

## The effect of adding dried egg whites and the enzyme lysozyme on improving some chemical properties of fresh and cold preservation sausages.

<sup>1</sup>Haider. K. Kazem  <sup>2</sup>Ghaidda. A. Makki  <sup>3</sup>Raghdan. H. Mohsin 

Department of Animal Production, College of Agriculture, University of Basrah, Iraq

E-mail: pgsagr.haider.kareem@uobasrah.edu.iq

### Abstract

The current study aimed to use dried egg whites at concentrations of 0.6, 0.4, and 0.2% in preserving beef sausage by cooling at a temperature of 4°C. The results showed that there was a significant decrease in the values of peroxide (TBA) and the percentage of free fatty acids for the meat samples treated with dried egg whites compared to the control treatment, which amounted to 3.74 mEq/kg, fat, 1.24 mg, malondialdehyde /kg, 0.58%, and on the seventh day of preservation, they were excluded due to their microbial contamination, While the addition samples continued within standard limits until day 21 of cryopreservation.

The results also showed a significant decrease in cholesterol concentration in beef sausages treated with dried egg whites compared to the group treated with the lysozyme enzyme. The averages in the fifth treatment reached 0.89 mg/g on day 21 of storage, while in the sixth treatment (adding the enzyme lysozyme) they reached 0.91 mg/g.

**Keyword:** Meat quality, Egg whites, Lysozyme enzyme, Fatty acids, Sausages

### I. INTRODUCTION

Food safety, extending its shelf life, preserving its nutritional value, and reducing the rate of loss during its handling, display, and storage are important issues that attract the attention of consumers and industrialists alike, recent studies have focused on human food, its components, and methods of preparing it, as it has a direct impact on his health. Recently, interest in natural antioxidants, especially the process of fat oxidation, has increased, as they have an impact on the physiological processes of the body's systems on the one hand and on the quality of food on the other hand. Due to the oxidation process, fatty materials become rancid, and oxidative rancidity is the main cause of food spoilage (Olaoye and Onilud , 2010).

The importance of these antioxidants lies in reducing the oxidation-rancidity reactions of oils, as they act as hydrogen donors or free radical receptors (Kalalou *et al.*, 2004; Mielnik *et al.*, 2008). In recent years, natural additives of plant origin have been sought to possess both antioxidant and antimicrobial activity, which work to maintain meat quality and prevent economic loss (Yin and Cheng, 2003; Mielnik *et al.*, 2008). Phenolic compounds are among the most prominent natural antioxidants, which include flavonoids, tannins, carotenoids, natural phenolic acids, vitamins, and other natural components of food, which are abundant in all plant parts, such as leaves, flowers, fruits, stems, roots, bark, and seeds, which can be used as natural additives in preserving foods, such as meat and others, and which are accepted by the consumer because they are natural and included in human food (Brewer, 2007), and in addition to their action as antioxidants, many studies have proven the role of phenolic compounds as antibacterial, antiviral, and antifungal agents (Cai *et al.*, 2004). Therefore, the research aimed to use different concentrations of dried egg whites in cold preservation of meat patties and to study the chemical and microbial changes.

## II. MATERIAL AND METHODS

### A. Materials used

**Egg whites:** Obtained by drying the egg whites after isolating them from the yolk at a temperature of 40°C until dry and the dried whites are collected and the yield is calculated.

**Meat:** Beef veal (thigh area) was purchased from local markets in Basra Governorate.

The process of manufacturing and processing beef sauce:

Meat samples were treated with dried egg whites at concentrations: 0.2, 0.4, 0.6%, while the control treatment was left without addition, and the samples were refrigerated at a temperature of 4 °C for specific time periods of 0, 7, 14, 21 days.

### B. The process of manufacturing and processing beef sauce:

Meat samples were treated with dried egg whites at concentrations: 0.2, 0.4, 0.6% while the control treatment was left without addition, and the samples were refrigerated at a temperature of 4 °C for specific time periods of 0, 7, 14, 21 days.

### C. Chemical tests:

**Peroxide number:** Peroxide number was measured according to the following equation ( Pearson, 1981):

$$\text{Peroxide value} = \frac{(\text{Na}_2\text{S}_2\text{O}_4 \text{ ml} \times \text{N} \times 1000)}{(\text{Wt. of Sample, gm})}$$

**Thiobarputeric acid (TBA):** The TBA value was estimated according to the method of Mehran,(1976).

Concentration of malonaldehyde ( $\text{mg kg}^{-1}$ ) = absorbance  $\times$  7.83

**Free Fatty Acid (FFA):** The percentage of free fatty acids was estimated according to the following of method of Pearson *et al.*, (1981).

$$\text{Free Fatty Acid \%} = \frac{\text{Titration (A-B)} \times \text{N} \times 282 \times 100}{1000 \times \text{Wt of Sample, gm}}$$

A= number of ml of Potassium hydroxide titrated with fat of oil sample

B= number of ml Potassium hydroxide titrated with blank sample

282= Oleic acid molecular weight

**Cholesterol Concentration:** The concentration of cholesterol was estimated according to the method of Al-Obaidi (1999), and the cholesterol concentration was calculated by applying the following equation:

$$\text{Cholesterol concentration (mg/g)} = \frac{\text{sample read}}{(\text{standard cholesterol reading})} \times 2$$

### D. Statistical analysis:

The results were analyzed statistically using a factorial experiment with three factors, using a completely randomized design (CRD). The data were analyzed statistically using the ready-made statistical program (SPSS, 2016). The results were compared using the adjusted least significant difference (R.L.S.D) at the probability level of 0.05:

### III. RESULTS AND DISCUSSION

**Peroxide number:** The results of Table (1) show a significant decrease ( $P < 0.05$ ) in the peroxide number values of beef sausage treated with dried egg whites at concentrations of (0.6, 0.4, 0.2)% and preserved by cryopreservation at a temperature of 4°C, compared to the control treatment with a continuation of the cryopreservation period. The PV value in the control treatment increased from 2.22 mEq/kg fat to 3.74 mEq/kg fat on the seventh day of cryopreservation, and the values continued to rise, and they were excluded due to their microbial contamination, while the PV values in treatments with dried egg whites on the seventh day of cryopreservation reached 2.16, 2.31, and 2.38 mEq/kg fat for concentrations (0.2, 0.4, 0.6)%, respectively. When comparing the treatments to which dried egg whites were added on the 14th day of cryopreservation with the second treatment (market sample), the average peroxide number values reached 2.25, 2.17, and 2.72 mEq/kg fat, respectively. While the peroxide values for the market sample reached 3.05 mEq/kg fat. The results of the table also showed that the meat samples were within the standard periods even after 21 days of preservation, as the averages reached 2.34, 2.35, and 2.63 mEq/kg fat for concentrations (0.2, 0.4, 0.6)%, respectively, for the treatments to which dried egg whites are added, when comparing the results with the peroxide values in sausage samples to which the enzyme lysozyme was added at concentrations of 0.3 and 0.4%, they reached 2.35 and 2.40 mEq/kg fat, respectively, on the 21st day of preservation. These results are encouraging for the use of dried egg whites in meat storage because they possess highly effective antioxidants, as it maintains the oxidation of meat within acceptable limits when preserved by cooling, this is due to the presence of some compounds that act as antioxidants possessed by dried egg whites, such as phenolic compounds (Nolasco *et al.*, 2020). These results are similar to what was found by Muthia *et al.*, (2012) when using a concentration of 4% of egg whites in the manufacture of duck meat sauce and measuring the antioxidant activity by keeping it at 4°C by refrigeration.

**Table (1): The effect of adding different concentrations of dried egg whites (0.2, 0.4, 0.6)% and the lysozyme enzyme (0.3, 0.4)% on the peroxide number in beef sausage preserved by refrigeration at 4°C.**

Treatment	Storage Periods				Average
	0	7	14	21	
T1	2.22	3.74			2.98
T2	2.22	2.54	3.05	3.12	2.73
T3	2.22	2.38	2.72	2.63	2.49
T4	2.22	2.31	2.17	2.35	2.26
T5	2.22	2.16	2.25	2.34	2.24
T6	2.22	2.15	2.27	2.35	2.25
T7	2.22	2.29	2.30	2.40	2.30
<b>Average</b>	2.22	2.51	2.46	2.53	

RLSD for storage period= 0.242

RLSD for concentrations=0.185

T1; control treatment, T2; market treatment (nitrate and nitrite), T3; adding dried egg whites 0.2%, T4; adding dried egg whites 0.4%, T5; adding dried egg whites 0.6%, T6; adding lysozyme enzyme 0.3%, T7; adding lysozyme enzyme 0.4%



**Thiobarputeric acid (TBA):** The results of Table (2) show a significant decrease ( $P < 0.05$ ) in the (TBA) values of beef sausages treated with dried egg whites at concentrations of (0.2, 0.4, 0.6)% and stored at a temperature of 4°C, compared to the control treatment with a continued duration of cryopreservation. The TBA values in the control treatment increased from 0.22 mg malondialdehyde /kg meat to 1.96 mg malondialdehyde/kg meat on the seventh day of cryopreservation, and the values continued to rise, it was excluded due to microbial contamination. While the values of (TBA) in dried egg white treatments on the seventh day of cryopreservation reached 0.71, 0.70, and 0.77 mg malondialdehyde/kg meat (0.2, 0.4, 0.6)%, respectively. When comparing the treatments to which dried egg whites were added on the 14th day of cryopreservation with the second treatment (market sample), the average TBA values reached 1.02, 0.95, and 0.94 mg malondialdehyde/kg, respectively. While the TBA values for the market sample reached 1.24 mg malondialdehyde/kg.

The results of the table also showed that the meat samples were within the standard range even after 21 days of preservation, as the averages reached 1.36, 1.42, and 1.56 mg malondialdehyde/kg for concentrations (0.2, 0.4, 0.6), respectively, for the treatments to which dried egg whites were added.

**Table (2): The effect of adding different concentrations of dried egg whites (0.2, 0.4, 0.6)% and the lysozyme enzyme (0.3, 0.4)% on the values of thiobarbituric acid in beef sausage preserved by refrigeration at 4°C.**

Treatment	Storage Periods				Average
	0	7	14	21	
T1	0.22	1.96			1.09
T2	0.22	0.85	1.24	1.73	1.01
T3	0.22	0.77	1.02	1.56	0.89
T4	0.22	0.70	0.95	1.42	0.82
T5	0.22	0.71	0.94	1.36	0.81
T6	0.22	0.69	0.92	1.38	0.80
T7	0.22	0.67	0.87	1.26	0.76
<b>Average</b>	20.25	24.56	26.53	1.45	

RLSD for storage period= 0.242

RLSD for concentrations=0.185

T1; control treatment, T2; market treatment (nitrate and nitrite), T3; adding dried egg whites 0.2%, T4; adding dried egg whites 0.4%, T5; adding dried egg whites 0.6%, T6; adding lysozyme enzyme 0.3%, T7; adding lysozyme enzyme 0.4%

When comparing the results with the peroxide values in the sausage samples to which the enzyme lysozyme was added at a concentration of (0.3 and 0.4)%, they reached 1.38 and 1.26 mg malondialdehyde/kg, respectively, on the 21st day of preservation. The reason for this may be attributed to the dried egg whites possessing active groups that have the ability to snatch up free radicals and stop the series of oxidation reactions through the contribution of (OH) groups, which work to break the oxidation chain (Chen *et*



*al.*, 2021). In addition, inhibiting the process of fat oxidation is By suppressing the activity of free radicals, the duration of the first stage of the oxidation process will increase and the formation of peroxides and then the formation of hydroperoxides will slow down, thus reducing the amount of malondialdehyde amalgam, which is one of the by-products of fat oxidation and the breakdown of peroxides in meat and its products (O'sullivan *et al.*, 2004). The results were in agreement with Marcinkowska-Lesiak *et al.*, (2023), where he observed a significant decrease in thiobarbituric acid (TBA) values compared to the control treatment, in a study on pig liver patties added to egg whites treated with plasma at a concentration of (80, 40) ppm. Of nitrogen dioxide stored at a temperature of 4°C for periods of (1, 3, 5, 7) days.

**Free fatty acid percentage (FFA):** The results of Table (3) show a significant decrease ( $P < 0.05$ ) in the percentage of free fatty acids (FFA) of beef sausage treated with dried egg whites at concentrations of (0.2, 0.4, 0.6)% and stored by refrigeration at a temperature of 4°C, compared to the control treatment with a continued duration of cryopreservation.

The percentage of free fatty acids (FFA) in the control treatment increased from 0.33% to 0.58% on the seventh day of cryopreservation, and the values continued to rise, they were excluded due to their microbial contamination.

While the percentage of (FFA) in dried egg white treatments on the seventh day of cryopreservation reached 0.39, 0.35 and 0.38% for concentrations (0.2, 0.4, 0.6)%, respectively, when comparing the treatments to which dried egg whites were added on the 14th day of cryopreservation with the second treatment (market sample), the averages for the (FFA) percentage reached 0.48, 0.47, and 0.44%, respectively, while the percentage of free fatty acids (FFA) for the market sample was 0.62%.

The results of the table also showed that the meat samples were within the standard periods even after 21 days of preservation, as the averages reached 0.52, 0.57, and 0.59% for the concentrations (0.2, 0.4, 0.6), respectively, for the treatments to which dried egg whites were added. When comparing the results with the peroxide values in the sausage samples to which the lysozyme enzyme was added at a concentration of (0.3 and 0.4)%, they reached 0.52 and 0.51%, respectively, on the 21st day of preservation.

The reason the treatments maintain their quality may be attributed to dried egg whites, which are a natural antioxidant because they contain active groups that prevent or obstruct the action of lipolytic enzymes, such as lipase, which works to liberate free fatty acids (Liu *et al.*, 2019). The results agreed with Marcinkowska-Lesiak *et al.*, (2023), where he noted a significant decrease in the values of thiobarbituric acid reactive substances, it is considered a known evidence of fat oxidation and represents the amount of (MDA), one of the breakdown products of fat hydroperoxides that are formed during the increase in polyunsaturated fatty acids compared to the control treatment (Wenjiao *et al.*, 2014), in a study on pig liver patties added to plasma-treated egg whites. At a concentration of (40, 80) parts per million of nitrogen dioxide and stored by refrigeration under 4°C for periods of (1, 3, 5, 7) days.

**Table (3): The effect of adding different concentrations of dried egg whites (0.2, 0.4, 0.6)% and the lysozyme enzyme (0.3, 0.4)% on the free fatty acid percentage in beef sausage preserved by refrigeration at 4°C.**

Treatment	Storage Periods				Average
	0	7	14	21	
T1	0.33	0.58			0.46
T2	0.33	0.41	0.62	1.00	0.59
T3	0.33	0.38	0.44	0.59	0.44
T4	0.33	0.35	0.47	0.57	0.43
T5	0.33	0.39	0.48	0.52	0.40

<b>T6</b>	0.33	0.36	0.42	0.52	0.37
<b>T7</b>	0.33	0.33	0.38	0.51	0.35
<b>Average</b>	0.33	0.58	0.47	0.62	

RLSD for storage period= 0.242

RLSD for concentrations=0.185

T1; control treatment, T2; market treatment (nitrate and nitrite), T3; adding dried egg whites 0.2%, T4; adding dried egg whites 0.4%, T5; adding dried egg whites 0.6%, T6; adding lysozyme enzyme 0.3%, T7; adding lysozyme enzyme 0.4%

**Cholesterol concentration:** The results of Table (4) indicate a significant decrease ( $p < 0.05$ ) in the cholesterol concentration of beef sausages treated with dried egg whites at concentrations of (0.2, 0.4, 0.6)%. It is stored at a temperature of 4°C, compared to the control treatment, while the cryopreservation period continues. The cholesterol concentration in the control treatment increased from 0.63 mg/g to 0.90 mg/g on the seventh day of cryopreservation, and the values continued to rise, and they were excluded due to microbial contamination, while the cholesterol concentration of the dried egg white treatment on the seventh day of cryopreservation reached 0.75 and 0.71 and 0.77 mg/g. When comparing the treatments to which dried egg whites were added on day 14 with the second treatment (market treatment), the cholesterol concentration reached 0.85, 0.81, and 0.80 mg/g, while in the market sample it reached 0.95 mg/g.

The results of the table also showed that the meat samples were within the standard periods even after 21 days of preservation, as the averages reached 0.89, 0.90, and 0.95 mg/g for concentrations (0.2, 0.4, 0.6)%, respectively. When comparing the results with the cholesterol concentration in sausage samples to which the enzyme lysozyme was added at a concentration of (0.3, 0.4)%, it reached 0.99 and 0.91 mg/g on the 21st day of preservation. The reason may be due to egg whites being highly effective as antioxidants, as they maintain the oxidation of meat within acceptable limits when kept cold. This is due to the presence of some compounds that act as antioxidants, such as phenolic compounds, prononothocyanidins,  $\beta$ -caroten, vitamin E, gallic acid, Linoleic acid and Flaconids (Nolasco *et al.*, 2020), since cholesterol is concentrated in cellular membranes and the oxidation process leads to the release of oxidized cholesterol compounds in meat and its products, antioxidants inhibit or reduce oxidation, which then leads to a decrease in the amount of cholesterol (Broncano *et al.*, 2009). In this context, Matsuoka and Sugano, (2022) noted that consuming 8 g/day of egg whites for 8 weeks significantly reduced total cholesterol concentrations in the blood compared to the control group in people suffering from hypercholesterolemia.

**Table (4): The effect of adding different concentrations of dried egg whites (0.2, 0.4, 0.6)% and the lysozyme enzyme (0.3, 0.4)% on the cholesterol concentration in beef sausage preserved by refrigeration at 4°C.**

Treatment	Storage Periods				Average
	0	7	14	21	
<b>T1</b>	0.63	0.90			0.77
<b>T2</b>	0.63	0.79	0.95	1.13	0.88
<b>T3</b>	0.63	0.77	0.85	0.94	0.80
<b>T4</b>	0.63	0.71	0.81	0.90	0.76



<b>T5</b>	0.63	0.75	0.80	0.89	0.77
<b>T6</b>	0.63	0.73	0.81	0.91	0.77
<b>T7</b>	0.63	0.75	0.83	0.90	0.78
<b>Average</b>	0.63	0.77	0.84	0.95	

RLSD for storage period= 0.242

RLSD for concentrations=0.185

T1; control treatment, T2; market treatment (nitrate and nitrite), T3; adding dried egg whites 0.2%, T4; adding dried egg whites 0.4%, T5; adding dried egg whites 0.6%, T6; adding lysozyme enzyme 0.3%, T7; adding lysozyme enzyme 0.4%

#### IV. CONCLUSION

The results showed a decrease in oxidation indicators in treatments of beef sausage added to dried egg whites compared to the control sample and the ready-made sample (market sample) and similar to the lysozyme enzyme treatment, which was cryopreserved at 4°C. The results also showed a significant decrease in cholesterol levels in beef sausage treatments to which dried egg whites were added by 0.6 and 0.4% compared to the control treatment.

#### V. REFERENCES

- Al-Obaidi, Fares Abdul Ali Mahdi. (1999). Assessment of the qualitative and chemical characteristics of Japanese quail eggs. PhD. College of Agriculture, University of Baghdad.
- Broncano, J. M.; Petrón, M. J.; Parra, V. and Timón, M. L. (2009). Effect of different cooking methods on lipid oxidation and formation of free cholesterol oxidation products (COPs) in Latissimus dorsi muscle of Iberian pigs. *Meat Science*, 83(3): 431-437.
- Brewer, M.S. (2007). The chemistry of beef flavor. An executive summary. National Cattlemen's Beef Association, Centennial, CO.
- Cai, Y., Luo, Q., Sun, M., & Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life sciences*, 74(17), 2157-2184.
- Chen, Y., Han, P., Ma, B., Wang, X., Ma, M., Qiu, N., & Fu, X. (2021). Effect of thermal treatment on the antioxidant activity of egg white hydrolysate and the preparation of novel antioxidant peptides. *International Journal of Food Science & Technology*, 57(5), 2590-2599.
- Kalalou, I.; Faid, M. and Ahomi, A. T. (2004). Extending the shelf life of fresh minced camel meat at ambient temperature by *Lactobacillus delbruekii* sub sp. *Delbruekii*. *Electronic Journal of Biotechnology*. 7: 251-246
- Liu, D., Chen, X., Huang, M., & Zhou, G. (2019). Antioxidant activity of peptides in postmortem aged duck meat as affected by cooking and in vitro digestion. *International journal of food properties*, 22(1), 727-736.
- Marcinkowska-Lesiak, M., Alirezalu, K., Stelmasiak, A., Wojtasik-Kalinowska, I., Onopiuk, A., Szpicer, A., & Poltorak, A. (2023). Physicochemical characteristics of pork liver pâtés containing nonthermal air plasma-treated egg white as an alternative source of nitrite. *Applied Sciences*, 13(7), 4464.
- Matsuoka, R., & Sugano, M. (2022). Health functions of egg protein. *Foods*, 11(15), 2309. Mehran, M. (1976). *Oil Testing*. First Edition, Tehran University Press, 263 PP.



- Mielnik, M. B., Sem, S., Egelanddal, B., & Skrede, G. (2008). By-products from herbs essential oil production as ingredient in marinade for turkey thighs. *LWT-Food Science and Technology*, 41(1), 93-100.
- Muthia, D., Huda, N., Ismail, N., & Easa, A. M. (2012). The effects of egg white powder addition with tapioca and sago flours on physicochemical and sensory properties of duck sausage. *International Food Research Journal*, 19(4).
- Nolasco, E., Jundt, E., Kramp, M. M., Dutta, E., Nishijima, N., Oda, Y., ... & Majumder, K. (2020). Application of dried egg white to enhance the textural properties of fresh and frozen pancakes. *ES Food & Agroforestry*, 2, 50-57.
- O'Sullivan, C. M., Lynch, A. M., Lynch, P. B., Buckley, D. J., & Kerry, J. P. (2004). Assessment of the antioxidant potential of food ingredients in fresh, previously frozen and cooked chicken patties. *International Journal of Poultry Science*, 3(5), 337-344.
- Olaoye, O. A., & Onilude, A. A. (2010). Investigation on the potential application of biological agents in the extension of shelf life of fresh beef in Nigeria. *World Journal of Microbiology and Biotechnology*, 26, 1445-1454.
- Wenjiao, F.; Yongkui, Z.; Yunchuan, C.; Junxiu, S.; Yuwen, Y. TBARS predictive models of pork sausages stored at different temperatures. *Meat Sci.* 2014, 96, 1-4
- Pearson, D. (1970). *The chemical analysis of food*. 6<sup>th</sup> ed. J. & A. Churchill, London.
- Pearson, D.; Egan, H.; Kirk, R. S. and Sawyer, R. (1981). *Chemical analysis of food*. Longman Scientific and Technical New York.
- SPSS (2006). *Statistical Packages of Social Sciences*. Version 15 for windows. SPSS. Inc. USA.
- Yin, M. C. and Cheng, W. S. (2003). Antioxidant and antimicrobial effect of four garlic-derived organosulfur compounds in ground beef. *J. Meat Sci.* 63: 23-28.

