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Original Research Article

Higher Extraction of Phytochemical Compounds from Tartary Buckwheat Seeds by the Application of Surfactant Formulation

Cheol Ho Park, Md Obyedul Kalam Azad, Jeong Won Heo and Wie Soo Kang*

Department of Bio-Health Technology, Kangwon National University, Chuncheon 24341, Korea

Abstract - The aim of this study was to determine the suitability of surfactant to extract higher phenolic compound, flavonoid and antioxidant activity from Tartary buckwheat and evaluate the potentiality of surfactant as a screening agent for breeding purpose. Primarily, we employed two types of surfactant (Hydrophilic: Tween 20 and Lipophilic: Span 80) to select the suitable surfactant agent for the extraction of optimum bioactive compounds. Between two surfactants, Tween 20 showed highest efficiency at 4 mM concentration to extract total phenolic content (TP), total flavonoid (TF) and antioxidant activity (AA). Tween 20 at 4 mM concentration was fixed for further analysis along with hot water (90℃) treatment as a control. In our findings, highest TP (118 ㎎/g), TF (38 ㎎/g) and AA (76%) was achieved in KW21 and KW22 among the fifteen accessions of Tartary buckwheat. In other way, TP, TF and AA was 200%, 120% and 110% higher in surfactant formulation compared with control treatment, respectively.

Key words - Antioxidant activity, Flavonoid, Phenolic compounds, Surfactant, Tartary buckwheat

Introduction

Plant phenolic compounds are considered as a strong antioxidant and health promoting phytochemicals. The antioxidant activity of phenolic compounds exhibits beneficial antibacterial, antiviral, anticarcinogenic and anti-inflammatory properties (Duthie *et al.,* 2000; Mattila and Hellstrom, 2006) which protect the body from various diseases such as cancer, brain dysfunction and cardiovascular diseases (Vattakandya and Chaudharib, 2013).

Tartary buckwheat is one of the important pseudo-cereal having a rich source of flavonoid along with proteins, carbohydrates, lipids, vitamins, minerals and amino acids. Tartary buckwheat grain contains higher flavonoids, proteins and amino acids more than common buckwheat (Li and Zhang, 2001; Morishita *et al.,* 2007). The most used extraction solvents are pure or aqueous mixtures solution containing methanol, ethanol or acetone. But the use of inorganic solvents causes toxicity to human health. Moreover, the efficiency of pure water extraction is very poor because of only hydrophilic molecules are dispersed in water. Previously enhanced antioxidant activity was achieved of barley plants by the application of enzymes treatment (Phouthaxay *et al.,* 2015) and anthocyanin content was extracted by water based acid mediated medium (Kim *et al.,* 2017). However, surfactant based extraction methods possess higher potentiality that could enhanced extraction of phenolic compounds.

An amphiphilic compound such as surfactants are currently being used in extracting phenolic compounds from fruits (Hosseinzadeh *et al*., 2013; Sharma *et al.,* 2015). Surfactant exhibits a strong affinity to polar and nonpolar substances and make a chemical crosslinked with the molecules in which dispersion forces are reduced. Surfactant moieties form micelles in a solution above their critical micellar concentration (CMC). These micelles are composed of hydrophilic head and lipophilic tail having capabilities of establishing chemical and physical interactions with both hydrophilic and lipophilic compounds (Berthod and Coque, 2000; Bordbar and Hosseinzadeh, 2006). Moreover, surfactant molecule gets absorbed onto the interface or the surface of the system and thereby alter the interfacial free energies (Rosen, 2004). However, surfactants are recognized as non-toxic or harmless reagents to human health until further branching (Lee *et al.,* 2001).

*Corresponding author. E-mail : kangwiso@kangwon.ac.kr
Tel. +82-33-250-6494

Buckwheat represents a minor crop and, hence, the main goals is to increase the yield and phytochemical profile of newly developed buckwheat genotypes. Despite numerous biochemical studies providing information on locally cultivated genotypes, there is still a lack of comprehensive information regarding the comparison of species and genotypes of different origin with their status of nutritional composition.

Therefore, the aim of this research was to evaluate the suitability of surfactant agent to extract higher phytochemical content from Tartary buckwheat seeds instead of conventional solvents.

Materials and Methods

Tartary buckwheat flour preparations

Fifteen accessions of Tartary buckwheat were collected from different locations at different times from Kangwon Do, Korea. Tartary buckwheat seeds were grinded using an electric blender (Model No. Blixer 5 plus, Robot coup, USA) and prepared find powder. The flour was stored in an air tide polyethylene bag at room temperature for further analysis.

Chemical and reagents

Surface active agent tween 20 (HLB: 16.9), span 80 (HLB: 4.3), phenolic reagent (Folin ciocalteu, 2N), sodium bi-carbonate (Na_2CO_3), aluminium nitrate $(AlNO_3)_3$, potassium acetate (CH_3CO_2K), DPPH (2, 2-diphenyl-1 picryl hydrazyl) were purchased from Sigma-Aldrich (Korea). All other chemicals used were of analytical grade and pursued from Merck. Deionized distill water (EC <0.3μS/㎝) was used for sample preparation.

Sample preparation of Tartary buckwheat

Five hundred milligram (0.5 g) of Tartary buckwheat powder was added with previously prepared different surfactant concentration in a 50 ㎖ tube. The sample was shaken at 150 rpm, 25℃ using shaking incubator (SI-900RF, JEIO TECH, Korea) for one hour. The sample was filtered through 125 ㎜ filter paper (Advantech 5B Tokoyo Roshi Kaisha, Japan) and then extract was collected. The extract was stored in the refrigerator at -20℃ for further analysis.

Determination of total phenolic contents (TPC)

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

Determination of flavonoid content (TF)

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

DPPH free radical scavenging activity (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 ㎖ of extract was added to 3 ㎖ of DPPH. The mixture was shaken vigorously and left to stand at room temperature in the dark for 30 mins. The absorbance was measured at 517 ㎚ using a spectrophotometer (UV-1800 240 V, 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)*100.

Statistical analysis

All data were expressed as Mean ± SD of triplicate measurements. The obtained results were compared among the different surfactants concentration and types using a paired t test in order to observe the significance differences at the level of 5%. The paired t test between mean values was analyzed by MINITAB (version 16.0).

Results and Discussion

Effect of surfactant types and concentration on extraction of total phenols, flavonoids and antioxidant activity of Tartary buckwheat

Two types of surfactant formulation was employed such as hydrophilic agent Tween 20 and lipophilic agent Span 80 with different concentration viz. 2, 4, 6, 8 and 10 mM. Methanol and water was used as a control treatment. It is found that Tween 20 had highest efficiency compared to span 80 in extraction phenolic compounds. Among the different concentration, Tween 4 mM extracted highest total phenol (94.5 ㎎/g), total flavonoid (42.79 ㎎/g) and antioxidant activity (83.69%) (Table 1) compared to control.

It is attributed that polar solvents (water and methanol) extracted only polar components, but non-polar and other protein binding matrix of active compounds were not extracted, thus least extraction was achieved in conventional solvents. On the other hand, surfactant based assemblies form colloidal dispersion and dissolve both polar and non-polar component of active compounds due to their amphiphilic nature (Perez *et al.,* 1997; Hosseinzaeh *et al.,* 2013). The linear molecular formula revealed that hydrophilic surfactant tween 20 has longer hydrophilic chain than span 80 surfactant. Due to long hydrophilic component of tween 20, an enhanced hydrophilic-hydrophilic interaction was facilitated thus highest phenolic compound was extracted (Hosseinzaeh *et al.,* 2013; Sharma *et al.*, 2015).

When plant materials mixed with surfactant formulation, the surfactant micelle interact with hydrophilic and lipophilic compounds of plant materials. Therefore, plant phytochemical which either hydrophilic or lipophilic both are strongly attracted by micelle core. When the solution is experienced by sonication, surfactant micelle form a shell type monolayer surrounded by active molecules are called chemistry based encapsulation process (Ladj *et al.,* 2013) (Fig. 1).

Surfactant concentration was determined by estimating the TP, TF and AA (%) from Tartary buckwheat. The increase in extraction efficiency has been obtained in 4 mM concentration (Table. 1). Further increase in surfactant concentration showed decreasing in extraction efficiency. Therefore, the surfactant concentration was fixed to 4 mM and assumed that this is the maximum efficient concentration for Tween 20 surfactant for

Table 1. Effect of surfactant types and concentration on total phenols, flavonoid and antioxidant activity of Tartary buckwheat

Treatment	Total phenols (㎎/g)	Total flavonoid (㎎/g)	DPPH (%)
Water (90℃)	70.1 ±3.1[z]	22.1 ±1.1	55.7 ±4.3
MeOH (80%)	78.2 ±1.4	35.9 ±2.1	62.2 ±3.7
Tween 2 mM	76.92±2.8	32.2 ±3.3	66.93±2.1
Tween 4 mM	94.5 ±2.3	42.79±4.1	83.69±1.8
Tween 6 mM	81.3 ±3.4	28.2 ±2.3	66.1 ±1.3
Tween 8 mM	76.9 ±1.6	25.79±3.2	67.7 ±4.1
Tween 10 mM	75.5 ±2.3	26.2 ±4.0	69.9 ±3.6
Span 2 mM	45.7 ±2.1	22.9 ±4.1	65.24±3.8
Span 4 mM	61.3 ±1.3	38.2 ±2.8	20.2 ±4.7
Span 6 mM	53.2 ±1.2	31.2 ±4.2	24.5 ±3.7
Span 8 mM	58.1 ±2.7	27.7 ±3.3	32.7 ±2.3
Span 10 mM	44.5 ±2.3	32.2 ±4.5	22.6 ±2.1

[z]Data was expressed as Mean ± SD (n=3).

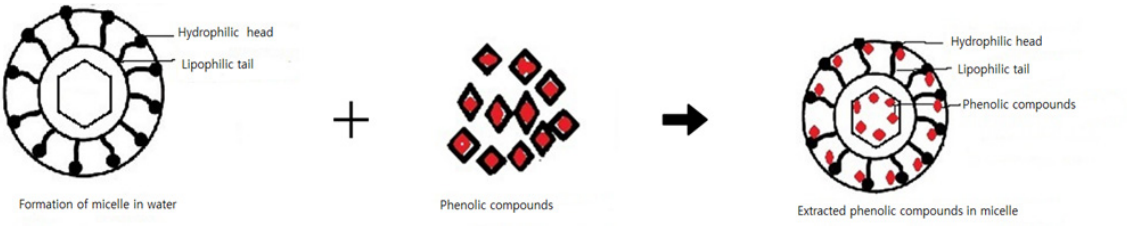

Fig. 1. Schematic illustration of extraction of phenolic compounds in surfactant micelle.

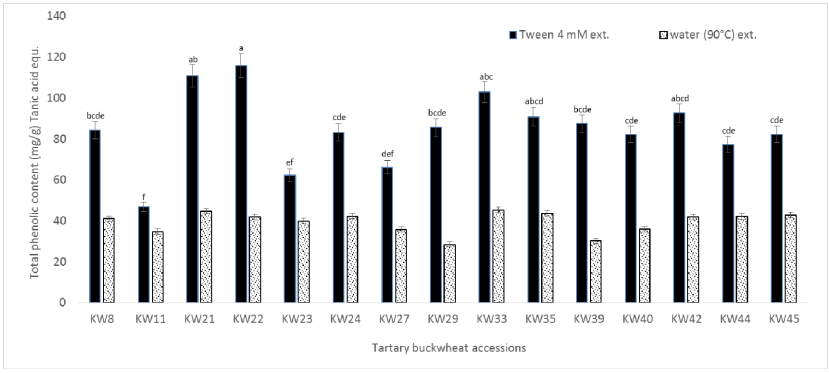

Fig. 2. Effect of surfactant formulation on total phenolic content of fifteen genotypes of Tartary buckwheat.

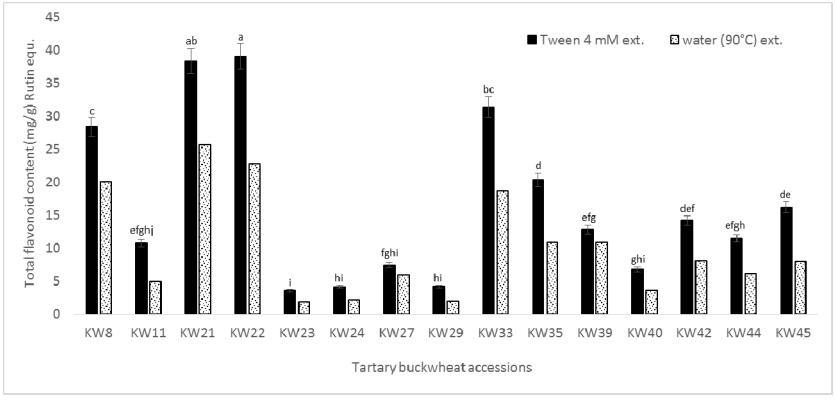

Fig. 3. Effect of surfactant formulation on total flavonoid content of fifteen genotypes of Tartary buckwheat.

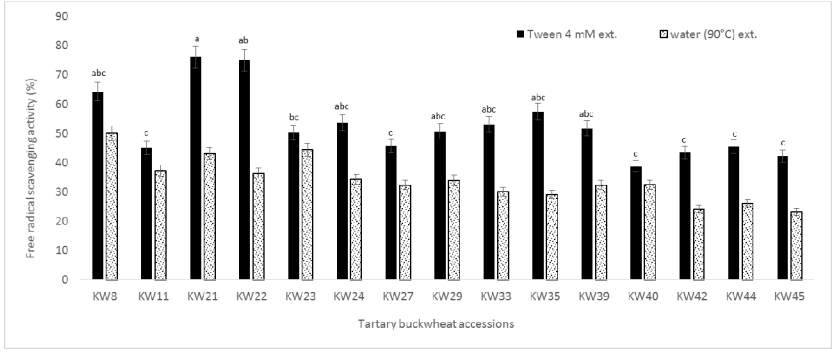

Fig. 4. Effect of surfactant formulation on antioxidant activity of fifteen genotypes of Tartary buckwheat.

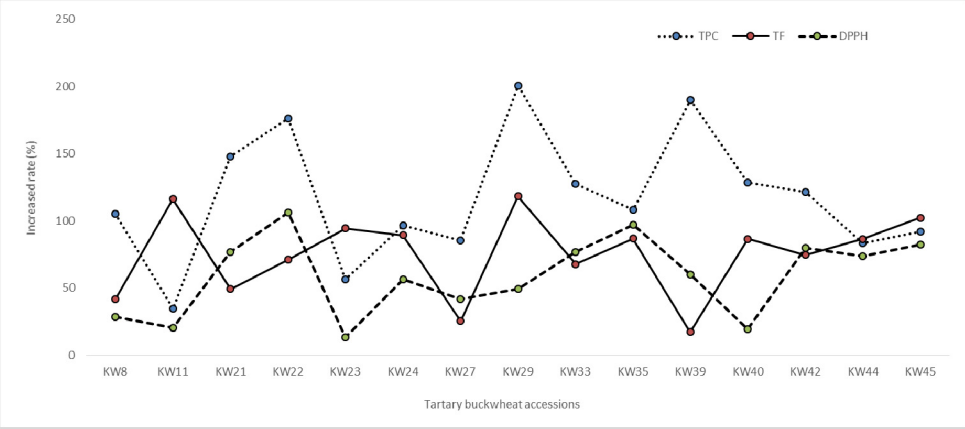

Fig. 5. Increases of Total phenolic content in surfactant formulation compared to water extraction.

the extraction of phytochemical compounds from Tartary buckwheat. Similarly, most efficient concentration was found 7 mM in extracting phytochemical content from apple juice (Sharma *et al.,* 2015). Therefore it might be assumed that optimum concentration of surfactant formulation is depended upon the extracting materials.

Extraction of total phenols, flavonoids and antioxidant activity of different accessions of Tartary buckwheat in surfactant formulation

Total phenolic profile and total flavonoid content and antioxidant activity were analyzed from fifteen accessions of Tartary buckwheat using hydrophilic surfactant Tween 20 at 4 mM concentration along with water as a control treatment (Fig. 2, 3, 4). Among the fifteen accessions of Tartary buckwheat KW22 showed the highest phenolic (115 ㎎/g) and flavonoid content (38 ㎎/g) among the accessions (Fig. 2 and Fig. 3) which was 200% and 120% higher compared to water extraction (Fig. 5). Antioxidant activity (76%) was found significantly higher in KW21 among the accessions (Fig.4) which was 110% higher compared to water extraction (Fig. 5).

It is clearly observed from our findings that hydrophilic surfactant formulation efficiently extracted phytochemical content compared to water extraction. Surfactant is a newly introduced agent in extraction technology. Surfactant getting more attention due to superior extraction efficiency and edibility (Lee *et al.,* 2001). Differences in secondary metabolites within Tartary buckwheat accessions are not well documented. However, fatty acids content was found different among the four different varieties of buckwheat (Pomeranze *et al.,* 1975; Kim et al., 2004; Tomotake *et al.*, 2006; Tang *et al.,* 2009). Tahir et al. (1985) reported that protein content and phenolic profile differ among the Tartary buckwheat cultivars followed by physiological and morphological traits. Variation of yield, nutritional characteristics in Tartary buckwheat cultivars are depends on their growing environment, maturity and harvesting time (Gabr *et al.,* 2012).

In brief, it is demonstrated that hydrophilic Tween 20 surfactant formulation successfully extracted higher total phenolic, total flavonoid and antioxidant activity from fifteen accessions of Tartary buckwheat. The efficiency of surfactant formulation to extract TP, TF and AA showed superiority over water and ethanol. The characterization of buckwheat accessions (KW21, KW22) and the introduction of buckwheat-based products from elite cultivars into the modern food chain are needed to fill the current gap. Finally, we would conclude that hydrophilic surfactant can be used for the screening of buckwheat accessions for further food and breeding purpose.

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