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USING OF POTASSIUM CHLORIDE WITH ULTRASOUND SONICATION TO ENHANCE THE HYGIENIC STATUS OF FROZEN SAUSAGE

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ABSTRACT
The meat industry has been increasing the strategies to produce and commercialize products where the reduction or even the replacement of NaCl is an important goal. In the replacement of traditional techniques as meat tenderization and assisting of traditional techniques as freezing and thawing, ultrasound has been effectively utilized in various areas of food technology and yet further research demand is recommended**.** This research was applied to replace partially and totally sodium chloride salt with potassium chloride in union with ultrasound technique for improvement the hygienic status and quality of frozen sausage. Regarding to results demonstrated that partial replacement of sodium chloride with potassium chloride up to 80% did not effect on sensory, chemical and microbiological properties of frozen sausage. In addition, Ultrasonic processing is still in its infancy and requires a great deal of future research in order to develop the technology on an industrial scale, and to more fully elucidate the effect of ultrasound on the properties of foods.

KEYWORD:- Potassium chloride – ultrasound sonication – frozen sausage.

1. INTRODUCTION
Concerns over the quality of meat, particularly its impact on health, have grown among consumers in recent decades. Because of its association with cardiovascular problems, consumers and health authorities have been very concerned about the amount of salt, especially sodium chloride, in processed beef products. As a result, the meat industry has been using more tactics to create and market healthier goods, with lowering or even eliminating salt as a key objective **(Domínguez et al., 2016).**

The World Health Organization **(WHO, 2021)** recommended that adults reduce their consumption of NaCl to less than 5 g NaCl/day or 2 g sodium/day. However, mean NaCl intake of most people worldwide is about 9– 12 g/day **(Ranilovi et al., 2019).** WHO encourages the industry to reduce the sodium content in processed foods towards a 30% reduction in global NaCl/ sodium intake by 2025 **(WHO, 2021)**

Numerous meat studies demonstrate that alternative salts, primarily potassium, calcium, and magnesium chlorides, could be used in place of sodium chloride for producing some meat products, such as Bologna sausages **(Dos Santos et al., 2017)** and pork sausages **(Rodrigues et al., 2020).**

Ultrasound has been successfully used in many areas of food technology to replace traditional methods (such as cutting, degassing, and tenderizing meat) and support traditional methods (such as brining, freezing and thawing, and filtering), but more research is still needed **(Boateng and Nasiru 2019).**

Ultrasound is generally defined as sound with a frequency above that to which the human ear can respond **(Chemat and khan, 2011).** Ultrasound is a pressure wave that is oscillating between the frequencies 20 kHz and 10 MHz which are the range of ultrasound applications. Ultrasound, used in processing is above the range of human hearing frequencies **(Ünver, 2016).**

Because of its exceptional qualities, ultrasonic technology is therefore highly regarded for processing and preservation purposes. Over the past 10 years, high hydrostatic pressure and ultrasound have been studied as potential new alternative strategies; however, high-intensity ultrasound has generated a lot of interest for use in food storage **(Sandra et al., 2017)**

Ultrasound is used in processes that combine other methods, including heat, refrigeration, packing in a changed atmosphere, and chemicals, to inactivate or reduce microorganisms. Some well-known benefits of using ultrasound in processes include increased productivity, yield, and selectivity; improved processing time; improved quality; less chemical and physical risks; and environmental friendliness **(Chemat et al., 2011).**

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 preservatives and packed inside natural casings on lamb intestine or industrial casing and preserved in frozen form **(ES, 1972/2005)**

In sight of these facts, this research aimed to determine the effect of partially and total replacement of sodium chloride with potassium chloride with ultrasound techniques on frozen sausage as a one of meat products. Assessment of organoleptic, chemical and aerobic plate counts s of sausage treatments were applied at the day of manufacture and at intervals during storage at freezer till the signs of spoilage appeared.

2. MATERIAL AND METHODS

2.1 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 with sodium chloride only as control one) (1), group 2 (sausage with sodium and potassium chloride (1:1) with ultrasound sonication) (2), group 3 (sausage with sodium and potassium chloride (1:4) with ultrasound sonication) (3) and group 4 (sausage with potassium chloride (100%) with ultrasound sonication) (4). The meat mixtures were stuffed into natural mutton casing. The ultrasound sonication was conducted by ultrasound sonication apparatus (Branson, model 3510 Mexico) for 30 minutes at low intensity power. 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 week for sensory, chemical and microbiological criteria until spoilage occurred.

2.2 Sensory evaluation

Sensory attributes for raw and cooked sausage sample (texture, odor, taste and overall) were examined according to the scheme adopted by **Asmaa et al. (2013)** Using the 9-point hedonic scale. Sensory examinations of samples were conducted by members from the Food Hygiene Department, Animal Health Research Institute until the end of the study.

2.3 Microbiological analysis

Microbiological analysis was performed on all control and treated sausage samples, for the presence of the main microorganisms such as Total aerobic bacterial count, total anaerobic bacterial count, Coliform count and Coagulase Positive Staphylococci and salmonella count. 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.

Total bacterial anaerobic count (FDA /2001)

Using Plate Count Agar (PCA, Oxoid) incubated anaerobically at 30°C for 72 hours.

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 **Detection of Salmonella spp. (ISO 6579-1:2017 (E)) Amd. 1:2020** Using Xylose Lysine Deoxycholate agar (XLD agar, Oxoid) incubated at 37 °C and examined after 24 hours.

2.4 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).

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.

2.5 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).**

3. RESULTS

Table 1: The organoleptic score (mean ± SD) of the examined sausage treatment groups during freezing storage period.

	Sausage treatment group s	Storage time (wks)												
		Zero	1st wk	2nd wk	3rd wk	4th wk	5th wk	6th wk	7th wk	8th wk	9th wk	10th wk	11th wk	12th wk
Texture	1	8 ±	8±	7.5	7.5 ±	7.5^{A}	7.5^{A}	7^{A} ±	7^{A} ±	6.5^{A}±	S^{A}	S^{A}	S	s

scores (10)		0.5	0.0	$\pm 0.5^{A}$	0.0^{A}	± 0.5	± 0.5	0.0	0.5	0.5				
	2	8± 0.0	8± 0.5	8 ± 0.5	7.5^{B}± 0.5	7.5^{B} ± 0.0	7.5^{B} ± 0.5	7^{B} ± 0.0	7^{B} ± 0.5	7^{B} ± 0.5	6.5^{B} ± 0.0	S^{B}	S	s
	3	8 ± 0.0	8.5 ± 0.5	8.5± 0.5^{a}	8.5^{ab} ± 0.5	8^{abC} ± 0.0	8^{C} ± 0.5	7.5^{C}± 0.5	7.5^{C} ±0.5	7^{C} ± 0.0	7±0.0	6.5 ± 0.0	S	s
	4	8 ± 0.5	8.5± 0.0	9± 0.0^{a}	9^{ab} ± 0.5	9^{abc} ± 0.0	9^{abc}± 0.5	8.5^{abc} ± 0.5	8.5^{abc} ± 0.0	8^{abc} ±0.5	7.5^{ab} ± 0.0	7^{ab} ± 0.0	S	s
Odour scores (10)	1	8 ± 0.0	8± 0.5	8.5± 0.5	8.5 ± 0.5	8± 0.0	7.5 ± 0.5	7.5 ± 0.5	7 ± 0.0	7 ± 0.5	S	S	S	S
	2	8 ± 0.0	8± 0.5	8.5± 0.5	8.5 ± 0.0	8± 0.5	7.5± 0.5	7.5± 0.0	7 ± 0.5	7 ± 0.0	7± 0.0	S	S	S
	3	8± 0.5	8± 0.0	8.5± 0.5	8.5± 0.5	8± 0.0	7.5 ± 0.0	7.5 ± 0.5	7 ± 0.0	7 ± 0.5	7± 0.5	6.5 ± 0.5	S	S
	4	8 ± 0.5	8± 0.0	8.5± 0.0	8.5 ± 0.5	8± 0.0	7.5 ± 0.5	7.5 ± 0.5	7 ± 0.0	7 ± 0.5	7± 0.5	6.5± 0.0	S	S
Taste scores (10)	1	8.5^{A} 0.0	8.5^{A}± 0.5	8.5^{A} ± 0.0	8^{A} ± 0.5	8^{A} ± 0.5	8^{A} ± 0.0	7.5^{A} ± 0.5	7.5^{A} ± 0.5	7.5^{A} ± 0.0	S^{A}	S^{A}	S	S
	2	8 ± 0.5	8 ± 0.0	8.5^{B} ± 0.5	8^{B} ± 0.5	8^{B} ± 0.5	8^{B} ± 0.0	7 ± 0.5	7 ± 0.5	7^{B} ± 0.5	7^{B} ± 0.5	S^{B}	S	S
	3	8 ± 0.0	8 ± 0.5	8^{C} ± 0.5	7.5 ± 0.5	7.5 ± 0.5	7.5 ± 0.5	7 ± 0.0	7 ± 0.5	7^{C} ± 0.0	7^{C} ± 0.5	7^{C} ± 0.5	S	S
	4	7.5^{a} 0.5	7.5^{a} ± 0.0	7^{abc} ± 0.5	7^{ab} ± 0.5	7^{ab} ± 0.0	7^{ab} ± 0.5	6.5^{a} ± 0.5	6.5^{a} ± 0.0	6.5^{abc} ± 0.5	6^{abc} ± 0.0	6^{abc} ± 0.0	S	S
Overall scores (30)	1	8± 0.0	8 ± 1	7.5± 0.0	7.5 ± 0.0	7.5 ± 0.5	7± 0.5	7± 0.5	7± 0.5	6.5 ± 1	S^{A}	S^{A}	S	S
	2	8 ± 1	8± 0.5	8± 0.0	7.5± 0.5	7.5 ± 0.0	7.5 ± 0.0	7.5 ± 0.5	7.5± 0.5	7 ± 0.5	7^{a}± 0.5	S^{B}	S	S
	3	8 ± 0.0	8± 0.5	8± 0.5	7.5 ± 0.5	7.5 ± 0.0	7.5 ± 0.0	7.5 ± 0.5	7.5± 0.5	7 ± 1	7^{a}± 0.0	6.5^{ab} ± 0.5	S	S
	4	8 ± 0.5	8± 0.0	8± 0.5	7.5 ± 0.5	7.5± 0.5	7± 0.5	7± 0.0	7± 0.0	6.5 ± 0.5	6.5^{a} ± 0.0	6^{ab}± 0.0	S	S

1: Control **2:** Nacl+Kcl(50%:50%) with ultrasound **3:** Nacl+Kcl(20%:80%) with ultrasound **4:** Kcl 100% with ultrasound
S: Spoiled samples SD: Standard Deviation
*: results shown are means of triplicates of each group.
There is sig. diff. (P<0.05) between means having the same capital and small letters (A, a) in the same column for each score.

Table 2: Protein % (mean ± SD) of the sausage treatment groups during frozen storage period.

Storage periods wks	Storage period/ wks*			
	1	2	3	4
Zero day	15.46^{A}± 0.02	16.86^{aB} ± 0.2	16.44^{ab} ± 0.2	16.39^{ab} ± 0.01
1st wk	15.4^{A} ± 0.3	16.8^{a} ± 0.2	16.4^{a}± 0.3	16.35^{a} ± 0.35
2nd wk	15.5^{A} ± 0.2	16.82^{a} ± 0.315	16.42^{a} ± 0.2	16.3^{a} ± 0.3
3rd wk	15.6^{A} ± 0.3	16.85^{a} ± 0.15	16.55^{a} ± 0.2	16.38^{a} ± 0.38
4th wk	15.8^{A} ± 0.2	16.77^{a} ± 0.2	16.6^{a} ± 0.2	16.4^{a} ± 0.2
5th wk	15.7^{A} ± 0.2	16.8^{a} ± 0.2	16.65^{a}± 0.2	16.45^{a} ± 0.45
6th wk	15.75^{A} ± 0.2	16.75^{a} ± 0.25	16.4^{a} ± 0.1	16.42^{a} ± 0.2
7th wk	15.7^{A} ± 0.3	16.77^{a} ± 0.2	16.42^{a} ± 0.2	16.5^{a} ± 0.5
8th wk	15.8^{A} ± 0.2	16.8^{a} ± 0.3	16.48^{a} ± 0.0	16.6^{a} ± 0.2
9th wk	15.85^{A} ± 0.15	16.82^{aB} ± 0.2	16.48^{ab} ± 0.0	16.2^{ab} ± 0.2
10 wk	s	16.82 ± 0.2	16.5 ± 0.5	16.3 ± 0.3
11th wk	s	s	16.55^{a} ± 0.2	16.4± 0.4
12th wk	s	s	s	s

1: Control **2:** Nacl+Kcl(50%:50%) with ultrasound **3:** Nacl+Kcl(20%:80%) with ultrasound **4:** Kcl 100% with ultrasound
S: Spoiled samples SD: Standard Deviation
There is sig. diff. (P<0.05) between means having the same capital and small letters (A. a) in the same raw.

*: results shown are means of triplicates of each group**.**

Table 3: Thiobarbituric acid values (mean ± SD) of the sausage treatment groups during frozen storage period.

Storage periods wks	**Storage period/ wks***			
	1	**2**	**3**	**4**
Zero day	0.48 ± 0.02	0.49 ± 0.01	0.48 ± 0.01	0.49 ± 0.01
1st wk	0.52 ± 0.02	0.51 ± 0.02	0.51 ± 0.02	0.5 ± 0.02
2nd wk	0.55 ± 0.03	0.53 ± 0.03	0.54 ± 0.02	0.53± 0.03
3rd wk	0.57 ± 0.03	0.56 ± 0.04	0.55 ± 0.05	0.56 ± 0.01
4th wk	0.62 ± 0.05	0.62 ± 0.02	0.61 ± 0.02	0.62 ± 0.05
5th wk	0.65 ± 0.02	0.64 ± 0.01	0.65 ± 0.01	0.65 ± 0.02
6th wk	0.68 ± 0.01	0.67 ± 0.03	0.69 ± 0.04	0.68 ± 0.04
7th wk	0.79 ± 0.02	0.78 ± 0.01	0.79 ± 0.02	0.78 ± 0.02
8th wk	0.81 ± 0.01	0.82 ± 0.02	0.82 ± 0.02	0.82 ± 0.02
9th wk	0.84 ± 0.01	0.84 ± 0.03	0.84 ± 0.04	0.84 ± 0.04
10 wk	1.05 ± 0.01(S)	0.91 ± 0.01	0.9 ± 0.02	0.92 ± 0.02
11th wk	S	1.04 ± 0.04 (S)	1.04 ± 0.04 (S)	1.03 ± 0.03 (S)
12th wk	S	S	S	S

1: Control **2:** Nacl+Kcl (50%:50%) with ultrasound **3:** Nacl+Kcl (20%:80%) with ultrasound **4:** Kcl 100% with ultrasound

S: Spoiled samples SD: Standard Deviation

*: results shown are means of triplicates of each group**.**

Table 4: Peroxide values (mean ± SD) of the sausage treatment groups during frozen storage period.

Storage periods wks	**Storage period/ wks***			
	1	**2**	**3**	**4**
Zero day	0.1 ± 0.005	0.11 ± 0.01	0.1 ± 0.005	0.09 ± 0.02
1st wk	0.1 ± 0.005	0.12 ± 0.01	0.11 ± 0.02	0.1 ± 0.01
2nd wk	0.15 ± 0.01	0.14 ± 0.01	0.14 ± 0.02	0.145 ± 0.02
3rd wk	0.17 ± 0.02	0.15 ± 0.02	0.15 ± 0.02	0.16 ± 0.02
4th wk	0.2± 0.02	0.18 ± 0.02	0.18 ± 0.02	0.18 ± 0.01
5th wk	0.21 ± 0.01	0.2 ± 0.02	0.2 ± 0.01	0.21 ± 0.01
6th wk	0.25± 0.01	0.24 ± 0.01	0.23 ± 0.02	0.24 ± 0.02
7th wk	0.25 ± 0.01	0.25± 0.02	0.25 ± 0.01	0.25 ± 0.03
8th wk	0.3 ± 0.02	0.29 ± 0.01	0.28 ± 0.01	0.29 ± 0.02
9th wk	0.33 ± 0.03	0.31± 0.02	0.32 ± 0.02	0.32 ± 0.02
10 wk	S	0.37 ± 0.02	0.36 ± 0.02	0.38 ± 0.03
11th wk	S	0.43 ± 0.03	0.41 ± 0.02	0.42 ± 0.02
12th wk	S	S	S	S

1: Control **2:** Nacl+Kcl (50%:50%) with ultrasound **3:** Nacl+Kcl (20%:80%) with ultrasound **4:** Kcl 100% with ultrasound

S:Spoiled samples SD: Standard Deviation

*: results shown are means of triplicates of each group

Table 5: Total volatile basic nitrogen (mean ± SD) of the sausage treatment groups during frozen storage.

Storage periods wks	**Storage period/ wks***			
	1	**2**	**3**	**4**
Zero day	11.2^{A} ± 0.0	11.9^{a} ± 0.28	11.9^{a} ± 0.0	12.04^{a} ± 0.0
1st wk	11.34^{A} ± 0.14	11.9 ± 0.0	11.9 ± 0.7	12.18^{a} ± 0.28
2nd wk	11.48 ± 0.14	11.76 ± 0.28	11.9 ± 0.7	12.18± 0.0
3rd wk	11.76^{A} ± 0.28	11.76^{B} ± 0.0	12.18^{ab} ± 0.0	11.9± 0.28
4th wk	11.9 ± 0.28	11.9± 0.28	12.32± 0.28	12.18 ± 0.28
5th wk	12.6 ± 0.0	12.18 ± 0.28	12.6 ± 0.0	12.6 ± 1.4
6th wk	12.88^{A} ± 0.28	12.32^{a} ± 0.28	12.6 ± 0.28	12.6 ± 0.0
7th wk	13.3^{A} ± 0.0	12.32^{aB} ± 0.0	12.88^{ab} ± 0.0	12.6^{ab} ± 0.28
8th wk	13.3^{A} ± 0.7	12.6^{a} ± 0.28	13.3 ± 0.28	12.88± 0.28
9th wk	13.58^{A} ± 0.28	12.88^{aB} ± 0.0	13.58^{bC} ± 0.0	13.3^{abc} ± 0.0

10 wk	14^{A} ± 0.42	12.88^{aB} ± 0.28	14^{bC} ± 0.42	13.3^{ac} ± 0.0
11th wk	16.8^{A} ± 0.0	13.3^{aB} ± 0.0	14^{bC} ± 0.0	13.58^{abc} ± 0.28
12th wk	21 ± 1.4 (S)^{A}	16.8^{aB} ± 0.28	18.2^{abC} ± 0.7	15.4^{abc} ± 1.4
13th wk	S^{A}	21^{a} ± 0.0 (S)	21^{a} ± 0.0 (S)	19.6^{a} ± 0.7

1: Control **2:** Nacl+Kcl(50%:50%) **3:** Nacl+Kcl(20%:80%) **4:** Kcl 100%
S: Spoiled samples SD: Standard Deviation
There is sig. diff. ($P<0.05$) between means having the same capital and small letters (A. a) in the same raw.
*: results shown are means of triplicates of each group

Table 6: Total plate count (log10 cfu/g ± SD) of the sausage treatment groups during frozen storage.

Storage periods wks	Storage period/ wks*			
	1	2	3	4
Zero day	3.447^{A} ±0.015	3.30^{aB} ± 0.0	3.079^{abC} ± 0.07	3^{abc} ± 0.0
1st wk	3.65^{A} ± 0.029	3.63 ^{B} ± 0.03	3.397 ^{abC} ± 0.03	3.176^{abc} ± 0.0
2nd wk	3.963^{A} ± 0.009	3.991^{aB} ± 0.004	3.892^{abC} ± 0.0	3.653^{abc} ± 0.009
3rd wk	4.390^{A} ± 0.035	4.457^{aB} ± 0.0	4.397^{bC} ± 0.03	4.255^{abc} ± 0.02
4th wk	4.638^{A} ± 0.03	4.633^{B} ± 0.020	4.556^{abC} ± 0.024	4.301^{abc} ± 0.0
5th wk	4.98^{A} ± 0.009	4.986^{B} ± 0.008	4.653^{abC} ± 0.019	4.477^{abc} ± 0.014
6th wk	5.518^{A} ± 0.026	5.505^{B} ± 0.027	4.963^{abC} ± 0.009	4.832^{abc} ± 0.006
7th wk	5.74^{A} ± 0.015	5.690^{B} ± 0.017	5.531^{abC} ± 0.0	4.959^{abc} ± 0.004
8th wk	5.892^{A} ± 0.011	5.90^{B} ± 0.0	5.819^{abC} ± 0.013	5.397^{abc} ± 0.017
9th wk	6.819^{A} ± 0.0(S)	5.977^{aB} ± 0.0	5.913^{abC} ± 0.0	5.681^{abc} ± 0.0
10 wk	S^{A}	S^{B}	5.954^{ab} ± 0.0	5.944^{ab} ± 0.0
11th wk	S^{A}	S^{B}	6.716 ^{ab} ± 0.0 (S)	6.698^{ab} ± 0.0 (S)
12th wk	S	S	S	S

1: Control **2:** Nacl+Kcl(50%:50%) **3:** Nacl+Kcl(20%:80%) **4:** Kcl 100%
S:Spoiled samples SD: Standard Deviation
There are sig. diff. ($P<0.05$) between means having the same capital and small letters (A. a) in the same raw.
*: results shown are means of triplicates of each group**.**

4. DISCUSSION

4.1 Sensory evaluation

The results of the sensory scores (texture, color, taste and overall acceptability) are shown in Table (1). For texture character, from 2nd and 3rd weeks there are significant difference between control sausage group fortified with 100 % sodium chloride and with sausage groups fortified with 100 % and 80 % potassium chloride with ultrasound sonication, respectively, till end of storage period. Moreover, partially or complete substitution of sodium chloride with potassium chloride did not influence on the color character of the products. In addition, for taste character there are significant difference between control sausage group fortified with 100 sodium chloride and with sausage groups fortified with 80 % and 100 % potassium chloride with ultrasound sonication, till end of storage period. High content of potassium chloride lead to slightly increasing in bitterness of sausage. On the other hand, the sausage group fortified with sodium and potassium chloride (1:1) has generally no significant difference with control sausage group in texture, color, taste and overall. This indicated that this treatment not influence on the overall sensory character on sausage product. Previously, different authors observed well acceptable sensory scores. **Dos Santos et al., 2017** concluded that the partial replacement of NaCl by KCl did not have negative impacts on physicochemical and technological, properties.

Teixeira et al., 2021 stated that the replacement of NaCl with 50% KCl did not affect the physicochemical and sensory properties of frozen goat sausage. **Nayak & Pathak (2021)** developed low sodium chevon patties by applying salt blend which constituted of 1% NaCl, 0.4% KCl, and 0.2% $CaCl_2$. This formulation was successfully reduced 37.5% NaCl and did not deteriorate quality and sensory acceptability.

From result showed in table (1) high content of potassium chloride lead to slightly increasing in bitterness of sausage. The slight bitterness can be solved by addition of other additives as yeast extract **Wang et al. (2021).** He concluded that yeast extract could enhance sensory defects due to the addition of KCl.

addition of KCl improved water holding capacity, rheological properties, and microstructure compared to gels with $CaCl_2$. These findings indicated that KCl was a potential replacer for NaCl to develop low- sodium surimi products **(Yu et al., 2017)**.

Da Silva- Araujo et al., 2021 evaluated the effect of using four different types of salt (100% NaCl, T1; 100% Himalayan salt, T2; 50% NaCl + 50% KCl, T3 or 50% NaCl + 25% KCl + 20% $MgCl_2$ + 5% $CaCl_2$, T4) on physicochemical, textural, microbiological and sensory characteristics of frozen goat sausage. There was no treatment effect (*P* > 0.05) on water activity and sensory analysis. The reduction of sodium by replacing NaCl with chloride salts at the studied levels had no negative influence on the physicochemical, microbiological and sensory characteristics of frozen goat sausage**.**

Other than that, the result showed that the application of ultrasound didn't influence negatively on all sensory character of sausage treated groups. **Barretto et al., 2018** concluded that ultrasound enhanced sensory acceptability of restructured cooked ham with 30% of sodium reduction.

Sánchez et al. (2000) and Contreras-Lopez et al. (2020) observed that the application of high intensity acoustic energy produce a product with uniform salt content. In addition, low-intensity ultrasound is commonly used in improving the taste, tenderness, and quality which are important to achieve consumer acceptability. Recent studies indicate the prospective application of high-intensity ultrasound on tenderizing **(Caraveo et al., 2015 and Peña-González et al., 2017)**

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 from zero day till the end of storage period weekly of storage at -18 °C for total bacterial count, while the microbiological analysis of anaerobes, coliforms, Staphylococcus coagulase positive and salmonella were performed only at day zero. As it is noted that anaerobes, coliforms, Coagulase-positive staphylococci and salmonella were found to be undetectable for all samples during analysis. The results presented in Table (6) 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 ($P \leq 0.05$) slightly decreased with increase in kcl concentration. There is a significant difference between control group and sausage fortified with 50, 80, 100% kcl with ultrasound sonication from the beginning of storage period till the end. Thus total aerobic bacterial count of control group exceeds the permissible limit in 9th week of storage period according to Egyptian standard no **(ES 1972/2005).** While, sausage fortified with 50% kcl with ultrasound sonication exceed the permissible limit in 10th week of storage period. In addition, sausage treated with 80 and 100 % kcl with ultrasound sonication exceed the permissible limit in TPC in 11th week of storage period.

Our results indicated that kcl has an equivalent antimicrobial effect against sodium chloride in meat products. This results was nearly similar to results demonstrated by **Bidlas and Lambert, 2008; Zanardi et al., 2010; Aaslyng et al., 2014; Dos Santos et al., 2017; Da Silva- Araujoet al., 2021 and Teixeira, 2021.** The results were obtained by authors concluded that the partial replacement of NaCl by other salts especially kcl in different meat products did not affect the growth of spoilage or pathogenic microorganism. Therefore, the correct salt reduction does not result in a major change in the composition of the microbial community. In addition, **Harper & Getty, 2012** stated that Potassium chloride had a similar effect against different spoilage and pathogenic species when used as salt replacer at certain conditions.

Ultrasound can be used for food preservation in combination with other treatments by improving its inactivation efficacy. There have been many studies combining ultrasound with either pressure, temperature, or pressure and temperature **(Ercan and Soysal 2013)**

The bactericidal effect of ultrasound is attributed to intracellular cavitation and demage of cell walls due to the mechanical effect of cavitation. Ultrasound increases the sensitivity of the cell against heat. Structure of proteins and enzymes changes and becomes more suspective for denaturation **(Sala et al., 1995).** There are some studies on cell destruction effect of ultrasound. Heat, pH, chlorination, presurising are the effective combinations for ultrasound that increase the lethality of the tested microorganisms. Heat and sonication togeather is known to increase the lethal effect on microorganisms but surprisingly, the lethal effect of the triple treatment was reported to be tenfold when compared to non-pressure thermosonication **(Kentish and Feng, 2014)**

the application of high intensity acoustic energy increased the overall salt concentration. Also, meat brining assisted by ultrasound enhances the mass transfer and salt diffusion into beef. It was observed that the content of salt content and water post-treatment were greater in the sonicated sample than the non-sonicated one. This process also produces a product with uniform salt content **(Sánchez et al., 2000 and Contreras-Lopez et al., 2020).**

in addition, our results finding that application of ultrasound sonication with salting has slightly significant effect of bacterial count that may be due to the research fact demonstrated by **Kang et al., 2016** who demonstrated that Ultrasound contributes to the antimicrobial effect as well as increases the shelf life of meat products due to the cavitation in liquid media. The antimicrobial effect is also dependent on the time of contact with microorganisms, the type of microorganisms, the quantity of food and its composition, and the temperature of treatment.

4.3 Chemical examination

The results in the table (2) showed that, the protein % of the control group was close to 15.75 %, while the other groups incorporated with kcl with ultrasound 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. This indicated that application of ultrasound and replacement of sodium chloride salt with potassium chloride had no negative influence on protein content. This results were nearly similar to results demonstrated by **Lorenzo et al., 2015** and **Da Silva- Araujo et al., 2021** who concluded that replacement of sodium chloride with potassium chloride did not influence on chemical properties of meat products.

Thiobarbituric acid and peroxide values are used as indicators of the degree of lipid oxidation. The results in the table (3,4) showed, the TBA and peroxide value of the control group started from (0.48 ± 0.02 mg malonaldehyde/kg and 0.10 ± 0.005 mg equivalent oxygen/kg) at the beginning of storage period (zero day) to (1.05 ± 0.02 and 0.38±0.02) at the end of storage periods. In addition, the TBA and peroxide value of the sausage with kcl 50%, 80% and 100% with ultrasound sonication started from 0.49 ± 0.01, 0.48± 0.01, 0.49 ± 0.01 and 0.11± 0.01, 0.10± 0.005, 0.09 ± 0.02 at the beginning of storage period (zero day), respectively to 1.04 ± 0.04, 1.04 ± 0.04, 1.03 ± 0.03 and 0.43 ± 0.02, 0.41 ±0.02, 0.42 ± 0.02 at the end of storage periods, respectively. The results of control group were in accordance with the Egyptian frozen sausage standard 1972/2005 until 9th week of storage while the results of other groups were in accordance with ES until 10th week of manufacture. Where, the TBA level was 0.9 mg/ Kg according to its basic requirements. These results were nearly similar to results concluded by **Lorenzo et al., 2015** and **Da Silva- Araujo et al., 2021** who concluded that replacement of sodium chloride with potassium chloride did not influence on lipid oxidation of meat products.

Total volatile basic nitrogen (TVB-N) is often used as a biomarker of protein and amine degradation and used as interpret meat freshness**.** The results in the table (5) showed that, the TVB-N of the control group started from 11.2 ± 0.0 mg/100g at the beginning of storage period (zero day) to 21 ± 1.4 at the 12th week of storage periods. Moreover, the TVB- N of the sausage with kcl 50%, 80% and 100% with ultrasound sonication started from 11.9 ±0.28, 11.9 ± 0.0, 12.04 ± 0.0 mg/100g at the beginning of storage period (zero day) to 21 ± 0.0, 21 ± 0.0, 19.6 ± 0.7mg/100g at the 13th day of storage periods. The results of control group were in accordance with the Egyptian frozen sausage standard 1972/2005 until 11th week of storage while the results of the sausage with kcl 50%, 80% and 100% with ultrasound sonication were in accordance with ES until 13th week of manufacture. Where, the TVB-N % was 20 mg/ 100g according to its basic requirements. These results were nearly similar to results concluded **Lorenzo et al., 2015** and **Da Silva-Araujo et al., 2021** who concluded that replacement of sodium chloride with potassium chloride did not influence on chemical properties of meat products.

Finally, in contrast to our findings on the protein content, total volatile basic nitrogen, lipid oxidation tests, the partial replacement of NaCl by KCl or ultrasound did not influence the proximate composition of frozen sausage.

Regarding to results demonstrated that partial replacement of sodium chloride with potassium chloride up to 80% did not effect on sensory, chemical and microbiological properties of frozen sausage. In addition Ultrasonic processing is still in its infancy and requires a great deal of future research in order to develop the technology on an industrial scale, and to more fully elucidate the effect of ultrasound on the properties of foods.

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