Fagopyrum 39 (1): 13-18 (2022)

Research paper

Incorporation of yeast and hot melt extrusion enhance contents of total polyphenol and flavonoids and antioxidant ability in buckwheat flour

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DOI https://doi.org/10.3986/fag0024

Received: March 16, 2022; accepted March 23, 2022.
Keywords: Common buckwheat, yeast, hot-melt extrusion, total polyphenol, rutin, antioxidants

ABSTRACT

A study was conducted to determine the degree of interaction between yeast and hot-melt extrusion on contents of total polyphenol, total flavonoid, and rutin and antioxidant ability in common buckwheat. The highest content of total polyphenol was 95mg/100g in buckwheat flour, which included 2% yeast and was dried for 8 hours after extrusion while that of total flavonoids was 181mg/100g. The highest content of rutin in this study was 4.23mg/100g (156% higher than control, non-extrusion without yeast) in buckwheat flour, included 2% yeast, and dried for 8 hours after extrusion. Antioxidant ability determined by DPPH free radical scavenging ability was higher in yeast-mixed buckwheat flour extruded and dried for 8 hours (23-61%) than non-extruded with or without yeast (19-40%). The highest antioxidant ability (61%) was shown in buckwheat flour mixed with 2% yeast, extruded, and dried for 8 hours after extrusion. These results may be attributed to incorporating extruded buckwheat flour with yeast powder during 8 hours of drying and fermentation followed.

INTRODUCTION

Buckwheat (*Fagopyrum esculentum* Moench.) is annual melliferous crop and a pseudo cereal. For many years, the cultivation of buckwheat declined, but recent interest in and demand for dietary health care have led to the revival of its cultivation.

As a recognized medicine-food source, buckwheat contains a lot of bioactive substances having a variety of antioxidants such as flavonoids and phenolic acid along with proteins, carbohydrates, lipids, vitamins, and minerals (Krkoskova & Mrazova, 2005). In recent years, flavonoids and phenolic acids have attracted increasing interests due to their various beneficial pharmacological effects including antioxidative, anti-inflammatory, antiallergic, antiviral, anticancer, and antihypertensive properties (Kreft et al., 1999; Chao et al., 2002). Buckwheat is a good source of rutin, a flavonol glycoside plant metabolite, able to antagonize the increase of capillary fragility associated with hemorrhagic disease or hypertension in humans (Kreft et al., 1999; Park et al, 2000).

The extrusion process is traditionally employed via ram extrusion and screw (single or twin screw) extrusion. Twin screw extrusion generates high shear stress with a higher degree of mixing capacity than the single screw extruder. The solvent-free hot melt extrusion (HME) equipped with twin screw is widely used in the pharmaceutical and food industries, to develop solid composites with a nano-sized particle of the active compounds (Azad et al., 2019). In general, the high temperature of HME softens the polymer and favors the diffusion of active compounds such that they are dispersed into the polymer matrix. The processing ability in the HME can be enhanced by adding plasticizer, which lowers the melt viscosity of the extrudate. Hydrophilic plasticizer has a significantly positive effect on the increase in dissolution rate (Azad et al., 2019; Koo et al., 2019). HME contributes to enhancing the bioaccesibility, functionality, and thermal stability of nutraceutical compounds (Lee et al., 2019; Azad et al., 2020; Azad et al., 2021).

An interesting method of obtaining food of improved bioactive potential, increased digestibility, high nutritional value and favorable taste is solid-state fermentation of plant seeds. Yeast cells derived from *Saccharomyces cerevisiae* and related species have gained increasing attention as oral delivery systems for bioactive agents, including pharmaceuticals, supplements, and nutrients (Ten et al., 2021). Yeast cells have a rigid outer cell wall comprised of dietary fibers and glycoproteins that provide unique features for these applications. Indeed, yeast cells have several potential advantages such as their ability to deliver both hydrophobic and hydrophilic compounds and protect encapsulated substances from heat, light, oxygen, and moisture. The food-related bioactive compounds that have been successfully encapsulated using yeast cells include flavors, vitamins, carotenoids, phenolics, and other nutraceuticals (Ten et al., 2021).

This study is a preliminary trial for buckwheat flour to enhance contents of total polyphenol and flavonoids, including rutin, and antioxidant ability by incorporating hot melt extrusion with yeast powder during processing buckwheat flour for food or cosmetic materials.

MATERIALS AND METHODS

Sample preparation of common buckwheat

For experiment 200g of common buckwheat flour (Chuncheon Makguksu Agricultural Cooperation, Maemilgaru) was mixed with 1 or 2 percent of yeast powder (Beoma Food. Co., Instant yeast). Control was not mixed with yeast powder. With those mixed flour, t Three trials were done for hot melt extrusion (Han Kook Tech., AL-300); flour was non-extruded, flour dried just after extrusion, and flour dried for 8 hours after extrusion. Drying of buckwheat flour was conducted at 70 °C of dryer. Hot melt extrusion was done with an extruder at a temperature of 95-115 °C and pressure of 15 bar. Screwing speed was 160 rpm and time to ejection after extruding was 60-90 seconds.

Determination of total phenolic contents (TPC)

The total phenolic contents (TPC) were determined by the Folin-Ciocalteu assay (Singleton and Rosi, 1963). A sample aliquot of 200 µl was added to a test tube containing 200 µl phenol reagent (1N). The volume was increased by adding 1.8 ml of distilled deionized water. The solution was allowed to stand for 3 min for reaction. To continue the reaction, 400 µl of Na_2CO_3(10% in water v/v) was added and vortexed (Daihan Scientific, VM-30). The final volume 4 ml was adjusted by adding 1.4 ml of distilled water. The prepared sample was then incubated for 1 hour at room temperature. The absorbance was measured at 725 nm using a spectrophotometer (UV-1800 240V, Shimadzu Corporation, Kyoto, Japan).

The total phenolic content was expressed as tannic acid equivalents (TAE) in dry weight basis (DW)

Determination of flavonoid contents (TF)

The total flavonoid content (TF) was determined according to Ghimery et al. (2009) with slight modification. Briefly, an aliquot of 0.5 ml of sample (1 mg/ml) was mixed with 0.1 ml of 10% aluminum nitrate and 0.1 ml of potassium acetate(1M). In the mixture, 3.3 ml of distilled water was added to make the total volume 4 ml. The mixture was vortexed and incubated for 40 minutes. The total flavonoid was measured using spectrophotometer (UV-1800 240V, Shimadzu Corporation, Kyoto, Japan) at 415 nm. Total flavonoid content was expressed as μg/g quercetin equivalent in dry weight basis.

DPPH free radical scavenging ability (AA)

The antioxidant activity was determined on the basis of the scavenging activity of the stable 2, 2-diphenyl-1 picryl hydrazyl (DPPH) free radical according to methods described by Braca et al. (2003) with slight modification. 1 ml of extract was added to 3 ml of DPPH. The mixture was shaken vigorously and left to stand at room temperature in the dark for 30 minutes. The absorbance was measured at 517 nm using a spectrophotometer (UV-1800 240V, Shimadzu Corporation, Kyoto, Japan). The percent inhibition activities of the purple potato sample were calculated against a blank sample using the following equation. Inhibition (%) = (blank sample extract sample/blank sample) x 100.

RESULTS AND DISCUSSION

Buckwheat flour processed by hot melt extrusion showed 43-89% higher contents of total polyphenol and 30-48% higher total flavonoid than control, non-extruded treatment without yeast (Table 1). Rutin content was also 43-47% higher than control. Addition of yeast powder to buckwheat flour induced higher contents of total polyphenol and flavonoid as well as rutin content. In the treatment of non-extrusion, 1% and 2% of yeast increased 29% and 51% of total polyphenol contents and 38% and 42% of total flavonoid contents. Rutin content in the treatment of non-extrusion increased from 1.65mg/100g without yeast to 1.96mg/100g (19% higher) in 1% of yeast and 3.96mg/100g (140% higher) in 2% of yeast. Two types of extrusion caused increases of total polyphenol, total flavonoid, and rutin. Drying 8 hours after extrusion induced higher contents of total polyphenol, total flavonoid, and rutin than those obtained just after extrusion, indicating that fermentation in buckwheat flour hot-melt extruded by solid-state yeast was continuously proceeded during drying of 8 hours after extrusion. Addition of 2% yeast induced higher contents of total polyphenol, total flavonoid, and rutin rather than addition of 1% yeast. The highest content of total polyphenol was 95mg/100g in buckwheat flour included 2% yeast and dried for 8 hours after extrusion wwhile that of total flavonoid was 181mg/100g. The highest content of rutin in this study was 4.23mg/100g (156% higher than control, non-extrusion without yeast) in buckwheat flour included 2% yeast and dried for 8 hours after extrusion. Antioxidant ability determined by DPPH free radical scavenging ability was higher in yeast-mixed buckwheat flour extruded and dried for 8 hours (23-61%) than non-extruded with or without yeast (19-40%). The highest antioxidant ability (61%) was shown in buckwheat flour mixed with 2% yeast, extruded and dried for 8 hours after extrusion. These results indicate that extruded buckwheat flour incorporates with yeast powder during 8 hours of drying and following fermentation. HME processing technology was applied to several crops to maintain higher nutraceutical or pharmaceutical compound contents. HME increased the reactive surface area of compounds and de-structures the fibre matrix, thereby causing enhance decursin and decursinol angelate to be released into the solution in *Angelica gigas* (Azad, et al., 2020). HME resulted in prolongation of the thermal stability of anthocyanins in a biopolymer-mediated purple potato (Azad et al, 2021). This study showed a high potential of improvement from nutraceutical points of view. Effect of fermentation on buckwheat flour was determined in several cases of study using diverse microbials including yeast, *Saccharomyces* spp. The total phenolic content and antioxidant potential of fermented buckwheat dough significantly increased using *Lactobacillus delbrueckii* (Gandhi & Dey, 2013). Amino acid, glutamate and tryptophan in buckwheat increased through the serial repitching of *Saccharomyces pastorianus* (yeast) on the composition of the barley, buckwheat and quinoa fermentation medium (Deželak et al., 2015). Buckwheat groats fermented with *Rhizopus oligosporus* are a potential source of flour of advantageous antioxidant parameters for application as a food additive (Starazynska-Janiszewska et al., 2016). Raw buckwheat flour fermented with liquid-state fermentation by lactic acid bacterial and

Table 1. *Total polyphenol, total flavonoid, and rutin in different conditions of yeast and hot melt extrusion on buckwheat flour*

Treatment		Total polyphenol content (mg/100g)	Total flavonoid content (mg/100g)	Rutin content (mg/100g)
Yeast (%)	*Extrusion*			
0.0	Non-extrusion (Control)	35	64	1.65
	Just after extrusion	50	83	2.36
	Drying for 8 hrs after extrusion	66	95	2.43
1.0	Non-extrusion	46	88	1.96
	Just after extrusion	52	101	2.96
	Drying for 8hrs after extrusion	77	117	3.21
2.0	Non-extrusion	53	91	3.96
	Just after extrusion	59	131	3.69
	Drying for 8 hrs after extrusion	95	181	4.23

Rhizopus oligosporus fungi contained higher amount of rutin and total polyphenol contents (Zielinski et al., 2019). The ethanolic extracts of buckwheat fermented with three strains of *Agaricus* exhibited the higher total phenolic contents and superoxide anion radical scavenging ability (Kang et al., 2017).

CONCLUSIONS

This study was attempted through the combination of biological (yeast) and mechanical (hot-melt extrusion) approaches for improving quality of buckwheat flour. In this preliminary study, we found a potential of incorpora-

Table 2. *Antioxidant ability in different conditions of yeast and extrusion on buckwheat flour*

Treatment		DPPH free radical scavenging ability (%)
Yeast (%)	*Extrusion*	
0.0	Non-extrusion (Control)	18.5
	Just after extrusion	23.1
	Drying for 8 hrs after extrusion	31.8
1.0	Non-extrusion	35.7
	Just after extrusion	47.5
	Drying for 8 hrs after extrusion	57.0
2.0	Non-extrusion	39.7
	Just after extrusion	50.9
	Drying for 8 hrs after extrusion	60.6

tion of yeast with hot melt extrusion to enhance antioxidant parameters including rutin content of buckwheat flour. Further studies are needed to clarify a decisive mechanism of the incorporation based both biological and mechanical approaches resulting in quality improvement of buckwheat flour.

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IZVLEČEK

Uporaba kvasa in vročega ekstrudiranja poveča vsebnost celotnih polifenolov, flavonoidov in antioksidacijsko sposobnost pri ajdovi moki

Namen raziskave je bil ugotoviti interakcijo dodanega kvasa in vročega ekstrudiranja mešanice ajdove moke s kvasom na vsebnosti celotnih polifenolov, flavonoidov in antioksidacijske sposobnosti pri moki navadne ajde. Ajdovi moki je bilo dodanega 2% kvasa, rezanci so bili sušeni 8 ur po ekstruziji. Najvišja vsebnost skupnih polifenolov je bila 95mg/100g z dodatkom 2% kvasa, vsebnost celotnih flavonoidov je bila 181mg/100g. Najvišja vsebnost rutina je bila 4,23mg/100g (156% več kot kontrola, brez ekstrudiranja, s kvasom) pri ajdovi moki z 2% kvasa in sušeno 8 ur po ekstruziji. Antioksidativna sposobnost, določena z DPPH, je bila višja pri uporabi kvasa, ekstruzije in sušenja 8 ur (23-61%), kot brez uporabe kvasa in ekstruzije (19-40%). Najvišja antioksidacijska sposobnost (61%) je bila določena pri ajdovi moki z dodatkom 2% kvasa, ekstrudirano in po ekstruziji sušeno 8 ur. Pričakovati je, da bodo rezultati te raziskave prispevali k uspešni uporabi ekstrudiranja ajdove moke z uporabo kvasa in osem urnega sušenja ter fermentacije.