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Using Mixture of Some Natural Preservatives to Prolong Shelf Life of Frozen Sausage

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ABSTRACT:

The meat industry has been increasing the strategies to produce and commercialize products focusing on the reduction or even the replacement of chemical preservatives. Therefore, the main goal of the current study was to evaluate the effect of mushroom and Celery powders on the sensory, chemical, and bacterial quality of the frozen Egyptian sausage. The experimental four groups were designed as follow: Control negative (sausage without any additives), Control positives (using nitrite in concentration 50ppm), Sausage with 1% celery powder and 2% mushroom powder and Sausage with 1% celery powder and 5% mushroom powder. Assessing the sensory, chemical and microbiological quality of the studied sausage groups. The four experimental sausage groups were kept frozen at – 18 ^{0}C until the appearance of signs of spoilage, during the storge period Frozen sausages were examined for sensory attributes, protein %, fat %, total volatile basic nitrogen, thiobarbituric acid reactive substances, peroxide values, total bacterial counts, psychrotrophic counts, clostridium perfringens count, coliform counts and Coagulase positive staphylococcus. The results showed that there were slightly increase in fat and protein content in celery and mushroom groups compared with control positive (sausage with nitrite 50 ppm). Moreover, a significant ($P < 0.05$) reduction in TBA, peroxide values, TVBN, total bacterial count and psychrotrophic counts were observed in all treated sausage with mushroom and celery additives. Coliform, staph and clostridium perfringens were not detected in all sausage groups. Furthermore, all sausage treated with a combination of mushroom and celery powder had acceptable sensory qualities. Thus, meat processors can safely employ these natural additives to enhance the quality and prolong the shelf life of frozen meat products.

INTRODUCTION

Sausage is among the most well-known foods in Egypt which is based on beef or chicken and commonly packed in a casing. According to Egyptian standards frozen sausage is one of meat products prepared from minced meat mixed with seasoning materials with or without fillers, natural improvers and preserva-

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tives and packed inside natural casings on lamb intestine or industrial casing and preserved in frozen form **(ES, 2005).**

The elevated fat content can trigger rapid lipid oxidation, potentially leading to a decline in meat quality and the production of cytotoxic and genotoxic substances **(Lapidot et al. 2005)**.

To prolong the shelf life of meat products, a variety of chemical additives have been added to sausage to stop unintended processes related to lipid oxidation. Nitrates and nitrite are two of the primary synthetic meat additives used in the production of sausages, these addition improve the end product's color, taste and shelf life **(Zahran and Kassem, 2011).**

Nitrites and nitrate are commonly employed in meat based products to promote an antibacterial impact against certain infections, particularly anaerobic bacteria such as clostridium spp., for the formation of distinctive colors and aromas, and for their antioxidant effect. Accord-
ing to the World Health Organization's cancer agecy, the Interntioal Agency for Research on Ccer (IARC), ntrites reacting with secondary o r tertiary amines in meat could generate carcin ogenic, teratogenic, and mutagenic compounds

As a result, food companies and academic s have been looking for new natural nitrite repl acers **(Pennisi et al. 2020 and Nader et al. 20 21).**

The creation of low-nitrite or nitrite-free meat products has gained momentum due to the present industry trend toward natural antioxidants or antimicrobials; nevertheless, ensuring customer safety and maintaining the normal organoleptic qualities of meat products must be ensured. **(Kumar et al. 2015).**

As a member of the apiaceous family, celery is a flavorful vegetable that is valued for both its nutritional and medicinal qualities **(Horsch et al. 2014)**. The leaves of celery have a high nitrate content **(Sebranek and Bacus, 2007)**. Current meat technology concerns center on using celery leaf powder in place of synthetic nitrite because it doesn't alter the final product's flavor or look. **(Sucu and Yildiz 2018 and Usinger et al. 2016).**

Mushroom is high in protein, amino acids, polysaccharides, dietary fiber, and other nutrients. Numerous researchers have demonstrated the biological properties of mushrooms, including anticancer, antioxidant, lipid and blood sugar reduction, and more **(Guillamon et al. 2010).**

Regardless of the extraction processes or active ingredients, additional research has demonstrated that mushrooms offer a number of biological advantages, including antioxidant and anticancer properties **(Dong et al. 2017)**. Furthermore, research has indicated that mushrooms are now considered a nutritious food since they are low in calories and high in proteins, minerals, and dietary fiber (including mannans, chitin, hemicellulose, and β-glucans) (**Kumari 2020**), which can enhance the quality of some food items (**Banerjee et al. 2020 and Wang et al. 2022**).

The aim of this work was to study the effects of reforming the frozen Egyptian sausage by replacing chemical preservatives with mushroom powder and celery powder as naturally one and assessing the sensory, chemical and microbiological quality of studied sausage groups.

2. MATERIALS and METHODS:

2.1. Raw Materials;

Fresh lean meat, beef fat, soy hydrate, skimmed milk, black pepper and seasonings mixture, were purchased from a local supplier in Cairo, Egypt.

2.2. Preparation of celery and mushroom powder:

Organic celery leaves were purchased from a local market in Cairo, Egypt. The leaves were washed, cut and dried as the following parameters: 2 h at 100°C and a further 4 h at 60°C. Dried celery was then ground and sieved, and the powder was pasteurized at 131 °C for 10 s **(Montiel-Flores et al. 2013).** Mushrooms (fruiting bodies) were purchased from a local market in Cairo, Egypt. They are cleaned of compost residue, halved and dried in an oven at 40°C for 4 hrs. All dried mushrooms were ground into powder using a BJ-

300 high-speed multifunctional mixer (Moulinex, France), then put into sealed plastic bags and stored in a desiccator at room temperature for later use.

2.3. Sausage manufacture and experimental design:

The sausage was manufactured in the lab according to the criteria stipulated by the Egyptian Organization for Standardization and Quality Control (ES 1972 (2005). The meat mixture was divided into four groups of 1 kg each: group 1 (sausage without any additives) (A), group 2 (sausage with nitrite 50ppm) (B), group 3 (sausage with 1% celery powder and 2.0% Mushroom powder) (C) and group 4 (1% celery powder and 5.0% mushroom powder) (D), the meat mixtures were stuffed into natural mutton casing. All control and treated samples were then packed in polyethylene bags and stored at -18 C for further examinations. During storage period, the control and treated samples were examined every 2 weeks for sensory, chemical and microbiological criteria until spoilage occurred.

2.4. Sensory evaluation:

Sensory attributes for raw (appearance, color, odour) and cooked sausage (taste) sample were examined according to the scheme adopted by **ISO, 16779:2015** using the 5-point assessment score according to the following scheme: 1= very bad, 2= bad, 3 = accepted, 4= very good and 5= excellent.

2.5. Microbiological analysis:

Microbiological analysis were performed on all control and treated sausage samples, for the presence of the main microorganisms such as Total bacterial count, Psychotropic count, Clostridium perfringens count, Coliform count and Coagulase positive Staphylococci. The preparation of the test samples, the initial suspension and the decimal dilutions for the microbiological analysis was carried out using the **(ISO 6887-1/ 2017)**. The methods were conducted as follow:

Total bacterial Count (TBC): ISO 4833-1-2013 Cor1:2014 Amd 1:2022

Using Plate Count Agar (PCA, Oxoid) incubated at 30°C for 72 hours. **Enumeration of clostridium perfringens (ISO 7937 /2004)**

Cycloserine agar (Oxoid) incubated at 35°C for 48 hours in anaerobic atmosphere.

Coliform count: **(FDA/ 2020)**

Using violet red bile agar and incubated at 35ºC for 24 hours.

Coagulase positive Staphylococci: (ISO 6888 -1/ 2021).

Using Baird Parker agar (Oxoid) incubated at 34-38°C for 24- 48 hours

Total Psychrotrophic count (ISO 17410/2019)

Using Plate Count Agar (PCA, Oxoid) incubated at 7°C for 10 days.

2.6 Chemical examination:

Determination of Protein Content (ISO 1871/ 2009):

Determination of protein content using kjeldahl digestion and distillation units (Manufactured by VELP Scientifica, model DK).

Determination of fat % (ISO 1443/1973).

Determination of fat content by digestion of the samples with concentrated hydrochloric acid (acid hydrolysis) followed by extraction with petroleum ether in soxhlet apparatus for 4 hrs.

Total volatile basic nitrogen: (ES 63-9/2006)

Determination of TVB-N according to method described by the Egyptian standard method.

Peroxide values (AOAC, 2016)

Determination of peroxide value was according to method described by AOAC, 2016 official method 965.33

Thiobarbituric acid values: (ES 63-10/2006)

Determination of TBA was according to method described by ES, 2006.

2.7 Statistical analysis:

Statistical comparisons were performed using one-way analysis of variance (ANOVA). The experiment was repeated three times. The data were logarithmically transformed and analyzed by SPSS software (version 20, IBM CO) **(Clark and Kempson, 1997).**

RESULTSs:

Table (1): Sensory attributes (mean ± SD) of frozen and cooked sausage (taste only) treated with different preservatives during storage at-18˚C.

	Treatment	Appearance	Color	Flavor (taste)	Odor	Overall
Zero day	A	5.0 ±0.0	4.7 ±0.6	4.7 ±0.6	4.7 ±0.6	4.7 ±0.5
	B	5.0 ±0.0	4.7 ±0.6	4.7 ±0.6	4.7 ±0.6	4.7 ±0.5
	C	5.0 ±0.0	5.0 ±0.0	5.0 ±0.0	5.0 ±0.0	5.0 ±0.0
	D	4.7 ±0.6	4.3 ±0.6	4.7 ±0.6	4.3 ±0.6	4.5 ±0.5
15 day	A	4.3 ±0.6	4.0±0.0	4.5±0.5	4.5±0.0	4.3^{A}±0.3
	B	5.0 ±0.0	5.0 ±0.0	4.8 ±0.3	4.8±0.3	4.9^{aB}±0.2
	C	5.0 ±0.0	5.0 ±0.0	5.0 ±0.0	5.0 ±0.0	5.0^{aC} ±0.0
	D	4.3 ±0.6	4.0±0.0	4.5±0.5	4.5±0.0	4.3^{bc} ±0.3
30 day	A	4.2 ±0.3	4.0 ±0.0	4.3 ±0.3	4.5 ±0.0	4.2^{A} ±0.0
	B	4.2 ±0.3	4.5±0.0	4.5 ±0.5	4.5±0.5	4.4 ±0.2
	C	4.8 ±0.3	5.0±0.0	5.0 ±0.0	4.7±0.3	4.9^{aC} ±0.1
	D	4.2 ±0.3	4.0 ±0.0	4.3 ±0.3	4.5 ±0.0	4.2^{c} ±0.0
45 day	A	4.0 ±0.0	4.0 ±0.0	4.0 ±0.0	4.0 ±0.0	4.0^{A} ±0.0
	B	4.0 ±0.0	4.2 ±0.3	4.3 ±0.3	4.0 ±0.0	4.1 ±0.1
	C	4.8 ±0.3	4.5 ±0.5	4.7 ±0.3	4.5 ±0.0	4.7^{aC} ±0.3
	D	4.0 ±0.0	4.0 ±0.0	4.0 ±0.0	4.0 ±0.0	4.0^{c} ±0.0
60 day	A	3.0 ±0.0	3.3 ±0.3	3.0 ±0.0	2.7 ±0.3	2.9^{A} ±0.1
	B	3.0 ±0.0	3.3 ±0.3	3.0 ±0.0	2.7 ±0.3	2.9 ±0.1
	C	4.0 ±0.0	4.0 ±0.0	4.2±0.3	3.8 ±0.3	4.0^{aC} ±0.2
	D	3.5 ±0.5	3.0 ±0.0	3.3 ±0.3	3.5 ±0.0	3.3^{c} ±0.1
75 day	A	2±0.0	2±0.0	2±0.0	2±0.0	2±0.0
	B	3.0 ±0.0	3.3 ±0.3	3.0 ±0.0	2.7 ±0.3	3.0 ±0.0
	C	3.5 ±0.0	3.5 ±0.5	3.5 ±0.0	3.2 ±0.3	3.5 ±0.0
	D	3.2 ±0.3	2.8 ±0.3	3.0 ±0.0	2.7 ±0.3	3.2 ±0.3
90 day	A	2±0.0	2±0.0	2±0.0	2±0.0	2±0.0
	B	3.0 ±0.0	3.3 ±0.3	3.0 ±0.0	2.7 ±0.3	3.0 ±0.0
	C	3.5 ±0.0	3.5 ±0.5	3.5 ±0.0	3.2 ±0.3	3.5 ±0.0
	D	3.2 ±0.3	2.8 ±0.3	3.0 ±0.0	2.7 ±0.3	3.2 ±0.3
105 day	A	1±0.0	1±0.0	1±0.0	1±0.0	1±0.0
	B	3.0 ±0.0	3.3 ±0.3	3.0 ±0.0	2.7 ±0.3	3.0 ±0.0
	C	3.5 ±0.0	3.5 ±0.5	3.5 ±0.0	3.2 ±0.3	3.5 ±0.0
	D	3.2 ±0.3	2.8 ±0.3	3.0 ±0.0	2.7 ±0.3	3.2 ±0.3
110 day	A	1±0.0	1±0.0	1±0.0	1±0.0	1±0.0
	B	1±0.0	1±0.0	1±0.0	1±0.0	1±0.0
	C	1±0.0	1±0.0	1±0.0	1±0.0	1±0.0
	D	1±0.0	1±0.0	1±0.0	1±0.0	1±0.0

There are significance differences ($P<0.05$) between means having the same capital and small letter in the same column in the same inspection time.

A: Control negative, **B**: Sausage with nitrite 50 ppm, **C**: Sausage with 1% celery powder and 2% mushroom powder, **D**: Sausage with 1% celery powder and 5% mushroom powder. S: spoiled sample

Table 2. Total bacterial count of sausage groups (mean ±SD) log cfu/g

Treat-ment	0 days	15 day	30 day	45 day	60 day	75 day	90 day	105 day	110 day
A	4.66 ± 0.043^{A}	$4.9^{A} \pm 0.1$	$5.5^{A} \pm 0.048$	$5.7^{A} \pm 0.013$	$5.7^{A} \pm 0.09$	S	S	S	s
B	$4.53^{B} \pm 0.075$	$4.66^{B} \pm 0.2$	$4.9^{aB} \pm 0.1$	$4.9^{aB} \pm 0.1$	$5.61^{B} \pm 0.037$	$5.72^{B} \pm 0.21$	$5.81^{B} \pm 0.14$	$6.0^{B} \pm 0.2$	s
C	$4.0^{ab} \pm 0.09$	$3.8^{ab} \pm 0.3$	$4.5^{abC} \pm 0.02$	$4.9^{aC} \pm 0.03$	$4.9^{abC} \pm 0.1$	$5.5^{C} \pm 0.20$	$5.5^{C} \pm 0.20$	$6.0^{C} \pm 0.05$	s
D	$4.0^{ab} \pm 0.09$	$3.8^{ab} \pm 0.2$	$3.8^{abc} \pm 0.2$	$4.37^{abc} \pm 0.18$	$4.41^{abc} \pm 0.42$	$4.9^{bc} \pm 0.1$	$4.9^{bc} \pm 0.1$	$5.7^{bc} \pm 0.013$	s

There are significances differences (P<0.05) between means having the same capital and small letters in the same column.

A: Control negative, **B**: Sausage with nitrite 50 ppm, **C**: Sausage with 1% celery powder and 2% mushroom powder, **D**: Sausage with 1% celery powder and 5% mushroom powder. S: spoiled sample

Table 3. Psychrotrophic count of sausage groups (mean ±SD) log cfu/g

Treat-ment	0 days	15 day	30 day	45 day	60 day	75 day	90 day	105 day	110 day
A	$4.5^{A} \pm 0.41$	$4.9^{A} \pm 0.1$	$5.3^{A} \pm 0.36$	$5.8^{A} \pm 0.09$	$6.1^{A} \pm 0.46$	S	S	S	s
B	$4.5^{B} \pm 0.41$	$4.9^{B} \pm 0.09$	$4.9^{B} \pm 0.03$	$5.4^{B} \pm 0.36$	$5.9^{B} \pm 0.09$	$5.9^{B} \pm 0.1$	$6.0^{B} \pm 0.46$	$6.1^{B} \pm 0.46$	s
C	4.0 ± 0.40	$4.5^{C} \pm 0.4$	$4.5^{aC} \pm 0.45$	5.1 ± 0.69	$5.2^{ab} \pm 0.40$	$5.3^{bC} \pm 0.2$	5.8 ± 0.04	$5.8^{C} \pm 0.04$	s
D	$3.6^{ab} \pm 0.27$	$3.6^{abc} \pm 0.27$	$3.8^{abc} \pm 0.2$	$4.4^{ab} \pm 0.39$	$4.8^{ab} \pm 0.13$	$4.8^{bc} \pm 0.1$	$5.5^{b} \pm 0.28$	$5.2^{bc} \pm 0.28$	s

There are significances differences (P<0.05) between means having the same capital and small letters in the same column.

A: Control negative, **B**: Sausage with nitrite 50 ppm, **C**: Sausage with 1% celery powder and 2% mushroom powder, **D**: Sausage with 1% celery powder and 5% mushroom powder. S: spoiled sample

Table 4. The mean values of protein % of sausage groups:

day	A	B	C	D
zero day	$15.46{\pm}0.2^{A}$	$15.86{\pm}0.2^{aB}$	$16.44{\pm}0.1^{ab}$	$16.39{\pm}0^{ab}$
15 day	$15.7{\pm}0.1^{A}$	$15.82{\pm}0.1^{B}$	$16.42{\pm}0.2^{ab}$	$16.3{\pm}0.2^{ab}$
30 day	$15.5{\pm}0.5^{A}$	$15.77{\pm}0.4^{B}$	$16.6{\pm}0.6^{ab}$	$16.4{\pm}0.4$
45 day	$15.55{\pm}0.2^{A}$	$15.75{\pm}0.1^{B}$	$16.4{\pm}0.2^{ab}$	$16.42{\pm}0.2^{ab}$
60 day	$15.78{\pm}0.1^{A}$	$15.8{\pm}0.4^{B}$	$16.48{\pm}0.0^{ab}$	$16.6{\pm}0.4^{ab}$
75 day	s	$15.82{\pm}0.5^{B}$	$16.5{\pm}0.2^{b}$	$16.3{\pm}0.2$
90 day	s	$15.82{\pm}0.5^{B}$	$16.58{\pm}0.2^{b}$	$16.35{\pm}0.0^{b}$
105 day	s	$15.82{\pm}0.5^{B}$	$16.58{\pm}0.2^{b}$	$16.35{\pm}0.0^{b}$
110 day	s	s	s	s

There are significances differences ($P<0.05$) between means having the same capital and small letters in the same raw.
A: Control negative, **B**: Sausage with nitrite 50 ppm, **C**: Sausage with 1% celery powder and 2% mushroom powder, **D**: Sausage with 1% celery powder and 5% mushroom powder. S: spoiled sample

Table 5. The mean values of fat % in sausage groups:

day	A	B	C	D
zero day	$21.6{\pm}0.2^{A}$	$21.8{\pm}0.6^{B}$	$22.35{\pm}0.35^{aC}$	$23.15{\pm}0.15^{abc}$
15 day	$21.56{\pm}0.56^{A}$	$21.7{\pm}0.7^{B}$	$22.2{\pm}0.2^{C}$	$23.5{\pm}0.5^{abc}$
30 day	$21.7{\pm}0.2^{A}$	$21.8{\pm}0.4^{B}$	$22.84{\pm}0.26^{ab}$	$23.6{\pm}0.6^{ab}$
45 day	$21.8{\pm}0.5^{A}$	$21.9{\pm}0.4^{B}$	$22.6{\pm}0.6^{C}$	$23.56{\pm}0.0^{abc}$
60 day	$22.15{\pm}0.15^{A}$	$21.65{\pm}0.0^{aB}$	$22.55{\pm}0.2^{abC}$	$23.71{\pm}0.2^{abc}$
75 day	s	$21.69{\pm}0.6^{B}$	$22.7{\pm}0.1^{bC}$	$23.5{\pm}0.0^{bc}$
90 day	s	$21.69{\pm}0.7^{B}$	$22.5{\pm}0.2^{bC}$	$23.2{\pm}0.2^{bc}$
105 day	s	$21.69{\pm}0.5^{B}$	$22.5{\pm}0.2^{bC}$	$23.2{\pm}0.2^{bc}$
110 day	s	s	s	s

There are significances differences ($P<0.05$) between means having the same capital and small letters in the same raw.
A: Control negative, **B**: Sausage with nitrite 50 ppm, **C**: Sausage with 1% celery powder and 2% mushroom powder, **D**: Sausage with 1% celery powder and 5% mushroom powder. S: spoiled sample

Table 6. The mean values of total volatile basic nitrogen in sausage groups (mg/ 100g).

day	A	B	C	D
zero day	11.9±0.28^{A}	12.32±0.14^{aB}	11.2±0^{abC}	11.62±0.28^{bc}
15 day	12.46±0.14^{A}	12.74±0.14^{B}	11.62±0.14^{ab}	11.48±0.28^{ab}
30 day	14.84±0.28^{A}	13.86±0.14^{aB}	13.3±0.269^{abC}	12.18±0.28^{abc}
45 day	16.8±0.28^{A}	15.4±0.49^{aB}	14.7±0.14^{ab}	14.28±0.28^{ab}
60 day	18.2±0.28^{A}	17.64±0.14^{B}	16.24±0.56^{ab}	15.68±0.28^{ab}
75 day	21±0.14^{A}	18.9±0.7^{aB}	17.78±0.14^{abC}	16.52±0.0^{abc}
90 day	s	20.16±0.14^{B}	19.6±0.28^{bC}	18.48±0.28^{bc}
105 day	s	s	20.16 ±0.28	20.16 ±0.28
110 day	s	s	s	s

There are significances differences (P<0.05) between means having the same capital and small letters in the same raw.
A: Control negative, **B**: Sausage with nitrite 50 ppm, **C**: Sausage with 1% celery powder and 2% mushroom powder, **D**: Sausage with 1% celery powder and 5% mushroom powder. S: spoiled sample

Table 7. The mean value of TBA in sausages groups (mg malonaldehyde/ kg):

day	A	B	C	D
zero day	0.597±0.046^{A}	0.363±0.05^{a}	0.355±0.02^{a}	0.313±0.013^{a}
15 day	0.663±0.035^{A}	0.568±0.02^{aB}	0.487±0.0085^{ab}	0.414±0.014^{ab}
30 day	0.625±0.025^{A}	0.599±0.0^{B}	0.512±0.012^{abC}	0.454±0.004^{abc}
45 day	0.712±0.014^{A}	0.675±0.025^{aB}	0.605±0.005^{abC}	0.512±0.012^{abc}
60 day	0.889±0.0^{A}	0.789±0.009^{aB}	0.714±0.014^{ab}	0.685±0.030^{ab}
75 day	1.25±0.025^{A}	0.956±0.025^{aB}	0.735±0.02^{ab}	0.712±0.014^{ab}
90 day	s	0.789±0.009^{B}	0.898±0.02^{b}	0.865±0.015^{b}
105 day	s	0.956±0.025^{B}	0.898±0.02^{b}	0.865±0.015^{b}
110 day	s	s	s	s

There are significances differences (P<0.05) between means having the same capital and small letters in the same raw.
A: Control negative, **B**: Sausage with nitrite 50 ppm, **C**: Sausage with 1% celery powder and 2% mushroom powder, **D**: Sausage with 1% celery powder and 5% mushroom powder. S: spoiled sample

Table 8. The mean values of peroxide values in sausage groups (mg equivalent .oxygen/ kg)

day	A	B	C	D
zero day	0.2±0.01^{A}	0.21±0.01^{B}	0.1±0.0^{ab}	0.09±0.01^{ab}
15 day	0.25±0.02^{A}	0.24±0.02^{B}	0.14±0.02^{ab}	0.145±0.0^{ab}
30 day	0.3±0.02^{A}	0.28±0^{B}	0.18±0.02^{ab}	0.18±0.01^{ab}
45 day	0.35±0.05^{A}	0.34±0.02^{B}	0.23±0.02^{ab}	0.24±0.02^{ab}
60 day	0.4±0.02^{A}	0.39±0.01^{B}	0.28±0^{ab}	0.29±0.02^{ab}
75 day	0.48±0.02^{A}	0.47±0.07^{B}	0.36±0.06^{ab}	0.38±0.03^{a}
90 day	s	0.61±0.01^{B}	0.52±0.02^{b}	0.53±0.03^{b}
105 day	s	0.61±0.01^{B}	0.54±0.02^{b}	0.55±0.03^{b}
110 day	s	s	s	s

DISCUSSION:

4.1 Sensory evaluation:

The results of the sensory scores (appearance, color, flavor, odour and overall acceptability) are shown in Table (1). No statistical differences (P>0.05) were observed between all sausage groups attributes to overall at zero day storage after manufacturing , while from 15th days to the end of storage, the sensory scores of sausage treated with celery 1%+ mushroom 2% were significantly (P < 0.05) higher than those treated with celery1%+ mushroom 5% formula and also with other groups (control negative and positive). At the 75th day of storage and till the end of storage period, the sensory scores of all attributes for the control negative formula were close to an unacceptable score while, the sensory scores of all treated formulas with celery and mushroom were still with acceptable score until the end of storage. Generally, the mean values for group treated with 1% celery and 2% mushroom % at zero day storage after manufacturing until 105 day showed the highest scores for all sensory attributes. On the other hand, the higher concentration of mushroom (MP 5%) interferes with the quality of the color and appearance during the storage period which considered the most crucial attribute of food quality and has a great influence on consumer impressions.

Previously, different authors observed well acceptable sensory scores after incorporation of natural additives, celery and mushroom. **Wan Rosli and Solihah, 2012** recommended incorporation of mushroom in beef patties and permit a reduction of the formulation cost without affecting sensory characteristics of the product to which the consumer is familiarized**.** **Shalaby et al. 2015** evaluated formulas of like -sausages produced from oyster mushroom as main ingredient. Obtained results showed that most values of organoleptic evaluation of the mushroom-pea formulas were higher than the corresponding other groups. In addition, **Zhang et al. 2016, Jo et al. 2018, Wang et al.2018 and Wang et al. 2022** concluded that addition of mushroom improve the sensory character especially texture, taste and flavor. More ever, **Jin et al. 2018, Hwang et al. 2018** and **Pennesi et al. 2020** concluded that celery powder had no effect on sensory character of incorporated product**s.**

4.2 Microbiological analysis:

The total bacterial count was recognized as an important parameter to evaluate the shelf-life stability .Therefore, The microbiological analysis of sausages in the control and treated groups were tested on days 0, 15, 30, 45, 60, 75, 90,105 and 110 of storage at -18 °C for total bacterial count and psychrotrophic microorganisms, while the microbiological analysis of coliforms, Staphylococcus coagulase positive and clostridium perfringens were performed only at day zero. As it is noted that coliforms, Coagulase-positive staphylococci and clostridium perfringens were found to be undetectable for all samples during analysis . The results presented in Table(2,3) offered that The microbial load of both control and treated samples of sausage had significantly ($P \leq 0.05$) increased with increase in storage period but it concomitantly ($P \leq 0.05$) decreased with increase in preservatives concentration .while the incorporation of celery and mushroom mixture during processing of sausages resulted in significant ($P < 0.05$) reduction in total bacterial count and psychotropic count after processing and during storage. Considering the values until the 105th day of storage for the treatments with celery and mushroom showed total bacterial count and psychotropic count lower than 6.0 log CFU/g and remained within the acceptable limit of microbial contamination compared to the negative control groups. The obtained results were close to that obtained by **Kisworo et al. 2020** and **Pennesi et al. 2020** who concluded that celery powder had antibacterial and antioxidant effect. Moreover, the antibacterial action may be due to its high content of nitrate and nitrite **(Sebranek and Bacus, 2007).** In addition, **Zhang et al. 2016** suggested that the antibacterial effect of mushroom due to its phenolic compound content (e.g., quercetin, chlorogenic acid, gallic acid, and protocatechuic acid).

4.3 Chemical examination:

The results in the table (4) showed that, the protein % of the control group and sausage added with nitrite was close to 15.8 %, while the other groups incorporated with mushroom and celery were close to 16.5 %. All these results were in accordance with the Egyptian frozen sausage standard 1972/2005 where the protein % was around 15% according to its descriptive criteria. In addition, there were significant differences ($P < 0.05$) in the protein contents between the groups incorporated with mushroom and control one. **Zhang et al. 2013** and **Dong et al. 2017** said that mushrooms have high levels of nutrients (protein, polysaccharides, fiber, and vitamins). Also it can be used as an alternative protein source to meat products **(Asgar et al. 2010).** These results were similar to results demonstrated by **Shalaby et al. 2015** who stated that manufacture of many formulas of like-sausages produced from mushroom as main ingredient, showed that the protein % of mushroom incorporated groups were higher than control one.

In addition, the fat % of the control negative group and sausage added with nitrite group was close to 21.6 %, while the other groups incorporated with mushroom and celery (1% celery+2%mushroom and 1% celery+5%mushroom) were close to 22.5 and 23.5 % respectively (table 5). All these results were in accordance with the Egyptian frozen sausage standard 1972/2005 where the fat % is around 30% according to its descriptive criteria. In addition, there were significant differences ($P < 0.05$) in the fat contents between the groups incorporated with mushroom and control one. Moreover, the sausage group incorporated with (5% mushroom+ 1% celery) was significally higher than the other group incorporated with 2% mushroom).This results were similar to results demonstrated by **Shalaby et al. 2015** who stated that manufacture of many formulas like-sausages produced from mushroom as main ingredient, the fat % of mushroom incorporated groups were higher than control one.

Total volatile basic nitrogen (TVB-N) is often used as a biomarker of protein and amine degradation and used as interpret meat freshness **(Bekhit, et al. 2021).** The results in the table (6) showed that, the TVB-N of the control negative group started from 11.9 ±0.28mg/100g at the beginning of storage period (zero day) to 21 ±0.14 at the 75th day of storage periods. Moreover, the TVB-N of the sausage nitrite group started from 12.32±0.14mg/100g at the beginning of storage period (zero day) to 20.16±0.14 at the 90th day of storage periods, while, the TVB-N of the (celery 1% and mushroom 2%) group and (celery 1% and mushroom 5%)started from 11.2±0±0.14 and 11.62±0.28 mg/100g at the beginning of storage period (zero day) to 20.16 ±0.28 and 20.16 ±0.28 at the 105th day of storage periods, respectively. there were significant differences ($P < 0.05$) in the TVB-N between the groups incorporated with mushroom and control one. Moreover, the sausage group incorporated with (5% mushroom+ 1% celery) was significally lower than the other group incorporated with 2% mushroom). The results of control group were in accordance with the Egyptian frozen sausage standard 1972/2005 until 75th day of storage while the results of natural added groups were in accordance with ES until 105th day of manufacture. Where, the TVB-N % was 20 mg/ 100g according to its basic requirements. These results were nearly similar to results concluded by **Jin et al. 2018** who suggest that celery powder effectively protects sausages from quality deterioration during storage and had no effect on TVB-N and also similar to results demonstrated by **Shalaby et al. 2015** and **Jo et al. 2018** who concluded that the TVB-N of mushroom incorporated groups were lower than control one.

Thiobarbituric acid and peroxide values are used as indicators of the degree of lipid oxidation. The results in the table (7,8) showed , the TBA and peroxide value of the control negative group started from (0.597±0.046 and 0.20±0.01)at the beginning of storage period (zero day) to (1.25±0.045 and 0.48±0.02) at the 75th day of storage periods. In addition, the TBA and peroxide value of the sausage with nitrite group started from 0.363±0.05 and 0.21±0.01 at the beginning of storage period (zero day) to 0.956±0.025 and 0.61±0.01 at the 105th day of storage periods , respectively.

While, the results of the celery 1% and mushroom 2% group and celery 1% and mushroom 5% started from 0.355±0.02, 0.1±0.0 and 0.313±0.013, 0.09±0.01 at the beginning of storage period (zero day) to 0.898±0.02, 0.54±0.02 and 0.865±0.015, 0.55±0.03 at the 105th day of storage periods, respectively. there were significant differences ($P < 0.05$) in the TBA and peroxide between the groups incorporated with mushroom and celery and control one. Moreover, the sausage group incorporated with 5% mushroom+ 1% celery was significally lower than the other group incorporated with 2% mushroom and 1% celery. The results of control group were in accordance with the Egyptian frozen sausage standard 1972/2005 until 75th day of storage while the results of natural added groups were in accordance with ES until 105th day of manufacture. Where, the TBA was 0.9 mg/ Kg according to its basic requirements. These results were nearly similar to results concluded by **Jin et al. 2018** who suggest that celery powder effectively protects sausages from quality deterioration during storage and reduce the lipid oxidation and also similar to results demonstrated by **Shalaby et al. 2015** and **Jo et al. 2018** who concluded that the lipid oxidation of mushroom incorporated groups were lower than control one. **Kisworo et al., 2020** explaind the antioxidant effect of celery as it contain about more than 2000 ppm of nitrate contents, in addition, there are many reports on the antioxidant activity of mushrooms. Mushrooms contain phenolic compounds (e.g., quercetin, chlorogenic acid, gallic acid, and protocatechuic acid) and flavonoids, which are well-known antioxidants **(Lee, et al. 2018** and **Banerjee et al. 2020).**

According to the results of this study, the mushroom powder and celery can serve as natural ingredient that have antibacterial and strong antioxidant effect and they can be used as nitrite substitution. So they can be used for improving the quality of frozen sausage and extension of its shelf life.

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