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Effect of Adding Lemon Peel Extract Fortified with Gelatin on Preserving Beef Meatballs During Refrigeration on Qualitative and Sensory Characteristics

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Abstract

The study aimed to evaluate the use of lemon peel extract at a concentration of 0.5%, 1%, 1.5% and 2 % in preserving beef meatballs under refrigeration at 4oC. The results showed that (TBA) and percentage of free fatty acid in meat samples that treated with lemon peel extract significantly reduced compared to the control, which reached 1.94 malno aldehyde/ 0.58 kg on the sixth day of preservation. While the treated samples remained within the standard limits until the period of refrigeration preservation, which reached to 1.68, 1.55, and 1.51. Also, the results showed that the capacity of water holding in beef meatballs that treated with lemon peel extract increased significantly compared to the control treatment. On the sixth day of refrigeration, the averages in the third, fourth and fifth treatment were 36.67%, 48.21%, and 43,11%, respectively, while in the control treatment reached 32.67% for the same period of storage.

Keywords: Beef meatballs, Lemon peel extract, Meat storage, Natural antioxidants, Shelf life extension.

1. Introduction

Meat and its products are high nutritional value, it is a major source of essential amino acids that are required for human body to build its tissues. Additionally, meat is a major source ofB vitamins and minerals, such as iron. Also, help reduces malnutrition and increase household food security (Platts-Mills et al., 2020).

Between the most prominent factors affecting the quality of meat is its chemical and biological nature, making it susceptible to spoilage during storage due to fat oxidation and microbial growth. Oxidation negatively affects the taste and flavor, which reduces the shelf life of meat and its products (kazemet. al., 2024), microbial growth also contributes to food poisoning and economic losses as a result of meat spoilage (Moustafa et. al., 2022). Therefore, many industrial preservatives have been used for centuries to preserve flavor, improve taste, and extend the shelf life of meat, which would reduce food spoilage (Haqueet. al., 2020).

Nowadays, many synthetic antioxidants, including Butylated Hydroxy Toluene (BHT) and Butylated Toluene Anisole (BHA)are widely used commercially. Because of antioxidants negative effects on consumer health, the safety of them have raised many doubts in the past years. It is known that synthetic antioxidants are carefully handled by the US Food and Drug Administration (FDA) due to concerns about their toxicity when consumed above certain limits (Badr El-Dinet. al., 2024). Lemon peels are an important source of biologically active compounds and have been used in folk medicine for centuries. The antioxidant activity of phenolic compounds from citrus and lemon peels has been reported in terms of reduced lipid per oxidation. Their extracts are rich sources of biologically active compounds, for example, flavonoids and vitamin C, are therefore used as additives inmeat products(Abd el-naeemet al., 2022).Due to the importance of the subject, the current study aimed to:

1- Water extraction of lemon peels.

2- Adding lemon peel extract to minced beef balls with a study of oxidation indicators and microbial contamination during the period of refrigeration storage at a temperature of 4°C over different time periods.

2. Materials and Methods

2.1. Water Extraction of Lemon Peels

The water extract was prepared according to Gülçin et al. (2003). A 25 g of each sample was mixed with 500 ml of boiled distilled water and placed on a magnetic stirrer for 30 minutes. The mixture was then filtered through filter paper using a vacuum and then the filtrate was concentrated using a rotary evaporator at 40 ℃ to remove the water. Afterward, the filtrate was left to dry at room temperature (25 ℃) then placed in opaque, tightly sealed bottles and stored in the refrigerator at 4 ℃ until use.

2.2. Manufacturing and Preparation of Beef Meatballs

Beef meat (thigh area) was obtained from the local markets of Basra Governorate and minced using electric meat grinder to prepare the samples. The minced meat samples were treated with lemon peel extract at concentrations of 0.5%, 1%, 1.5%, 2% while the control samples were untreated. The meat was formed into balls

weighing 50 g each .All samples were stored for specific periods of time 0, 2, 4, 6, 8, 10 days in a refrigerator at 4°C.

2.3. Chemical Tests

2.3.1. Estimation of Thio Barbituric Acid (Tba)

Thio barbituric acid was estimated based on method of Mehran (1976), and it was calculated according to the following equation:

Malon aldehyde concentration (mg/kg) = optical absorbance × 7.83

Estimation of free fatty acids (FAA)

Free fatty acids were estimated using the method of Pearson et. al. (1981),with the following equation:

$$Free\ Fatty\ acid\% = \frac{Titration\ (A-B) \times N \times 282}{1000 \times weight\ of\ sample\ (g)} \times 100$$

Where:

A = volume (mL) of KOH used for titration with the fat or oil sample.

B = volume (mL) of KOH used for the blank sample

282 = molecular weight of oleic acid

Estimation of myoglobin pigment concentration

The myoglobin concentration was estimated based on the method of Zessinet. al, (1961). The optical absorbance of the filtrate was measured at 525 nm, and the concentration was calculated according to the equation:

$$Myoglobin\ concentration = \frac{absorbance \times 2.4}{sample\ weight \times 0.425}$$

Where;

0.452 represents the absorbance coefficient at (525 nm) and 2.4 represents the dilution coefficient.

Estimation of water holding capacity

The water holding capacity of meat samples stored under refrigeration and freezing was estimated according to the method of Dolatowski and Stasiak (1998). The percentage of water holding capacity was calculated as follows:

$$Water\ holding\ capacity = \frac{weight\ of\ added\ water - weight\ of\ water\ after\ centrifuge}{sample\ weight} \times 100$$

Estimation of weight loss during cooking weight loss during cooking was calculated by grilling minced meat sausages on a hot plate for six minutes, turning for three minutes to ensure the internal temperature reached 70°C according to the following equation (Rasmussen and Mast, 1989).

$$Loss\ during\ cooking\ \% = \frac{sample\ weight\ before\ cooking - sample\ weight\ after\ cooking}{sample\ weight\ before\ cooking} \times 100$$

2.4. Statistical Analysis

SSPS software was used to analyze all data. A three-factor factorial experiment was conducted with a completely randomized design (CRD). Means were separated using the least significant difference (LSD) test at a probability level of 0.05.

3. Results and Discussion

3.1. Thio Barbituric Acid (TBA)

The results of Table (1) showed a significant decrease ($p \leq 0.05$) in the value of thio barbituric acid in beef meatballs treated with different concentrations of lemon peel extract (0.5, 1, 1.5)% compared to the control sample throughout the preservation period.The TBA value in the control treatment increased from 0.28 to 1.94 by the sixth day of preservation, while the average TBA values for treatments with lemon peel extract reached (1.68, 1.55, 1.51, 1.51)% for concentration of (0.5, 1, 1.5, 2)%, respectively, during the same period. As for the eighth day of preservation, the control sample exceeded the limits of the standard specifications that confirm the upper limit of the value of TBA in preserved meat is 2 mg malon di aldehyde / kg meat (Central Organization for Standardization and Quality Control, 1987).Therefore, The control sample was excluded. while the meatball samples treated with lemon peel extract at concentrations of 0.5%, 1%, 1.5% and 2% maintained their permissible TBA limits until the eighth day of storage, the TBA values were 1.82, 1.73 and 1.68 respectively. On the tenth day of refrigeration ,the TBA averages for beef meatballs were 1.92 and 1.91 for the 0.5% and 1%concentrations,respectively, while the values reached 1.74 and 1.77% for the (1.5% and 2%concentrations, respectively. These results confirm that the use of lemon peels was effective in preserving meatball samples from oxidation for an additional period compared to the control sample, the reason for this is effect may attribute to the active substances present in lemon peels (Sabuet. al., 2020).

Table 1. Effect of different concentrations of lemon peel extract on TBA values (mg malondialdehyde/kg) of meatballs stored at 4°C in refrigeration.

Treatments	Periods (day)						Rate of treatments
	0	2	4	6	8	10	
T1	0.28	0.99	1.36	1.94			1.37
T2	0.28	1.14	1.51	1.68	1.82	1.92	1.39
T3	0.28	0.99	1.22	1.55	1.73	1.91	1.28
T4	0.28	0.95	1.17	1.51	1.68	1.74	1.22
T5	0.28	0.99	1.18	1.51	1.68	1.77	1.23
Rate of periods	0.28	1.01	1.29	1.64	1.74	1.84	

Note: LSD for treatments = 0.02

LSD for Periods = 0.09

3.2. Percentage of Free Fatty Acids

The results of Table 2 showed a significant decrease (p≤ 0.05) in the value of free fatty acid in beef meatballs treated with different concentrations of lemon peel extract (0.5, 1, 1.5)% compared to the control sample throughout the preservation period.In the control treatment, the percentage of free fatty acidsincreased from 0.35 to 1.63 by the sixth day of storage, thus exceeding the standard limits of 1.2% set by the Iraqi standard specification (Central Organization for Standardization and Quality Control, 1987), while the percentage for the treatments with lemon peel extract on the sixth day reached (0.67, 0.57, 0.47, 0.47)% for concentrations of (0.5, 1, 1.5, 2)%, respectively. On the 10th day of cold storage,the average percentages for beef meatballs treated withlemon peel extract reached (0.84, 0.84) for concentrations of (0.5, 1)%, while they reached (0.82, 0.82)% for concentrations of (1.5, 2)%, respectively.The results in Table 2 show that lemon peel extract significantly reduced the average percentage of free fatty acids. The reason for the quality of the treatments may be attributed to lemon peel, which is a natural antioxidant because it contains active compounds that inhibit fat-dissolving enzymes such as lipase, thereby reducing the release of free fatty acids (Khan et al., 2020). These results were consistent with those of Athauasiadis et al.(2024) who reported the presence of antioxidants in lemon peel extract.

Table 2. Effect of different concentrations of lemon peel extract on the free fatty acids of meatballs stored under refrigeration at 4°C.

Treatments	Periods (Day)						Rate of treatments
	0	2	4	6	8	10	
T1	0.35	0.59	0.97	1.63			0.89
T2	0.35	0.34	0.43	0.67	0.82	0.84	0.58
T3	0.35	0.35	0.44	0.57	0.81	0.84	0.56
T4	0.35	0.35	0.42	0.47	0.78	0.82	0.53
T5	0.35	0.35	0.42	0.47	0.78	0.84	0.54
Rate of periods	0.35	0.4	0.54	0.77	0.79	0.84	

Note: LSD for treatments = 0.02
LSD for Periods = 0.03

3.3. Concentration of Myoglobin Pigment

The results in Table 3 showed a significant increase (p≤ 0.05) in the concentration of Myglobin pigment in beef meatballs treated with different concentrations of lemon peel extract 0.5%, 1%, 1.5% compared to the control sample during the storage period. The Myglobin pigment concentration in the control treatment decreased from 3.17% to 1.71% by the sixth day of storage, while the values of Myglobin pigment in the treatments with lemon peel extract on the sixth day reached 2.43%, 2.44%, 2.48%, 2.49% for concentration of 0.5%, 1%, 1.5%, and 2% respectively. The meatball samples treated with concentrations of 0.5, 1, 1.5, and 2 lemon peel extract, they maintained their stability within the permissible limits until the tenth day of storage, as the averages reached 2.11 and 2.03 for concentrations of 0.5% and 1%, while they reached 2.26% and 2.27% for concentrations (1.5, 2)%, respectively.

The improved stability of Myglobin pigment in meatballs treated with lemon peel extract may be due to the extract containing flavonoid and phenol active substances. These substances are able to delay the formation of the met myoglobin pigment, which is formed when Myglobin combines with oxygen, leading to the oxidation of ferrous ions (Fe+2) in Myglobin to ferric ions (Fe+3), which give the dark pigment, which is one of the important substances in meat processing (Al-Ameri and Nasser., 2021).

Table 3. Impact of different concentrations of extract of lemon peel on myoglobin pigment of meatballs stored at 4°C in refrigeration.

Treatments	Periods (day)						Rate of treatments
	0	2	4	6	8	10	
T1	3.17	2.81	2.42	1.71			2.36
T2	3.17	2.83	2.53	2.43	2.22	2.11	2.55
T3	3.17	2.87	2.64	2.44	2.22	2.03	2.56
T4	3.17	2.91	2.77	2.48	2.37	2.26	2.66
T5	3.17	2.91	2.76	2.49	2.38	2.27	2.66
Rate of periods	3.17	2.87	2.63	2.31	2.25	2.13	

Note: LSD for treatments = 0.01
LSD for periods = 0.02

3.4. Water Holding Capacity

The results presented in Table 4 show a significant increase (p≤ 0.05) in the Water holding capacity (WHC) of beef meatballs treated with different concentrations of lemon peel extract (0.5, 1, 1.5, 2)% compared to the control sample ethroughout the storage period. The WHC value decreased in the control treatment from 57.11 to 32.67 by the sixth day of storage. while the WHC values for the treatments with lemon peel extract on the sixth day for concentrations (0.5, 1, 1.5, 2)% reached (35.67, 36.67, 48.21, 43.11)%, respectively. The meatball samples treated with concentrations 0.5, 1, 1.5, and 2 of lemon peel extract, they maintained their permissible limits until the tenth day of storage. The averages reached (32.45,31.78) for concentrations 0.5% and 1% while it reached (40.92,38.11)% for concentrations (1.5% and 2% respectively. The results in Table 4 also show that the concentrations 1.5% and 2% were significantly more effective (p<0.05) in increasing the WHC compared to the concentration 0.5% and 1% for all treatments.

This enhancement in WHC may be attributed to the presence of collagen protein and the functional compounds in lemon peels, which contribute to retaining fluids within the meat and leads to raising the PH. These results were consistent with Rafat (2023) whoobserved similar improvements in WHC when using lemon peel powder and rosemary powder in preparing chicken burgers.

Table 4. Impact of different concentrations of extract of lemon peel on Water holding capacity (WHC) of meatballs stored at 4°C in refrigeration.

Treatments	Periods (Day)						Rate of treatments
	0	2	4	6	8	10	
T1	57.11	41.67	38.67	32.67			42.53
T2	57.11	42.22	38.67	35.67	33.33	32.45	39.9
T3	57.11	42.67	39.11	36.67	34.32	31.78	40.27
T4	57.11	54.32	50.11	48.21	43.11	40.92	48.97
T5	57.11	50.33	47.32	43.11	40.66	38.11	46.1
Rate of periods	57.11	46.24	42.77	39.26	37.12	35.81	

Note: LSD for treatments = 0.37
LSD for periods = 1.41.

3.5. The Percentage of Weight Loss During Cooking

The results in Table 5 showed that the average weight loss during cooking of beef meatballs treated with different concentrations of lemon peel extract significantly decreased ($p \leq 0.05$) compared to the control sample when stored at 4 °C. With the progression of the storage period, the average weight loss during cooking in the control treatment increased from 26.21 to 29.98 by the sixth day of storage, while, the average weight loss during cooking for the treatments with lemon peel extract on the sixth day were (28.21, 26.22, 24.11, 24.11)% for the concentrations of (0.5, 1, 1.5, and 2)% respectively.

On the tenth dayof storage, the weight loss averages were (29.22, 27.22) for the concentrations 0.5% and 1%, while it reached (25.63,25.82)% for concentrations 1.5% and 2% respectively. The reason for reduction in weight loss during cooking in the lemon peel treatments may be attributed to the effect of the active compounds in extract on meat proteins and the increase in water-holding sites. Additionally, the ability of lemon peel to bind water and retain moisture contributes to the decrease weight loss during cooking. Many studies have showed that the correlation between weight loss during cooking and the ability to hold water is inverse. This characteristic is very important in meat processing to minimize product shrinkage during cooking and thus making the product demand consumer (Basati and Hosseini, 2018). The results were consistent with Kumar et al. (2015) which observed that the WHC value increased using lemon and ginger extracts.

Table 5. Effect of different concentrations of extract of lemon peel on the percentage of weight loss during cooking of meatballs stored at 4°C in refrigeration.

Treatments	Periods (day)						Rate of treatments
	0	2	4	6	8	10	
T1	26.21	27.55	28.32	29.98			28.01
T2	26.21	26.22	27.55	28.21	28.73	29.22	27.69
T3	26.21	25.33	25.61	26.22	36.71	27.22	26.22
T4	26.21	23.22	23.55	24.11	25.45	25.63	24.69
T5	26.21	23.41	23.74	24.11	25.56	25.82	24.81
Rate of periods	26.21	25.15	25.75	26.53	26.61	26.97	

Note: LSD for treatments = 0.12
LSD for periods = 0.08

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