

Processing Effects on Anti-nutrient Factors of Tartary Buckwheat (*Fagopyrum tataricum*)

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ABSTRACT--- *Steaming, boiling, baking, and se-enrichment germination processing were used to improve the edible value of tartary buckwheat and eliminate the anti-nutritional factors. The content of trypsin, α -amylase activity, polyphenols and phytic acid were researched and compared in different treatments. The results showed the content of trypsin inhibitor, α -amylase inhibitor, polyphenol, phytic acid in tartary buckwheat processed by different methods. Among these physical treatments, boiling had the highest inhibitory effects followed by steaming. In contrast, the baking methods had the lowest effect in inhibition. The Se-enrichment germination could greatly reduce the content of trypsin inhibitor, however, it would increase the content of α -amylase inhibitor, phytic acid and polyphenols. Furthermore, prolonged Se-enrichment time could reduce not only the content of trypsin inhibitor but also the content of phytic acid.*

Keywords--- Tartary buckwheat; Anti-nutritional factors; Trypsin inhibitor; α -amylase inhibitor; Polyphenol; Phytic acid

1. INTRODUCTION

A tartary buckwheat is a dicot genus Polygonaceae with 30-60 cm high. Its triangular or hastate shape seeds, wrapped by a thick crust, can be ground to eat after peeled. Tartary buckwheat also could be processed to make buckwheat tea and other health care products by baking or other methods. However, there is a certain amount of anti-nutritional factors in tartary buckwheat, the digestion and absorption rate of the tartary buckwheat is lower than of other cereals. and some people even cause Symptoms such as nausea and vomiting, could happen in some people after consumption. Anti-nutritional factors (ANF) are referred to the number material contained in food which can produce adverse physiological reactions on people and animals in digestion, absorbance and utilizing of nutrients (Enechi, Odo, & Oburu, 2014). ANF mainly includes protease inhibitors, phytic acid, lectins, erucic acid, gossypol, tannin (Kumar & Singh, 1984), glucosinolates, non-starch polysaccharides, antioxidant vitamins and other factors (P & S, 2007). Previous studies showed that anti-nutritional factors have effects like antioxidant, anti-cancer, lowering blood glucose and cholesterol and so on (Soetan, 2008). Therefore anti-nutritional factor attracted more and more people's attention. The anti-nutritional factors of buckwheat are mainly trypsin inhibitor, α -amylase inhibitor, polyphenols, phytic acid and so on. At present, research on anti-nutritional factors are mainly focus on legumes and oil crops such as soybean and rapeseed (E, 1994; R, Reddy, 1982; Udensi, Ekwu, & Isinguzo, 2007), while research on anti-nutritional factors of tartary buckwheat are less. In previous studies, germination process of soybean could enrich selenium by transform inorganic selenium into organic selenium. Selenium in the body can play a role as antioxidant which scavenging free radical. Moreover, not many studies were focused on the effect of Se-enrichment germination processing on anti-nutrient factors production.

In this study, the tartary buckwheat of Shanxi specialty, a rich source of anti-nutrition was used raw material and analyzed. Amount changing of anti-nutritional factors in tartary buckwheat: trypsin activity, α -amylase activity and polyphenols, phytic acid were compared after steaming, boiling, baking or enriched-Se sprouted treatments. The proper way that how to choose tartary buckwheat food depends on different groups of people, and a theoretical basis for the deep processing of buckwheat were provided.

2. MATERIAL AND METHODS

2.1 Material

Tartary buckwheat were supplied from Datong Lingqiu, Shanxi, China and were stored in the laboratory in September 2014.

Pretreatment

Physical treatment: 4 kinds of treatment groups were included in the experiment. Each group was 250 g. After peeled, the first group without any treatment was control, three other groups treated by steaming, baking, boiling process. Four groups were powdered respectively after 50-60 °C drying.

Biological treatment: The experiment also had 4 groups. 250 g of each group was washing with a 1% available chlorine NaClO for 5 min and soaked in distilled water (weight:volume is 1: 3) for 10 h. The first group was dried at 60 °C directly; three other group subsequently placed on filter paper and germinated by dipped in 20 µg / mL Na₂SeO₃ solution (Zhu, 2014), which were incubated in the dark and added Na₂SeO₃ solution once every 4 h. After sprouting 1 d, 2 d, 3 d respectively, Se-enrichment germination samples milled after 60 °C drying.

2.2 Trypsin activity analysis

The method described by M L Kakade was used (L, J, E, & A, 1974). 1.000 g tartary buckwheat powder were weighed, 10 mmol/L PBS buffer according to a volume ratio of 1: 4 was added (containing 0.15 mol/L NaCl pH 7.5) to extract for 2 h at room temperature. The mixture was centrifuged at 3000 r/min for 20 min. The supernatant was denatured by heating at 68 °C for 10 min, then rapidly cooled and centrifuged at 3000 r/min for 20 min to discard precipitate. The supernatant was added (NH₄)₂SO₄ up to saturation, stand at 4 °C overnight. The liquid was centrifuged at 10000 r/min for 30 min, the precipitate was collected and dissolved in a small amount of 0.05 mol/L pH 7.8 PBS buffer, concentrated to give crude trypsin inhibitor product. Accurately weighed trypsin 2.000 g, using 10mL of 30 °C distilled water to dissolve for 30 min, filtered with filter paper and the filtrate was diluted 10-fold.

1.0 mL diluted enzyme solution were added in four test tubes respectively, wherein one was blank, three parallel test tube. Placed all of tubes at 40 °C for 3 min. In three other test tube, 1.0 mL 2% casein solution were added, then a protease inhibitor 0.2 mL, accurate insulation 10 min. 2 mL 0.4 mol/L Na₂CO₃ solution and 1.0 mL Folin-phenol reagent were in sequence added, shaken, reacted at 40 °C water bath for 20 min. In Blank tube adding the 2.0 mL 0.4 mol/L trichloroacetic acid solution to stop the reaction, and then adding 1.0 mL 2% casein solution, after 15 min filtrating with a filter paper. The following operation is the same test tube parallel to the blank tube as a control measure absorbance at 680 nm wavelength.

Tyrosine standard curve : $y = 0.0927x - 0.1044$, $R^2 = 0.9886$

Protease activity unit = $(A / 10) \times 4 \times N \times [1 / (1-w)]$; (at 40 °C pH 2.5 , the amount of enzyme hydrolyzed casein 1.0 g tyrosine release is defined as a per minute protease units.)

Formula where: A was the absorbance value of sample measured, quite tyrosine quality checked the standard curve, g;
4 of the total volume of the tube , the reaction solution , mL;

10 is a reaction time , 10 min;

N is the dilution factor ;

w is water content of the sample.

2.3α- amylase activity analysis

α- amylase activity was analyzed according to the method decribed by Jiang *et al.* (Xiaodong, Jing, & Gang-gang, 2013) Take 3 clean test tubes, 1.0 mL amylase liquid were added in numbered 1, 2, 3, placed them in (70±0.5) °C water bath for 15 min, then cooled, adding 2.0 mL 3,5- dinitrosalicylic acid in 1 tube, and the starch solution was placed in (40 ± 0.5) °C temperature water bath for 10 min, each added 1.0 mL 10 g/L starch solution, then add α-amylase inhibitor, (40 ± 0.5) °C accurate thermal water bath for 5 min, added 2.0 mL 3,5-dinitrosalicylic acid in 2, 3 tube. After shaking each test tube, the absorbance was measured at 540 nm. Maltose as standard, with distilled water as reference solution, the regression equation:

$y = 0.0793x - 0.1034$, $R^2 = 0.9894$

α- amylase [mg maltose / (g fresh weight • min)] = $[\alpha \text{ maltose content (mg / mL)} \times \text{amylase stock solution total volume (mL)}] \div [\text{sample weight (g)} \times 5 \text{ (min)}]$

2.4 polyphenol content analysis

solid-liquid ratio 1:40 (g: mL) 95% ethanol were added and placed in 50 °C water bath shaker to extract for 2 h, after completion of the extraction to filtrate, the filtrate use a rotary evaporator to concentrate the sample, the sample solube in the same volume centrifuged at 6000 r / min for 10 min to obtain polyphenol extracts.

Polyphenol content assay was performed as described previously with some adjustment (Khanizadeha, Tsaob, Rekikaa, Yangb, Charlesa, & Rupasinghec, 2008). 0.1 mL polyphenol extract was added into a test tube with 95% ethanol as reference solution, adding 0.1 mL Folin-Ciocalteu's phenol and 2.8 mL of distilled water, stand for 8 min, adding 2 mL 7.5% Na₂CO₃ solution, sealed and reacted in dark for 2 h. The absorbance is measured at 765 nm wavelength, repeated three times. Gallic acid as standard, the regression equation: $y = 0.0028x + 0.0042$, $R^2 = 0.9996$.

2.5 Phytic acid content analysis

1.0 g samples in a 50 mL conical flask and 0.01 mol / L HCl were added to extract, acid extraction conditions were: solid-liquid ratio 25: 1 (v / w, ml / g), extract temperature 34 °C extraction time 68 min. centrifuged at 6000 r / min for 10 min, the supernatant volume to 10 mL to determine the content.

Phytic acid content analysis was conducted a published method with some modification(Beiying & Qun, 2013). Take the sample 2.5 mL, 10 mL distilled water and 4.0 mL reagent was added, shaken and centrifuge at 3000 r / min for 10 min, stand at room temperature for 20 min, the absorbance value of supernatant was measured at 500 nm. Sodium phytic acid as standard, with distilled water as reference solution, the regression equation: $y = 0.2020x - 0.2174$, $R^2 = 0.9984$.

3. RESULTS AND ANALYSIS

3.1 Effect of different processing on trypsin activity inhibitor

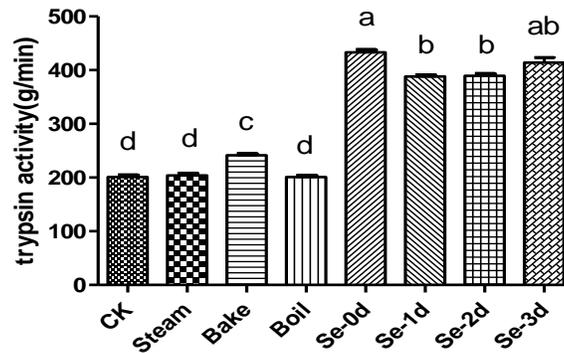


Fig.1 Changes of trypsin activity with different treatments

In different treatment, the higher trypsin activity was on behalf of the less content of trypsin inhibitor in tartary buckwheat. As can be seen from Figure 1, effect of steaming, baking, boiling treatment on trypsin activity is significantly less than enrichment-Se germination, indicating that Se-enrichment greatly reduces the content of protease inhibitors. Compared with the control, it can be seen in the steaming, baking, boiling three processing modes, boiling manner had lowest trypsin activity, baking had the highest trypsin activity. In another words, boiling process can be derived the maximum content of trypsin inhibitor, and baking-processing was lower content of trypsin inhibitor in physical methods. Suggesting that baking process to some extent undermined trypsin inhibitor of buckwheat and thus was helpful to the body to digest and absorb. In the Se-enrichment germination trial, as the length of Se-enrichment time prolonged, trypsin activity first increased and then decreased trend. Trypsin activity on the first 1d, 2d were lower than the first initial and the 3d activity. It can be seen from the significant differences, an appropriate extension of se-enrichment time can reduce the content of trypsin inhibitor. Thus facilitating handling for the body to digest protein were baking, steaming, boiling, enrichment-Se germination in turn.

3.2 Effect of different treatments on α -amylase activity inhibitor

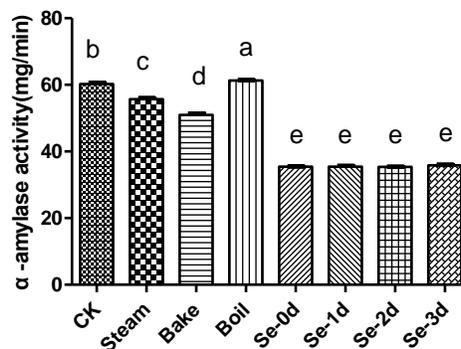


Fig.2 Changes of α -amylase activity with different treatments

In different treatment, the greater α -amylase activity reflects the less α -amylase inhibitor content in tartary buckwheat. As can be seen from Figure 2, compared with the control, baking treatment approached the lowest α -amylase activity in steaming, baking, boiling treatment, Which was highest content of α -amylase inhibitor by baking treatment in tartary

buckwheat. It can be seen α -amylase activity by boiling manner was slightly higher than the control, it can draw boiling process destroy α -amylase inhibitor, and α -amylase inhibitor content by steaming also increased but the increase content is lower than baking. The α -amylase activity by Se-enriched germination trial is greatly reduced, indicating that Se-enriched sprouted treatment increased α -amylase inhibitors in tartary buckwheat, but with sprouted time prolonged, it can be seen there were no significant difference in α -amylase activity. People who digestive system is not good and allergic to buckwheat are recommend eating boiling buckwheat, people with superfluous nutrition and diabetes eat selenium-enrich foods, reducing the utilization of food intake.

3.3 Effect of different treatments on poly-phenol content

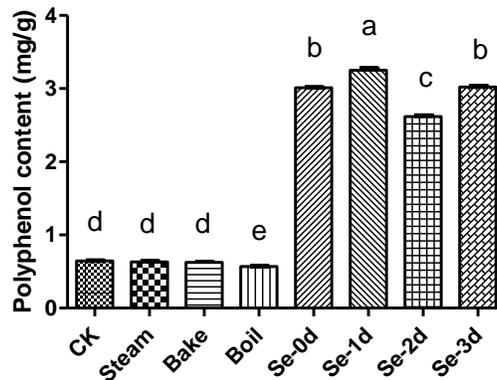


Fig.3 Changes of poly-phenol content with different treatments

As can be seen from Figure 3, steaming, baking, boiling treatments had little effect on polyphenols content in buckwheat, there is no clear trend. Se-enrichment manner increase about 4.1-5.7 times in the polyphenol content than control and render first increased then decreased trend, the lower content on 2d. Compared with the control, we can see steaming, baking, boiling three ways had no significant difference in changing the polyphenol content. Se-enrichment germination on 1d, 2d, 3d were significantly greater than that of non Se-enrichment, which consistent with the findings of (Yimei, 2006) and (Zhang, 2004). Therefore, it is better for population with superfluous nutrition to consume enriched selenium sprouted buckwheat.

3.4 Effect of different treatments on phytic acid content

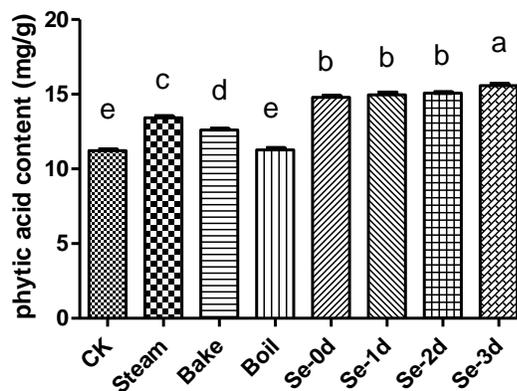


Fig.4 Changes of phytic acid content with different treatments

As can be seen from Figure 4, phytic acid content after various processing all increased, while phytic acid content by boiling ways had not significantly different with the phytic acid content of control. Se-enrichment handling obviously increased phytic acid content more than some other physical process. There was significant difference can be seen in Se-enrichment treatment, with time of Se-enrichment prolonged, phytic acid content showed an increasing trend. In the physical handling content of phytic acid was the smallest in boiling way, so boiling buckwheat is the most suitable to indigestion crowd.

4. DISCUSSION

In previous research, there are more study on anti-nutritional factors from soybean, rapeseed, but less research on anti-nutritional in tartary buckwheat. Most of paper studied anti-nutritional factors of soybean, rapeseed and cancellation anti-nutritional factors method as well. Previous study had showed that the main method to remove the inhibiting proteins factor have heating, puffing and secondary granulating and sprouting process and microbial fermentation, heating divided into dry heat and moist heat, dry heat methods include roasting, baking, microwave radiation, infrared radiation, moist heat methods include boiling, hot pressing. When the heating is not enough, anti-nutritional factors can not be completely eliminated, but excessive heat would affect the efficiency of protein and vitamins. The heating effect are closely related with particle size and raw materials, heating time, temperature and humidity. In this experiment, ultimately similar conclusions also came to, which were the different effects of steaming, boiling, baking on nutritional factors for the heating temperature was different. After the seeds germinate, anti-nutritional factors are destroyed by endogenous enzymes (Bau, Villaume, & Nicolas, 1997). In this study, only protease inhibitor has decreased with enriched selenium sprouted tartary buckwheat treatment, amylase inhibitors, polyphenols and phytic acid content increased, and the increase content were very high. Fengfeng Wu has a similar result that buckwheat trypsin inhibitor may be damaged by endogenous enzyme, but many anti-nutritional factors such as polyphenols has been synthesized in the enriched sprouted process (Wu, 2013). This test used the physical, biological treatment to analyze the quantitative of anti-nutritional factors by steamed, boiled, grilled, enriched sprouted in tartary buckwheat. The test results provide a theoretical basis for the comprehensive utilization of buckwheat.

5. CONCLUSION

ANF content of buckwheat: trypsin inhibitor, polyphenols, phytic acid, α -amylase inhibitors, had changed after different treatments. By contrast, in the physical treatment, buckwheat inhibitors after boiling process were greatest damaged, except levels of trypsin inhibitors elevated, three other inhibitors decrease, so the product had good digestible and absorbed. Baking treatment in the physical processing was worst, followed by steaming effect. Biological treatment can significantly reduce the amount of trypsin inhibitor, also increases inhibitors content of polyphenols, phytic acid, α - amylase. To extend the time of selenium-rich had little effect on trypsin inhibitor and α -amylase inhibitors, but can increase the phytic acid content and changes polyphenol content, which can be provide a reference for future tests. In summary, it is good for pepole with bad digestive system to eat boiling buckwheat food, best suits to their consumption. Pepole with nutrition and three high-population was proposed Se-enriched food for reducing food availability.

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