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# THE DIGESTIVE INHIBITION OF CASTELLA WITH CHLOROPHYLL INGREDIENTS

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## ABSTRACT

This study analyzes the degree of inhibitory effects of α-amylase and α-glucosidase when vegetable ingredients such as mugwort, green tea, and chlorella are added to castella cakes. They have a lot of dietary fiber as food ingredients. After preparing castella with these ingredients, water and ethanol were used as solvents, respectively, to extract the composition affecting these enzymes. α-amylase inhibitory activity of water extract for mugwort, green tea, and chlorella castellas were evaluated 60.2%, 54.9%, and 38.1% at 1.0 mg/ml, respectively. α-amylase inhibitory activity of ethanol extract for mugwort, green tea, and castella were evaluated 62.1%, 56.5%, and 41.3% at 1.0 mg/ml, respectively. The α-amylase inhibitory activity of the mugwort was higher than those of green tea and chlorella. There was no significant difference among three groups ($p$>0.05). α-glucosidase inhibition of water extract for mugwort castella evaluated 12.6% at 0.25 mg/ml and 57.0% at 1.0 mg/ml. α-amylase inhibitory activity of ethanol extract for mugwort, green tea, and castella were evaluated 58.2%, 54.9%, and 52.0% at 1.0 mg/ml, respectively. Vegetable materials such as mugwort, green tea, and chlorella are believed to be able to slightly control the factors of obesity by delaying or inhibiting digestive absorption to give a feeling of satiety.

**KEYWORDS:** α-amylase, α-glucosidase, Castella, Chlorella, Green tea, Mugwort.

## INTRODUCTION

Castella (Japanese is also called kasutera) is a kind of wagashi (a Japanese traditional confectionery) originally developed in Japan.[1] In the 16th century, the Portuguese reached Japan. In the Edo period, in part due to the cost of sugar, castella was an expensive dessert to make despite the ingredients sold by the Portuguese. The name is derived from Portuguese Bolo de Castela, meaning "cake or bread from Castile". Castella has gained popularity worldwide due to its distinctive pillowy, Soufflé-like texture, rich aroma, and slightly sweet taste.[2]

Castella is such a simple dessert. There are now many varieties made with ingredients such as powdered green tea, brown sugar, and honey. A traditional Japanese sweet made from bread flour, eggs, sugar and mizuame, a type of sugar syrup which gives the cake a moist texture, Japanese castella is a denser and slightly gooier in texture with a darker crust. This castella is the definition of a perfect sponge cake.

Traditional casetella cakes have high calories while containing little dietary fiber.[3] Dietary fiber is the portion of plant-derived food that cannot be completely broken down by human digestive enzymes and are nondigestible and provide beneficial health effects.[4] Dietary fiber is found in plants, typically eaten whole, raw or cooked, although fiber can be added to make dietary supplements and fiber-rich processed foods. Dietary fiber increases food volume without increasing caloric content to the same extent as digestible carbohydrates, providing satiety which may reduce appetite.[5] In addition, it acted resistant to the action of human alimentary enzymes.[6] Therefore, adding fiber to castella cakes is necessary to improve their nutritional value. Fibre was utilized bakery products such as integral breads and cookies.[7, 8]

*Artemisia princeps* (mugwort) is an Asian plant species in the Asteraceae family. The species is native to China, Korea and Japan. Mugwort is widely used in Korean foods as well as in traditional medicine.[9] Mugwort has been used in traditional Asian medicine for the treatment of inflammation, diarrhea, carbuncles, bacterial infection, and circulatory disorders.[10, 11]

Green tea is a type of tea that is made from *Camellia sinensis* leaves and buds. Green tea originated in China, and since then its production and manufacture has spread to other countries in East Asia. Green tea is widely available across the globe. There are many compounds, nutrients, vitamins, and minerals in green tea leaves.[12] Numerous claims have been made for the medicine or health benefits of green tea.[13] Green tea is mainly used for drinks, but powders are also used for cooking.

Numerous studies indicate that tea has versatile health benefits, and attempts are being made to use it as a food additive.[14]

Chlorella is a genus of about thirteen species of single-celled algae off the division Chlorophyta and grows in fresh water. It's sometimes called seaweed. It's used for nutrition, human health, and as medicine.[15] Chlorella is a good source of protein, fats, carbohydrates, fiber, chlorophyll, vitamins, and minerals.[16] It is commonly used as a super food and can be found as an ingredient in certain liquid-based cocktails.

## MATERIALS AND METHODS

### Preparation of castella

Materials for making castella were used mainly 5 basic ingredients: bread flour, eggs, sugar, milk, and honey.

I foamed the whites to a certain extent, then added salt, and whipped the sugar three times to make meringue. I whipped until the meringue was made, until it showed a soft bending firmness. The ingredients were mixed evenly by whipping at low speed while adding yolk one by one to the finished white meringue. Whipped at low speed and mixed evenly while pouring milk, honey, and oil little by little. If you whip it for too long, the foam won't hesitate, so you poured it little by little so that it wouldn't sink, and whipped it quickly with a whisk. The powder ingredients were sifted in and quickly mixed evenly, scratching the floor with a spatula. A few drops of vanilla oil were dropped and mixed evenly to complete the dough. About 60% of the dough was filled in a mold with parchment paper, the top was evenly organized, and the pan was hit on the floor to remove the remaining air bubbles. It was baked in the bottom of the oven preheated to 170-180 degrees, and after 10 minutes, the temperature was lowered to 150-160 degrees and baked for 30 to 40 minutes. When fully cooked, hit the pan on the floor, take it out of the mold, turn it over on the Teflon sheet, and cool it.

### Preparationt of aditive dietary fiber materials

Flour is also an ingredient of carbohydrates, but rice powder was added as a source of carbohydrate nutrition to reduce flour and increase rice consumption. Fish collagen powder was added as a protein source. Mugwort, green tea, and chlorella powder were added as sources of blue and inorganic salts.

### Sample preparation of α-amylase inhibitory assay

Casetella (1,000 g) were added to 3,000 ml of distilled water or 80% ethanol and a grinding mixer. They were squeezed out with the muslin cloth. An aliquot of the mixture was further mixed with 100 mM Tris-HCl buffer (pH 7.4). The mixture of boiling group was further stirred with a magnetic bar at 60℃ for 30 minutes. The sample was treated with ultrasound at room temperature for 60 minutes. The ultrasound extraction was carried out using an ultrasonic bath (5510, Branson, USA). The mixture was shaken vigorously for one hour at room temperature. Extracted sample was filtered. The sample was evaporated to remove solvent under reduced pressure and controlled temperature by using rotary vacuum evaporator (N-1001S-W, Eyela, Tokyo, Japan). To get dry powder, samples placed in a low temperature vacuum chamber.

### α-amylase inhibitory assay

The α-amylase inhibitory assay was determinated by the method described Apostolidis and Lee[17] with slight modification. α-amylase inhibitory activity was carried out by quantifying the reducing sugar (maltose equivalent) liberated under assay conditions. The inhibitor, different extracts, and fractions of water or solvent (control) were added to different well of 96 well microplate. The assay mixture containing 25 µl of 50 mM phosphate buffer pH 6.8, 2.5 µl extract and 0.25 U/ml pre-incubated porcine α-amylase (Sigma Aldrich Chemical Co, Steinheim, Germany) were incubated at 37℃ for 10 min. After pre-incubation, 25 µl of 0.5% starch solution was added. The reaction mixtures were then incubated at 37℃ for 10 min. The reaction was terminated with the addition of 150 µl of 90 mM 3,5-dinitrosalicylic acid (DNS) reagent and placed in boiling water bath for 10 minutes. The extract was then cooled to room temperature until use. Absorbance (A) was measured at 540 nm. Acarbose (4,6-Dideoxy-4-([1S]-[1,4,6/5]-4,5,6-trihydroxy-3-hydroxymethyl-2-clohexenylamino)-maltotriose) (Sigma Aldrich Chemical Co) was used as reference standard (positive control).

Control incubations represent 100% enzyme activity and were conducted in a similar way by replacing extracts with vehicle. For blank incubation (to allow for absorbance produced by the extract), enzyme solution was replaced by buffer solution and absorbance recorded. Separate incubation carried out for reaction t = 0 was performed by adding samples to DNS solution immediately after addition of the enzyme. Experiments were performed in triplicate.

### α-glucosidase inhibitory assay

The bioassay method of multiwell plate system was applied for α-glucosidase inhibitory activity assay as described by Deutschlander et al.[18] with some modification. Extracts and catechins were prepared as described above. The test compound and 2 mU of Yeast α-glucosidase (Cat. No: G 5003, Sigma Aldrich Chemical Co, Steinheim, Germany) was dissolved at a concentration of 0.1 U/ml in 100 mM sodium acetate buffer (pH 5.6). Enzyme source was prepared bovine serum albumin 2000 mg/ml and sodium azide 200 mg/ml in 100 mM sodiumacetate buffer (pH 5.6). Paranitrophenyl-α-D-glucopyranoside (pNPG) (Cat. No: N1377, Sigma Aldrich Chemical Co, USA) was used as substrate. A total of 20 ul from each extract were diluted to 97 µL in 0.1 M sodium acetate buffer (pH 5.6) and pre-incubated in 96-wellplates at 37℃ for 10 min. The reaction was initiated by adding 3 µL of 3 mM pNPG as a substrate. The plate was incubated for an additional 10

min at 60℃, followed by addition of 100 µL 1 M NaOH to stop the reaction. All test compounds were prepared in DMSO as described above. The final concentrations of extracts and catechins were between 0.03-10 µg/mL and 5–1000 µM, respectively. The final concentration of α-glucosidase was 20 mU/mL. The optical density (OD) of the solution was read using the Microplate Reader (VersaMax, Califonia, USA) at the wavelength 410 nm. The reaction system without tea extracts was used as control and system without α-glucosidase was used as blank for correcting the background absorbance. Acarbose was used as reference standard (positive control). Acarbose, known as BAY g 5421, is an α-glucosidase inhibitor that prevents absorption of sucrose and maltose. All samples were prepared in triplicate.

**Inhibitory analysis**

Data was conducted using Microsoft Excel and SPSS 21.0 for Windows (Chicago, IL, USA). A one-way and a two-way analysis of variance (ANOVA) followed by the Tukey post hoc test were used to analyze statistical significance ($p < 0.05$). All analysis was carried out at least in triplicate. The results were expressed as the mean±SD. Significance and confidence level were estimated at $p < 0.05$.

The percent inhibition was calculated as the decolourization percentage of the test sample using the following formula:

Inhibition (%) = (IA-As)/IA×100

Where IA is the absorbance of the 100% initial and As is the absorbance of the sample. IA and As were the values which were subtracted the average absorbance of the blank wells.

## RESULTS

**α-amylase inhibitory effects**

In this study, the inhibitory effects against α-amylase for water and ethanol extracts of castella ingredients were investigated. The percentage inhibition of three materials within castella showed a concentration-dependent reaction in percentage inhibition (Table 1). α-amylase inhibitory activity of water extract for mugwort castella was evaluated 14.7% at 0.25 mg/ml and 60.2% at 1.0 mg/ml. The α-amylase inhibitory activity of the mugwort was higher than those of green tea and chlorella. There was no significant difference among three groups ($p>0.05$). α-amylase inhibitory activity of the green tea castella was evaluated 54.9% at 1.0 mg/ml and that of the chlorella castella was 38.1% at same concentration.

α-amylase inhibitory activity of ethanol extract for mugwort, green tea, and castella were evaluated 62.1%, 56.5%, and 41.3% at 1.0 mg/ml, respectively. The α-amylase inhibitory activity of the ethanol mugwort extract was also higher than those of green tea and chlorella. Although ethanol extracts were slightly higher in α-amylase inhibitory activity than those of water extracts, there was no significant difference between two extract groups ($p > 0.05$). Figure 1 was shown the rate of α-amylase inhibitory of Acarbose (positive control) and relative inhibitory rate for three castellas on four different concentrations. The values of water extract for mugwort castella, green tea castella, and chlorella castella were 77.6%, 70.8%, and 49.1%, respectively. The values for mugwort castella, green tea castella, and chlorella castella extracts were 77.6%, 70.8%, and 49.1%, respectively (Figure 2).

**Table 1: The degree of α-amylase inhibition (%) of aqueous and ethanol extracts of castella ingredients.**

| Ingredient | Concentration (mM) | Solvent | | *t*-test |
|---|---|---|---|---|
| | | Water | Ethanol | |
| Mugwort | 0.25 | 14.66±3.85 | 17.27±2.81 | 0.177 |
| | 0.50 | 26.97±1.01 | 30.96±2.06 | |
| | 0.75 | 46.81±3.62 | 48.40±3.48 | |
| | 1.0 | 60.24±2.71 | 62.05±1.73 | |
| Green tea | 0.25 | 7.99±1.91 | 10.94±2.78 | 0.185 |
| | 0.50 | 23.33±1.52 | 26.56±2.82 | |
| | 0.75 | 36.07±2.86 | 38.58±3.50 | |
| | 1.0 | 54.94±2.93 | 56.48±3.18 | |
| Chlorella | 0.25 | 8.58±2.14 | 11.39±4.69 | 0.366 |
| | 0.50 | 19.67±2.65 | 23.68±2.31 | |
| | 0.75 | 31.26±2.44 | 34.81±2.59 | |
| | 1.0 | 38.10±1.19 | 42.28±0.68 | |
| *F*-test | | 0.049 | 0.036 | |

Data represented the mean ± SD from three replicates.

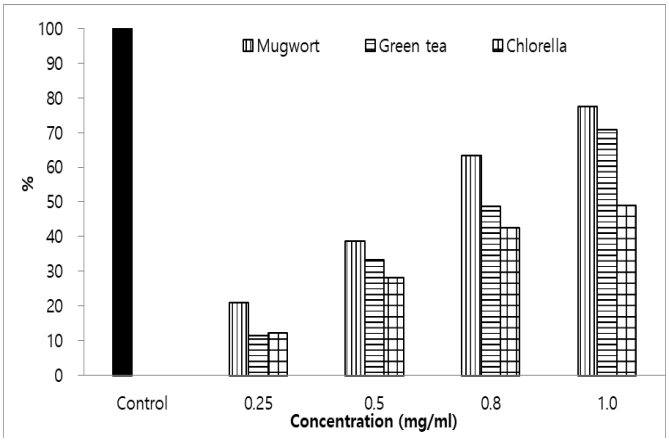


**Figure 1: The rate of α-amylase inhibitory of Acarbose (Positive control) and Relative inhibitory rate for water extractions of castella ingredients at different concentrations.**

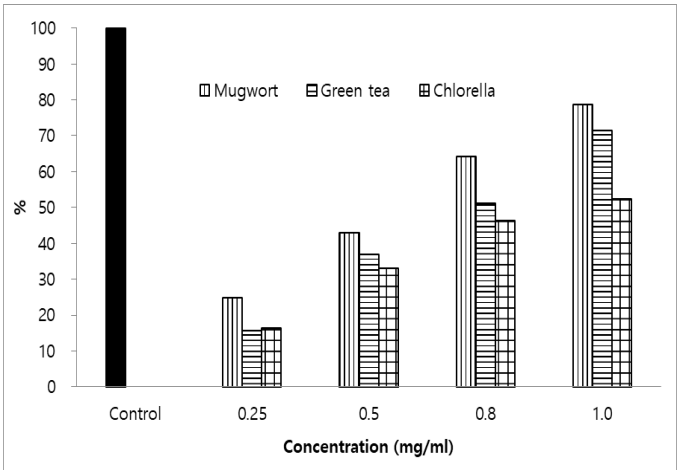


**Figure 2: The rate of α-amylase inhibitory of Acarbose (Positive control) and Relative inhibitory rate for ethanol extractions of castella ingredients at different concentrations.**

**α-glucosidase inhibitory effects**

The results of the α-glucosidase inhibitory effects of water extracts were shown in Table 2. It was observed that inhibition percentage values go on increasing with enhancements in concentration of three castella extracts in the assay mixture. α-glucosidase inhibition of water extract for mugwort castella evaluated 12.6% at 0.25 mg/ml and 57.0% at 1.0 mg/ml. The values of α-glucosidase inhibition for green tea and chlorella evaluated were 53.4% and 50.3% at 1.0 mg/ml, respectively. The all values of α-glucosidase inhibitory for ethanol extracts were higher than those of water extracts. However, they are not showed a statistically significant difference ($p<0.05$).

Figure 3 was shown the rate of α-glucosidase inhibitory of Acarbose (positive control) and relative inhibitory rate for three water extracts on four different concentrations. The relative values of water extract for mugwort castella, green tea castella, and chlorella castella were 73.6%, 69.0%, and 66.9%, respectively.

α-amylase inhibitory activity of ethanol extract for mugwort, green tea, and castella were evaluated 58.2%, 54.9%, and 52.0% at 1.0 mg/ml, respectively. Figure 4 was shown the rate of α-glucosidase inhibitory of Acarbose (positive control) and relative inhibitory rate for three ethanol extracts on four different concentrations. The values for mugwort castella, green tea castella, and chlorella castella of ethanol extracts were 74.8%, 70.6%, and 66.9%, respectively (Figure 4).

**Table 2: The degree of α-glucosidase inhibition (%) of Aqueous and Ethanol extracts of castella ingredients.**

| Ingredient | Concentration (mM) | Solvent | | *t*-test |
|---|---|---|---|---|
| | | Water | Ethanol | |
| Mugwort | 0.25 | 12.58±3.45 | 15.79±3.22 | 0.163 |
| | 0.50 | 27.73±1.94 | 29.95±1.04 | |
| | 0.75 | 39.16±3.10 | 40.97±2.57 | |

| | | | | |
|---|---|---|---|---|
| | 1.0 | 56.97±1.14 | 58.15±1.35 | |
| Green tea | 0.25 | 9.77±1.69 | 10.27±4.41 | 0.106 |
| | 0.50 | 24.57±2.52 | 26.20±1.75 | |
| | 0.75 | 35.49±2.38 | 37.42±4.37 | |
| | 1.0 | 53.42±2.53 | 54.94±2.45 | |
| Chlorella | 0.25 | 8.60±1.05 | 5.93±4.95 | 0.013 |
| | 0.50 | 20.69±0.36 | 22.01±2.64 | |
| | 0.75 | 34.26±1.73 | 33.23±4.02 | |
| | 1.0 | 50.30±4.84 | 52.91±4.70 | |
| *F*-test | | 0.001 | 0.017 | |

Data represented the mean ± SD from three replicates.

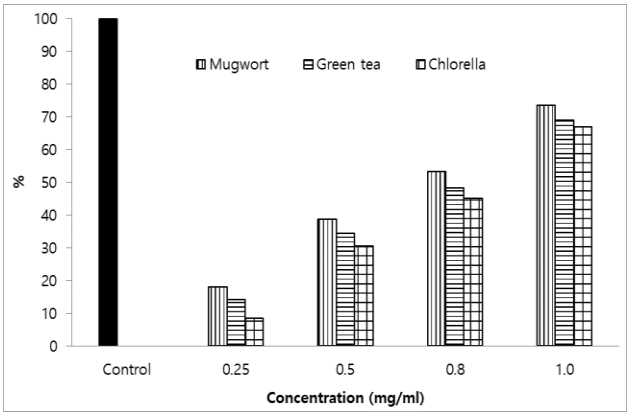


**Figure. 3: The rate of α-glucosidase inhibitory of Acarbose (Positive control) and Relative inhibitory rate for water extractions of castella ingredients at different concentrations.**

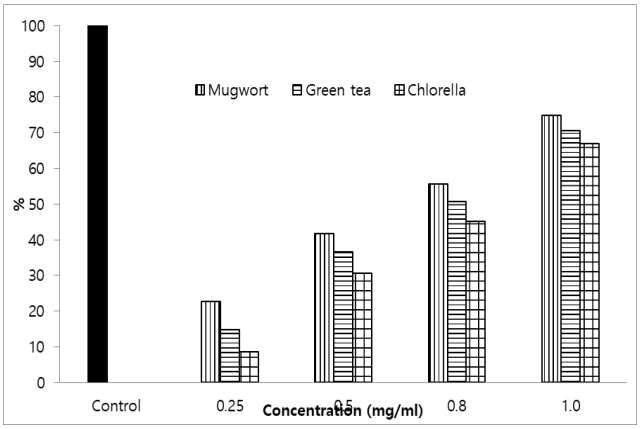


**Figure. 4: The rate of α-glucosidase inhibitory of Acarbose (Positive control) and Relative inhibitory rate for ethanol extractions of castella ingredients at different concentrations.**

## DISCUSSION

Bread is used all over the world and has been plays a significant role in our diet for thousands of years.[19] There are dozens of different breads including castella cakes. Bread contains complex carbohydrates, mainly starch which provide about 90% of the total carbohydrate content of bread.[20, 21]

Amylase breaks down the starch, acting on amylose or amylopectin. Alpha – amylase (α-amylase) breaks the starch chain into small pieces and beta-amylase (β-amylase) breaks off maltose molecules.

Glucoamylase hydrolyzes long-chain polysaccharides best, while α-glucosidase prefers short malto-oligosaccharides.[22] It is found in the pancreas and salivary gland and plays an important role in the conversion of dietary starches into glucose for energy in the human body. Inhibiting amylase action in the digestive system will limit carbohydrate degradation, resulting in less energy.

Chlorophyll is a pigment in plants. There could be minor effects on the stomach/intestines, like nausea/vomiting from chlorophyll supplements. chlorophyllin is a chlorophyll derivative that manufacturers use as a food

additive and natural. Chlorophyll supplements generally do not carry risks, and people absorb them in liquid supplements more easily. However, a person who is pregnant or lactating can speak with a doctor before taking chlorophyll and its derivatives, as its effects are not fully clear.

Mugwort, green tea, and chlorella have been mainly used to represent green pigments in the manufacture of castella. However, they also have a lot of dietary fiber as food ingredients. Dietary fibre is a complex mixture of polysaccharides with many different functions and activities as it passes through the gastrointestinal tract.[5] Vegetable materials such as mugwort, green tea, and chlorella are believed to be able to slightly control the factors of obesity by delaying or inhibiting digestive absorption to give a feeling of satiety.

## CONCLUSIONS

The addition of dietary fibers such as mugwort, green tea, and chlorella in the manufacture of castella cakes can reduce the obesity effect caused by carbohydrate intake.

## CONFLICT OF INTEREST

Author declares that they do not have any conflict of interest.

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