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The Current Food Landscape in China

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Review Article

The Types, Regional Distribution, and Consumption Trend of Chinese Traditional Wheat-Based Foods

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Chinese wheat-based foods have a long history and a wide range of varieties, which is representative of Chinese food culture. Pasta and bread are made of wheat flour, and the characteristics of pasta and bread are closely related to the quality of wheat flour. The quality of wheat is mainly affected by environmental conditions, and different varieties of wheat are suitable for planting in different regions, so the regionalization of wheat is formed. Due to the different quality of wheat and eating habits in different regions of China, the same kind of wheat-based foods has different flavors in different regions, such as steamed bread, noodles, and stuffed buns. The regional characteristics of food are also formed between different regions. For example, Naan. With the changes in Chinese people's eating habits and consumption level, there are more and more types of wheat-based foods, which are developing in the direction of industrialization. This review clarifies the wheat planting regionalization in China, giving an insight into the relationship between different wheat quality and the variety of traditional wheat-based foods, describing the types and regional distribution of traditional wheat-based food products in China. Moreover, the types of wheat-based foods are classified and whose characteristics are introduced, and the consumption trend of wheat-based foods in China is elaborated.

1. Introduction

Wheat is one of the most important food crops in the world, accounting for about 30% of the global food crops. It is the food crop with the largest planting area, the highest total yield, and the richest variety of food processing in the world [1]. In China, wheat, rice, and maize are known as the three major food crops, among which wheat has been cultivated in China for more than 5,000 years. The planting area of wheat in China accounts for about 1/7 of the world's total wheat sown area, and the total output accounts for 17% of the world's total output all year round [2].

The main component of wheat grain is starch (amylose and amylopectin), which accounts for about 75% of the grain weight, and is the second largest source of starch [3]. In addition, there are biological macromolecules such as protein and fat and small amounts of minerals, sugars, and vitamins in wheat grains [4]. Wheat starch has the characteristics of good thermal paste stability, low gelatinization temperature, heat resistance and stirring resistance, high gel strength, and so on [5]. Amylopectin can improve the edible quality of steamed bread, enhance the palatability of noodles, and shorten the cooking time. The protein content of wheat is 12–14%, and its quality and value are high [6]. Wheat proteins can be divided into four categories: gliadin, glutenin, globulin, and albumin. As storage proteins, gliadin and glutenin are the main components of gluten, accounting for about 80% of wheat grain proteins, and they are closely related to the elastic viscosity and ductility of dough [7, 8].

Due to the long history of wheat cultivation in China, Chinese people have formed the dietary habit of making wheat flour products as the staple food through cooking methods such as steaming, boiling, baking, and frying for thousands of years. Up to now, Chinese traditional wheat flour products include steamed bread, noodles, dumplings, stuffed buns, and fried fritters [9].

The edible quality of flour products is closely related to the quality of wheat, the raw material for flour production,

Table 1: Wheat planting regionalization in China.

Main areas	Subregions	Suitable wheat varieties for planting	
Spring wheat production area	Northeast spring wheat area	Red grain	Strong gluten
	Northern spring wheat area	Red grain	Medium gluten
	Northwest spring wheat area	White grain, red grain	Strong gluten
Winter wheat production area	Northern winter wheat area	White grain	Strong gluten
	Huang-Huai winter wheat area	White grain	Medium gluten, strong gluten
	Yangtze river winter wheat area	Red grain	Medium gluten, weak gluten
	Southwest winter wheat area	Red grain	Medium gluten, weak gluten
	South China winter wheat area	Red grain	Medium gluten, weak gluten
Winter-spring wheat production area	Xinjiang winter-spring wheat area	White grain	Medium gluten, strong gluten
	Qinghai-Tibet spring-winter wheat area	Red grain	Medium gluten

and the quality of wheat flour, which is the secondary processing product [10, 11]. The yield and quality of wheat directly affect the safety and satisfaction of people's food demand, as well as the nutritional balance of human beings and the development of the flour and the food processing industry [12]. In recent years, the yield of wheat in China has increased year by year, and the goal of wheat cultivation and breeding has changed from focusing only on yield in the past to paying equal attention to increasing yield and quality [13]. With the rapid development of the national economy and the continuous improvement of people's living standards, higher requirements have been put forward for the type and quality of wheat products [14–16]. The quality of wheat grain is of great significance to flour and other deep-processed wheat products, which directly affects the quality and use of flour [17–19].

This paper introduces the quality regionalization of wheat, the types and regional distribution of wheat-based foods, and the trend of consumption in China.

2. Wheat Planting Regionalization in China

Wheat in China has a wide geographical distribution and complex ecological types, and there are great differences in wheat quality among different regions. This difference is not only determined by the genetic characteristics of wheat varieties themselves, but also affected by environmental conditions such as climate, soil, tillage system, cultivation measures, and the interaction between varieties and the environment [20–23]. The influence of the difference of environmental conditions on wheat quality is greater than that of wheat varieties, and the regional difference of wheat quality reflects the regional distribution law of wheat quality [24].

Countries with developed wheat industries, such as the USA, Canada, Australia, and so on, have already carried out quality regionalization of their wheat producing areas to meet the needs of the international market [25]. Generally speaking, the wheat planting area in China is divided into winter wheat, spring wheat, and winter-spring wheat production area and further divided into ten subregions [2, 26] (Table 1).

There are few high-quality strong gluten and weak gluten wheat in the main wheat producing areas of China, while the number of medium-gluten wheat is larger, accounting for about 65% [27]. The spring wheat area of China is suitable for the production of bread flour, and it can also be matched with other flour to produce noodle flour and northern steamed bread flour [28]; the fertile land in Huang-Huai and northern winter wheat areas can grow strong gluten wheat and develop bread flour; other places are more suitable for the production of northern steamed bread and noodle flour; the Southwest winter wheat area and Yangtze River winter wheat area are suitable for the production of southern steamed bread flour, pastry powder, biscuit powder, and so on [29].

Table 2: Standard for wheat flour for steamed bread.

Project	Refined grade	General grade
Moisture (%)	≤14.0	
Ash (%)	≤0.55	≤0.70
Wet gluten content (%)	25.0~30.0	
Silty curve stabilization time (min)	≥3.0	
Falling number (s)	≥250	

3. The Types and Regional Distribution of Traditional Wheat-Based Food Products in China

Chinese pasta and bread have a long history and a wide variety, which is representative of Chinese food culture. After a long period of evolution, the wheat-processing culture in China, which includes the preparation of steamed bread, noodles, stuffed buns, dumplings, and fried fritters, is developing steadily. Due to the different qualities of wheat suitable for development in different regions and different eating habits formed over thousands of years, even the same kind of pasta has a different flavor, and different regions have formed their own unique pasta culture and regional characteristics.

3.1. Steamed Bread. Steamed bread refers to a kind of food made by fermenting and steaming the dough, which originated in China and has a history of more than 1700 years [30]. Steamed bread is a traditional staple food of the Chinese people, especially in the north. The consumption of steamed bread accounts for about 2/3 of the northern pasta

Table 3: Classification of Chinese steamed bread.

Classification	Characteristic	Distribution
Northern-style steamed bread (Chiang mian mantou)	Chewy, firm, and elastic	Shandong, Shanxi, Shaanxi, Henan, and other northern provinces
Southern-style steamed bread (Xiao mian mantou)	Soft, white, and palatable	Southern provinces and cities, some northern provinces and cities
Cantonese-style steamed bread (Guangdong mantou)	Unique flavor	Guangdong, Hainan, Fujian, Taiwan and Southeast Asian countries

structure and nearly 50% of the national wheat products [31, 32].

The special wheat flour for steamed bread is regulated in China [33]. The indexes of the special wheat flour are shown in Table 2.

Steamed bread was classified by Zuoji Lin in *food processing and wheat quality improvement* (1994). According to this classification, Chinese steamed bread can be divided into Northern-style steamed bread (Chiang mian mantou) and Southern-style steamed bread (xiao mian mantou). Steamed bread has been classified by some foreign experts, which is roughly the same as that of Zuoji Lin. According to the method of phenomenological classification, steamed bread is divided into Northern-style steamed bread, Southern-style steamed bread, and Cantonese-style steamed bread [34, 35]. The characteristics and distribution of all kinds of steamed bread are shown in Table 3.

Steamed bread accounts for about 60% of the pasta structure of people in northern China and occupies an important position in the diet of northerners. Due to different production methods in different regions, a variety of northern steamed buns with different flavors have been formed. Famous steamed buns such as Shandong gaozhuang mantou, Xi'an guanguan mo, Jinnan non-alkali steamed buns, Henan gangzi mantou, and so on [36]. The variety of steamed bread made in the south of China is made from not only flour, water, and yeast but also baking powder, sugar, and shortening. Because of its fluffy structure, soft texture, delicate texture and non-sticky teeth, it is also very popular in the north [37]. Compared with the northern steamed bread as the staple food of northerners, Cantonese-style steamed bread is usually used as an afternoon tea snack or dessert, which is a popular pastry.

The quality of high-quality food is affected by the raw materials, and different flour products have different requirements for wheat flour. Therefore, special wheat flour should be produced according to different quality characteristics and requirements of pasta food [38].

Due to the differences in eating habits between the north and the south, there is a difference in the quality of steamed bread between the north and the south, so there is also a difference in the quality of steamed bread flour for making steamed bread [39]. Generally, medium-gluten flour and medium high gluten flour are suitable for making northern steamed bread, while low-gluten flour is suitable for southern steamed bread. Compared with the steamed bread flour suitable for making steamed bread in the south, the steamed bread flour suitable for making northern steamed bread has the advantages of long stability time, low tensile resistance to extension, and high extensibility. [40, 41] (Table 4).

The quality of wheat is the key to the quality of wheat flour. Zhao [39] and others pointed out that due to the different regions in the south and the north, most of them are weak gluten wheat in the south and medium and strong gluten wheat in the north, which leads to the different characteristics of steamed bread in the south and north. The effect of wheat quality on steamed bread quality can be analyzed from the aspects of grain characters, protein quality, starch characteristics, and so on [42].

Previous studies have found that the bulk density of wheat grain has a positive effect on the elasticity of steamed bread [43, 44]. Protein content is an important factor in determining the quality of steamed bread, which has a significant effect on the volume and appearance of steamed bread. When the protein content of wheat flour is 10%–13%, the quality of steamed bread is better [45, 46]. The quality of protein in gluten is also closely related to the quality of steamed bread [47]. The ratio of glutenin to gliadin determines the strength of gluten. When the glutenin content is high, the upright degree and elasticity of steamed bread are better, but when the glutenin content is too high, the quality of steamed bread decreases; the content of gliadin was positively correlated with the volume and softness of steamed bread [43]. When the ratio of glutenin to gliadin is appropriate, the elongation and elasticity of gluten are better, the fermentation time of dough is moderate, and the quality of steamed bread is better [48]. The steamed bread made from wheat with high amylose content is characterized by the small size, poor toughness, and sticky teeth. Amylopectin is beneficial in improving the edible quality of steamed bread, and a higher ratio of amylopectin to amylose is better [49, 50].

3.2. Noodles. Noodles are the traditional staple food in China. 35% of the wheat flour consumed every year is used for noodle processing. Noodle food can be traced back to the Neolithic Age. It has a history of more than 4000 years in China, and now it has become a common pasta in China and some countries and regions in Asia [51, 52]. For example, about 1200 years ago, Chinese handmade noodles were introduced into Japan and developed into noodles with local characteristics [53].

A great deal of research has been done on the factors affecting the quality of noodles. Black et al. [54] found that the starch and protein composition of the wheat was an important factor in the quality of the noodles.

Table 4: Quality characteristics of wheat flour for making different steamed bread.

Classification	Wet gluten content	Whiteness	Ash	Stability time	Resistance to extension	Extensibility
Northern steamed bread	26.5%–33.5%	≥80%	0.48%~0.6%	3.7–7 min	260~420 EU	130~210 mm
Southern steamed bread	≤25%	≥80%	≤0.56%	≤2 min	480~630 EU	≤110 mm

Table 5: Appearance and distribution of different noodles.

Classification	Exterior characteristics	Distribution
Regular salted noodles	With white color and with a smooth, glossy surface after boiling	Most of China
Alkaline noodles	With yellow color, and with a characteristic aroma and flavor	Guangdong, Fujian, Taiwan, Hong Kong

The content, composition, pasting, and swelling properties of starch have significant effects on the quality of noodles [55]. The wheat varieties with lower amylose content had higher swelling power and peak viscosity, and the comprehensive score of noodles was higher [56]. It is generally believed that the noodles made from wheat flour with high peak viscosity are of better quality [55, 57]. Swelling power and expansion volume reflect the expansion ability of starch [57]. The study of Martin et al. [58] showed that the swelling power of wheat flour was positively correlated with elasticity and cohesion and negatively correlated with noodle hardness.

According to Oh et al. [59], protein content is an important index affecting the hardness and elasticity of noodles, and the internal texture of noodles with high-protein content is harder. The higher the glutenin content of the flour is, the greater the dough strength will be [60]. Soluble glutenin and insoluble glutenin can increase the maximum resistance to extension, extension distance, and extension energy of noodles, while the extensibility of gliadin can improve the tensile length, tensile resistance, and tensile resistance of noodles [61].

There are many kinds of noodles in China, which are mainly made of flour, water, and salt. Noodles can be divided into Regular salted noodles and Alkaline noodles according to whether alkaline substances (Na_2CO_3 or K_2CO_3) are added or not [62]. The differences between them are shown in Table 5.

The quality of high-quality noodles primarily depends on the quality of wheat flour. China has stipulated the special wheat flour for noodles [63]. The indexes of the special wheat flour are shown in Table 6.

Noodles are divided into cut noodles, dried noodles, hand-extended noodle, hand rolling noodles, and so on [64]; from the processing method, it can be divided into fresh noodles, dried noodles, steamed noodles, boiled noodles, frozen boiled noodles, and sterilized boiled noodles, steamed and deep-fried instant noodles, steamed and hot-air dried noodles [63]. In terms of regional characteristics, thousands of varieties can be divided, such as the famous Shanxi Sliced Noodles, Chongqing Street noodles, Shaanxi Biangbiang Mian (BBM), Lanzhou Hand-Pulled Noodles, and so on [51].

The unique flavor of different kinds of noodles is closely related to the characteristics of the wheat flour used to make noodles, so according to the characteristics of different noodles, predecessors have studied some special wheat flour for noodles (Table 7) [65–68].

Table 6: Standard for wheat flour for noodles.

Project	Refined grade	General grade
Moisture (%)	≤14.5	
Ash (%)	≤0.55	≤0.70
Wet gluten content (%)	≥28	≥26
Silty curve stabilization time (min)	≥4.0	≥3.0
Falling number (s)	≥200	

A study of Zhu et al. [65] demonstrated that ash and wet gluten content had no significant effect on the quality of BBM when studying the effect of wheat flour characteristics on BBM quality.

3.3. Other Chinese Pasta

3.3.1. Dumplings. Dumpling is an important traditional wheat flour food in China, which has a history of more than 1000 years [69]. It is also one of the most important and popular staple foods in the daily life of residents in northern China [70]. Now they are popular not only in China but also in Japan, South Korea, and Southeast Asian countries [71].

The development of special flour for dumplings has been studied in China [72]. The special wheat flour for dumplings was stipulated in 1993 [73]. The index of special wheat flour is shown in Table 8.

The characteristic of flour is one of the factors affecting the quality of dumpling wrapper [74]. The formation time of flour has a negative effect on the hardness of the dumpling wrapper, and the peak viscosity and rebound value mainly affect the elasticity and smoothness of the dumpling wrapper [75]. The higher the sedimentation value and the better the rheological properties of the dough, the better the texture quality of the dumpling skin made from wheat flour [76]. Gluten strength and starch pasting properties significantly promote the elasticity and smoothness of raw dumplings [77]. It is considered that the protein content of the flour, dough development time, stable time, and weakening degree are the main factors affecting the quality of quick-frozen dumplings, according to the study of Zhang et al. [78].

3.3.2. Stuffed Buns. There are many kinds of stuffed buns in China. Due to the different quality of raw wheat used in different regions, consumers have different taste preferences.

Table 7: Quality index of wheat flour for making different kinds of noodles.

	Ash (%)	Wet gluten content (%)	Protein concentration (%)	Stable time (min)	Weakening degree (FU)	Resistance to extension (BU)
Shanxi sliced noodles	<0.55	27.80~32.50	12.70~15.90	3.90~6.70	67.70~108.30	222.60~303.00
Chongqing Street noodles	0.37~0.55	23.85~26.66	10.17~11.24	3.90~5.67	81.33~91.67	255.00~420.00
BBM			11.02~12.66	5.40~7.86	55.17~89.83	416.18~564.82
Lanzhou alkaline pulled noodles	<0.65	>30.00	11.50~13.00	>9.00	<65.00	>400.00

Therefore, according to the regional classification, stuffed buns can be divided into northern stuffed buns, Yangtze River valley stuffed buns, and southern stuffed buns. The wrappers of stuffed buns in the north are generally more gluten, and most of them choose medium-strong gluten flour; the wrappers in the Yangtze River valley stuffed buns taste soft and are mostly made of medium-gluten flour or medium-low gluten flour; the southern stuffed buns require a softer taste, so low-gluten flour is used [79].

According to Li et al. [80], the quality of northern fermented stuffed buns is mainly affected by wet gluten content and dough rheological properties.

The Yangzhou stuffed buns are typically stuffed buns in the Yangtze River Basin. There are five factors affecting the quality of Yangzhou stuffed buns, including sedimentation value, protein content, wet gluten content, dough stability time, mixing tolerance index, and so on [81].

Barbecued pork buns are traditional Cantonese-style pastries. The quality of protein and starch are important factors affecting the quality of barbecued pork buns. The dough strength is weak, the flour expansion rate is low, and the low-gluten flour with a protein content of 7.5% to 8.0% is suitable for making barbecued pork buns [82, 83].

3.3.3. Fried Fritters. Fried fritters are mainly composed of wheat flour, expanding agent, oil, salt, sugar, water, and other raw materials. As the most important raw material for making fritters, wheat flour plays a key role in the quality of fritters [84]. The quality of protein in wheat flour is very important to the quality of fritters. Fritters made from flour with medium and weak gluten strength have a higher sensory evaluation value [85]. Glutenin and increasing the content of amylopectin and amylose in appropriate proportion were beneficial to improving the specific volume and elasticity of fritters [86]. The quality index of fritters special flour has not been stipulated in China, but predecessors have conducted research on this aspect and provided the reference index of fritters' special flour quality [86–88] (Table 9).

3.3.4. Naan. Flatbread is widely distributed in the world and is popular in many countries and regions, whose origin is very ancient [89]. As a type of flatbread with a unique flavor, Naan (Xinjiang flatbread) is one of the main foods of the Uygur nationality, and it is also a popular traditional pasta food of other ethnic groups in Xinjiang, China [90]. It has a history of more than 2000 years, according to textual research [91]. According to the eating habits and quality types of wheat in different regions, Cao [92]and others classified Naan into "high gluten Naan," "ordinary Naan," and "weak gluten Naan" and recommended wheat quality standards for different types of wheat (Table 10).

Table 8: Standard for wheat flour for dumplings.

Project	Refined grade	General grade
Moisture (%)	≤14.5	
Ash (%)	≤0.55	≤0.70
Wet gluten content (%)	28.0~32.0	
Silty curve stabilization time (min)	≥3.5	
Falling number (s)	≥200	

Table 9: Quality reference index of special flour for fried fritters.

Index	Numerical value
Ash (%)	<0.55
Moisture (%)	≤14
Wet gluten content (%)	29~32
Formation time (min)	4.0~6.0
Stable time (min)	4.4~8.8
Water absorption (%)	58.0~64.7
Resistance to extension (BU)	346.6–525.4
Weakening degree (BU)	41.2~70.5
Amylose content (%)	17.2~23.7
Ratio of amylose to amylopectin	0.315~0.545
Extensibility (mm)	≥180
Chromaticity L value	93.43~94.62

4. The Consumption Trend of Traditional Wheat-Based Foods in China

The production of flour food requires three links: wheat planting, flour processing, and food processing, which must be linked up with each other; otherwise, the development of the flour food industry will be restricted [93]. For a long time, the goal of wheat breeding in China has been based on yield, but the selection of quality characters has been neglected. The variation range of wheat varieties is wide, there are many quality types, and the cultivation measures are still inadequate, so it is difficult to give full play to the potential of improved varieties. The natural environment also restricts the development of the wheat industry [94].

There are many middle types of wheat varieties in China, which are suitable for handmade steamed bread and noodles. However, there are few varieties suitable for machine-made noodles and steamed bread with the characteristics of rapid mixing resistance of dough. There is a lack of hard,

Table 10: Quality index of special wheat varieties with different types of Naan.

Project		Index		
		High gluten naan	Ordinary naan	Weak gluten naan
Grain	Hardness index	≥60.0	50.0~59.0	<50.0
	Protein content/%	≥13.0	12.5~12.9	<12.5
	Bulk density (g/L)	≥700.0	≥700.0	≥700.0
Wheat flour	Wet gluten content (14% water base)/%	≥28	26.0~27.9	<26.0
	Hydroscopic rate (%)	≥58.0	≥56.0~57.9	<56.0
	Stable time/min	≥6.0	3.0~5.9	<3.0
	Maximum resistance to extension/EU	≥300.0	200.0~299.0	
	Extension area/cm^2	≥65.0	50.0~64.0	

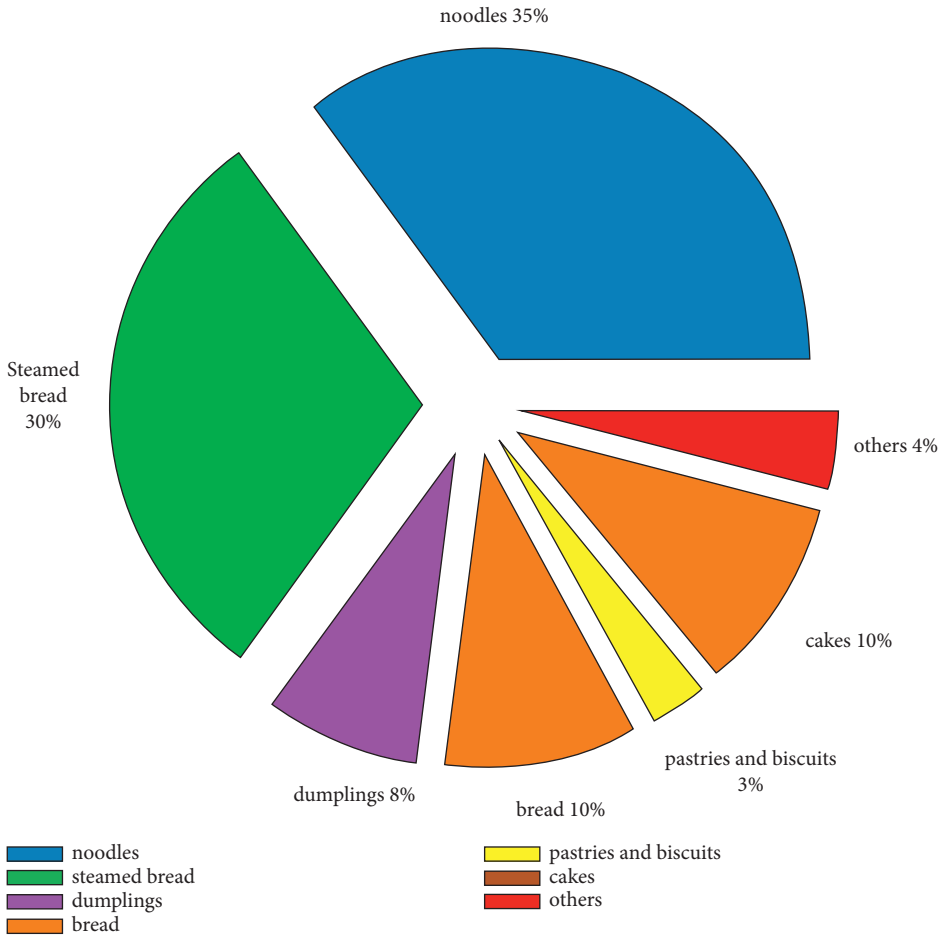

Figure 1: Proportion of pasta in flour circulation.

high-protein, and strong-gluten wheat for making high-quality bread and soft, low-protein, and weak-gluten wheat for making high-quality biscuits and cakes.

Chinese consumers' demand for bread and biscuits has increased, but the main demand is still for traditional staples such as steamed bread and noodles. The development of suitable wheat varieties according to local conditions can improve the development potential of improved wheat varieties and improve the quality of wheat. In order to meet the market demand, from the point of view of the whole country, it is necessary to vigorously develop medium-strong gluten wheat suitable for making machine-made noodles and steamed bread, plant strong gluten wheat in spring wheat areas and some areas with suitable climate and fertile soil, and appropriately develop soft wheat in specific areas of the south. Improve existing varieties of gluten quality, dough formation time and stability time, dough extensibility, starch properties, protein content, and quality of existing varieties.

Chinese traditional pasta is the accumulation product of Chinese national excellent traditional diet culture, with a wide variety and rich nutrition, which is the basis of national nutrition and health, and is accepted and loved by the majority of consumers [95, 96]. According to data, among

the annual consumption of more than 70 million tons of flour, staple food consumption accounts for more than 83%, of which steamed bread accounts for 30%, noodles account for 35%, and dumplings account for 8%. It has a huge market capacity and a broad market demand space [97, 98] (Figure 1).

However, at present, the development level of China's staple food industry is still low, and there is a problem of uneven development between urban and rural areas. The vast rural is the main area where staple foods such as steamed bread and noodles are consumed. Generally, it is the mode of manual workshop production and stall sales, and the quality, hygiene, and safety of raw materials and products cannot be guaranteed. There have been some large-scale and mechanized pasta processing plants in large and medium-sized cities, which have improved in processing efficiency and food safety. However, due to the limitation of technology and equipment, the indexes of influencing factors such as fermentation method and dough structure to determine the quality of staple food cannot be reflected, and there is a lack of market competitiveness [97].

The industrialization of pasta food in developed countries such as Europe and the USA has developed rapidly, and at a high level, the industrialization of pasta has generally reached 70%, while that in China is only 15% to 20% [99, 100]. Promoting the industrialization of pasta can promote the large-scale production of pasta, which is suitable for the fast-paced life of modern people, and can produce wheat flour and pasta in accordance with scientific standards to achieve conservation and nutrition. Therefore, promoting the industrialization of pasta is of great significance in promoting the gradual modernization of residents' dietary consumption and improving people's consumption patterns.

Promoting the industrialization of pasta can extend the wheat industry chain and actively develop new products to meet the consumption needs of different people. For example, convenience foods, frozen pasta, and specialty customized noodles can be developed. Instant noodles and dried noodles are the representatives of instant food in China. In recent years, the total consumption of dried noodles in China has stabilized at about 1.7 million tons, and the per capita consumption of instant noodles in China has ranked among the top five in the world [101]. However, instant noodles are faced with nutritional deficiencies, inadequate innovation, and quality and safety issues. There is a problem of uneven quality of noodles between north and south and large differences in consumption of dried noodles [102]. It is necessary to increase the intensity of product innovation and look for alternatives to food additives that may be harmful to the human body. Corn, soybeans, potatoes, and miscellaneous grains can be added on an original basis to change the flavor and taste of traditional instant noodles and make their nutrition more balanced. Develop whole-grain noodles, miscellaneous grain noodles, soybean noodles, potato noodles, and vegetable noodles to meet the needs of different people [103].

With the rapid development of China's food industry, other instant pasta types has also made breakthroughs, such as egg yolk pies, soft bread, biscuits, steamed slices, and so on. More kinds of convenience foods will appear in the market in the future. The scale of the frozen food industry in China is expanding rapidly, with frozen dumplings, noodles, steamed bread, and stuffed buns accounting for more than 30% of the total frozen food [104]. Frozen dough technology has also developed rapidly, and it has more remarkable advantages than traditional technology. The chain management of noodle food can meet the needs of consumers and the market and has a wide application prospect [105].

With the demand for healthy food, health care pastry food has a vast market. For special patients, great efforts should be made to develop healthy pastry, dietotherapy pastry, and nourishing pastry [106]. For infants and young children, middle-aged and elderly people, and the three high groups, the market has launched foods such as children's dumplings, infant noodles, whole wheat steamed bread, miscellaneous grain steamed bread, etc. According to the needs of consumers, leisure foods such as seasoning pasta food and expanded pasta products with different flavors can be developed. For different groups of people, featured customized pasta food can be developed to meet the needs of the market.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

Classification, Processing Procedures, and Market Demand of Chinese Biscuits and the Breeding of Special Wheat for Biscuit Making

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With the improvement of living standards, consumers' demand for wheat food is gradually diversified. Biscuit, as a kind of convenience food, becomes a consumer's leisure snack due to its characteristics such as low processing cost, easy-to-carry and convenient-to-eat traits, long shelf life, diverse varieties, and rich tastes, which have attracted more and more people. Biscuits are composed of four main ingredients, which are flour, fat/oil, sugar, and water, whereas several secondary ingredients also are important sources of high molecular carbohydrates, plant proteins, vitamins, and minerals for human beings. In this study, we systematically summarized the related research of biscuits, including the main types of China's biscuits, the market demands, and statistics of wheat planting, production, and import in recent ten years, as well as the research of soft wheat breeding for biscuit. The flour consumption of biscuit industry has been maintained at more than 4 million tons, accounting for more than 30% of the flour consumption in food industry. The planting area of wheat in China has stabilized around 22.8 million hectares in 2010–2020, while the yield of wheat has increased 18.0% (20.86 million t) due to the increase of yield per unit of wheat. China's total annual pastry import bill increased 5 times and the gap between import and export bill of pastry has been increased more than 7 times from 2010 to 2020, suggesting the strong demand of the national pastry market. This research also provides a direction for the future breeding of special soft wheat for biscuits in China.

1. Introduction

The developed navigation industry in the 19th century made Europeans accustomed to carry one kind of food that was low in moisture and easy to store and could fight hunger, so biscuit, a simple food made of flour and water, was born. The word "biscuit" comes from the Latin word "Panis biscotus" meaning twice-cooked bread usually made for sailors and is named "marine biscuit." The biscuits were defined as "hard dry bread that transported to sea" by Dr. Samuel Johnson. The British were the first to make this kind of biscuit, including crackers, cookies, and wafers [1].

Biscuit is a kind of convenience food, which belongs to the category of baked food. Biscuit is a crisp food with less than 6% moisture content, which is made by main raw material weak gluten wheat flour with other auxiliary materials such as sugar (or without), oil, and other excipients through flour blending, molding, baking, and other processes. Due to its mature production technology, variety, and rich taste, biscuits can meet the needs of different consumers compared with other kinds of ready-to-eat snacks. Biscuit has attracted a wide consumption base due to its characteristics such as low processing cost, easy-to-carry and convenient-to-eat, and long shelf life [2, 3]. The good eating quality has led to the development of new type of biscuits of different categories and improved nutrition. Biscuits turn flour, fat, and sugar into nutritious and energy-rich snack foods that people accept at a low-cost way. It has become a new trend in the biscuit

industry to study compound flour baked cookies rich in special ingredients or to develop fortified biscuits. Biscuit can be used as strategic reserves in emergency situations such as food planning, earthquake resistance, and disaster prevention due to its good nutrition and storage characteristics.

Since the reform and opening up of China, people's living standards have been gradually improved with the development of economy and the influx of western technology and culture. As a classification of baked goods, biscuits began to develop rapidly. A large number of small enterprises with backward productivity go bankrupt or have been merged by big ones with the development of production technology. Likewise, the biscuit industry continues to merge into large-scale production with a healthily and orderly developed biscuit market [4]. The annual per capita consumption of biscuits in the United States, Britain, and western European countries is more than 10 kg, while in southeast Asian countries or regions, such as Singapore, Hong Kong, Thailand, and Indonesia, that is more than 4.25 kg. The annual per capita biscuit consumption of Japan and China is estimated at 7.5 kg and <2 kg, respectively [5]. Therefore, China's biscuit industry and market has strong potential. With the continuous growth of China's economy and the improvement of people's living standards, the biscuit manufacturing industry has developed from labor-intensive handicraft industry to technology-intensive industrial production, and biscuits have also developed from hunger-fighting snacks to leisure, nutrition, and health care food. A variety of biscuit products with rich nutritional value are constantly introduced to the market, showing the new vitality in the biscuit market. This study summarizes the related research of China's biscuits in the aspects of raw materials, types, production technology, domestic biscuit demand, and special wheat breeding for biscuits.

2. Biscuit Ingredients and Their Influence on Biscuit Texture

Biscuits consist of main and secondary ingredients. Among them, flour, fat/oil, sugar, and water are the basic main ingredients, whereas salt, egg, emulsifier, starter (sodium bicarbonate, ammonium bicarbonate), milk powder, and seasoning spices are optional secondary ingredients [6]. The main ingredients of different biscuits vary a lot, and according to their characteristics, there are different special secondary ingredients. Therefore, it is difficult to compare the use of each ingredient; here we mainly describe the role of the most commonly used ingredients in biscuit production. Wheat flour dough is a complex and heterogeneous system consisting of three main components: flour, sugar, and fat. Each of these ingredients plays a different role, contributing to its taste, texture, color, and flavor. The type and proportion of ingredients affect the quality of the final product.

2.1. Flour. Flour is one of the main raw materials of bread, cakes, biscuits, and other baking products. It is mainly composed of starch, protein, and water. Unlike protein, starch does not form a large network of gluten in biscuit dough, but acts as the main filler in the dough. Soft wheat flour with low protein content (8–11%) is considered to be an ideal flour for making biscuits because gluten forms a smaller network structure [7]. The addition of protein increases the hydration property of the mixture and the consistency of the dough, reduces the baking time of biscuits, and results in lower hardness and darker color [8]. Changing in flour protein content between 14% and 20% can lead to significant changes in dough rheological properties and biscuit size and texture [9]. In addition, the moisture content of flour and the amount of water added in dough have different effects on the properties of biscuit dough [7]. The increase of flour moisture will result in the increase of the fluidity and adhesion of the dough and biscuit diameter [10]. The particle size of flour is an important factor affecting the quality of biscuits, and its effect varies with different types of dough. The high extension of biscuits is related to the fine particles of soft wheat flour. The finer the flour is, the higher the density of biscuits and the lower the expansion are [6]. The average particle size of the flour used in most biscuit production is about 50 μm, of which less than 10% of the flour is larger than 130 μm [1]. However, glutenin in flour can cause immune response in some people, leading to celiac (CD), non-celiac gluten intolerance (NCGS), and wheat allergy. The market demand for gluten-free products is increasing rapidly. In addition, the higher content of carbohydrates in flour can easily lead to consumer obesity. Therefore, people use different kinds of flour to replace wheat flour or to add different nutrients to biscuits, such as multi-grain mixture [11], rice flour [8], millet or adzuki bean flour [12], oat flour [13], soybean and cassava flour [14], and so on.

2.2. Sucrose. Sugar can improve the structure and enhance the flavor of biscuits. Sucrose is the most commonly used sugar in biscuit baking. The sweetness of sucrose is provided by transformed fructose during baking. Sugar has a significant effect on the phase transition of biopolymers such as starch and gluten in biscuit dough [15]. Sugar disperses proteins and starch macromolecules through hydration to prevent the formation of gluten networks that make biscuits crispy. Based on the macro and micro data of dough and biscuits, Chevallier et al. [16] proposed that the structure of wheat biscuits is formed by the bridge between protein aggregates and lipids due to the melting of sugars during baking process. After cooling, the continuous glassy state of sugar and the intact or almost undamaged starch particles are embedded one after another. Regardless of the composition of wheat biscuits, both glutenin and sucrose are glassy [17]. On the contrary, Gallagher et al. [18] believe that the texture of wheat biscuit is mainly affected by starch gelatinization and supercooled sugars because of the rarely formed gluten networks in wheat biscuits. Maillard reaction occurs during the baking process of sugar, which enhances the color and flavor of cookies. Due to the differences of water content in dough, the sugar is partially or completely dissolved and recrystallized during cooling to increase the hardness and surface crack of the biscuit [19]. During

baking, the undissolved sugar gradually melts to extend the dough and cools into amorphous glass, giving the biscuit a crisp taste [20]. In addition, the crystal size and dissolution rate of sugar also affect the characteristics of biscuits. Extra coarse sugar crystal particles reduce the extensibility of biscuit dough and increase surface cracking due to the decrease of dissolution rate [21].

The amount of sugar also has a great effect on the characteristics of biscuits. Increasing sugar content can reduce the viscosity and fermentation time of the dough and increase the hardness of biscuits [19]. Therefore, high concentration and small particles of sugar is conducive to the extension of biscuits; however, excessive sugar reduces the degree of dough softening and the consistency and cohesion of the dough, which in turn results in the tiling of biscuits, and affects the baking time of biscuits [15]. The difference in sugar content produces two very different kinds of biscuits: one is low-sugar biscuits with a sugar content of 17% to 25%, which is with the least spread of dough during baking, such as ordinary tough biscuits; the other is sweet crisp biscuits containing about 25% to 60% sugar (based on flour) and the dough will swell significantly during baking [7].

In the baking process, 10–20% of the reducing sugar in the biscuit dough combines with the amino acids, which contributes to the formation of biscuit color. Due to their high calorie content, sugars are replaced by artificial sweeteners in biscuit making, such as saccharin, acesulfame-K, aspartame, sucralose, cyclic esters, and fructose [22]. These sweeteners are very sweet, and they are good substitutes of sugar.

2.3. Fat. Fat is the third major ingredient in biscuit making, providing the tenderness and crisp taste of biscuits. Fat destroys the continuity of protein and starch by isolating water from protein and starch granules, thus preventing the dough from producing stickiness and the formation of large gluten networks [23]. It also inhibits the fermentation of carbon dioxide diffusion in the dough by forming a coating around the flour particles [24]. Fat can significantly affect the gelatinization performance of flour and increase the gelatinization temperature.

The mechanical properties of biscuits largely depend on the fat composition in dough. The type and proportion of fat in biscuit dough will affect the rheological properties of biscuit dough and the change of dough size during baking and finally affect the characteristics of baked products [25]. The type of fat affects the texture of dough, and biscuits with high solid fat content have higher breaking ability [26]. It is found that reducing fat content or using liquid fat to instead of solid fat can decrease the hardness and increase the viscosity and elasticity of biscuit dough and make biscuits with higher breaking strength [27, 28]. Many studies have found that the biscuit dough made from liquid oil with lower content of saturated fatty acids (such as sunflower oil) has better malleability during baking and larger biscuit diameter [27, 29]. Fats such as butter and vegetable oils are often called shortening. Shortening acts as a lubricant by blocking the formation of gluten and the adhesion of starch particles, making cookies more tender and uniform in shape after baking [29], resulting in better surface properties and higher brittleness [26].

Although fat plays an important role in baked goods, eating too much fat can lead to a variety of diseases, such as obesity, cancer, high cholesterol, and coronary heart disease [19]. In addition, baked goods are one of the main sources of trans fatty acids in the diet [30]. The study found that the content of trans fatty acids in biscuits varied from 0.1 to 3.2 g per 100 g. Glucose biscuits, sweet biscuits, and salt biscuits had the lowest trans fatty acid content (0.1 g /100 g), while butter biscuits had the highest trans fatty acid content (17.1 g /100 g) [31]. World Health Organization (WHO) recommends limiting trans-fat intake to less than 1% of total energy intake [32].

Therefore, it is necessary to select appropriate fat substitutes to reduce the fat content and trans fatty acids in biscuits. Fat substitutes can be divided into carbohydrates, proteins, lipids, emulsifiers, and synthetic fats. At present, many ingredients (emulsifier, sorbitol [33], poly (dextran) [34, 35], and crystalline fiber, etc.) are used as fat substitutes [36]. Carbohydrate-based fat substitutes, such as dextrin, modified starch, and hydrophilic colloids, form a gel-like matrix by combining water, providing the required smooth taste to mimic fat [35, 37]. Compared with polyglucose, using maltodextrin instead of fat can improve the color and appearance of biscuits and make cookies with a softer taste [35, 38]. Tarancón et al. [38] have developed healthy biscuits with fewer trans fatty acids using olive oil. Using vegetable oils instead of fat can shorten the shelf life of biscuits due to the taste, flavor instability, and oil oxidation [29]. However, it is an unsolved problem that fat substitutes have a great influence on the texture of biscuit; when reducing the fat in biscuit, the biscuit will become hard and brittle [34].

2.4. Water. Water plays an important role in biscuit baking because it provides the necessary media for the physical, chemical, biological, and biochemical reactions needed for the conversion of raw materials into the final baked goods. In addition, it also has a decisive impact on the overall quality and taste of biscuits [39]. Water hydrates gluten protein in the mixing process, gelatinizes starch in the baking process, and acts as a dispersing medium for other components [40]. The salt in the water will affect the characteristics of the dough. Hard water containing magnesium and calcium ions may have a "tightening effect" on the dough, while soft water may have a "relaxation effect" on the dough.

2.5. Salt. Salt is used as a surface condiment and decoration for delicious biscuits. Depending on the weight of the flour, a salt concentration of less than 2% produces a satisfactory taste [41]. Salt hardens gluten, increases the consistency of the dough, makes the dough easy to process, and slows fermentation and Maillard reaction. In addition, it is also conducive to the formation of hard shells.

2.6. Starter. Another important ingredient for biscuit making is the starter. Adding starter to the dough produces fermenting gases, which can cause holes in the cookie during baking. The most typical chemical starters are baking powder (a mixture of sodium bicarbonate and acid), sodium pyrophosphate, sodium bicarbonate ($NaHCO_3$), and ammonium bicarbonate (NH_4HCO_3). Sodium bicarbonate dissolves in the dough and reacts with the acid to produce carbon dioxide. When heated, ammonium bicarbonate decomposes to form carbon dioxide, ammonia, and water. Both the loosening agent and salt in the dough can affect the formation of hydroxymethylfurfural (HMF) and acrylamide in biscuits. The type of starter has a great influence on the formation of acrylamide and hydroxymethyl cellulase. In addition, compared with sodium salt, ammonium salt accelerates the degradation of sucrose, so it plays an important role in promoting the formation of acrylamide and HMF. Replacing ammonium bicarbonate with sodium bicarbonate can reduce the content of acrylamide to about 70% [42] and the production of HMF to 95% [43]. With the addition of NH_4HCO_3 (pH 7.82), the production of acrylamide after 15 min baking at 205°C increased by 6 times compared to the control without any raising agent (pH 5.82). On the contrary, in the presence of $Na_4P_2O_7$ (pH 6.78), the final acrylamide concentration of the samples was similar to that of the control, while in the presence of $NaHCO_3$ (pH 8.10), the final concentration of acrylamide even decreased by 52% [44].

3. Classification of Biscuits

3.1. Classification of Biscuit Based on the Formula, Process, and End Products. Generally speaking, according to the characteristics of biscuit formula, process, and end products, biscuits can be divided into five categories: coarse biscuit, tough biscuit, crisp biscuit, sweet crisp biscuit, and fermented biscuit.

3.1.1. Coarse Biscuits. The amount of fat and sugar is very small (0 : 10), and the main material is flour, with a ratio of 1 : 5 for the sugar and flour. The biscuit is formed by stamping or roller cutting. The biscuits are compact, crisp, and dry and can be used to make compressed biscuits.

3.1.2. Tough Biscuits. They have low sugar and oil content, good dough toughness, and forming by stamping and roll cutting. The end product has a large block type and a concave pattern on the surface, which is smooth. The cross section has layers, brittleness is prominent, and crispness is poor. This biscuit has a strong layered sense and a crisp taste. The higher the content of wet gluten, the better the toughness of the dough. Therefore, the flour used in tough biscuits requires a wet gluten content of 22% to 26% [45]. However, the content of oil and sugar is relatively low, and the ratio of oil to sugar is generally required to be 1 : 2.5, while the ratio of oil and sugar to flour is 1 : 2.5. The dough is mixed by hot powder technology with high extensibility and smooth surface. Due to the high-water content of the dough, it is suitable to bake at low temperature for a long time, which is beneficial to the dehydration of biscuit.

3.1.3. Crisp Biscuits. They have high content of sugar and oil, adding dairy products, eggs, and other ingredients. The dough is semi-soft, with little elasticity and strong plasticity, so it is formed by roll cutting. The end product is small and thick, and the surface pattern is clear and mostly convex. The cross section is a loose and porous structure without layers, so that biscuits taste crumbly.

In crisp biscuits, the ratio of oil sugar to flour is 1 : 2, and the ratio of oil to sugar should also be controlled 1 : 2. The wheat flour used is weak gluten flour, and the wet gluten content is about 20%. The oil should be shortenings with good crispness to prevent the occurrence of oil running. Because the temperature of crisp dough is close to room temperature, cold flour technology is used to mix flour. The biscuits with high oil content are molded by roll printing, and the temperatures of bottom fire and surface fire are higher about 300°C, in order to facilitate product shaping and avoid oil stalls (the surface area can be expanded in a regular shape). The varieties with low oil content have low fire temperature and high bottom fire temperature after entering the furnace, which is conducive to its volume expansion and the formation of a hard shell on the surface.

3.1.4. Sweet Crisp Biscuits. They have high oil and sugar content, low water content, more milk, eggs, and other accessories. In sweet crisp biscuits, the ratio of oil and sugar to flour is 1 : 1.35, and the ratio of oil to sugar is also 1 : 1.35. The dough is small in elasticity, strong in crispness and soft, and is formed by extrusion or steel wire cutting. The end product is thicker, the cross section is compact porous structure, and the taste is crisp.

3.1.5. Fermented Biscuits. The content of sugar and oil is less, with a ratio of 10:0–0.5, and the gluten is formed more, with a ratio of 1:4–6 for the sugar and oil to flour. The gluten strength will be reduced by excessive fermentation of dough, the inclusion of shortening, and so on, and the dough is finally formed by roller cutting or printing. The surface of end product is generally unpatterned, and the hierarchical structure of cross section is clear, the brittleness is prominent, and the fermentation flavor is obvious. Soda cracker, a type of fermented biscuits, needs to go through secondary fermentation. Fermented dough is formed by rolling, crisping, and lamination. When baking, it is necessary to avoid the formation of a hard shell on the surface of the cake prematurely; otherwise it will not be conducive to the escape of carbon dioxide from the dough and the accumulation and expansion of the dough. After the cake billet expands to the maximum volume, the surface fire temperature should be strengthened to prevent the cake billet from collapsing for the low temperature, resulting in the biscuits not crisp enough.

3.2. Different Types of Biscuits in China. According to the processing technology and the biscuit industry standard GB/T20980-2007 "biscuit" in China, biscuit can be divided into 12 types of biscuits, such as crispy biscuits, cookies, fermented biscuits, tough biscuits, compressed biscuits, Sandwich biscuits, wafers, waffles, egg rolls, pancakes, decorative biscuits, sponge biscuits, and so on.

3.2.1. Crispy Biscuits. Crisp biscuits are made of wheat flour, sugar, and oil as the main raw materials, adding loosening agent and other auxiliary materials, and the surface patterns are mostly convex flowers and porous structures made by cold powder process, rolling or non-rolling, forming, and baking. The taste of the biscuits is crispy or crunchy, and the fragrance is mellow.

3.2.2. Cookies. Cookies are made of wheat flour, sugar, syrup, oil, and dairy products as main raw materials, adding loosening agent and other auxiliary materials, mixing powder by cold powder process, and forming by one of the methods of extrusion, wire cutting, or roll stamping. Crisp biscuits are baked with stereoscopic patterns or regular ripples on the surface.

3.2.3. Fermented Biscuits. Fermented biscuits are made of wheat flour, oil, and sugar (or sugar-free) as main raw materials, yeast as puffing agent, adding various excipients, mixed with flour, fermented and extended, roll cutting, molding, and baking. Biscuits are crisp or crumbly with unique flavor of fermented products.

3.2.4. Tough Biscuits. Tough biscuits are made by hot powder process, taking wheat flour, sugar (or sugar-free), and fat as the main raw materials, adding loosening agents, modifiers, and other auxiliary materials. The surface patterns are mostly concave flowers with smooth appearance. The cross section of the biscuit is layered, and its surface is flat and usually with pinholes. Biscuits are crispy.

3.2.5. Compressed Biscuits. Using wheat flour, sugar, oil, and dairy products as the main raw materials, adding other auxiliary materials, mixing, roller printing, and baking to make biscuits, then crushing, adding oil, sugar, nutritional fortifier, or adding other dried fruits, meat floss, dairy products, etc.

3.2.6. Sandwich Biscuits. They are biscuits with adding stuffing such as sugar, cream, dairy products, chocolate jam, various compound sauces, or jams between the cookie pieces (or the hollow part of the biscuits).

3.2.7. Wafers. Using wheat flour (or glutinous rice flour) and starch as the main raw materials, adding emulsifier, puffing agent, and other auxiliary materials, a porous sheet is made by mixing, pouring, and baking. Two or more layers of biscuits are usually added between the sheets with materials such as sugar, grease, and other stuffing.

3.2.8. Waffles. A biscuit made of wheat flour, sugar, and eggs as the main raw materials, adding loosening agents, flavors, and other excipients, then squeezing and baking biscuits.

3.2.9. Egg Roll. Take wheat flour, sugar, and eggs as the main raw materials, and add or not add fat. In addition, leavening agents, modifiers, and other auxiliary materials are added, followed by mixing, pouring or hanging, baking, and rolling.

3.2.10. Pancakes. A biscuit made of wheat flour (glutinous rice flour, starch, etc.), sugar, and eggs as the main raw materials, with or without oil, adding leavening agents, and other auxiliary materials, mixing, pouring, or hanging and baking.

3.2.11. Decorative Biscuits. A biscuit with a coating, line, or pattern on the surface of a biscuit coated with chocolate jam, jam, and other accessories or sprayed with seasonings or framed candy flowers.

3.2.12. Sponge Biscuit. The loose and light biscuits with strong egg flavor are made by flour blending, multiple rolling, molding, hot water blanching, cold water soaking, and baking using wheat flour, sugar, and eggs as the main raw materials and adding loosening agent.

4. Biscuits' Consumption and Demands

In 2019, the total output of wheat flour was 83.192 million t, the total import 284,000 t, the total supply 84.056 million t, the total domestic consumption 83.073 million t, and the total export 305,000 t. In the total consumption of wheat flour in China, the consumption of catering and baking food occupied 41.8% (34.704 million tons), and the food industry consumption occupied 16.6% (13.812 million) (Figure 1(a)). In the food industry consumption of wheat flour, the flour consumption of biscuit industry is 4.362 million t occupying 31.6% (Figure 1(b)), which is 10 percent higher than noodles while a few percent than instant noodles. Although the consumption of biscuit industry declined slightly from 2016 to 2109 (4.675 million t in 2018, 4.964 million t in 2017, and 4.936 million t in 2016) [46], the biscuit industry still plays an important role in the food industry market.

According to the statistics of the National Bureau of Statistics (https://data.stats.gov.cn/index.htm), China's total annual pastry export bill increased 73.2% with an average annual rate of 7.73%, while the import bill increased 5 times with an average annual rate of 50.0% from 2010 to 2020 (Figure 2). The gap between import and export bill of pastry has been increased more than 7 times with an annual rate of 77.2% (from 0.61 to 5.32 billion dollars) from 2010 to 2020, suggesting the strong demand of the national pastry market. The scale of the baked food market showed a steady expansion trend [47]. The total production of biscuits in China

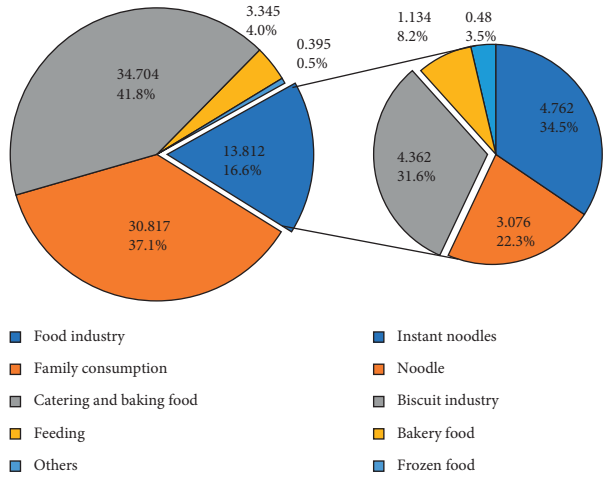

FIGURE 1: The consumption of China's wheat flour in 2019 from the statistics of Ma [46]. (a) The consumption and proportion of wheat flour in different industries of China; (b) the consumption and proportion of wheat flour in Food industry.

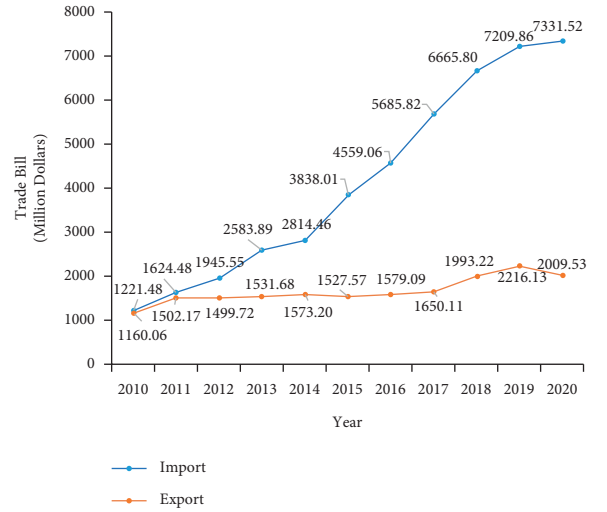

FIGURE 2: The total annual pastry import and export bill of China from 2010 to 2019.

increased at an average annual rate of 25.90% from 2005 to 2009, among which the output of biscuits in China was 1.3675 and 3.431 million t in 2005 and 2009, respectively, an increase of 2.5 times in 5 years [48]. At present, China's per capita consumption of biscuits is still keeping in a quite low state. The per capita annual consumption of biscuits in China is less than 2 kg, showing a big gap to that of developed countries (>10 kg), which should be related to the different eating habits [5]. From the perspective of long-term developing, China has a large population, and the people's living standards are gradually improving, and the demand for ready-to-eat leisure food is increasing, suggesting a great potential for the development of the biscuit industry. At this stage, function, nutrition, and fashion are still the main direction for biscuit development.

5. The Main Research of Soft Wheat for Biscuit

5.1. The Definition of Soft Wheat. The definition of soft wheat varies in different countries. For example, European countries call common wheat "soft wheat" and durum wheat "durum wheat," while in the United States, Australia, and Japan, those that have softer texture and lower protein content are called soft wheat,which is suitable for making biscuits and cakes.

At present, the representative weak gluten wheat abroad is American soft red winter wheat, American soft white wheat, and Australian soft wheat [49]. In China, according to the physical and chemical characteristics, wheat is divided into strong gluten wheat, medium gluten wheat, and low (weak) gluten wheat, among which the dough of low-gluten wheat has a short stability time (≤1.5 min) and is suitable for processing and baking food.

5.2. Characteristics of Soft Wheat and Its Distribution in China. Grain softness was an important trait of interest in wheat because of its role in producing flour suitable for making high-quality biscuits, cookies, cakes, and some other products. As early as the 1950s, the breeding and planting regionalization of soft wheat varieties were carried out abroad. At the same time, the relationships between grain texture, gluten protein content, rheological properties of dough, solvent retention and processing quality of cakes, biscuits, and other foods were studied deeply and systematically. Therefore, the breeding and production of high-quality weak gluten wheat varieties and the research of special flour have been greatly developed.

Hexaploidy wheat is usually divided into soft wheat and hard wheat. The flour produced by soft wheat is suitable to be used for making biscuits and cakes for its smaller particle size, less broken starch, and lower water absorption [50]. Durum wheat is more suitable for bread making because of its higher pentosans and protein content, higher lipid, and dry gluten content, as well as better ability to form elastic dough [51, 52].

In China, the early stage of wheat breeding focused on hard wheat with high protein content, which was mainly used for the improvement of bread and noodles, while the quality improvement of soft wheat started late. The hardness and quality of 1,361 commercial wheat samples and 687 wheat varieties collected in China in 2006 were analyzed by single grain analysis system (SKCS). The results show that, among the commercial wheat in China, hard wheat and soft wheat account for 35.2% and 7.2%, respectively, the proportion of soft wheat is low, and the wheat with different hardness is seriously mixed. The hard wheat and soft wheat in the variety wheat are 60.1% and 10.4%, respectively, and the proportion of soft wheat is still on the low side [53]. Soft grain, good extensibility, low protein content, and weak gluten strength are the main characteristics of high quality and weak gluten wheat [54]. China has formulated a national standard for weak gluten wheat (GB/T 17893–1999), with a flour content not less than 70%, a grain crude protein not more than 11.5%, a falling quality not less than 300 s, and a wet gluten content of wheat flour no more than 22%.

Low-gluten wheat generally grows in areas with excessive rainfall in the later stage. Strong gluten and medium gluten hard wheats are mainly grown in the north due to sufficient sunshine and less rainfall at mature stage, while more rain water is in the middle and lower reaches of the Yangtze River, where mainly medium and weak gluten soft wheat is grown. Weak gluten wheat in China is mainly distributed in Xinyang of Henan Province, and Jiangsu areas.

5.3. The Quality Index of Biscuit Special Powder and Breeding of Biscuit Special Soft Wheat. The bubble blowing is closely related to the quality of biscuits, so the bubble blowing indicator is often used to determine and control the quality index of biscuit special powder. Solvent retention capacity (SRC), proposed by Slade and Levine [55], is widely used in the prediction and evaluation of wheat flour quality, such as water SRC (WSRC), sucrose SRC (SSRC), lactic acid SRC (LASRC), and sodium carbonate SRC (SCSRC). It comprehensively reflects the physical and chemical properties of flour, such as gluten, pentosan, damaged starch, and water absorption and can predict the baking properties of flour as well. It is the leading method to evaluate the quality of soft wheat [55]. Studies have shown that SRC is less affected by environmental effects, which is mainly determined by genotypes [56, 57]. The correlation coefficient between biscuit quality and the parameters of farinograph and extensometer was small, while the elasticity/extensibility of blister, alkaline water retention capacity (AWRC), SCRC, and SSRC were closely related to the diameter of biscuits, which could be used as screening indexes of biscuit quality [56]. Therefore, it is feasible to use genetic improvement to reduce the SRC of selected varieties to obtain weak gluten wheat with high-quality [57]. Hardness can be used as an efficient and practical selection criterion in breeding; weak gluten wheat must use parents of corresponding quality types [58]. On the basis of the selection of grain hardness and protein content, reducing gluten strength and comprehensive water absorption of flour is the main goal of soft wheat breeding. WSRC, LASRC, and bubble blowing indicator are the most important screening indexes for soft wheat breeding [59].

The specific quality indexes of high-quality weak gluten wheat varieties for biscuit were put forward by Zhang [56], named SKCS. The grain hardness of high-quality weak gluten wheat varieties is 40, grain protein content ranges from 9.0% to 11.5%, flour protein content ranges from 8.0% to 10.0%, blister elasticity $\leq$40 mm, elasticity/extensibility $\leq$0.5, blower energy $\leq 7.5 \times 10{-}3$ J, AWSRC $\leq$ 59%, WSRC $\leq$ 53%, SCSRC $\leq$ 66%, LASRC $\leq$ 83%, and SSRC $\leq$ 87%. In addition, the contents of water-soluble pentosans and total pentosans were significantly negatively correlated with the diameter of biscuits [60]. At present, the high-quality weak gluten wheat cultivars in China basically are "Yangmai 15," "Yangmai 13," "Mianmai 51," "Zhengfeng 5," "Guangmai 1311," "Nongmai 126," "Wanxi 0638," and so on.

5.4. Grain Softness Genes and Their Unitization in the Breeding of Biscuit Special Soft Wheat. Endosperm texture is the main factor affecting the final use of wheat. It is controlled by genes located on a major locus called Ha that controls grain hardness (Ha) [61, 62]. The Ha locus is located on the chromosome arms 5DS; its wild-type form (Ha) and the mutated form (ha) alleles are present in the soft and hard wheats of hexaploidy bread wheat varieties, respectively [61, 63]. The Ha locus consists of 10 closely linked genes, among which 3 genes in an interval of approximately 70 kb comprising of puroindoline a (Pina-D1), puroindoline b (Pinb-D1), and Grain Softness Protein-1 (Gsp-D1) are associated with grain texture [61, 64, 65]. Pina-D1 and Pinb-D1 encode 2 low molecular weight, tryptophan, and cysteine enriched proteins, called puroindolines, PINA, and PINB, respectively. It has been shown that these 2 proteins constituted a major fraction of a 15 kDa protein originally named "friabilin," which are presented on the surface of water-washed starch granules. They are normally accumulated in the starchy endosperm cells and aleurone cells of the developing kernels [66]. They are found in abundant and minimal amounts in soft and hard wheats, respectively [67]. The presence of the wild-type alleles (Pina-D1a and Pinb-D1a) encoding PINA and PINB, respectively, results in a soft phenotype whereas mutations in Pina-D1 or Pinb-D1 results in a hard phenotype [68]. As durum wheat contains only the A and B genome, its grain mechanical properties are the hardest. The most prevalent allele associated with grain hardness is the Pinb-D1b, corresponding to a glycine-to-serine point mutation in position 46. This is classified as a hard phenotype in bread wheat [69].

Ma et al. [70] analyzed the puroindoline allelic variations and their association with kernel hardness in a diverse panel of wheat accessions (including 1539 Chinese cultivars and 107 landraces, and another 141 foreign accessions). The frequencies of wild type allele of Pina-D1a (Pina) and Pinb-D1a (Pinb) accounted for 90.4% and 41.1%, respectively, and the kernel hardness varied from 1.4 to 102.7, suggesting that there is a huge number of germplasms that can be used in soft wheat breeding [70]. Marker-assisted selection (MAS) is a fast and effective method widely used in wheat breeding [71], which was also deployed to develop advanced wheat lines with soft grains [62]. The Pina-D1a gene was transferred from the donor parent Australian soft-grained variety Barham to an Indian variety, DBW14, a hard grain wheat with PinaD1b and PinbD1a genes, to improve its grain softness using Marker-assisted backcross breeding strategy in Rai et al. [62]. Meanwhile, the function markers for the alleles of the Pina-D1a and Pinb-D1a have be effectively and widely used in the selection of soft wheat and this accelerated the breeding progress in many researches [62, 71].

Puroindoline polymorphism (Pina and Pinb) explains over 60% of the variation in kernel hardness. However, the other genetic factors except Puroindolines have be exploited over the past 2 decades and numerous kernel hardness-associated quantitative trait loci (QTLs) have been identified on almost every chromosome in wheat [72–77]. For example, 1 major QTL on 4BS as well as 3 minor QTLs on 1BS and 5AL affecting super soft kernel texture were identified

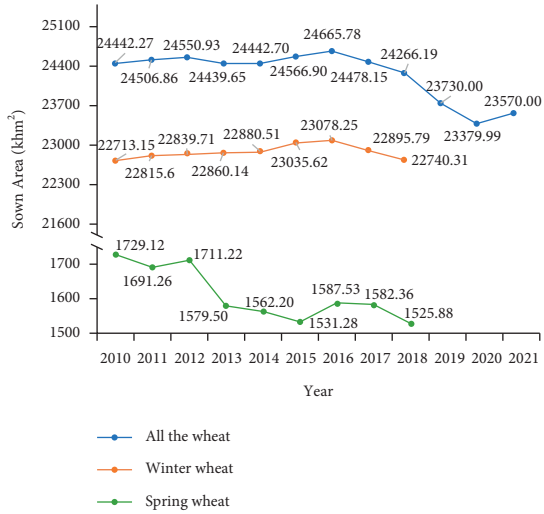

Figure 3: The sown area of wheat in China from 2010 to 2021. The sown area data of winter and spring wheat in 2019–2021 was with no records in the website of National Bureau of Statistics of China (https://data.stats.gov.cn/index.htm).

using a population of 268 F6 recombinant inbred lines (RILs) derived from the cross between Alpowa ("normal" soft) and "Super Soft" line (BC2SS163) in Kumar et al. [74], and 2 molecular markers significantly associated with kernel texture were identified and effectively used in MAS of soft wheat. Therefore, the multiple QTLs and the molecular markers significantly associated with kernel texture will enhance our understanding of kernel softness (hardness) and provide perspectives for future fine-tuning of grain texture and breeding the special soft wheat with high-quality to meet the needs of different biscuits.

6. Guidance on the Breeding and Demand of Soft Wheat for Biscuit

With the improvement of living standards, consumers' demand for wheat food is gradually diversified. According to the data from National Bureau of Statistics of China (https://data.stats.gov.cn/index.htm), we have obtained some statistics of Chinese wheat, including planting area, annual yield, import and export trade, etc. Most of China's wheat is winter wheat; the planting area of winter wheat in China has stabilized around 22.8 million hectares from 2010 to 2018; however, the sown area of spring wheat in China was decreased about 2.0 million hectares from 2010 to 2018 (Figure 3). The yield of wheat has increased 18.0% (20.86 million t) (Figure 4(a)) despite a slight decrease of the sown area for all the wheat (Figure 3), which is due to the increase of yield per unit of wheat (Figure 4(b)). The yield per unit of winter and spring wheat is increased about 700 and 550 kg/hm^2 from 2010 to 2018, respectively, and the average annual increase in yield per unit of all the wheat is about 96.5 kg/hm^2 from 2010 to 2021 (Figure 4(b)). Although the wheat production of China has been increasing, it is still unable to meet the domestic demand and has to be imported in large quantities from abroad every year (Figure 5). The total annual wheat imports amount and bill of China has increased to some extent from 2010 to 2020. In particular, the wheat imports amount and bill in 2020 was more than two times that in 2019 (Figure 5), which may be due to the impact of COVID-19 on China in 2020. With the decreasing of weak gluten wheat in wheat production in China, processing enterprises need to import a large number of foreign high-quality wheat to meet the pastry market demand (especially for biscuits) (Figure 2).

At present, China's per capita consumption of biscuits is still keeping in a quite low state, while the demands of biscuits are gradually increasing, suggesting a great potential market for biscuits industry. Soft wheat with weak gluten is the suitable wheat for biscuits and cakes making. For the current production, it is difficult for most varieties to reach the stable standard of high quality and weak gluten wheat, and the planting quality is unstable between years and regions [78]. The quality and yield of soft wheat are greatly affected by cultivation techniques and environmental conditions. Fertilizer and water management measures directly affect the yield and quality [79, 80]. The national standard requires that the protein content of weak gluten wheat is less than 11.5%. It is necessary to introduce high-quality weak gluten wheat germplasm resources with low protein content for genetic improvement. Contrary to the requirements of many quality indexes of soft wheat with weak gluten, the use of subunits with little effect on the quality improvement of strong gluten wheat or silencing of high-quality subunits may be beneficial to the improvement of weak gluten quality. Therefore, the deletion of high molecular weight glutenin subunits can be used to cultivate high-quality soft wheat with weak gluten [81]. In addition, the grain softness genes and QTLs can be effectively and widely used in the selection of soft wheat. With the decreasing of soft wheat in wheat production of China, it is necessary to optimize the current wheat quality structure, strengthen the improvement of wheat quality, and select soft wheat varieties with high quality and stable yield to meet the needs of the biscuit market.

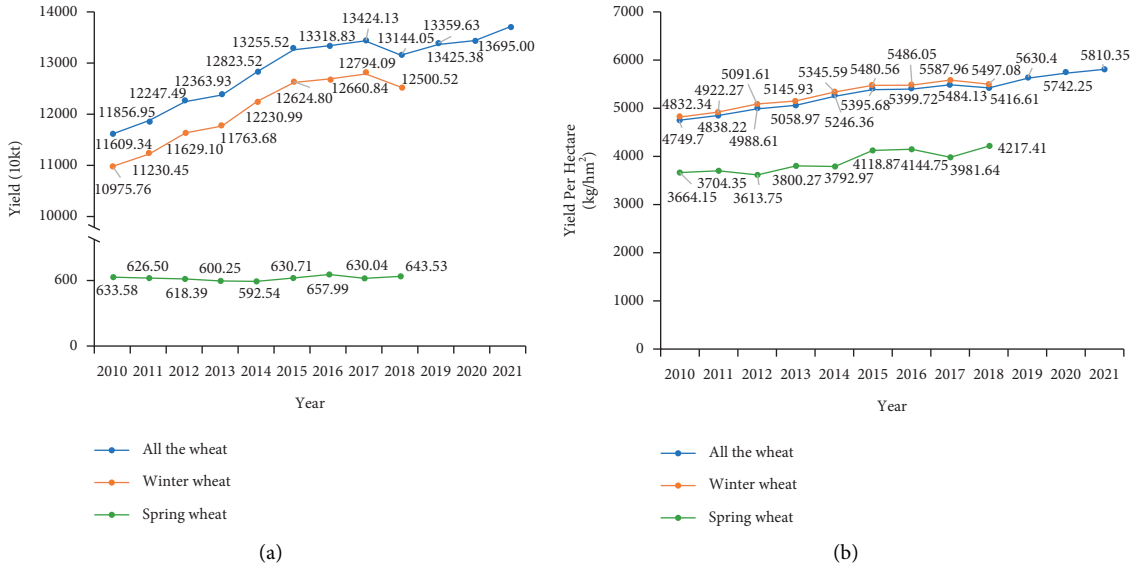

FIGURE 4: The total annual yield and average yield per hectare of wheat in China from 2010 to 2021. (a) The total annual yield of wheat in China from 2010 to 2021; (b) the average yield per hectare of wheat in China from 2010 to 2021. The annual yield and average yield per hectare of winter and spring wheat in 2019–2021 was with no records in the website of National Bureau of Statistics of China (https://data.stats.gov.cn/index.htm).

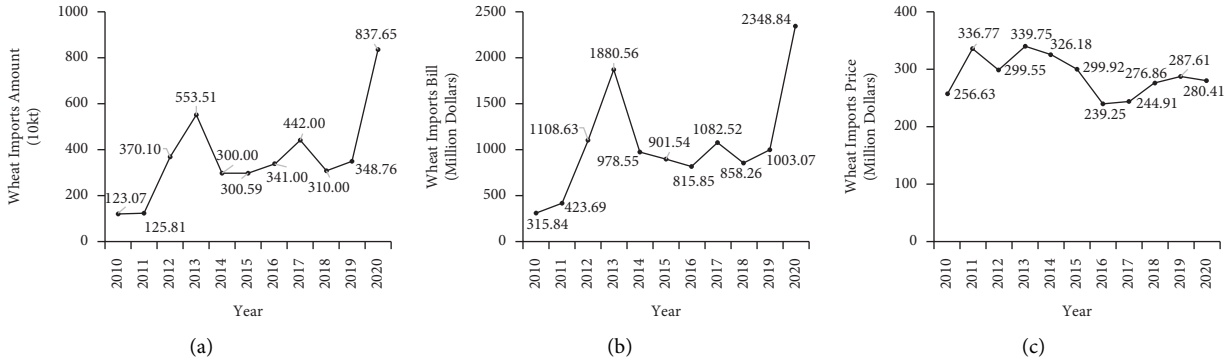

FIGURE 5: The total annual wheat imports amount, bill, and price of China from 2010 to 2020. (a) The total annual imports amount of wheat in China from 2010 to 2020; (b) the total annual imports bill of wheat in China from 2010 to 2020; (c) the total annual imports price of wheat in China from 2010 to 2020.

7. Conclusion

Biscuits, as a kind of convenience food, have attracted more and more people and become a consumer and leisure snack for their low processing cost, easy to carry and eat, long shelf life, diverse varieties, and rich taste. Biscuits are made up of four main ingredients like flour, fat or oil, sugar, and water and several secondary ingredients. The ingredients play a different role, contributing to their taste, texture, color, and flavor. The type and proportion of ingredients affect the quality of the final product. According to the characteristics of biscuit formula, process, and end products, biscuits can be divided into five categories, such as coarse, tough, crisp, sweet, and fermented. The types of biscuits in China vary a lot, which were with different processing technology and texture. At present, China's per capita consumption of biscuits is still keeping in a quite low state, while the demands of biscuits are gradually increasing, suggesting a great potential market for biscuits industry. Soft wheat with weak gluten is the suitable wheat for biscuits and cakes making. With the decreasing of weak gluten wheat in wheat production in China, it is necessary to optimize the current wheat quality structure, strengthen the improvement of wheat quality, and select soft wheat varieties with high quality and stable yield to meet the needs of the biscuit market.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

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

Authors' Contributions

Xin Hu and Lejia Hu contributed equally to this work.

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Research Article

Comprehensive Evaluation of Salt Tolerance in Asparagus Germplasm Accessions (*Asparagus officinalis* L.) at Different Growth Stages

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The screening and cultivation of salt-tolerant crops are becoming more and more important owing to the constant increase in the saline soil area worldwide. *Asparagus* (*A. officinalis* L.) is a highly nutritious vegetable crop and widely consumed globally for a long time; however, little research has been done on asparagus. In this study, the salt tolerance of 95 asparagus germplasm accessions was evaluated at three growth stages (germination, seedling, and adult stages) under both salt-stressed and control conditions. Results showed that the growth parameters of most germplasm accessions were obviously inhibited by salt stress. The mean value of the seed germination rate at the germination stage decreased by half under salt-stressed conditions, the mean salt-injury index at the seedling stage reached 57.68%, and the fresh weight of the aboveground part (FWA) and the dry weight of the aboveground part (DWA) decreased the most among the traits determined at the adult stage by more than 60%. Our study screened out 30, 19, and 18 tolerant germplasm accessions (including highly salt-tolerant and salt-tolerant germplasm accessions) at the germination stage, seedling stage, and adult stage, respectively. Among them, two germplasm accessions (Ji08-2 and Jx1502) were simultaneously identified to be tolerant in all three growth stages, while other germplasm accessions were tolerant only at one or two stages. Thus, the salt tolerance of asparagus has periodic characteristics and changes throughout the lifecycle, and the identification of salt tolerance at all the main growth stages facilitates adequate assessment and application of tolerant germplasm accessions.

1. Introduction

Vegetables are necessary food in people's daily life. With the improvement of living standard, people have more and more demand on the quantity and nutrition of vegetables. However, vegetables often suffer great damage from salt stress during their growth thus leading to the decrease of yield and quality [1]. Therefore, screening and cultivating salt-tolerant germplasm is set to become an alternative way to make full use of large areas of saline soil and to stabilize vegetable yield. Asparagus *(Asparagus officinalis* L.*)* is considered high in nutrients, including vitamins, steroidal saponins, flavonoids, minerals, and amino acids [2]. It has been widely consumed globally for a long time, making it an economically valuable plant. China is the largest asparagus producer and exporter in the world, which accounts for over 40% of the global planting area and yield of asparagus. Asparagus has strong salt tolerance and can grow normally in moderate saline-alkali soil below 0.3% [3]. In this environment, the impediment of upward transport of Na^+ by asparagus roots and the redistribution of Na^+, K^+, and Ca^{2+} on the organ level play key roles in asparagus adaptation to salt stress [3]. In addition, the enhanced ROS-scavenging capacity and carbon metabolism were also demonstrated as

important salt tolerance mechanisms in asparagus plants [4]. However, there are great differences in salt tolerance among varieties in severe saline-alkali soil, where only some varieties can grow normally [3, 5]. Therefore, it is important to identify and screen salt-tolerant asparagus germplasm accessions for research on the salt resistance mechanisms, breeding, and cultivation of salt-tolerant varieties in saline-alkali soil.

In recent years, considerable research on the identification of salt-tolerant germplasm has been carried out in multiple vegetables around the world, such as cucumber, tomato, and chilli [6–8], from which some success and experience have been achieved. However, in asparagus, the research is still in the initial stage and lacks depth and breadth. From many previous studies, we know that plant salt tolerance was evaluated at the germination stage and seedling stage due to their prerequisite roles in determining yield [6–8] while the results were usually different in the entire growth period, including the adult stage, another critical stage closer to actual production. Thus, salt-tolerant identification at the adult stage must be conducted before plants are applied in actual production in saline-alkali soil, which will be more reliable combined with identification at the germination stage and seedling stage.

The aims of this study were to screen the different germplasm accessions of asparagus for salt tolerance and to determine more reliable screening stages. In the present study, 95 asparagus germplasm accessions were evaluated and screened at the germination stage, seedling stage, and adult stage for salt tolerance, which further builds a foundation for research on asparagus salt resistance mechanisms and the breeding of salt-tolerant varieties.

2. Materials and Methods

2.1. Plant Materials. A total of 95 germplasm accessions of asparagus (*Asparagus officinalis* L.) were collected from various countries, including 31 from the United States, 11 from Holland, 6 from New Zealand, 5 from Italy, 3 from Germany, 3 from Japan, 1 from the United Kingdom, and 35 from China (Table 1). Seeds of these germplasm accessions were provided by the Institute of Cash Crops, Hebei Academy of Agriculture and Forestry Sciences.

2.2. Identification of Salt Tolerance at the Germination Stage. Seeds were first disinfected in ozone for 30 minutes. Next, 50 plump seeds of similar size were selected from each germplasm for germinating in Petri dishes (10 cm diameter) with three layers of filter paper soaked in 10 ml of 250 mM NaCl solution. In control, the distilled water was used. The experiment was arranged in a randomized complete block design with three replicates. The Petri dishes were sealed with parafilm and cultured in an artificial climate chest at 27°C with 75%–80% relative humidity. After 12 days, the germination rate (GR) of each genotype was determined. Then, the relative salt-injury rate (RSR) at the germination stage was calculated via the following formulae [9], and salt tolerance was correspondingly divided into five grades (Table 2):

$$\mathrm{RSR} = \frac{\mathrm{GR_{ck}} - \mathrm{GR_{st}}}{\mathrm{GR_{ck}}} \times 100\%, \quad (1)$$

where RSR is the relative salt-injury rate at the germination stage and GR_{st} and GR_{ck} represent the value of GR for germplasm under salt-stressed and control conditions, respectively.

2.3. Identification of Salt Tolerance at the Seedling Stage. For each asparagus germplasm, the germinated seeds were sown in plastic pots (15 cm diameter, one seedling/pot) with garden soil, vermiculite, and peat (4 : 3 : 3, v/v/v) in an artificial weather room at an ambient temperature of 28°C/19°C (day/night), the light intensity of 40000 lux, and a photoperiod of 10 h light/14 h dark. Then, 35-day-old seedlings were subjected to salinity stress and control treatments with nine replicates in each treatment on each asparagus germplasm. For the salt treatment group, seedlings were irrigated with 300 ml of 200 mM NaCl solution, while the controls were irrigated with distilled water. Plant injury was visually ranked at the termination of the 14-day stress treatment after the first salt treatment. Ranking of the standard and calculation of the salt-injury index (SI) followed the method of Zhen et al. [10], with some modification. The ranking criteria of salt-injury are presented in Table 3. The salt-injury index (SI) was calculated using the following equation:

$$\mathrm{SI} = \left[\sum \frac{(N_0 \times 0 + N_1 \times 1 + N_2 \times 2 + N_3 \times 3 + N_4 \times 4)}{N \times 4}\right] \times 100\%, \quad (2)$$

where N is the number of total seedlings; N_0 is the number of level 0 seedlings; N_1 is the number of level 1 seedlings; N_2 is the number of level 2 seedlings; N_3 is the number of level 3 seedlings; and N_4 is the number of level 4 seedlings. According to the value of SI, salt tolerance at the seedling stage was divided into five grades. The grading standard is shown in Table 2.

2.4. Identification of Salt Tolerance at the Adult Stage

2.4.1. Experimental Treatment and Trait Measurement. The experiment at the adult stage was conducted in the artificial weather room of the Institute of Cash Crops, Hebei Academy of Agriculture and Forestry Sciences, China. On February 20, 2020, 30 70-day-old asparagus seedlings for each genotype were transplanted in pots containing 15 kg fertile soil (1.1% organic matter, 347 mg/kg total N, 17.5 mg/kg available P, and 152 mg/kg available K), with the tray at the bottom preventing water leakage. After seven days, each pot was watered with 2 L 200 mM NaCl solution every 24 hours for three days, or with an equal volume of distilled water for controls. After these, all pots with seedlings were

Table 1: Name, origin, and the salt tolerance grades of the 95 asparagus accessions at each growth stage.

Number	Name	Origin	Germination stage		Seedling stage		Adult stage	
			RSR (%)	Grade	SI (%)	Grade	MVFS	Grade
1	Apllo	The United States	23.43	2	41.5	3	0.76	1
2	UC115	The United States	18.34	1	57.6	3	0.65	2
3	UC157	The United States	11.24	1	53.5	3	0.52	3
4	UC800	The United States	83.44	5	66.5	4	0.36	3
5	NJ800	The United States	27.07	2	56.5	3	0.76	1
6	NJ857	The United States	13.03	1	26.5	2	0.60	3
7	NJ951	The United States	50.87	3	67.8	4	0.55	3
8	NJ956	The United States	81.16	5	51.8	3	0.30	4
9	NJ978	The United States	71.07	4	78.6	4	0.41	3
10	NJ1089	The United States	8.85	1	68.2	4	0.36	3
11	NJ1123	The United States	83.61	5	38.5	2	0.29	4
12	NJ1156	The United States	42.49	3	78.8	4	0.63	2
13	NJ1189	The United States	23.55	2	62.5	4	0.55	3
14	NJ1191	The United States	41.72	3	63.8	4	0.65	2
15	Grande	The United States	22.46	2	71.8	4	0.59	3
16	Walker pioneer	The United States	40.04	3	66.7	4	0.65	2
17	Walker deluxe	The United States	63.50	4	39.2	2	0.48	3
18	Purple passion	The United States	53.38	3	68.9	4	0.46	3
19	Trillon	The United States	66.01	4	28.5	2	0.58	3
20	Patron	The United States	37.79	2	60.1	4	0.48	3
21	Jersey night	The United States	20.99	2	12.8	1	0.61	3
22	Jersey supreme	The United States	51.61	3	70.8	4	0.44	3
23	Jersey giant	The United States	37.51	2	72.5	4	0.45	3
24	Jersey knight	The United States	42.50	3	61.2	4	0.37	3
25	Male crown	The United States	28.69	2	42.7	3	0.52	3
26	Atlas	The United States	13.30	1	68.7	4	0.68	2
27	Florida	The United States	83.76	5	47.9	3	0.40	3
28	Early Califomia	The United States	94.20	5	59.2	3	0.63	2
29	Imperial	The United States	74.71	4	99.2	5	0.52	3
30	Wb-210	The United States	43.93	3	67.1	4	0.49	3
31	WB-215	The United States	52.65	3	77.6	4	0.49	3
32	Ariane	Germany	58.33	3	69.2	4	0.20	5
33	Eposs	Germany	35.66	2	50.4	3	0.19	5
34	European man	Germany	30.32	2	62.5	4	0.39	3
35	Pacific purple	New Zealand	34.65	2	77.3	4	0.50	3
36	Pacific challenger	New Zealand	33.71	2	65.4	4	0.46	3
37	Taramec	New Zealand	54.16	3	38.9	2	0.29	4
38	Pacific green	New Zealand	14.66	1	43.9	3	0.52	3
39	JWC1	New Zealand	21.85	2	63.7	4	0.47	3
40	Pacific 2000	New Zealand	61.56	4	29.5	2	0.36	3
41	Vittorio	Italy	53.98	3	81.6	5	0.28	4
42	Fo5030115	Italy	57.75	3	33.5	2	0.31	4
43	Fo5030215	Italy	31.56	2	16.7	1	0.60	3
44	Italo	Italy	10.59	1	64.1	4	0.48	3
45	Enos	Italy	64.48	4	74.8	4	0.51	3
46	Franklim	Holland	48.36	3	87.5	5	0.65	2
47	Gynlim	Holland	29.27	2	70.8	4	0.48	3
48	Boolim	Holland	73.14	4	38.9	2	0.47	3
49	Gijnlim	Holland	55.80	3	45.6	3	0.26	4
50	Limbras 10	Holland	37.25	2	67.2	4	0.52	3
51	Thielim	Holland	67.93	4	82.9	5	0.41	3
52	Taramea	Holland	34.92	2	61.4	4	0.41	3
53	James	Holland	52.00	3	51.3	3	0.26	4
54	Diamonds	Holland	53.45	3	97.2	5	0.26	4
55	Crown 300	Holland	41.83	3	73.6	4	0.55	3
56	Granno	Holland	33.26	2	15.2	1	0.43	3
57	Welcom	Japan	57.53	3	63.5	4	0.69	2
58	Accell	Japan	67.22	4	33.8	2	0.38	3
59	Shower	Japan	67.24	4	68.4	4	0.45	3

Table 1: Continued.

Number	Name	Origin	Germination stage		Seedling stage		Adult stage	
			RSR (%)	Grade	SI (%)	Grade	MVFS	Grade
60	Mondeo	The United Kingdom	62.97	4	18.9	1	0.39	3
61	Shuofeng	China	56.01	3	72.5	4	0.44	3
62	Zhefeng-4112	China	63.93	4	58.8	3	0.20	5
63	Jiahui	China	67.85	4	49.6	3	0.19	5
64	Prince	China	83.40	5	42.6	3	0.34	3
65	Shengju-1	China	92.28	5	69.2	4	0.36	3
66	Sheng96-8	China	81.18	5	55.1	3	0.35	3
67	A11	China	64.14	4	67.2	4	0.43	3
68	Ji08-2	China	4.17	1	0	1	0.85	1
69	Jilulvwang	China	47.23	3	66.5	4	0.41	3
70	Jinggang red	China	73.20	4	46.6	3	0.65	2
71	Jinggang-701	China	61.45	4	26.6	2	0.53	3
72	Jing lv-1	China	45.47	3	52.4	3	0.32	3
73	Jing lv-2	China	47.18	3	62.8	4	0.33	3
74	Jing lv-3	China	50.42	3	91.2	5	0.26	4
75	Jing lv-1042	China	70.59	4	45.8	3	0.64	2
76	Jing lv-1040	China	76.35	4	86.4	5	0.45	3
77	Wei2014-1	China	73.23	4	58.9	3	0.36	3
78	Wei2014-2	China	67.95	4	95.8	5	0.38	3
79	TC	China	44.21	3	32.6	2	0.80	1
80	Green spears	China	71.86	4	98.5	5	0.44	3
81	Lu asp-1	China	33.65	2	43.8	3	0.54	3
82	Green foison	China	48.09	3	55.3	3	0.63	2
83	Lu2000-3	China	55.59	3	64.6	4	0.45	3
84	Jx1502	China	4.39	1	21.8	2	0.82	1
85	Gold crown	China	69.48	4	83.6	5	0.59	3
86	Jadeite Pearl	China	56.34	3	43.8	3	0.30	4
87	New2030	China	44.16	3	65.1	4	0.47	3
88	Champion	China	54.80	3	23.6	2	0.77	1
89	JK113	China	50.76	3	87.5	5	0.31	4
90	JK1125	China	14.60	1	57.6	3	0.32	3
91	BJ14001	China	35.79	2	48.6	3	0.36	3
92	BJ14003	China	68.03	4	52.8	3	0.43	3
93	BJ14004	China	70.18	4	70.9	4	0.37	3
94	BJ14006	China	61.02	4	68.5	4	0.46	3
95	C9-12	China	51.55	3	38.5	2	0.77	1

1, 2, 3, 4, and 5 in the last three columns represent the germplasm accessions of highly salt-tolerant, salt-tolerant, moderately salt-tolerant, salt-sensitive, and highly salt-sensitive, respectively. RSR: the relative salt-injury rate; SI: the salt-injury index; MFVS: the membership function value of salt tolerance.

Table 2: Grading standard of salt tolerance at the germination stage and seedling stage.

Grade	Salt tolerance	RSR (%)	SI (%)
1	Highly salt-tolerant	0.0–20.0	0.0–20.0
2	Salt-tolerant	20.1–40.0	20.1–40.0
3	Moderately salt-tolerant	40.1–60.0	40.1–60.0
4	Salt-sensitive	60.1–80.0	60.1–80.0
5	Highly salt-sensitive	80.1–100.0	80.1–100.0

[z]RSR: the relative salt-injury rate at the germination stage; SI: the salt-injury index at the seedling stage.

watered with 2 L distilled water every 10 days and cultured in an artificial weather room at the temperature of 28°C/19°C (day/night), the light intensity of 50000 lux, and a photoperiod of 12 h light/12 h dark.

On September 10, 2020, the whole plant was dug out and nine traits were determined, including plant height (PH), the diameter of the stalk (DS), the diameter of the basal plate (DBP), the number of stalks (NS), the number of roots (NR), the fresh weight of aboveground part (FWA), the dry weight of aboveground part (DWA), the fresh weight of underground part (FWU), and the dry weight of underground part (DWU).

2.4.2. Data Analysis. Nine traits as indicators of salt tolerance were evaluated based on analysis of the genetic coefficient of variation, heritability, salt-tolerant coefficient, and membership function value of all the investigated traits with Microsoft Excel 2013 and SPSS 20.0 software [11]. ANOVA, Duncan's multiple comparison, and Pearson correlation analysis in our study were performed with SPSS software.

The genetic coefficient of variation (CV_g) of each trait was calculated using the following equation:

Table 3: The ranking criteria of seedlings after salt treatment.

Level	Ranking criteria
0	Normal growth without symptoms of injury
1	Approximately normal growth but with chlorosis and etiolation symptoms at the top of newborn spears
2	Inhibited growth and with etiolated leaves (cladodes) in all newborn spears and less than 20% dried stalks
3	Severely inhibited growth and with completely etiolated leaves aboveground and between 20% and 60% dried stalks
4	Almost dead or certified death and with more than 60% dried stalks

$$\mathrm{CV}_g = \frac{\sqrt{V_g}}{\overline{X}} = \sqrt{\frac{\left(G_k - SG_{jk}\right)/ij}{\overline{X}}} \times 100\%, \tag{3}$$

where V_g is the genotypic variance; $\overline{X}$ is the average value of the trait under the two salt treatments; G_k is the mean square of the genotype under the two salt treatments; $\mathrm{SG_{jk}}$ is the mean square of the interaction between salt treatments and the genotype; i is the number of replications; j is the number of salt treatments; and k is the number of genotypes.

The broad-sense heritability (H^2) of each trait was calculated using the following equation:

$$H^2 = \frac{V_g}{V_p} = \frac{V_g}{V_g + V_{sg} + V_\varepsilon} = \frac{\left(G_k - \mathrm{SG_{jk}}\right)/ij}{\left(G_k - \mathrm{SG_{jk}}\right)/ij + \left(\mathrm{SG_{jk}} - \varepsilon\right)/i}, \tag{4}$$

where V_p is the phenotypic variance; V_{sg} is the interactive variance between salt treatments and the genotype; V_ε is the variance of error; and ε is the mean square of the error.

The salt-tolerant coefficient (SC) was calculated using the following equation [12, 13]:

$$\mathrm{SC_{tg}} = \frac{X_{\mathrm{tgst}}}{X_{\mathrm{tgck}}}, \tag{5}$$

where $\mathrm{SC_{tg}}$ is the salt-tolerant coefficient of trait (t) for genotype (g) and X_{tgst} and X_{tgck} are the values of the trait (t) for the genotype (g) evaluated in the salt treatment and control group, respectively.

Asparagus salt tolerance was evaluated by the membership function value (MFV) using the fuzzy comprehensive evaluation method [11]. The modified membership function value of salt tolerance (MFVS) was calculated using the following equation:

$$\mathrm{U_{tg}} = \frac{\mathrm{SC_{tg}} - \mathrm{SC_{tmin}}}{\mathrm{SC_{tmax}} - \mathrm{SC_{tmin}}},$$
$$U_g = \frac{1}{n}\sum_{g=1}^{g} \mathrm{U_{tg}}, \tag{6}$$

where U_{tg} is the membership function value for salt tolerance of trait (t) for genotype (g); $\mathrm{SC_{tmin}}$ and $\mathrm{SC_{tmax}}$ are the respective minimum and maximum values for the salt-tolerant coefficient of trait (t); and U_g is the average value of the membership function of nine traits for the genotype (g) for salt tolerance.

Salt tolerance was divided into five grades according to the average value ($\overline{U}$) and standard deviation (SD) of MFVS. The grade standard for the salt tolerance of asparagus was established based on a study by Chen et al. [11]: if $U_g \geq \overline{U} + 1.64\mathrm{SD}$, highly salt-tolerant; $\overline{U} + 1\mathrm{SD} \leq U_g < \overline{U} + 1.64\mathrm{SD}$, salt-tolerant; $\overline{U} - 1\mathrm{SD} \leq U_g < \overline{U} + 1\mathrm{SD}$, moderately salt-tolerant; $\overline{U} - 1.64\mathrm{SD} \leq U_g < \overline{U} - 1\mathrm{SD}$, salt-sensitive; and $U_g < \overline{U} - 1.64\mathrm{SD}$, highly salt-sensitive.

3. Results

3.1. Salt Tolerance Evaluation of Asparagus at the Germination Stage. The effects of salt stress on GR of 95 asparagus germplasm accessions were evaluated. The germination of seeds of the 95 asparagus germplasm accessions was generally normal under control conditions with an average GR of 94.51% and standard deviation of 4.15%, whereas the mean value under the salt-stressed conditions (47.71%) decreased by half. ANOVA further revealed a highly significant variation between the two salinity conditions for GR tested at $P < 0.001$, which showed that salt stress significantly affected the seed germination of asparagus. Additionally, ANOVA revealed highly significant variations among genotypes for GR at $P < 0.001$. To reflect the differences in salt tolerance among different germplasm accessions, the RSR value of GR was calculated, which had a relatively high coefficient of variation (0.43) among genotypes and followed a normal distribution. Thus, the germplasm accessions with different salt tolerance were effectively distinguished under our experimental conditions according to the grading standard in Table 2. As a result, 10 germplasm accessions were highly salt-tolerant, 20 germplasm accessions were salt-tolerant, 32 germplasm accessions were moderately salt-tolerant, 25 germplasm accessions were salt-sensitive, and eight germplasm accessions were highly salt-sensitive (Table 1). The 10 highly salt-tolerant germplasm accessions are Ji08-2, Jx1502, NJ1089, Italo, UC157, NJ857, Atlas, JK1125, Pacific green, and UC115.

3.2. Salt Tolerance Evaluation of Asparagus at the Seedling Stage. At the seedling stage, all germplasm accessions grew normally, with SI values of 0 under control conditions. However, the SI values of most germplasm accessions under salt-stressed conditions showed an obvious increasing trend with a coefficient of variation of 0.35 among different genotypes and followed a normal distribution. It showed the varying degrees of injury caused by salt stress. According to the grading standard (Table 2), five germplasm accessions were highly salt-tolerant, 14 germplasm accessions were salt-

tolerant, 26 germplasm accessions were moderately salt-tolerant, 39 germplasm accessions were salt-sensitive, and 11 germplasm accessions were highly salt-sensitive (Table 1). The five highly salt-tolerant germplasm accessions are Ji08-2, Jersey night, Granno, Fo5030215, and Mondeo. The SI of Ji08-2 was 0; in other words, all the Ji08-2 seedlings grew normally without obvious symptoms of injury under salt stress, which is worthy of our particular attention for its application in subsequent research and production.

3.3. Salt Tolerance Evaluation of Asparagus at the Adult Stage

3.3.1. Response of Traits to Salt Stress. Phenotypic variation was confirmed by the average phenotypic value, SD, and SC of each investigated trait at the adult stage (Table 4). The mean values of all nine traits decreased under salt treatment. ANOVA revealed highly significant variations between two salinity conditions for all nine traits tested at $P < 0.001$ (Table 5). Among the nine traits, FWA and DWA decreased by more than 60% under salt-stressed conditions compared with the control. Further variance analysis and Duncan's multiple comparison indicated that there was a significant difference ($P < 0.05$) between the SC of these two traits and other traits, indicating that these two traits were very sensitive to salt stress while the decreased ratio of other traits (DS, DBP, NS, FWU, DWU, NR, PH, FWA, and DWA) compared with the control was in the range from 42% to 51%. Additionally, ANOVA revealed highly significant variations among the genotypes for all nine tested traits at $P < 0.001$ (Table 5), and the interactions between genotype and salt treatment for all traits were also significant ($P < 0.001$).

3.3.2. Genetic Variation and Broad-Sense Heritability of the Investigated Traits. The CV_g values for the nine investigated traits ranged from 13.34% to 39.84% (Table 5). The CV_g values of DWA, FWA, and NS exceeded 30%, i.e., 39.84%, 38.94%, and 30.44%, respectively. For six other traits (PH, DS, NR, DBP, DWU, and FWU), the CV_g values were in the range of 13.34% to 29.28%. These results indicated that there were significant genotypic variations among the 95 accessions with respect to the investigated traits under both salinity conditions.

The H^2 values of the nine investigated traits were analyzed. Their values reached medium to high levels, ranging from 0.36 to 0.82, suggesting these traits are stable and suitable to be applied in the assessment systems. The heritability values of DS, NS, NR, DWU, and FWA were relatively high, i.e., 0.82, 0.71, 0.64, 0.61, and 0.60, respectively. Among all the traits, PH had the lowest heritability of 0.36. FWU, DWU, and DBP had moderate heritability with values of 0.56, 0.44, and 0.43, respectively.

3.3.3. Identification of Salt Tolerance among Asparagus Germplasm Accessions. MFVS was used as a comprehensive index to evaluate the salt tolerance of the asparagus germplasm accessions. The estimated MFVS values of the 95 asparagus accessions based on the traits under the two salinity conditions are presented in Table 1, and their distribution was normal. The minimum and maximum values of MFVS were 0.19 (Eposs) and 0.85 (Ji08-2), respectively. Among the 95 asparagus accessions, seven accessions (Apllo, Ji08-2, Jx1502, TC, Champion, C9-12, and NJ800) were highly salt-tolerant; 11 accessions including Welcom, Atlas, Franklim, and NJ1191 were salt-tolerant. Results indicate that these 18 accessions with MVFS values ranging from 0.62 to 0.84 are optimal in saline soils. By contrast, 4 accessions and 11 accessions were classified as highly salt-sensitive and salt-sensitive, with MFVS values ranging from 0.19 to 0.32, which demonstrates that these asparagus accessions are not suitable for planting in saline soils. The remaining 62 accessions were regarded as moderately salt-tolerant, with MFVS values ranging from 0.33 to 0.61.

Table 4: The mean value and standard deviation (SD) of traits investigated under two salinity conditions and the salt-tolerant coefficient (SC) of each trait.

Traits	Mean ± SD (salt)	Mean ± SD (control)	Mean ± SD (SC)
DS (mm)	4.24 ± 0.98	7.32 ± 1.24	0.58 ± 0.08A
DBP (cm)	4.26 ± 1.40	7.78 ± 1.33	0.55 ± 0.16AB
NS	12.13 ± 5.13	22.10 ± 6.95	0.55 ± 0.13AB
FWU (g)	1052.54 ± 348.12	1954.78 ± 414.59	0.54 ± 0.14ABC
DWU (g)	375.12 ± 142.13	740.10 ± 190.87	0.52 ± 0.17BC
NR	125.62 ± 49.38	245.18 ± 81.66	0.52 ± 0.13BC
PH (cm)	93.22 ± 37.90	187.41 ± 21.85	0.49 ± 0.18C
FWA (g)	691.88 ± 469.87	1733.36 ± 720.78	0.39 ± 0.17D
DWA (g)	112.60 ± 82.54	299.83 ± 124.21	0.36 ± 0.18D

DS: the diameter of the stalk; DBP: the diameter of the basal plate; NS: the number of stalks; FWU: the fresh weight underground; DWU: the dry weight underground; NR: the number of roots; PH: plant height; FWA: the fresh weight of the aboveground part; DWA: the dry weight of the aboveground part. Values with different capital letters in the last column indicate a significant difference ($P < 0.05$).

3.4. Comparison of Salt Tolerance among Germination, Seedling, and Adult Stages. After the evaluation of the salt tolerance of 95 asparagus accessions at each growth stage, we obtained some tolerant germplasm accessions, including highly salt-tolerant and salt-tolerant germplasm accessions that had high potential for further application in saline soils and breeding. There were a total of 30 tolerant germplasm accessions at the germination stage, 19 at the seedling stage, and 18 at the adult stage. In order to further explore the relationships among salt tolerance identified from the three growth stages, Venn diagrams were constructed to detect both unique and overlapping sets of the tolerant germplasm accessions in different stages (Figure 1). Among them, there were six germplasm accessions common to the germination stage and seedling stage, five common to the seedling stage and adult stage, and six common to the adult stage and germination stage, while only two germplasm accessions (Ji08-2 and Jx1502) were simultaneously detected in all three

Table 5: Analysis of variance, genetic coefficient of variation (CV_g), and broad-sense heritability (H^2) of each trait under salt-stressed and control conditions.

Variation source	Df	Means of squares								
		PH (cm)	DS (mm)	NS	NR	DBP (cm)	DWA (g)	FWA (g)	DWU (g)	FWU (g)
Replication (R)	2	26.71	.05	1.53	75.43	0.01	44.37	17039.19	1460.31	482.03
Salt (S)	1	1264305.31***	1351.20***	14170.11***	2037027.41***	1758.60***	4995244.91***	154566150.95***	18982007.59***	115999914.11***
(S × R)	2	32.95	0.01	0.49	15.350	0.03	73.64	9023.88	389.79	4582.44*
Genotype (G)	94	3921.08***	6.91***	193.40***	22499.76***	8.01***	53612.32***	1779336.98***	122896.07***	687700.54***
(S × G)	94	1819.63***	0.63***	30.61***	4817.57***	3.16***	13114.50***	441580.74***	47000.49***	191507.74***
Error	376	14.09	0.03	0.96	46.04	0.02	46.41	5936.49	244.04	1277.95
CVg (%)		13.34	17.70	30.44	29.28	14.93	39.84	38.94	20.17	19.12
H^2		0.36	0.82	0.71	0.64	0.43	0.61	0.60	0.44	0.56

*Significant difference at the 0.05 level. **Significant difference at the 0.01 level. ***Significant difference at the 0.001 level.

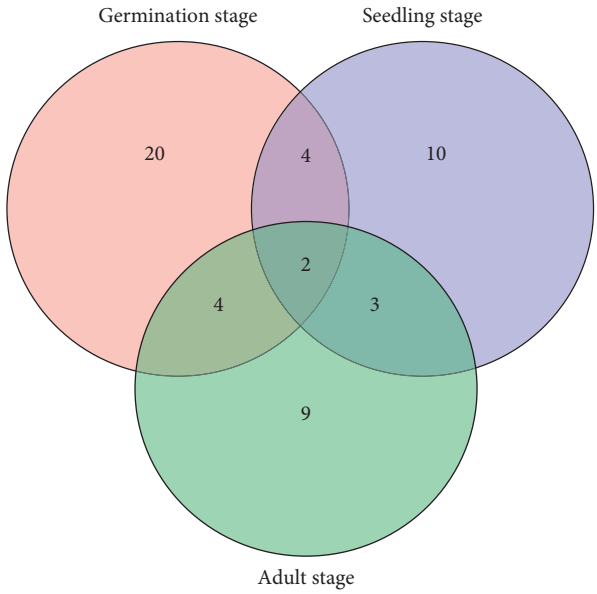

Figure 1: Venn diagrams showing specific and common tolerant germplasm accessions in the three growth stages.

growth stages. In general, most of the tolerant germplasm accessions were specific to one growth stage. Additionally, the correlations of the salt tolerance grades of 95 asparagus accessions between any two growth stages were analyzed and none showed a significant correlation with the P values of 0.346, 0.081, and 0.141, respectively. This result indicated that there may be no direct connection between the salt tolerance identified at different growth stages, and the identified results at each stage may show their own biological significance.

4. Discussion

It is well known that salt stress severely impacts crop production worldwide by reducing growth and yield [14, 15]. Specifically, the growth parameters of different germplasms show significantly different responses to salt stress, such as the germplasms of cucumber, tomato, and chilli [6–8]. In asparagus, we also found that 95 germplasm accessions exhibited different salt tolerance with a large variation. Overall, the growth of most germplasm accessions was obviously inhibited by salt stress. For instance, the mean value of the seed germination rate decreased by half under the salt-stressed condition compared to the control; the mean SI at the seedling stage reached 57.68%; and FWA and DWA at the adult stage decreased by more than 60%. Therefore, it is crucial for future research and production to screen salt-tolerant asparagus germplasm accessions through appropriate means.

Differences in salt tolerance occur not only among different germplasms but also at different developmental stages of the same germplasm [16, 17], which may be related to different salt tolerance mechanisms. A large number of studies have shown that germination and seedling stages are the preferred periods when evaluating the salt tolerance of crops due to their sensitivity to salt and important roles in crop stand and eventual crop yield [18–21]. Another critical stage closer to actual production, the adult stage, has often been overlooked. Several studies have proved the predictive role of some adult asparagus characteristics on future yield [22], which simultaneously resolves the problem of great workload inherent in continuously measuring yield throughout the entire harvest season. Thus, identifying salt-tolerant adult plants will be more reliable when combined with the identification at the germination and seedling stage. In our study, the identified results of asparagus salt tolerance at the three above-mentioned growth stages were different and showed no significant correlation. Therefore, the identification of salt tolerance based on only one growth stage may be one-sided, and it is necessary to identify all the main growth stages in order to adequately assess the salt tolerance of asparagus.

Salt stress usually affects plant morphological development, which provides some visual and measurable appearances for the identification of salt tolerance. The germination stage of asparagus plants is characterized by the germination of seeds. At the germination stage, GR is an important indicator, and its RSR is a reliable indicator to distinguish germplasm accessions with different salt tolerances [23]. Plants at the seedling stage are vulnerable; salt-sensitive and highly salt-sensitive germplasm accessions made up more than half of our germplasm accessions; and the symptoms of injury caused by salt stress were obvious, such as leaf chlorosis, growth inhibition, and even death. In this period, SI is suggested as an index for salt tolerance identification of perennial plants, based on the classification of the salt-injury level and the number of plants with a corresponding level [10], and can reflect the extent and intensity of salt injury to plants. Additionally, multiple indicators including the ground and underground parts of adult asparagus are correlated with future yield potential [22]. These traits in our asparagus germplasm accessions had adequate genetic variations and heritabilities, which are necessary guides for the breeding value and the trait utility within the selection process. In the case of multitrait evaluation, a comprehensive evaluation method, MFVS that combines the SC values of morphological traits, is often used [11, 18, 23]. In our study, the salt tolerance of 95 asparagus germplasm accessions was effectively distinguished according to the above methods, and their corresponding indicators (RSR, SI, and MFVS) for tolerance identification followed a normal distribution, indicating the rationality of our results. We obtained two germplasm accessions (Ji08-2 and Jx1502) that were simultaneously identified to be tolerant in all three growth stages and some other tolerant germplasm accessions tolerant at only one or two stages, which have a significant value for salt tolerance improvement in asparagus.

5. Conclusion

Ninety-five asparagus germplasm accessions were evaluated for their morphological traits under both salt and control conditions to screen the germplasm accessions for salt resistance at different growth stages. This work made it clear

that a difference in the salt tolerance of asparagus occurred not only among different germplasms but also at different growth stages of the same germplasm. There were 30, 19, and 18 germplasm accessions that were identified to be tolerant (including highly salt-tolerant and salt-tolerant germplasm accessions) at the germination stage, seedling stage, and adult stage, respectively. Among them, only two germplasm accessions (Ji08-2 and Jx1502) were simultaneously identified to be tolerant at all three growth stages and have great potential for further use in saline soils and germplasm improvement. Other germplasm accessions were tolerant only at one or two stages, indicating that these germplasm accessions could be used to improve germplasm through a careful choice of crossing parents with complementary salt tolerance at certain stages.

Data Availability

All data generated or analyzed during this study are included in this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Huimin Gao and Xuhong Zhang contributed equally to this work.

Acknowledgments

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Research Article

Efficacy and Mechanism of Ultrasound Combined with Slightly Acidic Electrolyzed Water for Inactivating *Escherichia coli*

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In the present study, the synergetic effect and mechanism of ultrasound (US) and slightly acidic electrolyzed water (SAEW) on the inactivation of *Escherichia coli* (*E. coli*) were evaluated. The results showed that US combined with SAEW treatment showed higher sanitizing efficacy for reducing *E. coli* than US and SAEW alone treatment. US and US combined with SAEW treatments resulted in smaller particle size of *E. coli* compared to the control and SAEW treatment. In addition, US combined with SAEW treatment induced the highest potassium leakage. However, the highest protein leakage was recorded in US treatment. Moreover, scanning and transmission electron microscopy analysis revealed that the greatest damage of the appearance and ultrastructure of *E. coli* was achieved after US combined with SAEW treatment. The synergetic effect was also confirmed by CLSM analysis. Fluorescence spectroscopy suggested that treatments of US, SAEW, and US combined with SAEW changed protein conformation of *E. coli*. Overall, the present study demonstrated that the sterilization mechanism of US combined with SAEW treatment was decreasing the particle size and disrupting the permeability of cell membrane and the cytoplasmic ultrastructure as well as changing protein conformation of *E. coli*.

1. Introduction

Escherichia coli (*E. coli*), a Gram-negative rod-shaped bacterium, is widely recognized as one of the major food-borne pathogens often from the consumption of contaminated foods [1, 2]. Therefore, a great variety of sanitization strategies, including heating, ultrasound, ultraviolet-C, sodium hypochlorite, sodium benzoate, potassium sorbate, chlorine dioxide, and so on, have been used to reduce the microbial population and prolong the shelf life of food products by the food industry [3–5]. Even so, a more effective and much safer sterilizing technology is crucial for environmental conservation and food preservation.

Ultrasound, as a promising non-thermal sterilization technique, can cause physical effects and/or chemical effects, thus decontaminating microorganisms from the surfaces of foods. Koda et al. [6] reported that inactivation of microorganisms by high frequency ultrasound was mainly dependent on the chemical effects. On the other hand, reactive oxygen species generated by cavitation also assisted sterilization during ultrasound processing [7]. In recent years, many literatures reported that sterilization effect of combined treatment of ultrasound with chemical sterilant was more effective than each used alone [8]. Our results showed that ultrasound produced micro-cracks in the bacterial cell membranes, allowing NaOCl into the cells, and thus

deactivated *E. coli* and reduced the usage amount of sodium hypochlorite [9]. In China, non- thermal sterilization has not been widely used in food industry. NaOCl also has been widely used in sterilization in our country, but its security is a concern.

The slightly acidic electrolyzed water (SAEW), as an alternative and novel method with great potential for sterilization, has recently received a great deal of attention for its sanitizing efficacy and environmentally friendly nature [10–13]. Hypochlorous acid (HClO) produced by SAEW can inactivate the microbial cell via improving the oxidation of lipids and protein compounds as well as resulting in a modification in the electron transfer mechanism of microorganisms [14]. The results of Naka et al. [12] indicated that, at the same chlorine concentration, SAEW is more effective than NaOCl in reducing or eliminating bacterial count. Recently, some studies investigated the synergistic action of electrolyzed water and ultrasound on microbial inactivation. José Cichoski et al. [14] reported that the combination of US and SAEW could effectively reduce some microorganisms, including enterobacteria, mesophilic bacteria, lactic acid bacteria, and psychrotrophic bacteria, and thus has a great potential to improve the prechilling of poultry carcasses. In addition, SAEW simultaneous with US treatment at 40°C for 3 min showed the synergic effects against *B. cereus* on potato [10]. Scanning and transmission electron microscopy analysis revealed that combined ultrasound-SAEW treatment resulted in greater damage of *Staphylococcus aureus* than either treatment alone [15]. However, little information is available on the effect of US combined with SAEW against *Escherichia coli* and the related mechanism.

Therefore, in this study, the effects of US combined with SAEW treatment on the antibacterial activity, membrane permeability, membrane integrity, cell morphology, intracellular organization, and protein conformation of *E. coli* were investigated to the antibacterial mechanism of US combined with SAEW.

2. Materials and Methods

2.1. Microbial Inoculation. *Escherichia coli* CICC 10899 was obtained from Chinese Center of Industrial Culture Collection). The stock cultures were transferred to 50 mL of nutrient broth (NB) (Hiabo Bio-Tech Co., Qingdao, China) and incubated at 37 °C in an air bath incubator with a reciprocal shaker for 16 h at 150 rpm. Following incubation, the microbial culture was sedimented by centrifugation at 6,000 × g for 10 min at 4°C. The supernatant was discarded and the bacterial cells were washed twice with 0.90% sterile saline solution and resuspended for following use. The final population in bacterial suspension of *E. coli* was approximately 10^6 CFU/mL.

2.2. SAEW Preparation. SAEW was produced by electrolysis with a continuous supply of dilute NaCl solution (0.9%) in a chamber without a membrane using an electrolysis device (Anywhere-320W, Rui Andre Environmental Equipment Co., Ltd., Beijing, China). SAEW pH and ORP values were determined immediately before sample treatment using a pH meter (Starter 300, Ohaus Co., USA) with pH and ORP electrodes. A colorimetric method with a digital chlorine test kit (RC-3F; Kasahara Chemical Instruments Corp., Saitama, Japan) was used to measure ACC. In this study, SAEW with a pH of 6.18, ORP of 827 mV, and available chlorine concentration (ACC) of 30 mg/L was used to sterilize.

2.3. Single or Combined Treatments with US and SAEW. US treatment was applied using a probe-style ultrasonic processor (Scientz-II D; Ningbo Scientz, Zhejiang, China). A total of 27 mL of cell suspension was added with 3 mL 0.90% sterile saline solution, and then the ultrasonic emitter was immersed 2.0 cm into the solution and ultrasonically treated for 10 min at a frequency of 20 kHz and 10 W/cm^3 energy density.

For SAEW treatment, the inoculated samples of 27 mL were mixed with 3 mL SAEW in a sterile glass beaker for 10 min. For combined treatment, after mixing 3 mL SAEW in 27 mL cell suspension, the US treatment followed immediately for 10 min under the above ultrasonic conditions. In this study, a thermostatic water bath (DC-1006, Safe Corporation, Ningbo, China) was used to maintain the temperature at 20°C in order to prevent a lethal thermal effect after US treatment.

2.4. Microbiological Analysis. Following treatments, microbiological analysis was conducted using plate counting method according to the previous procedure [9]. After incubation, microbial colonies were counted with an automated plate counter (ProtoCOL, Synoptics, Cambridge, UK). All analyses were conducted in duplicate with 3 replicates for each experiment.

2.5. Microbiological Size Measurement. The Malvern Mastersizer 2000 (Malvern Instruments, UK) was used to measure the particle size measurement of bacterial suspensions according to the method described by Gao et al. [16].

2.6. Measurement of the Intracellular Protein Leakage and Potassium Leakage. After treatments, the suspension was centrifuged at 10000 g for 10 min at 4°C. The protein content in the supernatant was according to the method of Bradford [17], using bovine serum albumin as standard.

The intracellular potassium leakage of the supernatant was determined using flame atomic absorption spectrophotometry (AAS) (AAnalyst 100, PerkinElmer Co., USA) as previously described by Tang et al. [18]. A linear relationship between potassium concentration and emission was obtained using potassium standards (analytical grade, Sigma-Aldrich, Poole, United Kingdom). The content of potassium was measured by AAS and calculated by the calibration.

2.7. Scanning and Transmission Electron Microscopy Analysis. Scanning electron microscopy (SEM) was used to observe the morphological changes in *E. coli* cells according to the method of Li et al. [15]. After centrifugation at 10000 g for 10 min at 4°C, the precipitates were collected and rinsed twice with 0.85% sterile saline solution. The samples were fixed with 2.5% glutaraldehyde for 24 h and then were washed three times with phosphate buffer solution (pH 7.2) and post-fixed with 1% osmium tetroxide for 2 h. Afterwards, the samples were dehydrated using a graded ethanol (30, 50, 70, 80, 90, 95, and 100%) series and transferred to a mixture of ethanol and tertiary butanol ($v:v=1:1$) for approximately 30 min. They were then placed in pure tertiary butanol. Finally, the dehydrated samples were coated with gold-palladium and observed using a JSM-7500F scanning electron microscope (JEOL Ltd., Tokyo, Japan).

For TEM analysis, the cells were infiltrated and embedded in Epon-812 after washing and dehydration. The prepared specimens were sliced to thin sections 70 nm and stained with uranyl acetate and alkaline lead citrate for 10 min. A HT7700 transmission electron microscope (Hitachi Ltd., Tokyo, Japan) was used to examine at 80 kV.

2.8. Confocal Laser Scanning Microscopy (CLSM) Analysis. To assess the damage to *E. coli* cell membranes following treatments with single and combination of US and SAEW, CLSM analysis was performed using the method of Kang et al. [7], with some modifications. Cell suspensions were incubated with dye buffer (LIVE/DEAD® BacLight Bacterial Viability Kits, L7012, ThermoFisher) and stained with 20 μM propidium iodide (PI) in the dark for 30 min at room temperature. The mixture was washed with 1 mL sterile HEPES buffer (pH 7.0) and then observed in a fluorescence microscope (TCS SP5, Leica, Germany).

2.9. Fluorescence Spectroscopy Experiments. All recordings of fluorescence, synchronous, and resonance light scattering spectra were carried out on a FL2700 luminescence spectrometer (Hitachi High-Technologies Corporation, Japan) with a quartz cell of 10 mm path length. The excitation and emission wavelength, excitation and emission bandwidths intervals, and scanning wavelength range were in accordance with our previous study [9].

2.10. Statistical Analyses. All experiments were performed in triplicate. Data were expressed as the mean ± standard deviation (SD). Significant differences were determined using one-way analysis of variance (ANOVA) and Duncan's multiple range tests (SPSS 19.0, SPSS Inc., Chicago, IL, USA) at $p < 0.05$.

3. Results and Discussion

3.1. Effect of US Combined with SAEW Treatment on the Microbicidal Efficiency and Particle Size Distribution. Microbial reduction values resulting from different treatments are shown in Figure 1(a). US treatment for 10 min decreased the number of *E. coli* by 0.48 log CFU/mL, indicating that the US treatment alone was not effective for inactivating *E. coli* CICC 10899. The similar phenomenon was also found in decontamination of *S. aureus* [15], *E. coli* ATCC 10536, and *V. Parahaemolyticus* KCTC 2471 [19]. The action of the US and SAEW is related to the species of bacteria. Park et al. [19] reported that SAEW treatment (chlorine 30 mg/L) showed the higher sterilizing effect for *V. Parahaemolyticus* KCTC but showed the lower sterilizing effect for *E. coli* ATCC 10536 than US treatment for 50 min. SAEW treatment also was not efficient in reducing *Staphylococcus* spp. [14]. However, in the present study, SAEW treatment led to 7.01-fold reduction of *E. coli* CICC 10899 when compared with US treatment for 10 min. This result indicated that SAEW was an effective disinfectant for inactivating *E. coli* CICC 10899. The different phenomena of US and SAEW treatments on *E. coli* ATCC 10536 and *E. coli* CICC 10899 might have resulted from the difference of ultrasonic time. It also needs further confirmation.

Cichoski et al. [14] reported that SAEW combined with the application of US at 25 kHz showed the synergistic effect on inactivating of enterobacteria, mesophilic bacteria, lactic acid bacteria, and psychrotrophic bacteria; however, SAEW combined with the application of US at 130 kHz had no synergistic effect on inactivating of all bacteria. Moreover, for *Staphylococcus* spp., SAEW combined with US treatment did not increase and even decreased the inactivation efficacy compared to single US and SAEW treatment [14]. These results suggested that the synergistic effect of US combined with SAEW was also related to ultrasonic frequency and microbial species. In addition, SAEW combined with US treatment also significantly improved the reductions in the populations of inoculated *S. aureus*, *B. cereus*, *E. coli* O157 : H7, and *A. fumigatus* in kashk compared to SAEW alone [20]. In the present study, US and SAEW treatment exhibited the synergistic effect in sterilization of *E. coli* and presented the highest reduction of *E. coli* with value of 3.64 log CFU/mL. The reason might be that cavitation resulted from US disrupted cell membrane, accelerating SAEW into microbial cells, and thus inactivated *E. coli* CICC 10899.

Monomodal was observed in control and SAEW treated samples (Figure 1(b)). However, a small volume distribution at 100–500 nm was found after US and US + SAEW treatments. The average Sauter diameters of control and SAEW treated samples were 1688 nm and 1436 nm, respectively. US and US + SAEW treatments caused significant reduction in particle size of *E. coli*, which was 718 nm and 762 nm, respectively. These results indicated that the small distribution and decrease in particle size of E. coli were mainly attributed to cavitation of ultrasound rather than SAEW. The similar phenomenon was also observed by combined treatment of US and NaOCl [9].

3.2. Effect of US Combined with SAEW Treatment on the Intracellular Protein and Potassium Leakage. The protein and potassium leakage can be used to investigate the damage of the cell membranes [21, 22]. As shown in Figure 2, all treatments led to the leakage of intracellular protein and

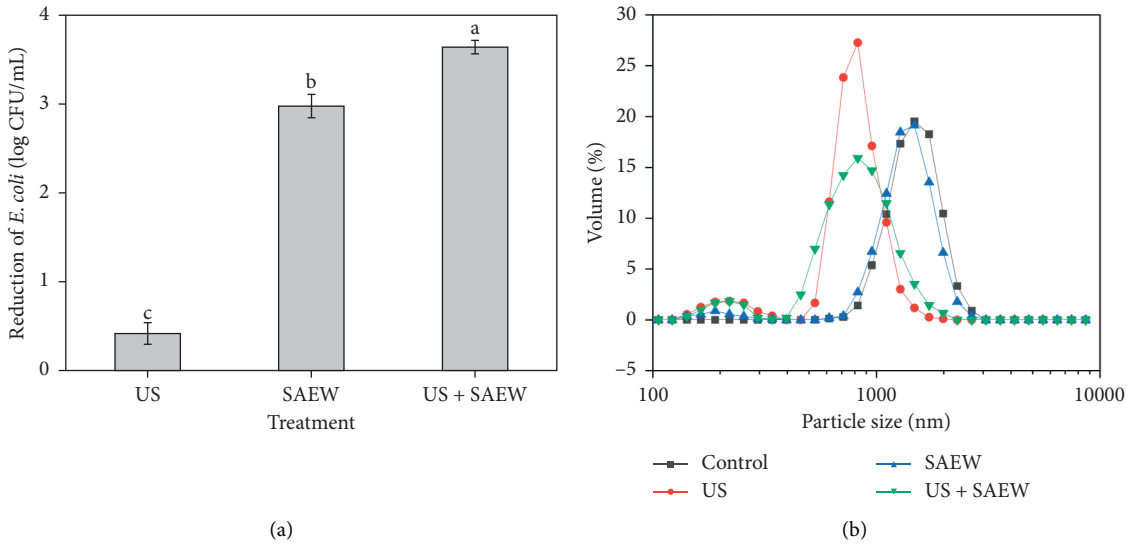

Figure 1: Reduction of *E. coli* (a) and particle size distribution (b) after the single and combined treatment of ultrasound and slightly acidic electrolyzed water. Values not sharing the same letter are significantly different at $p < 0.05$. US: ultrasound, SAEW: slightly acidic electrolyzed water, and US + SAEW: ultrasound combined with slightly acidic electrolyzed water.

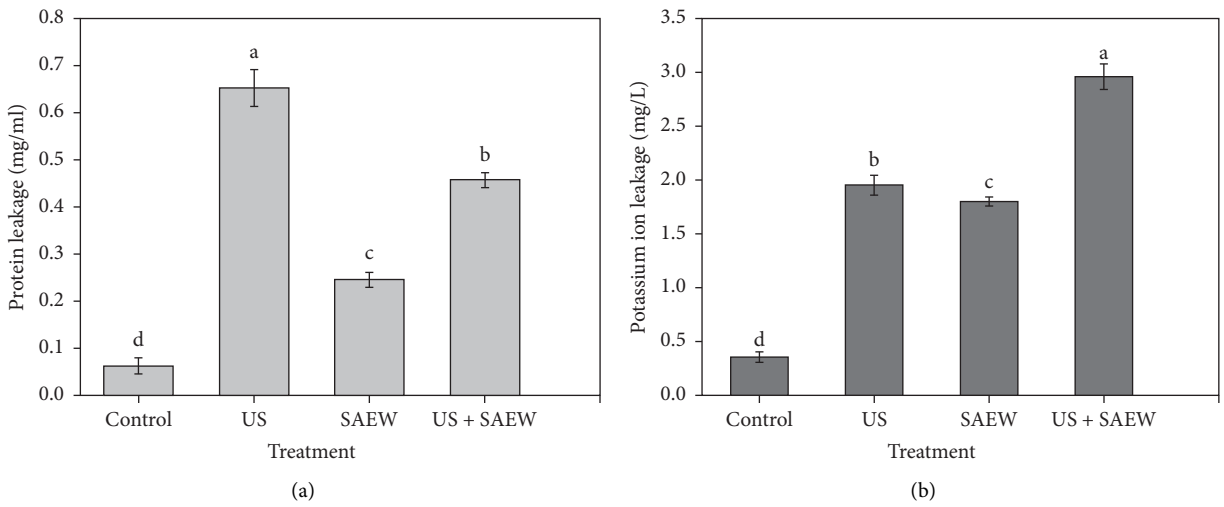

Figure 2: Protein leakage (a) and potassium ion leakage (b) of *E. coli* after the single and combined treatment of ultrasound and slightly acidic electrolyzed water. Values not sharing the same letter are significantly different at $p < 0.05$. US: ultrasound, SAEW: slightly acidic electrolyzed water, and US + SAEW: ultrasound combined with slightly acidic electrolyzed water.

potassium of *E. coli*. After US, SAEW, and US + SAEW treatments, the protein concentrations in suspension increased to 0.65, 0.25, and 0.46 mg/mL, respectively. On the other hand, US + SAEW treatment caused the highest potassium leakage, which was increased by 44.2% and 64.3% compared to US and SAEW treatment, respectively, indicating that US + SAEW treatment led to the most serious damage of the cell membranes of *E. coli*.

3.3. Morphological Changes Revealed by Electron Microscopy. Morphological changes of *E. coli* induced by US and SAEW were observed using SEM and TEM. SEM micrographs revealed that the cells of control samples maintained intact shapes, but with markedly deformation after SAEW treatment. *E. coli* was found markedly shrunk and cell wall was collapsed (Figure 3). This phenomenon could be due to the oxidative damage and the permeability of the cell membrane, thus resulting in the leakage of intracellular protein and potassium, and throwing the osmotic pressure out of balance [15, 23]. While US and US + SAEW treatments resulted in more serious damage compared to SAEW treatment, in addition to shrink and collapse, cell membrane and cell wall of *E. coli* were also damaged.

The TEM micrographs of *E. coli* after treatments with US, SAEW, and US + SAEW are shown in Figure 4. For control samples, cell wall and membrane of *E. coli*. were continuous and intact and well defined. SAEW treatment

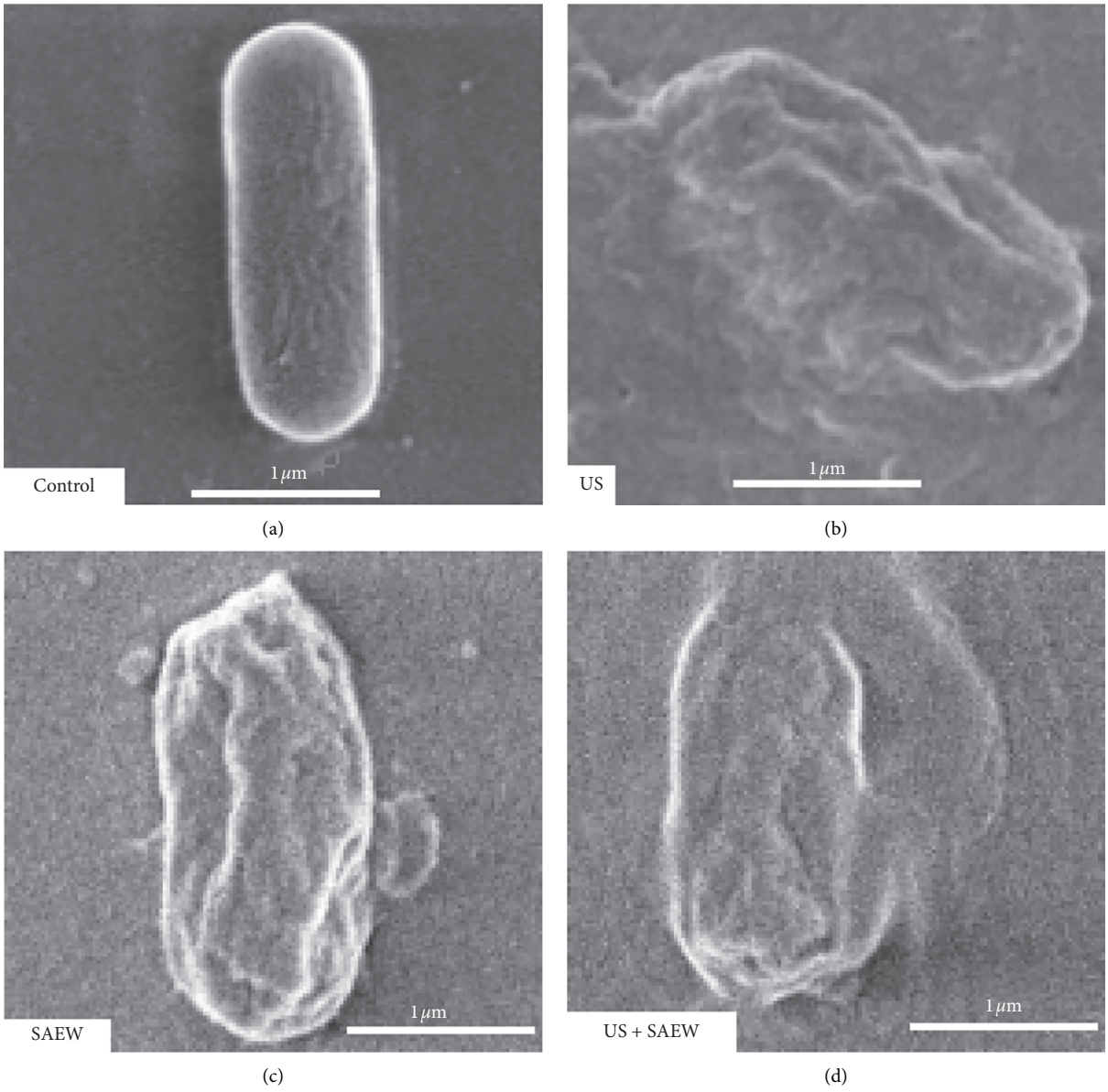

Figure 3: Scanning electron microscopy of *E. coli* after the single and combined treatment of ultrasound and slightly acidic electrolyzed water. Images were taken at magnification of ×20 K. US: ultrasound, SAEW: slightly acidic electrolyzed water, and US + SAEW: ultrasound combined with slightly acidic electrolyzed water.

resulted in slight damage of cell wall. However, after US treatment, cell wall and membrane of *E. coli* were damaged and indefinite. US + SAEW treatment led to the most serious damage; meanwhile, a plenty of intracellular compounds also leaked. US could rupture the chemical bonds between molecular components in cell membranes, thus accelerating SAEW into bacteria [15, 24]. Hence, the release of cell contents and disintegration of the cell wall was mainly attributed to the action of ultrasound on the damaged cells.

3.4. CLSM Analysis of E. coli Under US Combined with SAEW Treatment. The cells of *E. coli* with intact cell membranes were stained with fluorescent green, whereas cells with a damaged membrane were stained by red PI [25]. Figure 5 shows the live and dead population of *E. coli* after different treatments. A small fraction of dead cells was found after US and SAEW alone treatment. However, after treatment of US combined with SAEW, nearly all cells of *E. coli* showed red, indicating cytoplasmic membrane of most treated cells was injured, which was in accordance with results of microbial reduction values, SEM, and TEM analysis (Figures 1, 3, and 4).

3.5. The Effect of US Combined with SAEW Treatment on the Membrane Protein of E. coli. The conformational changes of proteins of *E. coli* can be successfully investigated by fluorescence spectroscopy [26, 27], since the intrinsic fluorescence of indol chromophores in Trp residues is sensitive to

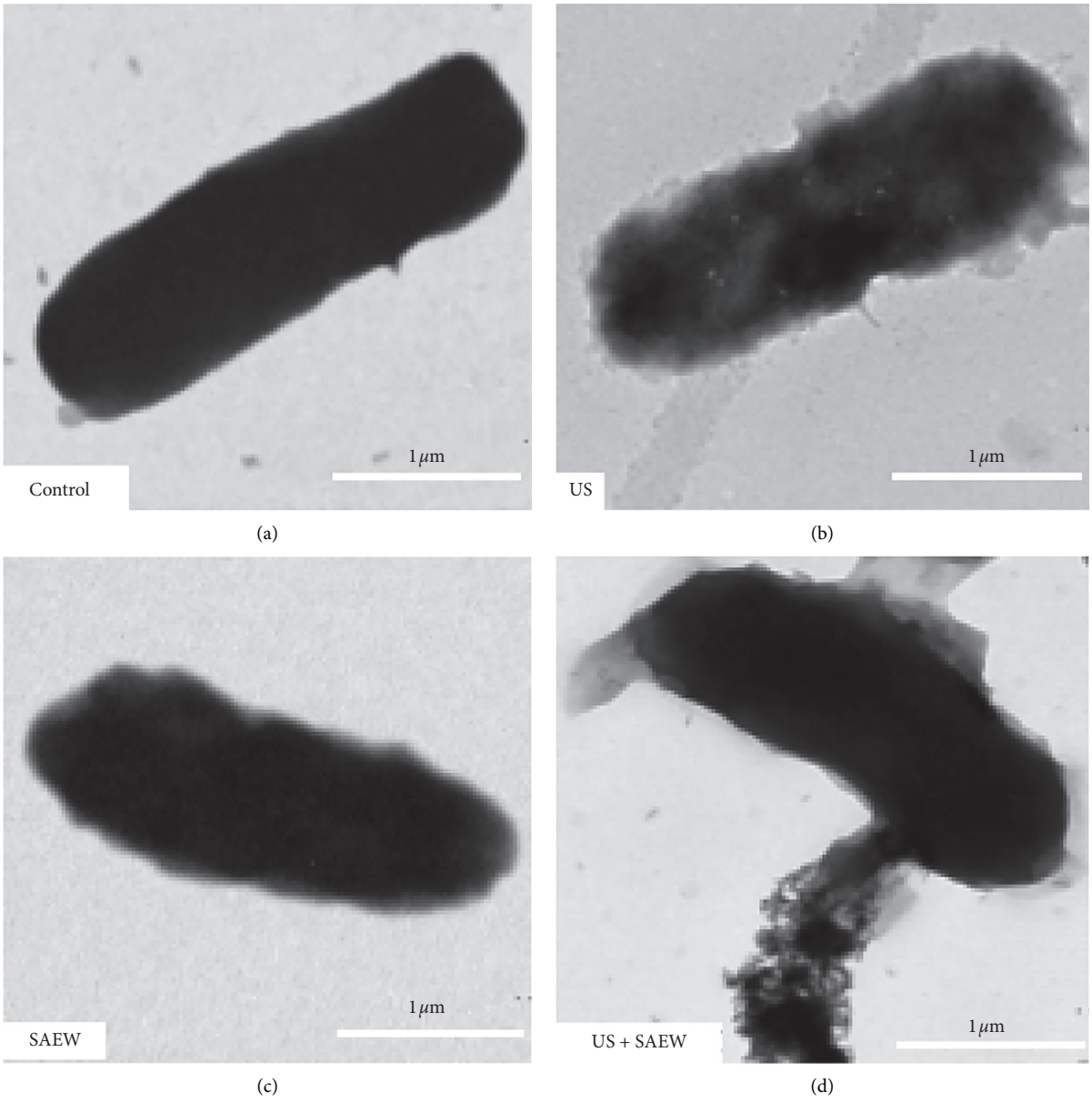

Figure 4: Transmission electron microscopy of *E. coli* after the single and combined treatment of ultrasound and slightly acidic electrolyzed water. Images were taken at magnification of ×5K. US: ultrasound, SAEW: slightly acidic electrolyzed water, and US + SAEW: ultrasound combined with slightly acidic electrolyzed water.

microenvironment particularities [27], which can provide information about the molecular microenvironment in the vicinity of the chromophores [28]. The spectrum of *E. coli* with different treatments is shown in Figure 6. Under the excitation wavelength of 278 nm, the maximum emission wavelength of protein in control and US treated samples was 332 nm. Nevertheless, the maximum emission wavelength of protein fluorescence was decreased after SAEW and US + SAEW treatments, which was 330 nm, which suggested that SAEW could result in a blue shift of the maximum emission peak. In addition, it has been reported that US treatment could increase the fluorescence intensity of *E. coli*, thus improving the hydrophobicity of *E. coli* protein [29]. In the present study, the similar result was also observed after US treatment. Nevertheless, SAEW and US + SAEW treatments reduced fluorescence intensity. The reduction of fluorescence intensity and the blue shift of the maximum emission peak implied the Trp residues transfer to a polar environment after SAEW and US + SAEW treatments [30].

The microenvironment of amino acid residues of biomolecules can be evaluated by synchronous fluorescence spectroscopy. The maximum emission wavelength of Tyr ($\lambda = 15$ nm) was 283 nm under control and US treatments. However, it was decreased to 281.5 nm after SAEW and US + SAEW treatments (Figure 7(a)). The similar change of Trp ($\lambda = 60$ nm) was also observed. After SAEW and US + SAEW treatments, the maximum emission wavelength of Trp shifted from 278 nm to 276 nm

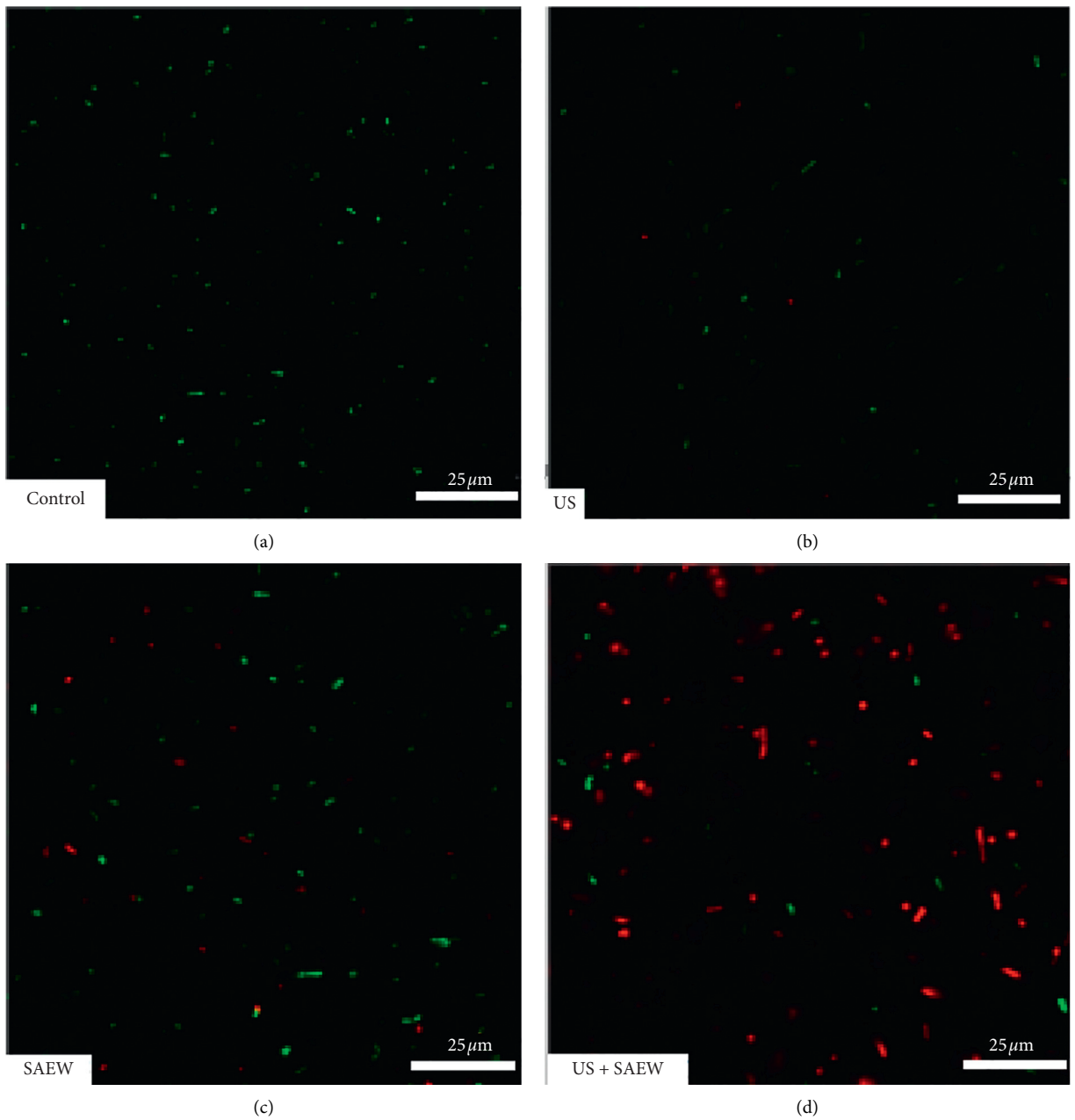

Figure 5: Confocal laser scanning micrographs of *E. coli* after the single and combined treatment of ultrasound and slightly acidic electrolyzed water. Images were taken at magnification of ×630. US: ultrasound, SAEW: slightly acidic electrolyzed water, and US + SAEW: ultrasound plus slightly acidic electrolyzed water.

(Figure 7(b)). These results indicated that the polarity around the Tyr residues and Trp residues of *E. coli* decreased and the hydrophobicity increased after SAEW and US + SAEW treatment [27]. Additionally, the enhancement of fluorescence intensity was detected in US-treated *E. coli*, whereas the decrease of fluorescence intensity was found in SAEW and US + SAEW treated samples. However, there was no significant difference between SAEW and US + SAEW treatment. The identical changes in resonance intensity of *E. coli* were also observed after US, SAEW, and US + SAEW treatments compared to the control (Figure 8). In the present study, US treatment enhanced the resonance

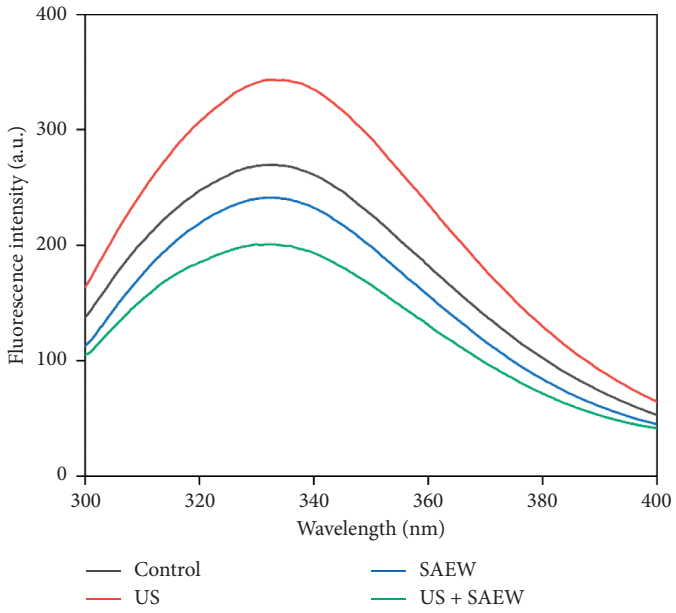

Figure 6: Endogenous fluorescence spectrometry of *E. coli* after the single and combined treatment of ultrasound and slightly acidic electrolyzed water. US: ultrasound, SAEW: slightly acidic electrolyzed water, and US + SAEW: ultrasound plus slightly acidic electrolyzed water.

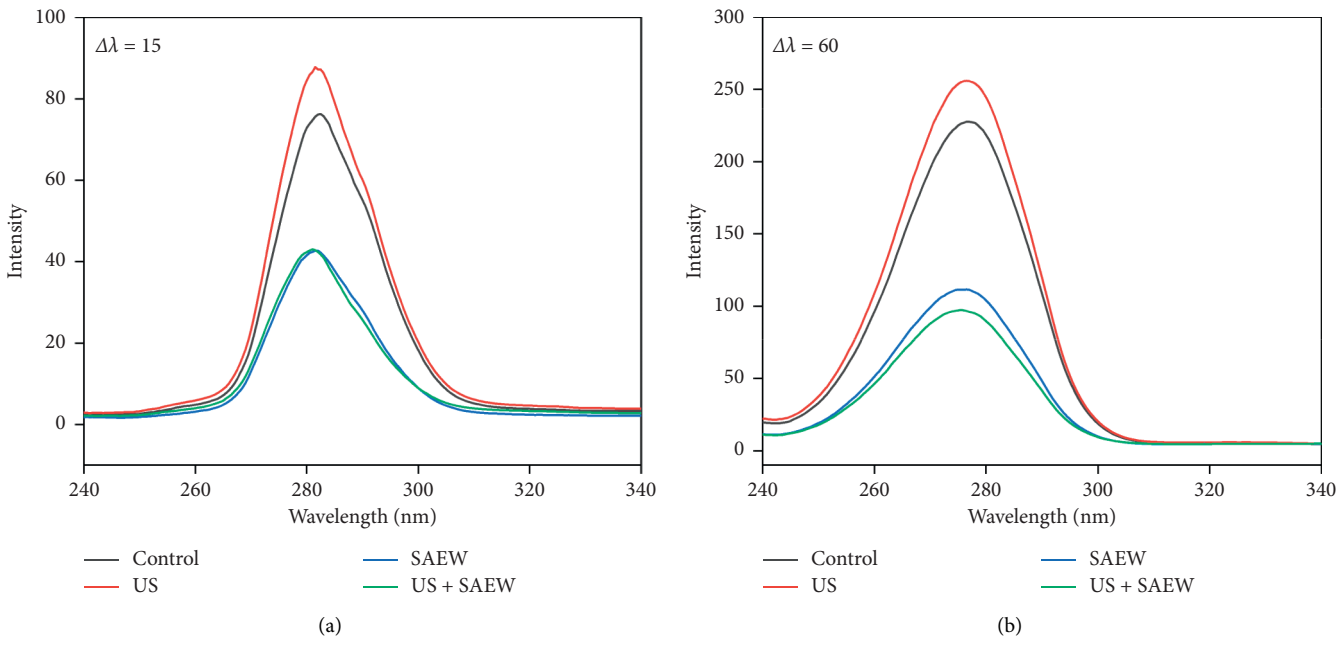

Figure 7: Synchronous fluorescence spectrometry of *E. coli* after the single and combined treatment of ultrasound and slightly acidic electrolyzed water. US: ultrasound, SAEW: slightly acidic electrolyzed water, and US + SAEW: ultrasound plus slightly acidic electrolyzed water.

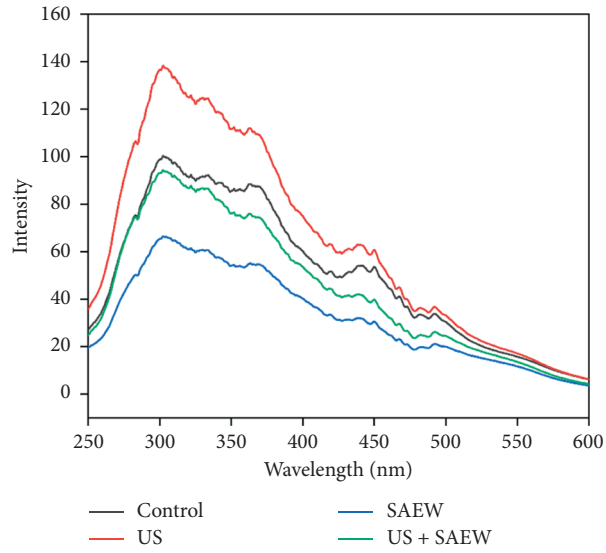

Figure 8: Resonance light scattering fluorescence spectrometry of *E. coli* after the single and combined treatment of ultrasound and slightly acidic electrolyzed water. US: ultrasound, SAEW: slightly acidic electrolyzed water, and US + SAEW: ultrasound plus slightly acidic electrolyzed water.

intensity of *E. coli,* indicating proteins of *E. coli* assembled. On the other hand, the decrease in resonance intensity of *E. coli* after SAEW treatment might be due to breakdown of protein [31].

4. Conclusion

US combined with SAEW showed the best sterilizing efficacy, with a significant reduction of survival cells compared with single US and SAEW treatments. Protein and potassium leakage tests as well as the morphologies of *E. coli* and CLSM analysis showed visible change under the combined treatment of US and SAEW. Fluorescence spectroscopy analysis found US and SAEW treatment changed membrane integrity and protein conformation of *E. coli*. In short, US treatment disrupted the cell membrane of *E. coli* and facilitated SAEW into the cells, thus improving the sterilizing effect. These results showed that US in combination with SAEW, as an environment friendly and safety sterilizing technology, could be developed as an effective and practical sterilizing method for food industry.

Data Availability

The data used to support the findings of this study are included within the article.

Disclosure

Xuecong Zhang and Liping Guo are the co-first authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Liping Guo and Xuecong Zhang contributed equally to this work.

Acknowledgments

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Research Article

Synergistic Effect of Heating pH and Transglutaminase on the Gelation Kinetics and Texture of Yak Skim Milk Gels

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Textural defects (including syneresis and poor consistency) often occur in yogurt gels produced from yak milk. In this research, the synergistic effects of transglutaminase (TGase) and heating pH on the textural properties of acidified yak skim milk gels, as well as the related mechanism of action, were investigated. The pH values of yak skim milk were adjusted to 6.3, 6.7, and 7.1, respectively. The samples were heated at 80°C for 30 min and then cooled to 42°C. After treatment with different contents of TGase (0, 3, and 10 U TGase per gram proteins), the samples were acidified with glucono-delta-lactone. For a given TGase content, the final storage modulus (G′) of gels was positively related to the heating pH, whereas the opposite trend was observed for the gelation time. This effect was obvious between pH 6.3 and 6.7. At a definite heating pH value, the final G′ of the gels was highest at 3 U TGase per gram proteins. The highest water holding capacity and firmness value were observed in gels prepared using pH 7.1 and 3 U TGase per gram proteins. In the samples treated with 3 U TGase per gram proteins (preheating pH 7.1), more rigid network structures were seen in the gel than 0 or 10 U TGase per gram proteins. Therefore, adjusting the heating pH values and TGase contents is an effective way of improving the textural properties of yak milk gels.

1. Introduction

The yak (*Bos grunniens*) is a unique animal in the Qinghai-Tibet Plateau area which has an altitude of over 3000 m in western China [1, 2]. Yak milk-based products have received considerable attention worldwide. Compared with cow milk, yak milk is richer in nutrients, easier to digest, and has lower allergenicity [2–4]. Set-type yogurt produced from yak milk has become one of the most popular dairy products in western China due to its unique sensory and flavor characteristics [5]. However, during storage and long-distance transport, textural defects often occur in set-type yogurt produced from yak milk. These defects can be reflected in the syneresis and poor consistency of yogurt, which greatly reduce its acceptability. Therefore, it is important to improve the quality of set-type yogurt produced from yak milk.

The syneresis and consistency of set-type yogurt gels are closely related to the structural characteristics of gel networks [6–8]. The building blocks of the yogurt gel networks are milk proteins, including whey proteins and caseins [9]. In native milk, the phosphorylated serines in caseins are crosslinked by calcium phosphate, forming colloidal particles of approximately 200 nm in diameter [10, 11]. These colloids are named casein micelles. During acidification, caseins in the micelles are gradually liberated and then are rearranged into a weak network structure through noncovalent bonds at pH 4.6 [12, 13]. To better improve the structural characteristics of gel networks, the introduction of covalent bonds into the yogurt gel network has been proved to be useful [14]. At present, two methods have been adapted to introduce covalent bonds: heat and transglutaminase (TGase) treatment of milk proteins.

Heat treatment before acidification has a significant impact on the gel properties [15, 16]. After heat treatment, denaturation of whey proteins occurs [17, 18]. It has been shown that by adjusting the pH of the milk before heat treatment, different amounts of denatured whey proteins are associated with the casein micelles [19]. When the milk pH before heating is lower than 6.7, most of the denatured whey proteins can interact with casein micelles. In this case, the denatured whey proteins are mainly in the micellar phase [20]. However, when the milk pH before heating is higher

than 6.7, most of the denatured whey proteins interact covalently with the serum phase κ-casein. In this case, most of the denatured whey proteins exist in the soluble phase. It has been shown that both the final storage modulus (G') and loss modulus (G'') are positively related to preheating pH in the range 6.2–6.9 [21]. TGase has been extensively used to produce covalent bonds among proteins. TGase catalyzes crosslinks between γ-carboxyl groups and ε-amino groups in different protein molecules. Following treatment with TGase, the syneresis and poor consistency of yogurt can be greatly improved [22].

Heat treatment and TGase might have a synergistic effect on acid gels. Native whey proteins are difficult to catalyze by TGase due to their folding structure. After heat treatment, the whey proteins are denatured and become unfolded. This results in the complete unfolding of all amino acid residues, which might easily be crosslinked by TGase treatment. Numerous studies have been carried out on the use of TGase and heat treatment on the gelation kinetics and texture of acid milk gels. However, few studies have been carried out on the effect of TGase and heating pH of yak skim milk in terms of the gelation kinetics and texture of acid-induced milk gels.

In the present study, in order to improve the syneresis and poor consistency of acidified yak milk gels, we investigated the effects of TGase and heating pH of yak skim milk on the gelation kinetics and texture of acid-induced milk gels.

2. Materials and Methods

2.1. Materials. Yak milk was collected from Pali grassland in northwest China. The altitude in this area is 4300 m. The milk contained 0.85% (w/v) ash, 18.42% dry matter, 5.91% protein, 7.22% fat, and 5.04% lactose, respectively. To inhibit the activity of plasmin and microbial growth, 0.03% trypsin inhibitor and 0.03% sodium azide were added to the samples [23]. The trypsin inhibitor, sodium azide, and glucono-delta-lactone (GDL) were purchased from Sigma Aldrich (St. Louis, USA). TGase was purchased from Kelong Biotechnology Co., Ltd. (Jiangmen, Guangdong). The enzyme activity was 200 U/g. The rennet Stamix 1150 was purchased from Chr. Hansen (Beijing, China).

2.2. Sample Preparation and Characterization. The experimental design in this study is shown in Figure 1. The sample treatment procedures and characterization methods are present in the following sections.

2.2.1. Preparation and Characterization of Heat-Treated Yak Skim Milk. Yak milk was defatted by centrifugation (5-5N, Hunan Hengnuo Instrument Equipment Co., Ltd., Changsha, China) at 5000 g for 20 min at 25°C, followed by adjustment of the pH to 6.3, 6.7, or 7.1 with 2 mol/L HCl or NaOH. The samples were heated at 80°C for 30 min and then cooled to 42°C in a waterbath. The rennet and acid precipitation method in combination with reversed phase high performance liquid chromatography (RP-HPLC) was used to evaluate the distribution of whey proteins, as reported previously [19, 24]. In this method, the heat-treated samples were first acidified with 2 mol/L HCl. Thirty minutes later, the dispersion was centrifuged at 5000g for 30 min at 25°C. The concentration of β-lactoglobulin in the supernatant was analyzed by RP-HPLC (Agilent 1100 Series, CA, USA) with a C4 column (4.6 × 250 mm, 300 Å, Phenomenex, CA, USA). Before the RP-HPLC determination, 0.3 mL of the supernatants was first added to 2.5 mL of reducing agents. The reducing agents contained 0.1 mol/L Tris, 6 mol/L urea, and β-mercaptoethanol (0.4%, w/v), followed by adjusted to pH 7.0 with 2 mol/L HCl. Renneting of the heat-treated milk samples was carried out based on the reported literature [25]. Following the addition of rennet for 30 min, the mixture was centrifuged at 5000g for 30 min at 25°C. The concentration of β-lactoglobulin in the supernatant was analyzed by RP-HPLC. Before the RP-HPLC determination, 0.3 mL of the supernatants was first added to 2.5 mL of reducing agents. Solvent A was distilled water (with 0.1% (v/v) trifluoroacetic acid); solvent B was acetonitrile (with 0.1% trifluoroacetic acid). A linear gradient from 35.0% to 55.0% of solvent B over 60 min was used.

Yak milk (300 μL was added to 2.7 mL of reducing agent solution (including 0.1 mol/L Tris, 6 mol/L urea, β-mercaptoethanol (0.4%, w/v), adjusted to pH 7.0 with 2 mol/L HCl). Solvent A was distilled water (with 0.1% (v/v) trifluoroacetic acid); solvent B was acetonitrile (with 0.1% trifluoroacetic acid). A linear gradient from 35.0% to 55.0% of solvent B over 60 min was used.

2.2.2. Preparation and Characterization of TGase-Treated Samples. After heat treatment, TGase was added to the samples of 0, 3, and 8 U/g milk proteins. The samples were magnetically stirred for 5 min and incubated at 42°C in a waterbath for 40 min. The TGase in the samples was inactivated at 75°C for 10 min. The pH values in the samples were readjusted to 6.7 before GDL addition, with 2 mol/L HCl or NaOH.

The degree of covalent crosslinking in the heat- and TGase-treated samples was measured by a spectrometer, as reported previously [26]. Briefly, 10 mL of the heat- and TGase-treated samples were freeze-dried (FD-1A-50, Hangzhou Chuanyi Experimental Instrument Co. Ltd., Hangzhou, China) to constant weight for 48 h. One milliliter of 4% $NaHCO_3$ solution and 1 mL of 0.2% (w/v) trinitrobenzene-sulfonic acid solution were added to 10 mg dried samples. After incubation at 42°C for 4 h, the samples were digested with 3 mL 6 mol/L at 65°C for 2 h. Following dilution with distilled water to 15 mL, the samples were added to cuvettes for absorbance determination (U-2900 spectrometer, Hitachi, Ltd., Tokyo, Japan). The crosslinking degree (DE) was calculated as follows:

$$\mathrm{DE}\,(\%) = \left(1 - \frac{A_S/m_s}{A_{NS}/m_{Ns}}\right) \times 100\%, \quad (1)$$

where A_S and A_{NS} is the absorbance of TGase-treated and non-TGase-treated samples, respectively. m_s and m_{Ns} is the mass of TGase-treated and non-TGase-treated samples, respectively.

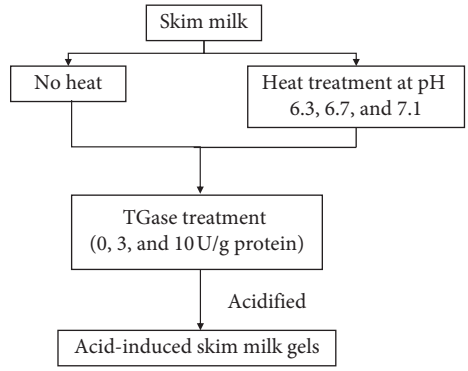


Figure 1: The experimental design of the study.

2.2.3. Preparation and Characterization of Acidified Milk Gels. The yak skim milk was acidified with 1.35% GDL at 37°C for 6 h. The final pH value of all the acidified skim milk gels was 4.4. The samples were stored at 4°C before use. The gelation kinetics of the samples were determined using a rheometer (AR 2000, TA Instruments, USA), equipped with a concentric cylinder. GDL was directly added to the TGase-treated samples to 1.35%. The samples were then added to the concentric cylinder after the addition of GDL for 2 min and were oscillated at 0.1 Hz and 37°C with a strain of 1%. Gelation time was defined as the point when G′ of the samples was more than 1 Pa.

The firmness of the gels was measured by a texture analyzer (TMS-Pro, Sterling, USA). The gels were stored at 25°C for 120 min before texture measurement. A probe (with a diameter of 25 mm) was vertically moved into the samples to 15 mm at 25 mm/min for penetration measurement.

The water holding capacity (WHC) of the acidified milk gels was measured according to a modified method [24]. Eight millimeters of the TGase-treated samples were acidified for 4 h at 37°C in 10 mL tubes. After acidification, the samples were centrifuged at 800g at 25°C for 10 min. The WHC was calculated as the percentage of the gel weight at the bottom of the centrifuge tubes compared to the initial weight.

The structural characteristics of the gels were determined by cryoscanning electron microscopy (S-3000N, Hitachi Co., Tokyo, Japan). Samples were added into the specimen holder and sublimated at −90°C for 25 min before observation (Quorum PP 3000T, UK).

2.3. Statistical Analysis. Independent experiments were repeated three times. Analysis of variance (ANOVA) was used to determine significant differences ($P < 0.05$). Statistical analyses were carried out using IBM SPSS 21 for Windows 10.0. Duncan's multiple range tests for differences were performed.

3. Results and Discussion

3.1. Extent of Denaturation of Whey Proteins and Their Distribution. The distribution of whey proteins can be evaluated with the rennet and acid precipitation method. This is because the casein micelles can be precipitated in the presence of rennet, while both casein micelles and denatured whey proteins can be precipitated at pH 4.6. The distribution of whey proteins including native whey proteins, whey protein aggregates in the soluble phase, and whey proteins associated with the casein micelles were determined, respectively. Table 1 gives the distribution of the highest content of whey protein in yak milk, β-lactoglobulin [27], after different heat treatments. No statistical differences were observed in the degree of undenatured β-lactoglobulin. This indicated that the degree of denaturation was little influenced by the heating pH of yak skim milk. However, the degree of denatured β-lactoglobulin present in the soluble or micellar phase was closely related to the heating pH of yak skim milk. When heated at pH 6.3, only 18.5% of β-lactoglobulin was present in the soluble phase; this value increased to 48.2% when the heating pH was 7.1.

3.2. Gelation Kinetics. The evolution of the storage modulus (G′) after the addition of GDL to skim milk samples treated with different preheating pH values and TGase contents is presented in Figure 2. G′ and gelation time are summarized in Table 2. Preheating pH had a significant impact on the final G′ and gelation time (except for the case for 0 U/g protein). For a given TGase content, the final G′ was positively related to the preheating pH as the preheating pH increased from 6.3 to 7.1, whereas the opposite trend was observed for the gelation time. This effect was obvious between pH 6.3 and 6.7 but was smaller between preheating pH 6.7 and 7.1. These results are generally consistent with previously reported results [26].

It was observed at a definite preheating pH value that the TGase content had a significant impact on the final G′ and gelation time. Surprisingly, when the preheating pH was 6.7, the maximum G′ value was observed in the sample treated with 3 U TGase per gram proteins, followed by 10 U TGase per gram proteins. Similar results were also observed when the preheating pH was 6.3 or 7.1. In summary, at a definite pH value, the maximum G′ value was observed in the 3 U TGase per gram proteins sample, whereas the opposite trend was found for the gelation time. During acidification, caseins in the micelles are gradually liberated and rearranged into a weak network structure through noncovalent bonds at pH 4.6. Although the introduction of covalent bonds is a useful

Table 1: The denaturation extent of β-lactoglobulin and its distribution.

Heating pH	Native (%)	Soluble phase (%)	Micellar phase (%)
pH 7.1	7.3 ± 1.8^{a}	48.2 ± 3.7^{c}	45.0 ± 5.1^{a}
pH 6.7	8.2 ± 1.6^{a}	33.2 ± 4.0^{b}	56.8 ± 7.6^{b}
pH 6.3	7.1 ± 0.6^{a}	18.5 ± 4.2^{a}	76.2 ± 5.9^{c}

Native, soluble phase, and micellar phase are represented by native whey proteins, whey protein aggregates in the soluble phase, and whey proteins associated with casein micelles, respectively. Values are mean ± standard deviation; means with different superscript letters within the same column are significantly different ($P < 0.05$).

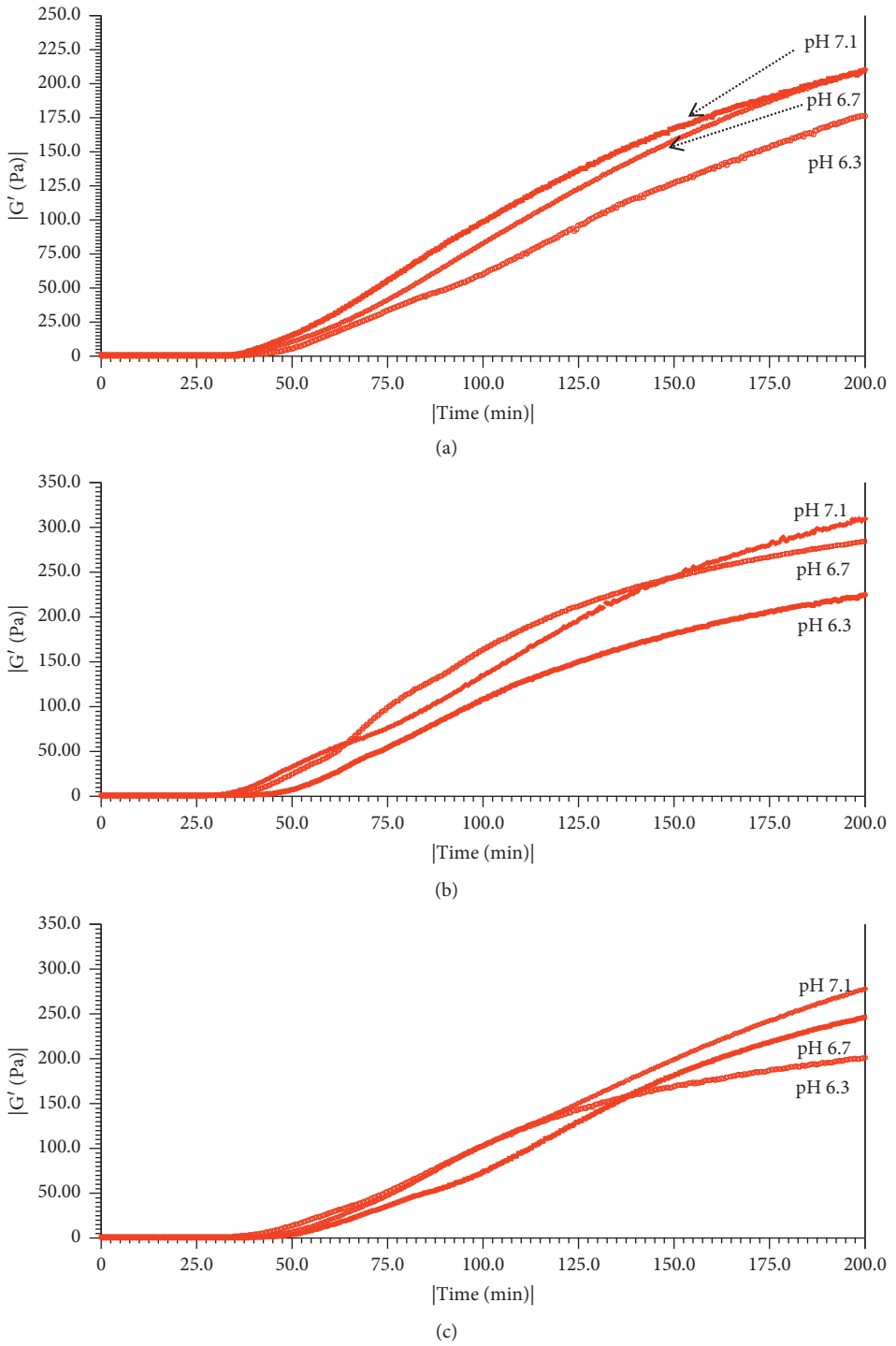


Figure 2: Effect of transglutaminase and preheating pH on the storage modulus of acid-induced gels prepared from 0 (a), 3 (b), and 10 (c) U transglutaminase per gram proteins.

Table 2: Effect of preheating pH and TGase contents on the G′ and gelation time of acid-induced yak milk gels.

	G′ (Pa)			Gelation (min)		
	0 U/g protein	3 U/g protein	10 U/g protein	0 U/g protein	3 U/g protein	10 U/g protein
Heating pH 7.1	221.3 ± 18.2^{a}	320.5 ± 15.1^{c}	275.7 ± 16.4^{b}	36.5 ± 2.5^{a}	27.5 ± 3.2^{a}	39.5 ± 2.2^{ab}
Heating pH 6.7	217.9 ± 22.4^{a}	281.6 ± 22.0^{b}	259.4 ± 20.1^{b}	37.5 ± 1.8^{a}	34.9 ± 2.8^{b}	42.5 ± 4.0^{b}
Heating pH 6.3	188.1 ± 17.0^{a}	230.2 ± 14.6^{a}	206.9 ± 18.5^{a}	41.2 ± 3.7^{b}	42.7 ± 1.4^{c}	36.2 ± 2.8^{a}

Values are mean ± standard deviation; means with different superscript letters within the same column are significantly different ($P < 0.05$).

Table 3: Effects of preheating pH and TGase contents on the firmness of acid-induced yak milk gels.

	Firmness (*N*)		
	0 U/g protein	3 U/g protein	10 U/g protein
Heating pH 7.1	2.9 ± 0.4^{b}	4.0 ± 0.5^{b}	3.5 ± 0.4^{a}
Heating pH 6.7	3.1 ± 0.6^{b}	4.5 ± 0.4^{b}	3.2 ± 0.6^{a}
Heating pH 6.3	2.2 ± 0.3^{a}	3.2 ± 0.4^{a}	3.6 ± 0.4^{a}

Values are mean ± standard deviation; means with different superscript letters within the same column are significantly different ($P < 0.05$).

Table 4: Effects of preheating pH and TGase contents on the WHC of acid-induced yak milk gels.

	WHC (%)		
	0 U/g protein	3 U/g protein	10 U/g protein
Heating pH 7.1	80.4 ± 2.4^{b}	91.3 ± 5.1^{b}	83.7 ± 6.7^{a}
Heating pH 6.7	83.4 ± 3.5^{b}	93.4 ± 6.2^{b}	88.5 ± 5.1^{a}
Heating pH 6.3	75.4 ± 4.7^{a}	85.5 ± 4.8^{a}	85.8 ± 5.8^{a}

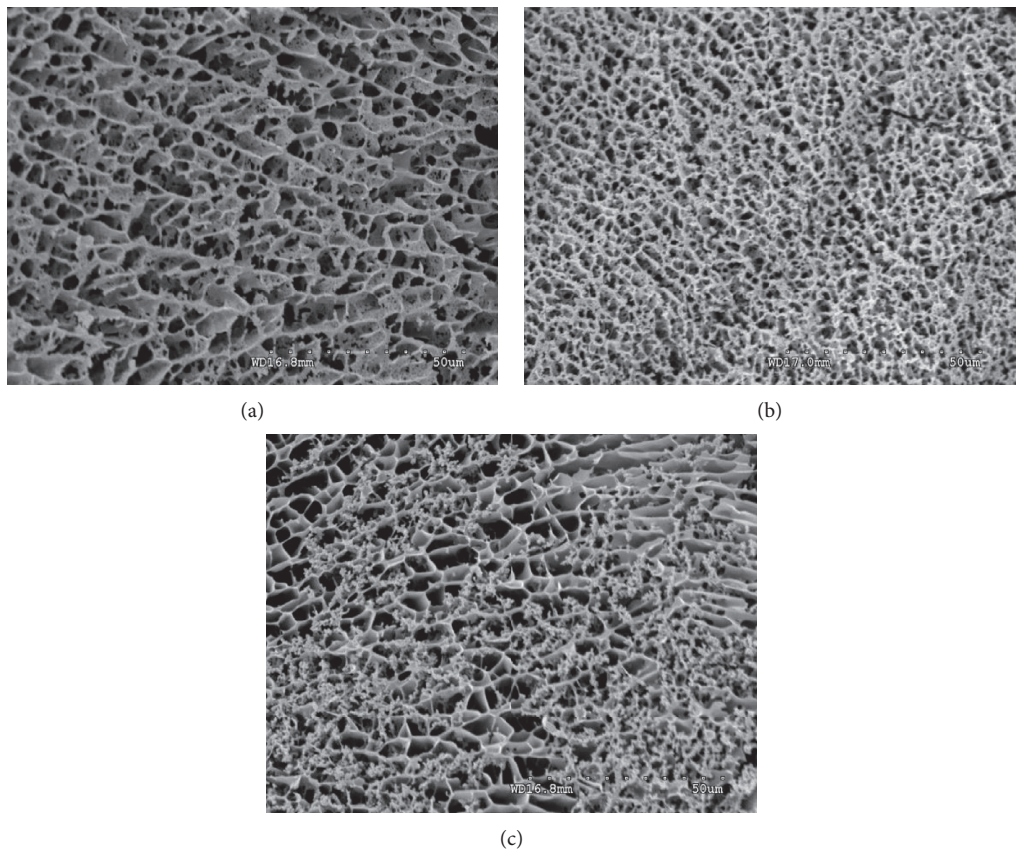
Values are mean ± standard deviation; means with different superscript letters within the same column are significantly different ($P < 0.05$).

method for improving the textural properties of the yogurt gel network, excess crosslinking of caseins in micelles can inhibit the adequate rearrangement of caseins during gelation [22]. This might explain why the samples treated with 3U TGase per gram proteins were higher than the samples treated with 10 U TGase per gram proteins.

3.3. Water Holding Capacity and Firmness. The firmness of the gels is given in Table 3. For the samples treated with 0 or 3 U TGase per gram proteins, firmness of the final gels was higher at preheating pH values of 6.7 or 7.1 than at 6.3, whereas no significant difference was observed between pH 6.7 and 7.1. However, when the samples were treated with 10 U TGase per gram proteins, no significant differences were observed among different preheating pH values. When the preheating pH values were definite, the TGase contents also had a significant impact on gel firmness. When the heating pH was 6.7, the maximum firmness value was seen in the sample treated with 3 U TGase per gram proteins. Similar results were also observed when the preheating pH was 7.1. At a definite pH value, maximum firmness was observed at 3 U TGase per gram proteins.

The WHC of the gels prepared with different preheating pH and TGase contents is given in Table 4. When the preheating pH values were definite, the WHC of samples treated with 0 and 10 U TGase per gram proteins showed no significant difference but were lower than the samples treated with 3 U TGase per gram proteins. When the TGase contents were 0 and 3 U TGase per gram proteins, the WHC of the samples treated with preheating pH 6.7 or 7.1 was higher than that with preheating pH 6.3, while the samples treated with pH 6.7 and 7.1 showed no significant difference. For the samples treated with 10 U TGase per gram proteins, the samples with different pH treatments showed no significant differences.

3.4. Microstructure and Crosslinking Degree. The microstructure of acid-induced yak milk gels prepared from yak skim milk with preheating at pH 7.1 and different TGase contents are shown in Figure 3. In the samples treated with 3 U TGase per gram proteins, more rigid network structures were observed in the gel than 0 or 10 U TGase per gram proteins. This was consistent with the results of the textural properties. The crosslinking degrees of proteins (catalyzed by TGase) in milk samples heated at pH 6.3, 6.7, and 7.1 were $15.3 \pm 3.7\%$, $33.2 \pm 5.2\%$, and $38.1 \pm 4.7\%$, respectively. This indicated that the number of covalent bonds introduced was positively related to the heating pH values. As mentioned above, more denatured whey proteins were present in the micellar phase when the heating pH of yak skim milk was lower, together with the fact that casein cannot be dissociated from the micelles [28]. This indicated that the attached whey proteins with casein micelles might hinder crosslinking between caseins, and thus, the crosslinking sites between proteins are limited. On the contrary, when the heating pH of yak skim milk was higher, dissociation of micelles may occur, and more sites catalyzed by TGase might be exposed, together with the fact that denatured whey proteins in the soluble phase cannot attach onto the surface of casein micelles which might hinder crosslinking. Therefore, the crosslinking degree of milk proteins (catalyzed by TGase) was higher when the yak skim milk pH value was high.



(a) (b) (c)

Figure 3: The microstructure of acid-induced yak milk gels prepared from skim yak milk with preheating pH 7.1 and 0 (a), 3 (b), and 10 (c) U TGase per gram proteins.

4. Conclusions

In this study, we investigated the effects of TGase and heat treatment of yak skim milk on the gelation kinetics and texture of acid-induced milk gels, as well as the related mechanism of action. For a given TGase content, the final G′ was positively related to the preheating pH when the preheating pH increased from 6.3 to 7.1, whereas the opposite trend was observed for the gelation time. The yak skim milk treated with a preheating pH value of 7.1 and 3 U TGase per gram proteins demonstrated the highest WHC and firmness and more rigid network structures.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Review Article

Molecular Characteristics, Synthase, and Food Application of Cereal β-Glucan

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Cereal β-glucan is a type of valuable dietary fiber that mainly exists in the aleurone, subaleurone, and endosperm of some cereal grains. β-Glucan is acknowledged as a functional food ingredient owing to its multiple health benefits, including the prevention of diabetes, reduction in the incidence of cardiovascular disease, antitumor effects, antioxidant activities, and immunostimulation. It is well documented that cellulose synthase-like *CslF/H/J* genes encode synthases responsible for β-glucan biosynthesis in cereal grains. Recently, β-glucan has been widely applied as an emulsion stabilizer, thickening agent, fat substitute, and bioactive ingredient in the food industry due to its water solubility, viscosity, gelation property, and health benefits. Therefore, the present paper aims to review the molecular characteristics, synthase gene family, and food application of cereal β-glucan in recent years.

1. Introduction

β-Glucan (also known as mixed linkage glucan) is a long-chain polysaccharide consisting of D-glucose monomers linked via β-glycosidic bonds. It is a type of valuable dietary fiber that is widely present in cereals, mushrooms, seaweeds, yeast, and some bacteria [1, 2]. The glycosidic linkages in cereal β-glucan are a combination of β-1, 3 and β-1, 4 glycosidic linkages; hence, it is called (1, 3; 1, 4)-β-glucan. The glycosidic linkages in β-glucan from other sources are a combination of β-1, 3 and β-1, 6 glycosidic linkages; thus, it is called (1, 3; 1, 6)-β-glucan [3]. On the basis of the above molecular structure, β-glucan has a high water binding capacity, resulting in its physicochemical properties, such as solubility, viscosity, and gelation.

β-Glucan is acknowledged as a functional food ingredient owing to its multiple health benefits, including the prevention of diabetes, reduction in the incidence of cardiovascular disease, antitumor effects, antioxidant activities, and immunostimulation [4]. β-Glucan has a positive effect on the management of diabetes by reducing postprandial plasma glucose and insulin levels [5, 6]. It is well documented that consuming β-glucan can lower the risk of coronary heart disease by significantly reducing serum cholesterol levels [7, 8]. Moreover, β-glucan has long been considered an important antitumor agent for its immune-stimulatory and immunomodulatory effects [9]. Recent studies have validated that β-glucan can inhibit the viability and metastasis of cancer cells [10, 11] and promote cancer cell apoptosis [12]. β-Glucan also exerts an immunostimulatory effect by the activation of the mucosal immune system through β-glucan receptor *dectin-1* on macrophages [13]. Due to the various health benefits of β-glucan, many countries, including the United States, the European Union, Canada, Australia, New Zealand, Brazil, and South Korea, have authorized claims to recommend the daily consumption of β-glucan at least 3 g per day or 0.6–1 g per serving [14]. To the best of our knowledge, China has not authorized similar health claims hitherto.

The reported functions of β-glucan have encouraged researchers to investigate the incorporation of β-glucan in various kinds of foods to make functional foods. Furthermore, there has been a breakthrough in the research of β-glucan synthase during the past fifteen years. A

comprehensive understanding of the synthase gene family will lay the foundation for improving the β-glucan content in cereals. Hence, the present paper aims to review the molecular characteristics, synthase gene family, and food application of cereal β-glucan in recent years.

2. Common Sources and Molecular Characteristics of Cereal β-Glucan

β-Glucan is predominantly found in the aleurone, subaleurone, and endosperm of some cereals (barley and oat). Cereal β-glucan is a linear polymer of a D-glucose unit that contains two to three consecutive β-1, 4 linkages separated by a β-1, 3 linkage. Many features of β-glucan are different among cereals, such as content, molecular size, and molar ratio (DP3/DP4) (Table 1).

2.1. Common Sources of Cereal β-Glucan. Among cereals, barley and oat have the highest β-glucan content, ranging from 2.2% to 19.8% and 2.2%–7.8%, respectively [3, 15, 16, 18]. Other cereals, such as wheat, rice, and maize, also contain β-glucan but in much lower amounts (Table 1). Previous studies have demonstrated that variations in β-glucan content are mainly caused by species and cultivars. Although the β-glucan content in ordinary barley cultivars is between 4% and 11% [26], a genotype containing as high as 19.8% has been reported [16]. In addition to species and cultivars, environmental conditions also have a significant effect on β-glucan content. Some studies have verified that warm and dry weather conditions enhance the β-glucan content [27, 28]. Given that a great amount of β-glucan is located in the outer layer of the grain, such as aleurone and subaleurone, the processing is another factor that affects the final β-glucan content in addition to the factors mentioned above. It is noteworthy to mention that β-glucan is distributed primarily in the endosperm in barley grains in comparison to in the aleurone layers of oat grains; thus, pearling has little effect on the β-glucan content in barley [29, 30].

2.2. Molecular Weight of Cereal β-Glucan. The molecular weight of β-glucan is reported to be scattered in the range of $31–2700 \times 10^3$ in barley, $65–3100 \times 10^3$ in oat, $21–1100 \times 10^3$ in rye, $43–758 \times 10^3$ in wheat, and 36×10^3 in sorghum [17, 19]. The big variations in the molecular weight of β-glucan are attributed to varietal and environmental factors, extraction and purification protocols, and analytical methodologies [2, 26]. The molecular weight of β-glucan can largely determine some other physical characteristics, such as viscosity and solubility, thereby affecting its functional properties. Previous research has indicated that high molecular weight (HMW) barley β-glucan can delay gastric emptying due to increased viscosity, resulting in a reduced glycemic response and diet-induced thermogenesis [31]. Otherwise, low molecular weight (LMW) barley β-glucan was ineffective in lowering glycemic responses [32]. Wang et al. demonstrated that the consumption of HMW barley β-glucan rather than that of LMW β-glucan altered the composition of gut microbiota and consequently reduced the risk markers of cardiovascular disease [33].

2.3. Molar Ratio of Cereal β-Glucan. The structure of cereal β-glucan, also known as (1, 3; 1, 4)-β-glucan, can be defined by digestion with specific (1, 3; 1, 4)-β-glucan endohydrolase, which only hydrolyzes the β-1, 4 linkage adjacent to the β-1, 3 linkage, releasing oligosaccharides with a degree of polymerization (DP) of mainly DP3 (cellotriose) and DP4 (cellotetraose) [34, 35]. The molar ratio of DP3/DP4 is quite variable among cereals (Table 1). In addition to species, the molar ratio is associated with genotype and growth environment. High β-glucan cultivar and drier environment have led to a lower molar ratio in oat [36]. The molar ratio is a unique feature of each cereal, and it affects the solubility and the viscosity of β-glucan in the solution. For instance, oat β-glucan with a lower molar ratio (1.5–2.3) is more soluble than barley and wheat β-glucan with a higher molar ratio (2.6 and 3.2) [25].

3. β-Glucan Synthase in Cereals

During the past fifteen years, considerable progress has been made in the synthesis mechanism of β-glucan. The quantitative trait loci (QTL) for the β-glucan content of barley grains have been identified extensively, such as the major QTL on chromosome 2H [37, 38], 3H [38], 4H [39], and 7H [40–43]. The β-glucan synthase gene families have been reported in rice, barley, wheat, oat, maize, and sorghum (Table 2). By comparative genomics analysis between rice and barley, six cellulose synthase-like *CslF* genes have been found in the syntenic region that is a major QTL for β-glucan content on barley chromosome 2H. After the introduction of two of these *CslF* genes (*OsCslF2* and *OsCslF4*) to *Arabidopsis*, a species without *CslF* genes and β-glucan, the low β-glucan levels have been detected by a β-glucan-specific antibody, indicating the participation of *OsCslF* genes in β-glucan biosynthesis [44]. Through a similar experimental approach, cellulose synthase-like *HvCslH1* [51] and cellulose synthase-like *HvCslJ* [52] have also been proved to be capable of directing β-glucan synthesis.

CslF6, the most highly and widely expressed *CslF* gene in barley, wheat, and rice [53–57], is the predominant gene for the synthesis of the majority of β-glucan in cereals. Three independent barley β-glucanless mutants have shown the cosegregation of the β-glucan deficiency phenotype with the single nucleotide mutation in *HvCslF6* coding sequence (CDS) region, demonstrating a unique role for *HvCslF6* in β-glucan biosynthesis [48, 49]. This has been further confirmed by the transient expression of wild-type *HvCslF6* (which can synthesize β-glucan) and mutant *HvCslF6* (which cannot synthesize β-glucan) in *Nicotiana benthamiana* (*N. benthamiana*) leaves [49]. Additionally, the overexpression of *HvCslF6* under the control of an endosperm-specific promoter has increased the β-glucan content and altered its fine structure in barley grains [47]. On the contrary, the downregulation of *TaCslF6* by RNA interference (RNAi) has resulted in decreased β-glucan content in

Table 1: Common sources and molecular characteristics of cereal β-glucan.

Source	Content (% w/w)	Molecular weight (g/mol)	Molar ratio (DP3/DP4)	References
Barley	2.2–19.8	$31–2700\times10^3$	1.8–3.5	[15–17]
Oat	2.2–7.8	$65–3100\times10^3$	1.5–2.3	[3, 17, 18]
Rye	1.0–2.7	$21–1100\times10^3$	1.9–3.0	[17, 19]
Wheat	0.18–1.8	$43–758\times10^3$	2.8–4.5	[17, 20–22]
Sorghum	0.1–1.7	36×10^3	2.1–3.0	[19, 23]
Maize	0.8–1.7	—	2.5	[24, 25]
Rice	0.02–0.13	—	1.18	[21, 24]

Table 2: List of genes confirmed to function in β-glucan synthesis in cereals.

Gene	Promoter	Gene source	Transgenic host	Approach	Function	Reference
OsCslF2	*CaMV 35S*	Rice	Arabidopsis	HE	Synthesize β-glucan ≤ 0.1% (w/w) in leaves.	[44]
OsCslF4	*CaMV 35S*	Rice	Arabidopsis	HE	Synthesize β-glucan ≤ 0.1% (w/w) in leaves.	[44]
OsCslF6	*CaMV 35S*; secondary cell wall-specific; senescence-associated	Rice	Arabidopsis	HE	Accumulate high-level β-glucan with poor growth by the 35S and secondary cell wall promoter; accumulate high-level β-glucan with normal growth by the senescence-associated promoter.	[45]
OsCslF6	—	Rice	—	Mutant	Reduce more than 97% β-glucan.	[46]
HvCslF4	*CaMV 35S*	Barley	Barley	OE	Increase up to 50% β-glucan in grain and raise the DP3 / DP4 ratio.	[47]
HvCslF6	—	Barley	—	Mutant	Completely lack β-glucan in the endosperm and aleurone layers of cell walls.	[48, 49]
HvCslF6	*CaMV 35S*; endosperm-specific	Barley	Barley	OE	Increase high-level β-glucan in leaves but has little effect on gain β-glucan by the 35S promoter; increase more than 80% β-glucan in grains and reduce the DP3/DP4 ratio by the endosperm-specific promoter.	[47]
HvCslF6	*CaMV 35S*	Barley	*N. benthamiana*	HE	Synthesize β-glucan about 1.62% (w/w) in leaves with a DP3/DP4 ratio of 1.40.	[21]
HvCslF6	—	Barley	Barley	Knockout	Reduce more than 97% β-glucan in grains.	[50]
HvCslH1	*CaMV 35S*	Barley	Arabidopsis	HE	Synthesize β-glucan 0.00015%–0.016% (w/w) in leaves and stems.	[51]
HvCslJ	*CaMV 35S*	Barley	*N. benthamiana*	HE	Synthesize β-glucan ≤ 0.1% (w/w) in leaves.	[52]
TaCslF6	Endosperm-specific	Wheat	Wheat	RNAi	Decrease β-glucan by 30%–52% in the endosperm.	[53]
TaCslF6	*CaMV 35S*	Wheat	*N. benthamiana*	HE	Synthesize β-glucan approximately 0.6%–2.0% (w/w) in leaves with a DP3/DP4 ratio of 1.60.	[21]
AsCslF6	*CaMV 35S*	Oat	*N. benthamiana*	HE	Synthesize β-glucan approximately 0.59% (w/w) in leaves with a DP3/DP4 ratio of 1.09.	[21]
ZmCslF6	*CaMV 35S*	Maize	*N. benthamiana*	HE	Synthesize β-glucan approximately 1.59% (w/w) in leaves with a DP3/DP4 ratio of 1.07.	[21]
SbCslF6	*CaMV 35S*	Sorghum	*N. benthamiana*	HE	Synthesize β-glucan approximately 3.8%–5.9% (w/w) in leaves with a DP3/DP4 ratio of 0.93.	[21]

HE, heterologous expression; OE, overexpression; RNAi, RNA interference.

the endosperm of wheat [53], and the *cslf6* knockout mutant has displayed more than 97% reduction of β-glucan in rice [46]. Recently, a series of CRISPR/Cas9-induced mutations in the members of the *CslF/H* gene family have been generated. β-Glucan has only been absent in the grain of *cslf6* knockout lines, whereas *cslf3*, *cslf9*, and *cslh1* knockout lines have similar β-glucan content to the wild-type [50]. Hence, *CslF6* is a crucial β-glucan synthase gene for engineering the accumulation of β-glucan in cereals.

However, there is a dosage effect negatively correlating β-glucan levels with plant growth. Transgenic plants overexpressing *CslF6* under the constitutive *CaMV 35S* promoter have accumulated high-level β-glucan with severe growth and developmental defect [45, 47]. The negative effects of elevated β-glucan accumulation on plant growth have been prevented by the spatial-temporal regulation of *CslF6* expression under the control of senescence-associated promoter or endosperm-specific promoter [45, 47].

As mentioned above, the molar ratio (DP3/DP4) of β-glucan varies and is a unique feature of each cereal. The *CslF6* from *Brachypodium*, wheat, and barley has produced β-glucan with a relatively high DP3/DP4 ratio, while *CslF6*

from maize, oat, rice, and sorghum has generated β-glucan with a relatively low DP3/DP4 ratio. By generating a series of chimeric constructs between four *CslF6* cDNAs, it has been found that the transmembrane helices 4 (TMH4) of the membrane pore region of CslF6 can control the DP3/DP4 ratio and the fine structure of β-glucan. Point mutation constructs have further confirmed the isoleucine-to-leucine (I/L) change in the TMH4 of CslF6 to be responsible [21]. Furthermore, the glycine-to-aspartic acid (G/D) difference between barley and sorghum in the catalytic region of CslF6 has also been defined to dramatically influence the DP3/DP4 ratio of β-glucan [58].

4. Food Application of Cereal β-Glucan

Recently, β-glucan has been widely applied as an emulsion stabilizer, thickening agent, fat substitute, and bioactive ingredient in the food industry due to its water solubility, viscosity, gelation property, and health benefits [1, 3, 59]. Some of these functional products are favored by consumers owing to their improved quality together with low cholesterol and hypoglycemia properties [60–62]. Notwithstanding, incorporating β-glucan into some food products is still a challenge due to the possible negative effects on the textural quality, sensory characteristics, and shelf life of foods [63, 64]. The effects of β-glucan on food quality and consumer acceptance in various food products will be discussed in the following sections.

4.1. Traditional Chinese Food. Noodle is a traditional Chinese food made from refined wheat flour that is low in dietary fiber, vitamins, minerals, and other important nutrients [65]. Thus, various ingredients including β-glucan are added to improve the health benefits of wheat flour noodles. Noodle incorporated with 30% banana flour and 10% oat β-glucan has exhibited an increase in total dietary fiber and essential minerals, thereby decreasing the glycemic index and carbohydrate digestibility rate [66]. Oat flour, famous for its high β-glucan content, has also been supplemented to wheat flour to produce oat-fortified noodles. Noodle formulations containing 10%–30% oat flour have led to increased β-glucan content and noodle firmness together with decreased noodle lightness and color stability [67]. In addition, more than 50% of wholemeal oat flour (with high-level β-glucan) has been added into wheat flour and other ingredients (compensating for diluted gluten) to make oat-based white salted noodles, resulting in increased pasting viscosities and noodle hardness [68].

Steamed bread (*Mantou*), another traditional Chinese staple food, is also made from refined wheat flour and accounts for 40% of wheat consumption in China [69]. Steamed bread incorporated with 30% barley flour has presented significant improvements in the amount of β-glucan (from 0.03% to 1.03%), hardness, and chewiness, but decreases in the specific volume, brightness, and whiteness index of steamed bread [70]. Steamed bread with the addition of less than 3 g/100 g oat β-glucan has produced a comparable overall consumer acceptance, while an oat β-glucan addition of 5 g/100 g has reduced the consumer acceptance but decreased the *in vitro* starch digestibility and predicted glycemic index [71].

4.2. Milk Products. β-Glucan is a functional bioactive component in the production of yogurt. Yogurt incorporated with β-glucan has exhibited faster proteolysis, lower release of large peptides, and more free amino acids [72]. The addition of barley β-glucan (0.5, 1, 1.5, and 2%, w/v) has significantly enhanced the separation, viscosity, texture profile, and sensory characteristics of full-fat yogurt during storage [62].

Oat milk has also increased the β-glucan content while maintaining the sensory evaluation similar to the control drink [73]. Nonetheless, the incorporation of oat β-glucan into milk is challenged by the thermodynamic incompatibility between milk proteins and β-glucan, thereby limiting its application [64, 74]. Additionally, β-glucan has been used as a fat substitute to produce low-fat cheese, which has resulted in softer cheese with decreased melt time and sensory scores [75].

4.3. Baking Products. Nowadays, β-glucan is preferred as a thickening and structure-making agent applied in gluten-free bakery products due to its prohealth benefits. The application of oat β-glucan (with an optimized percentage of 2.63%) in gluten-free yeast-leavened cake has achieved positive effects on texture, volume, and sensory acceptance [61]. Similarly, gluten-free yeast-leavened cake with 5%–20% high-in-β-glucan oat fiber powder has shown improved springiness, cohesiveness, porosity, and volume [76].

β-Glucan-enriched biscuits, containing 5.2 g/100 g β-glucan from barley flour, have been more acceptable to consumers, with sensory responses being similar to the control [77]. The addition of β-glucan to bread has also been widely tested, but its effect on loaf volume, bread firmness, rate of staling, and consumer acceptance has varied depending on certain conditions, including molecular weight, concentration, and source of β-glucan [63, 78–81].

4.4. Meat Products. β-Glucan is applied as a fat substitute in some meat products, such as beef patties, burgers, and sausages. Oat β-glucan gel (13.45%) can be effectively applied as a fat replacer in low-fat beef patties by retaining fat and moisture, thereby increasing the cooking yield [82]. The addition of inulin gel (IG) and oat β-glucan (βG) mixtures (3%-IG and 0.3%-βG, 6%-IG and 0.6%-βG) could be a valuable alternative to improve the stability, texture, and adhesiveness of low-fat meat emulsions [83]. Low-fat beef burgers, containing 2.2% β-glucan, have exhibited improved texture parameters and cooking properties along with enhanced nutritional characteristics [84]. Oat β-glucan (OG) and marine collagen peptide (MCP) mixed gel (OG/MCP ratio 10 : 1) in low-fat sausage (50% fat reduced) has significantly increased the springiness and chewing, while the taste and overall palatability of such sausage have been comparable with those of the control [85].

5. Conclusions and Future Perspectives

β-Glucan from cereal grains is a valuable dietary fiber that has numerous health-promoting applications. Its health benefits and physicochemical characteristics are conducive to its application in various food products. In China, people pay considerable attention to health and functional food nowadays. The application of β-glucan in traditional Chinese food, such as noodles and steamed bread, is a trend. Nevertheless, wheat and rice, as the main food sources of humans, have a less amount of β-glucan compared with barley and oat. Therefore, future research should focus on improving the β-glucan content in wheat and rice grains. Molecular weight and molar ratio should also be considered due to their effects on the solubility and viscosity of β-glucan and subsequently on the final food products. With further studies on the β-glucan synthase gene family, it is promising to improve the β-glucan content and modify its molar ratio by marker-assisted breeding and molecular design breeding in the future.

Data Availability

All the data generated or analyzed during this study are included in this article.

Conflicts of Interest

All the authors declare that there are no conflicts of interest.

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Research Article

Effects of Drying Temperature and Relative Humidity on Quality Properties of Chinese Dried Noodles

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The influence of the drying conditions on protein structural properties and its impact on Chinese dried noodles (CDN) quality properties is addressed in this study. The CDN were produced under nine different drying conditions utilizing combination of three temperatures (40°C, 60°C, and 80°C) and three relative humidities (65%, 75%, and 85%). The color, texture profile analysis of uncooked and cooked noodles, shrinkage ratio, and cooking quality of CDN were assessed. SEM and FTIR microimaging were investigated to determine the changes in the gluten structural properties. Drying temperature and relative humidity have significant effects on quality characteristics of CDN. However, the influences on different indicators were different. Drying temperature was the main influencing factor of the quality of CDN and protein microstructure. After the drying temperature exceeded 60°C, proteins began to aggregate, and the surface protein distribution became uneven. Compared with cross section, the uniformity of protein distribution on the surface of noodles showed a significant decrease. A high temperature (60°C) could improve the quality of CDN products. The quality of CDN products could be adjusted by the combination of drying temperature and relative humidity.

1. Introduction

Chinese dried noodles (CDN) are one of the most popular noodle types all over the world due to their convenience, variety, long shelf life, and nutritional values. The total production yield of CDN has reached over 8 million tons per year in China, which consumes about 35% of the total flour consumption in mainland China [1, 2]. Drying process is a key step of CDN production. The process design and parameter control largely determine the quality of product, production efficiency, and energy consumption [3].

Temperature and relative humidity (RH) in drying chamber are the two main conditions during drying process. A reasonable drying temperature can promote the evaporation of moisture in noodles, improve the quality of noodles, shorten drying time, and reduce production costs [4]. At present, the manufacturers generally use the low- or medium-temperature (lower than 45°C) drying process relying on air heating (coal or gas), while the drying condition regulation and control are mainly based on personal experiences [3]. The effects of drying conditions and process on the quality of spaghetti and dried udon noodles have been extensively explored [5–13]. Inazu et al. found that the effect of relative humidity on the apparent moisture diffusivity of Japanese noodle (udon) was smaller than that of temperature, but its impact could not be neglected [6]. Mercier et al. reviewed 66 studies on enriched pasta and showed that high drying temperatures generally improve the cooking properties of enriched pasta [10]. Padalino et al. suggested that spaghetti cooking quality was positively affected by drying temperature increase and found that the improvement of sensory and cooking quality properties of spaghetti was

directly related to the density increase in both chemical crosslink of protein matrix and physical crosslink of starch granules [11]. Bruneel et al. implied that an optimal degree of protein polymerization during drying and/or the subsequent cooking led to the high-quality pasta [14]. Verbauwhede et al. studied the impact of heat on microstructure of heating nonfermented dough and found out that the gluten microstructure was affected from 65°C onwards [15]. Wang et al. observed that gluten content mainly affected the drying rate in the middle drying period of Chinese dried noodle [16]. The effects of drying parameters on moisture content, moisture state, or energy consumption have been researched [3, 17]. However, the effect of drying temperature, relative humidity, and their interactions on quality characteristics of Chinese dried noodles, the changes in protein microstructure under different drying conditions, and the influences of these changes on the quality characteristics of CDN products were rarely explored.

The aims of this study were to investigate the effects of various drying temperatures and different relative humidity on the color, cooking, and texture characteristics of CDN, explore the changes in protein microstructure, and reveal the correlation among these parameters and product quality.

2. Materials and Methods

2.1. Materials. The wheat variety Ningchun 4 was used in this the experiment, which is a good noodle wheat variety. Ningchun 4 is a hard spring type, with low protein and wet gluten contents (12.3% and 28.5%) and medium to strong gluten strength (6.6 min of stability time and 206 BU of resistance). The flour was produced in the MLU 202 experimental flour mill (Bühler, Switzerland) with the flour yield of 71.2%. The edible salt used in the noodles was from the local supermarket. Other reagents used were of analytical grade.

2.2. Preparation of Fresh Noodles. Ningchun 4 flour (14% wet weight) of 1000 g was mixed with 350 g distilled water and 1% edible salt for 8 min in a dough mixer (Henan Dongfang Food Machinery Equipment Co., Ltd., China) to make dough crumbs. The obtained dough crumbs were rolled twice on the MT5-215 sheet rolling machine (Nanjing Yangzi Cereals, Oils and Food Machinery Co., Ltd., China) to obtain a 4 mm thick noodle sheet, which was put into ziplock bags, sealed, and rested at room temperature for 30 min. Thereafter, the dough sheet was then rolled four times to obtain a 1 mm thick sheet, which was then cut into 2 mm wide wet raw noodles.

2.3. Drying Conditions Design. The fresh noodles were hung in a drying chamber (BLC-250-III, Beijing Land and Technology co., Ltd., Beijing, China) which had been equilibrated in advance. The drying parameters including temperature and relative humidity were set to 40, 60, 80°C, and 65%, 75%, 85%, respectively. In the present work, nine different combinations of drying conditions were implemented. The drying temperature and relative humidity were adjusted to different combinations before operations. Drying time was 300 min. After drying, the noodles were cut into a length of 25 cm and put into ziplock bags for subsequent use.

2.4. Color of Dried Noodles. Twenty noodle sticks were randomly selected and then measured at five positions on each noodle stick for the colors using the DigiEye Digital-Imaging System (VeriVide Limited, UK). In total, 100 measurements were obtained for each sample and the average value was calculated as the color of dried noodle samples. According to the CIE Lab system, three color indexes of L^*, a^*, and b^* were obtained. Each sample was tested for three times.

2.5. Noodle Shrinkage Ratio. Firstly, ten straight dried noodle sticks were randomly selected. Then, the width and thickness in the middle and two ends (2 cm away from the ends) of selected noodles were measured with a vernier caliper. With the width (a_0, 2 mm) and thickness (b_0, 1 mm) of wet raw noodles as the standard, the shrinkage ratio (ψ) of dried noodles was calculated as follows:

$$\psi(\%) = (S_0 - S_1) \times \frac{100}{S_0},$$
$$S_0 = a_0 \times b_0, \quad (1)$$
$$S_1 = a \times b,$$

where S_0 is the cross-sectional area of the wet noodle, mm^2, S_1 is the cross-sectional area of the dried noodle, mm^2, a_0 is the width of the wet raw noodle, mm, b_0 is the thickness of the wet raw noodle, mm, a is the width of the dried noodle, mm, and b is the thickness of the dried noodle, mm.

2.6. Texture Characteristics of Uncooked CDNs. Twenty noodle sticks were randomly selected from each batch of CDN product and then cut into the length of 18 cm. The TAXT plus Texture Analyzer (Stable Micro System, UK) was used to measure the bending resistance of dried noodles. The A/SFR probe dropped at a speed of 1.00 mm/s until the noodle stick was broken. The maximum resistance encountered during the process when the probe pressed the noodle represented the bending strength of the dried noodle. The distance from the point where the probe was in contact with the noodle to the point where the noodle was broken indicated the breaking distance. The area of the region surrounded by the force and time in the period from contacting time between the noodle and the probe to breaking time was considered breaking work.

2.7. Water Absorption Rate (WAR) and Cooking Loss Ratio (CLR). According to AACCI 66–50.001 [18] with minor modifications, 20 CDN sticks were placed into 500 mL of boiling distilled water. When the white core disappeared, heat was removed, and the time was recorded as the optimal cooking time (OCT) of the CDN. In a separate test, 10.00 g of

noodles was weighed, and 500 mL of distilled water was added in a stainless-steel pot and heated to the boiling point with an induction cooker. The weighed sample was added into the pot and the boiling state was maintained. After OCT was reached, noodles were obtained from the pot immediately. When no obvious water could be observed on the noodle surface, the noodles were weighed to calculate water absorption ratio (WAR, %). The remaining noodle soup in the steel pot was heated until all liquid had evaporated. The stainless-steel pot was put in an oven and baked at 105°C to constant weight. The total mass of the stainless-steel pot and the remaining material was weighed to calculate the cooking loss ratio (CLR, %).

2.8. Texture Characteristics of Cooked CDNs. According to the method described by Liu et al. [19], twenty noodles were randomly selected, cooked for optimal cooking time, fished out immediately, rinsed with tap water for 30 s. A set of five sticks were placed in parallel on the loading platform in a TA. XT plus Texture Analyzer (Stable Micro System, UK). Testing parameters were set as follows: TPA measurement mode; the probe, A/LKB-F; the speed before the measurement, 2 mm/s; the measurement speed, 0.8 mm/s; the speed after the measurement, 2 mm/s; the compression ratio, 70%; the interval between two compressions, 10 s; the starting induction force, initial value, 10 g; and the data acquisition rate, 200 pps. Three parallel experiments were performed for each sample.

2.9. Scanning Electron Microscopy (SEM). The CDN samples were cut, then respectively fixed with glutaraldehyde for 48 h and osmic acid for 2 h, and dried at the supercritical point of CO_2. The dried samples were then cut off with pliers. The cut samples with a flat surface were selected and fixed on the sample platform. Gold particles were sprayed on the samples with the Hitachi IB-5 Ion Coating Apparatus, and the samples were then placed under a SU 8010 scanning electron microscope (HITACHI, Japan) at 300× and 800× magnification, observed, and photographed.

2.10. Infrared Microscopic Imaging Analysis. The surface and cross section of the dried noodles were examined by a LUMOS stand-alone FTIR microscope (Bruker, Saarbrucken, Germany) to analyze the protein content distribution on the surface and cross section of noodles. The measurement parameters are as follows: ATR mode, MCT detector, wavenumber range (4000–600 cm^{-1}), air as the reference, the infrared spectral resolution (4 cm^{-1}), and 16 scans. Area array sampling was performed in 15×15 points (imaging area, $375\,\mu m \times 300\,\mu m$; spatial resolution, $25\,\mu m \times 25\,\mu m$). The air background was automatically removed from the sample spectra in the OPUS 8.1 software. The peaks in the amide I and amide II regions (1715–1484 cm^{-1}) were integrated to generate a 2D pseudocolor plot, which was used to represent the protein distribution in noodle samples [20, 21]. The bright purple and red in the scale indicate a high value of protein content while blue indicates a low value.

2.11. Statistical Analysis. All the experiments were repeated at least 3 times and experimental data was expressed as the mean ± SD. Statistical analysis was carried out by SPSS for Windows version 18.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (one-way ANOVA) and multiple comparison analysis were carried out to estimate the statistically significant differences between different temperatures, RH, and their combinations, and multiway analysis of variance (multiway ANOVA) was carried out to find out the contributions rate of each factor. Differences were considered to be significant at $P < 0.05$.

3. Results

3.1. Quality Properties of Uncooked Noodles

3.1.1. Color of CDN. The colors of CDN under different drying temperature and relative humidity are shown in Table 1. Under the conditions of different drying temperatures, with the increase in relative humidity, the L^* values of noodles showed the decreasing trend, whereas the a^* values showed the increasing trend. The L^* values combination under the relative humidity of 85% were significantly lower than those under 65% and 75%, whereas the a^* values under the relative humidity of 85% were significantly higher than those under 65% and 75%. Under the same relative humidity, the L values or a^* values of CDN under the conditions of different temperatures showed no significant difference $P < 0.05$. Under the combination condition of 40°C, 65%, the b^* values were significantly lower than those under other combination conditions ($P < 0.05$). The b^* values under the drying temperature of 60°C were significantly lower than those under 80°C.

3.1.2. Shrinkage Ratio. The shrinkage ratio of CDN showed no regular variation with temperature or relative humidity. Under the combination condition of 40°C and 65%, the shrinkage ratio of dried noodles was the lowest, which was significantly lower than that under other combination conditions, while under the combination conditions of 80°C and 65%, the shrinkage ratio was the highest (Table 1). There was no significant difference in the shrinkage ratio between other combination conditions of temperature and relative humidity.

3.1.3. TPA of Uncooked CDN. Under the drying temperatures of 40°C and 60°C, with the increase in relative humidity, the bending strength of CDN firstly decreased and then increased. Under the drying temperature of 80°C, the bending strength showed an increasing trend with increasing humidity. Under the combination condition of 80°C and 85%, the bending strength of dried noodles was the highest (Table 2).

Under the drying temperatures of 60°C and 80°C, with the increase in relative humidity, the breaking distance

Table 1: Effects of drying temperature and relative humidity on quality characteristics of uncooked CDN.

Temperature (°C)	Relative humidity (%)	Color of CDN			Shrinkage ratio (%)
		L*	a*	b*	
40	65	89.80 ± 0.35ab	0.41 ± 0.04cd	13.62 ± 0.35d	12.96 ± 0.83c
	75	89.43 ± 0.21bc	0.61 ± 0.08bc	14.79 ± 0.23bc	18.17 ± 1.40ab
	85	88.78 ± 0.26d	0.81 ± 0.10ab	14.75 ± 0.39bc	16.04 ± 1.93b
60	65	89.63 ± 0.23ab	0.41 ± 0.04cd	14.60 ± 0.20c	18.20 ± 1.46ab
	75	89.63 ± 0.15ab	0.47 ± 0.07cd	14.61 ± 0.09c	17.12 ± 1.24b
	85	88.95 ± 0.45cd	0.57 ± 0.02bc	14.54 ± 0.29c	18.40 ± 1.71ab
80	65	90.03 ± 0.35a	0.25 ± 0.01d	15.18 ± 0.19b	20.51 ± 1.83a
	75	89.43 ± 0.25bc	0.41 ± 0.02cd	16.52 ± 0.27a	17.33 ± 0.78ab
	85	89.00 ± 0.23cd	0.93 ± 0.38a	16.70 ± 0.33a	17.12 ± 1.75b

Note. Data in this table are expressed as means ± standard deviations and the data followed by different letters in the same column mean significant differences ($P < 0.05$).

Table 2: Effects of drying temperature and relative humidity on TPA characteristics of uncooked CDN.

Temperature (°C)	Relative humidity (%)	Bending strength (g)	Broken distance (mm)	Broken power (10^{-3}J)
40	65	15.21 ± 0.50ab	45.26 ± 2.26a	4.47 ± 0.10a
	75	13.76 ± 0.39c	35.01 ± 3.83cd	3.39 ± 0.31bc
	85	14.38 ± 0.76bc	29.10 ± 2.40e	3.01 ± 0.05cd
60	65	14.67 ± 0.17bc	38.08 ± 0.84bc	3.70 ± 0.13b
	75	14.41 ± 0.44bc	29.63 ± 0.75e	3.04 ± 0.20cd
	85	15.09 ± 0.31ab	31.17 ± 3.27de	3.29 ± 0.30c
80	65	14.05 ± 0.73c	32.08 ± 0.50de	3.15 ± 0.34cd
	75	14.39 ± 0.77bc	27.95 ± 3.43e	2.81 ± 0.16d
	85	15.80 ± 0.64a	39.89 ± 3.55b	4.18 ± 0.22ab

Note. Data in this table are expressed as means ± standard deviations and the data followed by different letters in the same column mean significant differences ($P < 0.05$).

and breaking power of CDN firstly decreased and then increased. Under the drying temperature of 40°C, the breaking distance and breaking power gradually decreased with increasing humidity. Under the combination condition of 40°C and 65%, the breaking distance and breaking power were the largest, which were significantly higher than those under other combination conditions ($P < 0.05$, Table 2).

Opposite changes of bending strength, broken distance, and broken power were observed between 40°C and 80°C. At 40°C, the noodle strength was decreased with increasing humidity; in contrast, opposite phenomenon was observed at 80°C.

3.2. Quality Properties of Cooked Noodles

3.2.1. Optimal Cooking Time (OCT). As shown in Table 3, the OCT of dried noodles obtained at drying temperatures of 40°C and 60°C showed no significant differences between different relative humidities. When the drying temperature was 80°C, OCT under the relative humidity of 65% was significantly shorter than that under 75% and 85% $P < 0.05$, but OCT obtained under relative humidities of 75% and 85% showed no significant difference ($P < 0.05$). Under the relative humidity of 65%, OCT obtained at drying temperatures of 60°C and 80°C was significantly shorter than that at 40°C, but OCT obtained at drying temperatures of 60°C and 80°C showed no significant difference. OCT obtained under relative humidities of 75% and 85% firstly decreased and then increased with the increase in drying temperature. Under different relative humidities, OCT obtained at the drying temperature of 60°C was the shortest.

3.2.2. Water Absorption Ratio (WAR). Temperature and relative humidity had little effect on the WAR of dried noodles. The WAR obtained under the combination condition of 80°C and 65% was the highest, which was significantly higher than that obtained under other combination conditions. The WAR of dried noodles obtained under other combination conditions showed no significant difference (Table 3).

3.2.3. Cooking Loss Ratio (CLR). When the drying temperature was 40°C, the CLR of dried noodles obtained under different relative humidities showed no significant differences. When the drying temperature was 60°C, with an increase in the relative humidity, the CLR firstly decreased and then increased. When the drying temperature was 80°C, the CLR under different relative humidities was the highest (Table 3).

3.2.4. TPA of Cooked CDN

(1) Hardness. Table 4 shows the one-way ANOVA results of the effects of drying temperature and relative humidity on the texture characteristics of cooked CDN. When the drying

Table 3: Effects of drying temperature and relative humidity on cooking quality characteristics of CDN.

Temperature (°C)	Relative humidity (%)	Optimal cooking time (s)	Water absorption ratio (%)	Cooked loss ratio (%)
40	65	343 ± 7a	162.62 ± 3.42b	6.86 ± 0.46bc
	75	337 ± 6a	167.92 ± 1.54ab	6.85 ± 0.23bc
	85	344 ± 7a	164.18 ± 5.91b	7.00 ± 0.18abc
60	65	314 ± 10c	165.00 ± 2.41b	6.73 ± 0.44b
	75	316 ± 6c	160.00 ± 2.59b	6.59 ± 0.51c
	85	318 ± 15bc	164.25 ± 4.30b	7.41 ± 0.27ab
80	65	316 ± 14c	176.5 ± 10.64a	7.00 ± 0.49abc
	75	349 ± 8a	167.65 ± 3.94ab	7.68 ± 0.17a
	85	333 ± 7ab	158.67 ± 9.07b	7.59 ± 0.37b

Note. Data in this table are expressed as means ± standard deviations and the data followed by different letters in the same column mean significant differences ($P < 0.05$).

Table 4: Effects of drying temperature and relative humidity on TPA of cooked CDN.

Temperature (°C)	Relative humidity (%)	Hardness/(g)	Adhesiveness	Springiness	Cohesiveness	Gumminess	Resilience
40	65	307.43 ± 3.94b	−0.53 ± 0.08a	1.10 ± 0.03a	0.67 ± 0.00a	204.84 ± 2.53ab	0.38 ± 0.01ab
	75	288.20 ± 1.31cd	−0.73 ± 0.66ab	1.11 ± 0.00a	0.67 ± 0.01ab	191.61 ± 1.23cd	0.39 ± 0.01a
	85	289.73 ± 11.13cd	−0.89 ± 0.42ab	1.05 ± 0.02b	0.67 ± 0.01a	193.75 ± 7.92bcd	0.40 ± 0.01a
60	65	278.02 ± 4.46d	−4.21 ± 1.33cd	1.01 ± 0.02bc	0.64 ± 0.00e	178.53 ± 3.13e	0.35 ± 0.01d
	75	289.48 ± 7.64cd	−3.00 ± 0.98bcd	1.01 ± 0.01c	0.66 ± 0.01bc	189.87 ± 7.36cde	0.37 ± 0.01bc
	85	289.67 ± 8.46cd	−5.38 ± 2.06d	1.01 ± 0.00c	0.63 ± 0.00d	182.95 ± 4.88de	0.34 ± 0.00e
80	65	304.33 ± 13.35bc	−4.99 ± 1.81d	1.02 ± 0.02bc	0.64 ± 0.00de	194.16 ± 8.46bcd	0.36 ± 0.00cd
	75	307.11 ± 5.60b	−3.49 ± 1.36cd	1.02 ± 0.02bc	0.64 ± 0.01d	197.71 ± 5.71bc	0.37 ± 0.01b
	85	328.31 ± 9.74a	−2.58 ± 0.22abc	1.03 ± 0.02bc	0.65 ± 0.00cd	212.57 ± 6.12a	0.38 ± 0.00b

Note. Data in this table are expressed as means ± standard deviations and the data followed by different letters in the same column mean significant differences ($P < 0.05$).

temperature was 40°C, the noodle hardness obtained under relative humidity of 65% was significantly higher than that obtained under 75% and 85%, but the hardness showed no significant difference between 75% and 85%. When the drying temperature was 60°C, the hardness under different relative humidities showed no significant difference. When the drying temperature was 80°C, the hardness obtained under relative humidity of 85% was significantly higher than 65% and 75%, but the hardness between 65% and 75% showed no significant differences. When the relative humidity was 65%, the hardness of the noodles obtained under the drying temperature of 60°C was significantly lower than that obtained under the drying temperatures of 40°C and 80°C, but the hardness of the noodles obtained under drying temperatures of 40°C and 80°C showed no significant differences. The hardness of dried noodles obtained under the conditions of drying temperature of 80°C and the relative humidity of 85% was the highest. The hardness obtained under the condition of 60°C, 65% was the lowest.

(2) Adhesiveness. When drying temperatures were, respectively, 40°C and 60°C, the adhesiveness of cooked noodles obtained under different relative humidities showed no significant difference. When the drying temperature was 80°C, the adhesiveness of noodles decreased with the increase in relative humidity (Table 4). When relative humidities were, respectively, 65% and 75%, the adhesiveness increased with the increase in temperature. When the relative humidity was 85%, the adhesiveness firstly increased and then decreased with the increase in drying temperature.

(3) Springiness. When the drying temperature was 40°C, the springiness of the cooked noodles under relative humidities of 65% and 75% was significantly higher than that under 85%. When drying temperatures were, respectively, 60°C and 80°C, the springiness under different relative humidities showed no significant difference. Under different relative humidities, when the drying temperature increased from 40°C to 60°C, the springiness decreased significantly; when the temperature continued to increase to 80°C, the springiness showed no significant change (Table 4). When the drying temperature was 60°C, the springiness of the noodles obtained under different relative humidities was the smallest.

(4) Cohesiveness. When drying temperatures were, respectively, 40°C and 80°C, the cohesiveness of cooked noodles under different relative humidities showed no significant difference. But when the drying temperature was 60°C, the cohesiveness firstly increased and then decreased with the increase in relative humidity. Under different relative humidities, the cohesiveness obtained under 60°C and 80°C was significantly lower than 40°C. When the relative humidity was 65%, the cohesiveness under drying temperatures of 60°C and 80°C showed no significant change. When the relative humidity was 75%, the cohesiveness obtained under 60°C was significantly higher than that obtained under 80°C. When the relative humidity was 85%, the cohesiveness obtained under 60°C was significantly lower than that obtained under 80°C. The cohesiveness obtained under 60°C and 85% was the lowest (Table 4).

(5) Gumminess. When the drying temperature was 40°C, the gumminess of the cooked noodles obtained under the relative humidity of 65% was significantly higher than 75% and 85%, but gumminess obtained under relative humidities of 75% and 85% showed no significant difference. When the drying temperature was 60°C, gumminess obtained under different relative humidities showed no significant differences. When the drying temperature was 80°C, the gumminess under 85% was significantly higher than that under 65% and 75%, but gumminess obtained under 65% and 75% showed no significant difference. Under different relative humidities, the gumminess firstly decreased and then increased with the increase in the drying temperature. The gumminess obtained at the drying temperature of 60°C was the lowest. The gumminess obtained under 60°C and 65% was significantly lower than other combination conditions (Table 4).

(6) Resilience. When the drying temperature was 40°C, the resilience obtained under different relative humidities showed no significant differences. When the drying temperature was 60°C, the resilience firstly increased and then decreased with the increase in relative humidity. When the drying temperature was 80°C, the resilience under the relative humidity of 65% was significantly lower than that under 75% and 85%, but the resilience obtained under 75% and 85% showed no significant difference. Under different relative humidities, the resilience obtained at the drying temperature of 40°C was significantly higher than that under 60°C and 80°C. When relative humidities were 65% and 75%, the resilience at drying temperatures of 60°C and 80°C showed no significant difference. Under the relative humidity of 85%, the resilience obtained at 60°C was significantly lower than that 80°C. The resilience obtained under 60°C and 85% was significantly lower than that of other combination conditions.

3.3. Images of Scanning Electron Microscopy (SEM). Figure 1 shows SEM images of noodles cross sections prepared under different conditions of drying temperature and relative humidity. Figures 1(a) and 1(b) are, respectively, images of 300X magnification and 1000× magnification. Dried noodles had a dense internal structure and the gluten network formed a continuous sheet structure, where large and small starch granules were tightly adhered or wrapped. The surface of partial starch granules shrank. The binding capacity between starch granules and gluten inside dried noodles obtained under different conditions of drying temperature and relative humidity showed the significant differences and starch granules fell away at the cross section of dried noodles to different degrees. In the noodles obtained at the drying temperature of 80°C, the falling phenomenon of starch granules was the most serious. The binding between starch granules and gluten network structure is related to bending strength, water absorption ratio, and cooking loss ratio of the noodles.

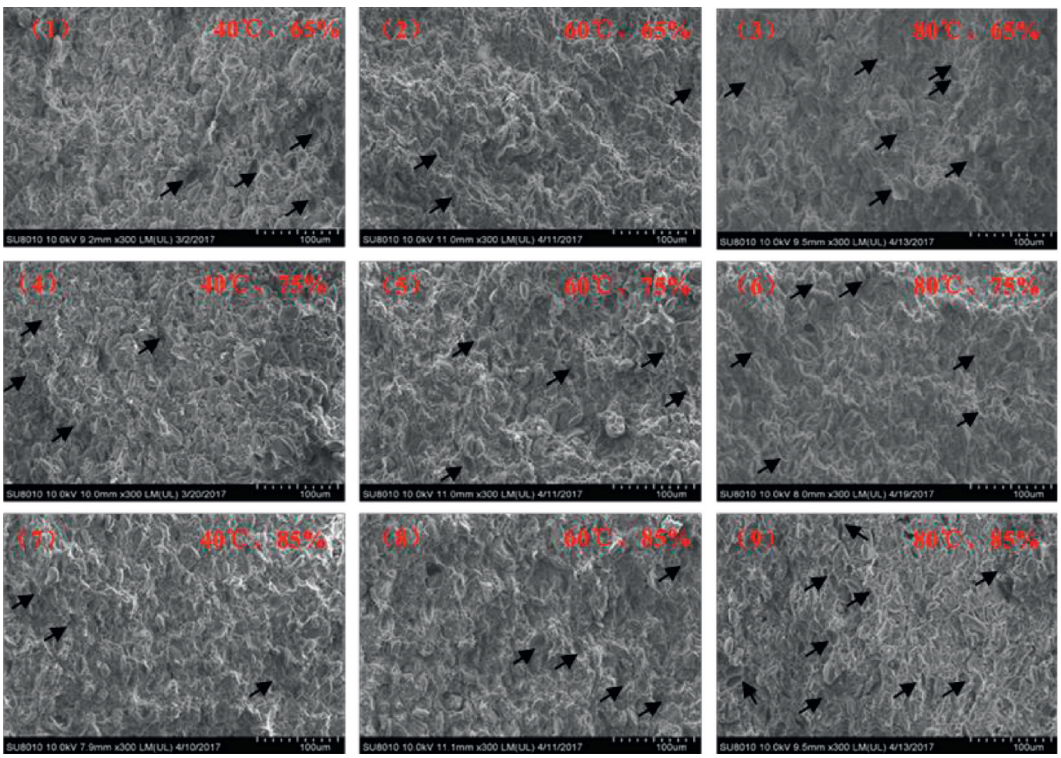
3.4. Fourier Transform Infrared (FTIR) Microscopy. The protein quantity distribution diagrams at the cross section and surface of the noodles produced under different conditions are shown in Figure 2. The protein quantity distribution at the cross section of the noodles was largely affected by drying temperature (Figure 2(a)). Especially at the drying temperature of 80°C, the protein aggregation was serious, and the aggregation phenomenon was more significant at the cross section. The falling phenomenon of starch granules observed by SEM may be related to protein aggregation.

Compared with cross section (Figure 2(a)), the protein distribution on the surface of noodles (Figure 2(b)) showed the decreased uniformity. Under the conditions of 80°C and 75%, the protein distribution on the noodle surface showed the worst uniformity and the obvious protein aggregation was observed. Protein aggregation may reduce its ability to wrap starch granules. Therefore, starch granules on the surface tended to fall off during cooking and the cooking loss ratio increased. The results were consistent with the variations of cooking loss ratio with drying temperature and relative humidity.

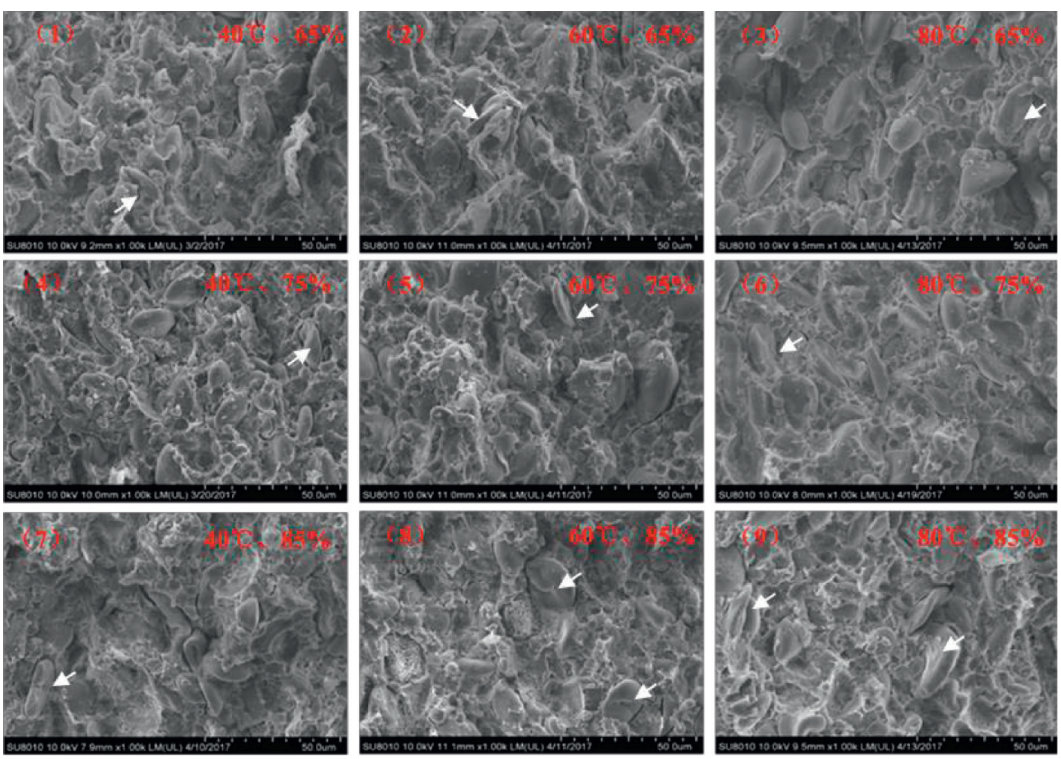
4. Discussion

Temperature and relative humidity are the main control factors in the drying process of noodles and affect the drying efficiency and quality of noodles [3–6]. At present, the common production process of noodles in China is the hot air convection drying process at medium temperature (≤45°C) [3]. Since 1970s and 1980s, the high-temperature drying process had been explored in the production of spaghetti in Italy and Japanese dried noodles and was widely applied in the production of spaghetti. Higher drying temperatures (>60°C) could improve the cooking characteristics of spaghetti [10, 11]. This study indicated that drying temperature and relative humidity had significant or extremely significant effects on the color, density, shrinkage, cooking characteristics, and texture characteristics of dried noodles. However, the influences on various indicators of dried noodles were different. The drying temperature had a great influence on OCT and TPA characteristics of CDN. The relative humidity had great effects on the L^* value, a^* value, and bending strength of CDN. In general, when the drying temperature was 60°C, OCT, hardness, cohesiveness, gumminess, chewiness, and resilience reduce, and the cooking characteristics of CDN can improve to a certain degree. This study confirmed that the quality of noodle products could be adjusted by regulating different combinations of drying temperature and relative humidity.

Bruneel et al. pointed out that the formation of a suitable protein network structure during drying largely determined the quality of spaghetti produced from durum wheat [12]. Verbauwhede et al. observed the changed microstructure of the gluten protein network, cleaved hydrogen bonds, protein recombination caused by hydrophobic interactions, and branched protein structure when nonfermented dough was heated above 65°C [13]. In this study, the SEM and FTIR results indicated that, with the increase in temperature, the internal microstructure or protein content distribution of dried noodles showed significant change. When the drying temperature increased to 60°C, due to protein aggregation,

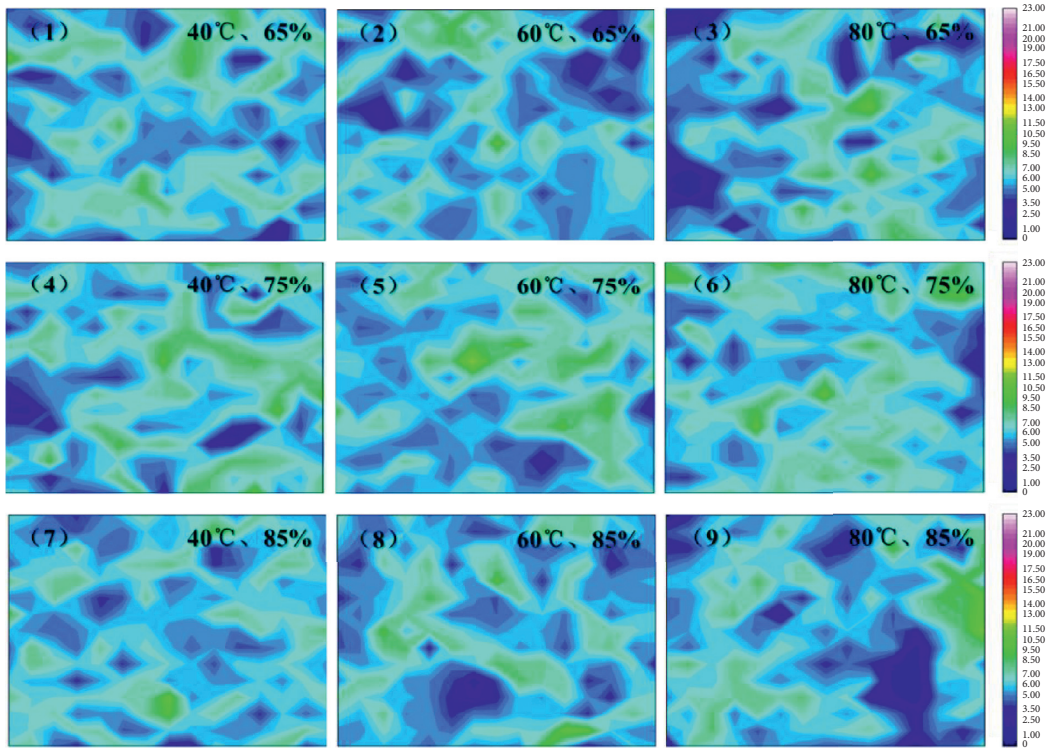


(a)

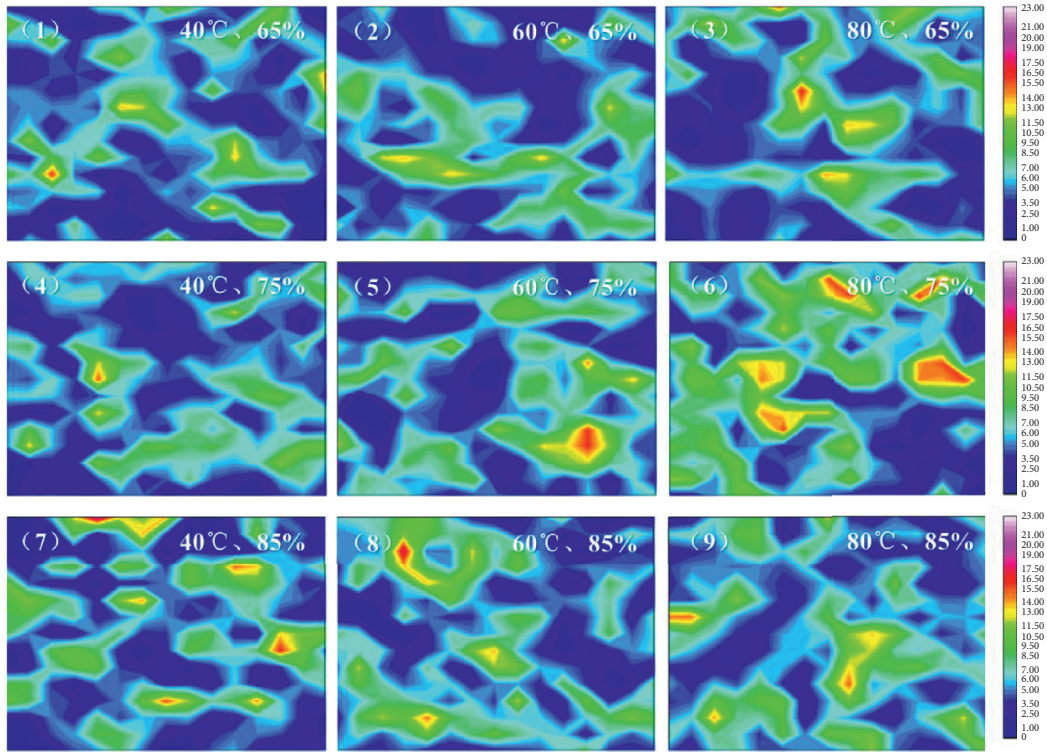


(b)

Figure 1: SEM of cross sections of CDN at different drying temperatures and relative humidities ((a) ×300; (b) ×1000).



(a)



(b)

Figure 2: Contour plots of the variation of protein quantity for cross sections (a) and surfaces (b) of dried noodles at different drying temperatures and relative humidities.

the surface protein distribution became uneven and starch swelling was not observed.

5. Conclusions

Drying temperature and relative humidity have significant effects on quality characteristics of CDN. However, the influences on different indicators were different. Drying temperature was the main influencing factor of the quality of CDN and protein microstructure. After the drying temperature exceeded 60°C, proteins began to aggregate, and the surface protein distribution became uneven. A high temperature (60°C) could improve the quality of CDN products. The quality of CDN products could be adjusted by the combination of drying temperature and relative humidity. But in the practical application process, the adjustment should be considered based on the performance of drying equipment, production targets, energy consumption requirements, and drying process parameters.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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