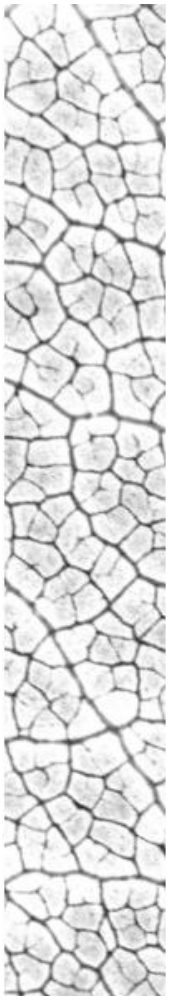

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The effect of adding different concentrations of hibiscus sabdariffa on prolong the cooling preservation period of chicken kofta

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Abstract
The study aimed to investigate the impact of adding Hibiscus sabdariffa to extend the shelf life of chicken kofta stored under cooling. Chemical tests (percent of free fatty acid and peroxide number value), and microbiological test (total bacterial number and psychrophilic bacteria) were conducted. The control sample was eliminated on the seventh day of the experiment because the FFAs value and peroxide value exceeded the limit allowed. Adding of Hibiscus sabdariffa to chicken kofta samples led to extending the shelf life of these samples until the twelfth day of the cold storage in comparison with control sample. During the storage time, control sample was eliminated on the seventh day as the total bacterial number and the psychrophilic bacteria exceeded the limit allowed according to standard specification, while the samples treated with Hibiscus sabdariffa kept on its acceptable microbial numbers until the twelfth day and the best concentration was 1.5%.

Keywords: hibiscus sabdariffa, antioxidant, antimicrobial, chicken kofta

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INTRODUCTION

Hibiscus sabdariffa belongs to Malvaceae which contains over 200 genera and 2300 species of plant. Hibiscus sabdariffa probably originated in Africa, but it has also been grown in Asia, America, and Australia (Mehdi *et al*., 2013). This plant is cultivated in Sudan, India, Malaysia, and Taiwan. It is an annual or perennial herb and is generally 2-2.5 m tall. Its flowers take months to mature into a bright red color (Singh *et al*., 2017; Hounkpè *et al.,* 2019). Manufacturing companies use Hibiscus sabdariffa for its beneficial effects to reduce blood pressure because its tea contains vitamin C, minerals, and different antioxidants which help lower blood pressure and relieve anxiety (Rao, 1996). It may also help to prevent cardiovascular and blood vessel diseases, reduce cholesterol, boost immune system, exhibit anti-inflammatory activity, and reduce of cancer diseases incidence (Islam, 2019). Hibiscus sabdariffa also contains medical traits.

Chemical preservatives, which are used to preserve and extend the shelf-time of food, may cause severe health problems and many consumers do not prefer high levels of preservatives in products as opposed to the use of natural preservatives, including Hibiscus sabdariffa to control *E. coli* bacteria and pH value during freezing time (Paim *et al*., 2017)

Food processing industry has started using natural ingredients to preserve meat products, including hamburger, meatballs, and sausages (Freire,2004) as well as dried and marinated meat using meat additives (Simões *et al*,.2001; Mariutti *et al*., 2008). Food additives are added to improve physical, chemical, biological, and sensory properies of food product during manufacturing, preparing, packaging, transporting and storage (Brasil, 2000). These additives preserve and improve food (Nespolo *et al*., 2015; Silva, 2005), because these additives such as Hibiscus sabdariffa contain phenolic compounds, which are source of strong antioxidant, can destroy free radicals, and protect cells from oxidative damage through its ability to give hydrogen which many scientists consider Hibiscus sabdariffa as antioxidant compound (Sáyagol *et al*., 2007; Bidie *et al*., 2011).

Hibiscus sabdariffa is rich in phenolic compound, particularly anthocyanins which are responsible for the red pigmentation in plants and are an excellent source of antioxidants (Cisse *et al*., 2009; TSAI *et al*., 2004).

Anthocyanins are water soluble flavonoid pigment depending on the pH percentage that may appear red, purple, or blue depending on the pH (Mendoza-Diaz *et al*., 2012).

The study aims to investigate the effect of adding Hibiscus sabdariffa on the chemical, physical and

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Table 1. The effect of adding Hibiscus sabdariffa on the peroxide value meq/Kg in chicken kofta samples preserved in cooling

Hibiscus Sabdariffa concentrations%	(Cold storage) days							
	0	2	4	6	8	10	12	Average
Control	1.29	2.68	3.74	4.74	excluded	excluded	excluded	3.11
0.5	1.16	1.46	2.64	2.82	3.92	3.98	4.16	2.87
1	1.14	1.34	2.41	2.56	2.74	3.94	4.08	2.60
1.5	1.09	1.34	2.43	2.68	2.96	3.34	4.04	2.55
Average	1.17	1.70	2.80	3.20	3.20	3.41	4.09	2.78

RLSD for storage period= 0.69
RLSD for concentrations=0.37

microbiological properties, and extending the shelf-life of food during freezing.

MATERIALS AND METHOD

Meat: Laying chicken breasts were purchased at 45 weeks of age from the poultry hall at the University of Basrah. After cutting the chicken breasts and fat, they were put in sealed polyethylene bags.

Plant: Hibiscus sabdariffa plant was purchased from the local market of Basrah province, was added in ratio of (0.5, 1, 1.5) % to meat samples during cold storage. Chemical, physical and microbiological tests were conducted at the animal production department, microbiology lab, biochemistry lab, meat lab.

Chemical tests

Determination of the peroxide value

Peroxide value was determined according to Pearson *et al.* (1981). 3 g of minced meat was weighed, a mixture of 30 ml (3 parts of glacial acetic acid and 2 parts of chloroform) was added with 5ml of saturated potassium iodide, 20ml of distilled water and few drops of starch indicator, the mixture then titrated with sodium thiosulfate solution (0.001)N until disappearing the blue color. Peroxide value was determined by using the following equation:

$$\text{Peroxide value} = \frac{(Na_2S_3O_4 \text{ ml} \times N \times 1000)}{(\text{Wt.of Sample,gm})}$$

Free Fatty Acid Percentage

Determination of Free fatty acids (FFA) was conducted according to Pearson *et al.* (1981), where three grams of minced meat was weighted, added to ethyl alcohol (50 ml) at (98%) concentration, then heated until boiling in a water bath, drops of phenolphthalein indicator were added, then the mixture titrated with potassium hydroxide solution (0.1N) till the solution turns to light pink. Free fatty acid percentage was calculated using the following equation: -

$$\text{Free Fatty Acid\%} = \frac{\text{Titration (A}-\text{B)x n x 282 x 100}}{1000 \text{ x wt.of sample gm}}$$

A= number of ml of KOH titrated with fat of oil sample
B= number of ml KOH titrated with blank sample
282= Oleic acid molecular weight

Bacteriological Tests

Bacterial total count and psychrophilic bacteria counts were conducted by using pour plate technique and nutrient agar. Required serial dilutions were achieved and petri dishes were incubated at 37 ° C for 24-48 h. for the bacterial total count. For psychrophilic bacteria, petri dishes were incubated in refrigerator at 4 ° C for 10 days.

Statistical Analysis

The data were analyzed by using Complete Randomized Design (CRD) and using the SPSS program. Version 24(2016) The averages were compared using the least significant difference of (LSD) at ($P<0.05$).

RESULTS AND DISCUSSION

Results in **Table 1** indicate that chicken kofta samples treated with Hibiscus sabdariffa at concentrations of 0.5,1, and1.5% showed a significant decrease in peroxide value which preserved at 4 ºC in comparison with the control sample while maintaining cooling. The peroxide value in the control sample increased from 1.29 meq/kg-2.68 meq/kg after two days of cooling. The values continued to rise and on the sixth day of the experiment they reached 4.74 meq/kgm and they were eliminated from the experiment due to microbiological contamination, while on the sixth day of cooling. the peroxide value in samples treated with Hibiscus sabdariffa reached 2.82, 2.56, and 2.68 meq/kg at concentrations of 0.5, 1and 1.5% respectively, since the Hibiscus sabdariffa works as an antioxidant (Paim *et al*., 2017). The results are consistent with the findings of Villasante *et al*. (2019) where natural additives including Hibiscus sabdariffa were added to extending the shelf life of sardines for six days in cooling storage, where Hibiscus sabdariffa reduced the oxidative value in comparison with the control sample.

Results in **Table 2** indicate that the percentage of FFAs significantly ($P<0.05$) decreased in samples treated with Hibiscus sabdariffa in comparison with the control sample with increasing the period of the cold storage. On the second day of storage, the FFAs in samples treated with (0.5, 1 and 1.5) % of Hibiscus sabdariffa reached %0.39, %0.40, 0.34 respectively, while the control sample reached %0.56. On the sixth day, the FFAs percentage in the control sample reached 1.66, exceeding the standard limit which is %1.5, while the sample treated with Hibiscus sabdariffa remained within the acceptable levels until the twelfth day of the cold storage, where the means reached 1.50,1.42, and 1.40% at concentrations of 0.5, 1, and 1.5%

Table 2. The effect of adding Hibiscus sabdariffa on the percentage of FFAs in chicken kofta samples preserved in cooling

Concentrations % Hibiscus Sabdariffa	(Cold storage) days							
	0	2	4	6	8	10	12	Average
Control	0.27	0.56	0.97	1.66	excluded	excluded	excluded	0.86
0.5	0.25	0.39	0.43	0.67	0.79	0.82	1.50	0.69
1	0.25	0.40	0.42	0.46	0.78	0.73	1.42	0.63
1.5	0.22	0.34	0.43	0.48	0.77	0.71	1.40	0.62
Average	0.24	0.42	0.56	0.81	0.78	0.75	1.44	0.70

RLSD for storage period= 0.18
RLSD for concentrations=0.12

Table 3. The effect of adding Hibiscus sabdariffa on the total bacterial (cfu /g) number in chicken kofta samples preserved in cooling

Hibiscus Sabdariffa Concentrations%	(Cold storage) days							
	0	2	4	6	8	10	12	Average
Control	30 $x10^4$	57.33 $x10^4$	73.66 $x10^4$	148 $x10^5$	33$x10^7$	excluded	excluded	77.24
0.5	29 $x10^4$	37.23 $x10^4$	42.11 $x10^4$	58.41 $x10^5$	61.11 $x10^5$	70.31 $x10^5$	90.12 $x10^6$	55.47
1	30 $x10^4$	34.00 $x10^4$	40.00 $x10^4$	51.23 $x10^5$	59.09 $x10^5$	68.34 $x10^5$	85.31 $x10^6$	52.56
1.5	29 $x10^4$	32.33 $x10^4$	40.33 $x10^4$	50.66 $x10^5$	59.33 $x10^5$	66.09 $x10_5$	80.22 $x10^6$	51.13
Average	29.5 $x10^4$	40.22 $x10^4$	49.02 $x10^4$	77.07 $x10^5$	59.84 $x10^5$	68.24 $x10^5$	85.21 $x10^6$	59.10

R.L.S.D for storage time= 15.22
R.L.S.D for concentrations= 19.20

Table 4. The effect of adding Hibiscus sabdariffa on psychrophilic bacteria (cfu /g) numbers in chicken kofta samples preserved in cooling

Hibiscus Sabdariffa concentrations %	(Cold storage) days							
	0	2	4	6	8	10	12	Average
Control	13.32 $x10^4$	33.43 $x10^4$	53.03 $x10^4$	190.30$x10^6$	excluded	excluded	excluded	52.27
0.5	11.11 $x10^4$	22.34 $x10^4$	30.07 $x10^4$	50.93 $x10^5$	88.33 $x10^5$	95.31 $x10^5$	120.66 $x10^6$	59.82
1	10.34 $x10^4$	21.35 $x10^4$	29.33 $x10^4$	49.23 x105	86.75 $x10^5$	90.13 $x10^5$	117.35 $x10^6$	57.78
1.5	10.02 $x10^4$	20.13 $x10^4$	29.00 $x10^4$	47.33 $x10^5$	84.03 $x10^5$	87.22 $x10^5$	114.66 $x10^6$	56.05
Average	11.19 $x10^4$	24.31 $x10^4$	35.35 $x10^4$	84.44 $x10^5$	86.37 $x10^5$	90.88 $x10^5$	117.55 $x10^6$	56.48

For periods L.S.D =16.22
For treatment type L.S.D =12.44

respectively. The results in the table show the effect of Hibiscus sabdariffa in reducing the percentages of FFAs in cold chicken kofta and this attributed to phenolic compounds and flavonoid in Hibiscus sabdariffa which act as antioxidant, and this result is consistent with Singh *et al*. (2017) and Olusola, (2011) who showed that Hibiscus sabdariffa contains anthocyanins and flavonoid which are sources of strong antioxidant.

Results in **Table 3** show the total number of bacteria in chicken kofta preserved in cooling. On the second day, the average number of bacteria in (0.5. 1 and 1.5) % concentrations reached (37.23 $x10^4$, 34.00 $x10^4$, 32.33$x10^4$)cfu /g respectively, while the control sample the bacterial number reached (57.33 $x10^4$) cfu /g. On the eighth day, the control sample exceeded the acceptable total aerobic bacteria number and reached (33$x10^7$) cfu /g where food product is unsafe for human consumption, while chicken kofta treated with Hibiscus sabdariffa remained within the standard limits until the twelfth day as it reached (90.12$x10^6$, 85.31 $x10^6$, 80.22 $x10^6$) cfu /g for the concentrations (0.5, 1, 1.5) % respectively.

Table 3 showed that the increase in Hibiscus sabdariffa concentrations reduces the number of bacteria and these results are consistent with Paim *et al*. (2017) who noticed that Hibiscus sabdariffa reduced the number of *E. coli* bacteria in the treated contaminated minced meat. The results are also consistent with Villasante *et al*. (2019) who found that Hibiscus sabdariffa extended the shelf life of sardines to seven days during cold storage.

Results in **Table 4** show significant differences ($p<0.05$) in the number of psychrophilic bacteria in chicken kofta samples treated with different concentrations of Hibiscus sabdariffa in comparison with the control sample, a significant increase was noticed in the number of bacteria in control sample on the fourth day of the experiment, where the average reached (53.03 $x10^4$) cfu/g, while there was a decrease in chicken kofta samples treated with Hibiscus sabdariffa in (0.5, 1 and 1.5)% concentrations where bacteria numbers reached (30.07 $x10^4$, 29.33 $x10^4$, 29.00 $x10^4$) cfu/g respectively. The number of psychrophilic bacteria continued to increase significantly in the control sample and reached (35$x10^7$) cfu /g on the seventh day, and this sample was eliminated as it exceeded the permissible standard. Chicken kofta samples treated with Hibiscus sabdariffa remained within acceptable limits until the twelfth day when the mean reached (120.66 $x10^6$, 117.35 $x10^6$, 114.66 $x10^6$) cfu/g at concentrations of (0.5, 1, and 1.5) %, respectively. This may be attributed to the presence of phenolics in Hibiscus sabdariffa which are able to act as antioxidant and antibacterial (Jung *et al*., 2013).

CONCLUSION

Results indicated that the use of different concentrations of Hibiscus sabdariffa improved the chemical and microbiological properties of chicken kofta

samples in comparison with the control sample. This is achieved by reducing the percentage of FFAs, peroxide value, and reducing the total bacterial number and psychrophilic bacteria during the cold preservation. Adding Hibiscus sabdariffa to chicken kofta led to extending the shelf life of the product to twelve days in comparison with the control sample.

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