Effect of Incorporation of Chia Seeds Flour in Chicken Sausage on TBA Values and Microbial Quality during Cooling Storage

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Abstract: In this study the effect of incorporation of chia seeds flour (CSF) by 2, 4 and 6% on storage stability of chicken sausage stored at 4±1°C for 21 days was investigated. Four formulations were developed using whole chicken meat as follows: Control (C), F1 (2% CSF), F2 (4% CSF) and F3 (6% CSF). The control and treated samples were filled in foam dishes and packed in polyethylene bags and stored immediately in a refrigerator (4±1°C) for 21 days, then were analyzed for TBA values and microbial quality. Chia seeds were analyzed for its total phenolic compounds (TPC) content which recorded 4.58 mg GAE/g CSF and antioxidant activity using DPPH radical assay presented 79.17% inhibition. TBA values were significantly increased ($p<0.05$) during storage period. At the end of storage, the TBA values recorded 1.853 mg MDA/Kg in F1, while it was 1.209 mg MDA/Kg in F3. Microbial measurements showed that, along storage period microbial counts significantly increased in control as well as treatments, but were within the standard limits. Control sample didn't reach the 14th day of storage and get spoiled. It was observed that, by increasing the percent of added CSF, decreasing in the microbial count statistically occurred; this indicates that chia seeds flour may have an antimicrobial effect.

***Keywords*:** Chicken sausage, incorporation, TBA values, microbial quality

INTRODUCTION

Sausage is a ground or minced meat integrated with different spices, salt and other ingredients, then glutted into a casing of specific shape and size. By changing meat source, the preparation method, spices and different ingredients, wide varieties of sausage can be produced. Chia known as *Salvia hispanica* L. is a 2 m tall herbaceous plant that belongs to the Lamiaceae family, fundamentally cultivated in southern Mexico and northern Guatemala (Jamboonsri *et al*., 2012). It considered as a functional food and worth to call: the miracle seed whereas rich in fatty acids especially polyunsaturated FA, dietary fiber and antioxidants (Capitani *et al.,* 2012). It could be used as flour, gel and whole seed. Ixtaina *et al.* (2008) mentioned that chia seeds contain about 25-38% oil which have the highest known percentage of alpha-linolenic fatty acid (18:3n-3), approximately 60% which considered healthier polyunsaturated fatty acid (PUFA). Parejo *et al.* (2002) and Hou *et al.* (2003) mentioned that, free radicals which form due to oxidation are the cause of neurological diseases, ageing, immunodeficiency, inflammations, heart diseases, strokes, Parkinson's diseases, cancers and Alzheimer's. Jin *et al.* (2012) and Ciftci *et al.* (2012) detected some substances in chia seeds such as polyphenolic compounds, for example, gallic, protocatechuic acid and p-coumaric acids, chlorogenic, caffeic acid, tocopherols and sterols.

Reyes-Caudillo *et al.* (2008) postulated that the defatted chia seed meal is a good source of phenolic compounds with antioxidant capacity. Yingbin *et al.* (2018) investigated the chemical characterization of chia seed oil, they found that the content of α-linolenic acid (63.64% of total fatty acids) was the highest, followed by linoleic acid (19.84%), and saturated fatty acid (less than 11%). Moreover, they postulated that the antioxidant capacity of chia seed oil estimated with ABTS and DPPH methods, presented modest antioxidant capacity when compared with Catechin and Tocopherol. Furthermore, Martinez-Cruz and Paredes-Lopez (2014) declared that chia seeds contains significant quantities of phytonutrients, includes tocopherols, phytosterols, and phenolic compounds. Other important research findings by Gazem *et al.* (2017) who showed that the chia seeds oil significantly inhibited the proliferation of human lymphoblastic leukemic cell lines, HeLa, and Michigan cancer foundation (MCF-7) cells.

The purpose of this research was to examine the capability of chia seeds flour as antioxidant and antimicrobial agent, therefore promoting food shelf life.

MATERIALS AND METHODS

Materials:

Chicken meat was obtained from local market in Assiut city during April 2018. Chia seeds were obtained from local market in Cairo during April 2018. Mixed spices, were prepared using (clove, black pepper, Chinese cubeb, Word press button, cinnamon, lura, ginger, seasonings and nutmeg, dried garlic), also salt were obtained from the local market during April 2018.

Sheep intestine*:* was obtained from a butcher shop in Heliopolis, Cairo city, (was cleaned with 1% acetic acid and preserved by large salt crystals).

Chemicals: all chemicals used in this investigation were obtained from EL-Gomhouria for Trading Chemicals and Drugs Co., Assiut city, Egypt.

DPPH, TBA and Gallic acid obtained from sigma-Aldrich Chemie Gmbh Munich, Germany.

Methods:

Preparation of ingredients:

Chicken meat was washed carefully, and then minced with meat mincer (HR2727 Philips, China), using disc 5mm. Chia seeds were finally grounded, then

every formula proportion of the seeds was weighed after that allowed to swell in 100 ml tap water.

Preparation of chicken sausage:

For the preparation of the chicken sausages, the procedures described by Al-Bachir and Othman (2013) with some modifications were taken into consideration (Table 1). The sausage formulae were formed by stuffing all batter into natural sheep casing mechanically. After preparation, the chicken sausage samples were filled in foam dishes and packed in polyethylene bags and were stored immediately in a refrigerator (4±1°C) until analysis.

Table (1): Composition of Chicken sausage formulae (% Total formula)

Ingredients %	Control	F1	F2	F3
Minced chicken meat	95.6	93.6	91.6	89.6
Chia seeds flour	-	2	4	6
Non meat ingredients (All spices and salt)	4.4	4.4	4.4	4.4

Chemical analysis:

Thiobarbituric acid: (TBA) values

The assessment of lipid oxidation of the sausages was conducted by the Thiobarbituric acid (TBA) test, according to (Lemon, 1975). TBA values were determined in triplicates for each sample after 1, 7, 14 and 21 days of storage at 4±1°C. Twenty grams of each chicken sausage sample were homogenized with 40 ml of trichloroacetic acid (7.5%) for 1 minute and left for 30 minutes. Filtration was carried out using whatman No. 1 filter paper. Five mls of the filtrate were mixed, with 5 ml of TBA solution (0.2883 g TBA/100 ml water) in a test tube. Blank was executed using 5 ml distilled water and 5 ml TBA solution. In boiling water bath, tubes were covered and heated for 40 min, then cooling quickly in ice bath, absorbance at 538 nm was assessed using ultraviolet visible scanner Spectrophotometer (LKB 4054 Cambridge, England). The TBA values were calculated by multiplying the absorbance by the factor of 7.8 and the result was represented as mg of malonaldehyde per 1000g sample.

Determination of total phenolic compounds content:

The Folin Ciocalteu method was used to determine the total phenolic compounds content in the methanol: water (80:20 v/v) extracts. This method was based on Cicco *et al.* (2009) with a few minor modifications. A standard curve was developed using gallic acid, which was linear between 10-100. To each of the standard samples, the appropriately diluted extract samples and a blank (methanol: water, 80:20 v/v) (all 300 µl), 300 µl of Folin Ciocalteu's reagent was added and left to equilibrate for 2 min. Then, 2.4 ml of 5% (w/v) sodium carbonate solution was added to each preparation and left to react in the dark at room temperature for 1hour. Absorbance was then read on a Carry 50 Spectrophotometer at a wavelength of 760 nm. The values were determined using a gallic acid standard curve (prepared each day). Results were expressed as mg Gallic acid equivalents (GAE) sample g^{-1}.

Determination of antioxidant activity using the DPPH method:

The antioxidant activities of the methanol: water (80:20 v/v) extracts were determined using the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical based on the procedure described by Thaipong *et al.* (2006). A standard curve using Trolox was prepared, which was linear between 20-200 µg ml^{-1}. sample extracts (150 µl)and standards at the appropriate dilutions were allowed to react with 0.1M DPPH (2.85 ml) for 24 h in the dark. The absorbance was then read at 515 nm on a Carry 50 spectrophotometer. Values were determined using the Trolox standard curve (prepared each day) and the results were expressed as mg Trolox Equivalents (TE) sample g^{-1} dry weight.

Microbiological examinations:

The microbiological examinations of the prepared chicken sausage samples includes the determination of total aerobic bacterial counts, yeasts and molds.

Sample preparation:

Ten grams of representative chicken sausage products sample were mixed with 90 ml of sterile saline solution (9 g NaCl/1L distilled water) in a blender, under sterile conditions, to give 1/10 dilution. Serial dilution was prepared to be used for counting total aerobic bacteria, yeasts and moulds (Osheba, 1998).

Microbiological methods:

Total aerobic plate counts:

The total aerobic bacterial count was determined using nutrient agar medium according to the procedure as described by APHA (1976) and Osheba (1998). The plates were incubated at 30ºC for 3 days as recommended by APHA (1976) and Difco Manual (1998).

Yeast and mould counts:

Yeasts and moulds were determined using Bacto yeast malt (Y.M) agar medium according to the method described by Difco Manual (1998). The plates were incubated at 30±2ºC for 5-7 days.

Statistical analyses:

Statistical analysis for data obtained from three replicates were analyzed by ANOVA using the SPSS 20.0 software statistical package program, and differences among the means were compared using the Duncan's Multiple Range test (SPSS, 2011). A significance level of 0.05 was chosen and continuous variables described by mean and standard deviation.

RESULTS AND DISCUSSION

Thiobarbituric acid values

The Thiobarbituric acid values (TBA) in the control and the three treatments were expressed as mg Malonaldehyde /kg of sample and the results are shown in Table (2).

Lima *et al*. (2013) mentioned that malonaldehyde (MDA) is a relatively stable secondary product of the oxidative degradation of polyunsaturated fatty acids (PUFAs). It can occur in diverse forms counting on the pH value. They postulated that hydroperoxyl, cyclic peroxides, and biclyclic endoperoxides are some of its main remnants. Moreover, Min and Ahn (2005) indicated that MDA has an importance for scientific research and industry which it can be used for determining lipid peroxidation through the TBARS test (Thiobarbituric acid reactive substances), the most common used examination to estimate the effects of meat products.

Data in Table (2) revealed that TBA values were significantly increased ($p<0.05$) among all samples including control over the course of storage (along storage period). The increase in TBA values means that a lipid oxidation occurred. Results agreed with Al-Bachir *et al.* (2010) they investigated the effect of storage on the chicken sausages at 1-4°C, they found that the longer storage period lasts the more lipid oxidation occurs. They explained the increasing in TBA that may be due to using minced chicken meat which speeds up the oxidation of myoglobin, besides adding salt and spices that affect the chicken sausage while preparing. Also, Badr (2007) mentioned that, cooking and salt addition increased the TBARS values after preparation and during storage of raw and cooked meat products significantly ($p<0.05$). Referring to the data in (Table 2), it can be noticed that TBA values increased significantly in control sample during the period of zero time and 7 days of storage at 4±1°C, then the sample getting spoiled after that period. TBA values increased significantly in F1 (2% chia seeds flour) along storage period.

Table (2): Changes in Thiobarbituric acid values (mg malonaldehyde/kg sample) of chicken sausage formulated with chia seeds flour during storage at 4±1°C up to 21 days:

Time of storage \ Sample	Control	F1	F2	F3
0	0.763^{aB}	0.564^{aD}	0.555^{aB}	0.415^{aB}
7 days	1.248^{aA}	0.913^{bC}	0.792^{cB}	0.661^{dB}
14 days	-	1.535^{aB}	1.434^{bA}	1.093^{cA}
21 days	-	1.853^{aA}	1.581^{bA}	1.209^{cA}

Different the capital letters in the same columns means significantly difference ($p<0.05$) between storage periods
Different the small letters in the same row means significantly difference ($p<0.05$) between storage treatments

On the other hand there were no significant increase in TBA values during the period starting from zero time until 7 days of storage in both F2 (4% chia seeds flour) and F3 (6% chia seeds flour). And more than that, there were no significant increase in TBA values during period from 14 day to 21 days of storage which reflects the affect of increasing the proportion of chia seeds. By comparing the different treatments with control, it's clear that, by increasing the chia seeds % there were significant decrease in TBA values at the same time of storage and along storage period. All treatments formulated with chia seeds flour in different concentrations succeeded to decrease the oxidation which means elongation the product life time. Regarding to TBA values in the three studied treatments up to 21 days of storage, it could be noticed that the values decreased from 1.853 MDA/Kg in F1 (which considered unsafe to human health) to 1.209 MDA/Kg in F3 which doesn't represent a danger to human health according to Torres and Okani (1997) who reported that TBA values up to 1.59 MDA/Kg couldn't be sensed by sensory analysis and considered safe and not a threat to human health.

Total phenolic compounds content and DPPH radical scavenging activity:

Chia seeds (*Salvia hispanica*) were analyzed for total phenolic compounds and antioxidant activity. Results in Table (3) declared the total phenolic content (TPC) and antioxidant activity measured by DPPH in chia seeds flour. TPC content were 4.58 mg GAE/g chia seed flour which were higher than those reported by Tunçil and Çelik (2019) who found that the total phenolic contents of white and black chia seeds were 3.52 and 3.42 mg GAE/g defatted chia seeds, respectively, also our result were higher than those announced by Martinez-Cruz and Paredes-Lopez (2014) who postulated that, the TPC content of Chilean chia seeds was 0.94 mg GAE/g sample, and Mexican chia seeds was 1.64 mg GAE/g sample, respectively. Oliveira-Alves *et al.* (2017) postulated that, TPC of chia seeds and fiber flour extracts were corresponding, but was very low in the oil. They reported that TPC content in chia seeds, chia fiber flour and chia oil were 1.16, 1.11 and 0.02 mg GAE/g respectively. However, Ayerza (1995); Ayerza and Coates (2004); Ayerza (2010) and Ayerza (2013) attributed the differences in chia seeds

TPC between different studies due to kind of sample, growing location which affects the composition of chia seeds significantly. On other hand, Scapin *et al.* (2016) mentioned other reason influences the TPC, the extraction methods of phenolic compounds vary among different studies, which significantly influence the total phenolic contents of chia seeds examined.

Regarding to data in (Table 3) the antioxidant activity of the total phenolic compounds using DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical assay presented 79.17% inhibition which was higher than that reported by Martinez-Cruz and Paredes-Lopez (2014) who assessed the chia seeds antioxidant activity using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical assay which showed 68.83% inhibition, moreover he postulated that his result was higher than the values reported formerly for chia and various plant foods. Also our result was higher than that mentioned by Coelho and Salas-Mellado (2014) who obtained that the chia phenolic extract (32.35 µg GAE ml^{-1} extract showed antioxidant activity that was effective in neutralizing more than 70% of the free radicals.

Table (3): Total phenolic compounds (mg GAE/g) and antioxidant activity (DPPH percent free radical scavenging activity) for chia seeds flour:

Sample	Total phenol	DPPH
Chia seeds flour	4.58	79.17

According to the findings, chia seeds present bioactive, natural antioxidant components which decrease the incidence of some diseases such as cardiovascular and prevent the rancidity of unsaturated fatty acids (Reyes-Caudillo *et al*. 2008).

Microbial measurements

Changes in total bacterial, molds and yeast counts of the prepared chicken sausage formulated with different percent of chia seeds flour during storage (4±1°C) up to 21 day are presented in Table (4).With progressive of the storage period the total bacterial, molds and yeast counts were significantly increased in control as well as treatments and that might be due to the temperature appropriate psychotrophic bacteria growing besides free water availability. Russell *et al.* (1995) postulated that chilling temperature at 3°C is suitable for growing psychrotrophic bacteria, such as the pseudomonads, and can multiply on the poultry meat surface by using glucose and other carbohydrates as energy sources.

Table (4): Changes in total bacterial count (cfu/g) of chicken sausage formulated with chia seeds flour during storage at 4±1°C up to 21 days

Storage time (days)	C	F1	F2	F3
		Bacterial count ($\times 10^4$)		
0	8.67^{aB}	7.00^{aC}	5.00^{bC}	4.33^{bB}
7	29.00^{aA}	25.00^{abB}	21.33^{abB}	16.00^{bB}
14	-	57.00^{aAB}	46.00^{abA}	38.00^{bA}
21	-	73.00^{aA}	51.00^{abA}	45.00^{bA}
		Molds count ($\times 10^2$)		
0	7.67^{aB}	5.33^{abC}	5.00^{abD}	4.00^{bC}
7	17.67^{aA}	14.00^{abBC}	12.00^{abC}	9.00^{bB}
14	-	25.00^{aAB}	21.00^{aB}	19.00^{aA}
21	-	37.00^{aA}	29.00^{bA}	23.00^{bA}
		Yeast count ($\times 10^3$)		
0	8.67^{aAB}	7.00^{abC}	6.00^{bC}	4.00^{cC}
7	17.00^{aA}	15.00^{aBC}	12.67^{aBC}	11.00^{aBC}
14	-	28.00^{aB}	25.00^{aAB}	23.00^{aAB}
21	-	41.00^{aA}	37.00^{aA}	30.00^{aA}

Different the capital letters in the same column means significantly difference ($p<0.05$) between storage period
Different the small letters in the same row means significantly difference ($p<0.05$) between treatment

The total bacterial count results in treated chicken sausage samples compared with control showed that, among treatments at zero time of storage there were no significant differences between control sample and F1 (2% chia seeds flour), but with increasing the percent of added chia seeds flour, we noticed significant decrease ($p<0.05$) in both F2 (4% CSF) and F3 (6% CSF) which didn't differ significantly among each other (5.00 and 4.33 × cfu/g respectively) compared to control and F1(8.67 and 7.00 × 10^4 cfu/g respectively).

After 7 days of storage, only F3 significantly had lower bacterial count ($p<0.05$) than control, meanwhile after 14 and 21 days of storage, control sample totally get spoiled and there was no significant difference between F1 and F2 either between F2 and F3, while F3 had lower counts significantly ($p< 0.05$) than F1 ranging from 57 to 38 × 10^4 cfu/g after 14 days and from 73 to 45 × 10^4 cfu/g after 21 days of storage.

Regarding to total molds count, data in Table (4) indicated that, at 0 and 7 days of storage, there was only significant difference between control and F3 which decreased ($p<0.05$) due to incorporation of 6% chia seeds flour in F3 which decreased from 7.67 to 4 × 10^2 cfu/g at 0 time of storage and from 17.67 to 9 × 10^2 cfu/g at 7th day of storage. After 14 days of storage there were no significant differences among all treatments formulated with chia seeds flour (control was spoiled), on the other hand, after 21 days of storage, both F2 and F3 molds count decreased significantly ($p<0.05$) compared to F1.

With regard to total yeast count in Table (4), data revealed that, at 0 time of storage control sample didn't differ significantly from F1 while there were significant difference between both of them and F2 and F3 which indicated that by increasing percent of chia seeds flour, total yeast count decreased significantly. We didn't find any significant difference among all treatments along the rest of the storage peri.

Data gave us an obvious trend that F3 which had the higher percentage of chia seeds flour, almost decrease the microbial count statistically and this indicates that chia seeds may have an antimicrobial effect. Our result agreed with Divyapriya *et al.* (2016) who mentioned that chia seed have antimicrobial activities against periodontal pathogens, including *Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans* and with Elshafie *et al.* (2018) who declared that chia seeds essential fatty acids has been detected to exhibit antimicrobial effect against some phytopathogenic fungi. Moreover, Zaki (2018) measured the total bacterial count and psychrotrophic bacteria of camel burger formulated with different levels of chia seeds, he found significant decrease in burgers microbial count formulated with chia seeds during 9 and 12 days of storage.

On the other hand Tunçil and Çelik (2019) couldn't observe any antimicrobial activity of chia seed extract and chia seed oil against previously untested human pathogens *Staphylococcus aureus* NCTC 8530, *Bacillus subtilis* NRRL-B209, and *Listeria monocytogenes* ATCC 7644 as well as an untested strain of *Escherichia coli*. Also, Scapin *et al.* (2015) declared that no significant difference was detected in control or treated pork sausage with chia seeds extract during 14days of cold storage at 4°C.

CONCLUSION

Chia seeds are rich in phenolic compounds thus showed a high antioxidant capacity in DPPH analysis. Also the seeds may have an antimicrobial activity so it would help in keeping food safe and healthy for a long time.

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تأثير إضافة دقيق بذور الشيا في سجق الدجاج على قيم TBA والجودة الميكروبية أثناء التخزين المبرد

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في هذه الدراسة تم دراسة تأثير إضافة دقيق بذور الشيا بنسب ٢، ٤، ٦ % على ثبات سجق الدجاج المخزن بالتبريد على درجة حرارة أربعة درجات مئوية لمدة ٢١ يوم. تم إعداد أربعة معاملات باستخدام لحم الدجاج الكامل كما يلي: كونترول، معاملة ١ (٢% دقيق بذور الشيا)، معاملة ٢ (٤% دقيق بذور الشيا) ومعاملة ٣ (٦% دقيق بذور الشيا). تم تعبئة العينات في أطباق فوم وتغليفها بأكياس بولي ايثيلين ثم تخزينها مباشرة في الثلاجة على درجة حرارة أربعة درجات مئوية لمدة ٢١ يوم وتم تحليلها لتقدير قيم حمض الثيوبربتيوريك والجودة الميكروبية. تم تحليل بذور الشيا لتقدير محتواها من المركبات الفينولية الكلية والتي سجلت ٤,٥٨ مجم جاليك أسيد لكل جم بذور شيا، ونشاطها كمضادات أكسدة باستخدام فحص (١,١ داى فينيل -٢- بيكريل هيدرازيل) والذى أعطى نتيجة قدرها ٧٩,١٩ %. ازدادت قيم حمض الثيوبربتيوريك معنويا ما بين كل عينة بما فيهم الكونترول طوال فترة التخزين. بنهاية فترة التخزين، سجلت قيم حمض الثيوبربتيوريك ١.٨٥٣ مجم مالونالدهيد لكل كجم في معاملة ١ بينما سجلت١.٢٠٩ مجم مالونالدهيد لكل كجم في معاملة ٣. أظهرت التقديرات الميكروبية إزدياد الأعداد الميكروبية معنويا في الكونترول كما في جميع المعاملات, لكنها كانت ضمن الحدود القياسية. لم تصل عينة الكونترول حتى اليوم الرابع عشر من التخزين وفسدت. لوحظ أنه بزيادة النسبة المئوية المضافة من دقيق بذور الشيا، انخفض عدد الميكروبات معنويا، مما يشير إلى احتمالية وجود تأثير مضاد للميكروبات لهذه البذور.