Evaluation of Anthocyanin Stability in Surfactant Formulation from Extrudate Purple Potato (*Solanum tuberosum* L.)

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**Abstract**

Anthocyanin has a strong antioxidant capacity but exhibit poor stability in water. Therefore, increasing stability of anthocyanin from purple potato (*Solanum tuberosum* L. var. love gold valley) was investigated in surfactant colloid followed by hot melt extrusion (HME). The hydrophilic Brij 35 and the lipophilic Span 80 were used to prepare association colloids of surfactant (ACS) and surfactant based purple potato extrudate (SPE). Result revealed that extraction of total phenol (TP), total flavonoid (TF), total anthocyanin (TA) and antioxidant activity (AA) was increased at 4, 2, 5 and 2 times, respectively, at 9 mM span 80 ACS compared to control (without surfactant). There was no significant reduction of anthocyanin was observed in SPE. Whereas, a significant reduction of anthocyanin was observed in control extrudate. The nano size particle (<500 nm) was achieved in SPE. The higher extraction of phenolic compound in ACS is may be due to chemical crosslink (adsorption and/or absorption) between surfactant micelle and anthocyanin molecule. It would be concluded that lipophilic association colloids can be used to protect anthocyanin from oxidation. However, further study needed to explain the mechanism and more understanding in this regard.

**Keywords:** Anthocyanin; Purple potato; Surfactant; Hot melt extrusion

**Introduction**

Purple potato (*Solanum tuberosum* L. var. love gold valley) is rich in anthocyanin and phenolic acids which have strong antioxidant capacities [1]. Dietary intake of anthocyanins reduces the risk of cancer, cardiovascular diseases, Alzheimer’s and diabetes [2]. Moreover, anthocyanins are also used as natural colorants in food, beverage, cosmetics and paint industries [3]. However, the use of anthocyanin is limited because of major technological challenges since anthocyanin have low stability in aqueous medium due to their high heat sensitivity [4-6]. Several studies reported a logarithmic course of anthocyanin destruction with an arithmetic increase in temperature [7,8].

In recent year extraction and separation of anthocyanin was investigated by the application of surfactant emulsion [9]. Studied reported that light, pH, temperature, NaCl, sugar, metal ion, and other factors affect anthocyanin solution stability [10,11]. Aditya et al. [12] prepared a surfactant emulsion to embed curcumin and catechin, and the results showed a remarkable enhancement of the stability of curcumin and catechin in a simulated gastrointestinal tract environment. McClements [13] reported that plant active compound can be incorporated into surfactant emulsion for longer stability without adversely affecting the food quality attributes.

Surfactant moiety form micelles in a solution above their critical micellar concentration (CMC). These micelles are composed of a hydrophilic head and lipophilic tail having capabilities of establishing chemical and physical crosslinked with both hydrophilic and lipophilic compounds [14,15]. Moreover, surfactant micelle alters the functional properties of biopolymers by binding with them or to displacing biopolymers from oil-water interfaces through competitive adsorption or modulating the crystallization in the given phases [16]. One of the most important aspects of surfactant is their ability to control stability and rheology of a food composition [14,17,18].

The aim of the study was to determine the efficacy of the surfactant formulation as an encapsulating agent for the protection of anthocyanin compound. This proposed encapsulating design might improve the stability of anthocyanin in food processing industries.

**Materials and Methods**

**Flour preparations**

Purple potato was purchased from the Chuncheon, Korea local market. Samples were cut into slices (2-3 mm thickness) and freeze dried (Ilishin BioBasae, FD 5510S- FD 5520S, Korea). The freeze dried potato was blended using an electrical blender (Model No. Bixer 5 plus, Robot coup, USA) and prepared coarse powder. Potato powder was milled by a pin crusher (JIC-P10-2; Myungsung Machine, Seoul, Korea) equipped with a 30-mesh sieve. The milled powder was fractionated using a sieve shaker (CG-213, Ro-Top, Chunghye Industrial Mfg. Co., Seoul, Korea) equipped with a series of sieves (20 cm). The powder was passed through 300 μm mesh size sieves, and unpassed particles were grinded again with the pin crusher. Potato fine powders were then stored in a desiccator for the further use.

**Chemical and reagents**

Brij 35 (HLB: 16.9), Span 80 (HLB: 4.3), Phenolic reagent (Folin ciocalteu, 2N), Sodium bi-carbonate (Na 2CO3), Aluminum nitrate (Al(NO3)3, potassium acetate (CH3CO2K), DPPH (2, 2-diphenyl-1 picryl hydrazyl), were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade and purchased from...
Merck Chemical Corp. (Darmstadt, Germany). Deionized, distilled water (EC value <0.3 µS·cm⁻¹) was used for sample preparation.

Surfactant formulation and sample preparation

Surfactant emulsions were prepared in water with different molar concentration followed by 3.5, 7.9, and 11 mM. Formulations were well mixed using electric stirrer (Ultra Torque, Model BDC1850) with high shear in order to breakdown the kinetic energy barrier to ensure as surfactant is adequately dispersed.

Extraction of phenolic compound from the purple potato

One gram of potato fine powder was added in 100 ml of different surfactant formulation in 200 ml conical flask. The sample was shaken at 150 rpm, using shaking incubator (SI-900RF, JEIO TECH, Korea) for one hour at room temperature. The particle size of the supernatant was achieved at 5669 nm, whereas the non-extrudate sample was achieved at 1584 nm in the extrudate sample. It is clearly observed that nanonization was enhanced of the particle size reduction strategy results in increased surface area, decreased diffusional distance, and increased dissolution rates [25-27].

Preparation of extrudate formulation and HME configuration

The extrudate of purple potato was developed 9 Mm of Brij 35 and Span 80 surfactant using an STS-25SH twin-screw HME (Hanook E.M. Ltd., Pyungtaek, Korea). The extruder was equipped with a round-shaped die (1 mm) and was operated at a feeding rate of 40 g/min, 150 rpm with high shear. The temperature profile from the feeding zone to die was 80/100/80/70°C. The potato extrudate was dried in an oven at 50°C and then ground for further analysis.

Particle size analysis

The potato powder (PP) and potato extrudate powder (PEP) (0.5 g) was suspended in 50 ml of distilled water. The supernatant was separated by centrifugation at 3,000 rpm for 10 min. The percent inhibition of the sample was calculated against a blank sample using the following equation: inhibition (%) = (blank sample-extract sample/blank sample)×100.

Determination of total flavonoid content

The total phenolic contents (TP) were determined by the Folin-Ciocalteu assay [19]. A sample aliquot of 200 µl was added to 200 µl 1N phenol reagent. The solution was allowed to stand for 3 min for reaction. To continue reaction, 400 µl of Na₂CO₃ (10% in water v/v) was added and vortexed. The prepared sample was then incubated for 1 hour at room temperature. The absorbance was measured at 725 nm using a spectrophotometer (UV-1800 240 V, Shimadzu Corporation, Kyoto, Japan). The TP was expressed as Gallic acid equivalents (GAE) in dry weight basis.

Determination of total flavonoid content

The total flavonoid content (TF) was determined according to Ghimeray et al. [20]. Briefly, an aliquot of 0.5 ml of sample (1 mg/ml) was mixed with 0.1 ml of 10% aluminium nitrate and 0.1 ml of potassium acetate (1M). The mixture was vortexed and incubated for 40 min. The total flavonoid was measured using spectrophotometer at 415 nm. The TF was expressed as µg/g quercetin equivalent in dry weight basis.

Determination of total anthocyanin content

The content of total anthocyanin (TA) was determined by the pH differential method. Each extract (0.5 ml of) was diluted with 2.5 mL of 0.025 M potassium chloride buffer, pH 1.0 and 0.4 M sodium acetate buffer, pH 4.5, separately. The diluted solutions were incubated at room temperature for 15 min. The absorbance was taken at 520 and 700 nm against a blank cell filled with distilled water using spectrophotometer. The TA was calculated according to equation described by Giusti and Wrolstad [21].

DPPH free radical scavenging activity

The antioxidant activity was determined on the basis of the scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical according to methods described by Braca et al. [22]. 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 mins. The absorbance was measured at 517 nm using a spectrophotometer. The percent inhibition activities of the 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 (Minitab Inc., State College, PA, USA).

Results and Discussion

Effect of surfactant on the particle size reduction of the extrudate and non extrudate potato powder

It is clearly observed that nanonization was enhanced of the extrudate solid by the hot melt extrusion process (Figure 1). The particle size of the non-extrudate sample was achieved at 5669 nm, whereas the size was 1584 nm in the extrudate sample in control treatment. It is reported that HME extrusion is the most suitable process to enhance the amorphization of crystal materials by reducing particle size [23,24]. The particle size reduction strategy results in increased surface area, decreased diffusional distance, and increased dissolution rates [25-27].

Among the formulations, the nano particle size (289 nm) was achieved in the lipophilic Span 80 mediated extrudate compared to Brij 35. The effect of the surfactant on the particle size, polydispersity, type of particle size distribution and structure of nanoparticles synthesized has been studied by Toton et al. [28]. It is also reported that surfactant emulsion produce a nanoparticle from a diverse variety of materials, including metals [29], silica [30], polymers [31] were studied. In previous experiment CuSO₄ nano composite was prepared by the surfactant mediated HME process [32]. Similarly, in this study for the purple potato powder the nano size particle was achieved in Span 80
agent. Further investigation is needed to clarify the mechanism behind this nanonization process.

Effect of surfactant and solvent (water and ethanol) on the extraction of the phenolic compound

It is clearly shown from Table 1 that surfactant efficiently extracted phenolic compound from the purple potato compared to water and ethanol. An associate colloidal of the dispersed active compound was prepared by the surfactant agent. The phenolic compound of the Purple potato was dispersed in water and ethanol. The content of the total phenol (1053, 1952 μg/g), total flavonoid (580, 721 μg/g), total anthocyanin (150,287 μg/g) and antioxidant activity (42%, 57%), respectively in water and ethanol solvent (Table 1). It is attributed that polar solvents (water and ethanol) 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.

The highest active compound was extracted at the 9 mM concentration which is selected as a critical micelle concentration (CMC) of the purple potato. Total phenolic compound (PC), total flavonoid (TF), total anthocyanin (TA) and antioxidant activity (AA) was increased 4, 2, 4 and 2 times, respectively at 9 mM concentration compared to water extraction. Lipophilic surfactant Span 80 showed highest efficiency to extract PC, TF, TA and AA compared to Brij 35 at the same concentration. It is observed that extraction efficiency for active compounds from purple potato linearly increased with increasing surfactant concentration till 9 mM. It is shown that total phenolic compound (PC), total flavonoid (TF), total anthocyanin (TA) and antioxidant activity (AA) was increased 4, 2, 4 and 2 times, respectively at 9 mM concentration compared to water extraction.

The maximum extraction efficiency has been obtained at 9 mM concentration and further increased in surfactant concentration showed declined trends in extraction efficiency. Surfactant concentration more than their CMC level makes an abrupt change in physicochemical

### Table 1: Effect of the surfactant concentration on the total phenol, flavonoid, anthocyanin and antioxidant activity of the purple potato.

<table>
<thead>
<tr>
<th>Phenol compound</th>
<th>Water</th>
<th>EtOH</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>11</th>
</tr>
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<td></td>
<td></td>
<td></td>
<td>Span 80</td>
<td>Brij 35</td>
<td>Span 80</td>
<td>Brij 35</td>
<td>Span 80</td>
</tr>
<tr>
<td>TPC</td>
<td>1053e</td>
<td>1952c</td>
<td>1608d</td>
<td>1552c</td>
<td>2343e</td>
<td>1112e</td>
<td>2516b</td>
</tr>
<tr>
<td>TF</td>
<td>580d</td>
<td>721c</td>
<td>703c</td>
<td>511</td>
<td>380c</td>
<td>312c</td>
<td>415c</td>
</tr>
<tr>
<td>TA</td>
<td>150e</td>
<td>267d</td>
<td>301c</td>
<td>210c</td>
<td>380c</td>
<td>312c</td>
<td>415c</td>
</tr>
<tr>
<td>DPPH</td>
<td>42e</td>
<td>57c</td>
<td>60b</td>
<td>39d</td>
<td>63b</td>
<td>48d</td>
<td>63b</td>
</tr>
</tbody>
</table>

Results shown are mean±SD (n=3). Same letters within the row are not statistically difference at p<0.05.

Figure 2: Effect of the surfactant concentration on the total phenolic compound from the purple potato.

Figure 3: Effect of the surfactant concentration on the total flavonoid compound from the purple potato.

Figure 4: Effect of the surfactant concentration on the anthocyanin compound from the purple potato.

Figure 5: Effect of the surfactant concentration on the free radical antioxidant activity of the active compound from the purple potato.
properties of surfactant such as surface tension, electrical conductivity and osmotic pressure. This is because monomers are amphiphilic and so are surface active whereas, micelles are hydrophilic and therefore have low surface activity [28]. Result demonstrated that lipophilic surfactant Span 80 showed highest efficiency in extracting TP, TF, TA, and AA compared to control and Brij 35 at 9 mM concentration.

Surfactant based assemblies form associated colloidal which dissolve both polar and non-polar matrix of active compounds due to their amphiphilic nature [33]. Span 80 surfactant mediated aqueous solution established a chemical croslinked with polar and non-polar molecules of polyphenol compounds and formed hydrogen bond between them, as a consequence, higher extraction was attained.

As the anthocyanin easily oxidized during extraction in Brij 35 mediated solution therefore TF contents were found lower than those achieved with Span 80 mediated assemblies. Studied showed that non-ionic hydrophilic surfactant Brij 35 effectively extracted active compounds from apple juice compared to lipophilic surfactant and conventional solvents [33,34]. However, in our study, Span 80 had an effective extracting moiety having a capacity of anthocyanin protection from oxidation in purple potato. Appropriate surfactant for each particular application depending on the type of active ingredient to be encapsulated, the nature of food matrix and functional attributes [13].

**Effect of melt extrusion on the extraction of the active compound**

Total phenolic, total flavonoid and anthocyanin was quantified from the extrudate purple potato (Figures 2-6). Result demonstrated that there were no significant changes of the content of the total phenol, total flavonoid and total anthocyanin in extrudate solid compared to non extrudate. The possible mechanism involved in this processes is the developing coating layer around the active compound in the aqueous media by the surfactant micelle. The chemical formula of Brij 35 [C12H25(OCH2CH2)23OH] and Span80 [(C24H44)O6] exposed that aqueous media by the surfactant micelle. The chemical formula of Brij 35 showed highest efficiency in extracting TP, TF, TA, and AA compared to control and Brij 35 at 9 mM concentration.

**Conclusion**

The lipophilic surfactant Span 80 showed higher capabilities to protect anthocyanin from oxidation during processing compared to hydrophilic Brij 35. There was no significant reduction of active compound observed in extrudate solid compared to non extrudate purple potato powder. This finding would help for better understanding to develop a processing technology to enhance anthocyanin stability from the purple potato.

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**References**


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**Figure 6:** Effect of the surfactant concentration on the particle size of the active compound.


