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Effect of Adding Lemon Peels Powder on Some Quality Properties of Minced Beef Patty during Refrigerated Storage

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A B S T R A C T

The effect of adding lemon peels powder on some quality properties of minced beef patties during refrigerated storage is the objectives of the present work. Control beef patty samples (LP) exhibited significant higher TBARS values during refrigerated storage. Whereas the prepared lemon peel patties (1% and2% LP) samples showed good quality with lower TBARS till 9 days at 4°C. Total volatile basic nitrogen (TVBN) of patty samples gradually increased with different rates. LP 2% samples exhibited higher antioxidant activity (AOA %) than those treated with 1% LP. The LP 2% sample gave the greatest DPPH followed by 1% LP compared to control at zero time. The DPPH **%** activity decreased during refrigerated storage in all patties samples. Concerning Met-myoglobin%; control sample revealed the highest met-myoglobin% estimated from 19.20% at the beginning day of storage to 76.47% at the end of the storage period. Meanwhile, 2% LP sample gave lowest % Met-myoglobin (40.74%) and 1% LP sample exhibited 47.42% Met-myoglobin at the end of storage time (12days). Noticeably the overall acceptability scores of the formulated samples increased with increasing levels of citrus peel [L.P]; where 2% LP sample gained great acceptability more than the 1%.

Introduction

Citrus processing generates a considerable amount of wastes which represent a serious environmental problem. Citrus wastes are promising sources with valuable technological and nutritional properties can be used as functional ingredients when designing healthy foods (functional foods) (Marı´n, *et al.,* 2002; Puupponen-Pimia *et al.,* 2002).They have high content of bioactive compounds and can be used as natural food additives, antioxidants, antimicrobials, colorants, flavorings and thickener agents (Ayala-Zavala *et al.,* 2011). Therefore, these wastes may add worth for food industry benefits from nutrition and economic viewpoints, where, they give potential health protection for the consumer

from their excellent nutritional value. On the other hand, the reused citrus waste is expected to minimize the environmental influence and add value to this waste. Natural preservatives can protect the human body from free radicals and could retard the progress of many chronic diseases as well as lipid oxidation and microbial growth in foods due to their phenolic compounds (Camo *et al.,* 2008; APHA, 2001). Several studies on citrus waste including lemon peels [LP] noticed that they may be viable functional ingredients for different products such as meat pastes, baked goods and yoghourt. So there is interest in using lemon peels "citrus waste" as natural functional ingredients through processing healthy meat products to enhance oxidative stability and preserve meat quality for longer shelf life as maintaining food safety according to consumers demand for natural and safe products.

Minced beef is one of the most popular and acceptable meat products, that are widely consumed and used as fast meals that are highly perishable (Michalczyk *et al.*,2012).. Microbial growth, lipid oxidation and color changes are important factors for shelf-life and consequently for consumer acceptance of fresh meat (Hayes *et al.,* 2010; Pavelková *et al.,* 2013). Minced meat undergo oxidative changes and develop rancidity more quickly than whole muscle, since grinding exposes more of the muscle surface to air and microbial contamination (Mitsumoto, *et al.,* 2005). Grinding meat leads to rapid formation of met-myoglobin, undesirable brown color and oxidative rancidity (Sahoo and Anjaneyulu 1997), seriously affecting consumer acceptance. Also, oxy-myoglobin and lipid oxidation appear to be interrelated in meat (Anton *et al.,* 1993) With prolonged storage, oxy-myoglobin oxidizes to met-myoglobin and gives meat an unattractive brown color (Djenane *et al.,* 2002). Oxidative stability is a central parameter in the estimation of meat quality because of the susceptibility of meat products to oxidative degeneration, which is one of the main causes of spoilage. In meat products oxidative reactions are affected by several factors, including lipid composition, processing and could be delayed by endogenous or exogenous antioxidants (Sacchetti *et al.,* 2008). In addition, lipid oxidation affects essential sensory traits of meat products, causing flavor, color and texture deterioration (Estevez *et al.,* 2005). The shelf life of meat is related to lipid oxidation reactions, which could affect its sensory properties, causing rancidity, as well as its nutritional characteristics through the formation of potentially toxic compounds (Sacchetti *et al.,* 2008). Moreover, oxidative processes are also associated with discoloration of meat products as lipid oxidation results in the formation of pro-oxidants which are capable of reacting with oxy-myoglobin and lead to formation of met-myoglobin (Frankel 1998). Therefore, color and lipid stability in meat are very important quality characteristics which influence consumer acceptability and the lipid oxidation problem has extensive economic importance for the meat industry. Several research studies indicated that oxidation in meat can be effectively controlled or at least, minimized by adding antioxidants (Djenane *et al.,* 2004). It is known that the antioxidant is a substance that delays oxidation by inhibiting initial free radical formation or by preventing them from producing more free radicals, which can perpetuate the reaction (Fennema 1996). Also, refrigeration storage is usually the most common preservative method of meat and meat products. In order to extend refrigerated storage time, antimicrobial and antioxidant additives are added to muscle foods (Solomakos, *et al.,* 2008). Therefore, the use of lemon peel wastes [LP] as natural food additives or supplements and as antioxidant additives for production of healthy beef products of high quality and safety as well as

high nutritional value have gained increasing interest because these are high-value products and their recovery may be economically attractive

So, the objective of the present study was to evaluate the effectiveness of adding lemon peels powder at two levels (1 and 2 %) on some quality properties of beef patties during refrigerated storage.

Materials and Methods

Citrus lemon (*Citrus aurantiifolia*) fruits were purchased from an Egyptian local market. Also, fresh beef lean (from the round) and beef back fat from the same beef carcasses were purchased from a slaughter house at Egyptian local market, Giza, Egypt.

Chemicals

Chemicals, solvents, standards and reagents were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). All other chemicals used were of analytical grade.

Preparation of lemon peels powder

Lemon fruits were washed by running tap water, peeled and their edible portions were carefully separated. The obtained fresh citrus peels were cut into small pieces before the drying process.

Microwave- Drying Method

A programmable domestic microwave oven (type Samsung,77 QH 400148,MF 2015), with a maximum output of 1500 W at 2450 MHz was used for drying the fresh lemon peel pieces samples for 6 min.

The dried lemon citrus peels (LP) were ground to a fine powder using a mechanical laboratory grinder and passed through a 24 - mesh sieve then packaged in polyethylene bags and stored at 4±1°C until required for use.

Preparation of Minced beef patties

The beef lean meat (from the round) and beef back fat were minced separately through 3/8 inch plate using a laboratory meat mincer for preparing the treated minced beef patties samples. Control sample was formulated without any lemon peels addition whereas the treated patties samples were prepared by adding 5% lemon peels suspension as follows in (Table 1). All ingredients were manually mixed by hand for 5 min, and then, re-minced through a 1/4 in. plate to be mixed uniformly. All ingredients were manually mixed by hand for 5 min, and then, re-minced through a 1/4 in. plate to be mixed uniformly. The homogenized meat mixtures were shaped into patties (approximately 50 g each) per treatment using a round shape patty maker (6 cm × 2 cm). Patties were analyzed as fresh samples on the manufacture day. Beef patties were placed on plastic foam meat trays, wrapped with polyethylene film then labeled and stored in a refrigerator at 4±1°C for 12 days. Patties were analyzed as fresh samples on the manufacture day. Each group of samples was withdrawn at three days intervals over storage period for analysis.

Analytical Methods

Thiobarbituric acid reactive substances (TBARS)

Determination of TBARS value has been found to be a good indicator for lipid oxidation in meat and meat products. TBARS values were determined in tested minced beef patties samples during refrigerated storage at 4±1°C to evaluate efficiency of the additives (lemon peels) as natural antioxidants according to (Pearson, 1991). Meat samples

(10 g) were mixed with 50mL distilled water. Then 2.5 mL of 4M HCl was added to get the pH to 1.5, followed by addition of antifoaming and a few glass beads. The flask was heated by means of an electric mantel and 50 mL distillate was collected in10 min from the time of boiling commences. Five mL of the distillate was mixed with 5 mL of TBA solution (0.2883 gm TBA/100 mL of 90% glacial acetic acid) in a glass-stopper tub.

Blank was carried out using 5 mL distilled water and 5 mL TBA solution. Tubes were covered and heated in boiling water bath for 35 min, then after rapid cooling in ice bath, absorbance at 538 nm was measured against the blank using ultraviolet visible scanner spectrophotometer (T80+ UV/V is Spectrophotometer PG instrument Ltd). The TBARS values were calculated by multiplying the absorbance by the factor of 7.8 and the result was represented as mg of malonaldehyde per kg sample.

Total volatile basic nitrogen (TVBN)

It is known that TVBN are products of bacterial decomposition and the content is extensively used as an index to assess the keeping quality and shelf life of meat and meat products (Pearson, 1991). The method recommended for determination of TVBN is based on a semi-micro distillation procedure. Weigh 10 gm of minced meat samples plus 2gm magnesium oxide (MgO) into a semi-micro-distillation apparatus, and then add 300 mL tap water and antifoam materials. Steam distilled collect in 25 mL 2% boric acid containing kjeldahl indicator solution which exhibited blue color. Kjeldahl flask was heated for 10 min, then distillate for another 15 min from the time of boiling commences, so ~ 150 mL distillate was collected into boric containing flask, the color became light green after fixing the ammonia "NH3". Titrate to a pale blue end point with 0.1N H_2SO_4 solution. The TVBN values were calculated by multiplying the reading minus the blank by the factor of 14.The results were represented as mg N per 100 gm sample.

$$\text{TVBN value} = \frac{\text{volume HCl} \times 14 \times \text{N HCl} \times 100}{\text{weight sample (g)}}$$

Antioxidant activity

The antioxidant potential expressed in terms of percentage of antioxidant activity (AOA %) was calculated by the following equation (Wijewickreme and Kitts, 1998):

$$\text{AOA \%} = \frac{\text{[TBARS value of the control} - \text{TBARS of the test sample]} \times 100}{\text{TBARS value of the control}}$$

Radical scavenging activity (DPPH)

The effect of used lemon citrus peel extracts on 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was estimated in order to assess the antioxidant capacity according to the procedure described by (Yi *et al.*, 2008)[3] with some modifications. The reaction mixture was incubated for 30 min in darkness at room temperature. The absorbance of the resulting solution was measured at 517 nm using spectrophotometer (T80 UV/ Visible - PG instrument Ltd, Made in Germany). For the control, the assay was conducted in the same manner using ethanol instead of sample solution. DPPH scavenging capacity of the tested samples was measured as a decrease in the absorbance and was calculated by using the following equation:

$$\text{Scavenging activity (\%)} = Ac - As / Ac \times 100$$

Where Ac and As are the absorbance's at 517 nm of the control and sample, respectively.

Microbiological preparation of medium used for T.P.C

Total plate count (T.P.C) of the control and treated beef samples were evaluated by plating on nutrient agar medium (N.A) according to the recommended method of (Frazier and Foster, 1950), However, its composition was prepared according to the method described by Difco Manual (1998) as follows: Beef extract (3gm), sodium chloride (5gm), peptone (5gm), agar (15gm), distilled water up to (1L) and modified pH of the medium to 6.8 ± 0.2.

These ingredients were mixed thoroughly, boiled to dissolve them then dispensed into flasks and autoclaved at 121°C for 15 min and then cool to about 45°C and pour into sterilized plates.

Ten grams of representative minced beef patty samples were mixed with 90 mL peptone in 250 mL flask under aseptic conditions.

The suspension was checked by hand for 5 min to give 1/10 dilution. Serial dilutions were prepared to be used for counting bacteria.

Total plate count (T.P.C)

One ml from each prepared dilution was plated on the above medium in triplicate and incubated at 37 °C for 48 hr. The count was then calculated and expressed as colonies per gram flesh.

Sensory evaluation of beef patties

The sensory attributes (appearance, odor, texture, taste tenderness and overall-acceptability) of the freshly cooked beef patties samples were evaluated using a10-point numerical scale; where ten corresponded to “the highest quality”, a score less than 4 indicate that the sample is "rejected" (Watts *et al.,* 1989).

Met-myoglobin content

Met-myoglobin percentages of the tested samples were estimated according to the method of Trout (1990).

Color measurements

Color of each tested beef patty sample was measured using a Hunter Lab. scan XE colorimeter (Hunter Lab. Inc., Reston, VA, USA) calibrated with a white standard tile: (X = 77.26, Y = 81.94 and Z = 88.14). Commission International d'Eclairage (CIE): L* (lightness), a* (redness) and b* (yellowness) saturation index were measured. Reflectance measurements were collected at 10 nm increments using illuminate A (Podolak *et al.,* 1997) and three random readings per sample were recorded.

pH value

Each minced beef patty sample (10 g) was blended with distilled water (100 ml) for 1 min. The pH values were measured using a standardized electrode attached to a digital pH meter (Cyber scan 500 pH meter -Serial No 112598).

Water and oil holding capacities

Water and Oil holding capacities of lemon peel was determined as described by Chau and Huang, (2003). One gram of powdered sample was weighed, added into 10 mL of distilled water or 10 mL of sunflower oil and stirred for 1 min. The suspensions were then centrifuged at 2200g for 30 min, and the supernatant volume was measured. WHC or OHC was expressed as gram of water or oil held per gram of sample.

Statistical analysis

All measurements were carried out in triplicate and the data were presented as mean

± SD. The effects of adding lemon peel and storage period were analyzed and the data obtained was subjected to one-way analysis of variance (ANOVA) and least significant differences (LSD) at $P < 0.05$ were calculated using PC-Stat Version IA procedures (PC-Stat 1985)

Results and Discussion

Thiobarbituric acid reactive substances (TBARS)

TBARS method has been used to determine the degree of lipid oxidation (Klangpetch *et al.*, 2016)[1]also used as an index of lipid oxidation in meat products during storage (Fernández-López *et al.*, 1997 and Pearson, 1991).

Data in Table (2) showed the changes in TBARS values of the prepared raw ground beef patties mixed with lemon peel powder (1% or 2 % LP samples) during refrigerated storage for 12 days at 4±1°C. The results indicated that TBARS values increased over the storage time for all patty samples. Control raw patties sample exhibited significantly higher TBARS values during refrigerated storage period as compared to the other tested lemon peel patties. These LP patties samples showed good quality with lower TBARS till 9 days at 4°C as indicated by their lower TBARS (i.e. less than 0.9 mg MDA kg^{-1} meat) according to the (ES 1694/2005)[4] for meat products, whereas opposed to only the nine and twelve days for control samples were up to 1.20 &1.45 respectively. Worthy to note that the tested beef patty with 2% LP samples showed good quality till the end of refrigerated storage (12days) under the same conditions. The obtained results of the present study agreed the findings of Klangpetch *et al.*, (2016)[1] who reported that TBARS values increased considerably in control (2.5 mg MDA/kg sample) but remained relatively low in lime peel-treated samples with no significant differences at all concentration (1.5 -1.8 mg MDA/kg sample) and at the end of storage (14 days) and lime peel (Citrus *aurantifolia* Swingle) reduced the lipid oxidation for more than 40%. However, the accumulation of malonaldehyde (MDA) in patties during refrigerated storage could be due to hydrolytic and oxidative processes in the lipid fraction (Brake and Fennema 1999)[5].

Total volatile basic nitrogen (TVBN)

TVBN is a biochemical method for assessing meat spoilage (Pearson, 1991). TVBN changes of beef patties sample are illustrated in (Table 3). At zero day of storage control, 1% and 2% LP samples exhibited 10.80, 10.50 and 10.30 mg N/100g sample; respectively. These values are an indication of good quality raw material used in this assay and they are similar to some reported previous results by EL-Desouky *et al.*, (2006) and Moawad *et al.*, *(*2000). Also it is noted that, TVBN of all patty samples gradually increased with different rates depending on addition amount and storage time.

At the end of storage, TVBN reached 25.04, 19.34, and 15.40 mg N/100g for control, 1% and 2% LP samples respectively. This increase in TVBN values is related to the activity of spoilage bacteria and endogenous enzymes (Pearson, 1991). The lower TVBN values with 1% or 2% LP may be due to the effectiveness of the bioactive compound in this citrus peel on microorganisms. Concerning TVBN values as a spoilage index for meat product; (ES 1694/2005) reported that a level above 20 mg N/100 g sample is usually considered spoiled minced meat. In the present study control beef patties seemed acceptable with TVBN index of storage and its level reached to 20.36 mg N/100 g sample at 9^{th} day. While, the TVBN of beef patties samples with lemon peels powder was lower

than the control sample during storage period and reached up to 12 days under the same conditions. These results confirmed the findings of Mexis *et al.,* (2012).

Antioxidant activity (AOA)

The total antioxidant activity has been generally recognized as a tool to test the antioxidant potential of a pure compound or a food extract (Aruoma 1996). The AOA of a food could be a useful index to predict oxidative stability (Sacchetti *et al.,* 2008).

Data on the antioxidant potential or activity (AOA) of lemon peel as antioxidants in the patty samples stored at 4°C for 12 days are depicted in Table 4. A significant difference between the AOA % of the tested patty samples as a result of adding 1 or 2% LP during storage was observed. It was noticed that beef patty samples treated with 2% LP exhibited a higher AOA% than that treated with 1% LP during the refrigerated storage. This pattern difference may be due to the phenolic content in 2%LP samples which exhibited a higher AOA% than other patty samples treated with 1% LP.

The data indicated that the antioxidant activity of 1 & 2% LP samples may be due to the result of their radical scavenging activity whereas may act in a similar trend as reductions by donating electrons and reacting with free radicals to convert them to more stable products and terminate free radical chain reactions as reported by Negi and Jayaprakasha, 2003.

Radical scavenging activity (DPPH)

The DPPH was used as a free radical to evaluate antioxidant activity present in natural sources (Schwarz *et al.,* 2001) the use of antibiotics as feed additives has been gradually restricted. Data in Table (5) showed that patties samples with 2% LP gave the greatest DPPH followed by 1% LP compared to control at zero time. The DPPH % activity decreased during refrigerated storage in all patties samples.

Sherwin, (1998) reported that antioxidants are believed to intercept the free radical chain of oxidation and to give hydrogen from the phenolic hydroxyl groups, thereby; forming a stable end product that does not initiate or propagate further oxidation of lipids.

Microbiological properties

The shelf-life of meat is usually limited by microbial spoilage. Depending on hygiene and preservation conditions, raw beef patties stored aerobically have a shelf-life of around 7-8 days in refrigerated conditions (Sallam and Samejima 2004; Hayes *et al.,* 2010).

In the present study total plate counts (TPC) of beef patties were evaluated and the counts (as log 10 CFU/gm) are presented in Table (6). Slight higher in TPC value was noticed in control beef patty samples as compared with other treated samples at zero time of cold storage; which can indicate that lemon peel caused sudden lethal effect for microorganisms. As might be expected, increase in storage time produced significant greatly increase in TPC whatever the treatment conditions, thus the storage time have a significant effect on TPCs of raw beef patties (Table 6), whereas by the day 9 of storage TPC value of control sample (7.77 log10 CFU/gm) exceeded the maximal recommended limit of 7 log10 CFU/gm for TPC in raw meat (ICMSF, 1986) indicating a shelf life of about 8 days. Meanwhile, 1% lemone peel (LP) treatments significantly delayed the microbial growth and extended the shelf life of the product up to 12 days at which the TPC was 5.66 versus 8.88 log10 CFU/gm in control samples. Also it was revealed that at the 12^{th} day of storage samples containing 2% LP had the lower TPC value (4.75 log10

CFU/gm) than the maximal recommended limit, while control samples exhibited 8.88 log10 CFU/gm, these indicating that as the concentration of LP increased in the formula; the TPC of the samples of LP increased in the formula; the TPC of the samples decreased. Similar results were achieved by (Alahakoon *et al.,* (2013)[18] who found that significant effects of addition citrus peel extract and onion peel extract to chicken breast meat sample on microbial growth inhibition during storage at different temperatures. Klangpetch *et al.,* (2016) reported also that total viable count (TVC) of all samples increased during storage. Lime peel reduced TVC soon after dipping, to be lower than control and water for 2 and 1.2 log-orders during 6 to 10 days of storage.

Water and oil holding capacities

Water-holding capacity(WHC) is one of the properties of fresh meat as it affects some major characteristics such as potential drip loss, technological quality, appearance and sensory properties (Das *et al.*, 2011). WHC of meat is an important factor affects tenderness, thawing drip and cooking loss of meat (Hamm, 1986). Also it is one of important measurements of quality attributes for possibility of using meat in manufacturing of any meat product. This property is largely affected by the muscles proteins and pH value level. Generally WHC decline continuously with the progression of storage period.

Oil-holding capacity (OHC) is a technological property related to the chemical structure of the plant polysaccharides and depends on surface properties, overall charge density, thickness, and hydrophobic nature of the fiber particle (Fernandez-Lopez *et al.,* 2009 and Figuerola *et al.,* 2005). Effect of adding lemon peels powder on WHC and OHC of the tested beef patties are presented in Table (7). The lowest OHC and WHC values were noticed for control beef patties samples compared to other formulated samples. The OHC and WHC of the tested pattie samples decreased slightly during storage due to the addition of powdered citrus peel. The OHC increased in beef patties samples contained 2% lemon peel followed by 1% lemon peel compared with the control. Similar trends were found in case WHC of the above meat samples.

Viuda-Martos *et al.,* (2012) reported that WHC is related to the soluble dietary fiber (SDF) content, and high levels of SDF produce a high WHC value. This could be explained by the higher WHC of soluble fibers, such as pectin and gums than cellulosic fibers. So, the higher WHC of LP samples could be due to the chemical structures, which possess a higher WHC than cellulosic fibers in citrus peel. Noticeably, all the tested samples showed decrease WHC during refrigerated beef patties. Such effects could be attributed not only to particle size reduction, but also to the altering of the fiber matrix structure. Kethireddipalli *et al.,* (2002) reported that WHC depends on matrix structure formed by polysaccharide chains which can hold large amounts of water through hydrogen bonds.

Also, Sangnark and Noomhorm, (2003) reported that particle size reduction of dietary fibers has been associated with a lower ability to retain water and a lower oil binding capacity.

Determination of pH value

Meat pH is considered as one of the most important technological properties as it alters pigment and lipid stability. Changes in pH values in ground beef meat during cold storage at 4±1°C for 12 days are presented in Table (8). pH value of control sample at zero time of storage was 6.01.

Table.1 Preparation of the Minced beef patties samples

Control beef patties sample	Beef patties samples with 1% lemon peels[LP]	Beef patties samples with 2% lemon peels[LP]
82% Beef Lean	80% Beef Lean	78% Beef Lean
15% Beef Fat	15% Beef Fat	15% Beef Fat
3% Cold water --------- 100 % = Total weight	5% Lemon peel suspension: (1%LP +4%ColdWater) -------- 100 % = Total weight	7%Lemon peel suspension: (2%LP +5% Coldwater) -------- 100 % = Total weight

Table.2 Thiobarbituric acid reactive substances (TBARS) of the tested patty samples mixed with 1 or 2 % LP during refrigerated storage.

Beef patty Samples	Refrigerated Storage(4±1 °C) per day				
	0	3	6	9	12
Control	0.46± 0.01^{a}	0.59± 0.01^{a}	0.80± 0.01^{a}	1.20± 0.01^{a}	1.45± 0.01^{a}
1% LP	0.39± 0.02^{b}	0.51± 0.00^{b}	0.62± 0.01^{c}	0.72± 0.01^{c}	0.90± 0.004^{c}
2%LP	0.35± 0.01^{d}	0.46± 0.004^{d}	0.55± 0.001^{e}	0.61± 0.01^{e}	0.77± 0.004^{e}

All values are mean of triplicate determinations ± standard deviation (SD). Mean values in the same column & raw bearing the same superscript do not differ significantly (P<0.05). Thiobarbituric acid reactive substances (TBARS) as mg of malonaldehyde per Kg sample . LP=Lemon Peel

Table.3 Total volatile basic nitrogen (mg N/100gm sample) changes of the prepared beef patties during refrigerated storage at 4±1 °C for 12 days

Beef patty Samples	Refrigerated Storage(4±1 °C) per day				
	0	**3**	**6**	**9**	**12**
Control	10.80^{a}± 0.01	13.27^{a}±0.01	16.46^{a}±0.02	20.36^{a}±0.02	25.04^{a}±0.04
1%LP	10.50^{c}± 0.02	11.50^{c}±0.00	12.75^{c}±0.01	16.07^{c}±0.04	19.34^{c}±0.03
2%LP	10.30^{c}± 0.02	10.40^{e}±0.01	11.45^{e}±0.01	13.46^{e}±0.02	15.40^{e}±0.02

All values are mean of triplicate determinations ± standard deviation (SD). Mean values in the same column bearing the same superscript do not differ significantly (P>0.05). LP =Lemon Peel

Table.4 Antioxidant activity (AOA %) of the tested beef patty mixed with1% LP and 2%LP samples during refrigerated storage at 4±1°C per day

Beef patty Samples	Refrigerated Storage (4±1 °C) per day				
	0	**3**	**6**	**9**	**12**
1% LP	15.22	13.56	22.50	40.00	37.93
2 % LP	23.91	22.03	31.25	49.17	46

All values are mean of triplicate determinations ± standard deviation (SD). LP= Lemon Peel

Table.5 Changes radical scavenging activity (DPPH %) values of beef patty samples during storage at 4±1 °C for 12 days

Beef patty Samples	Refrigerated Storage(4±1 °C) per day				
	0	3	6	9	12
Control	1.83^{e}±0.03	0.90^{e}±0.03	0.08^{e}± 0.03	0.02^{e}± 0.04	0.00^{e}±0 .00
1% LP	23.61^{c}± 0.03	18.39^{c}±0.45	13.98^{c}±0.47	9.80^{c}± 0.07	3.69^{c}± 0.03
2 % LP	34.01^{a}± 0.1	29.03^{a}± 0.2	25.19^{a}± 0.2	21.17^{a}±0.07	16.38^{a}± 0.1

All values are mean of triplicate determinations ± standard deviation (SD). Mean values in the same column bearing the same superscript do not differ significantly (P<0.05).LP= lemon peel

Table.6 Total plate count changes of beef patty samples during refrigerated storage at 4±1 °C for 12 days (as log10 CFUgm^{-1})

Beef patty Samples	Refrigerated Storage(4°C) per day				
	0	3	6	9	12
Control	4.97^{a}± 0.04	6.05^{a}± 0.05	7.43^{a}± 0.06	7.77^{a}± 0.15	8.88^{a}± 0.14
1%LP	3.27^{b}± 0.13	3.48^{b}± 0.11	4.72^{b}± 0.08	5.56^{b}± 0.06	5.66^{b}± 0.05
2%LP	1.35^{c}± 0.91	2.87^{c}± 0.2	3.39^{c}± 0.17	4.63^{c}± 0.07	4.75^{c}± 0.11

All values are mean of triplicate determinations ± standard deviation (SD). Mean values in the same column bearing the same superscript do not differ significantly (P<0.05). Total plate count as log10 CFUgm^{-1}, LP: Lemon Peel.

Table.7 Water and oil holding capacity values changes of beef patties sample during refrigerated storage at 4±1 °C for 12 days

Beef patties Sample	Storage(day)				
	0	3	6	9	12
	Water Holding Capacity				
Control	1.50^{c} ±0.04	1.35^{c} ±0.02	1.11^{c} ±0.10	0.50^{c} ±0.03	0.11^{e} ±0.01
1%LP	3.87^{b} ±0.03	3.74^{b} ±0.02	3.59^{b} ±0.02	3.13^{b} ±0.10	2.94^{c} ±0.02
2%LP	4.04^{a} ±0.11	3.90^{a} ±0.03	3.77^{a} ±0.01	3.47^{a} ±0.04	3.29^{a} ±0.02
	Oil Holding Capacity				
Control	0.70^{c} ± 0.02	0.60^{c} ±0.03	0.42^{e} ±0.01	0.18^{e} ±0.02	0.05^{e} ±0.00
1%LP	1.07^{b} ± 0.04	0.92^{b} ±0.01	0.91^{c} ±0.01	0.78^{c} ±0.01	0.69^{c} ±0.03
2%Lp	1.59^{a} ± 0.03	1.51^{a} ±0.04	1.47^{a} ±0.04	1.37^{a} ±0.06	1.28^{a} ±0.04

All values are mean of triplicate determinations ± standard deviation (SD). Mean values in the same column bearing the same superscript do not differ significantly (P> 0.05) and LP=Lemon Peel

Table.8 pH changes of beef patties during refrigerated storageat 4±1° for 12 days

Beef patties Sample	Refrigerated Storage (day)				
	0	3	6	9	12
Control	6.01^{a} ±0.01	6.20^{a}±0.00	6.33^{a}±0.00	7.31^{a}±0.01	7.82^{a}±0.00
1% LP	5.80^{bc} ±0.01	5.95^{bc}±0.00	6.03^{c}±0.00	6.30^{b}±0.00	6.58^{c}±0.00
2% LP	5.71^{c} ±0.01	5.85^{d} ±0.00	5.97^{c}±0.00	6.22^{c}±0.01	6.37^{e}±0.00

All values are mean of triplicate determinations± standard deviation (SD). Mean values in the same column bearing the same superscript do not differ significantly (P> 0.05). LP= Lemon Peel

Table.9 Instrumental color values of beef patties as affected by addition of lemon peel powder during refrigerated storage at 4±1 °C for 12 days

Beef patties Samples	Refrigerated Storage (day)					
	Parameter	**0**	**3**	**6**	**9**	**12**
Control	*L**	52.07±0.02	53.88±0.04	56.03±0.01	56.75±0.02	56.96±0.04
	*a**	9.11±0.01	7.72±0.02	6.41±0.03	6.33±0.03	6.02±0.01
	*b**	18.69±0.11	17.31±0.01	16.91±0.02	16.68±0.03	16.03±0.02
1% LP	*L**	58.35±0.01	58.57±0.01	59.37±0.01	59.78±0.03	59.91±0.07
	*a**	11.16±0.05	9.11±0.05	8.46±0.06	8.37±0.04	8.14±0.03
	*b**	19.3±0.03	17.47±0.0	17.31±0.01	17.27±0.03	17.03±0.06
2%LP	*L**	59.30±0.01	58.61±0.05	63.07±0.04	63.26±0.04	63.34±0.02
	*a**	13.27±0.03	9.72±0.03	9.09±0.05	8.96±0.03	8.73±0.02
	*b**	20.78±0.0	18.68±0.02	18.21±0.06	18.05±0.05	17.89±0.02

All values are mean of triplicate determinations ± standard deviation (SD). Mean values in the same column bearing the same superscript do not differ significantly (P≤ 0.05) LP=Lemon Peel

Table.10 Met-myoglobin% of beef patties as affected by addition of lemon peel powder during refrigerated storage at 4±1 °C for 12 days

Beef patties Samples	Refrigerated Storage(4±1 °C) per day				
	0	**3**	**6**	**9**	**12**
Control	19.20	38.00	53.11	70.09	76.47
1%LP	18. 83	33.05	41.01.	43.19	47.42
2%LP	18.75	**31.33**	33.32	37.45	40.74

Table.11 Sensory evaluation of the beef patties containing powder of lemon peels during refrigerated storage at 4±1 °C

Beef patty Samples	Color	Appearance	Odor	Texture	Taste	Tenderness	Overall-Acceptability
Zero time							
Control	7.56^{e} ±1.5	7.19^{d} ±1.76	7.25^{c}±1.39	7.31^{c}±1.40	7.19^{c}±1.63	7.13^{e} ±1.63	7.15^{d} ±0.55
1%LP	7.69^{d}±0.10	7.69^{c} ±0.95	7.44^{b}±1.05	7.13^{d}±1.36	7.06^{d}±1.61	7.56^{d} ±1.21	7.54^{c} ±0.78
2%IP	8.50^{a}±1.21	8.19^{a} ±1.47	8.13^{a}±1.41	7.81^{a}±1.17	8.38^{a} ±1.20	8.19^{a} ±1.11	8.38^{a} ±0.77
The third day							
Control	7.00^{e}±1.00	7.23^{e} ±1.00	6.92^{c}±0.95	6.77^{e}±1.79	6.85^{c}±1.34	6.77^{d} ±1.42	6.17^{e} ±0.58
1%LP	7.46^{c}±0.78	7.54^{c} ±1.05	6.92^{c}±1.19	7.69^{c}±0.75	6.69^{d}±1.38	7.38^{b} ±1.39	7.54^{c} ±0.78
2%LP	8.31^{a}±1.03	8.31^{a} ±0.85	8.15^{a}±0.80	8.00^{a}±1.08	7.77^{b}±1.17	8.08^{a} ±0.64	8.38^{a} ±0.77
The sixth day							
Control	5.75^{e}±1.66	6.17^{e} ±1.47	5.83^{e}±1.34	5.83^{e}±1.59	5.50^{d}±1.57	5.67^{e} ±1.50	6.17^{e} ±0.58
1%LP	7.21^{c}±1.34	7.58^{c} ±0.51	7.00^{c}±1.21	7.58^{c}±0.79	7.00^{c}±1.04	7.50^{c} ±1.17	7.50^{c} ±0.48
2%IP	8.25^{a}±1.08	8.58^{a} ±0.51	8.17^{a}±0.72	8.17^{a}±0.72	7.42^{b}±1.24	8.17^{a} ±0.72	8.58^{a} ±0.47

All values are mean of eleven repeated determinations ± standard deviation (SD). Mean values in the same column bearing the same superscript do not differ significantly (P > 0.05) LP=Lemon peel

The pH values of raw samples (mixed with 1 or 2% LP) obviously decreased to be less than 6.00 (slight acidic values). This decrease in pH values could be due to the presence of some organic acids found in LP which shifted the control sample to be less slightly acidic.

Braddock, (1995) reported that the pH values of samples with added lemon albedo were lower than control samples. This decrease in pH could be related to the presence of some organic acid in albedo. Generally, it was shown the pH value increased gradually with increasing storage time (Table 8). Abd El-Aal (2005) and Das *et al.*,(2011) also reported that an increase in pH during the storage period may be due to growth of Gram-negative bacteria and due to accumulation of metabolites by bacterial action on protein and amino acids.

Instrumental color measurement due to the concentration of myoglobin, hemoglobin and their chemical Color is an important factor which observed by consumers to indicate meat freshness and healthiness in order to purchase. The color of meat is caused states (Brewer, 2004) Instrumental color evaluation (Table 9) in the present is study revealed a significant ($P \leq 0.05$) effect of 1 or 2% of lemon peel powder on Hunter color parameters values of the formulated beef patties samples.

Color (L^*) values of the control sample was increased all over the storage period and the reverse pattern occurred for a*(redness) and b*(Yellowness) color values. Mixing of LP affected all color values relative to the control. Addition of lemon peel to the tested patties sample (1% or 2% LP) affected the color parameters values during the storage period. Lightness (L^*) values was increased to reach (59.91 & 63.34) respectively, at the 12^{th} day (end of storage). Meanwhile, a^*(redness) values decreased compared to control along the storage period to be (8.14 & 8.73) respecively. Also, the b* (Yellowness) were of lower values than the control to be (17.03 & 17.89) at the end of storage period. A decrease in (b*) and (a*) values of beef patties containing natural antioxidants has been reported by Ashour *et al.,* (2014) who reported that over storage time, the a* values of all patties sample decreased and becoming less red or brown due to met-myoglobin. The yellowness (b*) values followed similar trend decreasing to day 12. Also, Rojas and Brewer, (2008) reported a decrease in (b*) values of beef patties containing natural antioxidants. Aleson-Carbonell *et al.,* 2005 cleared that addition of any type of citrus albedo at any concentration increased surface b* and a* values. This increase in b* value is related to the incorporation of yellowness and redness components present in albedo to a studied burgers.

Met-myglopin Content

The oxidative changes are more intense in minced meat due to increased surface area and exposure to air during grinding and processing than intact meat. Das *et al.,* (2006) cleared that ground buffalo meat tends to become brown and rancid more rapidly than whole retail cuts. Trout (1990) the meat color varies depending on the state of myoglobin. Myoglobin has three natural colors depending on its exposure to oxygen and the chemical state of the iron. If no oxygen is present, the meat appears purple red, and it is in the deoxy-myoglobin state. Bright red color indicates oxymyoglobin is present. Meat appears tan or brown when only very small amounts of oxygen are present.

Met-myoglobin is the state when the iron has oxidized and is tan or brown in color. Met-myoglobin is responsible for the undesirable brown color of fresh meat. Meat can also appear brown when the meat color life is exhausted late in display when the iron in the pigment becomes oxidized. Although

brownish-red colored meat can indicate spoilage, it doesn't always mean that meat meat is spoiled.

In the present investigation, effect of adding Lemon peel on met-myoglobin % of raw ground meat was studied and compared with control beef meat patty over 12 days of refrigerated storage (Table10). Generally, the present investigation indicated that met-myoglobin percentages of the control beef meat patties (LP samples) were higher than those of the tested samples with Lemon Peel (1% LP and 2% LP samples) during the refrigerated storage period (Table 10). Also, it was noticed when the refrigerated storage period increased, met-myoglobin accumulation in all the meat samples increased consistently. The control sample revealed the highest met-myoglobin% estimated from 19.20% at the zero day of storage to 76.47% at the end of the storage period. Noticeably, Sahoo and Anjaneyulu (1997) also reported that highly significant effects of the storage period by increasing met-myoglobin in ground buffalo meat were observed.

In the present study beef patties samples mixed with 2% LP gave lowest percentage of Met-myoglobin that (40.74%) at the end of storage period(12days). Meanwhile beef patty samples (1% LP sample) exhibited Met-myoglobin percentage 47.42% at the end of storage time

Sensory evaluation

The results of the sensory evaluation are presented in Table (11). Beef patties incorporating 1 or 2% lemon peel powder were of high sensory attributes than control sample at zero time. Meanwhile, the sensory attributes were increased by increasing the storage time. At 3rd day of storage, of the LP patty samples were noticed to be more than the sensory attributes corresponding 1% formulations. Also, at 6th day of storage 2% LP samples were more acceptable than the corresponding 1% samples. Regarding the overall-acceptability of the studied samples it was found that at zero day of storage the control sample was (7.69), this overall-acceptability increased gradually for the formulated samples. At 3rd day of storage the overall-acceptability of the control sample decreased relative to its corresponding at zero days. Worthy to note that the 2% LP formulated patty samples were more accepted than 1% LP samples; also 2% LP sample was of more acceptable sample.

At six days of storage the overall-acceptability of the control sample was of lowest acceptability (6.17), this acceptability increased for the formulated samples but still the 2% LP samples having great acceptability followed with 1% corresponding samples. Thus, it can be concluded that overall acceptability scores of the formulated samples increased with the increasing levels of citrus peel.

From the present investigation it was concluded that adding Lemon peel powder to minced beef patties could inhibit oxidation changes, retard development of rancidity and discoloration, enhance color quality and minimize, as possible, met-myoglobin formation in meat during the applied storage period. Samples of 2% LP gained the great acceptability more than the 1% samples.

Also, overall acceptability scores of the formulated samples increased with increasing levels of citrus peel. Thus, samples of the suggested 1% or 2% Lemon peel powdered could be employed as cheap food additives and used as a substitute for synthetic antioxidants for enhancing the shelf life of minced beef meat patties. Also, can be used as functional ingredients when designing healthy meat products

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