

## Original Research

## Effect of partial replacement of nitrate with annatto seeds powder in the physical and chemical properties of chilled beef sausages

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**ABSTRACT:**

The objective of the present study is to evaluate the partial replacement of nitrate with annatto seeds powder at different percentages in the physical and chemical characteristics of chilled beef sausages under 4°C for 21 days. In this study, six different percentages of annatto seed powder treatments were analyzed viz., T<sub>1</sub> (100% nitrite sodium); T<sub>2</sub> (80% sodium nitrite, 20% annatto powder); T<sub>3</sub> (60% sodium nitrite, 40% annatto powder); T<sub>4</sub> (40% sodium nitrite, 60% annatto powder); T<sub>5</sub> (20% sodium nitrite, 80% annatto powder) and T<sub>6</sub> (100%, annatto powder). The treatments were stored for the storage periods of 1, 7, 14 and 21 days. Then results were analyzed after the physical and chemical tests conducted. The following results were obtained:

- The addition of annatto seed powder to chilled beef sausages led to a significant increase (P<0.01) in the moisture, protein and fat percentages during the cold storage period as compared with sodium nitrite treatments.
- The treatments with the levels of annatto seed powder was higher than the sodium nitrite levels which have showed a higher percentages in water holding capacity in meat.
- The results of the addition treatments of annatto seed powder showed a significant decrease (P<0.01) in peroxide value in fresh chilled beef sausages as compared than to the sodium nitrite treatment during the storage period.
- The addition of annatto seed powder to chilled beef sausages have a significant increase (P<0.01) in myoglobin concentration as compared to the sodium nitrite treatment during the storage period.

It can be concluded that the addition of annatto seed powder to fresh beef sausages, in cold storage and with different storage periods has achieved higher antioxidant efficiency through lower oxidation indicators such as peroxide value, increased myoglobin concentration and improved water holding capacity in meat.

**Keywords:**

Annatto seeds, Sodium nitrate, Beef sausages, Chemical, Physical properties.

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## INTRODUCTION

Meat and their products are deteriorated during the storage process because of the chemical and biological composition due to oxidation of fat and bacterial growth, which are the main factors affecting the quality of food and decrease in its biological value. Fat oxidation leads to the deterioration of taste, flavour and texture of meat and their products which reduces shelf life (Yolmeh *et al.*, 2014). Oxidation rancidity is one of the main causes of food damage, including meat and meat products and the resulting degradation of unsaturated fatty acids and oxidant cholesterol products have adverse effects on the consumer health (Andres *et al.*, 2014). For this purpose, several substances were used to reduce the harmful effects of oxidation and microbial contamination and to preserve their colour, including nitrates and nitrite during the process of meat processing. However, their use has become the subject of controversy and studies because these substances cause many problems that affect the health of the consumer (Al-Rudaiman, 2000).

At present, attention has been drawn to the extraction of natural substances or additives from plants, fruits and grains for use in food (Cardarelli *et al.*, 2008). Annatto plant seeds have been used as natural ingredients in foods commonly used in Asia. Also, it is the second largest natural food colouring in the world as well as used as an anti-microbial and anti-oxidant. (Yolmeh *et al.*, 2014). It is a red or orange substance used to colourize food and extracted from tree seeds (*Bixa orellana*) (Achiote). It is often used to enhance the red or orange colour of the food, but is sometimes used as a flavour or aromatic substance for food and also has a fragrant aroma (Aubrey, 2013).

Because of the lack of existing studies that indicated to the use of natural alternatives to different local or global nitrate, so this study aimed to find natural alternatives that are safer for the consumer and replacing them with nitrate, which is a serious danger to the con-

sumer health. Therefore, different percentages of annatto seed powder instead of sodium nitrite were applied to study its effect in some physical and chemical characteristics of freshly chilled beef sausages.

## MATERIALS AND METHODS

### Manufacturing of sausages

External fat and heavy connective tissue were trimmed off from top round of local beef. Then the meat was cut into small cubes of 2-3 cm<sup>3</sup> to facilitate the chopping process. Pelvic and kidney fat were cut into small cubes of 2-3 cm<sup>3</sup> size before starting the process of making the sausages to facilitate the process of chopping. Various types of non-grated spices were used which are obtained from the local markets in Baghdad. Natural casings were used to fill the sausages and the large intestines of the sheep were obtained. The process of cleaning and preparation of sausages were done according to Alrubeii and Alalqa (2018). The weights of the lean and fat were prepared in the ration of 3:1. Nitrate and annatto powder were added to the mixture of minced meat with different proportions according to the treatments used, T<sub>1</sub>: 100% sodium nitrite without the addition of annatto; T<sub>2</sub>: 80% sodium nitrite with 20% annatto powder; T<sub>3</sub>: 60% sodium nitrite with 40% sodium nitrite; T<sub>4</sub>: 60% nitrate powder with 20% sodium nitrite; T<sub>5</sub>: 80% nitrate powder and T<sub>6</sub>: 100% annatto powder without sodium nitrite. The sausages manufactured were stored for 1, 7, 14 and 21 days and were refrigerated under 4°C.

Chemical analysis (moisture, protein, fat, ash percentages) was done according to the method given in AOAC (2005) also the peroxide value was estimated according to the method given by Alrubeii and Alalqa (2018). The myoglobin concentration (Mb) was measured by the method of Zessin *et al.* (1961). The method adopted by Dolatowski and Stasiak (1998) was used to determine the water holding capacity. The data were analyzed according to the factorial experiment (4×6)

applied with respect to the Complete Randomized Design (CRD). To study the effect of treatment and the duration of storage in treatments using the program . SAS was used (SAS, 2012). The averages are compared using Duncan's Multiple Range Test.

**RESULTS AND DISCUSSION**

**Chemical composition of the chilled beef sausages**

**Moisture**

Table 1 shows the effect of the interaction between the different treatments and the period of storage by refrigerating in the moisture percent of the chilled beef sausages. The results of the table show a significant increase (P<0.01) in the moisture percent in T<sub>6</sub> (100% annatto) in the storage period of 1 day, which did not differ significantly from treatment T<sub>2</sub> (80% sodium nitrite and 20% annatto) in the same storage period as compared to treatment T<sub>1</sub> (100% sodium nitrite), which gave the lowest moisture percent that reached 51.72% in the period of 21 days of storage. The results also showed significant differences between the interaction treatments (treatments and different storage periods). The results of the same table indicated that the treatment T<sub>6</sub> (100% annatto) gave a significant increase in moisture that reached 56.25% which was superior when compared to all the treatments in all storage periods followed by treatment T<sub>5</sub> (20% sodium nitrite and 80% annatto) and in turn, followed by treatment T<sub>2</sub> 80% so-

dium nitrite and 20% annatto), which gave a moisture percent that reached 55.46% and 55.26% respectively in all the storage periods. The lowest moisture percent obtained from the treatment T<sub>1</sub> (100% sodium nitrite) which gave general mean in all the storage periods was 54.22%. Some studies have indicated that the increase in the moisture level is evident when some medical plants or their extracts were added to the meat. Huang *et al.* (2011) explained that the addition of lotus plant extract and barley extract to pork meat resulted in a significant increase in the moisture percent as compared to the use of sodium nitrite alone (100% sodium nitrite).

With regards to the effect of the storage periods, the results of the statistical analysis in Table 1 showed that the moisture percent was significantly affected (P<0.01). The moisture was at the highest level in the storage period of 1 day then began to decrease with the increase of the storage period until it reached the lowest level in storage period of 21 days. This decrease in moisture percent is natural with increasing storage time as the moisture decreases and the percentages of dry matter that contains protein, fat and ash increases. These results were in agreement with the results of Alrubeii *et al.* (2008).

**Protein**

The results of Table 2 indicated to significant the increase (P<0.01) in the protein percent of fresh beef sausages at all storage periods, 1, 7, 14 and 21 days. The

**Table 1. The effect of interaction between the treatments and the period of cold storage on moisture percentage in freshly chilled beef sausages (mean± SE)**

S. No	Treatment	Period of cold storage				Average
		Day1	Day 7	Day 14	Day 21	
1	T <sub>1</sub>	56.64±0.001 <sup>F</sup>	55.81±0.005 <sup>L</sup>	53.41±0.005 <sup>R</sup>	51.72±0.0005 <sup>X</sup>	54.22±0.72 <sup>F</sup>
2	T <sub>2</sub>	58.14±0.001 <sup>B</sup>	56.22±0.001 <sup>H</sup>	53.71±0.005 <sup>Q</sup>	52.97±0.0005 <sup>U</sup>	55.26±0.77 <sup>C</sup>
3	T <sub>3</sub>	57.06±0.005 <sup>D</sup>	55.93±0.0005 <sup>J</sup>	54.22±0.001 <sup>O</sup>	52.98±0.001 <sup>T</sup>	54.94±0.54 <sup>D</sup>
4	T <sub>4</sub>	56.95±0.005 <sup>E</sup>	56.47±0.0005 <sup>G</sup>	54.02±0.001 <sup>P</sup>	51.81±0.001 <sup>W</sup>	54.65±0.73 <sup>E</sup>
5	T <sub>5</sub>	55.80±0.005 <sup>M</sup>	55.94±0.001 <sup>I</sup>	55.75±0.001 <sup>N</sup>	52.43±0.001 <sup>X</sup>	55.46±0.66 <sup>B</sup>
6	T <sub>6</sub>	58.35±0.001 <sup>A</sup>	57.56±0.001 <sup>C</sup>	55.90±0.005 <sup>K</sup>	53.35±0.0005 <sup>V</sup>	56.25±0.68 <sup>A</sup>
7	Average	57.16±0.26 <sup>A</sup>	56.32±0.17 <sup>B</sup>	54.45±0.29 <sup>C</sup>	52.54±0.18	.....
8	Significance of effects	**	**	**	**	**

The averages that carries different letters are significantly different (P<0.01) among them; T<sub>1</sub>: 100% sodium nitrite; T<sub>2</sub>: 80% sodium nitrite, 20% annatto powder; T<sub>3</sub>: 60% sodium nitrite, 40% annatto powder; T<sub>4</sub>: 40% sodium nitrite, 60% annatto powder; T<sub>5</sub>: 20% sodium nitrite, 80% annatto powder and T<sub>6</sub>: 100% annatto powder.

**Table 2. The effect of interaction between the treatments and the period of cold storage on protein percentage in freshly chilled beef sausages (mean± SE)**

S. No	Treatment	Period of cold storage					Average
		Day1	Day 7	Day 14	Day 21		
1	T <sub>1</sub>	20.71±0.001 <sup>T</sup>	21.23±0.0005 <sup>P</sup>	21.92±0.00 <sup>K</sup>	23.25±0.0005 <sup>E</sup>	21.75±0.3 <sup>C</sup>	
2	T <sub>2</sub>	20.27±0.0005 <sup>X</sup>	21.14±0.000 <sup>S</sup>	21.75±0.0005 <sup>L</sup>	23.05±0.0005 <sup>F</sup>	21.57±0.37 <sup>F</sup>	
3	T <sub>3</sub>	20.42±0.001 <sup>V</sup>	21.28±0 <sup>N</sup>	22.03 ±0 <sup>J</sup>	23.27±0.001 <sup>D</sup>	21.75±0.39 <sup>E</sup>	
4	T <sub>4</sub>	20.36±0.000 <sup>W</sup>	21.28±0.001 <sup>R</sup>	22.18 ±0.0005 <sup>I</sup>	23.29 ±0 <sup>C</sup>	21.75±0.4 <sup>D</sup>	
5	T <sub>5</sub>	20.47±0.0005 <sup>U</sup>	21.60±0.001 <sup>M</sup>	22.33±0.0005 <sup>H</sup>	23.58±0.0005 <sup>B</sup>	21.99±0.4 <sup>B</sup>	
6	T <sub>6</sub>	21.18±0.001 <sup>Q</sup>	21.25±0.0005 <sup>O</sup>	22.46±0 <sup>G</sup>	23.70±0.001 <sup>A</sup>	22.14±0.3 <sup>A</sup>	
7	Average	20.57±0.09 <sup>D</sup>	21.28±0.04 <sup>C</sup>	22.11±0.07 <sup>B</sup>	23.35±0.06 <sup>A</sup>	.....	
8	Significance of effects	**	**	**	**	**	

The averages that carries different letters are significantly different (P<0.01) among them; T<sub>1</sub>: 100% sodium nitrite; T<sub>2</sub>: 80% sodium nitrite, 20% annatto powder; T<sub>3</sub>: 60% sodium nitrite, 40% annatto powder; T<sub>4</sub>: 40% sodium nitrite, 60% annatto powder; T<sub>5</sub>: 20% sodium nitrite, 80% annatto powder and T<sub>6</sub>: 100% annatto powder.

treatment T<sub>6</sub> (100% annatto) gave the highest protein percent in sausages at 14 and 21 days that reached to 22.46 and 23.70% respectively, followed by T<sub>5</sub> (20% sodium nitrite and 80% annatto) which gave 21.99% while the treatment T<sub>2</sub> (80% sodium nitrite and 20% annatto) came at last by giving the lowest percent of protein at all storage periods which reached 21.57%. Some studies indicated that the addition of some natural substances to meat and meat products led to an increase in the protein percent in meat during the storage by refrigerating or freezing (Alrubeii *et al.*, 2008). As for the effect of treatments on the protein percent, the results of Table 2 indicated to a significant increase in the protein percent (P<0.01) in the addition treatments as compared to the treatment T<sub>1</sub> (100% sodium nitrite). This is in agreement with the results of Huang *et al.* (2011), which

pointed out that the addition of lotus plant and barley plant extracts to pork meat led to a significant increase in the protein percent as compared to the treatment of 100% sodium nitrite.

With regard to the effect of storage periods, the results of the statistical analysis in Table 2 showed that there were significant differences (P<0.01) in the protein percent among all the addition treatments as compared to the treatment T<sub>1</sub> (100% sodium nitrite). The protein percent was the lowest in the storage period of 1 day then increased with the increase of storage period until it reached the highest level in the storage period of 21 days. This increase is natural, when the storage periods increase, the moisture will decrease and the percentages of dry matter, which includes protein, fat and ash will be increased. This is in agreement with results

**Table 3. The effect of interaction between the treatments and the period of cold storage on fat percentage in freshly chilled beef sausages (mean± SE)**

S. No	Treatment	Period of cold storage					Average
		Day1	Day 7	Day 14	Day 21		
1	T <sub>1</sub>	19.26±0.000 <sup>W</sup>	20.52± 0.001 <sup>V</sup>	21.00±0.0005 <sup>U</sup>	21.92±0.0005 <sup>I</sup>	20.6±0.36 <sup>F</sup>	
2	T <sub>2</sub>	21.16±0.0005 <sup>R</sup>	21.55±0.0005 <sup>M</sup>	22.43±0.0005 <sup>C</sup>	22.76±0.0005 <sup>B</sup>	21.97± 0.24 <sup>C</sup>	
3	T <sub>3</sub>	21.45±0.0005 <sup>O</sup>	21.52±0.005 <sup>N</sup>	21.73±0.0005 <sup>K</sup>	22.34± 0.001 <sup>E</sup>	21.76±0.13 <sup>E</sup>	
4	T <sub>4</sub>	21.14±0.001 <sup>S</sup>	21.65± 0.001 <sup>L</sup>	22.27±0.0005 <sup>F</sup>	23.27± 0.001 <sup>A</sup>	22.08±0.13 <sup>A</sup>	
5	T <sub>5</sub>	21.43±0.0005 <sup>Q</sup>	21.37±0.001 <sup>P</sup>	22.02± 0.0005 <sup>H</sup>	23.27±0.0005 <sup>A</sup>	22.00±0.29 <sup>B</sup>	
6	T <sub>6</sub>	21.07±0.0005 <sup>T</sup>	21.88±0.0005 <sup>J</sup>	22.24±0.0005 <sup>G</sup>	22.36±0.0005 <sup>D</sup>	21.89± 0.19 <sup>D</sup>	
7	Average	20.90±0.22 <sup>D</sup>	21.41± 0.12 <sup>C</sup>	21.95±0.14 <sup>B</sup>	22.65± 0.15 <sup>A</sup>	.....	
8	Significance of effects	**	**	**	**	**	

The averages that carries different letters are significantly different (P<0.01) among them; T<sub>1</sub>: 100% sodium nitrite; T<sub>2</sub>: 80% sodium nitrite, 20% annatto powder; T<sub>3</sub>: 60% sodium nitrite, 40% annatto powder; T<sub>4</sub>: 40% sodium nitrite, 60% annatto powder; T<sub>5</sub>: 20% sodium nitrite, 80% annatto powder and T<sub>6</sub>: 100% annatto powder.

**Table 4. The effect of interaction between the treatments and the period of cold storage on ash percentage in freshly chilled beef sausages (mean± SE)**

S. No	Treatment	Period of cold storage					Average
		Day1	Day 7	Day 14	Day 21		
1	T <sub>1</sub>	0.96±0.0005 <sup>J</sup>	1.04±0.002 <sup>I</sup>	1.33± 0.003 <sup>C</sup>	1.90±0.002 <sup>A</sup>	1.21±0.02 <sup>A</sup>	
2	T <sub>2</sub>	0.52±0.001 <sup>Q</sup>	0.88 ± 0.002 <sup>N</sup>	1.22±0.001 <sup>F</sup>	1.34±0.002 <sup>B</sup>	0.86±0.10 <sup>E</sup>	
3	T <sub>3</sub>	0.85±0.001 <sup>O</sup>	0.94±0.002 <sup>K</sup>	1.05±0.002 <sup>H</sup>	1.33±0.002 <sup>C</sup>	1.11± 0.14 <sup>D</sup>	
4	T <sub>4</sub>	0.89±0.003 <sup>M</sup>	1.27±0.001 <sup>E</sup>	1.31±0.002 <sup>D</sup>	1.34±0.003 <sup>B</sup>	1.20± 0.06 <sup>B</sup>	
5	T <sub>5</sub>	0.95± 0.002 <sup>J</sup>	1.11±0.001 <sup>G</sup>	1.32±0.02 <sup>F</sup>	1.86± 0.002 <sup>O</sup>	1.16±0.05 <sup>C</sup>	
6	T <sub>6</sub>	0.47± 0.003 <sup>R</sup>	0.71±0.002 <sup>P</sup>	0.89±0.001 <sup>M</sup>	0.91±0.001 <sup>L</sup>	0.79±0.11 <sup>F</sup>	
7	Average	0.84±0.02 <sup>D</sup>	0.88±0.02 <sup>C</sup>	1.11±0.08 <sup>B</sup>	1.33 ± 0.05 <sup>A</sup>	.....	
8	Significance of effects	**	**	**	**	**	

The averages that carries different letters are significantly different (P<0.01) among them; T<sub>1</sub>: 100% sodium nitrite; T<sub>2</sub>: 80% sodium nitrite, 20% annatto powder; T<sub>3</sub>: 60% sodium nitrite, 40% annatto powder; T<sub>4</sub>: 40% sodium nitrite, 60% annatto powder; T<sub>5</sub>: 20% sodium nitrite, 80% annatto powder and T<sub>6</sub>: 100% annatto powder.

of Alrubeii *et al.* (2009).

**Fat**

The results of Table 3 showed the effect of the interaction between different treatments and the period of storage by refrigerating in the fat percent of freshly chilled beef sausages. There was a significant decrease (P<0.01) in the fat percent that reached to 19.26% in the treatment T<sub>1</sub> (100% nitrite sodium) in the storage period of 1day as compared to the treatment T<sub>4</sub> (60% annatto, 40% sodium nitrite) and T<sub>5</sub> treatment (20% nitrite sodium, 80% annatto), which recorded the highest fat percentage which reached 23.27% and 23.27%, respectively in the storage period of 21 days using the refrigeration storage. There were significant differences between treatments for different storage periods.

It is noted from the results of same tables that there was a significant decrease in the fat percent of the treatment T<sub>1</sub> (100% sodium nitrite) as compared to all the treatments and for all periods of storage 1, 7, 14 and 21 days that reached 20.60%, while the highest fat percent was found among the treatments in the treatment of T<sub>4</sub> (40% sodium nitrite, 60% annatto) in the storage period of 21 days that reached 22.08% and T<sub>5</sub> treatment (20% sodium nitrite, 80% annatto) which gave 22.00%. Studies have indicated that the highest percentage of fat in the treatments included some medicinal plants or extracts addition, this is in agreement with results of Jaworska *et al.* (2016). The results of Table 3 also

showed a significant increase (P<0.01) in the fat percent of the added treatments as compared to the treatment T<sub>1</sub> (100% sodium nitrate). This is due to the presence of natural additives in the superior treatments including the carnosic acid which has the ability to protect the tissues and prevent active oxygen creation and attack free radicals (Naveena *et al.*, 2013). As for the effect of the storage period, the statistical analysis in Table 3 showed that there was a significant superiority (p <0.01) in the fat percent of the added treatments as compared to the treatment of T<sub>1</sub> (100% sodium nitrite), and that the fat percent was at the lowest level in the storage period of 1 day, then increasingly started with the increase of storage periods to reach the highest level in the storage period of 21 days and this is normal as with increase of the storage periods. The moisture decreased and the dry matter increased, which includes all of protein, fat and ash, this is in agreement with results

**Ash**

The result of Table 4 show the effect of the interaction between the different treatments and the period of storage by refrigerating in the ash percent of the freshly chilled beef sausages, the treatment T<sub>1</sub> (100% sodium nitrite) gave the highest percentage of ash reached 1.90% in the storage period of 21 days using refrigerating followed by T<sub>4</sub> (40% sodium nitrite, 60% annatto) which gave 1.34% for the same period while the treatment T<sub>6</sub> (100% annatto) gave the lowest percent

of ash which reached 0.47% for one day storage period. There were also significant differences between the treatments and for different storage periods. The results of Table 4 also showed a significant decrease ( $P < 0.01$ ) in the ash percent in the treatments as compared with the treatment T<sub>1</sub> (100% annatto). Among all the treatments in all storage periods 1, 7, 14 and 21 days that gave 0.79% was the superior followed by T<sub>2</sub> treatment (80% sodium nitrite, 20% annatto) with 0.86%. The highest percentage of ash was obtained from T<sub>1</sub> (100% sodium nitrite) with 1.21%. Some studies have indicated that the percentage of ash decreased in the treatments which included some medicinal plants or their extracts as compared with the treatment of control (Nieto *et al.*, 2010; Moran *et al.*, 2012). While the effect of the storage period, the statistical analysis results in Table 4 showed that the percentage of ash is at the lowest level in the period of 1 day then increases with the increase of the storage period until reaching the highest level in the period of 21 days. This is natural, because the increase of dry matter (include both protein, fat and ash) and decrease of moisture with increase of storage periods and this is in agreement with the results of (Alrubeii *et al.*, 2008).

**Myoglobin concentration**

The results of Table 5 shows the effect of the interaction between the different treatments and the period of storage using refrigerating in the concentration

of myoglobin mg/g in freshly chilled beef sausages. There was a significant increase ( $P < 0.01$ ) in myoglobin concentration that reached 5.65 mg/g meat in the treatment of T<sub>6</sub> (100% annatto) in the one day storage period as compared with T<sub>1</sub> (100% sodium nitrite) which gave the lowest value of myoglobin concentration that reached 4.12 Mg/g meat in the storage period of 21 days with refrigeration. There were also significant differences between different treatments and for storage periods.

The results of the same table showed that the treatment of T<sub>6</sub> (100% annatto) gave a high myoglobin concentration as compared to all treatments and in all storage periods *viz.*, 1, 7, 14 and 21 days, by 5.65, 5.49, 5.34 and 5.24 mg/g, respectively, with the mean of 5.43 mg/g meat, followed by the treatment of T<sub>5</sub> (80% sodium nitrate, 20% annatto) by 5.34 mg.g<sup>-1</sup> meat, while the lowest concentration of myoglobin obtained from the control treatment T<sub>1</sub> (100% sodium nitrite) in all of storage periods reached 4.95, 4.62, 4.42, and 4.12 mg.g<sup>-1</sup> respectively, with a mean of 4.53 mg/g meat. Studies have indicated a high concentration of Myoglobin when adding some medicinal plants or extracts to meat (Alrubeii *et al.*, 2008).

Yolmeh *et al.* (2014) pointed the role of annatto seeds in improving the colour of the processed meat and decreased the colour oxidation as compared with T<sub>1</sub> (100% sodium nitrite). Table 5 showed a significant

**Table 5. The effect of interaction between the treatments and the period of cold storage on myoglobin concentration (mg/g meat ) in freshly chilled beef sausages (mean± SE)**

S. No	Treatment	Period of cold storage				Average
		Day1	Day 7	Day 14	Day 21	
1	T <sub>1</sub>	4.9±0.005 <sup>N</sup>	4.62±0.005 <sup>Q</sup>	4.42±0.005 <sup>S</sup>	4.12±0.005 <sup>T</sup>	4.53±0.11 <sup>F</sup>
2	T <sub>2</sub>	5.25±0.001 <sup>H</sup>	5.08±0.001 <sup>K</sup>	4.81±0.001 <sup>O</sup>	4.55±0.005 <sup>R</sup>	4.92±0.09 <sup>E</sup>
3	T <sub>3</sub>	5.36±0.005 <sup>E</sup>	5.15±0.001 <sup>J</sup>	4.98±0.005 <sup>M</sup>	4.75±0.001 <sup>P</sup>	5.06±0.08 <sup>D</sup>
4	T <sub>4</sub>	5.44±0.001 <sup>D</sup>	5.28±0.005 <sup>G</sup>	5.18±0.005 <sup>I</sup>	5.05±0.001 <sup>L</sup>	5.24±0.05 <sup>C</sup>
5	T <sub>5</sub>	5.59±0.005 <sup>B</sup>	5.36±0.005 <sup>E</sup>	5.27±0.001 <sup>G</sup>	5.16±0.001 <sup>J</sup>	5.34±0.06 <sup>B</sup>
6	T <sub>6</sub>	5.65±0.001 <sup>A</sup>	5.49±0.001 <sup>C</sup>	5.34±0.001 <sup>F</sup>	5.24±0.005 <sup>H</sup>	5.43±0.05 <sup>A</sup>
7	Average	5.37±0.06 <sup>A</sup>	5.16±0.008 <sup>B</sup>	5.00±0.09 <sup>C</sup>	4.81±0.11 <sup>D</sup>	.....
8	Significance of effects	**	**	**	**	**

The averages that carries different letters are significantly different ( $P < 0.01$ ) among them; T<sub>1</sub>: 100% sodium nitrite; T<sub>2</sub>: 80% sodium nitrite, 20% annatto powder; T<sub>3</sub>: 60% sodium nitrite, 40% annatto powder; T<sub>4</sub>: 40% sodium nitrite, 60% annatto powder; T<sub>5</sub>: 20% sodium nitrite, 80% annatto powder and T<sub>6</sub>: 100% annatto powder.

**Table 6. The effect of interaction between the treatments and the period of cold storage in peroxide value (meq O<sub>2</sub> / kg oil) in freshly chilled beef sausages (mean± SE)**

S. No	Treatment	Period of cold storage				
		Day1	Day 7	Day 14	Day 21	Average
1	T <sub>1</sub>	1.33±0.001 <sup>K</sup>	3.29±0.0005 <sup>M</sup>	3.74±0.005 <sup>G</sup>	5.10±0 <sup>A</sup>	3.34±0.52 <sup>A</sup>
2	T <sub>2</sub>	1.26±0.0005 <sup>H</sup>	3.29±0.001 <sup>M</sup>	3.70±0 <sup>H</sup>	4.95±0.001 <sup>C</sup>	3.30±0.50 <sup>B</sup>
3	T <sub>3</sub>	1.21±0.001 <sup>V</sup>	2.48±0.001 <sup>Q</sup>	3.42±0.005 <sup>L</sup>	4.79±0.005 <sup>F</sup>	3.01±0.48 <sup>F</sup>
4	T <sub>4</sub>	1.22±0.0005 <sup>U</sup>	2.79±0.001 <sup>P</sup>	3.48±0.001 <sup>K</sup>	4.88±0.005 <sup>E</sup>	3.10±0.50 <sup>E</sup>
5	T <sub>5</sub>	1.29±0 <sup>S</sup>	3.09±0 <sup>O</sup>	3.53±0 <sup>J</sup>	4.92±0.001 <sup>D</sup>	3.21±0.49 <sup>D</sup>
6	T <sub>6</sub>	1.22±0.001 <sup>U</sup>	3.15±0 <sup>N</sup>	3.62±0.002 <sup>I</sup>	5.04±0.0005 <sup>B</sup>	3.28±0.50 <sup>C</sup>
7	Average	1.25±0.01 <sup>D</sup>	3.04±0.08 <sup>C</sup>	3.58±0.03 <sup>B</sup>	4.95±0.03 <sup>A</sup>	.....
8	Significance of effects	**	**	**	**	**

The averages that carries different letters are significantly different (P<0.01) among them; T1: 100% sodium nitrite; T2: 80% sodium nitrite, 20% annatto powder; T3: 60% sodium nitrite, 40% annatto powder; T4: 40% sodium nitrite, 60% annatto powder; T5: 20% sodium nitrite, 80% annatto powder and T6: 100% annatto powder.

effect (P<0.01) of the treatment on myoglobin concentration. The concentration of the myoglobin was significantly superior in the treatments of T<sub>6</sub>, T<sub>5</sub>, T<sub>4</sub>, T<sub>3</sub>, and T<sub>2</sub>, respectively as compared to T<sub>1</sub> the treatment. this is in agreement with the results of Moran *et al.* (2012). This may be due to the presence of natural antioxidants which contains phenolic type compounds which probably function as free radical scavengers similar to the synthetic antioxidants, that give the hydrogen a reduced air that does not allow oxidation of meat pigments. It also maintains the unsaturated fatty acids from the rancidity oxidative and prevent the formation of free radicals resulting from the reactions of oxidation, thus improves the stability of colour and fat (Al-rubeii *et al.*, 2009). Natural antioxidants are readily acceptable by consumers as they are considered to be more powerful and safe than the synthetic antioxidants .

Also the results of Table 5 showed a significant superiority (P<0.01) for the storage period on the myoglobin concentration and it was at the highest level in the period of one day then began to decrease with the progress of the storage period until reaching its lowest level in the period of 21 days. This is may be due to many factors such as enzymatic activity and temperature of storage, packing method, muscle type, light intensity, difference of meat type, as well as the type of antioxidant used according to Liu *et al.* (2009) that when adding rosemary plant extract to beef burger led

to the stability of myoglobin concentration while retaining the desired color during the storage of the product for 180 days in freezing as compared with the control treatment. Zhang *et al.* (2010) noted that the carnation plant is highly adaptable to maintain the color of pork meat cooked during storage at room temperature.

**Peroxide value**

The results of Table 6 indicated a significant decrease (P<0.01) in the value of peroxide (1.21 mEq O<sub>2</sub>/kg oil) in treatment T<sub>3</sub> (60% sodium nitrite, 40% annatto) in storage period of one day which did not differ from T<sub>4</sub> treatment (40% sodium nitrite, 60% annatto) in the same storage period as compared to the treatment of T<sub>1</sub> (100% sodium nitrite), which recorded the highest peroxide value that reached 5.10 mEq O<sub>2</sub>/kg oil in the storage period of 21 days using refrigeration. Significant differences were observed between the treatments and for different storage periods.

From the results of the same table, there is a significant decrease (P<0.01) in the peroxide value of the treatment T<sub>3</sub> (60% sodium nitrite, 40% annatto) as compared to all treatments in all storage periods by mean that reached 3.01 mEq O<sub>2</sub>/Kg oil followed by T<sub>4</sub> (40% sodium nitrite, 60% annatto) with an average reach of 3.10 mEq O<sub>2</sub>/kg oil while the highest peroxide value obtained from the control treatment T<sub>1</sub> (100% sodium nitrite) in all storage periods that reached 1.33, 3.29, 3.74 and 5.10 mEq O<sub>2</sub>/kg oil respectively, with the

average of 3.34 mEq O<sub>2</sub>/kg oil, the permissible peroxide value shall not exceed mEq O<sub>2</sub>/kg oil for oil or fat according to the Egan *et al.* (1981) as well as the Iraqi standard specifications of the COSQC (1987).

As for the effect of treatments, the data in table 6 indicated to a significant effect (P<0.01) on the value of peroxide of added treatments. it decreased in all annatto treatments, which include T<sub>3</sub>, T<sub>4</sub>, T<sub>5</sub>, T<sub>6</sub> and T<sub>2</sub> respectively and the highest value of peroxide obtained from T<sub>1</sub> control treatment. The reason for the decrease in peroxide values is due to the effect of natural additives of the treatments in inhibiting fat oxidation and decrease in peroxide value, according to Obrien, (2008) who observed a decrease in the peroxide value in meat samples which included additives of annatto powder as compared to the free additives, samples during the storage periods using refrigeration, as it has an inhibitory effect in inhibiting fat oxidation in fresh meat (Kim *et al.*, 2013). The results of the statistical analysis in Table 6 showed that the storage period has a significant effect on peroxide value (P<0.01) and that the peroxide values are at their lowest value in the storage period of one day then start to rise with the increase of storage period to reach the highest value in the period of 21 days. This is in agreement with the results of (Al rubeii *et al.*, 2012).

**Water holding capacity**

The results of Table 7 indicated to the significant increase (P<0.01) in water holding capacity as it

was 45.61% in the treatment of T<sub>6</sub> (100% annatto) in the storage period of one day using refrigeration as compared to the treatment of T<sub>1</sub> (100% sodium nitrite) which reached 32.95% during the storage period of 21 days using refrigeration. There were also significant differences between the treatments in different storage periods. The results of the same table showed that the treatment T<sub>7</sub> (100% annatto) gave a significant increase (P<0.01) in water holding capacity as compared with other treatments in all storage periods 1, 7, 14 and 21 days, by means of 39.35% followed by treatment T<sub>5</sub> (80% annatto, 20% sodium nitrite) by 38.21%, while the lowest value of the water holding capacity obtained from the control treatment T<sub>1</sub> (100% sodium nitrite) in all the above storage periods by a mean of 36.87%. The results of the statistical analysis of Table 7 showed a significant difference (P<0.01) in water holding capacity, which was significantly superior in the treatments of T<sub>6</sub>, T<sub>5</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>2</sub>, respectively, as compared with T<sub>1</sub> (100% sodium nitrite). This may be due to the presence of these compounds, which have the ability to protect cellular membranes from the damage thus protect the proteins from decomposition and prevent the losing of water and retention inside cell and associated with protein by protein-water bonds, or may be due to these compounds contributed to raise the pH of meat resulting in an increased water holding capacity (Viuda-martos *et al.*, 2015).

**Table 7. The effect of interaction between the treatments and the period of cold storage in water holding capacity percentage in freshly chilled beef sausages (mean± SE)**

S. No	Treatment	Period of cold storage				Average
		Day1	Day 7	Day 14	Day 21	
1	T <sub>1</sub>	41.06±0.005 <sup>F</sup>	37.25±0.005 <sup>L</sup>	35.43±0.005 <sup>R</sup>	32.95±0.005 <sup>X</sup>	36.87±1.57 <sup>F</sup>
2	T <sub>2</sub>	42.16±0.01 <sup>D</sup>	37.86±0.005 <sup>K</sup>	35.68±0.005 <sup>P</sup>	33.34±0.005 <sup>V</sup>	37.26±1.22 <sup>E</sup>
3	T <sub>3</sub>	41.55±0.005 <sup>E</sup>	38.71±0.005 <sup>I</sup>	34.42±0.01 <sup>T</sup>	33.75±0.005 <sup>U</sup>	37.51±0.83 <sup>C</sup>
4	T <sub>4</sub>	42.44±0.005 <sup>C</sup>	38.53±0.001 <sup>J</sup>	35.77±0.005 <sup>O</sup>	33.14±0.001 <sup>W</sup>	37.47±1.30 <sup>D</sup>
5	T <sub>5</sub>	42.63±0.005 <sup>B</sup>	38.74±0.005 <sup>H</sup>	36.92±0.01 <sup>N</sup>	34.41±0.005 <sup>S</sup>	38.21±1.44 <sup>B</sup>
6	T <sub>6</sub>	45.61±0.005 <sup>A</sup>	40.29±0.005 <sup>G</sup>	37.10±0.005 <sup>M</sup>	35.65±0.001 <sup>Q</sup>	39.35±1.02 <sup>A</sup>
7	Average	42.57±0.43 <sup>A</sup>	38.56±0.28 <sup>B</sup>	35.87±0.28 <sup>C</sup>	33.87±0.27 <sup>D</sup>	.....
8	Significance of effects	**	**	**	**	**

The averages that carries different letters are significantly different (P<0.01) among them; T<sub>1</sub>: 100% sodium nitrite; T<sub>2</sub>: 80% sodium nitrite, 20% annatto powder; T<sub>3</sub>: 60% sodium nitrite, 40% annatto powder; T<sub>4</sub>: 40% sodium nitrite, 60% annatto powder; T<sub>5</sub>: 20% sodium nitrite, 80% annatto powder and T<sub>6</sub>: 100% annatto powder.

Also significant differences ( $P < 0.01$ ) were observed among the storage periods on the water holding capacity according to the results in Table 7 and that the highest level during the storage period of one day then begins to decrease with the progress of storage period until reaching the lowest level in the storage period of 21 days. This may be due to the decomposition of meat proteins by increasing storage periods thus inability to hold water (Al-Rubeii *et al.*, 2009).

## CONCLUSION

The results of this study showed that the addition of annatto seed powder to the fresh chilled beef sausages with reducing the addition of sodium nitrite leads to an increase in the moisture, protein and fat levels and decrease of peroxide value. It also improved the water holding capacity in meat sausages. as well as the addition of annatto seed powder that has reduced the oxidation of myoglobin pigment to metmyoglobin during storage as compared than sodium nitrite addition treatment.

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