Influence of Lemon (*Citrus limon*) on Lipid Oxidation and Keeping Quality of Chicken crumbs

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ABSTRACT

Lipid oxidation is a major cause of quality deterioration in meat products. However, attention is shifting to natural antioxidants in controlling lipid oxidation in meat, due to hazards linked to synthetic antioxidants. This study therefore, sought to evaluate the influence of lemon juice on lipid oxidation and keeping quality of chicken crumbs. In a completely randomized, 6x3 factorial design; hygienically harvested fresh chicken meat, was deboned, cut into smaller pieces and marinated inside freshly squeezed lemon juice at different concentrations of 0%, 10%, 20%, 30%, 40%, 50% in 100 mls of water respectively. Marinated chicken samples were dried in an electric oven for 1hour at 105^{0}C to constant weight. Thereafter, they were removed from the oven, cooled, packed per treatment in Ziploc bags and stored for 90 days (3 months) at room temperature. Evaluations were done at days 30, 60 and 90 for pH, water holding capacity (WHC) and oxidative rancidity using standard procedures. Data obtained were analysed using ANOVA at $\alpha_{0.05}$. pH during the first month had the highest value (6.19) with the least observed in the third month (5.99). WHC had the highest value (96.11%) in the first month, with least value in the third month (91.67%). Oxidative rancidity (mg/g) in the third month had the highest value (2.70 mg/g). Effect of lemon juice on physicochemical quality of chicken crumbs showed differences among treatments. pH was significantly higher in chicken crumbs without lemon juice (6.34) with least pH observed in chicken crumbs marinated in 2% lemon juice. WHC of chicken crumbs marinated in 20% and 40% lemon juice had the same value (97.22%) which was significantly higher than control which had the lowest value (90.00%). For oxidative rancidity, control had the highest value of 3.77 mg/g and 10.00% lemon juice had the lowest value of 1.63 mg/g. Interaction effects of storage duration and lemon juice on pH of chicken crumbs showed significant differences ($p<0.05$) across treatments. It can be concluded from this study that chicken crumbs can be marinated in lemon juice of concentrations between 20% to 40% before drying to improve its shelf life for up to three months.

Keywords: Lemon juice, Chicken muscle, Chicken crumbs, Oxidative rancidity, Keeping quality

1.0 INTRODUCTION

Meat constitutes a crucial component of the majority of human diets, providing essential amino acids, highly bioavailable iron, zinc, selenium, and B vitamins, notably vitamin B_{12} (Neufingerl & Eilande, 2021). Many of these nutrients are globally deficient and can be addressed by consuming just a few ounces of beef per week (Neufingerl & Eilande, 2021). However, meat is highly perishable, susceptible to microbial growth, chemical changes, and breakdown by endogenous enzymes, rendering it unfit for consumption and potentially hazardous to health (Ronald *et al.*, 2024). Various measures can be taken to mitigate these processes, including reducing temperatures to slow microbial growth, cooking or canning to destroy organisms and enzymes, or employing methods like drying or osmotic control to remove water. Additionally, chemicals or, more recently, ionising radiation (though restricted in some countries) can be used to inhibit microbial growth (Ronald *et al.*, 2024). Meat can be consumed in various forms including fresh, cured, dried, or processed, with approximately one-third of all meat being processed. Consumer perception of processed meat products is a pivotal concern for the meat industry (Oshibanjo, 2017).

During the cooking of meat, hydro-peroxides are formed, which can break down into volatile organic compounds, impacting sensory quality and leading to oxidative flavours, pigment and vitamin loss in meat products (Macho-González *et al.*, 2020; Huang *et al.*, 2019). The heating process disrupts muscle cell structure, deactivates anti-oxidative enzymes, and generates catalytic iron from myoglobin, creating a pro-oxidant environment affecting both lipids and proteins (Huang *et al.*, 2019). Grinding further exacerbates lipid oxidation by disrupting muscle cell membranes (Huang *et al.*, 2019). Meat is a complex food with a vulnerable composition prone to oxidation (Amaral *et al.*, 2018). The oxidative stability of meat hinges on the interaction between endogenous anti-oxidant and pro-oxidant substances, as well as the composition of substrates prone to oxidation, including polyunsaturated fatty acids (PUFA), cholesterol, proteins, and pigments (Macho-González *et al.*, 2020). Lipid oxidation leads to the formation of reactive oxygen species (ROS), affecting both lipids and proteins and resulting in changes to sensory attributes and nutritional quality (Macho-González *et al.*, 2020; Cheng, 2016). Antioxidants, whether synthetic or natural, can be added to meat and meat products during processing to delay lipid oxidation (Domínguez *et al.*, 2019; Bellucci *et al.*, 2022; Petcu *et al.*, 2023). Natural antioxidants, such as plant polyphenols and essential oils (EOs), are increasingly favoured over synthetic options due to safety concerns (Bellucci *et al.*, 2022; Petcu *et al.*, 2023). Their addition has been shown to delay lipid oxidation, prevent off-flavour development, improve colour stability, enhance microbiological quality, and extend shelf life without compromising sensory or nutritional properties (Tomović *et al.*, 2017).

This study seeks to evaluate the efficacy of lemon as a natural antioxidant in enhancing the quality and shelf-life of meat products throughout processing, storage, distribution, and consumption, offering potential solutions to health concerns associated with their consumption.

2.0 MATERIALS AND METHODS

2.1 Experimental Site

This experiment was carried out at Animal Products Laboratory of Animal Production Department Faculty of Agricultural Science University of Jos, Jos, Plateau State Nigeria located between latitude 9^0 E55' North of the Greenwich meridian and longitude 8^0 E54' East of the Equator (Haruna *et al.,* 2007).

2.2 Experimental Design

6×3 arrangement in a completely randomized design. 6 = 6 lemon juice concentrations 0, 10%, 20%, 30%, 40%, 50%, in 100 mls of water 3 = 3 evaluation intervals, 30 days (First month), 60 days (Second month), and 90 days (Third month).

2.3 Lemon Juice Preparation

Fresh lemons were harvested, washed thoroughly, then juiced with the aid of juice squeezer. The juice was then added to 100 ml of water at different concentrations thus; 0, 10%, 20%, 30%, 40%, and 50% respectively.

2.3.1 Crumbing Process

Hygienically harvested chicken muscles were cut into smaller pieces averaging 100g, washed thoroughly with distilled water and marinating with the different concentrations of lemon juice. The marination was allowed to stand for one hour before drying the samples to constant weight in the oven at 105^0C.

2.3.2 Storage procedure

The chicken samples were removed from the oven and allowed to cool at room temperature before packing each treatment in Ziploc bags and stored for 90 days. Evaluations were done at days 30, 60 and 90.

2.4 Chicken Crumbs quality evaluation

2.4.1 pH

The pH was determined by weighing 1gram of sample into a blender with 9 ml of distilled water and homogenized until smooth slurry was formed. The digital pH meter was placed in a buffer solution in order to allow equilibrium for two minutes before placing it into prepared slurry. An average of two readings taken gave the pH value according to method described by AOAC (2000)

2.4.2 Water Holding Capacity

Water Holding Capacity (WHC) was determined according to Wardlaw *et al.,* (1973). Minced meat (20 g) was placed in a centrifuge tube containing 30 ml of 0.6 M NaCl and was stirred with glass rod for 1min. The tube was then kept at 4 ± 1 °C for 15 min, stirred again and centrifuged at 3000 g (R-24, Remi Instruments, India) for 25 min. The supernatant was measured and WHC was expressed in percentage.

2.4.3 Oxidative Rancidity

Thiobarbituric acid value (TBA) was estimated by modified methods of Buege and Aust (1978). 3mls each of glacial acid and 1% TBA solution were added to test tubes appropriately labelled blank and tests. 0.6 ml of distilled water was added to the blank, while 0.6 ml of the homogenised sample was added to each of the test tubes. These were thoroughly mixed, incubated in a boiling water bath for 15 minutes, then allowed to cool, after which they were centrifuged and their supernatants collected. The supernatant from the blank was used to zero the

spectrophotometer (preset at 532 nm) before reading the absorbance of the supernatant from the test solutions. The amount of TBARS was expressed as milligrams of malondialdehyde per gram of sample.

$$TBA = \frac{O.D \times V \times 1000}{A \times V \times I \times Y}$$

Where:

O.D = Absorbance of test at 532 nm.

V= Total volume of the reaction mixture = 6.6 mL

A= Molar extinction coefficient of the product, and according Buege and Aust (1978) is equal to 1.56x105

I = Length of light path =1 cm.

Y= mg of tissue in the volume of the sample used.

V= volume of tissue juice used =0.6 ml

2.5 Ethical approval

No ethical approval was required and requested for this study, because it did not involve any dangerous procedures or the use of live animals.

2.6 Statistical Analysis

The collected data were statistically analysed using one-way ANOVA and two-way ANOVA. ANOVA was selected because it is a tool that makes it more straightforward to compare means and determine if significant differences exist between them. A 95% confidence interval was used to determine the statistical difference between the control and treatments and between groups.

3.0 RESULTS

Table 1 presents the effects of storage duration on the physicochemical properties of chicken crumbs. In the first month, the pH value was highest at 6.19, followed by the second month at 6.15, while the third month recorded the lowest pH value of 5.99. The water holding capacity in the first month was 96.11%, slightly higher than the second month with a value of 94.17%. The third month exhibited the lowest water holding capacity at 91.67%. In terms of oxidative rancidity (measured in mg/g), the third month showed the highest value at 2.70, followed by the second month at 2.51. The first month had the lowest oxidative rancidity value at 1.85 (mg/g).

3.1 Effect of lemon juice on physicochemical quality of chicken crumbs

Table 2 shows the effects of lemon juice on the physicochemical quality of chicken crumbs. Regarding pH levels, it was observed that the chicken crumbs without lemon juice (control) had the highest pH value of 6.34. Conversely, the chicken crumbs marinated in 20% lemon juice exhibited the lowest pH value. In terms of water holding capacity, crumbs with both 20% and 40% lemon juice concentrations showed the highest value at 97.22%, which was significantly greater than the values obtained for 30.0%, 50.00%, 40%, and the control, all of which had the lowest value at 90.00%. Analysing oxidative rancidity, it was noted that the control sample had the highest value at 3.77 mg/g. On the other hand, the samples treated with 40%, 20%, 10%, and 50% lemon juice demonstrated the lowest values, all at 1.63 mg/g.

Table 1: Effect of storage duration on physicochemical quality of chicken crumbs

Parameters	1st Month	2nd Month	3rd Month	SEM	P-value
pH	6.19^{a}	6.15^{b}	5.99^{c}	0.01	0.000
Water holding capacity (%)	96.11a	94.17^{b}	91.67^{c}	1.39	0.004
Oxidative rancidity (mg/g)	1.85^{c}	2.51^{b}	2.70^{a}	0.16	0.036

$^{a, b, c}$ means with different superscripts on the same row differ significantly ($p<0.05$)
SEM - Standard error of mean.

Table 2: Effect of lemon juice on physicochemical quality of chicken crumbs

Parameters	Control	10%LJ	20%LJ	30% LJ	40% LJ	50% LJ	SEM	P-value
pH	6.34^{a}	5.98^{e}	6.10^{b}	6.11^{b}	6.08^{c}	6.05^{d}	0.01	0.000
Water holding capacity (%)	90.00^{d}	97.22^{a}	97.22^{a}	95.00^{ab}	91.11^{cd}	93.33^{bc}	2.43	0.000
Oxidative rancidity (mg/g)	3.77^{a}	1.92^{c}	2.43^{b}	1.74^{cd}	2.64^{b}	1.63^{d}	0.28	0.000

$^{a, b, c}$ means with different superscripts on the same row differ significantly ($p<0.05$)
LJ - Lemon juice
SEM - Standard error of mean.

Table 3: Effect of storage duration and lemon juice on pH of chicken crumbs

Parameters	Months	Control	10%LJ	20%LJ	30%LJ	40%LJ	50% LJ	SEM	P-value
pH	1st month	6.32^{bi}	6.18^{ak}	6.15^{bcl}	6.22^{aj}	6.17^{ak}	6.14^{al}	0.01	0.000
	2nd month	6.48^{ai}	5.78^{cl}	6.24^{aj}	6.13^{bk}	6.11^{bk}	6.13^{ak}	0.05	0.000
	3rd month	6.24^{ci}	5.98^{bj}	5.91^{cl}	5.99^{cj}	5.95^{ck}	5.89^{bl}	0.03	0.000
	SEM	0.04	0.06	0.05	0.03	0.03	0.04		

$^{a, b, c, d}$ means with different superscripts on the same column differ significantly ($p<0.05$)
$^{i, j, k, l}$ means along the same row with different superscripts differ significantly ($p<0.05$)
LJ - Lemon juice
SEM - standard error of mean.

3.2 Effect of storage duration and lemon juice on pH of chicken crumbs

Table 3 shows the interaction effect of storage duration and lemon juice on the pH of chicken crumbs. The results indicate significant differences ($p<0.05$) across and among the treatment groups. Specifically, in the first month, the chicken crumbs marinated in 0% lemon juice (control) recorded the highest pH value at 6.32. In contrast, the lowest pH value was observed in chicken crumbs marinated in 20% lemon juice during the second month, measuring 5.78.

Table 4: Effect of storage duration and lemon juice on water holding capacity of chicken crumbs

Parameters	Months	Control	10% LJ	20% LJ	30%LJ	40% LJ	50% LJ	SEM	P-value
Water holding capacity (%)	1st month	91.67^{aj}	100.00^{ai}	100.00^{ai}	98.33^{ai}	90.00^{aj}	96.67^{ai}	1.04	0.000
	2nd month	93.33^{aij}	96.67^{abi}	96.67^{abi}	96.67^{ai}	90.00^{aj}	91.67^{bj}	0.72	0.000
	3rd month	85.00^{bk}	95.00^{bi}	95.00^{bi}	90.00^{bj}	93.33^{aij}	91.67^{bij}	0.88	0.000
	SEM	1.55	0.79	0.79	1.30	0.79	0.92		

[a, b, c, d] means with different superscripts on the same column differ significantly ($p<0.05$)

[i, j, k, l] means along the same row with different superscripts differ significantly ($p<0.05$)

LJ - Lemon juice

SEM - standard error of mean.

Table 4 shows the interaction effect of storage duration and lemon juice on the water holding capacity of chicken crumbs. The results indicate a significant difference ($p<0.05$) in the interaction between months and inclusion levels. In detail, during the first month, chicken crumbs marinated in 10% and 20% lemon juice exhibited the highest water holding capacity. Conversely, the chicken crumbs marinated in 0% lemon juice (control) demonstrated the lowest water holding capacity in the third month. Table 4 shows the interaction effect of storage duration and lemon juice on the water holding capacity of chicken crumbs. The results indicate a significant difference ($p<0.05$) in the interaction between months and inclusion levels. In detail, during the first month, chicken crumbs marinated in 10% and 20% lemon juice exhibited the highest water holding capacity. Conversely, the chicken crumbs marinated in 0% lemon juice (control) demonstrated the lowest water holding capacity in the third month. Table 5 displays the interaction effect of storage duration and lemon juice on the oxidative rancidity of chicken crumbs. The indicate significant differences ($p<0.05$) among the treatments. Specifically, in the control group, the chicken crumbs at the third month exhibited the highest oxidative rancidity value at 5.07 (mg/g). On the other hand, the chicken crumbs marinated in 10% lemon juice showed the lowest oxidative rancidity value at 1.15 (mg/g) in the second month.

Table 5: Effect of storage duration and lemon juice on oxidative rancidity of chicken crumbs

Parameters	Months	Control	10% LJ	20%LJ	30% LJ	40% LJ	50% LJ	SEM	P-value
Oxidative rancidity (mg/g)	1st month	1.95^{cij}	1.65^{bjk}	1.93^{bij}	2.05^{aij}	2.35^{bi}	1.17^{bk}	0.09	0.000
	2nd month	4.30^{bi}	1.15^{cm}	2.78^{ak}	1.77^{abl}	3.57^{aj}	1.50^{blm}	0.28	0.000
	3rd month	5.07^{ai}	2.96^{aj}	2.59^{ajk}	1.39^{bm}	2.00^{bm}	2.21^{akl}	0.29	0.000
	SEM	0.47	0.27	0.13	0.10	0.24	0.20		

[a, b, c, d] means with different superscripts on the same column differ significantly ($p<0.05$)
[i, j, k, l] means along the same row with different superscripts differ significantly ($p<0.05$)
LJ - Lemon juice
SEM - Standard error of mean.

4.0 DISCUSSION

4.1 Physicochemical quality of chicken crumbs on effect of storage duration

4.1.1 pH

Meat pH is one of the most important meat quality parameters, which is also associated with the rate of glycolytic pathway during post slaughtering. The flesh of animals prior to slaughter has a pH value of 7.1 after slaughter some of the glycogen in the meat turns into lactic acid. After slaughtering, the anaerobic glycolysis results in accumulation of lactate, which contribute to the increased concentration of hydrogen ions (H+), as well as the protein denaturation, which release H+, thus leading to the changes in muscle pH value (salwani *et al.,2015)*. The pH also affects water holding capacity. Denaturation of protein, which is caused by a reduction in pH, contributes to the loss of ability for the meat to hold water. The reduction in pH could by the release of amino acids and carbonyls from the protein denaturation, which could have changed the myofibrilla protein isoelectrical point, thus also the loss of bounded water, and reduced water holding capacity in meat after long storage duration

(Leygonie *et al.,*2012). Significant interaction was noted between storage duration and lemon juice concentration in pH of chicken crumbs. Storage duration had effect on chicken crumbs pH because the the concentration of lemon juice the lower the pH value and these was consistently observed at all evaluation intervals. The results suggest increased acidity of the chicken crumbs samples over the storage duration (Table 3). Similar decreasing trend in pH was reported by Verma *et al.* (2013) in mutton nuggets incorporated with guava powder and Banerjee *et al.* (2012) in goat meat nuggets incorporated with broccoli powder juice. Also, the result obtained is in line with reports by Disha *et al.* (2020), who stated that a slight decrease in raw pH values for all treatments and an increase in the acidity values for all samples along with storage time during 90 days of storage as a result of increasing of free fatty acids due to rancidity.

4.1.2 Water Holding Capacity

The water capacity (WHC) is the ability of meat to hold all or part of it water and one of the most important traits of meat quality. Weight loss due to purge or drip loss range from 2% to 10% when meat is cut into chops. Water holding capacity in meat is influenced by the changes in muscle cellular and extracellular components (Leygonie *et al.,*2012). Generally, a reduction in meat water holding capacity could be resulted from storage duration (Sabow *et al.,*2016).

This study demonstrated the interaction effect of storage duration (after 3 months storage) and lemon juice concentration levels, which were related to the decrease in water holding capacity. The effect was also observed in the crumbs, indicating reduction in meat water holding capacity after being stored longer period of time. This would also increase water movement from intracellular into extracellular spaces, contribute to the increases in the concentration of the solutes in the cells, leads to protein denaturation and reduces the meat ability to retain water (Saelin *et al.,*2017). Meanwhile, the presence of oxygen could also contribute to the lipid and protein oxidation, which may cause structural damage to the meat, and thus increase water loss after longer storage duration. (Marcinkowska-lesiak *et al.,2016)* Therefore, it has been shown that there was a significant interaction ($p < 0.05$) between storage duration and inclusion levels of lemon juice in the chicken crumbs. The higher value percentages were observed on inclusion levels after longer storage duration. Significant differences ($p < 0.05$) in the inclusion levels were noticed among the chicken crumbs stored in different months, with the highest value of 100.00 % recorded while the least value of 85.00% (Table 4). The chicken crumbs stored for 3 months showed significant differences in water holding capacity across all storage duration.

4.1.3 Oxidative rancidity

Lipids are important components of all types of meat and are responsible for many desirable characteristics of meats. They are important for the flavor and aroma profile of meats and contribute to tenderness and juiciness. Oxidative rancidity is best controlled by the addition of antioxidants. The heat treatment experienced during extrusion is usually sufficient to destroy these enzymes. Most antioxidants are added before extrusion even though there may be minor destruction during the process depending on the time and temperatures employed. There is a growing trend to utilize natural antioxidants such as mixed lemon juice, rosemary, and other unique ingredients.

The results obtained in this study showed that there was significant ($p<0.05$) difference and interaction between the storage time and lemon juice concentration. A trend was observed as the lemon juice inclusion levels increase, there was a decrease in oxidative rancidity. Similarly, as the storage duration increases, there was an increase in the oxidative rancidity. The result obtained shows that lemon juice has antioxidant activities which caused a reduction in the oxidative rancidity as the concentration levels increased. The result obtained in this study was in agreement with the finding of (Chidanandaiah *et al.,* 2009) who reported TBARS values increased significantly ($p<0.05$) during refrigerated storage period. Similar findings were stated by Yadav *et al*. (2018) who found a significant increase in TBARS value of control and fiber enriched sausage with an increase in storage period. (Disha *et al.,* 2020) also obtained similar results obtained in this study where different level of lemon juice on quality and shelf life of chicken meatballs during frozen storage. The results obtained could be due to the presence of bioactive compounds found in lemon juice that is ascorbate acid which has antioxidant activity and oxygen barrier properties that contributed to lipid oxidation-reduction. The oxidative rancidity value in lemon treated crumbs was significantly lower than in control without lemon juice, which could be due to the inhibition of oxygen permeation and synergistic effects of lemon juice (Kostaki *et al.,* 2009; Lu *et al.,* 2009; Chytiri *et al.,* 2004).

5.0 CONCLUSION

The study was carried out to improve the keeping quality of chicken crumbs marinated with lemon juice as a good source of natural antioxidant. The lemon juice improved the keeping quality and antioxidant activity of the chicken crumbs. Also, the results show that the addition of lemon juice caused a significant ($p < 0.05$) decrease in pH, water holding capacity and oxidative rancidity. In addition, chicken crumbs can be produced with the addition of lemon juice between 4.00% to 6.00% because of their higher physicochemical quality.

RECOMMENDATION

Generally, this study recommends the use of lemon juice between 20.00% to 40.00% inclusion in the development of chicken crumbs and keeping quality of meat product. Further research should be carried out on microbial evaluation and sensory properties.

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