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Extracting Beta-Glucan from Pumpkin Seeds and Studying its Effect on Some Meat Quality Characteristics

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Abstract

The current study aimed to use beta-glucan extracted from pumpkin seeds and add it at 1 and 2% concentrations to preserve the minced beef discs in addition to studying its qualitative characteristics during different preservation periods (0, 2, 4, 6, 8, and 10) days at a temperature of 4 °C.The study concluded that adding beta-glucan extracted from pumpkin seeds by 1 and 2% led to a significant decrease in the peroxide values of meat samples to which beta-glucan was added. In comparison to the control treatment, which had a meat quantity of 6.95 mEq/kg on the sixth day of preservation, it was removed from the analysis due to microbial contamination. The meat samples exhibited mEq/kg values of 2.76 and 2.69, respectively. The samples treated with beta-glucan maintained levels within the acceptable range throughout the ten-day preservation period. The samples treated with beta-glucan also exhibited a notable reduction in the proportion of free fatty acids.. The research results showed a substantial reduction in cholesterol and the volume of the liberated liquid in the samples treated with beta-glucan compared with the control sample.

Keywords: Beef discs, Beta-glucan, Meat preservation, Pumpkin seeds.

1. Introduction

Beta-glucan polymers are an essential functional element in various diets because of their impact on product quality and functional properties and their significant effects on human health (AHMAD ET AL., 2012).

Beta-glucan has become the focus of attention in many foods, chemical, and medical industries. It has been used in the food products industry as a gelling or thickener, as a substitute for fats, or as a source of dietary fiber and was also used in the manufacture of low-calorie food products (WORRASINCHAI et al., 2006).

The process of isolating dietary fiber,including beta-glucan, and incorporating it into food constituents yields advantageous outcomes by altering the structural characteristics and enhancing the stability of emulsions over the course of storage. Furthermore, it possesses the capability to enhance the viscosity of solutions in water and produce enduring gel structures. (SANTIPANICHWONG&SUPHANTHARIKA, 2009).Beta-glucans have proven to be a highly effective alternative to fat in the formulation of processed meat and various other dietary items.(MORIN et al. 2002).The presence of saturated fats in meat products can contribute to various health issues, including elevated cholesterol levels, cardiovascular diseases, diabetes, and several forms of cancer. However, it is possible to mitigate these detrimental consequences by reducing the unhealthy components in meat products. Consumers and meat processors are becoming more knowledgeable about the benefits of a healthy diet (MORA-GALLEGO et al., 2016; VAN et al., 2018).

The meat industry holds significant importance globally, either due to consumer demand or intense competition within the sector. Meat is a palatable and highly nutritious food item (GRASSO et al., 2014; JIANG &XIONG, 2016).Given the limited amount of existing research on the incorporation of beta-glucan in processed meat products and the importance of this topic, the current study aimed to achieve the following aims :

1. Extraction of beta-glucan from pumpkin seeds.
2. Beta-glucan is used to improve some qualitative characteristics of meat preserved for different refrigeration periods.

The materials used in the study

1.1. Pumpkin Seeds

The plant was acquired from the local markets of Basra Governorate, where dry and finely powdered pumpkin seeds were obtained.

1.2. The Meat

The beef was obtained from the thigh region and was acquired from the local markets of Basra city during the early morning hours. In a similar manner, the beef fat was obtained from the identical carcass and transported in containers that had been sanitized, so ensuring the preservation of its quality before undergoing further processing.

1.3. Spices

The pre-packaged spices used in the making of birker were sourced from local markets in the city of Basra, namely from the Karbala factory in Iraq, which specializes in the manufacturing of food powders and spices.

1.4. Starch and Salt

A 5% concentration of starch and a 1.5% concentration of salt were incorporated into the processed beef, based on its weight.

1.5. Extraction of β-Glucan from Pumpkin Seeds

The extraction of pumpkin seeds was carried out according to the method of Byron (1993).

1.6. The process of Manufacturing and Processing Minced Meat Tablets

The meat underwent mincing through the utilization of an electric mincing machine, followed by the addition of minced fat to certain treatments at a proportion of 15%. The mixture was thoroughly blended. Different concentrations of β-glucan were included, and the treatments were subsequently allocated as follows.:

1. The control treatment involved the addition of 15% fat alone to the beef, without the inclusion of β-glucan.
2. The second intervention was the incorporation of 15% fat into the meat along with 1% β-glucan.
3. The third experimental condition was the addition of 1% β-glucan to the beef sample, without any concurrent addition of fat.
4. The fourth treatment was the addition of 2% β-glucan to meat with a fat content of 15%.
5. The fifth treatment involved the addition of 2% β-glucan to the meat, without the inclusion of any fat.Berker made meat weighing 100 gm per disc and kept it refrigerated at 4°C until tests were carried out.

1.7. Chemical Tests

1.7.1. Free Fatty Acid Ratio

The estimation of free fatty acids (F.F.A) was conducted following the procedure outlined by Pearson et al. (1981). The estimation of the proportion of free fatty acids was conducted by employing the subsequent equation:

The percentage of free fatty acids = (100 x 282 x N x (B-A) smoothness) / (1000 x sample weight)

A = number of milliliters of KOH swabbed with the fat or oil sample.

B = number of milliliters of KOH swabbed with the plank sample

282 = molecular weight of oleic acid.

1.7.2. Peroxide Value

The estimation of the peroxide number was conducted using the approach proposed by Pearson et al. (1981). the peroxide number was determined utilizing the subsequent equation:

Peroxide number = (Na2S3O41000 x N x ml) / sample weight

1.7.3. Estimation of Cholesterol Concentration in Meat

The study examined the cholesterol levels of the beef pillswas assessed using the Al-Obaidi method (1999), which was subsequently improved by AL-SALIHI (2012).

The cholesterol concentration of the meat was determined by applying the subsequent equation: Cholesterol concentration (mg/g) = (model reading)/(standard cholesterol reading) x 2

1.8. Physical Tests

1.8.1. Value (pH)

The pH measurement was conducted using the methodology described by John et al. (1975) with a pH meter.

1.9. Extract Release Volume (ERV)

The method indicated by Pearson (1970) was followed by placing 15 g of minced meat in a 100 ml beaker containing a homogenizing lever and adding 60 ml of extraction reagent to it, shaking it well for two minutes, then filtering using a Whatman N0.1 filter paper. 15 minutes at 25°C which is the opposite characteristic of water holding capacity WHO

The extraction reagent was prepared from (50 ml of potassium dihydrogen phosphate N 0.2 KH2PO4 + 3.72 ml of sodium hydroxide NaOH 0.2N). It was supplemented with distilled water to 200 ml, shaken well, and the pH of the solution was fixed at 5.8.

1.10. Statistical Analysis

The data obtained from the experiment were subjected to statistical analysis using a two-factor design in a Complete Randomized Design (CRD). The statistical analysis was performed using the SPSS software program (SPSS, 2012). The data were compared utilizing the mean least significant difference (R.L.S.D) at a significance threshold of $p<0.05$. In the study conducted by Al-Rawi and Khalaf Allah (2000), the authors examined

1.11. Chemical Properties

1.11.1. Free Fatty Acids %

During the cryopreservation period at a temperature of 4°C, the meat tablets treated with different quantities of β-glucan generated from pumpkin seeds exhibited a statistically significant decrease (P<0.05) in the proportion of free fatty acids compared to the control sample. The data reported in Table 1 provides support for this finding. The recorded mean percentage of free fatty acids on the sixth day of cryopreservation was 1.38%.Consequently, the specimen surpassed the established thresholds of the standard and was subsequently eliminated from consideration owing to its elevated oxidation indications. However, when β-glucan was added, the meat tablets were kept within the permissible limits for the percentage of free fatty acids until their exclusion. The exclusion duration differed according to the difference in concentration, which averaged 0.74% and 0.62% for concentrations of 1 and 2% β-glucan without fat on the eighth day, respectively.The concentrations of 1% and without fat were excluded on the tenth day of cryopreservation due to microbial contamination. The treatments retain their quality due to the β-glucan of yeast, a natural antioxidant (Thondre et al., 2011).The reason may be due to the inhibitory ability of β-glucan of yeast to lipolytic bacteria, which are responsible for spoilage and spoilage of meat and its by-product during cold storage. Thus, β-glucan has prolonged the shelf life of meat tablets compared to the control sample (Ozcan&Ertan, 2018).

From the results of Table (1), it was also observed that there were no significant differences in the percentage of free fatty acids in meat patties containing 1 and 2% β-glucan concentrations. The average was 0.74 and 0.62% on the eighth day of the treatments, to which 1 and 2% β-glucan were added without fat, respectively. This may be attributed to β-glucan as a natural antioxidant whose effect increases with increasing concentration (Omana et al., 2011).

The study did not yield statistically significant variations in the proportion of free fatty acids in meat patties whether incorporating or excluding 15% fat. The table reveals that the mean percentage of free fatty acids was recorded as 0.68 and 0.66 when incorporating 2% β-glucan in the absence of fat or in the presence of 15% fat, respectively, on the tenth day. These results are positive and essential to the consumer because of the negative effects of fat on the product due to the development of oxidation indicators very quickly during cryopreservation. Thus, β-glucan has reduced the proportion of free fatty acids.

Table 1. Effect of different concentrations of β-glucan from pumpkin seeds on the percentage of free fatty acids in frozen beef burger discs at 4°C.

Storage date (one day)					Parameters
10	**8**	**6**	**2**	**0**	
		1.38	0.70	0.33	% (0) control
	0.77	0.72	0.54	0.32	β-glucan 1% + 15% fat
	0.74	0.69	0.52	0.32	β-glucan 1%
0.68	0.65	0.63	0.51	0.32	β-glucan 2% + 15% fat
0.66	0.62	0.62	0.51	0.32	β-glucan 2%
3.16					RLSD0.05

1.12. Peroxide Value

The results presented in Table 2 indicate a statistically significant (p<0.05) reduction in the peroxide number of beef tablets following treatment with varying amounts of β-glucan. In comparison to the control treatment, cryopreservation at a temperature of 4°C exhibited certain differences. The control sample exhibited an average measurement of 3.43 mEq/kg meat on the fourth day and 6.95 mEq/kg meat on the seventh day of preservation. Consequently, the peroxide number beyond the prescribed thresholds outlined in the standard standards, leading to its exclusion due to the elevated oxidation signs present. As for the meat tablets to which 1 and 2% β-glucan of pumpkin seeds were added were kept within the permissible limits until the eighth day for the 1% concentration, the tenth day for the 2% concentration, and the eleventh day for the 4% concentration. The averages were (3.44), (3.04) and 2.67 (equivalents/kg meat, respectively. Thus, the results showed significant superiority of 4% β-glucan concentration in pumpkin seeds to prolong the storage period of meat tablets in refrigeration until the eleventh day. The reason for this may be attributed to This was confirmed by (Thondre et al., 2011) and confirmed by (Omana et al., 2011) when studying poultry meat fed with β-glucan and stored for some time. Seven days, it was found that the oxidation indices decreased through the decrease in the values of peroxide in poultry meat compared to the control sample.

The table revealed a notable reduction in the peroxide number of meat patties held at 4 °C as the concentration of β-glucan increased. This decline can be attributed to the inherent antioxidant properties of β-glucan. Its effect increases with increasing concentration (2010 (Omanaet al.). Alternatively, the reason may be due to the presence of natural antioxidant compounds in β-glucans, which can dislodge free radicals (the key to the oxidative chain reaction), and that the concentration of 1% was not sufficient to produce the desired effect and reduce the development of the peroxide number. The concentrations were 2 and 4% Very effective in controlling oxidation.

Based on the findings presented in Table 2, it was observed that the inclusion of 15% fat in beef burger discs, along with the presence of β-glucan, did not have a significant impact on the peroxide number values. Despite the presence of fat, which is typically associated with higher oxidation indices, the meat discs remained within the acceptable limits specified by the standard regulations throughout the duration of cold storage. This is noteworthy as previous studies have indicated that meat products containing high levels of fat during cryopreservation and freezing tend to exhibit elevated peroxide numbers. Furthermore, β-glucan has been suggested as a potential mitigating factor in this regard, as several studies have reported its ability to reduce peroxide levels. According to (Youssef 2014),. The obtained outcome presents a positive outlook for the utilization of β-glucan. Due to the adverse health implications associated with fat consumption, particularly in relation to heart disease and hypertension, there exists a need for an alternative to fat in the production of meat-based products. Several studies have shown evidence for the capacity of β-glucan to function as an antioxidant by inhibiting free radicals and diminishing their activity through its antioxidative impact on lipid peroxidation. (Jaehrig et al., 2007).

Table 2. Effect of using different concentrations of β-glucan from pumpkin seeds in peroxide number equivalent/kg for frozen beef stocked beef burger discs at 4°C.

Storage date (one day)					Parameters
10	**8**	**6**	**4**	**0**	
	-	6.95	3.43	2.23	(%0) control
	3.52	3.15	2.50	2.22	β glucan 1% + 15% fat
	3.44	12.3	2.39	2.22	β glucan 1%
3.02	3.03	2.76	2.38	2.21	β glucan 2% + 15% fat
3.04	2.98	2.69	2.23	2.21	β glucan 2%
0.022					RLSD0.05

1.13. Cholesterol

The results shown in Table 3 indicate a statistically significant impact, with a significance level of $P<0.05$, on the cholesterol content of meat pellets treated with varying quantities of β-glucan derived from pumpkin seeds. At the onset of the trial, the frozen treated beef tablets exhibited the lowest mean cholesterol levels. Specifically, the concentrations of 1%, 2%, and 4% yielded average cholesterol values of 0.89, 0.69, and 0.56 mg/g, respectively. Furthermore, the observed cholesterol content in the experimental sample is comparatively lower than the mean cholesterol content found in the control sample, which exhibited an average cholesterol content of 1.23 mg/gm. The findings of this study indicate that the extended cryopreservation time has revealed the effectiveness of β-glucan derived from pumpkin seeds in reducing cholesterol levels in samples. Specifically, it was noted that the addition of β-glucan at concentrations of 1 and 2 resulted in a significant reduction in cholesterol content. The samples obtained on the eleventh day exhibited cholesterol levels of 0.83 and 0.65 mg/g, respectively, surpassing the control sample's average cholesterol level of 1.12 mg/g after a mere seven days of cryopreservation. The control sample was excluded due to microbial contamination, high oxidation indicators, and a decline in its cholesterol content. The inclusion of β-glucan in treatments has been found to be of considerable importance..Moreover, fat decrease increases with increasing concentrations and the length of time of preservation. This may be attributed to β-glucan being an antioxidant that can inhibit free radicals and reduce fat oxidation and the ability of β-glucan to reduce cholesterol (El-Sherbiny et al., 2003).

These results are encouraging for the use of β-glucan from pumpkin seeds in preserving meat and its products, to which different ratios are added to the ability of β-glucan to have the negative effects of fats as a result of high cholesterol in consumers, thus preserving consumer health from diseases related to high cholesterol.

It was concluded from the studies that β-glucan extracted from pumpkin seeds is characterized by its ability to inhibit free radicals and also show antioxidant activity, and can prevent cholesterol oxidation (Jaehrig et al., 2007). This study observed that 2% was superior to 1% concentration in reducing the amount of cholesterol in frozen meat patties. Some studies on β-glucans in humans and animals have shown that the decrease in cholesterol is due to the interaction between β-glucans, fat, and cholesterol (Sima et al., 2018).

Table 3. Effect of using different concentrations of β-glucan from pumpkin seeds on cholesterol mg/gm of Berker's beef broiler discs at 4°C.

Date of storage			parameters
8	**6**	**2**	
-	1.12	1.23	(%0) Control
1.01	1.04	1.09	β glucan 1% + 15% fat
0.83	0.86	0.89	β glucan 1%
0.72	0.75	0.77	β glucan 2% + 15% fat
0.65	0.67	0.65	β glucan 2%
3.16			RLSD0.05

1.14. PH Value (pH)

The results of Table No. (4) showed a significant decrease in the pH values of the meat discs treated with different concentrations of β-glucan in pumpkin seeds. The average was 6.08 when 1% β-glucan was added on the eighth day of preservation and 6.09 when 2% β-glucan was added on the tenth day of preservation compared to the control sample, whose average pH was 6.74 on the sixth day of preservation and was excluded as a result of high Indicators of oxidation and microbial contamination.

The lower pH in the treated meat tablets was attributed to the lower pH of β-glucan, which had a pH of 5.6. It is also noted from the table results that the pH increased during the periods of cryopreservation. This rise may be attributed to protein decomposition due to proteolytic enzymes, the production of volatile bases, the production of ammonia, and the homologous tripartite amine (Abboud, 2015), and the addition of fat by 15% to the meat tablets did not affect the value of the number pH of treatments to which β-glucan was added in equal concentrations. The study agreed with the results (2008) of Pinero et al., who demonstrated a decrease in the pH of β-glucan in a study of chicken meat fed different levels of β-glucan in barley ration.

Table 4. Effect of different concentrations of β-glucan from pumpkin seeds on the pH values of frozen beef burger discs at 4°C.

Storage date (one day)						Parameters
10	**8**	**6**	**4**	**2**	**0**	
		6.76	6.74	6.31	6.13	(%0) control
	6.13	6.13	6.11	5.98	5.91	β glucan 1% + 15% fat
	6.09	6.08	6.08	5.96	5.90	β glucan 1%
6.09	6.08	6.07	6.06	5.93	5.88	β glucan 2% + 15% fat
6.06	6.06	6.06	6.03	5.90	5.86	β glucan 2%
5.61						RLSD0.05

1.15. Extract Release Volume (ERV)

The results from Table 5 demonstrate a statistically significant reduction ($P<0.05$) in the amount of released liquid in the meat discs subjected to cryopreservation at 4 °C and treated with varying quantities of β-glucan derived from pumpkin seeds, in comparison to the control sample. The average liberated liquid reached 16.11% when adding 1% β-glucan on the ninth preservation day. These samples were excluded on the tenth day due to the high indicators of microbial contamination in them. When adding 2% of β-glucan from pumpkin seeds, the liberated liquid decreased to 13.76%. As for the meat tablets in the control sample, the average liberated liquid in it reached its highest average on the seventh day of cryopreservation, 20.16%, and they were excluded because they exceeded the standard specification for microbial contamination with high oxidation indicators.

The decrease in the liberated liquid when adding β-glucan from pumpkin seeds is due to its remarkable ability to bind and retain water, which increases the WHC. Since there is an inverse relationship between the carrying capacity of water and the liberated liquid, it was observed from the results that the amount of liberated liquid decreased with the high water carrying capacity, anessential characteristic of meat producers and processors (Omanaet al., 2011).

These results agree with the results of Thondre et al. (2011), which confirmed the risingwater-carrying capacity in chicken meat fed with different levels of β-glucan and cryopreserved for seven days.

The results also showed that the increase in the amount of liberated liquid during the periods of cryopreservation was significant in the control sample, as its average increased from 17.46% at the beginning of the experiment to 20.13% after seven days of cryopreservation.

While the increase in the level of the amount of liquid released in the β-glucan-treated meat discs was minimal, as the average amounted to 15.11% at the beginning of the experiment when using a concentration of 1% β-glucan, and it reached its highest average of 16.14%. The averages reached 13.80% when using 2% concentrations atthe beginning of the experiment and 13.78 at the end of cryopreservation, respectively.

The addition of fat to the meat discs did not significantly affect the amount of liberated liquid, and this result is one of the encouraging results of using β-glucan in the manufacture of meat products because of the ability of β-glucan to bind the fat in the product, which leads to the discs not shrinking during cooking.

Table 5. Effect of different concentrations of β-glucan from pumpkin seeds on the decreased volume of liberated liquid (ml) for broiler beef patties at 4°C.

Storage date						Parameters
10	**8**	**6**	**4**	**2**	**0**	
			20.16	18.18	17.46	(%0) control
	16.20	16.20	15.73	15.50	15.30	β glucan 1% + 15% fat
	16.14	16.11	15.52	15.31	15.11	β glucan 1%
14.65	14.65	14.60	14.56	14.20	14.00	β glucan 2% + 15% fat
13.78	13.78	13.78	13.73	13.72	13.72	β glucan 2%
RLSD0.05						RLSD0.05

2. Conclusions

1. According to studies, adding beta-glucan derived from pumpkin seeds led to a decrease in oxidative stress indicators through peroxide number in meat that was stored by refrigeration.
2. A significant decrease in the percentage of free fatty acids in meat samples to which beta-glucan was added compared to the control sample.
3. A significant decrease in cholesterol levels in meat samples to which beta-glucan was added compared to the control sample

3. Recommendations

1. The study recommends using beta-glucan extracted from other plants as well, and not just extracting it from pumpkin seeds, to help improve the quality characteristics of meat, as it is a cheap, available, and safe extract from a human health standpoint.
2. Conducting a study on the use of beta-glucan on frozen meat and studying the qualitative characteristics and microbial tests to determine the standard period for maintaining meat type and quality.

The results of the sensory evaluation showed that the use of beta-glucan extracted from pumpkin seeds led to improving the sensory characteristics of the meat with regard to colour, flavour, juiciness and general acceptability compared to the control sample.

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