Slow hydrolysis of amylose in soluble starch and amylopectin in suspendable starch liberated from non-glutinous rice flour heated with a sorghum extract

Umeo Takahama a,*, Toshihiro Ansaib, Sachiko Hirota c

a Emeritus Professor of Kyushu Dental University, Kitakyushu 803-8580, Japan
b Division of Community Oral Health Development, Kyushu Dental University, Kitakyushu 803-8580, Japan
c Sanyo-Gakuen College, Okayama 703-8501, Japan

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ABSTRACT

Polyphenols in plant can interact with amylose and amylopectin in different ways affecting their hydrolysis by α-amylase. Pancreatin liberated starch from non-glutinous rice flour heated with and without an aqueous extract of sorghum seeds, and hydrolyzed the liberated starch. The hydrolysis of the liberated starch was slowed down by the sorghum extract. Then, the liberated starch was fractionated into soluble starch and suspendable starch. In the soluble starch, amylose hydrolysis was slowed down more significantly than amylopectin hydrolysis, and in the suspendable starch, the hydrolysis of amylopectin was slowed down efficiently by the sorghum extract. It is discussed that (i) the slowdown in the former might be due to the binding of sorghum components including procyanidins to amylose, and that (ii) the slowdown in the latter might be due to the complex formation between amylopectin and shorter amylose combined with the sorghum components. The contribution of amylose to the slowdown was supported by the result that the sorghum extract inhibited the starch hydrolysis only slightly in glutinous rice flour, the starch of which was almost composed of amylopectin. It was proposed a possible mechanism of the slowdown of amylopectin hydrolysis in suspendable starch by shorter amylose combined with the sorghum components.

1. Introduction

In Japan, glutinous rice flour (mochi-ko) and non-glutinous rice flour (joshin-ko) are commonly used to prepare rice cakes. During the preparation, the flours are kneaded with mugwort leaf paste, sesames, walnut, and so forth, and then heated in a steamer or a microwave oven. On the other hand, the kneaded rice flours are cooked in boiling water to prepare the dumplings. Seeds of sorghum (Sorghum bicolor (L.) Moench) can also be used to prepare the cakes and the dumpling using mochi-ko and joshin-ko.

Sorghum seeds contain procyanidin oligomers and polymers (Awika et al., 2003; Dykes and Rooney, 2006; Luca et al., 2020), in addition to hydroxy benzoic acids, hydroxy cinnamic acids, flavones, flavanones, and so forth (Girard and Awika, 2018; Li et al., 2020; Salazar-Lopez et al., 2018). The procyanidins have antioxidant activity in general, and the antioxidant activity is paid great attention in the fields of nutrition and medicine because the activity has potential to protect the human body from various diseases such as cardiovascular ailments, diabetes, cancer, and so forth (Rauf et al., 2019).

In addition, it has been reported that proanthocyanidins are effective to slow down starch hydrolysis by inhibiting starch digestive enzymes such as α-amylase (Barret et al., 2013; Gonçalves et al. 2011; Lee et al., 2007), and by forming complexes with amylose and amylopectin (Amoako and Awika, 2019; Barros et al., 2012; Takahama and Hirota, 2021; Takahama et al., 2019). If proanthocyanidins can slow down the hydrolysis of starch in the intestine, the rise of blood sugar can be reduced by taking food, which were prepared from cereals such as rice and wheat and proanthocyanidin-rich ingredients. Thus, sorghum seeds can be used as an ingredient to prepare food, which can prevent diabetes and obesity protecting human body from oxidative damages. It has been reported that although the amount of sorghum procyanidin decreases accompanying their preparations, 16–87% of the original amount are remained in the cookies and bread (Awika et al., 2003).
In previous papers, it is shown that pancreatin-induced amylose hydrolysis is slowed down by forming amylose/procyanidin complexes using adzuki bean, which mainly contains dimeric procyanidins (Morina et al., 2020; Takahama et al., 2021a, 2021b), and it is discussed that trimeric procyanidin C1 can also slow down amylose hydrolysis by forming double helical structure between two amylose molecules (Takahama and Hirota, 2021).

In this study, a joshin-ko suspension was heated with and without an aqueous extract of sorghum seeds, and the heated flour was incubated with pancreatin. Pancreatin liberated soluble starch and suspendable starch and hydrolyzed the liberated starch species. Sorghum extract slowed down the hydrolysis of not only the soluble starch but also suspendable starch. Taking the above and other results obtained in this study into account, it is proposed that the slowdown of the hydrolysis of soluble starch was due to the binding of sorghum components including procyanidins to amylose, and that the slowdown of the hydrolysis of suspendable starch was due to the binding of short amylose, which combined with the sorghum components, to amylopectin.

2. Materials and methods

2.1. Ingredients, reagents, and equipment

Sorghum seeds (Sorghum bicolor (L.) Moench) produced in Iwate Prefecture was obtained from Suzuya-Kokumotu (Sendai, Japan). Joshin-ko (flour produced from polished japonica non-glutinous rice) and mochi-ko (flour produced from polished japonica glutinous rice) were from Maruse-Seifunn (Shimoseki, Japan). The amylose contents of the former and the latter are 17–20% (w/w) and below 1% (w/w), respectively (Aoki et al., 2012; Hamashiro et al., 2004; Tran et al., 2001; Wu et al., 2010). Porcine pancreatin were from FUJIFILM Wako Pure Chemicals (Osaka, Japan). According to their data, the activities of digestive enzymes in the pancreatin are following: protease (26–46 units mg⁻¹, pH 8), α-amylase (3–5 units mg⁻¹, pH 7), lipase (0.75–1.4 units mg⁻¹, pH 8). Two types of reagent amylose (synthetic) BAR-5K (average molecular weight, 4500) and BAR-30K (average molecular weight, 31, 800) were obtained from PS-Biotec Inc. (Osaka, Japan). Iodine solution (100 mM) was prepared as described previously (Hirota and Takahama, 2017).

Absorption spectra were measured using a spectrophotometer (UV-2450) equipped with an integrating sphere (ISR-240A) (Shimadzu Cooperation, Kyoto, Japan). The path-length of the measuring beam was 2 mm.

2.2. Starch hydrolysis of mochi-ko and joshin-ko heated with sorghum extract

Seeds of sorghum (2 g) suspended in 10 mL of 0.1 M sodium phosphate (pH 7.0) with 0.15 M NaCl was stood for about 16 h at room temperature (about 20 °C). After the standing, the buffer solution separated from the grains was centrifuged at 1830 x g for 5 min. Joshin-ko (20 mg) or mochi-ko (20 mg) was suspended in 2 mL of the supernatant, and then heated in gently boiling water for 10 min covering the test tube with a glass ball. As the control, the rice flours were heated in the buffer solution for 10 min.

After cooling the heated flours to 37 °C in a water bath and adjusting the volume to 2 mL with water, an aliquot (50 μL) of the suspension was withdrawn. Starch hydrolysis was initiated by adding pancreatin (10 μg per mL) to the suspension. After the addition, aliquots (50 μL) of the reaction mixture were withdrawn at 10 min intervals for 90 min to estimate the starch hydrolysis.

2.3. Estimation of starch hydrolysis

Pancreatin-induced starch hydrolysis was estimated as following. The aliquot (50 μL) withdrawn (see above) was added to 1 mL of 0.1 M sodium phosphate (pH 7.0) with 0.15 M NaCl, and then 0.1 mL of 100 mM iodine solution was added to the buffer solution. The mixture was stood for 10 min at room temperature (about 25 °C), and the absorption spectra of the fractions prepared as shown Scheme 1 were measured.

During the standing for 10 min, large rice flour particles (precipitate-1, in the following PPT-1) were precipitated. The upper phase of the mixture (supernatant-1, in the following SUP-1), which contained soluble and suspendable starch, was taken away remaining PPT-1, and the absorption spectrum of SUP-1 was measured. The PPT-1 was suspended in 1 mL of 0.1 M sodium phosphate (pH 7.0) with 0.15 M NaCl to measure the absorption spectrum. The SUP-1, the absorption spectrum of which had been measured, was centrifuged at 810 x g for 10 min to prepare the precipitate-2 (PPT-2) and the supernatant-2 (SUP-2), which were mainly composed of suspendable starch and soluble starch, respectively. After measuring the absorption spectrum of SUP-2, the absorption spectrum of PPT-2 was estimated by taking the difference spectrum of SUP-1 minus SUP-2 at each incubation period. In addition, the PPT-2 was suspended in the above buffer solution to measure the absorption spectrum.

Accompanying the incubation with pancreatin, the absorption spectrum of starch-iodine complexes in each fraction was changed decreasing the absorbance at all wavelengths examined. It has been reported that the absorbance of starch-iodine complexes decreases in a first-order like reaction in a range of incubation time (Takahama et al., 2021a, 2021b; Takahama and Hirota, 2021). Then, the absorbance decrease was plotted semi-logarithmically at 500, 550, and 700 nm, which would reflect the concentration of starch with shorter, middle, and longer glucose chains, respectively. The linear region of the plot was determined using the least squares method (r > 0.95), and the half-live was calculated from the slope.

2.4. Estimation of procyanidins bound to joshin-ko

Sorghum extract was prepared as described in Section 2.2. Joshin-ko (30 mg) was added to 3 mL of the sorghum extract, heated in boiling water, and then cooled as Section 2.2. An aliquot (0.4 mL) of the heated joshin-ko was withdrawn prior to the addition of pancreatin, and then withdrawn at 30 and 60 min after the addition of pancreatin (10 μg per mL). The aliquot was mixed with 1 mL of 0.1 M sodium phosphate (pH 7.0) with 0.15 M NaCl. The mixture was stood for 10 min, and then PPT-1, PPT-2, and SUP-2 were prepared as shown in Scheme 1.

The prepared PPT-1 and PPT-2 were kept on ice, and suspended in 1 mL of water just before the addition of reagents to estimate procyanidins bound to the PPTs. Ethanol was added to SUP-2 immediately after its preparation by centrifugation making its concentration 70% (v/v). After

Scheme 1. Preparation of various fractions from a mixture of starch-iodine complexes.
leaving for more than 10 min at room temperature, ethanol insoluble precipitate (ppt of SUP-2) was collected by centrifuging at 1830 \( \times g \) for 10 min. The precipitate was also suspended in 1 mL of water as above. The amounts of procyanidins in the precipitates were estimated as following. Two mL of 1-butanol, 0.065 mL of ammonium iron (III) sulfate (2%, w/v) dissolved in 2 M HCl, and 0.2 mL of concentrated HCl were added in this order to the above precipitates suspended in 1 mL of water, and then the mixtures were heated in boiling water for 30 min (Amarowicz and Pegg, 2006). During the heating, the color of the butanol layer turned into reddish. The reddish pigment was identified to be cyanidin by HPLC. It has been reported that cyanidin is formed from procyanidins by this procedure (Amarowicz and Pegg, 2006). The concentration of cyanidin in the butanol layer was expressed as the absorbance at 545 nm, because sorghum grains contained various procyanidin species (Awika et al., 2003).

2.5. Reddish sorghum components bound to heated joshin-ko

Joshin-ko (30 mg) was suspended in 3 mL of the sorghum extract and heated as described in Section 2.4. An aliquot (1 mL) of the suspension was withdrawn prior to the addition of pancreatin, and then at 30 and 60 min after the addition of pancreatin (10 \( \mu \)g per mL). The aliquot was added to 2 mL of 0.1 M sodium phosphate (pH 7.0) with 0.15 M NaCl. After standing the mixture for 10 min, PPT-1, PPT-2, and SUP-1 were prepared as Scheme 1. The prepared PPT-1 and PPT-2 were kept on ice, and the ppt of SUP-1 was prepared as described in Section 2.4.

The PPTs and the ppt of SUP-2 were suspended in 1 mL of the above buffer solution to measure the absorption spectra. Prior to the measurement of the spectra of PPT-1 suspensions, the suspensions were sonicated for 2–3 s to disperse large joshin-ko particles using an Ultrasonic Disruptor UD-200 (Tomy Seiko Co. Ltd., Tokyo, Japan).

The absorption spectra of the precipitate suspensions did not have any clear peaks but had a shoulder around 500 nm. Therefore, the concentrations of the reddish component were estimated from the first differential spectra (\( d\lambda = 20 \) nm).

2.6. Statistical analysis

Each experiment was repeated more than three times. Data were presented as means with standard deviations. Significant difference between two samples was determined using the Student’s t-test with the significance threshold set to 0.05.

3. Results and discussion

3.1. Pancreatin-induced starch hydrolysis in heated joshin-ko

3.1.1. Supernatant-1 and precipitate-1

An aliquot of joshin-ko suspension mixed with iodine was fractionated as shown in Scheme 1. The absorption spectrum of SUP-1 had a peak around 600 nm and a shoulder around 700 nm before the addition of pancreatin (Figure 1A, trace 0), suggesting the presence of amylose,
which might be leached out from the flour particles by heating, in the SUP-1. Pancreatin increased the absorbance at all wavelengths examined during the incubation for 10 min, and the absorption spectrum had a peak around 540 nm (trace 10). The absorbance increase was accompanied by the absorbance decrease in PPT-1 (Figure 1B, compare traces 0 and 10), indicating that the absorbance increase in Figure 1A (trace 10) was due to the liberation of starch from PPT-1. The absorption spectrum of the liberated starch, namely, the difference spectrum of trace 0 minus trace 10 of PPT-1 (Figure 1B, dashed line) was similar to the absorption spectrum of trace 0 of PPT-1 with a peak and a shoulder around 580 and 700 nm, respectively, but different from the spectrum of Figure 1A (trace 10).
result indicates that the starch liberated from PPT-1 was hydrolyzed into soluble starch and suspendable starch decreasing the degree of polymerization (DP) of glucose.

After the incubation for 10 min, the absorbance of SUP-1 decreased shifting the peak to shorter wavelengths (Figure 1A). The absorbance of PPT-1 was also decreased (Figure 1B), but the decrease was much slower than the decrease in absorbance in SUP-1 at any wavelengths, indicating the rapid hydrolysis of the soluble and suspendable starch, which was liberated from PPT-1 during the incubation for 10 min.

3.1.2. Supernatant-2

Figure 1C shows pancreatin-induced changes in absorption spectra of SUP-2, which might contain soluble starch. Before the addition of pancreatin (trace 0), the spectrum did not have any bands, and the absorbance in the wavelengths shorter than 600 nm was below the baseline. This spectrum indicates that almost all starch-iodine complexes contributing to trace 0 of SUP-1 (Figure 1A) was precipitated by the centrifugation, decreasing the iodine concentration in SUP-2, and suggests that the absorption spectrum of trace 0 of PPT-2 or trace 0 of SUP-2 minus SUP-1 should be similar to that of trace 0 of SUP-1 (see below).

Pancreatin increased the absorbance at all wavelengths examined during the initial incubation for 10 min. The increase might be due to the solubilization of the suspendable starch contributing to trace 0 of SUP-1 and the solubilization of starch liberated from trace 0 of PPT-1. After the incubation for 10 min, the soluble starch in SUP-2 was hydrolyzed.

3.1.3. Supernatant-1 minus supernatant-2

Figure 1D shows the difference spectrum of SUP-1 minus SUP-2 at each incubation period, which corresponded to the absorption spectra of PPT-2. Before the addition of pancreatin (trace 0), the difference spectrum had a peak around 580 nm and a shoulder around 700 nm, indicating the spectrum was similar to that of trace 0 of SUP-1. After the addition of pancreatin, (i) the ΔA decreased rapidly at all wavelengths examined, and the decrease was significant at 600–700 nm (trace 10), (ii) the decreased ΔA increased at almost all wavelengths (trace 30), and then (iii) the increased ΔA decreased remaining a shoulder at 540–560 nm (traces 30–90).

The ΔA decrease at 600–700 nm in (i) might due to the rapid hydrolysis of suspendable amylose into soluble amylose, the ΔA increase in (ii) might be due to the liberation of PPT-2 from PPT-1, and the ΔA decrease in (iii) could attribute to the hydrolysis of the liberated PPT-2. The shoulder in (iii) suggested the presence of shorter hydrolysable starch complexes made from shorter amylose and amylopectin in PPT-2 (Ottenhof and Farhat, 2004; Vanademan et al., 2014; Zhou et al., 2011).

3.1.4. Precipitate-2 suspension

The PPT-2 prepared as Scheme 1 was suspended in 1 mL of 0.1 M sodium phosphate (pH 7.0) with 0.15 M NaCl. The absorption spectrum had a peak and a shoulder around 590 and 700 nm, respectively (Figure 1E, trace 0), which might correspond to the absorption bands around 600 and 700 nm, respectively, in Figure 1A (trace 0).

The absorbance of trace 0 was decreased at the wavelengths longer than approximately 550 nm but increased by 0–50% at the wavelengths shorter than 550 nm by incubating with pancreatin for 10 min (trace 10) (Figure 1E). The change in absorption spectrum supports the rapid hydrolysis of amylose in PPT-2 as described in Section 3.1.3.

After the absorbance decrease, the absorbance was increased during the incubation period from 10 to 40 min, supporting the liberation of PPT-2 from PPT-1 (see Section 3.1.3). The increased absorbance decreased after the incubation for 40 min, keeping the peak around 550 nm. This peak might correspond to the shoulder at 540–560 nm (Figure 1D).

The absorbance of starch-iodine complexes decreased with the decrease in wavelength from 550 to 450 nm in Figure 1E, but only slightly in Figure 1B. It has been reported that iodine bound to shorter helical structures of starch is dissociated when iodine concentration is decreased (Hollo and Szefi, 1958), suggesting that the iodine bound to amylopectin branches in PPT-2 was dissociated when suspended in a buffer solution. On the other hand, amylose with DP > 12 can bind to iodine in an iodine solution (Bailey and Whelan, 1961). Thus, the absorption spectra of Figure 1D suggest that the DP of glucose in amylopectin branches in PPT-2 might be higher than 12. Such shorter branches could be formed by the hydrolysis of amylopectin in PPT-2 liberated from PPT-1. Taking the above discussion into account, the absorption spectra in Figure 1E (traces 40–90) represented the spectra of shorter amylose that was still combined with iodine, even after PPT-2 was suspended in the buffer solution.

3.1.5. Characterization of amylose in precipitate-2

Amylose reagents BAR-5K (average DP = 28) and BAR-30K (average DP = 196) were dissolved and suspended in boiling water, respectively. The former had a peak around 570 nm, and the latter had a broad peak around 650 nm and shoulders around 600 and 700 nm, when complexed with iodine in 0.1 M sodium phosphate (pH 7.0) with 0.15 M NaCl. The peak wavelengths suggest that the absorption bands at 580–600 nm and around 700 nm of PPT-2 (Figure 1D and 1E, trace 0) might be derived from shorter and longer amylose, respectively.

After the incubation for 40 min, the absorbance peak around 550 nm was retained in Figure 1D and 1E, and the absorbance at 550 nm (A550) relative to that at 700 nm (A700), namely, the A550/A700 was 2.0–2.3 (traces 40–90) in the both figures. The A550/A700 in trace 10 of SUP-1 of
heated joshin-ko was approximately 2.4 (Figure 1A). On the other hand, the $A_{550}/A_{700}$ in trace 0 of SUP-1 prepared from heated mochi-ko, which contained soluble and suspendable amyllopectin, was approximately 4.5 (this study). In addition, amyllopectin-iodine complexes have been reported to have an absorption peak at 510–540 nm (Wang et al., 2010, 2019). The values of $A_{510/540}/A_{700}$ calculated from the spectra in the above references were 3.4–4.6.

The above data support the presence of shorter amyllose in PPT-2 prepared after the incubation for 40 min. Taking the report by Bailey and Whelan (1961) and the peak wavelength (540–560 nm) of the PPT-2, DP of glucose of the shorter amyllose was assumed to be 30–40.

3.2. Slower hydrolysis of joshin-ko heated with sorghum extract

Figure 2 shows the absorbance change of starch-iodine complexes, which was plotted semi-logarithmically, at 500 (Figure 2A), 550 (Figure 2B), and 700 (Figure 2C) nm. In SUP-1 and SUP-2, the linear regions were observed at 20–70, 20–60, and 20–70 min at 500, 550, and 700 nm, respectively, and the sorghum extract decreased the slope. In PPT-1, the absorbance decreased greatly in the initial 10 min, thus the slope was determined from the absorbance decrease under the postulation that the decrease was a first-order like reaction. After the incubation for 10 min, the effects of sorghum extract were unclear.

In SUP-1 minus SUP-2 and PPT-2, the absorbance at the three wavelengths was increased during the incubation from 10 to 40 min in the control joshin-ko and from 10 to 60 min in joshin-ko heated with the sorghum extract attaining to the maximal value. The absorbance increase was slowed down by the extract.

After attaining to the maximal value, the absorbance decreased. In SUP-1 minus SUP-2, the slope was linear at 40–70 min at the three wavelengths in the control joshin-ko, and linear at 40–90 min at 500 nm and at 60–90 min at 550 and 70 nm in joshin-ko heated with the sorghum extract. In PPT-2, the slope was linear at 40–90 min in the control and at 60–90 min in joshin-ko heated with the sorghum extract at the three wavelengths. Table 1 show half-lives estimated from the slopes.

3.3. Longer half-life of starch hydrolysis in joshin-ko heated with sorghum extract

3.3.1. Supernatant-1

In the control SUP-1, the half-life increased with the decrease in the wavelength from 700 to 500 nm. The increase might be due to the formation of shorter starch, which was hydrolyzed further, from longer starch. The sorghum extract increased the half-lives, and the increase seemed to be greater at 700 nm than at 500 nm.

3.3.2. Precipitate-1

There was no significant difference in the half-life of PPT-1 between 500, 550, and 700 nm (Table 1). The sorghum extract increased the half-lives, and degree of the increase was similar at each wavelength. The result could be explained if sorghum components inhibited the liberation of PPT-2, namely, amyllose/amyllopectin complexes, from PPT-1.

3.3.3. Supernatant-2

In the control SUP-2, the half-life also increased with decrease in the wavelength (Table 1). The increase could be explained as described in Section 3.3.1. The sorghum extract increased the half-life in the order of 500 ≤ 550 < 700 nm, suggesting the more efficient inhibition of soluble amyllose hydrolysis than soluble amyllopectin hydrolysis by the extract. The efficient inhibition was possible if sorghum components more readily combined with amyllose than with amyllopectin. The combining with amyllose might result in the formation of double helical structures between the two amyllose strands, which were less hydrolysable than the single helical structures (Gidley et al., 1995; Takahama and Hirota, 2021; Tamura and Ogawa, 2012). It has been reported that caffeic acid, quercetin, epigallocatechin gallate, and procyanidins can assemble two amyllose strands (Chang et al., 2020; Yu et al., 2021; Ekaette and Saldaña, 2020; Li et al., 2020; Takahama and Hirota, 2021).
3.3.4. Supernatant-1 minus supernatant-2

The ΔA of control SUP-1 minus SUP-2, which represented the absorbance of PPT-2, decreased after the incubation for 40 min (Figure 2). The half-life determined after the incubation period was significantly longer than that of SUP-2. This might be due to the interaction between shorter amylose and amylopectin with shorter branches in PPT-2. It has been reported that (i) amylose/amylopectin complexes can be formed during the retrogradation (Wang et al., 2015), that (ii) the hydrolysis property of amylose/amylopectin complexes is depend on the amylose structure (Zhou et al., 2014), and that (iii) shorter amylose can form less hydrolysable amylose/amylopectin complexes (Ottenhof and Farhat, 2004; Vamadevan et al., 2014; Zhou et al., 2011).

Sorghum extract increased the half-life, and the increase was in the order of 700 < 550 ≤ 500 nm (Table 1), indicating that sorghum components inhibited the amylopectin hydrolysis more significantly than the amylose hydrolysis in PPT-2, although the extract did not efficiently inhibit the hydrolysis of soluble amylopectin in SUP-2. A possible explanation for the greater inhibition is the liberation of amylopectin complexed with shorter amylose, which was combined with sorghum components.

To evaluate the above explanation, effects of the sorghum extract on pancreatic-induced starch hydrolysis was studied using mochi-ko. The sorghum extract increased the half-life in mochi-ko SUP-1, but the increase was approximately 30% of the increase in joshin-ko. The above results support the important role of amylose for the inhibition of amylopectin hydrolysis by the sorghum extract, and suggest that even if sorghum components were bound to amylopectin, their binding was not strong enough to prevent the access of α-amylase to amylopectin. The above idea may be supported by the report that a procyanidin-rich black soybean extract inhibits slightly the starch hydrolysis in mochi-ko but significantly the starch hydrolysis in joshin-ko (Takahama et al., 2021a, 2021b).

It has been reported that α-amylase activity is affected by various phenolic components including proanthocyanidins (Barret et al., 2013; Gonçalves et al. 2011; Lee et al., 2007; Takahama and Hirota, 2018). The different effects, however, of sorghum extract on starch hydrolysis between mochi-ko and joshin-ko (see above) suggest that the slow hydrolysis of starch in joshin-ko heated with the sorghum extract was not mainly due to the inhibition of α-amylase activity by sorghum components. The different effects of the sorghum extract on the kinetics of starch hydrolysis between SUP-2 and SUP-1 minus SUP-2 supports the important role of the interaction of sorghum components with starch for the inhibition of its hydrolysis by the extract.

3.3.5. Precipitate-2 suspension

It was discussed that the absorption spectrum of the control PPT-2 suspended in the buffer solution represented the spectrum of shorter amylose (DP < 30–40) after the incubation for 40 min (Section 3.1.4). Then, the half-life of the PPT-2 suspension after the incubation period could be attributed to the hydrolysis of shorter amylose. The half-life of the PPT-2 suspension was similar to that of the control SUP-1 minus SUP-2 (Table 1).

The half-life of the PPT-2 suspension was increased by the sorghum extract to similar extent at the three wavelengths, suggesting that the hydrolysis of amylose combined with sorghum components was slow. The degree of the increase in PPT-2 suspension was similar to that in SUP-1 minus SUP-2 at 700 nm, but smaller in PPT-2 suspension than in SUP-1 minus SUP-2 at 500 and 550 nm (Table 1). The difference might be explained if shorter amylose combined with sorghum components prevented the access of α-amylase to amylopectin in PPT-2 (see below).

3.4. Procyanidin present in precipitate-1, precipitate-2, and supernatant-2

It was discussed that sorghum components could slow down the starch hydrolysis in joshin-ko by combining with amylose. Then, it was investigated whether a sorghum component, procyanidin, was present in PPT-1, PPT-2, and ppt of SUP-2 of joshin-ko heated with the sorghum extract.

Cyanidin was formed from the three precipitates prepared before the addition of pancreatin by the butanol/Fe(III)/HCl-treatment (Table 2, time 0), suggesting the binding of procyanidins to starch in PPT-1 and PPT-2. Although cyanidin was formed in ppt of SUP-2, starch-iodine complexes could not be detected in SUP-2 at 0 min (traces 0, Figure 1C). These results might be explained if procyanidins in SUP-2 (traces 0) were combined with shorter starch, which could not make complexes with iodine, but could be precipitated by 70% ethanol.

The amount of cyanidin formed was decreased in PPT-1, while increased in PPT-2 and ppt of SUP-2 during the incubation with pancreatin for 30 min, indicating that starch combined with procyanidins was liberated from PPT-1 into SUP-2 and PPT-2. The changes in the cyanidin amount were small during the incubation from 30 to 60 min. The small changes might be due to the slow liberation of starch from PPT-1 (Figures 1 and 2).

3.5. Sorghum pigments in precipitate-1, precipitate-2, and supernatant-2

A joshin-ko suspension heated with sorghum extract was pale red, and the absorption spectrum of the suspension had a shoulder around 500 nm. The precipitate prepared by centrifugation (810 × g for 10 min) had also a shoulder around 500 nm when the suspended in the buffer solution (Section 2.5). Not all the reddish components in the precipitate were extracted by 70% ethanol, indicating that part of the reddish pigments were strongly combined with the precipitate. The reddish components can be formed by the oxidation of procyanidins (Takahama et al., 2019). Actually, the reddish color intensity of the sorghum extract increased by heating with joshin-ko for 10 min.

Figure 3 shows absorption spectra of the suspensions of PPT-1 (Figure 3A), PPT-2 (Figure 3B), and ppt of SUP-2 (Figure 3C). The spectra also had a shoulder around 500 nm, and the first differential spectra had a positive around 485 nm and a negative peak around 530 nm. Then, the concentrations of the reddish pigments could be estimated from the differential values (Table 3). The value of PPT-1 decreased, and the value of PPT-2 and ppt of SUP-2 increased during the incubation for 30 min. During the incubation, the differential value increased by 4 ± 11 and 67 ± 12% in PPT-2 and ppt of SUP-2, respectively, while the amount of cyanidin increased by 186 ± 53 and 34 ± 19% in PPT-2 and ppt of SUP-2, respectively (Table 2). The data suggest that procyanidins could more readily bind to PPT-2, and the reddish pigments to ppt of SUP-2.

Table 3. Reddish pigments bound to precipitates.a

| Incubation time (min) | ΔA/dt (arbitrary unit) (n = 4) | PPT-1 | PPT-2 | ppt of SUP-2 |
|-----------------------|-------------------------------|-------|-------|-------------|
| 0                     | 56.5 ± 8.5 (100)              | 37.8 ± 2.4 (100) | 50.3 ± 8.4 (100) |
| 30                    | 30.0 ± 2.7 (55 ± 14)          | 39.3 ± 3.0 (104 ± 11) | 84.0 ± 12.9 (167 ± 12) |
| 60                    | 29.5 ± 9.4 (52 ± 12)         | 48.3 ± 3.1 (128 ± 12) | 99.4 ± 14.3 (198 ± 9) |

a Details are found in Section 2.5.

b Values are difference between 485 nm (a positive peak) and 530 nm (a negative peak) in the first differential spectra.

c Precipitate prepared from SUP-2.
combined with the above components, which might be contained in PPT-2 liberated from PPT-1. In addition to the procyanidin and reddish pigments, other phenolic components in the sorghum extract may also contribute to the inhibition of the starch hydrolysis in joshin-ko. Further studies are required to elucidate sorghum components other than procyanidins that can slow down the starch hydrolysis.

4. Conclusion

Pancreatin liberated soluble starch and suspendable starch from joshin-ko heated with and without sorghum extract, and hydrolyzed the liberated starch species. The extract increased the half-life of the soluble starch hydrolysis, and degree of the increase was in the order of 500 < 550 < 700 nm. The increase was discussed to be due to the liberation of soluble amylase combined with procyanidins and reddish pigments, which was hydrolyzed slowly.

The extract also increased the half-life of the suspendable starch hydrolysis in the order of 700 < 550 < 500 nm, indicating that the sorghum components could inhibit the hydrolysis of amyl popectin more effectively than that of amylase. The more effective inhibition was discussed to be due to the formation of complexes between amylpectin and shorter amylose combined with procyanidins and reddish pigments, decreasing the accessibility of α-amylase to amylpectin by the shorter amylose.

The above conclusion suggest that we can prepare food, the glycemic index of which is low, using an amylase-containing foodstuff and a procyanidin-rich foodstuff.

Declarations

Author contribution statement

Umeo Takahama: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Toshihiro Ansai: Analyzed and interpreted the data.

Sachiko Hirota: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interest’s statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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