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***THE EFFECT OF ADDITION OF CHIA SEEDS ON STORAGE PERIOD OF PROCESSED  
MEAT AND ITS EFFECT ON THE FINAL PRODUCT QUALITY***

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**THE EFFECT OF ADDITION OF CHIA SEEDS ON STORAGE PERIOD OF PROCESSED MEAT AND ITS EFFECT ON THE FINAL PRODUCT QUALITY**

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**Abstract:**

This study aimed to verify the effect of chia seed (*Salvia hispanica*) at concentrations of 1%, 3% and 5% as an antimicrobial in sausage frankfurter and to evaluate pH, lipid stability, and microbiological stability and sensory attributes during refrigerated storage, measurement of lipid stability by TBARs values; microbiological; and sensory analysis. The results regarding the pH of the sausage increased over time in storage. After 14 days of storage, TBARs values were 1.62 mg MDA/ kg for the treatment with 3% chia extract and 2.87 mg MDA/kg for the control treatment. Microbiological analyzes on day 14 recorded a noticeable decrease in all samples prepared with chia seeds, especially at a concentration of 5%. While samples on day 21 showed an increase in their numbers at the end of storage. The sensory evaluation characteristics were maintained. The extract of chia seed (*Salvia hispanica*) at a concentration of 3% was shown to inhibit lipid oxidation of sausages frankfurter and microbial inhibition of Enterobacteriaceae bacteria during the storage period.

**Keywords:**

Chia seeds, smoked meats, storage condition and Antioxidant.

**Introduction**

Sausages are one of the most popular foods among all people with different ages. Frankfurter is a smoked sausage, the fat particles are distributed inside it, and manufactured Frankfurter is found in local stores. In addition, frankfurters-type sausages are the most widespread type of emulsified meat product in the world (Safaa et al., 2019; Ashura Katunzi-

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**Kilewela et al., 2021**), mainly differentiated by the seasonings used and to regional preferences (**Jandásek, 2014**). On the other hand, the consumption of this type of product is related to negative health concerns, even if it is manufactured according to international or local specifications (**Zettel and Hitzmann, 2013; Grasso et al., 2014**). The reason is because its technical components are rich in fats (**Novakovic et al., 2019**). Hence, the contamination in meat products such as Sausages, especially by foodborne bacteria, are a major concern for meat producers, high fat content it results in a decrease in quality (flavor and texture) during storage (**Campos et al., 2016 ;Alirezalu et al., 2019**). Hence researchers endeavor to reduce contamination in sausages using natural antimicrobials (**Fernandes et al., 2018; Das, 2018**). The antimicrobial effect of chia seeds in combination with natural antioxidants was studied according to **Falowo et al., (2014); Oliveira-Alves et al., (2017)** in Frankfurter sausages on the quality characteristics and stability of frankfurter sausages during storage. In this context, chia offers considerable potential for the development of healthier foods, and with it has been widely used as an ingredient in foods such as bread and cakes (**Fernandes and Salas-Mellado, 2017; Muhammad Abdullah Bin Masood, 2022**), cookies, snacks, ready-to-eat meals, beverages, etc (**Coelho et al., 2015; Ding et al., 2018**). Chia (*Salvia Hispanica L.*) is an annual herbaceous plant belonging to the Lamiaceae or Labiatae family. Chia seed is composed of protein (15–25%) according to **Grancieri et al., (2019)**, fats and omega-3 (30–33%) (**Villanueva-Bermejo et al., 2019**), carbohydrates (26–41%), dietary fiber (18– 30%), and ash (4–5%), it also contains a high amount of vitamins, minerals, dry matter (90–93%) and antioxidants (**Engy F. Zaki., 2018; Barros et al., 2018; Bartosz Kulczy nski et al., 2019**). This research was aimed to Effect of addition of chia seeds on storage period of processed meat and effect on the final product quality.

## 2. Material and Methods

### 2.1. Preparing frankfurters



**Fig.4.** manufactured Sausages



**Fig.3.** processed sausages



**Fig.2.** Sausage filling



**Fig.1.**Chia seeds

The frankfurters are made according to a traditional formula (**Bover et al., 2000**) (Only meat percentages up to 100%) but other ingredient percentages are meat related): beef (70%) and lamb fat (30%), 15% water (ice form w/w), 3% potato starch (w/w), 2% sodium Chloride (w/w), 300 mg/kg, casein 1.5%, 0.2 ml/kg smoke liquid sauce and spices (mixture white pepper, mace, and coriander) and Chia seeds were previously grinded, chia seeds, Chia seeds were previously provided from Those samples were purchased from Egyptian Bio Aloe Vera & Organic. Casings to fill your sausage you need a natural sheep casing caliber 20/22. Batches of 2kg of each formula were handily mixed and formed by using a filling attachment for your meat grinder. This original mixture was divided in four batches (500 g approx.). Batch 1 was used as control, while chia products were added to the other three batches: batch 2 contained 1% chia seeds; batch 3 contained 3% of chia seeds; and batch 4 had 5% chia seeds. The products were prepared in Fayoum University laboratories according to ((**Bover et al., 2000**)). After homogenization, the resulting meat batter was stuffed using a piston stuffer into natural sheep casing caliber 20/22. Samples: were hand linked and cooked in a water bath (90 °C) and monitor product temperature, the sausages were immediately chilled in ice for 5 min. in plastic bags (high barrier film of water vapour permeability). All samples were stored immediately after packing at (4°C ± 1°C) under darkness conditions. For frankfurter characterization (proximate composition, texture, and sensorial). Further analyses (pH, colour, lipid oxidation, residual nitrite level, and

microbiological analysis) were carried out on days 0, 7, 14 and 21 to monitor the effect of storage on quality characteristics.

## **2.2. Technological properties**

### **2.2.1. P<sup>H</sup>**

**P<sup>H</sup>** Determination of frankfurter Cubes, frankfurter cubes were weighed approximately 2-2.5 g and mixed with distilled water (1:10 w/v) and homogenized at 12000 rpm for 2 min. The **P<sup>H</sup>** of samples was measured in duplicate by a **P<sup>H</sup>** meter (Hanna Instruments, Portugal).

### **2.2.2 Determination of Oxidative Stability of frankfurter**

For the determination of oxidative stability of refrigerated stored frankfurter at days 0, 7, 14, and 21 thiobarbituric acid-reactive substances (TBARs) assay was performed with a slightly modified method of **Bekhit et al., (2003)**. For analysis, 2.5 g sample was placed into a beaker containing 25 mL of 0.38 % TBA and 15 % TCA prepared in 0.25 N HCl solution. The sample was homogenized at 10000 rpm for 3 min and three 5 mL aliquots obtained from homogenate was heated for 10 min in boiling water bath to develop a pink color and then cooled in tap water. The boiled samples were then clarified by centrifugation at 5000 rpm for 10 min and their absorbance was measured at 532 nm by using a Shimadzu (Model 2450, Japan) spectrophotometer. Average of three absorbance values was used to determine the oxidative stability of stored samples.

## **2.3. Microbiological analysis:**

Samples of chia different concentration were prepared to be used for microorganisms tests. Microbiological analysis of frankfurters was carried out by duplicate as follows: for each sample, 10 g was taken and placed in flask with 90 ml of Distilled water. Appropriate decimal dilutions were pour plated on the following media: plate count agar for the total viable count (TVC) (37 °C for 48 h); for lactic acid bacteria (LAB) (37 °C for 48 h); and Enterobacteriaceae (37 °C for 24 h). Results are expressed as logarithms of colony forming units per gram (log cfu/g) were determined according to **Ercolini et al., (2009)**.

## 2.4. Sensory evaluation

A sensory panel consisting of 20 individuals (between males and females) aged between (18-55 years) and without specific training in sensory analysis. Frankfurter (smoked sausage) is among the employees and students at Fayoum University. Sensory analysis protocols were pre-approved Scientific Ethics Committee at Fayoum University.

## 2.5. Statistical analysis

One way analysis of variance (ANOVA) was performed to evaluate the statistical significance ( $P \leq 0.05$ ) of the effect of sample formulation (product characterization) and two-way ANOVA as a function of formulation and storage time (product shelf-life) using the SPSS program v. 27 for Windows

## 3. Results and Discussion

### 3.1- Technological properties:

#### 3.1.1- $P^H$ value

**Table 1: Evolution of  $P^H$ , respect to with different levels of Chia seeds, during storage of the frankfurters at 4°C for 21 days.**

Samples	Storage periods (days)			
	0	7	14	21
	$P^H$			
<b>Control</b>	5.48±0.39 <sup>Ab</sup>	5.39±0.04 <sup>Aa</sup>	5.37±0.04 <sup>Ab</sup>	5.31±0.13 <sup>Ab</sup>
<b>1% Chia seeds</b>	5.45±0.22 <sup>Aa</sup>	5.34±0.12 <sup>Ab</sup>	5.20±0.04 <sup>Aa</sup>	5.06 ± 0.03 <sup>Ab</sup>
<b>3% Chia seeds</b>	5.50±0.01 <sup>Aa</sup>	5.36±0.03 <sup>Aa</sup>	5.22±0.08 <sup>Aa</sup>	5.22±0.08 <sup>Aa</sup>
<b>5% Chia seeds</b>	5.47±0.05 <sup>Ab</sup>	5.33±0.02 <sup>Aa</sup>	5.29±0.03 <sup>Ab</sup>	5.29±0.15 <sup>Ab</sup>

Each value represents the mean  $\pm$ SD (n-3). Means within the same row with different superscripts letters are significantly different ( $p \leq 0.05$ ).

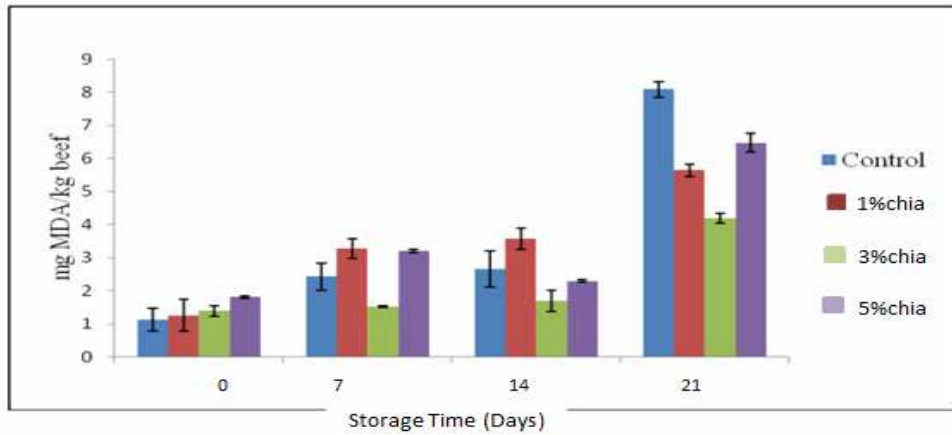
Data of  $P^H$  value of smoked sausage (frankfurter) formulated with different levels of chia seeds during storage are shown in Table 1. No significant differences were found in  $P^H$  value between control and samples at zero time. In other words, the addition of chia (in any of its presentation forms) did not have any positive or negative effect on  $P^H$  changes for

frankfurters at day 0. This study agreed with (Fernández-Ginés, FernándezLópez, Sayas-Barbera, Sendra, & Pérez-Álvarez, 2004), the  $P^H$  value of all samples slightly decreased during the first 7 days of storage. But at 14 and 21 days differences were noted between the  $P^H$  of the control frankfurters and those with added with different concentrations chia seeds ( $P < .05$ ). The decreasing trend in  $P^H$  could be due to carbohydrates Contained in frankfurter formulations during preparation may promote this growth. Similar data have been reported by many from authors on some of the processed meat products. (Pintado, et al., 2016, Engy F 2018; Fernández-López et al., 2018). ), while the results differed (Kerem et al., 2010) as they showed that the 7<sup>th</sup> day pH values increased until 21 day except for the sample treated with 3%.

### 3.1.2- Effect of chia seeds on Oxidative Stability of frankfurter:

Lipid oxidation is one of the most undesirable change that affect the quality of foodstuff during storage e due to deterioration of polyunsaturated fatty acids (PUFA) (de Falco et al., 2017). The oxidative stability of frankfurter was examined by conducting, thiobarbituric acid-reactive substances. (TBARs) assay. Absorbances obtained from these assays at 532 nm, were then used to calculate amount of TBARs. Results are given in Figure (5) as mg malonaldehyde equivalent/kg beef. The initial TBARs value of fresh frankfurter were determined as  $0.91 \pm 0.063$  mg MDA/kg beef. Level of lipid oxidation was increased in all control and treated samples during refrigerated storage. Although treatment with 5% chia seeds reduced the lipid oxidation of frankfurter by 0.7, the best result against lipid oxidation was obtained from frankfurter treated with 3% chia seeds after 14 days of cold storage; 2% chia seeds kept TBARs value of beefs. This result may be attributed to the amount of hydroxyl groups within the phenolic structures of constituents present in crude chia seeds mainly. It is assumed that inhibition of lipid oxidation and hydrogen donor ability is enhanced with the increasing amount of hydroxyl groups (Ö Yüncü et al., 2021).





(Fig.5)

### 3.1.3- Lipid oxidation

TBARs values in all the samples were below the level of incipient rancidity ( $\geq 1.0$ ) throughout the storage period are presented in Table 2. While there were no differences between all samples At day 0, frankfurters containing chia seeds 5% showed the best TBARs value ( $P < .05$ ) Long storage period compared with the other samples. Taking into account that chia products contain highly unsaturated fatty acids, which are very susceptible to lipid oxidation, a higher oxidation rate in the frankfurters containing chia products was to be expected. This pattern of lipid oxidation could be related to the presence of antioxidant compounds in chia products (Park et al., 2017; Fernández-López et al., 2018; Kulczyński et al., 2019; Maša Knez Hrnčič et al., 2020).

**Table (2) Evolution of lipid oxidation (TBAR<sub>s</sub> values, expressed as mg MA/kg) and residual nitrite level (mg/kg) during storage of the frankfurters**

Treatments	Storage periods (days)			
	0	7	14	21
	Lipid oxidation			
Control	0.26± 0.02 <sup>bv</sup>	0.43 ± 0.02 <sup>aw</sup>	0.58 ± 0.02 <sup>ax</sup>	0.64 ± 0.03 <sup>ay</sup>
1% Chia seeds	0.28± 0.01 <sup>bv</sup>	0.35 ± 0.01 <sup>cw</sup>	0.41± 0.02 <sup>bx</sup>	0.42± 0.02 <sup>bx</sup>
3% Chia seeds	0.39 ± 0.02 <sup>av</sup>	0.41 ± 0.03 <sup>bv</sup>	0.41± 0.02 <sup>bw</sup>	0.41± 0.02 <sup>bw</sup>
5% Chia seeds	0.29 ± 0.03 <sup>bv</sup>	0.31 ± 0.01 <sup>dw</sup>	0.35 ± 0.02 <sup>cx</sup>	0.42± 0.03 <sup>by</sup>

Each value represents the mean ±SD (n-3). Means within the same row with different superscripts letters are significantly different (p≤0.05).

**3.2.1- Microbiological analyses during storage:**

**Table.3 *Enterobacteriaceas* bacteria and Lactic acid bacteria count of smoked sausage (frankfurter) formulated with different levels of chia seeds during storage at 4°C for 21 days:**

Treatments	Storage periods (days)			
	0	7	14	21
	Enterobacteriaceas bacteria (Log CFU/g)			
Control	2.41 ± 0.02 <sup>av</sup>	2.8 ± 0.04 <sup>aw</sup>	3.55 ± 0.03 <sup>az</sup>	4.41 ± 0.04 <sup>ay</sup>
1% Chia seeds	2.38 ± 0.06 <sup>av</sup>	2.89 ± 0.06 <sup>aw</sup>	3.60 ± 0.04 <sup>ax</sup>	4.49 ± 0.06 <sup>av</sup>
3% Chia seeds	2.50 ± 0.05 <sup>av</sup>	2.99 ± 0.09 <sup>aw</sup>	3.87 ± 0.09 <sup>bx</sup>	4.46 ± 0.03 <sup>ay</sup>
5% Chia seeds	2.59 ± 0.02 <sup>av</sup>	3. 1 ± 0.05 <sup>aw</sup>	4. 01± 0.05 <sup>bx</sup>	4. 5 ± 0.05 <sup>ay</sup>

Each value represents the mean ±SD (n-3). Means within the same row with different superscripts letters are significantly different (p≤0.05).

Different levels of chia seeds were shown in Table 3. The microbiological stability of processed meat products depends on internal factors, such as its composition, and external factors, especially the packaging and storage temperature, different levels of chia seeds were shown in Table 3. No significant differences were found in total bacterial

count of fresh samples (control and products formulated with different concentration chia seeds). Total bacteria count for all samples decreased slightly on 7 days of cold storage, while on day 14 was a noticeable decrease in all samples processed by chia seeds specially 5% concentration. While samples on day 21 showed increase numbers at the end of storage. The end of the experiment was below 5.0 log cfu/g, which is the level that was taken into account insufficient to enhance the quality property of the product. *Enterobacteriaceae* were detected in the frankfurter only on the 21th day Storage. Bacterial count values for frankfurter samples were slightly higher than reported by **Engy F. (2018)** but are comparable for those **Viuda Martos et al., 2010; Silva et al., 2015; Ángel et al., 2017; Ranucci et al., 2018; Rabail et al., 2021).**

### 3.3- Sensory evaluation

As regards the sensory analysis of the frankfurters, samples generally scored significantly Acceptance for all the sensory parameters considered except texture, for which scores were similar ( $P > .05$ ) in all the samples. Only texture was not modified by the addition of any chia product, all the samples obtaining similar scores to the control. For the rest of the attributes evaluated (colour, flavour, taste and general acceptability).

**Table (4): Organoleptic evaluation of laboratory frankfurter treated with different concentrations of chia seeds.**

Properties	Score	Samples		
		A	B	C
appearance	10	5.42±1.05 <sup>a</sup>	4.76±1.01 <sup>a</sup>	5.15±1.28 <sup>a</sup>
Color	10	5.27±1.17 <sup>a</sup>	5.32±1.27 <sup>a</sup>	5.25±1.13 <sup>a</sup>
Taste	10	5.42±1.05 <sup>ab</sup>	5.82±0.82 <sup>a</sup>	5.10±1.31 <sup>a</sup>
Odor	10	5.23±1.13 <sup>a</sup>	5.39±1.07 <sup>a</sup>	5.05±1.22 <sup>a</sup>
Softness	10	5.39±1.04 <sup>a</sup>	5.45±1.05 <sup>a</sup>	5.42±1.07 <sup>a</sup>
Overall acceptable	50	3.71±0.76 <sup>a</sup>	3.92±1.09 <sup>a</sup>	3.71±0.74 <sup>a</sup>

Each value represents the mean ±SD (n-3). Means within the same row with different superscripts letters are significantly different ( $p \leq 0.05$ ).

## Conclusion:

The chemical composition of chia seeds with added different concentrations Helped raise Product Quality. The mean values of pH in the different analyzed treatments increased 14 day during the period of storage. TBARs levels tended to increase over time in storage. The extract of chia seeds (*Salvia hispanica*) was efficient regarding the oxidative stability of the frankfurter sausage, during 14 days of storage, when all treatments with added chia extract had lower oxidation than the control treatment; the treatment with 1% added chia extract had the lowest lipid oxidation for all the treatments In addition to proven non-resistance to *Enterobacteriaceae* bacteria. In the sensory analysis, the mean values of the scores of the analyzed attributes were “moderately liked.” sausages frankfurter containing 3% chia seed showed the best results compared to the other concentrations with regard to lipid oxidation and microbial inhibition.

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## تأثير إضافة بذور الشيا على مدة التخزين للحوم المصنعة وأثرها على جودة المنتج النهائي المخلص العربي:

هدفت هذه الدراسة إلى التحقق من تأثير بذور الشيا (*Salvia hispanica*) بتراكيزها ١% و ٣% و ٥% كمضاد للميكروبات في نقائق فرانكفورتر ولتقييم درجة الحموضة واستقرار الدهون والاستقرار الميكروبيولوجي والصفات الحسية خلال تخزين مبرد. قياس استقرار الدهون بقيم TBARS؛ الميكروبيولوجية والتحليل الحسي. النتائج المتعلقة بالرقم الهيدروجيني للسجق زيادة مع مرور الوقت في التخزين. بعد ١٤ يوماً من التخزين، كانت قيم TBARS 1.62 ملجم /MDA كغم للمعاملة بمستخلص الشيا ٣% و ٢.٨٧ ملجم /MDA كغم للمعاملة المقارنة. وسجلت التحاليل الميكروبيولوجية في اليوم ١٤ انخفاضا ملحوظا في جميع العينات المحضرة ببذور الشيا وخاصة عند التركيز ٥%. بينما أظهرت العينات في اليوم ٢١ زيادة في أعدادها عند نهاية التخزين. التقييم الحسي تم الحفاظ على الخصائص. خلاصة بذور الشيا (*Salvia hispanica*) بتركيز تبين أن ٣% يثبط أكسدة الدهون في نقائق فرانكفورتر ويمنع البكتيريا Enterobacteriaceae خلال فترة التخزين.

الكلمات المفتاحية: بذور الشيا، اللحوم المدخنة، ظروف التخزين و مضادات الأكسدة.