Hydrocolloids Decrease the Digestibility of Corn Starch, Soy Protein, and Skim Milk and the Antioxidant Capacity of Grape Juice.

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ABSTRACT: Hydrocolloids have many applications in foods including their use in dysphagia diets. We aimed to evaluate whether hydrocolloids in foods affect the digestibility of starch and protein, and their effects on antioxidant capacity. The thickening hydrocolloids: locust bean gum and carboxymethyl cellulose, and the gel-forming agents: agar agar, konjac-glucomannan, and Hot & Soft Plus were blended with corn starch and soy protein, skim milk, or grape juice and were examined for their in vitro-digestibility by comparing the reducing sugar and trichloroacetic acid (TCA)-soluble peptide, for antioxidant capacity by total polyphenol contents and the 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity. The hydrocolloids resulted in a decrease in starch digestibility with the gel-forming agents. Hydrocolloids diminished TCA-soluble peptides in skim milk compared to soy protein with the exception of locust bean gum and decreased free radical scavenging capacities and total phenolic contents in grape juice. Our findings may provide evidence for the use of hydrocolloids for people at risk of nutritional deficiencies such as dysphagia patients.

Keywords: hydrocolloids, in vitro-digestability, locust bean gum

INTRODUCTION

Food hydrocolloids can be classified into two groups (9), thickening agents that increase viscosity and gel-forming agents. Thickening agents include xanthan gum, LBG, carboxymethyl cellulose (CMC), starch, modified starch, and guar gum. Gel-forming agents include agar agar (AA), carrageenan, gelatin, pectin, gellan gum, and konjacglucomannan (KG) (9). CMC is a derivative of cellulose formed through a reaction with alkali or acid. CMC rapidly dissolves in both cold and hot water and results in a high-viscosity solution (10). LBG is a polysaccharide obtained from the seeds of the carob tree Ceratonia siliqua (11). LBG dissolves in cold water and is widely used as thickener and stabilizer in ice cream (12). AA is derived from red algae, and its main components are a mixture of agarose and agaropectin (13-15). Agarose is stable between pH 2.5 ~ 10 and forms a gel at temperatures between 35 and 45°C (16). KG is a polysaccharide found in the root stem of konjac and is composed of a mixture of glucose and mannose. Because of its fatty mouth feel when hydrated, KG is used as an ingredient in many commercial products such as ice cream, chocolate, and beverages. Gellan gum is a high-
molecular weight polysaccharide composed of two molecules of D-glucose, one molecule of D-glucuronic acid, and one molecule of L-rhamnose, which are produced by the microbe Sphingomonas elodea during fermentation (17). Gels formed by gellan have high resistance to acid, heat, and enzyme activity (17).

During both food preparation and digestion, interactions among the constituents of the food affect the digestibility and absorption of nutrients such as starch (18). In addition, the absorption of nutrients is dependent on the concentration of the nutrients in the food and their availability to intestinal absorption. These processes are affected by the chemical and physical characteristics of the food such as its dietary fiber content (19,20). The inhibitory effects of food hydrocolloids on the digestion of foods have been reported (21). The addition of resistant polysaccharides has been shown to reduce the digestibility of foods by 10% compared to that of foods without these additives (22). LBG has been shown to reduce the availability of minerals such as zinc in milk (19). Guar gum has postprandial blood glucose-lowering effects in individuals with diabetes and results in a subsequent reduction in urine glucose concentration (23). Gelatin has been shown to reduce the absorption of digested starch and postprandial blood glucose levels (24). Glucomannan, which is rich in KG, also has blood cholesterol-lowering effects (25).

Despite the benefits of food hydrocolloids for individuals with dysphagia, their effects on food digestibility, nutrient absorption, and the availability of polyphenols and antioxidants need to be considered for the use of hydrocolloids in commercial products. The selection of foods with high antioxidant capacity has been implicated in reducing the risk for cardiovascular diseases (26). It was proposed that the accessibility of ingested antioxidants for intestinal absorption is important (27). There are limited studies assessing the impact of food hydrocolloids on the digestion of starch and protein, as well as on their antioxidant capacity. In order to evaluate the effects of the addition of hydrocolloids in foods, we chose several different food sources for starch, protein, and polyphenols, which were corn starch, skim milk, soy protein, and grape juice, respectively. Our study evaluated the digestibility of starch and protein, total phenolic content available for absorption, and the free radical scavenging capacity of these food matrices after being blended with various types of hydrocolloids using an in vitro digestion system.

**MATERIALS AND METHODS**

**Materials**
Commercially available food ingredients were used for the study: corn starch powder that consists of 85% (w/w) corn starch and 10% (w/w) wheat flour (Choyafood, Eumseong, Korea), skim milk containing 35% (w/w) milk protein (Seoulmilk, Seoul, Korea), soy protein consisting of 90% isolated soy proteins (SUPRO® 710 IP, Solae, Pryor Creek, OK, USA), and grape juice (Del Monte, Lotte Chilsung, Seoul, Korea). The thickening agents included LBG (Cargill Deutschland GmbH, Krefeld, Germany) and CMC (Bolak Co., Hwaseong, Korea). The gel-forming agents used for the study were AA (Myeng Shin Agar Agar Mfg. Co., LTD., Yangsan, Korea), KG (Hubei YiZhi Konjac Biotechnology Co., Ltd., Yichang, China), and Hot&Soft (Korea Medical Foods Co., Seoul, Korea). The main components of Hot&Soft Plus are gellan gum, dextrin, and α-amylase. Purified pepsin from porcine gastric mucosa was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Pancreatin from porcine pancreas and bovine bile were purchased from Sigma-Aldrich Co.

The preparation of food specimens using thickening or gel-forming agents
Each food specimen was prepared by cooking or mixing with one of the thickening or gel-forming agents at specified ratios (Table 1). Corn starch powder was used at 2% (w/w) of the total weight of the specimen. To prepare the test samples, 2% (w/w) corn starch powder, 4% (w/w) skim milk, 4% (w/w) soy protein, or 20% (v/v) grape juice was used. To prepare the thickened specimens, corn starch was mixed with each of the thickening agents and warmed in a boiling water bath for 5 min. The skim milk and soy protein were cooked for 2 min in a boiling water bath after being mixed with a thickening agent. The grape juice specimen was prepared by mixing with a thickening agent without warming. To prepare the gel-form ed specimens, each of the food ingredients, including corn starch powder, skim milk, soy protein, and grape juice, were mixed with a gel-forming agent and then warmed in boiling water for 5 min. During the preparation of the specimens, distilled water

<table>
<thead>
<tr>
<th>Type of hydrocolloid</th>
<th>2% corn starch</th>
<th>4% soy protein</th>
<th>4% skim milk</th>
<th>20% grape juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CMC (%)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>LBG (%)</td>
<td>1</td>
<td>1</td>
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<td>1</td>
</tr>
<tr>
<td>AA (%)</td>
<td>0.5 or 0.8</td>
<td>0.5 or 0.8</td>
<td>0.5 or 0.8</td>
<td>0.5 or 0.8</td>
</tr>
<tr>
<td>KG (%)</td>
<td>0.5 or 0.8</td>
<td>0.5 or 0.8</td>
<td>0.5 or 0.8</td>
<td>0.5 or 0.8</td>
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<tr>
<td>Hot &amp; Soft (%)</td>
<td>0.5 or 0.8</td>
<td>0.5 or 0.8</td>
<td>0.5 or 0.8</td>
<td>0.5 or 0.8</td>
</tr>
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</table>

1Control, 20% grape juice with no addition of hydrocolloids: CMC, carboxymethyl cellulose; LBG, locust bean gum; AA, agar agar; KG, konjacglucomannan; Hot & Soft, hot & soft plus.
was added to replace lost water, and the specimen was homogenized using a Braun hand blender (Braun Española S.A, Barcelona, Spain). The control specimen was prepared by adding distilled water without thickening or gel-forming agent. Hot&Soft which main components are gelan gum, dextrin and α-amylase was recommended to be used at 0.8% (w/w) according to the manufacturer’s protocol. In order to compare the effects of different types of gel-forming hydrocolloids we used 0.8% of AA, KG, and Hot&Soft and included lesser concentrations of 0.5%. In our preliminary tests, 0.8% concentrations of the thickening agents (CMC and LBG) did not thicken the specimens enough for the comparison with the gel-forming agents. Thus, we decided to use 1% of CMC and LBG for the study.

**Viscosity measurements**

Solutions consisting of 100 mL of each specimen was used to measure the viscosity using a Viscometer (RVDV-II+Pro, Brookfield Engineering Laboratories, Middleboro, MA, USA). The measurements were conducted using a RV5-No.4 spindle at 100 rpm at room temperature (16±2°C) for 5 min. All measurements were performed in triplicates.

**In vitro digestion of the food specimens**

The in vitro digestion system used in this study mimics digestion in the stomach and small intestine. For the tests, 10 g of each food specimen was added to distilled water with a final volume of 100 mL. The pH of the solution was adjusted to 2.0 using a 6 M HCl solution. After the addition of 0.3 mL of pepsin solution (10 g pepsin/100 mL of 0.1 M HCl), the sealed sample was incubated in a shaking incubator (IS-917R, JEIO THCH, Gimpo, Korea) for 2 h at 37°C and 120 rpm. After the solution was adjusted to pH 7.5, 1.5 mL of a mixture of pancreatic and bile (3 g/L of pancreatic and 7 g/L of bile in 0.1 M NaHCO₃) was added for incubation in a shaking incubator for 2.5 h at 37°C and 120 rpm. After incubation, the samples were centrifuged at 12,000 g for 20 min, and the supernatants were stored until use.

**Determination of reducing sugars and peptides**

To measure the content of reduced sugars in the supernatants, a solution containing 3,5-dinitrosalicylic acid (DNS: 10 g 3,5-dinitrosalicylic acid, 0.5 g sodium sulfite, 10 g sodium hydroxide in 1 L of water; Bio Basic, Markham, Canada), sodium sulfite (Sigma-Aldrich Co.), and sodium hydroxide (Duksan Pure Chemicals, Seoul, Korea) was mixed in a 1:1:1 (v/v/v) ratio. After 5 min of boiling, the tube was cooled in an ice water bath, and the absorbance was measured at 595 nm with a UV-Vis plate reader (VersaMAX, The Lab World Group, Boston, MA, USA). A standard curve was generated using maltose. The total amount of reducing sugar was calculated by multiplying the concentration of reducing sugar and the total volume of the supernatant from each food sample.

For skim milk and soy protein, 10 mL of 20% trichloroacetic acid (Duksan Pure Chemicals) was added prior to the final centrifugation. The digestibility of the protein was examined by measuring the TCA-soluble peptides in the supernatant from the food samples using a bicinchoninic acid protein assay kit (Thermo Scientific, Hudson, NH, USA) according to the manufacturer’s instructions.

**DPPH free radical scavenging ability and total polyphenol content**

The supernatant of the grape juice samples was diluted with distilled water in a 1:1 (v/v) ratio and then mixed with DPPH solution containing 2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich Co.) in methanol. After 30 s of shaking, the samples were protected from light and incubated for 30 min at room temperature. The absorbance was measured at 517 nm using a microplate reader. A 70% ethanol solution was used as the control solution. The free radical scavenging capacity of the sample was calculated based on the relative value compared to the control using the following formula:

$$\text{free radical scavenging capacity (\%) } = \frac{\text{absorbance of control } - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

To determine the total polyphenolic content, the colorimetric Folin-Ciocalteu method was used (28,29). For this test, 100 µL of the supernatants from the in vitro digested grape juice samples were diluted with 1 mL of distilled water. Next, 100 µL of Folin-Ciocalteu’s reagent was added to the mixture and agitated for 3 min at room temperature. After the addition of 200 µL of 1 N Na₂CO₃, the tubes were incubated for 1 h in the dark. At the end of the incubation, the absorbance was measured at 725 nm with a UV-Vis plate reader (VersaMAX, The Lab World Group). All measurements were carried out in triplicates. The calibration curve was constructed using gallic acid (Sigma-Aldrich Co.). The total phenolic compound amount was determined by comparison to the curve, and the results are expressed as mg equivalents of gallic acid/g of sample.

**Statistical analyses**

SPSS was used to perform statistical analyses (Statistical Package for the Social Sciences; SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by Duncan’s multiple comparisons test was used to determine statistically significant differences among the
**RESULTS**

**Viscosity of food samples prepared with a thickening agent**

Corn starch, soy protein, skim milk, and grape juice were examined for viscosity after mixing and quick boiling with CMC or LBG at a 1% (v/v) concentration (Fig. 1). In the case of CMC, all viscosities of the tested food types were similar, in a range between 2,110.7 and 2,671.0 cP. On the other hand, LBG showed a wide range of viscosities between 261.1 cP and 2,248.0 cP. The viscosities of CMC and LBG were similar in corn starch; however, in soy protein, skim milk, and grape juice, LBG was less viscous than CMC. The viscosity of soy protein with LBG was 52.9% compared to that with CMC. The viscosity of grape juice with LBG was 12.4% compared to that with CMC. These results indicate that CMC results in a relatively consistent viscosity, whereas the viscosity with LBG depends on the food source.

**Reducing sugar content after in vitro digestion**

In order to investigate the effects of various thickening (CMC and LBG) or gel-forming (AA, KG, and Hot&Soft) agents on starch digestibility, the degree of reducing sugar formation was measured in the supernatant fraction of the corn starch sample after in vitro digestion and centrifugation. Compared to the control, which did not contain any agents, the use of thickening or gel-forming agents reduced the amount of reducing sugar formed (P<0.05) (Fig. 2). CMC and LBGs showed similar reductions of 11.7% and 13.2%, respectively, in reducing sugar content after in vitro digestion compared to that of the control. With 0.5% or 0.8% concentrations of AA, KG, and Hot&Soft, there were greater reductions in starch digestion, which ranged from a 28.4% decrease with 0.5% AA to a 46.3% decrease with 0.8% AA. AA dose-dependently reduced the content of reducing sugars. Our data indicated that in the digestion of starch, thickening agents (CMC and LBGs) resulted in a greater hydrolysis of starch than did the gel-forming agents (AA, KG, and Hot&Soft). The gel-forming agents AA, KG, and Hot & Soft showed greater inhibitory effects on starch digestion, with larger reductions in reducing sugars compared to that of the control, even at lower concentrations (0.5% and 0.8%), compared to the effects of CMC and LBG (1%).

**TCA-soluble peptide content after in vitro digestion**

Soy protein and skim milk were examined for protein digestibility by measuring the TCA-soluble peptide content (Figs. 3A and 3B). Addition of CMC or LBG resulted in minor 14.7% and 14.4% respective reductions in the TCA-soluble peptide content of soy protein compared to those of the control. In skim milk, CMC resulted in greater reductions than LBG (30.8% and 15.8%, respectively). LBG had comparable reduction rates in both soy protein and skim milk (14.4% and 15.8%, respectively), but all of the other tested agents had much greater reduction rates in skim milk than in soy protein. On the other hand, 0.8% of AA and KG resulted in a greater decrease in TCA-soluble peptide content than with the 0.5% concentrations. In addition, 0.5% and 0.8% of Hot&Soft resulted in reductions in both soy
protein and skim milk. Our results suggested that overall the hydrocolloids resulted in a 14.6% to 27.6% reduction in the protein hydrolysis of soy protein and a 27.6% to 45.1% reduction in the protein hydrolysis in skim milk. The tested hydrocolloids, with the exception of LBG, showed a greater inhibitory effect on the protein digestibility of skim milk than on soy protein. In addition, AA and KG were inhibitory in a dose-dependent manner.

**Total phenolic content and the free radical scavenging capacity of grape juice prepared with thickening or gel-forming agents**

After *in vitro* digestion, the soluble fractions of the samples were examined for total phenolic content and free radical scavenging capacity. The results showed that 1% LBG and 1% CMC decreased the total phenolic content by small margins of 3.2% and 9.5%, respectively, compared to that of the control (Fig. 4A) and diminished the DPPH free radical scavenging capacity greatly by 40.7% and 54.4%, respectively (Fig. 4B). Among the gel-forming agents, 0.5% KG resulted in a higher total phenolic content and greater free radical scavenging capacity than after the addition of AA or Hot&Soft (Figs. 4A and 4B). The addition of 0.8% KG resulted in a slight reduction of 12.7% in the soluble total phenolic content but greatly decreased the free radical scavenging capacity by 51.6%, which was even lower than those observed with 0.8% AA or 0.8% Hot&Soft (39.0% and 36.2%, respectively) (Figs. 4A and 4B). In addition, AA and Hot&Soft did not significantly affect the free radical scavenging capacity. Overall, the total polyphenol content was
not conspicuously reduced by most hydrocolloids except Hot&Soft, and the free radical scavenging capacities were decreased by all hydrocolloids compared to the control. Thus, it appeared that hydrocolloids produced a small reduction in total phenolic contents in the soluble fraction after in vitro digestion but greatly decreased the antioxidant capacity of associated food sources.

**DISCUSSION**

High viscosity has been shown to reduce the digestibility of foods in animal models by altering the interactions between enzymes and digested food components (30). Therefore, it was assumed that the addition of any thickening or gel-forming agent would reduce digestibility. In this study, all of the thickening and gel-forming agents tested reduced the levels of reducing sugar and peptides after the in vitro digestion of corn starch, soy protein or skim milk. However, the degree of reduction differed with the type of hydrocolloid tested and the food source.

The digestion of corn starch was less affected by the thickening agents CMC and LBG than the gel-forming agents AA, KG, and Hot&Soft. These findings are consistent with a previous report in which CMC reduced the total starch hydrolysis in a corn starch food matrix (31). The mechanism of action for hydrocolloids in the inhibition of starch digestion was suggested to be due to the formation of a physical barrier that limits digestive enzyme attack (32,33). These results suggest that, when the slow digestibility of starch is beneficial, such as in the case of diabetes, gel-forming agents such as AA or Hot&Soft might be a good choice of hydrocolloids because they seem to slow the speed of intestinal absorption of glucose. An in vivo experiment demonstrated that the use of dietary fibers and fiber analogues lowered glucose production as indicated by decreased insulin responses (34). However, in individuals who need to maintain a high digestibility of starchy food, such as elderly individuals with dysphagia who are at risk for malnutrition, CMC or LBG may be better choices for hydrocolloids rather than the gel-forming agents like AA, KG, and Hot&Soft. In a previous study where corn starch was blended with a hydrocolloid, the researchers found that guar gum did not affect the resistant starch fraction after in vitro starch digestion, although xanthan gum and hydroxypropylmethylcellulose lowered the resistant starch fraction (31). Our results might not be able to be extended to other thickening or gel-forming agents because of the diverse effects of different types of hydrocolloids on the digestibility of starchy foods.

In accordance with a previous finding that plant hydrocolloids lowered the digestion of proteins (35), we also showed that the protein digestibility of soy protein and skim milk was decreased by all of the tested hydrocolloids, although with varying extents. Furthermore, the reduction rates were influenced by the type of food matrix. Differences in protein digestibility between soy protein and skim milk might be in part due to the presence of other nutrients that might have interfered with protein digestion. The protein content in skim milk was 58% (w/w) as compared to 90% (w/w) in soy protein, indicating that there were other nutrients that might have contributed to the inhibitory effects of the hydrocolloids. As previously suggested, the mechanism of a hydrocolloid’s inhibitory effect on protein hydrolysis may be due to its direct binding to the enzyme or substrate, thus weakening the interaction between enzyme and substrate, or through an indirect mechanism by changing the conformation of the enzymes (36,37). On the other hand, there was no clear association between the degree of viscosity and the reduction in protein digestibility; 1% LBG increased the viscosity of soy protein to levels that were half that of 1% CMC. However, protein digestibility did not differ between 1% LBG and 1% CMC (149.0 and 149.2 mg peptide/g, respectively). Skim milk that was treated with LBG had 80% the viscosity of the milk that was treated with CMC and demonstrated better digestibility, suggesting that skim milk with lower viscosity may be more easily digested. Interestingly, it appeared that 1% LBG hydrocolloid resulted in the smallest inhibition of protein digestion in skim milk. In general, it seems that the source of the food, and the type of hydrocolloid were more likely than viscosity to determine the digestibility of protein in a food matrix.

As for grape juice specimens, it appears that there is no association between polyphenol content and free radical scavenging capacity of in vitro digested grape juice mixed with hydrocolloids. The hydrocolloids did not decrease the total polyphenol contents available after in vitro digestions. However there were great reductions in the free radical scavenging capacities in hydrocolloids-containing grape juice. Thus, it is likely that hydrocolloids may reduce the total antioxidant capacity in foods rather than lower the polyphenol content available for intestinal absorption. Hydrocolloids seemed to greatly lower the total antioxidant capacity as assessed by the free radical scavenging capacity but did not affect the content of absorbable total polyphenols.

There are several limitations in our study. The specimens with gel-forming agents failed to be tested for their viscosities. Thus, our in vitro digestion data were not accurately explained by the effects of viscosities differentially caused by the gel-forming agents. In addition, despite of differences in the viscosities caused by CMC and LBG, these agents displayed similar responses in total polyphenol contents and free radical scavenging capacity in the grape juice food matrix. Additional antioxi-
dant assays are warranted to corroborate these findings. Because of the in vitro digestion system used in this study, our findings need to be tested in an in vivo system.

CONCLUSIONS

We demonstrated that the addition of hydrocolloids reduced the digestibility of starch and protein as well as the total antioxidant capacity of grape juice. Our data provides evidence of the possible adverse effects of hydrocolloids on the digestibility of nutrients and antioxidant capacity in individuals who may be at risk for malnutrition. On the other hand, the use of hydrocolloids may be desirable for the development of foods with a low glycemic index.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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