A Thesis for the Degree of Doctor of Philosophy

Studies on the Effect of Coffee Creamer with Different Emulsion Properties on Instant Coffee Polyphenols

커피 크리머의 유화특성이 인스턴트 커피 폴리페놀에 미치는 영향에 관한 연구

August, 2022

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지도교수 장 판 식

이 논문을 농학박사학위논문으로 제출함

2022년 8월

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전 혜 주

전혜주의 박사학위논문을 인준함

2022년 8월

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Abstract

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Coffee is among the most widely consumed beverages worldwide and a good source of polyphenols, which prevent oxidative-stress related diseases. This health benefit was identified in epidemiological investigations and has been evaluated in terms of the biological activity of phenolic compounds interacting with milk proteins in dairy matrices (due to frequent consumption of coffee simultaneously with phenolic-rich foods and dairy products).

The objectives of this study are to evaluate effects of creamers containing milk proteins on the bioaccessibility of chlorogenic acids, and the antioxidant activity of instant coffee, considering the consumption pattern of instant coffee in Korea. Creamer is an oil-in-water emulsion that contains milk proteins as emulsifiers, which impart long-term stability. The effects of the types and contents of milk proteins on antioxidant activity were investigated.

Firstly, creamer A and creamer B, differing in contents and types of milk proteins, were prepared and their emulsion characteristics were compared. Creamer

A, which had a low protein content, had high emulsion stability due to the high emulsifying ability of acid casein. Creamer B, which had a high protein content, being mainly composed of aggregated milk proteins, showed low emulsion stability.

In creamer emulsion systems, creamer A was more stable than creamer B to the droplet aggregation caused by pH changes in coffee, but there was no significant difference in the mean particle size or distribution associated with emulsification. Creamer A was more stable than creamer B because the rates of aggregation and creaming (following clarification) in creamer B were faster. Creamer A had a higher proportion of adsorbed to non-adsorbed protein than creamer B. Therefore, the adsorption ability of acid casein in creamer A was higher than that of skim milk and milk protein concentrate in creamer B, although creamer B had a higher total protein content than creamer A.

Second, complexation between the polyphenols in instant coffee and milk proteins in the creamers could vary depending on changes during *in vitro* digestion, which affect the bioavailability of polyphenol. Therefore, changes in the distribution of particles in instant coffee alone (IC), instant coffee with creamer A (CRA), instant coffee with creamer B (CRB), and instant coffee with skim milk (SM), were evaluated by Turbiscan analysis. In addition, the bioaccessible contents and bioaccessibility of chlorogenic acids were compared at each step of *in vitro* digestion.

Changes in delta-backscattering (Δ-BS) profiles during gastric digestion indicated that flocculation, coalescence, and creaming at the top occurred in CRA and CRB due to low pH and protein hydrolysis by digestive enzymes. In SM, which had

the highest protein content, sedimentation of protein aggregates and complexes involving structured clots occurred at the gastric phase. At the pancreatic phase, changes in Δ-BS profiles indicated sedimentation at the bottom and clarification at the top by lipid and protein hydrolysis, and milk proteins were fully hydrolyzed after pancreatic digestion. There were no significant differences ($p > 0.05$) in chlorogenic acid contents among IC, CRA, and CRB, but the total chlorogenic acid content in SM was significantly lower than that of the other samples ($p < 0.05$) at the gastric phase. Pancreatic digestion caused a significant decrease ($p < 0.05$) in the total chlorogenic acid content in all samples, except SM.

The bioaccessible content of CRA and CRB was not significantly changed ($p > 0.05$) during gastric digestion, suggesting that chlorogenic acids in CRA and CRB retained their structures in the gastric phase. After the pancreatic phase, the bioaccessibility of CGAs in IC, CRA, CRB, and SM was 26.4%, 40.5%, 51.7%, and 83.6%, respectively, indicating that bioaccessibility increased significantly with increasing protein concentration. Moreover, CRA and CRB increased the bioaccessibility of chlorogenic acids as a result of prevention of degradation by the milk proteins in creamers.

Finally, the effects of creamer addition on the total polyphenols and antioxidant activity of instant coffee were evaluated during *in vitro* digestion. The total phenolic (TP) contents of CRA and CRB were significantly higher ($p < 0.05$) than that of IC, at all digestion stages. The TP content of IC decreased after pancreatic digestion compared to that before digestion and after gastric digestion. These results were

attributed to the low stability of phenolic compounds in coffee at neutral pH. In addition, the changes in 5-caffeoylquinic acid (5-CQA), the most abundant chlorogenic acid in instant coffee, were analyzed during digestion. The results after pancreatic digestion were similar to those of IC, suggesting that coffee polyphenols were degraded at the pancreatic phase.

The antioxidant activity of IC, CRA, CRB, and SM during *in vitro* digestion was measured by $ABTS^{+}$ and ferric reducing antioxidant power (FRAP) assay. The antioxidant activities of CRA, CRB, and SM were higher than those of IC after pancreatic digestion and showed smaller decreases than IC during pancreatic digestion. IC showed the greatest decrease (25.6%) in FRAP compared to that before digestion; smaller decreases were observed for CRA (12.7%), CRB (16.2%), and SM (13.8%). These results suggest that milk proteins preserved the antioxidant activity of phenolic compounds during digestion, particularly at the pancreatic phase.

As it was not elucidated how the protein in creamers had effects on enhancing the antioxidant activity of instant coffee, it is necessary to clarify the exact mechanism in terms of the complexation between proteins and polyphenols. Furthermore, the correlation of *in vitro* result with *in vivo* effects in human seems to be a further work to provide more exact and practical results.

This study demonstrated that the addition of creamers to instant coffee can significantly affect the bioaccessibility of chlorogenic acids and antioxidant activity, possibly attributable to complexation of milk proteins and coffee phenolics. Therefore, the consumption of instant coffee with creamer enhances the biological activity of

coffee phenolics after gastro-pancreatic digestion, compared to the consumption of instant coffee alone.

Key words: instant coffee, creamer, milk protein, emulsifying properties, antioxidant activity, bioaccessibility, chlorogenic acids, *in vitro* digestion.

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Chapter I.

General Introduction

I-1. Creamer emulsion system

Coffee creamers give coffee a desirable color and form a complex with coffee tannins, reducing its astringency and alleviating the bitter and sour taste (Golde & Schmidt, 2005). Coffee creamers are commonly called coffee whiteners because they impart a white color. In addition, creamers are often added to tea and cocoa products, which are rich in polyphenols, providing desirable sensory attributes. In terms of creamer functionality, it is important that coffee creamers increase stability against de-oiling and feathering in hot coffee of low pH (*i.e.*, 4.8–5.2) (Khatkar & Gupta, 2014; Tran & Einerson, 1987). Because protein and oil impact final product functionality, they are major ingredients of commercial creamers.

Emulsions are a class of dispersion systems that contain two or more immiscible liquid phases. In food applications, these two liquids generally consist of an oil phase and an aqueous (water) phase that is stabilized by the addition of emulsifiers. Emulsions are classified as oil-in-water (O/W) or water-in-oil (W/O). Creamers made from dairy or plant proteins are popular oil-in-water emulsion foods (Golde et al., 2005).

Milk-based proteins and surfactants are commonly used to formulate stable creamer emulsions. Surfactants reduce the surface tension of oil droplets to facilitate droplet disruption and reduce their size. Milk proteins are adsorbed to the surface of oil droplets, and inhibit their cohesion and movement by increasing the viscosity of

the aqueous phase, resulting in long-term stability of creamers (Meena, Singh, Panjagari, & Arora, 2017).

Acid casein and sodium caseinate, which have high heat stability, are commonly used as emulsifiers in creamers, while whey protein and milk protein concentrate, which show low emulsion stability, are generally used for their sensory and foaming attributes, among other properties (Meena, Singh, Gupta, Borad, Arora, & Tomar, 2018). For this reason, approaches to increase the stability of milk proteins for use in white coffee have been investigated.

I-2. Coffee polyphenols and antioxidant activity

Coffee is a good source of polyphenols, which have at least one hydroxy group bonded to an aromatic ring. Polyphenols can be classified into phenolic acids and flavonoids, according to the nature of their carbon skeleton (Guo, Kong, & Meydani, 2009). Phenolic acids include caffeic acid, ferulic acid, and hydrolysable tannins. Flavonoids can be divided into several classes according to the degree of oxidation in the heterocycle. Flavonoids include flavonols, flavones, isoflavones, flavanones, anthocyanins, and flavanols (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004; Scalbert & Williamson, 2000).

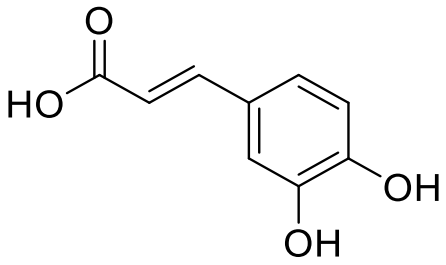
Polyphenols have health benefits because of their antioxidant properties, which are associated with a reduced risk of oxidative stress-related diseases (Chen et al., 2011; Weng & Yen, 2012; Di Chen, Yang, Yuan, Chan, & Dou, 2011).

Oxidative stress is created by an increase of free radicals in cells, which oxidize blood vessel walls, proteins, DNA, carbohydrates, and lipids. These radicals alter cell membranes and break bonds in DNA, thereby damaging the cell (Menshchikova, Lankin, Zenkov, Bondar, Krugovykh, & Trufakin, 2006; Yashin, Ryzhnev, Yashin, & Chernousova, 2009). Prolonged oxidative stress from excessive free radicals may contribute to premature aging and the development of cancer, heart diseases, and diabetes (Harman, 1999). Free radicals can be eliminated by antioxidants acting as

free-radical scavengers, which are supplied in foods or supplements (Marian Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006; Valko, Morris, & Cronin, 2005).

The most abundant phenolic compounds in coffee are caffeic acid and chlorogenic acid derivatives and a cup of coffee contains approximately 70–350 mg of chlorogenic acids (Figure I-1).

(A)



(B)

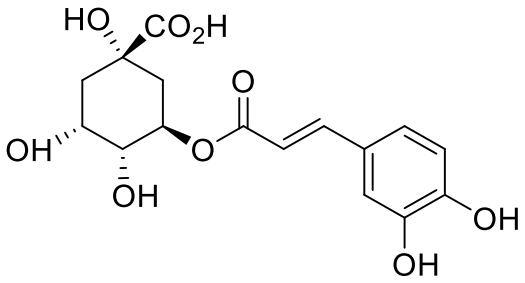


Figure I-1. Chemical structure of major phenolic compound present in coffee. (A) Caffeic acid and (B) chlorogenic acid.

Chlorogenic acids are esters of trans-hydroxycinnamic acids and quinic acid, and are classified as caffeoylquinic acids (CQAs), feruloylquinic acids (FQAs), and di-caffeoylquinic acids (di-CQAs). 5-O-Caffeoylquinic acid (5-CQA) is the most abundant chlorogenic acid in coffee. Chlorogenic acids are formed by esterification of trans-cinnamic acids (caffeic, ferulic, and p-coumaric) with quinic acid.

The structures of the major chlorogenic acids in coffee, including 3- and 4-CQA, isomers of 5-CQA, and isomers of dicaffeoylquinic acids (3,4-, 3,5-, and 4,5-diCQA) and feruloylquinic acids (3-, 4-, and 5-FQA) are shown in Figure I-2.

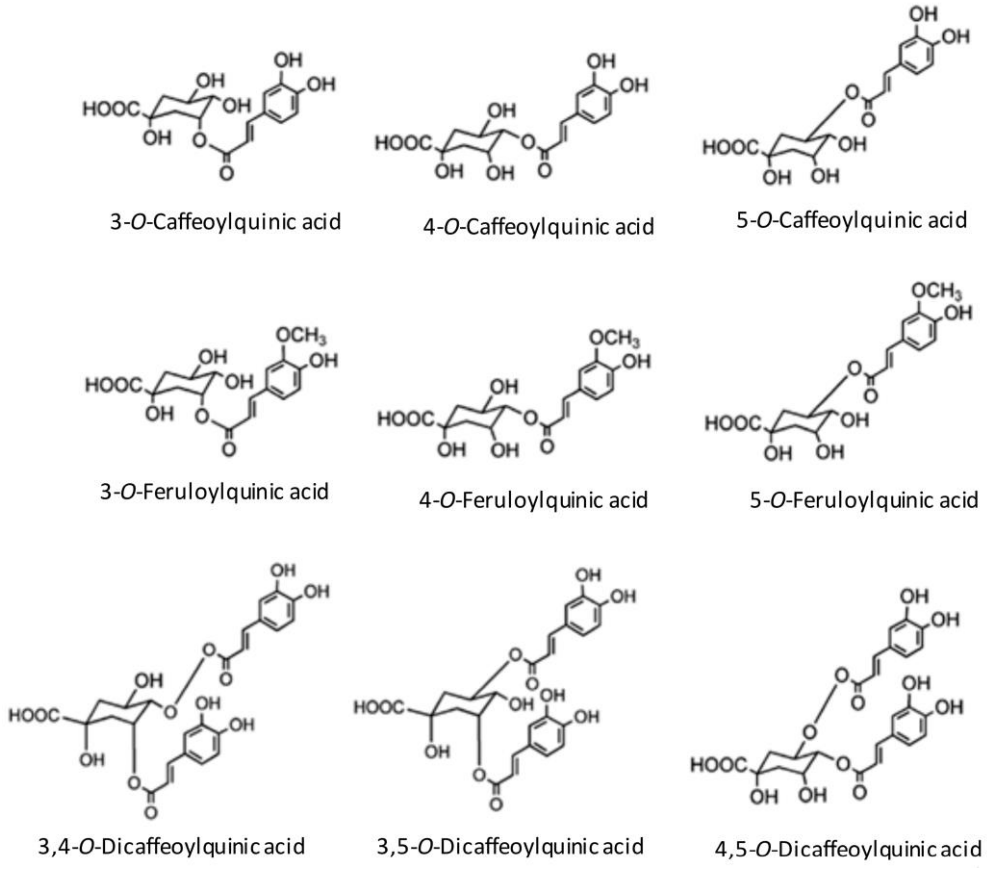


Figure I-2. Structures of chlorogenic acids present in coffee (Adapted from reference Stalmach, Mullen, Nagai, & Crozier (2006)).

I-3. Interactions between polyphenols and milk proteins

Foods rich in polyphenols are often consumed with milk products. Examples of high-antioxidant foods and beverages paired with dairy products include green and black tea with milk, coffee with milk, cocoa with milk, wine with cheese, and berries with yogurt. Research has shown that milk proteins affect the nutritional properties of polyphenols, such as their inherent antioxidant activity, bioavailability, and digestibility.

Proposed mechanisms of complex formation include noncovalent interactions such as hydrogen bonds, hydrophobic interactions, and van der Waals attractions, as well as covalent bonding with nucleophilic groups of proteins, such as $-NH_2$ and -SH, under specific conditions. Noncovalent interactions and covalent bonds mediate reversible and irreversible binding, respectively (Yuksel, Avci, & Erdem, 2010; Prodpran, Benjakul, & Phatcharat, 2012).

Hydrogen bonds are formed between electronegative atoms of amino ($-NH_2$) and hydroxyl (-OH) groups of a protein with a positively charged hydrogen atom of the neighboring hydroxyl group of a polyphenol molecule. Hydrophobic interactions occur between the aromatic amino acids of proteins and nonpolar aromatic rings of polyphenols (Yildirim-Elikoglu & Erdem, 2018).

Polyphenols have strong affinity for proteins with a high proline content, such as casein and gelatin, and interact predominantly via hydrogen bonding and

hydrophobic interaction. β-lactoglobulin in whey protein interacts not only with phenolic groups of coffee polyphenols via noncovalent interactions, but also with various polyphenols in flavonoids (Stojadinovic et al., 2013; Zhang et al., 2014).

Important characteristics of polyphenols for protein binding include their structural flexibility, molecular weight, and number of hydroxyl groups. High-molecular-weight polyphenols tend to bind strongly to proteins (Frazier et al., 2010). For example, larger polyphenols, such as the theaflavins and thearubigins in black tea, showed high binding affinity to milk proteins (J. Ye, Fan, Xu, & Liang, 2013). Structurally flexible polyphenols have the same binding affinity to various proteins, and the number of hydroxyl groups in the polyphenol structure is associated with the strength of binding to proteins (Frazier et al., 2010; Hasni, Bourassa, Hamdani, Samson, Carpentier, & Tajmir-Riahi, 2011; Kanakis, Hasni, Bourassa, Tarantilis, Polissiou, & Tajmir-Riahi, 2011).

Other characteristics of food matrices, such as temperature, pH, ionic strength, and protein type and content, affect polyphenol-protein binding mechanisms. Also, various brewing methods and processes (*e.g.*, instantizing and decaffeination), and additives (*e.g.*, milk and instant creamer), are studied for coffee (Ziyatdinova, Nizamova, & Budnikov, 2011; Sánchez-González, Jiménez-Escrig, & Saura-Calixto, 2005; Niseteo, Komes, Belščak-Cvitanović, Horžić, & Budeč, 2012)

Few studies have evaluated the mechanism by which proteins affect polyphenols in foods considering dietary patterns and circumstances (Trombley,

Loegel, Danielson, & Hagerman, 2011). Most studies focused on the effect of polyphenol-protein interactions on antioxidant activity and the bioavailability of polyphenols (Le Bourvellec & Renard, 2012).

In general, proteins significantly decrease the antioxidant activity of polyphenols. However, the data are contradictory. Because antioxidant activity cannot be measured based only on binding capacity, it needs to be evaluated based on the bioaccessibility of phenolic compounds (Le Bourvellec et al., 2012).

Total phenolic and flavonoid contents are decreased by polyphenol-protein interactions, but the antioxidant capacity of phenolic compounds is maintained or increased during digestion. This could be due to differences in the food matrix containing phenolic compounds or proteins, or to the method used to assay antioxidant capacity, which may be based on hydrogen atom transfer or electron transfer (Huang, Ou, & Prior, 2005).

I-4. Structural properties of milk proteins as emulsifiers

I-4-1. Acid casein

Casein, making up 80% (w/w) of milk protein, is a mixture of phosphoproteins of differing molecular weights that shows excellent emulsifying properties because of its hydrophilic and hydrophobic groups (Bhat, Dar, & Singh, 2016). Casein in milk is present in 80–300 nm diameter micelles and composed of αs_1-, αs_2-, β-, κ-casein in an approximate 3:1:3:1 ratio (Swaisgood, 1985).

αs_1- and β-casein, which constitute 85% of total caseins, are responsible for emulsion stability. αs_1-casein is a phosphoprotein sensitive to calcium that has a high proline content and three hydrophobic regions (Bingham, Farrell, & Carroll, 1972). β-casein has a molecular weight of about 24 kDa (higher than αs_1-casein), and a higher initial reduction rate of interfacial tension and stronger surface-active properties than αs_1-casein.

Acid casein is produced by the acidification of skim milk to pH 4.6, and precipitated casein curd is separated by washing process (Figure I-3). Next, acid casein is obtained by drying the insoluble acid casein gel and converting insoluble acid casein into a soluble form (Davis, 1980).

Acid casein is neutralized by sodium hydroxide or calcium hydroxide to produce sodium caseinate and calcium caseinate, respectively (Srinivasan, Singh, &

Munro, 2003). Casein particles undergoing phase conversion, like sodium caseinate, are 10–20 nm in diameter and promote emulsion stability. Casein, which is soluble at pH 6.0, imparts constant viscoelasticity with high viscosity, increasing the heat stability of emulsions.

I-4-2. Milk protein concentrate

Milk protein concentrate products are processed directly from skim milk by a combination of diafiltration and ultrafiltration or microfiltration, which are pressure-driven membrane processes (Figure I-3). During ultrafiltration, low-molecular-weight substances (*e.g*., lactose, minerals and vitamins) pass through the membrane into the permeate (Singh, 2007).

Milk protein concentrate production does not involve severe heat and chemical treatments. The product has a casein to whey protein ratio equal to that of natural milk. However, the emulsifying ability of milk protein concentrate is lower than that of acid casein and sodium caseinate, because milk protein concentrate is changed into "aggregated form" passed through ultrafiltration, homogenization, and heat treatment (Meena et al., 2017).

Casein micelles encompass αs_1-, αs_2-, β-, and κ-caseins, and whey proteins such as β-lactoglobulin and α-lactalbumin have good emulsifying properties (Dickinson, 1998), although these are diminished in an aggregated or micellar (Mulvihill & Murphy, 1991). The smaller the droplet size, the larger the surface area to be covered by emulsifiers. A higher concentration of aggregated milk protein concentrate is required to cover the surface of oil droplets. Therefore, additional treatments to overcome the low emulsifying ability are required. For example, calcium-depleted milk protein concentrate products have a reconstituted casein micelle form that is dissociated to some extent by chelating the calcium linked to casein (A. Ye, 2011).

When milk protein concentrate is used as an emulsifier, calcium chelating agents such as sodium phosphate and citrate are added to dissociate the aggregates by calcium depletion and reduce their size to 20–200 nm. These smaller micellar caseins have better emulsifying ability and could expand its applications in specific applications.

When emulsions with high protein contents are heated, hydrophobic interactions of unabsorbed proteins with casein micelles and heat-induced aggregation of whey proteins result in emulsion instability and poor solubility. To increase emulsion heat stability, creamer products containing milk protein concentrate are manufactured by controlling the ionic strength and pH with appropriate buffer salts.

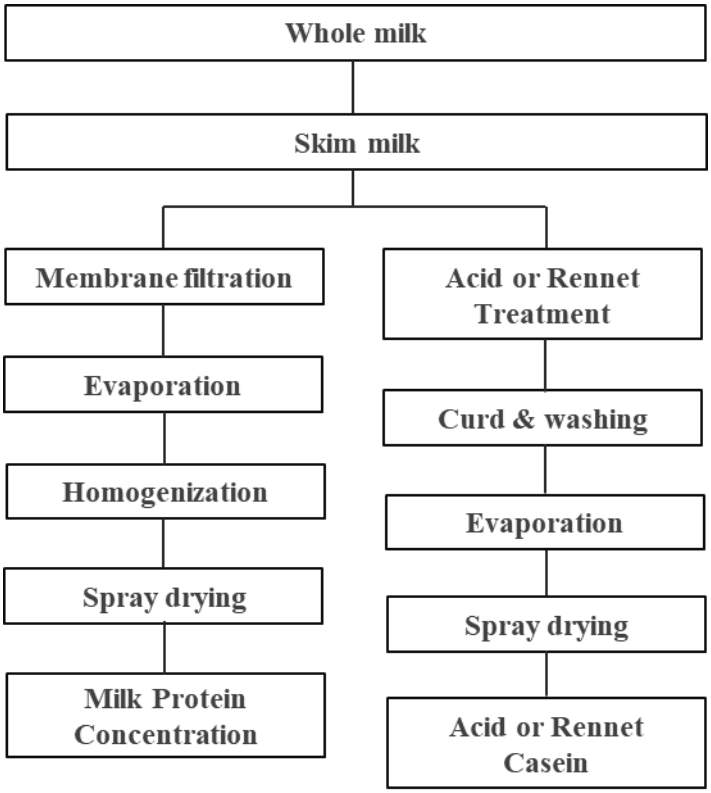


Figure I-3. Process flow diagram of acid casein and milk protein concentrate production from whole milk.

I-4-3. Milk protein in skim milk

In the case of creamers used as milk substitutes, liquid whole milk or skim milk may be used to impart a real milk flavor and mouthfeel. However, most powdered creamers that are spray-dried use skim milk or skim milk powder because of the oxidative stability of milk fat.

Casein proteins in skim milk, as self-assembled colloidal particles, are larger than acid casein. There are considerable structural differences between the casein proteins in skim milk and acid casein (Schlesser, 2007).

Milk proteins in natural skim milk are similar in type and structure to those in milk protein concentrate. Because milk protein concentrate is produced only by fractionation and concentration according to molecular size through ultrafiltration (Meena et al., 2018).

Therefore, milk protein micelles in skim milk should be dissociated to some extent by physical and chemical treatments, such as homogenization and pH adjustment.

I-5. Effect of milk protein on the antioxidant activity of instant coffee

In Europe and the United States, where coffee is commonly consumed in the form of lattes as well as instant coffee, many studies of polyphenol antioxidant activity and bioavailability have been conducted.

Consumption with milk decreases the antioxidant activity of coffee due to hydrogen bonding with polar groups of milk proteins and phenolic groups of coffee polyphenols. The formation of polyphenol-protein complexes does not significantly alter the antioxidant activity of coffee *in vivo*, due to protein and polyphenol degradation during digestion.

Although several studies have evaluated the influence of milk on the bioaccessibility and antioxidant activity of phenolic compounds in coffee, few have assessed the effects of creamer (Keogh, McInerney, & Clifton, 2007; Renouf, Marmet, Guy, Fraering, Longet, Moulin, Enslen, Barron, Cavin, Dionisi, et al., 2010).

The demand for ready-to-drink and instant “3-in-1” products (combination of coffee, creamer and sugar) has grown due to their ease of use, and sweet and mellow taste (Quan et al., 2020). Creamer is an oil-in-water emulsion containing a smaller quantity of milk proteins than natural milk, and is used as an additive in instant coffee (Koo, Chung, Fu, Sher, Rousset, & McClements, 2019).

Previous studies highlighted that food matrix properties are important for the bioaccessibility of polyphenols (Sun-Waterhouse, Zhou, & Wadhwa, 2013; Trigueros, Wojdyło, & Sendra, 2014; Han et al., 2011; Rashidinejad, Birch, Sun-Waterhouse, & Everett, 2013). Accordingly, creamer products can be evaluated in terms of polyphenol-protein interactions in a processed format intended to form an oil-in-water emulsion.

A little are present in the literature evaluating the bioaccessibility of chlorogenic acids in coffee and creamer formulations. An *in vivo* study on the bioavailability of chlorogenic acids in coffee demonstrated that the addition of 10% (v/v) whole milk had no significant effect on the absorption of chlorogenic acids, measured based on plasma concentration. By contrast, the addition of creamer delayed their detection in plasma without affecting bioavailability (Renouf, Marmet, Guy, Fraering, Longet, Moulin, Enslen, Barron, Cavin, & Dionisi, 2010).

Therefore, the bioaccessibility and bioavailability of coffee polyphenols should be considered based on the complexity of creamer products with different contents of milk proteins (and other macronutrients), pH values, and ionic strengths.

I-6. References

Bhat, M. Y., Dar, T. A., & Singh, L. R. (2016). Casein proteins: structural and functional aspects. *Milk Proteins; From Structure to Biological Properties and Health Aspects,* 1-17. London, England: InTech.

Bingham, E. W., Farrell, H. M., & Carroll, R. J. (1972). Properties of dephosphorylated αs_1-casein. Precipitation by calcium ions and micelle formation. *Biochemistry, 11*(13), 2450-2454.

Davis, P. F. (1980). Rennet casein and method for treatment thereof. US Patents: US4213896A.

Di Chen, S. B. W., Yang, H., Yuan, J., Chan, T. H., & Dou, Q. P. (2011). EGCG, green tea polyphenols and their synthetic analogs and prodrugs for human cancer prevention and treatment. *Advances in Clinical Chemistry, 53*, 155.

Dickinson, E. (1998). Proteins at interfaces and in emulsions stability, rheology and interactions. *Journal of the Chemical Society, Faraday Transactions, 94*(12), 1657-1669.

Frazier, R. A., Deaville, E. R., Green, R. J., Stringano, E., Willoughby, I., Plant, J., & Mueller-Harvey, I. (2010). Interactions of tea tannins and condensed tannins with proteins. *Journal of Pharmaceutical and Biomedical Analysis, 51*(2), 490-495.

Golde, A., & Schmidt, K. (2005). Quality of coffee creamers as a function of protein source. *Journal of Food Quality, 28*(1), 46-61.

Guo, W., Kong, E., & Meydani, M. (2009). Dietary polyphenols, inflammation, and cancer. *Nutrition and Cancer, 61*(6), 807-810.

Han, J., Britten, M., St-Gelais, D., Champagne, C. P., Fustier, P., Salmieri, S., & Lacroix, M. (2011). Polyphenolic compounds as functional ingredients in cheese. *Food Chemistry, 124*(4), 1589-1594.

Harman, D. (1999). Aging: minimizing free radical damage. *Journal of Anti-Aging Medicine, 2*(1), 15-36.

Hasni, I., Bourassa, P., Hamdani, S., Samson, G., Carpentier, R., & Tajmir-Riahi, H.-A. (2011). Interaction of milk α-and β-caseins with tea polyphenols. *Food Chemistry, 126*(2), 630-639.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*(6), 1841-1856.

Kanakis, C., Hasni, I., Bourassa, P., Tarantilis, P., Polissiou, M., & Tajmir-Riahi, H.-A. (2011). Milk β-lactoglobulin complexes with tea polyphenols. *Food Chemistry, 127*(3), 1046-1055.

Keogh, J. B., McInerney, J., & Clifton, P. M. (2007). The effect of milk protein on the bioavailability of cocoa polyphenols. *Journal of Food Science, 72*(3), S230-S233.

Khatkar, S. K., & Gupta, V. K. (2014). Physicochemical and functional quality

attributes of dairy whitener prepared from ultrafiltration process. *Journal of Food Processing and Preservation, 38*(3), 1145-1154.

Koo, C. K., Chung, C., Fu, J.-T. R., Sher, A., Rousset, P., & McClements, D. J. (2019). Impact of sodium caseinate, soy lecithin and carrageenan on functionality of oil-in-water emulsions. *Food Research International, 123*, 779-789.

Le Bourvellec, C., & Renard, C. (2012). Interactions between polyphenols and macromolecules: quantification methods and mechanisms. *Critical Reviews in Food Science and Nutrition, 52*(3), 213-248.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *The American Journal of Clinical Nutrition, 79*(5), 727-747.

Meena, G. S., Singh, A. K., Gupta, V. K., Borad, S., Arora, S., & Tomar, S. K. (2018). Effect of pH adjustment, homogenization and diafiltration on physicochemical, reconstitution, functional and rheological properties of medium protein milk protein concentrates (MPC70). *Journal of Food Science and Technology, 55*(4), 1376-1386.

Meena, G. S., Singh, A. K., Panjagari, N. R., & Arora, S. (2017). Milk protein concentrates: opportunities and challenges. *Journal of Food Science and Technology, 54*(10), 3010-3024.

Menshchikova, E., Lankin, V., Zenkov, N., Bondar, I., Krugovykh, N., & Trufakin, V. (2006). *Oxidative stress: Prooxidants and Antioxidants*, 136. Slovo, Moscow,

Russia.

Mulvihill, D., & Murphy, P. (1991). Surface active and emulsifying properties of caseins/caseinates as influenced by state of aggregation. *International Dairy Journal, 1*(1), 13-37.

Niseteo, T., Komes, D., Belščak-Cvitanović, A., Horžić, D., & Budeč, M. (2012). Bioactive composition and antioxidant potential of different commonly consumed coffee brews affected by their preparation technique and milk addition. *Food Chemistry, 134*(4), 1870-1877.

Prodpran, T., Benjakul, S., & Phatcharat, S. (2012). Effect of phenolic compounds on protein cross-linking and properties of film from fish myofibrillar protein. *International Journal of Biological Macromolecules, 51*(5), 774-782.

Quan, W., Qie, X., Chen, Y., Zeng, M., Qin, F., Chen, J., & He, Z. (2020). Effect of milk addition and processing on the antioxidant capacity and phenolic bioaccessibility of coffee by using an in vitro gastrointestinal digestion model. *Food Chemistry, 308*, 125598.

Rashidinejad, A., Birch, E. J., Sun-Waterhouse, D., & Everett, D. W. (2013). Effects of catechin on the phenolic content and antioxidant properties of low-fat cheese. *International Journal of Food Science & Technology, 48*(12), 2448-2455.

Renouf, M., Marmet, C., Guy, P., Fraering, A.-L., Longet, K., Moulin, J., Dionisi, F. (2010). Nondairy creamer, but not milk, delays the appearance of coffee

phenolic acid equivalents in human plasma. *The Journal of Nutrition, 140*(2), 259-263.

Sánchez-González, I., Jiménez-Escrig, A., & Saura-Calixto, F. (2005). *In vitro* antioxidant activity of coffees brewed using different procedures (Italian, espresso and filter). *Food Chemistry, 90*(1-2), 133-139.

Scalbert, A., & Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *The Journal of Nutrition, 130*(8), 2073S-2085S.

Schlesser, J. (2007). *Dairy Science and Technology*, Pieter Walstra, Jan T.M. Wouters, Tom J. Geurts (2nd ed.). Boca Raton, FL: CRC Press.

Singh, H. (2007). Interactions of milk proteins during the manufacture of milk powders. *Le Lait, 87*(4-5), 413-423.

Srinivasan, M., Singh, H., & Munro, P. (2003). Influence of retorting (121° C for 15 min), before or after emulsification, on the properties of calcium caseinate oil-in-water emulsions. *Food Chemistry, 80*(1), 61-69.

Stalmach, A., Mullen, W., Nagai, C., & Crozier, A. (2006). On-line HPLC analysis of the antioxidant activity of phenolic compounds in brewed, paper-filtered coffee. *Brazilian Journal of Plant Physiology, 18*, 253-262.

Stojadinovic, M., Radosavljevic, J., Ognjenovic, J., Vesic, J., Prodic, I., Stanic-Vucinic, D., & Velickovic, T. C. (2013). Binding affinity between dietary polyphenols and β-lactoglobulin negatively correlates with the protein susceptibility to digestion and total antioxidant activity of complexes formed.

Food Chemistry, 136(3-4), 1263-1271.

Sun-Waterhouse, D., Zhou, J., & Wadhwa, S. S. (2013). Drinking yoghurts with berry polyphenols added before and after fermentation. *Food Control, 32*(2), 450-460.

Swaisgood, H. (1985). Characteristics of edible fluids of animal origin: milk. *Food Chemistry, 2*, 796.

Tran, K. M., & Einerson, M. A. (1987). A rapid method for the evaluation of emulsion stability of non-dairy creamers. *Journal of Food Science, 52*(4), 1109-1110.

Trigueros, L., Wojdyło, A., & Sendra, E. (2014). Antioxidant activity and protein–polyphenol interactions in a pomegranate (Punica granatum L.) yogurt. *Journal of Agricultural and Food Chemistry, 62*(27), 6417-6425.

Trombley, J. D., Loegel, T. N., Danielson, N. D., & Hagerman, A. E. (2011). Capillary electrophoresis methods for the determination of covalent polyphenol–protein complexes. *Analytical and Bioanalytical Chemistry, 401*(5), 1523-1529.

Valko, M., Morris, H., & Cronin, M. (2005). Metals, toxicity and oxidative stress. *Current Medicinal Chemistry, 12*(10), 1161-1208.

Valko, M., Rhodes, C., Moncol, J., Izakovic, M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-biological Interactions, 160*(1), 1-40.

Yashin, Y. I., Ryzhnev, V. Y., Yashin, A. Y., & Chernousova, N. (2009). *Natural*

Antioxidants: Their Content in Foods and Impact of Human Health and Aging. TransLit: Moscow, Russia.

Ye, A. (2011). Functional properties of milk protein concentrates: Emulsifying properties, adsorption and stability of emulsions. *International Dairy Journal, 21*(1), 14-20.

Ye, J., Fan, F., Xu, X., & Liang, Y. (2013). Interactions of black and green tea polyphenols with whole milk. *Food Research International, 53*(1), 449-455.

Yildirim-Elikoglu, S., & Erdem, Y. K. (2018). Interactions between milk proteins and polyphenols: Binding mechanisms, related changes, and the future trends in the dairy industry. *Food Reviews International, 34*(7), 665-697.

Yuksel, Z., Avci, E., & Erdem, Y. K. (2010). Characterization of binding interactions between green tea flavanoids and milk proteins. *Food Chemistry, 121*(2), 450-456.

Zhang, H., Yu, D., Sun, J., Guo, H., Ding, Q., Liu, R., & Ren, F. (2014). Interaction of milk whey protein with common phenolic acids. *Journal of Molecular Structure, 1058*, 228-233.

Ziyatdinova, G., Nizamova, A., & Budnikov, H. (2011). Novel coulometric approach to evaluation of total free polyphenols in tea and coffee beverages in presence of milk proteins. *Food Analytical Methods, 4*(3), 334-340.

Chapter II.

Emulsifying Properties of Creamers

II-1. Introduction

The milk proteins used as emulsifiers impart long-term stability to creamers. Acid casein and sodium caseinate, which have good emulsifying and stabilizing properties, are commonly used, particularly for powdered creamer, which requires high heat and acid resistance for use in coffee (Vega & Roos, 2006). Milk protein concentrate, whey protein, skim milk (SM) powder, and micellar casein are used to impart sensory attributes to creamers and have emulsifying activity.

Creamers should remain stable when added to hot and acidic coffee, and exert a whitening effect and impart desirable mouthfeel properties (Golde & Schmidt, 2005). Therefore, phenomena indicating instability, such as feathering, creaming and sedimentation, should be considered when formulating creamers.

Instability typically occurs during the processing and storage of creamers, which are thermodynamically unstable. Such changes may be the result of several factors, including thermal aggregation caused by heat treatment; isoelectric aggregation caused by the pH of coffee being similar to the isoelectric points of proteins; and electrostatic aggregation caused by electrostatic repulsive interactions in the presence of soluble salts (Hamboyan, Pink, Klapstein, MacDonald, & Aboud, 1989; Oldfield, Teehan, & Kelly, 2000; Tran & Einerson, 1987). These are largely the result of interactions between proteins and oil droplets, different proteins, and different oils in the emulsion.

The structure, conformation, and aggregation state of milk proteins in creamer products are affected by the processing conditions; *e.g.*, temperature, homogenization pressure, type of spray drying, and types and contents of buffer salts. Buffer salts are used to minimize heat damage and chelate calcium ions, facilitating thermal aggregation and resistance to electrostatic repulsion (Meena, Singh, Gupta, Borad, Arora, & Tomar, 2018).

In this chapter, the emulsion characteristics and stability of creamers (according to milk protein types and contents) were evaluated and used to predict creamer stability in an *in vitro* digestion model.

II-2. Materials and Methods

II-2-1. Materials

Maltodextrin dextrose equivalent (DE) 20 and vegetable oil were supplied by Ingredion Incorporated (Incheon, Republic of Korea) and Dongsuh Oil & Fats Co., Ltd. (Changwon, Republic of Korea). Buffer salts including potassium phosphate dibasic and potassium citrate were obtained from Youngjin Core-Chem Company (Yesan, Republic of Korea) and mono-di-glycerin fatty acid esters and sodium stearyl lactylate as emulsifiers were obtained from Kerry Ingredients (Penang, Malaysia). Milk protein concentrate and acid casein were obtained from Fonterra Co-operative Group Ltd. (Auckland, New Zealand) and skim milk were purchased from Maeil Dairies Co., Ltd. (Pyeongtaek, Republic of Korea).

Instant coffee, a commercial product made from mixed coffee beans (*Coffea arabica: Coffea canephora* = 7:3, w/w), was provided by Dong Suh Foods Corp. (Seoul, Republic of Korea) and stored at −18°C until use.

II-2-2. Creamer preparation and composition

Vegetable oil and emulsifiers were heated to 60℃ in a water bath, and acid casein was dissolved into a buffer salt solution. For creamer A, Maltodextrin DE 20 and distilled water were heated to 60℃ in a water bath. Then, acid casein (3%, w/w) solubilized with buffer salts was added, followed by stirring for at least 30 min to ensure complete dispersion. Next, a mixture of vegetable oil and emulsifiers was blended and stirred for 30 min at 60–65℃.

Creamer B was prepared similarly, but milk protein concentrate and skim milk were used as protein sources. Milk protein concentrate and skim milk were dissolved in hot water but were used after dissociated in the buffer salts solution to chelate calcium ions.

The coarse emulsions were homogenized using a two-stage high pressure valve homogenizer at a first-stage pressure of 20 MPa and second-stage pressure of 4 MPa, and spray-dried using a spray dryer (Mobile Minor; GEA, Düsseldorf, Germany) at an inlet air temperature of 175℃ and outlet air temperature of 80℃.

The major components and milk protein sources used in creamer A and creamer B are listed in Table II-1. The main difference between two samples was the type and content of milk protein, but there were some adjustments in oil composition to impart the emulsion stability considering the emulsifying ability of each protein.

Table II-1. Major components (%, w/w) and protein sources of creamer A and creamer B

	Creamer A	**Creamer B**
Components (%, w/w)		
Protein	2.7	8.5
Vegetable oil	30.0	26.0
Matodextrin DE 20	62.0	57.0
Buffering salts	< 3.0	< 3.0
Others	< 2.5	< 6.0
Protein source	Acid casein	Milk protein concentrate skim milk

II-2-3. Determination of particle size distribution

The mean droplet diameter (nm) and particle size distribution of creamer A, creamer B, instant coffee with creamer A (CRA), and instant coffee with creamer B (CRB) were measured by static light scattering (Malvern Mastersizer, Malvern Instruments, Worcestershire, UK).

Creamer A and creamer B were prepared by dissolving 5 g of creamer samples in hot distilled water. For CRA and CRB, 5 g of creamers and 1.6 g of instant coffee powder were added to hot distilled water. Before analysis, samples were shaken gently to ensure uniformity.

A refractive index ratio of 1.08 was used to calculate the particle-size distribution. The particle size distribution was calculated by finding the best fit between the predictions of Mie theory and the measured light-scattering pattern. The particle diameter (μm) was reported as the volume-weighted mean diameter ($d_{4,3}$), with $d_{4,3}$ being defined as ($d_{4,3} = \sum n_i d_i^4 / \sum n_i d_i^3$), where n_i is the number of droplets of diameter d_i. The mean diameter $d_{4,3}$ was selected to represent the average of all particle diameters, because $d_{4,3}$ is more sensitive to the presence of particles of diameter di (McClements, 2004). The mean particle diameter, $d_{4,3}$ was calculated as the average of triplicate measurements.

II-2-4. Zeta potential measurement

The electrical charge (zeta potential; ζ-potential) of the droplets in creamer A, creamer B, CRA, and CRB was determined by particle electrophoresis (Zetamaster; Malvern Instruments, Worcestershire, UK) to determine the surface charge (mV). The zeta potential is determined by measuring the direction and velocity of droplet movement in the applied electric field.

Creamer samples, CRA, and CRB were diluted in distilled water at 1/1,000 and placed in a clear disposable zeta shell (Model DTS 1060C; Malvern Instruments) without air bubbles to assess particle electrophoretic mobility. The cell was maintained at 60℃.

The zeta potential was calculated from the average of three measurements of diluted emulsion and expressed in millivolts (mV).

II-2-5. Turbiscan analysis

The emulsion stability of creamers A and B was evaluated using a vertical scan analyzer (Turbiscan MA 2000; Formulaction, Toulouse, France). Creamer samples were dissolved to about 5% (w/w; a standard intake amount) in distilled water and 20 mL was transferred to a glass cylindrical cell and analyzed using a near-infrared (800 nm) light beam scanning vertically from bottom to top. The detection head scans the entire length of the sample, acquiring transmission and backscattering data every 40

μm (Lemarchand, Couvreur, Vauthier, Costantini, & Gref, 2003). The temperature of the glass cell was maintained at 60℃ during the 2 h assay.

Two synchronous optical sensors receive light transmitted through the sample (180° from the incident light) and light backscattered by the sample (45° from the incident radiation). The detection head scanned the entire length of the sample (about 50 mm), acquiring backscattering data every 10 min for 2 h, and the time-dependent behavior of the emulsion was analyzed quantitatively.

In this study, the change in delta backscattering (Δ-BS) profiles was calculated as the difference between backscattering flux at 0 h and a given time. The average changes in Δ-BS over time in the bottom, middle and top phase were analyzed using Turbiscan MA 2000 software. The Turbiscan stability index (TSI) was calculated based on backscattering changes using Turbiscan MA 2000 software to assess the stability of creamer emulsions over time.

II-2-6. Protein composition and interfacial proteins in creamer

Each 5.0 g of creamer A or creamer B was dissolved in 100 mL distilled water and 62.5 mL skim milk in 37.5 mL distilled water at 80 rpm for 10 min at 45°C. Then, the serum and cream phases in 1.0 mL samples were separated by centrifugation at 30,000 × g for 1 h at 4°C, followed by withdrawing the serum phase using a syringe.

The total protein content (mg) in 100 mL each solution was as follows: creamer A, 120 mg; creamer B, 729 mg; skim milk, 1,875 mg. Skim milk was used as a control to confirm the compositions of milk proteins in this experiment. The protein content in the serum phase (non-adsorbed proteins) and cream phase (adsorbed protein) were determined using a Bradford assay (Kruger, 2009). The protein composition of total proteins (without separation) and in the serum and cream phases was identified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) analyses under reducing conditions.

The separation and stacking gel contained 12% and 4% (w/v) polyacrylamide, respectively. For the analysis, 15 μL aliquots of each sample and 15 μL buffer solution (Tris 0.5 M, SDS 10% (w/v), glycerol 20% (w/v), bromophenol 0.05% (v/v), and β-mercaptoethanol 10% (v/v)) were mixed and boiled for 10 min. Then, 15 μL samples were applied to each well. Electrophoresis was conducted at a constant current of 20 mA in a Hoefer SE 250 mini-gel system (GE Healthcare, Chicago, IL, USA) at room temperature. The gels were stained with Coomassie blue R-250 and destained in acetic acid/methanol/water (1:4:5, v/v) solution for 24 h.

II-2-7. Statistical analysis

All experiments were performed in triplicate, unless stated otherwise, and the results are presented as means and standard deviations. The experimental data were analyzed by Two sample t-test and the significant differences among the means were determined at a 95% confidence level ($p < 0.05$).

II-3. Results and Discussion

II-3-1. Particle size and size distribution

The particle size distribution of re-dispersed creamer emulsions containing acid casein or milk protein concentrate and skim milk at 5% (w/w) are shown in Figure II-1. The emulsions prepared using creamer A and creamer B had similar bimodal size distributions. Creamer A and creamer B showed good emulsification in the beginning stages of powdered creamer in a narrow size range of 0.1–1.0 μm.

The size distribution by volume (%) versus particle size showed that creamer A had a smaller particle size distribution than creamer B. Therefore, the size of the oil droplets is mainly affected by the types and concentrations of milk proteins used as emulsifiers under same processing conditions. The higher stability caused by the formation of smaller micelles likely protected bioactive compounds from degradation during digestion (Otemuyiwa, Williams, & Adewusi, 2017).

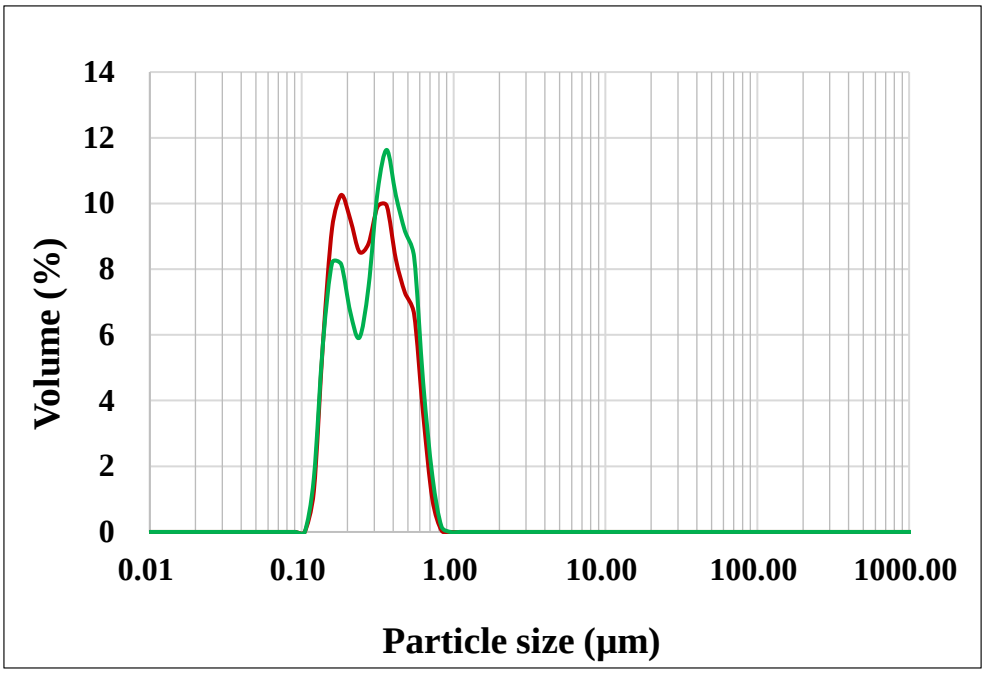


Figure II-1. Droplet size distributions of creamers (5%, w/w) containing acid casein (creamer A, red), and milk protein concentrate and skim milk (creamer B, green).

Acid casein has excellent emulsifying ability and can create stable emulsions at a low protein-to-oil ratio (~1:50) (Taneja, Ye, & Singh, 2015). Therefore, creamer A, which has a higher protein-ratio compared to oil content, showed a stable emulsification state.

In contrast, the emulsifying ability of aggregated milk protein products, such as milk protein concentrate and heat-treated skim milk, is lower than that of acid casein and the emulsions have larger droplets (Taneja et al., 2015). In emulsion using milk protein concentrate, the coverage of the oil droplet surface by protein is hampered by the presence of colloidal calcium phosphate (Euston & Hirst, 1999; Singh & Ye, 2020).

For creamer B, the types and contents of calcium chelating salts to increase the dissociation of milk proteins were considered, resulting in a relatively stable emulsion. There were no significant differences in mean particle diameter ($d_{4,3}$) values (creamer A, 0.305 μm; creamer B, 0.33 μm). These results are consistent with a report that the $d_{4,3}$ of commercial creamer was 0.34 μm from pH 5.5 to 7.0 (Chung, Sher, Rousset, & McClements, 2017b).

The addition of creamer B, but not creamer A, to coffee affected the mean particle diameter (Figure II-2). Therefore, creamer A, which contains acid casein, was more stable than creamer B, which contains milk protein concentrate and skim milk, under hot and acidic conditions.

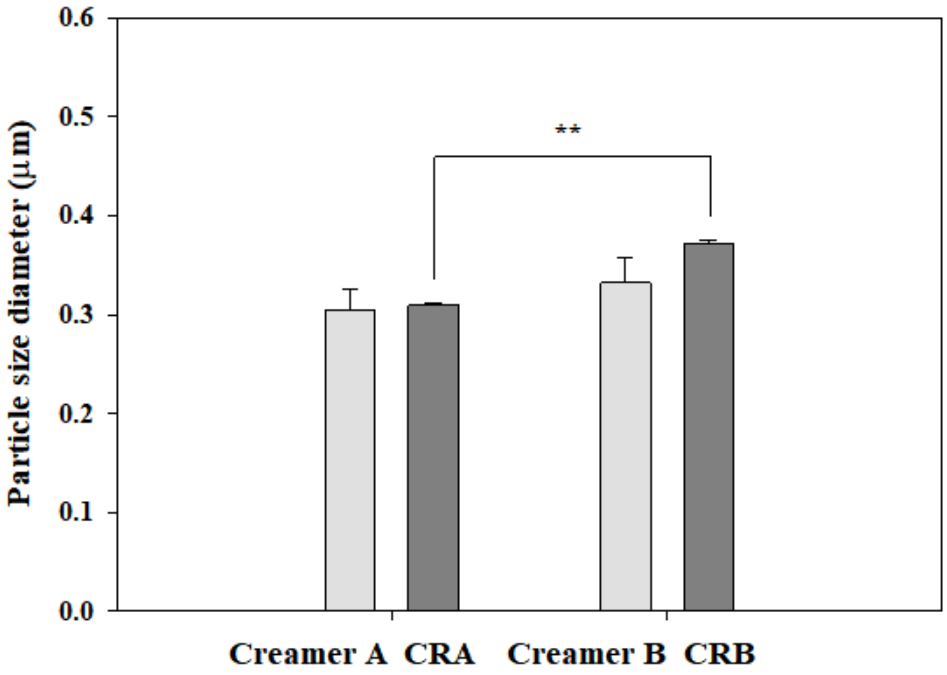


Figure II-2. Effect of creamer types on the mean particle size diameter ($d_{4,3}$) of creamer A, creamer B, instant coffee with creamer A (CRA), and instant coffee with creamer B (CRB). CRA and CRB were prepared by dissolving 1.6 g coffee powder with 5.0 g creamer powder in the hot distilled water. The data are presented as means with standard deviations (n=3). An asterisk indicates a significant difference in the mean particle size diameter depending on the creamer types ($p < 0.01$).

II-3-2. Zeta potential

Zeta potential is widely used to evaluate emulsion stability to aggregation depending on the electrical potential of the oil droplets (Chung et al., 2017b).

The electrical properties of creamer samples, CRA, and CRB were measured by particle electrophoresis to evaluate emulsion stability. The zeta potential of droplets in creamer A and creamer B was -33.4 and -34.7 mV, compared to -31.5 and -31.6 mV for CRA and CRB, respectively (Figure II-3). The differenced may be due to changes in the pH and ionic conditions caused by coffee addition (Chung, Sher, Rousset, & McClements, 2017a).

It is known that a high negative zeta potential prevents droplet aggregation due to electrostatic repulsion, and larger droplets having a smaller surface area to cover typically have a high zeta potential (McClements, 2004). However, there was no significant difference between creamers A and B, or between CRA and CRB.

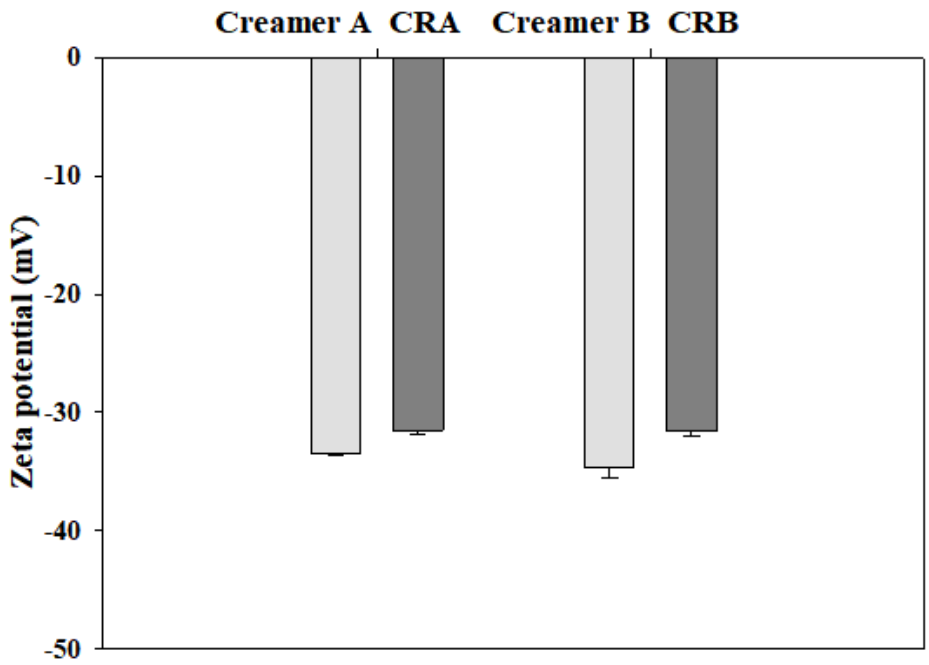


Figure II-3. Effect of creamer types on zeta potential of creamer A, creamer B, instant coffee with creamer A (CRA), and instant coffee with creamer B (CRB). CRA and CRB was prepared by dissolving 1.6 g coffee powder with 5.0 g creamer powder in the hot distilled water. The data are presented as means with standard deviations (*n*=3)

Chung *et al*. reported that caseinate-stabilized emulsions had a surface potential of -28 to -33 mV, and that the zeta potential of droplets in commercial creamer was highly negative (-44 mV) at pH 7.0 (Chung, Koo, Sher, Fu, Rousset, & McClements, 2019; Chung et al., 2017b).

The similar zeta potential of creamer B could be caused by the removal of calcium ions from the casein micelle of milk protein concentrate by calcium-chelating salts. Also, negatively charged and dissociated proteins (*e.g*., non-micellar acid casein) were incorporated on the oil droplet surface.

Colloids of -30 to -40 mV zeta potential are electrically stable and resist droplet aggregation and creaming (Kumar & Dixit, 2017). Despite the use of different milk protein types and contents, there was no significant difference in the stability of the colloidal dispersions.

II-3-3. Emulsion stability

The Δ-BS profiles are shown in Figure II-4. Turbiscan analysis enables identification all destabilization phenomena caused by particle migration (sedimentation, creaming, etc.) and granulometric variation (flocculation, coalescence, etc.) at an early stage (Lemarchand et al., 2003; McClements, Decker, Park, & Weiss, 2009).

The TSI was used to evaluate emulsion stability, and was calculated using the following formula:

$$\mathrm{TSI} = \sqrt{\frac{\sum_{i=1}^{n} (x_i - x_{\mathrm{BS}})^2}{n-1}}$$

where x_i is the average value of the scattered light intensity at each time, x_{BS} is the average of $x_{i,}$ and n is the number of scans. The lower the TSI value, the higher the stability of the emulsion (Lu et al., 2017). The TSI values of creamer emulsions with acid casein and milk protein concentrate at pH 7.4 were 1.34 and 1.27, respectively.

As shown in Figure II-4, the backscattering flux in creamer A at the top of the tube increased significantly compared to creamer B, and the TSI of creamer A was slightly higher than that of creamer B. Therefore, the creamer A emulsion was more

unstable than creamer B. However, clarification at the bottom occurs with creaming, and the decrease in Δ-BS profile of creamer B was greater than that of creamer A.

For further precise interpretation, the cell was divided into top, middle, and bottom zones. The first zone is classified into the bottom of the cell which sedimentation phenomena occurs and the second can find whether the flocculation occurs at the middle of the cell. Finally, the third one is the top of the cell which clarification occurs. The changes in average intensity in backscattering flux over time are shown in Figures II-5, II-6, and II-7.

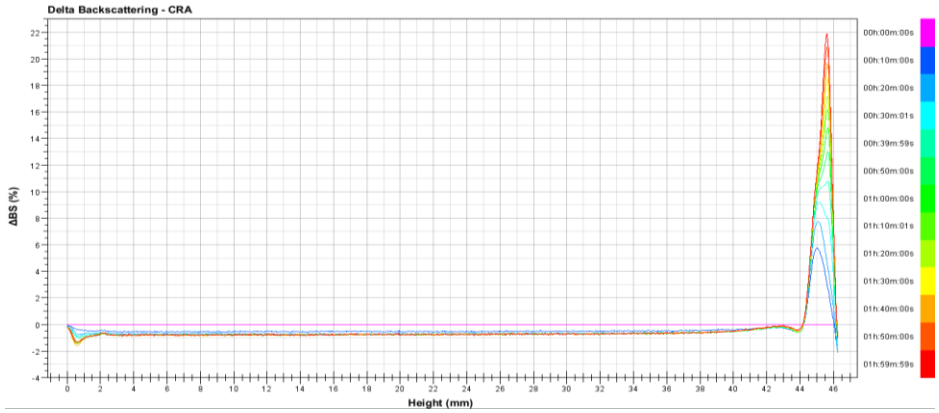
Coalescence for creamer B occurred more rapidly than for creamer A in the middle of the tube, and the Δ-BS profile of creamer B increased after 30 min, likely leading to the entanglement phenomenon.

As shown in Figure II-7, BS varied significantly over time in the top zone. Creamer B showed a more rapid decrease in Δ-BS profile at the bottom of the tube. However, low backscattering variation was observed at the top, indicating that the particles were closer together and became more closely packed over time. This could be explained by the low fat and high protein contents, promoting creaming at a lower height and a closer-packed, denser layer at the top.

Therefore, the creamer A emulsion was more stable over time because its TSI value was higher than that of creamer B. However, the variation of aggregation and creaming (following clarification) in creamer B was greater. The different kinetics of

the Δ-BS profiles of creamer B are attributed to greater amounts of protein being adsorbed at the oil-water interface, which slows degradation.

(A)



(B)

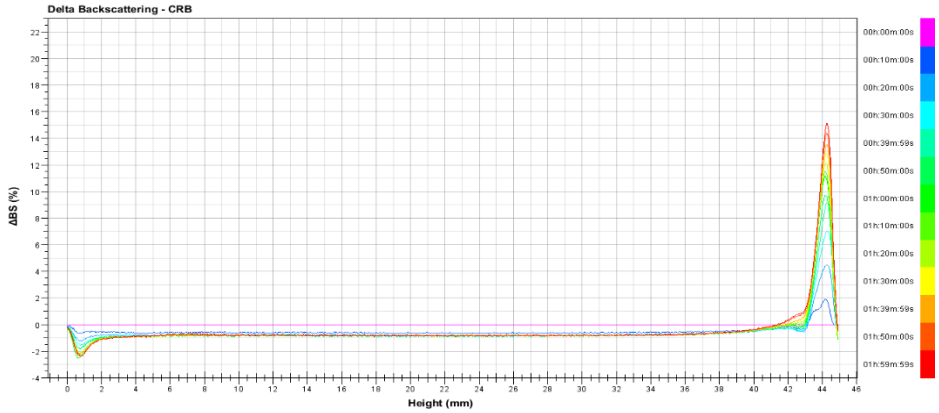
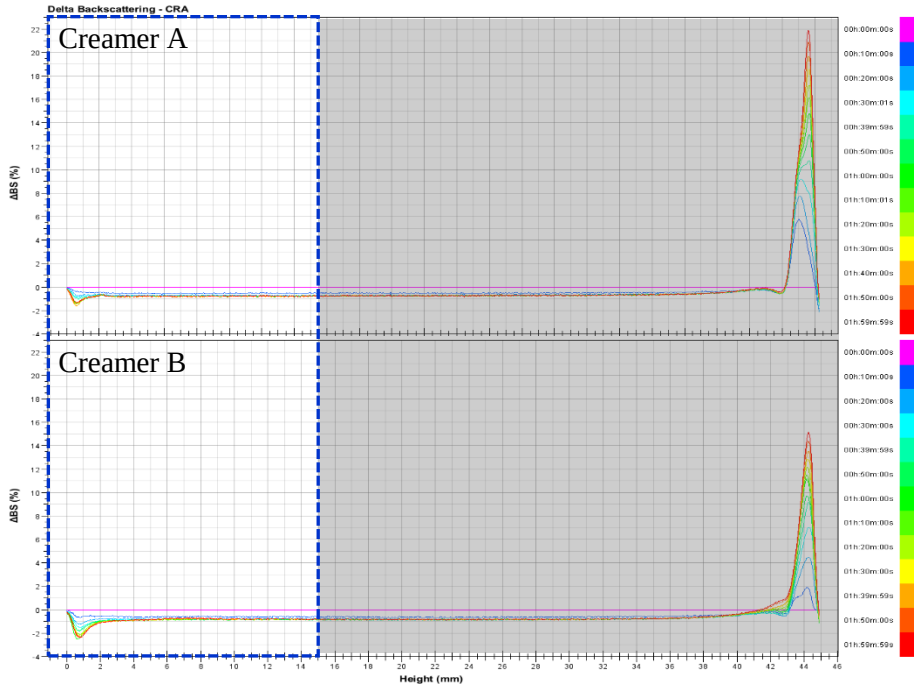


Figure II-4. The Δ-BS profiles of creamer A emulsion (5%, w/v) and creamer B emulsion (5%, w/v) as a function of time (0-2 h) and of sample height (mm). (A) Creamer A and (B) creamer B.

(A)



(B)

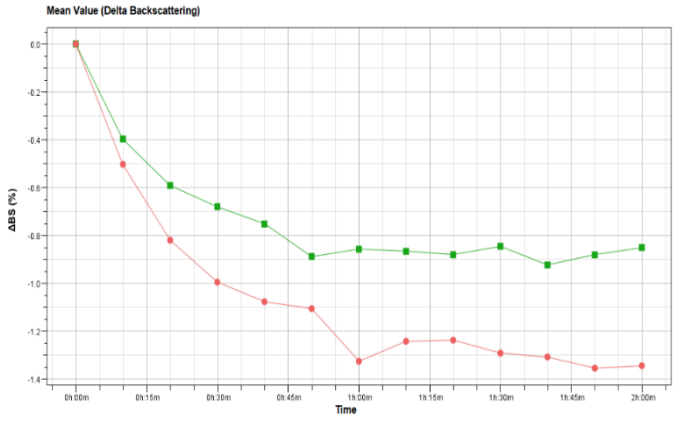
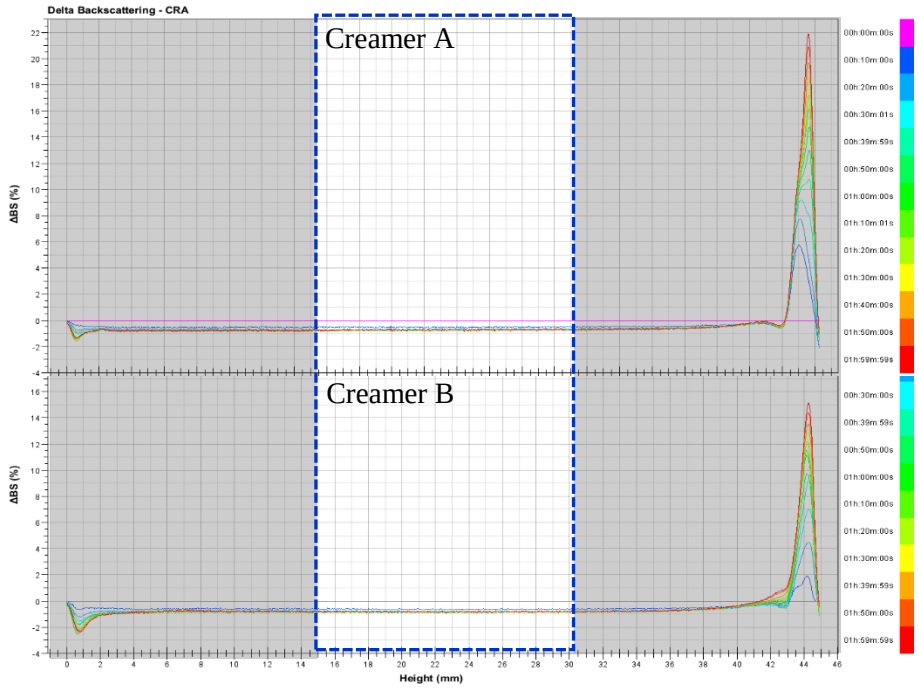


Figure II-5. (A) The Δ-BS profiles at the bottom of the cell for creamer A and creamer B (5%, w/v) and (B) the mean Δ-BS showing destabilization of creamer A (green) and creamer B (red).

(A)



(B)

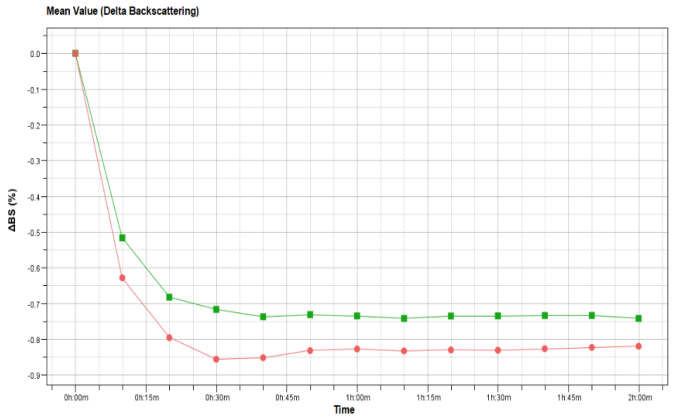
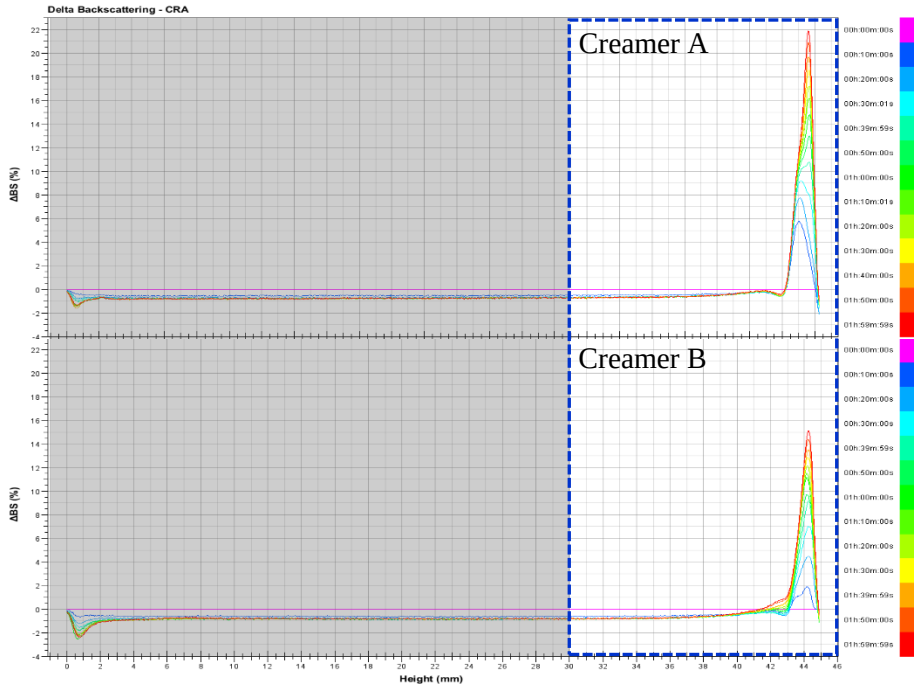


Figure II-6. (A) The Δ-BS profiles at the middle of the cell for creamer A and creamer B (5%, w/v) and (B) the mean Δ-BS showing destabilization of creamer A (green) and creamer B (red).

(A)



(B)

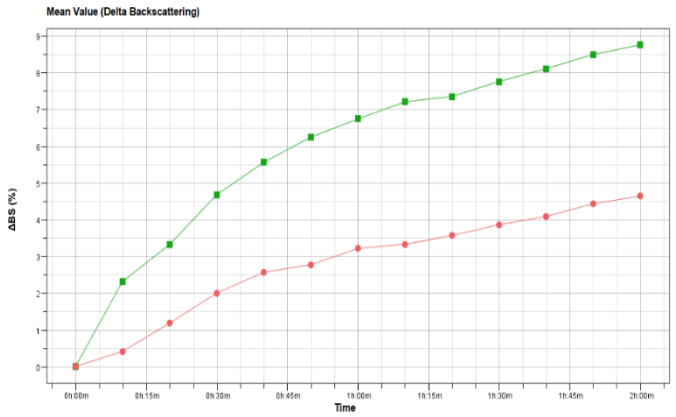


Figure II-7. (A) The Δ-BS profiles at the top of the cell for creamer A and creamer B (5%, w/v) and (B) the mean Δ-BS showing destabilization of creamer A (green) and creamer B (red).

II-3-4. Protein composition and interfacial proteins in creamer and skim milk

As described in Chapter I-1, it is assumed that the nature of the milk proteins at the oil-water interface plays a critical role in determining the overall emulsion stability. Not only that, protein types and adsorption properties at oil-water interfaces in creamers could affect protein digestibility in the gastro-pancreatic environment.

Digestibility causes variation in the complexation between protein and phenolic compounds during gastric and pancreatic digestion, which in turn affect the bioaccessibility and antioxidant activity of phenolic compounds (Macierzanka, Sancho, Mills, Rigby, & Mackie, 2009). Therefore, protein compositions and adsorption properties at oil-water interfaces among creamer A, creamer B, and skim milk were analyzed and compared.

The content of adsorbed and non-adsorbed proteins in creamer A, creamer B and skim milk are shown in Table II-2.

Table II-2. Content of adsorbed and non-adsorbed proteins at the oil-water interface in creamer A, creamer B, and skim milk. Values in parentheses are percentages (%) of adsorbed and non-adsorbed protein content with respect to total protein content

	Protein content (mg/100 mL)	
	Adsorbed protein	**Non-adsorbed protein**
Creamer A	42.8 ± 5.4 (35.9 ± 4.5)	72.0 ± 7.8 (60.0 ± 6.5)
Creamer B	178.0 ± 4.6 (24.4 ± 0.6)	567.4 ± 9.9 (77.8 ± 1.4)
Skim milk	N.D.*	1923.9 ± 64.1 (102.6 ± 3.4)

Data are means ± standard deviations (n = 3).

* N.D.: not determined.

As shown in Table II-1, the proportion of adsorbed protein to non-adsorbed protein was higher in creamer A than that of creamer B, which was possibly attributed to the good adsorption ability of acid casein and the lower lipid amount but the higher total protein amount in creamer B than in creamer A. In skim milk, which contains tiny amounts of lipids, all proteins were recovered from the serum phase, indicating that all proteins were evenly dispersed in the serum phase.

The composition of total proteins, adsorbed proteins and non-adsorbed proteins in creamer A, creamer B, and skim milk was identified by SDS-PAGE analyses (Figure II-8). The whey proteins lactoferrin (76.1 kDa) and serum albumin (66.4 kDa), and caseins (24-35 kDa) were observed in total protein fraction in all samples (lane 2, 5, and 8). Meanwhile, the differences in the compositions of total proteins in creamer A, creamer B, and skim milk were the presence of β-lactoglobulin (18.4 kDa) and α-lactalbumin (14.2 kDa). Creamer A containing lactic casein showed no bands of β-lactoglobulin and α-lactalbumin. By contrast, in creamer B containing milk protein concentrate and skim milk powder, β-lactoglobulin was observed, whereas α-lactalbumin was not identified, which was possibly due to its lower protein content than that in skim milk.

In case of skim milk used as a control to confirm the protein composition of milk (lane 8), bands with high intensity were observed in a broad range from 24 to 35kDa, likely from the casein fraction, which includes αs_1-, αs_2-, β-, and κ-caseins with different molecular weights. Among total proteins in creamer A (lane 2) and

creamer B (lane 5), we observed a sharper band of casein than in skim milk. These results indicated that casein conformation in creamer A and creamer B differed from that in skim milk. As described in Chapter I, the conformational changes of casein in creamer A were caused by the acidification process during the manufacturing of lactic casein from milk (Dalgleish & Corredig, 2012). Casein conformation in creamer B is mainly changed to the non-micellar form due to the addition of calcium chelators and other dissociation process during preparation (Liang, Matia-Merino, Gillies, Patel, Ye, & Golding, 2017).

In terms of protein adsorption behavior, a high-intensity casein band was observed in adsorbed proteins in creamer A (lane 3), indicating that casein was the major protein adsorbed at oil-water interfaces in creamer A. However, lactoferrin and serum albumin were also located at the interfaces, suggesting that adsorption was non-specific among the proteins in creamer A. Similarly, no differences in the protein compositions of adsorbed (lane 6) and non-adsorbed proteins (lane 7) were observed in creamer B, which contains lactoferrin, serum albumin, caseins, β-lactoglobulin, and α-lactalbumin.

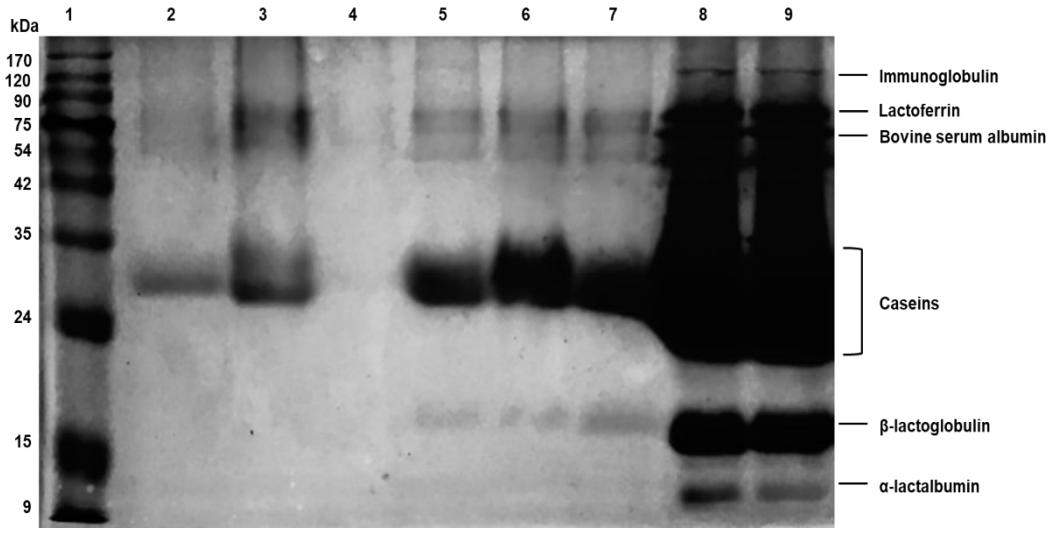


Figure II-8. SDS-PAGE patterns of total proteins in creamer A, creamer B, and skim milk at oil and aqueous phases under reducing conditions. The pattern of proteins at the oil phase in skim milk was not determined due to the absence of protein. Lanes: 1, molecular mass standard; 2, total proteins in creamer A; 3, proteins at oil phase in creamer A; 4, proteins at aqueous phase in creamer A; 5, total proteins in creamer B; 6, proteins at oil phase in creamer B; 7, proteins at aqueous phase in creamer B; 8, total proteins in skim milk; 9, proteins at aqueous phase in skim milk.

II-4. Conclusions

Creamer A, which has a low protein content, had high emulsion stability; this was attributed to acid casein. Creamer B, which has a high protein content and is mainly composed of aggregated milk proteins, showed low emulsion stability.

The creamer A emulsion was more stable than the creamer B emulsion to droplet aggregation caused by pH changes, but there was no significant difference in mean particle size or distribution. This could be attributed to the different protein types, protein contents and calcium chelating effect of the creamer B.

II-5. References

Chung, C., Koo, C. K., Sher, A., Fu, J.-T. R., Rousset, P., & McClements, D. J. (2019). Modulation of caseinate-stabilized model oil-in-water emulsions with soy lecithin. *Food Research International, 122*, 361-370.

Chung, C., Sher, A., Rousset, P., & McClements, D. J. (2017a). Influence of homogenization on physical properties of model coffee creamers stabilized by quillaja saponin. *Food Research International, 99*, 770-777.

Chung, C., Sher, A., Rousset, P., & McClements, D. J. (2017b). Use of natural emulsifiers in model coffee creamers: Physical properties of quillaja saponin-stabilized emulsions. *Food Hydrocolloids, 67*, 111-119.

Dalgleish, D. G., & Corredig, M. (2012). The structure of the casein micelle of milk and its changes during processing. *Annual Review of Food Science and Technology, 3*, 449-467.

Euston, S. R., & Hirst, R. L. (1999). Comparison of the concentration-dependent emulsifying properties of protein products containing aggregated and non-aggregated milk protein. *International Dairy Journal, 9*(10), 693-701.

Golde, A., & Schmidt, K. (2005). Quality of coffee creamers as a function of protein source. *Journal of Food Quality, 28*(1), 46-61.

Hamboyan, L., Pink, D. A., Klapstein, D., MacDonald, L., & Aboud, H. (1989).

Ultraviolet spectroscopic studies on the feathering of cream in instant coffee. *Journal of Dairy Research, 56*(5), 741-748.

Kruger, N. J. (2009). The bradford method for protein quantitation. In *The Protein Protocols Handbook* (3rd ed.). Totowa, NJ: Humana Press.

Kumar, A., & Dixit, C. K. (2017). Methods for characterization of nanoparticles. In *Advances in Nanomedicine for the Delivery of Therapeutic Nucleic Acids*, 43-58. Sawston, England: Woodhead Publishing.

Lemarchand, C., Couvreur, P., Vauthier, C., Costantini, D., & Gref, R. (2003). Study of emulsion stabilization by graft copolymers using the optical analyzer Turbiscan. *International Journal of Pharmaceutics, 254*(1), 77-82.

Liang, Y., Matia-Merino, L., Gillies, G., Patel, H., Ye, A., & Golding, M. (2017). The heat stability of milk protein-stabilized oil-in-water emulsions: A review. *Current Opinion in Colloid & Interface Science, 28*, 63-73.

Lu, Y., Kang, W., Jiang, J., Chen, J., Xu, D., Zhang, P., Wu, H. (2017). Study on the stabilization mechanism of crude oil emulsion with an amphiphilic polymer using the β-cyclodextrin inclusion method. *RSC Advances, 7*(14), 8156-8166.

Macierzanka, A., Sancho, A. I., Mills, E. C., Rigby, N. M., & Mackie, A. R. (2009). Emulsification alters simulated gastrointestinal proteolysis of β-casein and β-lactoglobulin. *Soft Matter, 5*(3), 538-550.

McClements, D. J. (2004). *Food Emulsions: principles, practices, and techniques* (3rd ed.), 690. Boca Raton, FL: CRC Press.

McClements, D. J., Decker, E. A., Park, Y., & Weiss, J. (2009). Structural design principles for delivery of bioactive components in nutraceuticals and functional foods. *Critical Reviews in Food Science and Nutrition, 49*(6), 577-606.

Meena, G. S., Singh, A. K., Gupta, V. K., Borad, S., Arora, S., & Tomar, S. K. (2018). Effect of pH adjustment, homogenization and diafiltration on physicochemical, reconstitution, functional and rheological properties of medium protein milk protein concentrates (MPC70). *Journal of Food Science and Technology, 55*(4), 1376-1386.

Oldfield, D., Teehan, C., & Kelly, P. (2000). The effect of preheat treatment and other process parameters on the coffee stability of instant whole milk powder. *International Dairy Journal, 10*(9), 659-667.

Otemuyiwa, I. O., Williams, M. F., & Adewusi, S. A. (2017). Antioxidant activity of health tea infusions and effect of sugar and milk on *in-vitro* availability of phenolics in tea, coffee and cocoa drinks. *Nutrition & Food Science*, *47*(4), 458-468.

Singh, H., & Ye, A. (2020). Interactions and functionality of milk proteins in food emulsions. In *Milk Proteins* (2nd ed.), 467-497. Cambridge, Massachusetts, United States: Academic Press

Taneja, A., Ye, A., & Singh, H. (2015). Influence of protein concentration on the stability of oil-in-water emulsions formed with aggregated milk proteins

during spray drying. *Dairy Science & Technology, 95*(3), 279-293.

Tran, K. M., & Einerson, M. A. (1987). A rapid method for the evaluation of emulsion stability of non-dairy creamers. *Journal of Food Science, 52*(4), 1109-1110.

Vega, C., & Roos, Y. (2006). Invited review: spray-dried dairy and dairy-like emulsions—compositional considerations. *Journal of Dairy Science, 89*(2), 383-401.

Chapter III.

Changes in Creamer Emulsions and Bioaccessibility of Chlorogenic Acids during *in vitro* Digestion

III-1. Introduction

It is well known that a sort of food products with the same components do not always show equivalent nutritional properties. “Food matrix” is a structure that surrounds the nutrients and structural components and is often evaluated negligible in the estimation of the real nutritional value of a food product (Lamothe, Rémillard, Tremblay, & Britten, 2017). But food matrix has a significant effect on the digestion extent (bioaccessibility) and the absorption (bioavailability) of bioactive compounds and nutrients (Turgeon & Rioux, 2011).

As described in former chapter, many studies considered the interaction of polyphenols with compounds included in foods, like carbohydrates, proteins, or lipids, because these macronutrients can give a variation in the bioactivity of polyphenols. In addition to this, the changes in the food matrix lead to alterations in the protein properties, which consequently affects the complexation between protein and phenolic compounds (Sęczyk, Sugier, Świeca, & Gawlik-Dziki, 2021; Wu, Guo, & Lin, 2020). Therefore, food matrix effect should be considered at diverse aspects including the changes of components themselves, their interactions, and their fate upon digestion as well.

In this study, changes in physical properties and dispersion stability of particles in food matrix such as creamer with instant coffee can be evaluated by Turbiscan analysis, which is measured by the concentration and size of particles. Some

alterations in the protein properties including adsorption properties at oil-water interface and protein aggregation can cause the changes in creamer products with different dairy and food matrices during digestion. As the emulsions are formed with very stable status, there were also presumed that proteins and phenolic compounds might been protected from degradation during digestion. To predict this, the bioaccessibility of chlorogenic acids at the different pH values of gastro pancreatic digestion was investigated. For comparison between the effects of milk protein in milk on coffee polyphenols and those in creamer, skim milk which exclude milk fat effects was used as a control.

The bioaccessibility refer to the release of a nutrient from food matrix and the amount of a nutrient solubilized in the small intestine available for absorption, namely the stability under the gastrointestinal condition (Palafox-Carlos, Ayala-Zavala, & González-Aguilar, 2011; Lamothe, Azimy, Bazinet, Couillard, & Britten, 2014; Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010).

This is considered important since only the components released from special food matrix and remain stable in the gastro-intestinal environment can be bioavailable to exert their nutritional and beneficial effects. Initial contents, phenolic properties, and digestive enzymes as well as food matrix can also influence the bioaccessibility (Ekbatan et al., 2016).

However, it is difficult to evaluate the bioaccessibility of dietary phenolics *in vivo* due to the cost and trial time etc. (Sengul, Surek, & Nilufer-Erdil, 2014).

Therefore, *in vitro* experiments have been conducted as speedy and useful methodologies to assess the bioaccessibility of dietary phenolics (Cardoso, Afonso, Lourenço, Costa, & Nunes, 2015).

This chapter was intended to correlate the changes in the protein and emulsion properties with the complexation between protein and phenolics or the interpretation of the bioaccessibility results.

III-2. Materials and Methods

III-2-1. Chemicals

Pepsin from porcine gastric mucosa (P6887; ≥3,200 units/mg protein), pancreatin from porcine pancrease (P7545, 8 × United States Pharmacopeia [USP] units), and bile extract from porcine (B8631) were purchased from Sigma-Aldrich (St. Louis, MO, USA) for the *in vitro* digestion model. High-performance liquid chromatography (HPLC)-grade methanol was purchased from J. T. Baker (Phillipsburg, NJ, USA) and Chlorogenic acids standards were purchased from Sigma-Aldrich.

III-2-2. Instant coffee and creamers

Instant coffee, a commercial product made of mixed coffee beans (*Coffea arabica: Coffea canephora* = 7:3, w/w) was provided from Dong Suh Foods Corp. (Seoul, Republic of Korea) and stored at -18°C until further use.

Detailed preparation method for Creamer A and Creamer B was described in Chapter II-2-2. Commercially available skim milk was purchased at local market (Maeil Dairies Corp., Seoul, Republic of Korea). The nutritional composition (%,

w/w) of skim milk within the total solid was as follows: skim milk, 38.7% protein, 1.1% fat, 52.2% carbohydrate.

III-2-3. Sample preparation

Instant coffee beverage (IC) was prepared by dissolving 1.6 g instant coffee in 100 mL distilled water. Instant coffee beverage with creamer A (CRA) or creamer B (CRB) was prepared by mixing 1.6 g instant coffee with 5.0 g creamer, followed by the addition of distilled water for a total volume of 100 mL. Instant coffee beverage with skim milk (SM) was prepared by mixing 37.5 mL distilled water, 1.6 g instant coffee, and 62.5 mL skim milk for a total skim milk solid amount of 8.0% (w/v). Each sample was mixed by magnetic stirring at 80 rpm for 10 min at 45°C to sufficiently dissolve solid substances while minimizing thermal damage to the components in the samples.

III-2-4. In vitro digestion model

In vitro digestion of the freshly prepared beverages was performed as previously described(Minekus et al., 2014), with slight modifications. Digestion was conducted in two consecutive steps of gastric and pancreatic digestion. For simulated gastric digestion, 10.0 mL aliquots of each beverage were withdrawn and mixed with 7.5 mL simulated gastric fluid (SGF) electrolyte solution. The pH of the mixture was

adjusted to 2.5 by adding 1.0 M HCl. Then we added 1.6 mL pepsin stock solution (25,000 units/mL) prepared in SGF electrolyte solution, followed by $CaCl_2$ dissolved in distilled water for a final concentration of 0.15 mM, and the total volume of the mixture was adjusted to 20.0 mL with distilled water. The reaction mixture was incubated at 37°C for 2 h in the dark with orbital shaking at 150 rpm.

After gastric digestion, 10.0 mL gastric chyme was pulled out and its pH adjusted to 7.5 by adding 1.0 M NaOH to arrest pepsin activity. The chyme was mixed with 5.5 mL simulated intestinal fluid (SIF) electrolyte solution and 2.5 mL pancreatin solution (800 units/mL, based on trypsin activity) and 1.25 mL bile extract solution (64.0 mM bile salts/mL), prepared in the SIF electrolyte solution were added to the mixture. Then $CaCl_2$ dissolved in distilled water was added to the mixture for a final concentration of 0.6 mM and the total volume was adjusted to 20.0 mL with distilled water. The reaction mixture was incubated at 37°C for 2 h in the dark with orbital shaking at 150 rpm.

III-2-5. Turbiscan analysis

Changes in the food matrix of each sample were assessed by analyzing delta backscattering (Δ-BS) profiles using a Turbiscan MA 2000 system (Formulaction, Toulouse, France) during *in vitro* digestion. Immediately after preparation, each 20 mL sample was placed in a cylindrical glass cell and gastric and pancreatic digestion was performed in the cell using the procedure described above. During digestion, the

samples were exposed to near-infrared light at 880 nm and the intensity of transmitted and backscattered light was recorded from the bottom layer to the top layer. The total scanning time was 2 h and the scanning interval was fixed at 10 min. The kinetics of coalescence/flocculation, creaming, and clarification were determined from the changes in backscatter intensity (ΔBS, %) as a function of the digestion time from the bottom layer to the top layer.

III-2-6. Quantitative analysis of chlorogenic acids

Quantitative analyses of chlorogenic acids, including caffeoylquinic acids (CQAs), feruloylquinic acids (FQAs), and di-caffeoylquinic acids (di-CQAs), were performed using an HPLC instrument (Waters 2695, Water Corp., Milford, MA, USA) with a photodiode array detector (Water 2998, Waters Corp.) and a reverse phase column (5 μm, 4.6 × 250 mm; Symmetry C_{18} column, Water Corp.). The analytes, filtered through a 0.45-μm membrane filter, were subjected to gradient elution with methanol (A) and a mixture of 10 mM citric acid and methanol (4:1, v/v) (B), as follows: 0-25 min, 100% (A); 25-50 min, 80% (A); 50-60 min, 60% (A); and 60-70 min, 100% (A). Samples of 20 μL were injected and the flow rate was set at 1 mL/min. Individual chlorogenic acids were quantified from peak areas at 325 nm using calibration curves of the 5-caffeoyl quinic acid standard combined with the molar extinction coefficients (Farah, de Paulis, Trugo, & Martin, 2005).

III-2-7. Determination of bioaccessible content and bioaccessibility of chlorogenic acids

To determine the bioaccessible content of chlorogenic acids in the different digestion phases, the beverages were filtered through a centrifugal ultrafilter unit at a cut-off size of 3 kDa (Amicon Ultra-4, Merck, Darmstadt, Germany) to separate chlorogenic acids bound to high-molecular-weight materials immediately after each digestion. It is hard to specify how small components can permeate the small intestine, since the absorption properties (permeation into small intestine) of components should be different depending on the physicochemical properties such as hydrophilicity and charge. However, it was considered that components <3 kDa are small enough to be absorbable through the intestinal mucosa in various previous studies (Sęczyk et al., 2021; Tagliazucchi, Helal, Verzelloni, & Conte, 2012; Valli, Danesi, Gianotti, Di Nunzio, Saa, & Bordoni, 2016)

Chlorogenic acids passing through the filter were considered bioaccessible and expressed as mg compound per 100 mL sample. 3.0 mL of samples was withdrawn before and after the digestion; these were transferred to a filter unit and centrifuged at 7,500 × g for 100 min at 4°C. The bioaccessibility of chlorogenic acids in the samples was calculated as a percentage of the ultrafiltrate content after pancreatic digestion with respect to that before digestion. This index indicates the amount of digested chlorogenic acids and became available for potential absorption in the simulated biological system.

III-2-8. Statistical analysis

All experiments were performed at least three times. The data are presented as means ± standard deviations. Analysis of variance (ANOVA) was performed using the SPSS ver. 25.0 software (IBM Corp., Armonk, NY, USA) and significant differences were evaluated using Tukey's test at a level of $p < 0.05$.

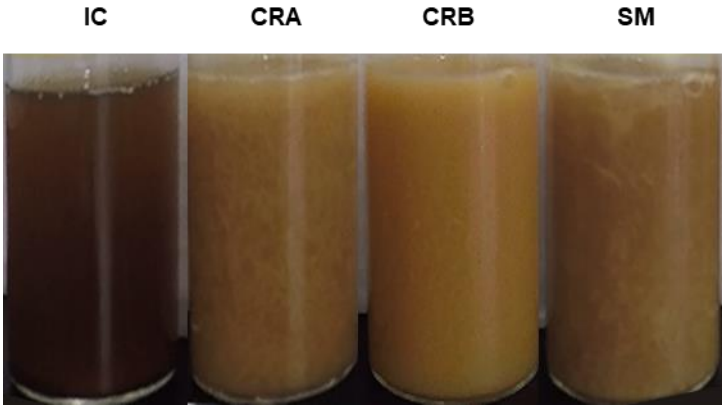
III-3. Results and Discussion

III-3-1. Changes in visual appearance during in vitro digestion

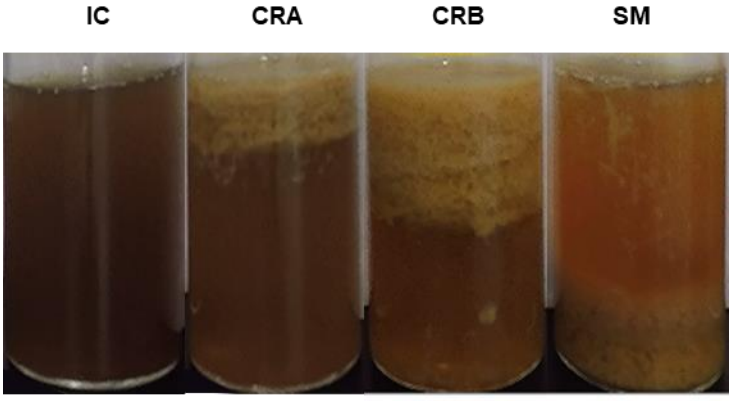
The emulsifying characteristics and the stability as a form of oil-in-water emulsion are important attributes during *in vitro* digestion in term of the complexation between protein and coffee polyphenols. Considering the emulsions consumed in liquid form, the variations of particles such as the changes of protein properties in the interfacial layer and oil droplets migration according to the protein changes are evaluated by Turbiscan analysis during subsequent digestion period.

The samples at each digestion steps were placed in a cylindrical glass cell and monitored by measuring Δ-BS as a function of cell height. Additionally, the visual appearances of IC, CRA, CRB, and SM before digestion and after gastric and pancreatic digestion were presented in Figure III-1.

[A]



[B]



[C]

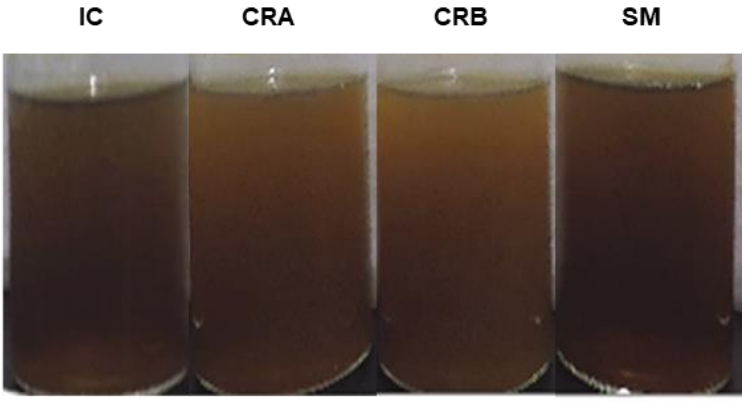


Figure III-1. Visual appearance of instant coffee alone (IC), instant coffee with creamer A (CRA), instant coffee with creamer B (CRB), and instant coffee with skim milk (SM) in different digestion phases; (A) before digestion, (B) after gastric digestion, (C) and after pancreatic digestion.

III-3-2. Changes in food matrix during gastric digestion

The Δ-BS profiles of CRA, CRB, and SM were determined to evaluate changes of particles migration and emulsion properties in the food matrix during gastric digestion (Figure III-2)

As shown in Figure III-2, exposure of CRA to gastric conditions resulted in increase of Δ-BS at the top layer (height, 39-42 mm) and decrease of Δ-BS from the bottom to middle layers (height, 2-38 mm) of the measuring cells during digestion for 20 min. The Δ-BS profiles did not significantly thereafter. These results indicated that flocculation and coalescence occurred from the bottom to the middle layers and creaming occurred at the top, causing destabilization of the emulsion.

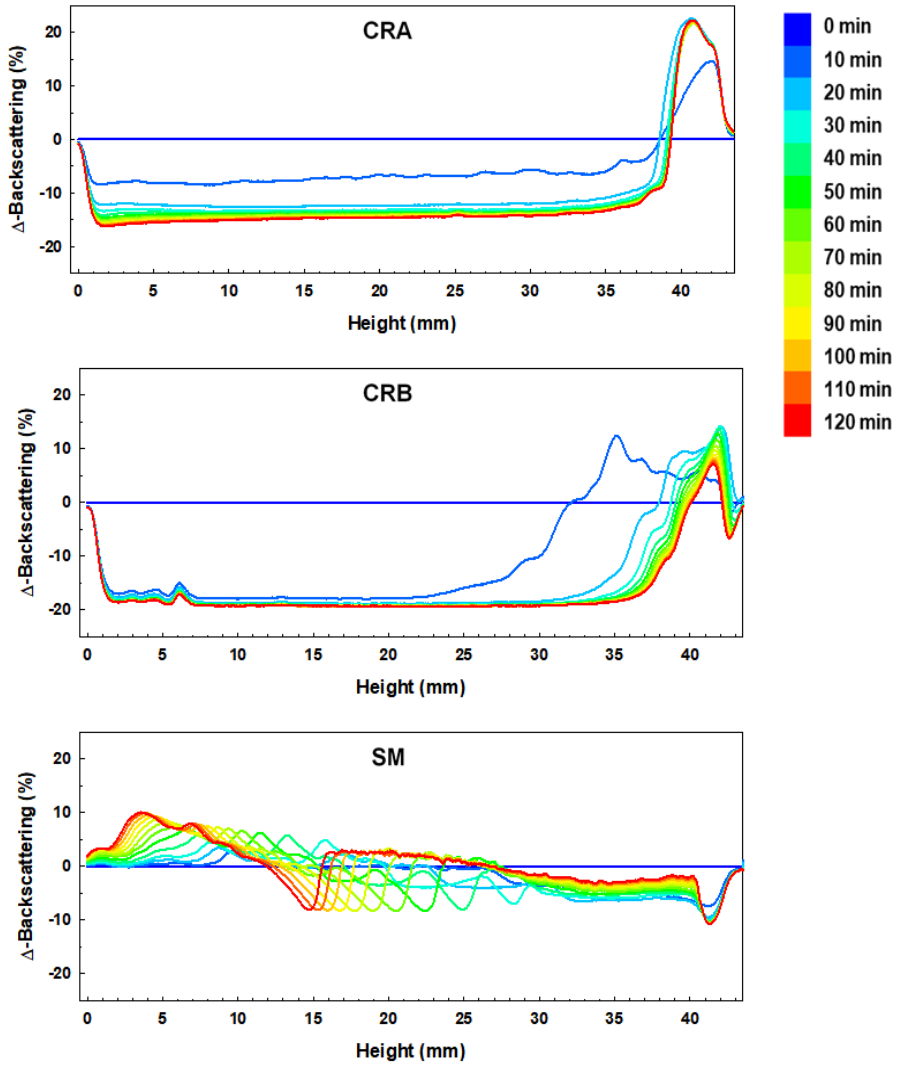


Figure III-2. The Δ-BS profiles of instant coffee with creamer A (CRA), instant coffee with creamer B (CRB), and instant coffee with skim milk (SM) during *in vitro* gastric digestion. Data are presented as a function of digestion time (0-120 min) and sample height (0-42 mm).

When coalescence and flocculation occurred, Δ-BS decreased throughout the sample height due to the reduced volume fraction of the oil phase. By contrast, creaming increased Δ-BS at the top of the sample because droplet concentration increases by oil rising (K. Wang, Li, & Zhang, 2018). Coalescence, flocculation, and creaming phenomena in protein-stabilized emulsions during gastric digestion are primarily attributed to the hydrolysis of adsorbed proteins at oil-water interfaces (Kenmogne-Domguia, Meynier, Viau, Llamas, & Genot, 2012). The hydrolysis by pepsin generally results in a loss of electrostatic repulsions at the droplet surface, and peptides produced by the hydrolysis are not sufficiently strong to overcome the attractive forces and steric effects between droplets (Singh, 2011).

During gastric digestion, CRB showed a decrease in Δ-BS from the bottom to the middle layers and an increase in Δ-BS at the top of the measuring cells, which was similar with the Δ-BS profiles of CRA. This result suggested that flocculation, coalescence, and creaming occurred in CRB, due to the hydrolysis of adsorbed proteins.

However, the kinetics of the Δ-BS profiles of CRB as a function of digestion time were different from those of CRA, with Δ-BS changing only during the first 20 min, and not during further digestion. Meanwhile, CRB showed significant changes in the Δ-BS profile throughout the entire digestion period (0-120 min). In CRB, the Δ-BS increase gradually shifted to the top of the measurement cell as digestion time increased from 0 to 60 min and the Δ-BS at the top (height, 39-42 mm) decreased

gradually from 20 to 120 min. This shift and decrease in Δ-BS suggested that the creaming layers at the top of the sample became more closely packed as digestion progressed. The different kinetics of the Δ-BS profile of CRB are attributed to higher amounts of adsorbed protein at oil-water interfaces, causing slower protein hydrolysis compared to CRA.

In SM, Δ-BS was increased at the bottom layer (height, 0-10 mm), but fluctuated in the middle layer (height, 15-28 mm) throughout the digestion period. These complicated Δ-BS profiles are attributed to protein aggregation and hydrolysis in skim milk, particularly in the middle layer. Increases in Δ-BS at the bottom layer suggested the sedimentation of protein aggregates, which increased as digestion progressed. Coagulation between milk proteins results in the formation of structured clots in a knitted network during gastric digestion of skim milk at an acidic pH, initially driven by the action of pepsin on κ-casein, which disrupts the protective effects of κ-casein on casein micelle aggregation (Ye, Cui, Dalgleish, & Singh, 2016).

Whey proteins including serum albumin, lactoferrin, and α-lactalbumin, are susceptible to hydrolysis by pepsin under gastric conditions, whereas β-lactoglobulin is largely resistant to hydrolysis because of its highly folded globular structure (Shimoni, Levi, Tal, & Lesmes, 2013). However, the digestibility of proteins in an emulsion system can change at the oil-water interface (Singh, 2011). β-lactoglobulin is particularly susceptible to hydrolysis by pepsin when it is adsorbed at the oil-water interface, causing conformational changes that expose pepsin cleavage sites

(Macierzanka, Sancho, Mills, Rigby, & Mackie, 2009). Meanwhile, casein is readily hydrolyzed by pepsin, regardless of adsorption, due to its flexible random structure.

In this regard, protein-phenolic complexes in CRA, in which casein is the major protein along with some lactoferrin and bovine serum albumin, could be broken down through proteolysis during gastric digestion (van der Burg-Koorevaar, Miret, & Duchateau, 2011). Similarly, most of the protein-phenolic complexes involving casein, β-lactoglobulin, lactoferrin, and bovine serum albumin in CRB might be disrupted; however, small amounts of a hydrolysis-resistant complex formed with non-adsorbed β-lactoglobulin could be remained (Dupas, Marsset-Baglieri, Ordonaud, Ducept, & Maillard, 2006).

In SM, most of the complexes involving structured clots were retained throughout gastric digestion because caseins, β-lactoglobulin, and α-lactalbumin, which are involved in clot formation, are not susceptible to hydrolysis due to low pepsin accessibility (X. Wang, Ye, Lin, Han, & Singh, 2018; Ye et al., 2016).

III-3-3. Changes in food matrix during pancreatic digestion

After gastric digestion, the Δ-BS profiles of CRA, CRB, and SM were determined to evaluate changes of particles migration and emulsion properties in the food matrix during pancreatic digestion (Figure III-3).

During pancreatic digestion, actions of pancreatic lipase and various proteases such as trypsin, chymotrypsin, and aminopeptidase are expected to cause substantial changes in the food matrix (Singh, 2011).

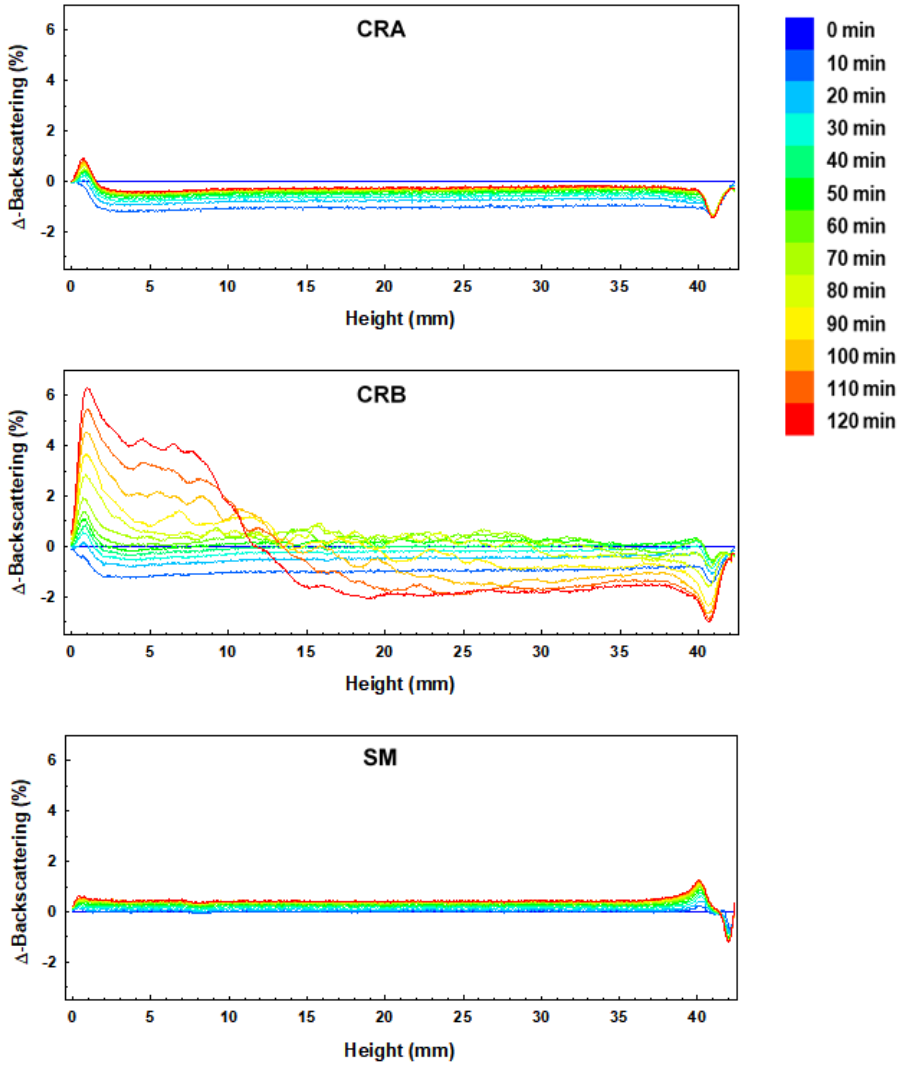


Figure III-3. The Δ-BS profiles of instant coffee with creamer A (CRA), instant coffee with creamer B (CRB), and instant coffee with skim milk (SM) during *in vitro* pancreatic digestion. Data are presented as a function of digestion time (0-120 min) and sample height (0-42 mm).

Pancreatic digestion of CRA caused slight changes in its Δ-BS profiles, such that Δ-BS decreased both at the bottom (height, 0-2 mm) and top (height, 40-42 mm) of the sample. These results indicated slight sedimentation and clarification at the bottom and top, respectively, mainly because of the hydrolysis of agglomerated lipids, catalyzed by the pancreatic lipase, in the creaming layers.

Subsequently, digested lipids including free fatty acids, monoglycerides, and diglycerides led to sedimentation (Golding & Wooster, 2010). In addition, polypeptides produced by pepsin in the gastric phase were digested as oligopeptides and amino acids by various proteases in pancreatic phase (Singh, 2011).

The Δ-BS profiles of CRB indicated sedimentation and clarification at the bottom (height, 0-10 mm) and top (height, 40-42 mm), respectively. As observed for CRA, these results suggested lipid digestion through hydrolysis and conversion of proteins to small peptides and amino acids. Changes in the Δ-BS profiles of CRB showed a higher intensity and broader region than those of CRA throughout digestion; these differences may be due to differences in emulsifying properties such as droplet size or zeta-potential under pancreatic conditions (Sarkar, Horne, & Singh, 2010).

Slight changes in the Δ-BS profiles of SM over time were observed, such that Δ-BS increased throughout the sample height as digestion progressed. Milk proteins were fully hydrolyzed by protease, producing oligopeptides and amino acids that were dispersed evenly throughout the sample (Singh & Ye, 2020), and clots forming

sediment in the bottom layers during gastric digestion were broken down after pancreatic digestion.

Hence, it was thought that protein-phenolic complexes in all samples might be completely disrupted after pancreatic digestion, regardless of protein content and compositions, due to complete protein digestion by the actions of various proteases under pancreatic conditions.

III-3-4. Bioaccessible content and bioaccessibility of chlorogenic acids

Bioaccessible content indicates the extent to which chlorogenic acids are bound to proteins, *i.e.*, the amounts of complexes formed between proteins and chlorogenic acids (Yildirim-Elikoglu & Erdem, 2018). Lower bioaccessible content indicates that more complexation occurred.

The bioaccessible contents of chlorogenic acids in the samples during *in vitro* digestion are shown in Table III-1. In all samples, FQAs and diCQAs constituted a small fraction of the total chlorogenic acids, and CQAs accounted for approximately 80% of the content of total chlorogenic acids, which is consistent with results of a previous study (Tagliazucchi et al., 2012).

Table III-1. Bioaccessible content of caffeoylquinic acids (CQAs), feruloylquinic acids (FQAs), di-caffeoylquinic acids (diCQAs), and total chlorogenic acids (total CGAs) in instant coffee alone (IC), instant coffee with creamer A (CRA), instant coffee with creamer B (CRB), and instant coffee with skim milk (SM) in different digestion phases

Digestion phase	Compounds	Bioaccessible content (mg/100 mL)			
		IC	CRA	CRB	SM
Before digestion	CQAs	54.77 ± 2.28 ^{Ab}	50.21 ± 3.80 ^{Ab}	51.09 ± 2.74 ^{Ab}	52.01 ± 3.56 ^{Aa}
	FQAs	9.17 ± 1.65 ^{Ab}	9.36 ± 1.33 ^{Ab}	9.21 ± 1.05 ^{Ab}	8.85 ± 1.32 ^{Aa}
	diCQAs	3.72 ± 0.19 ^{Cb}	3.10 ± 0.18 ^{Bb}	3.12 ± 0.18 ^{Bb}	1.95 ± 0.35 ^{Aab}
	Total CGAs	67.66 ± 0.90 ^{Bb}	62.67 ± 2.78 ^{Ab}	63.42 ± 2.09 ^{Ab}	62.81 ± 1.92 ^{Ab}
Gastric digestion	CQAs	50.83 ± 5.83 ^{Ab}	49.80 ± 3.95 ^{Ab}	49.95 ± 1.57 ^{Ab}	45.96 ± 4.76 ^{Aa}
	FQAs	9.92 ± 1.82 ^{Bb}	9.50 ± 0.24 ^{Bb}	9.44 ± 0.59 ^{Bb}	6.77 ± 1.95 ^{Aa}
	diCQAs	3.67 ± 0.56 ^{Bb}	3.17 ± 0.18 ^{ABb}	3.20 ± 0.29 ^{ABb}	2.43 ± 0.60 ^{Ab}
	Total CGAs	64.42 ± 4.46 ^{Bb}	62.48 ± 4.09 ^{Bb}	62.59 ± 1.19 ^{Bb}	55.16 ± 3.19 ^{Aa}
Pancreatic digestion	CQAs	12.61 ± 1.77 ^{Aa}	20.40 ± 8.38 ^{ABa}	26.78 ± 7.11 ^{Ba}	44.62 ± 3.03 ^{Ca}
	FQAs	4.85 ± 0.49 ^{Aba}	4.56 ± 1.33 ^{Aa}	5.32 ± 1.13 ^{Aba}	6.66 ± 0.45 ^{Ba}
	diCQAs	0.40 ± 0.04 ^{Aa}	0.58 ± 0.35 ^{Aa}	0.80 ± 0.42 ^{Aba}	1.29 ± 0.36 ^{Ba}
	Total CGAs	17.86 ± 1.34 ^{Aa}	25.55 ± 6.88 ^{ABa}	32.90 ± 6.63 ^{Ba}	52.57 ± 3.75 ^{Ca}

Data are means ± standard deviations (n = 3). Uppercase letters in the same row indicate significant differences ($p < 0.05$) among compounds in different samples in the same digestion phase. Lowercase letters in the same column indicate significant differences ($p < 0.05$) among compounds in the same sample in different digestion phases.

Before digestion, bioaccessible content of CQAs and FQAs did not significantly differ among samples ($p > 0.05$), whereas the content of diCQAs and total chlorogenic acids were significantly higher in IC than in all other samples ($p < 0.05$). At the gastric phase, no differences in the content of CQAs, FQAs, diCQAs, or total chlorogenic acids were found in IC, CRA, or CRB before and after digestion. However, SM had significantly lower content of total chlorogenic acids ($p < 0.05$) after digestion. At the gastric phase, there were no significant differences in the content among IC, CRA, and CRB ($p > 0.05$), but the content of total chlorogenic acids in SM was significantly lower than that of the other samples ($p < 0.05$). Meanwhile, pancreatic digestion caused a significant decrease in the content of CQAs, FQAs, diCQAs, and total chlorogenic acids among all samples except SM, compared to the gastric phase ($p < 0.05$). After pancreatic digestion, IC and SM showed the lowest and the highest content of total chlorogenic acids, respectively.

Before digestion, CRA, CRB, and SM, which contain milk proteins, had significantly lower bioaccessible content of total chlorogenic acids than IC, suggesting the formation of complexes between milk proteins and chlorogenic acids. However, the no significant difference in biaccessible content of total chlorogenic acids among CRA, CRB, and SM indicated that the amounts of complexes among total chlorogenic acids were not dependent on protein content or type.

Meanwhile, significant differences in the content of diCQAs among CRA, CRB, and SM indicated that diCQA content decreased as protein concentration increased,

suggesting that structure of phenolic compounds affected complexation, possibly due to differences in binding affinity between diCQAs and either CQAs or FQAs (Ozdal, Capanoglu, & Altay, 2013).

In the gastric phase, no significant changes in the bioaccessible content of IC before and after digestion, which suggested that chlorogenic acids were retained under gastric conditions. These results can be explained by the high stability of chlorogenic acids under acidic conditions and gastric enzymes (*e.g.*, gastric proteases and lipases) did not affect the structure of chlorogenic acids (Bermúdez-Soto, Tomás-Barberán, & García-Conesa, 2007).

Significant decreases in the content of total chlorogenic acids in SM at the gastric phase suggested that more complexes formed between proteins and chlorogenic acids during gastric digestion. Although complexes produced before digestion were retained due to low protein digestibility, undigested proteins formed additional complexes under gastric conditions. These results may be due to conformational changes of protein caused by pH, which in turn affected the binding affinity of chlorogenic acids on proteins. Coffee phenolic extracts have higher binding affinity to β-lactoglobulin at pH 2.5 than at pH 7.2 due to disruption of its tertiary structure under acidic conditions, which diminishes structural requirements for binding (Stojadinovic et al., 2013). The formation of structured clots in SM may also have caused protein conformational changes to promote interaction with chlorogenic acids.

Meanwhile, in CRA and CRB, protein-phenolic complexes were mainly broken down due to the digestion of the proteins during gastric digestion (described in Chapter III-3-2). Therefore, the results that the bioaccessible content in CRA and CRB was not changed during gastric digestion and there was no significant difference on the content among IC, CRA, and CRB suggested that most of chlorogenic acids in CRA and CRB were released from the complex and retained with intact structures in the gastric phase, as observed for IC.

In the pancreatic phase, additional protein-phenolic complex could not form because the food matrix was degraded by digestive enzymes. Hence, significant reductions in the bioaccessible content of chlorogenic acids in the samples during the pancreatic digestion were associated with the degradation of chlorogenic acids released from the food matrix. Chlorogenic acids have low stability at neutral and alkaline pH values. Irreversible degradation of chlorogenic acids induced by pH is involved in oxidation, polymerization, and transformation under pancreatic conditions (Quan et al., 2020).

After pancreatic digestion, bioaccessibility of total chlorogenic acids in the samples was determined (Figure III-4). The bioaccessibility of total chlorogenic acids among the samples as follows: IC (26.4 ± 1.7%), CRA (40.5 ± 9.4%), CRB (51.7 ± 9.0%), and SM (83.6 ± 3.6%), indicating that the bioaccessibility increased significantly as protein concentration in the samples increased.

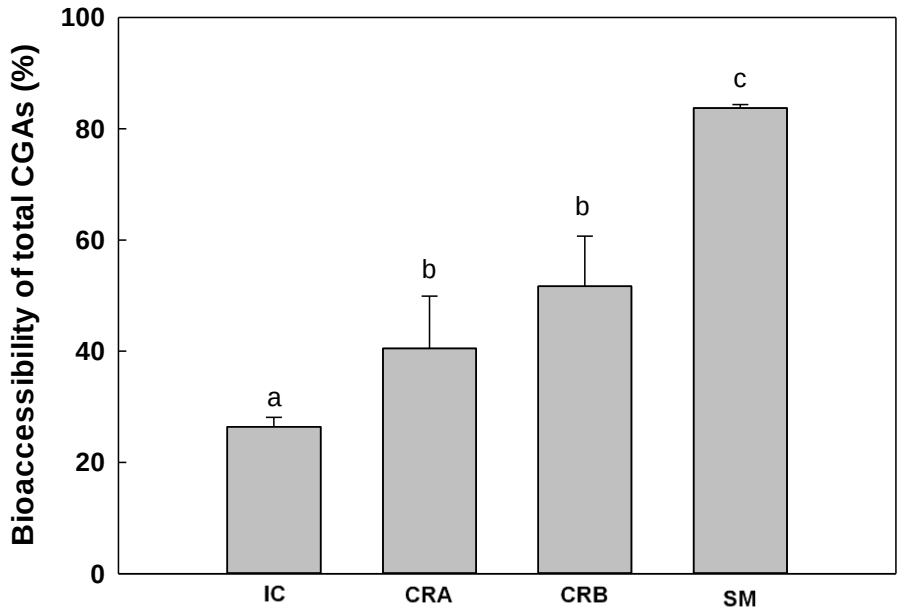


Figure III-4. Bioaccessibility of total chlorogenic acids (CGAs) in instant coffee alone (IC), instant coffee with creamer A (CRA), instant coffee with creamer B (CRB), and instant coffee with skim milk (SM) after *in vitro* digestion.

A high correlation (R^2 = 0.9757) was observed between bioaccessibility of chlorogenic acids and protein concentrations in the samples (Figure III-5).

These results clearly demonstrated that milk proteins enhanced the bioaccessibility of chlorogenic acids, which suggested that changes in the bioaccessibility of chlorogenic acids caused by milk proteins were critically determined by protein concentrations at the end of pancreatic digestion. Furthermore, protein types and adsorption properties of proteins in creamers and skim milk may not cause significant differences in the enhancement of the bioaccessibility of chlorogenic acids after pancreatic digestion.

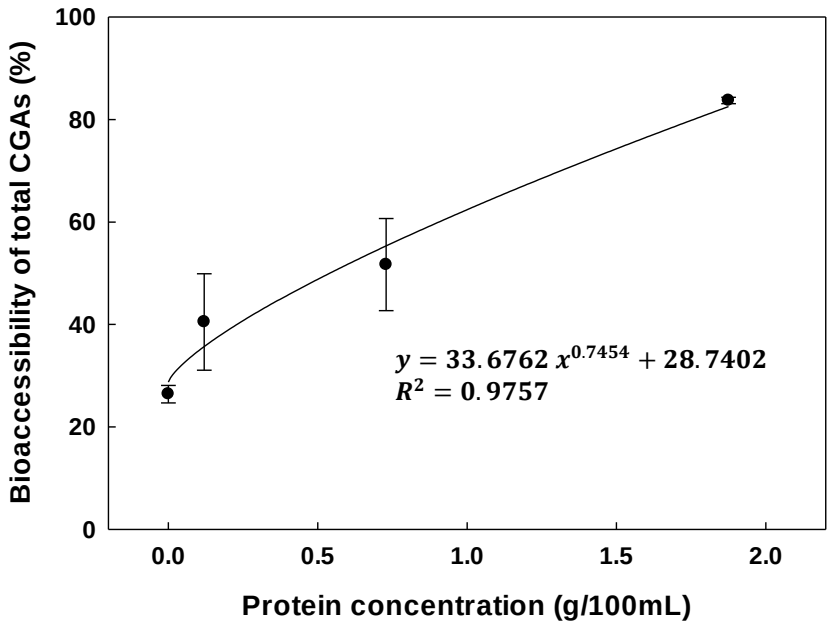


Figure III-5. Correlation between bioaccessibility and total protein concentrations in the samples. Data are means ± standard deviations (n = 3). Different letters indicate significant differences ($p < 0.05$).

The influences of creamers and skim milk addition on the enhancement of the bioaccessibility of chlorogenic acids may be associated with the protection from degradation and delayed release of chlorogenic acids from the dairy matrix, caused by complexation between milk proteins and chlorogenic acids (Moser, Chegeni, Jones, Liceaga, & Ferruzzi, 2014; Sabouri, Arranz, Guri, & Corredig, 2018).

This protective effect is attributed to the antioxidant activity of various peptides and/or amino acids produced through proteolysis of milk proteins during gastro-pancreatic digestion (Lamothe et al., 2014). In addition, the delayed release of chlorogenic acids reduced the exposure time for chlorogenic acids to be degraded under pancreatic conditions. Chlorogenic acids are partially absorbed in the small intestine, but predominantly after microbial metabolism in the colon (de Morais, Pessato, Rodrigues, Mallmann, Mariutti, & Netto, 2020). Thus, chlorogenic acids are required to retain intact structures in the gastrointestinal tract before absorption.

Accordingly, the complexation allows chlorogenic acids to reach locations where absorption can occur with lower compound loss, which thereby enhances the absorption (Moser et al., 2014).

Consequently, the creamer addition to instant coffee exhibited similar effects on the bioaccessibility of chlorogenic acids to those of skim milk at the end of pancreatic digestion. However, the enhancement of the bioaccessibility of chlorogenic acids is less effective for creamer addition than for skim milk addition because creamers have lower protein concentrations.

III-4. Conclusions

CRA and CRB, having relatively low protein content, showed the dispersion stability properties that coalescence, flocculation, and creaming occurred in gastric phase, and sedimentation during pancreatic phase. It was thought that their protein-phenolic complexes might be completely broken down by the actions of various digestive enzymes regardless of protein content and compositions.

The influences of CRA and CRB on the bioaccessibility of chlorogenic acids have a positive effect due to the protection from degradation of chlorogenic acids caused by milk proteins in creamers.

III-5. References

Bermúdez-Soto, M.-J., Tomás-Barberán, F.-A., & García-Conesa, M.-T. (2007). Stability of polyphenols in chokeberry (Aronia melanocarpa) subjected to *in vitro* gastric and pancreatic digestion. *Food Chemistry, 102*(3), 865-874.

Cardoso, C., Afonso, C., Lourenço, H., Costa, S., & Nunes, M. L. (2015). Bioaccessibility assessment methodologies and their consequences for the risk–benefit evaluation of food. *Trends in Food Science & Technology, 41*(1), 5-23.

de Morais, F. P., Pessato, T. B., Rodrigues, E., Mallmann, L. P., Mariutti, L. R., & Netto, F. M. (2020). Whey protein and phenolic compound complexation: Effects on antioxidant capacity before and after *in vitro* digestion. *Food Research International, 133*, 109104.

Dupas, C. J., Marsset-Baglieri, A. C., Ordonaud, C. S., Ducept, F. M., & Maillard, M. N. (2006). Coffee antioxidant properties: effects of milk addition and processing conditions. *Journal of Food Science, 71*(3), S253-S258.

Ekbatan, S. S., Sleno, L., Sabally, K., Khairallah, J., Azadi, B., Rodes, L., Kubow, S. (2016). Biotransformation of polyphenols in a dynamic multistage gastrointestinal model. *Food Chemistry, 204*, 453-462.

Farah, A., de Paulis, T., Trugo, L. C., & Martin, P. R. (2005). Effect of roasting on the formation of chlorogenic acid lactones in coffee. *Journal of Agricultural and*

Food Chemistry, 53(5), 1505-1513.

Golding, M., & Wooster, T. J. (2010). The influence of emulsion structure and stability on lipid digestion. *Current Opinion in Colloid & Interface Science, 15*(1-2), 90-101.

Kenmogne-Domguia, H. B., Meynier, A., Viau, M., Llamas, G., & Genot, C. (2012). Gastric conditions control both the evolution of the organization of protein-stabilized emulsions and the kinetic of lipolysis during *in vitro* digestion. *Food & Function, 3*(12), 1302-1309.

Lamothe, S., Azimy, N., Bazinet, L., Couillard, C., & Britten, M. (2014). Interaction of green tea polyphenols with dairy matrices in a simulated gastrointestinal environment. *Food & Function, 5*(10), 2621-2631.

Lamothe, S., Rémillard, N., Tremblay, J., & Britten, M. (2017). Influence of dairy matrices on nutrient release in a simulated gastrointestinal environment. *Food Research International, 92*, 138-146.

Macierzanka, A., Sancho, A. I., Mills, E. C., Rigby, N. M., & Mackie, A. R. (2009). Emulsification alters simulated gastrointestinal proteolysis of β-casein and β-lactoglobulin. *Soft Matter, 5*(3), 538-550.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Dupont, D. (2014). A standardised static *in vitro* digestion method suitable for food– an international consensus. *Food & Function, 5*(6), 1113-1124.

Moser, S., Chegeni, M., Jones, O. G., Liceaga, A., & Ferruzzi, M. G. (2014). The

effect of milk proteins on the bioaccessibility of green tea flavan-3-ols. *Food Research International, 66*, 297-305.

Ozdal, T., Capanoglu, E., & Altay, F. (2013). A review on protein–phenolic interactions and associated changes. *Food Research International, 51*(2), 954-970.

Palafox-Carlos, H., Ayala-Zavala, J. F., & González-Aguilar, G. A. (2011). The role of dietary fiber in the bioaccessibility and bioavailability of fruit and vegetable antioxidants. *Journal of Food Science, 76*(1), R6-R15.

Quan, W., Qie, X., Chen, Y., Zeng, M., Qin, F., Chen, J., & He, Z. (2020). Effect of milk addition and processing on the antioxidant capacity and phenolic bioaccessibility of coffee by using an *in vitro* gastrointestinal digestion model. *Food Chemistry, 308*, 125598.

Sabouri, S., Arranz, E., Guri, A., & Corredig, M. (2018). Sodium caseinate stabilized emulsions as a delivery system for epigallocatechin-gallate: Bioaccessibility, anti-proliferative activity and intestinal absorption. *Journal of Functional Foods, 44*, 166-172.

Sarkar, A., Horne, D. S., & Singh, H. (2010). Pancreatin-induced coalescence of oil-in-water emulsions in an *in vitro* duodenal model. *International Dairy Journal, 20*(9), 589-597.

Sęczyk, Ł., Sugier, D., Świeca, M., & Gawlik-Dziki, U. (2021). The effect of *in vitro* digestion, food matrix, and hydrothermal treatment on the potential

bioaccessibility of selected phenolic compounds. *Food Chemistry, 344*, 128581.

Sengul, H., Surek, E., & Nilufer-Erdil, D. (2014). Investigating the effects of food matrix and food components on bioaccessibility of pomegranate (Punica granatum) phenolics and anthocyanins using an *in vitro* gastrointestinal digestion model. *Food Research International, 62*, 1069-1079.

Shimoni, G., Levi, C. S., Tal, S. L., & Lesmes, U. (2013). Emulsions stabilization by lactoferrin nano-particles under *in vitro* digestion conditions. *Food Hydrocolloids, 33*(2), 264-272.

Singh, H. (2011). Aspects of milk-protein-stabilised emulsions. *Food Hydrocolloids, 25*(8), 1938-1944.

Singh, H., & Ye, A. (2020). Interactions and functionality of milk proteins in food emulsions. In *Milk Proteins* (2nd ed.), 467-497: Academic Press

Stojadinovic, M., Radosavljevic, J., Ognjenovic, J., Vesic, J., Prodic, I., Stanic-Vucinic, D., & Velickovic, T. C. (2013). Binding affinity between dietary polyphenols and β-lactoglobulin negatively correlates with the protein susceptibility to digestion and total antioxidant activity of complexes formed. *Food Chemistry, 136*(3-4), 1263-1271.

Tagliazucchi, D., Helal, A., Verzelloni, E., & Conte, A. (2012). The type and concentration of milk increase the *in vitro* bioaccessibility of coffee chlorogenic acids. *Journal of Agricultural and Food Chemistry, 60*(44),

11056-11064.

Tagliazucchi, D., Verzelloni, E., Bertolini, D., & Conte, A. (2010). *In vitro* bio-accessibility and antioxidant activity of grape polyphenols. *Food Chemistry, 120*(2), 599-606.

Turgeon, S. L., & Rioux, L.-E. (2011). Food matrix impact on macronutrients nutritional properties. *Food Hydrocolloids, 25*(8), 1915-1924.

Valli, V., Danesi, F., Gianotti, A., Di Nunzio, M., Saa, D. L. T., & Bordoni, A. (2016). Antioxidative and anti-inflammatory effect of *in vitro* digested cookies baked using different types of flours and fermentation methods. *Food Research International, 88*, 256-262.

van der Burg-Koorevaar, M. C., Miret, S., & Duchateau, G. S. (2011). Effect of milk and brewing method on black tea catechin bioaccessibility. *Journal of Agricultural and Food Chemistry, 59*(14), 7752-7758.

Wang, K., Li, G., & Zhang, B. (2018). Opposite results of emulsion stability evaluated by the TSI and the phase separation proportion. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 558*, 402-409.

Wang, X., Ye, A., Lin, Q., Han, J., & Singh, H. (2018). Gastric digestion of milk protein ingredients: Study using an *in vitro* dynamic model. *Journal of Dairy Science, 101*(8), 6842-6852.

Wu, C.-S., Guo, J.-H., & Lin, M.-J. (2020). Stability evaluation of pH-adjusted goat milk for developing ricotta cheese with a mixture of cow cheese whey and

goat milk. *Foods, 9*(3), 366.

Ye, A., Cui, J., Dalgleish, D., & Singh, H. (2016). Formation of a structured clot during the gastric digestion of milk: Impact on the rate of protein hydrolysis. *Food Hydrocolloids, 52*, 478-486.

Yildirim-Elikoglu, S., & Erdem, Y. K. (2018). Interactions between milk proteins and polyphenols: Binding mechanisms, related changes, and the future trends in the dairy industry. *Food Reviews International, 34*(7), 665-697.

Chapter IV.

Influence of Creamer Addition on Antioxidant Activity of Instant Coffee during *in vitro* Digestion

IV-1. Introduction

It can be inferred through previous reviews that coffee is a good source of antioxidants and provides relatively high amounts of beneficial polyphenols, but the food matrices paired with coffee (including instant coffee) might influence the health-promoting effect of coffee negatively or positively.

As the reflection of consumers needs on various types of coffee-related beverages, many processing technologies have been developed for these demands and diversified products could be put on the market. Coffee is often formulated and provided with the supplements such as whole milk, skim milk, milk cream as well as the sweeteners (Alongi, Calligaris, & Anese, 2019; Ferruzzi, 2010).

For ready-to-drink (RTD) beverages containing coffee and milk-based ingredients, commercial processing methodologies such as retort, HTST and UHT sterilization applicable to packaging types are applied to fulfill the expiry date and consumer's demands for nutritious and safe products (Murakami et al., 2010). In case of 3-in-1 typed products, each ingredient is manufactured through the drying process and mixed in powder form with relatively long shelf life.

In general, instant coffee is a product prepared by extraction and drying of water-soluble materials from roasted beans, mixed and roasted with different species of green beans (Sánchez-González, Jiménez-Escrig, & Saura-Calixto, 2005). Spray-

drying process or freeze-drying process is applied for commercial instant coffee production. For creamers, it is manufactured by mixing raw materials and spray drying following the sterilizing process. Homogenization process is a typical and common step for making the emulsifying foods (Paquin, 1999) and pH adjustment of coffee-milk based beverages is an important as well to ensure milk stability in coffee of low pH during heat sterilization (Kumazawa & Masuda, 2003).

These various compositions and processing methods can exert significant differences in the structures of nutrients composing with food matrix, which will undergo different pathways during the digestive process (Alongi et al., 2019; Tagliazucchi, Helal, Verzelloni, & Conte, 2012). Therefore, it is important to evaluate how the composition of milk-based ingredients and different processing conditions affect the antioxidant activity of instant coffee.

In this chapter, it is assessed that how creamers with different contents and types of milk proteins applied affect the antioxidant activities and total phenolic content of instant coffee during *in vitro* digestion. These results may contribute to optimize and develop the formulations applied to those types of coffee-milk based beverages by evaluating the effects on the health benefits.

IV-2. Materials and Methods

IV-2-1. Chemicals

Pepsin from porcine gastric mucosa (P6887; ≥3,200 units/mg protein), pancreatin from porcine pancreas (P7545, 8 × United States Pharmacopeia [USP] units), and bile extract from porcine (B8631) were purchased from Sigma-Aldrich (St. Louis, MO, USA) for the *in vitro* digestion model. Folin–Ciocalteu phenol reagent, tannic acid, and other reagents including 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS), 2,4,6-tris-(2-pyridyl)-s-triazine (TPTZ), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) for the evaluation of antioxidant activity were purchased from Sigma-Aldrich. 5-caffeoylquinic acid (5-CQA) standard (CAS No. 327-97-9) for control experiment was purchased from Sigma-Aldrich. All other chemicals were of analytical grade and used without further purification.

IV-2-2. Sample preparation and in vitro digestion

Instant coffee beverage (IC) was prepared by dissolving 1.6 g instant coffee in 100 mL distilled water. CRA and CRB was prepared by mixing 1.6 g instant coffee with 5.0 g of each creamer samples, then adding the distilled water to adjust a total volume of 100 mL. Instant coffee beverage with skim milk (SM) was prepared by

mixing 37.5 mL distilled water, 1.6 g instant coffee, and 62.5 mL skim milk for a total skim milk solid amount of 8.0% (w/v). Each beverage was mixed by magnetic stirring at 80 rpm for 10 min at 45°C to sufficiently dissolve it.

Aliquots of freshly prepared samples were subjected to consecutive simulated gastric and pancreatic digestion as previously described (Minekus et al., 2014). *In vitro* digestion experiments of each sample are conducted as described in the Chapter III-2-4. At the end of 2 hours of gastric digestion and pancreatic digestion (both are post treatment samples), 1.0 mL of beverage was withdrawn. Samples removed after the pancreatic digestions were immediately acidified to pH 2.0 to ensure the stability of the phenolic compounds.

In other experiments, the effect of digestion on 5- caffeoylquinic acid (5-CQA) standard was independently evaluated but at the same conditions for quantitative analysis and FRAP analysis.

IV-2-3. Total phenolic contents

The TP content of each sample was determined using the Folin–Ciocalteu method with slight modifications (Muhammad, Praseptiangga, Van de Walle, & Dewettinck, 2017). Each 1.0 mL sample was supplemented with 7.5 mL distilled water. After adding 0.5 mL Folin–Ciocalteu reagent, the mixture was allowed to stand at 25°C, and then 1.0 mL 35.0% (w/v) sodium carbonate solution was added

into the reaction tube and mixed. After reaction for 1 h at 25°C, the absorbance of the reaction solution was measured at 760 nm using a spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Waltham, MA, USA). The results are expressed as mg tannic acid equivalents (TAE) per 100 mL sample using a tannic acid calibration curve.

IV-2-4. $ABTS^+$ radical scavenging activity

$ABTS^+$ radical scavenging activity of samples was evaluated as previously described (Dubeau, Samson, & Tajmir-Riahi, 2010), with slight modifications. To produce $ABTS^+$ radical stock solution, 7.0 mM ABTS diammonium was dissolved in the distilled water and was mixed with 2.45 mM potassium persulfate in distilled water. The mixture was incubated in the dark at 4 °C for 16 h. The stock solution was diluted with 50 mM sodium phosphate buffer (pH 7.4) to an absorbance at 734 nm of 0.70 ± 0.02. 50 μL samples was added before and after digestion to 200 μL ABTS solution and the absorbance was measured at 734 nm using the spectrophotometer. The reaction was performed at 25 °C for 6 min in the dark. The $ABTS^+$ radical-scavenging activity was determined as the percentage of inhibition of $ABTS^+$ radicals and was expressed as Trolox equivalent antioxidant capacity (TEAC) values as mmol Trolox equivalent (TE)/100 mL sample using a calibration curve of a linear regression of Trolox.

IV-2-5. Ferric reducing antioxidant power (FRAP)

FRAP of samples was evaluated as previously described (Wootton-Beard, Moran, & Ryan, 2011). FRAP reagent was prepared by mixing 1.0 mL 10 mM TPTZ solution in 40 mM HCl and 1.0 mL 20 mM $FeCl_3$ with 10.0 mL 300 mM sodium acetate buffer (pH 3.6). Then, 190 μL FRAP reagent was mixed with 10 μL aliquots of each sample (withdrawn before and after digestion). The mixture was incubated at 37°C for 30 min in the dark, and absorbance at 595 nm was measured using the spectrophotometer. The results are expressed as the TEAC values according to a Trolox calibration curve.

IV-2-6. Statistical analysis

All experiments were performed at least three times. The data are presented as means ± standard deviations. Analysis of variance (ANOVA) was performed using the SPSS ver. 25.0 software (IBM Corp., Armonk, NY, USA) and significant differences were evaluated using Tukey's test at a level of $p < 0.05$.

IV-3. Results and Discussion

IV-3-1. Total phenolic contents

The total phenolic (TP) content of IC, CRA, CRB and SM was measured during *in vitro* digestion (Figure IV-1). Before digestion, the TP content of IC was significantly different from those of CRA and CRB ($p < 0.05$). The TP content of IC was the lowest among the samples, and SM showed the highest TP content before digestion and after gastric and pancreatic digestion.

Decreases in TP content of IC were found after pancreatic digestion, compared to those before digestion and after gastric digestion. Interestingly, the TP content of SM was significantly increased ($p < 0.05$) during gastric and pancreatic digestion, compared to that before digestion.

To confirm the changes of 5-CQA, the most abundant chlorogenic acids in instant coffee, through *in vitro* digestion system, only 5-CQA is analyzed and quantified using the same HPLC conditions after same digestive conditions. As a result, there was no change in a 5-CQA content after gastric condition and decreased significantly after pancreatic digestion compared to before digestion (Figure IV-2).

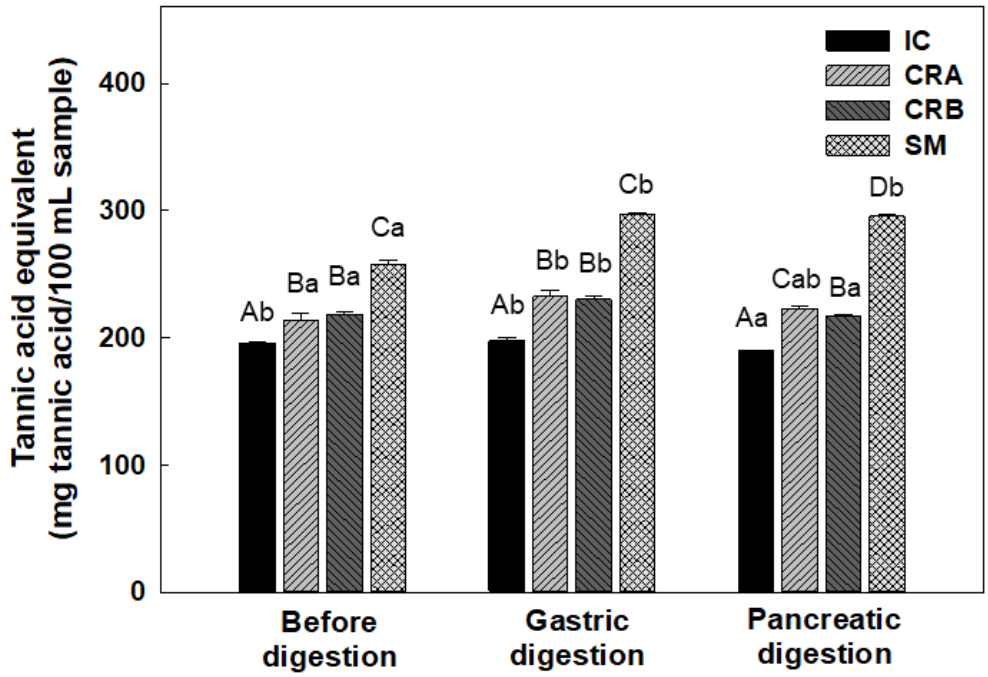


Figure IV-1. Total phenolic contents of instant coffee alone (IC), instant coffee with creamer A (CRA), instant coffee with creamer B (CRB), and instant coffee with skim milk (SM) during *in vitro* gastric and pancreatic digestion. The values of CRA, CRB, and SM were obtained by including intrinsic values of creamer A, creamer B, and skim milk, respectively, at each digestion phase. Data are means ± standard deviations ($n = 3$). Uppercase letters indicate significant differences ($p < 0.05$) among different samples in the same digestion phase. Lowercase letters indicate significant differences ($p < 0.05$) among the same samples in different digestion phases.

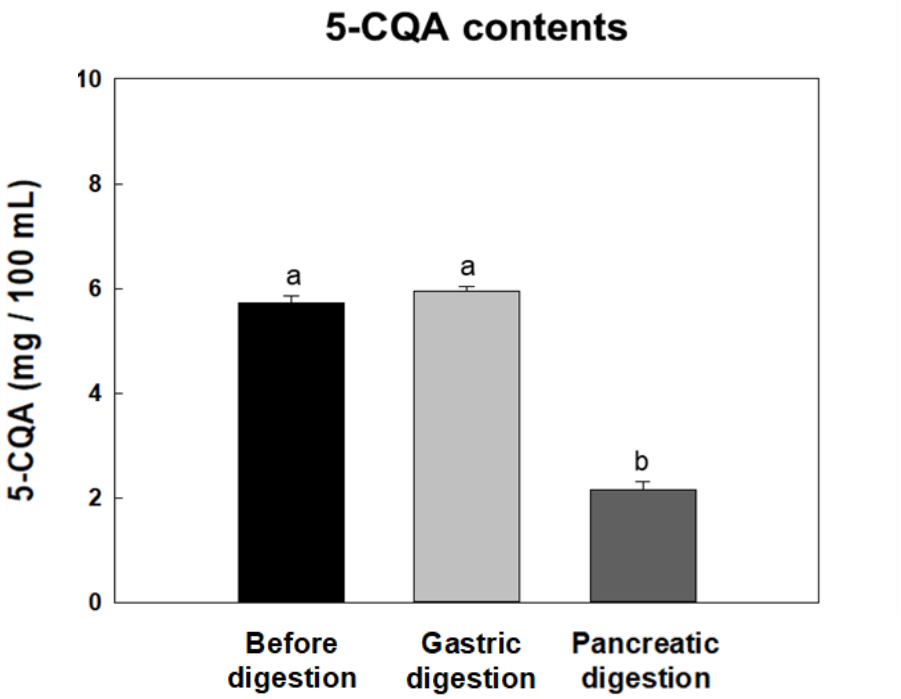


Figure IV-2. Individual 5-caffeoylquinic acid (5-CQA) contents during *in vitro* gastric and pancreatic digestion. Data are means ± standard deviations (n = 3). Different lowercase letters indicate significant differences ($p < 0.05$) in the different digestion phases.

Meanwhile, The TP content of CRA and CRB was significantly higher ($p < 0.05$) than that of IC before digestion and after gastric and pancreatic digestion. TP content of CRA and CRB was not significantly ($p > 0.05$) different during digestion, except those at gastric phase.

Generally, it is known that total polyphenolic compound doesn't indicate the antioxidant activity in food sample, but usually inferred that the high phenolic compounds are closely related to high antioxidant capacity. It is reported that many non-phenolic compounds such as sucrose, peptides and amino acids can reduce can react with the Folin-Ciocalteu (FC) reagent because it is not only specific to the phenolic compounds (Prior, Wu, & Schaich, 2005). In fact, the TP content of creamers and skim milk were substantially increased as digestion progressed, which might be attributed to peptides and amino acids produced through the proteolysis.

IV-3-2. Comparison on ABTS$^+$ radical scavenging activities at each digestion step

As shown in Figure IV-3, ABTS$^+$ radical scavenging activity of IC was the lowest among the samples at all digestion stage but was not significantly different from those of CRA at before digestion and gastric digestion. The ABTS$^+$ radical scavenging activity of IC was not changed at gastric phase but was significantly decreased ($p < 0.05$) at pancreatic phase, compared to that before digestion.

In case of SM, it has shown the highest ABTS$^+$ radical scavenging activity at all digestion stages and was significantly increased ($p < 0.05$) during gastric and pancreatic digestion compared to before digestion.

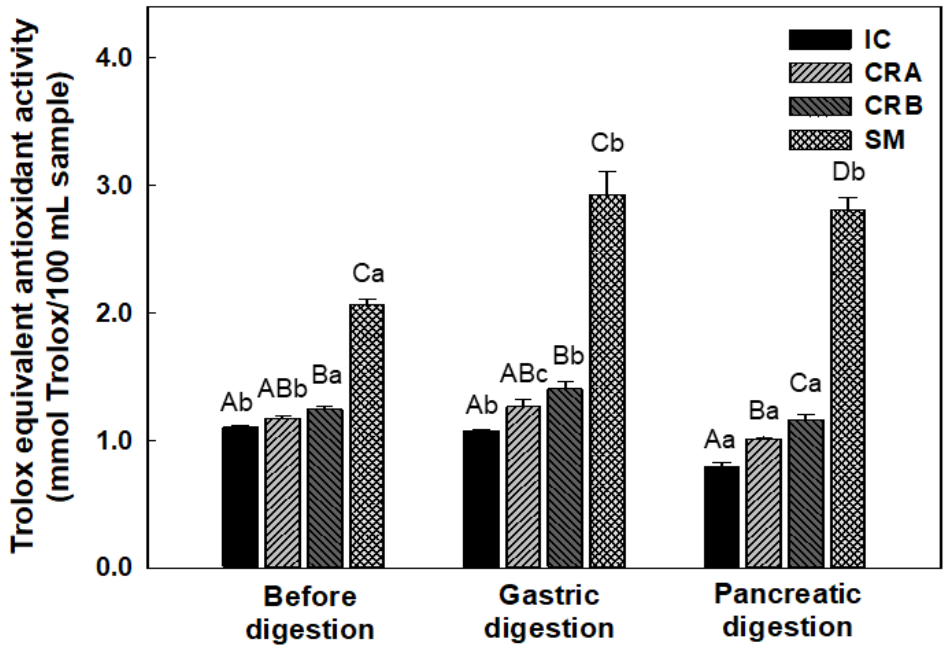


Figure IV-3. ABTS^{+} radical scavenging activity of instant coffee alone (IC), instant coffee with creamer A (CRA), instant coffee with creamer B (CRB), and instant coffee with skim milk (SM) during *in vitro* gastric and pancreatic digestion. The values of CRA, CRB, and SM were obtained by including intrinsic values of creamer A, creamer B, and skim milk, respectively, at each digestion phase. Data are means ± standard deviations (n = 3). Uppercase letters indicate significant differences (p < 0.05) among different samples in the same digestion phase. Lowercase letters indicate significant differences (p < 0.05) among the same samples in different digestion phases.

CRA and CRB showed the higher $ABTS^+$ radical scavenging activity than IC before and after digestion overall and the lower $ABTS^+$ radical scavenging activity than SM at all digestion steps significantly ($p < 0.05$).

In addition, CRA showed the significant increase and decrease ($p < 0.05$) in the $ABTS^+$ radical scavenging activity at gastric phase and pancreatic phase, respectively. But the $ABTS^+$ radical scavenging activity of CRB was significantly increased after gastric digestion ($p > 0.05$) and decreased slightly at pancreatic phase.

Similar with the TP analysis, $ABTS^+$ radical scavenging activity of creamers and skim milk were also increased as digestion progressed for reasons mentioned in TP analysis. Shahidi and Zhong reported that other substances such as sugar, peptides, and amino acids, are reactive with $ABTS^+$ radical and can interfere with the measuring antioxidant activity and overestimate its antioxidant activity in food sample (Shahidi & Zhong, 2015)

Nevertheless, the antioxidant activity of skim milk has significantly increased compared to those of creamers during the digestion process. Dubeau et al. obtained similar results and reported that the antioxidant activity milk itself contributes to 4-14.8% of the antioxidant activity of milk tea beverages in $ABTS^+$ analysis (Dubeau et al., 2010).

IV-3-3. Comparison on FRAP at each digestion step

Changes in FRAP values of the samples during *in vitro* digestion are shown in Figure IV-4. Before digestion, FRAP values of CRA, CRB, and SM were not significantly different ($p > 0.05$) from that of IC, whereas that of SM was lower than those of CRA and CRB at gastric and pancreatic digestion, overall.

Gastric digestion did not decrease FRAP values of all samples, but pancreatic digestion reduced the FRAP values of all samples. In the pancreatic phase, IC had the lowest FRAP value among the samples, and FRAP values of CRA, CRB, and SM did not significantly differ ($p < 0.05$). Creamers and skim milk showed no intrinsic antioxidant values in the FRAP assay.

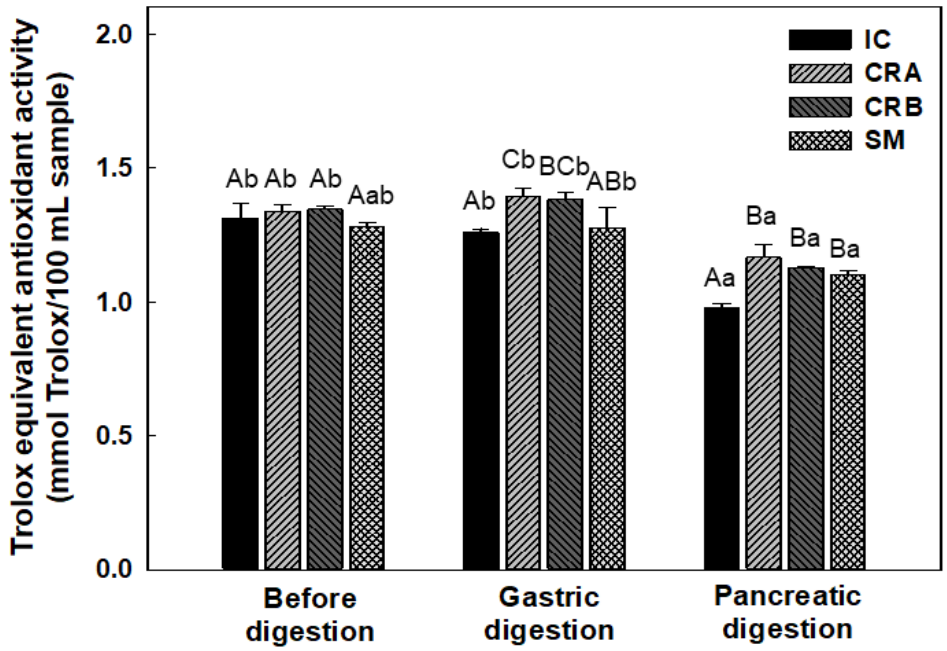


Figure IV-4. Ferric reducing antioxidant power of instant coffee alone (IC), instant coffee with creamer A (CRA), instant coffee with creamer B (CRB), and instant coffee with skim milk (SM) during *in vitro* gastric and pancreatic digestion. The values of CRA, CRB, and SM were obtained by including intrinsic values of creamer A, creamer B, and skim milk, respectively, at each digestion phase. Data are means ± standard deviations (n = 3). Uppercase letters indicate significant differences (p < 0.05) among different samples in the same digestion phase. Lowercase letters indicate significant differences (p < 0.05) among the same samples in different digestion phases.

Unlike the TP and $ABTS^+$ analysis, it was not found that creamers and skim milk increased in FRAP value during digestion, probably due to low electron transfer ability of peptides and amino acids under FRAP assay conditions (Babini, Tagliazucchi, Martini, Dei Piu, & Gianotti, 2017).

This can be explained by the fact that the differences in the antioxidant activity obtained by $ABTS^+$ and FRAP may be due to the different principles and mechanisms of the measuring methods.

IV-3-4. Total polyphenols and antioxidant activity

In this chapter, the effects of coffee creamer on the phenolic content and antioxidant activity of phenolics of instant coffee were investigated by an *in vitro* digestion process.

Overall, the TP content and the antioxidant activity ($ABTS^+$ radical scavenging activity and FRAP value) of IC was not changed during gastric digestion but decreased during pancreatic digestion. These results suggest that phenolic compounds in coffee, mainly chlorogenic acids, are maintained at gastric phase because they have high stability under the acidic conditions. In contrast, the phenolic compounds are degraded during pancreatic digestion, which is attributed to the low stability under the neutral condition (Narita & Inouye, 2013), as confirmed the 5-CGAs changes during the *in vitro* digestive process (Figure IV-2)

CRA and CRB showed similar TP content and antioxidant activity before digestion and gastric digestion. In addition to, the TP content and the antioxidant activity of CRA, CRB, and SM were higher than those of IC after pancreatic digestion. CRA, CRB, SM showed lower decreases in the TP content and the antioxidant activity than IC during pancreatic digestion. These results suggest that milk proteins preserve the antioxidant activity of phenolic compounds during digestion, particularly at pancreatic phase rather than at gastric phase.

However, the results of TP content and antioxidant activity were contradictory depending on the measuring methods; SM showed increases in the TP content and

activity but decrease in FRAP value after pancreatic digestion. These discrepancy in the results is caused by the difference in the measuring principles and measuring conditions of the TP content and antioxidant activity. Determination of TP content, measured by Folin-Ciocalteu assay, is based on single electron transfer mechanism, which is associated with the reducing power of phenolic compounds determined by FRAP assay (Apak, Özyürek, Güçlü, & Çapanoğlu, 2016). Meanwhile, $ABTS^{+}$ radical can be neutralized by either direct reduction via single electron transfer or by radical quenching via hydrogen atom transfer (Gulcin, 2020). Considering that different results were obtained despite of the same measuring mechanisms between TP content and FRAP value, it was thought that the different measuring conditions such as pH and ionic strength caused a significant difference in the results.

Thus, it was considered that FRAP values are reliable to evaluate the effects of creamer and skim milk addition on antioxidant activity of instant coffee after gastro-pancreatic digestion. Therefore, the antioxidant activity of 5-CGAs through *in vitro* digestion process was evaluated using the FRAP assay (Figure IV-5).

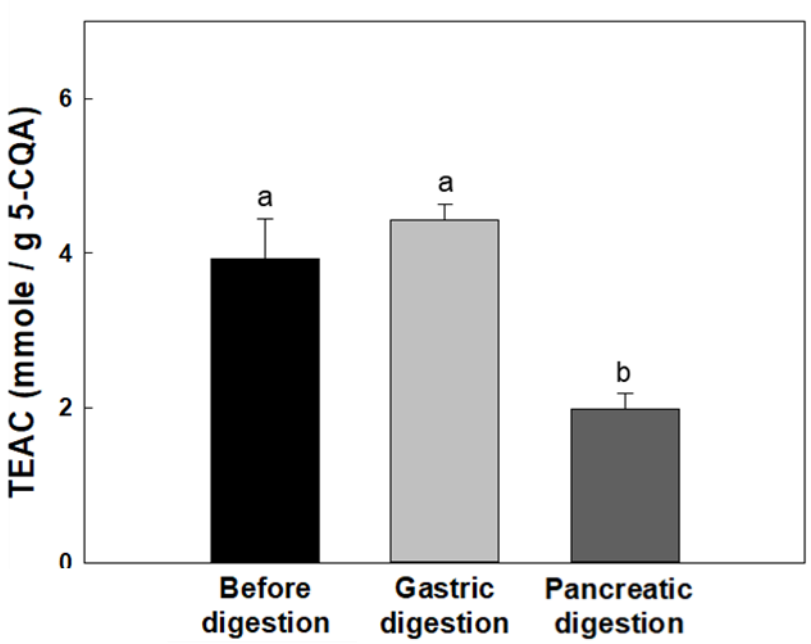


Figure IV-5. Ferric reducing antioxidant power of 5-caffeoylquinic acid (5-CQA) during *in vitro* gastric and pancreatic digestion. Data are means ± standard deviations (n = 3). Different lowercase letters indicate significant differences ($p < 0.05$) in the different digestion phases.

FRAP value of only 5-caffeoylquinic acid (5-CQA) was also not significantly different from that of before digestion, but, in the pancreatic phase, the ferric reducing antioxidant activity of 5-CQA was decreased significantly due to its instability under the neutral condition.

Meanwhile, the results of the TP content and $ABTS^{+}$ radical scavenging activity analyses were used to understand changes in the antioxidant activity in instant coffee caused by adding creamers and skim milk only before digestion.

At pancreatic phase, decreases in FRAP values of the samples, with respect to those before digestion, were as follows: IC, 25.6%; CRA, 12.7%; CRB, 16.2%; and SM, 13.8%. During digestion, IC showed the greatest decrease among the samples, whereas CRA, CRB, and SM showed similar decreases in FRAP values, regardless of their protein concentrations. These results may be due to the increased stability of phenolic compounds during digestion caused by the protective effect of the complexation between protein and phenolic compounds (De Morais, Pessato, Rodrigues, Peixoto Mallmann, Mariutti, & Netto, 2020).

However, in this study, it was not found how the protein concentration and type in creamers and skim milk had influence on enhancing the antioxidant of coffee beverages. Moreover, the antioxidant properties of the coffee beverages, found in this study, can be more susceptible to temparature changes, compared to coffee beverages prepared at higher temperature than that applied in this study. Further research is required to elucidate the exact mechanism of the milk proteins on preserving

antioxidant activity of coffee beverages during digestion, in terms of the complexation between proteins and phenolic compounds.

IV-4. Conclusions

The total phenolic (TP) contents of CRA and CRB were significantly higher ($p < 0.05$) than that of IC, at all the digestion stage. Decrease in total phenolic content of instant coffee after pancreatic digestion was attributed to the low stability of phenolic compounds under the neutral condition. The antioxidant activities of CRA and CRB were higher than those of IC after pancreatic digestion and showed lower decreases than IC during pancreatic digestion

After all, creamer A and creamer B had a positive effect on preserving the antioxidant activity of instant coffee in the gastrointestinal tract, particularly in the pancreatic phase, which was similar with the effects of skim milk.

As it was not elucidated how the protein with different types and concentration in creamers had effects on enhancing the antioxidant activity of instant coffee in this study, it is necessary to clarify the exact mechanism in terms of the complexation between proteins and polyphenols. Furthermore, the correlation of *in vitro* result with *in vivo* effects in human seems to be a further work to provide comparable and more accurate results.

IV-5. References

Alongi, M., Calligaris, S., & Anese, M. (2019). Fat concentration and high-pressure homogenization affect chlorogenic acid bioaccessibility and α-glucosidase inhibitory capacity of milk-based coffee beverages. *Journal of Functional Foods, 58*, 130-137.

Apak, R., Özyürek, M., Güçlü, K., & Çapanoğlu, E. (2016). Antioxidant activity and capacity measurement: classification, physicochemical principles, mechanisms, and electron transfer-based assays. *Journal of Agricultural and Food Chemistry, 64*(5), 997-1027.

Babini, E., Tagliazucchi, D., Martini, S., Dei Piu, L., & Gianotti, A. (2017). LC-ESI-QTOF-MS identification of novel antioxidant peptides obtained by enzymatic and microbial hydrolysis of vegetable proteins. *Food Chemistry, 228*, 186-196.

De Morais, F. P. R., Pessato, T. B., Rodrigues, E., Peixoto Mallmann, L., Mariutti, L. R. B., & Netto, F. M. (2020). Whey protein and phenolic compound complexation: effects on antioxidant capacity before and after *in vitro* digestion. *Food Research International, 133*, 109104.

Dubeau, S., Samson, G., & Tajmir-Riahi, H.-A. (2010). Dual effect of milk on the antioxidant capacity of green, darjeeling, and english breakfast teas. *Food Chemistry, 122*(3), 539-545.

Ferruzzi, M. G. (2010). The influence of beverage composition on delivery of phenolic compounds from coffee and tea. *Physiology & Behavior, 100*(1), 33-41.

Gulcin, İ. (2020). Antioxidants and antioxidant methods: an updated overview. *Archives of Toxicology, 94*(3), 651-715.

Kumazawa, K., & Masuda, H. (2003). Investigation of the change in the flavor of a coffee drink during heat processing. *Journal of Agricultural and Food Chemistry, 51*(9), 2674-2678.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Dupont, D. (2014). A standardised static *in vitro* digestion method suitable for food– an international consensus. *Food & Function, 5*(6), 1113-1124.

Muhammad, D. R. A., Praseptiangga, D., Van de Walle, D., & Dewettinck, K. (2017). Interaction between natural antioxidants derived from cinnamon and cocoa in binary and complex mixtures. *Food Chemistry, 231*, 356-364.

Murakami, K., Akiyama, M., Sumi, M., Ikeda, M., Iwatsuki, K., Nishimura, O., & Kumazawa, K. (2010). Differences in flavor characteristics of coffee drinks originating from thermal sterilization process. *Food Science and Technology Research, 16*(2), 99-110.

Narita, Y., & Inouye, K. (2013). Degradation kinetics of chlorogenic acid at various pH values and effects of ascorbic acid and epigallocatechin gallate on its stability under alkaline conditions. *Journal of Agricultural and Food Chemistry, 61*(4), 966-972.

Paquin, P. (1999). Technological properties of high pressure homogenizers: the effect of fat globules, milk proteins, and polysaccharides. *International Dairy Journal, 9*(3-6), 329-335.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food chemistry, 53*(10), 4290-4302.

Sánchez-González, I., Jiménez-Escrig, A., & Saura-Calixto, F. (2005). *In vitro* antioxidant activity of coffees brewed using different procedures (Italian, espresso and filter). *Food Chemistry, 90*(1-2), 133-139.

Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of Functional Foods, 18*, 757-781.

Tagliazucchi, D., Helal, A., Verzelloni, E., & Conte, A. (2012). The type and concentration of milk increase the *in vitro* bioaccessibility of coffee chlorogenic acids. *Journal of Agricultural and Food Chemistry, 60*(44), 11056-11064.

Wootton-Beard, P. C., Moran, A., & Ryan, L. (2011). Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after *in vitro* digestion measured by FRAP, DPPH, ABTS and Folin–Ciocalteu methods. *Food Research International, 44*(1), 217-224.

국문 초록

전 세계적으로 물을 제외하고 가장 섭취빈도가 높은 음료인 커피는 항산화 활성을 나타내는 폴리페놀이 풍부한 기호식품으로 널리 알려져 있다. 따라서, 커피의 건강기능성 및 여러 질병예방 효과 등이 최근 여러 영양역학 연구결과를 통해 지속적으로 입증되어 왔다. 또한, 커피에 대한 관심의 증가로 2000년대 초기부터 항산화물질이 풍부한 식품을 우유와 동반 섭취할 경우 식품내 항산화물질의 항산화력 및 체내 생체 이용률에 미치는 연구가 활발히 진행되어 오고 있다.

본 논문에서는 국내 인스턴트 커피의 섭취 유형으로 가장 빈도가 높은 커피믹스 형태를 고려하여, 크리머와 혼합하여 섭취할 때 인스턴트 커피의 항산화 활성에 미치는 영향을 비교 분석하고자 하였다. 특히, 수중유적형 유화액으로 알려진 크리머 제품에 있어서 유화를 구성하는 우유단백질의 종류 및 함량을 달리함으로써 식품 구성형태에 따른 항산화활성에 미치는 영향을 동시에 확인하였다.

첫번째로, 유화능 및 열안정성이 높은 산 카제인을 우유단백질원으로 사용하되 단백질 함량이 낮은 크리머 A와 상대적으로 유화능 및 열안정성이 낮은 농축 우유단백분말 및 무지방우유를 사용하되 단백질 함량이 높은 크리머 B의 유화특성을 비교하였다.

크리머 유화액 상태에서 지방구 입자의 크기뿐 아니라 지방구 응집에 대한 전하적 안정성, 터비스캔을 통한 유화시스템의 안정성을 비교해 보았다. 크리머 A가 전반적으로 크리머 B 대비해서는 안정적인 유화액을 구성한 것으로 평가되었으나 유의적인 차이를 나타내지는 않았다. 이는 크리머 B의 단백질 함량이 크리머 A 대비 높을 뿐 아니라 유화능이 낮은 농축 우유단백분말의 유화특성을 높인 제조특성 때문으로 분석된다.

그러나, 유화 안정성 지수인 TSI (Turbiscan stability index) 뿐 아니라 측정 테스트 튜브내 각 위치별로 지방구 입자의 변화양상을 분석한 결과, 농축 우유단백분말과 무지방 우유로 유화계를 구성하는 크리머 B가 크리밍 및 합일 (Coalescence)과 같은 입자의 이동현상에 있어 크리머 A 대비 빠르게 발생되었다. 이는 계면에 미 흡착된 단백질이 유화액내 입자의 이동에 영향을 준 것으로 추정된다. 실제 유화 계면에 흡착된 단백질과 미흡착된 단백질의 조성 및 함량을 비교한 결과, 크리머 B는 산 카제인만으로 구성된 크리머 A 대비 계면에서의 흡착율은 상대적으로 낮은 것으로 확인되었다.

두번째로, 인스턴트 커피의 폴리페놀과 크리머에 함유된 우유 단백질의 결합은 소화과정 중 유화액의 변화양상에 따라 달라질 것이며 이는 폴리페놀의 생체이용율에 영향을 미칠 것으로 예상하였다. 따라서, 인스턴트 커피 단독, 인스턴트 커피에 크리머 A 또는 크리머 B, 그리고 대조구로 무지방 우유를 첨가한 샘플을 IC, CRA, CRB, SM으로 하여 *in vitro* 위장관

소화모델을 통해 유화액의 변화과정을 살펴보고 각 소화단계별 인스턴트 커피유래 폴리페놀의 함량변화 및 생체 이용률을 비교 분석하였다.

위 소화과정에서 낮은 pH 및 소화효소에 따라 단백질의 분해가 발생되어 CRA와 CRB 모두 응집과 합일 그리고 크리밍 현상이 발생되었다. 단백질 함량이 가장 많은 SM의 경우, 위 소화 환경에서 다량의 단백질 응집 및 일부 단백질 분해를 통한 응집 형성으로 침전되는 현상이 관찰되었다.

소장 소화모델에서는, 유화특성의 차이에 따른 시간적인 차이는 있었지만 대부분 지방 및 단백질 분해효소에 의해 분해가 이루어져 침전현상이 관찰되었다. 각 시료별 폴리페놀과 단백질 복합체의 경우, 단백질의 함량 및 조성에 따라 다소 차이가 있지만 위장 소화모델 이후 모두 분해된 것으로 추정된다.

소화과정 중, 인스턴트 커피내 주요 폴리페놀인 클로로겐산 (Chlorogenic acids, CGA) 함량 분석을 통해 생체 이용률을 분석한 결과, 소화 전 및 위 소화 단계에서 시료 간의 유의적인 차이는 없었지만 인스턴트 커피의 총 클로로겐산이 수치적으로 가장 높았고 SM이 가장 낮았다. 그러나 소장 소화 이후에 IC, CRA 및 CRB의 총 클로로겐산 함량은 감소하였으나 SM의 경우 변화가 없는 것으로 나타났다. 이는 클로로겐산이 소장소화 조건에서 낮은 안정성을 나타내기 때문이며 상대적으로 우유단백질이

함유된 경우 이를 일부 보호해 주는 것을 확인할 수 있었다. 위장관 소화모델을 거친 후 총 클로로겐산의 생체 이용률은 IC의 경우 26.4%, CRA 40.5% 그리고 CRB가 51.7%로 나타났고 SM은 83.6%로 가장 높게 분석되어 단백질의 함량에 따른 유의적인 영향이 있는 것으로 확인되었다.

세번째로, 위장관소화과정을 통해 인스턴트 커피의 총 폴리페놀의 함량 변화 및 비교, 항산화활성을 비교함으로써 크리머가 미치는 영향을 평가하였다. 인스턴트 커피의 총 폴리페놀 함량은 소화전, 위 소화과정 이후 대비 소장소화 이후 감소되었으며 이는 중성 조건에서의 폴리페놀의 불안정성으로 추정된다. 추가적으로 커피내 가장 함량이 높은 5-caffeoylquinic acid (5-CQA)를 동일 처리조건 후 분석한 결과 소장소화 후 동일하게 감소하는 것을 확인하였다.

인스턴트 커피 유래 항산화활성을 $ABTS^{+}$ 및 FRAP 항산화 활성법을 통해 비교한 결과, 소화 전의 CRA, CRB의 항산화활성은 IC와 유사하지만 소장소화 후 IC 대비 높았다. 또한 소장소화 후 IC의 항산화활성 감소 폭 대비 적은 폭으로 감소된 것으로 볼 때 우유단백질이 소화과정 중 부분적으로 소장소화단계에서 커피의 폴리페놀의 항산화활성 보호에 도움을 주는 것으로 평가되었다.

그러나 본 연구에서는 크리머에 함유된 단백질이 어떻게 인스턴트 커피의 항산화 활성에 긍정적인 영향을 미치는 지에 대하여 밝히지는 못했다.

향후에 종류 및 함량이 다른 단백질과 폴리페놀과의 복합체 형성의 관점에서 정확한 메커니즘을 규명하는 추가 연구가 필요하며 또한, *in vivo* 실험 등을 통해 *in vitro* 실험과의 비교 등을 제시하는 것이 바람직할 것으로 보인다.

본 논문을 통해, 인스턴트 커피에 크리머를 첨가하여 섭취할 경우 소화과정을 거치면서 인스턴트 커피가 가지는 항산화물질인 클로로겐산의 생체이용률 뿐 아니라 항산화활성에 긍정적으로 영향을 미치는 것으로 확인되었다.

주제어: 크리머, 커피 항산화활성, 유화특성, 우유단백질, 생체 이용률, 총 폴리페놀, 클로로겐산, 위장관 소화모델

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