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Mannan extracted from (*Brassica oleracea*) and its use to reducing microbial contamination and oxidation indicators in frozen meat

Jameel. Z. N. Al-Atabi

Department of Animal Production, College of Agriculture, University of Basrah, Iraq

Corresponding author email: zailjameel@gmail.com

Amira. K. Nasse

Department of Animal Production, College of Agriculture, University of Basrah, Iraq.

Abstract---The study was conducted in the laboratories of the College of Agriculture at the University of Basrah. In order to determine the microbial level, which included the numbers of total bacteria and cold-loving bacteria, and the level of oxidation indicators, which included the level of free fatty acids and the peroxide number when adding mannan sugar, extracted from the outer layer's leaves and applied to the meat in the freezer. The results of the study showed that the average number of total bacteria decreased when adding different levels of mannan sugar compared to the control treatment. The results of the table also indicated a significant decrease in the number of total bacteria with the increase in storage periods. The average was 617.5 CfU/g10³. Before storage, it decreased to 463.1 CfU/g10³ after 90 days. The results also showed that the addition of mannan sugar at different levels led to a decrease in the numbers of cold-loving bacteria. It was also noted that the storage period of meat recorded a significant decrease for the 1-3 months, in which the number of bacteria reached 37.57, 31.01, and 24.34 CfU/g10³, respectively, compared to the zero period in which the number of bacteria was 44.52 CfU/g10³. The study showed a decrease in the number of peroxide and free fatty acids when adding mannan to meat stored in freezing, where the average peroxide reached 2.73, 2.12, 2.11, and 1.92 mEq/kg when using concentrations of 0.5, 1, 1.5 and 2 %, respectively, compared to the control treatment, whose average peroxide was 2.87 mEq/kg. As for the storage period, it was also noted that the average period of meat storage for the months (1-3) had a significant increase for all months at 2.04, 3.03, and 3.99 mEq/kg compared to the average duration of zero (0.34 mEq/kg of peroxide

number until the third month of freezing, As for free fatty acids, a significant decrease was also observed when adding different levels of mannan. In terms of storage duration, an increase in the proportion of free fatty acids was observed compared to the control treatment.

Keywords---reducing microbial, oxidation, Mannan extract.

Introduction

Meat and its products are characterized by their high nutritional value as a major source of the essential amino acids needed by the human body, as well as containing a group of B-complex vitamins and some minerals such as iron (Kalalou and Touhami Ahami, 2004). Depending on the chemical and biological nature of the meat, it is subject to spoilage during storage as a result of fat oxidation and microbial growth, which are two of the most important factors affecting food quality (Ravyts and Leroy, 2012; Yolmeh et al., 2014). Which leads to food poisoning and economic losses as a result of spoiling meat (Andres et al., 2014). Therefore, research focused on the use of antioxidants and antimicrobials in the meat industry, especially natural materials (McCarthy et al., 2001), which are distinguished from industrial antioxidants and antibiotics in that they are safer to use and more acceptable by consumers, does not adversely affect human health (Meyer et al., 2002). The ability of plants to inhibit the activity of free radicals and microorganisms is due to the presence of chemical compounds and their active groups, such as phenolic compounds, which have the ability to inhibit microorganisms' enzymes, especially bacteria (Tapiero et al., 2002). Compounds that contain an (OH) group in their chemical structure, such as alcohols (Epicatachin, Catachin) found in some plant extracts, have the ability to inhibit various microorganisms due to their direct effect on the proteins of the microbial cell. It causes denaturation of cell membrane proteins and thus increases the permeability of the cell membrane (Jahodar, 1993). Mannan is a polysaccharide that is a biomolecule of all living organisms, which is structurally composed of homogeneous or heterocyclic monosaccharides and uronic acids linked by glycosidic bonds (Zhang et al., 2015). They are mostly found in various parts of plants, animals, fungi, bacteria, and seaweed, and they play an important role in many physiological functions of life (Zong et al., 2012). Polysaccharides are defined as bioactive sugars that are produced by living organisms, or employed by sugar-based substances that have biological effects on living organisms. Moreover, during the past decades, bioactive polysaccharides have been investigated as therapeutic agents against several chronic diseases due to their biodegradability, non-toxic nature, and biocompatibility (Colegate et al., 2007). Studies have shown that polysaccharides possess a wide range of pharmacological benefits such as antioxidant, antitumor, antimicrobial, antiobesity, hypolipidemic, antidiabetic, and hepatoprotective properties (Zhang et al., 2017; Dong et al., 2017). It is also widely used to develop new products in the fields of cosmetics, food, pharmaceuticals, petrochemicals, and paper (Jung et al., 2017). In particular, in the medical industry, polysaccharides are mostly used as medicines and biomedical materials (hypoglycemic products, anti-osteoporosis, and anti-cancer products) to reduce the effects of related metabolic syndromes (Chen et al., 2015). As for mannan, it is a hemicellulose polysaccharide present in

the primary cell wall of the plant and has two main physiological roles: it is a storage polysaccharide that provides energy for growing seedlings; and it is a structural component of the hemicellulose-cellulose network and has a similar function to that of xyloglucans (Schröder et al., 2009). Mannan is also known as mannose-based oligomers linked together by β -1,4 glycosidic bonds. It is found naturally in some plants, beans, and a manoprotein portion of the cell wall of the yeast *Saccharomyces cerevisiae* (Pourabedin et al., 2016). About 90% of plant cell wall material consists of cellulose, hemicellulose, and lignin cross-linked together in a ratio of 2:1:1 (Srivastava et al., 2017). This matrix structure is useful in making the plant cell wall resistant to microbial degradation and flexible enough to facilitate growth and movement. Hemicellulose makes up 20% of the dry weight in softwoods while only 5% in hardwoods. Hemicelluloses are the second most abundant hetero-polymers in nature after cellulose and are linear or branched polysaccharides that are assembled based on the major component polysaccharides found in the polymer backbone, such as xylan, mannan, galactans, and arabinans. (Regmi et al., 2016).

Materials and methods

Bacteriological tests

Bacteriological tests were carried out for Berker meat samples stored for each period of freezing storage in the Microbiology Laboratory of the College of Agriculture, University of Basra, which included total bacteria count and numbers of cryophilic bacteria according to the method mentioned by Eaton et al. (2005)

Chemical tests

Peroxide value

The peroxide number of freeze-stored meat samples was estimated according to the method of Pearson et al. (1981) in the Meat Science Laboratory of the College of Agriculture, University of Basrah.

$$\text{Peroxide value} = \frac{(\text{Na}_2\text{S}_2\text{O}_3 \text{ ml} \times \text{N} \times 1000)}{(\text{Wt. of Sample, gm})} \quad \text{milieq./ gm}$$

Free Fatty Acids %

The free fatty acid content of frozen-stored meat samples was estimated based on the method of Pearson et al. (1981) in the Meat Science Laboratory of the College of Agriculture, University of Basrah.

$$\text{Free Fatty Acid \%} = \frac{\text{Titration (A-B)} \times \text{N} \times 282 \times 100}{1000 \times \text{Wt of Sample, gm}}$$

Results and Discussion

Total count bacteria:

The results of Table (1) indicated that there was a significant increase ($p \leq 0.05$) in the total number of bacteria in the control treatment, in which the average number was 595.8 CfU/g with a decrease in the number of bacteria in Berker meat treated with concentrations of 0.5, 1, 1.5 and 2% of The extracted mannan

as the mean numbers were 560.0, 516.8, 504.0 and 519.8 CfU/ respectively. The reason for this is due to the ability of mannan to reduce the total numbers of bacteria and improve the ability to preserve meat burger due to the inhibiting ability to work of enzymes responsible for basic metabolic reactions, which leads to protein denaturation and then the inability of bacteria to persist and this is what Al-Rubeii et al (2009) found. The use of horseradish (rosemary) in ground beef leads to a significant decrease in the logarithm of the total bacterial number compared to the control treatment and with the other treatments. The results of the table also indicated a significant decrease in the number of total bacteria with increasing storage periods, as the average amounted to 617.5 CfU/g before storage, it decreased to 463.1 CfU/g after 90 days. The reason for the decrease in the number of total bacteria in mannan sugar treatments may be attributed to the fact that adding polysaccharides to meat stored in freezing leads to an increase in the shelf life of meat compared to ascorbic acid as a food preservative by reducing the growth rate of the total bacteria number (Hajji et al., 2021). While (Al-Shouki and Nasser (2019) indicated the inhibitory ability to work the enzymes responsible for basic metabolic reactions, which leads to protein denaturation and then the inability of bacteria to persist. It is also noted that the number of bacteria decreased significantly compared to the entire preservation period, starting from the zero preservation period. The first, second, and third month.

Psychrophilic total count

From the results of Table 2, the effect of using different concentrations of mannan sugar in preparing cryophilic bacteria for Berker meat samples preserved in freezing at -18°C . was observed. Its treatments with various concentrations of mannan sugar, T3, T4, T5, and T6. The average numbers of CfU/g for the treatments were 35.74, 33.00, and 24.59 CfU/g, respectively, compared with the average of the control treatment, which had 40.91 CfU/g. It was noted that there was no significant difference between the mean treatment T2, which amounted to 37.57 CfU/g compared to the control treatment T1, which amounted to 40.91 CfU/g, and that there was no significant difference between the mean treatment T3, which amounted to 35.74 CfU/g bacteria compared to the T2 treatment, which amounted to 35.74 CfU/g. Bacterial count (CFU/g): 37.57. It was noted that there was no significant difference between the mean of treatment T4 in which the number of bacteria reached 33.00 CfU/g and the treatment of T3 in which the number of bacteria reached 35.74 CfU/g. It was also noted that there was a significant decrease ($p < 0.05$) in the mean of treatment T5 compared to the mean of treatment T4, which reached the number of bacteria at 33.00 CfU/g. The reason for the decrease in the numbers of cryophilic bacteria may be attributed to the good anti-bacterial activity of sucralmanan. may work to disrupt and destroy the cell walls of microorganisms and then penetrate the cell and disrupt its work (Bhawana et al., 2011; Andrcs et al., 2014). The addition of mannan sugar at different levels led to a decrease in the numbers of cryophilic bacteria, and this result was in agreement with Al-birawee and Nasser (2019) when using adult jelly, which led to a decrease in the numbers of cryophilic bacteria in cold-preserved meat samples. It was also noted that the storage period of meat recorded a significant decrease for the months (0–3) in which the number of bacteria reached 37.57, 31.01, and 24.34 CfU/g, respectively, compared to the period of zero, in which the number of bacteria reached 44.52 CfU/g. The results

agreed with Al-Zoba'i (2010). It showed the effectiveness of lactic acid bacteria in reducing the microbial growth of cold-loving bacteria in a relative manner and contributed to keeping the numbers of bacteria within the acceptable limits of less than 105.

Table (1)
Effect of mannan use and storage period on average total bacterial numbers (cfu/g 10^3) for meat products stored in freezing at -18°C

Treatments	Time / month				average treatments
	0	1	2	3	
T1	707.0	632.7	558.7	484.7	595.8
T2	637.0	570.7	534.3	498.0	560.0
T3	596.0	548.7	490.3	432.0	516.8
T4	598.0	535.0	472.7	410.3	504.0
T5	549.3	529.3	510.0	490.7	519.8
average time	617.5	563.3	513.2	463.1	

R.L.S.D per treatments: 15.91 R.L.S.D time: 14.23 R.L.S.D Overlap: 31.82

Table (2)
Effect of mannan use and storage period on average numbers of total cryophilic bacteria (cfu/g 10^3) for meat products stored in freezing at -18°C

Treatments	time / month				average treatments
	0	1	2	3	
T1	53.65	45.00	36.62	28.37	40.91
T2	49.00	41.10	33.87	26.31	37.57
T3	46.00	39.00	32.33	25.64	35.74
T4	40.67	35.37	30.58	25.39	33.00
T5	33.32	27.39	21.66	16.00	24.59
average time	44.52	37.57	31.01	24.34	

R.L.S.D per treatments: 3.771 R.L.S. D time: 3.372 R.L.S.D Overlap: 7.541
peroxide number

The results in Table (3) showed the effect of using different concentrations of mannan sugar on the average peroxide number of meat Burkholder samples stored at -18°C for 3 months. With different concentrations of mannan sugar compared to the average of the control treatment, the average peroxide number was 2.73, 2.12, 2.11, and 1.92 mEq/kg when using a concentration of 0.5, 1, 1.5, and 2% of mannan meat for treatments T2, T3, T4, and T5, respectively, compared with the control treatment (T1) which averaged peroxide It has 2.87 mEq/kg of meat. The reason for the decrease in the peroxide number when adding polysaccharides is the presence of natural antioxidant compounds that have the ability to displace free radicals. While Al-Shouki and Nasser (2019) indicated The decrease in the peroxide number when adding different levels of polysaccharides is due to the polysaccharides' having high-efficiency antioxidants that preserve the meat during storage. The reason for the decrease in the peroxide number of the transactions may be the action of mannan in removing free radicals and stopping the chain of oxidation reactions through the synthesis of mannan and peroxide. It

should not exceed 10 mEq/kg of fat for fat or oil, according to the Iraqi standard specifications (Central Organization for Standardization and Quality Control 1987). According to what was mentioned by (Al-Alwani, 2017), the reason for this decrease is that the addition of mannan has a significant effect in inhibiting free radicals, or that the addition of mannan has a significant effect in curbing fat oxidation and decreasing the value of peroxide, and the obtained results also showed a significant improvement in the stability of meat color. Overall, these results indicate that raw polysaccharides are worthy of development as functional and biologically active components of the food and nutraceutical industries (Hamed et al., 2020). It was noted from the results of the table that the average period of meat storage for the months (1–3) had a significant increase for all months compared to the average period of zero (0.34) from the peroxide number until the third month of freezing, and the reason for the increase in the peroxide number was the high oxidation indicators in the meat stored for freezing. Al-Shouki and Nasser (2019)

Free fatty acids

The results in Table (4) show the effect of using different concentrations of mannan sugar, which led to a decrease in the value of fatty acids when using different concentrations of mannan sugar for Berker meat samples stored at -18 °C. From the results of the table, it is noticed that there is a significant decrease ($p \leq 0.05$) in the percentage of free fatty acids in the meat Berker treated with different concentrations of mannan sugar compared with the mean of the control treatment T1, where the average percentage of free fatty acids was 0.74, 0.64, 0.52, 0.49% when using mannan concentrations of 0.5, 1, 1.5, and 2% for the average treatments T2, T3, T4, and T5, respectively. While the average percentage of free fatty acids was 0.79% in the control threshold, it was also noted that there was a significant decrease ($p \leq 0.05$) for all the averages of the treatments among them. It inhibits or impairs the action of lipolytic enzymes such as lipase, which leads to the release of free fatty acids (Khan et al., 2009). This result is in agreement with that of Dev et al. (2021), which showed that mannan sugar possesses natural antioxidants that lead to the reduction of free fatty acids. The results of the study agreed with the findings of Al-birawee and Nasser (2019), who noted a decrease in the percentage of fatty acids when using different concentrations of polysaccharides (adult plant gel in meat preservation). As for Al-Shouki and Nasser (2019), it was shown that the decrease in the percentage of free fatty acids was caused by the adult plant jelly, which is a polysaccharide, as well as its inhibitory activity for bacteria that produce the lipase enzyme responsible for decomposing fats and releasing free fatty acids. From the results of the table, it is also noted that the average free fatty acid increased with the periods of frozen storage, as the averages reached (0.52), (0.87), and (1.08%) for the storage periods of 1, 2, and 3 months, respectively, Free Fatty (Al-Alwani 2017)

Table (3)

The effect of the use of mannan and the storage period on the average peroxide number (mEq / kg) of meat products stored in freezing at a temperature of -18 C

treatments	time / month				average treatments
	0	1	2	3	
T1	0.41	2.87	3.86	4.36	2.87
T2	0.27	2.36	3.92	4.37	2.73
T3	0.31	1.84	2.54	3.80	2.12
T4	0.40	1.78	2.50	3.76	2.11
T5	0.34	1.39	2.33	3.64	1.92
average time	0.34	2.04	3.03	3.99	

R.L.S.D per treatments: **0.032** R.L.S. D time: **0.014** R.L.S.D Overlap: **0.016**

Table (4)

The effect of the use of mannan and the storage period on the average percentage of free fatty acids % for meat products stored by freezing at a temperature of -18 C

treatments	time / month				average treatments
	0	1	2	3	
T1	0.08	0.73	1.04	1.33	0.79
T2	0.09	0.62	0.98	1.26	0.74
T3	0.07	0.57	0.88	1.07	0.64
T4	0.09	0.34	0.74	0.92	0.52
T5	0.10	0.32	0.71	0.85	0.49
average duration	0.08	0.52	0.87	1.08	

R.L.S.D per treatments: 0.015 R.L.S. D time: 0.013 R.L.S.D Overlap: 0.030

Conclusion

The current study indicates that the use of mannan extracted from the plant Cabbage has an important role in reducing the indicators of microbial contamination and oxidation in meat products.

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