



저작자표시-비영리-변경금지 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:



저작자표시. 귀하는 원저작자를 표시하여야 합니다.



비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.



변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 [이용허락규약\(Legal Code\)](#)을 이해하기 쉽게 요약한 것입니다.

[Disclaimer](#)

**Master's Thesis of Science in Agriculture**

**Effects of Ginseng (*Panax ginseng*) Saponin on  
Storage Stability of Soymilk**

인삼(*Panax ginseng*) 사포닌이 두유의 저장 안정성에 미치는 영향

**February 2019**

**Jongheon Kim**

**Department of International Agricultural Technology  
Graduate School of International Agricultural Technology  
Seoul National University**

## Abstract

### Effects of Ginseng (*Panax ginseng*) Saponin on Storage Stability of Soymilk

Jongheon Kim

Department of International Agricultural Technology  
Graduate School of International Agricultural Technology  
Seoul National University

The soybean (*Glycine max*), which contains 35-40% plant proteins, and health functional substances such as saponin, isoflavone, and oligosaccharide, is major source of plant protein and in the human food. However, it also contains trypsin inhibitor, phytate and lectins as anti-nutritional components. Consequently, soybean must be subjected to proper treatment such as thermal, acidic, and ultra-sonication etc. before consumption by human beings to reduce these anti-nutritional contents Soy processed foods may be divided into two classes: fermented foods (soy sauce, soybean paste etc.) and non-fermented foods (soymilk, tofu, soybean flour etc.) Especially, soymilk is the extract obtained after soaking, grinding, cooking and filtering the soybean. It has gained much popularity as a healthy plant-based beverage and easily digestive products. However, soymilk often shows some precipitation during long-term storage and this is susceptible to quality loss, which may adversely affect physicochemical properties (particle size, microstructure, and turbidity), textural, sensory properties of soymilk and limit soymilk utilization. According to Stoke's law:  $V = 2\Delta\rho g\alpha^2/9\eta$ , the particle precipitation of soymilk influences the particle size, density and viscosity of soymilk. Also, it can improve the storage stability of soymilk by controlling these three factors. To improve storage stability of soymilk,

previous studies were mostly focus on using the ultra-high pressure homogenization equipment, and the addition of hydrocolloids. Ultra-high pressure homogenization in soymilk can enhance shelf-life, colloidal stability, and reduce the average particle size and settling. However, it can cause protein denaturation and degradation of several compounds. Adding hydrocolloids in soymilk showed that it interacted with soymilk particles. It can inhibit the particle aggregation and decreased particle precipitation. However, some kinds of hydrocolloids reported that may cause lesions or cancer in the gastrointestinal tract. And the hydrocolloid is sensitive to environmental factors, such as pH, thermal treatment, and ion species. Many researches show that surfactant also can improve stability of emulsion to interact with particles and form the complexes in beverages. Also, this complex can inhibit the protein aggregation and precipitation, thereby the storage stability of the beverage improved. Recently, researchers have reported that ginseng (*Panax ginseng*) saponin as one of the natural surfactant, also can interact with protein, and make the complex that can improve emulsion stability and change interfacial properties. In addition, it was well known for their health benefits, anti-cancer, and anti-aging.

The aim of this study is to characterize an influence of ginsenosides as a natural surfactant on storage stability of soymilk during stored under various conditions (4, 25, 37 °C). The study was divided into two categories: “soymilk interface model system (chapter 2)” to understand the soymilk stabilization mechanism induced by ginsenosides, and “soymilk products (chapter 3)” to investigate the effect of GSP on storage stability of soymilk.

Interfacial characteristics of mixed soy protein isolate (SPI, purity $\geq$ 90%) and ginseng saponin powder (GSP, purity $\geq$ 80%) was studied at air/water interface (Chapter 2). To investigate the change of interfacial properties of mixed GSP-SPI depending on the mass ratio of GSP/SPI, Mixed protein-surfactant system was divided into three concentration profiles: (1) “unsaturated interface layer”: fixed 10<sup>-5</sup>%, w/w SPI, (2) “saturated GSP interface layer”: fixed 10<sup>-2</sup>%, w/w GSP, and (3) “saturated SPI layer”: fixed 10<sup>-1</sup>%, w/w SPI. Dynamic interfacial tension

on the timescale 0-5 h was measured using a wilhelmy plate method. The dynamic interfacial tension and extrapolated equilibrium interfacial tension are discussed from the point of view of a mixed GSP-SPI, with interfacial properties of the mixed GSP-SPI changing with the ratio of GSP/SPI. At both lower concentration of GSP and SPI, Mixed GSP-SPI can form a GSP/SPI complex, which has synergistic effects. At both lower and higher ratio of GSP/SPI, The interfacial tension is similar value of each SPI and GSP at interface. It means that GSP and SPI are competitive adsorption relationships, which may control the adsorption behavior depending on the ratio of GSP/SPI at interface. Also, this might be useful for formation of suspension or emulsion food systems, if properly used, might in fact widen them.

The effect of ginseng saponin on the storage stability of soymilk was investigated at soymilk products. Soymilk was prepared according to general manufacturing process. And ginseng saponin powder (GSP) was added to soymilk at 0, 0.5, 1, 1.5, 2, 3%, w/w. Storage stability of soymilk was analyzed by examining: (1) “shelf-life”: pH value and microbial test, (2) “storage stability”: visual appearance, particle size distribution, and zeta potential, (3) “rheological property” during storage at 4, 25, and 37 °C stored 35 days. In case of shelf-life of soymilk, the pH value remained constant at pH 6.2-6.4. And microbial load was not detected. In case of storage stability of soymilk, the particle precipitation of soymilk decreased with increasing storage temperature, thereby the storage stability of soymilk was improved. It suggested that the soymilk particles, stored at lower temperature, have a larger electrostatic repulsive force according to the zeta potential results. Therefore, the particle aggregation of soymilk was inhibited during storage. In addition, according to the rheological properties of soymilk results, the soymilk showed a higher viscous behavior at lower storage temperature. It was expected that the precipitation rate of soymilk was decreased induced by Stoke’s law. Also, the soymilk showed a higher viscous behavior with increasing the GSP concentration. It was expected to be due to the increase in the total solid of soymilk or the formation of inter-molecular links, which

is binding surfactant with micelle-several peptide. However, the addition of GSP had little effect on the storage stability of soymilk, including the particle precipitation and aggregation. This might be because soymilk is a complex system that contains high protein contents, many kinds of components. To understand the specific effect of ginsenoside on the storage stability of soymilk, additional experiments are necessary, such as making the soymilk suspension or/and emulsion model system that simplified the soymilk.

This study systematically investigated the effects of various conditions (various GSP concentration and storage temperature) on storage stability of soymilk, and the interaction mechanism between ginsenoside and colloidal particles of soymilk at interface model system. It can help to explain the stabilization and storage stability of food dispersion model systems such as foams, emulsions, and its interface systems. Also, it can help to increase the utilization of soymilk processing and consumption as well as the food industrial utilization.

.....  
**Keywords:** Soymilk, Soymilk interface model, Storage stability, Ginsenosides, Soy protein isolate, Protein-saponin complex

**Student Number:** 2016-26563

# Contents

<b>Abstract</b> .....	i
<b>Contents</b> .....	v
<b>List of Tables</b> .....	viii
<b>List of Figures</b> .....	ix

## Chapter 1

### Research background

1. Soybean nutritional values and soybean-based foods .....	1
2. Soymilk .....	2
2.1. Nutritional values and composition.....	2
2.2. Particle aggregation and precipitation during storage.....	4
2.3. Prevention methods of particle precipitation.....	6
2.3.1. Ultrahigh pressure homogenization process .....	6
2.3.2. Adding hydrocolloids.....	6
2.3.3. Adding surfactant as a new approach.....	8
3. Ginseng ( <i>Panax ginseng</i> ) saponin.....	8
3.1. Compositions and health functionality.....	8
3.2. Saponin as a natural surfactant .....	11
4. Overall objective.....	12

## Chapter 2

### Interfacial properties of ginseng saponin and soy protein isolate mixtures at air/water interface

<b>Introduction</b> .....	13
<b>Materials and Methods</b> .....	14
1. Preparation of suspension .....	14
2. Interfacial tension measurement using wilhelmy plate method .....	15
<b>Results and Discussion</b> .....	15
1. Interfacial properties of ginseng saponin .....	16
1.1. Kinetics adsorption of saponin .....	16
1.2. Adsorption isotherm of saponin .....	17
2. Interfacial properties of soy protein isolate .....	18
2.1. Kinetics adsorption of protein .....	18
2.2. Adsorption isotherm of protein .....	18
3. Interfacial properties of mixed ginseng saponin and soy protein isolate .....	19
<b>Conclusions</b> .....	29

### Chapter 3

#### Effects of ginseng (*Panax ginseng*) saponin on storage stability of soymilk

<b>Introduction</b> .....	30
<b>Materials and Methods</b> .....	31
1. Soymilk elaboration .....	31
2. Shelf-life of soymilk .....	32
2.1. Microbiological test .....	32
2.2. pH .....	32
3. Storage stability of soymilk .....	33
3.1. Visual appearance .....	33
3.2. Particle size distribution .....	33

3.3. Zeta potential.....	33
4. Rheological properties of soymilk.....	33
<b>Results and Discussion</b> .....	34
1. Shelf-life of soymilk.....	34
2. Storage stability of soymilk.....	34
2.1. Visual appearance.....	35
2.2. Particle size distribution.....	35
2.3. Zeta potential.....	36
3. Rheological properties of soymilk.....	37
<b>Conclusions</b> .....	50
<b>Overall conclusions</b> .....	51
<b>References</b> .....	52
<b>Abstract in Korean</b> .....	60

# List of Tables

## Chapter 1

**Table 1.** Nutritional value of soymilk ..... 3

## Chapter 2

**Table 1.** Values of the CMC, surface excess concentration,  $\Gamma_{\text{CMC}}$ , the area per molecule at adsorption layer,  $A_{\text{CMC}}$ , as determined from the Gibbs adsorption isotherm. .... 23

## Chapter 3

**Table 1.** Flow behavior index (n) and consistency index (k) values of soymilk samples with 1% GSP or without GSP at various storage temperature 4, 25 and 37 °C. .... 47

**Table 2.** Flow behavior index (n) and consistency index (k) values of soymilk samples for various concentration of GSP (0-3%) at stored 25 °C. .... 49

# List of Figures

## Chapter 1

- Figure 1.** Precipitation process of soymilk particles during storage..... 5
- Figure 2.** Prevention methods of soymilk particle precipitation: (A) Ultra high pressure homogenization, (B) Adding hydrocolloids. .... 7
- Figure 3.** Chemical structure of 7 major ginsenosides. .... 10

## Chapter 2

- Figure 1.** Dynamic interfacial tension of GSP as a function of time at air/water interface during 12 hours measurements. GSP concentrations in following solutions: 0% (dotted black line),  $1 \times 10^{-5}\%$  (solid blue line),  $1 \times 10^{-4}\%$  (solid purple line),  $5 \times 10^{-4}\%$  (solid dark green line),  $1 \times 10^{-3}\%$  (solid cyan line),  $5 \times 10^{-3}\%$  (solid dark yellow line),  $1 \times 10^{-2}\%$  (solid gray line),  $1 \times 10^{-1}\%$  (solid green line),  $5 \times 10^{-1}\%$  (solid dark blue line),  $1 \times 10^0\%$  (solid yellow line),  $2 \times 10^0\%$  (solid red line)..... 22
- Figure 2.** Influence of ginsenoside concentration on the equilibrium interfacial tension measured at air/water interface. The solid dash lines represent interfacial tension for the phosphate buffer solution (0% GSP). The lines below CMC correspond to the best fits of the experimental data by Gibbs adsorption model (dash red lines). .... 23
- Figure 3.** Dynamic interfacial tension of SPI as a function of time at air/water interface during 12 hours measurements. SPI concentrations in following solutions: 0% (dotted black line),  $1 \times 10^{-5}\%$  (solid blue line),  $1 \times 10^{-4}\%$  (solid purple line),  $1 \times 10^{-3}\%$  (solid cyan line), ,  $1 \times 10^{-2}\%$  (solid gray line),

$1 \times 10^{-1}\%$  (solid green line),  $1 \times 10^0\%$  (solid yellow line),  $2 \times 10^0\%$  (solid red line). ..... 24

**Figure 4.** Influence of SPI concentration on the equilibrium interfacial tension measured at air/water interface. The solid dash lines represent interfacial tension for the phosphate buffer solution (0% SPI). ..... 25

**Figure 5.** Dynamic interfacial tension for Mixed GSP-SPI (top): “GSP with SPI” (left panel, solid line, Fig 5-(A)), “GSP without SPI” (right panel, short-dash line, Fig 5-(B)). And interfacial tension isotherm (Fig 5-(C)) for without (open symbols) and with (solid symbols) SPI. GSP concentrations in following mixtures: 0% (dotted black line),  $1 \times 10^{-5}\%$  (blue line),  $1 \times 10^{-4}\%$  (purple line),  $5 \times 10^{-4}\%$  (dark green line),  $1 \times 10^{-3}\%$  (cyan line),  $1 \times 10^{-2}\%$  (gray line),  $5 \times 10^{-1}\%$  (solid dark blue line). The SPI concentration was fixed at  $10^{-5}\%$ , w/w (solid dash line). ..... 26

**Figure 6.** Interfacial tension isotherm for without (open symbols) and with (solid symbols) GSP. The GSP concentration was fixed at  $10^{-2}\%$ , w/w (solid dash line). ..... 27

**Figure 7.** Interfacial tension isotherm for without (open symbols) and with (solid symbols) SPI. The SPI concentration was fixed at  $10^{-1}\%$ , w/w (solid dash line). ..... 28

### Chapter 3

**Figure 1.** Changes in pH of soymilk prepared without or with different concentrations of soymilk during 35 days of storage. (A) stored at 4 °C, (B) stored at 25 °C, (C) stored at 37 °C. ..... 39

**Figure 2.** Soymilk precipitation process during 35 days of storage at 25 °C. .... 40

**Figure 3.** Changes in precipitate layer ratio of soymilk prepared without or with different concentrations of soymilk during 35 days of storage. (A) stored at 4 °C, (B) stored at 25 °C, (C) stored at 37 °C. .... 41

**Figure 4.** Changes in particle size distribution of the top 10% of soymilk prepared without or with different concentrations of soymilk during 35 days of storage at 25 °C. .... 42

**Figure 5.** Changes in particle size distribution of the bottom 20% of soymilk prepared without or with different concentrations of soymilk during 35 days of storage at 25 °C. .... 43

**Figure 6.** Changes in zeta potential of the top 10% of soymilk prepared without or with different concentrations of soymilk during 35 days of storage. (A) stored at 4 °C, (B) stored at 25 °C, (C) stored at 37 °C. .... 44

**Figure 7.** Changes in zeta potential of the bottom 20% of soymilk prepared without or with different concentrations of soymilk during 35 days of storage. (A) stored at 4 °C, (B) stored at 25 °C, (C) stored at 37 °C. .... 45

**Figure 8.** Shear stress (Pa) for soymilk samples with 1% GSP (full symbol) or without GSP (empty symbol) as a function of shear rate (1/s) at various storage temperature 4 °C (blue symbol), 25 °C (black symbol), and 37 °C (red symbol). .... 46

**Figure 9.** Shear stress (Pa) for various concentration of soymilk samples (0-3%) as a function of shear rate (1/s) at stored 25 °C. .... 48

# Chapter 1

## Research background

### 1. Soybean nutritional values and soybean-based foods

The soybean (*Glycine max*) is one of the major crops in the world. It is a major source of plant protein and in the human food and has been utilized in the formulation of the animal's rations (Salimi and Moradi 2012). In general, it contains 35-40% protein, 20-25% carbohydrates, and 15-20% oil depending on the soybean species (Messina 1995). Furthermore, soybeans contain many minor substances, such as isoflavone, saponin, isoflavones and oligosaccharides, are known to be health functionality and to prevent human cancers and other diseases (Messina, Persky et al. 1994). However, it also contains trypsin inhibitor, phytate and lectins as anti-nutritional components that can damage digestion activities in the stomach (Gu, Pan et al. 2010; McCue and Shetty 2004). Consequently, soybean must be subjected to proper treatment such as thermal, acidic, and ultra-sonication etc. before consumption by human beings or animals to reduce these anti-nutritional contents (Pusztai and Grant 1998).

Soy processed foods were developed in oriental countries and accepted in western countries recently (He and Chen 2013). These products may be divided into four classes: soy ingredients, traditional soy foods, second-generation soy foods, and foods where soy is used as a functional ingredient. Soy ingredients are processed soybean protein products (soy protein concentrates and isolate) and soybean oil. Traditional soy foods include soymilk, tofu, tempeh, natto, miso and soy source. Second-generation soy foods include meat extenders, soy burgers, soy sausage, imitation chicken and soy cheese. Foods in which soy is used as functional ingredient include baked goods to which soy flour is added (Alejandro, Granato et al. 2011).

## **2. Soymilk**

### **2.1. Nutritional values and composition**

Soymilk is the extract obtained after soaking, grinding, cooking and filtering the soybean. It has gained much popularity as a healthy plant based beverage and easily digestive products (Cruz, Capellas et al. 2007;Giri and Mangaraj 2012). From ancient times, soymilk has been an important high-quality protein source in the diet of Asian people. In western countries, soymilk has been used as a important plant-based protein sources for vegetarian and replacer of milk for lactose-intolerant peoples, as well as a low-cost source of protein and energy source, mainly in developing countries (Kwok and Niranjana 1995;Liu 1997). In general soymilk contains around 8-10% total solids, mostly including 3.6% protein, 2% fat, 2.9% carbohydrates, and 0.5% ash. Its contents depend on the soybean species used and processing conditions (Liu 1997). Most importantly, soymilk possesses many bioactive compounds such as polyunsaturated and monounsaturated fats, isoflavone, saponin, vitamins, and lecithin. These components provide health benefits, to reduce blood cholesterol level, the risk of cardiovascular disease, and to be strong antioxidants (Peng, Ren et al. 2016).

**Table 1.** Nutritional value of soymilk

<b>Components</b>	<b>Amounts (%)</b>
<b>Water</b>	93
<b>Carbohydrates</b>	3.1
Sugar	1.08
Fiber	1.3
<b>Protein</b>	2.8
$\beta$ -conglycinin (7S)	1.12
glycinin (11S)	0.84
<b>Lipid</b>	
Triglycerides	1.83
Phospholipids	0.02-0.06
<b>Ash</b>	0.5
<b>Minor ingredients</b>	
Saponin	0.022-0.39
Isoflavone	0.01

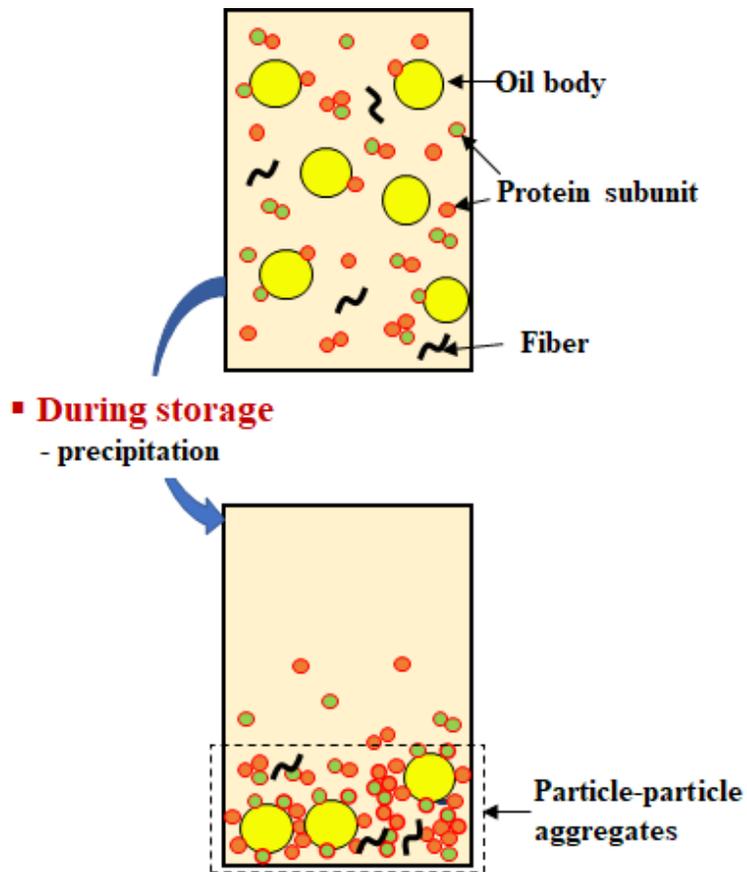
(Adapted from Giri and Mangaraj 2012;Chen and Ono 2014)

## 2.2. Particle aggregation and precipitation during storage

Soy milk is a colloidal system, which is low-fat suspension containing oil bodies, protein particles, dissolved proteins, and ash (Idogawa and Fujii 2015). In case of a colloidal system, for a colloidal suspension to be functional, it must be capable of suspending the dispersed phase during storage of product and/or be easily dispersed should precipitation occur. Brownian motion is usually important to maintain the particles in the dispersed phases. For example, for larger particles the effect of gravity becomes significant if there is a significant difference in density between the dispersed phase and the continuous phase (Idogawa and Fujii 2015; Larsson, Hill et al. 2012). In this case, it can be predicted from the ratio of gravitational to Brownian forces using Equation (1):

$$\alpha^4 \Delta \rho g / k_B T \quad (1)$$

Where  $\alpha$  is the particle radius,  $\Delta \rho$  is the difference density between the dispersed and continuous phases,  $g$  is acceleration due to gravity,  $k_B$  is the Boltzmann constant and  $T$  is the temperature. If this ratio is less than unity, the suspension is likely to indicate a stable system, while a ratio greater than unity indicates that some degree of sedimentation can be expected (Larsson, Hill et al. 2012). When soy milk is stored at an elevated temperature during long-term storage, the soy milk particles continually collide with each other and consequently particles may become aggregated induced by van der Waals attractive force and hydrophobic interaction. This can lead to the formation of aggregated particles of much larger size, and therefore a larger gravitational contribution according to Eq. (1), with consequent sedimentation. This is susceptible to quality loss, which may adversely affect physicochemical properties (particle size, microstructure, and turbidity), textural, sensory properties of soy milk and limit soy milk utilization (He, Yuan et al. 2016; Mukherjee, Chang et al. 2017; Shimoyamada, Tsushima et al. 2008). The soy milk precipitation process is outlined in Figure 1.



**Figure 1.** Precipitation process of soymilk particles during storage.

## **2.3. Prevention methods of particle precipitation**

### **2.3.1. Ultra-high pressure homogenization process**

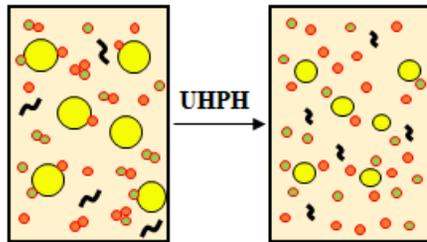
Ultra-high pressure homogenization (UHPH) is widely used in various areas of chemical, biotechnology, pharmaceutical industry, and recently, is being investigated for application in the food industry (Cruz, Capellas et al. 2007). The most common application of UHPH is to make a stable fine emulsions and suspension. In addition, some advantages of UHPH have been reported, for example, inhibition of microbial, inactivation of anti-nutrient factors and enzymes (Datta, Hayes et al. 2005;Diels, Callewaert et al. 2005), increasing emulsion viscosity, and improve sensory parameters (Cruz, Capellas et al. 2007;Poliseli-Scopel, Hernández-Herrero et al. 2012). However, for the application of UHPH to food industry, it needs to solve several problems: (1) degradation of components due to viscous stress, (2) inefficient to inhibit particle aggregation, (3) complex equipment, and (4) higher cost when set the equipment (Georget, Miller et al. 2014;Marszalek, Wozniak et al. 2017).

### **2.3.2. Adding hydrocolloids**

Hydrocolloids are a heterogeneous group of long chain polymers (polysaccharides and proteins) and have a wide array of functional properties in foods. These include gelling, thickening, emulsifying, and stabilization though the basic properties for which hydrocolloids find extensive use are thickening (Saha and Bhattacharya 2010). In soymilk industry, commercial products used carrageenan, and gums to enhance soymilk body and prevent precipitation during shelf storage (Mukherjee, Chang et al. 2017;Liu and Chang 2013). However, some kinds of hydrocolloid reported that may cause lesions or cancer in the gastrointestinal tract (Tobacman 2001). And the hydrocolloid is sensitive to environmental factors, such as pH, thermal treatment, and ion species (Saha and Bhattacharya 2010).

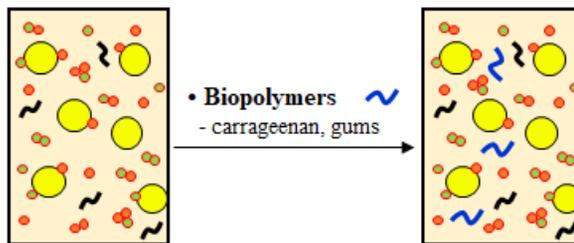
**(A) Ultra-high pressure homogenization (UHPH)**

- decreasing particle size



**(B) Adding hydrocolloids**

- increasing soymilk viscosity
- inhibition of particle aggregation



**Figure 2.** Prevention methods of soymilk particle precipitation: (A) Ultra-high pressure homogenization, (B) Adding hydrocolloids

### **2.3.3. Adding surfactant as a new approach**

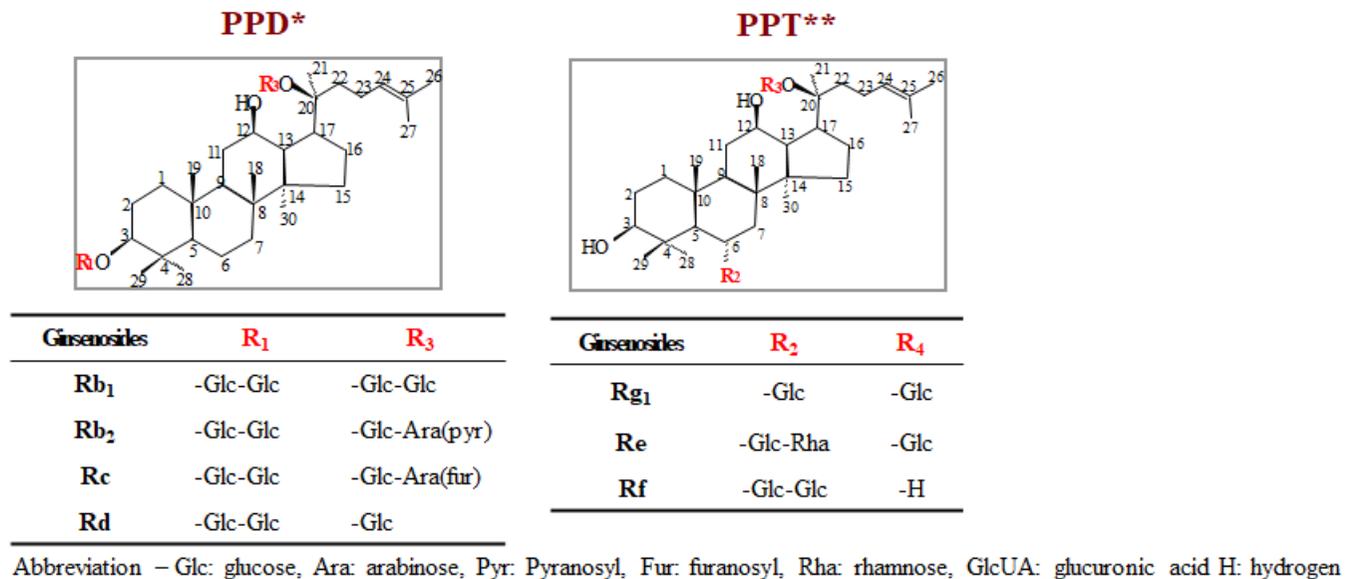
Surfactants (surface active agents) are an amphiphilic compound that contain a polar group and a hydrophobic group, typically alkyl chain. By these structural characteristics, surfactant, which are known as detergents, wetting agents, foaming agents, and emulsifiers, play a major role in controlling the microstructure, structural, and textural stability of products in industry (Otzen 2011;Kotsmar, Pradines et al. 2009). In food industry, surfactant usually used as emulsifiers for the preparation of many food product systems (mayonnaise, salad dressing, deserts etc.). It can decrease interfacial tension at the interface and make a protective coating around droplet. Finally, the food structure improves stability (Kotsmar, Pradines et al. 2009;Yang, Leser et al. 2013). In addition, surfactant can interact with other colloidal particles (oil droplets, proteins, polysaccharides, etc.) that can modify the adsorption layer properties at food interface and colloidal system, and was emphasized in modern technologies, such as food processing, cosmetics, pharmaceutical industry (Kotsmar, Pradines et al. 2009;Ringgenberg 2011). In soymilk system, especially soy protein interacts with each other during storage, and promote the protein-protein association due to hydrophobic interaction. When the surfactant is added to the protein solution, the surfactant and protein interact each other (higher tendency of protein-protein association), can make the surfactant-protein complex (Kotsmar, Pradines et al. 2009). These complex can enhance the solubility of soy protein providing a much more hydrophilic surface than original proteins, therefore protein aggregation and precipitation could be prevented (Malhotra and Coupland 2004).

## **3. Ginseng (*Panax ginseng*) saponin**

### **3.1. Compositions and health functionality**

Ginseng is one of the world's most widely used and valuable medicinal plants. The name ginseng is derived from a Chinese term referring to the “man-like” shape of the root (Qi, Wang

et al. 2011). Currently, the ginseng is a members of the genus *Panax*, which have 14 plants, including 12 species and two infraspecific taxa. In this case, the *panax ginseng* is one of the most commonly used and researched of the ginseng, also called Korean ginseng (Kiefer and Pantuso 2003). It contains approximately 200 substances, such as ginsenosides (called saponin extracted from ginseng), polysaccharides, peptides, and amino acids. Of these, the major active compounds of ginseng are ginsenosides (Liu and Xiaob 1992;Attele, Wu et al. 1999). About 200 kinds of ginsenosides have been reported, including major ginsenosides, such as Rb1, Rb2, Re, Rc, Rg1, and minor ginsenosides Rg3, Rh1, Rh2 (Qi, Wang et al. 2011) By chemical structure, ginsenosides are classified into two groups: protopanaxadiol (PD), and protopanaxatriol (PT). These ginsenosides share a four-ring hydrophobic triterpene aglycone with hydrophilic sugar moieties, but differ in the carbohydrate moieties at C3, C6 and C20. The chemical structure of ginsenosides was expressed at Figure 3. (Kim 2012). In addition, the health functionality of ginsenosides has been widely reported, including prevention of cardiovascular disease, enhancement of vasomotor tone, amelioration of lipid profile, and improvement in blood circulation (Kim 2012;Kim 2018).



**Figure 3.** Chemical structure of 7 major ginsenosides.

\*PPD: protopanaxadiol

\*\*PPT: protopanaxatriol

### 3.2. Saponin as a natural surfactant

Ginseng saponin (ginsenosides) are amphipathic agent, structurally have one or more hydrophilic sugar moieties combined with hydrophobic triterpene derivative (Mesgarzadeh, Akbarzadeh et al. 2017). Due to its amphiphilic molecular structure, ginsenosides have strong surface activity at interface. Also ginsenosides reported high surface elasticity of saponin adsorption layers at the interface (Golemanov, Tcholakova et al. 2013). These properties express that saponin can be a highly effective emulsifier or stabilizers that forms emulsion and foam, also which are stable against a wide range of environment stress (Yang, Leser et al. 2013; Ralla, Herz et al. 2017). Recently, many studies reported that saponin can interact with protein, mixture of saponin and protein may have synergistic behavior, which leads to the formation of complexes in bulk (foam, emulsion), and interface systems, and are of great concern for many industries (Otzen 2011; Golemanov, Tcholakova et al. 2013; Kezwon and Wojciechowski 2014; Reichert, Salminen et al. 2017; Piotrowski, Lewandowska et al. 2012). In food research, various studies examined the interactions of Quillaja bark saponin with food relevant proteins like  $\beta$ -lactoglobulin,  $\beta$ -casein, and hen egg lysozyme. (Kezwon and Wojciechowski 2014; Piotrowski, Lewandowska et al. 2012; Böttcher, Keppler et al. 2017). Mixture of Quillaja bark saponin and  $\beta$ -lactoglobulin (negative and net charge) enables both electrostatic attraction and hydrophobic interaction, however hen egg lysozyme (positive and net charge) usually interact with Quillaja bark saponin by hydrophobic interaction (Kezwon and Wojciechowski 2014). These differences generally depend on the presence of specific binding sites, charge group, and specific structure. And it can modulate the simple electrostatic and hydrophobic interaction available in mixture of protein with saponin. (Dan, Kotsmar et al. 2012). However, there are no reported studies interaction between ginsenosides and food relevant proteins at both interface and bulk systems.

## 4. Overall objective

The aim of this study is to characterize an influence of ginsenosides as a natural surfactant on storage stability of soymilk during stored under various conditions (4, 25, 37 °C). And then, to understand the soymilk stabilization mechanism induced by ginsenosides, studied the investigation of interaction between ginsenosides and soymilk colloidal particles (mainly soy proteins) and its effect of the resulting interaction at soymilk interface model systems. To accomplish this, the study was divided into two categories:

(1) Interfacial properties of ginseng saponin and soy protein isolate mixtures at air/water and oil/water interface – **Chapter 2**

(2) Effects of ginseng (*Panax ginseng*) saponin on storage stability of soymilk – **Chapter 3**

It can obtain a better understanding of the storage stability of soymilk and interaction between ginsenosides and colloidal particles at soymilk model system, in addition, it can help to explain the stabilization and storage stability of food dispersion model systems such as foams, emulsions, and its interface systems. Also, it can help to increase the utilization of soymilk processing and consumption as well as the food industrial utilization.

## **Chapter 2**

# **Interfacial properties of ginseng saponin and soy protein isolate mixtures at air/water interface**

## **Introduction**

Mixed protein-surfactant system widely used in modern technologies, such as food processing, cosmetics, and pharmaceutical industry. This system can modify the adsorption layer properties at fluid/fluid interface, which contribute the storage influence on the formation of foam and emulsion, also affect the strong influence on the stability of foam and emulsion system. (Kezwon and Wojciechowski 2014;Piotrowski, Lewandowska et al. 2012;Böttcher, Keppler et al. 2017). Recently, the mixed protein-surfactant reported that can interact each other and have synergistic or antagonistic behavior, which affects to the formation of complexes in food bulk (foam, emulsion), and interface systems (Otzen 2011;Golemanov, Tcholakova et al. 2013;Kezwon and Wojciechowski 2014;Reichert, Salminen et al. 2017;Piotrowski, Lewandowska et al. 2012). For example, Mixture of Quillaja bark saponin and  $\beta$ -lactoglobulin (negative net charge) enables both electrostatic attraction and hydrophobic interaction, however hen egg lysozyme (positive net charge) usually interact with Quillaja bark saponin by hydrophobic interaction. This interaction can modulate the modulate the simple electrostatic and hydrophobic interaction available in mixture of protein with saponin, which can modify the formation and stability of food system (Kezwon and Wojciechowski 2014;Dan, Kotsmar et al. 2012).

Ginseng saponin (ginsenosides) are extracted from ginseng, is a bioactive compounds, having many health functionality that include significant effects on cardiovascular disease, enhancement of vasomotor tone, improvement in blood circulation, and amelioration of lipid profile (Kim

2018). For this reason, ginsenosides is widely used and study not only natural surfactants but also bioactive compounds in medicine and food industry. Also, ginsenosides are amphiphilic agent, consisting the glycone (one or more hydrophilic glycoside moieties) combined with aglycone (hydrophobic triterpene derivative) (Mesgarzadeh, Akbarzadeh et al. 2017). Due to its amphiphilic molecular structure, ginsenosides can play surfactant, which have strong surface activity at interface, and can be a highly effective emulsifier or stabilizers that forms emulsion and foam. Because of this, ginsenosides is upcoming components as a natural surfactant in food industry and research. (Yang, Leser et al. 2013;Golemanov, Tcholakova et al. 2013;Ralla, Herz et al. 2017).

Soy protein is an important food ingredient as a low cost bulking gent, high biological value, and good emulsifiers in food industry (Xu and Liu 2016). Soy protein isolate (SPI) are the commercial soy protein product. SPI contains at least 90% protein, is amphipathic agents (hydrophobic amino acid + hydrophilic amino acid). Owing to these structural properties, SPI can ben enhance the physicochemical properties of food structure. For example, SPI can attach the oil/water or air/water interface, and forming highly viscoelastic film. As a result, it can play emulsifiers that have foaming and emulsifying abilities (Malhotra and Coupland 2004;Zhu, Liu et al. 2015;Wan, Wang et al. 2014).

In this study, we investigate the effect of mixed soy protein isolate with ginsenosides on interfacial properties at air/water interface.

## **Materials and Methods**

### **1. Preparation of suspensions**

The ginseng (*Panax ginseng*) saponin powder (GSP,  $\geq 80\%$ , w/w ginsenosides) was purchased from Amax NutraSource, Inc. (OR, Eugene, USA). Soy protein isolate (SPI) ,

which contains 90% proteins, 7% moisture, 1% fat, 8% ashes, and 0.5% crude fiber, was purchased from ES food (Gyeonggi-do, Gunpo, Korea). All chemicals were purchased from Daejung Chemical CO. (Gyeonggi-do, Siheung, Korea).

The GSP 2.0% was prepared as a stock suspension by dispersing GSP powder in phosphate buffer solution (pH 7) prepared by mixing appropriate amounts of 0.01M  $\text{NaH}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4$  without purification, and stirred at least for 2 hours at room temperature. Deionized water (18.3  $\Omega$ ) was used to prepare the all solutions. The various concentration (0.00001 to 2.0%, w/w) of GSP suspension was prepared by diluted the stock suspension. In case of SPI suspension, SPI 4.0% was prepared as a stock suspension by dispersing SPI powder in phosphate buffer solution (pH 7) and stirred at least for 2 hours, after then kept overnight (12 h) at 2 °C for completed hydration of soy protein isolate. The various concentration (0.00001 to 2.0%, w/w) of SPI suspension was prepared by diluted the stock suspension. The mixture of SPI and GSP suspension was prepared by mixing each SPI and GSP suspension, and stirred at least for 30 min. To investigate the interfacial properties of mixed SPI and GSP depending on the mass ratio of SPI and GSP at interface, the mass ratio of mixed SPI and GSP was divided into three profile: (1) 0.00001% SPI (constant) with various GSP concentration, (2) 0.1% SPI (constant) with various GSP concentration, (3) 0.01% GSP (constant) with various SPI concentration. All samples were stirred at least for 30 min at room temperature before measurement.

## **2. Interfacial tension measurement using wilhelmy plate method**

The wilhelmy plate method was used to determine the dynamic interfacial tension of the suspensions. The measurements were performed on a K100 tensiometer (Kruss GmbH, Hamburg, Germany) by using a platinum plate. Before each measurement, the plate was cleaned by heating on a flame, followed by abundant rinsing with deionized water. After then, the suspension was contained in a SV20 glass vessel and the plate was then positioned at the

suspension. Dynamic surface tension was monitored for at least 16 h at 25 °C. The equilibrium interfacial tension ( $\gamma_{eq}$ ) values were calculated using a long-time extrapolation of dynamic interfacial tension (Kezwon and Wojciechowski 2014).

$$\gamma_{eq} = \gamma - \frac{RT\Gamma^2}{c_o} \sqrt{\frac{\pi}{4Dt}} \quad (2)$$

Where  $\gamma$  is the interfacial tension, R is the gas constant, T is the temperature, D is the diffusion coefficient, t is the measurement time, and  $c_o$  is bulk concentration. When the subsurface concentration approaches that in the bulk solution, Equation (2). is valid for  $t \rightarrow \infty$ . Prior to interfacial properties of mixed SPI and GSP, firstly we investigate the interfacial properties of GSP and SPI at air/water interface.

## Results and Discussion

### 1. Interfacial properties of ginseng saponin

#### 1.1 Kinetics adsorption of saponin

The dynamic interfacial tension decayed for the same set of GSP concentrations in the aqueous phase at air/water interface (Figure 1). In all cases, two groups of the dynamic interfacial tension curves could be distinguished around the concentration of 0.025% GSP. Below 0.025% GSP concentration, the rate of interfacial tension decay started to increase significantly until 2 h, after then maintains a constant value relatively. In contrast, above 0.025% GSP concentration, the interfacial tension of GSP increased after 0.5 h, then it was maintained during measurement. This phenomenon might be due that ginsenosides at specific concentration can forms strong viscoelastic interfacial films induced by hydrogen bonds between neighboring sugar residues in saponins (Böttcher, Keppler et al. 2017).

## 1.2. Adsorption isotherm of saponin

For the most part, The dynamic interfacial tension data equilibrated after measuring 5 h, consequently the data from this point was used for estimation of equilibrium values by extrapolation of the dynamic data to  $t \rightarrow \infty (t^{-1/2} \rightarrow 0)$ , using the asymptotic solution of the Ward-Tordai equation (Wojciechowski 2013; Mitra and Dungan 1997). Figure 2 shows the interfacial tension versus GSP concentration profiles at air/water interface. For interface system, The interfacial tension decreased with increasing GSP concentration and was the lowest at 0.01% GSP ( $34.89 \pm 0.27$  mN/m). However, the interfacial tension values slightly increased until 0.1% GSP ( $40.95 \pm 1.98$  mN/m) and maintained the values above 0.1% GSP. It might be due that the ginsenosides can make the formation of film induced by hydrophobic interaction or hydrogen bonds between adjacent GSP molecules at interface. (Böttcher, Keppler et al. 2017).

In order to obtain more information of interfacial properties, the critical micelle concentration (CMC), surface excess concentration ( $\Gamma$ ), and area per molecule ( $\text{\AA}$ ) were calculated and presented in Table 1. Although there was no zero slope from interface profile, the apparent CMC was determined using previous method (Mitra and Dungan 1997). According to Figure 2, the apparent CMC of GSP was determined at 0.011% (w/w). These values are similar with reported CMC values of 0.02% (w/w) for ginsenoside (80% saponin contents) solution (Pagureva, Tcholakova et al. 2016). The surface excess concentration ( $\Gamma$ ) can be calculated using the Gibbs adsorption isotherm equation, we fitted experimental data below CMC.

$$\Gamma = -\left(\frac{1}{RT}\right) \frac{d\gamma}{d \ln c} \quad (3)$$

Where R is the gas constant, T is the absolute temperature, C is the surfactant concentration in the bulk solution, and  $\gamma$  is the surface tension (Yang, Leser et al. 2013; Pagureva, Tcholakova et al. 2016). From the slope of  $\gamma/\ln c$ , we determine the surface excess concentration around the CMC. The surface excess concentration of GSP was  $2.15 \mu\text{mol}/\text{m}^2$ . These values was similar to  $3.20 \mu\text{mol}/\text{m}^2$  from previous studies for ginsenosides (80% saponin contents) solution.

(Pagureva, Tcholakova et al. 2016). The area per molecule ( $A$ ) was calculated from the surface excess concentration values.

$$A = \frac{1}{\Gamma N_A} \quad (4)$$

where  $N_A$  is the avogadro's number. The area per molecules of GSP was  $77.2 \text{ \AA}^2$  per molecule at air/water. The value GSP is in good agreement with the value of  $51.9 \text{ \AA}^2$  per molecule from reported previous studies for ginsenosides (80% saponin contents) solution (Pagureva, Tcholakova et al. 2016). As a result, the interfacial properties of GSP is similar to the other types of saponin reported previous studies. In addition, Ginsenosides have the interfacial activity at air/water interface, which contributes more to foam formation than the emulsion system.

## **2. Interfacial properties of soy protein isolate**

### **2.1 Kinetics adsorption of protein**

The dynamic interfacial tension values decayed for the same set of SPI concentrations in the aqueous phase at air/water interface (Figure 3). In all case the dynamic interfacial tension values decreased sharply within 1 hour, then interfacial tension values of SPI decreased during 12 h measurement. This result might be expected that the protein may change the adsorption structure induced by layer rearrangements, such as formation of multilayer or interfacial gelation (Kezwon and Wojciechowski 2014;Yano 2012;Beverung, Radke et al. 1999).

### **2.2. Adsorption isotherm of protein**

In fact, the dynamic interfacial tension of SPI did not reach the equilibrium and slightly decreased during 12 h measurement according to Figure 3. In spite of this result, we attempted to discuss the equilibrium interfacial tension from the dynamic interfacial tension values on the timescale of around 5 hours, as some importance for possible practical applications of protein. (Kezwon and Wojciechowski 2014). Figure 4 shows the interfacial tension versus SPI

concentration profiles at air/water interface. Likewise, the Figure 2 result, the interfacial tension values decreased with increasing SPI concentration at air/water and interface. However, there was no SPI concentration range in which the interfacial tension values was relatively constant. Also, the SPI could not obtain the interfacial parameters (CMC,  $\Gamma$ , and  $\text{\AA}$ ). It might be due that the SPI may forms multi-layer within the setting SPI concentration range (0-2%, w/w) or layer rearrangement during measurement (Yano 2012). Nevertheless, the SPI has lower interfacial tension values at air/water interface ( $44.37 \pm 0.83$  mN/m at 1% SPI) than negative control ( $66.91 \pm 2.10$  mN/m). It means that the SPI was highly effective at reducing the interfacial tension at interface system, which contributes to the formation of colloidal systems.

### **3. Interfacial properties of mixed ginseng saponin and soy protein isolate**

For the purpose of studying the interfacial properties of mixed GSP-SPI and interaction between GSP and SPI, mixed GSP-SPI system was divided into three concentration profile: (1) unsaturated layer (10<sup>-5</sup>% w/w SPI with various concentration of GSP) to investigate the synergistic or antagonistic effect of mixed SPI-GSP. The interfacial tension values at 10<sup>-5</sup>% SPI did not significantly decrease during 5 hours measurements. (2) saturated GSP layer (10<sup>-2</sup>% (w/w) GSP with various concentration of SPI, (3) saturated SPI layer (10<sup>-1</sup>% (w/w) SPI with various concentration of GSP). In case of (2) and (3), we investigated the change of interfacial properties and relationship between GSP and SPI.

#### **(1) Unsaturated layer: 10<sup>-5</sup>%, w/w SPI with various concentration of GSP**

The comparison of dynamic interfacial tension and interfacial tension isotherm of mixed GSP/SPI were shown in Figure 5. The dynamic interfacial tension results (Figure 5-(A) and 5-(B)), All sample remained the constant interfacial tension values until 1 hour measurements. The

presence of GSP reduced the time required to reach apparent equilibrium values of dynamic interfacial tension to about 30 min at lower GSP concentration ( $\geq 10^{-4}\%$  GSP). Above the  $10^{-3}\%$  GSP, the rate of reaching equilibrium was significantly constant regardless of GSP.

The equilibrium interfacial tension of mixed GSP-SPI determined within the 5 hours measurements, similarly to the previous GSP and SPI results. At Figure 5-(C), the presence of GSP had a synergistic effect, which reduced the equilibrium interfacial tension of suspension, below  $5 \times 10^{-3}\%$  GSP. The most likely reason for this synergistic effect and adsorption behavior might be the formation of GSP-SPI complex. The complex might be generated by the interaction between the hydrophobic amino acid of SPI and hydrophobic aglycone of GSP (Malhotra and Coupland 2004). Above the  $5 \times 10^{-3}\%$  GSP, interfacial tension values with GSP showed similar interfacial value of without GSP. It means that the presence of GSP has no further effect on interfacial tension isotherm for the prior to the GSP than SPI. This is because the GSP molecules as smaller and low molar mass surfactant may penetrate from bulk to interface, while the SPI molecule would be desorption from interface to bulk (competitive adsorption).

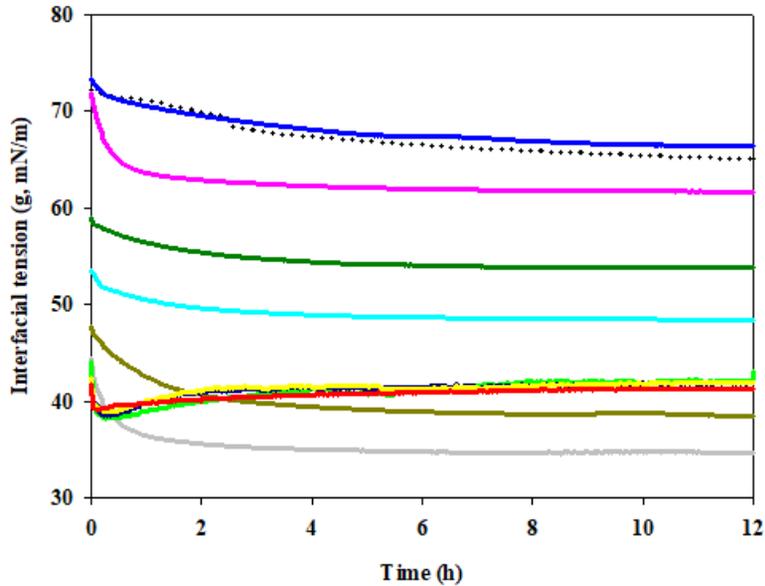
## **(2) Saturated GSP layer: $10^{-2}\%$ , w/w GSP with various concentration of SPI**

The comparison of interfacial tension isotherm of mixed GSP/SPI was shown in Figure 6. Likewise Figure 5 results, The equilibrium interfacial tension was attained until 5 hours measurements (dynamic interfacial tension results were not shown). The interfacial behavior of mixed GSP/SPI was divided into two concentration ranges: (a) below  $10^{-1}\%$  SPI, (b) above  $10^{-1}\%$  SPI concentration. At the range of (a), SPI had little effect on the adsorption behavior at interface. However, at the range of (b), the interfacial tension increased with increasing SPI. It suggested that the SPI molecule are bigger than GSP molecule, it is hard to penetrate into the GSP saturated interface at low concentration (below  $10^{-1}\%$  SPI). However, at higher

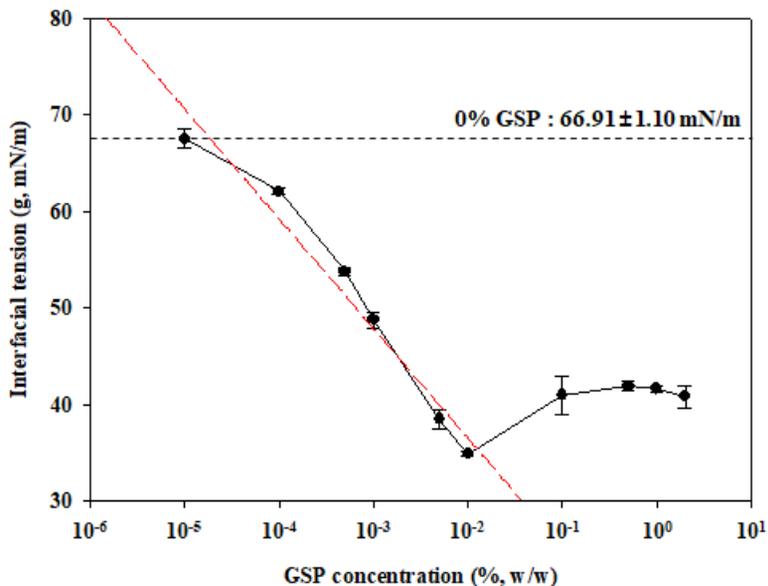
concentration (above  $10^{-1}\%$  SPI) the SPI might do the displacement of GSP as a competitive adsorption relationship.(Fainerman, Zholob et al. 2004).

**(3) Saturated SPI layer:  $10^{-1}\%$  w/w SPI constant with various concentration of GSP.**

The comparison of interfacial tension isotherm of mixed saturated SPI and GSP was shown in Figure 7. (dynamic interfacial tension result was not shown). The interfacial tension behavior of mixed GSP/SPI was also divided into two concentration range: (a) below  $10^{-2}\%$  GSP, (b) above  $10^{-2}\%$  GSP concentration. In contrast the Figure 5-(C) results, mixed saturated SPI/GSP had little effects at (a) range of GSP. This is because that the GSP might be difficult to penetrate the SPI at SPI saturated layer. At the range of (b), the interfacial tension isotherm of mixed SPI/GSP has similar interfacial properties as original GSP. That suggest that GSP adsorbs to the interface, which SPI may desorb from interface to bulk.



**Figure 1.** Dynamic interfacial tension of GSP as a function of time at air/water interface during 12 hours measurements. GSP concentrations in following solutions: 0% (dotted black line),  $1 \times 10^{-5}\%$  (solid blue line),  $1 \times 10^{-4}\%$  (solid purple line),  $5 \times 10^{-4}\%$  (solid dark green line),  $1 \times 10^{-3}\%$  (solid cyan line),  $5 \times 10^{-3}\%$  (solid dark yellow line),  $1 \times 10^{-2}\%$  (solid gray line),  $1 \times 10^{-1}\%$  (solid green line),  $5 \times 10^{-1}\%$  (solid dark blue line),  $1 \times 10^0\%$  (solid yellow line),  $2 \times 10^0\%$  (solid red line).

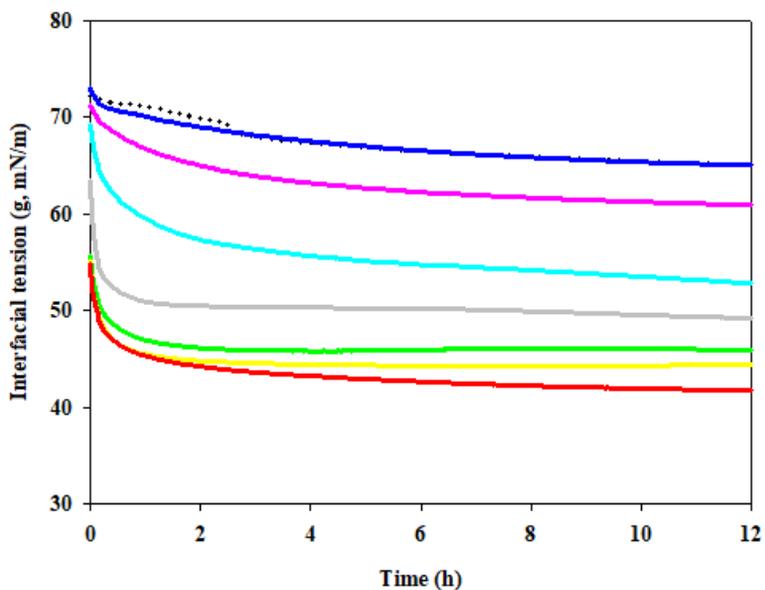


**Figure 2.** Influence of ginsenoside concentration on the equilibrium interfacial tension measured at air/water interface. The solid dash lines represent interfacial tension for the phosphate buffer solution (0% GSP). The lines below CMC correspond to the best fits of the experimental data by Gibbs adsorption model (dash red lines).

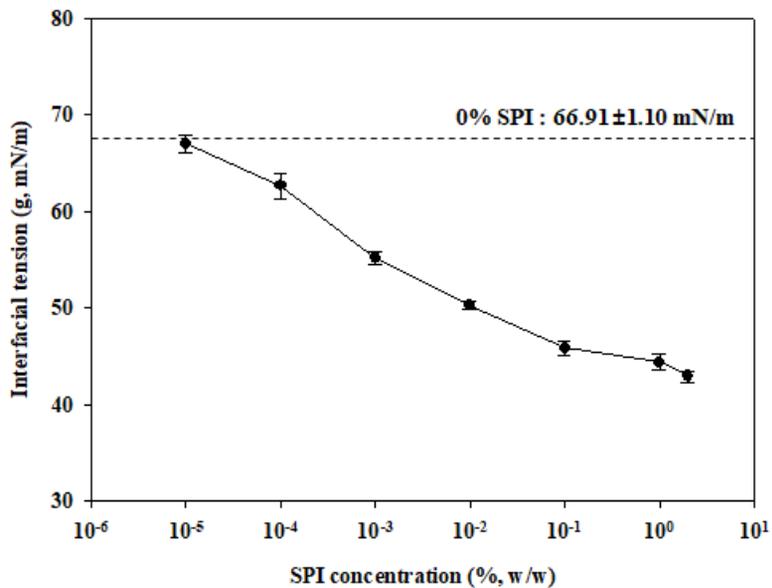
**Table 1.** Values of the CMC, surface excess concentration,  $\Gamma_{\text{CMC}}$ , the area per molecule at adsorption layer,  $A_{\text{CMC}}$ , as determined from the Gibbs adsorption isotherm.

Parameter	GSP solution	Reference
CMC* (% w/w)	0.011	0.02
$\Gamma_{\text{CMC}}$ ( $\mu\text{mol}/\text{m}^2$ )	2.15	3.20
$A_{\text{CMC}}$ ( $\text{nm}^2$ )	77.2	51.9

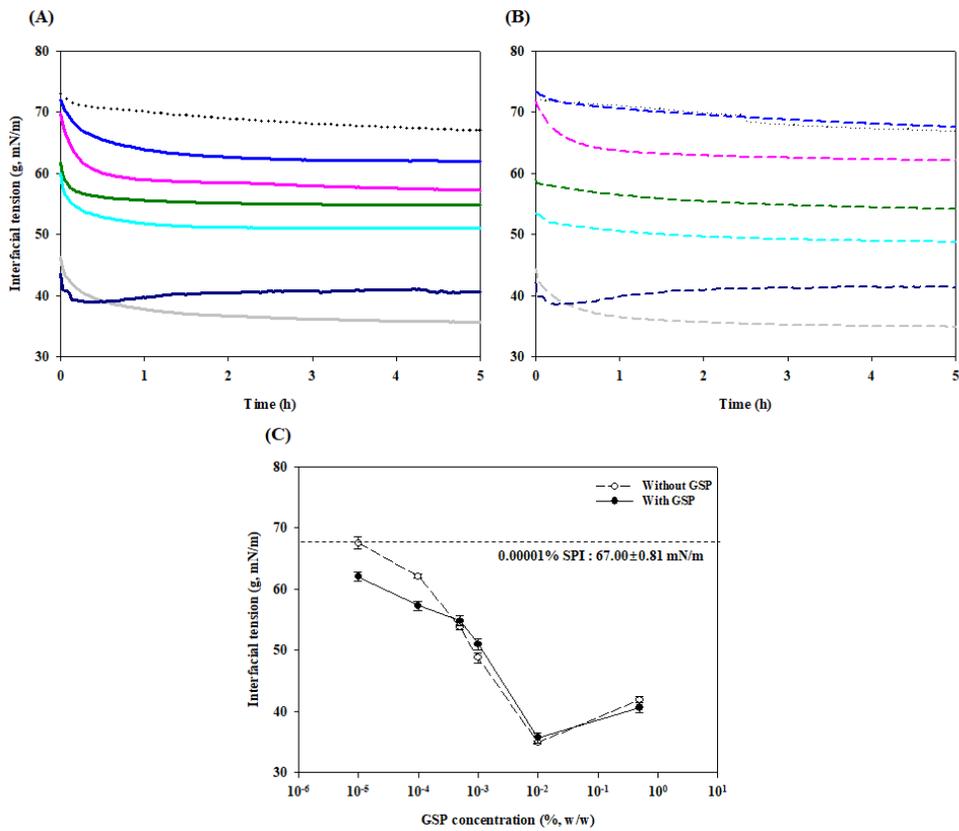
\* CMC : critical micelle concentration



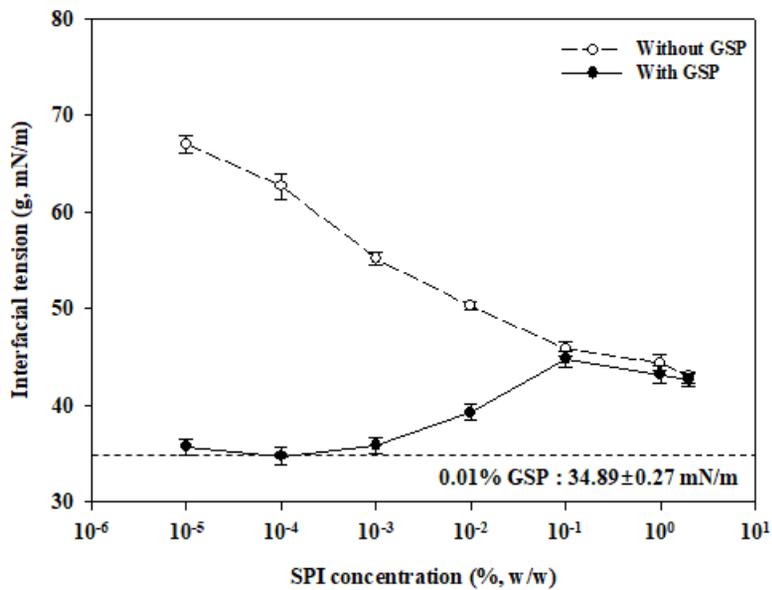
**Figure 3.** Dynamic interfacial tension of SPI as a function of time at air/water interface during 12 hours measurements. SPI concentrations in following solutions: 0% (dotted black line),  $1 \times 10^{-5}\%$  (solid blue line),  $1 \times 10^{-4}\%$  (solid purple line),  $1 \times 10^{-3}\%$  (solid cyan line),  $1 \times 10^{-2}\%$  (solid gray line),  $1 \times 10^{-1}\%$  (solid green line),  $1 \times 10^0\%$  (solid yellow line),  $2 \times 10^0\%$  (solid red line).



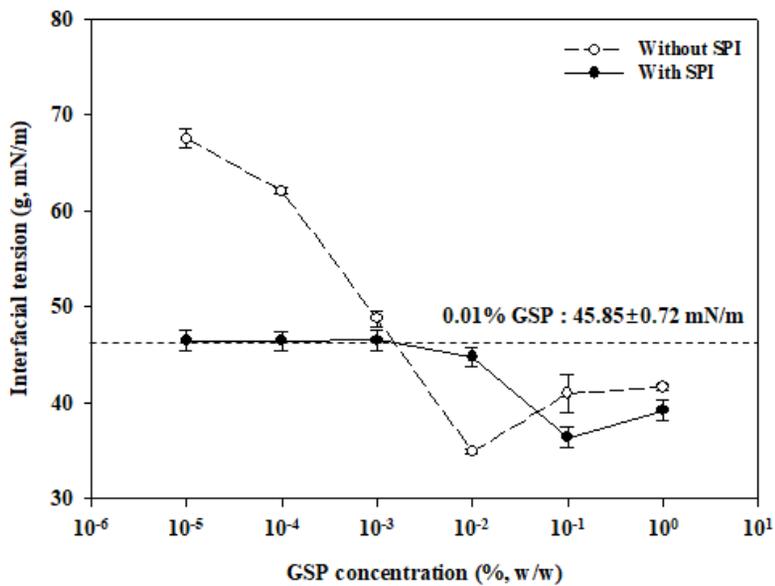
**Figure 4.** Influence of SPI concentration on the equilibrium interfacial tension measured at air/water interface. The solid dash lines represent interfacial tension for the phosphate buffer solution (0% SPI).



**Figure 5.** Dynamic interfacial tension for Mixed GSP-SPI (top): “GSP with SPI” (left panel, solid line, Fig 5-(A)), “GSP without SPI” (right panel, short-dash line, Fig 5-(B)). And interfacial tension isotherm (Fig 5-(C)) for without (open symbols) and with (solid symbols) SPI. GSP concentrations in following mixtures: 0% (dotted black line),  $1 \times 10^{-5}$  % (blue line),  $1 \times 10^{-4}$ % (purple line),  $5 \times 10^{-4}$ % (dark green line),  $1 \times 10^{-3}$ % (cyan line,  $1 \times 10^{-2}$ % (gray line),  $5 \times 10^{-1}$  % (solid dark blue line). The SPI concentration was fixed at  $10^{-5}$ %, w/w (solid dash line).



**Figure 6.** Interfacial tension isotherm for without (open symbols) and with (solid symbols) GSP. The GSP concentration was fixed at  $10^{-2}\%$ , w/w (solid dash line).



**Figure 7.** Interfacial tension isotherm for without (open symbols) and with (solid symbols) SPI. The SPI concentration was fixed at 10<sup>-1</sup>%, w/w (solid dash line).

## Conclusions

Ginsenosides as a natural surfactant can interact with soy protein isolate, consequently the mixed GSP-SPI make the complex, which have synergistic effects within lower concentration of both GSP and SPI. As the concentration of GSP and SPI was increased, the mixed GSP-SPI had little effect, and showed similar interfacial behavior to the more dense species. It means that the GSP and SPI are competitive adsorption relationship, but the GSP as smaller molecules is better adsorption into the interface. Mixed GSP and SPI can control the interfacial properties depending on the ratio of GSP/SPI at air/water interface. This may be useful for formation of suspension or emulsion food systems, if properly used, might in fact widen them.

## **Chapter 3**

# **Effects of ginseng (*Panax ginseng*) saponin on storage stability of soymilk**

## **Introduction**

Soy milk has gained much popularity as a healthy plant based beverage (Cruz, Capellas et al. 2007; Giri and Mangaraj 2012). It possesses rich bioactive compounds such as polyunsaturated and monounsaturated fats, isoflavone, saponin, vitamins, and lecithin. These components provide health benefits, to reduce blood cholesterol level, the risk of cardiovascular disease, and to be strong antioxidants (Peng, Ren et al. 2016). However, soy milk often shows some precipitation during long-term storage and this is susceptible to quality loss of products. Quality loss of soy milk may adversely affect physicochemical properties (particle size, microstructure) of soy milk (Mukherjee, Chang et al. 2017). Particle aggregation or coagulation of protein, a result of complex interactions occurring between various colloidal particles, can be considered to be major contributing factors to the change of physicochemical properties, and overall quality of soy milk during storage (Ringgenberg 2011; Poliselí-Scopel, Hernández-Herrero et al. 2013).

To improve storage stability of soy milk, previous studies were mostly focus on using the ultra-high pressure homogenization equipment, and the addition of hydrocolloids, such as carrageenan and gums (Giri and Mangaraj 2012; Sidhu and Singh 2016; Wang, Xing et al. 2017). Ultra-high pressure homogenization in soy milk can enhance shelf-life, colloidal stability, and reduce the average particle size and settling. However, it can cause protein denaturation and degradation of several compounds (Ringgenberg 2011; Giri and Mangaraj 2012; Poliselí-Scopel, Hernández-Herrero et al. 2013). Adding hydrocolloids in soy milk showed that it interacted with

soymilk particles. It can inhibit the particle aggregation and decreased particle precipitation (Mukherjee, Chang et al. 2017; Babbar, Aggarwal et al. 2015). However, some kinds of hydrocolloid reported that may cause lesions or cancer in the gastrointestinal tract (Tobacman 2001). And the hydrocolloid is sensitive to environmental factors, such as pH, thermal treatment, and ion species (Saha and Bhattacharya 2010). Many researches show that surfactant also can improve stability of emulsion to interact with particles and form the complexes in beverages (Chaudhari, Pan et al. 2015). These complex can enhance the solubility of protein providing a much more hydrophilic surface than original proteins, therefore protein aggregation and precipitation could be prevented (Kotsmar, Pradines et al. 2009; Malhotra and Coupland 2004; Kralova and Sjöblom 2009). Recently, researchers have reported that saponin also can interact with protein, and it make the complex that can improve emulsion stability and change interfacial properties (Kezwon and Wojciechowski 2014; Böttcher, Keppler et al. 2017).

Ginsenosides (called saponin extracted from ginseng) are the main active constituents in ginseng (Ralla, Herz et al. 2017). These active constituents are well known for their health benefits, anti-cancer, and anti-ageing. Besides, ginsenosides exhibit surface-active properties due to amphipathic structure, including hydrophilic sugar moieties combined with hydrophobic triterpene derivative (Mesgarzadeh, Akbarzadeh et al. 2017). In addition, it also can interact with soy protein and make the saponin-protein complex (the complex was confirmed in Chapter 2).

In this study, we characterized an influence of ginsenosides on stability of soymilk during stored under various conditions (4, 25, 37 °C).

## **Materials and Methods**

### **1. Soymilk elaboration**

Whole soymilk was obtained from the Daehak soymilk Inc. (Pyeongchang, South Korea).

Ginseng saponin powder (GSP, Purity  $\geq$  80%) was purchased from Amax NutraSource Inc. (Eugene, OR, USA). All chemicals used in this work were purchased from Daejung Chem (Siheung, South Korea). Distilled water for high-performance liquid chromatography (HPLC) was purchased from Fisher Scientific (Hampton, NH, USA).

Soy milk was prepared according to the method developed by Giri and Mangaraj 2012 and Ringgenberg, Corredig et al. 2012 with some modifications. The whole soy milk was heated (100 °C for 15 min) for inactivation of anti-nutrient factors such as protease inhibitors, phytates, and trypsin inhibitors. After then the whole soy milk filtrated by 5-layer gauze to remove coarse material, which is mainly composed of insoluble fiber material. Sodium azide (0.04% w/w) was added to prevent microbial growth. The filtrated soy milk was mixing with or without ginseng saponin powder (GSP) at 0, 0.5, 1, 1.5, 2, 3% (w/w) and stirring at room temperature during 2 hours. The filtrated soy milk with GSP was firstly homogenized using high speed homogenizer (Ultra-Turrax T25, IKA, Staufen im Breisgau, Germany) with 10,000 rpm for 3min at 25 °C. Then it was homogenized using high pressure homogenizer (MN250A, Micronox, Seongnam, South Korea) at 34.47 MPa and 25 °C for four time passes. Soy milk was transferred into 30 mL glass vials and placed in a water bath set at 121 °C for 5 min. After heating, the vials were then immediately cooled to room temperature in an ice-water bath. All sample was stored at 4 (refrigerator condition), 25 (room temperature), and 37 °C (tropical and heating cabinet conditions). And all samples were analysed in triplicate at day 0, 1, 7, 14, 21, 28, and 35 days of storage.

## **2. Shelf-life of soy milk**

### **2.1. Microbiological test**

For the sterility test of soy milk, total counts bacteria were determined in plate count agar (PCA, Difco) incubated at 37 °C for 48 h. Values were measured in triplicate.

## **2.2. pH**

pH meter (S220-K, Mettler Toledo International Inc., Shanghai, China) calibrated to pH of 4.01, 7.00, and 9.21 was used to measure the pH of soymilk. Values were measured in triplicate.

## **3. Storage stability of soymilk**

### **3.1. Visual appearance**

Visual appearance of soymilk was observed by digital camera (EOS 77D, Canon, Tokyo, Japan).

### **3.2 Particle size distribution**

The Particle size distribution (% volume distribution) was analysed by laser light-scattering particle size analyser (Cilas 1190, Cilas, Orléans, France), supported by the Fraunhofer theory, which provides accurate results in the 0.04 and 2000  $\mu\text{m}$  regions. Soymilk sample was diluted until obscuration from 10 to 15%. All sample was analysed in three times and averages were recorded.

### **3.3 Zeta potential**

The zeta potential of the soymilk were determined with a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, U.K.) using Smoluchowski model. Sample were injected into disposable plastic cell and measured. The equilibrium time was 10 s.

## **4. Rheological properties of soymilk**

Shear rate-shear stress measurements were carried out using a rotational shear rheometer (DHR-3, TA instruments, New castle, DE, USA) Flow sweeps were conducted using recessed concentric cylinders (standard cup diameter of 30.39 mm and a bob diameter of 27.99 mm). A

shear rate ranging from 0.1 to 100 1/s was applied to investigate the shear stress of each soymilk sample. To determine the fluid type of soymilk, the power law model (Equation (5)) was used:

$$\tau = K(\dot{\gamma})^n \quad (5)$$

where  $\tau$ : shear stress (Pa),  $K$ : consistency coefficient ( $\text{Pa}\cdot\text{s}^n$ ),  $\dot{\gamma}$ : shear rate (1/s), and  $n$ : flow behaviour index. If  $n < 1$  the fluid shows pseudoplastic behaviour, if  $n = 1$  the fluid shows newtonian behavior, and if  $n > 1$  the fluid shows dilatant behaviour.

## **Results and Discussion**

### **1. Shelf-life of soymilk**

Microbiological growth and pH are an important factor that contributes to the physicochemical changes during storage and determines the shelf-life of products. Figure 1 shows pH measurement of soymilk without GSP and with various concentration of GSP during 35 days of storage at 4, 25, 37 °C. The natural pH of soymilk is approximately 6.40, and pH values of all soymilk slightly decreased with increasing the storage temperature. In addition, all sample remained constant nutrient pH values around pH 6.19 to 6.4 during 35 days of storage. However, there was no significantly difference with different GSP concentration. The microbial results, no microorganisms were shown in all samples during storage (data not shown).

### **2. Storage stability of soymilk**

Soymilk is an emulsion and colloidal suspension, which may experiment destabilization phenomena such as creaming and solid precipitation during storage. (Ringgenberg 2011). These problems adversely affect the soymilk quality depending on particle compositions, contents, and particle size distribution. To understand the possible changes in storage stability during storage, particle precipitation and aggregation were evaluated.

## **2.1 Visual appearance**

Figure 2 shows soymilk precipitation process during 35 days of storage at 25 °C. All soymilk sample separated into two layers (precipitate layer and soluble layer) with well-defined boundary within 1 days. After then, the height of precipitate layer was slightly increased with increasing the storage time. For investigation of effect on the degree of precipitation of particles specifically, the height of the precipitate layer in soymilk was measured during the storage. Figure 3 shows the precipitate layer ratio of soymilk during 35 days of storage. According to Figure 3, the precipitate layer ratio of soymilk increased to around 8.79-12.22% rapidly during 1 day of storage., and then gradually increased to around 20-23.08% during 35 days of storage. The precipitate layer ratio of soymilk increased with increasing storage temperature, however there was no significantly difference with different GSP concentration. The precipitated particles are mostly big particles or aggregates, such as insoluble fiber, proteins, and ash (Sidhu and Singh 2016). Especially, soy protein interacts with each other during storage, and can make protein aggregates due to mainly hydrophobic interaction (Cruz, Capellas et al. 2007;Poliseli-Scopel, Hernández-Herrero et al. 2012). As the temperature increased, the hydrophobic interaction of protein might be accelerated by increasing in the movement of water molecules (Chou and Morr 1976). To investigate the specific precipitation phenomenon of soymilk, based on the visual appearance results, the other parameters were measured by divided into two parts: top 10% volume and bottom 20% volume layer in soymilk. The layer was collected and diluted with 0.01 M phosphate buffer solution (pH 6.4) as ratio of 1:10.

## **2.2 Particle size distribution**

Analyzing particle size distribution is useful to determine the individual particle size, also to detect particle aggregates, which are fat globules, protein-fat globule, and some protein aggregates (Cruz, Capellas et al. 2007;Poliseli-Scopel, Hernández-Herrero et al. 2012). Figure 4

shows particle size distribution of top 10% volume of soymilk during 0, 1, and 35 days of storage at 25 °C. All samples exhibited a triple-peak of particle distribution, which have the first peak in the value of 3  $\mu\text{m}$ , second small peak (11  $\mu\text{m}$ ), and the third peak (36  $\mu\text{m}$ ) at 0 day of storage. At 1 day of storage, the second and the third peaks disappeared, but the remained one peak (3  $\mu\text{m}$ ) gradually shifted to the left side with increasing storage period and temperature. Based on the visual appearance results, this phenomenon may be expected that the precipitate layer consists to be mostly due to precipitation of larger particles ( $>11 \mu\text{m}$ ) at 1 day of storage, after then the smaller particle ( $<3 \mu\text{m}$ ) or its aggregates induced by long-term storage gradually precipitated during storage. However, there was no significantly difference with different GSP concentration. (data not shown).

Figure 5 shows particle size distribution of bottom 20% volume of soymilk during 0, 1, and 35 days of storage at 25 °C. Likewise, the Figure 4, all samples exhibited a triple-peak of particle distribution. At 1 day of storage, the first peak (3  $\mu\text{m}$ ) area decreased with increasing storage period. However, the second (11  $\mu\text{m}$ ) and third (36  $\mu\text{m}$ ) peak area increased, after then the second (11  $\mu\text{m}$ ) and the third (36  $\mu\text{m}$ ) peak area slightly increased with increasing storage period and temperature, it was expected to be due to particle precipitation or particle aggregation according to the visual appearance results. However, there was no significantly difference with different GSP concentration.

### **2.3. Zeta potential**

$\zeta$ -potential, which is a measure of the colloidal particles, offers a determinant of the type and magnitude of particle interactions, and the indication of potential stability of a colloidal system. And the increased absolute  $\zeta$ -potential values indicated and prevent further aggregates formation (Mukherjee, Chang et al. 2017; Sui, Bi et al. 2017). Figure 6 shows zeta potential values of the top 10% volume of soymilk during 35 days of storage. All soymilk samples showed

negatively charged particle with a relatively low  $\zeta$ -potential. Around absolute 20-30mV are usually low to consider electrostatic repulsion as the main repulsive force preventing particle aggregation. (Menon and Wasan 2006). These suggested that soymilk particle during storage can form particle aggregates by interaction between particle each other. During storage  $\zeta$  - potential value of all soymilk samples is constant, regardless of storage time and GSP concentration. However, as the storage temperature increased, the absolute of  $\zeta$  -potential values slightly decreased to around -20 mV stored at 25°C and around -17 mV stored at 37°C. It means that the lower storage temperature, the less particle aggregation process occurs. The zeta potential results are similar tendency to the previous results. Likewise, the bottom 20% volume of soymilk also show negatively charged particle, and slightly decreased the  $\zeta$  -potential values with increasing storage time and temperature (Figure 7). These results indicated that the absolute charge of particle is decreased. The decrease might be expected to be caused by the formation of protein aggregation or ionic species interactions with protein during storage. The reason for the decrease is not known and requires further experiments about particle-particle interaction in a soymilk system. However, there was no effect of GSP concentration on the absolute zeta potential. These results expected that the GSP is a non-ionic surfactant and so can only interact with a protein. By hydrophobic interaction, which little affect the net charge of proteins (Malhotra and Coupland 2004).

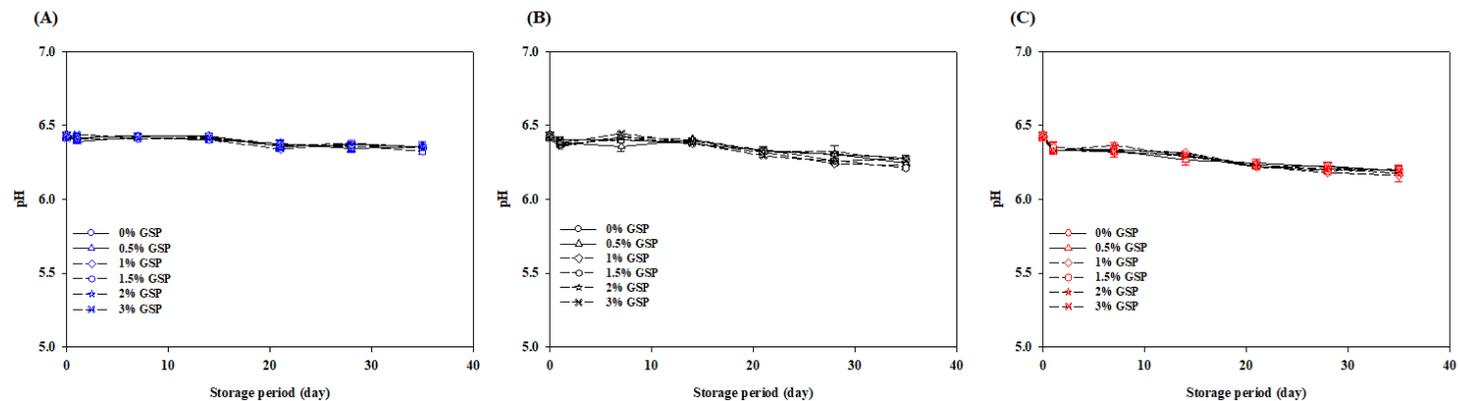
### **3. Rheological properties of soymilk**

Increasing the viscosity of soymilk can slow down the particle settling rate of soymilk. The rate of precipitation can then be estimated from Stoke's law (Equation 6)

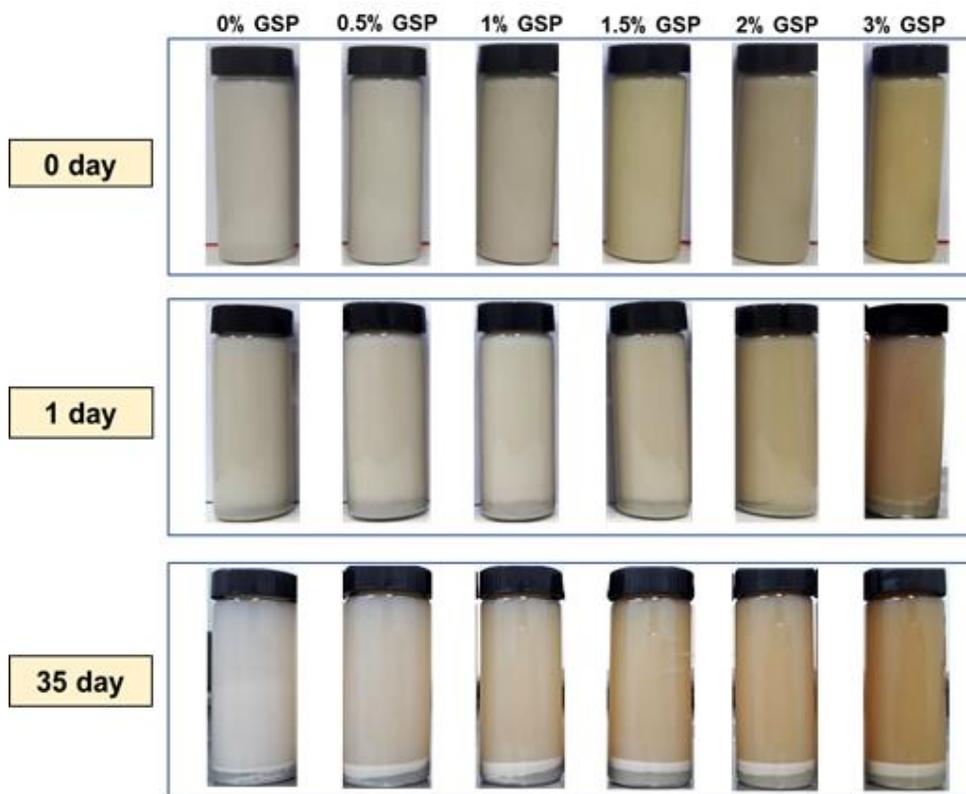
$$V = 2\Delta\rho g\alpha^2 / 9\eta \quad (6)$$

Where  $V$  is the precipitation velocity,  $\alpha$  is the particle radius,  $\Delta\rho$  is the density difference between the dispersed and continuous phases,  $g$  is acceleration due to gravity, and  $\eta$  is the shear

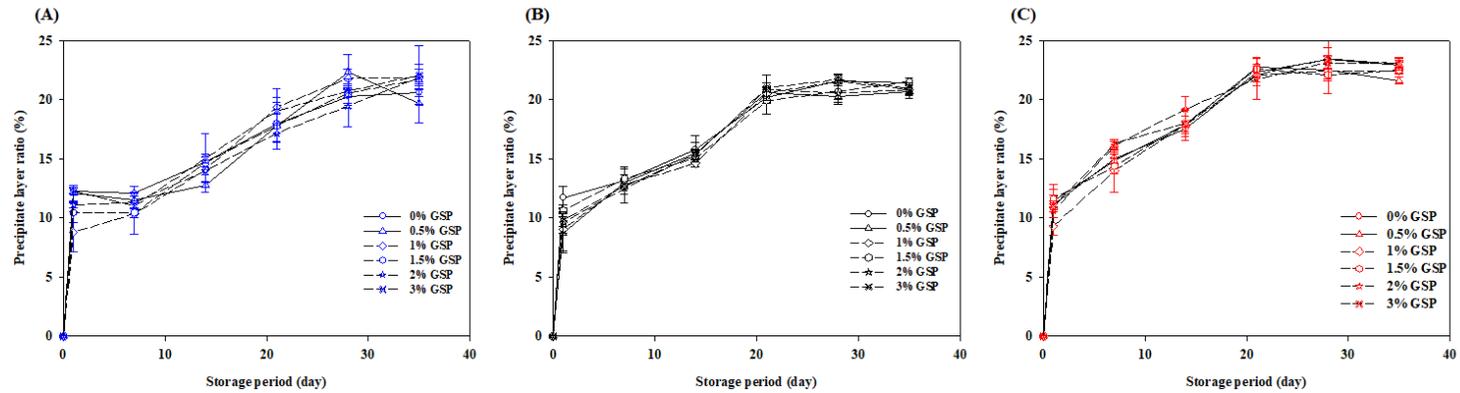
viscosity of continuous phase (Larsson, Hill et al. 2012). Figure 8 shows fluid behavior of soymilk stored at 25 °C. And the rheological parameter (k, n) of soymilk obtained from the power law model (Table 1 and 2). All soymilk was shown to a pseudoplastic fluid ( $n < 1$ ). As the storage temperature decreased, the flow behaviour index decreased from 0.9557 (stored at 37 °C) to 0.8426 (stored at 4 °C). Also, the consistency index(k) increased from 0.0023 (stored at 37 °C) to 0.0070 Pa.s<sup>n</sup> (stored at 4 °C). This is due to decreasing intermolecular interaction, and similar results were reported by Sivanandan, Toledo et al. 2010. In case of GSP concentration effect, the GSP concentration increased, the flow behaviour index increased from 0.9743 (0% GSP) to 0.9129 (3% GSP). And the consistency index slightly increased from 0.0020 (0% GSP) to 0.0040 (3% GSP) expressed Figure 9. These results caused by three factors: (1) Interaction between protein-surfactant: When surfactant concentration is above CMC, surfactant can make the micelles, which could simultaneously bind several charged peptide chains and serve as intermolecular links, thus lead to an increase in viscosity (Greener, Contestable et al. 1987). (2) Total solid : When the concentration of GSP as glycoside was increased, the soymilk may make the thicker solution that can decrease the sedimentation (Ringgenberg 2011;Sivanandan, Toledo et al. 2010). As a result, the soymilk has higher viscous behavior at lower storage temperature and higher GSP concentration, which indicate less particle precipitation.



**Figure 1.** Changes in pH of soymilk prepared without or with different concentrations of soymilk during 35 days of storage. (A) stored at 4 °C, (B) stored at 25 °C, (C) stored at 37 °C.

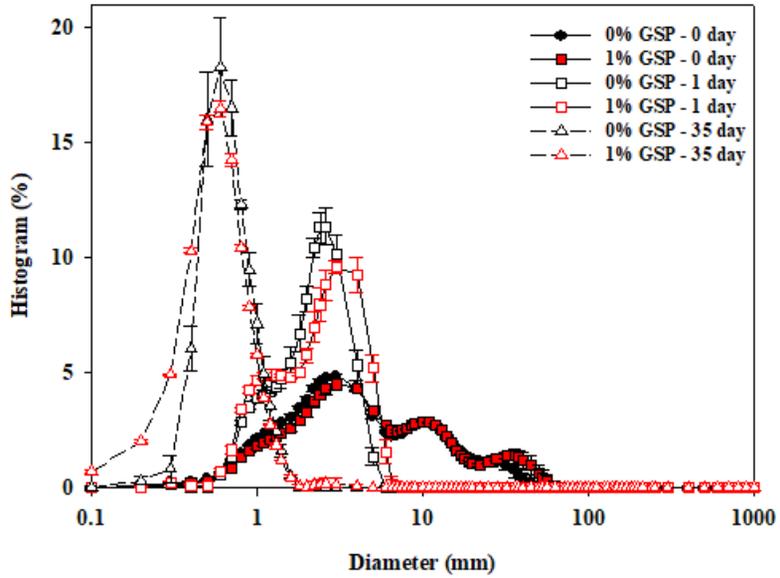


**Figure 2.** Soymilk precipitation process during 35 days of storage at 25 °C.

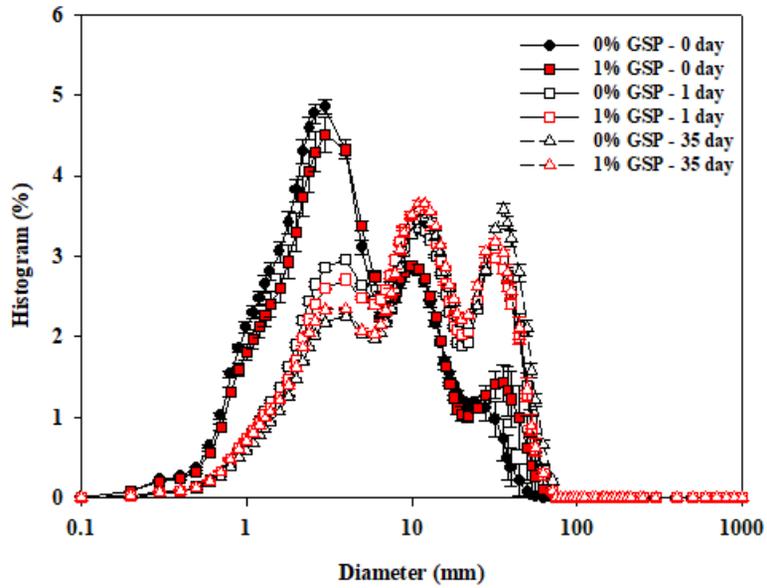


**Figure 3.** Changes in precipitate layer ratio of soymilk prepared without or with different concentrations of soymilk during 35 days of storage. (A) stored at 4 °C, (B) stored at 25 °C, (C) stored at 37 °C.

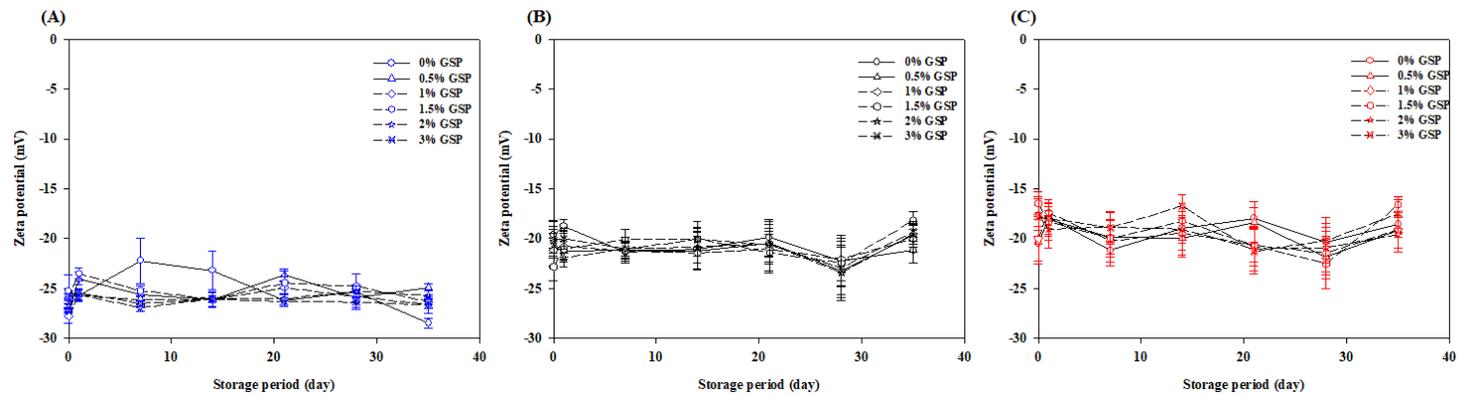
$$\text{※ Precipitate layer ratio (\%)} = \frac{\text{Precipitate layer height of soymilk}}{\text{Total height of soymilk}} \times 100(\%)$$



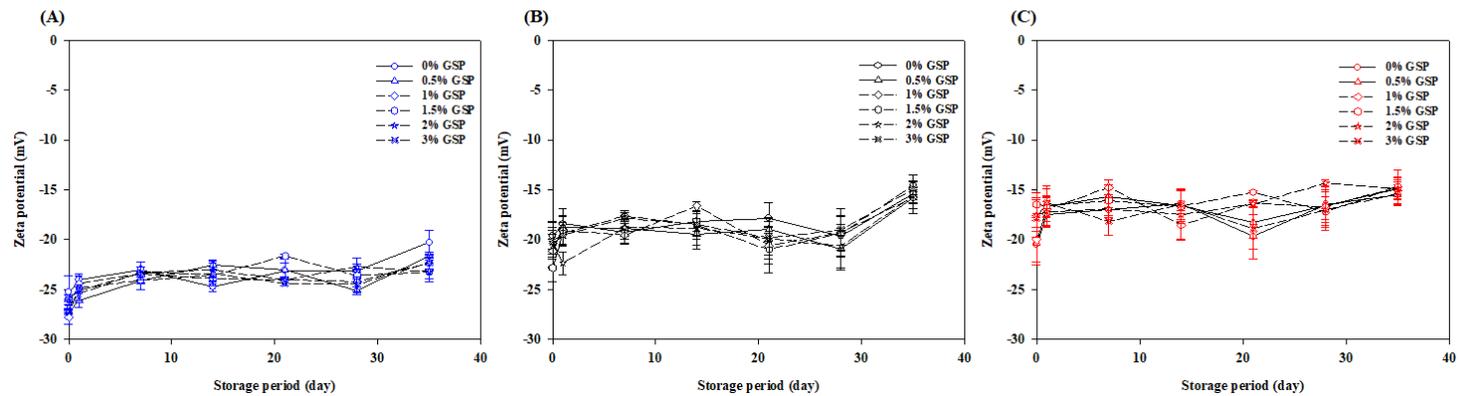
**Figure 4.** Changes in particle size distribution of the top 10% of soymilk prepared without or with different concentrations of soymilk during 35 days of storage at 25 °C.



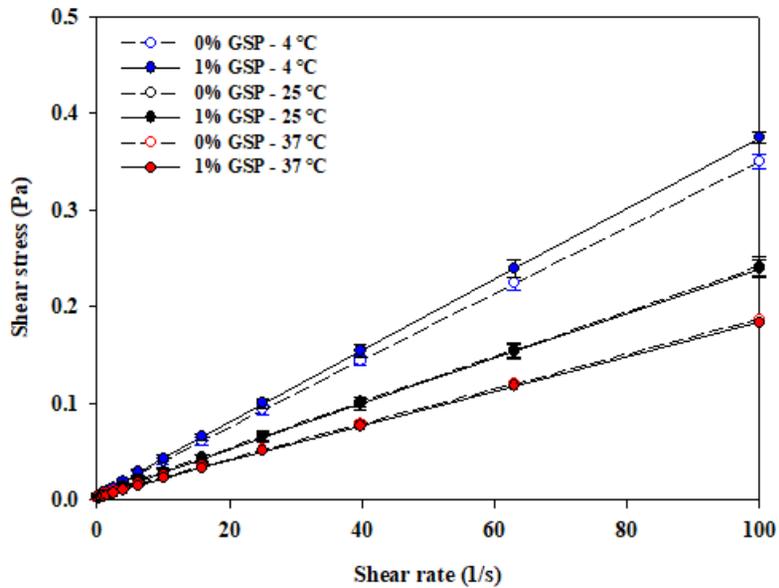
**Figure 5.** Changes in particle size distribution of the bottom 20% of soymilk prepared without or with different concentrations of soymilk during 35 days of storage at 25 °C.



**Figure 6.** Changes in zeta potential of the top 10% of soymilk prepared without or with different concentrations of soymilk during 35 days of storage. (A) stored at 4 °C, (B) stored at 25 °C, (C) stored at 37 °C.



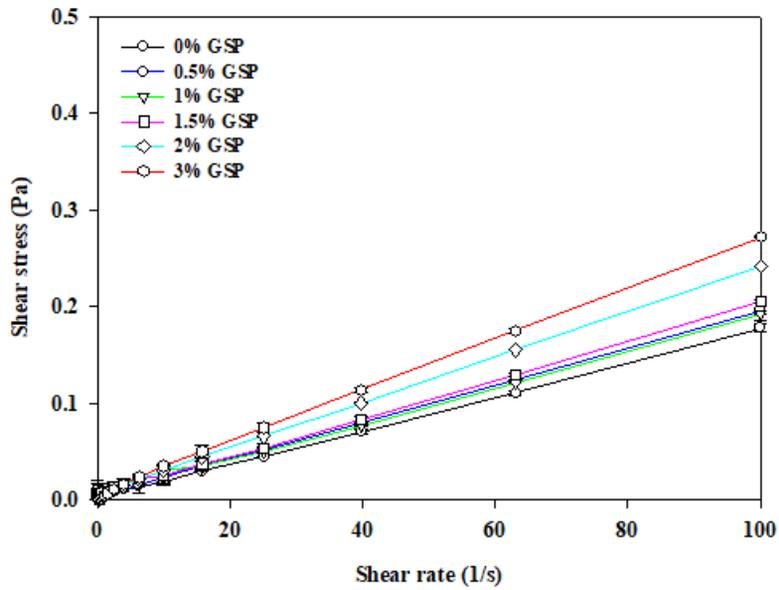
**Figure 7.** Changes in zeta potential of the bottom 20% of soymilk prepared without or with different concentrations of soymilk during 35 days of storage. (A) stored at 4 °C, (B) stored at 25 °C, (C) stored at 37 °C.



**Figure 8.** Shear stress (Pa) for soymilk samples with 1% GSP (full symbol) or without GSP (empty symbol) as a function of shear rate (1/s) at various storage temperature 4 °C (blue symbol), 25 °C (black symbol), and 37 °C (red symbol).

**Table 1.** Flow behavior index (n) and consistency index (k) values of soymilk samples with 1% GSP or without GSP at various storage temperature 4, 25 and 37 °C.

<b>Storage temperature</b>	<b>n</b>	<b>k</b>	<b>R<sup>2</sup></b>
<b>4 °C</b>			
<b>0% GSP</b>	<b>0.8426±0.014</b>	<b>0.0070±0.0004</b>	<b>0.9938</b>
<b>1% GSP</b>	<b>0.8351±0.021</b>	<b>0.0078±0.0002</b>	<b>0.9938</b>
<b>25 °C</b>			
<b>0% GSP</b>	<b>0.8401±0.018</b>	<b>0.0050±0.0002</b>	<b>0.9949</b>
<b>1% GSP</b>	<b>0.8437±0.025</b>	<b>0.0048±0.0003</b>	<b>0.9945</b>
<b>37 °C</b>			
<b>0% GSP</b>	<b>0.9557±0.080</b>	<b>0.0024±0.0001</b>	<b>0.9989</b>
<b>1% GSP</b>	<b>0.9592±0.011</b>	<b>0.0023±0.0001</b>	<b>0.9992</b>



**Figure 9.** Shear stress (Pa) for various concentration of soymilk samples (0-3%) as a function of shear rate (1/s) at stored 25 °C.

**Table 2.** Flow behavior index (n) and consistency index (k) values of soymilk samples for various concentration of GSP (0-3%) at stored 25 °C.

<b>GSP Conc.</b>	<b>Total solid (%, w/w)</b>	<b>n</b>	<b>k</b>	<b>R<sup>2</sup></b>
<b>0% GSP</b>	<b>4.05±0.03</b>	<b>0.9743±0.011</b>	<b>0.0020±0.0003</b>	<b>0.9979</b>
<b>0.5% GSP</b>	<b>4.55±0.01</b>	<b>0.9471±0.008</b>	<b>0.0025±0.0004</b>	<b>0.9991</b>
<b>1% GSP</b>	<b>4.98±0.05</b>	<b>0.8642±0.041</b>	<b>0.0035±0.0010</b>	<b>0.9844</b>
<b>1.5% GSP</b>	<b>5.50±0.01</b>	<b>0.9104±0.021</b>	<b>0.0030±0.0005</b>	<b>0.9930</b>
<b>2% GSP</b>	<b>6.04±0.05</b>	<b>0.9206±0.027</b>	<b>0.0035±0.0007</b>	<b>0.9994</b>
<b>3% GSP</b>	<b>7.04±0.05</b>	<b>0.9129±0.021</b>	<b>0.0040±0.0008</b>	<b>0.9987</b>

## Conclusions

This study indicated an improvement of the storage stability of soymilk stored at 4 °C, verified from the analysis of visual appearance and particle size results. In addition, the particle aggregation was inhibited by higher electrostatic repulsion at 4°C according to  $\zeta$  -potential results. Also the particle precipitation rate was reduced by higher viscous behavior applied to stokes law. However, the addition of ginsenosides as a natural surfactant little affect the storage stability of soymilk. This is because the soymilk is a much more complex system, which contains various types and high amount of particle. This study specifically described the soymilk precipitation process at various conditions such as storage temperature and adding natural surfactant (ginsenosides) during storage, and it was new approaches using ginsenosides as a natural surfactant to improve the storage stability of soymilk. However, more systematic research is needed on the storage stability mechanism of soymilk.

## Overall Conclusions

This study was to characterize an influence of ginsenosides as a natural surfactant on storage stability of soymilk during stored under various conditions. To accomplish this, the study was divided into two categories: “soymilk interface model system (chapter 2)” to understand the soymilk stabilization mechanism induced by ginsenosides, “soymilk products (chapter 3)” to investigate the effect of GSP on storage stability of soymilk. In case of chapter 2, mixed ginsenosides (GSP) as a natural surfactant and soy protein isolate (SPI) can form the complex by hydrophobic interaction within lower concentration of both SPI and GSP. Also, at higher concentration of SPI and GSP, mixed GSP-SPI, which have competitive adsorption relationship, can control the interfacial properties depending on the ratio of GSP/SPI at air/water interface. This might be useful for formation of suspension, if properly used, might in fact widen them. When applied to soymilk as a real food system (chapter 3), the soymilk had more viscous behavior as the GSP concentration increased. It might be due to the increasing total solid or formation of inter-molecular links, which is binding surfactant with micelle-several peptide. However, GSP have little effect on the storage stability of soymilk, including inhibition of particle aggregation (mainly soy protein) and precipitation during various storage conditions (4, 25, and 37 °C). This might be because soymilk is a complex system that contains high protein contents, many kinds of components. To understand the specific effect of ginsenoside on the storage stability of soymilk, additional experiments are necessary, such as making the soymilk suspension or/and emulsion model system that simplified the soymilk.

## References

- Salimi, S. and S. Moradi (2012). "Effect the correlation, regression and path analysis in soybean genotypes (*Glycin Max L.*) under moisture and normal condition." International journal of Agronomy and Plant Production **3**(10): 447-454.
- Messina, M. (1995). "Modern applications for an ancient bean: soybeans and the prevention and treatment of chronic disease." The Journal of Nutrition **125**(suppl\_3): 567S-569S.
- Messina, M. J., V. Persky, K. D. Setchell and S. Barnes (1994). "Soy intake and cancer risk: a review of the in vitro and in vivo data." Nutr Cancer **21**(2): 113-131.
- Gu, C., H. Pan, Z. Sun and G. Qin (2010). "Effect of soybean variety on anti-nutritional factors content, and growth performance and nutrients metabolism in rat." Int J Mol Sci **11**(3): 1048-1056.
- McCue, P. and K. Shetty (2004). "Health benefits of soy isoflavonoids and strategies for enhancement: A review." Crit Rev Food Sci Nutr **44**(5): 361-367.
- Pusztai, A. and G. Grant (1998). "Assessment of lectin inactivation by heat and digestion." Methods Mol Med **9**: 505-514.
- He, F.-J. and J.-Q. Chen (2013). "Consumption of soybean, soy foods, soy isoflavones and breast cancer incidence: Differences between chinese women and women in Western countries and possible mechanisms." Food Science and Human Wellness **2**(3-4): 146-161.
- Alezandro, M. R., D. Granato, F. M. Lajolo and M. I. Genovese (2011). "Nutritional aspects of second generation soy foods." J Agric Food Chem **59**(10): 5490-5497.
- Cruz, N., M. Capellas, M. Hernández, A. J. Trujillo, B. Guamis and V. Ferragut (2007). "Ultra high pressure homogenization of soymilk: Microbiological, physicochemical and microstructural characteristics." Food Research International **40**(6): 725-732.

- Giri, S. K. and S. Mangaraj (2012). "Processing influences on composition and quality attributes of soymilk and its powder." Food Engineering Reviews **4**(3): 149-164.
- Kwok, K.-C. and K. Niranjana (1995). "Review: Effect of thermal processing on soymilk." International Journal of Food Science and Technology **30**: 263-295.
- Liu, K. (1997). Soybeans: Chemistry, technology, and utilization. Chapman & Hall.
- Peng, X., C. Ren and S. Guo (2016). "Particle formation and gelation of soymilk: Effect of heat." Trends in Food Science & Technology **54**: 138-147.
- Chen, Y. and T. Ono (2014). "Protein particle and soluble protein structure in prepared soymilk." Food Hydrocolloids **39**: 120-126.
- Idogawa, S. and T. Fujii (2015). "Rheological analysis of the aggregation behavior of a soymilk colloidal system." Food Science and Technology Research **21**(3): 479-487.
- Larsson, M., A. Hill and J. Duffy (2012). "Suspension stability; Why particle size, zeta potential and rheology are important." Annual Transactions of the Nordic Rheology Society **20**: 209-214.
- He, X. T., D. B. Yuan, J. M. Wang and X. Q. Yang (2016). "Thermal aggregation behaviour of soy protein: Characteristics of different polypeptides and sub-units." J Sci Food Agric **96**(4): 1121-1131.
- Mukherjee, D., S. K. C. Chang, Y. Zhang and S. Mukherjee (2017). "Effects of ultra-high pressure homogenization and hydrocolloids on physicochemical and storage properties of soymilk." J Food Sci **82**(10): 2313-2320.
- Shimoyamada, M., N. Tsushima, K. Tsuzuki, H. Asao and R. Yamauchi (2008). "Effect of heat treatment on dispersion stability of soymilk and heat denaturation of soymilk protein." Food Sci. Technol. Res. **14**(1): 32-38.
- Datta, N., M. G. Hayes, H. C. Deeth and A. L. Kelly (2005). "Significance of frictional heating for effects of high pressure homogenisation on milk." J Dairy Res **72**(4): 393-399.

- Diels, A. M., L. Callewaert, E. Y. Wuytack, B. Masschalck and C. W. Michiels (2005). "Inactivation of *Escherichia coli* by high-pressure homogenisation is influenced by fluid viscosity but not by water activity and product composition." Int J Food Microbiol **101**(3): 281-291.
- Poliseli-Scopel, F. H., M. Hernández-Herrero, B. Guamis and V. Ferragut (2012). "Comparison of ultra high pressure homogenization and conventional thermal treatments on the microbiological, physical and chemical quality of soymilk." LWT - Food Science and Technology **46**(1): 42-48.
- Georget, E., B. Miller, M. Callanan, V. Heinz and A. Mathys (2014). "(Ultra) high pressure homogenization for continuous high pressure sterilization of pumpable foods - a review." Front Nutr **1**: 15.
- Marszalek, K., L. Wozniak, B. Kruszewski and S. Skapska (2017). "The effect of high pressure techniques on the stability of anthocyanins in fruit and vegetables." Int J Mol Sci **18**(2).
- Saha, D. and S. Bhattacharya (2010). "Hydrocolloids as thickening and gelling agents in food: A critical review." J Food Sci Technol **47**(6): 587-597.
- Liu, Z.-S. and S. K. C. Chang (2013). "Nutritional profile and physicochemical properties of commercial soymilk." Journal of Food Processing and Preservation **37**(5): 651-661.
- Tobacman, J. K. (2001). "Review of harmful gastrointestinal effects of carrageenan in animal experiments." Environmental Health Perspectives **109**(10): 983-994.
- Otzen, D. (2011). "Protein-surfactant interactions: A tale of many states." Biochim Biophys Acta **1814**(5): 562-591.
- Kotsmar, C., V. Pradines, V. S. Alahverdijeva, E. V. Aksenenko, V. B. Fainerman, V. I. Kovalchuk, J. Kragel, M. E. Leser, B. A. Noskov and R. Miller (2009). "Thermodynamics, adsorption kinetics and rheology of mixed protein-surfactant interfacial layers." Adv Colloid Interface Sci **150**(1): 41-54.

- Yang, Y., M. E. Leser, A. A. Sher and D. J. McClements (2013). "Formation and stability of emulsions using a natural small molecule surfactant: Quillaja saponin (Q-Naturale®)." Food Hydrocolloids **30**(2): 589-596.
- Ringgenberg, E. (2011). "The physico-chemical characterization of soymilk particles and gelation properties of acid-induced soymilk gels, as a function of soymilk protein Concentration." Degree of Master of Science in Food Science, The University of Guelph.
- Malhotra, A. and J. N. Coupland (2004). "The effect of surfactants on the solubility, zeta potential, and viscosity of soy protein isolates." Food Hydrocolloids **18**(1): 101-108.
- Qi, L. W., C. Z. Wang and C. S. Yuan (2011). "Isolation and analysis of ginseng: advances and challenges." Nat Prod Rep **28**(3): 467-495.
- Kiefer, D. and T. Pantuso (2003). "Panax ginseng." American Family Physician **68**(8): 1539-1542.
- Liu, C.-X. and P.-G. Xiaob (1992). "Recent advances on ginseng research in china." Journal of Ethnopharmacology **36**: 27-38.
- Attele, A. S., J. A. Wu and C.-S. Yuan (1999). "Ginseng pharmacology: Multiple constituents and multiple actions." Biochemical Pharmacology **58**: 1685-1693.
- Kim, D. H. (2012). "Chemical Diversity of Panax ginseng, Panax quinquifolium, and Panax notoginseng." J Ginseng Res **36**(1): 1-15.
- Kim, J. H. (2018). "Pharmacological and medical applications of Panax ginseng and ginsenosides: A review for use in cardiovascular diseases." J Ginseng Res **42**(3): 264-269.
- Mesgarzadeh, I., A. R. Akbarzadeh and R. Rahimi (2017). "Surface-active properties of solvent-extracted Panax ginseng saponin-based surfactants." Journal of Surfactants and Detergents **20**(3): 609-614.

- Golemanov, K., S. Tcholakova, N. Denkov, E. Pelan and S. D. Stoyanov (2013). "Remarkably high surface visco-elasticity of adsorption layers of triterpenoid saponins." Soft Matter **9**(24).
- Ralla, T., E. Herz, H. Salminen, M. Edelmann, C. Dawid, T. Hofmann and J. Weiss (2017). "Emulsifying properties of natural extracts from *Panax ginseng* L." Food Biophysics **12**(4): 479-490.
- Kezwon, A. and K. Wojciechowski (2014). "Interaction of Quillaja bark saponins with food-relevant proteins." Adv Colloid Interface Sci **209**: 185-195.
- Reichert, C. L., H. Salminen, J. Utz, G. Badolato Bönisch, C. Schäfer and J. Weiss (2017). "Aging behavior of Quillaja Saponin – pea protein interfaces." Colloid and Interface Science Communications **21**: 15-18.
- Piotrowski, M., J. Lewandowska and K. Wojciechowski (2012). "Biosurfactant-protein mixtures: Quillaja bark saponin at water/air and water/oil interfaces in presence of beta-lactoglobulin." J Phys Chem B **116**(16): 4843-4850.
- Böttcher, S., J. K. Keppler and S. Drusch (2017). "Mixtures of Quillaja saponin and beta-lactoglobulin at the oil/water-interface: Adsorption, interfacial rheology and emulsion properties." Colloids and Surfaces A: Physicochemical and Engineering Aspects **518**: 46-56.
- Dan, A., C. Kotsmar, J. K. Ferri, A. Javadi, M. Karbaschi, J. Krägel, R. Wüstneck and R. Miller (2012). "Mixed protein–surfactant adsorption layers formed in a sequential and simultaneous way at water–air and water–oil interfaces." Soft Matter **8**(22).
- Xu, Y.-T. and L.-l. Liu (2016). "Structural and functional properties of soy protein isolates modified by soy soluble polysaccharides." Journal of Agricultural and Food Chemistry **64**(38): 7275-7284.
- Zhu, C.-Y., H.-F. Liu, M. Fu and X.-H. Zhao (2015). "Structure and property changes of soybean

- protein isolates resulted from the glycation and cross-linking by transglutaminase and a degraded chitosan." CyTA - Journal of Food **14**(1): 138-144.
- Wan, Z.-L., L.-Y. Wang, J.-M. Wang, Q. Zhou, Y. Yuan and X.-Q. Yang (2014). "Synergistic interfacial properties of soy protein–stevioside mixtures: Relationship to emulsion stability." Food Hydrocolloids **39**: 127-135.
- Wojciechowski, K. (2013). "Surface activity of saponin from Quillaja bark at the air/water and oil/water interfaces." Colloids Surf B Biointerfaces **108**: 95-102.
- Mitra, S. and S. R. Dungan (1997). "Micellar properties of Quillaja Saponin. 1. Effects of temperature, salt, and pH on solution properties." J. Agric. Food Chem. **45**(5): 1587-1595.
- Pagureva, N., S. Tcholakova, K. Golemanov, N. Denkov, E. Pelan and S. D. Stoyanov (2016). "Surface properties of adsorption layers formed from triterpenoid and steroid saponins." Colloids and Surfaces A: Physicochemical and Engineering Aspects **491**: 18-28.
- Yano, Y. F. (2012). "Kinetics of protein unfolding at interfaces." J Phys Condens Matter **24**(50): 503101.
- Beverung, C. J., C. J. Radke and H. W. Blanch (1999). "Protein adsorption at the oil/water interface: characterization of adsorption kinetics by dynamic interfacial tension measurements." Biophysical Chemistry **81**: 59-80.
- Fainerman, V. B., S. A. Zholob, M. Leser, M. Michel and R. Miller (2004). "Competitive adsorption from mixed nonionic surfactant/protein solutions." J Colloid Interface Sci **274**(2): 496-501.
- Poliseli-Scopel, F. H., M. Hernández-Herrero, B. Guamis and V. Ferragut (2013). "Characteristics of soymilk pasteurized by ultra high pressure homogenization (UHPH)." Innovative Food Science & Emerging Technologies **20**: 73-80.

- Sidhu, J. and R. Singh (2016). "Ultra high pressure homogenization of soy milk: Effect on quality attributes during storage." Beverages **2**(2).
- Wang, Y., J. Xing, R. Wang and S. Guo (2017). "The analysis of the causes of protein precipitate formation in the blanched soymilk." Food Chem **218**: 341-347.
- Babbar, N., P. Aggarwal and H. S. Oberoi (2015). "Effect of addition of hydrocolloids on the colloidal stability of litchi (*Litchi chinensis* Sonn.) juice." Journal of Food Processing and Preservation **39**(2): 183-189.
- Chaudhari, A., Y. Pan and N. Nitin (2015). "Beverage emulsions: Comparison among nanoparticle stabilized emulsion with starch and surfactant stabilized emulsions." Food Research International **69**: 156-163.
- Kralova, I. and J. Sjöblom (2009). "Surfactants used in food industry: A review." Journal of Dispersion Science and Technology **30**(9): 1363-1383.
- Ringgenberg, E., M. Corredig and M. Alexander (2012). "Physico-chemical characterization of soymilk particles as a function of their volume fraction: Comparison with theoretical systems." Food Biophysics **7**(3): 244-257.
- Chou, D. H. and C. V. Morr (1976). "Protein-water interactions and functional properties." J. AM. OIL CHEMISTS' SOC. **56**: 53A-62A.
- Sui, X., S. Bi, B. Qi, Z. Wang, M. Zhang, Y. Li and L. Jiang (2017). "Impact of ultrasonic treatment on an emulsion system stabilized with soybean protein isolate and lecithin: Its emulsifying property and emulsion stability." Food Hydrocolloids **63**: 727-734.
- Menon, V. B. and D. T. Wasan (2006). "A review of the factors affecting the stability of solids-stabilized emulsions." Separation Science and Technology **23**(12-13): 2131-2142.
- Sivanandan, L., R. T. Toledo and R. K. Singh (2010). "Rheological and ultrastructural properties and particle size distribution of soymilk as affected by processing methods." International Journal of Food Properties **13**(3): 580-598.

Greener, J., B. A. Contestable and M. D. Bale (1987). "Interaction of anionic surfactants with gelatin: Viscosity effects." Macromolecules **20**: 2490-2498.

## Abstract in Korean

대두 (*Glycine max*)는 35-40%의 높은 식물성 단백질을 함유하고 있으며, 또한 saponin, isoflavone 및 oligosaccharide와 같은 건강기능성 물질을 가진 작물로서, 세계인의 식생활에서 중요한 작물 중 하나이다. 그러나 대두는 trypsin inhibitor, phytate 및 lectin과 같은 영양 저해 인자를 함유하므로 대두는 섭취 전에 열처리 또는 가공공정이 필요하다. 대두가공식품은 발효 유무에 따라서 발효식품(간장, 된장, 청국장 등)과 비발효식품 (두유, 두부, 콩가루 등) 2가지로 분류된다. 특히 비발효식품 중 하나인 두유는 대두를 물에 불린 뒤, 갈아 만든 음료로서, 예로부터 고품질 식물성단백질의 공급원이면서, 유당불내증 환자를 위한 우유 대체 식품으로 전세계적으로 많이 소비되고 있는 건강기능성 음료이다. 그러나 두유는 저장 중 (1) 회분, 불용성 단백질, 섬유질과 같은 불용성 물질 (2) 두유 단백질 입자 응집에 의한 침전이 발생하며, 이는 두유의 물리화학적 성질(입도크기, 미세구조 및 탁도), 조직감 및 감각과 같은 두유의 품질을 손상시킨다. 두유의 입자 침전은 Stoke's law:  $V = 2\Delta\rho g r^2 / 9\eta$ 에 따라 두유 입자의 크기, 두유의 밀도, 점도에 영향을 미치며, 다음의 요인을 조절하여, 두유의 저장 안정성을 향상시킬 수 있다. 두유의 저장 안정성을 향상시키는 방법은 (1) 초고압균질 공정, (2) Hydrocolloid 첨가 등이 있다. (1) 초고압균질 공정의 경우, 두유를 초고압을 가하여 노즐을 통과시키고, 이때 발생하는 cavitation, 전단력 등을 두유 입자에 작용하여, 입자를 미세화, 분산시키는 방법이다. 그러나 초고압균질의 경우 (a) 초고압에 의해 발생한 마찰열에 의한 두유 미량성분의 파괴, (b) 고가의 설비 및 생산비용의 문제점이 있다.(2) Hydrocolloid 첨가의 경우, 두유에 hydrocolloid(자당 지방산에스테르, 모노글리세라이드, 카라기난 등)를 첨가, 분산시켜 두유의 점도 또는 겔 형성 특성을 변화시키는 방법이다. 그러나 특정 hydrocolloid는 pH 및 염과 같은 환경적 요인에 영향을 많이 받으며, 또한 hydrocolloid 섭취 시 위장관에서 병

변이나 암을 유발할 수 있다고 보고되었고, 또한 여전히 식품안정성에 대해 입증되지 않았다. 최근 계면활성제가 콜로이드 입자와 상호작용하여, 식품 계면 특성을 변형, 입자 응집 억제, 및 복합체를 형성할 수 있으며, 또한 음료의 저장 안정성을 향상시킬 수 있다고 보고되었다. 계면활성제 중 인삼(*Panax ginseng*) 사포닌은 천연 계면활성제로써, 항암, 노화방지, 면역 체계 향상, 혈관 운동 개선 등의 건강기능성과 더불어 식품안전성이 입증된 물질이다. 또한 사포닌은 단백질과 상호작용하여, 복합체를 형성하며, 또한 단백질의 친수성을 향상시켜, 저장 중 단백질 응집을 억제하는 기능이 보고되었다.

본 연구는 천연 계면활성제인 인삼 사포닌이 우유의 저장 안정성에 미치는 영향에 대해 조사하였다. 우유 연구는 인삼 사포닌에 의해 유도되는 우유 안정화 메커니즘을 알아보려고 우유 계면 모델 시스템(Chapter 2)에서 계면 특성 실험을 진행하였고, 또한 인삼 사포닌이 우유의 저장 안정성 향상 유무를 확인하고자 실제 우유 시스템(Chapter 3)에서 저장 안정성 실험을 진행하였다.

우유 계면 모델의 계면 특성 분석은 분리대두단백(SPI, 순도 $\geq 90\%$ )과 인삼 사포닌 분말(GSP, 순도 $\geq 80\%$ )을 각각 인산완충용액(pH 7)에 첨가하고, 혼합, 수화시킨 용액을 이용하였다. 혼합된 SPI-GSP 계면 시스템은 (1) “불포화 계면층”:  $10^{-5}\%$ , w/w SPI 고정, (2) “포화 GSP 계면층”:  $10^{-2}\%$ , w/w GSP 고정, (3) “포화 SPI 계면층”:  $10^{-2}\%$ , w/w GSP 고정, 총 3가지 농도에서 wilhelmy plate 방법을 사용하여 동적 계면장력을 측정하였다. 평형 계면장력은 동적 계면장력 결과를 외삽하여 결정하였다. 혼합된 SPI-GSP 용액은 GSP-SPI 복합체를 형성할 수 있으며, GSP와 SPI가 낮은 농도 범위 내( $\leq 10^{-3}\%$ , w/w GSP and SPI)에서 상승 효과를 나타낸다. 반면에 높은 농도 범위 내( $\geq 10^{-3}\%$ , w/w GSP and SPI)에서 혼합용액의 계면 특성은 GSP/SPI 비율에 따라 변화한다. GSP / SPI의 각각 낮은 비율과 높은 비율에서 계면 장력은 각 SPI와 GSP의 계면장력과 유의적으로 일치한 값을 나타낸다. 이는 GSP와 SPI가 경쟁 흡착 관계이며, GSP / SPI의 계면에서의 비율에 따라

흡착 거동을 조절할 수 있음을 의미한다. 또한 이는 현탁액 또는 유화액 식품 시스템의 형성 및 안정성 조절에 유용하게 이용할 수 있을 것이다.

실제 두유 시스템에서 인삼 사포닌이 두유의 저장 안정성에 미치는 영향을 조사하였다. 두유 샘플은 일반적인 두유 제조공정을 이용하였고, 두유에 인삼 사포닌 분말(GSP)을 0, 0.5, 1, 1.5, 2, 3%, w/w로 첨가한 샘플을 실험에 사용하였다. 두유의 저장 안정성 분석은 저장온도 4, 25, 37 °C에서 35일 저장기간 동안 각 (1) 저장 수명: 미생물 성장 및 pH, (2) 저장 안정성 확인: 두유 외관, 입도크기, 제타포텐셜, (3) 유변학적 특성 총 3분류로 나뉘어 분석하였다. 두유의 저장 수명은 저장 온도 및 GSP 농도에 관계없이 저장 기간 중 미생물이 확인되지 않았으며, 또한 pH는 6.2-6.4 값의 일정한 값을 유지하였다. 두유의 저장 안정성은 저장 온도가 낮을수록 두유의 침전이 천천히 발생하였고, 저장 안정성이 향상되었다. 이는 제타포텐셜 결과에 따라 두유를 4 °C에서 저장한 경우, 더 큰 정전기적 반발력이 발생하여 두유의 입자 응집이 억제되기 때문으로 예상되며, 또한 두유의 유체 특성 결과에 따라 저장 온도 4 °C에서 두유는 더 큰 점성특성을 나타내었고, 따라서 Stoke's law에 의해 두유의 입자 침전속도가 천천히 발생하기 때문으로 예상된다. 마찬가지로 GSP 농도가 증가함에 따라 두유는 더 큰 점성 특성을 나타내었다. 이는 두유의 총 고형물의 증가 또는 Inter-molecular links (ginsenoside 미셀과 두유 단백질 내 펩타이드와 결합)를 형성하기 때문으로 예상된다. 그러나 GSP의 첨가는 두유 입자 응집 및 침전을 포함하여 두유의 저장 안정성에 거의 영향을 미치지 않았다. 이는 두유가 고 단백질 함량 및 많은 종류의 성분을 함유하는 복잡한 시스템이기 때문으로 예상된다. Ginsenoside가 두유의 저장 안정성에 미치는 구체적인 효과를 확인하기 위해 두유 현탁액 또는 유체 모델과 같은 단순한 시스템을 만드는 등 추가 실험이 필요하다.

이 연구는 저장 온도 및 저장 중 천연 계면 활성제 (ginsenosides) 첨가와 같은 다양한 조건에서 두유 입자 침전 과정을 구체적으로 기술하였으며, 또한 두유 계

면 모델 시스템에서 ginsenoside가 두유 콜로이드 입자와의 상호작용 메커니즘을 확인하였다. 이는 두유 가공 및 소비의 활용도를 높이고 식품 산업 활용도를 높일 수 있으며, 또한 식품 인터페이스 및 분산 (거품, 유제) 시스템의 안정화 및 저장 안정성을 설명하는데 도움이 될 수 있습니다.

.....  
**주요어:** 두유, 두유 계면 모델, 저장 안정성, 진세노사이드, 분리대두단백, 단백질-사포닌 복합체

**학번:** 2016-26563