Assessment of the quality of *Polygonati Odorait Rhizoma* powder prepared by different processes and investigation of its additive properties in steamed buns

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ABSTRACT

Background: *Polygonati Odorait Rhizoma (PO), the dried rhizome of Polygonatum odoratum (Mill.) Druce is a popular traditional health food ingredient in China and Southeast Asia.* PO is primarily processed into strips and sheets, limiting its healthcare applications. Powder milling technology enhances the application value and overall utilization of PO.

Objective: This study aimed to investigate the effects of various processing methods on the physicochemical properties of PO powders, with a particular focus on enzymatically modified PO powder (EM). In addition, the study examined the additive properties of PO powder in steamed buns.

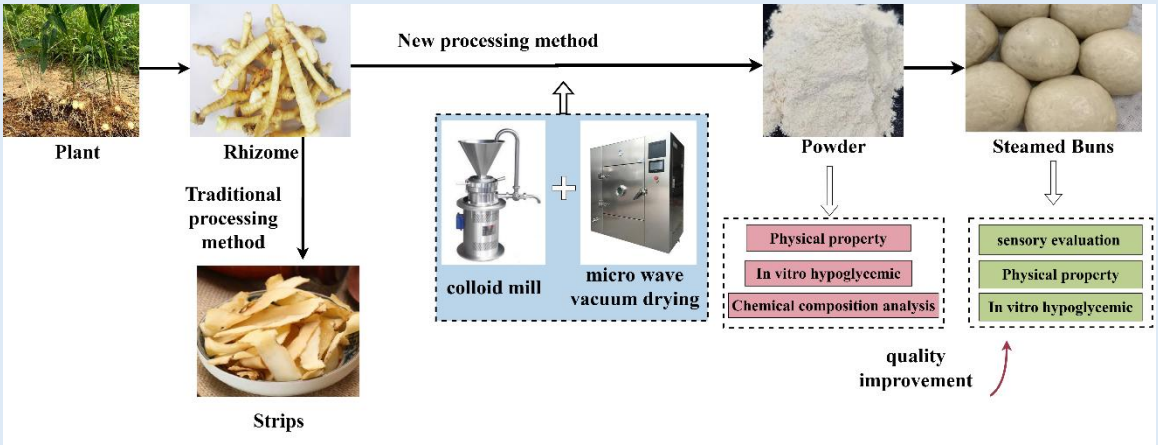
Methods: In this study, four different PO powders were prepared using different processes named Enzyme Modified PO Powder (EM), Vacuum Microwave-Dried PO Powder (VM), Vacuum Freeze-Dried PO Powder (VF), and Traditionally Processed PO Powder (TP). Comparison of the physical properties, chemical composition, and inhibitory activity against α-amylase of four different PO powders. The effects of adding the four PO powders on the quality of steamed buns were also compared.

Results: The results revealed significant increases in the functional components of EM compared to TP, with total saponin, total polysaccharide, total polyphenol, and total flavonoid contents rising by 74%, 127.8%, 91.2%, and 125.4%, respectively. With TP. Among the steamed buns with PO powder added, the steamed buns with EM had the best sensory score, aging, and fermentation characteristics. All four PO powders reduced the steamed buns' sugar content (RSC) to different extents than the blank ones. EM reduced the RSC of the steamed buns by 27.2% compared to the blank group.

Conclusion: Overall, complex enzymatic digestion could effectively improve PO powder's functional quality and application. EM powder showed the best performance regarding steamed buns' functional quality and additive properties.

Novelty: This experiment was the first to compare PO powders' physical properties and functional content in different processing methods. It was found that enzymatic modification could effectively improve the physical properties and functional content of PO powders. The addition of PO powders can enhance the sensory qualities of steamed bread.

Keywords: *Polygonati Oodrait Rhizoma* powder, steamed buns, enzymatic modification, physicochemical properties, quality characteristics



INTRODUCTION

Polygonati Odorait Rhizoma (PO) is the dried rhizome of *Polygonatum odoratum* (*Mill.*) Druce is a plant of the Liliaceae family that is widespread in the temperate regions of Eurasia. The use of PO as a functional food was first recorded in the ancient Chinese medical text named ShenNongBenCaoJing (Traditional Chinese Medicine Publications), which has been used for over 1,800 years. In the Ming Dynasty book JiuHuangBenCao (One of the earliest famine relief agricultural books in Chinese history), PO was mainly used to feed people during famine. The main active constituents of PO are compounds such as polysaccharides [1], flavonoids [2] and saponins [3]. These components have been reported to have antioxidant [4], intestinal flora regulation [5], anti-inflammatory [6], and hypoglycemic [7] effects, making it a functional food well suited to the needs of modern health management. In traditional Chinese medicine (TCM), PO is believed to have the effect of nourishing yin, moisturizing dryness, increasing body fluid, and quenching thirst. It has a mellow taste but is not greasy, and it is widely used in diet therapy (daily

soup or tea) and traditional medicine as a functional food in China and Southeast Asia. Although PO has been consumed for thousands of years, it is still processed by the conventional method of repeated steaming and baking, which is said to improve the health effects of PO. Due to the simplicity of the traditional processing method, PO is mainly presented in the market as strips or slices, which is challenging to meet the diversified needs of modern consumption and application scenarios, such as functional addition and efficient extraction. Traditional processing methods are produced in the past backward production conditions, often with low production efficiency and poor quality control. If modern engineering technology and equipment are adopted, the quality and efficiency problems will be effectively solved, and the diversification of PO products will also be realized.

Powdered raw material has the advantages of convenient storage, transportation, downstream processing, and application compared with unpulverized raw materials. PO is rich in soluble dietary fiber, flavonoids, saponins, and other active ingredients and has a good taste, which is very suitable for development into functional food ingredients. The high polysaccharide content and excessive viscosity of traditionally processed PO make it challenging to convert into powder using conventional techniques, highlighting the need for fresh processing methods. In a previous study, different methods, including ultrafine grinding, wet-heat, and compound enzymatic hydrolysis, were compared and optimized the enzymatic process conditions [8]. Based on previous studies, this study aims to comprehensively evaluate the physicochemical properties and health-promoting activities of the composite enzymatic PO powder, providing a scientific basis for its application development. To assess whether the compound enzyme-modified PO powder and the compound enzyme-modification process offer comparative advantages, four process samples, PO powder prepared by traditional processing (TP), PO powder prepared by vacuum freeze-drying from fresh PO slurry (VF), PO powder prepared by vacuum microwave drying from fresh PO slurry (VM), and PO powder prepared by compound enzymolysis from VM (EM) were prepared and assessed. Of the four samples, three were reference samples, respectively, TP served as a traditionally processed product, VF served as fresh PO (its quality being close to that of fresh PO), and VM served as an un-enzyme product.

As a plant resource with health care functions, the health benefits of PO-processed products should be the focus of attention. Improving the function and characteristics of staple food by compound addition is the direction of future food development. The additive characteristics of four kinds of PO powder in steamed buns were preliminarily studied to study the application value of PO powder further. As one of the traditional Chinese staple foods, steamed buns typically include wheat powder, yeast, and water. The taste and nutritional value of steamed buns from conventional wheat powder are highly unique [9]. Zhu et al. added beetroot powder to wheat powder to prepare steamed buns, and this addition significantly reduced the aging rate of the buns from 4.14% to 2.59% and increased their hardness and chewability [10]. Zhu et al. [11] developed a linseed powder-enriched steamed bun, and with the increase in linseed powder content, the hardness and chewiness of the buns improved, and their antioxidant capacity increased by nearly 300%. There have been no reports on the effects of adding PO powder to steamed buns. This study will compare the differences between the four PO powers in physical quality, chemical quality, α-amylase inhibitory activity, and the compound addition properties in steamed buns.

MATERIALS AND METHODS

Materials: *Polygonatum odoratum (Mill.)* Druce, provided by Hunan Tianhong Pharmaceutical Co., Ltd; glucose standard (batch NO: S21J12|138537, Shanghai Yuanye Biotechnology Co., Ltd); cellulase (batch NO.: H23A10S95964, enzyme activity 15000 U/g), pectinase (batch no.: L03J11L117193, enzyme activity 50 U/g), papain (batch NO: P15J11B118475, enzyme activity 800 U/mg), Shanghai Yuanye Biotechnology Co., Ltd; Wheat flour (Inner Mongolia Hengfeng Food Industry Group Co., Ltd); Angie's high active dry yeast (Angie's yeast Co., Ltd); superior sugar (Hunan Kanglid Food Co., Ltd); anhydrous methanol, anhydrous ethanol, and other reagents were analytically pure(SIinopharm Chemical Reagent Co., Ltd).

Experimental Equipment: JM-LB100 Colloid mill, Zhe Jiang Hao Star Machiner Equipment Manufacturing Co. Ltd; WB-5 Vacuum Microwave Drying Oven, Fuzhou Famouk Machinery Technology Co. Ltd; Ltd; KQ-1000DE CNC Ultrasonic Cleaning Machine, Kunshan Ultrasonic Instrument Co. Ltd; DZKW-4 Electronic Thermostatic Water Bath, Beijing Zhongxing Weiye Instrument Co. Ltd; Spectrophotometer CS-580, Hangzhou Color Spectrum Science and Technology Co. Ltd; TMS-PRO Multifunctional Mass Smeter, FTC Corporation.

Preparation of PO powder and steamed buns adding PO powder: Fresh PO rhizomes were washed, and non-edible parts were removed. Unqualified rhizomes, such as those with small volume, insect damage, or mold, were discarded, and debris was sieved out. The rhizomes were then cut into 2 cm segments. After adding distilled water at a ratio of 1:1, the PO segments were processed using a colloid mill (Zhe Jiang, China) for 1 minute to obtain a PO homogenate.

The vacuum Freeze-Dried PO Powder method was slightly modified from the method described by Li et al. [12]. An appropriate amount of PO homogenate was placed in a vacuum freeze-drying oven and dried until the moisture content was reduced to 10% or less. The dried material was then powdered and passed through a 50-mesh sieve to obtain vacuum freeze-dried PO powder (VF).

An appropriate amount of PO homogenate was vacuum microwave-dried at 60°C and -0.08 MPa until the moisture content reached 10% or less. The dried material was powdered and passed through a 50-mesh sieve to obtain vacuum microwave-dried PO powder (VM)[13].

One hundred grams of VM were mixed with five times the volume of distilled water. After dissolution, the pH was adjusted to 5.5 with 0.1 mol/L citric acid, and 3% (w/w) of a composite enzyme mixture (cellulase: papain: pectinase = 1:1:1 by mass) was added. The mixture was incubated at 40°C for 80 minutes to allow the enzyme reaction to proceed. Following the reaction, the enzyme was inactivated by heating the mixture in a water bath at 80°C for 10 minutes. The sample was then dried using a vacuum microwave under the same conditions as in 2.3.3. The dried sample was crushed and passed through a 50-mesh sieve to obtain enzyme-modified PO powder (EM) [14].

PO rhizomes were dried in an atmospheric pressure blast drying oven at 50°C until they became soft. The rhizomes were then repeatedly kneaded and dried until the cross-section appeared translucent, and the interior was not rugged, following traditional production methods. The traditionally dried PO was removed from the oven, powdered, and passed through a 50-mesh sieve to obtain traditional processing PO powder (TP). Figure 1 shows the photos of four different processes of PO powder.

Figure 1. Photographs of four different processes of PO powder

Based on the preliminary study results, incorporating PO powder at a ratio of 14% in wheat flour could yield steamed buns with the best sensory quality. Therefore, 35 g PO powder, 215 g wheat flour, 5 g sugar, and 120 mL of distilled water were well mixed. 2.5 g of activated yeast, dissolved in 35°C warm water, was added in advance and then poured into the pasta machine for 15 min to make the dough. The dough was then placed in a bowl, covered, and fermented at 37 ℃ for 60 minutes in a constant temperature incubator. Then, the dough was kneaded continuously to expel the gas. The dough was kneaded until its surface was smooth, then divided into equal weights and shaped.

The divided dough was placed back in the incubator at 37°C for 20 minutes, then transferred into a stainless-steel pot and steamed on an induction cooker. Steaming continued for 20 minutes after steam was observed to come out.

Physical quality Evaluation of four PO powders: A Spectrophotometer CS-580 was used to measure the L*, a*, and b* values of the samples, and their color difference values were analyzed in comparison with a white standard. The L* value indicated brightness (0 = black, 100 = white), the a* value indicated the red-green degree (-a* = green, +a* = red), and the b* value indicated the yellow-blue degree (-b* = blue, +b* = yellow). Each sample was measured six times, and the average value was recorded. The integrated color difference value ΔE was calculated using formula (1), with a smaller ΔE value indicating a closer color match to the white standard [15].

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (1)$$

Dispersibility was selected as the evaluation index for solubility [16]. A beaker containing 50 mL of distilled water at 50°C was placed on a magnetic stirrer with a speed of 500 rpm. Exactly 0.5 g of the sample was quickly and evenly dispersed in the water, and the time required to be wholly dispersed was recorded.

The method was conducted as described in the literature [17]. A funnel was fixed on a tabletop, and a glass plate was placed beneath it to ensure the funnel was perpendicular to the table. The end of the funnel was positioned 2 cm above the glass plate. The powder sample was slowly added through the funnel, allowing it to flow vertically onto the glass plate, forming a cone until the tip of the powder cone touched the lower mouth of the funnel. The radius d of the bottom surface of the

cone was measured, and the angle of repose was calculated using formula (2).

$$\theta(^\circ) = arc\ tan\ tan\ (\frac{2}{d})\ (2)$$

Ten grams of the sample were weighed and placed in a beaker. It was then dissolved with 150 mL of distilled water at 50°C, stirred evenly until completely dissolved and dispersed, and the viscosity was determined using an NDJ-8S digital display viscometer after cooling to room temperature.

As an evaluation index, moisture absorption was evaluated using Critical Relative Humidity (CRH). It was determined by referring to the method of Ahiduzzaman et al. [18]. Saturated solutions of $MgCl_2$-$6H_2O$, K_2CO_3-$2H_2O$, NaBr-$2H_2O$, $SrCl_2$-$6H_2O$, NaCl, and KCl were prepared separately. Five milliliters of each saturated salt solution were placed in the outer chamber of a Conway microdiffusion dish. A 0.5-g sample, dried to constant weight, was placed in a pre-dried glass dish, accurately weighed, and then transferred to the inner chamber of the Conway microdiffusion dish. The dish was then covered with its lid. The sample was placed at 25°C for 72 hours, after which it was reweighed. The moisture absorption rate of the sample under different relative humidity conditions was calculated using Equation (3), where M_1 represents the mass after moisture absorption and M_2 represents the mass before moisture absorption.

$$hygroscopic(\%) = \frac{M_1 - M_2}{M_2} \times 100\ (3)$$

Take the A_W value of each standard saturated salt solution at 25 ℃ as the horizontal coordinate and the moisture absorption rate as the vertical coordinate, plot the moisture absorption curve, and determine and calculate the CRH value.

Chemical composition analysis of four PO powders: The total polysaccharides were measured as described in the literature [19]. Glucose was used as the standard, and the total polysaccharide content was determined using the phenol-sulfuric acid method at 490 nm following the 2020 edition of the Chinese Pharmacopoeia.

The total flavonoids were extracted as described in the literature for ultrasound-assisted extraction of total flavonoids [20]. Using rutin as the standard, the total flavonoid content was determined at 510 nm.

The total polyphenol content was determined as described in the literature for ultrasound-assisted extraction of total polyphenols [21]. Total polyphenol content was determined at 760 nm using gallic acid as the standard, and the method was performed as described in the literature [22].

The total saponins were extracted as described in the literature [23]. Briefly, the extraction of total saponins was carried out using the ultrasound-assisted method. The total saponin content was determined at 518 nm using diosgenin as the standard, the Zang et al. 2017 technique.[24].

The above content determination results were calculated dryly after the sample's moisture content was deducted. The content (mg/g DW) was calculated based on the standard curve.

Determination of hypoglycemic function of four PO powders in vitro: 2.5 g of PO powder was accurately weighed, added with 125 ml of distilled water (75% ethyl alcohol), and extracted by ultrasonication for 35 min at 60 ℃ and 450 W. The extract was centrifuged at 4000 r/min for 15 min, and then the supernatant was concentrated at 50 ℃ under reduced pressure. Then, the volume of distilled water was fixed to 25 mL to obtain the aqueous extract with a mass concentration of 100 mg/mL PO powder. The alcohol solution extract was evaporated to dryness, re-dissolved in distilled water, and reconstituted to 25 ml to obtain the ethyl alcohol extract with a 100 mg/mL PO powder mass concentration. The extract was diluted to 20, 40, 60, and 80 mg/mL and stored in a refrigerator at 4 ℃.

Pipette 200 µL of the sample solution and 200 µL of α-amylase solution (20 U/mL) into a 10 mL centrifuge tube and mix well. Place the centrifuge tube in a water bath at 37°C for 10 min to activate the enzyme. Then, add 400 µL of a 1% soluble starch solution and conduct the reaction in a water bath at 37°C for an additional 10 min. After that, add 400 µL of DNS colorant, and terminate the reaction by cooling with running water immediately following a 5 min boiling water bath. Next, 6 mL of distilled water is added to dilute the mixture, and the absorbance is measured at 540 nm. To determine the absorbance of the blank (A'), replace the sample solution with distilled water and repeat the measurement [25].

$$\boldsymbol{inhibitionrate}\ (\%) = \frac{(\boldsymbol{A'} - \boldsymbol{A})}{\boldsymbol{A'}} \times \mathbf{100}\ (\mathbf{4})$$

Quality evaluation of steamed buns adding PO power: Following removal from the steamer, the steamed buns were cooled for 30 minutes. Subsequently, a team of sensory-trained experts (5 males and 5 females, aged between 18 and 50 years old, who are competent panelists from the food industry, general consumers, and students majored in Food Science), well-versed in sensory evaluation, assessed the steamed buns based on established sensory scoring criteria. The scores were averaged to obtain the sensory evaluation conducted regarding surface condition and color, tissue structure, flavor, and texture, respectively [26]. The sensory scoring criteria for steamed buns with the addition of PO are presented in Table 1[27].

Table 1. Sensory scoring criteria of steamed buns adding PO

Parameters	Evaluation Rules	Score
Skin condition and color	Smooth skin, no folds or bubbles, white and shiny (18-25)	25
	Medium (9-17)	
	Rough skin, not smooth, yellowish color (0-8)	
Organizational structure	Uniform internal organization, consistent particle size, tiny pores, soft and delicate (15-20)	20
	Medium (8-14)	
	Large and uneven pores (0-7)	
Flavor	The aroma of fermented flour products and PO (18-25)	25
	Medium (9-17)	
	Unusual odor (0-8)	
Taste	Chewing and chewing without sticking to teeth, elasticity (20-30).	30
	Medium (10-19)	
	Not chewy, sticky, poor elasticity (0-9)	

Using a 50 mL measuring cylinder, take an equal mass of dough from each sample group and allow it to rise in a constant temperature incubator set at 37°C. Record the volume at 30-minute intervals and cease readings after 120 min. Plot the curves with time on the horizontal axis and volume on the vertical axis to compare the effects of different PO powders on the fermentation volume of the dough.

This experiment was performed as described in the literature [28]. The steamed buns were cooled naturally

at room temperature (approximately 18-25°C) for 60 min. The buns were then cut into uniformly thick slices, 15 mm in thickness, in the vertical direction. Two slices near the center were selected to assess the texture using a texture meter. The test probe used was type P/36R, and the test conditions were set as follows: 50% compression rate, 5 s interval between compressions, 1.0

mm/s pre-test speed, 1.0 mm/s test speed, 1.0 mm/s post-test speed, auto trigger type with a starting load of 5 g, and data acquisition at 200 pps. The experimental curves of Texture Profile Analysis (TPA) yielded five parameter values: hardness, springiness, cohesiveness, adhesiveness, and chewiness.

Steamed buns were naturally cooled at room temperature (around 18-25 ℃) for 60 min. The buns were put into a sealed bag and stored at room temperature. The texture tests were performed at 0, 1and 3 d, respectively, where 0 d was the steamed buns naturally cooled for 60 min after the samples were steamed [29]. 0.5 g of the pretreated sample was weighed and homogenized by adding 10 mL of distilled water. Subsequently, 10 mL of HCl-KCl buffer (0.02 mol/L, pH 2.0) and 0.02 g of pepsin were added to the sample, and the mixture was placed in a 37°C thermostatic water bath for 1 h. After hydrolysis, a phosphate buffer solution (0.2 mol/L, pH 2.0) was added to bring the volume to 25 mL. Then, 0.03 g of α-amylase was added, and the mixture was incubated in a 37°C thermostatic water bath for an additional 1.5 h. The reduced sugar content (RSC) of the steamed buns after digestion was determined in this experiment. Blank steamed buns were used as the control, with the RSC value of the blank steamed buns set at 100%. The effect of the addition of PO powder on the RSC value of the steamed buns was analyzed by calculating the ratio of the reducing sugar content of the PO steamed buns after in vitro digestion to that of the blank group [30]. The calculation formula was as follows:

$$RSC = \frac{(A_1 - A_2 \times W)}{A_0} \times 100 \quad (4)$$

Where: A_1 is the reduced sugar content in PO steamed buns; A_2 is the reduced sugar content in PO powder; A_0 is the reduced sugar content in blank steamed buns; W is the mass fraction percentage of PO powder in PO steamed buns.

Statistics Analysis: Each data set was repeated 3 times except for hygroscopicity and sensory evaluation. The data were analyzed by ANOVA using SPSS 20.0, and $P<0.05$ indicated significant differences. Origin Pro 2015 was used for graphing.

RESULTS AND DISCUSSION

Physical property analysis of four PO powders: The ΔE value obtained from the color analysis indicates the overall color difference between the sample and a white plate, where a smaller ΔE value represents a lighter powder color [31]. As shown in Table 2, the ΔE value of VF is significantly lower than those of TP, VM, and EM, suggesting that the color of VF is closer to that of the white plate. This is attributed to the material's low temperature during the vacuum freeze-drying process, during which the material is not exposed to oxygen in the air due to the vacuum state, thereby better preserving the material's color [32]. The colors of VM and EM were the second lightest, like VF, with no significant differences observed. The darkest color of TP can be attributed to the use of hot air-drying technology in the traditional processing method, where prolonged heating may promote both enzymatic and non-enzymatic browning reactions [33].

Table 2. Color analysis results of four PO powders

Sample	L*	a*	b*	ΔE
TP	83.03±0.08b	2.11±0.02a	17.14±0.22a	18.46±0.22a
VF	91.80±0.19a	0.31±0.01b	8.00±0.14c	6.72±0.56c
VM	80.19±2.01c	-0.10±0.21c	10.87±0.81b	15.53±1.46b
EM	80.59±1.15c	-0.47±0.05d	10.92±0.17b	15.18±0.91b

Means of triplicate determination ± S.D with the letters in the row within each property are significantly different ($p < 0.05$).

A shorter dispersion time indicates better powder solubility [34]. As shown in Table 3, EM exhibited the shortest dispersion time among all groups, suggesting better solubility than VM. This enhanced solubility of EM may be due to enzymatic hydrolysis, which exposed more hydrophilic groups [35]. VF displayed the poorest solubility among the four samples, likely because the non-thermal process reduces macromolecular degradation. Larger molecules are more prone to agglomeration, which can result in a longer dispersion time for the powder in water.

Table 3. Solubility, flowability, and viscosity of four PO powders

Sample	Solubility/s	Flowability /°	Viscosity /mPa·S
TP	17.78±1.22b	47.77±2.61a	66.67±0.51a
VF	35.94±1.32a	47.07±0.93a	18.33±1.53c
VM	16.22±0.60b	44.87±0.97a	26.73±0.50b
EM	6.23±0.10c	45.83±2.11a	7.60±0.72d

Means of triplicate determination ± S.D with the letters in the row within each property are significantly different ($p < 0.05$).

The flowability was evaluated by measuring the angle of repose, with a smaller angle indicating better flowability. As shown in Table 3, VM exhibited the poorest flowability among the four PO powders, although there was no significant difference among the groups. These results suggest that the different processes had a minimal effect on the fluidity of the PO powder.

The internal friction of the solution influences the magnitude of viscosity. Higher internal friction results in greater viscosity. As shown in Table 3, the viscosity of TP was significantly higher than that of the other groups ($P<0.05$), which might be attributed to the increased content of carbohydrates and oligosaccharides in the PO powder after traditional processing. EM exhibited the lowest viscosity among the four PO powders, which might be attributed to the hydrolysis of macromolecules and the reduction in average molecular mass. The smaller the relative molecular mass, the weaker the intermolecular forces and, thus, the lower the viscosity [36]. There are significant differences in viscosity among the four PO powders (Table 2), indicating that traditional processing can increase the viscosity of PO powder, while enzymatic hydrolysis can reduce it.

Critical relative humidity (CRH) is the relative humidity that causes a sharp change in equilibrium moisture absorption, the relative humidity corresponding to the point where the moisture absorption equilibrium curve starts to absorb moisture rapidly. The higher the CRH value, the better the storage stability; therefore, the CRH is essential for the powder storage stability of PO power. As shown in Table 4, the CRH of TP, VF, VM, and EM is 67.2%, 63.99%, 66.78%, and 70.57%, respectively, EM showed higher CRH than VM and exhibited the highest CRH among four PO powders, indicating that complex enzymatic hydrolysis is beneficial to reduce the hygroscopicity of PO powder.

Table 4. Hygroscopicity of four PO powders

Hygroscopicity /%	Relative humidity (25℃)/%						CRH/%
	$MgCl_2·6H_2O$	$K_2CO_3·2H_2O$	NaBr·$2H_2O$	$SrCl_2·6H_2O$	NaCl	KCl	
	33.0	42.7	57.7	70.8	75.2	84.2	
TP	6.19	9.09	14.06	20.35	24.31	33.05	67.20
VF	7.40	8.93	12.42	19.39	22.59	31.32	63.99
VM	7.17	9.04	12.98	18.52	21.81	30.66	66.78
EM	7.11	11.25	13.93	20.57	24.35	34.70	70.57

Chemical composition analysis of four PO powders: The total polysaccharide contents are shown in Fig. 2A. The total polysaccharide content of TP（16.84mg/g）and VM（20.14mg/g） was higher than VF（14.52mg/g）, but there was no significant difference. The total polysaccharide content of EM (38.27 mg/g) was significantly elevated (P<0.05) compared to the other three groups. This may be because enzymolysis can promote the release of polysaccharide components, increasing total polysaccharide solubilization. Our results were consistent with enzyme-assisted extraction of *American ginseng* polysaccharides [37]. In Fig. 2A, it can be found that the total polysaccharide of EM was 91%, 129%, and 165% higher than that of the VM, TP, and VF groups, respectively. The highest total polysaccharide content in EM makes it a good candidate as a functional food ingredient.

The total saponin content is shown in Fig. 2B. The total saponin content in EM (8.46 mg/g) was significantly higher than that of the other groups, which may be related to the fact that enzymatic hydrolysis increased the solubilization of saponins in PO powder [38].

The total polyphenol content is shown in Fig. 2C. Compared with VF, the total polyphenol content in VM and TP was significantly lower (P<0.05), indicating that heat efficiently degraded polyphenols. However, the total polyphenol content of VM (0.94 mg/g) was significantly higher than that of TP (0.68 mg/g), indicating that vacuum microwave drying technology protects polyphenols better than hot air drying. This is because polyphenols are easily oxidized in air, and the vacuum microwave process isolates the oxygen to preserve the polyphenols [39] better. The significant (P<0.05) increase in total polyphenol content in EM (1.30 mg/g) compared to VM (0.94 mg/g) is related to the fact that the complex enzyme treatment increases the glycosidic bond breaking and releases bound polyphenols, which in turn increases the polyphenol content. This agrees with the study's results by Xu et al. [40].

The total flavonoid content is shown in Fig. 2D. Compared with VF, the total flavonoid content in TP and VM was significantly lower due to the easy degradation of flavonoids by heat. The vacuum freeze-drying environment isolated oxygen and lowered the temperature, effectively protecting the flavonoids in terms of temperature and oxygen. The total flavonoid content in EM (1.30 mg/g) was significantly higher than (P<0.05) VM (0.8 mg/g) and TP (0.59 mg/g), which indicates that the leaching of flavonoids from the PO powder was significantly increased after enzymatic modification, which agrees with the method of Hou et al. [41]. In conjunction with Fig. 2D, it was found that the difference between the total polyphenol content and the total flavonoid content in the PO powder was not significant, indicating that the polyphenols in PO are mainly flavonoids.

In conclusion, the contents of total polysaccharides and total saponins were significantly higher in EM than in the other three processing methods, and the contents of total flavonoids and total polyphenols were considerably higher than those in VM and TP. The main reason for this result was that the enzyme digestion process could significantly increase the dissolution of functional constituents in PO [42].

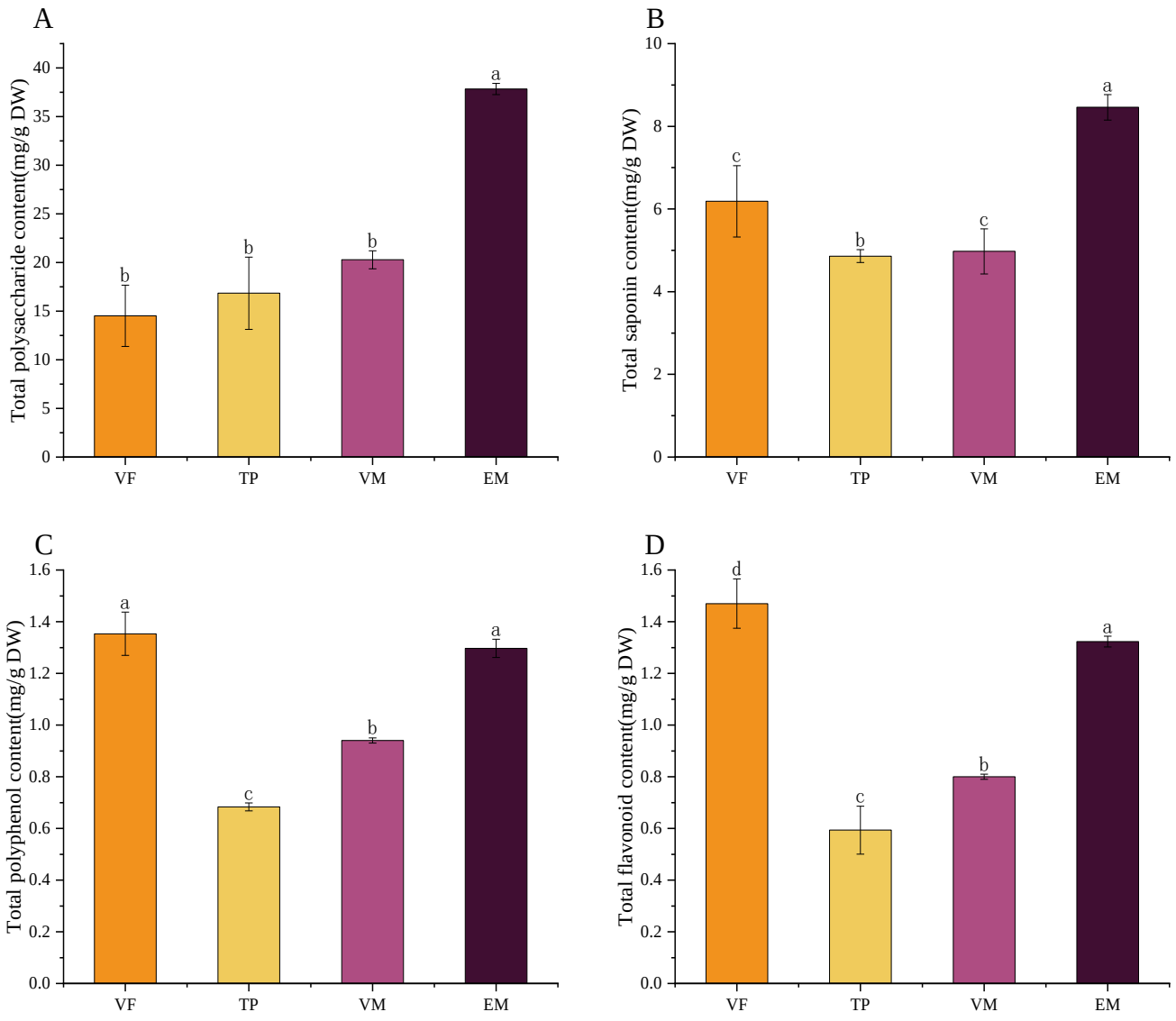


Figure 2. Chemical composition of four PO powders: (A) Total polysaccharide content; (B) total Saponin content; (C) Total polyphenol content; (D) Total flavonoid content, different letters indicate significant differences at different process methods ($p < 0.05$).

In vitro hypoglycemic function of different processes of four PO powders: As can be seen from Fig. 3A, the inhibitory effect of the aqueous extract of PO powder on α-amylase activity showed a dose-response relationship. The inhibition rate of α-amylase activity by VF, TP, VM, and EM at 100 mg/ml was 50.88%, 36.71%, 61.51%, and 83.94%, respectively. The aqueous extracts of EM showed the highest inhibitory activity on α-amylase among the four groups. It can be speculated that the active ingredients are more easily released after enzymatic hydrolysis of PO powder, resulting in an increase in substances with α-amylase inhibitory activity in the aqueous extract. This result is consistent with total polysaccharide and saponin contents in 3.2 (Functional ingredient content of PO powder). Therefore, it may be speculated that the polysaccharides and saponins are the active substances inhibiting α-amylase.

As shown in Fig. 3B, the alcohol extract of PO powder at different concentrations could inhibit the activity of α-amylase in a dose-dependent manner. VM and EM powder had similar inhibitory effects on α-amylase activity. Comparing Fig. 3A, the aqueous extract of EM was more effective in inhibiting α-amylase activity. These results indicated that most substances inhibiting α-amylase activity in EM were water-soluble.

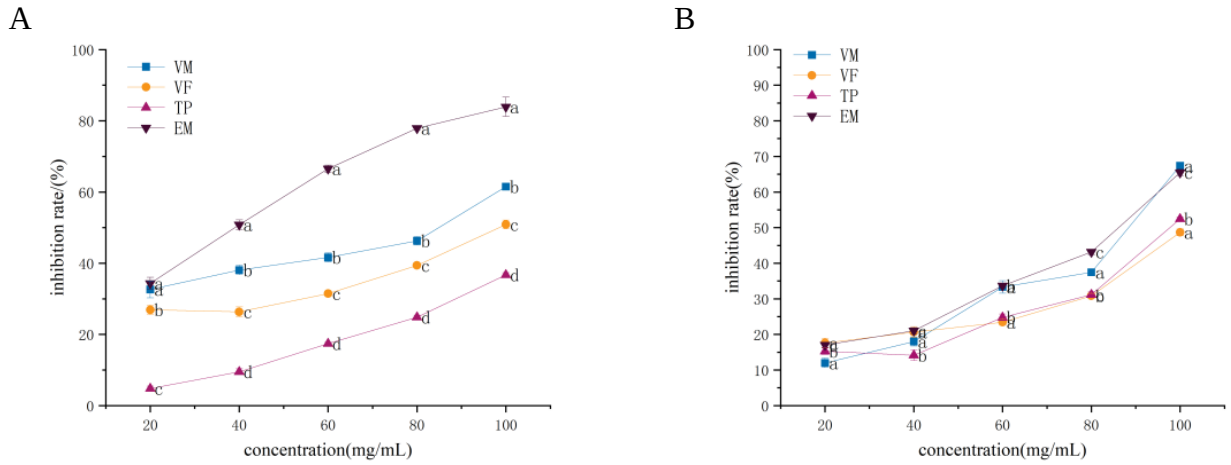


Fig 3. Inhibition rate of α-amylase activity by four PO powders: (A) Aqueous extract; (B) Alcohol extract, different letters indicate significant differences at different process methods ($p < 0.05$).

Correlation between the chemical composition of extract of PO powder and inhibition rate of enzyme activity: The correlation between the functional components and the inhibition rate of α-amylase activity was analyzed. As shown in Fig. 4, the four functional components were positively correlated with the inhibition rate of α-amylase activity. These results indicated that all four functional substances could inhibit the activity of α-amylase. A significant positive correlation was found between the total polysaccharide content and the inhibition rate of α-amylase activity. This indicates that polysaccharides in PO directly affect the activity of α-amylase. This is consistent with the results of Miranda et al., who found that polysaccharides in *Lentinus tigrinus* CCMB 553 could inhibit α-amylase activity [43]. In another study, polysaccharides in PO significantly lowered fasting blood glucose levels. It also reduces blood glucose concentration by regulating related enzyme activities and glutathione content in the liver, thereby protecting the liver [44].

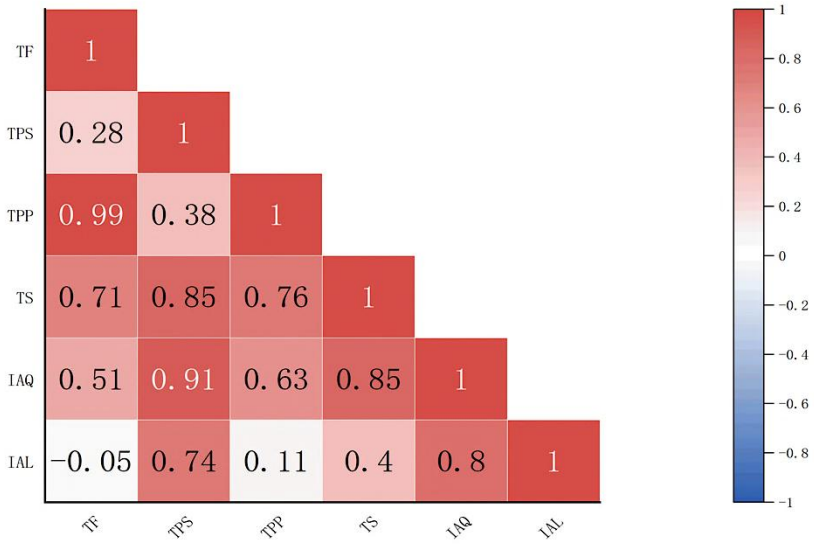


Figure 4. Correlation analysis between functional components and enzyme inhibition rate: the abbreviations are as follows: Total polysaccharide (TPS); total Saponin (TS); total polyphenol (TPP); total flavonoid (TF) Inhibition rate of α-amylase activity of aqueous extracts (IAQ); Inhibition rate of α-amylase activity of alcohol extracts (IAL)

Effects of four PO powders addition on the quality characteristics of steamed buns: The scoring of each item of steamed buns is shown in Fig. 5, and the total ranking is EM > VM = BG > TP > VF. Except for VF, the epidermal condition, color, and internal organization of the steamed buns, which added PO for the remaining groups, were better than those of the blank group (BG). Sensory evaluation panel members agreed that the EM composite buns were visually superior in color and were perceived as having a uniform, soft, and delicate internal texture. Regarding flavor and taste scores, all groups of buns with added PO outperformed the BG group. This indicated that the composite steamed buns could maintain their original texture and flavor well after adding PO powder, and there was no adverse effect on their texture and taste.

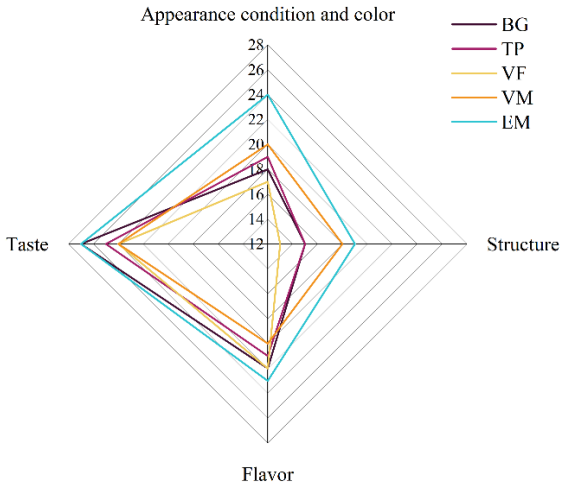


Fig 5. Results of sensory evaluation of steamed buns

The texture of food chiefly refers to its organizational properties, which are associated with food's sensory and edible properties. In the texture properties of steamed buns, hardness, adhesion, and chewability are negatively correlated with the quality of the steamed buns.[45] When the values of these three indicators are large, the steamed buns will be hard, difficult to chew, and sticky when eaten. The cohesion and elasticity of the steamed buns are positively correlated with their quality.

As can be seen from Fig 6A, B, and C, Compared with BG, the Steamed buns adding PO were improved to varying degrees in three aspects: hardness, adhesion, and chewability. Studies have shown that the higher the starch content, the higher the hardness and adhesiveness of the steamed buns. Moreover, increasing the proportion of dietary fiber in the raw materials can significantly reduce the hardness and adhesiveness of the steamed buns [46]. This is consistent with our results, as the dietary fiber in the PO powder can lower the hardness and adhesiveness of the steamed buns. Compared with VM steamed buns, EM steamed buns' hardness, chewiness, and adhesiveness were further improved, with values of 23.18±0.89, 0.08±0.00, and 79.01±5.90, respectively. This improvement may result from the enzymatic hydrolysis of large molecules into smaller ones, which yeast can utilize more efficiently [47]. The addition of EM allows for better dough fermentation, further reducing the hardness, chewiness, and adhesiveness of the steamed buns, as can be seen in Fig 6D, E. Compared with BG, the elasticity and cohesiveness of the four types of steamed buns with added PO powder were reduced, which may still be due to the influence of dietary fiber in the PO powder replacing starch. The gluten network plays a crucial role in the elasticity and cohesiveness of steamed buns [46].

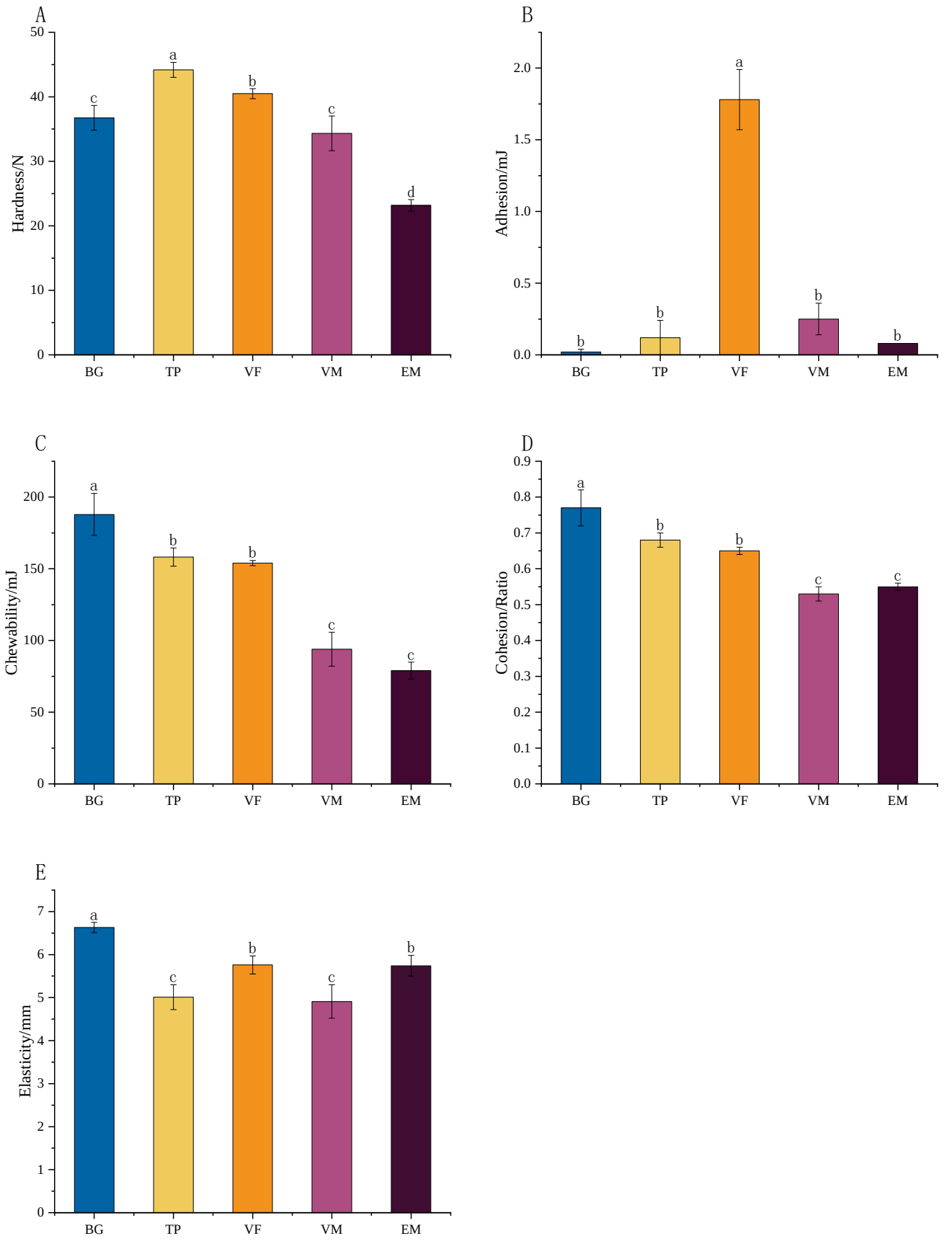


Figure 6. The texture of steamed buns: (A) Hardness, (B) Adhesion, (C) Chewability, (D)Cohesion, (E) Elasticity; different letters indicate significant differences at different process methods ($p < 0.05$).

For fermented flour products, hardness and chewiness are two essential quality indicators [48]. The smaller the values of hardness and chewiness, the better the palatability of the steamed buns. Among them, the changes in hardness and chewiness of steamed buns during short-term storage showed the aging degree of steamed buns. The difference in hardness and chewiness of steamed buns after 3 days of storage showed the aging rate of steamed buns with different steamed buns. So, this study used the hardness and chewiness of steamed buns as the main criteria to judge the aging performance of steamed buns.

As shown in Fig. 7A, the hardness and chewiness of the steamed buns increased gradually with the extension of storage time. VM and EM addition decreased the hardness of steamed buns compared with BG steamed buns. The hardness of EM steamed buns was significantly lower than other groups. Compared with the BG steamed buns, adding PO powder improved the chewiness of steamed buns and reduced the growth of steamed buns' chewiness, which became poor. As can be seen in Fig. 7B. Compared with TP and VF, VM and EM added to the chewiness of steamed buns and have a certain degree of improvement, of which EM has the most substantial improvement effect on the chewiness of steamed buns, which may be due to the enzyme treatment-producing small molecule sugar can inhibit the starch ordering and recrystallization, so that the starch aging degree is reduced. It is easy to chew [49].

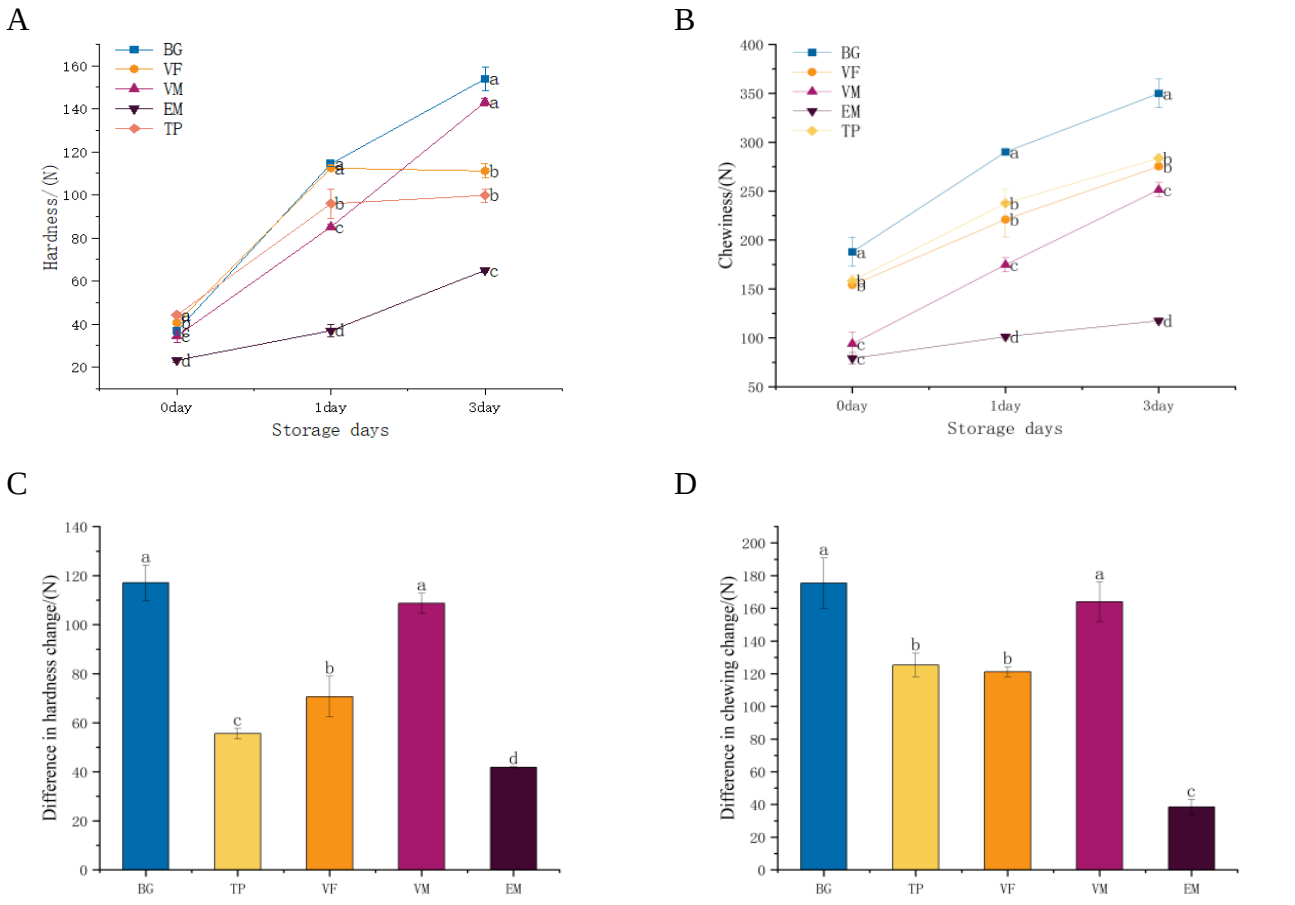
As shown in Fig. 7C, the hardness change values of steamed buns with PO powder addition were reduced after 3 days of storage compared with BG. This indicates that adding PO powder enhances the aging performance of steamed buns. Among them, the difference in hardness change value of EM (41.8 N) was much lower than that of BG (117.11 N). These results indicated that adding EM had the best mitigation effect on the aging rate of buns.

As shown in Fig. 7D, in terms of the change in chewiness, the addition of TP, VF, and EM significantly improved the change in chewiness of the steamed buns after 3 days of storage compared to BG, while the addition of VM had no significant effect on the shift in chewiness.

However, after PO powder was enzymatically modified, it significantly inhibited the degree of increase in the chewiness of steamed buns.

As can be seen from Fig.8, the trend of fermentation volume change of the dough after adding each PO powder was consistent with that of the BG group, with the dough volume increasing significantly in the first 60 minutes and then increasing slowly thereafter. Compared to the BG group, the addition of PO powder, except for the enzyme-modified PO powder group, resulted in different degrees of reduction in the volume of the steamed buns, which may be related to the obstruction of gluten protein formation in the steamed buns. The addition of EM promoted the fermentation of the dough, and this may be because the PO powder, after enzymatic modification, was hydrolyzed, which is beneficial for the growth and reproduction of yeast [50].

Figure 7. (A, B): Effect of the addition of PO powder on the short-term storage of steamed buns: (A hardness; (B) Chewiness, different letters indicate significant differences at different process methods ($p < 0.05$). (C, D): Changes in PO steamed buns after 3 days of storage: (C) Difference in hardness change; (D) difference in chewing change, different letters indicate significant differences at different process method ($p < 0.05$).



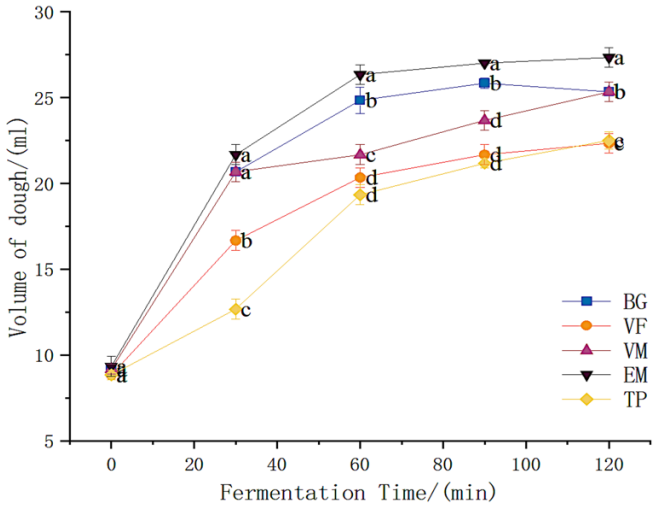


Figure 8. Effect of PO powder on the volume of dough fermentation, different letters indicate significant differences in process methods ($p < 0.05$).

In recent years, more and more studies have shown that a diet based on low glycemic index foods can reduce the risk of diabetes. As can be seen from Table 5, the RSC value of steamed buns was decreased by four PO powders in addition to different extents. This may be because PO powder has many ingredients that inhibit the activity of α-amylase, which inhibits the starch hydrolysis process and then decreases the sugar content in vitro digestion. Combined with the correlation analysis in section 3.4, it is speculated that the substance in PO powder that acts to reduce the RSC value is polysaccharide. The PO soluble non-starch polysaccharides in the PO powder can form a complex with the starch and encapsulate the starch gel, restricting the movement of water to the starch granules in steamed buns, thus interfering with the degradation of some pasted starch granules [51]. Compared with BG, adding VF, VM, EM, and TP significantly reduced the RSC value of steamed buns by 30.26%, 28.58%, 27.2%, and 24.77%, respectively.

Table 5. Effect of PO powder on RSC value of steamed buns

Sample	A1/(mg/g)	A2/(mg/g)	RSC value
BG	383.57±1.25a(A0)	227.19±2.39a	100.00±0.33a
TP	300.40±1.88b	118.01±1.89d	75.23±0.52b
VF	282.71±2.59d	162.49±2.97c	69.74±0.60d
VM	289.46±1.17c	159.38±3.14c	71.42±0.23c
EM	296.99±6.18b	183.80±1.41b	72.80±1.58c

Means of triplicate determination ± S.D with the letters in the row within each property are significantly different ($p < 0.05$).

CONCLUSIONS

Enzymatic modification treatment can improve the functional quality and application performance of PO powder prepared by vacuum microwave drying, especially regarding functional components and α-amylase inhibition activity. The total saponins, polysaccharides, polyphenols, and total flavonoid contents of EM increased by 74%, 127.8%, 91.2%, and 125.4%, respectively, compared with TP. The inhibition of α-amylase activity by EM extract was the highest among all the samples, with 61.51% at an extract concentration of 100 (mg/ml). Adding the four types of powders improved the quality of the PO steamed buns to different degrees; adding EM significantly improved the hardness, chewability, and adhesion. The PO powder had a varying degree of lowering effect on the RSC value of the steamed buns. The PO steamed buns exhibited a better hypoglycemic effect than traditional buns. The comprehensive evaluation indicated that the EM composite steamed buns had superior physical properties, chemical composition, and hypoglycemic function compared to other products. Compound enzymatic hydrolysis can effectively enhance the functional quality of PO powder. The results provide a scientific basis for modifying and applying PO powder.

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Ethics statement: Our research does not engage in research beyond sensory evaluation that may have implications for medical ethics. All materials used for the sensory assessment in our study were edible food. The informed consent was obtained from all human subjects in our research, and the privacy rights of our volunteers were consistently observed.

Competing Interests: None of the authors have any financial interests or conflicts of interest to report.

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