

## Article

# Effects of Different Amylose Contents of Foxtail Millet Flour Varieties on Textural Properties of Chinese Steamed Bread

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**Abstract:** In order to improve the nutritional value and quality of steamed bread, and promote the industrial development of the whole-grain food industry, a texture analyzer was used to study the effects of cultivars of whole foxtail millet flour (WFMF) on the texture of Chinese steamed bread (CSB). Orthogonal partial least squares discriminant analysis (OPLS-DA) was also conducted. The addition of different cultivars of WFMF significantly altered the height–diameter ratio, specific volume, hardness, cohesiveness, gumminess, and chewiness of CSB ( $p < 0.05$ ). Large amounts of foxtail millet flour significantly increased the hardness, gumminess and chewiness of the bread ( $p < 0.05$ ), and the bread height–diameter ratio, specific volume, cohesiveness and springiness significantly decreased ( $p < 0.05$ ). We screened sensory evaluation, chewiness, specific volume, and hardness as the signature differences in the quality components according to the variable influence on the projection (VIP) values. OPLS-DA could distinguish the addition levels of different samples.

**Keywords:** whole foxtail millet flour; amylose content; japonica and glutinous; Chinese steamed bread; texture properties

## 1. Introduction

Foxtail millet (*Setaria italica* L. Beauv) is rich in proteins, fats, carbohydrates, dietary fiber, vitamins and minerals. It is good for the spleen and stomach, and has been applied to food therapy for thousands of years [1,2]. After shelling and milling, millet is suitable for human consumption. However, the consumption of millet porridge alone cannot support the millet production and processing industry. The demand for whole grain food is growing worldwide. The sale of whole grain bread in the USA market has surpassed that of ordinary white bread [3,4]. Foxtail millet is an important whole grain that suffers relatively little loss of nutrients from processing and provides high nutritional value [5].

The raw materials used for whole grain food processing and their nutritional values are receiving great attention [6,7]. Chinese steamed bread (CSB) is a traditional staple food in China. It is convenient and nutritious [8]. The development of an improved CSB has been studied during the development of the whole-grain food industry. Quinoa flour [9,10], buckwheat flour [11,12], finger millet, and red kidney bean flour [13] have been added to CSB and the results have been documented. As whole grains have been proven to reduce the risk of diabetes, obesity, colorectal cancer, and cardiovascular disease [14], the use of composite flour to make bread is also a recent global development. Furthermore, due to the development of some social, economic, and health-related concepts, the use of whole-grain flour is a strategy for developing healthy food. Whole-grain cereals are the

focus of much research and an important component of a healthy diet. Foxtail millet has also been studied, but the results are limited to a single cultivar of milled millet flour [15,16]. Since the millet bran contains a variety of nutrients, the nutritional value of millet is greatly reduced. Compared with commercial refined flour, there is a large amount of dietary fiber in whole grains [3], which affects product quality; thus, it is necessary to develop a whole-grain millet that combines the characteristics of being good for health and possessing good texture quality. There is no information on the production of CSB using a variety of cultivars of WFMF. The numerous foxtail millet varieties have different physical and chemical properties. Their characteristics must be evaluated to select the most appropriate varieties and additive amounts for their use in CSB.

Thus, we determined the effects of different varieties and amounts of WFMF via the height–diameter ratio and the specific volume of CSB. Texture characteristic changes in hardness, cohesiveness, springiness, gumminess and chewiness were analyzed. We also used OPLS-DA to identify differences between the varieties and determine the most suitable varieties for the production of CSB. This information could increase the commercial use of foxtail millet in CSB manufacturing.

## 2. Materials and Methods

### 2.1. Materials

Special first-grade multi-purpose wheat flour (WF) was obtained from Jinsha River Noodle Group Co., Ltd., Xingtai, China; Angel high active dry yeast was obtained from Angel Yeast Co., Ltd., Yichang, China. The materials (japonica: Jigu-19, Taixuan-17, Yugu-18, An11-5365, and Ji0626-4; glutinous: Chifeng-1, Fente-5, and N101) were supplied by the Institute of Millet Crops, Hebei Academy of Agriculture and Forestry Sciences, China, and planted in Mazhuang Experimental Station, Shijiazhuang, China (Table 1). Each foxtail millet sample was harvested, dried, and stored at  $-20^{\circ}\text{C}$ .

**Table 1.** Foxtail millet varieties.

Sample	Name Code	Amylose (%)	Type of Cultivar
Jigu-19	JG19	$32.25 \pm 0.68$	Japonica
Taixuan-17	TX17	$24.64 \pm 0.37$	Japonica
Yugu-18	YG18	$15.19 \pm 0.81$	Japonica
An11-5365	A11-5365	$25.50 \pm 0.62$	Japonica
Ji0626-4	J0626-4	$12.36 \pm 0.53$	Japonica
Chifeng-1	CF1	$7.40 \pm 0.34$	Glutinous
Fente-5	FT5	$7.46 \pm 0.65$	Glutinous
N101	N101	$5.25 \pm 0.47$	Glutinous

### 2.2. Preparation of WFMF

All of the samples were dehulled using a SY88-TH cereal huller (Korea Ssangyong Machinery Industry Co., Ltd., Incheon, Korea) and then milled into powder using a M3100 automatic cyclone mill (Perten Instruments, Hägersten, Sweden). They were then sieved through a 100 mesh screen sieve (Henan Xinxian Fasite Instrument Co., Ltd., Xinxian, China) to prepare WFMF.

### 2.3. Amylose Content of WFMF

An Amylose/Amylopectin Assay Kit (Megazyme International Ltd., Bray, Ireland) was used to determine the amylose content of the samples.

### 2.4. CSB Preparation

CSB was prepared according to the method of Li et al. [17] with some modifications: 300 g amounts of mixed flour were prepared (each containing 0%, 10%, 20%, 30%, 40%, and 50% of WFMF) and mixed using a KVC3100 mixer (Kenwood, UK). Then, 150 mL of  $35^{\circ}\text{C}$  water and 2 g yeast were added, and the mixing was conducted at a low speed for 1 min,

and continued for another 9 min at a high speed. The smooth dough was fermented at a temperature of 35 °C and 75% RH in a constant temperature incubator (Percival Technology, Perry, IA, USA) for 60 min. The dough was divided to six equal portions and molded into semi-circular forms, which were placed in a plastic sealing box at room temperature for 10 min and then into a steamer and steamed for 30 min. The CSB was cooled at room temperature for 1 h before analysis. Each CSB treatment was prepared in triplicate.

## 2.5. CSB Evaluation

The specific volume ( $\text{cm}^3/\text{g}$ ) by volume displacement method is discussed in [18], and the height–diameter ratio (height/diameter, cm/cm) of the CSB was measured. Sensory evaluation was determined according to a previously published study [19] with some modifications.

The texture analysis of CSB samples was performed using a TMS-Pro texture analyzer (Food Technology Corporation, Sterling, VA, USA) using texture profile analysis (TPA) test mode. A whole CSB (hemispherical, height  $\times$  diameter = 4.5 cm  $\times$  7.5 cm) sample was placed in the center of the platform and compressed by a pressure plate probe  $p/75$  mm at the speed of 30 mm/min to 50% of the original thickness, with a pre-test speed of 30 mm/min and a post-test speed of 30 mm/min. The TPA test starting point's trigger force was 1 N at a data acquisition frequency of 100 Hz. All of the experiments were performed three times at room temperature. The parameters obtained from the TPA experiment were cohesiveness, hardness, gumminess, springiness, and chewiness.

## 2.6. Statistical Analysis

All measurements were performed at least three times. The results were analyzed using SPSS 17.0 (Chicago, IL, USA) and are expressed as mean  $\pm$  SD (standard deviation). We used one-way analysis of variance (ANOVA) to compare the treatment data. Duncan's test was used to compare the differences among means, and a  $p < 0.05$  was considered to indicate a significant difference. Simca13.0 (Umetrics, Umea, Sweden) was used to perform orthogonal partial least squares discriminant analysis (OPLS-DA) and to calculate the variable influence on projection (VIP) values.

# 3. Results and Discussion

## 3.1. Height–diameter Ratio and Specific Volume of the CSB

The height–diameter ratio measurement results of CSB are shown in TPA 2. The different amounts of the different foxtail millet flour cultivars added to CSB affected the final product. The height–diameter ratio of CSB significantly differed ( $p < 0.05$ ) in accordance with the different additions. The differences appeared in the height–diameter ratio of the same variety of millet and in different additive amounts, except for in the cases of TX 17 and A 11-5365. The height–diameter ratio represents the CSB's shape. The bread forms a uniform sponge-like hole during the steaming process, which props up its fullness without producing wrinkles [12]. The height–diameter ratio of the glutinous varieties was consistent, and that of the glutinous cultivar N101 was 0.82 when the addition was 20%, which was significantly lower than that of the other cultivars. This may have occurred due to the different contents of amylose and amylopectin in the powder. After gelatinization and expansion, the starch filled the gluten protein's skeletal structure in a non-uniform manner and, thus, could not support it, resulting in partial collapse and agglomeration. This made the shape of the CSB unacceptable and altered the height–diameter ratio.

The specific volume measurement results of CSB are shown in Table 2. The specific volumes of the different cultivars of WFMF added to CSB are significantly different ( $p < 0.05$ ). Within the same cultivar, the CSB specific volume decreased with an increased amount of WFMF ( $p < 0.05$ ). Specific volume indicates the degree of CSB fluffiness and reflects the gas production within the dough [20]. The specific volume of the commercially available CSB standard is generally  $>1.7 \text{ cm}^3/\text{g}$ . The theoretical addition of WFMF is within 30%. The specific volume of CSB is related to the gas-holding property of the dough [21]. As

the addition of WFMF increased, the gluten content in the dough decreased, ductility and springiness decreased, the internal voids of CSB were reduced and the specific volume decreased [22]. Usually, adding WFMF reduces the specific volume of CSB, and different cultivars of WFMF can change the specific volume of CSB. The results showed that the reduction in the glutinous cultivar was minimal, indicating that the gas-holding capacity of the japonica cultivars was lower than that of the glutinous cultivars.

The sensory evaluation results of CSB are shown in Table 2. The sensory evaluations of the different cultivars of WFMF added to CSB are not significantly different ( $p < 0.05$ ). Within the same cultivar, the CSB sensory evaluation increased at first and then decreased with an increased amount of WFMF ( $p < 0.05$ ). Almost every cultivar with the 30% addition showed better sensory evaluation, except when the additive amount was 0%. Sensory evaluation is a form of subjective, human evaluation, including appearance, smell, and taste. However, it reflects the preferences of consumers to a certain extent. In the sensory evaluation index, smell and taste are negatively correlated with hardness, and total acceptance is positively correlated with springiness and negatively correlated with hardness [18]. Moreover, because the color of steamed bread is bright, the varieties of whole grains with a minimal addition showed similarly high sensory evaluation scores, and it could be observed that the brightness of appearance is the main indicator.

### 3.2. CSB Texture Analysis

The hardness measurement results of CSB are shown in Table 3. The hardness values of the various CSB treatments were significantly different ( $p < 0.05$ ). Hardness refers to the maximum force peak of the first compression cycle. Under the same added amount of different cultivars, the difference in the hardness of CSB of the same cultivar WFMF was significant ( $p < 0.05$ ). With increasing amounts of WFMF, the hardness of most CSB samples increased by more than 50%. Among these, the hardness of YG 18, J 0626-4, and CF 1 reached  $(83.66 \pm 9.09)$  N,  $(82.41 \pm 10.10)$  N and  $(88.41 \pm 8.26)$  N, respectively. In contrast, under the same addition, glutinous cultivars FT 5 and N 101 only showed a moderate increase in hardness. This may be related to the different contents of amylose, as a high content of amylose causes the CSB to become hard. CSB with high toughness and high elasticity has a better taste. Hardness is an important sensory indicator that reflects the quality of CSB, and it affects consumer evaluations. CSB hardness is generally negatively correlated with CSB quality [23]. Increased hardness lowers the texture quality of CSB and affects springiness and chewiness.

The results of the CSB cohesiveness measurement are shown in Table 3. Cohesiveness was significantly different among treatments when the same amounts of different cultivars of WFMF were added ( $p < 0.05$ ). Cohesiveness is the contraction force inside the sample. It represents how well the product withstands a second deformation relative to its resistance under the first deformation (the larger the value, the stronger the cohesiveness and the less likely the sample will be destroyed by extrusion). Within the same cultivar of WFMF when the added amount was increased, the cohesiveness significantly decreased ( $p < 0.05$ ). WFMF contains relatively less gluten protein than that of wheat flour [24,25], so the greater the amount added, the lower the stability of the gluten network structure in the dough. Therefore, as the addition of WFMF increased, the cohesiveness significantly decreased. Cohesiveness may be related to the synergistic effect of the gluten protein network structure and the gelatinized starch in the steaming process [26]. The glutenin and gliadin crosslink through the disulfide bond to generate the gluten network structure. The gelatinized starch can be better wrapped, and the gas produced is maintained. The addition of WFMF caused an imbalance of gluten and starch. The gluten protein was insufficient to wrap the gelatinized starch. The starch compromised the protein network structure during the second compression test and made it difficult for the CSB cross-linking structure to rebound to its original shape. As the addition of WFMF increased, the springiness and cohesiveness decreased.

**Table 2.** Effect of different cultivars and addition of whole foxtail millet flour on height–diameter ratio, specific volume, and sensory evaluation of CSB ( $n = 3$ ).

Level (%)	Japonica					Glutinous		
	JG19	TX17	YG18	A11-5365	J0626-4	CF1	FT5	N101
Height–Diameter Ratio								
0	0.87 ± 0.05 Aa	0.87 ± 0.05 Aa	0.87 ± 0.05 Aab	0.87 ± 0.05 Aa	0.87 ± 0.05 Aab	0.87 ± 0.05 Ab	0.87 ± 0.05 Aab	0.87 ± 0.05 Aa
10	0.87 ± 0.01 Aa	0.90 ± 0.07 Aa	0.86 ± 0.02 Aab	0.85 ± 0.01 Aa	0.86 ± 0.03 Aab	0.86 ± 0.01 Ab	0.92 ± 0.03 Aa	0.82 ± 0.02 Aab
20	0.81 ± 0.03 ABab	0.82 ± 0.03 ABa	0.83 ± 0.01 ABab	0.84 ± 0.03 Aa	0.88 ± 0.05 Aa	0.89 ± 0.04 Ab	0.84 ± 0.03 Ab	0.82 ± 0.10 ABab
30	0.79 ± 0.03 Cab	0.85 ± 0.02 BCa	0.87 ± 0.02 ABa	0.81 ± 0.03 BCa	0.81 ± 0.01 BCb	0.93 ± 0.07 Aab	0.88 ± 0.01 ABab	0.84 ± 0.03 BCab
40	0.79 ± 0.02 Bab	0.86 ± 0.01 Ba	0.81 ± 0.03 Bb	0.82 ± 0.01 Ba	0.84 ± 0.03 Bab	0.95 ± 0.08 Aab	0.85 ± 0.03 Bb	0.86 ± 0.00 Ba
50	0.83 ± 0.02 Cb	0.83 ± 0.01 Ca	0.84 ± 0.02 BCab	0.82 ± 0.03 Ca	0.84 ± 0.02 BCab	1.00 ± 0.04 Aa	0.86 ± 0.01 BCab	0.89 ± 0.00 Ba
Specific Volume (cm <sup>3</sup> /g)								
0	2.25 ± 0.15 Aa	2.25 ± 0.15 Aa	2.25 ± 0.15 Aa	2.25 ± 0.15 Aa	2.25 ± 0.15 Aa	2.25 ± 0.15 Aa	2.25 ± 0.15 Aa	2.25 ± 0.15 Aa
10	2.10 ± 0.10 Aab	2.09 ± 0.04 Aab	1.52 ± 0.12 Bb	1.96 ± 0.14 Ab	1.91 ± 0.17 Ab	2.02 ± 0.08 Aab	1.92 ± 0.11 Ab	2.03 ± 0.20 Aab
20	2.00 ± 0.15 Aab	1.93 ± 0.14 ABb	1.54 ± 0.11 Cb	1.97 ± 0.03 ABb	1.84 ± 0.12 ABbc	1.74 ± 0.08 BCbc	1.87 ± 0.03 ABbc	1.98 ± 0.04 ABb
30	1.90 ± 0.08 Ab	1.58 ± 0.08 Bc	1.58 ± 0.10 Bb	1.75 ± 0.06 ABc	1.88 ± 0.05 Abc	1.74 ± 0.18 ABbc	1.78 ± 0.03 ABbcd	1.87 ± 0.02 Abc
40	1.56 ± 0.07 BCc	1.50 ± 0.07 Cc	1.67 ± 0.08 ABCb	1.60 ± 0.06 ABCc	1.64 ± 0.07 ABCcd	1.75 ± 0.14 Abc	1.69 ± 0.01 ABcd	1.77 ± 0.04 Abc
50	1.46 ± 0.09 BCc	1.38 ± 0.08 Cc	1.52 ± 0.07 ABCb	1.60 ± 0.05 ABc	1.56 ± 0.02 ABd	1.65 ± 0.09 Ac	1.66 ± 0.05 Ad	1.68 ± 0.04 Ac
Sensory Evaluation								
0	88.10 ± 2.46 Aa	88.10 ± 2.46 Aa	88.10 ± 2.46 Aa	88.10 ± 2.46 Aa	88.10 ± 2.46 Aa	88.10 ± 2.46 Aa	88.10 ± 2.46 Aa	88.10 ± 2.46 Aa
10	76.67 ± 3.46 Ac	70.33 ± 4.63 Acd	76.70 ± 3.14 Ab	74.33 ± 5.61 Ab	72.00 ± 3.84 Acd	74.63 ± 2.80 Ab	74.20 ± 4.27 Ab	75.13 ± 5.46 Acd
20	75.90 ± 3.03 ABc	80.33 ± 3.88 ABab	78.27 ± 3.67 ABb	74.13 ± 2.25 Bb	79.77 ± 4.98 ABabc	80.77 ± 4.15 ABab	78.30 ± 3.73 ABb	81.40 ± 2.10 Aabc
30	84.13 ± 3.69 Aab	85.40 ± 0.95 Aab	85.77 ± 4.39 Aa	86.43 ± 4.61 Aa	81.93 ± 2.18 Aab	81.07 ± 3.88 Aab	85.4 ± 3.73 Aa	85.20 ± 5.66 Aab
40	80.47 ± 5.00 Abc	77.83 ± 5.51 Abc	74.90 ± 2.19 Abc	75.03 ± 3.56 Ab	73.97 ± 4.93 Abcd	75.23 ± 4.71 Ab	78.83 ± 2.74 Ab	78.67 ± 0.65 Abcd
50	66.10 ± 1.82 Ad	67.00 ± 6.08 Ad	69.93 ± 1.48 Ac	68.07 ± 6.20 Ab	66.27 ± 6.98 Ad	64.90 ± 6.07 Ac	74.03 ± 2.12 Ab	71.87 ± 3.37 Ad

Note: The data in the table are the mean ± SD. Different uppercase letters in the same row indicate that there is a significant difference in mean ( $p < 0.05$ ). Different lowercase letters in the same column indicate that there is a significant difference in mean ( $p < 0.05$ ).

**Table 3.** Effect of different cultivars and addition of whole foxtail millet flour on texture properties of CSB ( $n = 3$ ).

Level (%)	Japonica				Glutinous			
	JG19	TX17	YG18	A11-5365	J0626-4	CF1	FT5	N101
Hardness (N)								
0	36.17 ± 3.26 <sup>Ad</sup>	36.17 ± 3.26 <sup>Ac</sup>	36.17 ± 3.26 <sup>Ae</sup>	36.17 ± 3.26 <sup>Ad</sup>	36.17 ± 3.26 <sup>Ae</sup>	36.17 ± 3.26 <sup>Ad</sup>	36.17 ± 3.26 <sup>Ac</sup>	36.17 ± 3.26 <sup>Ab</sup>
10	38.16 ± 0.49 <sup>Dd</sup>	45.87 ± 3.47 <sup>BCb</sup>	40.97 ± 1.45 <sup>CDde</sup>	37.12 ± 2.41 <sup>Dd</sup>	40.70 ± 2.87 <sup>CDde</sup>	47.24 ± 3.00 <sup>Bcd</sup>	57.76 ± 5.38 <sup>Aa</sup>	36.69 ± 1.48 <sup>Db</sup>
20	48.23 ± 6.56 <sup>Bc</sup>	45.75 ± 3.76 <sup>BCb</sup>	48.61 ± 2.78 <sup>Bcd</sup>	47.29 ± 2.71 <sup>BCc</sup>	51.12 ± 2.96 <sup>ABcd</sup>	58.98 ± 7.87 <sup>Abc</sup>	49.00 ± 0.21 <sup>Bab</sup>	39.23 ± 2.78 <sup>Cb</sup>
30	75.30 ± 4.27 <sup>Aa</sup>	49.48 ± 3.27 <sup>Cb</sup>	55.87 ± 6.48 <sup>BCbc</sup>	52.39 ± 2.99 <sup>Bbc</sup>	54.28 ± 4.93 <sup>Cc</sup>	65.07 ± 2.42 <sup>Bb</sup>	48.75 ± 9.41 <sup>Cab</sup>	46.51 ± 6.38 <sup>Cab</sup>
40	72.44 ± 7.55 <sup>ABab</sup>	75.05 ± 5.97 <sup>Aa</sup>	60.80 ± 5.30 <sup>BCDb</sup>	59.23 ± 4.11 <sup>BCDab</sup>	69.05 ± 5.88 <sup>ABCb</sup>	84.01 ± 18.81 <sup>Aa</sup>	43.58 ± 7.01 <sup>Dbc</sup>	53.76 ± 8.58 <sup>CDa</sup>
50	63.56 ± 3.22 <sup>BCb</sup>	76.42 ± 5.49 <sup>Aa</sup>	83.66 ± 9.09 <sup>Aa</sup>	64.23 ± 9.87 <sup>BCa</sup>	82.41 ± 10.10 <sup>Aa</sup>	88.41 ± 8.26 <sup>Aa</sup>	39.43 ± 1.05 <sup>Dbc</sup>	52.86 ± 6.08 <sup>CDa</sup>
Cohesiveness								
0	0.77 ± 0.01 <sup>Aa</sup>	0.77 ± 0.01 <sup>Aa</sup>	0.77 ± 0.01 <sup>Aa</sup>	0.77 ± 0.01 <sup>Aa</sup>	0.77 ± 0.01 <sup>Aa</sup>	0.77 ± 0.01 <sup>Aa</sup>	0.77 ± 0.01 <sup>Aa</sup>	0.77 ± 0.01 <sup>Aa</sup>
10	0.74 ± 0.01 <sup>BCb</sup>	0.74 ± 0.02 <sup>BCb</sup>	0.76 ± 0.01 <sup>Aba</sup>	0.77 ± 0.01 <sup>Aba</sup>	0.77 ± 0.02 <sup>Aa</sup>	0.75 ± 0.01 <sup>ABCb</sup>	0.74 ± 0.02 <sup>Cb</sup>	0.77 ± 0.01 <sup>Aa</sup>
20	0.71 ± 0.01 <sup>Dc</sup>	0.73 ± 0.01 <sup>ABb</sup>	0.74 ± 0.01 <sup>Ab</sup>	0.73 ± 0.00 <sup>ABCb</sup>	0.73 ± 0.01 <sup>ABCDb</sup>	0.72 ± 0.01 <sup>CDc</sup>	0.74 ± 0.01 <sup>Ab</sup>	0.72 ± 0.00 <sup>BCDb</sup>
30	0.67 ± 0.02 <sup>Cd</sup>	0.70 ± 0.01 <sup>ABc</sup>	0.72 ± 0.01 <sup>Ac</sup>	0.71 ± 0.00 <sup>Ab</sup>	0.68 ± 0.02 <sup>BCc</sup>	0.68 ± 0.01 <sup>BCd</sup>	0.69 ± 0.01 <sup>Bc</sup>	0.69 ± 0.00 <sup>Bc</sup>
40	0.61 ± 0.03 <sup>Df</sup>	0.65 ± 0.00 <sup>ABCd</sup>	0.67 ± 0.01 <sup>ABd</sup>	0.68 ± 0.01 <sup>Ac</sup>	0.64 ± 0.02 <sup>CDd</sup>	0.66 ± 0.03 <sup>ABCd</sup>	0.64 ± 0.02 <sup>BCd</sup>	0.65 ± 0.02 <sup>ABCd</sup>
50	0.64 ± 0.01 <sup>ABe</sup>	0.59 ± 0.02 <sup>Ce</sup>	0.61 ± 0.03 <sup>BCe</sup>	0.67 ± 0.04 <sup>Ac</sup>	0.59 ± 0.03 <sup>Ce</sup>	0.62 ± 0.02 <sup>BCe</sup>	0.61 ± 0.02 <sup>BCe</sup>	0.58 ± 0.01 <sup>Ce</sup>
Springiness (mm)								
0	5.65 ± 0.08 <sup>Aa</sup>	5.65 ± 0.08 <sup>Aa</sup>	5.65 ± 0.08 <sup>Aa</sup>	5.65 ± 0.08 <sup>Aa</sup>	5.65 ± 0.08 <sup>Aa</sup>	5.65 ± 0.08 <sup>Aa</sup>	5.65 ± 0.08 <sup>Aa</sup>	5.65 ± 0.08 <sup>Aa</sup>
10	5.51 ± 0.14 <sup>Aab</sup>	5.52 ± 0.68 <sup>Aa</sup>	5.73 ± 0.07 <sup>Aa</sup>	5.30 ± 0.29 <sup>Aa</sup>	5.47 ± 0.28 <sup>Aa</sup>	5.75 ± 0.31 <sup>Aa</sup>	5.51 ± 0.12 <sup>Aab</sup>	5.51 ± 0.31 <sup>Aab</sup>
20	5.70 ± 0.21 <sup>Aa</sup>	5.50 ± 0.26 <sup>Aa</sup>	5.51 ± 0.66 <sup>Aab</sup>	5.78 ± 0.23 <sup>Aa</sup>	5.50 ± 0.24 <sup>Aa</sup>	5.62 ± 0.11 <sup>Aa</sup>	5.44 ± 0.19 <sup>Aab</sup>	5.44 ± 0.27 <sup>Aab</sup>
30	5.27 ± 0.16 <sup>Abc</sup>	5.48 ± 0.08 <sup>Aa</sup>	5.48 ± 0.30 <sup>Aab</sup>	5.63 ± 0.93 <sup>Aa</sup>	5.55 ± 0.27 <sup>Aa</sup>	5.47 ± 0.09 <sup>Aa</sup>	5.20 ± 0.17 <sup>Ac</sup>	5.35 ± 0.28 <sup>Aab</sup>
40	5.15 ± 0.11 <sup>Ac</sup>	5.04 ± 0.45 <sup>Aa</sup>	5.49 ± 0.27 <sup>Aab</sup>	5.43 ± 0.05 <sup>Aa</sup>	5.42 ± 0.30 <sup>Aa</sup>	5.37 ± 0.45 <sup>Aa</sup>	5.41 ± 0.03 <sup>Abc</sup>	5.18 ± 0.40 <sup>Aab</sup>
50	5.12 ± 0.16 <sup>ABc</sup>	5.03 ± 0.37 <sup>ABa</sup>	5.08 ± 0.17 <sup>ABb</sup>	5.23 ± 0.18 <sup>Aa</sup>	5.15 ± 0.16 <sup>ABa</sup>	5.31 ± 0.11 <sup>Aa</sup>	4.78 ± 0.12 <sup>Bd</sup>	4.96 ± 0.11 <sup>ABb</sup>
Gumminess (N)								
0	27.91 ± 2.37 <sup>Ad</sup>	27.91 ± 2.37 <sup>Ac</sup>	27.91 ± 2.37 <sup>Ad</sup>	27.91 ± 2.37 <sup>Ad</sup>	27.91 ± 2.37 <sup>Ac</sup>	27.91 ± 2.37 <sup>Ac</sup>	27.91 ± 2.37 <sup>Ab</sup>	27.91 ± 2.37 <sup>Ab</sup>
10	28.43 ± 0.35 <sup>Dd</sup>	34.05 ± 1.94 <sup>BCb</sup>	31.29 ± 0.64 <sup>CDcd</sup>	28.32 ± 1.62 <sup>Dd</sup>	31.36 ± 1.61 <sup>CDbc</sup>	35.30 ± 2.32 <sup>Bbc</sup>	42.66 ± 3.03 <sup>Aa</sup>	28.21 ± 1.48 <sup>Db</sup>
20	34.28 ± 4.08 <sup>BCc</sup>	33.49 ± 2.87 <sup>BCDb</sup>	36.02 ± 1.88 <sup>Bbc</sup>	34.52 ± 1.87 <sup>BCc</sup>	37.12 ± 1.75 <sup>ABb</sup>	42.19 ± 5.14 <sup>Ab</sup>	28.95 ± 0.62 <sup>CDb</sup>	28.22 ± 2.01 <sup>Db</sup>
30	50.13 ± 1.88 <sup>Aa</sup>	34.68 ± 1.84 <sup>DEb</sup>	39.95 ± 4.15 <sup>BCb</sup>	37.28 ± 2.09 <sup>CDbc</sup>	37.13 ± 2.46 <sup>CDb</sup>	44.29 ± 2.14 <sup>Bb</sup>	33.82 ± 0.45 <sup>DEb</sup>	32.00 ± 4.45 <sup>Eab</sup>
40	43.80 ± 3.12 <sup>BCb</sup>	48.86 ± 3.63 <sup>ABa</sup>	40.97 ± 3.45 <sup>BCb</sup>	40.11 ± 2.49 <sup>BCab</sup>	43.82 ± 2.84 <sup>BCa</sup>	54.98 ± 10.37 <sup>Aa</sup>	27.89 ± 3.85 <sup>Db</sup>	34.73 ± 4.92 <sup>CDa</sup>
50	40.31 ± 2.17 <sup>Cb</sup>	45.35 ± 1.79 <sup>BCa</sup>	51.19 ± 7.28 <sup>ABa</sup>	42.72 ± 4.40 <sup>BCa</sup>	48.62 ± 4.66 <sup>ABCa</sup>	55.18 ± 4.03 <sup>Aa</sup>	29.54 ± 4.93 <sup>Db</sup>	30.76 ± 2.90 <sup>Dab</sup>
Chewiness (mJ)								
0	157.70 ± 12.26 <sup>Ad</sup>	157.70 ± 12.26 <sup>Ac</sup>	157.70 ± 12.26 <sup>Ad</sup>	157.70 ± 12.26 <sup>Abc</sup>	157.70 ± 12.26 <sup>Ac</sup>	157.70 ± 12.26 <sup>Ac</sup>	157.70 ± 12.26 <sup>Ab</sup>	157.70 ± 12.26 <sup>Aa</sup>
10	156.75 ± 5.79 <sup>Dd</sup>	188.24 ± 29.09 <sup>BCbc</sup>	179.47 ± 4.49 <sup>BCDbcd</sup>	150.4 ± 16.86 <sup>Dc</sup>	171.34 ± 2.91 <sup>CDc</sup>	203.27 ± 23.87 <sup>Bbc</sup>	235.14 ± 19.58 <sup>Aa</sup>	155.54 ± 15.98 <sup>Da</sup>
20	188.58 ± 25.97 <sup>BCDcd</sup>	184.66 ± 24.01 <sup>BCDc</sup>	199.34 ± 34.01 <sup>ABCcd</sup>	199.88 ± 17.40 <sup>ABCab</sup>	203.85 ± 3.38 <sup>ABb</sup>	236.91 ± 26.68 <sup>Aab</sup>	157.52 ± 4.84 <sup>CDb</sup>	154.07 ± 18.12 <sup>Da</sup>

Table 3. Cont.

Level (%)	Japonica					Glutinous		
	JG19	TX17	YG18	A11-5365	J0626-4	CF1	FT5	N101
40	225.49 ± 18.13 <sup>ABb</sup>	246.84 ± 34.85 <sup>ABa</sup>	225.55 ± 28.9 <sup>ABab</sup>	217.96 ± 14.97 <sup>ABa</sup>	237.73 ± 23.95 <sup>ABa</sup>	298.12 ± 76.19 <sup>Aa</sup>	150.69 ± 20.29 <sup>Cb</sup>	179.92 ± 27.75 <sup>BCa</sup>
50	206.68 ± 17.35 <sup>Cbc</sup>	227.64 ± 7.49 <sup>BCab</sup>	260.61 ± 45.96 <sup>ABa</sup>	223.74 ± 28.44 <sup>BCa</sup>	250.22 ± 16.61 <sup>ABCa</sup>	293.33 ± 23.95 <sup>Aa</sup>	141.41 ± 26.97 <sup>Db</sup>	152.41 ± 14.98 <sup>Da</sup>

Note: The data in the table are the mean ± SD. Different uppercase letters in the same row indicate that there is a significant difference in mean ( $p < 0.05$ ). Different lowercase letters in the same column indicate that there is a significant difference in mean ( $p < 0.05$ ).



The springiness measurement results of the CSB are shown in Table 3. The springiness of CSB among the different cultivars was not significantly different ( $p > 0.05$ ), except for when the additive amount was 50%. The difference in the japonica and glutinous cultivars was significant from 10% to 50% ( $p < 0.05$ ), and the value was between 4.78 and 5.75. Springiness measures the elasticity extent of recovery between the first and second compressions and indicates the ability of a substance to return to its original shape after an external force has been removed. The springiness of CSB is a side effect of its softness. Depending on the gluten content and the gas-holding capacity of CSB [12], springiness affects the choice of consumers. The predominant gluten proteins in wheat flour are glutenin and gliadin. Glutenin determines the springiness and extensibility of gluten [25]. The protein content in millet is low, and adding WFME reduced the total gluten content and decreased the CSB's springiness. This may be due to the increase in the additive amount, which increased the fiber content, and the decrease in gliadins and glutenins in dough, which led to a decrease in fermentation and ductility [27].

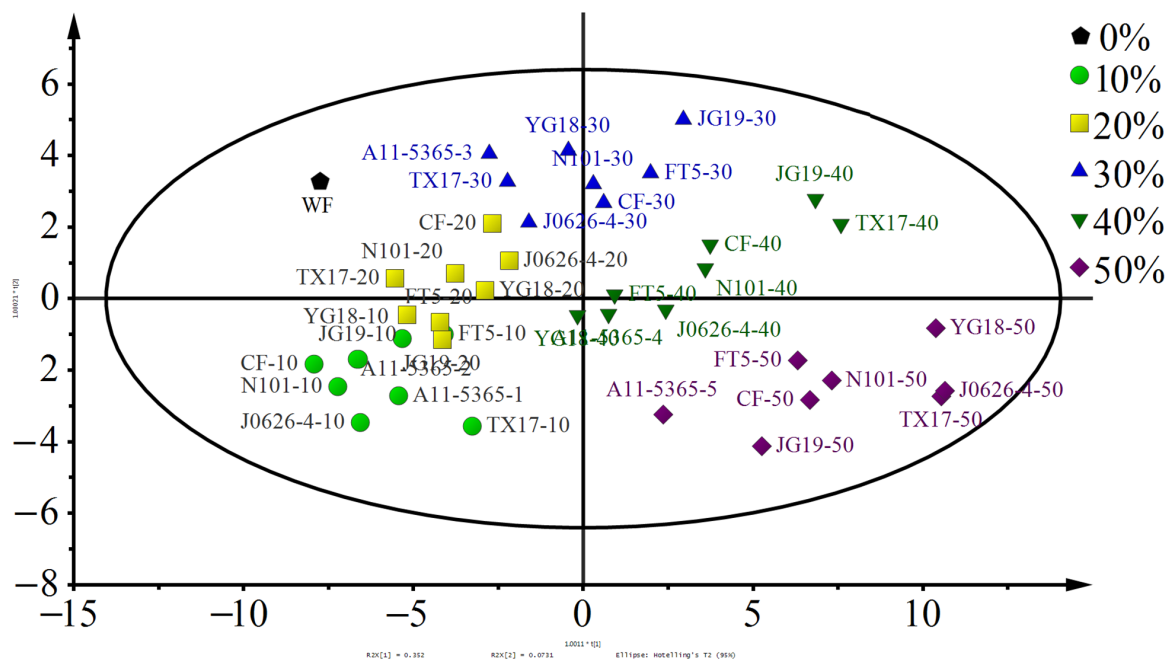
The results of the measurement of the gumminess of CSB are shown in Table 3. There was a significant difference in the gumminess of CSB when the same amounts of different cultivars of WFME were added ( $p < 0.05$ ). Gumminess describes the multiplication of hardness and cohesiveness and refers to the energy required to break down a semi-solid food before it is swallowed. As the addition of japonica WFME increased, the gumminess significantly increased ( $p < 0.05$ ). However, in the glutinous cultivar CSB, the gumminess value and the change trend in CF 1 were similar to those in the japonica CSB. The gumminess values of FT 5 and N101 were lower than those of japonica CSB. The gumminess of CSB may be related to the nature of the starch that it contains. The gelatinization properties of starch are mainly determined by its size, proportion, and content of amylose [28]. The starch granules gelatinized and expanded, and the gluten protein supported the structure of the CSB. A previous study found that the higher the amylopectin content, the lower the adhesiveness of brown rice bread [29]. Brown rice and oat substitution significantly increased the gumminess of CSB [30]. In a previous study, the final viscosity of buckwheat mixed powder was positively correlated with the hardness and gumminess of buckwheat steamed bread [31], which is consistent with the results of this study.

The chewiness results of CSB are shown in Table 3. The different cultivars of WFME added to CSB produced significantly different chewiness values ( $p < 0.05$ ). The japonica cultivars produced better results than those of the glutinous cultivars. There was a significant difference related to the amount of added millet flour ( $p < 0.05$ ). Chewiness is the energy required to chew a solid sample. With the increase in the additive amount of WFME, the chewiness of the CSB increased in different cultivars. As the amount of WFME from cultivar FT 5 increased, the CSB's chewiness first increased and then decreased. The chewiness decreased to 141.41 mJ when the amount added was 50%. Chewiness reflects the amount of energy required to chew a food into a swallowable state and it is correlated with cohesiveness and springiness [30]. The addition of whole quinoa flour is positively correlated with the chewiness of steamed bread [9,32], findings which are consistent with the results of the current study. Previous findings regarding the chewiness of buckwheat bread are also consistent with the results of the current study. A 30% brown rice and oat substitution significantly increased the gumminess of CSB [30]. The addition of a certain proportion of WFME maintains the water absorption and water-holding capacity of dough [33]. The gluten network structure has good cross-linking properties, suitable hardness and acceptable chewiness. However, when the addition of WFME is excessive, too many millet starch granules will compete with the protein and starch in the wheat flour, thereby weakening the gluten network structure. This results in an increase in hardness and chewiness.

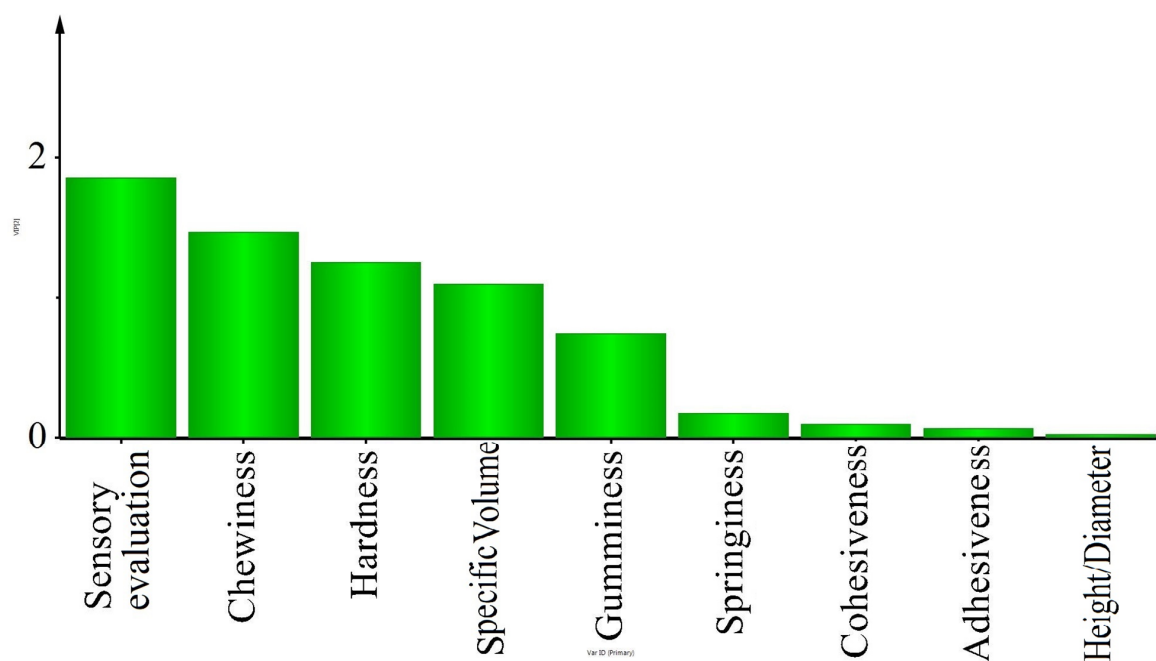


### 3.3. OPLS-DA of CSB

First, we performed OPLS-DA, as shown in Figure 1a, which is an objective method by which classification can be conducted and the trend among samples can be observed. We constructed this model by adding the texture properties and evaluation of the steamed bread to each group. The model fit the independent variable  $R^2X$  (cum) = 0.996,  $R^2Y$  (cum) = 0.365, and  $Q^2$  (cum) = 0.196, which indicates that it has a reliable predictive ability. The results show that the model was stable. The obvious aggregation tendency is reflected in the sample population, achieving better separation.



(a)



(b)

Figure 1. Cont.

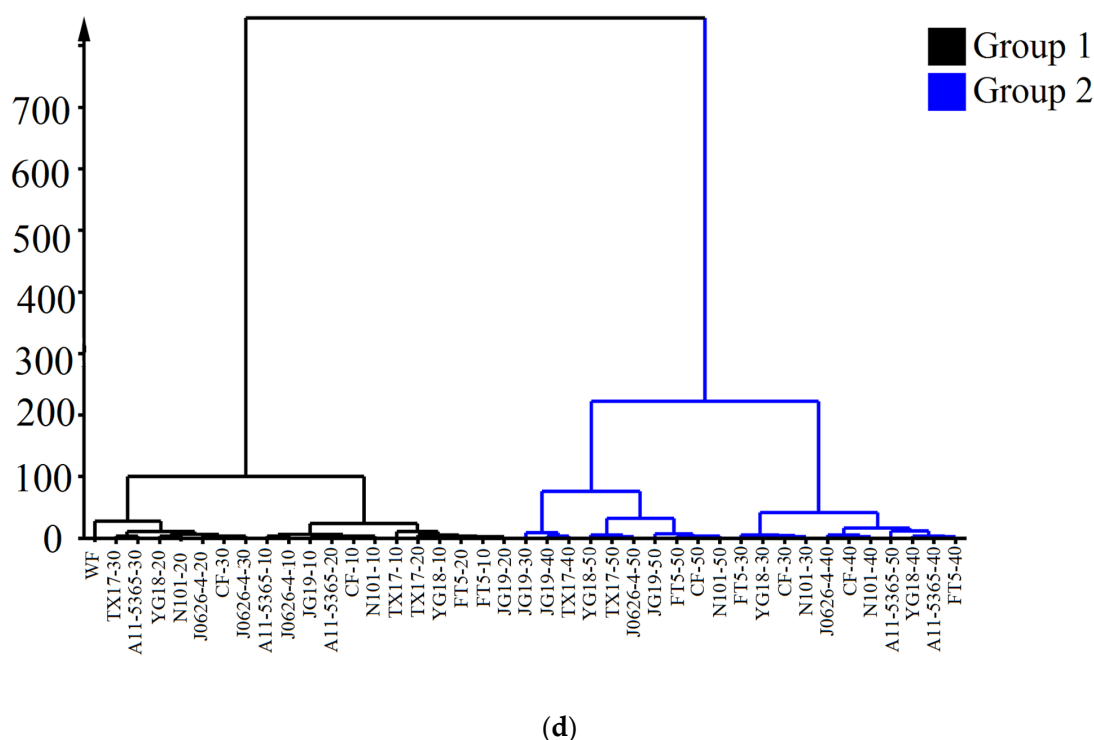
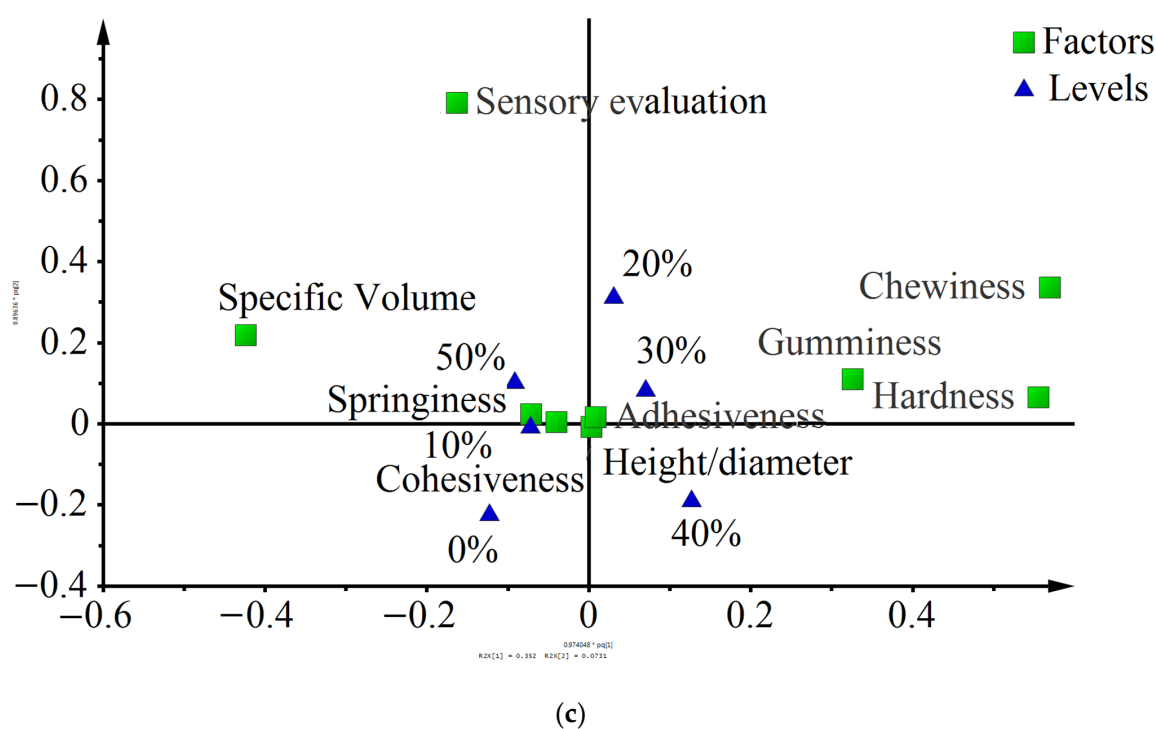


Figure 1. (a) OPLS-DA score plot; (b) VIP; (c) loading plot; (d) clustering results.

The VIP value was detected to further determine which variables significantly contribute to the OPLS-DA prediction model. Sensory evaluation, chewiness, specific volume, and hardness ( $VIP > 1$ ) were considered to be contribution indicators of CSB (Figure 1b). The loading-plot diagram in Figure 1c demonstrates that the addition level indicators gathered near the origin, whereas some evaluation indicators that had a considerable contribution to the prediction of the model classification were scattered at the two ends of the plot. The loading-plot diagram supports the determination of the VIP value.

The clustering results of different cultivars and different additive amounts, as shown in Figure 1d, indicate that they could be divided into two groups: CSB with mostly 10–30% cultivars and that with mostly addition levels of 40–50%. This indicates that the addition level is the key to the production of CSB. With an increase in the total amount of WFMF, all cultivars can cause the volume to decrease. The molecular structure of amylopectin destroyed the network structure during the manufacturing process for CSB [34] due to the higher content of amylopectin in the glutinous cultivars. Compared with the japonica cultivar, glutinous CSB exhibited increasing hardness and chewiness and a large reduction in volume [35]. When the amount of WFMF added was 20–30%, CSB had improved evaluation and texture properties. When the addition of all millet cultivars exceeded 40%, the CSB's specific volume and texture were considerably degraded.

#### 4. Conclusions

The effects of cultivars and the addition of various amounts of WFMF on the quality characteristics of CSB were studied. The specific volume and score of sensory evaluation significantly decreased as the amount of WFMF increased and the height–diameter ratio significantly changed. As the amount of WFMF increased, the hardness and chewiness of CSB increased, and the cohesiveness and the springiness gradually decreased. The texture properties of CSB were different to those of the millet cultivars and addition levels. This might be because the large addition causes the dough network structure to lack gluten, and because of the amylose content of the different cultivars. OPLS-DA was performed to carry out the classification and examine the trend among the samples. Four indicators (VIP > 1), sensory evaluation, chewiness, specific volume, and hardness, were considered to be the contribution indicators of CSB. All samples were divided into two groups by cluster analysis. The cultivars at an addition level of 20–30% featured improved quality and resulted in good sensory evaluation, which could provide a theoretical basis for the industrialization of steamed bread made of whole foxtail millet flour.

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