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Quality Evaluation of Cistanchedeserticola-potato Composite Rice Based on Principal Component Analysis and Cluster Analysis

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Abstract:
In order to obtain the best processing conditions for Cistanchedeserticola-potato extruded rice (CDPR), principal component analysis (PCA) and cluster analysis were used to screen 17 CDPR. First of all, the quality indexes of these 17 kinds of CDPR were measured (hardness, adhesiveness, springiness, cohesiveness, chewiness, resilience, water absorption index, water solubility index, content of echinacoside and sensory quality), and then the data were collected for PCA. The first three components were selected, explaining 89.406% of the total variance. By calculating the factor score, the highest factor score of CR3 was 1.146, and the lowest comprehensive score of CR1 and CR9 was -0.924 and -1.961, respectively. Cluster analysis also found that the three kinds of rice with comprehensive score greater than 0.5 were in the second category. Good quality and tasty CDPR can be extruded under certain twin-screw extrusion processing conditions (screw speed 140 r/min, barrel temperature 115°C, moisture content 29% and addition of Cistanchedeserticola 0.8%), which was characterised by its sweet taste, structural integrity and high echinacoside content (90.65 mg/100 g).

***Key Word**: Cistanchedeserticola; Composite rice, Principal component analysis; Cluster analysis; Twin-screw extruder*

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I. Introduction

Composite rice was a product made from broken rice which was reshaped at high temperature. It can significantly reduce the cooking time compared to ordinary rice. The research on composite rice can be traced back to the 1980s. Dip coating and film coating methods were used to strengthen the specific ingredients in the early days[1,2]. In the later research, the method of adding nutrients or substances rich in nutrients was used to make functional composite rice by combining with an extrude [3,4,5,6,7].

Functional compound rice mostly used materials rich in protein or fat in the selection of raw materials [8,9,10,11,12], but there was little exploration on medicinal and food homologous substances[13], especially Cistanchedeserticola. Cistanchedeserticola was one of the Nine Immortal Herbs of China, which was as famous as ginseng and cordyceps. Cistanchedeserticola, originally recorded in Shennong's Herbal Classic which was the earliest book on materia medica in the world. Cistanchedeserticola contained various types of components such as phenylethanoid glycosides, iridoids, lignin, alkaloids, trace elements, sugars, etc[14,15]. It had a variety of effects such as anti-oxidation, anti-aging, anti-fatigue, bone protection, kidney tonifying, immune regulation and so on[16,17,18,19]. Among them, echinacoside (phenylethanoid glycosides) had the best antioxidant activity[18]. At present, researches on Cistanchedeserticola mainly focused on cultivation methods, composition, and pharmacological effects, but few studies on its food application. Therefore, Cistanchedeserticola was selected as one of the raw materials to prepare the composite rice rich in echinacoside.

The fine processing of rice inevitably produced a large amount of broken rice, and the by-products of these processing were generally used as feed [20]. The effective reuse of resources can be realized by using broken rice as the main raw material. Substituting a part of broken rice with potato flakes had a certain positive impact on the development of potato staple food, and had good economic value and broad market prospects.

A combination of principal component analysis (PCA) and cluster analysis was often used to study on multiple indicators of multiple individuals[21]. PCA can minimize variables while retaining the original data information to the greatest extent. Cluster analysis was to group and categorize a large amount of data to explore the internal connection of the data set. In order to screen out the appropriate processing conditions and obtain the products with good taste and properties, 10 quality indicators of 17 Cistanchedeserticola-potato composite rice (CDPR) were evaluated and analysed by PCA and cluster analysis. This can provide methodological basis and practical reference for the development of functional composite rice.

II. Material And Methods

Materials

Broken rice (Peizheng Grain Store, Zibo, Shandong, China), potato flakes (Xisen Potato Industry Group Co., Ltd., Zibo, Shandong, China) and Cistanchedeserticola (Urumqi, Xinjiang, China) were used.

Sample preparation

All crushed materials with the particle size of 147 μm were mixed. The mixing ratio of crushed broken rice flour and potato flakes flour was 7:3 (w/w). The addition of Cistanchedeserticola was shown in Table 1. The solid feeding rate was 15 kg/h. The temperature of zone II, III, V and VI of the UVTE36-24 twin-screw extruder (Changsha Chuangheng Food Technology Co., Ltd, Changsha, Hunan, China) was set at 60, 90, 50 and 50°C, respectively. Then the samples were prepared according to Table 1. All extrudates were sheared at 2200 r/min.

Table no 1: Experimental arrangements of Cistanchedeserticola-potato composite rice.

Type	Screw speed (r/min)	Barrel temperature (Zone Ⅳ°C)	Moisture content (%)	Addition of Cistanchedeserticola (%)
CR1	140	95	29	0.6
CR2	140	105	29	0.6
CR3	140	115	29	0.6
CR4	140	125	29	0.6
CR 5	140	135	29	0.6
CR6	140	115	25	0.6
CR7	140	115	27	0.6
CR8	140	115	31	0.6
CR9	140	115	33	0.6
CR10	120	115	29	0.6
CR11	130	115	29	0.6
CR12	150	115	29	0.6
CR13	160	115	29	0.6
CR14	140	115	29	0.2
CR15	140	115	29	0.4
CR16	140	115	29	0.8
CR17	140	115	29	1.0

Texture quality

Texture qualities of samples were determined by a TA. XT Plus texture analyzer (Stable Micro System, London, UK). The cooked sample (50 g) was pressed by a P/36R probe at 0.5 mm/s until reaching 80% of sample thickness. More specific method referred to previous studies [22]. Each sample was tested five times in parallel. Items included hardness, adhesiveness, springiness, cohesiveness, chewiness and resilience.

Water absorption index and water solubility index

Sample (2.0 g) with particle size of 180-250 μm was immersed in distilled water (25 mL), and the mixture was swirled by a SHA-BA water bath constant temperature oscillator (Changzhou Ronghua Instrument Manufacturing Co., Ltd, Changzhou, Jiangsu, China) at 30 ℃ for 20 min. The resulting dispersion was centrifuged by DL-5-B centrifuge (Shanghai Anting Scientific Instrument Factory, Shanghai, China) at 4 000 r/min for 20 min. The supernatant transferred into the weighing bottle was weighed by drying. The precipitation quality was determined. Water absorption index (WAI) and water solubility index (WSI) were calculated by Eq. 1 and 2, respectively.

$$\mathrm{WAI(g/g)} = \frac{\text{Weight of precipitation}}{\text{Weight of sample}} \#(1.)$$

$$\mathrm{WSI(\%)} = \frac{\text{Weight of supernatant after drying}}{\text{Weight of sample}} \times 100\#(2.)$$

Content of echinacoside

The HPD300 macroporous resin (Beijing Solarbio Science & Technology Co., Ltd, Beijing, China) was soaked in ethanol (95%, Yantai Shuangshuang Chemical Co., Ltd, Shandong, China) for 24 h to swell fully and remove the impurities in the resin. The resin was washed with ethanol until there was no white turbidity when an equal volume of deionized water was added. Then the resin was washed with deionized water until there was no alcohol smell. Subsequently, the resin was soaked in hydrochloric acid solution (5%, Yantai Shuangshuang Chemical Co., Ltd, Shandong, China) for 3 h, and washed with deionized water until the pH of filtrate was neutral. The resin was soaked in sodium hydroxide (5%, Yantai Shuangshuang Chemical Co., Ltd, Shandong, China) for 3 h, and washed with deionized water until the pH of filtrate was neutral. The resin was loaded into the adsorption column by ethanol wet method, and the liquid level of ethanol was kept about 10 mm higher than the resin surface, soaking in balance for 12 h. The resin layer was washed with ethanol (95%) until there was no white turbidity after adding twice the deionized water. Then deionized water was used to wash until there was no alcoholic smell.

Crushed Sample with particle size of 250 μm was extracted by ethanol and purified by HPD300 macroporous resin. The standard substance of echinacoside (Beijing Hongyue Innovation Technology Co., Ltd, Beijing, China) was dissolved in methanol solution (Shanghai Yien Chemical Technology Co., Ltd, Shanghai, China), and the standard curve was drawn at 334 nm by the SP-Max2300A2 microplate reader (Shanghai Shanpu Biotechnology Co., Ltd, Shanghai, China). The content of echinacoside in each sample was determined according to Eq. 3. Each sample was tested 5 times in parallel.

$$\text{Content of echinacoside (mg/100 g)} = \frac{C \times V \times N \times 10^{-5}}{W} \#(3.)$$

where, C is the concentration of echinacosidein the sample extract (μg/mL), V is the volume of the sample extract (mL), N is the dilution multiple, and W is the weight of the sample (g).

Sensory evaluation

The sensory quality was conducted by a total of ten trained personnel based on the 9-point sensory evaluation criteria. Items included taste, appearance and structure, aroma and palatability. The specific evaluation criteria were shown in Table 2.

Table no 2: Sensory scoring criteria of Cistanchedeserticola-potato composite rice.

Item		Scoring criteria	Score
Taste	Purity and permanence	There is no flavour when chewing.	0-1.0
		There is a slightly sweet flavour when chewing.	1.1-2.0
		There is a rich, sweet flavour when chewing.	2.1-3.0
Appearance and structure	Lustre	Lustre of the composite rice is distinct.	0-0.4
	Colour	Colour of the composite rice is slightly poor and the presentation is uneven.	0-0.2
		Colour of the composite rice is characteristic, even, faintly pale yellow.	0.3-0.5
	Integrity	The composite rice is incomplete.	0-0.3
		The composite rice is structurally intact.	0.4-0.6
Aroma	Purity and permanence	Aroma of the composite rice is not strong.	0-0.7
		The composite rice is richly aromatic.	0.8-1.5
Palatability	Viscosity	The composite rice is sticky to the teeth, or not viscous.	0-0.5
		The composite rice is viscosity and basically non-stick toothy.	0.6-1.0
	Elasticity	The composite rice is sparse and not chewy.	0-0.5
		The composite rice is moderately elastic.	0.6-1.0
	Softness	The composite rice is slightly soft, or slightly hard.	0-0.5
		Hardness of the composite rice is moderate.	0.6-1.0

Statistical analysis

Statistical analysis was examined by IBM SPSS STATISTICS 23.0 (IBM Corp., Armonk, New York State, USA) and Origin 2021 (OriginLab Co., Ltd, Northampton, Massachusetts, USA) was used for drawing.

III. Result and discussion

Qualities of CDPR

There were differences in the texture characteristics of samples under different processing conditions (Figure 1).

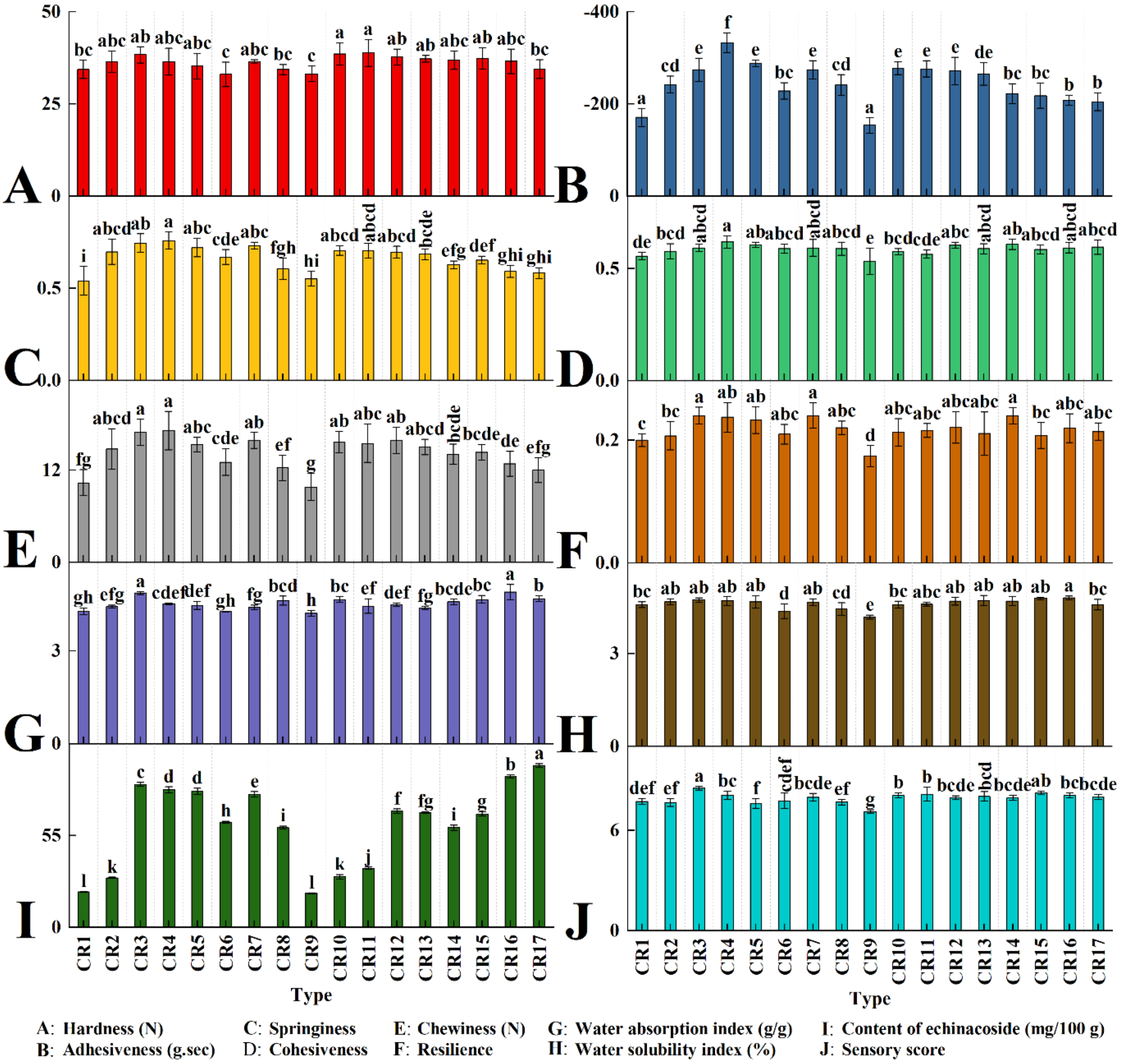

Figure 1: Effect of different processing conditions on (A) hardness, (B) adhesiveness, (C) springiness, (D) cohesiveness, (E) chewiness, (F) resilience, (G) water absorption index, (H) water solubility index (I) content of echinacoside and (J) sensory score of 17 varieties of Cistanchedeserticola-potato extruded rice.
Different letters mean significant difference ($P < 0.05$).

Hardness of CR6 was the smallest, while that of CR11 (38.85 N) was the largest, and the difference between the two types of rice was significant. If the rice was too soft or too hard, the taste was not good, so good CDPR cannot be prepared under the conditions of too low screw speed and too low moisture content. CR9 had the least adhesiveness and CR4 had the greatest adhesiveness, and the differences between the two kinds of CDPR were significant. Figure 1B showed that the adhesiveness of CR3 was not significantly different from that of CR10, CR11, CR12 and CR13, indicating that the change of screw speed had little effect on the adhesiveness of CDPR in this experiment. Among the 17 kinds of CDPR, springiness of only four kinds of them was lower than 0.60, namely CR1 (0.54), CR9, CR16 (0.59) and CR17. The springiness of other CDPR ranged from 0.60 to 0.76, among which CR3 and CR4 had the highest springiness of 0.75 and 0.76, respectively. Figure 1C indicated that the change of screw speed had little effect on the springiness, as shown by the non-significant differences between CR3 and CR10, CR11, CR12 and CR13. It can be seen from Figure 1D that cohesiveness of CDPR changed little (0.53-0.62) with different processing conditions and the addition of Cistanchedeserticola had no significant effect on the springiness of CDPR. Chewiness of CR9 and CR1 was lower than 12 N. Figure

1E revealed that chewiness of CDPR can be improved with the increase of a certain degree of temperature. Resilience of other samples was higher than 0.2 except for CR9 (0.17).

It can be seen from Figure 1G and Figure 1H that WAI and WSI of CR9 were 4.22 g/g and 4.18%, while those of CR16 were 4.91 g/g and 4.80%, respectively. The change of processing conditions (barrel temperature, moisture content, screw speed and addition of Cistanchedeserticola) had a significant impact on the WAI of CDPR. The effect of temperature and moisture content on WSI was not significant. As can be seen from Figure 1I, the content of echinacoside in different processing conditions were significantly different. The maximum and minimum contents of echinacoside were 97.16 mg/100 g (CR17) and 20.48 mg/100 g (CR9), respectively. Figure 1J demonstrated that sensory score of CDPR were higher than 7.66 except for CR9 (7.13).

Principal component analysis

As can be seen from Figure 2A, the sensory quality of CDPR was correlated with the other nine indicators ($p < 0.05$), especially hardness ($r = 0.75$, $p = 0.000$), chewiness, resilience, WAI and WSI ($r = 0.74$, $p = 0.000$). Both resilience and WSI had strong correlation with the other nine indicators. The content of echinacoside showed a strong correlation with cohesiveness, resilience and WAI ($p < 0.01$), but the correlation with hardness and springiness was not significant ($p < 0.1$). In addition, the correlation between WAI and adhesiveness, springiness, chewiness was not significant, and the relationship between hardness and cohesiveness was not significant ($p < 0.1$). This showed that correlation analysis cannot fully and completely explain the original variables and there were limitations to exploring multiple index rules only by correlation analysis.

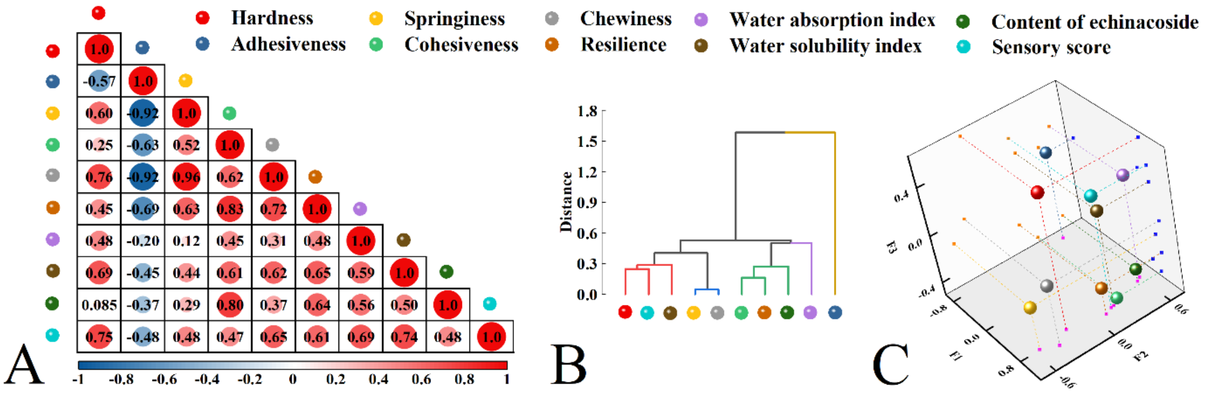

Figure2. Results of principal component analysis and cluster analysis of indicators. (A) Correlation between quality indicators of Cistanchedeserticola-potato extruded rice. (B) Hierarchical cluster of quality indicators. (C) Loading matrix of first three principal components.

The applicability test of factor analysis was performed after standardizing the original data according to Eq. 4.

$$X_{ij} = \frac{x_{ij} - \overline{x_j}}{\sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(x_{ij} - \overline{x_j}\right)^2}} \#(4.)$$

where, x_{ij} is the original value of the i type of composite rice in the j indicator, X_{ij} is the converted value of the i type of composite rice in the j indicator, and $\overline{x_j}$ is the average value of the j indicator.

The following indicators were selected for principal component analysis: hardness (X_1), adhesiveness (X_2), springiness (X_3), cohesiveness (X_4), chewiness (X_5), resilience (X_6), WAI (X_7), WSI (X_8), content of echinacoside (X_9) and sensory score (X_{10}). It was found that the KMO value was 0.729, and the significance of Bartlett sphere test was 0.000, indicating that there was a certain correlation between the indicators, and the data was suitable for factor analysis method. It can be seen from Table 3 that the eigenvalues of the first three common factors were 6.104, 1.575 and 1.261 (> 1), respectively. The variance contribution rate of the first three factors was 89.406% (≥ 85%), that is, the first three principal components reflected 89.406% of the information of the original indicator. Therefore, three factors can be extracted to make the best explanation for the analysed problem.

The greater the absolute value of the principal component loading, the more important the influence on the corresponding principal component. It can be seen from Figure 2C that the load distribution of each index on the principal component 1 was all greater than 0.5, which can be called the comprehensive factor. Among them, chewiness had the greatest effect, followed by resilience. The load of WAI was the lowest on principal component 1, while it showed the greatest loading on principal component 2, followed by content of echinacoside and springiness. The influence of hardness was mainly dominant on principal component 3.

The linear combination of F_1, F_2 and F_3 was obtained according to the three principal component coefficients:

$$F_1 = 0.120X_1 - 0.135X_2 + 0.129X_3 + 0.130X_4 + 0.149X_5 + 0.141X_6 + 0.098X_7 + 0.131X_8 + 0.105X_9 + 0.133X_{10}\#(5.)$$
$$F_2 = -0.157X_1 + 0.288X_2 - 0.350X_3 + 0.168X_4 - 0.254X_5 + 0.069X_6 + 0.383X_7 + 0.140X_8 + 0.356X_9 + 0.119X_{10}\#(6.)$$
$$F3 = 0.478X_1 + 0.179X_2 - 0.121X_3 - 0.371X_4 - 0.007X_5 - 0.228X_6 + 0.268X_7 + +0.219X_8 - 0.322X_9 + 0.324X_{10}\#(7.)$$

The scores of each composite rice on three principal factors can be calculated respectively through the above three formulas. Then, according to the ratio of the contribution rate of the variance to the cumulative variance contribution rate, the factor scores are weighted and aggregated to obtain the synthesis score of each composite rice:

$$F = 0.683F_1 + 0.176F_2 + 0.141F_3\#(8.)$$

The score of each factor and the synthesis score of composite rice were obtained and ranked. The top five CDPR scoring on the 1st principal component were CR3 (1.422), CR4 (1.262), CR7 (0.673), CR12 (0.594) and CR5 (0.480). The top five CDPR scoring on the 2nd principal component were CR16 (2.161), CR17 (1.959), CR14 (0.715), CR15 (0.698) and CR8 (0.645). The top five CDPR scoring on the 3rd principal component were CR10 (1.396), CR11 (1.359), CR15 (1.234), CR3 (0.818) and CR16 (0.812). It can be seen from Figure 3E that the top five composite rice were CR3 (1.149), CR16 (0.688), CR4 (0.563), CR15 (0.487) and CR14 (0.322). CR3, which had the highest synthesis score (Figure 3E), ranked in the top 6 on the three principal components, of which the score on principal component 1 (1.422) was the highest (Figure 3B). Higher adhesiveness, springiness and resilience made the hardness and chewiness of CDPR higher. CR3 had the highest content of echinacoside, which can reach 85.79 mg per 100 g of CDPR, with the same amount of Cistanche deserticola added. At an appropriate screw speed (140 r/min), the macromolecules in the raw materials can be better degraded, and at the same time, the effective ingredients in the raw materials can be further released. CR3 had the highest sensory score (8.519) due to its full shape and good texture quality, and was significantly different from other 16 kinds of CDPR. This showed that the appropriate addition of Cistanche deserticola can improve the edible quality of CDPR. In general, the synthesis score of composite rice was the highest under the specific screw speed (140 r/min), barrel temperature (115 ℃) and moisture content (29%). CR9 and CR1 performed the worst, occupying the last two positions.

Cluster analysis

The cluster analysis of the quality indicators of the CDPR revealed that the 10 indicators can be classified into three categories at a Euclidean distance of 0.529. As can be seen from Figure 2B, adhesiveness was classified into the third category. It can be seen from Figure 2A that the correlations between the indicators in the first category (hardness, sensory quality, WSI, springiness and chewiness) were strong ($p < 0.05$), and the indicators in the second category (cohesiveness, resilience, content of echinacoside and WAI) also had a strong positive correlation ($p < 0.05$).

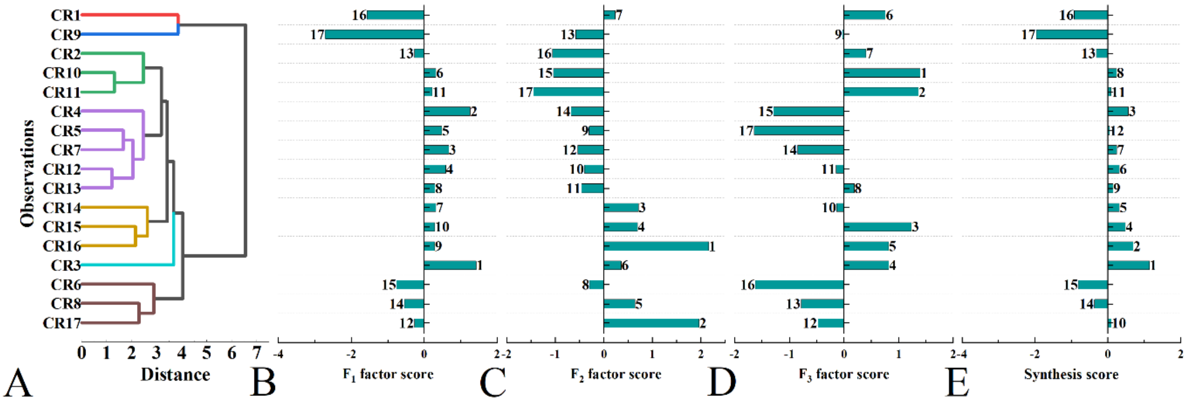

Figure 3: Results of cluster analysis and factor scores of Cistanchedeserticola-potato extruded rice. (A) Hierarchical cluster of 17 varieties of Cistanchedeserticola-potato extruded rice. (B) Scores and sorts on the first principal component. (C) Scores and sorts on the second principal component. (D) Scores and sorts on the third principal component. (E) Synthesis scores and sorts of 17 varieties of Cistanchedeserticola-potato extruded rice.

In order to screen appropriate process parameters for the CDPR, a systematic cluster analysis was conducted on the types of composite rice. The Euclidean distance and the average link between groups were used to cluster the composite rice under different processing parameters, and the results were shown in Figure 3A. All composite rice can be divided into three categories, the first category included CR1 and CR9 at the

Euclidean distance of 4.054. The first category of CDPR had the lowest synthesis score value, mainly in the 1st principal component factor. In the limited residence time, low extrusion temperature (95℃, CR1) may cause the material to not absorb enough heat, thus affecting the quality of the product. Water molecules do not move violently at low temperatures, preventing shearing action from penetrating inside the molecules, resulting in a low springiness and a low WAI of the composite rice. Water acted as a lubricant during the extrusion process and played an important role in the formation of a uniform melt. The ordered molecular structure in the raw material was dispersed into a disordered state, and the original crystal structure was destroyed, so that the shape of the CDPR was complete and the texture was uniform[23,24,25]. However, the excessively high moisture content (33%, CR9) made the material stay in the cavity of the extruder too short, and the crystal structure was not easily destroyed, which affected the gelatinization effect of the CDPR. The low adhesiveness, cohesiveness and resilience of CDPR resulted in low sensory score. Moisture reduced the friction between screw and materials, which caused that echinacoside in Cistanche deserticola can not be completely released by shearing in a short time. It can be seen from Figure 3E that the synthesis scores of this two kinds of composite rice was -0.924 and -1.961, respectively. The comprehensive ranking of the two was low, indicating that the corresponding extrusion parameters cannot meet the general taste requirements. The screw speed should not be less than 95 r/min and the moisture content should not be more than 33% in practical operation.

CR6, CR8 and CR17 were classified into the third category. The third category of CDPR had a low synthesis score, mainly in the 1st and 3rd principal component factors. The moisture content of CR6 was low (25%), and the fluidity of the material was reduced, resulting in low springiness and adhesiveness of the product. The hardness of CDPR was low and the chewiness was not high, which affected the taste of the product. CR8 ranked fifth in the 2nd factor score (Figure 3D), that is, it performed well in terms of water absorption. WAI mainly refleced the swelling capacity of starch[26,27]. Shear stress can cause water transfer into the interior molecules faster, which made the composite rice had a higher water absorption capacity[28]. CR17 had a higher score in the 2nd factor (1.959), which was mainly reflected in the WAI, content of echinacoside and springiness of CDPR. WAI was closely related to the hydrophilic capacity of large substances such as starch and protein in the raw materials. Starch molecules in raw materials were sheared, hydrogen bonds were broken, hydrophilic groups were exposed, resulting in the improvement of water absorption of CDPR[29,30]. In addition, the protein may be denatured and decomposed under the high temperature and high shear of the twin screw, and some fibers may also be pyrolyzed by extrusion [31]. The content of echinacoside increased with the increase of Cistanche deserticola, and the difference of echinacoside content in different types of CDPR was significant (Figure 1I). In this experiment, a maximum of 97.16 mg of echinacoside can be retained in every 100 g of CDPR, and the sensory score can still reach 8.

The three kinds of rice (CR3, CR16, CR4) with a synthesis score greater than 0.5 obtained by the principal component analysis were all concentrated in the second category, indicating that the composite rice, with similar taste quality, had the characteristics of a complete structure in appearance, high content of functional components and high sensory score. CR16, which had the second-highest synthesis score, had the highest score on principal component 2 (2.161) and had a high sensory score (8.100). It can be seen from Figure 1G that the WAI and WSI of CR16 were the largest, and they wee significantly different from those of other CDPR. Both potato flakes and Cistanche deserticola contained a small amount of fibre, which may have contributed to a certain degree of improvement in the water absorption properties of the CDPR. Dietary fiber contained a lot of hydrophilic groups and had strong hydrophilicity. Potato flakes retained the integrity of the potato cells during production process, allowing it retained the natural potato flavour, so the smell of the CDPR can still be retained. The taste of CDPR was not only the mellow flavor of rice, the rich taste of potato, but also the sweet taste of Cistanche deserticola, which may be caused by the polysaccharides in Cistanche deserticola (heteropolysaccharides composed of rhamnose, galactose, fructose, glucose) (Gong et al., 2007). CR4, with the third highest synthesis score, had the second highest score on principal component 1 (1.262), but performed poorly on principal components 2, and 3. The increase in temperature contributed to the adhesiveness and springiness of CDPR, which made CDPR had a suitable taste. The increase of temperature may lead to the degradation of fiber and the denaturation of protein, resulting in the increase of water-soluble substances in the product and the increase of WSI. However, too high temperature can cause Maillard reaction of the protein in the materials (Bjoerck et al., 1983), causing the loss of nutrients, so the barrel temperature should be lower than 135°C.

IV. Conclusion

Correlation analysis, principal component analysis and cluster analysis were used to discuss various indicators of different types of CDPR to obtain the best processing conditions. The results showed that there were significant differences in the same index among different types of CDPR ($p < 0.05$). The results of principal component analysis showed that the three principal components can explain all 10 indicators well. Through cluster analysis, 17 kinds of CDPR were divided into three categories. The three kinds of CDPR with good characters, good taste and high content of functional components were all concentrated in the same category. This study provided a theoretical basis and reference value for the establishment of the evaluation mechanism and the exploration of the technology of composite rice.

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