Journal of Chemistry

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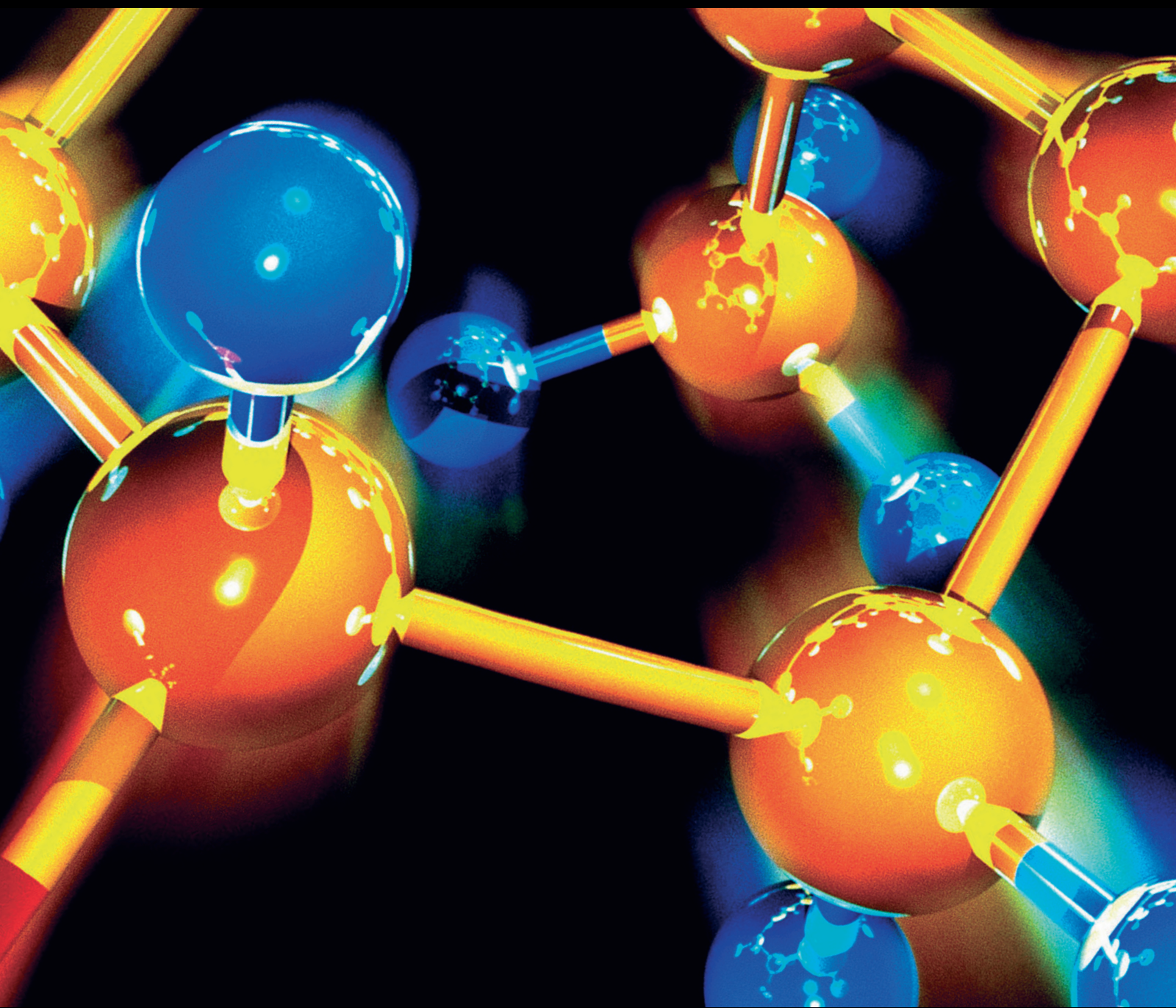

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Hindawi
Journal of Chemistry
Volume 2018, Article ID 1328797, 2 pages
https://doi.org/10.1155/2018/1328797

Editorial

Dietary Fiber: Chemistry, Structure, and Properties

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Received 18 July 2018; Accepted 18 July 2018; Published 26 September 2018

Dietary fiber is defined as "the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine." It includes mainly non-starch polysaccharides, oligosaccharides, resistant starch, and lignins. Health benefits of dietary fiber including promoting laxation, attenuating blood cholesterol and glucose, and preventing certain cancers have been extensively studied by many human and animal studies. These health benefits may be attributed to several physiological properties such as bulking, water-holding capacity, cation- and cholesterol-binding properties, and fermentation properties. However, the detailed mechanisms of its health benefits are still not clear which deserve more investigations. In addition, the food application of dietary fiber is still facing many challenges including poor texture and unpleasant mouthfeel of the fiber-fortified food products. *Journal of Chemistry* set out to publish a special issue devoted to the topic "Dietary Fiber: Chemistry, Structure, and Properties." The result is a collection of ten outstanding articles. These papers covered physicochemical properties of dietary fiber, its food applications, and novel fiber exploration. We believe these studies could make great contribution to dietary fiber in areas of both research and industrial applications.

Zhang et al. studied a novel dietary fiber material: bamboo shoot dietary fiber (BSDF). The effects of BSDF on the mechanical properties, moisture distribution, and microstructure of frozen dough were investigated. The results showed that the BSDF significantly improved the viscoelasticity and extensibility of frozen dough after thawing in a dose-dependent manner.

Wheat bran is rich in dietary fiber, which improves the flour nutritional content and also endows the flour with a richer flavor. However, a high content of insoluble dietary bran fiber may badly compromise the processing and edible quality of flour products. One study was conducted to explore ways to decrease the negative effects by using fermented bran, which showed longer dough extensibility and stability and better overall steam bread texture. This provided an innovative way to increase the dietary fiber content of steam bread.

Li et al. demonstrated how the complex additives including sodium carboxymethyl cellulose (CMC-Na) affect the storage property of steamed bread during frozen dough storage. The results showed that adding complex additives could enhance gas-holding capacity of gluten and maintain yeast activity upon frozen storage.

Another research was performed to investigate the quality of fresh wet noodles made from different flour milling streams. The basic composition, texture properties, cooking characteristics, and moisture status of the noodles were compared. The results indicated that as storage time increased, the springiness of fresh wet noodles gradually decreased, while the hardness increased.

Yang et al. reviewed the modification and application of dietary fiber in foods with respect to definition, classification, and methods for measurement, extraction, and modification. The supplementation of dietary fiber for flour, meat, and dairy products is also included. The benefits and risks of increasing consumption of dietary fiber are discussed.

Conflicts of Interest

The editors declare that they have no conflicts of interest.

Qingbin Guo
Ji Kang
Yanjie Bai
Feng Xu

Hindawi
Journal of Chemistry
Volume 2018, Article ID 7464297, 8 pages
https://doi.org/10.1155/2018/7464297

Research Article

Relationship of Moisture Status and Quality Characteristics of Fresh Wet Noodles Prepared from Different Grade Wheat Flours from Flour Milling Streams

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Received 6 August 2017; Revised 8 December 2017; Accepted 18 May 2018; Published 5 August 2018

Academic Editor: Qingbin Guo

This study was performed to investigate the quality of fresh wet noodles made from flour milling streams. The basic composition, texture properties, cooking characteristics, and moisture status were measured to evaluate the qualities of noodles. The results indicated that as storage time increased, the springiness of fresh wet noodles gradually decreased, but the hardness increased. Additionally, the cooking loss rate was increased obviously, and the water absorption rate generally decreased. The relaxation times T_{21} and T_{22}, analyzed by low-field nuclear magnetic resonance, showed a downward trend that proton mobility became poor and bound water changed into intermediate water. Noodles made from reduction flour exhibited better quality. Compared to that with ambient temperature storage, the wet noodles under frozen storage showed better quality. The relaxation time T_{21}, and T_{22} showed a positive correlation with noodle quality.

1. Introduction

Free water, intermediate water, and bound water are three forms of water in food [1]. As an important component of many foods, water has a decisive influence on food's rheological characteristics and its chemical and physical properties [2, 3]. The presence, distribution, and concentration of water strongly influence the processing characteristics, stability, and preservation properties of food [4].

Low-field nuclear magnetic resonance (NMR) technology is an effective tool to study the water status of food, mainly by determination of the proton relaxation behavior [5, 6]. The relaxation process occurs through fluctuations in the magnetic field caused by random molecular motions, both rotational and translational. The rate and the characteristics of these motions both affect the decay of the NMR signal, which is observed by the T_1 and T_2 relaxation times [7]. The NMR signal is commonly analyzed in terms of two main parameters, T_1 and T_2. The spin-lattice (T_1) relaxation involves the transfer of energy between the spin system and the environment, and spin-spin relaxation (T_2) processes involve the dephasing of nuclear spins, which are entropic processes [8]. The T_2 can be used to analyze the interactions between water and dough. The T_2 value is sensitive to water distribution with different mobility states [9]. Two transverse relaxation time constants, T_{21} and T_{22}, are spin-spin relaxation time constants and were identified from the NMR experiments using the Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence. The existence of these time constants indicates the presence of two distinct fractions of water. T_{21} is the portion of water that is strongly associated with other molecules by hydrogen bonding, almost "bound" water. However, T_{22} is more mobile water with a high molecular mobility. T_{21} and T_{22} have different relaxation rates and degrees of mobility. Generally, shorter T_{21} values indicate less mobile water, and longer T_{22} values indicate more mobile water [10].

A model of water distribution is useful to enable real-time monitoring and control of food quality during production and storage [11, 12]. Water mobility is mainly depended on the changes of hydrogen bonding structure. Hydrophilic materials such as proteins and carbohydrates can form hydrogen bonds with water molecules to influence the water mobility. Higher contents of proteins or carbohydrates decrease water mobility and vice versa [13].

Compared with dry noodles, wet noodles are fresher, with stronger boiling fastness, stronger gluten, better taste, and better flavor. However, the high moisture of fresh wet noodles can easily lead to spoilage, browning, rancidity, and deterioration, damaging appearance, quality, and flavor. At the same time, the change of both content and distribution of water during the milling of wheat flour contribute to the loss and migration of moisture and changes in flour characteristics. When noodles were stored at different temperatures (37°C, 45°C, or 55°C), T_{21}, T_{22}, and the content of free water increased, which indicated that migration and redistribution of water occurred [10]. Lai and Hwang found that the T_2 changed regularly with the moisture distribution and movement in noodles during cooking and storage. Additionally, the surface and the interior water showed different migration behaviors during storage, and moisture migration played a decisive role in the hardening of noodles [14]. Sekiyama et al. found that when the storage time was between 10 min and 120 min, the T_2 value on the surface of noodles decreased gradually with the extension of storage time, which was completely contradictory with the T_2 value of the center of noodles. This difference was mainly attributed to the redistribution of water. After 120 minutes, the water in different regions presented a certain moisture gradient, and the relaxation time T_2 was related to the microstructure and the degree of starch gelatinization of noodles [15].

The objectives of this research were to study the quality of fresh wet noodles made from different grade wheat flours of flour milling streams under different storage conditions, and the influence of storage temperature on the change of water status were observed by low-field NMR. Our finding was able to correlate water status and noodle quality, suggesting water status can be used to predict changes in noodle quality.

2. Materials and Methods

2.1. Materials. Wheat powders were obtained from the flour production workshop of Henan Zhonghe Co. Ltd. (Henan, China). Representative online wheat milling streams of break flour (2B, the number "2" represents the second time of milling, similarly hereinafter), reduction flour (1M, 2M, and 3M), and sizing flour (1S) were selected for the study. The basic physical and chemical indicators of the wheat milling streams are shown in Table 1.

2.2. Noodle Preparation. Fresh wet noodles were prepared as described previously [16]. Briefly, 100 parts of wheat flour and 35 parts of deionized water were mixed for 7 min using a pin mixer. The dough pieces were then hand kneaded into a stiff mass and passed through a laboratory noodle machine 4-5 times to form and compound a noodle sheet at a gap setting of 3.5 mm. The dough was then sheeted through five different roll gaps (3.0, 2.5, 2.0, 1.5, and 1.0 mm). Next, the sheet was cut into fresh noodle strands (15.0 cm length, 2.0 cm width, and 1.0 cm thickness) with cutting rollers.

2.3. Noodle Storage. Noodles were stored at room temperature (25°C) or in the cold (4°C) and were covered with a plastic wrap. Samples were then removed regularly and subjected to testing.

2.4. Chemical Analysis. Moisture and protein were measured following standard AACC methods (AACC, 2000). The starch content was determined by 1% hydrochloric acid polarimetry. Damaged starch (DS) content was determined using the SDmatic procedure [17]. The farinograph test was performed according to standard AACC methods (2000). The whiteness was determined according to GB/T 12097. The falling number was measured following GB/T10361-2008. D50 was determined using a laser particle size analyzer (BT-2002, Dandong BT Instrument Co. Ltd.). All analyses were performed in triplicate.

2.5. Texture Properties. The TA-XT2i type texture analyzer (Stable Micro Systems, UK) was used for texture property analysis (TPA), and a set of three strands of cooked noodles were placed parallel to each other on a flat metal plate. Hardness and springiness was determined. The experimental parameters were set as follows: pretest speed: 2 mm/s, test speed: 0.8 mm/s, posttest speed: 0.8 mm/s, minimum inductive force: 5 g, compression rate: 70%, and the time interval between two compression tests: 1 s.

2.6. Water Absorption. 20 g of noodles was cooked in 500 mL of boiling distilled water until the white core of the noodles disappeared, and a colander was used to separate the noodles from the water. The noodles were transferred to a filter paper, drained for 5 min at room temperature, and then weighed. The final results are the mean of triplicate determinations. The formula of water absorption index of dry matter was calculated according to the Chinese Standard Method GB 5497–1985.

2.7. Cooking Loss Ratio. Noodles and cooking water were cooled to room temperature and then transferred to a 500 mL volumetric flask and measured. Next, 50 mL of the above solution was poured into a 250 mL beaker of constant mass and then evaporated to dryness over a water bath. This evaporation procedure was performed as described above four times, drying a total 200 mL of the above solution. The dried material was then transferred to a hot air oven that was maintained at 105 ± 2°C and dried to constant mass. The cooking loss rate of dry matter (%) was calculated according to Zhang's method [18].

Table 1: The basic physical and chemical indicators of the wheat milling streams.

Sample	Moisture (%)	Protein (%)	Starch content (%)	Whiteness	D_{50} (μm)	Damaged starch (UCD)	Falling number (s)
1S	15.11	10.63	69.87	75.7	20.37	25.3	470
2B	15.11	12.69	66.85	68.5	23.76	19.0	423
1M	14.24	10.20	71.19	73.1	25.28	16.5	496
2M	13.52	10.43	70.66	71.9	25.92	15.6	508
3M	13.64	11.46	69.16	73.3	24.99	21.6	521

Note. S, sizing flour; B, break flour; M, reduction flour.

2.8. Nuclear Magnetic Resonance (NMR). NMR (NMR variable temperature analysis system, VTMR20-010V, Shanghai NM electronic Science & Technology Co., Ltd.) was used to assess the water properties of the noodles. The Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence was used to determine the transverse relaxation time T_2 of samples. The test parameters were set as follows: number of echo (NECH) = 2000, number of scans (NS) = 16, and echo time (ET) = 0.1 ms. The NMR spectra and T_2 of samples were processed with T_2-FitFrm software.

2.9. Statistical Analysis. All the data obtained in the study were expressed as the mean of at least two determinations. Analysis of variance was performed, and the data were analyzed using Duncan's test (level of significance, $P < 0.05$) with SPSS software (SPSS Institute, Cary, NC, USA).

3. Results and Discussion

3.1. Textural Properties. As shown in Table 2, the hardness of fresh wet noodles showed an overall upward trend as the storage time was extended. When stored for the same length of time, the hardness of noodles stored at room temperature was greater than that of noodles subjected to cold storage. It was probably because the noodles stored under normal temperature were more likely to loss moisture, causing noodles to become relatively dry and hard [19]. Noodles made from 1M showed the largest hardness value, followed by 2M; the hardness of 1s was close to 3M, and noodles made from 2B showed the lowest hardness value. This was probably due to the variation of starch content in the noodle. Previous studies reported that starch content was positively related to hardness [20], and our experimental results are consistent with this finding, as shown in Table 1. The variation of protein content might also affect the hardness of the noodle. Flour with higher protein content had higher water holding capacity, preventing the moisture loss of the noodle during storage [21].

Table 3 shows that the springiness of fresh wet noodles decreased as storage time increased under different storage conditions. Additionally, the springiness of noodles stored at cold temperature was lower than for those stored at room temperature. This was probably caused by the further development of the gluten network at room temperature, but in the cold condition, this development process was more restricted [22]. The springiness of sizing and the reduction flour was greater than that of the break flour, due to the higher content of bran speck in break flour, which hindered the formation of the gluten network structure. The reduction and sizing flour properties were determined by the presence of the endosperm, evaluation of the flour quality, and more complete formation of the gluten network structure [23]. Li et al. studied the textural properties of noodles during storage and found that the hardness increased and the springiness decreased from 0 h to 24 h, and then the springiness decreased again at 36 h and 48 h, consistent with our results [24]. Other reports suggested that with the extension of storage time, the brittleness increased, and that had an effect on springiness. With storage time being increased, the alpha helix content in the gluten protein secondary structure decreased and random coil content increased, resulting in a decrease of springiness and cohesiveness in noodles [25, 26].

3.2. Cooking Properties. Table 4 shows that under different storage conditions, the cooking loss rate of all fresh wet noodles showed an obvious increasing trend with the extension of storage time. This change may be due to the starch retrogradation process and gluten network disruption during storage [27, 28]. This can lead to loosening of the starch and other small molecular compounds and the dissolution of small particles embedded in the gluten protein network, resulting in increased cooking loss rate. Noodles stored in the cold showed lower cooking loss rate than that stored at room temperature. As seen from Table 5, the water absorption rate of dry matter showed a downward trend during storage. The water absorption rate of dry matter was greater in the cold, which indicated the cold storage was helpful to maintain noodle quality. With increased storage time, the binding force between starch and the gluten network structure in fresh wet noodles weakened gradually. Subsequently, the dissolution of starch in the cooking process increased, leading to a gradual increase of cooking loss rate and a decrease of water absorption rate as storage time increased [16]. The water absorption rate of 1S presented the opposite trend under different storage temperatures. We speculated that, at higher temperature, there was more extensive contact between molecules, facilitating the formation of intermolecular chemical bonds. Moreover, the high content of damaged starch in 1S has great ability of combining with water. During cooking, the noodles showed a greater ability to swell, and the water absorption ability increased.

For the same amount of storage time, the largest cooking loss rate was exhibited for noodles made with 1M, followed by 3M noodles. Noodles made from 2B flour showed the smallest cooking loss rate, probably because in the reduction

Table 2: Hardness of fresh wet noodles made from different flours during storage.

Storage temperature	Flour variety	Storage time						
		0 h	1 h	3 h	6 h	12 h	24 h	48 h
25°C	1S	10763.5^{a}	10857.9^{ab}	11045.6^{abc}	10994.8^{abc}	11082.6^{bc}	11228.4^{c}	11286.2^{c}
	2B	9351.2^{a}	9466.1^{a}	9547.0^{a}	9993.8^{b}	10144.0^{bc}	10480.4^{c}	10508.8^{c}
	1M	13581.0^{a}	13915.9^{b}	14370.6^{c}	14881.0^{e}	14755.4^{e}	14708.3^{de}	14563.5^{d}
	2M	12105.5^{a}	12415.3^{b}	12526.7^{b}	12650.5^{bc}	12883.3^{c}	13322.6^{d}	13429.8^{d}
	3M	10354.7^{a}	10649.4^{ab}	10760.5^{bc}	10879.4^{bcd}	11032.9^{cde}	11160.8^{de}	11271.2^{e}
4°C	1S	10763.5^{bc}	10596.1^{b}	10621.0^{b}	10741.5^{bc}	10119.8^{a}	10765.8^{bc}	10848.0^{c}
	2B	9351.2^{a}	9386.0^{a}	9552.9^{ab}	9921.4^{c}	9834.9^{bc}	9916.4^{c}	10199.4^{d}
	1M	13581.0^{a}	13662.7^{a}	13715.3^{a}	13927.6^{b}	14024.8^{b}	13913.5^{b}	14366.8^{c}
	2M	12105.5^{a}	12222.6^{ab}	12299.4^{ab}	12597.1^{bc}	12608.8^{bc}	12897.0^{cd}	13028.7^{d}
	3M	10354.7^{a}	10467.5^{ab}	10510.6^{ab}	10652.8^{ab}	10845.3^{bc}	10819.6^{abc}	11205.6^{c}

Values for a particular column followed by different letters differ significantly ($P < 0.05$). S, sizing flour; B, break flour; M, reduction flour.

Table 3: Springiness of fresh wet noodles made from different flours during storage.

Storage temperature	Flour variety	Storage time						
		0 h	1 h	3 h	6 h	12 h	24 h	48 h
25°C	1S	0.638^{a}	0.639^{a}	0.633^{ab}	0.621^{ab}	0.589^{c}	0.609^{bc}	0.617^{ab}
	2B	0.605^{c}	0.604^{c}	0.581^{b}	0.576^{b}	0.567^{b}	0.575^{b}	0.552^{a}
	1M	0.641^{a}	0.634^{a}	0.607^{a}	0.611^{a}	0.598^{a}	0.623^{a}	0.608^{a}
	2M	0.635^{a}	0.647^{a}	0.653^{ab}	0.670^{b}	0.712^{c}	0.730^{c}	0.710^{c}
	3M	0.754^{a}	0.749^{a}	0.750^{a}	0.746^{a}	0.726^{a}	0.712^{a}	0.721^{a}
4°C	1S	0.638^{d}	0.632^{cd}	0.629^{cd}	0.646^{e}	0.615^{b}	0.624^{bc}	0.592^{a}
	2B	0.605^{c}	0.585^{bc}	0.575^{b}	0.550^{a}	0.537^{a}	0.539^{a}	0.550^{a}
	1M	0.641^{c}	0.611^{ab}	0.608^{ab}	0.615^{ab}	0.622^{bc}	0.612^{ab}	0.594^{a}
	2M	0.635^{a}	0.675^{bc}	0.666^{abc}	0.645^{ab}	0.696^{c}	0.662^{ab}	0.659^{ab}
	3M	0.754^{b}	0.743^{b}	0.753^{b}	0.754^{b}	0.732^{b}	0.708^{a}	0.704^{a}

Values for a particular column followed by different letters differ significantly ($P < 0.05$). S, sizing flour; B, break flour; M, reduction flour.

Table 4: Cooking loss rate of fresh wet noodles made from different flours during storage.

Storage temperature	Flour variety	Storage time						
		0 h	1 h	3 h	6 h	12 h	24 h	48 h
25°C	1S	4.14^{a}	4.23^{a}	4.38^{a}	4.58^{ab}	4.83^{bc}	5.10^{cd}	5.50^{d}
	2B	3.63^{a}	3.78^{b}	3.85^{bc}	3.91^{cd}	4.02^{de}	4.10^{e}	4.19^{f}
	1M	4.39^{a}	4.50^{a}	4.61^{a}	4.98^{b}	5.22^{c}	5.44^{c}	5.82^{d}
	2M	4.12^{a}	4.26^{a}	4.44^{a}	4.60^{a}	4.74^{a}	4.83^{a}	4.96^{b}
	3M	4.23^{a}	4.33^{a}	4.50^{b}	4.74^{c}	4.90^{d}	5.05^{d}	5.26^{e}
4°C	1S	4.14^{a}	4.19^{a}	4.36^{ab}	4.47^{ab}	4.66^{bc}	4.99^{cd}	5.13^{d}
	2B	3.63^{a}	3.68^{ab}	3.77^{abc}	3.84^{bcd}	3.91^{cde}	4.00^{de}	4.09^{e}
	1M	4.39^{a}	4.42^{a}	4.51^{ab}	4.63^{bc}	4.74^{cd}	4.85^{de}	5.00^{e}
	2M	4.12^{a}	4.22^{ab}	4.35^{bc}	4.46^{cd}	4.58^{de}	4.67^{e}	4.73^{e}
	3M	4.23^{a}	4.29^{a}	4.40^{b}	4.54^{c}	4.65^{d}	4.71^{d}	4.84^{e}

Values for a particular column followed by different letters differ significantly ($P < 0.05$). S, sizing flour; B, break flour; M, reduction flour.

flour, the protein colloidal particles failed to fully contact water molecules during dough kneading and fermentation due to the relatively limited amount of water. Therefore, the gluten network structure was unable to fully form in the reduction flour. Furthermore, the cooking loss rate was negatively correlated with protein content and wet gluten content. The higher the protein content, the lower the loss rate of cooking [29].

3.3. Water Properties. The relaxation time was positively correlated with the mobility of water molecules. The T_{21} expressed by the relaxation time of water was related to the presence of nonaqueous material, including gluten protein, starch, and other macromolecular substances, also known as "deep binding water" [30, 31]. The T_{22} represents the water associated with the starch/arabinoxylans, as the gelatinization process includes the absorption of water [32]. Figures 1(a) and 1(b) shows that, under different storage conditions, the relaxation time T_{21} of the bound-water of fresh wet noodles decreased as storage time increased. The T_{21} values of noodles stored at cold temperature were generally larger than those for noodles stored at room temperature, probably because the noodles stored at room

Table 5: Water absorption rate of fresh wet noodles made from different flours during storage.

Storage temperature	Flour variety	Storage time						
		0 h	1 h	3 h	6 h	12 h	24 h	48 h
25°C	1S	127.54^{e}	126.23^{e}	124.53^{de}	121.10^{d}	115.30^{c}	93.63^{b}	75.96^{a}
	2B	131.06^{e}	127.06^{de}	120.56^{de}	111.40^{cd}	101.37^{bc}	84.31^{b}	51.43^{a}
	1M	137.86^{d}	135.11^{d}	133.11^{d}	130.09^{d}	120.93^{c}	98.20^{b}	71.56^{a}
	2M	143.57^{g}	141.71^{f}	139.47^{e}	135.14^{d}	128.37^{c}	122.86^{b}	120.16^{a}
	3M	131.24^{e}	130.06^{de}	128.59^{de}	127.72^{d}	123.95^{c}	120.98^{b}	110.61^{a}
4°C	1S	127.54^{c}	123.12^{abc}	119.28^{a}	121.53^{ab}	124.81^{bc}	139.07^{d}	144.48^{e}
	2B	131.06^{d}	133.17^{d}	136.72^{e}	130.05^{d}	125.87^{c}	120.67^{b}	97.04^{a}
	1M	137.86^{b}	143.73^{c}	149.51^{d}	147.88^{d}	142.72^{c}	136.94^{b}	128.98^{a}
	2M	143.57^{cd}	146.70^{de}	148.95^{e}	146.10^{cde}	142.97^{bc}	139.67^{ab}	136.73^{a}
	3M	131.24^{c}	135.76^{d}	142.50^{f}	139.66^{e}	132.67^{c}	126.93^{b}	120.78^{a}

Values for a particular column followed by different letters differ significantly ($P < 0.05$). S, sizing flour; B, break flour; M, reduction flour.

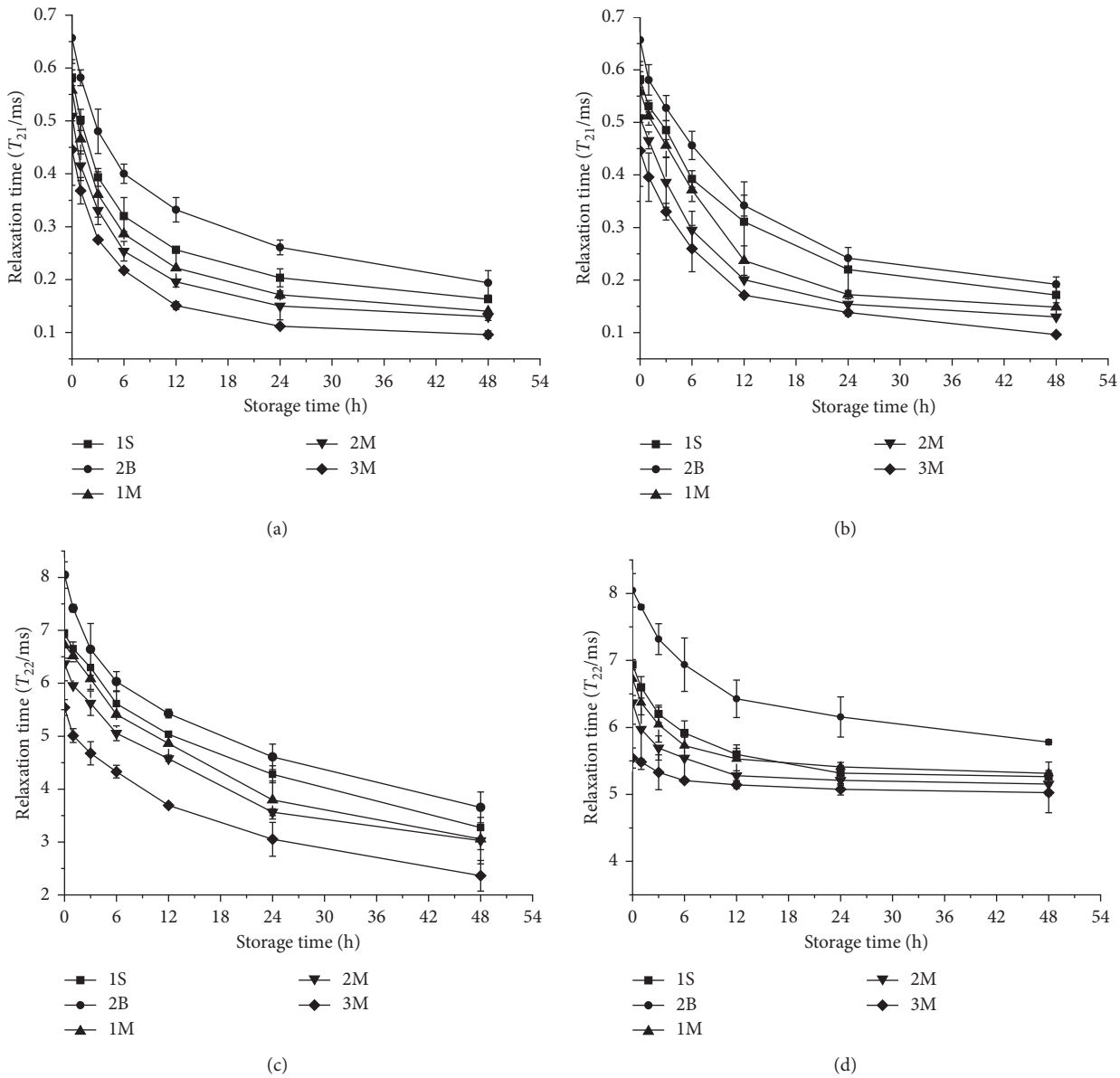

Figure 1: The water status of fresh wet noodles during storage. Relaxation time T_{21} at 25°C (a); T_{21} at 4°C (b); T_{22} at 25°C (c); T_{22} at 4°C (d).

Table 6: Correlation analysis between moisture and quality indicators of wheat flour.

Flour variety	Storage temperature	Water status	Hardness	Springiness	Water absorption rate	Cooking loss rate
1S	25°C	T_{21}	−0.964**	0.757*	0.826*	−0.939**
		T_{22}	−0.940**	0.646	0.957**	−0.999**
	4°C	T_{21}	−0.043	0.726	−0.760*	−0.988**
		T_{22}	0.050	0.657	−0.626	−0.953**
2B	25°C	T_{21}	−0.976**	0.942**	0.922**	−0.992**
		T_{22}	−0.971**	0.933*	0.961**	−0.991**
	4°C	T_{21}	−0.924**	0.902**	0.804*	−0.993**
		T_{22}	−0.950**	0.924**	0.787*	−0.995**
1M	25°C	T_{21}	−0.989**	0.724	0.806*	−0.947**
		T_{22}	−0.948**	0.506	0.954**	−0.993**
	4°C	T_{21}	−0.935**	0.557	0.536	−0.980**
		T_{22}	−0.899**	0.655	0.319	−0.938**
2M	25°C	T_{21}	−0.951**	−0.931*	0.946**	−0.993**
		T_{22}	−0.992**	−0.920**	0.991**	−0.969**
	4°C	T_{21}	−0.969**	−0.328	0.717	−0.996**
		T_{22}	−0.923**	−0.454	0.555	−0.979**
3M	25°C	T_{22}	−0.882**	0.877**	0.818*	−0.972**
		T_{22}	−0.704	0.902**	0.950**	−0.988**
	4°C	T_{21}	−0.905**	0.836*	0.584	−0.991**
		T_{22}	−0.889**	0.771*	0.498	−0.981**

*, **Correlation coefficient is significant at $P < 0.05$ and 0.01, respectively. S, sizing flour; B, break flour; M, reduction flour.

temperature suffered a greater loss of moisture content, the mobility of protons decreased, and there was diminished signal amplitude of the corresponding protons [33]. For the fresh wet noodles made from different flours, 2B showed the maximum relaxation time T_{21}, and 3M showed the minimum value. This difference was likely due to the high moisture content of 2B and the low moisture content of 3M.

Figures 1(c) and 1(d) show that, under different storage conditions, the extension of storage time for all fresh wet noodles gradually decreased the relaxation time T_{22} of the intermediate state water, and the water transformed from the combinative state to the intermediate state, for an overall decrease of total water content. Wang et al. also determined the water status in the noodle drying process using a low-field nuclear magnetic resonance analyzer, and reported that the weakly bonded water with transverse relaxation time T_{22} accounted for the largest proportion of water and the T_{22} value decreased gradually with drying time [34]. The relaxation time T_{22} was larger for noodles subjected to cold storage compared to noodles stored at room temperature. These differences are likely because of the higher moisture content, stronger proton mobility, and larger proton signal amplitude of the noodles stored in the cold. The largest relaxation time T_{22} was for 2B, and the 3M flour noodles showed the smallest value of T_{22}, in agreement with the observed relaxation time T_{21}.

The relaxation time T_2 reflects the number of water molecules with spin-spin relaxation time in the range of proton mobility. The decrease of relaxation time T_{22} and T_{21} is related to the migration and redistribution of water molecules in different states. For fresh wet noodles stored under different storage conditions, the hardness, acidity, and cooking loss rate increased with the decrease of the relaxation time T_{21} and T_{22}, and the whiteness, cohesiveness, springiness, and the water absorption of dry matter decreased. He et al. reported changes in the NMR parameters related to aging and moisture redistribution in steamed bread, and changes in moisture distribution were key to the aging process of steamed bread [35].

3.4. Correlation Analysis. The correlation analysis of quality characteristics and NMR parameters of fresh wet noodles made from different flours and stored under different storage conditions were determined and are shown in Table 6. The hardness values of all system powders were significantly negatively correlated with T_{21} and T_{22}. Thus, the lower the water content (including both the bound water and the mobile water), the greater the hardness of noodles. The springiness showed positive correlation with both T_{21} and T_{22} values for 2B and 3M, with significant correlation. This result indicated that sufficient moisture content in noodles can help maintain the springiness of noodles. The cooking loss rate of all fresh wet noodles showed a highly significant negative correlation with T_{21} and T_{22}, and the correlation coefficients were above 0.9. Under room temperature storage, most water absorption rates for the powders showed a significant positive correlation with T_{21} and T_{22}. However, the correlations between the water absorption of dry matter and T_{21} and T_{22} were not significant during cold storage. Thus, under different storage conditions, the water status was significantly correlated with the quality of the flour in fresh wet noodles and the migration changes of different states of water affected the quality characteristics of noodles. Therefore, it may be feasible to predict changes in noodle quality through changes in the state of water.

4. Conclusions

Overall, the noodles made from 2B flour had lower whiteness, hardness, springiness, cooking loss rate, and

water absorption rate of dry matter, compared with noodles made from reduction flour or sizing flour. With extended storage time, the relaxation time T_{21} and T_{22} decreased over 24 hours for all noodles. The relaxation time of noodles after storage at cold temperature was greater than that for noodles stored at room temperature. Noodles made from 2B flour showed the maximum relaxation time, and 3M noodles exhibited the minimum value. Clearly, the migration changes of different states of water influenced the quality characteristics of the noodles. In sum, 2M is the best type of flour for making wet noodles.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The present research was financially supported by the State Key Research and Development Plan "Modern Food Processing and food Storage and Transportation Technology and Equipment" (2017YFD0400200), National Natural Science Foundation of China (nos. 31571873 and U1704118), Henan Province Colleges and Universities Young Backbone Teacher Plan (2016GGJS-070), Key Scientific and Technological Project of Henan Province (172102110008), National University Students' Innovation and Entrepreneurship Training Program (201710463002), and Opening Foundation of Province Key Laboratory of Transformation and Utilization of Cereal Resource in Henan University of Technology (PL2016003).

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Hindawi
Journal of Chemistry
Volume 2018, Article ID 1597308, 7 pages
https://doi.org/10.1155/2018/1597308

Research Article

Effects of Fermented Wheat Bran on Flour, Dough, and Steamed Bread Characteristics

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Received 31 August 2017; Revised 13 December 2017; Accepted 12 April 2018; Published 19 June 2018

Academic Editor: Yanjie Bai

Wheat bran is rich in dietary fiber, which improves the flour nutritional content and also endows the flour with a richer flavor. However, a high content of insoluble dietary bran fiber can easily and significantly reduce the processing and edible quality of flour products. This study was conducted to explore ways to decrease the negative effects of adding bran to dough. Basidiomycete strain BS-01 was used to ferment the wheat bran. The surface structure of the bran was examined by scanning electron microscopy, and the fermented bran was incorporated into the wheat flour at various concentrations. The mixed flour farinographic and extensographic characteristics, dough rheological properties, and the specific volume, color, and textural properties of the steamed bread were determined and analyzed. The results suggested that adding an appropriate quantity of fermented bran improves the characteristics of the dough and the quality of the steamed bread compared to those with unfermented bran. The fermented bran effectively decreased the negative impacts exerted on the farinographic and extensographic characteristics of the mixed flour and exerted a positive influence on the dough viscoelasticity and bread specific volume.

1. Introduction

Wheat bran is the outer layer of the wheat kernel and an excellent source of dietary fiber (DF), which contains several nutrients, such as starch, protein, fat, minerals, and vitamins [1]. It has a wide range of applications in the modern food industry; for example, many popular foods of cereal origin, such as noodles, breads, and cookies, are good carriers of wheat bran DF [2]. However, due to the fibrous external layer, adding wheat bran can decrease the product sensory quality [3].

The content of DF in wheat bran is 35–50%, whereas the content of soluble DF is only 2-3% [4]. However, for wheat bran DF to be considered as a high-quality DF supplement, it requires a soluble DF content >10%; otherwise, it is only considered as filling DF [5]. Thus, many researchers have been dedicated to modify wheat bran DF so as to improve the soluble DF content in wheat bran. Nevertheless, there is still a lack of studies on the impact of insoluble DF on food eating quality. Moreover, insoluble DF impairs gas cell stabilization while making flour products, which reduces the products quality [6]. Adding raw wheat bran has a detrimental effect on the rheological properties of dough and steamed bread quality, dough stretching energy, elongation, and tensile resistance. For instance, the maximum tensile resistance decreases gradually as the amount of wheat bran added increases. Also, the hardness, gumminess, and chewiness of steamed bread increase gradually, whereas resilience, cohesiveness, and recovery decrease [7, 8].

To improve the eating quality and nutrition, many researchers have focused on modifying the properties of wheat bran, and several microorganisms have been adopted for fermentation [9–11]. Fermented bran increases the content and bioavailability of several functional compounds, such as water-extractable arabinoxylans, total free phenols, and soluble fiber, but only has a slight impact on physical properties. As a result, when forming the dough, the pH and

total acidity values increase, and the maximum height, gas retention, and stabilization time of the dough decrease after adding wheat bran fermented by *Lactobacillus* [12]. Even after processing by complex microbial agents (yeast, *Lactobacillus*, and the sweet wine koji), dough extensibility and the texture of steamed bread become worse after mixing with fermented bran [13]. These properties make it very difficult to improve the functional quality of flour products by mixing in wheat bran.

The insoluble DF content includes up to 90% hemicellulose and lignin [4]. Lignin fiber is strongly chemically resistant because of the conserved units, complex structure, random chemical bonding, and high molecular weight. Lignin and hemicellulose are linked by covalent bonds in the wheat bran and form a dense structure with cellulose embedded in the structure [14]. This solid structure prevents the use of common methods to sufficiently modify wheat bran and greatly reduces the nutritional value and eating quality of wheat bran and the food products containing it [15]. The soluble DF content in edible wheat bran can be increased, and the quality and nutrition can be effectively improved by targeted removal of the structural barrier formed by lignin and hemicellulose and disassembly of the interspace reticular structure. Accordingly, the Basidiomycetes which have been proved to exhibit a wide range of lignin and hemicellulose degradation activities become the optimal choice in wheat bran DF modification [16].

In this study, the Basidiomycete strain BS-01 was used to ferment wheat bran. Scanning electron microscopy (SEM) analysis was used to evaluate the effect of fermentation on the bran surface structure. In addition, the fermented bran was incorporated at various concentrations into wheat flour and steamed bread. Then, the flour farinographic and extensographic characteristics, dough rheological properties, technological qualities, and the correlations between them were determined. Additionally, the specific volume, color, and textural properties of bran mixed with steamed bread were also evaluated. This study may stimulate further interest in the use of fermentation to modify edible wheat bran.

2. Materials and Methods

2.1. Materials. Wheat flour (11.59% moisture, 0.59% ash, and 12.02% protein) was obtained from ZhengZhou TDR Flour Industrial Co., Ltd. (Zhengzhou, China). Wheat bran was obtained from Guangdong Baiyan Grain & Oil Industrial Co., Ltd. (Foshan, China). Yeast powder was purchased from Angel Yeast Co. (Yichang, China). BS-01 (CGMCC 3.7572) was obtained from the China General Microbiological Culture Collection Center (Beijing, China). α-Amylase A4551 (10 U/mg) and protease K P-6556 (30 U/mg) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemical reagents were purchased from Luoyang Chemical Reagents Factory and used directly.

2.2. Stock Culture Conditions. The stock culture was maintained on potato dextrose agar (PDA) slants at 4°C with periodic transfer. The PDA medium contained 200 g potato infusion, 20 g glucose, 3 g KH_2PO_4, 1.5 g·$MgSO_4$, and 15 g agar per liter. The mycelium from the slant was transferred to PDA plates and incubated at 28°C for 7 days to produce the inoculum. Five mycelial discs (5 mm diameter) were removed from the peripheral region of the PDA plates and used to inoculate Erlenmeyer flasks (250 mL) containing 50 mL of liquid potato dextrose (PD) medium. The cultures were incubated at 28°C on a rotary shaker incubator at 160 rpm/min [17].

2.3. Bran Fermentation. The solid culture medium contained 2 g sugar, 2 g $CaSO_4{\cdot}2H_2O$, and 196 g raw wheat bran per 200 g [18]. The moisture content of the medium was adjusted to 55 mL/100 g, and the medium was loaded into an autoclavable gusseted breathable plastic bulk mushroom grow bag. The loaded bags were prepared in triplicate and autoclaved at 121°C and 15 psi for 120 min. After cooling the bags to room temperature, they were inoculated with 2 ml of the BS-01 spore suspension and then incubated under standard conditions (22 ± 2°C and 60% relative humidity) for 10 days. The raw and fermented wheat bran was dried in a vacuum drier at 60°C until the moisture content was 3-4% and then pulverized and filtered through a 100-mesh sieve, and the screen residue were collected for SEM analysis.

2.4. Scanning Electron Microscopic Analysis. SEM was used to evaluate the effect of fermentation on wheat bran characteristics. The bran samples were suspended in 250 mL of 0.5 M sodium phosphate buffer (pH 6.5), and α-amylase was added to 3% and incubated in a water bath at 60°C for 1 h. Then, the pH value of the suspension was adjusted to 4.5, protease was added to in 3%, and the samples were incubated in a water bath at 60°C for 2 h. After the enzyme treatment, the samples were rinsed three times with the same buffer and dehydrated (twice in each solution) through a graded ethanol series (20, 40, 60, 80, 95, and 100%) for 10 min at each concentration. After drying to constant weight (48°C for 72 h), the samples were sputter-coated with palladium gold in the Emitech K550 and observed under a Zeiss DSM 940 A SEM system (Carl Zeiss, Oberkochen, Germany) at 2,000x magnification.

2.5. Steamed Bread Preparation. Steamed bread was prepared according to the sponge dough method described by Song et al. [19] with some modifications. Batches of 100 g of mixed flour (14.0% moisture base mixed with 0, 3, 6, 9, and 12% fermented or unfermented wheat bran powder by weight), 1 g active dry yeast, and water were mixed at low speed for 10 min using a flour mixer (JHMZ200, East Fude Technology Development Center, Beijing, China). Water content was about 85% of the water absorption of flour. Dough was leavened in a fermenting box for 1 h at 38°C and 85% relative humidity after mixing. The dough was sheeted 15 times at 3.5 mm (YMG110, Jiangsu Hengyue Co. Ltd., Jiangsu, China), rolled into a long cylindrical shape by hand, and divided into pieces of a specified weight (100 g pieces) and round in shape. Ultimately, the dough pieces were steamed

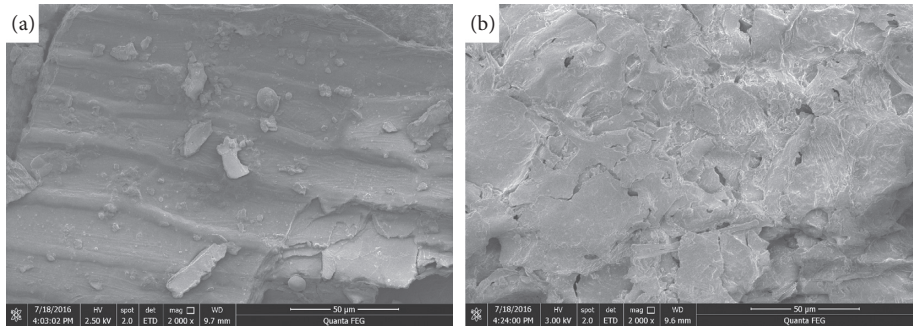

Figure 1: Scanning electron microscopic images of the surface structure of wheat bran: (a) unfermented wheat bran and (b) fermented wheat bran.

for 30 min in a steamer and boiling water (WSYH26A, Midea Co., Ltd., Guangdong, China). The steamed bread was covered with gauze and cooled at room temperature for 60 min before the quality evaluation. Each dough treatment was performed in duplicate.

2.6. Farinographic and Extensographic Properties. The rheological properties of the flour samples of nonfermented dough (flour water system) were measured with a farinograph (Farinograph-E, Brabender, GmbH & Co. KG, Duisburg, Germany) and an extensograph (Brabender Extensograph-E) following the AACC 54-21 and 54-10 standard methods.

2.7. Rheological Measurement. The dynamic rheological properties of the dough samples were determined with a rotational rheometer (MARSIII, Haake, Vreden, Germany) using the measuring system plate-plate (HPP 20) following the method of Stojceska et al. [20].

2.8. Steamed Bread Volume and Color Measurements. The bread volume, expressed as mL/g, was determined according to AACC Method 10-05 (AACC, 2000). Color values were measured with a Minolta CM-508i spectrophotometer (Minolta Co. Ltd., Tokyo, Japan) using the D65 standard illuminant and the 2° standard observer.

2.9. Steamed Bread Texture Measurements. The textural analysis was performed with a Texture analyzer (Model: TA-XT2i, Texture Technologies Corp., Scarsdale, NY, USA), equipped with a 25 mm diameter aluminum cylindrical probe. The steamed bread was sliced horizontally, and a bottom piece of 25 mm in height was compressed to 50% of the original height. Test conditions were as follows: pretest speed 3 mm/s, test speed 1 mm/s, and posttest speed 5 mm/s. The analysis was performed in triplicate.

2.10. Statistical Analysis. For all analyses, two technical replicates were used, and the data were statistically compared by analysis of variance (ANOVA). The significant differences between two samples were analyzed by Duncan's test using the SPSS software (version 15.0, SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered significant.

3. Results and Discussion

3.1. SEM Microstructure Analysis. SEM was used to examine the effect of fermentation on the surface microstructure of wheat bran.

The microstructure of the surface of two bran samples is shown in Figure 1. Specifically, raw wheat bran (Figure 1(a)) had a compact, smooth surface structure with many sharp edges. In comparison, due to the effective decomposition of the bran fiber by fermentation, the surface of the fermented bran (Figure 1(b)) was porous and rugged, with the Basidiomycete mycelia occupying the interspaces between the bran cells and concentrated in the microholes of the bran surface. The bran cell walls were broken down from the lumens to a compound middle lamella, while the bran cellulose, hemicellulose, and lignin were degraded by microbial metabolites. After fermentation, the porous surface structure was favorable to improve the rehydration and water absorption capacity of the bran mixed products. Moreover, the unsharpened edges helped reduce the negative effect of bran on the gas-holding capacity of the dough.

3.2. Farinograph Characterization of Bran Mixed Flour. The farinograph dough development time (DDT) represents the formation speed of the gluten network after wheat flour is combined with water [21]. The results listed in Table 1 show that the DDT was constantly prolonged as the proportion of wheat bran increased, but the DDT of the dough mixed with fermented bran was shorter than that of raw bran in most cases.

The dough stability time (ST), breakdown time (BT), and the quality index (QI) were used to assess the mixing tolerance of the bran mixed flour [22]. The protein concentration in the mixed flour was lower when the bran content was higher, which prevented formation of a good viscoelastic gluten network. The results show that the ST, BT, and the three QIs of the fermented bran mixed flour were highest when the bran content was 3% and then decreased gradually. The rate of decrease of the mixed fermented bran sample was slower than that of the unfermented bran. The softening degree (SD) of the mixed flour dough increased correspondingly with the increase of the bran content, and the extent of the increase was

Table 1: Different amounts of added bran and the farinographic properties of the mixed flour.

Added amount	Bran state	DDT (min)	ST (min)	SD (FU)	BT (min)	QI
0%		5.2^{b}	7.6^{a}	76^{d}	9.5^{a}	95^{a}
3%	Fermented	6.1^{a}	7.9^{a}	89^{c}	10.0^{a}	100^{a}
	Raw	4.8^{b}	7.1^{a}	79^{d}	9.2^{a}	92^{a}
6%	Fermented	4.1^{b}	7.5^{a}	102^{b}	8.9^{a}	89^{a}
	Raw	6.0^{a}	6.7^{b}	95^{c}	8.9^{a}	89^{a}
9%	Fermented	5.4^{b}	6.9^{a}	116^{a}	8.6^{b}	86^{b}
	Raw	5.7^{a}	5.8^{b}	105^{b}	8.5^{b}	85^{b}
12%	Fermented	6.8^{a}	6.9^{a}	123^{a}	9.1^{a}	91^{a}
	Raw	6.6^{a}	5.7^{b}	110^{b}	8.7^{b}	87^{b}

Values for a particular column followed by different letters differ significantly ($p < 0.05$). DDT, dough development time; ST, stability time; SD, softening degree; BT, breakdown time; QI, qualitative index.

Table 2: Different amounts of added bran and the extensographic properties of the mixed flour.

Added amount	Bran state	EE (cm^2)	ES (mm)	RE (FU)	MR (FU)	SR	Maximum SR
0%		70.5^{a}	147.0^{a}	301.0^{b}	371.0^{a}	2.10^{b}	2.55^{e}
3%	Fermented	73.0^{a}	125.0^{a}	355.5^{a}	416.5^{a}	3.00^{b}	3.35^{a}
	Raw	58.5^{b}	115.0^{b}	341.5^{a}	370.0^{a}	3.00^{b}	3.25^{b}
6%	Fermented	58.0^{b}	114.0^{b}	340.0^{a}	358.0^{a}	2.80^{b}	3.20^{c}
	Raw	51.5^{c}	104.5^{c}	339.0^{b}	355.5^{a}	3.25^{a}	3.40^{a}
9%	Fermented	46.5^{c}	94.5^{c}	349.0^{a}	352.5^{a}	3.70^{a}	3.70^{a}
	Raw	45.0^{d}	97.0^{c}	331.5^{b}	341.5^{b}	3.45^{a}	3.50^{a}
12%	Fermented	39.5^{d}	93.0^{c}	291.0^{b}	292.0^{b}	3.10^{b}	3.10^{c}
	Raw	42.5^{d}	96.5^{c}	319.5^{b}	322.5^{b}	3.35^{a}	3.35^{a}

Values for a particular column followed by different letters differ significantly ($p < 0.05$). EE, extension energy; ES, extensibility; RE, resistance to extension; MR, maximum resistance; SR, stretching ratio.

more evident when the mixed bran was fermented. Taken together, the farinograph properties of the fermented bran mixed flour were relatively lower than those of the unfermented flour with equal amounts of added bran.

3.3. Extensographic Characterization of Bran Mixed Flour. Resistance to extension (RE) indicates the strength of the gluten network and gas-holding capacity of the dough [23]. The results shown in Table 2 indicate that adding more than 3% bran caused a gradual decline in the RE. However, the RE and maximum resistance (MR) values of the fermented bran mixed flour were higher than those in the unfermented group, when the amount of bran added was <12%.

Extensibility (ES) is indicative of the ductility and plasticity of the dough, and the extension energy (EE) provides information about the strength of the gluten and the baking properties of the flour [24]. The results show that although the ES and EE decreased steadily with the increase of the amount of bran added, but when the amount added was <12%, both two indices are relatively higher in the fermented sample than in the unfermented one under the same bran added amount.

The dough stretching ratio is the maximum resistance to the extensibility ratio. The results in this study show that the stretching ratio of the bran mixed dough increased steadily, as unfermented bran was added, while the ratio of the fermented sample initially increased and then decreased. This extensographic properties analysis showed that adding fermented bran enhanced the strength of the dough network and had a better effect on the dough than unfermented bran with less bran was added.

3.4. Rheological Characterization of Bran Mixed Dough. The dough viscoelasticity represents the structural strength and degree of combination with water and has important effects on the flour product making process [25]. The average values of the elastic modulus (G'), viscous modulus (G''), and $\tan\delta$ (G''/G') for the fermented and unfermented bran mixed dough are shown in Figures 2–4.

The data presented in Figures 2 and 3 reveal that when the same amount of bran was added, the G' and G'' values of the fermented samples were higher than those of the unfermented samples, and both values were relatively higher, when the amount of bran added was 6% or 12%. This indicates that adding fermented bran favors the formation of the gluten network and enhances dough viscoelasticity. Moreover, the G' and G'' values of the 9% fermented bran samples were all higher than those of the 3% samples, demonstrating that adding fermented bran improved the gas-holding capacity of the dough. However, similar trends in the G' and G'' values were not observed for the unfermented groups.

The $\tan\delta$ indicates the relative contributions of the viscous (G'') and elastic (G') characteristics of the material. When the material behaves more like a solid, that is, when deformation within the linear range is essentially elastic and recoverable, the elastic character, G', exceeds the viscous character, G'', and $\tan\delta < 1$. On the other hand, when the material behaves more like a liquid or viscous system, then

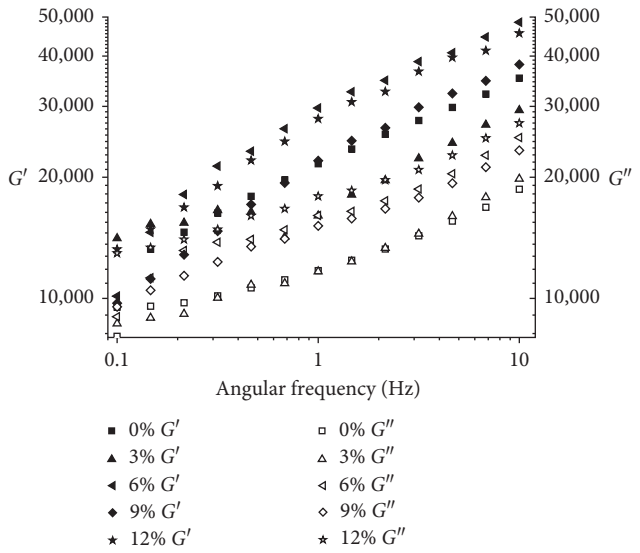

Figure 2: The effect of fermented bran addition on the G' and G'' of bread dough.

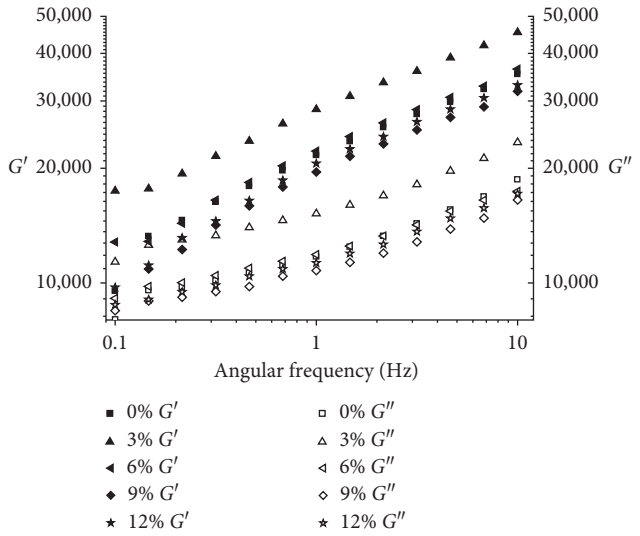

Figure 3: The effect of unfermented bran addition on the G' and G'' of bread dough.

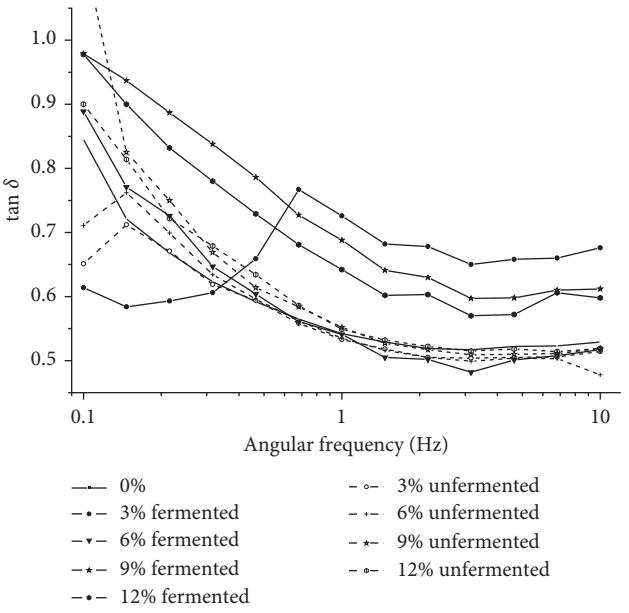

Figure 4: The effect of fermented and unfermented bran addition on the tan δ of bread dough.

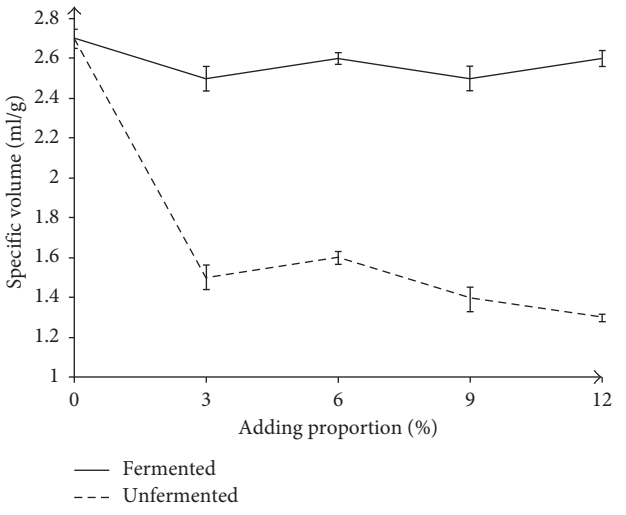

Figure 5: The effect of bran addition on the specific volume of steamed bread.

the viscous character, G'', predominates and tan $\delta > 1$ [26]. The results presented in Figure 4 indicate that the tan δ value of the bran mixed dough gradually decreased as the amount of the unfermented bran was increased, and the tan δ value of the fermented bran mixed dough changed from randomly scattered to consistent with the increase of the shear rate. This probably occurred because the fermentation changed the physical structure and chemical composition of the bran, and these complex physical and chemical changes led to the irregular shifts in the dough viscoelasticity.

3.5. Bread Volume, Color, and Texture of Bran Mixed Flour. The specific volume is an important indicator used to estimate gas-holding capacity of steamed bread dough. Wheat bran contains a high raw fiber content, which can damage the gluten network and break the gas-holding cells in the dough. Accordingly, adding bran likely affects the specific volume negatively, as confirmed by the results shown in Figure 5.

The specific volume of steamed bread initially increased and then decreased as the amount of unfermented bran added increased (Figure 5). The increase was steady when fermented bran was used and was more than twice that of the unfermented bran mixed sample with the same amount of added bran. This finding indicates that fermentation diminished the negative effect of adding bran on the quality of steamed bread.

The color values of steamed bread were recorded as L^* (0, black; 100, white), a^* (−100, green; +100, red), and

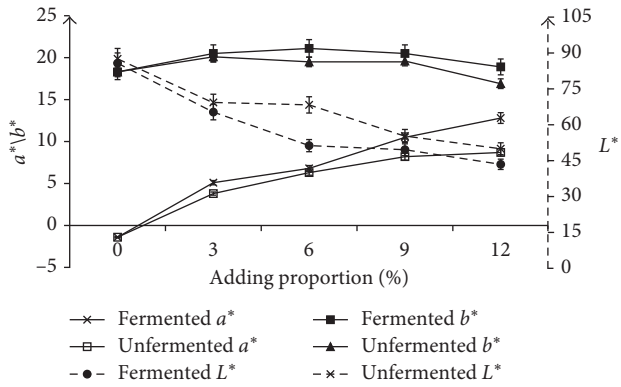

Figure 6: The effect of bran addition on the color of steamed bread.

Table 3: Different amounts of added bran and the texture of mixed steamed bread.

Addition amount	Bran state	Hardness (g)	Resilience	Cohesiveness	Springiness	Adhesiveness	Chewiness
0%		2528.89^b	41.19^a	0.76^a	94.28^a	1921.77^b	1811.13^b
3%	Fermented	11775.18^a	32.29^b	0.67^a	88.17^b	7875.12^a	6967.19^a
	Raw	3359.18^b	40.67^a	0.77^a	90.33^a	2594.86^b	2344.12^b
6%	Fermented	17017.31^a	29.68	0.61	85.98^b	10326.57^a	8880.28^a
	Raw	5432.35^b	42.90^a	0.82^a	91.96^a	4445.68^a	4089.49^a
9%	Fermented	13627.58^a	26.74	0.58^b	80.03^b	7938.69^a	6347.52^a
	Raw	5244.33^b	41.31^a	0.79^a	88.28^b	4143.02^b	3659.05^a
12%	Fermented	10350.28^a	31.65^b	0.64^b	49.81	6165.72^a	3716.70^a
	Raw	4183.14^b	41.01^a	0.79^a	87.59^b	3323.32^b	2910.51^b

Values for a particular column followed by different letters differ significantly ($p < 0.05$).

b^* (−100, blue; +100, yellow). As shown in Figure 6, the L^* value of steamed bread steadily decreased as the amount of bran added was increased and significantly decreased in the fermented bran mixed group. The a^* and b^* values of steamed bread increased with the increase of the amount of bran added. All fermented bran mixed sample values were relatively higher, but within acceptable limits. Fermentation deepened the color of the wheat bran.

The hardness, adhesiveness, and chewiness values represent the chewy texture of steamed bread, and higher values indicate a worse texture. On the other hand, the springiness, cohesiveness, and resilience represent the chewing quality of steamed bread, and higher values mean chewier bread [27]. The results listed in Table 3 show that bran had a strong effect on the textural quality of steamed bread, regardless of bran fermentation. The hardness, adhesiveness, and chewiness values increased as the amount of bran added increased, whereas springiness, cohesiveness, and resilience significantly decreased, due to a decline in the bread specific volume and water-absorbing capacity of gluten caused by the addition of fiber-rich bran.

The increases in bread hardness, adhesiveness, and chewiness and the decreases in springiness, cohesiveness, and resilience were sharper as the amount of fermented bran added increased, compared with the unfermented bran mixed samples. The changing tendencies of the hardness, adhesiveness, and chewiness of steamed bread were similar. Bread resilience improved slightly when the amount of fermented bran added was ≤9%.

4. Conclusion

The results obtained in this study suggest that adding fermented wheat bran improved the characteristics of the dough and improved some quality characteristics of steamed bread compared to unfermented bran. Fermentation by the BS-01 strain modified the bran structure, efficiently decreased the negative effects on the farinographic and extensographic characteristics of the mixed flour caused by the addition of bran, and exerted a positive influence on the dough viscoelasticity and bread specific volume. These results indicate that modifying wheat bran by fermentation is an effective method to improve the quality of high fiber flour products.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The present research was financially supported by National Natural Science Foundation of China (nos. 31671892 and U1704118), National College Students Innovation and Entrepreneurship Training Program Project (201710463002), Opening Foundation of National Engineering Laboratory for Wheat & Corn Further Processing in Henan University of Technology (NL2016004), and Foundation Research

Funds for the Henan Provincial Colleges and Universities in Henan University of Technology (2016QNJH18).

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Hindawi
Journal of Chemistry
Volume 2018, Article ID 3082026, 10 pages
https://doi.org/10.1155/2018/3082026

Research Article

Acetylation Modification Improves Immunoregulatory Effect of Polysaccharide from Seeds of *Plantago asiatica* L.

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Received 17 October 2017; Revised 13 December 2017; Accepted 8 January 2018; Published 13 February 2018

Academic Editor: Ji Kang

The current study was conducted to investigate effects of acetylated *Plantago asiatica* L. polysaccharides (Ac-PLCPs) on their immunoregulatory activities in bone-marrow-derived dendritic cells (DCs) model. Influences of natural *Plantago asiatica* L. crude polysaccharide (PLCP) and Ac-PLCPs on inducing phenotypic and functional maturation on DCs were determined. The results showed that Ac-PLCPs with degree of substitution (DS) from 0.06 to 0.1 could not only stimulate the expression of surface molecules major histocompatibility complex class II (MHC II), cluster of differentiation 86 (CD86), and CD80 on DCs ($P < 0.01$) but also increase the secretion of cytokine IL-12p70 ($P < 0.01$). The endocytosis activity of DCs was attenuated by Ac-PLCPs treatment ($P < 0.01$), while the mRNA expressions of chemokine receptors CCR7 and CXCR4 in DCs were significantly increased ($P < 0.01$). Besides, DCs treated with the Ac-PLCPs showed extremely strong T cell proliferation stimulating activity ($P < 0.01$). These data showed that Ac-PLCPs had higher maturation-stimulating activity on DCs than PLCP, which indicated that acetylation modification improved the immunoregulatory effect of PLCP.

1. Introduction

Bioactive polysaccharides have attracted extensive attention worldwide, and they are now considered as one functional component of traditional medicine. A great deal of researches indicate that bioactivity of polysaccharide is closely correlated with various structural parameters, such as molecular weight, monosaccharide composition, branches, conformation, and substituent group. Recently, a growing body of research implicates that appropriate structure modification can improve bioactivity of polysaccharide [1]. So far, lots of chemical modifications have been applied to modulate physicochemical or biological properties of polysaccharides, such as carboxymethylation [2, 3], sulfation [4], acetylation [5], phosphorylation [6], and oxidation [7] modification.

Acetyl content in some natural polysaccharides has been demonstrated to play an important role in their bioactivities. For example, the lymphocyte proliferation stimulating activity of polysaccharide from *Dendrobium nobile* mainly depended on the acetyl groups [8, 9]. Acetylated *Ulva pertusa* [10], *Laminaria japonica* polysaccharide [11], and *Enteromorpha linza* polysaccharide [12] have higher antioxidant activity. Acetylated *Grifola frondosa* polysaccharide peptides can inhibit glioma C6 cell line *in vitro*, and the combination treatment of cyclophosphamide and the acetylated polysaccharide peptides can significantly decrease total sialic acid levels in tumor-bear mice [13]. Acetyl fucoidan from *Cladosiphon okamuranus* could activate murine macrophage cell line, RAW 264.7 cells, through membrane receptor, toll-like receptor 4 (TLR4), cluster of differentiation 14 (CD14), and scavenger receptor class A [14].

Psyllium is a common name used for the plant genus *Plantago*. In western countries, dietary fiber from psyllium has been used extensively as both pharmacological supplements and food ingredients. Ripe seeds of *Plantago asiatica* L. are a traditional medicine used as antipyretic, diuretic, and expectorant treatment in China. Our previous study showed that polysaccharide from seeds of *Plantago asiatica* L. was a high branched heteroxylan consisting of β-1,4-linked Xyl*p* backbone with side chains attached to O-2 or O-3 position. And its side chains consist of β-T-linked Xyl*p*, α-T-linked Ara*f*, α-T-linked GlcA*p*, β-Xyl*p*-(1→3)-α-Ara*f*, and α-Ara*f*-(1→3)-β-Xyl*p* [15]. In particular, the polysaccharide was a potential immune adjuvant since it could promote

maturation of mouse bone-marrow-derived dendritic cells *in vitro* [16].

In the present study, *Plantago asiatica* L. crude polysaccharide (PLCP) were acetylated using acetic anhydride as acetylation reagent, and bioactivities of the acetylated polysaccharides (Ac-PLCP) were investigated on dendritic cells model.

2. Materials and Methods

2.1. Preparation of Acetylated Plantago asiatica L. Polysaccharides. PLCP was prepared as previously described [17]. Briefly, water-soluble polysaccharide was extracted from the seeds of *Plantago asiatica* L. by boiling water for 2 h and precipitated with 80% (v/v) ethanol. Then the polysaccharide was deproteinised with sevage reagent, a mixture of chloroform and n-butyl alcohol at a ratio of 4 : 1 (v/v), dialysed against double distilled water for 48 h (Mw cut-off, 8000–14,000 kDa), and obtained by freeze-drying.

Acetylation modification of PLCP was carried out using acetic anhydride as acetylation reagent [18]. One hundred milligram PLCP was firstly dissolved in 10 mL distilled water (pH 9.0). The solution was maintained at 30°C with continuous stirring. Acetic anhydride (400–2000 μL) was added to the solution dropwise within 2 h (pH 8.5–9.0). After the modification reaction, the mixture was neutralized with 1 M HCl and dialysed against double distilled water for 48 h (Mw cut-off: 8000–14,000 kDa). The acetylated PLCP (AC-PLCP) was obtained by freeze-drying.

The degrees of substitution (DS) of the acetyl group in the Ac-PLCPs were determined as described [19]. Briefly, 40 mg Ac-PLCP was dissolved in 20 mL 0.01 M NaOH. The solution was maintained at 50°C for 2 h, then excess NaOH was back-titrated with 0.01 M HCl using phenolphthalein indicator. Four Ac-PLPCs with gradient degrees of substitution, that is, DS of 0.027 (Ac-PLCP I), 0.059 (Ac-PLCP II), 0.082 (Ac-PLCP III), and 0.11 (Ac-PLCP IV), were selected to investigate the immune regulatory activity in dendritic cell model.

In addition, the bacterial endotoxins in PLCP and Ac-PLCPs were determined using tachypleus amebocyte lysate test kit (Chinese Horseshoe Crab Reagent Manufactory, Co., Fujian Province, China). The quantity of endotoxin was estimated to be ≤0.015 endotoxin unit (EU) per mg in all polysaccharide samples.

2.2. Animals. 4- to 6-week-old BALB/c (H-2K^d and I-A^d) and C57BL/6 (H-2K^d and I-A^d) mice were purchased from Hunan SJA Laboratory Animal, Co. (Hunan Province, China). All animals were kept according to the Care and Use of Laboratory Animals Guidelines published by the United States National Institute of Health (NIH Publication 85-23, 1996). All experimental procedures were approved by the Animal Ethnics Committee, Nanchang University.

2.3. Generation of Bone-Marrow-Derived Dendritic Cells (DCs). Bone marrow of BALB/c mice was sacrificed to obtain dendritic cells. According to our previous report [16], bone-marrow cells were flushed out with RPMI 1640 medium, then recombinant murine granulocyte macrophage-colony stimulating factor (rmGM-CSF, 10 ng/mL) combined with recombinant murine interleukin-4 (rmIL-4, 10 ng/mL, both from R&D system) was used to generate immature dendritic cells.

2.4. Determination of Phenotypic and Functional Maturation of DCs. Immature DCs were stimulated with the PLCP or Ac-PLCPs (100 μg/mL) for 48 h, and then the phenotypic and functional maturation of DCs were determined according to the methods reported previously [20].

Briefly, the cells were collected and incubated with FITC-conjugated monoclonal antibody [anti-major histocompatibility complex (MHC) class II, anti-CD80 (B7-1), and anti-CD86 (B7-2), eBioscience] for 1 h, and then the mean fluorescence intensity was determined by using FACSCalibur flow cytometer (BD Biosciences, USA). The endocytosis activity of DCs was also determined by flow cytometry using FITC-dextran (40,000 Da, Sigma). The quantity of the cytokine IL-12p70 in the culture supernatant was measured using Mouse IL-12p70 Enzyme Immunoassay kit (Wuhan Boster Biological Technology, China).

The migration activity of DCs was investigated by determining the mRNA expression of chemokine mRNA of CCR7 and CXCR4. Total RNA was extracted using Trizol Reagent (Invitrogen, Carlsbad, CA, USA). Then cDNA was synthesized using RevertAid ™ First Strand cDNA Synthesis Kit (Thermo Scientific, Maryland, USA). PCR products were analyzed by GoldView-agarose gel electrophoresis on 2% (w/v) agarose gel and visualized under UV light. The signals of target genes were measured by scanning densitometry and normalized to β-actin using Quantity One software. The forward and reverse PCR primers used for CCR7 were 5′-GCCTTCCTGTGTGATTTCTACAG-3′ and 5-TCACCTTCTCTCCTTTCTGTCAC-3′; for CXCR4 were 5′-TGTTGCCATGGAACCGATCA-3′ and 5′-GGATCC-AGACGCCCACATAG-3′; for β-actin were 5′-TGGCAC-CACACCTTCTACAATG-3′ and 5′-CCTGCTTGCTGA-TCCACATCTG-3′, respectively. Condition of reverse transcription system was 91°C for 5 min, 4°C for 5 min, 30°C for 10 min, 42°C for 30 min, 99°C for 5 min, and 4°C for 5 min. Condition of PCR system was initial denaturation at 94°C × 3 min, followed by 25 cycles of denaturation at 94°C × 30 s, annealing at 60°C × 30 s, extension at 72°C × 2 min, and final extension at 72°C for 10 min.

Besides, splenic T lymphocytes were purified from C57BL/6 (H-2K^d and I-A^d) mice using Pan T Cell Isolation kit II (Miltenyi Biotec, Inc., Auburn, USA) and then cocultured with DCs for another 48 h. The proliferation of T cells was determined using MTT Cell Proliferation and Cytotoxicity Assay kit (Beyotime, Shanghai, China).

2.5. Statistical Analysis. Results data were analyzed using SPSS statistical software (version 17.0) and expressed as mean ± standard deviation (SD) of the indicated number of experiments. The statistical significance was estimated using a Student's t-test. $P < 0.05$ and $P < 0.01$ were considered as statistically significant and highly significant, respectively.

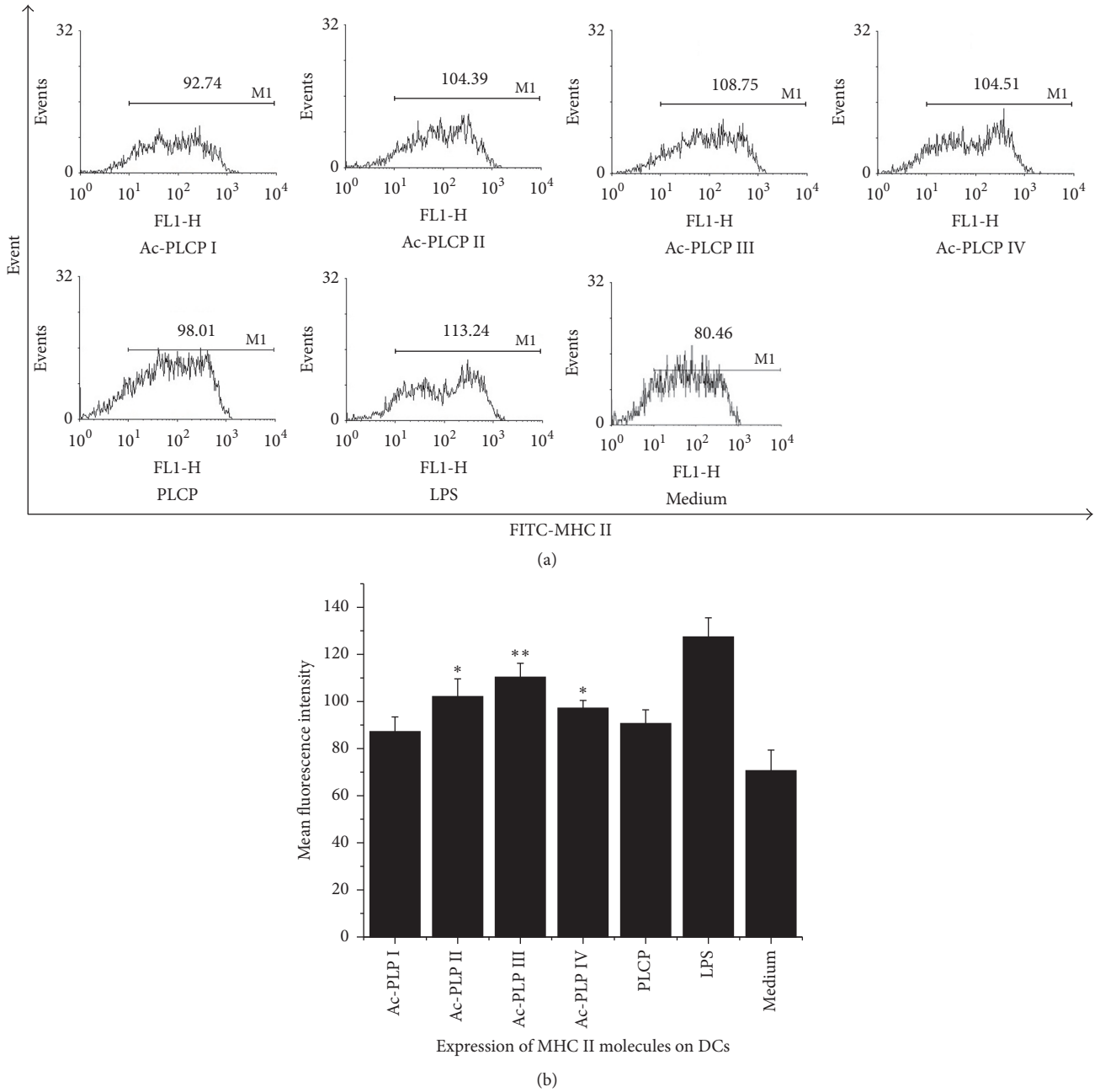
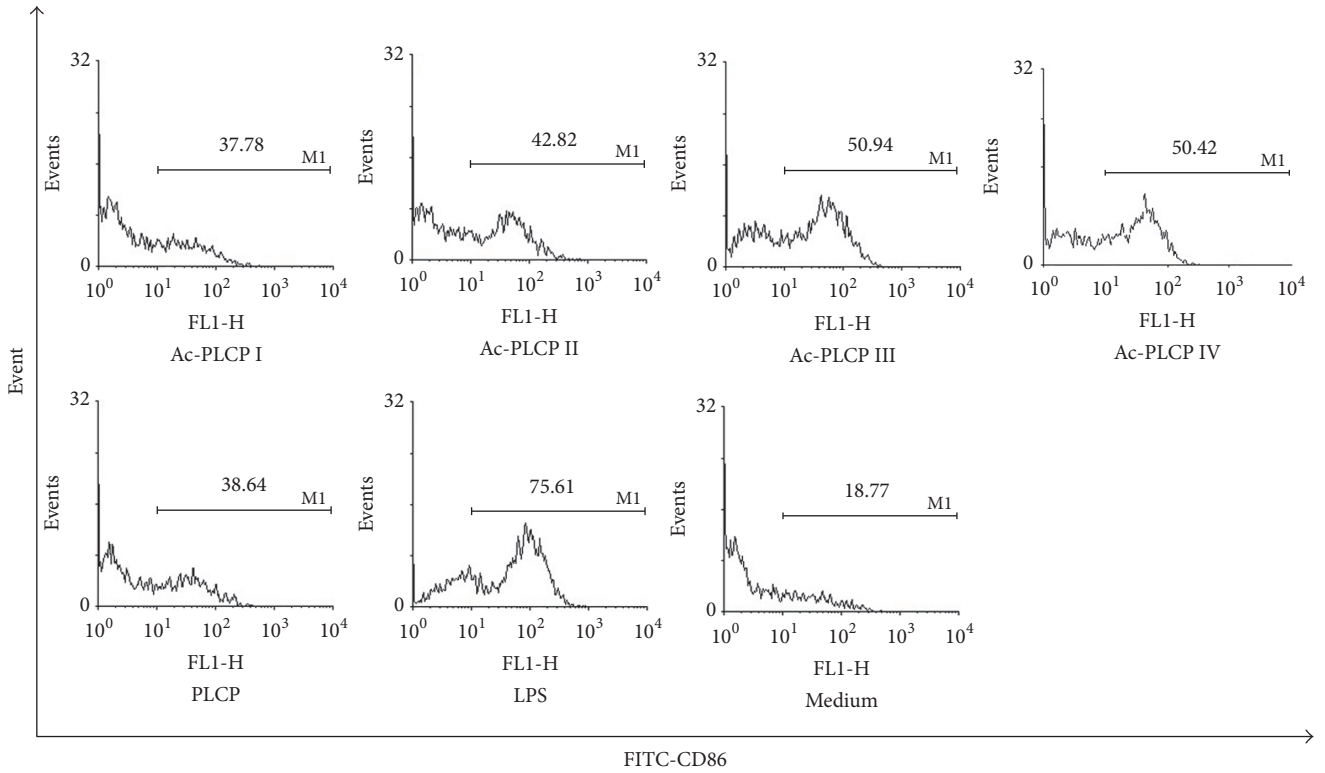


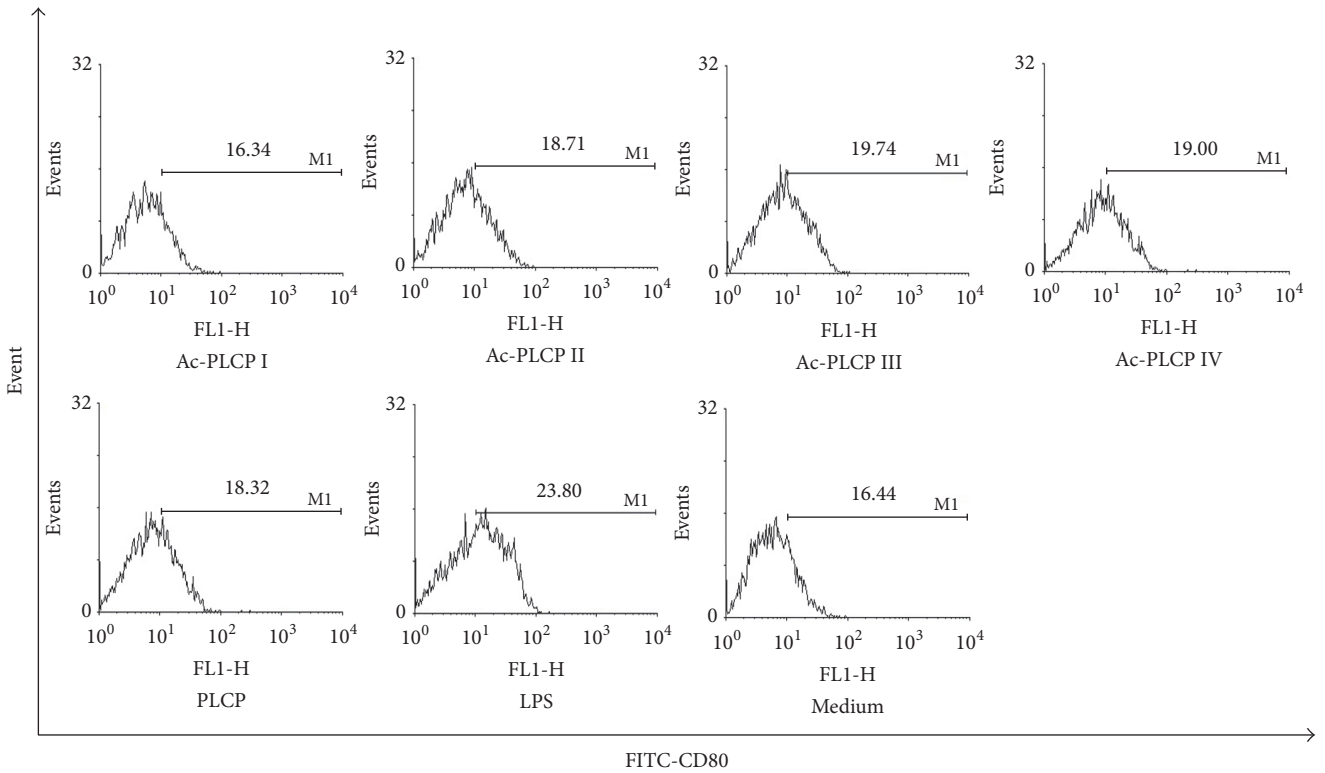
Figure 1: Ac-PLCP increased the expression of MHC II molecules on DCs. Immature DCs were treated with 100 μg/mL PLCP or Ac-PLCPs for 48 h. Then, the expression levels of MHC II molecules were determined by flow cytometry. The data were shown as mean fluorescence intensity (MFI). Compared with medium group, DCs treated with PLCP expressed higher level of MHC II molecules. While compared with the PLCP group, significant increases in MFI were observed in Ac-PLCP II, III, and IV groups. The results shown were from one representative experiment of three independent experiments performed. $^{*}P < 0.05$ compared to the PLCP group; $^{**}P < 0.01$ compared to the PLCP group.

3. Results

3.1. Ac-PLCP Enhanced Expression of MHC II, CD80, and CD86 Molecules in DCs. Immune responses were initiated through the specific recognition of antigens by lymphocytes, and this progress required stable adhesion of the T cells to the antigen-presenting cells (APCs), efficient antigen presentation, and transduction of signals from the cell surface to the nucleus of T cell. In particular, T cells could only recognize and respond to peptide antigens bound to and displayed by MHC molecules. Flow cytometry was applied to determine the expression of MHC II on DCs, and the data were shown as mean fluorescence intensity (MFI) in Figure 1. After incubation with PLCP, the expression of MHC II on DCs was increased from 80.46 to 98.01. Meanwhile, the expressions of MHC II in Ac-PLCP II, III, and IV groups were 104.39 ($P < 0.05$), 108.75 ($P < 0.01$), and 104.51 ($P < 0.05$), respectively, which were significantly higher than that of PLCP

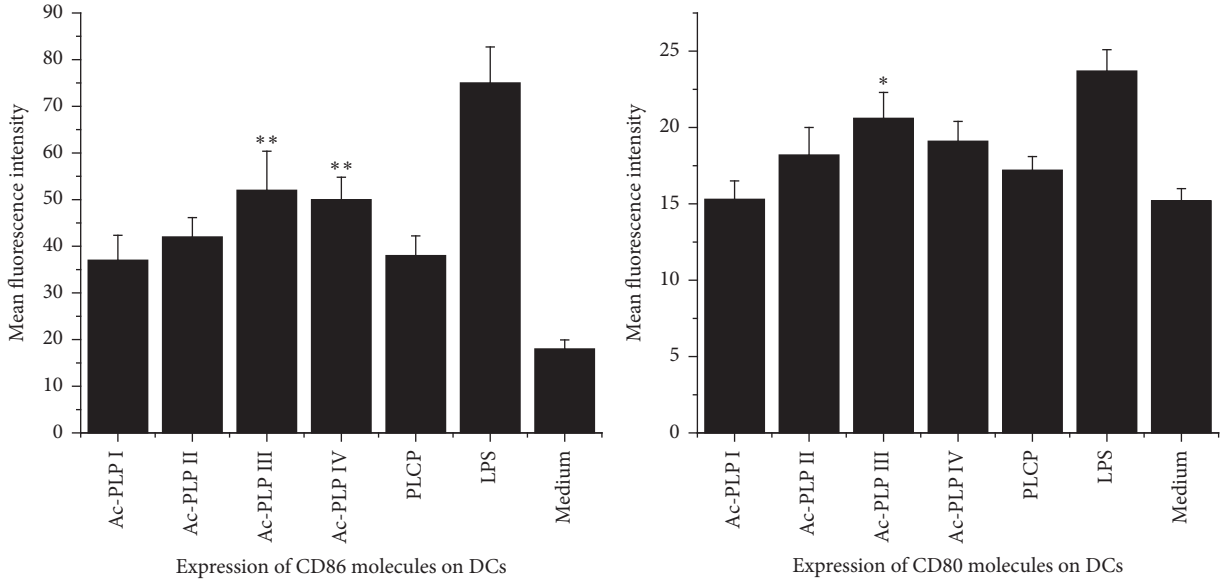


(a)



(b)

Figure 2: Continued.



(c)

Figure 2: Ac-PLCP increased the expression of costimulating molecules on DC. DCs treated with Ac-PLCPs III and IV expressed a higher level of CD86 (a) and CD80 (b) molecules than PLCP group. The results shown were from one representative experiment of three independent experiments performed. $^{*}P < 0.05$ compared to the PLCP group; $^{**}P < 0.01$ compared to the PLCP group.

group. Costimulating molecules are necessary to ensure an effective immune response. The expressions of CD86 and CD80 costimulating molecules were also measured using flow cytometry. As shown in Figure 2(a), the expression levels of CD86 in DCs in Ac-PLCP I, II, III, and IV groups were 37.78, 42.82, 50.94, and 50.42, respectively, which were much higher than that in the control group (18.77) ($P < 0.01$). In particular, the expression levels of CD86 in Ac-PLCPs III and IV were significantly higher than PLCP group ($P < 0.01$). As shown in Figure 2(b), the expression of CD80 molecules was notably stimulated by Ac-PLCP III (19.74) compared with PLCP group (16.44) ($P < 0.05$).

3.2. Ac-PLCP Stimulated Secretion of IL-12p70 in DCs. Beyond interaction with T cells through surface molecules, DCs also regulate the immune response by secreting cytokines. As shown in Figure 3, PLCP increased the secretion of IL-12p70 in DCs from 20.38 pg/mL (control group) to 49.97 pg/mL (PLCP group), while the cytokine levels of IL-12p70 in the acetylated polysaccharides-treated groups were 33.11 pg/mL (Ac-PLCP I), 60.51 pg/mL (Ac-PLCP II), 96.43 pg/mL (Ac-PLCP III), and 81.06 pg/mL (Ac-PLCP IV), respectively. Compared with PLCP group, Ac-PLCP III and Ac-PLCP IV groups were significantly enhanced ($P < 0.01$).

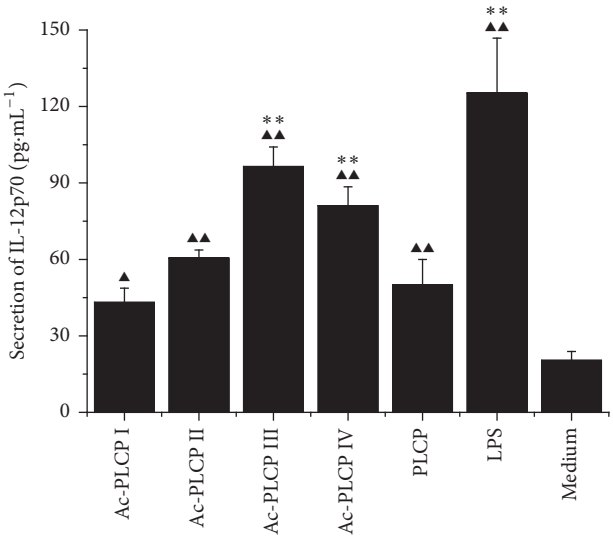


Figure 3: Ac-PLCP enhanced the secretion of IL-12p70 cytokine on DCs. Quantity of IL-12p70 in the culture supernatant was determined by ELISA method. Compared with PLCP group, the secretion of DCs in the Ac-PLCPs III and IV was significantly higher than that in PLCP group. $^{**}P < 0.01$ compared to the PLCP group; $^{▲}P < 0.05$ compared to the medium group; $^{▲▲}P < 0.01$ compared to the medium group.

3.3. Ac-PLCP Attenuated the Endocytosis of DCs. Although DCs are found in almost all tissues in the body, most of the cells exist in immature states. The immature DCs are not professional in antigen peptide presenting but antigen uptaking. However, once the immature DCs receive mature signal, they would develop maturation and transform from antigen-uptake cells into antigen-presenting cells. As shown in Figure 4, DCs in the control group (medium, 37°C) showed a dramatically high uptake of FITC-dextran. Compared with the control group, DCs in the PLCP and Ac-PLCP groups exhibited an attenuated endocytosis. Particularly, the MFI determined in the Ac-PLCP III (55.17) and IV groups (56.28) was significantly lower than PLCP group (67.60) ($P < 0.01$).

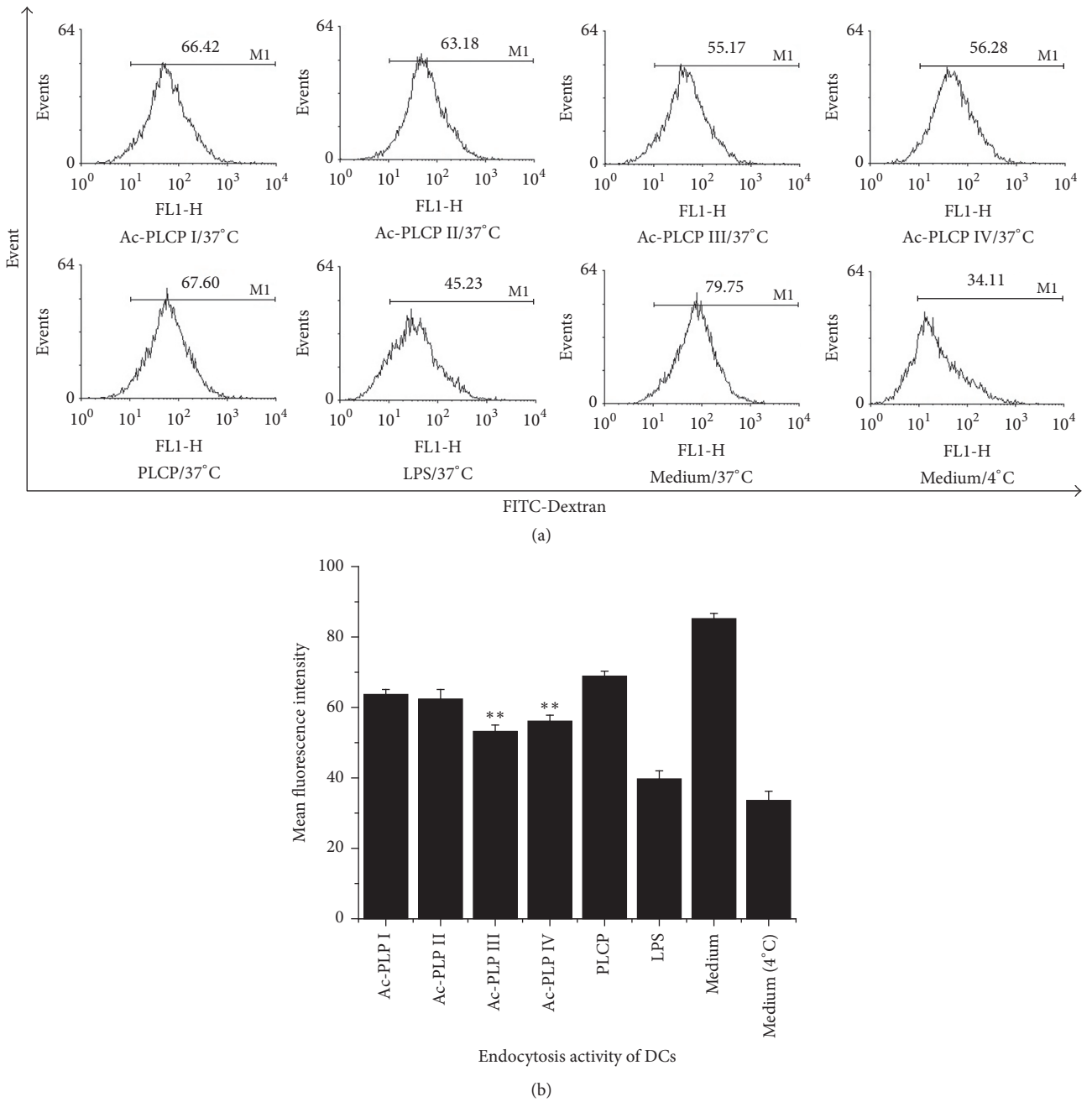


Figure 4: Ac-PLCP attenuated the endocytosis activity of DCs. The endocytosis activity was also determined by flow cytometry. The uptake of FITC-dextran of DCs in medium group was extremely high. However, endocytosis activity was significantly attenuated by PLCP treatment. In particular, DCs in Ac-PLCP III and IV groups exhibited much weaker uptake of FITC-dextran. The results shown were from one representative experiment of three independent experiments performed. $^{**}P < 0.01$ compared to the PLCP group.

3.4. Ac-PLCP Increased Expressions of CCR7 and CXCR4 Chemokine mRNA in DCs. The mRNA expression levels of chemokine receptors CCR7 and CXCR4 were analyzed using reverse transcription PCR to investigate the migration capability of DCs. As shown in Figure 5, notable increase was observed in the expressions of both CCR7 and CXCR4 mRNA in Ac-PLCP III and Ac-PLCP IV groups. In particular, the chemokine mRNA expression level in Ac-PLCP III or IV group was significantly higher than PLCP group ($P < 0.01$).

3.5. Ac-PLCP Treated DCs Stimulated T Lymphocyte Proliferation. Splenic T lymphocyte was purified and cocultured with DCs at a ratio of 10 : 1 for 48 h, and then the proliferation of T cells was determined by MTT assay. The proliferation index was calculated as absorbance ratio of polysaccharide group to control group. As shown in Figure 6, compared with control group, the proliferation index was significantly increased in all the polysaccharide groups ($P < 0.01$). The proliferation indexes in Ac-PLCP I and Ac-PLCP II groups were 124.21%

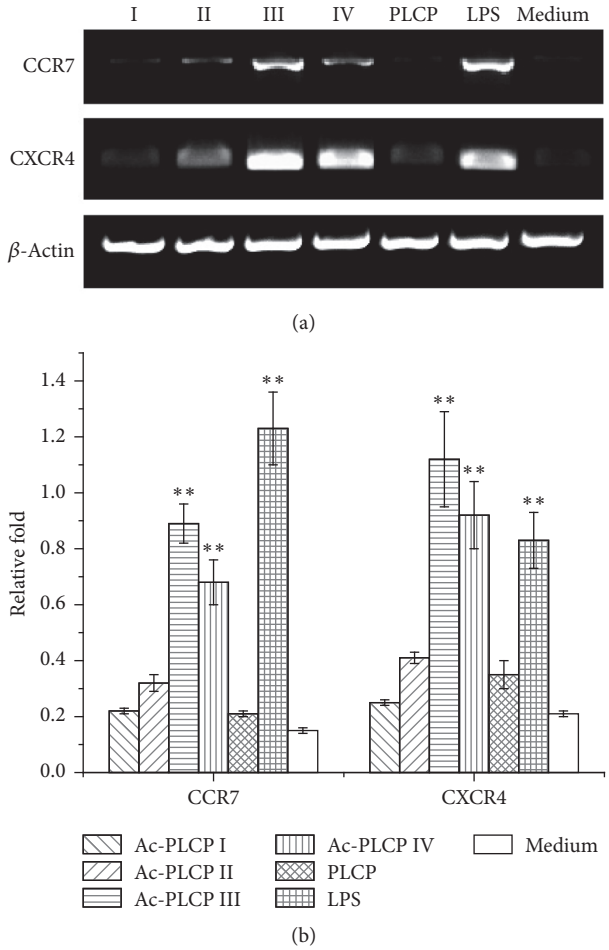


Figure 5: mRNA synthesis levels of CCR7 and CXCR4 were determined through reverse transcriptase PCR. The PCR product was analyzed by 1.5% agarose gel electrophoresis. The mRNA expression levels of both CCR7 and CXCR4 were significantly increased in Ac-PLCP III and IV groups. Besides, the expression of β-actin in each group was determined as internal control, and there was no significant difference among all the groups. $^{**}P < 0.01$ compared with PLCP group.

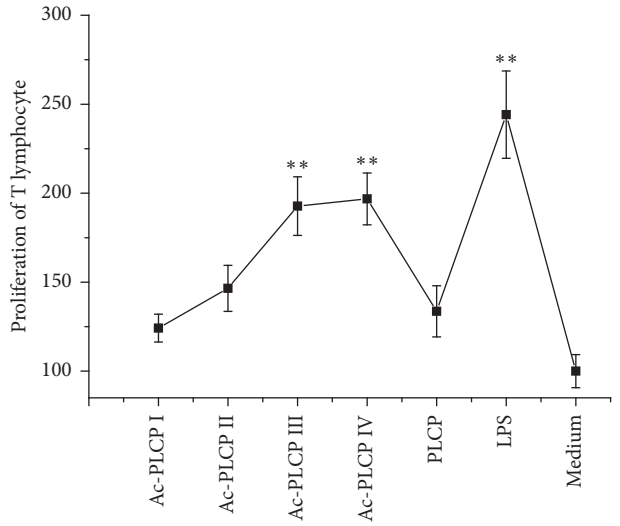


Figure 6: DCs treated with Ac-PLCPs showed stronger activity on stimulating T lymphocyte proliferation. T lymphocytes were obtained from C57BL/6 (H-2K^d and I-A^d) mice and cocultured with DCs in 96-well plate at the ratio of 10 : 1 for 48 h. The absorption value of the medium group was set at control values in the calculation of % proliferation. All the polysaccharide-treated DCs showed extremely strong T cell proliferation stimulating activity. Compared with PLCP group, DCs in Ac-PLCPs III and IV exhibited significantly enhanced T cell proliferation stimulating activity. $^{**}P < 0.01$ compared to the PLCP group.

and 146.52%. And the proliferation indexes in Ac-PLCP III and IV groups were 192.75% and 196.81%, respectively, which were dramatically higher than that in PLCP group (133.62%) ($P < 0.01$).

4. Discussion

DCs are professional antigen-presenting cells and essential mediators of innate and adaptive immune response, as well as tolerance [21]. Immature DCs are strategically located in dermis or mucosal system to capture antigen and then transport these antigens to regional lymph nodes, where they develop into mature and activate lymphocyte to initial immune response. However, immature DCs can weakly activate naïve T lymphocyte for lacking of antigen-presenting molecules. MHC molecules T cell receptor interaction and CD80(B7-1)/CD86(B7-2)-CD28 surface molecules interaction between APCs and T cells have been regarded as a key point to induce T cell activation [22]. Though immature dendritic cells synthesize large amounts of MHC II molecules, such MHC II molecules form $\alpha\beta$-dimers that exhibit intracellular distributions. The $\alpha\beta$-dimers are targeted to late endosomes and lysosomes where they reside unproductively with internalized antigens; however, after exposure to inflammatory mediators or microbial products, the newly formed immunogenic MHC II complexes are transported from lysosomes to plasma membrane [23]. In particular, the half-life of MHC II molecules increases from about 10 h to over 100 h during this process [24]. Ac-PLCPs III and IV were more effective in inducing DCs maturation than PLCP, since the expressions of MHC II molecules on DCs of the Ac-PLCP III and IV groups were significantly higher than that of PLCP group. Besides the primary signal delivered to T cells through MHC II molecules, the B7-CD28 interaction is one of the dominant costimulatory signals. Particularly, for naïve T cells that express only limited number of costimulatory receptors, CD28 is an indispensable receptor required for T cell priming [25]. The B7-CD28 costimulation signals could direct the development and function of T cells. Lenschow et al. found that effector and regulatory T cell responses were impaired in mice lacking CD28 or in mice lacking CD80 and CD86. [26]. Gimmi et al. reported that antigen presentation in absence of B7 costimulation results in human T cell clonal anergy [27]. We found that Ac-PLCP III and IV incubation significantly promoted the expression of CD86 molecules on DCs as well, which strongly suggests that the acetylated polysaccharides

could induce phenotypic maturation on DCs. Meanwhile, an obvious decline of endocytosis activity on DCs was also observed, which indicates that the immature dendritic cells transform from antigen-capturing cells into the mature antigen-presenting cells.

Besides the ligand-receptor interaction, DCs could regulate the polarization of T lymphocytes by secreting various cytokines. IL-12 is a proinflammatory molecule produced primarily by monocytes/macrophages and DCs. And IL-12 principally activates natural killer cells and induces the differentiation of native $CD4^+$ T cells to become interferon-γ-producing T helper 1 (Th1) effectors in cell-mediated immune responses [28]. IL-12 could also synergize with B7/CD28 interaction in inducing efficient proliferation and cytokine production of human T cells [29]. It was reported that $CD1c^+$ myeloid DC but not plasmacytoid DC (pDC) in humans can induce high level of cytotoxic molecules in naïve T cells by producing high amount of IL-12 [30]. The secretion of IL-12p70 of DCs was promoted by Ac-PLPC III and IV treatments, and the levels of IL-12p70 secretion in these two groups were significantly higher than PLCP group. Consistently, we also found that DCs incubated with Ac-PLPC III or IV could significantly stimulate proliferation of naïve T cell *in vitro*. Furthermore, DCs in Ac-PLCP III and IV groups showed an extremely strong effect on provoking sizeable mixed lymphocyte reaction when cocultured with splenic T cells, which indicates that the acetylated polysaccharides stimulated functional maturation on DCs. All these findings support that DCs treated with Ac-PLCPs III and IV may possess much more powerful immune regulatory activity than the natural polysaccharide.

Optimal encounter with naïve T cells for the presentation of antigens requires DCs to migrate to secondary lymphoid organs, which is governed by chemokine. The key chemokines directing DCs migration are chemokine (C-C motif) ligand 19 (CCL19), CCL21, and chemokine (C-X-C motif) ligand 12 (CXCL12) [31, 32]. As receptor for CCL19 and CCL21, CC chemokine receptor CCR7 has been found to mediate the migration of DCs from skin into lymphatic vessels [33]. However, the migration of skin DC was initiated by CXCL12-CXCR4 engagement [34]. For plasmacytoid DCs, the migration into splenic white pulp was regulated by CCR7 signal coordinate with CXCR4 signal [35]. And the chemokine signal also enhanced DCs maturation and survival [36]. The migration capability of DCs was enhanced by Ac-PLCP III and IV treatments evidenced by the increased mRNA expression levels of CCR7 and CXCR4.

Dendritic cells are equipped with a battery of pattern-recognition receptors (PRRs) that can detect molecular patterns of invading microorganisms or endogenous signals and alter the immune response. The most widely studied family of PRRs on DCs is Toll-like receptors. In particular, TLR-4 expressed on the surface of DCs was demonstrated to be one to recognize polysaccharides [37, 38]. DCs in intestinal mucosa tissue were found to be able to open the tight junctions between epithelial cells without breaking the integrity of the epithelial barrier [39], which provides a probable route for the polysaccharide to interact with DCs. We have also found that TLR-4 mediates the maturation of DCs induced by PLCP [16]. The acetylation modification may enhance the immunoregulatory activities of PLCP by improving the solubility of PLCP and decreasing the viscosity of the polysaccharide.

In conclusion, Ac-PLCPs exhibited higher maturation-stimulating activities on DCs compared with the natural polysaccharide. In particular, the Ac-PLCPs of DS ranging from 0.06 to 0.1 showed the best immunoregulatory activities. The current study also provided a new insight into the structure-activity relationship of PLCP and the utilization of the seeds of *Plantago asiatica* L.

Abbreviations

CD: Cluster of differentiation
Ac: Acetylated
DCs: Dendritic cells
DS: Degree of substitution
MHC: Major histocompatibility complex
PLCP: *Plantago asiatica* L. crude polysaccharide
IL-12: Interleukin-12.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This study is supported by the National Key Technology R&D Program of China (2012BAD33B06), the National Natural Science Foundation of China (21062012, 31260364), and the Program for New Century Excellent Talents in University (NCET-12-0749), which are gratefully acknowledged.

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Hindawi
Journal of Chemistry
Volume 2018, Article ID 6459243, 9 pages
https://doi.org/10.1155/2018/6459243

Research Article

Distribution of Phenolic Acids and Antioxidant Activities of Different Bran Fractions from Three Pigmented Wheat Varieties

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Received 4 September 2017; Accepted 26 December 2017; Published 28 January 2018

Academic Editor: Yanjie Bai

Phenolic acid profiles and antioxidant activities of outer bran, coarse bran, and shorts from blue, black, and purple wheat were analyzed. Phenolic acids were mainly in the bound form in pigmented wheat bran fractions. Phenolic acid content decreased in the order of outer bran, coarse bran, and shorts for the three pigmented wheat varieties. HPLC analysis of phenolic extracts demonstrated that the bound form of phenolic acids contained more ferulic, isoferulic, and p-coumaric acids compared to their free counterparts. Among the three pigmented wheat varieties, the bran fractions from blue wheat contained higher bound phenolic acids than the other two pigmented wheat bran fractions, except for purple coarse bran. The blue wheat outer bran had the highest total bound phenolic acid of 3458.71 μg/g while the purple wheat shorts had the lowest of 1730.71 μg/g. The contribution of bound phenolic acids to the total phenolic content and antioxidant activity was significantly higher than that of free phenolic acids. Blue wheat bran fractions had the highest radical scavenging activity against DPPH• while those of purple wheat gained the highest $ABTS^{\bullet+}$ scavenging activity. High correlations were observed between TPC and radical scavenging capacities for DPPH and ABTS ($R^2 > 0.85$, $P < 0.05$).

1. Introduction

Oxidative damage to important biomolecules such as DNA, proteins, and membrane lipids has been considered as causative of carcinogenesis, coronary heart disease, and many other age-related health problems [1–3]. Antioxidants can modulate cellular oxidative status and reduce the risk of these diseases and health problems [4–6]. Many previous researches demonstrated that wheat grain contained significant level of natural antioxidants [3, 7–12].

Wheat contains a wide range of chemical substances with antioxidant property [13]. Growing evidence indicated that a significant portion of the antioxidant property of wheat is attributed to phytochemicals instead of traditional vitamins [3, 14, 15]. Genotypes and culturing environment of wheat had significant influences on the phytochemical compositions and antioxidant activities [3, 16–18]. The antioxidant mechanisms may include acting as free radicals scavengers to end the radical chain reaction, transition metals chelators to inhibit the initiation of radical formation, reducing agents, the antioxidative enzyme systems stimulators, or quenchers of singlet oxygen [19]. Phenolic acids in wheat are supposed to significantly contribute to the antioxidant abilities and health benefits of whole wheat ingestion observed in many epidemiological studies [8, 16, 20]. There are mainly two groups of phenolic acids in wheat bran: hydroxybenzoic and hydroxycinnamic acid derivatives. Vanillic and salicylic acids are mainly hydroxybenzoic acid derivatives while ferulic acids and p-coumaric are the most common derivatives of hydroxycinnamic acid [21]. Phenolic acids in cereal grains are in free, soluble conjugates, and bound forms. The majority of them existed in bound form, esterified to cell wall material in bran [16, 22].

Phenolics were not equally distributed in the wheat grains but concentrated in the outer layers [10]. Wheat bran fractions enriched in phenolics and antioxidants can be obtained by debranning and used as functional food ingredients or nutrient supplement [8, 14]. Pigmented wheat, such as blue, black, and purple wheat, is currently gained increasing attention due to their anthocyanin pigments [23–26]. However,

most of these studies focused on the anthocyanin and their properties. Distribution of phenolic acids and antioxidant activities of bran fractions from different pigmented wheat has not been reported.

Pigmented wheat has great potential as functional foods (such as whole grain products) or functional food ingredients (such as anthocyanin-rich fractions) for disease prevention and health promotion [24]. However, too much bran in flour may lead to bad influences on food processing performance and acceptability by consumers [27]. Debranning of wheat before milling has been widely accepted to improve roller-milling property and obtain ingredients rich in special nutrients [8]. The present study obtained three bran fractions (outer bran, coarse bran, and shorts) by combining debranning and milling of three pigmented wheat varieties. Phenolic acid profiles and antioxidant activities of both free and bound phenolic acids in pigmented bran fractions were analyzed.

2. Materials and Methods

2.1. Materials. Three pigmented wheat varieties (blue, black, and purple wheat) were harvested from demonstration field of Tai'an Subcenter of National Wheat Improvement Center, Shandong province, China, during 2010 season. The demonstration field is located at 36.18°N latitude and 117.13°E longitude. The purple wheat was ShanNong Zimai 1. The blue and black wheat were one of breeding lines. Gallic, protocatechuic, p-hydroxybenzoic, chlorogenic, vanillic, caffeic, syringic, p-coumaric, ferulic, salicylic, and trans-cinnamic acids were purchased from Sigma–Aldrich (Shanghai, China). All other chemicals and solvents were of analytical or HPLC-grade purity.

Three pigmented wheat varieties were debranned by a friction debranning machine (6MT-5, Shandong Agriculture Machinery Institute, China) to obtain 8% outer bran. The debranned wheat were milled by a laboratory mill (JNFB70×30, Chengdu Grain Storage Research Institute, China) and yielded coarse bran (10% based on raw wheat), shorts (6% based on raw wheat), and flour (76% based on raw wheat). The wheat bran fractions were further milled with addition of liquid nitrogen and sieved through a 40-mesh screen and then stored at −20°C before analysis.

2.2. Extraction of Free Phenolic Acids. Free phenolic acids were extracted according to the procedure of Kim et al. [16] with some modification. The pigmented wheat bran fractions were defatted twice with hexane at a 10 : 1 ratio (v/w) in an ultrasonic cleaner for 50 min at room temperature. The mixture was centrifuged at 4000 rpm for 15 min to recover the bran fractions. The defatted samples were dried in vacuum drying oven at room temperature. The defatted bran fractions were extracted twice with 80% methanol at a 10 : 1 ratio (v/w) for 50 min at room temperature and then centrifuged at 4000 rpm for 15 min. The supernatants were transferred to pear shape flasks and rotary evaporated to dryness at 40°C. The extracts were redissolved in 4 mL of HCl solution (pH 2.0) and extracted with 4 mL of ethyl acetate/ethyl ether (1 : 1, v/v) three times. The organic layers were combined and rotary evaporated to dryness at 40°C. The solid extracts were reconstituted to 10 ml with methanol and frozen at −20°C before further analysis.

2.3. Extraction of Bound Phenolic Acids. The residues (1 g) after methanol extraction were dispersed with 40 mL NaOH solution (2.0 mol/L) in a 150 mL conical flask. The conical flask was purged with nitrogen to reduce the oxidation of phenolic compounds. The mixture was hydrolyzed in a mechanical shaker for 4 h at room temperature. The suspension was adjusted to pH 7.0 with 4.0 mol/L HCl and centrifuged at 4000 rpm for 15 min. The supernatant was adjusted to pH 2.0 and reextracted with ethyl acetate/ethyl ether (1 : 1, v/v). After evaporation of ethyl acetate and ethyl ether, the phenolic acid extract was reconstituted to 10 mL with methanol and frozen at −20°C until further analysis within a three-month period.

2.4. HPLC Analysis. The samples prepared above were filtered through a 0.45 um syringe filter before HPLC analysis. HPLC analysis was carried out using a Shimadzu LC-20AT liquid chromatograph equipped with an autosampler and a diode-array detector. The phenolic acids were separated on a reverse phase C18 column Inertsil ODS-SP (250 mm × 4.6 mm, 5 μm). The temperature of column oven was 40°C. The mobile phase consisted of water (solvent A) and acetonitrile (solvent B), both containing 0.1% formic acid (v/v). The flow rate was kept at 1.0 mL/min and the gradient elution was employed as follows: 0% B to 10% B in 20 min, 10% B to 50% B in 10 min, 50% B to 100% B in 10 min, 100% B to 10% B in 5 min, and 10% B in 15 min. The injection volume was 10 μL. Identification of phenolic acids was accomplished by comparing the retention time of peaks in the samples to that of the standards under the same HPLC conditions. Phenolic acids were quantified by comparing the peak area of the samples with the peak area of the calibration curves for all of the phenolic acids (all $R^2 > 0.999$).

2.5. Determination of Total Phenolic Content. Total phenolic content (TPC) of defatted wheat bran fractions was determined using the Folin– Ciocalteu reagent according to a modified procedure [16]. An aliquot of the methanolic extract (0.1 mL) was added to 0.5 mL Folin–Ciocalteu reagent. After equilibration for 5 min, 1.5 mL sodium carbonate solution was added to the mixture and mixed and made up to a final volume of 10 mL with distilled water. The mixture was allowed to stand at room temperature for 120 min, and then the absorbance was measured at 765 nm against a blank of distilled water. The total phenolic content in each extract was determined using a standard curve prepared using gallic acid and expressed as gallic acid equivalents (μg GAE/g defatted bran).

2.6. Determination of Antioxidant Activity

2.6.1. DPPH Radical Scavenging Capacity. The DPPH• scavenging capacity of the bran extracts was determined according to Verma et al. [28] with some modifications. The initial concentration was 50 μM for DPPH in all antioxidant-radical

reactions, freshly prepared in methanol before measurement. The bran extracts (0.1 mL) were reacted with 3.9 mL DPPH• solution to initiate antioxidant-radical reaction. After 60 min of reaction, the absorbance of the reaction mixture was measured at 517 nm against a blank of methanol. Control group was prepared in a similar way as for the experimental group except for the replacement of the testing sample solution with the corresponding extraction solvent. DPPH radical scavenging activity (%) = [1 − absorbance of sample/absorbance of control] × 100.

2.6.2. $ABTS^{\bullet+}$ Scavenging Activity. The $ABTS^{\bullet+}$ scavenging activity of the bran extracts was determined using a commercial kit from Beyotime Institute of Biotechnology (Shanghai, China). Trolox (6-hydroxy-2,5,7,8-tetra-methyl-chroman-2-carboxylic acid) was used as an antioxidant standard. Eighty percent ethanol was used to prepare the solutions of Trolox and to determine the reagent blank. The Trolox equivalent antioxidant capacity (TEAC) values of the extracts were calculated using a Trolox standard curve on the basis of the absorbance at 734 nm after 30 min of reaction and expressed in μmol Trolox/g defatted sample. The range of concentration of Trolox used for standard curve was 0.15–1.5 mmol/L. The tests were conducted in triplicate for each sample extract.

2.7. Statistical Analysis. Data were reported as mean ± standard deviation for triplicate determinations. ANOVA and Tukey's tests were performed with SPSS18.0 for Windows to identify differences among means. Statistical significance was declared at $P < 0.05$.

3. Results

The content of lipids in wheat bran fractions ranged from 2.9% to 3.8% at 12% moisture level. Samples were defatted to remove lipids and lipid-soluble ingredients which may influence phenolic acids and their antioxidant activities.

3.1. Distribution of Phenolic Acids in the Bran Fractions. Previous studies demonstrated that the widespread phenolic acids in wheat are gallic, protocatechuic, p-hydroxybenzoic, chlorogenic, vanillic, caffeic, syringic, p-coumaric, ferulic, and salicylic acids [29]. Gallic, protocatechuic, p-hydroxybenzoic, vanillic, syringic, p-coumaric, ferulic, isoferulic, salicylic acids were detected in this study (Table 1).

The content of free phenolic acids in the bran fractions was shown in Table 1. Gallic acid, ferulic acid, and salicylic acid were the major free phenolic acids in all bran fractions. Gallic acid and protocatechuic acid were only identified in the free form. On the whole, the content of phenolic acids presented a descending trend in the order of outer bran, coarse bran, and shorts, with the exception of p-coumaric acid and ferulic acid, which had the highest concentration in shorts fractions. Ferulic acid was the majority and it accounted for 16.81–23.01% of the total detectable free phenolic acids. In the outer bran of three pigmented wheat varieties, the concentration of salicylic acid was higher than that of ferulic acid, respectively. The content of free phenolic acids in purple wheat bran fractions was higher than blue and black wheat.

Table 2 showed the concentration of bound phenolic acids which were released during the alkaline hydrolysis process. Cereals were rich in substituted cinnamic acids such as ferulic acid which was esterified to arabinoxylan and arabinogalactan in the aleurone layer and pericarp [30]. Alkaline hydrolysis was often used to cleave the ester bond to separate and identify particular phenolic compounds [31]. Different wheat bran fractions contained different phenolic acid profiles. Gallic acid was not detectable in bound phenolic acids but was in free phenolic acids, while protocatechuic acid was only found in purple wheat shorts fraction in bound phenolic acids but was in all free phenolic acids, suggesting that the optimum method to obtain these phenolics is by 80% aqueous methanol extraction. Similarly, salicylic acid, which was found in free phenolic acids, was only detected in outer bran fractions in bound phenolic acids. The other phenolic acids were prevalent in the bound form. However, only ferulic, isoferulic, p-coumaric acids were predominant in the various fractions of wheat bran. The results demonstrated that the hydroxycinnamic acid derivatives can be easily liberated from the bound form by alkaline hydrolysis. They are the most common phenolic acids in wheat and hold promise as antioxidants, whereas only ferulic acid was released in significantly higher amount than any others in all bran fractions and accounted for about 49–89% of the total identified phenolic acids. From outer bran, coarse bran to shorts, amount of ferulic acid decreased progressively in blue wheat. Nevertheless, the coarse bran of black wheat and purple wheat had larger amount of ferulic acid than the other fractions. The difference in the distribution of ferulic acid might be due to the different colors and varieties. The outer bran of blue wheat had the greatest concentration of total phenolic acids (3459 μg/g), while the shorts of purple wheat had the least amount of total phenolic acids (1731 μg/g). There was a significant increase of phenolic acids on the basis of alkaline hydrolysis, 16-fold for blue wheat outer bran, 13-fold for black wheat outer bran, 8-fold for purple wheat outer bran.

3.2. Folin–Ciocalteu (FC) Determination of Total Phenolic Content. Total phenolic content of free and bound phenolic acids of the bran fractions, expressed as microgram gallic acid equivalents per gram defatted bran, was presented in Table 3. The antioxidant extracts differed significantly in their total phenolics contents. The total phenolic contents of both free and bound phenolic acids gradually decreased from the outer to the inner fractions, ranged from 7737.17 to 4387.99 μg GAE/g defatted bran for blue wheat, from 8269.97 to 4647.64 μg GAE/g defatted bran for black wheat, and from 8012.64 to 3942.18 μg GAE/g defatted bran for purple wheat. These results showed that the phenolics of wheat were mostly concentrated in the outer layer of wheat grains. Blue wheat outer bran contained the greatest level of total phenolics, whereas purple wheat shorts contained the least level of total phenolics. In the present study, black wheat and purple wheat bran exhibited larger amounts of phenolic acids than blue wheat. In the three pigmented wheat varieties, the bound phenolic concentration was significantly higher than free phenolic. It appeared that the dominating phenolic acids in

Table 1: The content (μg/g defatted bran) of free phenolic acids in the bran fractions of three pigmented wheat varieties.

Phenolic acids	Blue wheat			Black wheat			Purple wheat		
	Outer bran	Coarse bran	Shorts	Outer bran	Coarse bran	Shorts	Outer bran	Coarse bran	Shorts
Gallic acid	32.77 ± 0.37	31.99 ± 0.41	29.70 ± 0.17	29.07 ± 0.34	32.97 ± 1.33	29.08 ± 0.18	30.87 ± 0.22	29.88 ± 0.97	29.30 ± 0.15
Protocatechuic acid	3.03 ± 0.06	1.43 ± 0.12	0.90 ± 0.08	2.09 ± 0.14	2.91 ± 0.14	2.32 ± 0.10	17.18 ± 0.94	5.69 ± 0.21	2.82 ± 0.11
p-Hydroxybenzoic acid	11.17 ± 0.31	8.40 ± 0.06	8.38 ± 0.08	9.78 ± 0.07	8.60 ± 0.05	8.52 ± 0.04	13.83 ± 0.20	9.96 ± 0.07	8.99 ± 0.09
Vanillic acid	11.82 ± 0.14	5.73 ± 0.09	5.40 ± 0.38	15.88 ± 0.66	6.03 ± 0.20	4.59 ± 0.09	34.98 ± 2.20	9.94 ± 0.59	4.11 ± 0.40
Syringic acid	18.24 ± 0.01	13.92 ± 0.08	13.44 ± 0.15	20.93 ± 0.49	15.19 ± 0.34	14.01 ± 0.04	31.59 ± 0.63	14.03 ± 0.53	12.37 ± 0.00
p-Coumaric acid	24.66 ± 0.45	25.84 ± 0.03	25.88 ± 0.14	24.89 ± 0.82	23.67 ± 0.06	24.93 ± 0.19	26.53 ± 0.73	24.34 ± 0.14	28.44 ± 0.02
Ferulic acid	40.99 ± 1.14	40.37 ± 0.45	42.46 ± 0.81	32.45 ± 0.86	34.84 ± 0.37	35.88 ± 0.60	33.23 ± 0.46	38.11 ± 1.59	39.72 ± 0.21
Isoferulic acid	26.61 ± 0.27	24.85 ± 0.17	24.92 ± 0.16	25.34 ± 0.08	24.70 ± 0.05	24.66 ± 0.06	28.23 ± 0.36	25.10 ± 0.01	24.70 ± 0.05
Salicylic acid	48.20 ± 7.62	33.14 ± 0.67	33.42 ± 0.98	32.59 ± 0.25	29.97 ± 0.19	30.48 ± 0.82	65.01 ± 2.12	37.70 ± 2.00	31.99 ± 0.13
Total phenolics (HPLC)	217.49	185.67	184.49	193.01	178.89	174.48	281.45	194.75	182.44

Table 2: The content (μg/g defatted bran) of bound phenolic acids in the bran fractions of three pigmented wheat varieties.

Phenolic acids	Blue wheat			Black wheat			Purple wheat		
	Outer bran	Coarse bran	Shorts	Outer bran	Coarse bran	Shorts	Outer bran	Coarse bran	Shorts
Gallic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd
Protocatechuic acid	nd	nd	nd	nd	nd	nd	nd	nd	11.77 ± 1.29
p-Hydroxybenzoic acid	12.81 ± 0.57	14.95 ± 0.39	12.60 ± 0.18	15.56 ± 0.66	19.57 ± 1.13	16.07 ± 0.23	25.29 ± 1.67	18.01 ± 0.50	12.68 ± 0.30
Vanillic acid	19.47 ± 2.44	8.96 ± 0.67	7.12 ± 0.32	32.18 ± 3.14	13.36 ± 2.60	9.00 ± 0.33	107.18 ± 8.68	31.40 ± 1.52	12.49 ± 0.95
Syringic acid	33.99 ± 2.50	18.57 ± 3.41	21.78 ± 0.31	32.37 ± 3.34	22.18 ± 4.07	17.49 ± 5.53	85.88 ± 13.05	41.53 ± 3.57	28.68 ± 0.77
p-Coumaric acid	112.31 ± 11.42	75.26 ± 3.38	64.04 ± 1.20	89.87 ± 5.14	85.88 ± 5.85	73.00 ± 2.86	87.58 ± 4.31	76.82 ± 1.93	52.24 ± 2.72
Ferulic acid	2467.47 ± 82.55	2336.69 ± 60.50	1948.56 ± 28.46	1251.72 ± 24.84	1810.91 ± 32.66	1611.53 ± 72.25	1316.92 ± 36.29	2753.67 ± 51.27	1499.20 ± 47.40
Isoferulic acid	277.21 ± 14.42	157.97 ± 10.25	151.63 ± 3.73	242.23 ± 21.07	220.82 ± 15.40	206.12 ± 9.33	189.43 ± 10.59	156.21 ± 4.13	113.65 ± 6.37
Salicylic acid	535.45 ± 15.18	nd	nd	906.02 ± 28.66	nd	nd	540.27 ± 36.68	nd	nd
Total phenolics (HPLC)	3458.71	2612.38	2205.73	2569.95	2172.73	1933.21	2352.55	3077.63	1730.71

Table 3: Total phenolic content (μg GAE/g defatted bran) of free and bound phenolic acids in the bran fractions of three pigmented wheat varieties.

Samples	FPA	AHPA	Total
Blue wheat outer bran	874.49 ± 21.73	6862.68 ± 93.41	7737.17
Blue wheat coarse bran	476.41 ± 23.50	4522.18 ± 84.82	4998.59
Blue wheat shorts	450.15 ± 10.69	3937.84 ± 44.30	4387.99
Black wheat outer bran	590.38 ± 26.33	7679.60 ± 127.91	8269.97
Black wheat coarse bran	495.25 ± 14.52	5179.15 ± 105.51	5674.40
Black wheat shorts	452.25 ± 11.01	4195.39 ± 91.88	4647.64
Purple wheat outer bran	1139.79 ± 25.11	6872.68 ± 71.99	8012.46
Purple wheat coarse bran	636.35 ± 22.53	5257.32 ± 150.55	5893.67
Purple wheat shorts	433.49 ± 18.15	3508.70 ± 267.75	3942.18

FPA, free phenolic acid; AHPA, alkaline-hydrolysable phenolic acids.

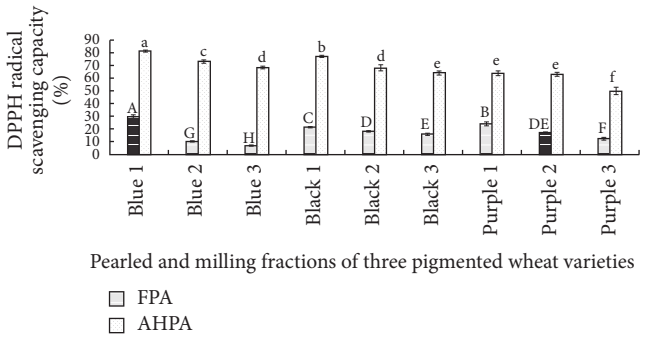

Figure 1: DPPH radical scavenging of the free and bound phenolic acids in the bran fractions of three pigmented wheat varieties. [a]Values are means of three determinations ± standard deviation, determined by HPLC analysis. [b]The same letters are not significantly different ($P < 0.05$). Blue: blue wheat; Black: black wheat; Purple: purple wheat; 1: outer bran; 2: coarse bran; 3: shorts; FPA, free phenolic acid; AHPA, alkaline-hydrolysable phenolic acids.

the pigmented wheat bran fractions were not extractable by aqueous methanol but released upon alkaline hydrolysis.

3.3. Antioxidant Capacity

3.3.1. DPPH Radical Scavenging Capacity. The scavenging capacity of the stable organic DPPH radical was used in the evaluation of antioxidant potential of free and bound phenolics of the bran fractions. The DPPH radical, with a dark purple color, has absorbance at 517 nm. It loses this absorption when accepting an electron or a free radical species and it is reduced to its nonradical form by antioxidants, which results in a colorless. It can detect numerous samples in a short period and is sensitive enough to measure active constituents at low contents [32]. The DPPH radical scavenging property of each wheat bran sample was reported as the percent of DPPH radical scavenging, with a lower value of percent of DPPH scavenging associated with a stronger DPPH scavenging ability [33]. Figure 1 showed the percentage of DPPH radical scavenging of the free and bound phenolics in the bran fractions. In the present study, remarkable DPPH radical scavenging capacity was obtained in all wheat bran extracts although the scavenging activities of phenolics were different among various wheat bran fractions. The bound phenolic acids were more effective than free phenolic acids in terms of DPPH radical scavenging activity. This indicated that the bound phenolics of all fractions had higher antioxidant activity than that of free phenolics. These results were derived from the higher total phenolic contents in the bound phenolics than those in the free phenolics. In agreement with that observed in TPC, the antioxidant activities of pigmented wheat bran were significantly decreased from outside to inside. The outer bran of the three wheat varieties exhibited the highest DPPH radical scavenging activity while the shorts fractions showed lowest scavenging efficacies. The result may be due to the fact that the endosperm diluted the antioxidant substances present in shorts resulting in lower antioxidant capacity compared to the outer bran and coarse bran. In general, DPPH radical scavenging activity of bound phenolic acids in blue wheat bran fractions was higher than black and purple wheat. However, blue wheat shorts possessed the lowest DPPH radical scavenging efficiency of free phenolic acids. The TPC and DPPH radical scavenging ability of pigmented wheat bran samples of both free and bound phenolics displayed a strong correlation ($R^2 = 0.91$ for blue wheat, $R^2 = 0.95$ for black wheat, and $R^2 = 0.94$ for purple wheat, $P < 0.05$).

3.3.2. $ABTS^{\bullet+}$ Scavenging Capacity. To better understand the radical scavenging properties, the antioxidant activity of the pigmented wheat bran fraction extracts was also detected using the TEAC (Trolox equivalent antioxidant capacity) method. The $ABTS^{\bullet+}$ scavenging capacity assay is a decolorization assay that examines the capacity of antioxidant substances to directly interact with (scavenge) $ABTS^{\bullet+}$, generated by a chemical way. $ABTS^{\bullet+}$ is a nitrogen centered radical having a typical blue-green color. It becomes colorless when reduced by antioxidant ingredients to its nonradical (ABTS) form [13]. The TEAC value of a compound corresponds to the content of Trolox (a water-soluble vitamin E analogue without the side-chain moiety) which is an antioxidant standard substance [34]. Wheat bran extracts were measured and compared for their radical scavenging activities against $ABTS^{\bullet+}$. Free and bound phenolics in all three pigmented

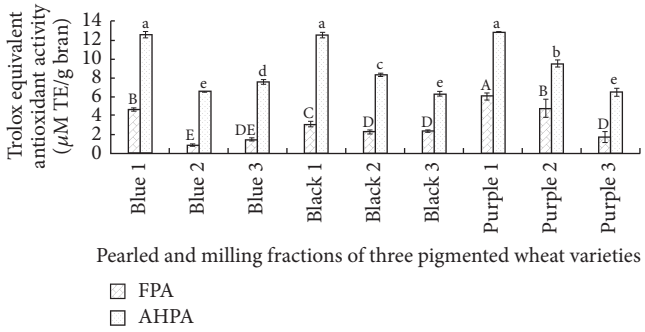

Figure 2: $ABTS^{\bullet+}$ scavenging capacity of the free and bound phenolic acids in the bran fractions of three pigmented wheat varieties. [a]Values are means of three determinations ± standard deviation, determined by HPLC analysis. [b]The same letters are not significantly different ($P < 0.05$). Blue: blue wheat; Black: black wheat; Purple: purple wheat; 1: outer bran; 2: coarse bran; 3: shorts; FPA, free phenolic acid; AHPA, alkaline-hydrolysable phenolic acids.

wheat varieties showed significant $ABTS^{\bullet+}$ scavenging capacity (Figure 2), although some differences among samples were noted. The TEAC values ranged from 0.81 to 6.01 and from 6.46 to 12.81 μmol TE/g of defatted bran for free and bound phenolics, respectively. Similar to DPPH radical scavenging, the TEAC values of bound phenolics were higher than those of the free phenolics, suggesting that the contribution of bound phenolics to total antioxidant activity was significantly higher than that of free phenolics in all wheat bran samples. TEAC values gradually decreased from the outer bran to shorts fractions in both black and purple wheat, with the exception of blue wheat bran fractions. The coarse bran of blue wheat gained the lowest antioxidant activity in all fractions. No difference in $ABTS^{\bullet+}$ scavenging activity of bound phenolics was observed among the fractions obtained from outer bran of three pigmented wheat varieties while significant difference was found in other fractions. These data indicated that color might be related to $ABTS^{\bullet+}$ scavenging activity of wheat. The highest TEAC value might be attributed to their highest total phenolic content. Both free and bound phenolics exhibited a strong correlation between TPC and TEAC ($R^2 = 0.85$ for blue wheat, $R^2 = 0.97$ for black wheat, and $R^2 = 0.85$ for purple wheat, $P < 0.05$).

4. Discussion

Phenolic acid compositions and concentration in the pigmented wheat bran fractions varied significantly. Liyana-Pathirana and Shahidi [12] have suggested that the majority of the phenolic acids were ferulic acid, p-coumaric acid, vanillic acid, and sinapic acid in whole wheat and all fractions examined. In this study, gallic, protocatechuic, p-hydroxybenzoic, vanillic, syringic, p-coumaric, ferulic, isoferulic, salicylic acids were present in free phenolic acids, but no gallic, protocatechuic acids were detected in bound phenolic acids. In this study, the majority of phenolic acids existed in bound form in the pigmented wheat bran and ferulic acid was the primary individual phenolic acids in bound form. The bound phenolics were supposed to improve health more effectively as they may avoid upper gastrointestinal digestion along with cell wall components and are absorbed into blood plasma during digestion of intestinal microorganism [35]. Meanwhile, a decreasing trend in phenolic acids content was found from outer bran to shorts. This observation is supported by other studies [29]. In wheat grains, phenolic acids are mainly found in the cell walls of their outer layers, mainly esterified to the arabinose side groups of arabinoxylans [36]. By debranning and milling, bran-rich fraction can be obtained and used to a source of natural antioxidant substance.

In agreement with other studies [16, 37], the effect of pigment on the concentration of phenolic acids was observed in this research. Kim et al. [16] reported that the total phenolic content of the red wheat bran was higher than that of the white wheat. According to Chandrasekara and Shahidi [38], millets with dark brown pigment had a higher phenolic content of soluble phenolic parts than those with white or yellow testa and pericarp.

According to Zhou and Yu [3], the TEAC ranged from 3.09 to 15.26 μmol TE/g for Akron wheat bran and from 2.74 to 12.04 μmol TE/g for Trego wheat bran. In present study, the consequences of the scavenging activity of wheat bran extracts against $ABTS^{\bullet+}$ were lower (0.81–12.81 μmol TE/g defatted bran) than the previous reports, which could be explained by different wheat varieties, locations, extraction methods, and the way used to prepare $ABTS^{\bullet+}$. Widespread used approaches for producing $ABTS^{\bullet+}$ include oxidation with potassium persulfate [29] or MnO_2 [39] and attenuation in ethanol or phosphate buffer. In this study, a commercial kit was used to determine the TEAC.

Total phenolic content has been found to be significantly associated with the antioxidant activity [8]. In this paper, significant correlations ($R^2 > 0.85$, $P < 0.05$) were observed between TPC and the antioxidant activity. Beta et al. [8] reported a strong correlation of total phenolic content and antioxidant activity for pearled wheat and milled fractions. However, Zhu et al. [40] reported that the relationship between TPC and free radical scavenging activities against both DPPH• and $ABTS^{\bullet+}$ was complex. In this study, all fractions of three pigmented wheat varieties had effective radical scavenging properties. Blue wheat bran fractions had the strongest radical scavenging activity against $DPPH^{\bullet}$ while those of purple wheat gained the strongest $ABTS^{\bullet+}$ scavenging activity. The scavenging activity of the same sample of DPPH radical was inconsistent with that of the $ABTS^{\bullet+}$ radical. The 70% ethanol extract showed the best DPPH radical scavenging activity but did not necessarily have the highest activity to quench $ABTS^{\bullet+}$ [40]. Several reasons could be provided for this observation: (1) DPPH and ABTS assays carried out in methanol and aqueous ethanol media, respectively; (2) different mechanisms related to the radical-antioxidant reactions; ABTS radical scavenging activity is a single electron transfer reaction, while the DPPH radical scavenging assay transfers hydrogen atom or electrons [41]; (3) stereoselectivity of the radicals or the solubility of wheat extracts in different estimating systems [42].

The bound phenolics exhibited significantly greater antioxidant activity than that of free phenolics in all samples. This may be due to the higher total phenolic content in the bound phenolic extracts than those in the free phenolic extracts. Consequently, the bound phenolic acids were the major antioxidant components of wheat bran and existed abundantly. These components were not extractable by aqueous methanol but released upon alkaline hydrolysis.

5. Conclusions

Three pigmented wheat varieties were debranned and milled to produce three bran fractions (outer bran, coarse bran, and shorts). The phenolic acids content and antioxidant capacity were investigated. In general, the content of phenolic acids and antioxidant activities both in free and bound forms gradually decreased in the order of outer bran > coarse bran > shorts. The content of bound phenolic acids and antioxidant capacities were significantly higher than free phenolic acids. The multiple differences existing in different fractions may be attributed to the differences in the composition and amount of phenolic acids. Ferulic acid was the predominant phenolic acid in the bound form. The total phenolics content and antioxidant capacity of both free and bound phenolics showed quite a strong correlation ($R^2 > 0.85$, $P < 0.05$). Pigmented wheat varieties have a considerable promise as a source of bioactive material for potential use in the functional food industry.

Abbreviations

TPC: Total phenolic content
GAE: Gallic acid equivalents
FC: Folin–Ciocalteu
HPLC: High performance liquid chromatography
DPPH: 2,2-Diphenyl-1-picrylhydrazyl
$ABTS^{\bullet+}$: (2,2′-Azino-bis[3-ethylbenzthiazoline-6-sulfonic acid])
TEAC: Trolox equivalent antioxidant capacity; Trolox (6-hydroxy-2,5,7,8-tetra-methyl-chroman-2-carboxylic acid)
FPA: Free phenolic acid
AHPA: Alkaline-hydrolysable phenolic acids
AAPH: 2,2′-Azobis-(2-methylpropionamidine) dihydrochloride.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Acknowledgments

The authors are appreciative of the financial support of the Development Program of Science and Technology in Shandong Province (2011GGA01072) and Funds of Shandong "Double Tops" Program.

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Hindawi
Journal of Chemistry
Volume 2018, Article ID 3805932, 9 pages
https://doi.org/10.1155/2018/3805932

Research Article

Gamma-Tocotrienol Stimulates the Proliferation, Differentiation, and Mineralization in Osteoblastic MC3T3-E1 Cells

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Received 11 October 2017; Accepted 6 December 2017; Published 15 January 2018

Academic Editor: Ji Kang

Gamma-tocotrienol, a major component of tocotrienol-rich fraction of palm oil, has been suggested to exhibit bone protective effects *in vivo*. However, the effects of γ-tocotrienol on osteoblast cells are still unclear. In this study, the effects of γ-tocotrienol on the proliferation, differentiation, and mineralization in osteoblastic MC3T3-E1 cells were investigated. Our results showed that γ-tocotrienol (2–8 μmol/L) significantly improved the cell proliferation ($p < 0.05$), but it did not affect cell cycle progression. γ-Tocotrienol significantly increased alkaline phosphatase (ALP) activity ($p < 0.05$), secretion levels of osteocalcin (OC) and osteonectin (ON), and mRNA levels of collagen type I (Col I) of MC3T3-E1 cells. Meanwhile, we found that γ-tocotrienol is promoted in differentiation MC3T3-E1 cells by upregulation of the expression of Runx2 protein. Moreover, the number of bone nodules increased over 2.5-fold in cells treated with γ-tocotrienol (2–8 μmol/L) for 24 d compared to control group. These results indicated that γ-tocotrienol at low dose levels, especially 4 μmol/L, could markedly enhance the osteoblastic function by increasing the proliferation, differentiation, and mineralization of osteoblastic MC3T3-E1 cells. Moreover, our data also indicated that Runx2 protein may be involved in these effects. Further studies are needed to determine the potential of γ-tocotrienol as an antiosteoporotic agent.

1. Introduction

Osteoporosis affects 1/3 of women and 1/5 of men over the age of 50 years worldwide; it affects 75% of aged population in Europe, USA, and Japan [1]; and in China alone, there are 0.21 billion of people with low bone density [2]. Osteoporosis is characterized by low bone density and deterioration of bone microarchitecture [3]. Osteoporosis causes progressive bone loss and arises from an imbalance of bone resorption and formation in the bone remodeling process. There are many factors which can cause osteoporosis such as menopause, aging, thyroid diseases, and calcium deficiency [4].

Currently, most drugs for the treatment of osteoporosis focus on improvement of bone resorption, via either reducing osteoclast number (such as bisphosphonates and estrogen) or inhibition of osteoclast activity (such as cathepsin K inhibitors). However, they have little ability to stimulate new bone synthesis [1, 5–7]. Since new bone formation depends primarily on the function of osteoblasts, the agents acting by either increasing the proliferation or inducing differentiation of the osteoblasts could enhance bone formation [8, 9]. Furthermore, the potential bone-forming agents or drugs currently available either may have serious side-effects or may not improve bone quality to reduce the susceptibility to fracture. Thus, the discovery of natural dietary compounds that promote bone formation may be able to avoid the occurrence of the adverse effects of traditional drug in humans and will be of great interest.

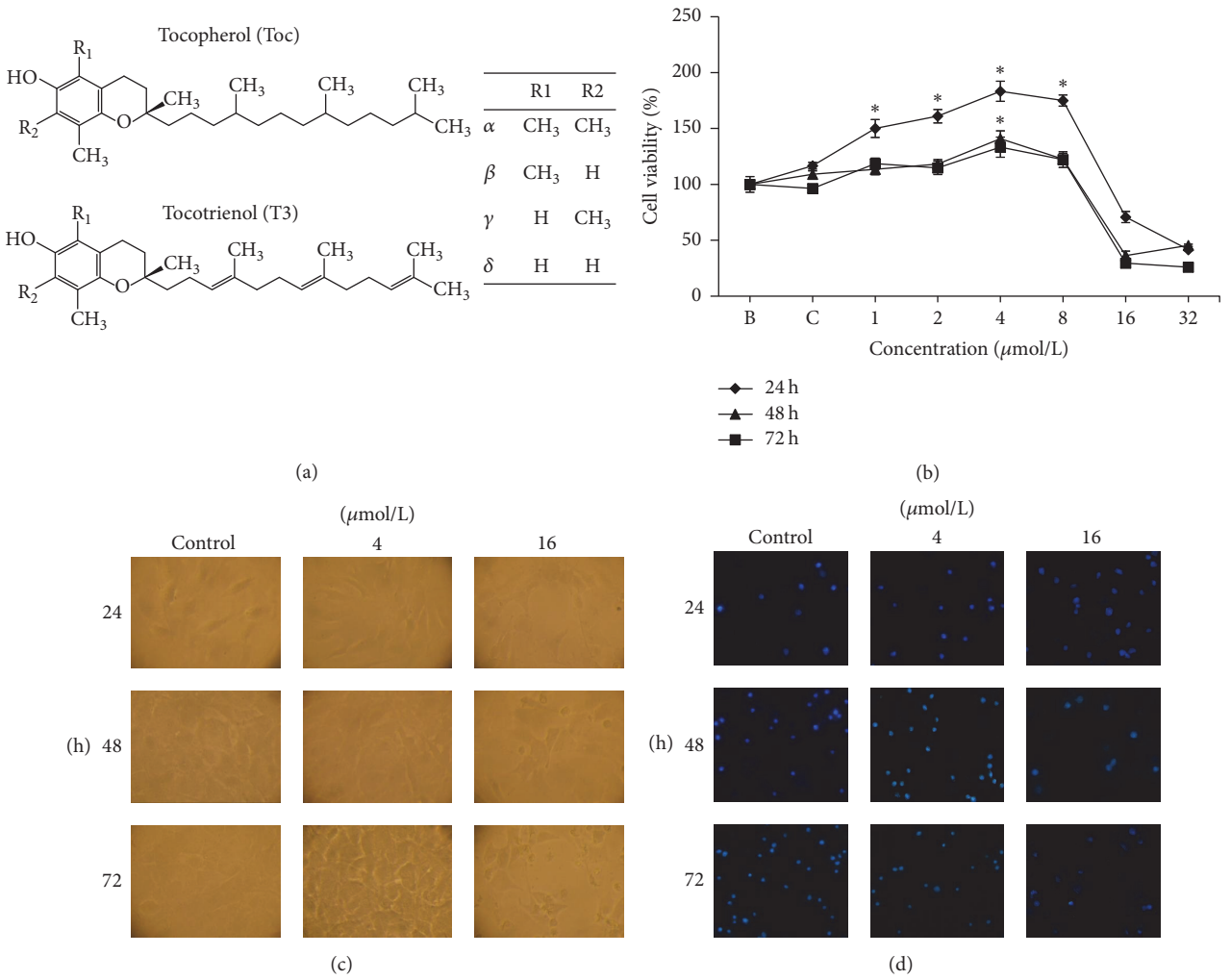

Figure 1: Effect of γ-tocotrienol on viability in MC3T3-E1 cells. (a) The structure of tocopherol and tocotrienol. (b) Cells were exposed to different doses of γ-tocotrienol for 24, 48, or 72 h. Cell viability was determined by MTT assay. (c) Cells were treated with different doses of γ-tocotrienol for 24, 48, and 72 h. Images were captured by phase contrast microscopy (200x). (d) Cells were stained with DAPI dye for measuring nuclear morphological alteration (DAPI, 200x). Data are expressed as mean ± SD ($n = 3$). $^*p < 0.05$, compared to the control group. B: blank control; C: ethanol control.

Tocotrienols and tocopherols, two subclasses of vitamin E, are abundant in food ingredients such as palm oil, rice bran oil, barley, corn, oats, rye, and wheat [10, 11]. Each of them has four stereoisomers, respectively, namely, α-, β-, γ-, and δ-tocopherols or tocotrienols (Figure 1(a)). Tocopherols contain a saturated phytol side chain in the chroman ring. Tocotrienols differ from the tocopherols in that they contain three double bonds in the side chain [12]. In previous studies, tocotrienols have been shown to have better bone protective effects when compared to α-tocopherol in animal osteoporosis models [13–19]. Furthermore, studies also showed that tocotrienols were able to prevent and even reverse osteoporosis in estrogen deficiency, testosterone deficiency, glucocorticoid excess, and nicotine exposure [15–17, 20–23]. Previous study demonstrated that palm tocotrienol is even more effective than calcium in preventing bone loss caused by estrogen deficiency [24]. Tocotrienols showed a better bone anabolic action than tocopherol in normal male rats [14, 25]. The protective mechanism of tocotrienols on bone was thought to be contributed by its antioxidant property [17, 18, 26].

Although *in vivo* studies have shown that tocotrienols exhibit bone protective activity, there is paucity of *in vitro* studies to determine the effect of tocotrienols on bone cells, especially tocotrienol isomers [15–17, 20–25, 27]. γ-Tocotrienol, the most abundant isomer in palm oil (up to 49% of the vitamin E) [28], was the most potent isomer of tocotrienols in promotion bone formation and protection in the *in vivo* studies [14, 29]. However, few studies had reported the direct evidence on the effect of γ-tocotrienol in osteoblast function. The purpose of the present study was to understand the effects of γ-tocotrienol on cell proliferation, differentiation, and mineralization in osteoblastic MC3T3-E1 cells.

Table 1: Sequences of primers for real-time PCR.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)	Size (bp)
GAPDH	CCCAGAAGACTGTGGATGG	GGATGCAGGGATGATGTTCT	81
Ki-67	GACAGCTTCCAAAGCTCACC	GTGTCCTTAGCTGCCTCCTG	228
PCNA	TGGAATCCCAGAACAGGAG	CCAATGTGGCTAAGGTCTCG	87
Col I	GCATGGCCAAGAAGACATCC	CCTCGGGTTTCCACGTCTC	83
Runx-2	GCCGGGAATGATGAGAACTA	TGGGGAGGATTTGTGAAGAC	155

2. Materials and Methods

2.1. Materials. Osteoblastic MC3T3-E1 cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Fetal bovine serum (FBS) and α-minimum essential medium (α-MEM) were purchased from Gibco Life Technologies Co. (Carlsbad, CA, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), LiCl, propidium iodide (PI), and 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI) were bought from Sigma Aldrich (Kansas, MO, USA). γ-Tocotrienol was purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). Alizarin Red staining reagent kit was purchased from GenMed Scientifics Inc. (USA). ALP reagent kit and ELISA kit were bought from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The MiniBEST Universal RNA extraction kit and PrimeScript™ RT reagent kits were purchased from Takara Biotech Co., Ltd. (Dalian, China). Rabbit polyclonal antibodies for NAPDH, PCNA, and Runx-2 and goat anti-rabbit secondary antibody were bought from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Cell Culture. Cells were cultured in α-MEM containing 10% FBS at 37°C in a humidified atmosphere containing 5% CO_2. When the cells reached subconfluence (about 24 h), various concentrations of γ-tocotrienol were added to the medium containing 5% FBS. Stock solutions of γ-tocotrienol were prepared in absolute ethanol and stored at −20°C. The final ethanol concentration in all cultures was 0.05%.

2.3. Viability Assay. The effect of γ-tocotrienol on cell proliferation was investigated by MTT assay as previously described with some modifications [30]. Briefly, MC3T3-E1 cells (1×10^4 cells/well) were seeded in the 96-well plates overnight. The medium was removed and the cells were treated with 200 μL of medium containing γ-tocotrienol (1, 2, 4, 8, and 16 μmol/L, resp.) for 24, 48, and 72 h. Each dose of γ-tocotrienol was repeated in five wells. Twenty microliters of MTT (5 mg/ml in PBS) was added to each well and incubated at 37°C for 4 h. The medium was carefully removed and 150 μL of dimethyl sulfoxide was added to each well. The plates were shaken for 10 min and the absorbance at 490 nm was measured in a microplate reader (BioTek Instruments, Inc., USA). The cell viability was indicated as the percentage of the OD of samples to that of the control group, taking the viability of the blank control cells as 100%.

2.4. Morphologic Observation. After treatment with various concentrations of γ-tocotrienol for the desired time, cell morphological changes were observed by inverted microscope. Changes of the nuclei were investigated by staining the cells with fluorescent DNA-binding dyes. Briefly, cells were harvested, washed with PBS, and fixed with 4% polyoxymethylene for 20 min at 4°C. After washing with PBS, cells were incubated with DAPI (15 μg/ml) for 15 min in the dark at room temperature. Images were taken using a fluorescence microscope (Eclipse 80i, Nikon, Japan).

2.5. Cell Cycle Analysis. The cells were harvested, washed three times with PBS, fixed with 70% cool ethanol for 2 h, and stained with PI solution (500 μL, 50 μg/ml PI, and 50 μg/ml RNase in 1% NP-40 solution) for 30 min in the dark at room temperature. Cells (1×10^5) were analyzed by FAC Sort flow cytometer (BD Biosciences, USA). The proportions in G_0/G_1, S, and G_2/M phases were analyzed using ModFit LT analysis software.

2.6. Quantitative Real-Time qPCR. Total RNAs were extracted from each treatment using MiniBEST Universal RNA Extraction kit according to the manufacturer's instruction. Total RNA was reverse transcribed into cDNA using PrimeScript™ RT reagent kits, following the manufacturer's instruction. Target gene expression was normalized to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The $2^{-\Delta\Delta Ct}$ method was applied to calculate relative gene expression when compared to the control group [31]. The primers [32–35] used for real-time PCR were listed in Table 1.

2.7. Western Blot Analysis. Protein expression levels were investigated by Western blot method according to our previous study [36]. The total protein concentrations of each sample were measured in a 550 Universal microplate reader (Bio-Tek Instruments, Inc.) at 562 nm. For Western blotting, 100 μg of protein was resolved on 10% polyacrylamide gels and transferred to a nitrocellulose membrane. The membrane was blocked in blocking buffer (1% BSA, 1% Tween 20 in 20 mM Tris-buffered saline (TBS), pH 7.6) for 30 min at 37°C in a hybridization oven, incubated with appropriate monoclonal or polyclonal primary antibody in blocking buffer for 2 h at 37°C or overnight at 4°C. The membrane was washed 3 × 5 times with Tris-buffered saline Tween 20 (TBST) followed by incubation with anti-mouse or anti-rabbit secondary antibody at 37°C for 1 h. The membrane was washed 3 × 5 times with TBST and then washed with TBS twice. Then the membrane was incubated with alkaline phosphatase until an appropriate signal level was obtained.

Protein bands were detected by FluorChem Imaging Systems (Bio-Rad, Hercules, CA, USA).

2.8. ALP Activity Assay. Cells were exposed to different concentrations of γ-tocotrienol (2, 4, 8, and 16 μmol/L) for 24, 48, and 72 h. Cells were harvested and incubated with 1% Triton-100 in PBS. ALP activity was measured by ALP assay kit according the manufacturer's instruction. The protein was also measured by the bicinchoninic acid (BCA) method (Applygen Technologies, Inc.). ALP activity was standardized as the relative percentage to control group.

2.9. OC, ON, and Col I ELISA Assays. After exposure to different concentrations of γ-tocotrienol (2, 4, 8, and 16 μmol/L) for 3 and 6 d, OC, ON, and Col I secretion were evaluated in the cultured medium by ELISA kit, according to manufacturer's instructions. The absorbance was measured at 450 nm with a microplate reader (Eon, Bio-Tek Instruments, USA).

2.10. Mineralization Analysis by Alizarin Red Staining. After being exposed to γ-tocotrienol (2, 4, 8, and 16 μmol/L) for 8, 16, and 24 d, cells were stained with Alizarin Red kit (GenMed Scientifics Inc., USA). Cells with orange red staining indicated calcium deposits. The results were observed with a phase contrast microscope at a magnification of ×200. Alizarin Red was quantified in a solution of 20% methanol and 10% acetic acid in water. After washing the cells for 15 min, the solution was transferred to a 96-well plate and absorbance of Alizarin Red was measured by a spectrophotometer at 450 nm.

2.11. Statistical Analysis. Statistical analysis was performed using SPSS version 14.0 (SPSS, Inc., Chicago, IL, USA). The data were expressed as mean ± SD. Differences between the control and treated groups were evaluated by the one-way analysis of variance (ANOVA) test with the Bonferroni post hoc multiple comparisons and considered significant at $p < 0.05$.

3. Results and Discussions

3.1. Effect of γ-Tocotrienol on the Proliferation of MC3T3-E1 Cells. The effect of γ-tocotrienol on cell viability was shown in Figure 1(b). Cell viability was increased significantly in γ-tocotrienol (1–8 μmol/L, especially at 4 μmol/L) groups when compared to control groups. After treatment with 4 μmol/L γ-tocotrienol for 24, 48, and 72 h, cell viability increased by 83.3%, 40.9%, and 33.3%, respectively ($p < 0.05$). However, cell viability was significantly inhibited by γ-tocotrienol at dosages above 16 μmol/L ($p < 0.05$). Morphological changes of control and treated cells are shown in Figure 1(c). In control cells, irregular shapes were observed, such as triangles, polygons, and long spindles with different length pseudopodia. The cytoplasm was abundant and clear, and the nuclei were large and clear. There was no significant morphological change in cells treated with γ-tocotrienol at doses of 4–8 μmol/L for 24, 48, and 72 h. However, MC3T3-E1 cells treated with γ-tocotrienol at doses above 16 μmol/L for 48 and 72 h began to show deformation, shrinking, and floating when compared with the control cells. The nuclear morphological alteration of MC3T3-E1 cells was shown in Figure 1(d); there were no nuclear morphological alteration between control cells and γ-tocotrienol- (2–8 μmol/L) treated cells. However, typically apoptotic changes such as chromatin condensation and nuclear fragmentation were found in cells treated with γ-tocotrienol at 16 μmol/L for 72 h or 32 μmol/L for both 48 and 72 h (data not shown).

Treatment with γ-tocotrienol (2–8 μmol/L) for 24 and 48 h did not change the ratios of G0/G1, S, and G2/M phase in MC3T3-E1 cells when compared to the control cells (Figure 2(a)). However, it was significantly affected if the dose of γ-tocotrienol was more than 16 μmol/L. The proportion in G_0/G_1 phase was changed from 57.85 to 64.61% in untreated cells and from 85.74 to 80.08% in treated cells for 24 and 48 h, respectively, while S phase was changed from 31.26 to 25.63% in the control cells and from 6.34 to 11.46% in treated cells for 24 and 48 h, respectively. The apoptotic rates were 4.55% ± 0.72% and 14.26% ± 1.91% in cells treated with γ-tocotrienol (16 μmol/L) for 24 and 48 h, respectively. Our results showed that γ-tocotrienol (2–8 μmol/L) could promote osteoblast proliferation did not affect the cell cycle distribution, and no cytotoxicity was observed in MC3T3-E1 cells. However, 16 μmol/L and above of γ-tocotrienol showed the toxicity to osteoblast cells. The results are consistent with previous studies [16, 37] which demonstrate that γ-tocotrienol, at low dosage, was better than α-tocopherol in protecting rat osteoblasts against H_2O_2 toxicity; but at high dosage it was toxic to osteoblasts in rats [16]. It may be caused by the fact that antioxidants, like γ-tocotrienol, at a certain dosage can become prooxidants [38]. The unsaturated side chains of tocotrienols (Figure 1(a)) allow them to penetrate more efficiently into the membrane lipid bilayer resulting in a high antioxidant activity in comparison with tocopherols [39].

PCNA, a nuclear protein that binds to DNA polymerase, is a cell cycle regulator expressed in the nucleus of proliferating cells. PCNA is an accepted hallmark for cellular proliferation [40, 41]. Ki-67, a protein expressed in proliferating cells, may indicate a more robust marker of cell proliferation than PCNA [16]. Ki-67 protein expression occurs during the G_1 phase, increases during the cycle cell, and rapidly declines after mitosis [42]. To further determine effects of γ-tocotrienol on the proliferation of MC3T3-E1 cells, levels of Ki-67 and PCNA were determined by real-time PCR and Western blot. The results were shown in Figures 2(b) and 2(c); mRNA levels of Ki-67 and PCNA were obviously upregulated by treatment with 2–8 μmol/L of γ-tocotrienol for 48 and 72 h. However, they were downregulated in cells treated with 16 μmol/L of γ-tocotrienol. Furthermore, treatment with 2–4 μmol/L of γ-tocotrienol for 24 and 48 h significantly increased the PCNA protein expression level in MC3T3-E1 in comparison with the control group. However, 16 μmol/L γ-tocotrienol decreased the expressive level of PCNA protein (Figure 2(d)). The result indicated that γ-tocotrienol at low dosage could indirectly regulate the activation of osteoblast proliferation and was consistent with MTT results.

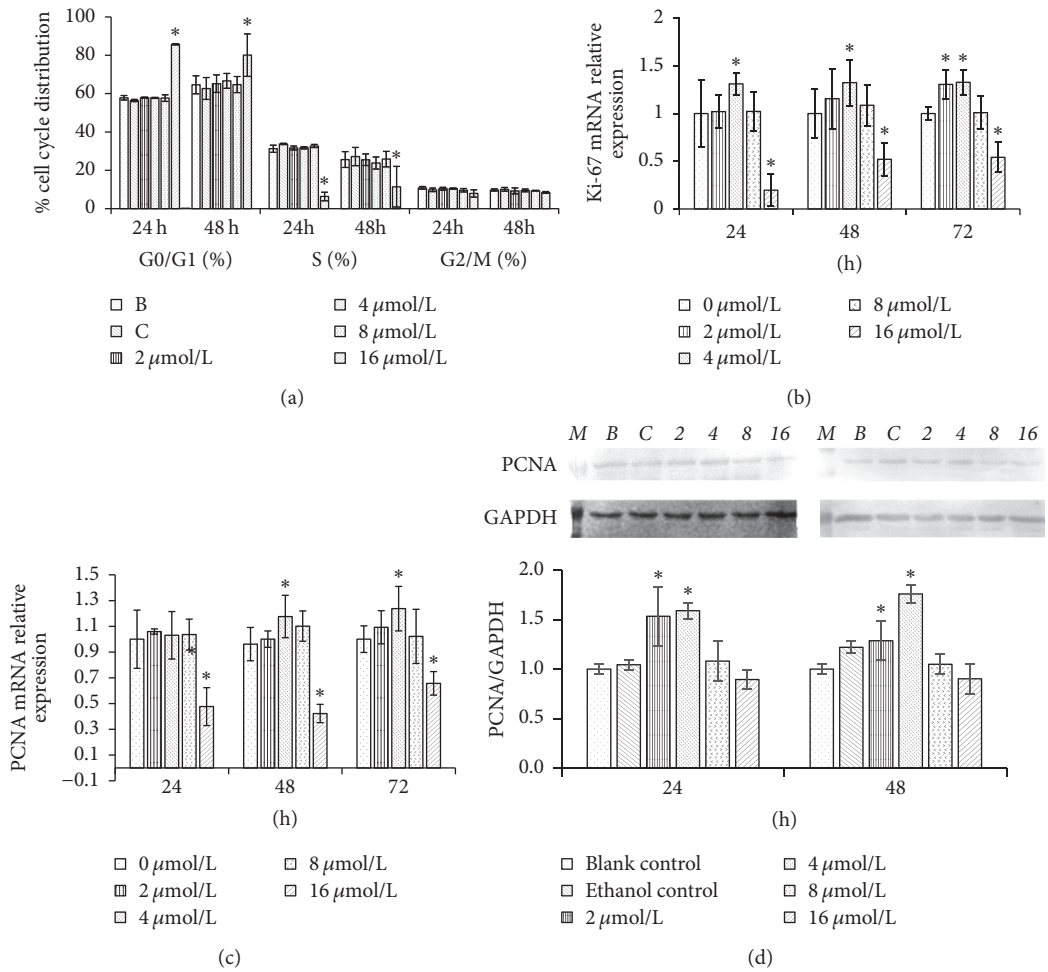

Figure 2: Effect of γ-tocotrienol on cell cycle progression, relative mRNA, and protein levels of Ki67 and PCNA in MC3T3-E1 cells. (a) Cells were treated with different doses of γ-tocotrienol for 24 and 48 h. Cells were fixed by 70% ethanol and stained by propidium iodide dye. The distribution of cell cycle was determined by flow cytometry. ((b), (c)) Cells were treated with different doses of γ-tocotrienol (2, 4, 8, and 16 μmol/L) for the desired time. mRNA levels of Ki67 and PCNA were determined by real-time PCR. (d) Protein levels of PCNA were determined by *Western blot*. Data are expressed as mean ± SD ($n = 3$). $^*p < 0.05$, compared to the control group. M: marker; B: blank control; C: ethanol control.

3.2. Effect of γ-Tocotrienol on the Differentiation of MC3T3-E1 Cells. ALP is a homodimeric glycoprotein secreted by osteoblasts, and the degree of secretion is related to the degree of differentiation of the osteoblasts [43]. ALP secretion is enhanced along with the process of osteoblasts differentiation [44]. Therefore, the appearance of ALP activity is an early phenotypic marker for osteogenic differentiation of osteoblasts. In this study, ALP activity in MC3T3-E1 cells treated with γ-tocotrienol for 24 h and 48 h did not significantly change (data not shown here). However, it was significantly increased from 15% to 194% in cells treated with γ-tocotrienol at 4–16 μmol/L for 72 h, when compared to the control group (Figure 3(a)). Furthermore, ALP activity in cells treated with 4 μmol/L of γ-tocotrienol for 72 h was significantly increased 1.55-fold when compared with control cells ($p < 0.05$). The results suggested that γ-tocotrienol could promote osteoblast differentiation.

OC, ON, Col I, and Runx2 are major phenotypic markers for preosteoblast differentiation during bone formation. OC is the most specific gene for the osteoblast differentiation and mineralization. OC is expressed during the postproliferative period and reaches its maximum expression during mineralization and accumulates in the mineralized bone [45]. ON is synthesized by cells of the osteoblastic lineage, and it also is a differentiation marker of bone cells [46]. Col I, an important component of the bone extracellular matrix, has been shown to be involved in the differentiation of the osteoblast phenotype [47]. LiCl is able to increase osteoblast differentiation [48]; thus it was used as a positive control. In this study, the secretion levels of OC and ON in the supernatants were shown in Figures 3(b) and 3(c). The treatment with γ-tocotrienol (2–8 μmol/L) for 3 d and 6 d obviously increased the secretion levels of both OC and ON in MC3T3-E1 cells. The maximal effect was observed in MC3T3-E1 cells treated with 4 μmol/L of γ-tocotrienol, which increased OC and ON secretion levels by 161.45% and 58.05% for 3 d and 122.97% and 69.77% for 6 d, respectively. Meanwhile, the Col I mRNA levels in cells treated with 2–8 μmol/L of γ-tocotrienol

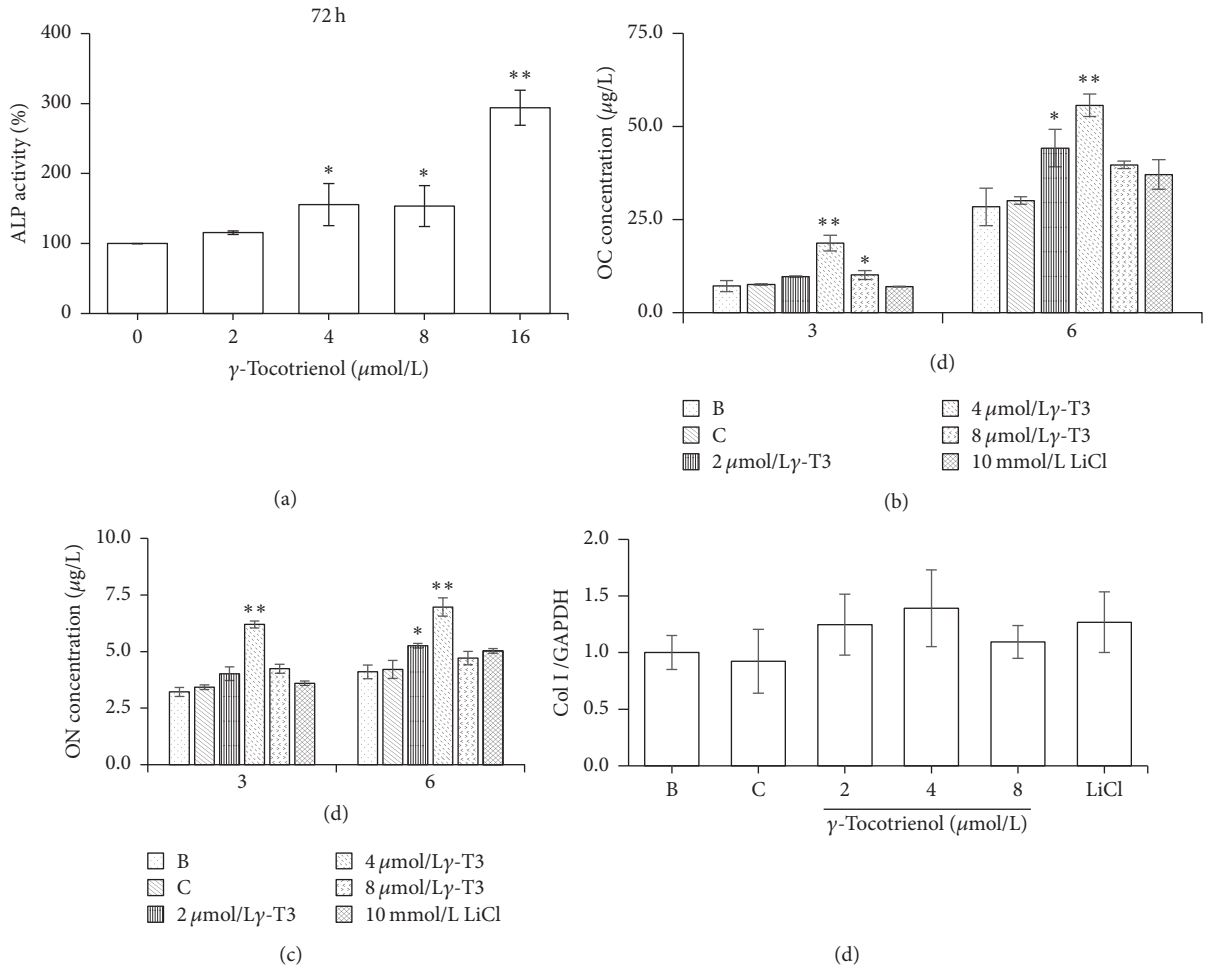

Figure 3: Effect of γ-tocotrienol on the differentiation in MC3T3-E1 cells. Cells were treated with γ-tocotrienol at different doses for the desired time. The ALP activity (a), OC and ON amount ((b), (c)), and Col I mRNA level (d) were determined by colorimetric analysis, ELISA, and real-time PCR analysis, respectively. Data are expressed as mean ± SD ($n = 3$). $^{*}p < 0.05$ and $^{**}p < 0.01$, compared to the control group. B: blank control; C: ethanol control.

for 3 d were obviously increased compared with the control cells (Figure 3(d)), and its protein level did not change in comparison with the control cells (data not shown). The result further confirmed that γ-tocotrienol at low dosage can stimulate osteoblast differentiation, and it also showed that γ-tocotrienol may contribute to the mineralization. In addition, other findings indicate that γ-tocotrienols delivered through nanoemulsion exhibit superior antioxidant properties and osteoblast differentiation [49], which is consistent with our findings.

Runx2, a member of the runt family of transcription factors, is important for osteoblast differentiation [50, 51]. In Runx2 knockout mice, no bone tissues or osteoblasts are generated, which indicates that osteoblast differentiation is completely blocked in the absence of Runx2 [52]. The results showed that both the mRNA and protein level of Runx2 (Figures 4(a) and 4(b)) were obviously upregulated in MC3T3-E1 cells treated with 4 and 8 μmol/L of γ-tocotrienol for 72 h when compared with the control cells ($p < 0.05$), which demonstrated that γ-tocotrienol promoted osteoblast differentiation by upregulation of the expression of Runx2.

3.3. Effect of γ-Tocotrienol on the Mineralization in Osteoblastic MC3T3-E1 Cells. Mineralization, an indispensable process for bone formation, occurs in the last stage following collagenous matrix maturation. It is a functional performance of the further differentiation and maturation of the cells. Osteoblasts are the main functional cells that promote bone mineralization. Mineralized nodule formation is a symbol of differentiation and maturation of osteoblasts and a morphological manifestation of osteogenesis [53]. In order to further clarify the effect of γ-tocotrienol on mineralization of osteoblasts, the level of mineralized nodule was observed by using Alizarin Red staining method. The quantities of Alizarin Red were analyzed by measuring the absorbance at 450 nm. As shown in Figure 5(a), the mineralized nodule formation was lower in the control group. The number of mineralized nodules was significantly increased in cells treated with 2–8 μmol/L of γ-tocotrienol for 24 days. The

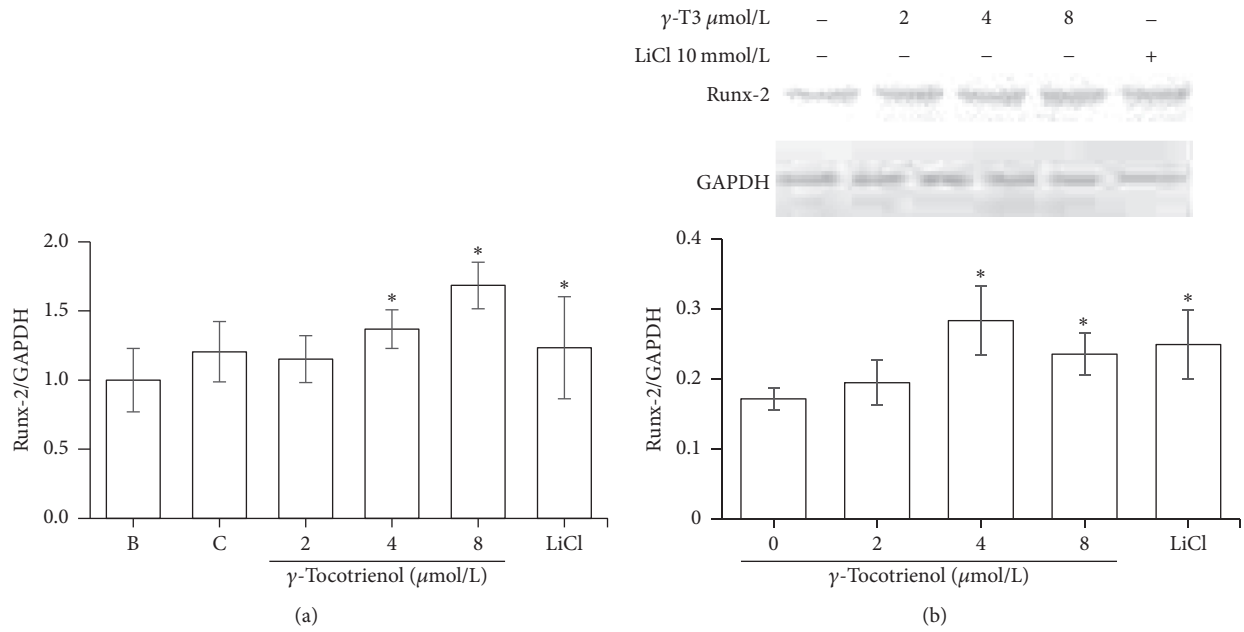

Figure 4: Effect of γ-tocotrienol on mRNA and protein levels of Runx2 in MC3T3-E1 cells. Cells were treated with different doses of γ-tocotrienol for 72 h. The mRNA (a) and protein expression (b) of Runx2 were determined by real-time PCR and *Western blot*. Data are expressed as mean ± SD ($n = 3$). $^{*}p < 0.05$, compared to the control group. B: blank control; C: ethanol control.

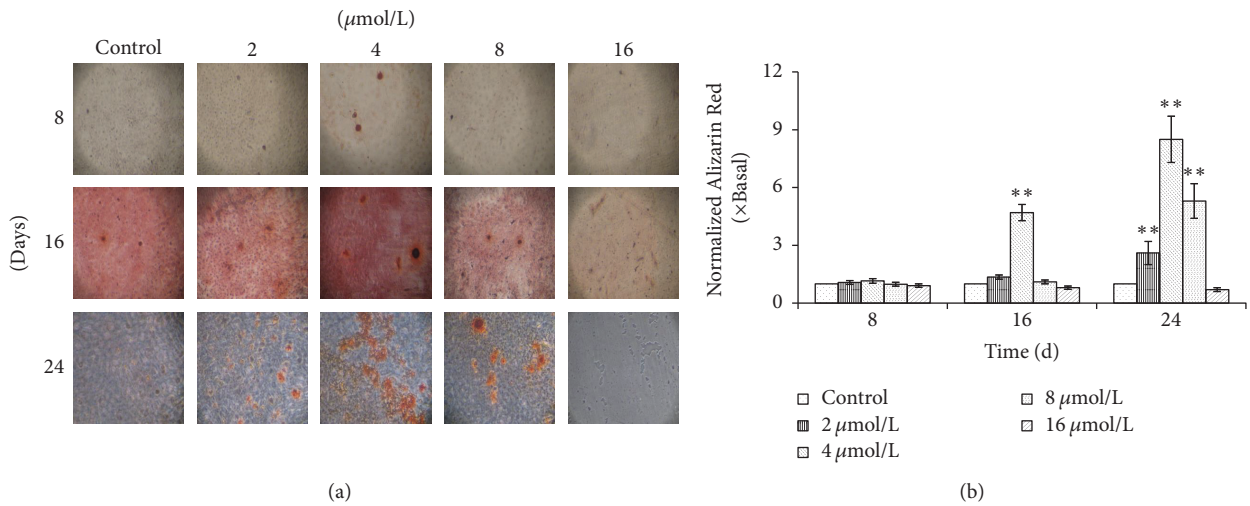

Figure 5: Effects of γ-tocotrienol on the mineralization in MC3T3-E1 cells. Cells treated with different doses of γ-tocotrienol for the desired time. The mineralization in cells was determined by Alizarin Red staining. (a) Formation of mineralized nodule (100x). (b) Alizarin Red was quantified by spectrophotometer. The results are expressed as means ± SD ($n = 3$). $^{**}p < 0.01$, compared to the control group.

formation of mineralized nodules was increased over 2.5-fold in cells treated with γ-tocotrienol at low concentration for 24 d compared with the control group (Figure 5(b)). No cells could be observed in the 16 μmol/L tocotrienol-treated group, which indicated that treatment with 16 μmol/L tocotrienol for 8 days or longer time showed a strong toxicity and inhibitory effect on the mineralization of osteoblast. Our results suggest that γ-tocotrienol at low doses (2–8 μmol/L) has a role in promoting mineralization and inhibits bone formation at doses of 16 μmol/L or over.

4. Conclusions

In summary, the data in this study showed that γ-tocotrienol (2–8 μmol/L) could promote the proliferation, differentiation, and mineralized nodule formation in MC3T3-E1 cells. Meanwhile, our data also indicated that Runx2 protein may be involved in the effect of γ-tocotrienol on bone formation through osteoblastic differentiation and subsequent mineralization. γ-Tocotrienol was effective for stimulating osteoblast bone formation and it might be useful for prevention of osteoporosis. However, the exact mechanism of γ-tocotrienol

on proliferation and differentiation of osteoblasts needs further investigation.

Abbreviations

Ki-67: Ki-67 antigen
PCNA: Proliferating cell nuclear antigen
MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
OC: Osteocalcin
ON: Osteonectin
ALP: Alkaline phosphatase
Col I: Collagen type I.

Conflicts of Interest

Authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

This project was supported by the National Natural Science Foundation of China (no. 31501481 and no. 81402666).

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Hindawi
Journal of Chemistry
Volume 2017, Article ID 8023610, 5 pages
https://doi.org/10.1155/2017/8023610

Research Article

Rheology of Sesame Pastes with Different Amounts of Water Added

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Received 9 August 2017; Revised 13 September 2017; Accepted 26 September 2017; Published 25 October 2017

Academic Editor: Ji Kang

Sesame paste, usually served as a dressing in some local dishes and the desserts in Southeast Asia and the Middle East, requires mixing with water before consumption to form a thin emulsion of the oil-in-water type. Usually water addition is performed empirically and it is difficult to keep the quality of water-mixed sesame paste consistent. In this study, the rheological behavior of sesame paste with different ratios of sesame paste to added water was investigated. The sesame paste tested contains 59.71% of crude fat and 17% of protein. The ratios of sesame paste to added water (w/v) were 1 : 0.75 (T1), 1 : 1 (T2), 1 : 1.25 (T3), and 1 : 1.5 (T4), respectively. All the samples showed the properties of the viscoelastic solids. The samples T1 and T2 behaved as a pseudoplastic material, whereas the apparent viscosity of T3 and T4 remained almost unchanged with the shear rate increasing. T2 had the moderate area of thixotropy loop (57.32 Pa/s) among the tested samples and scored the highest in the sensory evaluation. Therefore, our study suggested that ratio of sesame paste to added water of 1 : 1 (w/v) is suitable for preparing dressings for different dishes.

1. Introduction

Sesame paste, a suspension of the hydrophilic particles dispersed in the oil, has an oil content of approximately 60% and is a typical oil condiment [1].

Commercial sesame paste is often viscous and required mixing with water before consumption [2]. Usually water addition is performed empirically and mainly judged by sensory taste, which is not suitable for preparing large amount of sesame paste; therefore, it is hard to meet the need in the hot-pot or dry noodle industry when homogeneous mixing and consistent taste are required for large amount of sesame paste. The previous studies of rheological properties of sesame paste allow us to understand not only the changes in the texture and structure of sesame paste, but also the variation pattern of mechanical properties related to processing [1, 3–7]. However, the contribution of water addition to the overall rheological properties of the sesame paste has not been studied. The objectives of this study were to investigate the rheological behaviour of sesame paste as a function of water addition and determine the suitable amount of water added to the sesame paste when being used as the dressing and other condiments.

2. Materials and Methods

2.1. Samples. The commercial sesame paste sample was obtained from "Ruifu Oil and Fat Co. Ltd" (Weifang, China), which was made from the white sesame seed; its proximate composition was shown in Table 1. A certain amount of water was slowly added to sesame paste of 10 g in the beaker, and then, the mixture was stirred with the glass-stick for about 5 min to get the homogeneous emulsion. The ratios of sesame paste to water (w/v) were 1 : 0.75, 1 : 1, 1 : 1.25, and 1 : 1.5, and labeled as T1, T2, T3, and T4, respectively; all the samples were prepared just before the rheological test and allowed to rest for 10 min prior to measurement. The sesame paste without adding water was used as the control, labeled as CK. All reagents used were of analytical grade.

2.2. Analytical Methods. The content of protein, moisture, ash, and fat in the sesame paste was determined using the

Table 1: Chemical components and acid values of the sesame paste sample* .

Sesame paste	Crude fat (%)	Protein (%)	Crude fibre (%)	Total sugars (%)	Total ash (%)	Moisture content (%)	Acid values (mgKOH/g)
CK	59.71 ± 0.26	17.00 ± 0.13	3.78 ± 0.10	7.70 ± 0.16	5.01 ± 0.01	0.12 ± 0.02	1.07 ± 0.10

*The components are expressed on dry weight basis except moisture content.

methods of the Association of Official Analytical Chemists (AOAC, 2000) [8]. The content of fibre was determined according to van Soest et al. [9].

2.3. Measurement of the Rheological Properties. The rheological properties of the sesame paste were measured using A HAAKE Rheostress 60 rheometer. A power law model was used to fit the data as follows:

$$\tau = k * \gamma^n, \quad (1)$$

where τ is the shear stress, Pa; γ is the shear rate, 1/s; k is the consistency coefficient, Pa·s; and n is the flow index.

2.4. Sensory Evaluation. The sensory evaluation was determined by ten semitrained panelists. Six attributes including color and lustre, smell, texture, and taste were used to describe the sensory characteristics of the sesame paste. Every attribute was scored from 0 (attribute bad or abnormal) to 5 (attribute good and characteristic), and the data presented were the average of ten scores.

2.5. Data Analysis. All the data were analysed with the HAAKE Rheowin Data Manager 4.63.000, SPSS 16.0, and Excel 2003.

3. Results and Discussion

3.1. Proximate Composition. Table 1 showed that the sesame paste tested in this study contains 59.71% of crude fat, 17.00% of protein, 3.78% of crude fibre, 7.70% of total sugars, and 5.01% of total ash, respectively. The acid value was 1.07 mgKOH/g. The content of crude fat was similar to the roasted sesame seeds of two kinds of sesame in Cameroon [10] but higher than that of the raw sesame seeds produced in Syria [11] and Sudan [12]. This may be due to the fact that the preparation of the sesame paste involves the roasting procedure, which resulted in somewhat dehulling and the loss of water, and thus the content of crude fat was relatively elevated.

3.2. Static Rheological Properties. The shear stress versus shear rate behaviour of the sesame paste with different amount of added water was measured at 25°C (Figure 1). The results showed that, at the same shear rate, T1 exhibited the greatest shear stress, whereas T4 exhibited the lowest shear stress. Interestingly, the initial shear stress of T2 was greater than that of CK; however, it was lower than that of CK

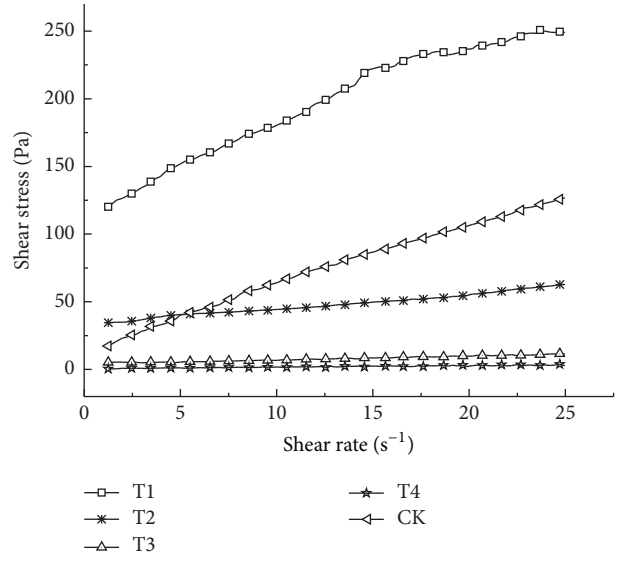

Figure 1: Flow curves of the sesame paste with different amounts of water (T1, T2, T3, and T4: the ratios of sesame paste to water (w/v) were 1 : 0.75, 1 : 1, 1 : 1.25, and 1 : 1.5, resp.; CK: the sesame paste without adding water).

when the shear rate was higher than 5 S^{-1}. The shear stress of T3 and T4 maintained the same level despite increasing the shear rates. We suspected that the molecular interactions of T3 and T4 were weak which made the samples flow easily.

The effect of the shear rate of the sesame paste on the apparent viscosity was also tested in this study (Figure 2). The results showed that samples T1, T2, and control CK performed a shear thinning behaviour like a pseudoplastic material in which the apparent viscosity decreased when the shear rates increased. This result was consistent with that of hulled and unhulled sesame paste [6], milled sesame [13], and sweetened sesame paste [7].

The apparent viscosity of T1 (ratio of paste/water was 1 : 0.75) was the highest at first but decreased fast with the shear rate increasing. The apparent viscosities of the rest of the sesame paste samples decreased slowly with increasing shear rates (Figure 2). The initial apparent viscosities of T3 (ratio of paste/water was 1 : 1.25) and T4 (ratio of paste/water was 1 : 1.5) were low and remained stable with increasing shearing rates, indicating that these two sesame paste samples were too dilute and thin; therefore, these two ratios of paste and water are not suitable for salad dressing. The initial apparent

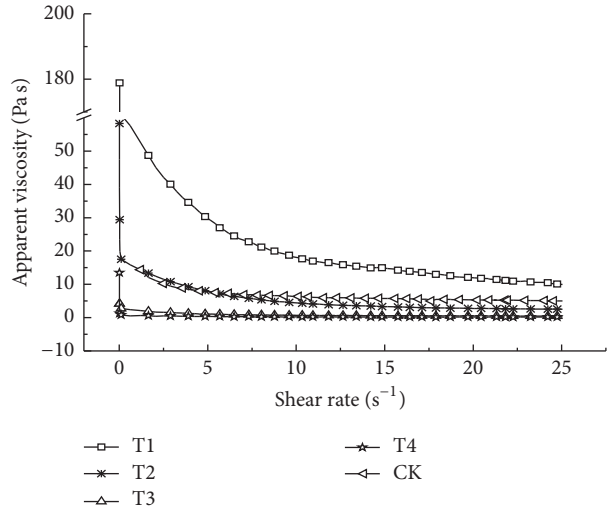

Figure 2: Relationship between the shear rate and apparent viscosity of the sesame paste with various amounts of water.

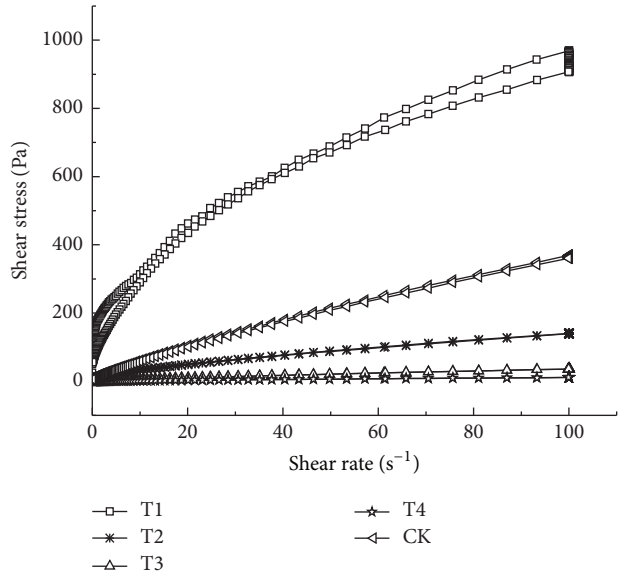

Figure 3: Thixotropy measurements of the sesame paste with different amounts of water.

viscosity of T2 (ratio of paste/water was 1 : 1) was similar to that of the original sesame paste; however, its apparent viscosity decreased rapidly when the shear rate increased, indicating that the ratio of paste and water at 1 : 1 has the excellent tackiness at stationary conditions and flows easily while stirring; thus, ratio of 1 : 1 is suitable for salad dressing and hot pot dishes.

3.3. Thixotropy. The thixotropy loop was obtained by increasing, holding, and decreasing the shear rate of samples (Figure 3). The areas of the thixotropy loops represent the energy of the samples to eliminate the time-dependent effect on rheological behaviour [14] (Table 2).

Table 2: Areas of thixotropy loops of sesame paste with different amounts of water.

Sample	T1	T2	T3	T4	CK
Area of thixotropy loop (Pa/s)	3500.00	57.32	21.00	8.14	500.00

The results showed that when the paste and water ratio was at 1 : 0.75 (sample T1), the shear stress increased fast with the shear rate increased, which reached as high as 970 Pa (Figure 3). Meanwhile, the area of the thixotropic loop was also the highest when the ratio of sesame paste and water was at 1 : 0.75, which was 61 times larger than that of 1 : 1 ratio (sample T2) (Table 2) and about 3 times larger than yogurts [15], which indicated that it may require longer time to recover to original structure when the sesame paste and water ratio was at 1 : 0.75. The sesame paste and water ratio of 1 : 1.5 (sample T4) showed the smallest area of the thixotropic loop, 8.14 Pa/s, which indicated that it is extremely easy to recover to original structure when adding 1.5 times the amount of water to the sesame paste. T2 showed a moderate thixotropy loop area of 57.32 Pa/s.

3.4. Dynamic Viscoelasticity. Dynamic oscillatory shear test was used to characterize the relationship between viscoelastic properties of the sesame paste samples and the changes of G' (the storage modulus) and G'' (the loss modulus) (Figure 4). G' represents the energy stored in the material; G'' indicates the measurement of energy dissipated during every cycle [16]. If $G' \geq G''$, the samples present characteristics of viscoelastic solids; if $G' \leq G''$, the samples show characteristics of fluids [17]. The results showed that 1 : 0.75 (T1) and 1 : 1.5 ratio (T4) of sesame paste and water displayed the highest and the lowest storage modulus G', respectively. However, all the samples showed higher G' than the loss modulus G'' when the frequency range was within 0.1–10 Hz (Figure 4), which demonstrated a behaviour of a gel and the properties of a viscoelastic solid [18].

3.5. Sensory Evaluation. The sensory evaluation of sesame paste with different ratio of water was assessed and the results were analysed (Table 3). The ratio of 1 : 0.75 (T1) and 1 : 1.5 (T4) showed the lowest texture and total sensory score. The ratio of 1 : 1 (T2) showed the highest total score which exhibited a light yellow color and lustre, a rich and typically aromatic smell of sesame paste, and appropriate viscosity. Therefore, the results of our study showed that the ratio of sesame paste and water at 1 : 1 is suitable for salad dressing and hot pot dishes.

Conflicts of Interest

The author declares that there are no conflicts of interest regarding the publication of this paper.

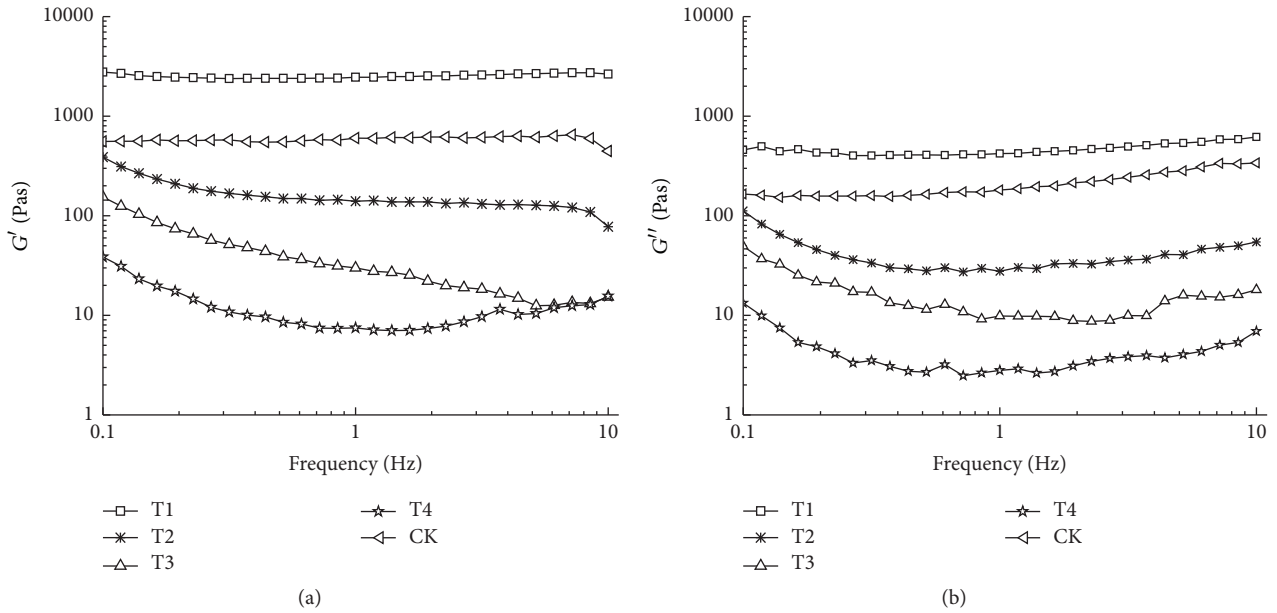

Figure 4: Frequency sweep charts of the sesame paste with different amounts of water: (a) storage modulus and (b) loss modulus.

Table 3: Sensory evaluation of different ratio of sesame paste and water.

Sample	Colour and lustre	Smell	Texture	Taste	Total score
T1	3	2	1	2	8
T2	4	5	5	5	19
T3	4	3	3	3	13
T4	2	2	1	2	7
CK	5	5	4	4	18

Acknowledgments

This work was supported by the Henan Provincial Scientific and Technological Research Project (Grant no. 152102210272, 2015).

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Hindawi
Journal of Chemistry
Volume 2017, Article ID 8948639, 9 pages
https://doi.org/10.1155/2017/8948639

Research Article

Physicochemical Characterisation of Polysaccharides from the Seeds and Leaves of Miracle Fruit (*Synsepalum dulcificum*) and Their Antioxidant and α-Glucosidase Inhibitory Activities In Vitro

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Received 21 November 2016; Revised 24 January 2017; Accepted 2 March 2017; Published 30 March 2017

Academic Editor: Feng Xu

Miracle fruit (*Synsepalum dulcificum*) has been well known and studied for its unique taste-modifying ability. In this study, the monosaccharide composition, molecular weight (Mw), and in vitro bioactivities (antioxidant, α-glucosidase inhibition) of polysaccharides from the seeds (MFP-S) and leaves (MFP-L) of miracle fruit were investigated. The results showed that MFP-S was a homogeneous polysaccharide (Mw 2804 Da) with glucose. MFP-L displayed three fractions (92093, 1496, and 237 Da) consisting of rhamnose, arabinose, galactose, glucose, and xylose. Moreover, the antioxidant and α-glucosidase inhibition of MFP-L were significantly greater than those of MFP-S. The α-glucosidase inhibition of MFP-L was remarkably better than the positive control, acarbose (an antidiabetes drug). More specifically, the 50% inhibitory concentration (IC50) values of α-glucosidase activities for MFP-S, MFP-L, and acarbose were 33, 0.01, and 1 mg mL^{-1}, separately. Therefore, MFP-L can be developed as a functional factor with both antioxidant and antidiabetes activities in food applications.

1. Introduction

The miracle fruit (*Synsepalum dulcificum*) is an ever green indigenous to tropical west Africa. The shrub yields ripe red berries called "miracle fruit" that exhibit an interesting and remarkable taste-modifying property of altering sour flavours to sweet [1]. The pioneering study on miracle fruit was proposed by Inglett et al. [2] whilst looking for a natural sweetener to replace saccharin and cyclamate. Kurihara and Beidler [3] first reported in *Science* that the active ingredient could be a special glycoprotein with a molecular weight of 44 kDa, and Brouwer et al. [4] named the glycoprotein "miraculin" in *Nature*. Miraculin was further extracted, and its genes have been transferred to *Aspergillus oryzae*, lettuce, and tomato [5–7] to promote the mass production of this unique glycoprotein. In addition to miracle fruit's well-known taste-modifying ability, the fruit could be used to improve food palatability for patients who are undergoing chemotherapy [8].

It is noteworthy that all parts of this plant have medicinal importance [9]. However, Swamy et al. [10] found that few studies had been done on the medical or clinical effects of miracle fruit aside from well-established studies on the sweetening effects [11]. Inglett and Chen [12] investigated the antioxidant properties of phenolics and flavonoids in the skin, pulp, and seeds of miracle fruit and suggested it as a good source of antioxidants for functional food applications. He et al. [13] studied the total phenolic content and DPPH radical

scavenging activities of extracts from the skin, pulp, and seed of miracle fruit. Du et al. [14] researched the phenolic and flavonoid content and antioxidant activity of miracle fruit flesh and seed methanol extracts, which could be used as an antioxidant-rich fruit to promote human health. Cheng et al. [15] also verified that polyphenols in seed extracts of miracle fruit were powerful antioxidants. Wang et al. [16] studied the antioxidant and antityrosinase effects of constituents of the stems of miracle fruit, suggesting the potential applications in food supplementation and medical cosmetology. Chen et al. [17] reported the inhibitory effects of aqueous extracts of miracle fruit leaves on oxidative and mutation damage and attributed them partially to its active phenolic components. Shi et al. [18] found that a butanol extract of miracle fruit exhibited equal effects to allopurinol, which is a commonly prescribed medication for gout and hyperuricemia. Therefore, they suggested that the butanol extract of miracle fruit could be used as a novel antihyperuricemia agent or health food. Moreover, Kaki Bale et al. [19] noted in a "Conference in Natural Medicine" that miracle fruit has been extensively used as an antidiabetes herbal medicine to protect the health of the Terengganu people in Malaysia, but the mechanism of effect has not been clarified. Chen et al. [20] suggested that miracle fruit could markedly raise insulin sensitivity in fructose-rich chow-fed rats, indicating that miracle fruit may be served as an adjuvant therapy for diabetic patients with insulin resistance.

Based on these very limited reports on the functional properties of polyphenol-enriched extracts from different parts of the miracle fruit plant, it was unexpected that polysaccharides have not yet been studied. However, it has been well reviewed that polysaccharides (such as tea polysaccharides) exhibit various bioactivities, including antioxidant, antidiabetes, anticancer, and immunological activities. Animal and clinical studies have suggested that tea polysaccharides play an important role in overall human health [21, 22]. Many studies have been done to search for safe and efficient antioxidants and α-glucosidase inhibitors from natural materials. Their purposes were to explore bioactive additives for functional foods or therapeutic compounds to prevent the development of diabetes and slow its progression. Nonstarch polysaccharides are also a type of dietary fiber, which can improve glycaemia and insulin sensitivity, lower good pressure, and drum cholesterol levels of individuals.

Therefore, we attempted to isolate polysaccharides from the seeds and leaves of miracle fruit. Their physicochemical properties (monosaccharide composition, molecular weight) and bioactivities, including antioxidant and α-glucosidase inhibitory activities in vitro, were investigated in this study.

2. Materials and Methods

2.1. Materials and Chemicals. The fresh seeds and leaves of miracle fruit were provided by the Shenzhen Miracle Fruit Co. Ltd. (Shenzhen, Guangdong, China). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Ferrozine, α-glucosidase, and dextran molecular weight standards were purchased from Sigma Chemical Co. (St. Louis, USA). *p*-Nitrophenyl α-D-glucopyranoside (*p*NPG) and acarbose were purchased from J&K Chemical Ltd. (Shanghai, China). All other chemicals and solvents were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China) and were of analytical grade or purer.

2.2. Extraction and Isolation of Miracle Fruit Polysaccharides. Polysaccharides are generally prepared with hot aqueous extraction followed by ethanol precipitation. The isolation processes of miracle fruit polysaccharides (MFPs) are presented in Figure 1. Both fresh seeds and leaves of miracle fruit were freeze-dried for 48 h in a Christ Alpha 1–4 LSC lyophiliser (Marin Christ, Osterode, Germany), were pulverised, passed through a 40-mesh sieve (0.43 mm), and were stored at −18°C in vacuum packaging. Each 60 g of the dried powder of seeds and leaves from miracle fruit was first processed ultrasonically with 5 volumes of 80% ethanol two times, in order to extract polyphenols, flavonoids, and oil. The dried residues were extracted with hot ultrapure water at 100°C at a ratio of 1 : 10 (w/v) for 5 h. Because the seeds contain a large amount of starch, which causes gelatinisation, 0.1% α-amylase was used to liquefy and decrease the viscosity of the extraction suspension at 70°C.

After extraction, the supernatant solutions were collected by centrifugation (6000*g*; 10 min; 25°C) and concentrated to about 100 mL at 50°C using a R-215 rotary evaporator (Buchi, Zurich, Switzerland). Sevag reagent (chloroform: n-butyl alcohol = 4 : 1, v/v) was added and shaken to remove the proteins in concentrated solutions at a ratio of 1 : 5 (v/v) for three times. The concentrated liquid extracts were mixed with 4 volumes of anhydrous ethanol at 4°C overnight for precipitation of MFPs. The resultant precipitates were collected by centrifugation (6000*g*; 15 min; 4°C). Polysaccharide precipitates were redissolved in water and precipitated with anhydrous ethanol and acetone twice again to remove other active compounds, soluble monosaccharide, and reducing sugars. At last, the polysaccharide precipitates were volatilised solvents and lyophilised to obtain the prepurified MFP fractions—those from seeds (MFP-S) and those from leaves (MFP-L).

2.3. Analysis of MFPs Monosaccharide Composition. The polysaccharide content of MFPs was determined by the phenol-sulfuric acid method using glucose as a standard [23]. The monosaccharide composition of MFPs was estimated by high-performance liquid chromatography (HPLC) after acid hydrolysis according to the method described by Wang et al. [24]. Briefly, each MFP sample (10 mg) was hydrolysed with trifluoroacetic acid (TFA, 2 M, 3 mL) at 121°C for 2 h in a sealed tube. Residual TFA was removed with a QGC-12T nitrogen blowing instrument (Quandao Co. Ltd., Shanghai, China) at 50°C. The hydrolysed samples were redissolved in ultrapure water and analysed with ICS 5000 ion chromatography (Dionex, Sunnyvale, CA) with a CarboPac PA20 analytic column (150 mm × 3 mm inner diameter) and a pulsed amperometric detector. The mobile phase consisted of 250 mM NaOH (2%) and water (98%) at a flow rate of 0.5 mL min^{-1}.

Figure 1: Preparation processes of MFP-S and MFP-L with aqueous extraction, followed by ethanol precipitation.

2.4. Determination of MFPs Molecular Weight. The molecular weight (Mw) of MFPs was determined by high-performance gel-filtration chromatography (HPGFC), according to the method of Li et al. [25]. A Waters 1525 HPLC system was equipped with an Ultrahydrogel™ Linear 300 mm × 7.8 mm inner diameter × 2 (Waters Corp., Milford, MA), a Waters 2414 Refractive Index Detector (RI), and an Empower3 workstation. $NaNO_3$ (0.1 M) was used as a mobile phase with a flow rate of 0.9 mL min^{-1}. Dextran molecular weight standards ranging from 2.7 to 135 kDa were used for calibration and calculation of the Mw of each peak.

2.5. Antioxidant Activities. In each of the following five different assays of antioxidant activities, the concentrations of MFP-S and MFP-L samples and the positive control of ascorbic acid (Vc) or ethylenediaminetetraacetic acid (EDTA) were set to be the same: MFP-S: 0.625, 1.25, 2.5, 5, 10, 20, 40, and 80 mg mL^{-1}; MFP-L: 0.00625, 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4, and 0.8 mg mL^{-1}; Vc/EDTA: 0.005, 0.01, 0.02, 0.04, 0.06, 0.08, 0.10, and 0.12 mg mL^{-1}.

2.5.1. DPPH Radical Scavenging Activity. The DPPH free radical scavenging activity of the MFPs was conducted according to a slightly modified method described by Li et al. [26]. In brief, 2 mL of the MFP samples at various concentrations was mixed with 4 mL of 0.2 mM DPPH dissolved in 95% ethanol. The mixtures were shaken adequately and incubated for 20 min at 25°C in the dark. The absorbance was measured at 517 nm against a blank group. Ascorbic acid (Vc) was used as a positive control. The DPPH scavenging activity was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = \left(1 - \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}}}\right) \times 100, \quad (1)$$

where A_{sample} is defined as the absorbance of the sample (sample + DPPH methanol solution), A_{blank} is the absorbance of the reagent blank (sample without DPPH methanol solution), and A_{control} is the absorbance of the control (DPPH methanol solution without sample).

2.5.2. ABTS Radical Scavenging Activity. The ABTS radical scavenging activity of the MFPs was examined according to the method of Siddhuraju and Becker [27] with slight

modification. The ABTS radical cation stock solution was prepared by mixing 88 μL of 140 mM potassium persulphate and 5 mL of 7 mM ABTS and incubating for 24 h at 25°C in the dark. The ABTS radical stock solution was then diluted with ultrapure water to obtain an absorbance of 0.70 ± 0.02 at 734 nm. MFP samples (0.4 mL) at various concentrations were excessively mixed with 6 mL of ABTS radical cation solution. The mixtures were left to stand for 1 h at 25°C in the dark, and the absorbance was tested at 734 nm. Ascorbic acid was also used as a positive control. The ABTS scavenging activity was calculated as follows:

$$\text{ABTS scavenging activity (\%)} = \left(1 - \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}}}\right) \times 100, \quad (2)$$

where A_{sample} is defined as the absorbance of the sample, A_{blank} is the absorbance of the reagent blank (sample without ABTS), and A_{control} is the absorbance of the control (ABTS without sample).

2.5.3. Hydroxyl Radical Scavenging Activity. The hydroxyl radical scavenging activity of the MFPs was investigated according to a modified method proposed by Li et al. [26]. MFP samples (3 mL) at various concentrations were mixed with 0.5 mL $FeSO_4$ (6 mM) and 0.5 mL H_2O_2 (6 mM), incubated for 10 min, and added to 0.5 mL salicylic acid (6 mM). The absorbance was measured at 510 nm after being left to stand for 30 min at 25°C. Ascorbic acid was also used as a positive control. The hydroxyl radical scavenging activity was calculated as follows:

$$\text{Hydroxyl radical scavenging activity (\%)} = \left(1 - \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}}}\right) \times 100, \quad (3)$$

where A_{sample} is defined as the absorbance of the sample, A_{blank} is the absorbance of the reagent blank (sample without reagents), and A_{control} is the absorbance of the control (reagents without sample).

2.5.4. Ferrous Ion Chelating (FIC) Activity. The chelating effect of the MFPs on Fe^{2+} was monitored by measuring the formation of a ferrous iron-Ferrozine complex according to the method of Chew et al. [28] with minor modification. MFP samples (4 mL) at various concentrations were mixed with 150 μL $FeCl_2$ (1 mM), incubated for 5 min, and added to 0.6 mL Ferrozine (1 mM). The absorbance was measured at 562 nm after being left to stand for 10 min at 25°C. EDTA was used as the positive control. The ferrous ion chelating ability was calculated with the following equation:

$$\text{Ferrous ion chelating activity (\%)} = \left(1 - \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}}}\right) \times 100, \quad (4)$$

where A_{sample} is defined as the absorbance of the sample, A_{blank} is the absorbance of the reagent blank (sample without reagents), and A_{control} is the absorbance of the control (reagents without sample).

2.5.5. Ferric-Reducing Antioxidant Power. The ferric-reducing antioxidant power (FRAP) assay was performed following a modified method of Wang et al. [24]. MFP samples (0.5 mL) at various concentrations were mixed with 2 mL phosphate-buffered saline solution (PBS; 0.2 M, pH 6.6) and 1 mL potassium ferricyanide (1%, w/v), separately, incubated in a 50°C water bath for 20 min and cooled immediately. After 2.5 mL trichloroacetic acid (10%, w/v) was added, the reaction mixture was centrifuged (5000g; 10 min; 25°C) and 1 mL of the supernatant was mixed with 2 mL ultrapure water and 0.5 mL $FeCl_3$ (0.1%, w/v). The absorbance was measured at 700 nm after being left to stand for 10 min at 25°C. Ascorbic acid was also used as a positive control. The higher absorbance indicated better reducing power.

2.6. α-Glucosidase Inhibition Assay. The α-glucosidase inhibitory activity of the MFPs was determined according to Xu et al. [29] with slight modification. A series of polysaccharide samples at various concentrations was prepared: 10, 20, 40, 80, 160, 320, and 640 mg mL^{-1} for MFP-S and 0.0025, 0.005, 0.01, 0.02, 0.04, and 0.08 mg mL^{-1} for MFP-L. MFP samples (0.6 mL) were mixed with 0.4 mL PBS (0.1 M, pH 6.9) containing α-glucosidase (0.1 U mL^{-1}) and incubated in a 37°C water bath for 10 min. Afterward, 170 μL of 1 mM *p*-nitrophenyl α-D-glucopyranoside (*p*NPG) as a substrate in PBS (0.1 M, pH 6.9) was added to the mixture. The reaction solution was incubated at 37°C for 30 min, and the reaction was stopped by adding 2 mL of 0.5 M Na_2CO_3. The α-glucosidase inhibitory activity was determined by monitoring the absorbance of *p*-nitrophenol release from *p*NPG at 405 nm. Acarbose (0.02, 0.2, 2, 4, 8, and 16 mg mL^{-1}) was used as the positive control. α-Glucosidase inhibitory activity was calculated with the following equation:

$$\text{Inhibition activity (\%)} = \left(1 - \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}}}\right) \times 100, \quad (5)$$

where A_{sample} is defined as the absorbance of the sample, A_{blank} is the absorbance of the reagent blank (sample + PBS instead of other reagents), and A_{control} is the absorbance of the control (PBS instead of sample).

2.7. Statistical Analysis. All experiments were performed at least twice each with triplicate sample analysis. The results are expressed as means ± SD. The method of analysis was based on the general linear model. Significant differences (set at $P < 0.05$) between means were distinguished by least significant difference procedures using Statistix software 9.0 (Analytical Software, Tallahassee, FL, USA).

3. Results and Discussion

3.1. Monosaccharide Compositions of MFP-S and MFP-L. The yield of the polysaccharides MFP-S and MFP-L was 15.5% and 1.0%, respectively. An HPLC chromatogram of mixed standard monosaccharides is presented in Figure 2(a). From left to right peaks 1–10 were amino glucose, rhamnose, arabinose, galactosamine, galactose, glucose, xylose, mannose,

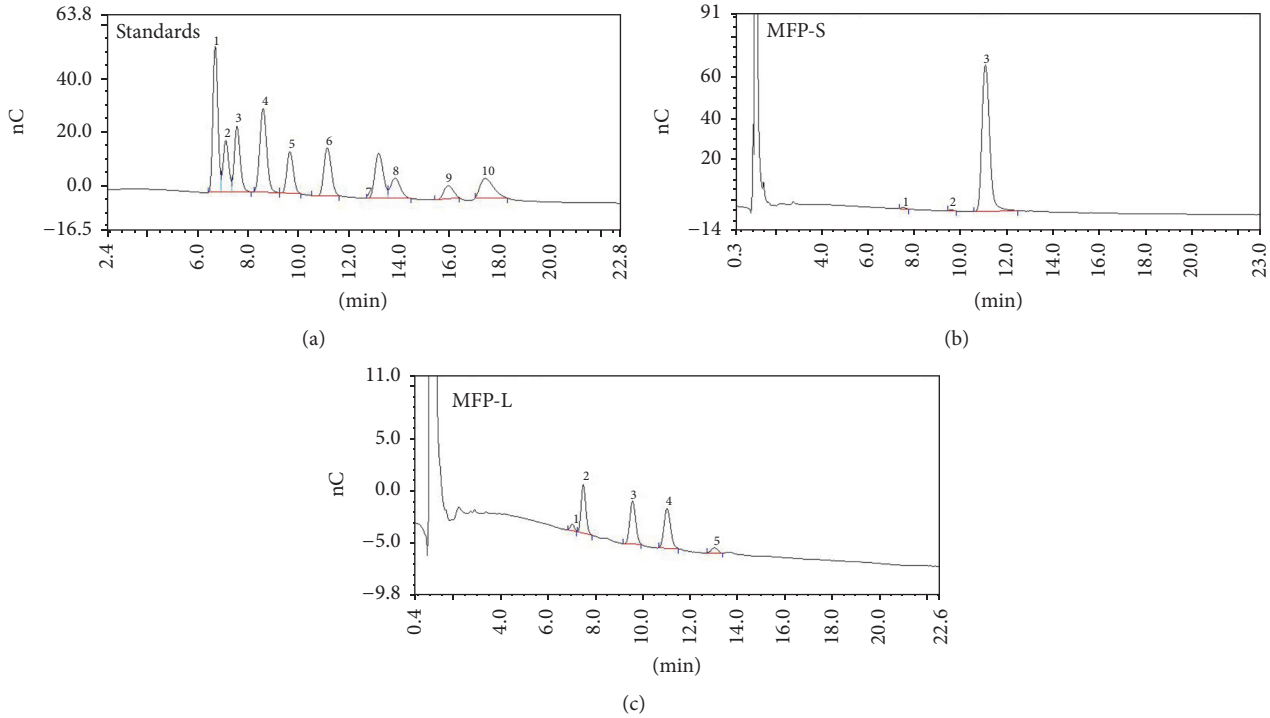

Figure 2: HPLC chromatograms of standard monosaccharides (a) and monosaccharide composition analysis of MFP-S (b) and MFP-L (c).

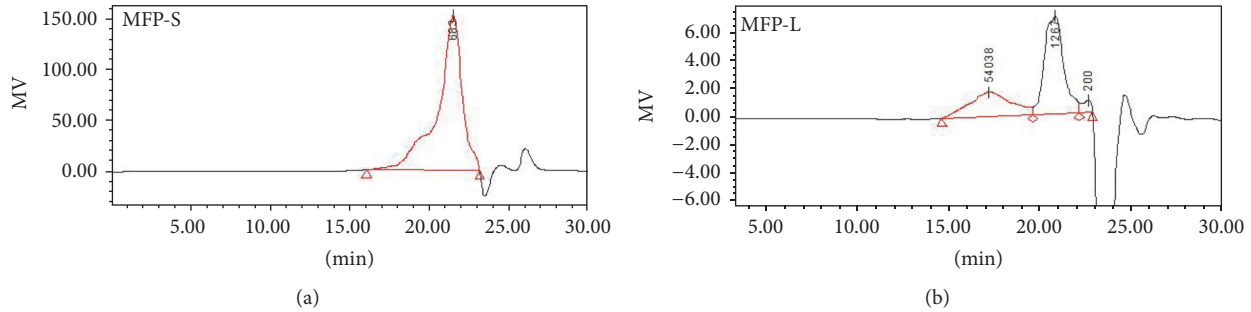

Figure 3: HPGFC profiles of MFP-S (a) and MFP-L (b) monitored with a refractive index detector.

fructose, and ribose, respectively. The results indicate that MFP-S and MFP-L were both heteropolysaccharides. MFP-S consisted of arabinose, galactose, and glucose in a ratio of 0.16 : 0.09 : 27.35 (Figure 2(b)). In contrast, MFP-L was composed of five monosaccharides, rhamnose, arabinose, galactose, glucose, and xylose, with ratios of 0.12 : 1.05 : 1.17 : 1.21 : 0.15 (Figure 2(c)). Actually, the differences in MFP-S and MFP-L on their monosaccharides could be predicted during the isolation process. That is, MFP-S exhibited high viscosity like glucose syrup, whilst MFP-L had a low viscosity.

3.2. Molecular Weight Distributions of MFP-S and MFP-L. The homogeneity and molecular weight (Mw) distributions of MFP-S and MFP-L were analysed by HPGFC. The average Mw of each elution peak was calculated according to the calibration curve derived from molecular weight standards. As shown in Figure 3(a), MFP-S presented a single, sharp, and symmetrical peak with an approximate Mw of 2804 Da (Mp: 683 Da), implying that MFP-S was a homogeneous polysaccharide. This result is consistent with its almost single monosaccharide composition with glucose (Figure 2(b)). Li et al. [25] reported a similar HPGFC profile for polysaccharide. MFP-L consisted of three major fractions with Mw of 92093, 1496, and 237 Da (Mp: 54038, 1267, and 200 Da) in proportion of 34%, 62%, and 4%, respectively (Figure 3(b)). The average Mw of three fractions in MFP-L was calculated to be 32249 Da.

3.3. Antioxidant Activities of MFP-S and MFP-L. The antioxidant activities of MFP-S and MFP-L with positive control were simultaneously estimated using five different methods, including DPPH, ABTS, hydroxyl radical scavenging activity, ferrous chelating ability, and ferric-reducing antioxidant power.

3.3.1. DPPH Radical Scavenging Activity. Because DPPH radicals are very stable but can be easily scavenged by antioxidants, they have been used to evaluate the free radical scavenging activity of natural compounds. As shown in Figure 4(a), all MFP-S, MFP-L, and positive control (Vc) scavenged

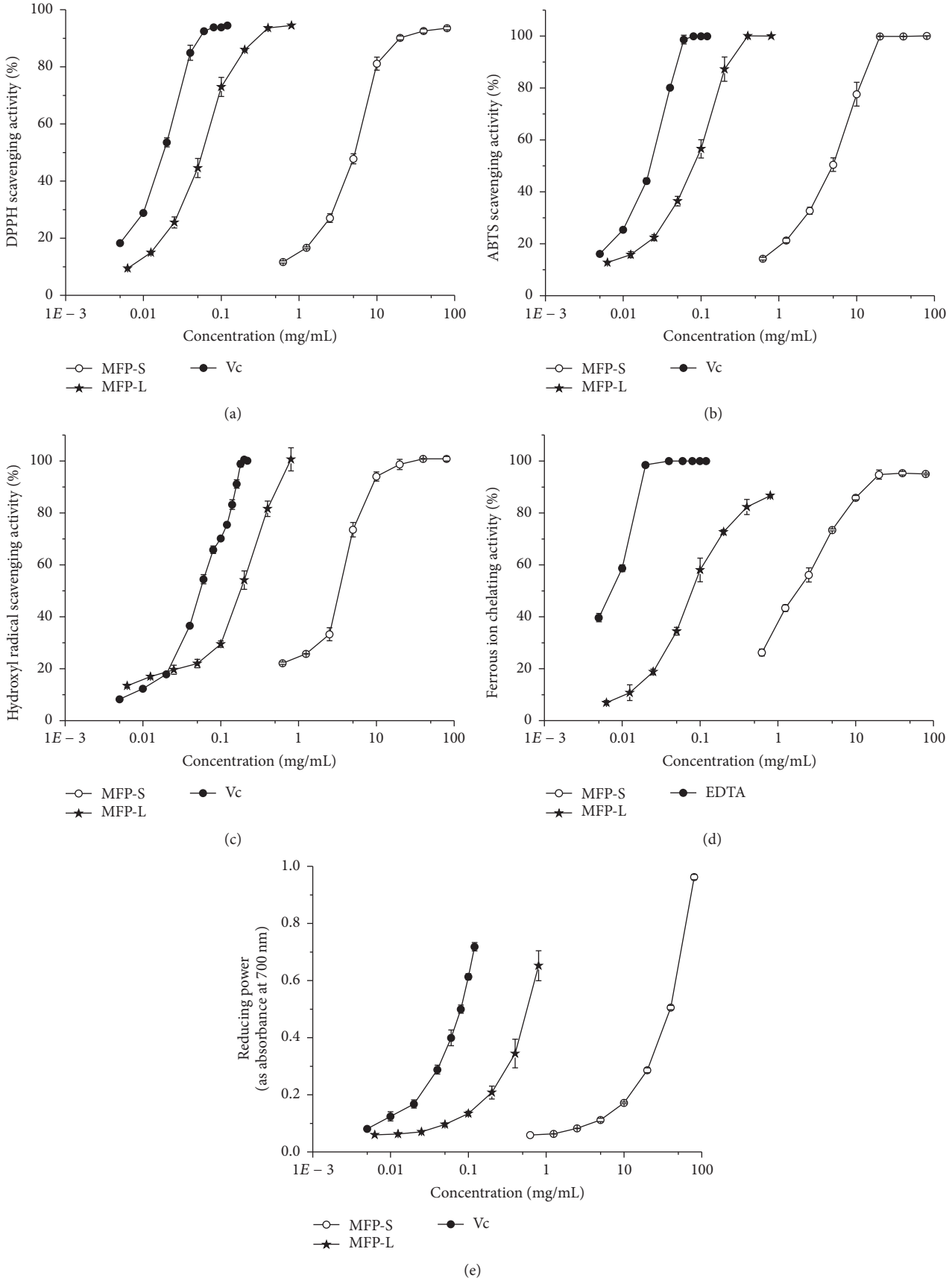

Figure 4: Antioxidant activities of MFP-S and MFP-L in vitro. DPPH (a); ABTS (b); hydroxyl radical scavenging activity (c); ferrous ion chelating activity (d); and ferric-reducing antioxidant power (e).

DPPH radicals in a concentration-dependent manner at their own appropriate concentration ranges. Remarkably, MFP-L presented much greater scavenging activity than MFP-S. Calculations were done to identify concentrations that caused 50% inhibition (IC50) of antioxidant activities. More specifically, the IC50 values of DPPH for MFP-S, MFP-L, and Vc were 5.3, 0.06, and 0.019 mg mL^{-1}, separately. Therefore, MFP-L could possibly act as a strong DPPH radical scavenger in the food application.

3.3.2. ABTS Radical Scavenging Activity. The ABTS radical scavenging activities of MFP-S, MFP-L, and Vc were determined, and the results are shown in Figure 4(b). Similar to the results of DPPH (Figure 4(a)), the ABTS radical scavenging activities of MFP-S, MFP-L, and Vc were also increased in a concentration-dependent manner. MFP-L was found to be a more effective ABTS radical scavenger than MFP-S. For example, the IC50 values of ABTS for MFP-S, MFP-L, and Vc were 5.0, 0.084, and 0.023 mg mL^{-1}, separately. It was implied that MFP-L could be used as a natural antioxidant to scavenge ABTS free radicals. This result also confirmed the positive correlation between ABTS and DPPH radical assays about antioxidant activity [30].

3.3.3. Hydroxyl Radical Scavenging Activity. Hydroxyl radicals are well known as some of the most reactive free radicals, which can induce severe damage to the organism [31]. Therefore, scavenging activities on the hydroxyl radical generated in a Fenton reaction of MFP-S, MFP-L, and Vc were investigated in this study. It could be seen that MFP-L still exhibited significantly better hydroxyl radical scavenging activity than MFP-S, as shown in Figure 4(c). In detail, the IC50 values of hydroxyl radical for MFP-S, MFP-L, and Vc were 3.5, 0.18, and 0.055 mg mL^{-1}, separately.

3.3.4. Ferrous Ion Chelating (FIC) Activity. The metal chelating ability was recognised as a correlative activity to antioxidant. As presented in Figure 4(d), at each considered concentration range, the FIC activities of MFP-S, MFP-L, and EDTA increased as the polysaccharide concentration increased. MFP-L also displayed a distinct advantage over MFP-S on FIC activity, whilst the overall FIC activity was not as strong as that of EDTA. Specifically, the IC50 values of FIC activity for MFP-S, MFP-L, and EDTA were 1.9, 0.083, and 0.008 mg mL^{-1}, separately.

3.3.5. Ferric-Reducing Antioxidant Power (FRAP). FRAP was expressed as absorbance (ABS) at 700 nm, and a higher absorbance value indicated stronger reducing power. The results of FRAP assay are shown in Figure 4(e). As expected, Vc possessed the best FRAP, followed by MFP-L and MFP-S. MFP-L still markedly exhibited much stronger activity for FRAP than MFP-S. The ABS of MFP-L at 0.00625 mg mL^{-1} was 0.060, which was already comparable to the ABS of MFP-S at initial 0.625 mg mL^{-1}. At the last point of MFP-L at 0.8 mg mL^{-1}, the right ABS was 0.652, whilst MFP-S was more than 40 mg mL^{-1}, and Vc was less than 0.12 mg mL^{-1}.

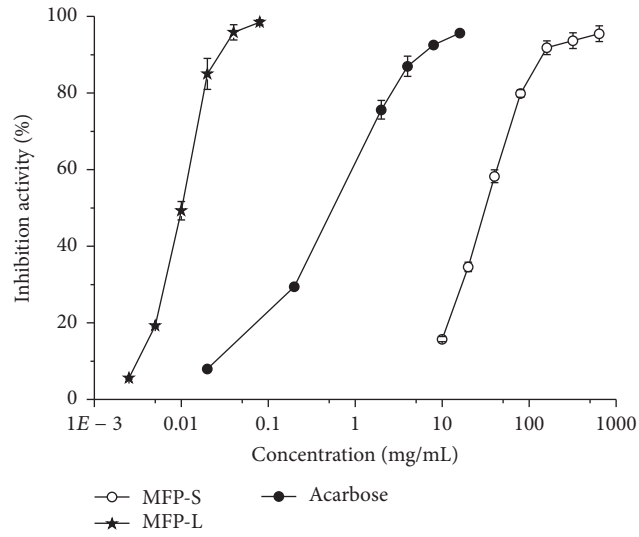

Figure 5: α-Glucosidase inhibitory activities of MFP-S and MFP-L with acarbose used as positive control.

3.4. α-Glucosidase Inhibitory Activity. Diabetes is characterised as a high concentration of blood sugar that can cause serious complications, such as damage to the eyes, kidneys, and cardiovascular and nervous systems. α-Glucosidase is an important carbohydrate digestion enzyme that has been considered as a therapeutic approach to adjust postprandial hyperglycaemia [29]. Many studies have focused on seeking effective and safe α-glucosidase inhibitors from natural products to develop functional foods to combat diabetes [32–36].

The dose-dependent effects on α-glucosidase inhibitory activities of MFP-S, MFP-L and the positive control acarbose are displayed in Figure 5. It was amazing to note that MFP-L presented remarkably greater α-glucosidase inhibitory activity than MFP-S and greater even than acarbose. More specifically, the inhibitory ability of MFP-L rose from 5.6% to 98.5% when the concentration was increased from 0.0025 to 0.08 mg mL^{-1}. More specifically, the IC50 values of α-glucosidase activities for MFP-S, MFP-L, and acarbose were 33, 0.01, and 1 mg mL^{-1}, separately. This finding indicates that MFP-L could have a promising inhibitory effect on α-glucosidase, even superior to that of the antidiabetes drug acarbose. Interestingly, it is coincident that the blood sugar values of some people with hyperglycaemia can be decreased to some extent by drinking tea made from the leaves of miracle fruit (data not shown).

Similarly, according to Xu et al. [29], the polysaccharide isolated from pu-erh tea stored for 5 years inhibits α-glucosidase three times better than that from acarbose. Chen et al. [32] considered that the diversities in antioxidant and α-glucosidase inhibitory activities amongst the three polysaccharides from green, oolong, and black tea appeared to be related to differences in their respective monosaccharide compositions and molecular weight distributions. They found that the high proportion of low molecular weight fractions in black tea polysaccharide contributed to its greater bioactivities of antioxidant and α-glucosidase inhibition than those in green and oolong tea. According to the results of

molecular weight distribution analysis of MFP-S and MFP-L (Figure 3), two fractions (Mw 1496 and 237 Da, resp.) of MFP-L accounted for 66%, whilst the single fraction of MFP-S had a higher average Mw of 2804 Da. It seems to be reasonable that low molecular weight fractions of MFP-L react easily with free radicals and other substrates to display wonderful bioactivity.

4. Conclusions

Overall, MFP-S and MFP-L have considerable differences in physicochemical properties (monosaccharide composition and molecular weight) and antioxidant and α-glucosidase inhibitory activities in vitro. The results demonstrate that MFP-S was a homogeneous polysaccharide that consisted almost entirely of glucose. However, MFP-L was a heterogeneous polysaccharide with three elution fractions, which were made up of five monosaccharides: rhamnose, arabinose, galactose, glucose, and xylose. MFP-L exhibited much better antioxidant abilities than MFP-S, although they were still weaker than those of the positive control (Vc/EDTA). The most exciting feature was that MFP-L showed notable inhibition of α-glucosidase, which was much better than that of the antidiabetes drug acarbose. It is suggested that MFP-L can be explored as a novel antidiabetes additive with both antioxidant and hypoglycemic bioactivities. The relationship between the structure of purified fractions from MFP-L and their bioactivities should be considered in further studies.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Acknowledgments

This research work was financially supported by the authors' home institution funds.

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Hindawi
Journal of Chemistry
Volume 2017, Article ID 2163218, 8 pages
https://doi.org/10.1155/2017/2163218

Review Article

Application and Development Prospects of Dietary Fibers in Flour Products

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Received 22 November 2016; Revised 13 February 2017; Accepted 5 March 2017; Published 16 March 2017

Academic Editor: Ji Kang

Dietary fibers are often characterized by high nutritional quality, as they are able to cure many chronic diseases and improve texture, sensory characteristics, and shelf life of foods. Here, the following aspects of dietary fibers have been reviewed: nutritional properties, including the ability to regulate blood sugar levels, effects on microorganisms, antioxidant effect, potential role in losing weight, ability to regulate blood lipids, application in flour products such as bread and Chinese noodles, challenges such as dark color, rough texture, and poor solubility, and potential solutions that include modification methods. The primary purpose of this review is to comprehensively evaluate potential applications of dietary fibers in flour products, addressing common problems and reviewing potential solutions to promote further study and applications of dietary fibers.

1. Introduction

With growing interest in health-promoting functional foods, the demand for natural bioactive additives has increased and the exploration for new sources is ongoing. The food processing industry in most countries generates large quantities of byproducts every year, which are frequently abandoned as wastes. However, many of these byproducts are dietary, functional, and potentially novel sources of nutrition. Of the many materials obtained, dietary fibers are particularly promising ingredients that has attracted considerable interest over the past few decades. The reason for this is their significant availability in most food byproducts, low costs, and positive effects for the prevention and treatment of a diverse range of diseases [1]. The fast growing food industry will likely generate an ever-growing amount of byproducts in future including bran, husk, peel, pomace, and other products that are rich in dietary fibers [2]. Therefore, finding optimal use of dietary fibers becomes increasingly imperative.

Ongoing scientific debates exist over the definition of dietary fibers. Currently, with growing understanding about the structure and composition of dietary fibers, this structure has been described as a series of polysaccharides with a degree of polymerization of three or more that are not digested or absorbed by the small intestine. Consequently, dietary fibers were previously regarded as a nonnutritive substance [3]. However, advancements in nutrition and medical science have revealed a promising potential of dietary fibers to reduce the risk of both common and chronic diseases. Due to the important role that dietary fibers play in maintaining human health, these are now recognized as the seventh largest nutrient group in the human body, after proteins, fats, carbohydrates, vitamins, minerals, and water.

Dietary fibers can be classified based on their solubility in water as soluble dietary fibers (SDF) and insoluble dietary fibers (IDF) [4–6]. IDF mainly consist of hemicellulose, cellulose, and lignin and cannot be degraded by enzymes in the human body or dissolved in water (neither warm nor hot). SDF include pectin, oligosaccharides, guar, and gums, most of which are dietary and healthy additives [7]. Currently, dietary fibers are widely added to a broader range of foods, including noodles, bread, milk, beverages, wine, and cookies [8]. These products have been generally acceptable among consumers and are thus profitable for food manufacturers. According

to published data, more than 50% of functional foods in supermarkets are fiber-fortified foods and this proportion is increasing [9].

The aim of this review is to summarize recent findings about the biological activity of dietary fibers in the human body, while simultaneously investigating its application in flour products. Substantial studies focused on the effects of various dietary fibers on flour products; however, few comprehensive reviews exist to date. Considering the important role that flour products play in the human diet, the influence of dietary fibers on bread and Chinese noodles has been described in detail and relevant mechanisms have been investigated. Additionally, with the ever-increasing types of fiber-fortified products, we carefully discussed problems that may limit the popularity of some of these and provide potential strategies to improve the acceptance of fiber-rich flour products.

2. Nutritional Properties of Dietary Fibers

Since the emergence of the hypothesis of the health function of dietary fibers, many comprehensive studies have been performed combining in vitro, animal, and human experiments. A growing number of reports have reported that dietary fibers can counter the effects of several chronic diseases, thus promoting human health.

2.1. Reducing Glycemic Response. In 1985, Harold et al. [10] first reported that dietary fibers have a hypoglycemic effect, and, since then, additional studies to this regard have been published [11, 12]. For example, the content of available carbohydrate in soybean-fortified flour products was lower than the respective level in normal flour products, suggesting that diabetics could consider dietary fibers as safe sugar substitutes and consequently increase their intake. Comparing the glycemic index (GI) of fiber-fortified foods and normal foods, fiber-fortified foods exhibited lower GI and dietary fibers were reported to present the major functional composition for these are undigested and can thus help lower blood sugar levels [13]. Furthermore, the inhibitory effect that dietary fibers exert on the absorption rate of digestible carbohydrates limits the postprandial blood glucose response, thus reducing plasma glucose levels. Hence, problems related to diabetes can be reduced due to the lowered insulin demand.

2.2. Induction of Microorganisms. Dietary fibers can be used as a fermentation substrate by several species of intestinal bacteria, helping to reduce the pH value in the gut, restraining the growth of saprophytic bacteria, reducing the accumulation of gut endotoxins, and inhibiting the generation of carcinogens [14]. Generally, dietary fibers promote a suitable microenvironment and create ideal conditions for intestinal flora.

2.3. Antioxidant Effect. Numerous health benefits have been considered to be partly attributed to the antioxidant capacity of phenolic compounds [15]. As a major food source, cereal grains contain abundant phenolic antioxidants, especially phenolic acids. Ferulic acid is one such special phenolic acid, which is the most abundant type of phenolic acid in cereal grains and can be found in cell wall components. Therefore, as the major constituents of plant cell walls, dietary fibers are regarded as an abundant source of phenolic acids. Furthermore, ferulic acid, sinapic acid, and several phenolic acid dehydrodimers were detected in dietary fiber fractions from a wide variety of cereal grains [16]. Due to the existence of phenolic antioxidants, positive correlations have been reported between dietary fibers and a radical scavenging activity [17]. Furthermore, dietary fibers are able to chelate iron and, in the absence of transition metal ions, hydrogen peroxide becomes fairly stable. Nandi and Ghosh [18] compared the antioxidant properties of dietary fibers extracted from defatted sesame husks, rice bran, and flaxseeds and found that these products exhibited different degrees of antioxidant capacity.

2.4. Weight Loss. Excessive caloric intake in the diet led to the currently observed obesity epidemic. Along with the high press, irregular lifestyle, and insufficient exercise of modern society, this tendency is widespread. Observational studies consistently demonstrate that the habitual increased intake of fiber-rich foods is associated with lower body weight [19]. According to a study, dietary fibers may increase fecal moisture and reduce its pH, which may benefit weight management by increasing volume and water content of feces, thus promoting fecal excretion and increasing defecation frequency [20]. Numerous studies found that dietary fibers can increase satiety and decrease appetite, hence indirectly reducing energy intake. Overall, the overwhelming majority of studies reported a positive correlation between fiber-rich food intake and weight loss [21, 22].

2.5. Regulating Blood Lipids. As reviewed in several studies, dietary fibers can reduce the risk of atherosclerosis, coronary heart disease, and hypertension [23]. This has been speculated to be related to mesh adsorption [24, 25]. More specifically, highly viscous dietary fibers have high resilience and strength, and, in the presence of water, reticulation will be formed and absorb certain substances [26]. Since the 1960s, this theory has been tested and several organic compounds such as cholesterol have been proposed to be absorbed by dietary fibers and subsequently eliminated from the body as feces.

3. Supplementation of Foods with Dietary Fibers

3.1. Effect of DF Enrichment on the Quality of Bread. Bread has a long history in the West and is currently the most common and popular principal food. Thus, the application of dietary fibers to bread has been studied. A wide spectrum of dietary fibers has been evaluated in order to discover a more suitable additive to improve both bread baking quality and nutritional properties. Pea and broad bean pod fibers were studied to understand their effect on dough and resulting bread at different supplementation [27]. The overall results

show that bread made with pea pod (PP) fibers and broad bean pod (BBP) fibers showed lower water retention capacity, which led to a slight increase in hardness. As for bread quality, increased addition of PP and BBP fibers darkened the bread and caused a large number of nonuniform large gas cells, which resulted in damage to the crumb structure. It was assumed that both fibers from PP and BBP have two opposite effects on bread at an addition level of 1 g/100 g. Overall, these materials are not ideal fiber enhancers. Other fibers were not so readily acceptable in bread such as hazelnut testa fibers [28]. Different qualities of hazelnut testae were added to wheat flour to increase the content of dietary fibers and investigate the effect on baking properties. Sensory descriptive analysis showed that many indexes were not as ideal as those of the control bread: darker color and lower loaf volume reduced overall acceptability. Detrimental effects of hazelnut testa fibers on bread properties were excessive and other fiber resources should be investigated. One study reported that the addition of flaxseed hulls (30% of dietary fibers), particularly in higher doses, could increase crumb hardness and depress loaf volume. However, nearly all indices were satisfied with addition of up to 4% flaxseed hull: the colors of both crumb and crust were slightly darker, the aroma and taste remained almost unchanged, and the texture was mildly influenced [29]. Furthermore, adding fenugreek fibers into the bread increased water absorbance and dough strength during farinograph mixing [30]. It was interesting to find that the moisture mobility of fenugreek fiber substituted bread was completely different from the control; thus fenugreek could help maintain bread quality during storage due to the increased water-holding capacity and the ability to prevent starch retrogradation of fenugreek fibers. Despite that, the incorporation of fenugreek fibers was reported to dilute both the gluten protein and wheat starch in flour compared to the control, which may be detrimental to bread texture. Wheat flour was planned to be completely replaced with several types of fibers-rich plant powders such as whole *Amaranthus cruentus* flour [31]. However, the color was significantly altered and the texture was barely satisfactory, suggesting that dietary fibers should only be used as a partial substitute for wheat flour due to its lack of gluten. Staling is detrimental to bread quality but occurs frequently in the food industry. Wheat flour with high water content was used to retard the staling of starch, and this study suggested fibers with high water-holding capacity such as potato peel to be an ideal additive to solve this particular problem [32]. The water activity of the crust increased during storage due to the migration of water from the crumb to the crust and, thus, the crust moisture content was significantly higher in potato fiber formula breads. In general, potato peel may produce a softer breadcrumb, even if supplied at low levels (0.4 g fibers/100 g flour). Similar results were reported in other papers: bread prepared by replacing 5% and 10% of the flour with banana peel fibers resulted in a better water-holding ability and oil-holding ability, thus promoting the production of high quality bread with a relatively long shelf life [33].

As for gluten-free (GF) produce, adding dietary fibers seems to result in improved quality. GF breads are often characterized by low nutritional quality as they merely contain vitamins, minerals, and, in particular, dietary fibers; therefore, an enrichment of GF baked products with dietary fibers seems to be necessary. Sabanis et al. [34] compared selected properties of GF bread formulation with the addition of different cereal fibers (wheat, maize, oat, and barley) and it became clear that all the dietary fibers at 3 g/100 g and 6 g/100 g improve all sensory properties of bread and adding 3 g/100 g maize fibers led to the highest score for overall acceptability. Similar results were also reported in other paper. It was reported that supplementing GF bread with rice bran helps to greatly improve the final bread quality, with fine taste, uniform crumb texture, brown color, and fresh appearance [35]. Hence, developing fiber-rich GF breads to increase acceptability and dietary fiber intake is a meaningful approach.

Tests with fiber resources resulted in different effects on the bread-making process. To summarize, diverse dietary fibers impact bread to varying degrees, and, possibly, dietary fibers with relatively high moisture content may produce improved quality. Apart from these beneficial effects, addition of fibers also exerted negative impacts that dramatically hampered the popularity of high-fiber breads. Therefore, the identification of the ideal dietary fiber resources and their correct dosages remains a necessary requirement.

3.2. Effect of DF Enrichment on the Quality of Chinese Noodles. Noodles are a popular traditional Chinese food and constitute the main part of the Chinese diet [36]. However, the nutritional value of noodles decreased due to increasingly sophisticated processing techniques. To improve the nutritional properties of noodles, it is feasible to add some nutrients, such as dietary fibers. In fiber-fortified noodles, many indexes such as hardness, adhesiveness, cohesiveness, chewiness, and resilience are likely to be significantly changed due to the functional characteristics of dietary fibers. To obtain an ideal formula, the amount of soluble fibers, dough mixing time, and different water levels were investigated, and the results showed that optimized values for soluble fiber level, water level, and mixing time were 3.4 g/100 g of flour, 36.0 mL/100 g of flour, and 5 min, respectively. Under these conditions, a softer texture, maximum cohesiveness, chewiness, resilience, and minimum adhesiveness of noodles were obtained, suggesting that the successful utilization of dietary fibers in noodles is possible [37]. Additionally, the effects of particle size and the amount of wheat bran on the quality of dry white Chinese noodles were studied and apparent trends were observed, suggesting significant correlations of the above two factors with noodle quality [38]. Generally, most of the values showed a decreasing trend with increasing addition and size of wheat bran, especially for high addition levels and for using coarse bran. An apparent decrease in peak viscosity and breakdown may alter the quality parameters of cooked dry white Chinese noodles, such as appearance, stickiness, and smoothness. However, the cooking time exhibited an increasing trend and this differed from another study, which reported a shorter required cooking time due to physical damage of the gluten network [39]. As for quality evaluation, remarkable decreasing trends

of color, taste, and appearance were caused by more than 5% addition of wheat bran or coarse bran [40]. Another study confirmed that supplementation with arabinoxylans from wheat bran in wheat flour should be relatively low and the appropriate quantity to be added should range within 0.25–1.0% to promote both cooking characteristics and texture of cooked noodles [41]. At this level, the cooking-loss rate was significantly suppressed and exhibited a distinct decreasing trend due to enhanced water absorbing capacity. As the amount of arabinoxylans increased, especially after 1% arabinoxylans addition, almost all the indicators declined, indicating that excessive addition of dietary fibers may cause undesirable effects on noodles. From the above studies, it can be deduced that the amount of dietary fibers should be controlled at a relative low level, or other food-quality-improving agents should be added to maintain noodle quality. Several studies suggested that a fineness degree was required; however, the opposite conclusion found that coarse bran was likely to have stronger water-holding power than fine bran [42]. Referring to Fan et al.'s work, arabinoxylans with a high molecular weight had a better effect on noodles than low molecular weight arabinoxylans. This is largely due to its remarkable ability to retain water and increase S–S content. Hence, finding the optimal granularity will require further evaluation.

3.3. Impact of DF Enrichment on Dough. Overall, research indicated two main reasons for dietary fibers to work in flour products: the unique physicochemical properties of dietary fibers and the effect on starch and protein in wheat flour. Many studies have been performed to evaluate how dietary fibers affect dough and the interaction among starch, protein, and dietary fibers. Among all the functional properties, hydroscopicity plays a crucial role in affecting dough properties such as hardness, cohesiveness, resilience, cooking characteristics, springiness, and uniformity. The porosity surface of dietary fibers could act as active carbon, which leads to high water absorption, thus increasing cooking characteristics of noodles [12]. There are many nonstarch polysaccharides such as mannan, phenolic groups, xylan, and pectin, whose molecular structures are characterized by a large number of hydroxyl groups that allow better water inhibition [43]. When adding a certain level of dietary fibers, a sticky gel network is formed via noncovalent interaction such as hydrogen bonds among main chains. This matrix can work similarly to a gluten network and expand the volume, thus improving uniformity and springiness, leading to a velvety texture of the resulting bread [44]. Evidence indicated that a high water maintaining ability promotes the maintenance of the gluten network and therefore strengthens the tensile performance of noodles [38–40]. Regarding staling, numerous tests showed that the addition of dietary fibers increased the water content of breadcrumbs and led to reduced deterioration during storage. The interaction of proteins, starches, and dietary fibers in cereal products could affect texture properties, and studies have reported a vulnerable fiber-starch-protein network structure. In the presence of dietary fibers, and particularly when the addition is below a relatively low level, this structure may emerge and thus retain more water and suppress excess expansion and diffusion of starch. However, this matrix may be easily destroyed when the addition exceeds a certain threshold. As a result, the stability of the gluten network may be hindered [41]. Dietary fibers can significantly affect the properties of dough in both positive and negative ways. As the amount, particle size, variety, and solubility vary, the influence of dietary fibers on flour products differs. Studies about the reverse impact of dietary fibers on gluten reported a dilution effect on the gluten network, which could reduce the gas-holding capacity and thus limit the expansion of air cells [45]. Other studies reported that the consistency of wheat gluten may be damaged by fibers, adversely affecting the specific volume of the bread [46]. Furthermore, considerable research findings supported by Fourier transform infrared spectroscopy, nuclear magnetic resonance, infrared spectroscopy, and Raman spectrum suggested that dietary fibers can compete with gluten to impede the formation of a gluten network and modify the secondary structure of gluten proteins [47–49]. Dietary fibers are chiefly composed of IDF including cellulose, hemicellulose, and xylogen, supplying more than three-quarters of some natural dietary fibers. As a consequence, the gluten matrix may be subjected to physical damage. Some studies indicated that IDF can break the bubble interface in fermented dough and damage the stability and uniformity of the gluten matrix [50, 51]. Based on the above analysis, dough is a complex system, and, given the sophisticated relationship of dietary fibers, starch, and proteins, further study is required to further our understanding of the effects of dietary fibers on dough properties.

4. Problems and Solutions of Dietary Fibers in Flour Products

4.1. Dark Color. Many studies reported a darker color of fiber-rich products. It is true that dietary fibers can create a dull color, thus reducing acceptability. Especially for principle foods such as noodles, steamed buns, and breads, the presence of unattractive color potentially limits the public acceptability of these products. Therefore, optimal strategies for decolorization are required. Since pypocholoride could produce residual chlorine and result in a undesired chlorine smell in the modern decoloration industry, hydrogen peroxide is more frequently used. Compared to pypocholoride, hydrogen peroxide is a more secure decolorizer that mainly ionizes hydrogen ions and peroxide ions in water. For example, apple pomace is a byproduct of the beverage industry; however, it is rich in highly active dietary fibers. During the manufacturing process, the integrity of cells may be greatly destroyed and many enzymes such as polyphenol oxidases may be released, leading to brown coloring and limiting its incorporation in food. An experiment was conducted to investigate the bleaching of apple pomace by hydrogen peroxide, showing that alkaline peroxide treatment allowed a more even and better bleaching effect compared to sodium chlorite [52]. The conditions of bleaching were also studied

and it was revealed that high pH and high concentrations of hydrogen peroxide affect bleaching, yields, and the swelling capacity. However, low yields were observed, suggesting alternative methods are required to improve the bleaching procedure without decreasing yields and sacrificing quality. A key reason for excessive addition of peroxide is that it is a labile compound that is easily affected by transition metals [53]. To solve this problem, experiments have been performed utilizing chitosan, the presence of which may absorb several transition metals such as iron, copper, and manganese and increase deacetylation [54]. These properties inhibit the degradation of peroxide and consequently accelerate the bleaching process. Further studies will be required to determine the ideal conditions to reduce the required amount of hydrogen peroxide and decrease bleaching time.

4.2. Rough Texture. As was mentioned above, dietary fibers are an indigestible substance with a hard and coarse texture. When added to food, particularly to flour products, a rough taste can be generated, consequently limiting the acceptance of these products. The reduction of particle size via micron technology could help to promote intestinal health and other functional properties, including water-holding capacity, water retention capacity, oil binding capacity, and swelling capacity. Additionally, with smaller particle sizes, the fiber structure could be broken down, thus exposing an increased surface area for bacterial growth and allowing a remarkable increase of bacterial mass and the amount of fecal output [55, 56]. Physical, chemical, and biological methods can all be applied to reduce particle size. Commonly used technologies include the superfine grinding technology, as well as enzyme, and fermentation approaches. Of these, various micronization methods have been applied for the processing of functional foods. Several common grinding methods exist: ball milling, jet milling, and high-pressure micronization, all used to reduce the particle sizes of dry material to different micron scales [55]. All treatments could successfully diminish the sizes of the insoluble fiber particles to different microsizes; however, there were significant differences in the required processing time. More specifically, ball milling and jet milling reduced particle sizes to 90.6% and 78.6%, respectively; however, jet milling took only a few seconds compared to ten hours of ball milling. Hence, jet milling seems a more efficient approach. High-pressure micronization treatment can pulverize a fine powder to 7.23 μm, but there are only few studies that utilize this technology [57]. Thus, further studies using this method are required. A further way to produce a smooth texture is the microfluidization treatment. This approach has been proven beneficial for the dispersion stability of wheat bran in water. With smaller particle size, better dispersion occurred due to the restrained formation of dense structures, which could help to produce uniform and stable dispersion in food [58]. Combined with the satisfying dispersion stability, an apparent decreasing trend of corn bran particle size was also observed; however, the degree of dissociation after microfluidization was lower than that after ball milling and jet milling. This suggests that microfluidization is not as good as both milling treatments to produce a finely ground bran [59]. The rough texture of fiber-enriched foods may be caused by many factors. In addition to fineness, dispersion and uniformity should also be considered, and combining methods may help to improve milling efficiency. As reported, several notable side effects such as improved water-holding capacity, swelling capacity, and oil-holding capacity were also observed, due to the substantially increased specific surface area and porosity caused by high-pressure microfluidization or other micronization methods. These results suggest ultrafine grinding to be an advantageous method to both produce smaller fragments and enhance other functional properties [60, 61].

4.3. Poor Solubility. The optimal mass ratio of SDF and IDF was reported to be 1 : 3 and real ratios in food can even reach 1 : 9, which severely affects its digestion and absorption in the human body as well as its physicochemical properties in cereal products [62]. To some extent, a high SDF content may be more efficient and acceptable due to it being more inclined to form gels and being readily incorporated into foods [63–67]. In contrast, IDF promotes gastrointestinal motility and fecal output; however, it also exerts adverse effects on foods [68]. Extrusion is a thermal processing method that involves the application of high heat, high pressure, and shear forces and can be used to treat uncooked masses such as cereal foods [69]. Compared to other treatments, it yields more SDF at a shorter processing time, low energy, and low cost [70]. Zhang et al. [71] optimized the extrusion conditions for yields of oat bran SDF and obtained 14.2 g/100 g at 140°C incubation temperature and 10% feed moisture. In addition to the frequently used extrusion processing, a modified technology named blasting processing has been introduced, which has shown great potential in extracting SDF from wheat bran and soybean residue [72, 73]. In Chen et al.'s work, using this technology produced almost 10-fold increased SDF values, which supplied a satisfactory result. The amount of soybean residue SDF was considerably enhanced from 2.6 ± 0.3% to 30.1 ± 0.6% with treatment at 170°C at 150 r/min extrusion screw speed, suggesting excellent efficacy of blasting processing. However, a relatively small change from 9.82 ± 0.16% (w/w, %) to 16.7 ± 0.28% (w/w, %) was reported for wheat bran [73] and the increment seemed not severe in contrast to Chen et al.'s work. This might be attributed to differences in raw materials. Accordingly, using blasting extrusion processing to produce SDF shows great potential [69]. Several IDF dissolved in water after ultrafine grinding treatment, suggesting that this technology could be used to simultaneously produce two types of effects: decreasing IDF particle sizes and improving solubilization. In previous studies, almost all micron technologies promoted solubility to varying degrees by increasing the surface area exposure and significantly damaging fiber structures [56]. Similarly, microfluidization may create a puffed morphology and a loose microstructure, thus enhancing the solubility of IDF. In addition to the above physical methods, some enzymes such as cellulose and hemicellulose can promote solubilization via collapsing the hard cell wall and lowering

molecular weight. Due to more sophisticated procedures of enzymatic process, physical means are more frequently used.

5. Conclusions

This paper summarizes the nutritive properties and applications of dietary fibers. Dietary fibers receive increasing interest due to their great potential for treating several diseases and, consequently, substantial studies have been performed to develop fiber-rich products. However, several problems such as dark color and rough texture have been severely limiting the popularity of fiber-fortified products; therefore, relevant strategies have also been discussed to address these problems. Generally, further studies should be conducted to effectively solve these problems and to better use dietary fibers in grain products.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

The present research was financially supported by Henan Province Colleges and Universities Young Backbone Teacher Plan (2016GGJS-070), Key Scientific and Technological Project of Henan Province (172102110008), and Opening Foundation of Province Key Laboratory of Transformation and Utilization of Cereal Resource in Henan University of Technology (24400036 and 24400031).

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Hindawi
Journal of Chemistry
Volume 2017, Article ID 9340427, 8 pages
https://doi.org/10.1155/2017/9340427

Review Article

Modification and Application of Dietary Fiber in Foods

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Received 5 December 2016; Revised 31 January 2017; Accepted 28 February 2017; Published 8 March 2017

Academic Editor: Qingbin Guo

Dietary fiber plays an important role in human health. The modification and application of dietary fiber in foods is reviewed with respect to definition and classification and methods for measurement, extraction, and modification of dietary fiber. The supplementation of dietary fiber for flour, meat, and dairy products is also reviewed. Finally, the benefits and risks of increasing consumption of dietary fiber are discussed.

1. Introduction

With the improvement of the standard of living, people's diet has become increasingly sophisticated. Many lifestyle diseases are caused by an imbalanced diet, such as diabetes, cardiocerebrovascular disease, obesity, intestinal cancer, constipation, and other disorders that have serious adverse effects on the health of human beings. Therefore, functional foods that can adjust the body function and prevent lifestyle diseases of civilization have attracted more attention in recent years. Dietary fiber has outstanding health promotion functions [1]. In this review, we describe our current knowledge on these aspects of dietary fiber: definition, determination, extraction, modification, and application.

2. Definition and Classification

The compositions of dietary fiber may be complicated, and detection methods have not been standardized. In the middle of the 20th century, the term "dietary fiber" was first used by Hipsley to refer to plant components that resist being decomposed by endoenzymes secreted by mammalian cells [2]. In the 1970s, the phrase "dietary fiber" was used by Trowell, to describe indigestible carbohydrates [3]. Currently, dietary fiber is defined as nonstarch polysaccharide that cannot be absorbed by humans or digested by enzymes in the human gastrointestinal tract [4]. These polysaccharides include cellulose, noncellulosic polysaccharides such as hemicellulose, pectic substances, gums, mucilage, and a noncarbohydrate component, and lignin [5]. It is important to note that the composition of dietary fiber cannot be completely determined, and the concept of dietary fiber will likely continue to evolve [6]. There have been recent suggestions that the oligosaccharides known as resistance oligosaccharides should also be considered dietary fiber [7, 8].

Dietary fiber plays a very important role in regulation of human bodies [9]. This material is not able to be decomposed in the human gut and affects the moisture absorption in the digestive system. It can increase the volume of food inside the intestines and stomach, increase satiety, and facilitate weight loss [10]. Dietary fiber can promote gastrointestinal peristalsis to alleviate constipation [11] and absorb the harmful materials in the gut, promoting their removal [12, 13]. Additionally, dietary fiber can improve the intestinal flora and provide energy and nutrition for probiotics proliferation [14]. Recent studies have shown that dietary fiber helps to reduce postprandial blood glucose, insulin, and triglyceride concentrations [15, 16] and can lower blood cholesterol levels [17, 18]. Decreased concentrations of faecal bile acids are correlated with reducing cancer risk [19–21].

Dietary fiber can include soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). SDF refers to fibers that cannot be digested or absorbed by human bodies but are

partly soluble in water. Examples of SDF are some gums, such as pectin, gum Arabic, guar gum, and glucan, and also include some biological polysaccharides and synthetic polysaccharides. IDF is a fiber that cannot be digested or absorbed by human bodies and is insoluble in water. IDF includes some components of the structure of cell walls, such as cellulose, hemicellulose, and lignin. Many scholars classify dietary fiber according to the source, emphasizing that the compositions of dietary fibers extracted from different sources are different. Dietary fiber includes plant dietary fiber, synthetic dietary fiber, animal dietary fiber, and microbial dietary fiber. Current studies on dietary fiber have mainly focused on plant dietary fiber, such as dietary fiber from soybean, rice bran, corn, wheat bran, fruit, and other sources. Additional sources will be exploited gradually; for example, there is a report of spent residue from cumin as a potential source of dietary fiber [22]. The dietary fibers obtained from different sources differ in total dietary fiber content, SDF content, physicochemical properties, and physiological properties [23].

3. Measurement and Extraction Methods

The measurement methods for dietary fiber are varied. Based on the emphasis, there are methods to determine the amount of dietary fiber, including Weende analysis, and solvent and enzymatic methods to determine the components of dietary fiber, such as chemical analysis and instrumental methods [24].

Weende analysis began in the early 19th century, and this method is still used in many factories to determine the fiber in foods and fodder. More recently, the crude fiber method has been used but is less widely utilized. In this method, dilute acid and dilute alkali are mixed with raw materials and subjected to continuous heating and boiling reaction until only the residue left is crude fiber, but crude fiber makes up only a small part of dietary fiber. During the acid-base reaction, hemicellulose and lignin loss will occur, so the results do not truly reflect the total fiber in food but are a rough indicator. With the development of the chemical industry, solvent methods have been developed for the measurement and extraction of dietary fiber from foods. The solvent method uses an appropriate solvent to remove protein and starch, leaving fiber as the remaining residue, mainly insoluble fiber. The solvent methods are divided into different methods, according to the use of different solvents, such as for alcohol insoluble fiber extraction, the neutral detergent method, or an acidic detergent method, for example. These various methods result in differing measurements. The disadvantage of these solvent methods is that there can be different degrees of damage to the fiber during processing by the solvent, which will affect the result of the measurement. However, current methodologies, in particular the approved method of analysis of the Association of Official Analytical Chemists 3207 991.43, are sufficient for many foods. Some foods require additional methods for quantification of the dietary fiber levels in foods, if they contain fructans (polymers and oligomers of fructose or inulin), modified dextrins, and/or synthetic dietary fiber analogues [25]. Enzymatic methods are also used to determine and extract dietary fiber. Enzymatic methods use enzymes to remove protein and starch, allowing the content of dietary to be obtained after drying and weighing. For example, enzymatic determination uses pepsin to remove protein to determine water-insoluble dietary fiber and uses pancreatin to remove polysaccharide. Modifications have been made to improve these methods. For instance, McCleary et al. used the Enzymatic-Gravimetric Method to determine high-molecular-weight dietary fiber (HMWDF), using Liquid Chromatography for low-molecular-weight dietary fiber (LMWDF). These methods provide results that are comparable to other official dietary fiber methods, and this method is recommended for adoption as Official First Action [26]. Chemical analysis is generally used to determine the components of dietary fiber, and is often combined with enzymatic methods by using enzymatic treatment to remove the starch and using anhydrous ethanol for precipitation to obtain total dietary fiber. The Fibertec tester (Sweden Tecator Company) and HPLC can be used to determine the composition of dietary fiber by chemical analysis. HPLC is a relatively mature method and shows good repeatability and high precision [27]. FT-IR spectroscopy can also be used to determine fruit dietary fiber composition [28].

Extraction can include dry processing, wet processing, chemical, gravimetric, enzymatic, physical, and microbial methods, or a combination. Different treatment methods have different effects on dietary fiber structure. The use of acid and alkali treatment will damage the molecular structure of the fiber, but the use of enzymatic approaches may result in incomplete extraction [29, 30]. Instead, a combination of enzymatic and solvent methods is usually used for the extraction of dietary fiber. For example, in the extraction of wheat bran dietary fiber, running water is first used to remove impurities such as dust and adherent starch in the wheat bran. 2% NaOH is added and then neutral proteinase removes the protein, resulting in relatively pure dietary fiber. Recent research has focused on the use of emerging technologies (ultrasound, microwave, and high pressure processing) for extraction to improve the fiber yield and also to maintain or enhance its functionality. These methods may reduce processing times and temperatures and optimize the usage of solvents [31, 32].

4. Modification

Modification methods are used to transform IDF into SDF for better physicochemical and physiological properties [33]. Modification methods include mechanical degradation, chemical treatment, enzymatic, and microbiological fermentation methods. Typically, a combined method may have greater effects than any single approach. For example, chemical-enzymatic, ultrasound-enzymatic, and microwave-enzymatic modification methods have been described.

4.1. Mechanical Degradation Treatment Method. Mechanical degradation treatment includes extrusion cooking, high pressure, heating, or novel technology to mechanically disrupt the fiber. This review describes current knowledge of the use of

extrusion cooking technology, instantaneous high pressure, and ultrahigh pressure treatment.

4.2. Extrusion Cooking Technology. Extrusion cooking technology subjects material to high temperature, high pressure, and high shear force, causing the internal moisture of the material to gasify quickly and extend and modify the fiber intermolecular and intramolecular spatial structure. At the moment of extrusion, the molecular structure of material is changed and forms a porous state. Studies have shown that extrusion cooking had a positive effect on total and soluble dietary fiber. The insoluble dietary fiber decreased appreciably with the varying processing parameters, probably due to disruption of covalent and noncovalent bonds in the carbohydrate and protein moieties leading to smaller and more soluble molecular fragments [34]. Additionally, the water solubility index was greatly enhanced by varying extrusion temperature and screw speed [35]. Some scholars used extrusion cooking technology-twin-screw extrusion to extract soluble dietary fiber from soybean residue. After a serial of orthogonal experiments, the optimum extrusion parameters were determined to be an extrusion temperature of 115°C, feed moisture of 31%, and screw speed of 180 rpm. Under these experimental conditions, the soluble dietary fiber content of soybean residue was 12.65%, 10.60% higher than the unextruded soybean residue. In addition, the dietary fiber in the extruded soybean residue had higher water retention, oil retention, and swelling capacity [36]. Another kind of modification method is blasting extrusion processing, and this method has great effect on dietary fiber modification. Chen et al. used blasting extrusion processing to modify the dietary fiber of bean dregs and found that, after extrusion modification at 170°C with an extrusion screw speed of 150 r/min, soluble dietary fiber content was increased by 27% [37].

4.3. Instantaneous High Pressure and Ultrahigh Pressure Treatment. Instantaneous high pressure treatment is performed using a high velocity jet homogenizer (microfluidizer) at pressures up to 300 MPa. Because the material quickly passes through the reaction chamber, the material is only briefly subjected to the high pressure and materials are ultra-micro-powderized. This treatment method was developed recently and allows even heating with low electricity costs. Liu et al. studied the use of instantaneous high pressure for soybean dreg dietary fiber and found significantly increased soluble dietary fiber content after treatment. Additionally, the physical characteristics (expansibility, water holding capacity, water-binding ability, and specific surface area) of the modified dietary fiber were different from those of the unmodified dietary fiber [38, 39].

Ultrahigh pressure treatment modifies raw material in an ultrahigh pressure reaction chamber in a certain time [40]. Wennberg and Nyman studied the use of ultrahigh pressure technology for Chinese cabbage SDF modification [41], and other scholars used this method to modify the dietary fiber of food like potato and apple. Ultrahigh pressure treatment can improve the content of soluble dietary fiber and its physicochemical properties and physiological characteristics. For example, Li et al. studied use of ultrahigh pressure for sweet potato residue IDF and found that the pressure, time and temperature of treatment had significant effects on blood sugar, blood fat regulation, and the capability of removing exogenous harmful substances. The optimal modification conditions by ultrahigh pressure for the ability to regulate blood sugar and blood fat were 600 MPa, 15 min, and 60°C. The treatment conditions for better removal of exogenous harmful material were 100 MPa, 10 min, and 42°C [42].

4.4. Chemical Method. Chemical methods use chemical reagents such as acid and alkali to break down dietary fiber. By controlling the amount of acid and alkali materials and the temperature and reaction time, some IDF is converted into SDF, with improved physiological characteristics. A recent study finds that hydrogen peroxide can improve the content of SDF in black soybean hull by about 10% and this material showed good ability of conjugating bile acid [43]. Carboxymethylation is also frequently used as a method of chemical modification and affects dietary fiber. A study reported improved SDF for dietary fiber extracted from whole grain barley by carboxymethylation [44]. Although chemical modification can improve the content of SDF, some chemical reagents may damage the molecular structure of the dietary fiber, reducing conversion efficiency or the physiological activity of dietary fiber.

4.5. Enzymatic and Microbial Fermentation Modification. Biological methods may be more environmental-friendly and healthy compared with other methods and include enzymatic and microbial fermentation. Enzymatic modification uses enzymes to degrade the dietary fiber, decreasing IDF and improving SDF. Enzymatic reactions use mild reaction conditions and have strong specificity. Additionally, there is typically little destruction of the composition and structure of the fiber, and this method does not result in chemical pollution, for high usability for the food industry. Enzymes used in dietary fiber modification are mainly xylanase, cellulose, and lignin oxidase. There are ongoing efforts to improve the enzymatic method. One study showed that using enzymes to hydrolyze bean dregs results in dietary fiber with higher biological activity and higher SDF is higher yield [45]. Some scholars used an enzymatic method to extract insoluble dietary fiber from *Dioscorea batatas* Decne residue and suggested that the method could improve the widespread use of Dioscorea [46].

Microbial fermentation uses organic acids and enzymes produced naturally by microorganisms to reduce the molecular weight and improve the solubility of dietary fiber. There are many sources of microbes and they are easily available. Nakajima et al. used fan anaerobic bacteria isolated from human faeces and showed that the bacteria can secrete an enzyme that can degrade fiber to generate oligosaccharides [47]. Another study reported that a filamentous fungus named CIsl6 has a significant effect on improving total dietary fiber and soluble dietary fiber of citrus dregs.

4.6. Mixed Treated Method. A combination of chemical, mechanical, enzymatic, and microbial fermentation methods

to modify dietary fiber often allow better experimental results than the use of the single methods. For instance, Zong-Cai et al. reported that using microbial fermentation alone improved the content of SDF by more than 15%, but the use of microbial fermentation followed by microfluidization resulted in 35% SDF, and the resulting dietary fiber modified by this hybrid method showed higher physiological activity. Additionally, performing fermentation first can reduce the homogeneous processing difficulty and economize the homogenization energy. [48]. There are many other hybrid methods of modifying dietary fiber, such as micronization technology combined with enzymatic modification and high hydrostatic pressure-enzyme treatment [49]. For instance, ultrasonic-assisted enzymatic extraction of soluble dietary fiber from pomelo peel was recently investigated by Tang et al. The modified dietary fiber showed better antioxidant activity [50].

5. Applications

Epidemiological studies have reported that the consumption of foods that are rich in dietary fiber may reduce the risk of cardiovascular diseases, various types of cancer, and type 2 diabetes and possibly improve body function regulation. At the same time, huge quantities of food processing by-products are generated and not utilized, creating considerable environmental pollution if not properly disposed. If these by-products could be used as a dietary fiber source, it would reduce pollution and add value [51]. Many foods with added dietary fiber have been introduced. At present, dietary fiber application research mainly includes addition of dietary fiber to flour products, meat products, and dairy products or use as additives [5].

5.1. Dietary Fiber Application in the Flour Products. Currently, flour products that are rich in dietary fiber are widely available. Compared with nonmodified food, this kind of improved dietary product has attracted consumer interest and is sold for higher prices. The dietary fiber is often added in flour products, such as whole grain bread, noodles, biscuits, and steamed bread.

In Asian countries, noodles constitute an integral part of the diet. Noodles are processed from refined wheat flour [52]. Fiber-rich noodles sold in markets are usually made by adding bran, rather than soluble dietary fiber. The use of partially hydrolyzed guar gum, as a soluble fiber source, has been tested for use in fiber-fortified noodles with health benefits. The effect of soluble fiber level (1–5 g/100 g of flour) was investigated by Mudgil et al. and shown to have a significant effect on hardness, adhesiveness, and cohesiveness of noodles [53]. Attempts have been made by scientists to improve the nutritional properties of food products [54] and improve the value of by-products of food processing [55]. Banana peel (BP) is a coproduct produced in large volumes annually by food-processing industries. Its disposal is of significant concern, but recent research suggests that BP is a valuable source of bioactive compounds [56, 57]. A recent research showed that the noodles with added banana flour, rich in dietary fiber, had good nutritional quality and sensory acceptability [58]. A similar finding was found for improving quality of chapatti using banana peel powder as dietary fiber source [59]. Wheat bran as a rich source of dietary fiber can be added into noodles and steamed bread. Researches show that when the added content of DF is 5%–10%, noodles with higher quality are produced [60].

Many other dietary fiber sources have been found and used in food processing. For instance, coffee grounds were added to cookies for increased dietary fiber source, resulting in more nutritious and more flavorful cookies with potential value in the prevention of diabetes [61]. Protein/fiber-enriched cake with good sensory quality was produced by the substitution of wheat flour by 5% of potato peel powder [62]. Dietary fiber has also been applied into other flour products. For example, De Delahaye et al. studied addition of soluble and insoluble dietary fiber into spaghetti noodles [63]. Another study found that addition of dietary fiber into pizza dough improves taste and allows 60 days storage at 18°C [64].

5.2. Dietary Fiber Application in the Meat Products. Meat is often a core part of daily meals, and consumption of meat products with good quality is important for a healthy diet. The primary importance of meat as food lies in the fact that it is a good source of high biological value protein and provides essential fatty acids, vitamins, minerals, and many essential micronutrients. However, most meat is deficient in essential dietary fiber. Thus, to improve the nutritional value, attempts have been made to add dietary fiber from different sources into sausages, surimi, meatballs, meat emulsion, and other meat products.

Fiber addition in meat products is becoming more common, and dietary fiber addition may effectively increase acceptability by giving meat products higher quality, improving the processing characteristics of meat products, improving the yield of meat products, and lengthening the shelf time [65, 66]. Sausage is a representative meat product, and if the fat in it is too high it may have adverse health effects. Dietary fiber has been used as a fat substitute and added into sausages [67]. Mendes and others found that adding dietary fiber from wine production into sausages may improve nutritional value [68]. The effects of pineapple dietary fibers were also tested for effects on the physical, chemical, and textural attributes of sausage which were investigated with different observed effects for the kind of fiber added [69]. Sausages made with oatmeal have different cooking yield and hardness, which may be due to the water-retention properties of different meats in response to heat treatment [70]. Scientists have made different attempts to add dietary fiber into other meat products to improve their quality. Addition of dietary fiber into meat emulsion can lower cooking loss and improve emulsion stability and viscosity [71], and dietary fiber may be correlated with the rheological properties of meat emulsion [72].

5.3. Dietary Fiber Application in the Dairy Produce. Yogurt is made of fresh milk that is fermented with bacterial cultures and is considered as a healthy food. Yogurt with better taste and higher nutritional value has a higher acceptability. The

physical attributes of yogurts, including the lack of visual whey separation and perceived viscosity, are crucial aspects of the quality and overall sensory consumer acceptance of yogurt [73]. Recent studies find that addition of dietary fiber may improve the nutritive value, but adding different amounts of dietary fiber into yogurt can influence texture, consistency, rheological properties, and consumer acceptability [74, 75]. For instance, Hashim et al. added different proportions of date fiber (DF; 0, 1.5%, 3.0%, 4.5%), a by-product of date syrup production into yogurt, or added 1.5% wheat bran (WB) into yogurt. Comparison of control yogurt with the yogurt showed that DF addition had a significant effect on yogurt acidity, and yogurt fortified with DF had firmer texture and darker color. Yogurt fortified with up to 3% DF had similar sourness, sweetness, firmness, smoothness, and overall acceptance ratings as the unmodified yogurt. The sensory ratings and acceptability of yogurt decreased significantly for 4.5% DF or 1.5% WB. Adding vanilla to yogurt fortified with 4.5% DF did not improve flavor or overall acceptance ratings. Thus, fortifying yogurt with 3% DF produced acceptable yogurt with beneficial health effects [76]. Goat milk is an excellent source of amino acids, fatty acids, and minerals and is widely used for processing fermented milks, such as yogurt. However, compared with cow milk yogurts, it is difficult to make goat milk yogurts with a good consistency. The fiber-rich pulp of Cupuassu (*Theobroma grandiflorum*) has different consistency than other fruit pulps. A recent study showed that adding Cupuassu (*Theobroma grandiflorum*) pulp into goat's milk for yogurt results in better consistency, texture, and higher nutritional value [77].

6. Development Prospect

Current tools to measure dietary fiber have low accuracy, and different measurements produce different results for dietary fiber content in food. For example, the dietary fibers of dried alga *Ulva lactuca* collected from the Tunisian littoral region were determined by the Prosky (gravimetric) and Englyst (enzymatic-chemical) methods. The two extraction methods resulted in approximately the same value of total fibers (54%) but had different insoluble and soluble fiber contents [78]. Therefore, improved measurement strategies are needed. Because different types of dietary fiber have different effects, a more precise definition of dietary fiber that describes composition and physical structure may be required [79].

The main extraction method for dietary fiber is chemical method, as this approach is easy, inexpensive, and easily applied for industrial production. However, the use of chemical reagents to extract dietary fiber may affect the physicochemical properties of the finer and could impact the resulting physiological benefits. The use of chemical reagents will also produce industrial wastewater that poses threats to the environment. In view of this, countries like the US and Japan are actively studying more eco-friendly extraction methods such as enzymatic method, membrane filtration, and fermentation. These extraction methods of dietary fiber are still in early development, and this is an important direction of future research.

Compared with IDF, more SDF is needed. Currently, there are no standards for the modification of dietary fiber. Industrial production of modified dietary fiber is still being developed. Future research is required for improved strategies to produce dietary fiber that is more suitable for the human body and to determine appropriate guidelines for dietary fiber intake. Previous studies of modified dietary fiber start with extraction, followed by modification, resulting in significant losses. If modification can be performed before extraction, these losses may be significantly decreased.

The stability of food can be obviously improved by adding dietary fiber into beverages. Dietary fiber may be added to food as a kind of additive agent. Dietary fiber can improve human health, but additional clinical studies are needed to determine the benefits and appropriate dosages.

7. Conclusion

Dietary fiber plays an important role in human health, with confirmed physiological benefits. However, some scientists and nutritionists believe that the benefits are overly exaggerated, and there may be confusion for consumers as to what they really need. Dietary fiber can prevent the absorption of harmful substances but will also prevent the absorption of proteins, inorganic salt and some minerals in food, a problem for people who need more of these nutrients, such as actively growing teenagers. Some people with intestinal tract disease may react poorly to supplementation with dietary fiber, resulting in irritation for the gastrointestinal tract. Better tools will elucidate the definition and physical properties of dietary fiber [80] and allow improved and more accurate determination of dietary fiber. Future methods for the extraction and modification of dietary fiber will be needed for large-scale industrial production. There are reports that supercritical CO_2 affects pear pomace insoluble dietary fiber physicochemical properties. For example, the glucose adsorption capacity and glucose retardation index of IDF were significantly higher after supercritical CO_2 treatment [81]. Additional studies are needed to expand the application of dietary fiber and improve its economic and practical value. Future work by nutritionists and scientists is also needed to determine the requirements for dietary fiber intake and to guide consumption of dietary fiber supplements.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

The present research was financially supported by Henan Province Colleges and Universities Young Backbone Teacher Plan (2016GGJS-070), Key Scientific and Technological Project of Henan Province (172102110008), Opening Foundation of Province Key Laboratory of Transformation and Utilization of Cereal Resource in Henan University of

Technology (24400036), and Support Program for the Natural Science Foundation of China in Henan University of Technology (31401035).

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Hindawi
Journal of Chemistry
Volume 2017, Article ID 4513410, 7 pages
https://doi.org/10.1155/2017/4513410

Research Article

Effects of Bamboo Shoot Dietary Fiber on Mechanical Properties, Moisture Distribution, and Microstructure of Frozen Dough

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Received 23 November 2016; Accepted 6 February 2017; Published 7 March 2017

Academic Editor: Qingbin Guo

In this paper, the effects of Bamboo shoot dietary fiber (BSDF) on the mechanical properties, moisture distribution, and microstructure of frozen dough were investigated. The state and distribution of water in frozen dough was determined by differential scanning calorimetry (DSC) and low-field nuclear magnetic resonance (LNMR) spectroscopy. The microstructure of frozen dough was studied. The structure of the gluten protein network found in wheat flour dough was studied by scanning electron microscopy (SEM). The result showed that the BSDF could significantly improve the viscoelasticity and extensibility of frozen dough after thawing in a dose-dependent manner. It was significantly improved with the increase in the addition amount of BSDF ($P < 0.05$). DSC analysis showed that the freezable water content and thermal stability of frozen dough were increased after the addition of BSDF. LNMR analysis showed that the appropriate (<0.1%) addition amount of BSDF could significantly ($P < 0.05$) decline the contents of bound water. Meanwhile, the loose bound water and free water were raised significantly ($P < 0.05$) after the addition of BSDF. Moreover, the addition of BSDF induces arrangement of starch granule and gluten network in frozen dough. BSDF can be used as a novel quality improver of frozen dough.

1. Introduction

Dietary fiber is the edible part of plants or analogous carbohydrates, which cannot be digested or absorbed in the human intestinal tract, and as such they proceed to the colon. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Many studies have demonstrated that the intake of dietary fiber can reduce the developing risk of cardiovascular diseases, hypertension, diabetes, obesity, cancer, and certain gastrointestinal disorders [1]. In addition to the various health benefits, dietary fiber also can improve the functional properties of many food products including water-holding capacity, oil-holding capacity, foaming capacity, emulsification and/or gel formation. When incorporated into foods (such as bakery and dairy products, jams, meats, and soups), dietary fibers can modify textural properties, avoid syneresis, stabilize high fat food and emulsions, and improve shelf life [2, 3]. Juvenile bamboo shoots have a long history of being used as nutritious food and medicine in many Asian countries like China, Korea, Japan, and Thailand. Bamboo shoots are a good source of edible fiber (6 to 8 g/100 g fresh weight). Bamboo shoot dietary fiber (BSDF) is available as a white tasteless powder containing little or no calories. Nowadays, BSDF has been widely used in various food products, such as bakery, meat, milk, sausage, beverages, spices, pasta, mustards, ketchups, and frozen dough [4].

Frozen dough technology began in the 1950s, which is widely used in food industry to facilitate the production of bread, Chinese steamed bread, dumplings, and several viennoiseries. Frozen dough technology can improve the work

efficiency, reduce the labor intensity of workers, increase the shelf life of products, and facilitate long-distance distribution of foods [5]. Two kinds of frozen dough are available, differing in whether or not the fermentation has occurred prior to freezing. Nonfermented frozen dough is fermented after thawing, and dough fermented before freezing is referred to as prefermented frozen dough [6]. However, frozen dough requires a longer proof time and produces products with lower specific volume and a texture that differs from freshly produced. The freezing process result in denaturation of protein, fat, starch, and other major components in dough. In addition, ice crystals will form during the freezing process, which can destroy the structure of dough. Moreover, the viscoelastic property of dough is also decreased with the freezing and thawing processes of dough, resulting in quality changes of final food products [7, 8]. Thus, it is necessary to improve the quality of frozen dough by appropriate food process technology.

The use of bamboo fiber as an ingredient can be effective for preserving the softness of the internal paste for longer time and help control moisture loss in high- and intermediate-moisture foods. It has been demonstrated that BSDF can be added into cookies, wheat flour, and cakes [9] to improve the texture properties and color [10]. However, there are few reports on the research and application of BSDF in frozen dough.

In our previous work, the functions and physical chemical properties of rice dietary fiber (RDF), soybean dietary fiber (SDF), and BSDF were compared. The result showed that the water holding capacity, oil holding capacity, swelling property, adsorption capacity of nitrite, and cholesterol of BSDF were significantly better than that of RDF and SDF. The BSDF was much more suitable to be used in foods. Therefore, the aim of this study was to investigate the influence of BSDF addition on the mechanical properties of frozen dough after thawing and the state and distribution of water in frozen dough. Furthermore, the microstructure of frozen dough was also characterized by scanning electron microscopy (SEM) to clarify the possible reasons for BSDF-mediated processing property changes of frozen dough.

2. Materials and Method

2.1. Materials. Bamboo shoot dietary fiber (BSDF) was obtained from Zhejiang Geng Sheng Tang Ecological Agriculture Co., Ltd. (Zhejiang, China). Wheat flour was purchased from China National Cereals, Oils, and Foodstuffs Corporation (Zhengzhou, China) with a protein content of 9.0%, moisture content 12.8%, and fat content 1.5%.

2.2. Preparation and Freezing of Dough. Wheat flour (1.0 kg), BSDF (0%, 1.0%, 1.5%, and 2.0% of the wheat flour weight), and salt (10 g) were mixed evenly. Then the mixed powder was hydrated by adding water (420 g) for 6.5 min and molded into dough. The doughs were put in the constant temperature box about 30 min (about 30°C, humidity 85%). Doughs were rounded and moulded into 400 g sausage shaped pieces. After mixing, dough pieces were frozen for 10 min in a mechanical blast freezer for 120 min at −35°C to a core temperature of −18°C. Immediately after freezing, dough pieces were placed in double high-density polyethylene (HDPE) bags and transferred to storage freezers at −20 ± 0.5°C.

2.3. Determination of Mechanical Properties of Frozen Dough

2.3.1. Determination of Tensile Properties. The frozen doughs were defrosted at 30°C in an incubator for 1 h and then subjected to the tension test with a tensile analyzer (TMS-Pro, Food Technology Co., Virginia, USA). The measurement was conducted with a Kieffer dough and gluten extensibility rig at a crosshead speed of 3.3 mm/s. The plots of force versus distance were recorded to obtain R_{max} and E values. R_{max} is the maximum peak force, which is a measure of the resistance of dough to stretching. E indicates the distance to rupture, which is the extensibility of dough [11].

2.3.2. Determination of Rheological Properties. The rheological properties were measured according to the methods of Wang et al. [12]. Briefly, the rheological behavior of dough was analyzed by a controlled stress rheometer (DSR200, Rheometric Scientific, USA) for small amplitude oscillation test. The measurement system was equipped with a parallel plate geometry (40 mm diameter) with a smart swap peltier plate temperature system to maintain the temperature at 25°C. Then, dough was loaded between the parallel plates and compressed to obtain a gap of 1 mm. Subsequently, dough was rested between the plates (5 min) before measurement. Stress sweep at 1 Hz frequency was carried out to determine the linear viscoelastic zone. Frequency sweeps test was carried out from 0.1 to 10 Hz to determine the elastic modulus (G'), viscous modulus (G''), and loss tangent ($\tan\delta$) as a function of frequency.

2.4. Analysis of Water State and Distribution in Frozen Dough

2.4.1. Differential Scanning Calorimetry (DSC). A differential scanning calorimeter (model DSC-Q200, TA Instruments, USA) was used to measure the freezable water in the heat treated samples. A slice subsample of 5–10 mg from each sample was encapsulated into an aluminum pan and cooled from 30°C to −40°C at 5°C/min using liquid nitrogen and then heated to 40 K at 5°C/min. According to the method of Yoshida et al. [13], the total amount of freezable water (W_f) in extrudate was calculated from the ratio of melting enthalpy at 0°C for per gram of sample and pure water. The amount of nonfreezable water (W_{nf}) in the extradite was defined as $W_{nf} = W_c - W_f$, where W_c was the total water content of samples, obtained by weight loss method. Two duplicate measurements were made for each sample.

2.4.2. Nuclear Magnetic Resonance (NMR). A low field pulsed NMI 20-Analyst (Shanghai Niumag Corporation, China) with 22.6 MHz was used in the experiment. Approximately 2 g of strip sample was placed in a 15 mm glass tube and inserted in the NMR probe. Carr-Purcell-Meiboom-Gill (CPMG) sequences were employed to measure spin-spin relaxation time, T_2. Typical pulse parameters were as follows: dwell time was 4 μs, echo time was 420.00 μs, recycle time

Table 1: Effects of BSDF addition on extensive ability of frozen dough*.

Addition amount of BSDF (%)	Tensile resistance (BU)	Extensibility (mm)	Ratio values
Control	493 ± 7^{c}	158 ± 2^{a}	2.9 ± 0.4^{b}
1.0	540 ± 5^{b}	139 ± 4^{b}	5.0 ± 0.4^{a}
1.5	611 ± 8^{a}	142 ± 5^{b}	4.8 ± 0.2^{a}
2.0	537 ± 4^{b}	132 ± 3^{b}	4.6 ± 0.3^{a}

*Means ± SD ($n = 3$). Within a column means with different superscript letters are significantly different ($P < 0.05$).

was 600 ms, echo count was 350, and scan repetitions were 16. Each measurement was performed in duplicate.

2.5. Microstructure Analysis of Frozen Dough. Microstructure analysis of frozen dough was referenced by Zhang et al. (2015) and Zounis et al. [14, 15]. The frozen dough was dried by a freeze dryer (ALPHA 1-2, Martin Christ Inc., Osterode, Germany). Then, a thin layer of the sample powder was mounted on the copper sample-holder with a double sided carbon tape and coated with gold of 10 nm thicknesses to make the samples conductive. The microstructure of frozen dough was observed using a scanning electron microscope (JSM-7001F, JEOL, Tokyo, Japan) at acceleration voltage of 15 kV.

2.6. Statistical Analysis. The results were analysed by one-way ANOVA under the significance level of $P < 0.05$ using SPSS 16.0 software (IBM Corporation, NY, USA). The graphs were drawn by OriginPro8 (OriginLab Corporation, MA, USA).

3. Results and Discussion

3.1. The Effects of BSDF on the Mechanical Properties of Frozen Dough

3.1.1. Tensile Properties. The tensile resistance and extensibility are the two important indicators of processing characteristics of dough [16, 17]. The resistance, extensibility, and draw ratio values of dough with different amount BSDF after thawing are shown in Table 1.

As seen from Table 1, the addition of BSDF significantly ($P < 0.05$) increased the resistance value and decreased extensibility of the dough. The addition of BSDF strengthen the gluten network structure of dough. Enhanced gluten network provides more flexibility for dough. The high extensibility indicates a high extend ability of dough, which collapses during the proofing stage. As shown in Table 1, the addition of BSDF significantly ($P < 0.05$) reduced the extensibility, which was indicating that the BSDF weakened the ductility of dough after thawing. BSDF has good water swelling properties; the swollen granules of BSDF were broken easily and impact the ductility of dough [18]. Compared with the control group, the ratio of tensile resistance and extensibility of dough with BSDF was significantly increased. The addition of BSDF improved the viscoelasticity, extensibility, and plasticity of frozen dough.

3.1.2. Rheological Properties. The G values were used to indicate the stiffness of viscoelastic properties and present the rheological properties of dough. The effects of BSDF on the rheological behavior of dough were shown in Figure 1.

According to Figures 1(a) and 1(b), the values of G' and G'' of dough were increased with the increasing levels of BSDF. These results suggested that the addition of BSDF strengthened the elasticity and viscosity abilities of dough, which could be beneficial to the products quality of dough [19]. The $\tan\delta$ value is another important indicator of dough quality. Compared with the control group (addition amount of 0%), the $\tan\delta$ was an obvious decrease regardless of containing different BSDF. The lowest value was obtained at the addition amount of 1.5% (Figure 1(c)). The lower $\tan\delta$ implied more elastic and lesser viscous behavior and the formation of a stable network structure [12].

3.2. Effects of BSDF on the Water State and Distribution in Frozen Dough

3.2. Effects of BSDF on the Water State and Distribution in Frozen Dough. The influences of BSDF on the rheological property of frozen dough may be attributed to the changes in the moisture distribution, microstructure, and aggregates formation [20]. Therefore, the effects of BSDF addition on moisture distribution and microstructure of frozen dough were studied.

3.2.1. DSC Analyses. The effects of BSDF addition on changes of ice crystals melting enthalpy and freezable and nonfreezable water contents of frozen dough were shown in Table 2. The enthalpy (ΔH) presents the ice crystals melting enthalpy [21].

As shown in Table 2 the enthalpy of frozen dough was increased with the increasing of BSDF addition amount. The higher enthalpy values represent a higher thermal stability of frozen dough. With the BSDF addition amount increased, the relative percentage content of freezable water was increasing. By contrast, the relative percentage content of nonfreezable water declined with the increase of BSDF addition amount. The differences and changes of water state in food could reflect the different interactions between molecular water and food components [22]. Hayashi et al. [23] found that the water molecules in polysaccharide hydrogels (namely, sodium salts of xanthan and hyaluronic acid) could be classified into nonfreezable, freezable bound, and free water by using DSC technique based on the difference in phase transition behavior of different water states and found that, below the critical quantity, all the water molecules were bound to protein or

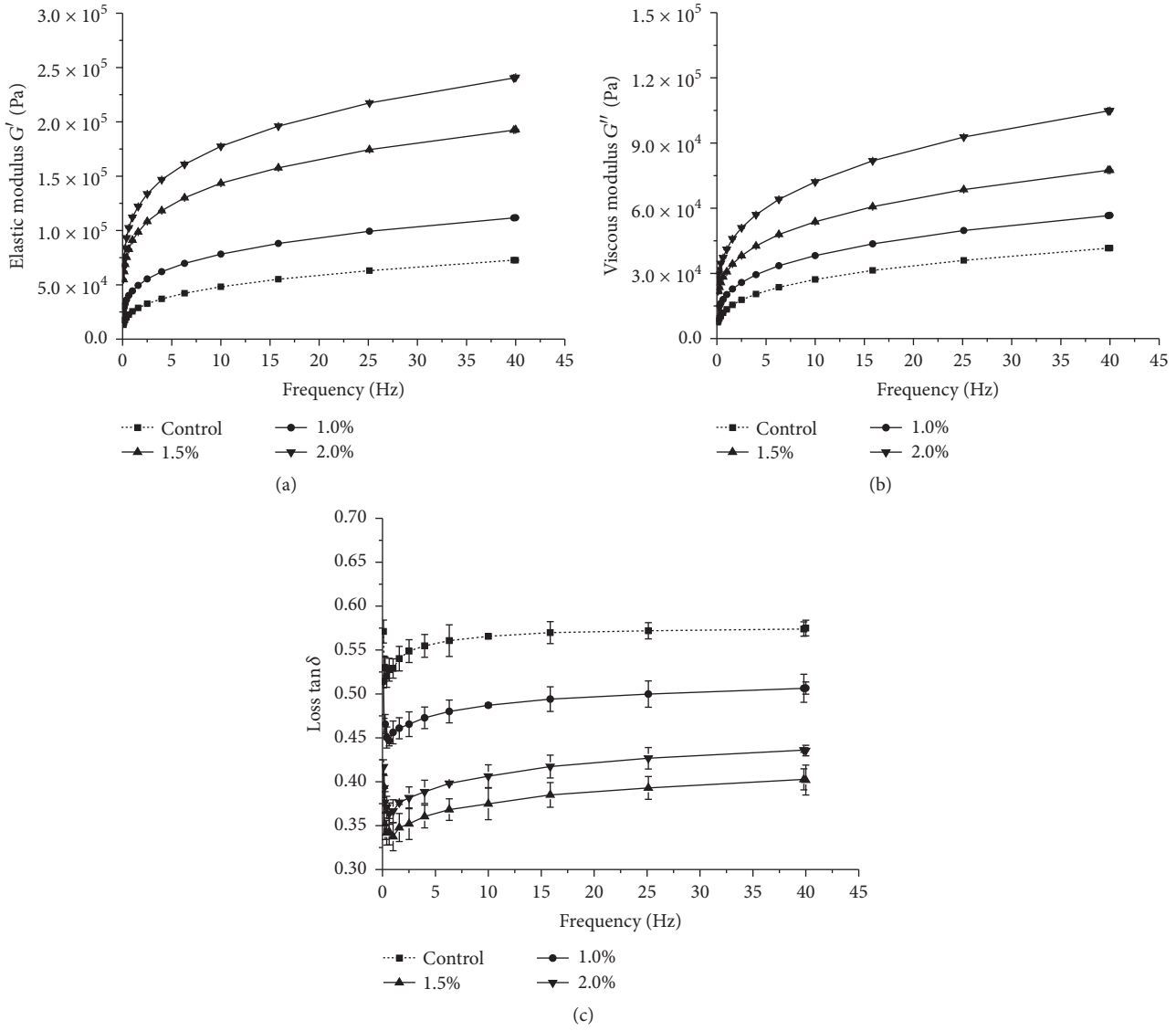

Figure 1: The effects of BSDF addition on the rheological property of frozen dough ((a) elastic modulus, (b) viscous modulus, and (c) loss tan δ.).

other macromolecules. Previous literature also found that when adding water into raw materials, nonfreezable water was necessary for protein plasticization [24], and freezable water worked as a lubricant to affect the flow and viscosity of protein dough [23].

Generally, the addition of BSDF increased the freezable water content of frozen dough and the thermal stability of frozen dough. This change maybe caused the decrease of extensibility of frozen dough.

3.2.2. NMR Analyses. Low-field nuclear magnetic resonance (LNMR) technique is widely used to investigate the phase and distribution characteristics of moisture in foods and food materials [25, 26]. T_{21} (peak 1 in Figure 2), T_{22} (peak 2 in Figure 2), and T_{23} (peak 3 in Figure 2) were the relaxation components, and PT_{21}, PT_{22}, and PT_{23} were the corresponding area fractions. It has been suggested that T_{21} component reflects water closely associated with micromolecules (bound water) [27] and T_{22} component reflects water trapped within the frozen dough microstructure (weak binding water), while T_{23} component corresponds to free water [28].

The peak 2 was the main peak (Figure 2), indicating that the water distribution of frozen dough was mainly based on loose binding water. The addition of the BSDF changed the water status and distribution in frozen dough. After the addition amount of BSDF, the area fractions of bound water declined significantly ($P < 0.05$), and decline by 57.26% at the addition amount of BSDF was 1% compared to the control (Table 3). After the addition of BSDF at 1.5% and 1.0%, the area fractions of loose bound water and free water

Table 2: Effects of content of BSDF on water content of frozen dough determined by DSC*.

Addition amount of BSDF (%)	ΔH (J/g)	Freezable water (%)	Nonfreezable water (%)
Control	73.00 ± 0.51^{d}	21.89 ± 0.38^{d}	18.20 ± 0.22^{d}
1.0	76.81 ± 0.63^{c}	23.00 ± 0.36^{c}	17.02 ± 0.32^{c}
1.5	80.78 ± 0.70^{b}	24.17 ± 0.23^{b}	15.77 ± 0.40^{b}
2.0	83.79 ± 0.51^{a}	25.14 ± 0.30^{a}	14.89 ± 0.31^{a}

*Means ± SD ($n = 3$). Within a column means with different superscript letters are significantly different ($P < 0.05$).

Table 3: Effects of content of BSDF on 3 kinds of state water content of frozen dough*.

Addition amount of BSDF (%)	T_{21} (%)	T_{22} (%)	T_{23} (%)
Control	7.51 ± 0.19^{a}	92.20 ± 1.11^{b}	0.24 ± 0.14^{c}
1.0	7.42 ± 0.09^{a}	92.10 ± 0.90^{b}	0.46 ± 0.21^{a}
1.5	3.21 ± 0.23^{b}	96.49 ± 0.77^{a}	0.29 ± 0.18^{b}
2.0	3.58 ± 0.25^{b}	96.11 ± 0.89^{a}	0.28 ± 0.19^{b}

*Means ± SD ($n = 3$). Within a column means with different superscript letters are significantly different ($P < 0.05$).

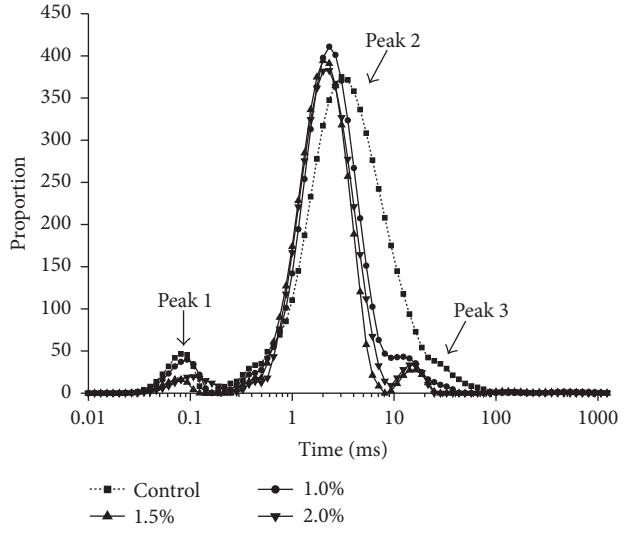

Figure 2: The effects of BSDF on the low-field nuclear magnetic resonance water distribution of frozen dough.

were increased by 4.65% and 91.67% ($P < 0.05$), respectively. When the addition amount of BSDF was more than 1.5%, the effects of BSDF on water distribution were not enhanced.

BSDF with a strong water holding ability could change the water distribution of frozen dough by competing with gluten protein and starch in dough. The change of water distribution in frozen dough has a direct impact on the characteristics of elasticity, viscosity, and rheological properties of frozen dough after thawing.

3.3. Effects of BSDF on Microstructure of Frozen Dough. In order to investigate the influence of BSDF on the microstructure of frozen dough, the SEM characterization was examined and the results were shown in Figure 3.

As shown in Figure 3(a), the starch granules in the dough with different sizes were wrapped in the network structure of gluten. Starch granules were featured as clear circular particles and the gap of starch granules were larger. Figures 3(b)–3(d) showed that the addition of BSDF changed the microstructure of frozen dough in a BSDF dose-dependent manner. Compared to control group (Figure 3(a)), the gap between starch granules of the BSDF added samples decreased, starch granules and dough matrix network structure began to appear adhesive state.

Summarily, the addition of BSDF changed the microstructure of frozen dough, causing a more delicate arrangement of starch granules and gluten network. The BSDF-induced changes in microstructure of frozen dough are the important reason for the changed of mechanical properties of frozen dough.

4. Conclusion

In this work, the effects of BSDF (1.0%, 1.5%, and 2.0%) on the processing properties, water distribution, and microstructure of frozen dough were investigated. The result showed that the viscoelasticity and extensibility of frozen dough after thawing were significantly ($P < 0.05$) improved with the addition of BSDF. The addition of BSDF also changed the water state and distribution in frozen dough. After adding the BSDF, the freezable water contents and thermal stability were significantly increased ($P < 0.05$). The bound water of frozen dough was significantly decreased ($P < 0.05$) at the

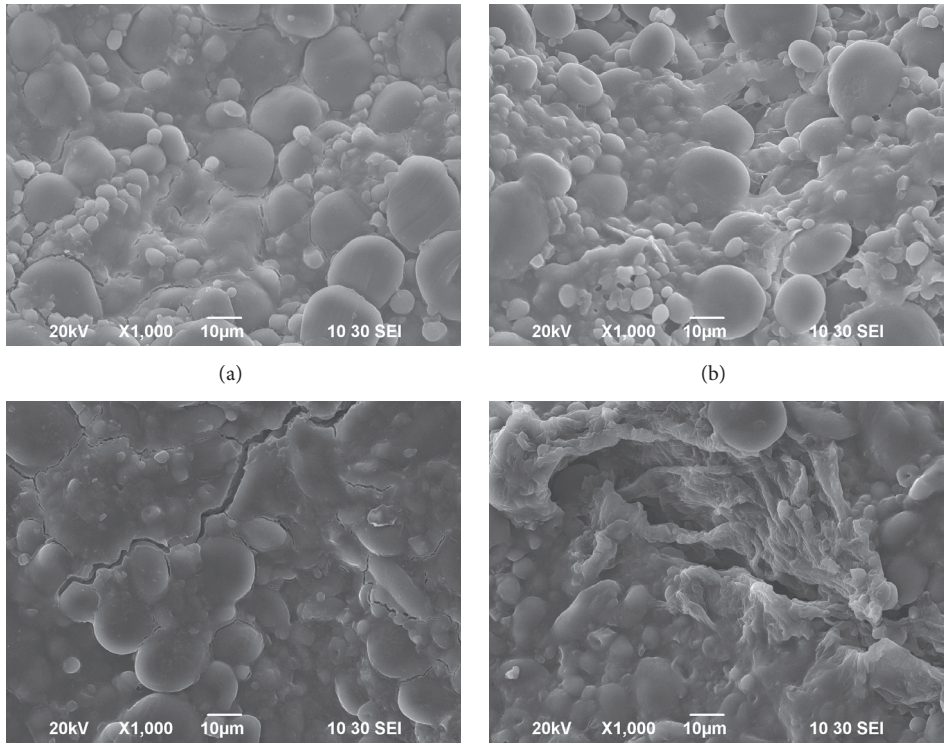

Figure 3: The effects of BSDF on the microstructure of frozen dough ((a) control, (b) BSDF addition amount of 1.0%, (c) BSDF addition amount of 1.5%, and (d) BSDF addition amount of 2.0%).

appropriate adding amount (<0.1%) of BSDF. Meanwhile, the loose bound water and free water were raised significantly ($P < 0.05$) by the addition of BSDF. SEM analysis found that the addition of BSDF induced obvious changes in the microstructure of frozen dough, such as the arrangement of starch granule and gluten network.

This work demonstrated that the addition of BSDF can increase the viscoelasticity, extensibility and plasticity of frozen dough, and improve the processing properties of frozen dough. The quality improvement effects of BSDF may be related with the increases in freezable water content, loose binding water content, and free water content the decrease in the binding water content and changes in the microstructure of frozen dough.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Acknowledgments

This research was supported by the Major Science and Technology Specific Projects in Henan Province (no. 14100110400).

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