Title page

Lycium barbarum polysaccharide inhibits lipid oxidation and protein degradation in Tan sheep meatballs during frozen storage

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Highlights

1. *Lycium barbarum* polysaccharide (LBP) was found to inhibit lipid oxidation and protein degradation in Tan sheep meatballs during frozen storage.

2. The major quality indicators of meatballs were determined by principal component analysis (PCA).

3. The results showed that the 0.02% LBP-treated group had a higher comprehensive score than the other groups.

Abstract The aim of the present study was to evaluate the effectiveness of *Lycium barbarum* polysaccharide (LBP) on lipid oxidation and protein degradation in Tan sheep meatballs during the frozen period. The meatballs were treated with LBP at 0.01%, 0.02% and 0.03% and stored at -18 ± 1 °C for 0, 3, 6, 9 and 12 weeks. The effects of LBP treatment were investigated using the contents of total volatile basic nitrogen (TVB-N),texture profile (TP), thiobarbituric acid reactive substances (TBARS), colour and pH values, compared with 0.2% butylated hydroxytoluene (BHT) treatment and the blank control. The results showed that LBP treatment significantly decreased TBARS content compared with the control, which confirmed LBP to be a highly effective component in preventing lipid oxidation of Tan sheep meatballs during storage, and protein degradation in Tan sheep meatballs had a significant inhibition effect because of TVB-N value reduction. In addition, the colour, TP and pH values of meatballs treated with LBP were improved dramatically. To further determine the quality changes of the blank control and all treated groups

during storage, the comprehensive score evaluation equation based on principal component analysis (PCA) was obtained: $Y = 0.51632Y_1 + 0.29589Y_2$ (Cumulative Contribution Rate = 81.221%), and the 0.02% LBP-treated group had a higher comprehensive score than the other groups, and the quality of LBP-treated meatballs was better as well. In summary, LBP may reduce or inhibit lipid oxidation and protein degradation , and enhance overall quality and shelf-life in prepared meat products.

Keywords Tan sheep meatballs, *Lycium barbarum* polysaccharide (LBP), Lipid oxidation, Protein degradation, Principal component analysis (PCA).

Introduction

With people's life rhythm getting quickly, people have become accustomed to enjoying instant food and prepared products. In recent years, the meatballs processed with rice, flour, vegetables, spices, seasonings, and other additives (Zhou, 2008) have become increasingly popular globally due to their unique flavour, nutritional balance, posh packaging, and convenient consumption (Oz and Cakmak, 2016). However, some deteriorations in quality and nutritional value of prepared meat products may occur during processing, storing and transporting, including colour and flavour changes, juice loss, lipid and protein oxidization caused by microbial growth and their physico-chemical reactions, which reduce consumer acceptance as well as sensory scores of prepared meat products.

Among them, the main reason for degradation in meat products is lipid oxidation and protein degradation, result in texture variation, formation of unpleasant tastes and odours, and unhealthy substances (Jia et al., 2012). At present, oxidative reactions of prepared meat products are restrained and delayed by adding synthetic and natural antioxidants (Cömert and Gökmen, 2018). However, the requirement for natural antioxidants has been augmented gradually by reason of their safety and health characteristics (Kumar et al., 2015). Natural antioxidants are extracted from roots, leaves, stems, fruits, seeds and peels of different plant parts (Guo et al., 2016), (Jeddou et al., 2016), (Kazemi et al., 2016). Thus, it is necessary to develop a new natural antioxidant to prolong shelf life and enhance qualities in prepared meat products.

Gouqi or Chinese wolfberry, *Lycium barbarum* L. (*L. barbarum*), has been generally planted in Northwest China over 2,000 years (Lu et al., 2019). It contains an abundance of active compounds, such as carotenoids, polysaccharides, organic acids, flavonoids, polyphenols, alkaloids, and fruit pigments (Zhou et al., 2017). In fact, LBP serves as the key bioactive substance of *L. barbarum* and has a high antioxidation activity among these constituents (Xia et al., 2019). Furthermore, LBP can enhance immune system function, prevent chronic diseases, and exhibit hypoglycaemic and hypolipidaemic effects (Amagase and Farnsworth, 2011; Fiedor and Burda, 2014; Zhao et al., 2015).

Therefore, this study was aimed to investigate the effectiveness of LBP on lipid oxidation and protein degradation in Tan sheep meatballs during frozen storage. The findings may provide theoretical guidance for natural antioxidant applications in prepared meat products.

Materials and methods

Preparation of materials

Tan mutton was obtained from Xinhai Food Limited Company in Yanchi, Ningxia, China. Other excipients were bought from a local market, such as carrots, salt, and garlic, for example. The LBP standard substance (Ultra Value = 80%) was purchased from Xi'an Shengqing Biotechnology Limited Company in Shaanxi, China.

The steps for making meatballs were as follows: First, the fresh Tan mutton was washed and connective tissues were removed. Second, the meat was minced and mixed with LBP at 0.01%, 0.02% and 0.03%, 0.2% BHT and other excipients (Table 1). Third, the meat was soused at 4 ℃ ± 1 ℃ for 20 h after mixing. Then, meatballs were made by using a forming machine in an approximate shape. Finally, the meatballs were precooked at 60 ℃ ± 1 ℃ for 10 min, boiled at 90 ℃ ± 1 ℃ for 10 min, and then stored at -18 ℃ ± 1 ℃ after cooling. The contents of TBARS, TVB-N, colour, texture profile and pH values were determined for comparing the effects of LBP treatment with 0.2% BHT treatment and the blank control at 0, 3, 6, 9 and 12 weeks.

Analysis of colour measurement

The colour of meatballs was analyzed (Ding et al., 2015) by using a Minolta Chromameter (CM-2300d, Konica Minolta Holding, Inc. Japan). The colour was measured at three separate locations of the each sample, and each measurement consisted of 3 readings. The average values of triplicate measurements of L*, a*, and b* were calculated.

Analysis of texture profile

The TP of the meatballs was obtained using a texture analyser (TA.XT Plus, Stable Micro System, Inc. UK) with P35 detector, 60% strain and 5 s residence time. The pre-test, mid-test and post-test velocities were 5 mm/s, 3 mm/s and 5 mm/s, respectively (Yu et al., 2021). All analyses were carried out in triplicate for each meatball.

Determination of pH

The pH value of the meatballs was determined (Al-Juhaimi et al., 2016) with a Meat digital pH metre (Testo 205; MerckKGaA Technology Co., Ltd., Shanghai, China). The means of three replicates were calculated for each treatment.

Determination of TBARS

TBARS was measured based on the China National Standard (GB/T35252-2017). The 5 g meatballs were weighed and minced. Then, the samples were homogenized and filtered after adding butyl alcohol. Five millilitres of sample fluid were blended with 5 ml thiobarbituric acid (TBA), and then the mixture was reacted in a water bath at 95 ℃ ± 1 ℃ for 2 h. The absorbance values at 530 nm were measured in a spectrophotometer (UC1902PC, Lengguang Technology Co., Ltd., Shanghai, China) after cooling at room temperature. The TBARS was obtained according to the following equation:

$$\textbf{TBARS}\left(\textbf{mgMDA/Kg}\right)=\left[\textbf{50}\times\left(A-B\right)\right]/\,m \qquad (1)$$

When the absorbance of the reagent blank was lower than 0.05, the equation was as follows:

$$\textbf{TBARS}\left(mgMDA\,/\,Kg\right)=\left(\textbf{50}\times A\right)/\,m \qquad (2)$$

Where A = absorbance of the sample solution, B = absorbance of the reagent blank, and m = weight (mg) of sample.

Determination of TVB-N

TVB-N content was measured by the China National Standard (GB5009.228-2016). Briefly, 20 g meatballs were minced, homogenized and filtered after adding distilled water. Then, 1 ml boric acid and 1 droplet compound indicator were added into the inter diffuser, while 1 ml sample fluid and 1 ml saturated potassium carbonate solution were mixed into the outer diffuser. Then, the diffuser was placed in an electrothermal incubator at 37 ℃ ± 1 ℃ for 2 h. The final sample fluid was titrated with hydrochloric acid after cooling at room temperature. At the same time, a reagent blank experiment was conducted (Goulas and Kontominas, 2007). The equation as follows:

$$\textbf{TVB-N}\left(\textbf{mg/100g}\right)=\left\{\left[\left(V1-V2\right)\times c\times\textbf{14}\right]/\,m\times\left(V\,/\,V0\right)\right\}\times\textbf{100} \qquad (3)$$

Where V1 = volume (ml) of hydrochloric acid consumed by test solution; V2 = volume (ml) of hydrochloric acid consumed by reagent blank; c is the concentration (mol/L) of hydrochloric acid; m is the weight (g) of sample; V = volume (1 ml) of filtrate; and V0 = volume (1 ml) of sample solution.

Statistical analysis

Data was expressed as the means ± standard deviations and calculated with Microsoft Office Excel 2018 software. Statistical analysis was performed using the SPSS 24.0 (IBM SPSS Inc., USA) and statistical significance was defined as two-sided $P < 0.05$. The PCA of the main quality indicators of meatballs during storage also used the SPSS.

Results and discussion

Sensory evaluation of meatballs

The five men and five women who are trained in food science were selected for sensory analysis of meatballs. The meatballs were evaluated from five aspects: colour, shape, tissue, odour, and impurities. The highest score for meatballs were ten. As shown in Tables 1 and 2, the sensory qualities of meatballs were affected by: soy protein isolate > carrageenan gel > pentasodium triphosphate > carrots puree. The best composition of the Tan sheep meatballs was: A3B2C1D2.

Effect of LBP treatment on colour of meatballs

As shown in Table 3, the a* values of each group gradually decreased with increasing storage time ($p < 0.05$). LBP may retard the oxidation of myoglobin and the formation of metmyoglobin by delaying lipid oxidation, preventing meatballs from discolouration. Otherwise, the discolouration of meatballs was caused by nonenzymatic browning reactions between lipid oxidation products and amines in meat (Xia et al., 2009). Gantner et al. (2018) reported that the polyphenols contained in *Salvia officinalis* L. extract might be compressed to form darkened meat, which brought about an intense colour of meat and the low a* values.

Overall, the LBP-treated meatballs had fine colour stabilization at 12 weeks comparedwith the control in Table 3 ($p < 0.05$). The a* values with 0.01% LBP, 0.02% LBP and 0.03% LBP at 0 week were 14.940, 15.500 and 14.443, respectively; The a* values of 0.01% LBP, 0.02% LBP and 0.03% LBP at 12 weeks were 11.517, 12.437 and 12.990, respectively. Simultaneously, the value of the control colour a* at 12 weeks was 13.333. Additionally, the a* values of all LBP-treated groups gradually decreased during storage ($p < 0.05$) compared with the control . The reason for these results might be the addition of LBP in meatballs and in accordance with Caglar et al. 2018. However, there were no significant correlations ($p > 0.05$) in the L* and b* values among all treated groups. Jayawardana et al. (2015) did not observe L* and b* values in chicken sausage by treatment with Moringa leaves, and these outcomes were entirely consistent with our study.

Effect of LBP treatment on the texture profile of meatballs

As shown in Table 4, the springiness of meatballs was higher in the LBP-treated groups than that in the control at the end of week 12 ($p < 0.05$). Moreover, hardness is among the most fundamental textural characteristic in meatballs. There were no significant differences among all groups for hardness during storage ($p > 0.05$). Li et al. (2013) found that tea polyphenols and grape seed extract having abundant polyphenolic compounds, could extend shelf life and improve the textural attributes of red drum. The gumminess and chewiness of LBP-treated groups were dramatically increased during frozen storage compared with the control group ($p < 0.05$). In summary, the rate and extent of pH decline might influence the changes of texture parameters, degradation of myofibrillar protein, and softening of the musculature (Li et al., 2012).

Effect of LBP treatment on the pH value of meatballs

pH is one of the critical indicators for real-time monitoring of freshness for chilled meat (Chang et al., 2019). As shown in Table 5, the pH values of the BHT- and LBP-treated groups before the 3rd week decreased significantly with increasing storage time ($p < 0.05$). Reduction in pH could be related to the accumulation of acidic substances during storage (Al-Juhaimi et al., 2017). However, pH values gradually increased ($p < 0.05$) from 3 to 12 weeks in all groups, of which LBP-treated groups were more stable than the control and BHT-treated groups. pH increase might be related to the activities of corruption bacteria and endogenous enzyme.

Effect of LBP treatment on the level of TBARS of meatballs

TBARS accumulated during frozen storage (Fig. 1). TBARS contents were lower in the LBP-treated groups than in the control group. The increases of TBARS contents were slight in the antioxidant-treated groups ($p < 0.05$) , while a more rapid increase was obtained from the control group, followed by the 0.02% BHT-, 0.01% LBP-, 0.02% LBP- and 0.03% LBP-treated groups, which reached their respective maximum values at the end of storage. The increase of TBARS contents might be put down to the denatured structure of the muscles during the storage (Cao et al., 2013). These appreciable results showed that LBP presented stable in lipid oxidation of meatballs compared with the control (Al-Juhaimi et al., 2018). However, the TBARS contents decreased in the 0.03% LBP-treated group from 3 to 6 weeks and in the control from 9 to 12 weeks ($p < 0.05$). Pereira de Abreu et al. (2011) also found that in Atlantic halibut samples, the TBARS values increased slightly until reaching a maximum value and then decreased slowly. The TBARS content could be degraded or interacted with other components , leading to the reduction of its content (Pereira de Abreu et al., 2010).

Freezing alone could not prevent the process of deterioration in meat products. The LBP on lipid oxidation might obtain the retardant effect due to its phenolic and other compounds, which contributed to the activities of antioxidants (Qin et al., 2013) and chelate metal ions (Nishad et al., 2018). Moreover, phenolic antioxidants may prevent the formation of free radicals, which account for the reaction or absorption of oxygen in the autoxidation process (Turgut et al., 2017).

Thus, LBP shows antioxidant activity by blocking radical chain reactions during the oxidation process and may be used as antioxidant to make meat and meat products against lipid oxidation (Morsy et al., 2018). In addition, TBARS slowly accumulated with the increasing of storage time, and the structural and functional changes of these proteins may be caused by their associated lipid oxidation products (Xiong et al., 2015).

Effect of LBP treatment on the level of TVB-N of meatballs

Protein degradation is one of the major causes of meat quality deterioration in addition to lipid oxidation. According to the China National Standard (GB2707-2016), the upper tolerable limit of TVB-N in fresh and frozen livestock products is 15 mg/100 g. The TVB-N values increased ($p < 0.05$) from 0.200 to 36.890 mg/100 g and 0.070-28.556 mg/100 g in the control and each treated group, respectively (Fig. 2). The formation of TVB-N is due to the enzymatic decarboxylation of specific amino acids (Balamatsia et al., 2007). Similarly, Guan et al. (2019) found that inhibition of fishy odour by extracts from sage leaves, oregano leaves and grape seeds (SOG) was directly associated with reducing TBARS content and TVB-N value, suggesting the potential advantages for SOG in retarding fishy odour formation and improving flavour of hairtail fish balls during storage. Consequently, the effect of phenolic compounds against protein oxidation was also correlated with their interactions with proteins, which might protect proteins from attracting free radicals (Viljanen et al., 2004).

lipid oxidation and protein degradation are catalyzed by the same compounds, which can progress independently or in parallel. Moreover, radicals, hydroperoxides and secondary oxidative compounds react with protein owing to lipid oxidation, thereby resulting in the destruction of protein functionality (Farvin et al., 2012). Therefore, lipid oxidation and protein degradation can interact.

The PCA of the main quality indicators of meatballs

To further determine the quality changes of the control and all treated groups during frozen storage, the pH value, a* value, springiness, TBARS value and TVB-N content were used as the main quality indices of the meatballs, and PCA was conducted. According to the idea of PCA dimensionality reduction, the change in the sum of the five main quality indicators of meatballs during frozen storage was divided into principal components, and the characteristic value of each principal component reflects the amount of original variable information. As shown in Table 6, the first two principal components were extracted by PCA, with eigenvalues greater than 1.000 and a cumulative contribution rate of 81.221%. Based on the load matrix and eigenvalues, the eigenvectors of each PCA1 and PCA2 were calculated, as shown in Table 7. The coefficients of two principal component linear equations were established by eigenvectors. The equations were as follows:

$$\text{PCA1: } Y_1 = 0.345X_1 - 0.308X_2 - 0.352X_3 + 0.568X_4 + 0.583X_5 \quad (4)$$

$$\text{PCA2: } Y_2 = 0.556X_1 + 0.636X_2 - 0.483X_3 - 0.217X_4 - 0.073X_5 \quad (5)$$

where Xi is standardized data, i = 0, 1, 2, 3, 4, 5.

Based on the variance contribution rate of the first two principal components as coefficients, the comprehensive score evaluation equation was obtained: $Y = 0.51632Y_1 + 0.29589Y_2$. As shown in Fig. 3, the measured values of the five main quality indicators of each group were standardized and substituted into the above equation to calculate the comprehensive score Y. The results showed that the 0.02% LBP-treated group had a higher comprehensive score than the other groups, and the quality of the LBP-treated meatballs was better as well.

Conclusions

In conclusion, lipid oxidation and protein degradation have a significant effect on the progress of oxidative reactions in Tan sheep meatballs. The results confirmed LBP to be a highly effective antioxidant in Tan sheep meatballs, it reduced TBARS contents, TVB-N values, and obtained stable colour, TP and pH during frozen storage

($p < 0.05$). Finally, the 0.02% LBP-treated group had a higher comprehensive score than the other groups, and the quality of LBP-treated meatballs was better as well. In a word, LBP as a natural antioxidant may have great potential to inhibit lipid oxidation and protein degradation in prepared meat products in the future.

Conflicts of interest

None.

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Tables

Table1 The compositions of meatballs.

Ingredients and excipients	Mass fraction (%, w/w)
Carrots puree	50.00
Salt	2.00
Garlic	0.50
Thirteen incense	0.50
Soy protein isolate	2.00
Carrageenan gel	0.30
Pentasodium triphosphate	0.05

Mass fraction of excipients was calculated according the quality of Tan mutton.

Table 2 Orthogonal experimental results of the excipients of meatballs.

Levels	Factors				
	A	B	C	D	Scores
1	1（1.00%）	1（0.20%）	1（0.05%）	1（45.00%）	6.89
2	1	2（0.30%）	2（0.10%）	2（50.00%）	7.01
3	1	3（0.40%）	3（0.15%）	3（55.00%）	7.22
4	2（1.50%）	1	2	3	7.54
5	2	2	1	3	8.49
6	2	3	2	1	7.94
7	3（2.00%）	1	3	2	8.11
8	3	2	3	1	8.35
9	3	3	1	2	8.40
K1	21.12	22.54	23.78	23.18	
K2	23.97	23.85	22.49	23.52	
K3	24.86	23.56	23.68	23.25	**Factors：**
k1	7.04	7.51	7.93	7.73	**A＞B＞C＞D**
k2	7.99	7.95	7.50	7.84	**Compositions：**
k3	8.29	7.85	7.89	7.75	**A3B2C1D2**
R	1.25	0.44	0.43	0.11	

A is soy protein isolate; B is carrageenan gel; C is pentasodium triphosphate; D is carrots puree; K1,K2 and K3 both are sum; k1, k2 and k3 both are range; R is variance.

Table 3 Effect of LBP treatment on colour of meatballs during frozen storage.

	Treated	Storage time				
	groups	0 week	3 weeks	6 weeks	9 weeks	12 weeks
L*	Control	60.200±0.396a	58.517±0.731a	59.463±2.423a	57.620±3.060a	59.427±2.413a
	0.02% BHT	59.037±1.390a	58.547±0.583a	59.237±1.277a	57.820±2.373a	58.147±1.331a

	0.01% LBP	60.160±3.618a	58.113±1.392a	57.670±2.393a	59.073±0.818a	58.977±1.784a
	0.02% LBP	56.443±1.947a	58.683±0.966a	60.577±0.588a	60.467±0.785a	56.507±3.151a
	0.03% LBP	59.820±1.811a	58.243±1.922a	59.257±1.717a	57.377±1.923a	56.733±0.828a
a*	Control	14.095±2.369a	12.700±1.459a	15.783±3.621a	11.073±1.280c	13.333±0.497a
	0.02% BHT	16.513±4.497a	12.753±2.880a	12.440±0.795b	13.393±0.676ab	12.173±0.997ab
	0.01% LBP	14.940±3.041a	12.580±1.427a	12.347±0.100b	13.073±1.126ab	11.517±0.871b
	0.02% LBP	15.500±1.385a	13.480±0.783a	11.537±0.358b	12.017±0.589bc	12.437±0.263ab
	0.03% LBP	14.443±1.426a	12.317±0.670a	12.757±0.323ab	13.877±0.397a	12.990±0.968a
b*	Control	33.245±0.545a	33.800±1.465a	37.633±4.015a	30.910±1.405a	34.070±3.631a
	0.02% BHT	35.507±4.438a	32.647±3.487a	31.600±0.470a	34.620±1.600a	30.280±5.119a
	0.01% LBP	34.580±2.782a	32.313±1.768a	34.307±5.000a	33.767±0.655a	30.110±1.633a
	0.02% LBP	34.030±2.544a	33.063±2.612a	34.493±2.171a	30.747±1.258a	30.127±2.437a
	0.03% LBP	33.030±0.494a	33.527±2.200a	34.010±3.220a	33.593±7.386a	30.873±2.535a

Values are means of triplicate samples (Means±SD). Means with different letters (a-c) at different storage time of the same treated group were different significantly ($p < 0.05$).

Table 4 Effect of LBP treatment on TP of meatballs during frozen storage.

	Treated groups	**Storage time**				
		0 week	**3 weeks**	**6 weeks**	**9 weeks**	**12 weeks**
Hardness	Control	12.384±0.992a	12.476±2.254a	12.061±0.113a	11.551±0.325a	11.285±0.520a
	0.02% BHT	11.211±0.989a	11.578±1.739a	12.054±1.621a	11.797±1.602a	11.651±1.399a
	0.01% LBP	11.321±2.021a	11.505±1.374a	13.025±0.078a	11.722±0.411a	11.468±1.067a
	0.02% LBP	10.552±1.710a	12.530±0.687a	11.716±0.559a	11.985±0.337a	11.248±1.311a
	0.03% LBP	10.845±0.444a	12.101±0.325a	12.677±1.430a	12.476±1.322a	13.043±1.128a
Springiness	Control	73.228±1.322a	63.001±0.105bc	46.854±1.537a	45.858±3.404a	39.074±9.485a
	0.02% BHT	32.854±3.295c	43.831±6.824d	38.351±1.482c	46.121±3.842a	40.499±3.179a
	0.01% LBP	47.039±10.653bc	55.696±1.199cd	46.085±1.008ab	42.996±0.161a	42.731±3.396a
	0.02% LBP	37.803±10.481bc	77.211±6.194ab	44.780±1.608ab	45.038±2.389a	40.490±4.612a
	0.03% LBP	56.425±20.973ab	84.536±7.729a	43.400±2.476b	41.164±3.959a	45.831±1.646a
Gumminess	Control	7.508±0.637a	5.124±0.158b	4.025±0.038a	3.610±0.510b	4.145±0.192a
	0.02% BHT	3.921±0.299b	3.881±0.828c	4.146±0.493a	4.057±0.490ab	4.459±0.356a
	0.01% LBP	4.989±2.548ab	5.360±0.481b	4.483±0.036a	4.347±0.256ab	4.158±0.562a
	0.02% LBP	4.008±0.769b	4.664±0.263bc	4.096±0.162a	4.638±0.687a	3.852±0.707a
	0.03% LBP	4.072±0.537b	7.083±0.269a	4.224±0.363a	4.226±0.383ab	4.508±0.142a
Chewiness	Control	5.493±0.368a	2.799±0.712b	1.981±0.202a	1.751±0.340a	1.612±0.340a
	0.02% BHT	1.282±0.036b	1.673±0.098b	1.590±0.206b	1.863±0.185a	1.812±0.053a
	0.01% LBP	2.601±1.966b	2.865±0.291b	2.060±0.079a	1.959±0.230a	1.784±0.352a
	0.02% LBP	1.726±0.747b	2.724±1.144b	1.871±0.197ab	1.861±0.195a	1.575±0.446a
	0.03% LBP	1.837±0.390b	6.083±0.196a	1.833±0.191ab	1.741±0.255a	2.068±0.140a

Values are means of triplicate samples (Means±SD). Means with different letters (a-d) at different storage time of the same treated group were different significantly ($p < 0.05$).

Table 5 Effect of LBP treatment on pH value of meatballs during frozen storage.

Storage time	Control	0.02% BHT	0.01% LBP	0.02% LBP	0.03% LBP
0 week	5.435±0.007bc	5.635±0.007a	5.460±0.014b	5.410±0.014c	5.255±0.007d
3 weeks	5.450±0.014a	5.295±0.007bc	5.325±0.021b	5.310±0.014bc	5.280±0.014c
6 weeks	5.540±0.014a	5.540±0.057a	5.475±0.021ab	5.450±0.028bc	5.385±0.021c
9 weeks	5.535±0.064a	5.495±0.021a	5.465±0.007ab	5.395±0.007b	5.305±0.035c
12 weeks	5.620±0.028a	5.530±0.099ab	5.355±0.007c	5.415±0.007bc	5.410±0.014bc

Values are means of triplicate samples (Means±SD). Means with different letters (a-d) at different storage time of the same treated group were different significantly ($p < 0.05$).

Table 6 The contribution rate and eigenvalues of each principal component.

Principal Component	Eigenvalues	Variance Contribution Rate（%）	Cumulative Contribution Rate（%）
1	**2.582**	**51.632**	**51.632**
2	**1.479**	**29.589**	**81.221**
3	0.529	10.584	91.805
4	0.303	6.064	97.869
5	0.107	2.131	100.000

Table 7 The load matrix and eigenvectors of each principal component.

Indicators	PCA1		PCA2	
	Load matrix	Eigenvectors	Load matrix	Eigenvectors
pH	0.555	0.345	0.676	0.556
a*	-0.495	-0.308	0.774	0.636
Springiness	-0.566	-0.352	-0.588	-0.483
TBARS	0.912	0.568	-0.264	-0.217
TVB-N	0.936	0.583	-0.089	-0.073

Figures

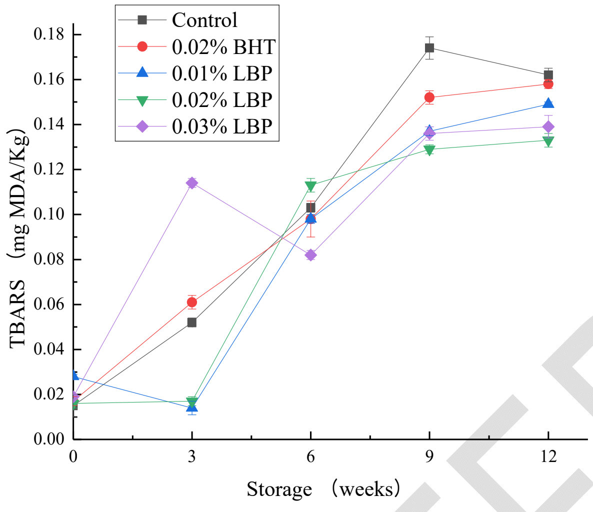

Fig. 1 Effect of LBP treatment on level of TBARS of meatballs during frozen storage. Error bars refer to the standard deviations obtained from triplicate sample analysis.

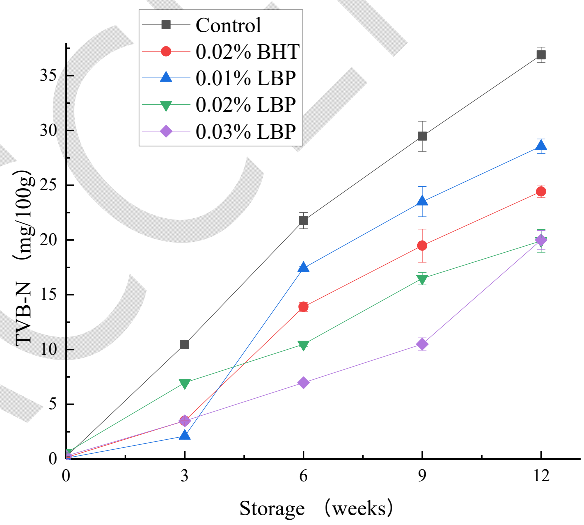

Fig. 2 Effect of LBP treatment on level of TVB-N of meatballs during frozen storage. Error bars refer to the standard deviations obtained from triplicate sample analysis.

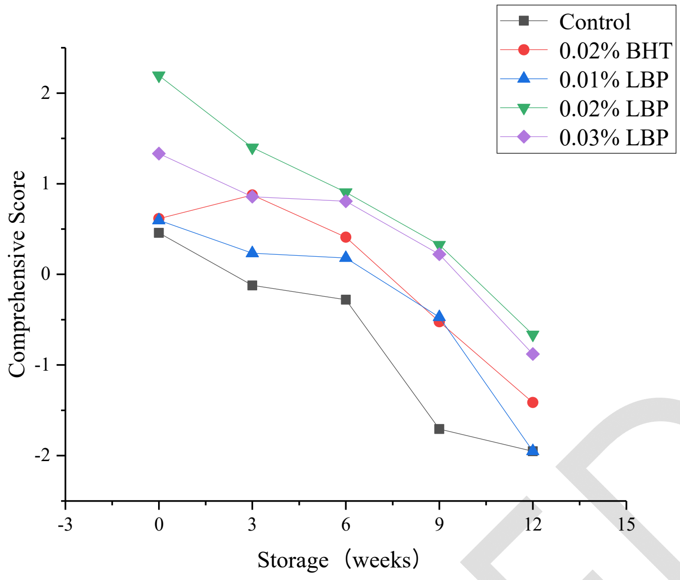

Fig. 3 The comprehensive score of meatballs during frozen storage.