meat preparations stored under refrigeration [21,40]. However, in this study, chitosan prevented neither lipid oxidation by raw chickpea on day 1 nor lipid oxidation during storage in all patties, although there was a statistical trend ($p < 0.1$) towards lower TBARS levels on days 4 and 7 in the patties with chickpeas. Overall, as an antioxidant agent, chitosan could be considered weak [44]. The antioxidant effect is based on chelating and radical scavenging activity, which depends on the molecular weight of chitosan [22,23,45]. In muscle products, chelating activity is more effective in cooked meat than in fresh meat. As for radical scavenging activity, it is lower for high molecular weight chitosan, the one used in this study, than for low molecular weight chitosan (<100 kDa) [21,40].

Table 5. Effect of the use of chitosan at two levels in control and chickpea-containing patties on their colour, pH, and lipid oxidation (TBARS) during refrigerated aerobic storage.

	Control Patties				Chickpea Paste Patties			
	CONP	**CONP$_{CH1}$**	**CONP$_{CH2}$**	***p*-Level**	**CP25P**	**CP25P$_{CH1}$**	**CP25P$_{CH2}$**	***p*-Level**
pH								
Day 1	6.04 ± 0.03^{c}	$6.63 \pm 0.02^{b,1}$	$6.97 \pm 0.20^{a,1}$	<0.001	6.03 ± 0.03^{b}	6.68 ± 0.17^{a}	6.83 ± 0.28^{a}	0.049
Day 4	6.02 ± 0.03^{c}	$6.52 \pm 0.01^{b,2}$	$6.86 \pm 0.04^{a,2}$	<0.001	6.05 ± 0.08^{c}	6.65 ± 0.01^{b}	6.85 ± 0.07^{a}	0.002
Day 7	5.92 ± 0.07^{c}	$6.45 \pm 0.02^{b,3}$	$6.74 \pm 0.01^{a,3}$	<0.001	5.96 ± 0.08^{c}	6.52 ± 0.01^{b}	6.77 ± 0.02^{a}	<0.001
p-level	0.056	0.003	0.007		0.47	0.34	0.49	
Colour								
L*								
Day 1	57.46 ± 2.49	57.67 ± 0.70	57.42 ± 0.59	0.99	56.62 ± 2.33	60.19 ± 1.26	60.58 ± 0.22	0.142
Day 4	58.13 ± 0.23	56.13 ± 1.95	56.91 ± 0.71	0.130	56.89 ± 0.90	60.02 ± 1.27	58.91 ± 0.71	0.69
Day 7	56.04 ± 2.59	56.34 ± 0.27	58.52 ± 1.16	0.65	58.73 ± 1.72	59.44 ± 0.84	59.52 ± 2.16	0.072
p-level	0.64	0.134	0.088		0.17	0.81	0.70	
a*								
Day 1	$8.01 \pm 0.11^{a,1}$	$5.82 \pm 0.25^{b,1}$	$6.09 \pm 0.56^{b,1}$	<0.001	7.80 ± 0.36^{1}	7.29 ± 0.3	6.70 ± 0.82	0.25
Day 4	5.79 ± 0.09^{2}	5.48 ± 0.24^{1}	5.77 ± 0.06^{1}	0.22	5.56 ± 0.35^{2}	6.34 ± 0.75	5.94 ± 0.47	0.46
Day 7	4.34 ± 0.37^{2}	3.87 ± 0.33^{2}	3.94 ± 0.37^{2}	0.056	$4.42 \pm 0.16^{b,2}$	5.69 ± 0.07^{a}	5.37 ± 0.32^{a}	0.019
p-level	<0.001	0.047	<0.001		0.004	0.077	0.21	
b*								
Day 1	17.66 ± 0.09^{a}	14.97 ± 0.52^{b}	15.15 ± 0.21^{b}	0.006	$17.24 \pm 0.1.69$	18.22 ± 0.64	18.92 ± 1.86	0.59
Day 4	15.94 ± 0.36	15.25 ± 0.63	16.32 ± 1.08	0.43	18.02 ± 0.07	17.32 ± 1.67	18.71 ± 0.49	0.48
Day 7	15.65 ± 0.79	16.12 ± 0.65	15.67 ± 0.32	0.72	16.77 ± 0.63	17.77 ± 0.23	18.22 ± 0.07	0.071
p-level	0.052	0.16	0.66		0.55	0.30	0.59	
TBARS								
Day 1	0.42 ± 0.11	0.44 ± 0.10^{2}	0.31 ± 0.15^{3}	0.23	3.42 ± 0.22^{2}	3.45 ± 0.16^{2}	3.31 ± 0.08^{2}	0.70
Day 4	0.56 ± 0.17	0.46 ± 0.04^{2}	0.39 ± 0.05^{2}	0.37	4.60 ± 0.31^{12}	4.08 ± 0.07^{12}	3.81 ± 0.24^{2}	0.089
Day 7	0.59 ± 0.29	0.68 ± 0.11^{1}	0.47 ± 0.02^{1}	0.78	5.72 ± 0.67^{2}	4.94 ± 0.37^{1}	4.58 ± 0.45^{1}	0.079
p-level	0.150	0.047	0.002		0.039	0.018	0.049	

TBARS: thiobarbituric reactive substances expressed as mg malondialdehyde equivalents/kg of patty. CONP: control patty; CP25P: patties with 25% of chickpea paste patty; $_{CH1}$ and $_{CH2}$: patties containing, respectively, 1 and 2 g chitosan/100 g of patty batter. ^{abc} Means within the same row showing any common letter superscript are significantly different ($p \leq 0.05$; Tukey post-hoc test). ^{123} Means within the same column showing any common number superscript are significantly different ($p \leq 0.05$; Tukey post-hoc test).

Table 6. Effect of the use of garlic at two levels in control and chickpea-containing patties on their colour, pH, and lipid oxidation (TBARS) during refrigerated aerobic storage.

	Control Patties				Chickpea Paste Patties			
	CONP	**CONP$_{FG1}$**	**CONP$_{FG2}$**	***p*-Level**	**CP25P**	**CP25P$_{FG1}$**	**CP25P$_{FG2}$**	***p*-Level**
pH								
Day 1	6.04 ± 0.03^{1}	6.06 ± 0.04^{1}	6.04 ± 0.01^{1}	0.70	6.03 ± 0.03	6.04 ± 0.06	6.04 ± 0.04	0.97
Day 4	6.02 ± 0.03^{12}	6.03 ± 0.01^{12}	6.00 ± 0.01^{1}	0.38	6.05 ± 0.08	6.02 ± 0.03	6.03 ± 0.04	0.80
Day 7	5.92 ± 0.07^{2}	5.91 ± 0.04^{2}	5.94 ± 0.01^{2}	0.38	5.96 ± 0.08	5.97 ± 0.08	5.94 ± 0.06	0.47
p-level	0.046	0.036	0.004		0.47	0.119	0.146	
Colour								
L*								
Day 1	57.46 ± 2.49	60.55 ± 0.01	57.11 ± 2.44	0.31	56.62 ± 2.33	60.57 ± 1.26	61.16 ± 0.28	0.108
Day 4	58.13 ± 0.23	57.33 ± 2.14	59.02 ± 3.51	0.79	56.89 ± 0.90	59.63 ± 0.99	59.70 ± 1.79	0.85
Day 7	56.04 ± 2.59	59.93 ± 0.31	58.68 ± 0.04	0.17	58.73 ± 1.72	61.80 ± 2.20	60.92 ± 2.80	0.89
p-level	0.64	0.154	0.74		0.17	0.48	0.74	
a*								
Day 1	$8.01 \pm 0.11^{a,1}$	$5.42 \pm 0.25^{b,1}$	$3.96 \pm 0.56^{c,1}$	0.006	$7.80 \pm 0.36^{a,1}$	$5.20 \pm 0.56^{ab,1}$	3.58 ± 0.64^{c}	0.024
Day 4	$5.79 \pm 0.09^{a,2}$	$1.98 \pm 0.66^{b,2}$	$2.44 \pm 0.56^{b,12}$	0.048	$5.56 \pm 0.35^{a,2}$	$4.48 \pm 0.12^{b,12}$	3.18 ± 0.37^{b}	0.010
Day 7	$4.54 \pm 0.07^{a,2}$	$1.73 \pm 0.06^{b,2}$	$1.28 \pm 0.04^{c,2}$	<0.001	$4.42 \pm 0.16^{a,2}$	$2.98 \pm 0.01^{b,2}$	2.89 ± 0.71^{b}	0.049
p-level	<0.001	0.049	0.044		0.004	0.048	0.57	
b*								
Day 1	17.66 ± 0.09	14.42 ± 1.51	16.69 ± 0.29	0.44	$17.24 \pm 0.1.69$	17.57 ± 0.40	17.59 ± 0.93	0.94
Day 4	15.94 ± 0.36	17.32 ± 1.86	16.67 ± 1.05	0.53	18.02 ± 0.07	18.17 ± 0.16	18.08 ± 0.93	0.96
Day 7	15.65 ± 0.79	17.53 ± 0.72	17.75 ± 1.14	0.079	16.77 ± 0.63	18.68 ± 0.68	18.82 ± 1.26	0.18
p-level	0.052	0.56	0.66		0.55	0.19	0.57	

Table 6. *Cont.*

	Control Patties				Chickpea Paste Patties			
	CONP	**CONP$_{FG1}$**	**CONP$_{FG2}$**	***p*-Level**	**CP25P**	**CP25P$_{FG1}$**	**CP25P$_{FG2}$**	***p*-Level**
TBARS								
Day 1	0.42 ± 0.11	0.59 ± 0.01 ^{3}	0.90 ± 0.02 ^{3}	0.23	3.42 ± 0.22 ^{2}	3.45 ± 0.82 ^{2}	3.45 ± 0.62 ^{2}	1.00
Day 4	0.56 ± 0.17 ^{a}	2.86 ± 0.06 ^{b,2}	4.24 ± 0.06 ^{c,2}	<0.001	4.60 ± 0.31 ^{12}	5.19 ± 0.05 ^{12}	5.72 ± 0.35 ^{12}	0.060
Day 7	0.59 ± 0.29 ^{c}	3.17 ± 0.11 ^{b,1}	4.75 ± 0.13 ^{a,1}	0.002	5.72 ± 0.67 ^{2}	6.97 ± 0.47 ^{1}	7.19 ± 0.47 ^{1}	0.085
p-level	0.150	<0.001	<0.001		0.039	0.018	0.031	

TBARS: thiobarbituric reactive substances expressed as mg malondialdehyde equivalents/kg of patty. CONP: control patty; CP25P: patties with 25% of chickpea paste; $_{FG1}$ and $_{FG2}$: patties containing, respectively, 1 and 2 g fresh garlic/100 g of patty batter. ^{abc} Means within the same row showing any common letter superscript are significantly different ($p \leq 0.05$; Tukey post-hoc test). ^{123} Means within the same column showing any common number superscript are significantly different ($p \leq 0.05$; Tukey post-hoc test).

Table 7. Effect of the use cumin at two levels in control and chickpea-containing patties on their colour, pH, and lipid oxidation (TBARS) during refrigerated aerobic storage.

	Control Patties				Chickpea Paste Patties			
	CONP	**CONP$_{CU0.5}$**	**CONP$_{CU1}$**	***p*-Level**	**CP25P**	**CP25P$_{CU0.5}$**	**CP25P$_{CU1}$**	***p*-Level**
pH								
Day 1	6.04 ± 0.03	6.03 ± 0.01 ^{1}	6.04 ± 0.32 ^{1}	0.92	6.03 ± 0.03	6.06 ± 0.05	6.06 ± 0.04	0.65
Day 4	6.02 ± 0.03	5.96 ± 0.01 ^{2}	6.02 ± 0.01 ^{1}	0.051	6.05 ± 0.08	6.03 ± 0.04	6.02 ± 0.03	0.90
Day 7	5.92 ± 0.07	5.88 ± 0.01 ^{3}	5.88 ± 0.08 ^{2}	0.60	5.96 ± 0.08	5.98 ± 0.07	5.99 ± 0.02	0.88
p-level	0.056	<0.001	0.042		0.47	0.40	0.17	
Colour								
L*								
Day 1	57.46 ± 2.49	56.19 ± 1.08	53.90 ± 0.61	0.23	56.62 ± 2.33	58.03 ± 0.76	57.05 ± 0.45	0.57
Day 4	58.13 ± 0.29	54.12 ± 1.70	53.97 ± 0.29	0.060	60.30 ± 0.82	58.36 ± 1.33	56.59 ± 0.07	0.059
Day 7	56.04 ± 2.59	56.65 ± 0.42	54.84 ± 0.14	0.55	58.73 ± 1.72	58.52 ± 0.91	56.79 ± 0.03	0.087
p-level	0.64	0.22	0.16		0.17	0.89	0.22	
a*								
Day 1	8.01 ± 0.11 ^{1}	7.55 ± 0.61 ^{1}	7.90 ± 0.47 ^{1}	0.61	7.80 ± 0.36 ^{1}	6.52 ± 0.53 ^{1}	5.53 ± 0.36 ^{12}	0.051
Day 4	5.79 ± 0.09 ^{2}	4.80 ± 1.05 ^{12}	4.32 ± 0.01 ^{2}	0.70	5.56 ± 0.35 ^{2}	5.98 ± 0.36 ^{12}	6.23 ± 0.08 ^{1}	0.22
Day 7	4.54 ± 0.07 ^{2}	4.32 ± 0.14 ^{2}	4.35 ± 0.14 ^{2}	0.40	4.42 ± 0.16 ^{2}	4.55 ± 0.14 ^{2}	4.59 ± 0.35 ^{2}	0.77
p-level	<0.001	0.021	0.002		0.004	0.045	0.026	
b*								
Day 1	17.66 ± 0.09 ^{b}	17.27 ± 0.20 ^{b}	18.90 ± 0.01 ^{a}	0.002	17.24 ± 0.69	18.51 ± 0.28	19.17 ± 0.76	0.33
Day 4	15.94 ± 0.36 ^{b}	18.06 ± 1.45 ^{ab}	18.30 ± 0.29 ^{a}	0.030	18.02 ± 0.07 ^{b}	18.07 ± 0.05 ^{b}	19.38 ± 0.34 ^{a}	0.011
Day 7	15.65 ± 0.79	15.91 ± 0.42	17.53 ± 0.70	0.117	16.77 ± 0.63	18.26 ± 1.08	19.17 ± 0.07	0.098
p-level	0.052	0.24	0.17		0.55	0.81	0.89	
TBARS								
Day 1	0.42 ± 0.11	0.27 ± 0.02 ^{3}	0.22 ± 0.01 ^{2}	0.113	3.42 ± 0.22 ^{2}	3.84 ± 0.94	3.74 ± 0.87	0.80
Day 4	0.56 ± 0.17	0.41 ± 0.02 ^{2}	0.47 ± 0.43 ^{1}	0.091	4.60 ± 0.31 ^{a,12}	3.55 ± 0.07 ^{b}	3.03 ± 0.04 ^{b}	0.008
Day 7	0.59 ± 0.29	0.65 ± 0.03 ^{1}	0.55 ± 0.01 ^{1}	0.59	5.72 ± 0.67 ^{a,1}	3.87 ± 0.46 ^{ab}	3.19 ± 0.68 ^{b}	0.048
p-level	0.150	0.002	<0.001		0.039	0.84	0.59	

TBARS: thiobarbituric reactive substances expressed as mg malondialdehyde equivalents/kg of patty. CONP: control patty; CP25P: patties containing 25% of chickpea paste; $_{CU0.5}$ and $_{CU1}$: patties containing, respectively, 0.5 and 1 g cumin/100 g of patty batter. ^{ab} Means within the same row showing any common letter superscript are significantly different ($p \leq 0.05$; Tukey post-hoc test). ^{123} Means within the same column showing any common number superscript are significantly different ($p \leq 0.05$; Tukey post-hoc test).

The addition of fresh garlic to the patties did not affect the pH on the first day (Table 6). The pH of the patties tended to decrease during storage, suggesting, as already mentioned, a growth of lactic acid bacteria. However, this effect was only significant in control patties with and without garlic. Neither L* nor b* were significantly affected by the addition of fresh garlic or storage time in any of the patties. In contrast, the use of fresh garlic proportionally and significantly reduced the redness (a* values) of the patties on the three days of storage. Discoloration due to garlic was also observed in in pork patties added with aqueous and methanolic garlic extracts and minced beef with 1 or 2% crushed garlic [27,46]. In this study, garlic produced a discoloration on day 1 of similar magnitude in patties with and without chickpeas. On days 4 and 7, however, the discoloration was greater in the patties without chickpeas. Specific compounds in garlic that interact with myoglobin and

accelerate iron oxidation, e.g., compounds that promote the generation of hydroxyl radicals, could be responsible for the negative effect of garlic on meat colour [47]. These results warrant further research to understand the reaction mechanism.

As for TBARS results, garlic promoted lipid oxidation of the patties during storage. Patties with garlic showed higher TBARS levels than patties without garlic on days 4 and 7, and TBARS levels tended to be higher in patties with higher levels of fresh garlic. Consistent with our results, Cózar et al. [48] found that 0.1% garlic powder increased discoloration and lipid oxidation in lamb patties during refrigerated aerobic storage, and Mariutti et al. [49] reported prooxidation of garlic, also 0.1% garlic powder, in chicken patties during frozen storage. In contrast, other studies showed how the use of garlic in fresh sausages or patties, at levels similar to those used in this study, acted as an antioxidant by reducing lipid oxidation [26,27]. The mechanisms and conditions by which garlic may act as a pro-oxidant or antioxidant agent do not seem to be clear [49].

Cumin did not change the pH of the patties on day 1 (Table 7). However, during storage, cumin promoted a significant decrease in pH in patties without chickpeas, but not in patties containing chickpeas. The effect of cumin on colour was limited to a significant increase in b* in patties without chickpeas on days 1 and 4 and in patties containing chickpeas on day 4. This change could be explained by the effect of the pigments provided by the spice. The addition of cumin did not prevent the discoloration of the patties caused by the use of chickpeas on day 1 of storage, i.e., TBARS values in CP25C were similar than those in $CP25P_{CU0·5}$ and $CP25P_{CU1}$. The Fe^{3+} reducing effect of cumin as a reducing agent and inhibitory activity of cumin on lipoxygenase described in vitro studies [30,50] were not effective under the conditions used in this study. However, cumin prevented, at the two levels used, the lipid oxidation (TBARS formation) that occurred during patty storage from day 1 to day 7 in patties containing chickpeas.

In summary, none of the tree natural ingredients used in this study were able to reduce the oxidation produced by the mixing raw meat and raw chickpea. However, chitosan, and more clearly cumin, could be useful in partially controlling the oxidation developed during storage of the chickpea patties. In contrast, garlic did not retard the oxidative processes of the chickpea patties during storage.

4. Conclusions

The use of raw chickpea as a meat extender in pork patties causes sudden oxidative discoloration and lipid oxidation in the patties, which might negatively affect the sensory quality. In addition, raw chickpea causes an increase in the lipid oxidation rate during subsequent aerobic storage of the patties. The degree of discoloration and lipid oxidation are interrelated and depend on the amount of chickpea used in patty formulation. The degree of oxidation caused by the use of raw chickpeas is not affected by temperature abuse (up to 15 °C) of the patty batter (up to 18 h) between mixing the ingredients and forming the patties. This suggests that strict temperature control is not necessary during the patty production process, as long as hygiene and sanitary standards are met. High molecular weight chitosan, garlic, and cumin, at the levels used, are not useful in inhibiting discoloration and lipid oxidation in patties containing raw chickpeas within 24 h of patty preparation. However, chitosan and cumin could be useful to partially control oxidation developed during subsequent storage of the patties. Garlic, in contrast, does not promote or retard oxidative processes in the patties containing chickpeas. In order to reduce the negative consequences of oxidation associated with the use of raw chickpeas as an ingredient in patties or similar meat products, further research is needed to find more effective antioxidant ingredients than those used in this study.

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