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Effects of konjac glucomannan and resistant starch on *in vitro* lipid digestion of non-dairy creamers

*Srikaeo, K. and Singchai, J.

Faculty of Food and Agricultural Technology, Pibulsongkram Rajabhat University, Muang, Phitsanulok, 65000, Thailand

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Abstract

The effects of konjac glucomannan (KG) and resistant starch (RS) on *in vitro* lipid digestion were examined on two types of non-dairy creamers, produced from palm and soybean oils. KG and RS were added to both types of non-dairy creamers and the samples were analyzed for particle size distribution. Sample emulsions were examined for their lipid digestion using an in vitro digestion model (pH 7.0: 2.0: 5.3: 7.5). Creaming stability, microstructure and free fatty acids (FFAs) were also analyzed. It was found that KG and RS showed different behaviors in controlling lipid digestion, as evidenced by droplet flocculation, phase separation (cream forming) and microstructural changes. In the presence of KG, the emulsions exhibited appreciable droplet flocculation and/or coalescence, resulting in phase separation of the emulsions. In contrast, the samples with RS exhibited no appreciable creaming. All emulsions appeared homogeneous and milky white. The amount of FFAs released after the digestion of the samples with KG was found to be lower than those with RS. Therefore, KG could be more effective in altering lipid digestion of the non-dairy creamers. The information found in this study could be used to create food emulsions with low caloric value or to optimize diets for individuals with lipid digestion problems.

Introduction

The lipids in food may be consumed in a wide variety of different physical structures such as oils, bulk fats or emulsified fats. Nevertheless, most fatty foods are broken down into oil-in-water emulsions in the mouth during mastication and within the stomach and small intestine during the digestion process (McClements and Li, 2010). Consequently, lipid digestion within the gastrointestinal tract typically involves digestion of emulsified fats. Lipid digestion involves several sequential steps that include various physicochemical and biochemical events (Torcello-Gomez *et al.*, 2011). In humans, the digestion of dietary fat commences in the stomach and continues within the small intestine, whilst the absorption of fat digestion products occurs primarily within the small intestine (Mu and Høy, 2004).

Overconsumption of fat is a major contributing factor to obesity, cardiovascular disease and diabetes (Bray and Popkin, 1998; Khogare, 2012). For this reason, there has been considerable interest in the development of effective strategies to reduce the caloric content of foods, or to reduce the spike in blood lipids that occurs after consuming a fatty meal. Several studies have suggested that certain types of dietary fibers can inhibit the digestion and absorption of lipids (Beysseriat *et al*., 2006; Edashige *et al*., 2008; Yonekura and Nagao, 2009). Numerous physicochemical and physiological mechanisms may contribute to this effect, including the ability of dietary fibers to alter the rheology of the gastrointestinal fluids, bind digestive components (such as bile salts and digestive enzymes), alter the aggregation state of lipid droplets, form protective coatings around lipid droplets and to be fermented within the large intestine by colonic bacteria (McClements *et al.*, 2008; Grabitske and Slavin, 2009; Lattimer and Haub, 2010). This benefit of dietary fibers has been confirmed in a number of animal and human feeding studies (Lairon, 1996; Carter *et al.*, 1998; Jenkins *et al*., 1998). Increased consumption of dietary fiber may, therefore, prove to be one method of reducing the effective caloric content of food products.

Dietary fiber, as a class of compounds, includes a mixture of plant carbohydrate polymers, both oligosaccharides and polysaccharides (e.g. cellulose, hemicelluloses, pectic substances, gums, resistant starch (RS), inulin), that may be associated with lignin and other non-carbohydrate components (e.g., polyphenols, waxes, saponins, cutin, phytates, resistant protein). Structurally, fiber can be sub-divided broadly into two forms, RS and non-starch polysaccharides (Elleuch *et al*., 2011). In this study,

*Corresponding author.
Email: *khongsak@live.psru.ac.th*

we examined two types of polysaccharides that act as dietary fibers, konjac glucomannan (KG) and RS. KG is a neutral polysaccharide composed of β-1,4 linked D-mannose and D-glucose, found abundantly in the konjac tuber. The content of KG varies between 8 and 10 g/100 g in the raw tuber. The refined konjac tuber powder contains KG in the range of 50–70 g/100 g, whereas purified powder or extracted KG has a content in excess of 90 g/100 g (Fang and Wu, 2004; Tatirat and Charoenrein, 2011). Several studies have proven the health benefits of KG (Chen *et al.*, 2005; Martino *et al*., 2005) and, consequently, it is now used in many industries such as food, pharmaceutical, biotechnology and fine chemical (Zhang *et al*., 2005).

RS is the fraction of starch that is not hydrolyzed to glucose in the small intestine, but which is fermented in the colon. Many studies have shown that RS is a linear molecule of α-1,4-D-glucan, essentially derived from the retrograded amylose fraction, and has a relatively low molecular weight. RS is composed of four groups: RS1 (physical inaccessible starch), RS2 (un-gelatinized starch granules), RS3 (retrograded starch) and RS4 (chemically modified starch). The physical properties of RS, particularly its low water-holding capacity, make it a functional ingredient that provides good handling and improves texture in the final product (Baixauli *et al.*, 2008; Fuentes-Zaragoza *et al*., 2010).

Non-dairy creamers were selected as the lipid food model as it contains more than 30% of vegetable lipids. It is gaining in popularity and being used in various foods and drinks. The fine powder form of non-dairy creamers is also suitable for fiber enrichment. In addition, research about this type of food product is mostly kept private for commercial use and, therefore, not much has been made available in academic literature. The information obtained from our study should prove beneficial for both commercial and academic uses.

Materials and Methods

KG and RS

KG was obtained in the form of konjac glucomanan powder (Konjac Foods Ltd.; USA), with a purity of >90 g/100 g dry solid. RS was obtained in the form of commercial RS2 (Hi-maize® 260) distributed by National Starch and Chemical (Thailand), with a minimum of 60 g/100 g dry sample total dietary fiber.

Non-dairy creamers and the sample mixtures

Spray dried non-dairy creamers from palm and soybean oils were produced on the commercial production facilities using the formula: 33% hydrogenated palm or soybean oil, 65% glucose syrup and 2% sodium caseinate. KG and RS were added to both non-dairy creamers at 0.5 g/100 g dry sample. This resulted in four mixture samples: Palm + KG, Palm + RS, Soy + KG and Soy + RS. Non-dairy creamers from palm and soybean oils without the addition of KG and RS were used as the control samples. The emulsions were prepared by dissolving 20 g of the mixture or control samples in 100 mL sodium bicarbonate buffer (pH 7.0) and mixed well using a homogenizer.

Particle size analysis

Particle size distribution of the non-dairy creamers and fiber mixtures (powder form) was measured by a Malvern Mastersizer 2000 equipped with a Scirocco 2000 dry dispersion unit (Malvern Instruments Ltd., UK). The measurement data were analyzed by the Mastersizer 2000 software Ver. 5.40.

in vitro *lipid digestion*

The *in vitro* digestion model used in this study was a modification of that described previously (Beysseriat *et al*., 2006; Klinkesorn and McClements, 2009). The model was designed to simulate the pH variations that food experiences during passage through the human digestive system. The procedure was as follows: emulsions were prepared as described above, adjusted to pH 7.0 with NaOH (if necessary) and stored at 37°C for 1 h. These emulsions were acidified to pH 2.0 with HCl, then incubated at 37°C for 1 h in a shaking incubator (95 rpm). The acidified emulsions were raised to pH 5.3 with NaOH, followed by addition of the Lipex® lipase solution (Novozymes, Denmark) to give a final concentration of 0.05 mg/mL sample and incubated for 2 h at 37°C. Finally, the pH of the emulsion was increased to 7.5 with NaOH and the samples were incubated at 37°C for 2 h, shaking at 95 rpm, to complete the intestinal phase of the in vitro digestion process.

Creaming stability

Fifteen milliliter aliquots of the solution mixtures were collected at the end of each stage of the digestion model (pH 7.0; 2.0; 5.3; 7.5) and transferred into test tubes. The solution emulsions were then stored for 24 h at 25°C, after which appreciable phase separation was observed. The height of each phase formed in the solution (serum phase, emulsion phase and creamed phase) was in accordance with Beysseriat *et al.* (2006).

Free fatty acid

The free fatty acids (FFAs) released after

Table 1. The parameters (v/v, µm) of the particle size distribution of the samples

Samples	Particle size distribution (v/v, µm)			
	10^{th} percentiles	50^{th} percentiles^{ns}	90^{th} percentiles	Volume weighted mean
Palm	26.05±1.11^{b}	121.61±3.14	264.44±4.34^{b}	137.00±4.09^{b}
Soy	26.06±1.10^{b}	122.85±3.85	266.08±5.11^{b}	138.29±5.18^{ab}
Palm + KG	27.75±0.82^{ab}	125.25±0.96	268.16±1.26^{b}	139.11±0.81^{ab}
Soy + KG	28.73±0.31^{ab}	126.38±0.46	282.15±2.75^{a}	144.00±1.03^{a}
Palm + RS	26.39±1.44^{b}	121.62±3.20	262.87±2.97^{b}	135.59±2.75^{b}
Soy + RS	31.17±5.14^{a}	127.81±5.08	278.19±4.94^{a}	144.08±4.20^{a}

Values are mean ± standard deviation (triplicate).
For each parameter (column), values with the same letters are not significantly different ($p>0.05$) and ns = not significant.

digestion were determined by titrimetry using 0.25 N NaOH and phenolphthalein as indicator. FFAs were expressed as oleic acid (Srikaeo and Pradit, 2011).

Microstructure

The microstructure of the emulsions was determined via optical microscopy using a Nikon Eclipse E400 microscope (Nikon Corporation; Japan). A drop of sample from each stage of the digestion model was placed on a microscope slide, covered by a coverslip and observed at a magnification of 200X. The observed images were acquired and analyzed using digital image processing software (UTHSCSA ImageTool, Ver. 3.0) as described by Srikaeo *et al.* (2011).

Statistical analysis

Standard t-test and Analysis of Variance (ANOVA) were performed by Minitab® ver. 16 with the confidence level of 95%.

Results and Discussions

Particle size distribution

Particle size distributions of all the samples are summarized in Table 1. Generally, all the samples showed similar particle size distribution patterns. Particle size affects the digestion and susceptibility to digestive enzymes, which could have been reduced in our analyses due to the similarity of particle size in all the tested samples.

in vitro *lipid digestion, creaming stability, FFAs and microstructure*

Images of the emulsions in the test tubes before and after passing through the digestion model (pH 7.0; 2.0; 5.3; 7.5) are shown in Figure 1. In addition, light microscopic images of the sample mixtures after passing through each step of digestion model are shown in Figure 2. It is clear that KG and RS display different patterns of results. For both types of

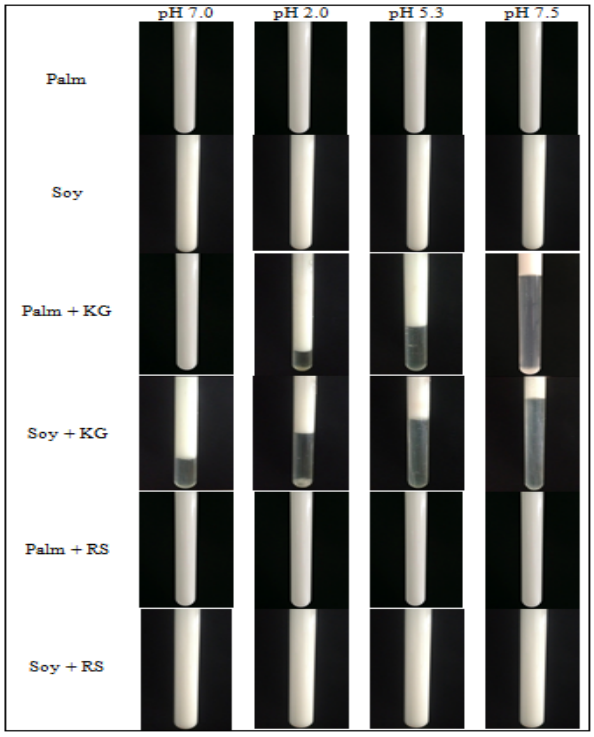

Figure 1. Images of the sample emulsions before and after passing through the digestion model (pH 7.0; 2.0; 5.3; 7.5)

samples (palm and soy), in the presence of KG and at all pH values, the emulsions exhibited appreciable droplet flocculation and/or coalescence. This resulted in creaming and phase separation of the emulsions (Figure 1). The creaming stability values, as determined by measuring the percentage of the height of the cream layer formed at the top of the emulsion after 24 h storage at 25°C, are shown in Table 2. However, emulsions in the presence of RS in both non-dairy creamers and at all pH values exhibited no appreciable creaming or phase separation, suggesting that no droplet flocculation, coalescence or creaming occurred throughout the digestion model. All suspensions appeared homogeneous and milky white (Figure 1).

Released FFAs are related to the rate of lipid digestion. KG was found to be a better inhibitor of lipid digestion, resulting in a lower amount

Table 2. Creaming stability as determined by measuring the percentage of the height of the cream layer

Samples	Creaming stability (%)			
	pH 7.0	pH 2.0	pH 5.3	pH 7.5
Palm	ND	ND	ND	ND
Soy	ND	ND	ND	ND
Palm + KG	ND	76.30±0.74	58.25±0.73	27.11±0.09
Soy + KG	76.54±0.71	61.63±0.39	46.63±0.72	19.55±0.39
Palm + RS	ND	ND	ND	ND
Soy + RS	ND	ND	ND	ND

Values are mean ± standard deviation (triplicate). ND = Not detected (no cream was formed).
For each pH (column), creaming stability values of palm and soy samples are statistically different as evaluated by t-test ($p<0.05$).

Table 3. Free fatty acids (FFAs) released after passing through the digestion model

Samples	FFAs (% as oleic acid)
Palm	0.70±0.09^{a}
Soy	0.78±0.02^{a}
Palm + KG	0.33±0.02^{c}
Soy + KG	0.34±0.03^{c}
Palm + RS	0.51±0.01^{b}
Soy + RS	0.54±0.01^{b}

For each parameter (column), values with the same letters are not significantly different ($p>0.05$).

of released FFAs (Table 3); these patterns were found in both types of non-dairy creamers. Light microscopic images of the microstructure confirmed the above results (Figure 2). After being subjected to the digestion model, all emulsions in the presence of KG became unstable, showing large droplet flocculation. In addition, emulsions in the presence of RS exhibited no appreciable creaming (Figure 1), but when observed by the light microscope, there was a slight increase of droplet size when the samples passed through the digestion model, especially at pH 5.3 and pH 7.5.

The instability of the emulsions to droplet aggregation as they passed through the in vitro digestion model may have occurred because of a number of different reasons. The influence of dietary fibers (pectin and chitosan) on the digestibility of emulsified lipids has been previously investigated (Beysseriat et al., 2006; Klinkesorn and McClements, 2009). These studies found that pectin promoted extensive droplet flocculation through a depletion mechanism, while cationic chitosan adsorbed to the droplet surfaces at some pH values and promoted droplet flocculation through a bridging mechanism. In this study, the non-ionic natural polysaccharides, KG and RS, with high molecular weights were studied. KG is water-soluble while RS appears to be a water-insoluble biopolymer (Yu *et al*., 2007; Fuentes-Zaragoza *et al*., 2010). We assumed that the droplet flocculation found in the emulsions in the presence of KG was flocculated through a depletion mechanism. It should be noted that fiber concentrations also influenced the droplet flocculation. The lowest concentration of a polymer required to promote depletion flocculation in an emulsion or the so-called critical flocculation concentration (CFC) decreases with increasing droplet size, as the magnitude of depletion attraction increases with droplet size (Beysseriat *et al*., 2006). At the concentration of KG used in this study (0.5 g/100 g), all of the droplets should exceed their CFC. In terms of RS, as they exhibited no appreciable creaming or phase separation, although a slight increase of droplet size was observed, we assumed that RS also promoted droplet flocculation but the interaction might not be strong enough to promote cream formation.

Recently, it has been reported that the characteristics of the droplet and composition, structure and physicochemical properties of the interfacial layer surrounding fat droplets, may play an important role in determining the extent and rate of lipid digestion and absorption (Armand *et al*., 1999). For example, the interfacial layer may form a protective coating around the lipid droplets that prevents lipase from accessing the emulsified triacylglycerol substrate (Klinkesorn and McClements, 2009). In this study, KG was able to promote droplet flocculation, leading us to assume that KG is more effective than RS in terms of preventing lipid digestion. The results of FFAs, as

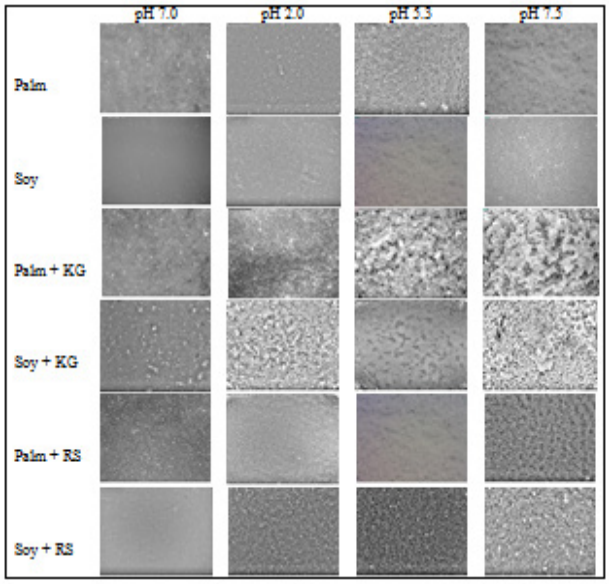

Figure 2. Light microscopic images of sample emulsions before and after passing through the digestion model (pH 7.0; 2.0; 5.3; 7.5)

presented above, also supported this assumption. The lower FFA content obtained from the samples with KG when compared to the samples with RS indicated that they were less digested (refers Table 3).

The results from this study indicate that the rate and extent of lipid digestion may be controlled by adding certain types of polysaccharides or dietary fibers, which may be useful for the rational design of functional foods and beverages. It should be noted that different fibers provide different patterns of droplet flocculation and, consequently, different alterations of lipid digestion. However, in order to assess lipid digestion in detail, new techniques may be required as other factors or properties may interfere with digestion as well (e.g. particle charges, concentrations, emulsifier types, particle sizes, etc.). For example, addition of a low level of pectin (0.025%) was found to increase the rate of lipid digestion in caseinate-stabilized emulsions, while high levels of pectin (0.5%) in Tween 80- and lactoferrin-stabilized emulsions was found to decrease the initial rate of lipid digestion. Various mechanisms have been proposed, including the increased lipid digestion rate being attributed to its ability to suppress droplet flocculation, while the decrease of lipid digestion rate was due to calcium binding or gel forming effects (Zhang *et al.*, 2015). In addition, xanthan gum at 0.2% and pectin at 0.1%, by weight, were found to significantly increase the rate of lipid digestion, which was attributed to their ability to inhibit droplet aggregation under gastrointestinal conditions (Qiu *et al*., 2015).

Conclusion

There has been considerable interest in the development of effective strategies to reduce the caloric content of foods. Certain types of polysaccharides, which act as dietary fibers, were found to inhibit the digestion and absorption of lipids. This study examined the effects of KG and RS on lipid digestion of non-dairy creamers containing glucose syrup, lipid and stabilizer. KG and RS provided different patterns of lipid digestion, as evidenced by droplet flocculation, phase separation (cream forming) and microstructural changes. Phase separation and cream formation were found in the non-dairy creamers with the addition of KG, while this phenomenon was not found in the samples with RS. This suggested that KG could be more effective than RS in controlling lipid digestion of non-dairy creamers. The information found in this study could, therefore, be used to create food emulsions with low caloric level, or to optimize diets for individuals with lipid digestion problems.

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