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Research Article

Production of Foxtail-Millet-Based Composite Antioxidant Nutritive Flour Using Coarse Grains and Fruit Flour

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Antioxidants are primarily responsible for the beneficial health effects of foxtail millet. This study evaluated a foxtail-millet-based composite antioxidant nutritive flour consisting of fermented foxtail millet, coarse grains, and fruit powders. The composition of the antioxidant nutritive flour was optimized, and it was found to be rich in antioxidant nutrients, such as phenolics (2.27 g/100 g), vitamin C (0.21 g/100 g), and polysaccharides (0.35 g/100 g). The results revealed that this composite nutritive flour has enhanced in vitro and in vivo physicochemical properties and higher antioxidant activities than commercial nutritive flour. The total antioxidant capability, superoxide dismutase (SOD) capability, and superoxide radical scavenging abilities were 0.84 mM Trolox equivalents/g, 38.29 U/g, and 42.02%, respectively. The activity of the antioxidant enzyme SOD and glutathione peroxidase increased, whereas malondialdehyde levels decreased in the liver, heart, and kidney of mice treated with the nutritive flour, indicating the potential antioxidant activity of this fermented foxtail-millet-based nutritive flour.

1. Introduction

Foxtail millet (*Setaria italica* (L.) P. Beauv.), a member of the *Poaceae* family and *Panicoideae* subfamily, is one of the earliest cultivated crops that is believed to have originated in northern China [1, 2]. It is widely cultivated in arid and semiarid regions of Asia and Africa owing to its excellent drought and salt tolerance [3, 4]. Foxtail millet is currently cultivated in 26 countries and is classified as a product derived from organic farming using conventional methods and no pesticides [3, 5, 6]. However, it is more commonly used as bird feed in some economically developed countries. Therefore, foxtail millet remains underutilized as a food source.

Foxtail millet contains numerous nutrients that are beneficial for health, such as proteins, fatty acids, fiber, and minerals [7, 8], making it a valuable food source with a unique balance of specific nutrients [3, 9]. Owing to its numerous health benefits, it is often used in China to prepare cereal porridge, pancakes, noodles, and nourishing gruel or soup [9, 10]. Additionally, foxtail millet has several health benefits, including preventing the development of hypoglycemia, hypolipidemia, and cancer [11]. It is frequently used as a nourishing food for pregnant women, postoperative patients, and patients with gastric diseases; moreover, it is used to treat insomnia and hepatopathy in China [12]. However, underinvestment and perceptions of foxtail millet as a livestock feed ingredient have restricted its commercial use, rendering it underexplored worldwide.

The health advantages of foxtail millet are mainly attributed to its antioxidant properties [3, 13]. Antioxidants in plant food materials can prevent genetic damage within the cell or cellular membrane damage by inhibiting free radicals [14, 15]. Therefore, foxtail millet may serve as a natural source of antioxidants as well as a nutraceutical and functional food ingredient for promoting health and reducing disease risk [16]. The preparation of foxtail millet antioxidant foods is crucial because they can support the commercialization of the plant and the economic growth of numerous Asian and African nations. However, the

development of antioxidant foods based on foxtail millet remains limited, and millet-based foods exhibit deficiencies in nutritional and antioxidant properties. Therefore, it is necessary to combine foxtail millet with other ingredients (such as coarse grains and fruits) to improve the nutritional and functional properties of millet-based foods.

Coarse grains, such as oats (*Avena sativa* L.) and rice beans (*Vigna umbellata*), are rich in protein with a balanced amount of essential amino acids, fiber, minerals, and antioxidant compounds (such as phenolic, flavonoid, and sterol compounds) [17–19]. Jujube (*Ziziphus jujuba* Mill.) is rich in flavonoids, polysaccharides, and triterpenic acid, which may be responsible for its antioxidant effects [20]. It was described as one of the five most valuable fruits in the ancient Chinese book on herbal medicine *Huangdi Neijing* (475–221 BC); moreover, it was considered a superior herbal medicine that prolonged the human lifespan by regulating the digestive system, nourishing the blood, and improving the quality of sleep, as mentioned in an earlier book on medicinal herbs (*Shennong Bencao Jing* [300 BC–200 AD]) in China [20]. Additionally, Goji (*Lycium* L.), which is considered a superfruit, is rich in health-promoting properties and chemical compounds, such as phenolic acids, flavonoids, coumarins, tannins, carotenoids, and anthocyanins. These compounds can delay the aging process; improve eyesight, liver, and kidney functions; and exert a positive effect on the well-being and immunity of the body [21, 22]. In this study, a foxtail-millet-based composite antioxidant nutritive flour was evaluated. A potential approach to enhancing the nutritional and functional properties of foxtail millet is to add coarse grains (oats and rice beans) and fruit (jujube and Goji) powder to the foxtail millet powder. This study was aimed at developing a foxtail-millet-based composite nutritive flour with antioxidant properties to expand the potential application of foxtail millet antioxidant foods.

2. Materials and Methods

2.1. Materials and Chemicals. Foxtail millet was obtained from Fengning County (Chengde, China). Oats, rice beans, jujube, Goji, white granulated sugar, and maltodextrin were purchased from the Yuanchen Supermarket (Langfang, China). The commercial nutritive flour was produced by Guangdong Baisha Food Technology Co., Ltd. (Guangdong, China). All commercial kits were obtained from the Jiancheng Bioengineering Institute (Nanjing, China). All other chemicals and reagents were obtained from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Deionized water was used throughout the experiment.

Pleurotus geesteranus was obtained from the Technical Innovation Center for Utilization of Edible and Medicinal Fungi in Hebei Province.

2.2. Solid-State Fermentation of Foxtail Millet. P. geesteranus was cultured on the solid medium of potato dextrose agar until the mycelium was full. Then, 100 mL of liquid potato dextrose broth was used for liquid-state fermentation. *P. geesteranus* mycelia were cultured at 25°C and shaken at 140 rpm for 7 days.

Foxtail millet was soaked for 12–24 h, and excess water was removed from the surface. After sterilization, the millet was inoculated with a homogeneous mycelial suspension (15% [*w*/*v*]) and incubated at 25°C for 31 days. Sterile water was used under identical conditions for control samples. After fermentation, the foxtail millet was heat-dried in a blast-drying oven (DHG-9240A, Shanghai Yiheng Scientific Instrument Co., Ltd., Shanghai, China) for 48 h at 60°C.

2.3. Processing of Raw Materials. The fermented foxtail millet, oat, rice bean, jujube, and Goji were stir-fried for 3–10 min using a kitchen stove at medium heat (180°C) until cooked, ground to powder using an AF-20S-type stainless-steel grinder (Auari, Zhejiang, China), and passed through a 150-mesh sieve (100 μm).

2.4. Sensory Analysis of Antioxidant Nutritive Flour. The foxtail-millet-based composite antioxidant nutritive flour was subjected to sensory evaluation for color, texture, taste, flavor, and overall acceptability by 20 trained panelists using a 9-point hedonic score system with individual scores [23].

2.5. Experimental Design. The effects of the fermented foxtail millet (25%, 30%, 35%, and 40%), jujube (20%, 23%, 26%, and 30%), oat (10%, 15%, 20%, and 25%), rice bean (10%, 13%, 16%, and 20%), and Goji (2%, 4%, 6%, and 8%), along with 12% white granulated sugar and 1.5% maltodextrin, were first investigated using a single-factor experiment design via sensory analysis of the antioxidant nutritive flour.

To further screen the experimental factors, an orthogonal experiment design L_{16} (4^5) was adopted based on the single-factor experiment design. The extraction was conducted using five factors at four levels (Table S1).

2.6. Polysaccharide and Total Soluble Sugar Content. The composite antioxidant nutritive flour was immersed in water (1 : 20, *w*/*v*) at 75°C in a thermostatic water bath (HWS-28, Shanghai Yiheng Scientific Instrument Co., Ltd., Shanghai, China) for 1 h and centrifuged at 5000 × *g* for 10 min. The supernatant was precipitated with 95% ethanol (1 : 4, *v*/*v*) for 30 min and centrifuged for 10 min at 5000 × *g*. The precipitate was dissolved in water, and the polysaccharide content was determined using the phenol sulfuric acid method as described previously [24].

The nutritive flour was dispersed in distilled water (1 : 10, *w*/*v*) at 100°C in a thermostatic water bath (HWS-28, Shanghai, China) for 30 min, and the extracts were collected and adjusted to 100 mL using distilled water. The total soluble sugar content was measured using the anthrone colorimetric method at a wavelength of 620 nm (L4 intelligent UV-Vis spectrophotometer, Shanghai Youke Instrument Co. Ltd., Shanghai, China), as described previously [25].

2.7. Determination of Crude Protein and Fat Content. The Kjeldahl nitrogen determination method was used to determine the flour's crude protein content (N × 6.25), as described previously [26].

Crude fat was extracted from the flour using the Soxhlet apparatus and petroleum ether for 5 h, and the solvent was removed at 50°C using a rotary vacuum evaporator

(Shanghai Rong Biochemical Instrument Co., Ltd., Shanghai, China).

2.8. Vitamin C (Vc) and Total Phenolic Content. Vc was extracted from the nutritive flour using a 2% (*w*/*v*) metaphosphoric acid solution. The mixtures were titrated against a 2,6-dichloroindophenol solution [27].

The total phenolic content of methanolic extracts obtained from the nutritive flour was determined using the Folin-Ciocalteu reagent, as described previously [28]. The phenolic content of the sample was determined based on a standard gallic acid curve. The data were reported as mg gallic acid equivalent (GAE)/100 g of samples (dry weight, DW).

2.9. Physicochemical Properties. Samples were heated in distilled water (1 : 100, *w*/*v*) in a water bath (HWS-28, Shanghai, China) for 30 min at 60°C with constant agitation and were centrifuged for 15 min at 1800 × *g*. The swelling power (SP) was calculated using the following formula [29]:

$$\text{SP (g/g)} = \frac{\text{M2}}{\text{M1}}, \tag{1}$$

where M1 and M2 indicate the dry and wet weights of samples, respectively.

The samples were thoroughly mixed after being dispersed in deionized water (1 : 20, *w*/*v*). The mixture was stored for 24 h at 25°C and centrifuged for 10 min at 2300 × *g*, after which the weight of the pellet was recorded. The water-holding capacity (WHC) was calculated using the following formula [30]:

$$\text{WHC (g/g)} = \frac{\text{M2} - \text{M1}}{\text{M1}}, \tag{2}$$

where M1 and M2 indicate the dry and wet weights of the samples, respectively.

Samples were dispersed in deionized water (1 : 30, *w*/*v*) and thoroughly mixed. The mixture was incubated for 20 h at 25°C, and the volume was recorded after water absorption. The water solubility capacity (WSC) was calculated using the following formula [30]:

$$\text{WSC (mL/mL)} = \frac{\text{V2} - \text{V1}}{\text{V1}}, \tag{3}$$

where V1 is the volume of the dry sample and V2 is the volume of the sample after water absorption.

The samples were dispersed in edible soybean oil (1 : 10, *w*/*v*) and thoroughly mixed. The mixture was maintained at 25°C for 1 h and centrifuged for 20 min at 5000 × *g*. The excess oil was discarded, and the residue was weighed. The oil-holding capacity (OHC) was calculated using the following formula [30]:

$$\text{OHC (g/g)} = \frac{\text{M2} - \text{M1}}{\text{M1}}, \tag{4}$$

where M1 and M2 indicate the weights of the dry sample and the sample after oil absorption, respectively.

2.10. Antioxidant Activity In Vitro. The total antioxidant capability (T-AOC) of polyphenolic extracts was determined using an antioxidant capacity assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The absorbance values (optical density [OD]) at 520 nm were recorded using an intelligent UV-Vis spectrophotometer (L4, Shanghai Youke Instrument Co. Ltd.) to determine the antioxidant capacity of phenolic extracts in the samples.

The superoxide dismutase (SOD) assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) was used to determine the SOD capability of polyphenolic extracts. The absorbance values at 450 nm (L4, Shanghai Youke Instrument Co. Ltd.) were recorded.

The superoxide anion radical ($\cdot O_2^-$) assay of polyphenolic extracts was conducted as previously described [31]. At 5 min, the absorbance of the reaction mixture at 320 nm (L4, Shanghai Youke Instrument Co. Ltd.) was measured. The scavenging rate was calculated as follows:

$$\text{Scavenging rate (\%)} = \left(1 - \frac{\text{A1} - \text{A2}}{\text{A0}}\right) \times 100, \tag{5}$$

where A0 is the autoxidation rate of pyrogallol for the control (the change of the absorbance), A1 is the oxidation rate of pyrogallol in samples, and A2 is the absorbance of the sample blank.

2.11. Animal Experiments to Measure the In Vivo Antioxidant Activity. This study was approved by the Animal Research Committee of Langfang Normal University (Langfang, China). Fifty Institute of Cancer Research (ICR) mice (weight, ~30 g) were purchased from SPE Biotechnology Co., Ltd. (Beijing, China). The mice were housed in a room at ~25°C with 50% humidity and 12 h light/dark. All procedures involving animals were conducted in strict accordance with the rules and guidelines for the use of laboratory animals, as adopted and promulgated by the Institutional Animal Ethical Committee.

The mice were randomly divided into five groups of 10 mice each after 1 week of acclimatization in the laboratory. The naive group (group AC) was provided with a normal chow diet purchased from SPE Biotechnology Co., Ltd. The negative control group (group NC) was provided with a diet made of a mixture of normal chow and unfermented foxtail millet, with a millet content of 400 g/kg processed by SPE Biotechnology Co., Ltd. The positive control group (group PC) was provided with a diet made of a mixture of normal chow and Vc, with a Vc content of 0.2 g/kg processed by SPE Biotechnology Co., Ltd. In addition, the two test groups were provided with a diet made of a mixture of normal chow and composite antioxidant nutritive flour and a mixture of normal chow and commercial grain fruit nutritive flour, respectively, with all of the flour content of 400 g/kg processed by SPE Biotechnology Co., Ltd. All mice were fed for 28 consecutive days, body weight was recorded weekly, and the total amount of feed consumed was

Table 1: Orthogonal experiment results.

Runs	A (foxtail millet) (%)	B (jujube) (%)	C (oat) (%)	D (rice bean) (%)	E (Goji) (%)	Overall
1	1(25)	1(20)	1(10)	1(7)	1(0.5)	7.20 ± 0.09^{f}
2	1	2(23)	2(15)	2(10)	2(1)	7.06 ± 0.01^{g}
3	1	3(26)	3(20)	3(13)	3(2)	7.49 ± 0.09^{de}
4	1	4(30)	4(25)	4(16)	4(3)	7.52 ± 0.07^{de}
5	2(30)	1	2	3	4	7.57 ± 0.11^{cd}
6	2	2	1	4	3	7.54 ± 0.08^{d}
7	2	3	4	1	2	7.26 ± 0.06^{f}
8	2	4	3	2	1	7.51 ± 0.09^{de}
9	3(35)	1	3	4	2	7.58 ± 0.07^{cd}
10	3	2	4	3	1	7.44 ± 0.12^{de}
11	3	3	1	2	4	7.40 ± 0.09^{e}
12	3	4	2	1	3	7.52 ± 0.06^{de}
13	4(40)	1	4	2	3	8.79 ± 0.09^{a}
14	4	2	3	1	4	7.69 ± 0.08^{c}
15	4	3	2	4	1	7.97 ± 0.06^{b}
16	4	4	1	3	2	7.42 ± 0.07^{de}
k1	7.32	7.79	7.39	7.42	7.53	
k2	7.47	7.43	7.53	7.69	7.33	
k3	7.49	7.53	7.57	7.48	7.84	
k4	7.97	7.49	7.57	7.65	7.55	
R	0.65	0.35	0.36	0.27	0.51	

Results are presented as means ($n = 20$) ± standard deviations. Values carrying different letters in the same column are significantly different ($P < 0.05$).

recorded daily during the experiment. All animals were sacrificed via decapitation at the end of the experimental period. The liver, heart, and kidney were immediately frozen in liquid nitrogen upon collection and then stored at −80°C until analysis.

2.12. Antioxidant Activity In Vivo. Mouse tissue (such as the liver, heart, and kidney) was homogenized in a precooled saline solution (1 mL per 100 mg of tissue). Commercial kits were used to determine the T-AOC, malondialdehyde (MDA) content, SOD content, and glutathione peroxidase (GSH-Px) activity (Nanjing Jiancheng Bioengineering Institute). The detection limits of T-AOC, MDA content, SOD content, and GSH-Px activity were 0.2–55.2 U/mL, 0–113.0 nmol/mL, 0.5–122.1 U/mL, and 20–330 U, respectively. Homogenized tissues were prepared using 0.9% cold saline in an ice bath and centrifuged at 5000 × g for 20 min to yield supernatants for biochemical analysis. The T-AOC, SOD content, and GSH-Px activity were expressed as units per milligram of protein (U/mg prot) in the tissues. The MDA activity was expressed as nanomoles per milligram of protein (nmol/mg prot) in the tissues.

2.13. Statistical Analysis. All experiments were performed in triplicate, and the data were presented as the mean ± standard deviation. Data were processed using SPSS v23.0, and statistical significance was determined using one-way analysis of variance (ANOVA). The fitting degree was evaluated using the coefficient of determination R^2, and the F-test and P value were used to assess the significance level of the regression coefficient. $P < 0.05$ was considered statistically significant. The mean values were compared using the Tukey test.

3. Results and Discussion

3.1. Effect of a Single Factor on the Sensory Analysis of Nutritive Flour. Tables S2–S6 present the mean organoleptic evaluation scores of the antioxidant nutritive flour containing several powders (foxtail millet, jujube, rice bean, oat, and Goji). The overall acceptability score of the nutritive flour was highest when the content of foxtail millet, jujube, oat, rice bean, and Goji was 35%, 23%, 20%, 13%, and 2%, respectively. The composition of the composite nutritive flour was further optimized via orthogonal analysis.

3.2. Orthogonal Analysis of the Nutritive Flour Composition. The orthogonal experiment L_{16} (4^5) was conducted to investigate the effects of other parameters on the organoleptic evaluation scores of the nutritive flour and further screen the experimental factors. The order of importance of the five experimental factors in terms of R (variance analysis) values was as follows: A > E > C > B > D or foxtail millet > Goji > oat > jujube > rice bean (Table 1). This result also indicated that $A_4B_1C_4D_2E_3$ was the optimal process combination. One-way ANOVA analysis (Table S7) showed that all the factors were statistically significant ($P < 0.05$), indicating that they significantly influenced the organoleptic evaluation of the antioxidant nutritive flour.

Table 2: Nutrient composition of foxtail-millet-based composite antioxidant nutritive flour.

Nutrient component	Content (g/100 g)
Polysaccharide	0.35 ± 0.06
Total soluble sugar	42.82 ± 1.76
Total phenolics	2.27 ± 0.24
Vitamin C	0.21 ± 0.03
Crude protein	16.12 ± 1.25
Crude fat	2.36 ± 0.33

Values are presented as means ($n = 3$) ± standard deviations.

Thus, the fermented foxtail-millet-based composite nutritive flour had the highest organoleptic evaluation score (8.79 ± 0.09) when the content of foxtail millet, jujube, oat, rice bean, and Goji was 40%, 20%, 25%, 10%, and 2%, respectively.

3.3. Nutritional Composition and Physicochemical Properties of Antioxidant Nutritive Flour. The nutrient compositions of the fermented foxtail-millet-based composite antioxidant nutritive flour are shown in Table 2. The content of natural antioxidants in this flour, such as polysaccharides (0.35 ± 0.06 g/100 g), Vc (0.21 ± 0.03 g/100 g), and total phenolics (2.27 ± 0.24 g/100 g) was determined. Phenolics were the major antioxidant active substances in the antioxidant nutritive flour, which act as antioxidants by reacting with various free radicals. The mechanisms underlying the antioxidant effects include single-electron transfer, hydrogen atom transfer, chelation of transition metals, and sequential proton loss electron transfer [32]. Additionally, the crude protein content of this antioxidant nutritive flour was 16.12 ± 1.25 g/100 g (Table 3). A previous study revealed that foxtail millet protein hydrolysates possess antioxidant activity similar to α-tocopherol but lower than butylated hydroxytoluene [33]. Therefore, the crude protein in this foxtail-millet-based nutritive flour may promote its antioxidant properties, especially after enzymatic hydrolysis [34, 35].

The functional properties of this fermented foxtail-millet-based antioxidant nutritive flour and commercial nutritive flour are presented in Table 3. These properties include SP, WSC, WHC, and OHC. SP, which depends on the composition of the sample, is an important parameter for determining sample consistency [36]. The swelling process of cereal starch is mainly driven by amylopectin, whereas amylose acts as a diluent and inhibits swelling after particle leaching [37]. The SP of this fermented foxtail-millet-based composite nutritive flour was 6.45 ± 0.39 g/g, which was approximately 1.26-folds higher than that of the commercial nutritive flour (5.13 ± 0.44 g/g). This indicated that the fermented foxtail-millet-based nutritive flour has greater amylopectin content or is more stable against shearing action when subjected to heat than commercial nutritive flour [38]. Similarly, the WSC of this fermented foxtail-millet-based flour was approximately 1.79-folds higher than that of commercial flour, indicating that the solubility of starch molecules in this fermented foxtail-millet-based nutritive flour is superior [39]. WHC plays an important role in developing ready-to-eat foods, and a high absorption capacity may ensure product cohesiveness. In this study, the WHC of the fermented foxtail-millet-based flour was 163.30 ± 5.77 g/g, which is lower than that of commercial flour (193.30 ± 11.54 g/g). This may be due to the higher fat content of this fermented foxtail-millet-based flour than that of commercial flour as lipids block the polar sites of proteins, attenuating water absorption [40]. The OHC of this foxtail-millet-based flour (60.00 ± 2.50 g/g) was also higher than that of commercial flour (53.30 ± 3.82 g/g), which may be due to the variation in protein concentration, degree of interaction with water and oil, and conformational characteristics [16]. OHC plays an important role in enhancing flavor retention and improving palatability and mouthfeel [41]. Therefore, a high OHC facilitated the better flavor and mouthfeel of this composite nutritive flour.

3.4. In Vitro Antioxidant Activity. It is well known that numerous potential factors prevent the activity of all antioxidants in a complex system from being adequately represented by a single antioxidant activity assay [42]. This study used the T-AOC assay, SOD capability, and superoxide radical ($\cdot O_2^-$) scavenging abilities to investigate the antioxidant properties of this fermented foxtail-millet-based composite nutritive flour and commercial nutritive flour (Table 4). This composite nutritive flour showed higher T-AOC and SOD capability (0.84 ± 0.02 mM Trolox equivalents (TE)/g and 38.29 ± 0.22 U/g, respectively) than commercial flour (0.51 ± 0.04 mM TE/g and 23.12 ± 0.19 U/g, respectively), which might be because it contains more antioxidants (such as polyphenol, polysaccharides, and Vc). To determine the contribution of the antioxidant capacity of each component in the composite nutritive flour, we measured the antioxidant ability of the fermented foxtail millet, one of the main ingredients in the nutritive flour. The T-AOC of the fermented foxtail millet was 0.99 ± 0.05 mM TE/g, and its SOD capability was 89.06 ± 0.31 U/g (Table 4). As the composite nutritive flour only contained 40% fermented foxtail millet, the T-AOC and SOD capability of foxtail millet in the composite nutritive flour were approximately 0.4 mM TE/g and 35.62 U/g, respectively. Other antioxidant properties could have been contributed by fruits and grains.

The superoxide anion plays an important role in the formation of other reactive oxygen species (ROS), such as singlet oxygen and hydrogen peroxide [43]. The superoxide anion radical, which is produced from molecular oxygen by the oxidative enzymes of the body and via nonenzymatic reactions, is the most common free radical generated in vivo. The fermented foxtail-millet-based composite nutritive flour and commercial nutritive flour exhibited scavenging effects of 42.02% ± 0.09% and 33.54% ± 0.11%, respectively. These results suggested that the fermented foxtail-millet-based composite nutritive flour had better antioxidant capability than commercial nutritive flour. Besides, the fermented foxtail millet exerted scavenging effects of 63.71% ± 0.17%, and its corresponding contribution to the composite nutritive

Table 3: Physicochemical properties of foxtail-millet-based nutritive flour.

Samples	SP (g/g)	WSC (mL/mL)	WHC (g/g)	OHC (g/g)
Commercial flour	5.13 ± 0.44^{b}	2.93 ± 0.41^{b}	193.30 ± 11.54^{a}	53.30 ± 3.82^{b}
This composite flour	6.45 ± 0.39^{a}	5.24 ± 0.36^{a}	163.30 ± 5.77^{b}	60.00 ± 2.50^{a}

SP: swelling power; WSC: water-solubility capacity; WHC: water-holding capacity; OHC: oil-holding capacity. Results are presented as means ($n = 3$) ± standard deviations. Values carrying different letters in the same column are significantly different ($P < 0.05$).

Table 4: In vitro antioxidant ability of foxtail-millet-based nutritive flour.

Samples	T-AOC (mM TE/g)	SOD capability (U/g)	($\cdot O_2^-$) scavenging abilities (%)
Commercial flour	0.51 ± 0.04^{c}	23.12 ± 0.19^{c}	33.54 ± 0.11^{c}
This composite flour	0.84 ± 0.02^{b}	38.29 ± 0.22^{b}	42.02 ± 0.09^{b}
Fermented foxtail millet	0.99 ± 0.05^{a}	89.06 ± 0.31^{a}	63.71 ± 0.17^{a}
This composite flour at 60°C	0.87 ± 0.04^{b}	36.75 ± 0.16^{b}	43.14 ± 0.21^{b}
This composite flour at 100°C	0.32 ± 0.03^{d}	18.45 ± 0.10^{d}	24.14 ± 0.06^{d}

T-AOC: total antioxidant capability; SOD: superoxide dismutase; ($\cdot O_2^-$): superoxide radical; TE: Trolox equivalent. Results are presented as means ($n = 3$) ± standard deviations. Values carrying different letters in the same column are significantly different ($P < 0.05$).

Table 5: Effect of nutritive flour on body weight gain and tissue index ($n = 10$ per group).

Group	Body weight gain (g)	Liver index (%)	Heart index (%)	Kidney index (%)
AC	4.70 ± 1.31^{a}	4.23 ± 0.06^{a}	0.71 ± 0.04^{a}	1.48 ± 0.15^{a}
PC	3.02 ± 0.82^{b}	4.08 ± 0.11^{a}	0.65 ± 0.18^{a}	1.50 ± 0.058^{a}
NC	4.88 ± 1.71^{a}	4.11 ± 0.09^{a}	0.63 ± 0.19^{a}	1.50 ± 0.09^{a}
This composite flour	3.42 ± 0.76^{b}	4.37 ± 0.19^{a}	0.73 ± 0.14^{a}	1.47 ± 0.11^{a}
Commercial flour	3.86 ± 1.12^{b}	4.58 ± 0.21^{a}	0.76 ± 0.08^{a}	1.49 ± 0.08^{a}

AC: the naive group was provided with a normal chow diet; PC: the positive control group was provided with a diet made of a mixture of normal chow and vitamin C, with a vitamin C content of 0.2 g/kg; NC: the negative control group was provided with a diet made of a mixture of normal chow and unfermented foxtail millet, with a millet content of 400 g/kg. This composite flour group was provided with a diet made of a mixture of normal chow and composite foxtail-millet-based antioxidant nutritive flour, with a composite flour content of 400 g/kg. The commercial flour group was provided with a diet made of a mixture of normal chow and commercial grain nutritive flour, with a flour content of 400 g/kg. All diets were processed by SPE Biotechnology Co., Ltd. Results are presented as means ($n = 10$) ± standard deviations. Values carrying different letters in the same column are significantly different ($P < 0.05$).

flour was 25.48%. The remaining ($\cdot O_2^-$) scavenging abilities of this composite nutritive flour were attributed to fruits and grains.

In addition, to comprehensively analyze the fermented foxtail-millet-based composite nutritive flour, we simulated the eating state and evaluated the antioxidant properties of the flour after heating at different temperatures. The antioxidant capacity of the composite nutritive flour did not change significantly ($P > 0.05$) after heating at 60°C, with T-AOC, SOD capability, and ($\cdot O_2^-$) scavenging ability of 0.87 ± 0.04 mM TE/g, 36.75 ± 0.16 U/g, and $43.14\% \pm 0.21$ %, respectively (Table 4). However, the antioxidant capacity of the nutritive flour significantly decreased ($P < 0.05$) after heating at 100°C, which may be because high temperatures destroy antioxidants (such as polyphenols and Vc) in the composite nutritive flour. Therefore, the optimal brewing temperature for this fermented foxtail-millet-based composite nutritive flour should not exceed 60°C, as the flour exhibits better antioxidant capability under this condition.

3.5. Antioxidant Activity of Nutritive Flour In Vivo. The initial weight of the experimental mice was stable at approximately 31 g (Table S8). The mice were randomized to different diets and fed for 4 consecutive weeks after weight stabilization. The body weight gain of mice in the AC and NC groups slightly increased compared with that in the other groups (Table 5), but there was no difference in the tissue index (liver, heart, and kidney) ($P > 0.05$). The total feed consumption of mice was generally similar (Table S8). These results indicated that supplementation with a normal chow diet, unfermented foxtail millet, Vc, fermented foxtail-millet-based nutritive flour, and commercial nutritive flour did not affect the growth and development of mice in this study.

Different indicators were selected to evaluate the antioxidant effects of nutritive flour in vivo. T-AOC is a comprehensive indicator of antioxidant ability, which prevents lipid peroxidation by eliminating ROS in the body and interrupting the peroxide chain [44]. The T-AOC in tissues was higher in the test groups (composite flour and commercial flour groups) than in the control groups (NC and AC groups) (Figure 1(a)). The T-AOC of this composite flour was 22.63%, 23.86%, and 37.29% higher than that of commercial flour in the heart, liver, and kidney of mice,

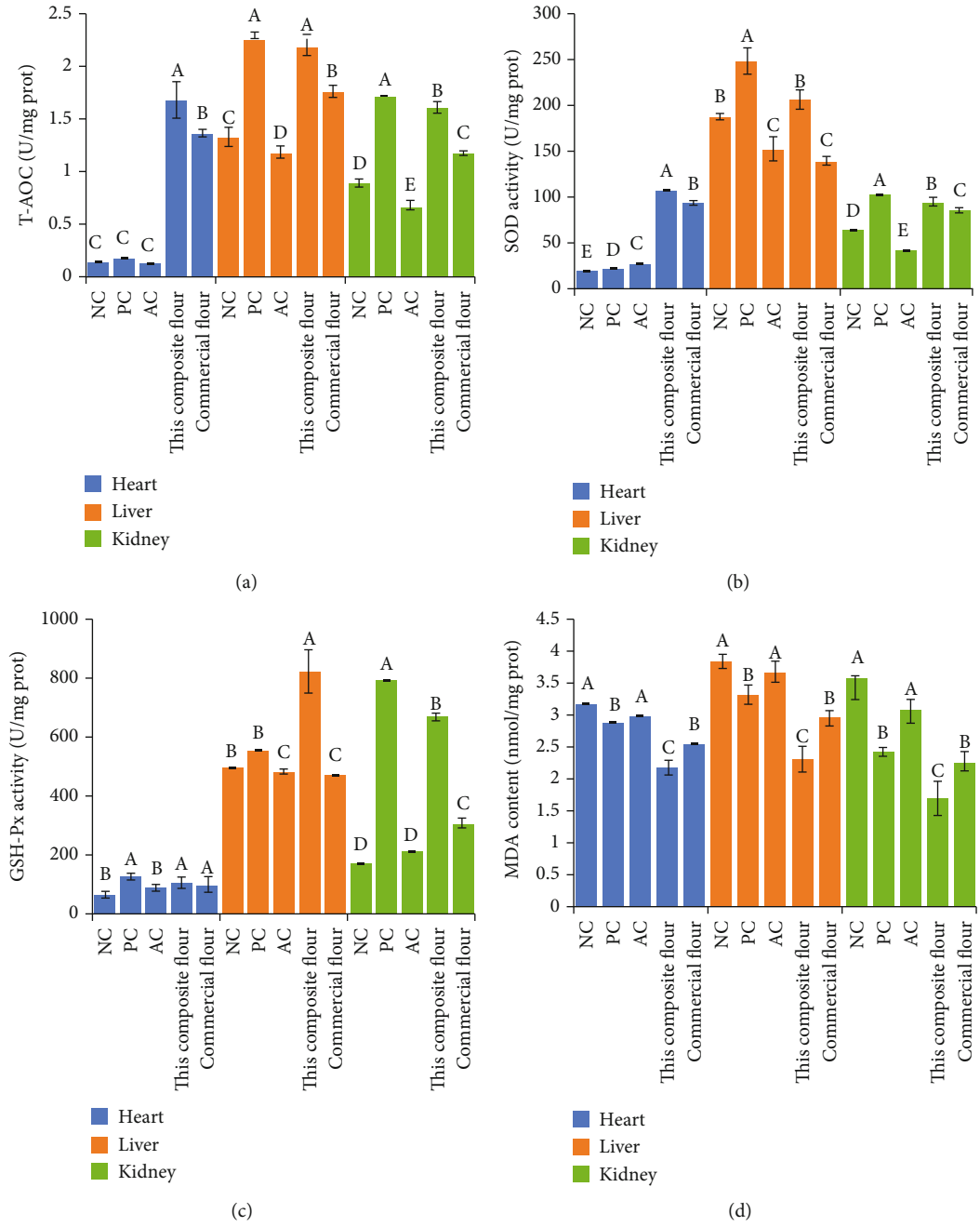

Figure 1: Detection of in vivo antioxidant activity in mice. (a) The total antioxidant capability (T-AOC) of different tissues (heart, liver, and kidney). (b) The superoxide dismutase (SOD) activity of different tissues (c) Glutathione peroxidase (GSH-Px) activities of different tissues. (d) Malondialdehyde (MDA) content of different tissues. Statistical analysis was performed between different groups in the same tissue, and the different letters represent significant differences ($P < 0.05$).

respectively, which may be because of the presence of additional antioxidants, such as polyphenols, polysaccharides, and Vc.

The SOD and GSH-Px activities and the MDA content are closely related to the aging process and oxidative stress [45]. SOD removes the hazardous substances that are created during the metabolism process. In this study, the SOD level in the heart of this composite flour group was significantly higher than that of the control (AC, PC, and NC) and commercial flour groups ($P < 0.05$) (Figure 1(b)). The SOD levels in the heart, liver, and kidney of this composite flour group were 1.13, 1.48, and 1.11 times higher than those of the commercial flour group, respectively. As shown in Figure 1(c), the GSH-Px activities in the heart, liver, and kidney of this composite flour group were 108.34 ± 18.45, 820.74 ± 71.62, and 669.75 ± 12.07 U/mg protein, which were 1.10-, 1.72-, and 2.17-folds those of commercial flour groups, respectively. SOD can transform superoxide radicals into hydrogen peroxide, and GSH-Px is an important peroxide decomposition enzyme that is widely distributed in the body. SOD and GSH-Px are important antioxidant

enzymes with a strong ability to eliminate free radicals [46]. Studies have shown that diets rich in polyphenols increase the antioxidant capabilities of serum SOD and GSH-Px [47, 48]. These results revealed that the fermented foxtail-millet-based composite antioxidant nutritive flour significantly increased ($P < 0.05$) the antioxidant activity of SOD and GSH-Px enzymes in mouse heart, liver, and kidney homogenates. This may be due to the antioxidant nature of phenolic compounds and polysaccharides.

MDA is a secondary product of lipid peroxidation that reflects the extent of lipid peroxidation and attacks free radicals in tissues and cells. As shown in Figure 1(d), these composite and commercial flour groups have lower MDA levels in all tissues (heart, liver, and kidney) than NC and AC groups, and the MDA levels of this composite flour group in the heart, liver, and kidney decreased by 14.40%, 21.55%, and 25.65% compared with those of the commercial flour group, respectively. These results show that this foxtail-millet-based composite nutritive flour can effectively reduce MDA concentration in tissues, which could reduce the damage of free radicals to tissues. The enhanced T-AOC, SOD activity, and GSH-Px activity as well as MDA content reduction in the animals indicated that this fermented foxtail-millet-based composite nutritive flour has better antioxidant activity in vivo than commercial nutritive flour.

4. Conclusions

The fermentation of foxtail millet and incorporation of coarse grains and fruit powders resulted in the formation of a composite flour with improved antioxidant properties, nutrient composition, and physicochemical properties. This study revealed that foxtail-millet-based composite antioxidant nutritive flour is rich in nutrients and possesses superior physicochemical properties as well as in vitro and in vivo antioxidant properties, exerting better effects than commercially available nutritive flour. However, further experiments are required to verify the process and mechanism underlying the in vivo antioxidant activity. This study suggests that foxtail millet is a food material with great potential and antioxidant properties for further development. This research serves as a guide for improving the added value of foxtail millet processing and increasing its viability as a food source.

Data Availability

The data are available from the corresponding author on reasonable request.

Additional Points

Novelty Impact Statement. The health benefits of foxtail millet are largely attributed to antioxidants, such as polysaccharides and phenolics. To further improve the added value of foxtail millet processing, a foxtail-millet-based composite antioxidant nutritive flour, consisting of fermented foxtail millet, coarse grains, and fruit powders, was evaluated in this study. Results revealed that this composite nutritive flour had higher in vitro and in vivo antioxidant properties, nutrient content, physicochemical properties, and antioxidant activities than commercial nutritive flour. This study could serve as a guide for improving the added value of foxtail millet processing, increasing its viability as an antioxidant food source.

Ethical Approval

The care and use of laboratory animals reported in this study were approved by the Langfang Normal University's Animal Care and Use Committee and the Ministry of Agriculture of China (GB/T 35892-2018).

Conflicts of Interest

There is no conflict of interest to declare.

Authors' Contributions

Tong Lin was responsible for the conceptualization, methodology, data curation, formal analysis, investigation, validation, and writing of the original draft. Gongjian Fan was responsible for the data curation, formal analysis, and investigation. Zhiguo Zhou was responsible for the data curation and validation. Chunyan Xie was responsible for the conceptualization, funding acquisition, investigation, project administration, supervision, and review and editing of the manuscript.

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Supplementary Materials

Supplementary Table S1: level of orthogonal factors. Supplementary Table S2: mean organoleptic evaluation scores of the antioxidant nutritive flour with different amounts of fermented foxtail-millet-based powder. Supplementary Table S3: mean organoleptic evaluation scores of the antioxidant nutritive flour with different amounts of jujube powder. Supplementary Table S4: mean organoleptic evaluation scores of the antioxidant nutritive flour with different amounts of oat powder. Supplementary Table S5: mean organoleptic evaluation scores of the antioxidant nutritive flour with different amounts of rice bean powder. Supplementary Table S6: mean organoleptic evaluation scores of the antioxidant nutritive flour with different amounts of Goji powder. Supplementary Table S7: one-way analysis of variance. Supplementary Table S8: body weight changes in mice fed with different diets. *(Supplementary Materials)*

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