Changes in Protein, Saponin Content and Starch Profiles of Quinoa (*Chenopodium quinoa* Willd) Seeds during Germination

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Abstract: Germination is an effective processing method to improve the texture and nutritional value of cereals. This study aimed to investigate changes in protein, saponin content and starch profiles of quinoa seeds during germination. Results showed that the crude and soluble protein content of quinoa seeds increased significantly by 5.38% and 17.55% during germination. As the germination time gradually increased, the total starch, amylose and amylopectin content decreased by 25.95%, 4.86% and 29.53%, respectively, while the reducing sugar content increased significantly by 26.60%. In addition, germination process significantly improved the *in vitro* starch digestibility of quinoa seeds. The percentage contents of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) were 38.45%, 43.64% and 17.90% in raw quinoa seeds, respectively. The RDS increased significantly to 53.46% after germination for 48h, while SDS and RS decreased to 40.42% and 6.11%, respectively. Moreover, the total saponin content of quinoa seeds increased significantly by 16.46% after germination. Therefore, germinated quinoa seeds have better nutritional value and digestibility, and can be used as functional food ingredient for human healthy.

Key words: quinoa seeds; germination; protein; saponin; starch digestibility

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藜麦发芽过程中蛋白质与皂苷及淀粉消化特性的变化规律

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摘要：发芽是改善谷物质构及其营养价值的一种高效的加工方式。本文研究了藜麦发芽过程中蛋白质与皂苷及淀粉消化特性的变化规律。结果显示，发芽显著地提高了藜麦中粗蛋白和可溶性蛋白含量，分别增加了 5.38%和 17.55%。随着发芽时间不断增加，藜麦总淀粉、直链淀粉及支链淀粉含量分别减少 25.95%、4.86%和 29.53%，而还原糖含量增加了 26.60%。发芽处理显著改善了藜麦种子的淀粉消化性能。发芽前藜麦种子中快消化淀粉、慢消化淀粉及抗性淀粉的百分含量分别为 38.45%、43.64%和 17.90%。发芽 48 h 后，快消化淀粉的百分含量显著地增加至 53.46%，而慢消化淀粉及抗性淀粉的百分含量分别下降至 40.42%和 6.11%。此外，发芽后藜麦种子中总皂苷含量显著增加了 16.46%。研究表明，发芽的藜麦种子含有更好的营养价值和消化性能，可以用于加工功能性食品，促进人体健康。

关键词：藜麦种子；发芽；蛋白质；皂苷；淀粉消化性

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Quinoa (*Chenopodium quinoa* Willd) has received increasingly attention in recent years due to its excellent nutritional and functional values. It is not only rich in macronutrients such as protein and starch, but also a large variety of phytochemicals such as saponin. Unlike other cereal proteins, quinoa proteins are accepted as high quality protein due to their balanced pattern of essential amino acids, and contain high level of lysine which is the limiting amino acids in conventional cereals[1]. Saponin represents another important family existed in quinoa, and is abundantly located in the outer layers of quinoa seeds. These compounds have recently showed a wide range of biological activity such as antifungal, anti-cancer, anti-inflammatory and antioxidant activities[2~4]. Therefore, numerous health benefits could be obtained from consumption of quinoa, such as antioxidant, anti-obesity, anti-diabetic and anti-cancer activities[1].

The process of germination not only softens the kernel structure, but improves the nutritional quality of cereals[5]. Recently, much attention has been paid to investigate the changes in nutritional compounds of cereals during germination. It has been reported that the protein content increased significantly in germinated oat, rice and black bean, and the protein properties were also improved after germination[6~8]. However, little information existed regarding changes in protein content of quinoa seeds during germination. In addition, effect of germination on starch profiles was evaluated in cereals. Tian et al.[8] found that the starch content decreased significantly in oat seeds after germination. Subsequently, germination process was reported to reduce the total starch content and increase the amylopectin /amylose ratio of brown rice[9]. A recent study reported that the digestibility of starch from brown rice increased after germination[10]. Several studies had been reported that the total starch content decreased significantly in quinoa seeds during germination[11~13]. However, the effect of germination on *in vitro* starch digestibility such as the percentage content of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) of quinoa seeds was not investigated in these studies. Moreover, it is not clear how the change in saponin content vary throughout germination process of quinoa seeds.

Therefore, the objectives of this study were to (1) investigate the changes in protein and saponin content of quinoa seeds during germination; and (2) evaluate the effect of germination on starch profiles of quinoa seeds.

1 Materials and methods

1.1 Chemicals and reagents

3,5-Dinitrosalicylic acid, porcine pancreatic *α*-amylase (30000 U/g) and amyloglucosidase (10000 U/g) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA); Coomassie Brilliant Blue assay kit was purchased from Nanjing Jiancheng Bioengineering institute (Nanjing, China); Oleanolic acid was obtained from Aladdin Reagents (Shanghai, China); All other reagents used were of analytical grade or above.

1.2 Sample preparation

Quinoa (*Chenopodium quinoa* Willd) seeds were purchased from Huaqing Quinoa Co. Ltd. (Shanxi Province, China) and stored at 4 ℃ until germination. Quinoa seeds were sterilized using 0.07% sodium hypochlorite with a ratio 1:5 (*W/V*) for 15 min, and then washed with distilled water to a neutral pH. Quinoa seeds were soaked in distilled water for 6 h at room temperature. The soaked seeds were placed in a tray with two layers of wet laboratory papers and germinated for 0, 12, 24, 36 and 48 h at 30 ℃, respectively. Wet laboratory papers were kept moist by spraying with distilled water every 8 h. All samples were freeze-dried and milled into powder through an 80-screen mesh and stored at -20 ℃ before analysis.

1.3 Determination of crude and soluble protein content

The nitrogen content was determined using the Kjeldahl method according to the national standard of People's Republic of China (GB 5009.5-2016). The nitrogen to protein conversion factor of 6.25 was utilized to calculate the crude protein content.

The soluble protein content was determined using

the Coomassie Brilliant Blue assay kit. Briefly, 1.0 g sample was mixed with 9.0 mL of distilled water, and then centrifuged at 2500 r/min for 10 min. The supernatants were collected, and diluted with distilled water to a final volume of 25 mL. Then, 50 μL of diluted solution or protein standard (0.563 g/L) or distilled water was mixed with 3.0 mL of Coomassie Brilliant Blue reagent. The mixture was incubated at room temperature for 10 min. The absorbance of final mixture was detected at 595 nm using a Shimadzu UV-1800 spectrometer (Shimadzu Inc., Kyoto, Japan). The soluble protein content was calculated as follows:

The soluble protein content of sample (g/L) = [(sample absorbance-blank absorbance)/(standard absorbance- blank absorbance)] × the protein content of standard (0.563 g/L).

1.4 Determination of total starch and amylose/amylopectin ratio

The total starch content was determined using the acid hydrolysis method based on the national standard of People's Republic of China (GB 5009.9-2016).

The amylose content was determined according to an iodine colorimetric method with slight modification[14]. Iodine solution was prepared by adding 2.0 g KI and 0.2 g I_2 to 100 mL distilled water and stored in dark. Sample was firstly defatted and desugared with hexanes and ethyl alcohol, respectively. Briefly, 0.1 g defatted and desugared sample was mixed with 10 mL of 0.5 M KOH solutions, and reacted in a 70 ℃ water bath for 10 min. After cooling, distilled water was added to bring the total volume to 50 mL. A reagent blank was prepared in the same way without adding starch. 2.5 mL of mixture was diluted with distilled water to 30 mL, and then the pH was adjusted to 3.0 with 0.1 M HCl. The mixture was mixed with 0.5 mL of iodine solution and distilled water to a final volume of 50 mL. After incubation at room temperature for 20 min, the absorbance of final mixture was detected at 630 nm. The amylose from rice was used as the standard. The amylose content was expressed as g/100 g of sample.

The amylopectin content was calculated as total starch content minus amylose content.

1.5 Determination of reducing sugar content

The reducing sugar content was determined using the 3,5-dinitrosalicylic acid (DNS) method[15]. Briefly, 2 g sample was diluted with 30 mL of distilled water, and then vortexed at room temperature for 30 min. After centrifugation at 2500 r/min for 10 min, the supernatants were diluted with distilled water to bring the total volume to 50 mL. 1 mL of diluted mixture was mixed with 0.75 mL of DNS solution, and incubated in a 70 ℃ water bath for 10 min. 10.75 mL of distilled water was then added to the cooling mixture. The absorbance of final mixture was detected at 540 nm, and the glucose was used as the standard. The reducing sugar content was expressed as mg/g of sample.

1.6 *In vitro* starch digestibility

In vitro starch digestibility was evaluated according the previous method with some modification [16]. The mixed enzyme solution consisted of porcine pancreatic *α*-amylase (290 U/mL) and amyloglucosidase (15 U/mL). Briefly, 0.3 g sample was diluted with 10 mL of acetate buffer (0.2 M, pH 5.2), and mixed with 10 mL of mixed enzyme solution. The mixture was incubated in a shaking water bath at 37 ℃ with the shaking speed of 120 r/min. After incubation for 0, 20 and 120 min, respectively, 1 mL enzymatic solution was taken out, and diluted with 1.5 mL distilled water. The enzymatic solution was then incubated in a boiling water bath to inactivate enzyme. After cooling, the enzymatic mixture was centrifuged at 6000 r/min for 10 min, and the supernatant was used to determine the reducing sugar content using DNS method.

In vitro starch digestibility was characterized by measuring the percentage content of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). The percentage content was calculated as follows:

$$RDS(\%)=[(G_{20}-G_0)\times0.9/TS]\times100$$

$$SDS(\%)=[(G_{120}-G_{20})\times0.9/TS]\times100$$

$$RS(\%)=100-RDS-SDS$$

Where G_0, G_{20} and G_{120} was the content of reducing sugar in enzymatic solution after digestion

for 0, 20 and 120 min, respectively, and the TS was the total starch content of sample.

1.7 Extraction and determination of total saponin content

Total saponin was extracted according to the previous method with slight modifications[17]. Quinoa samples were hydrolysed in reflux at 80 ℃ for 1 h with methanol (1:10, *W/V*). The hydrolysd solution was then centrifuged at 2500 r/min for 10 min to remove supernatant, and this process was repeated once. The combined supernatants were concentrated using an Eyelan-1100 rotary evaporator at 45 ℃ (Tokyo Rikakikai Co. Ltd., Tokyo, Japan) to a final volume of 10 mL. The final extract solution was stored at -20 ℃ until use.

Total saponin content was analysed based on a previous method with some modifications[18]. Briefly, 0.2 mL of the saponin extract solution was put in a 70 ℃ water bath to remove solvent. After cooling, the extract was mixed with 0.2 mL of 5% (*W/V*) vanillin (dissolve in acetic acid). Then, 0.8 mL perchloric acid was added and reacted in a 70 ℃ water bath for 10 min. The cooled mixture was then mixed with 5 mL acetic acid and incubated in room temperature for an additional 10 min. The absorbance was detected at 550 nm, and oleanolic acid was used as the standard. Total saponin content was expressed as mg oleanolic acid equivalents (OAE) per g dry weight (DW) of quinoa sample.

1.8 Statistical analysis

The results were expressed as the mean ± standard deviation (SD) for triplicate measurement of each sample. The means were analyzed for significance using one-way ANOVA followed by the SNK-q test ($p < 0.05$). All of statistical analysis was calculated using SPSS 19.0 software (SPSS Inc. Chicago, IL, USA).

2 Result and discussion

2.1 Effect of germination on protein content

Effect of germination on crude and soluble protein content of quinoa seeds are shown in Fig. 1. The crude protein content of raw quinoa seeds was 13.95 g/100 g. A significant increase in the crude protein content was observed after germination. The highest crude protein content was obtained at 36 h (14.7 g/100 g), which increased by 5.38% compared with initial content. Consistent with our results, Tian et al.[8] reported that protein content of oat seeds increased from 18.98 to 22.02 g/100 g after germination for 144 h. Kupkanchanakul et al.[6] also found a significant increase in the crude protein content of pre-germinated brown rice for three cultivars. These results could be attributed to that the metabolism of quinoa during germination resulted in the dry weight losses, thus, the germinated quinoa would contain more nitrogen than raw sample.

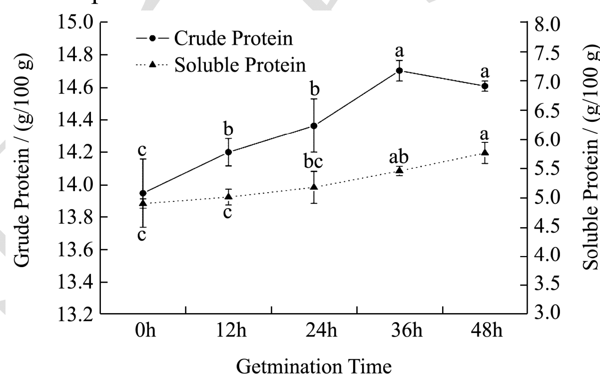

Fig.1 Effect of germination on crude and soluble protein content of quinoa seeds

Note: Means with different letters are significantly different (p<0.05).

In addition, the results showed that germination process resulted in an increase of soluble protein content of quinoa seeds. As the germination time increased from 0 to 48 h, the soluble protein content increased significantly from 4.90 to 5.76 g/100 g. The germinated quinoa seeds for 48 h shown highest soluble protein content, and was 1.18 times higher than that of quinoa before germination. The probable reason is that the protein in quinoa seeds was degraded and converted into a soluble form during germination[8].

2.2 Effect of germination on starch profiles and *in vitro* digestibility

Effect of germination on total starch and reducing sugar content of quinoa seeds are shown in Table 1. The total starch content ranged from 59.27 to 43.89

g/100 g, and the reducing sugar content ranged from 6.09 to 7.71 mg/g. As the germination time increased from 0 to 48 h, the total starch content decreased significantly from 59.27 to 43.89 g/100 g with a greater declining rate observed after 24 h, while the reducing sugar content increased significantly from 6.09 to 7.71 mg/g. These results could be due to that the α-amylase activity increased along with the prolongation of germination time, and increased rapidly after 24 h. Thus, starch was hydrolyzed into dextrin and other sugar with small molecular weight. Furthermore, the vigorous respiratory metabolism of germinated quinoa seeds could also utilize starch[12]. Therefore, germination process resulted in the decrease of total starch and increase of reducing sugar in quinoa seeds.

Table 1 Effect of germination on starch properties of quinoa seeds

Time/h	Total Starch/(g/100 g)	Amylose/(g/100 g)	Amylopectin/(g/100 g)	Reducing Sugar/(mg/g)
0	59.27 ± 1.96^{a}*	8.64 ± 0.27^{a}	50.63 ± 1.69^{a}	6.09 ±0.35^{c}
12	58.71 ± 2.20^{a}	8.51 ± 0.26^{a}	50.20 ± 2.04^{a}	6.25 ± 0.12^{c}
24	56.15 ± 1.04^{a}	8.30 ± 0.20^{a}	47.86 ± 1.24^{a}	6.38 ± 0.10^{c}
36	51.77 ± 0.69^{b}	8.18 ± 0.20^{a}	43.59 ± 0.80^{b}	7.15 ± 0.22^{b}
48	43.89 ± 1.03^{c}	8.22 ± 0.33^{a}	35.67 ± 1.25^{c}	7.71 ± 0.32^{a}

Note: * Values with different letters in each column are significantly different with respect to different germination time ($p<0.05$).

Effect of germination on amylose and amylopectin content of quinoa seeds are shown in Table 1. The amylose and amylopectin content decreased from 8.64 to 8.22 and 50.63 to 35.67 g/100 g, respectively, whereas the decrease of amylose did not show the statistically significant difference. The amylose/amylopectin ratio of quinoa seeds increased from 0.17 to 0.23 during germination. In accordance with our results, Wu et al.[9] reportd that total starch, amylose and amylopectin content of three rice cultivars decreased significantly after germination. However, they found that the amylose/amylopectin ratio decreased with prolonged germination time. This difference was partly attributed to that the enzymolysis rate of amylose by amylase was lower than that of amylopectin[19,20], so that the amylopectin in germinated quinoa seeds was hydrolyzed faster than amylose. Therefore, the amylose/amylopectin ratio of quinoa starch increased during germination.

Effect of germination on percentage content of rapidly digestible starch (RSD), slowly digestible starch (SDS) and resistant starch (RS) in quinoa seeds are shown in Table 2. The results showed that the percentage content of RSD increased from 38.45% to 53.46%, while the SDS and RS decreased from 43.64% to 40.42% and 17.90% to 6.11%, respectively. Similar results have been reported previously in lentil and brown rice[10,21]. The probably reason was that germination caused the damages and pores on the surface of starch particles, and thus improved the efficiency of enzymatic hydrolysis[10]. Al-Rabadi et al.[22] reported that the starch digestibility might be affected by the particle size and structures of starch including amylose content. Therefore, as germination time gradually increased, the increase of amylose/amylopectin ratio might also be the reason for the increase of starch digestibility in quinoa seeds. There results indicate that germination process could significantly improve the starch digestibility of quinoa seeds. The results of present study can provide useful information with potential application of germinated quinoa seeds for an industrial scale.

Table 2 Effect of germination on *in vitro* starch digestibility of quinoa seeds

Time/h	RSD/%	SDS/%	RS/%
0	38.45±0.63^{c}*	43.64±1.13^{a}	17.90±1.24^{a}
12	38.87±0.80^{c}	44.60±1.20^{a}	16.53±1.89^{a}
24	40.08±0.75^{c}	44.48±1.58^{a}	15.44±2.32^{a}
36	46.22±1.85^{b}	43.67±0.74^{a}	10.11±1.95^{b}
48	53.46±2.65^{a}	40.42±1.81^{b}	6.11±1.54^{b}

Note: RSD: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch.* Values with different letters in each column are significantly different with respect to different germination time ($p<0.05$).

2.3 Effect of germination on total saponin content

Effect of germination on total saponin content of quinoa seeds are shown in Fig. 2. The total saponin content of raw quinoa seeds was 9.78 mg OAE/g DW. Then, the saponin content increased significantly in a time-dependent manner from germination for 0 to 48 h. As the germination time increased from 0 to 12, 24, 36 and 48 h, the total saponin content increased significantly by 2.56%, 6.03%, 8.90% and 16.46%, respectively. Similar results were also observed in soybean, lentil and black bean that the total saponin content increased significantly after germination[23~25]. In addition, it was reported that germination process has effect on compounds involved in the plant defence system[23]. Meanwhile, saponins are widely present in quinoa seeds, and their natural function is to defend the plant from the external medium[26]. Therefore, the increase of total saponin content in this study could be partly interpreted as the accumulation of plant defensins during germination.

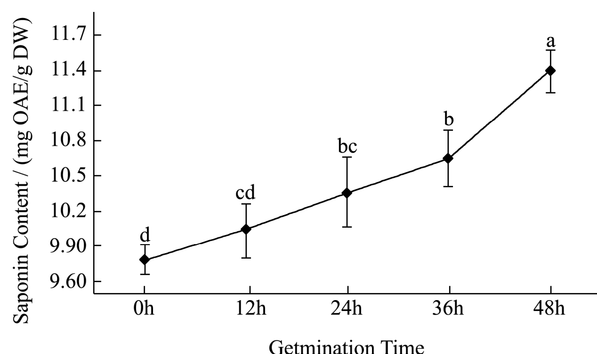

Fig.2 Effect of germination on total saponin content of quinoa seeds

Note: Means with different letters are significantly different ($p<0.05$). OAE: oleanolic acid equivalents; DW: dry weight.

3 Conclusion

Germination is an effective process to improve the texture and nutritional value of cereals. In the present study, germinated quinoa seeds induced substantial changes in protein. The results showed that the crude and soluble protein content of quinoa seeds increased significantly after germination for 48 h. In addition, germination process had a great influence on the losses of total starch, amylose and amylopectin content, but could significantly improve the digestibility of quinoa seeds. The percentage content of RDS in quinoa seeds increased significantly, while the SDS and RS decreased during germination, respectively. The amylase / amylopectin ratio was also increased with prolonged germination time. Moreover, a significant increase was observed in total saponin content of quinoa seeds after germination. In summary, germination process can improve the nutritional value and digestibility of quinoa seeds. Therefore, germinated quinoa seeds can be used as a natural source for functional food ingredients.

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