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Effect of conditions in the production of highly soluble powder from tomatoes using microbial enzyme preparation

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This study develops functional food ingredients made from tomatoes. In particular, the cellulose contained in tomatoes was saccharified using microbial enzyme preparations, and the possibility of producing highly soluble tomato powder was investigated. First, the study investigated the conditions for saccharification of Avicel using enzyme preparations A, B and C. A result of saccharifying tomato powder under best conditions for Avicel, release of glucose and reducing sugar was confirmed for all enzyme preparations. Total polyphenol content increased by saccharification compared to before saccharification for all enzyme preparations. DPPH radical scavenging activity was reduced for all enzyme preparations compared before saccharification. From these results, it is feasible to see the possibility of using discarded tomatoes as a new food material at the 24 h saccharification time. In particular, for enzyme A, hydrolysis to glucose and maintenance of antioxidant activity were higher than for enzymes B and C at 24 h of saccharification. Therefore, among the enzyme preparations used in this study, enzyme A was found to be the most suitable enzyme preparation for the saccharification of tomatoes.

Key words: Tomato, saccharification, cellulose, cellulase, glucose, reducing sugar, food ingredients, functional food.

INTRODUCTION

According to the World-Wide Fund for Nature (WWF), approximately 40% of the food produced for consumption worldwide, or about 2.5 billion tons of food, is wasted every year. The cost and environmental burden of processing these food wastes are becoming a worldwide problem. It is stated that Japan produces 16.7 million tons of food waste annually. Of this amount, 5.23 million tons per year is food that is still edible but is discarded, or so-called food waste. Additionally, about 1.98 million tons of harvested vegetables and fruits are being disposed of as substandard (Data were obtained from a statistical survey and mandatory reporting under the Food Recycling Law published by the Ministry of Agriculture, Forestry, and Fisheries in Japan (MAFF, 2021). It is mentioned that approximately 40% of vegetables and fruits that are not even harvested are thrown away. Generally, substandard

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vegetables are directly discarded, but in some cases, they are sold at low prices or used as livestock feed or processed foods. In addition, in recent years, research has been conducted to use such substandard vegetables as biomass for energy and bioplastics (Talan et al., 2021) (Tsang et al., 2019). Fruits and vegetables contain water, and their quality deteriorates rapidly unless they are thoroughly temperature-controlled, but drying and powdering them solve these problems and enable long-term preservation (Chua and Chou, 2003). In addition, vegetable powder is widely used as a food material from the viewpoint of ease of preservation and processing and is also attracting attention as a natural food coloring agent (Karam et al., 2016). On the other hand, there is a problem that simply powdering vegetables as they are leaves a large amount of dietary fiber, which affects the texture of food when vegetable powder is used as a food ingredient (Fernandez-Garcia et al., 1997). Therefore, research has been conducted to solve this problem by physically reducing the size of the powder particles (Grygorczyk and Blake, 2023), but there are few examples of solving this problem by decomposing dietary fiber. Saccharification of plant cell walls by microbial enzymes is a sustainable and clean method that has a much lower impact on the environment than chemical hydrolysis because no harmful waste is released (de Aguiar et al., 2020). Therefore, the laboratory has been working to solve this problem by degrading dietary fiber itself using microbial enzymes. Work has been on to develop a fully saccharified powder from discarded substandard vegetables, especially to saccharify cellulose among the dietary fibers of plant resources, to improve the solubility of vegetable powders, and to develop sweeteners that maintain the functionality of plant resources.

The main plant cell walls of fruits and vegetables are composed of cellulose, hemicellulose, and pectin, and it has been reported that oligosaccharides are released by enzyme produced by the *Aspergillus* and *Trichoderma* sp. (Sabater et al., 2020). Apple pomace also contains cellulose, hemicellulose, and pectin. It has been reported that the combination of enzymes promotes the saccharification of these polysaccharides contained in apple pomace, producing galacturonic acid, glucose, arabinose, and galactose (Gama et al., 2015). However, the polysaccharide composition contained in each plant resource is different, and it was necessary to establish saccharifying enzyme and conditions for each.

Kumamoto prefecture, where the university is located, ranks first in Japan in the production of tomatoes (*Solanum lycopersicum* L.). Tomatoes are rich in phenolic compounds (phenolic acid and Flavonoids), carotenoids (lycopene and α- and β-carotenes), and vitamin A and C (Tan et al., 2010). These functional components present in tomatoes have antioxidant, antimutagenic, antiproliferative, anti-inflammatory, and antiatherogenic properties, and are effective in preventing cancer and cardiovascular diseases (Chaudhary et al., 2018). On the other hand, large quantities of tomatoes are also discarded as substandard.

Therefore, in this study, as part of the solution to food waste, a study was conducted with the aim of developing new food materials, especially sweeteners, made from discarded tomatoes. The ultimate goal is to develop a soluble powder by saccharification of discarded substandard vegetables with a commercial enzyme preparation. First, this study was conducted to examine the conditions for saccharification of tomatoes so that it could be applied to similar plant resources. This paper investigated the conditions for saccharification of tomatoes using various commercially available enzyme preparations, especially the influence of different saccharification times, in order to produce highly soluble tomato powder. Avicel, a microcrystalline cellulose was used as a model substrate, to determine optimal conditions of saccharification temperature, time, and enzyme concentration, and attempted to saccharify tomatoes under these conditions. Furthermore, changes in phenolic compound content and antioxidant activity were evaluated.

MATERIALS AND METHODS

Chemicals

Avicel (Avicel PH-101), *p*-nitrophenyl-β-D-glucopyranoside, Somogyi's copper reagent, Nelson color reagent, DPPH (1, 1-diphenyl-2-picrylhydrazyl) and Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma-Aldrich. Glucose quantification reagent was purchased from FUJIFILM Wako Pure Chemical Corporation. Phenol reagent solution, gallic acid and sodium carbonate were purchased from NACALAI TESQUE, INC. And ethanol was purchased from Japan Alcohol Trading Co., Ltd.

Tomato

The tomatoes (cv.; *Reiyo*) used in the experiment were substandard tomatoes donated by a farmer in Kumamoto Prefecture. After crushing raw tomatoes with mixer, they were freeze-dried and powdered. Freeze-drying was performed using an eggplant-shaped flask with Freeze Dryer Fd-1 (Eyela Tokyoricakikai Co Ltd).

Enzyme preparation

A commercially available enzyme preparation was used for saccharification of tomato powder. Three types of enzyme preparations were used: *Trichoderma*-derived enzyme A (cellulase) and B (hemicellulse) from Mitsubishi Chemical Co., Ltd., and Enzyme preparation produced by *Talaromyces* sp. enzyme C (cellulase, hemicellulse) from Kyowa Kasei Co., Ltd. Table 1 shows the cellulase activity and β-glucosidase activity of each enzyme preparation.

Enzyme activity

Cellulase activity

A mixture of 2 ml of 1% (w/v) Avicel suspended in 0.05 M acetate

Table 1. Microbial origin of enzyme preparation and enzyme activity.

Enzyme preparation	Microbial origin	β-Glucosidase (U/g）	Cellulase (U/g）
A	*Trichoderma* sp.	144	33
B	*Trichoderma* sp.	185	31
C	*Talaromyces* sp.	1,530	189

buffer at pH 4.5 and 2 ml of 0.05% (w/v) enzyme solution was incubated at 40℃ for 1 hour. After that, the reaction solution was centrifuged (3,000 rpm, 5 min), and reducing sugars in the supernatant were measured (Takao et al., 1985). 1 µmol of glucose released in 1 minute was defined as 1 U, and the activity was evaluated as the activity per 1 g of enzyme preparation,

β-Glucosidase activity

A mixture of 0.9 ml of 0.01% (w/v) *p*-nitrophenyl-β-D-glucopyranoside and 0.1 ml of 0.05% (w/v) enzyme solution suspended in 0.05 M acetate buffer at pH 4.5. The mixture was incubated at 40℃ for 30 min. The reaction was terminated by adding 2 ml of 0.25 M Na_2CO_3, after standing at room temperature for 10 min, the amount of liberated *p*-nitrophenol was measured (Berghem et al., 1974). 1 µmol of *p*-nitrophenol released per minute was defined as 1 U, and the activity was evaluated as per 1 g of enzyme preparation.

Saccharification experiment of Avicel

30 ml of each enzyme solution of 0.01 to 0.1% (w/v) suspended in 0.05 M acetate buffer at pH 4.5 added to 0.3 g of Avicel, and incubated in an incubator (Bio-Shaker-BR 300L, Taitec) at 30 to 50℃ for 24 and 48 h. After incubation, the reaction solution was centrifuged (3,000 rpm, 5 min), and the glucose and reducing sugar concentration contained in the supernatant were measured.

Saccharification experiment of tomato

30 ml of each enzyme solution at 0.1% (w/v) was added to 0.3 g of tomato powder. Saccharification was performed at 45℃ for enzyme A and at 50℃ for enzymes B and C at stand, respectively, for 24 h and 48 h at the optimum saccharification temperature. After saccharification, the reaction solution was centrifuged, and the glucose concentration and reducing sugar concentration contained in the supernatant were measured (test). On the other hand, a blank (reaction 0 min) was obtained by mixing tomato powder and an enzyme solution and immediately measured the sugar in the supernatant, and the different between the test and blank was defined as the sugar concentration after saccharification. Additionally, 30 ml of deionized water was added to 0.3 g of tomato powder, and the sugar concentration was measured, and the sugar concentration before saccharification contained in the tomato itself was used as a control. Similar experiments were conducted three times.

Analysis of sugar concentration

To determine the glucose concentration of the saccharification reaction mixture, 0.1 ml of the supernatant of mixture and 3.0 ml of glucose quantitative reagent were mixed and incubated at 40℃ for 20 min. The absorption value of the reaction solution at 505 nm was measured. And glucose concentration was calculated from the standard curve. The reducing sugar concentration was calculated from standard curve using the Somogyi-Nelson method (Somogyi, 1952) (Nelson, 1944). The concentration of glucose and reducing sugar were determined and calculated as the amount of sugar per 1 g of tomato powder.

Total phenolic concentration

Using a phenol reagent solution adjusted to 0.9 N, the total polyphenol concentration was measured according to the method of Hamasaka et al. (2004), To 1 mL of saccharification reaction mixture, 1 mL of 0.9 N phenol reagent solutions was promptly added. Then, 1 mL of 10% (w/v) sodium carbonate solution was added and allowed to stand at room temperature in the dark for 1 hour. Then, the supernatant was measured for absorbance value at 750 nm on a spectrophotometer (U-1800, Hitachi Co., Ltd). and calculated as gallic acid equivalent per 1 g of tomato powder.

Measurement of DPPH radical scavenging activity

Test samples were prepared by mixing the saccharification reaction mixture and ethanol (1:1) to achieve an ethanol concentration of 50% (v/v). The DPPH radical scavenging activity was measured according to the method of Oki et al. (2002). 2 mL of 400 µM DPPH solution was added to 2 mL of test sample, mixed well, and allowed to stand at room temperature while shaded from light for 2 min. 2 min later, the reaction solution was measured for absorbance value at 520 nm on a spectrophotometer (U-1800, Hitachi Co., Ltd) and calculated as Trolox equivalent per 1 g of tomato powder.

RESULTS

As a result of measuring the cellulase activity of enzyme preparations, the enzyme C was the highest, with 189 U/g powder. On the other hand, A and B were close to each other at 33 and 31 U/g powder, respectively. As a result of measuring β-glucosidase activity, the enzyme C was the highest, with 1,530 U/g powder. On the other hand, A and B were 144 and 185 U/g powder, respectively (Table 1).

This time, the conditions for saccharification of Avicel, a microcrystalline cellulose, using enzyme A, B, and C were investigated. As a result, the saccharification rate reached its maximum at 45℃ for A, and at 50℃ for B and C. Furthermore, for all enzyme preparations, the saccharification rate reached its maximum at an enzyme concentration of 0.1% (w/v) and an incubation time of 48 h (Figure 1).

Saccharification of tomato powder was attempted using the saccharification conditions that maximized the

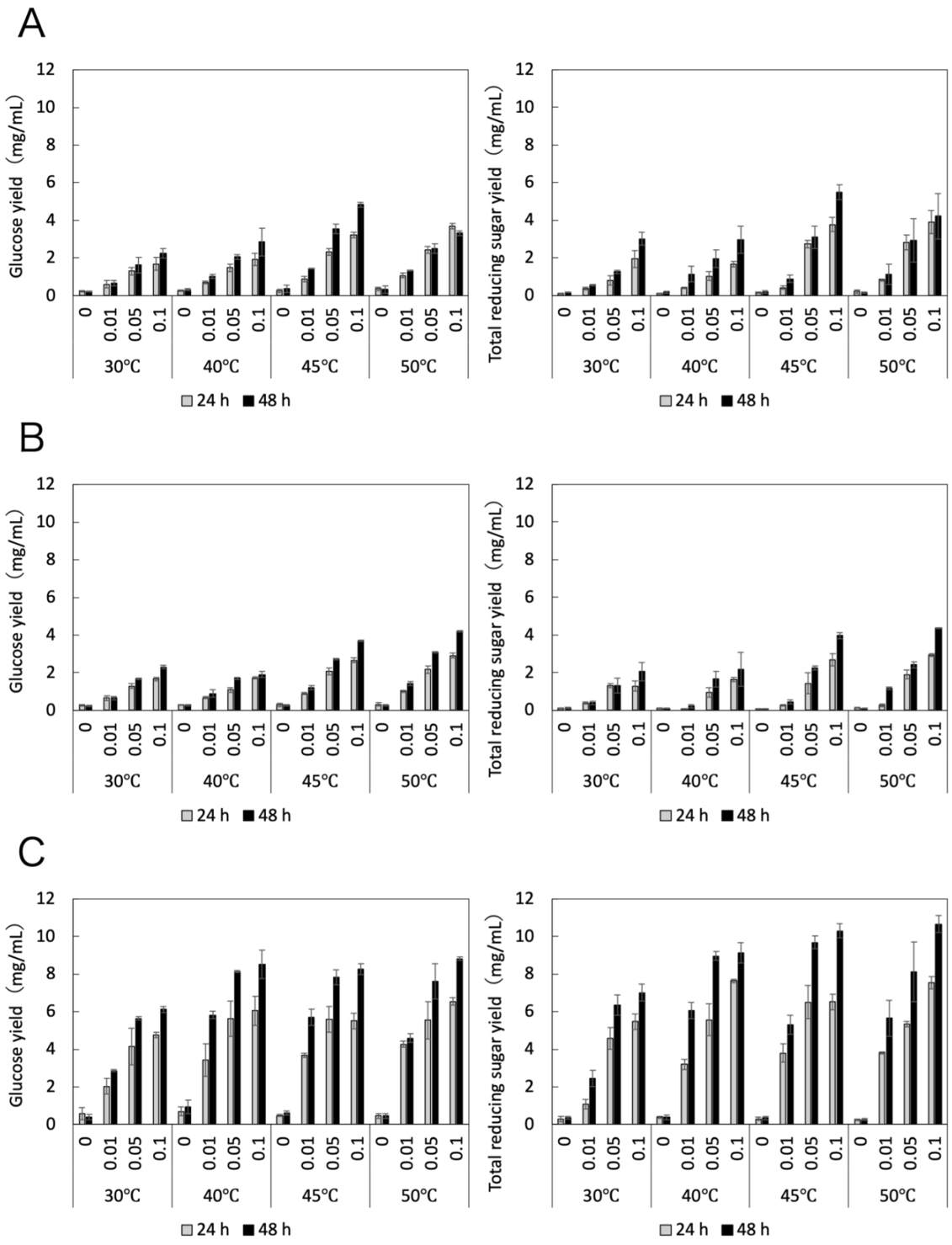

Figure 1. Saccharification of Avicel by enzyme preparation at various conditions. Error bars mean standard deviation (SD). The horizontal axis shows saccharification temperature and enzyme concentration (0, 0.01, 0.05, 0.1%).

saccharification rate. Tomato was saccharified under the same conditions as Avicel. As a result, it was confirmed that glucose and reducing sugar were released in all enzymes by saccharification treatment. However, no significant difference ($p < 0.05$) was observed in the release of glucose and reducing sugars due to differences

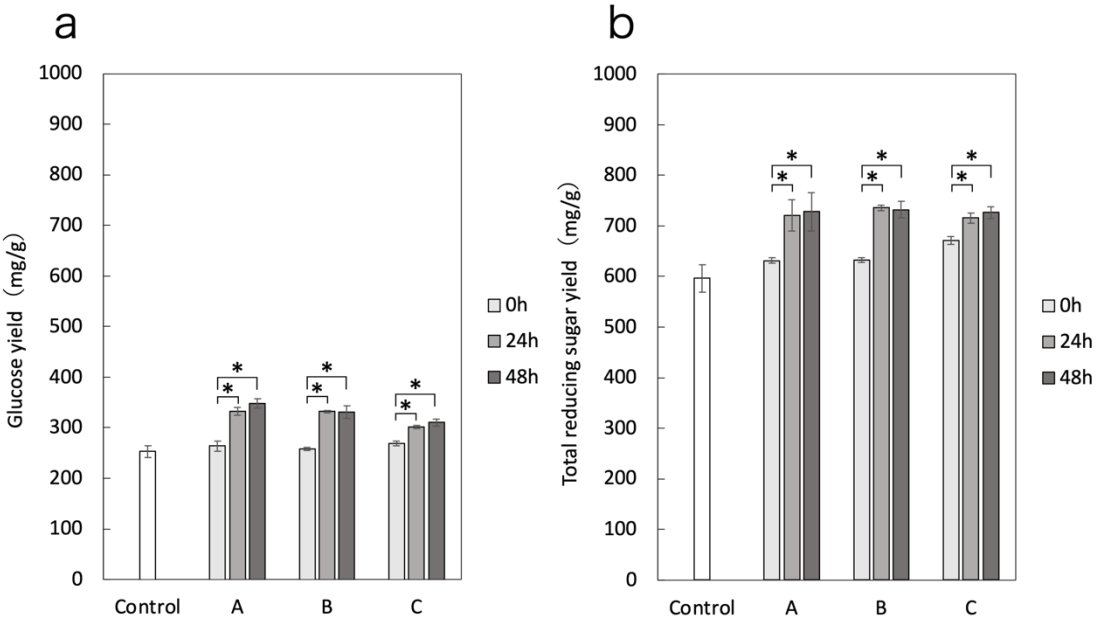

Figure 2. Effects of saccharification time on producing of glucose and reducing sugar. Error bars mean standard deviation (SD). “a” shows the release of glucose, and “b” shows the released reducing sugars per g of tomato powder. Significant differences from time 0 ($p < 0.05$) determined by T-test are indicated ＊.

in saccharification time for any of the enzyme preparations. Enzyme A had the highest glucose yield; the glucose concentration before saccharification was 264 mg/g powder, but it was 333 mg/g powder at 24 h and 348 mg/g powder at 48 h. Furthermore, since the cellulose content of the tomatoes used was 146 mg/g powder (data not shown), the glucose yield was 81.2％ in 24 h and 84.9％ in 48 h of saccharification. The enzyme with the highest yield of reducing sugar was enzyme B, which had a reducing sugar yield of 632 mg/g powder before saccharification, 736 mg/g powder after 24 h saccharification, and 732 mg/g powder after 48 h saccharification. The cellulose content was 146 mg and the pectin content was 40 mg per 1 g of tomato powder used. Therefore, the yield of reducing sugar was 90.0％ in 24 h and 89.5％ in 48 h (Figure 2).

The saccharification treatment increased the concentration of phenolic compounds in tomato powder. In enzyme B, the phenolic compound content before saccharification was 3.57 mg/g powder, but it increased by up to 1.29 times to 4.56 mg/g powder after 24 h and 4.62 mg/g powder after 48 h. The total polyphenol content increased by up to 1.25 times using enzyme A and up to 1.11 times using enzyme C. On the other hand, no significant difference ($p < 0.05$) was observed in the increase in phenolic compound content due to differences in saccharification time (Figure 3).

As a result of measuring the DPPH radical scavenging activity, the antioxidant activity was decreased for all enzyme preparations compared to before saccharification. There was no significant difference ($p < 0.05$) in the decrease in antioxidant activity due to the difference in saccharification time. Antioxidant activity decreased the most when saccharified with enzyme C for 48 h. Before saccharification, the equivalent amount of Trolox per g of tomato powder was 3.82 mg, but after saccharification it was 1.86, which was 48.8％ retained. On the other hand, after 24 h of saccharification, the amount of Trolox was 2.19 mg, maintaining 57.2％ of the antioxidant activity compared to before saccharification. Enzyme A was able to maintain relatively high antioxidant activity during the saccharification process. When saccharified for 24 h, the amount was 2.45 mg, which was maintained at 67.1％ compared to 3.65 mg before saccharification (Figure 4).

DISCUSSION

Plant resources contain various useful components, and sugar is one of them. In order to recover sugar from plant resources, it was necessary to efficiently saccharify the plant resources. It has been reported that the combination of commercially available enzyme preparations promotes the saccharification of polysaccharides, and that galacturonic acid, glucose, arabinose, and galactose are released in apple pomace (Gama et al., 2015). However, each plant resource has a different polysaccharide content, and it was necessary to establish saccharifying enzyme and conditions for each plant resource (Nawirska and Kwaśniewska, 2005). Until now, there has been little

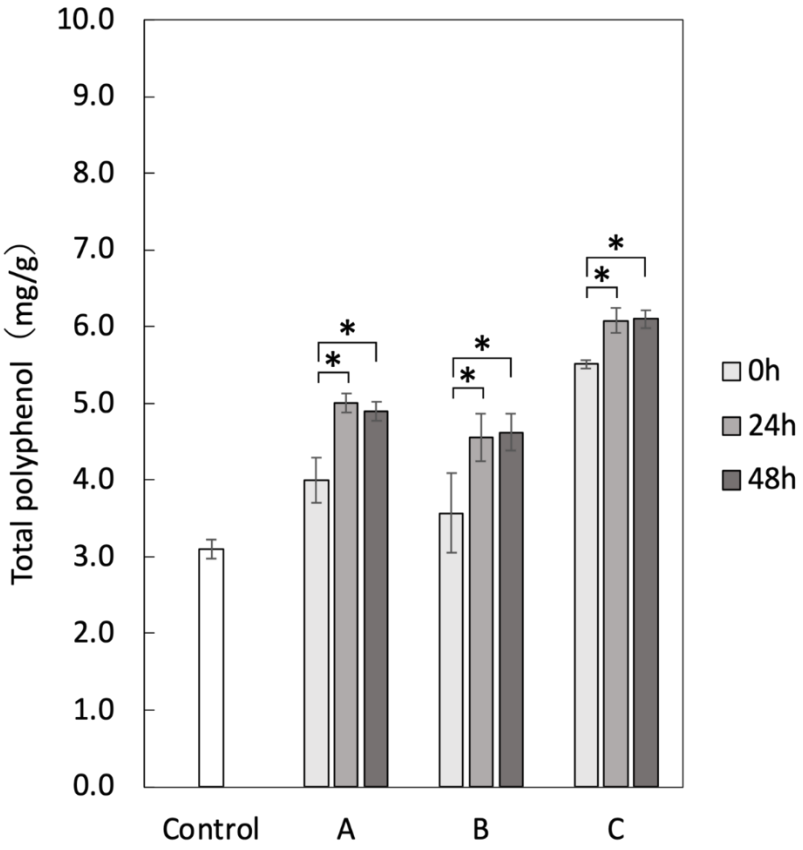

Figure 3. Effects of saccharification time on released polyphenol in tomato powder. Error bars mean standard deviation (SD). Calculated as total polyphenol content per g of tomato powder. Significant differences from time 0 ($p < 0.05$) determined by T-test are indicated by ⋆.

research on the enzymatic hydrolysis of tomato fruits using commercially available enzyme preparations. Therefore, in this study, commercially available enzyme preparations with high cellulase and β-glucosidase activities were used.

Saccharification conditions were investigated using Avicel as a model substrate, and tomatoes were saccharified under conditions that promoted saccharification. As a result of measuring the cellulase and β-glucosidase activity of three types of enzyme preparations, cellulase and β-glucosidase activity were observed in enzyme A, B, and C. In particular, cellulase activity of C was 5.7 to 6.1 timed higher than that of A and B, and β-glucosidase activity was 8.3 to 10.6 times higher than that of A and B. It has been reported that the *Talaromyces* sp. (previously classified as *Acremonium* sp.), which is the origin of enzyme C, exhibits higher cellulase and β-glucosidase activity than the *Trichoderma* sp., which is the origin of enzyme A and B (Fujii et al., 2009). In addition, as a result of actually saccharifying Avicel, enzyme C released more glucose (8.8 mg/ml) than A (4.8 mg/ml) and B (4.2 mg/ml), indicating that β-glucosidase was suggested to be important for the hydrolysis of cellulosic biomass. Cellulose hydrolysis usually involves the synergistic effect of endoglucanase (EG,EC 3.2.1.4), exoglucanase (EC 3.2.1.91, also called cellobiohydrolase: CBH), and β-glucosidase (BGL, EC. 3.2). It is decomposed by Endoglucanases randomly hydrolyze β-1,4 glycosidic bonds in cellulose. Exoglucanases liberate cellobiose from cellulose terminals, and β-glucosidase hydrolyzes the β-1,4 bonds of cellobiose to generate glucose (Singhania et al., 2013, Teugjas and Väljamäe, 2013). However, most of the reported β-glucosidase is sensitive to glucose, and the action of β-glucosidase is inhibited by glucose, which is the final hydrolysis product of cellulose. Thereby, cellobiose and oligosaccharides accumulate and inhibit the activity of endoglucanases and exoglucanases, thus stopping the entire process of cellulose degradation (Sørensen et al., 2013). This time, as a result of saccharifying tomatoes, there was no difference in the release of glucose and reducing sugars due to difference in saccharification time, so it was possible that the saccharification reaction had already finished at 24 h. The glucose yield of Enzyme A was 81.2% after 24 h of saccharification and 84.9% after

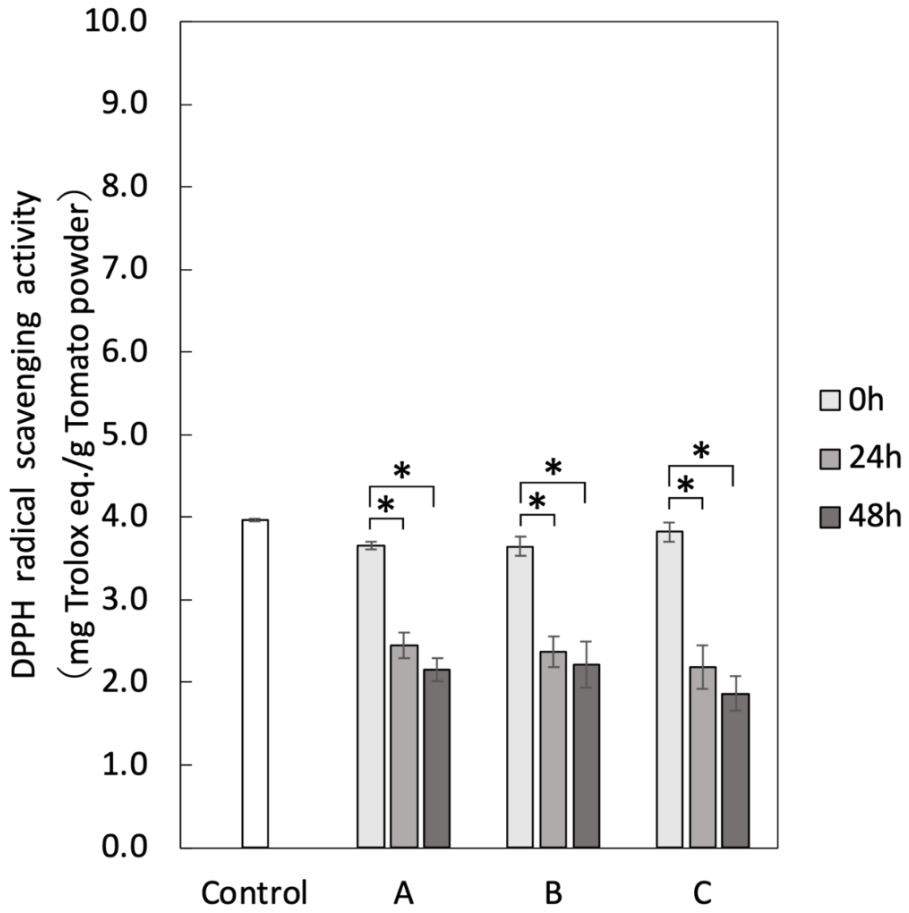

Figure 4. Effect of DPPH radical scavenging activity on different saccharification times of tomato powder. Error bars mean standard deviation (SD). Calculated as mg Trolox equivalent per g of tomato powder. Significant differences from time 0 ($p < 0.05$) determined by T-test are indicated by ＊.

48 h of saccharification. Since there was still room for saccharification at 18.8％ and 15.1％, respectively, it was also suggested that β-glucosidase activity may be inhibited by glucose. Additionally, there is a report that monosaccharides other than glucose inhibit β-glucosidase activity (Hsieh et al., 2014), but in reality, 264 mg of 1g tomato powder used was glucose. On the other hand, it also contained 230 mg of fructose (data not shown), and about 50％ was monosaccharides. Regarding the yield of reducing sugars, enzyme B was 90.0％ at 24 h and 89.5 ％ at 48 h, however, due to the inhibition of β-glucosidase activity, cellobiose and cellooligosaccharides accumulated, resulting in decrease in endoglucanase activity, suggesting that exoglucanase activity was inhibited.

In addition, phenolic compounds also inhibit the hydrolysis of cellulose by endoglucanases and exoglucanases and the hydrolysis of cellobiose by β-glucosidase, but β-glucosidase from the *Trichoderma* sp. are known to be less susceptible to inhibition (Ximenes et al., 2010). In this experiment, in tomato hydrolysis, enzyme A gave a glucose yield of 84.9％, enzyme B 81.9％, and C 74.7％ in 48 h, was found to be susceptible to inhibition by phenol.

Tomato cell walls are rich in ascorbic acid, caffeic acid, and chlorogenic acid, which are particularly abundant in the peel than in the pulp (George et al., 2004). Although immersion methods have traditionally been used to extract phenolic compounds from cell walls (Navarro et al., 2011), ultrasonic extraction is now common in laboratories and industrial applications (Ma et al., 2009). Regarding the release of phenolic compounds from cell walls due to tomato saccharification, Pirozzi et al. (2022) reported that phenolic compounds and carotenoids were released from cell walls in tomato pomace through chemical acid hydrolysis. The results of this study also showed that phenolic compounds are liberated during the saccharification of tomatoes by enzymatic hydrolysis. There was no change in the amounts of phenolic compounds due to the difference in saccharification time between 24 and 48 h. On the other hand, the antioxidant

activity due to DPPH radical scavenging activity decreased. This was inferred to be due to saccharification at high temperatures for long time, as phenolic compounds are stable to high temperature and ascorbic acid is unstable to high temperature (Navarro-Gonzalez et al., 2011).

This study investigated the effects of different saccharification times on the release of sugar and phenolic compounds and on the antioxidant capacity of tomato. As a result, saccharification using an enzyme preparation containing cellulase and β-glucosidase liberated phenolic compounds as well as sugars, but no effect was observed due to the difference in time. On the other hand, the antioxidant due to DPPH radical scavenging activity decreased with saccharification time. From these results, we were able to discover the possibility of using tomatoes, which are discarded during the 24 h saccharification time, as a new food material. In addition, particularly for enzyme A, the rate of hydrolysis to glucose and maintenance of antioxidant activity during 24 h saccharification were higher than for enzyme B and C. Furthermore, the increase rate of phenolic compounds was the second highest after enzyme B, so among the enzyme preparation used this time, enzyme A was the most suitable for tomato saccharification.

Conclusion

This paper succeeded in recovering sufficient sugar and phenol even in the saccharification of tomatoes at 24 h. Enzyme A was shown to be the most suitable among the enzyme preparations used in this study for the saccharification of tomatoes. These results also indicated the possibility of improving the solubility of tomato powder. The results of this study were obtained for one tomato variety, but there is room for further studies on other tomato varieties. The results show the potential for reducing food waste and contributing to the SDGs through the effective use of unused plant resources.

In this study, phenolic compounds were released by saccharification of tomatoes, but the bioactivity of the released phenolic compounds needs to be studied in the future. On the other hand, antioxidant activity was reduced. Tomatoes are one of the vegetables rich in nutrients, but the loss of these nutrients by saccharification is a major issue. Enough sugar and phenol were recovered at 24 h in this study. There is need for further investigation on how long the antioxidant activity can be maintained by reducing the saccharification time. Alternatively, it is necessary to lower the saccharification temperature to maintain heat-unstable components such as ascorbic acid. Saccharification of Avicel by enzyme C is possible at temperatures as low as 40°C and enzyme concentration as low as 0.05% (w/v), and it would be worthwhile to attempt saccharification of tomatoes under similar conditions.

If antioxidant activity can be maintained and a highly soluble tomato powder can be developed, storage conditions for highly soluble tomato powder with respect to temperature, humidity, and light should also be considered.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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