

Research paper

Changes of physicochemical properties and correlation analysis of common buckwheat starch during germination

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ABSTRACT

In order to clarify the physicochemical properties of starch during germination of common buckwheat, Xinong9976 was selected as the experimental material to study the main nutrients, particle structure, particle size distribution, transparency, aging value, pasting properties and the correlation between pasting properties and starch composition and main nutrients. The results showed that main nutrients were significantly different. The diameter of starch granules ranged from 2.36 to 8.89 μ m, and the shapes of starch granules were irregular with obvious holes and cracks on the surface. There were significant differences in starch transparency, aging value and pasting properties at different germination stages. Peak viscosity, through viscosity and final viscosity of germinated common buckwheat was significantly positive correlated with amylopectin content ($P < 0.05$) and breakdown, final viscosity and setback were significantly negatively correlated with amylose content ($P < 0.05$). The correlation analysis of starch pasting properties and main nutrients showed that breakdown, setback and crude fat content were significantly negatively correlated ($P < 0.01$), peak viscosity, through viscosity and final viscosity were significantly negatively correlated with crude fat content ($P < 0.05$), while the starch pasting properties had no significant correlation with other nutrients.

INTRODUCTION

Common buckwheat (*Fagopyrum esculentum*) belongs to Polygonaceae, it is an important minor grain crop in China (Nam et al. 2018). Common buckwheat is rich in nutrients, containing 10.6% -15.5% of protein, 1.2% - 2.8% of fat, 63% -71.2% of starch, as well as flavonoids, mineral elements and cysteine (Gimenez-Bastida and Zielinski 2015). Recently, with the improvement of people's living standard, it has become fashionable to pursue a balanced diet with scientific based nutrition. Therefore, common buckwheat products with the reputation of "the same origin of food and medicine" will be widely welcomed by people. Besides, the development and research of common buckwheat healthy food have broad market prospects, high economic value and social value (Yoshimoto et al. 2004). There have been many studies on the cultivation methods, yield and protein content of common buckwheat in China and abroad (Salehi et al. 2018, Fang et al. 2018, Khan et al. 2012) and studies on the starch properties have also been reported. However, changes of physicochemical properties of germinated common buckwheat starch is not reported. Starch is the main nutritional component of common buckwheat. Its physicochemical properties affect the nutritional properties of related products and are also related to the development of new uses of common buckwheat starch (Stibilj et al. 2004).

Germination is a dynamic process where plants come from resting state to the state with many physiological activities. Germination treatments can enhance the respiration of plants and significantly increase the species and number of enzymes (Mamilla and Mishra 2017). After germination, the starch content of mung bean decreased (Liu et al. 2014), while the amylase activity of kidney bean increased, leading to the changes in starch structure and composition (Yanli et al. 2018). Studies have showed that buckwheat has a high peak viscosity, hot paste stability and cold paste stability (Hara et al. 2007). Tartary buckwheat flavone content significantly increased after germination (Xiao-Peng et al. 2015). In addition, germination treatment can improve the edible and health value of buckwheat, enhance the resistance of starch paste and improve the stability of starch paste (Hara et al. 2007). Therefore, the study on the characteristics of germinated common buckwheat starch is of great significance to the development and utilization. In this experiment, the main nutrients, particle structure, physicochemical properties and the correlation between

gelatinization characteristic value and main nutrients of germinated common buckwheat starch were studied by using cv. Xinong9976 as material to provide basis for the development and utilization of common buckwheat sprouts and bean flour and the deep processing of starch.

MATERIALS AND METHODS

1. Experimental materials

Cv. Xinong9976, a common buckwheat variety, provided by small grain laboratory of Northwest A&F University, was used for the experiment. Common buckwheat seeds with full grain and no disease were selected and were sterilized with 0.1% H₂O₂ for 10 min and soaked in the distilled water for 24 h. Then seeds were placed in a petri dish with two layers of filter paper, the cultivation of the sample under 25°C in dark for germination, respectively taking sample after 2, 4, 6 d, removed shell, dried at 40°C.

The dried sample was grinded and passed through a 0.100 mm mesh. Added 80% ethanol, 50°C, with the ultrasonic treatment (500 w) 30 min to remove flavonoids, then added the volume of distilled water, heated under the condition of 30°C for 24 h. Centrifuged (4000 rpm, 10 min) three times, scraped off the grayish-brown soft layer. Finally, dried at 40°C for 48 h and sieved with a 0.150 mm mesh.

2. Measurement of physicochemical properties

The morphology of starch granules was observed by scanning electron microscope (JSM-6390, Jeol Ltd, Tokyo Japan) at 2000 x magnification, the particle size distribution was determined by a laser diffraction particle size analyzer and the pasting properties were measured using a rapid visco analyzer (RVA) (Newport Scientific, Pty Ltd, Warriewood, Australia).

Starch transparency was determined using a modified version of (Zhou et al. 2014). 0.5g of the starch sample was blended with 50 mL distilled water and heated in a boiling water bath for 30 min. After the starch was completely gelatinized by stirring, the starch was removed and cooled to room temperature. Distilled water was used as a blank for zero adjustment and the transparency was measured at the wavelength of 620 nm with a visible-light spectrophotometer.

The starch aging value was determined as follows: 0.5g of the starch sample was blended with 50 mL distilled wa-

Table 1 Changes of main nutrients of common buckwheat before and after germination

Germination time/d	Water/%	Ash/%	Crude fat/%	Crude protein/%	Total starch/%	Amylose/%	Amylopectin/%
0	12.26 ± 0.09 a	1.12 ± 0.03 c	1.37 ± 0.01 a	9.22 ± 0.06 c	68.41 ± 0.11 a	24.11±0.28 a	44.30±0.39 a
2	11.21 ± 0.03 b	1.36 ± 0.02 b	1.04 ± 0.01 b	9.42 ± 0.10 c	58.77 ± 0.81 b	22.89±0.17 b	35.88±0.97 b
4	9.78 ± 0.01 c	1.44 ± 0.01 b	0.92 ± 0.01 c	10.83 ± 0.17 b	52.80 ± 0.09 c	21.85±0.25 c	30.95±0.32 c
6	7.73 ± 0.01 d	1.69 ± 0.03 a	0.84 ± 0.01 d	13.79 ± 0.20 a	47.66 ± 0.11 d	19.77±0.20 d	27.89±0.31 d

Note: different letters in the same column mean significant difference of $P < 0.05$, the same as below.

ter and heated in a boiling water bath for 30 min and added distilled water to keep the total volume constant. Removed and cooled to room temperature, refrigerated for 24h, and defrosted at room temperature, and then centrifuged at 4000 rpm for 10 min, finally weighed the sediment quality. The starch aging value index was determined as follows: starch aging value = (weight of starch paste before centrifugation – weight of sediment quality) X 100.

3. Data analysis

Three parallel tests were conducted in the experiment. SPSS 17.0 was used for statistical analysis, Origin 9.0 was used for drawing, and LSD minimum significant difference test was used for the determination of significance of differences.

RESULTS AND DISCUSSION

Changes of the concentration of main nutrients

Main nutrients of common buckwheat were shown in table 1. The results showed that after germination, the contents of water, crude fat, total starch, amylose and amylopectin decreased significantly ($P < 0.05$), while the contents of ash and crude protein increased significantly ($P < 0.05$). Among them, the crude fat mass fraction decreased the most, from 1.37% to 0.84%, a decrease of 38.69% while the ash quality score increased the most, from 1.12% to 1.69%, increasing by 51%. In addition, the relative standard deviations of main nutrients in water, ash, crude fat, crude protein, total starch, amylose and amylopectin at different stages were 19.14%, 16.76%, 22.38%, 19.50%, 15.66%, 8.30% and 20.61%, respectively, indicating that the nutritional composition of the main nutrients in different stages of the germinated common buckwheat was significantly different.

After germination, the crude protein mass fraction increased exponentially, which may be caused by the decreased protease activity in the seeds of common buckwheat during the germination process, which effectively weakened the hydrolysis of related proteins and thus promoted the protein accumulation (Ikeda et al. 1984). The decrease of total starch mass fraction may be due to the activation of α -amylase and β -amylase in the sprouting of common buckwheat, which could promote the degradation of starch and provide part of sugars needed for the germination (Mohan et al. 2010). The decrease of fat mass fraction might be due to the action of lipase, which could decompose part of the fat into the energy required for the germination and growth of common buckwheat seeds.

Starch grain structure

As can be seen on Fig. 1a, the starch particles of mature common buckwheat seeds were complete with clear gaps, mostly spherical and oval in shape, with smooth surface and no holes or cracks. After the germination of 2 d, most starch granules were irregular in shape, while a few were spherical in shape. In addition, some of the crystalline structures of starch were destroyed, and a few starch granules showed cracks on the surface (Fig. 1b). In 4 d, the starch granules were disordered. A small number of starch granules were spherical in shape, while some starch granules were deformed and condensed together with the surrounding granules (Fig. 1c). And in 6 d, the starch granules were polygonal in shape with few of them being spherical. The crystalline structures of most starch granules were destroyed, and obvious cracks and holes appeared on the surface of most granules (Fig. 1d).

The table 2 showed that in the process of germination, starch granule size distribution was more dispersed, which indicated that the size of starch granules had ob-

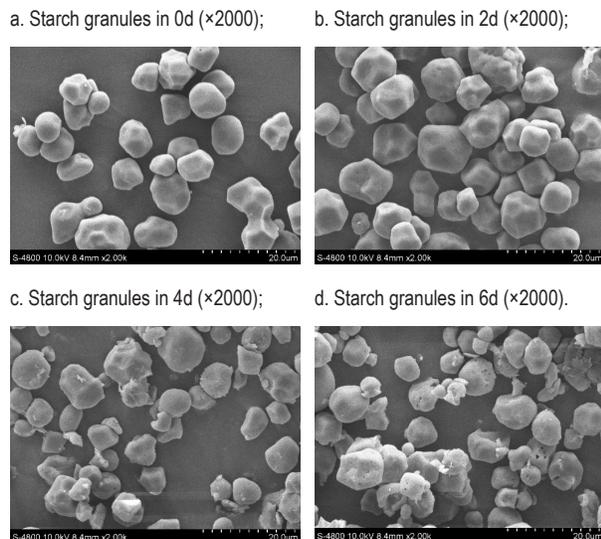


Fig.1. Scanning electron microscopy of starch granules of common buckwheat at different germination stages

vious differences. Chang found that the corn starch size ranged from 5.76 to 8.64 μm (Chang et al. 2004). Common buckwheat starch particles between 4.00 to 9.00 microns in diameter, which was smaller than the corn starch granules. After germination, the diameter, average sphericity and average aspect ratio of starch granules decreased significantly with the increase of germination time. In 6 d, the average diameter of starch granules decreased from 7.24 μm to 4.08 μm , a decrease of 43.65%. The average sphericity and the average aspect ratio decreased by 32.14% and 5.52%, respectively.

Starch transparency

Transparency is one of the important external characteristics of starch, which is directly related to the appearance and use of starch products (Wang et al. 2017).

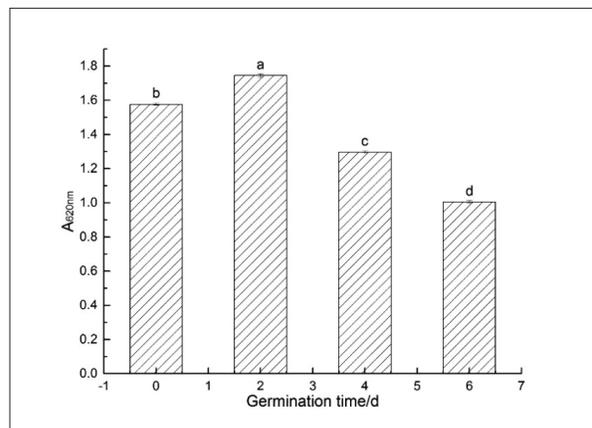


Fig.2. Changes of the transparency of common buckwheat starch at different germination stages

Transparency can reflect the mutual solubility of starch and water, and the transparency of buckwheat starch is positively proportional to the absorbance value, the higher the absorbance value is, the higher the transparency of the starch is (Li et al. 1997). As can be seen from Fig. 2, the transparency of common buckwheat starch first increased and then decreased with the extension of time in the germination process. And the transparency in different stages was significantly different ($P < 0.05$), the most transparent stage was 2 d and the absorbance value was 1.75, indicating that starch particles were completely expanded at this time, and there was no mutual association among the starch molecules after gelatinization. While the lowest transparency period was 6 d and the absorbance value was 1.00, which decreased by 36.30% compared with that of mature seeds. The average absorbance of starch in different germination stages was 1.41. After germination of 2d, starch transparency decreased significantly ($P < 0.05$), which may be due to the starch retrogradation, rearrangement of starch molecules and

Table 2 Starch particle size distribution during the germination of common buckwheat

Germination time/d	D10	D50	D90	Average sphericity	Average aspect ratio
0	4.97	7.86	8.89	0.84	1.45
2	4.68	6.64	7.98	0.76	1.43
4	3.29	5.17	6.97	0.68	1.40
6	2.36	4.05	5.84	0.57	1.37

Note: D_{10} , D_{50} and D_{90} represented the critical particle size values when the minimum particle size was added up to 10%, 50% and 90% of the sample.

scattering of light, thus reducing light transmission and starch transparency (Zhou et al. 2017).

Starch aging value

The essence of starch aging is that gelatinized starch molecules re-form hydrogen bonds during the cooling process (Jiamjariyatam et al. 2014). The aging process of starch can be regarded as the reverse process of gelatinization, but the degree of starch crystallization decreases after aging (Verma et al. 2018). It could be seen from Fig. 3 that there were significant differences in the aging value of common buckwheat starch in different germination stages ($P < 0.05$). The maximum aging value was 71.20% in mature grains. Subsequently, the aging value decreased gradually with the extension of germination time. Both 4d and 6d, the aging value decreased by 28.84%, 39.35%, respectively, which might be related to the weakened ability of buckwheat starch molecules to form hydrogen bonds again after germination (Liu et al. 2006). Starch aging not only makes food taste worse, but also reduces the digestibility (Verma et al. 2018). However, the aging value of common buckwheat gradually decreased during germination, indicating that common buckwheat sprouts were good in taste, easy to digest and had broad market development value.

Pasting viscosity

Starch granules rapidly absorb water in aqueous solution due to thermal expansion, resulting in the fracture of intramolecular and intermolecular hydrogen bonds, and the process of gradual diffusion of starch granules is called starch paste (Jane et al. 1992). The pasting temperature was different in different germination stages due to the size of starch granules. The starch pasting properties of common buckwheat in the process of germination were shown in table 3, the results showed that starch pasting viscosities gradually decreased and significantly different ($P < 0.05$) in different period. After germina-

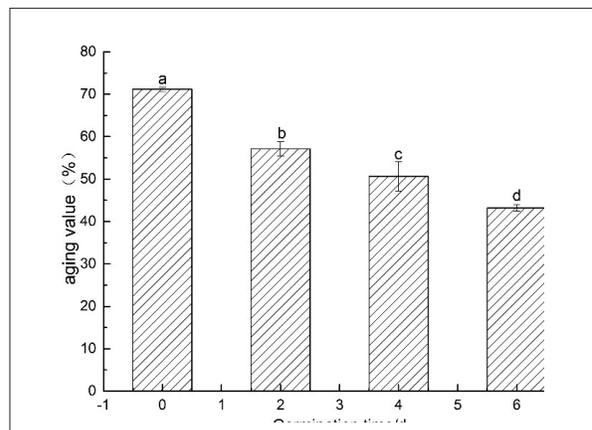


Fig.3. Changes of the aging value of common buckwheat starch at different germination stages

tion, peak viscosity, through viscosity, breakdown, final viscosity, setback, peak time and pasting temperature were lower than those of the mature grain starch.

Peak viscosity refers to the increase in the viscosity of starch paste caused by the friction between starch particles after full water absorption and expansion, which can reflect the expansion capacity of starch (Xiao-Li et al. 2008). As shown in table 3, the peak viscosity was 1394 - 2982 cp, the average peak viscosity was 2242 cp, and the difference was great. Through viscosity is caused by the sharp decrease in the viscosity of starch paste due to the fact that starch particles no longer friction with each other after they have expanded to the limit (Xiao-Li et al. 2008). The through viscosity was between 1335 and 2819 cp, and the average was 2131 cp. The breakdown is the difference between peak viscosity and through viscosity, which can reflect the thermal stability of starch paste. The smaller the breakdown is, the better the thermal stability is (Karim et al. 2000). The average breakdown was 111 cp, and the breakdown in mature grains was 2.76 times higher than that after germination, indicating that the starch

Table 3 Characteristic values of gelatinization of common buckwheat starch during germination

Germination time/d	peak viscosity /cp	Through viscosity /cp	breakdown/cp	final viscosity /cp	setback/cp	peak time /min	pasting temperature /°C
0	2982±37.64 a	2819±32.67 a	163±4.98 a	4765±34.43 a	1946±3.53 a	6.41±0.08 a	74.29±0.03 a
2	2587±18.50 b	2455±16.50 b	132±2.03 b	3982±41.51 b	1527±25.01 b	5.36±0.05 b	68.50±0.06 b
4	2005±7.84 c	1914±6.69 c	91±1.16 c	2933±17.53 c	1019±11.24 c	4.06±0.06 c	60.63±0.40 c
6	1394±11.29 d	1335±10.41 d	59±0.88 d	1988±18.02 d	654±7.62 d	2.24±0.04 d	50.73±0.34 d

had good thermal stability after germination and was suitable for the development of noodles and thickening agents. The final viscosity can reflect the retrogradation property of starch (Xiao-Li et al. 2008). After germination, the final viscosity decreased significantly, reaching 58.28% in 6d. The setback is the difference between final viscosity and through viscosity, which can reflect the stability of cold paste of starch. It can be seen from table 3 that the starch was not easy to age after germination and was suitable for making food such as common buckwheat instant noodles.

Correlation analysis of pasting properties and starch composition

Starch is the main component of common buckwheat grain, accounting for 60 -75% of the grain. The contents, composition and properties of buckwheat starch directly affect the processing technology of buckwheat food (Xin-Hua et al. 2009). The table 4 showed that peak viscosity, through viscosity and final viscosity of germinated common buckwheat was significantly positive correlated with amylopectin content ($P < 0.05$), which was the same as the relationship between the pasting viscosity and starch composition of the rice starch or germinated brown rice starch reported in previous studies, that was, the higher the content of amylopectin was, the higher the peak viscosity, through viscosity and final viscosity were. Break-

down, final viscosity and setback were significantly negatively correlated with amylose content ($P < 0.05$). Studies have shown that the short-term retrogradation of starch is mainly caused by the gelation order and dehydration crystallization of amylose molecules. However, setback was significantly negatively correlated with amylose content ($P < 0.05$), indicating that germinated common buckwheat with low amylose content was easy to retrograde.

Correlation analysis of pasting properties and other nutrients

The correlation analysis of starch pasting properties and other nutrients was shown in table 5. In 6d, pasting properties was positively correlated and negatively correlated with the main nutrients. Breakdown and setback were significantly negatively correlated with crude fat content ($P < 0.01$), indicating that the thermal stability and cold paste stability gradually increased after germination with the decrease of crude fat content. Peak viscosity, through viscosity and final viscosity were negatively correlated with crude fat content ($P < 0.05$). There was no significant relationship between pasting viscosity and water content, which may be due to the fast growth rate and the need to consume more water for growth, resulting in low water content. Similarly, pasting viscosity had no significant relationship with ash content and crude protein content.

Table 4 Correlation coefficient between pasting properties and starch composition (r/p)

Varieties	peak viscosity /cp	Through viscosity /cp	breakdown/cp	final viscosity /cp	setback /cp	peak time /min	pasting temperature /°C
Total starch	0.973/0.149	0.974/0.147	0.960/0.180	0.967/0.164	0.956/0.189	0.910/0.273	0.872/0.326
Amylose	-0.996/0.059	-0.995/0.062	-0.999/0.028*	-0.998/0.044*	-1.000/0.019*	-0.995/0.064	-0.983/0.118
Amylopectin	1.000/0.017*	1.000/0.014*	0.997/0.048*	0.999/0.032*	0.996/0.057	0.976/0.141	0.954/0.194

Note: Linear correlation coefficient between r. **: Significant correlation at the level of 0.01, *: Significant correlation at the level of 0.05, the same below.

Varieties	peak viscosity /cp	Through viscosity /cp	breakdown/cp	final viscosity /cp	setback /cp	peak time /min	pasting temperature /°C
Water	0.769/0.442	0.771/0.439	0.737/0.472	0.753/0.457	0.727/0.482	0.631/0.565	0.564/0.618
Ash	-0.798/0.412	-0.795/0.415	-0.826/0.381	-0.812/0.397	-0.834/0.372	-0.899/0.288	-0.933/0.235
Crude fat	-0.998/0.039*	-0.998/0.042*	-1.000/0.009**	-0.999/0.024*	-1.000/0.000**	-0.991/0.084	-0.977/0.137
Crude protein	-0.772/0.438	-0.770/0.441	-0.802/0.407	-0.787/0.423	-0.811/0.398	-0.880/0.315	-0.917/0.261

Table 5 Correlation coefficient between pasting properties and main nutrients (r/p)

CONCLUSIONS

The results showed that in comparison to non-germinated grain, after germination, the concentration of main nutrients of common buckwheat were significantly different, where the content of crude fat, total starch, amylose and amylopectin decreased significantly while the content of ash and crude protein increased significantly. Starch granules were arranged in a disorderly manner, most of which were irregular in shape, few of which were spherical in shape. Moreover, the crystal structure of most starch granules was destroyed, and obvious cracks and voids appeared on the surface. In addition, starch size of mature common buckwheat was 4-9 μm . Pasting properties were closely related to the starch composition, and peak viscosity, through viscosity, final viscosity and setback were sig-

nificantly positively correlated with amylopectin content, while breakdown, final viscosity and setback were significantly negatively correlated with amylose content. In addition, breakdown, setback and fat content were significantly negatively correlated, and peak viscosity, through viscosity and final viscosity were significantly negatively correlated with fat content, while pasting properties were not significantly correlated with other nutrients.

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IZVLEČEK

Za raziskavo fizikalno kemijskih lastnosti škroba tekom kalitve navadne ajde je bil izbran kultivar 'Xinong9976'. Raziskava je vključevala glavna hranila, zgradbo delcev, razporeditev velikosti delcev, prosojnost, vrednost staranja, lastnosti gnetenja ter korelacijo med lastnostmi gnetenja, sestavo škroba in glavnimi nutrienti. Pri glavnih nutrientih so bile tekom kalitve značilne razlike. Premer škrobnih zrn je bil od 2.36 do 8.89 μm , škrobna zrna so bila nepravilnih oblik in na površini z vidnimi odprtini in razpokami. V različnih fazah kalitve so bile razlike glede prosojnosti, vrednosti staranja in končne viskoznosti. Najvišja vrednost viskoznosti, prava viskoznost in končna viskoznost so bile pozitivno povezane z vsebnostjo amilopektina ($P < 0.05$), več parametrov viskoznosti je bilo negativno povezanih z vsebnostjo amiloze ($P < 0.05$). Lastnost gnetljivosti škroba ni bila značilno povezana z vsebnostjo hranil.