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

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In this study, functional food ingredients have been developed from tomatoes. Specifically, this paper investigates the possibility of producing tomato powder with high solubility by decomposing (saccharification) the cellulose contained in tomatoes using three different microbial enzyme preparations. Additionally, the paper examines the differences in the concentration of sugar produced with and without shaking during saccharification. No effect of shaking on the production of glucose and reducing sugars was observed in either enzyme formulation. However, enzyme preparations A and B produced more glucose than C, with enzyme A producing up to 92.1 mg of glucose. Furthermore, for enzyme preparation C, up to 56.1 mg of reducing sugars other than glucose were produced. Regarding antioxidant activity, the DPPH radical scavenging activity was reduced by saccharification, while the inhibitory activity of liquid peroxidation increased up to 2.02-fold using enzyme preparations A and B. Considering the high solubility and maintenance of functionality, the usefulness of enzyme preparations A and B was suggested.

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 food produced for consumption worldwide, or about 2.5 billion tons, is disposed of as food loss and waste (FLW) every year. FLW has been disposed of by landfilling, incineration, composting, or use as livestock feed (Lin et al., 2013), but the cost and environmental impact of disposal are a global problem (De Abreu et al., 2023; Lahiri et al., 2023). The composition of FLW varies by region, but in Asia (especially Japan, South Korea, and China), 56% of FLW is characterized by vegetables and fruit, compared to 40% in Europe (Braguglia et al., 2018). Agricultural products are also discarded at the production stage, with 5.23 million tons of FLW per year in Japan, but approximately 1.98 million tons of vegetables and fruits (VF) are discarded at the production stage as out-of-specification (Data were

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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).

Recently, studies have been conducted to utilize these substandard VF as biomass for energy and new materials such as bioplastics (Talan et al., 2021; Tsang et al., 2019). However, it is not only about reducing FLW, but also about sustainability and minimizing environmental burden, which is equally important (Roy et al., 2023). VF is an important nutritional source, rich in high antioxidant components such as vitamin C and E, β-carotene, polyphenols (mainly flavanols, flavones, anthocyanins, and phenolic acids), and dietary fiber. VF is a significant source (Kaur and Kapoor, 2002; Robards, 2003; Kazemi et al., 2022).

The main plant cell wall of VF is composed of cellulose, hemicellulose, and pectin (Szymańska et al., 2017), and polyphenols are bound to these dietary fibers (Jakobek and Matić, 2019). Zeyada et al. (2008) reported that among the vegetable peels they examined, olive leaves > tomato peels > cucumber peels > watermelon peels > potato peels were richer in phenolic content in that order. VF contains moisture, and its quality deteriorates quickly if the temperature is not strictly controlled, but drying and powdering solve these problems and enable long-term storage (Chua and Chou, 2003). VF powder is also used in various ways as a food ingredient for ease of storage and processing and is attracting attention as a natural coloring (Karam et al., 2016).

On the other hand, there is a problem that simply powdering VF as it leaves a large amount of dietary fiber, which affects the texture when VF powder is used as a food ingredient (Fernandez-Garcia and McGregor, 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 this problem being solved by decomposing the dietary fiber. Decomposition (saccharification) of plant cell walls by microbial enzymes is a sustainable, clean method that produces no harmful waste and has a much lower environmental impact than chemical hydrolysis (De Aguiar et al., 2020).

Therefore, we have been attempting to address the issue of VF powder texture by employing microbial enzymes (a compound enzyme containing cellulase and β-glucosidase) to break down the dietary fibers themselves. Specifically, our goal is to develop a fully saccharified powder that enhances the solubility of VF powder while preserving its functional properties by saccharifying the cellulose in the VF dietary fibers and releasing glucose. The region where our research facility is situated is the most productive in the country for tomatoes (*Solanum lycopersicum L.),* yielding approximately one-fifth of all tomatoes shipped nationwide. Tomatoes are abundant in phenolic compounds (phenolic acids, flavonoids), carotenoids (lycopene, α- and β-carotene), vitamin A, and vitamin C (Tan et al., 2010). These functional components in tomatoes exhibit antioxidant, anti-mutagenic, anti-proliferative, anti-inflammatory, and anti-atherosclerotic effects and are effective in preventing cancer and cardiovascular disease (Chaudhary et al., 2018).

In our recent study, we investigated saccharification time as one of the conditions for enhancing solubility in tomatoes and found that 24 h of saccharification was adequate to release glucose (Hirata et al., 2024). It has been frequently reported (Ganesh et al., 2000; Gunjikar et al., 2001) that agitating cellulase during the enzymatic reaction leads to the formation of a gas-liquid interface in the reaction solution, thereby reducing cellulase activity. However, there are limited reports on the impact of agitation conditions on cellulases during the actual saccharification of VF, and the studies currently available on the saccharification of VF through agitation are sparse.

The ultimate objective of this study was to develop a highly soluble powder through the saccharification of discarded substandard VF using a commercially available enzyme preparation. Initially, saccharification conditions for tomatoes were explored, with the intention of applying these findings to similar plant resources. This paper investigates the effects of saccharification conditions, particularly shaking, on tomatoes using various commercially available enzyme preparations, aiming to produce a highly water-soluble tomato powder. Avicel, microcrystalline cellulose, was employed as a model substrate to investigate the conditions under which cellulase activity was suppressed during shaking. The impact of shaking on the saccharification of tomatoes under these conditions was also examined. Furthermore, changes in phenolic compound content, antioxidant activity, and inhibitory activity of lipid peroxidation after saccharification were evaluated.

MATERIALS AND METHODS

Chemicals

Avicel (Avicel PH-101), *p*-nitrophenyl-β-D-glucopyranoside, Somogyi copper reagent, Nelson color reagent, DPPH (1, 1-diphenyl-2-picrylhydrazyl) and Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) and β-carotene were purchased from Sigma-Aldrich (St. Louis, MO, USA). Glucose quantification kit and sodium hydroxide were purchased from Fujifilm Wako Pure Chemicals Corporation (Osaka, Japan). Phenol reagent solution, gallic acid, sodium carbonate, potassium dihydrogen phosphate, chloroform, linoleic acid and Tween 40 and BHT (2, 6 - di- butyl - p - cresol) were purchased from Nakalai Tesque Inc (Kyoto, Japan). Ethanol was sourced from Japan Alcohol Alcohol Trading Co., Ltd (Tokyo, Japan).

Tomato

The tomatoes (cv.; *Reiyo*) utilized in the experiment were substandard tomatoes donated by a farmer in Kumamoto Prefecture. Following the crushing raw tomatoes with mixer, they underwent freeze-drying and were powdered. Freeze-drying was conducted using an eggplant-shaped flask with Freeze Dryer Fd-1 (Eyela

Tokyoricakikai Co Ltd, Tokyo, Japan).

Enzyme preparation

A commercially available enzyme preparation was used for saccharification of tomato powder. Three types of enzyme preparations were used: *Trichoderma* sp. derived enzyme A (cellulase) and B (hemicellulase) from Mitsubishi Chemical Co., Ltd., (Tokyo, Japan) and Enzyme preparation produced by *Talaromyces* sp. enzyme C (cellulase, hemicellulase) from Kyowa Kasei Co., Ltd (Saitama, Japan). The cellulase and β-glucosidase activities of each enzyme preparation were previously reported (Hirata et al., 2024).

Saccharification experiment of Avicel

30 ml of each enzyme solution suspended 0.1% (w/v) in 0.05 M acetate buffer at pH 4.5 was added to 0.3 g of Avicel in a 50 mL centrifuge tube and incubated in an incubator (Bio-Shaker-BR-300L, TAITEC, Saitama, Japan) at temperature optimised for each enzyme preparation (enzyme A: 45ºC, enzyme B and enzyme C: 50ºC) (Hirata et al., 2024) for 24 and 48 h with the culture vessel on its side with reciprocal shaking at 0 and 100 rpm. After incubation, the reaction solution was centrifuged at 3,000 rpm for 5 min at room temperature using a centrifuge (Kubota, KN-70, Kubota Shoji Co., Ltd., Tokyo, Japan) and the glucose and reducing sugar concentration in the supernatant was determined.

Saccharification experiment of tomato

30 ml of each enzyme solution at 0.1% (w/v) was added to 0.3 g of tomato powder in a 50 mL centrifuge tube. Saccharification was carried out in an incubator (Bio-Shaker-BR-300L, TAITEC, Saitama, Japan) with the culture vessel on its side with reciprocating shaking at 0 and 100 rpm for 48 h at the optimal temperature for saccharification at 45°C for enzyme A and 50°C for enzyme B and C, respectively.

Saccharification was carried out. After saccharification, the reaction solution was centrifuged and the glucose and reducing sugar concentrations in the supernatant were measured and calculated as the amount of sugar per gram of tomato powder (test). On the other hand, for the control (0 h reaction), the sugar in the supernatant was measuring immediately after mixing the tomato powder and enzyme solution.

Analysis of sugar concentration

A glucose quantitative kit was utilized to determine the glucose concentration in the saccharification reaction mixture, following the protocol provided with the kit. For the determination of glucose concentration, 0.1 ml of the supernatant of the mixture was mixed with 3.0 ml of glucose quantitative reagent and incubated at 40°C for 20 min. The absorption value of the reaction solution at 505 nm was then measured, and the glucose concentration was calculated from the standard curve.

Similarly, the reducing sugar concentration was determined using the Somogyi-Nelson method (Somogyi, 1952; Nelson, 1944), and the concentration of glucose and reducing sugar were calculated as the amount of sugar per 1 g of tomato powder.

Total phenolic concentration

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

Measurement of antioxidative activity

Inhibitory activity of lipid peroxidation

Test samples were prepared by mixing the saccharification reaction mixture with ethanol (1:1) to achieve an ethanol concentration of 50% (v/v). The inhibitory activity of lipid peroxidation was assessed using a partially modified method based on Hamasaka et al. (2004). Solutions of 0.2 M potassium dihydrogen phosphate and 0.2 M sodium hydroxide were prepared separately. A 0.2 M sodium phosphate buffer at pH 7.0 was then prepared by combining the two solutions, and its pH was adjusted.

Linoleic acid-β-carotene emulsions were prepared by mixing 0.2 mL of 10% (w/v) linoleic acid solution, 1.0 mL of 20% (w/v) Tween 40 solution, and 0.5 mL of 0.2% (w/v) β-carotene solution suspended in chloroform. This mixture was shaded from light, and nitrogen gas was used to remove the chloroform.

Then, 100 mL of deionized water and 8.9 mL of 0.2 M sodium phosphate buffer at pH 7.0 were added and thoroughly mixed.

For the test samples, 0.1 mL of the sample (containing 50% (v/v) ethanol) was mixed with 4.9 mL of the linoleic acid-β-carotene emulsion. The sample was quickly stirred, and the absorbance value (A470) at 470 nm was measured using a spectrophotometer (U-1800, Hitachi, Ltd., Tokyo, Japan). The solution was then promptly returned to the test tube, capped with a marble, and allowed to react in a thermostatic bath at 50°C for 60 min. After the reaction, the absorbance value at 470 nm was measured again, and the difference in absorbance was calculated as the BHT equivalent per gram of tomato powder.

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

First, Avicel, microcrystalline cellulose, was saccharified by reciprocal shaking at 100 rpm at saccharification times of 24 and 48 h. The results showed that shaking significantly ($p < 0.05$) reduced glucose release in all enzyme preparations, irrespective of saccharification time. At 24 h of saccharification, glucose release by shaking was 9.39, 5.14 and 7.39% lower than static for enzymes A, B, and C, respectively. At 48 hours, shaking for enzymes A, B, and C reduced glucose release by 20.9, 18.8 and

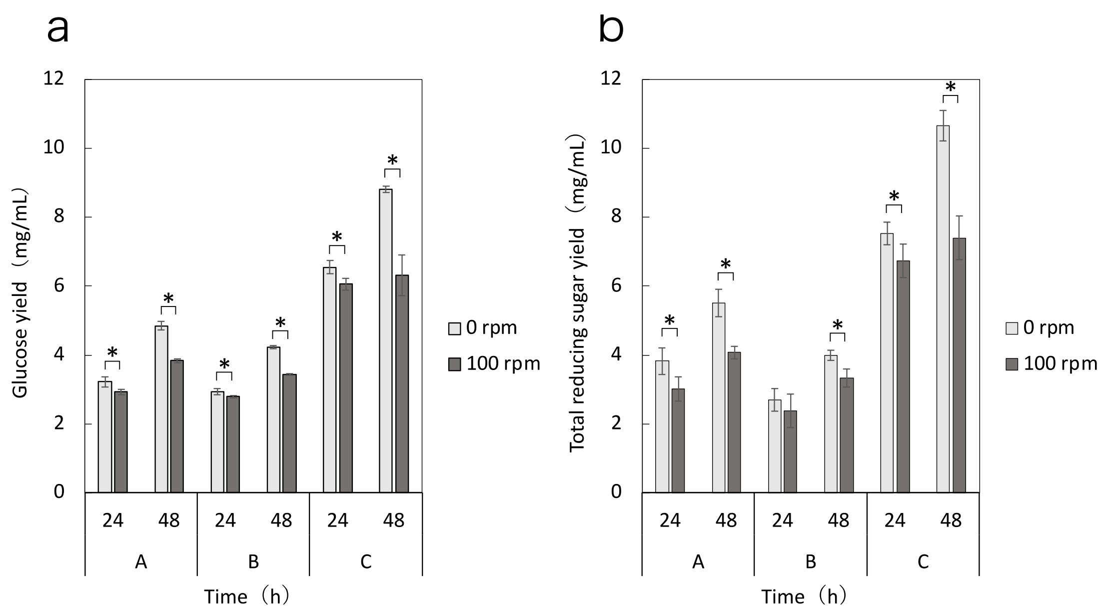

Figure 1. Saccharification of Avicel by enzyme preparation at shaking and different time. The same analysis was performed three times, and the average value is shown. Error bars mean standard deviation (SD). “a” shows the release of glucose, and “b” shows the released reducing sugars. The horizontal axis shows enzyme preparation and saccharification time (24 and 48 h). Significant difference ($p < 0.05$) determined by T-test are indicated by *.

28.4%, respectively, further than at 24 h of saccharification, indicating an even greater reduction in glucose release (Figure 1a). After saccharification by shaking, reducing sugars were measured, and the release of reducing sugars was similarly suppressed by shaking. At 24 h of saccharification, shaking resulted in a 21.1, 12.0 and 10.5% reduction in reducing sugars release for enzymes A, B, and C, respectively, compared with static saccharification, and a 26.1, 16.6 and 30.6% reduction at 48 h of saccharification, respectively (Figure 1b). The results showed that the release of glucose and reducing sugars was reduced at 48 h rather than 24 h of saccharification for both enzyme preparations. Saccharification of tomatoes by shaking was attempted at 48 h of saccharification, when the release of glucose and reducing sugars was most suppressed. The results showed that saccharification resulted in glucose and reducing sugar release with and without shaking (Figure 2). On the other hand, there was no significant difference ($p < 0.05$) between the shaking and no shaking: although enzyme C released the most glucose from Avicel, enzymes A and B released more glucose than enzyme C in the saccharification of tomatoes.

Enzyme A increased the amount of glucose per g of tomato powder by 92.1 mg under static conditions and 72.1 mg under shaking conditions compared to before saccharification (265 mg). Similarly, enzyme B increased the amount of glucose per gram of tomato powder by 87.9 mg under static conditions and 80.6 mg under shaking conditions compared to before saccharification (275 mg). Enzyme C also increased by 37.9 mg under static conditions and 28.2 mg under shaking conditions compared to before saccharification (255 mg). The enzyme that released the most glucose was enzyme A.

The amount of reducing sugars per gram of tomato powder after saccharification showed that saccharification released reducing sugars, but there were no significant differences ($p < 0.05$) between the presence and absence of shaking. While enzyme A released the most glucose, enzyme B released the most reducing sugars, increasing the amount of reducing sugars per gram of tomato powder by 157 mg during saccharification in static conditions and by 151 mg during shaking compared to before saccharification (652 mg). Enzyme C increased by 91.8 mg during saccharification in static conditions and by 84.3 mg during shaking compared to before saccharification (656 mg). The results of saccharification of tomatoes by shaking for 48 hours, which has been shown to have the greatest effect on sugar release in Avicel saccharification, showed no effect of shaking on the release of glucose and reducing sugars. Sedimentation of the reaction solution after saccharification was also observed. Sedimentation decreased with sugar release, but again, there was no difference in sedimentation with or without shaking (Figure 3). Furthermore, dissolving the saccharified and untreated tomato powder, this was further freeze-dried and powdered from the saccharification reaction solution and precipitation, in an equal volume of water showed improved solubility (Figure 4a). Additionally, when the supernatant of the saccharified tomato solution was

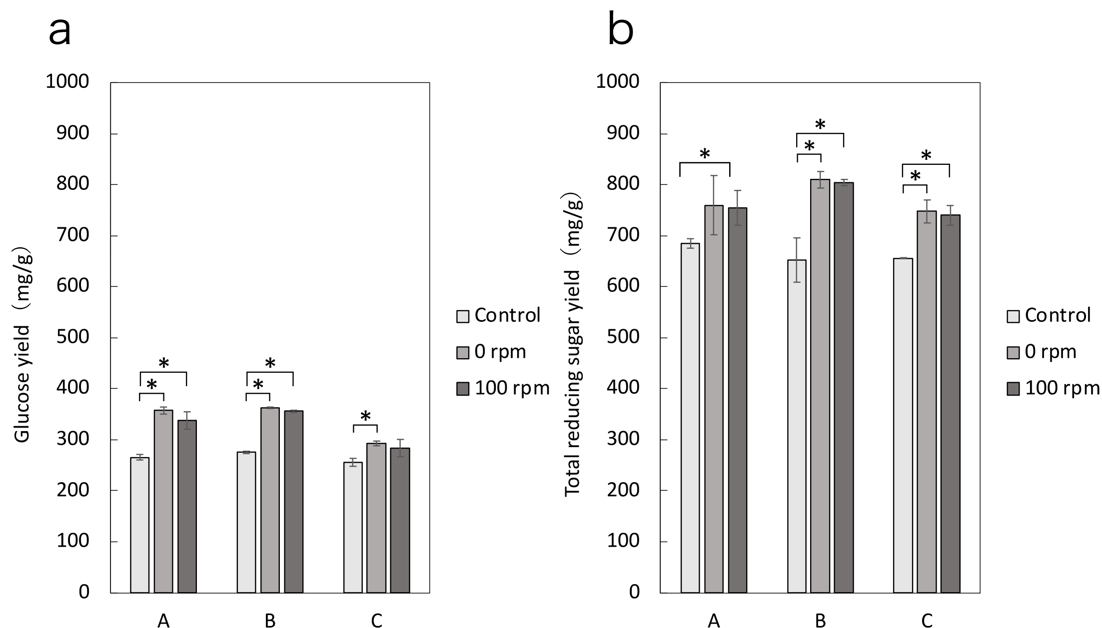

Figure 2. Effects of shaking reaction on sugar concentration in tomato powder. The same analysis was performed three times, and the average value is shown. Error bars mean standard deviation (SD). Control indicates the sugar concentration before the reaction. "a" shows the release of glucose, and "b" shows the released reducing sugars per g of tomato powder. Significant difference ($p < 0.05$) determined by T-test are indicated by ∗.

Figure 3. Effects of shaking reaction on tomato solubilization using enzyme preparation A. Shown left to right are the control, without shaking (0 rpm), and with shaking (100 rpm), control shows tomato powder suspension before reaction.

freeze-dried, the water absorbency increased, and the powder turned from powder to syrup-like when left in room conditions (Figure 4b). Shaking had no effect on the release of glucose and reducing sugars, but measurements on total polyphenol content showed that saccharification with enzyme B significantly ($p < 0.05$) increased polyphenol content with shaking (Figure 5a). The total polyphenol content per gram of tomato powder increased 1.25-fold (4.23 mg) under standing conditions and 1.36-fold (4.60 mg) with shaking compared to before saccharification (3.38 mg).

Enzyme A also increased the total polyphenol content the most, although there were no significant differences between with and without shaking. It increased 1.35-fold (4.71 mg) under standing conditions and 1.30-fold (4.52 mg) under saccharification with shaking, compared to before saccharification (3.49 mg). There was no significant difference ($p < 0.05$) in the total polyphenol content after

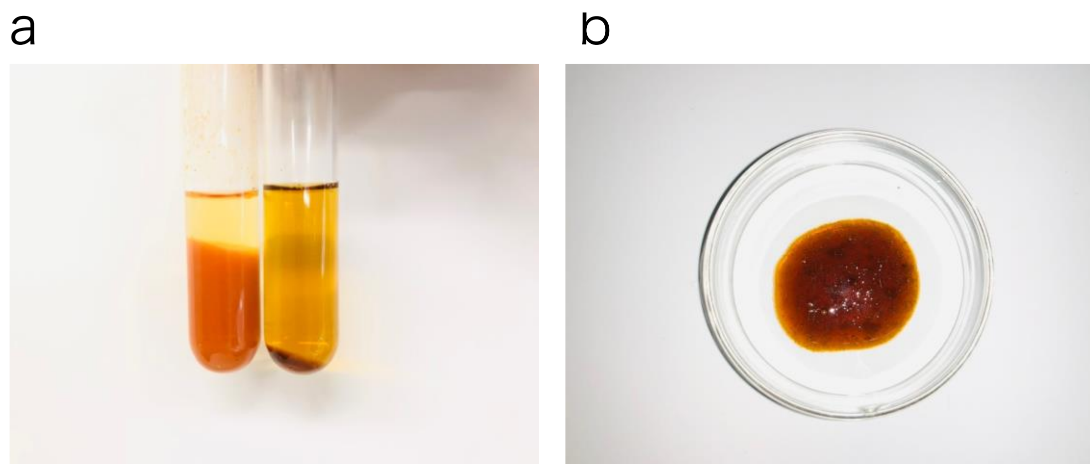

Figure 4. Solubilization and properties of after saccharification. “a” shows the photograph of improved solubility of the powder after saccharification. On the left, untreated tomato powder was dissolved in water. On the right, the reaction solution and precipitates after saccharification are lyophilized, further powdered, and dissolved in water. “b” shows the supernatant of the freeze-dried reaction solution left in the room. Left in the room, it went from powder to a syrupy consistency.

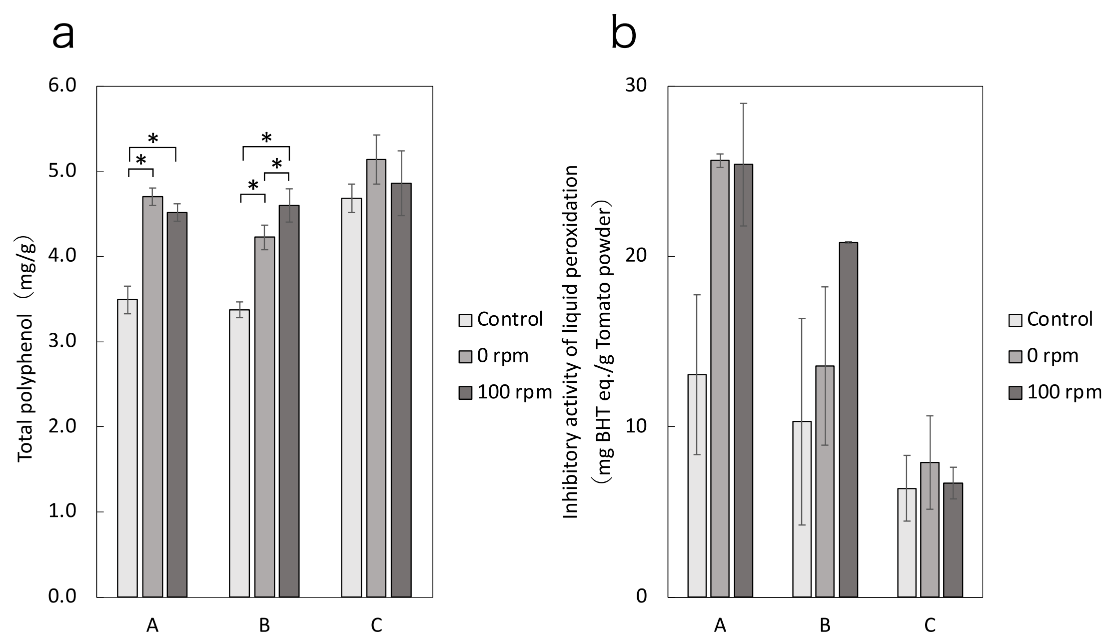

Figure 5. Effect of shaking reaction on phenolic components release and inhibitory activity of lipid peroxidation in tomato powder. The same analysis was performed three times, and the average value is shown. Error bars mean standard deviation (SD). Control indicates the phenolic components content and inhibitory activity of lipid peroxidation before the reaction. “a” shows the release of phenolic components, and “b” shows the inhibitory activity of lipid peroxidation as mg BHT equivalent per g of tomato powder. Significant difference (*p* < 0.05) determined by T-test are indicated by ∗.

saccharification with enzyme C.

Measurements of inhibitory activity of lipid peroxidation showed no negative effects due to saccharification in all enzyme preparations (Figure 5b). Furthermore, there was a correlation between total polyphenols and inhibitory activity of lipid peroxidation. Similar to the results for total polyphenols, saccharification by shaking increased the inhibitory activity of lipid peroxidation in enzyme B. Shaking increased the BHT equivalent per gram of tomato powder 2.02-fold (20.82 mg) after saccharification

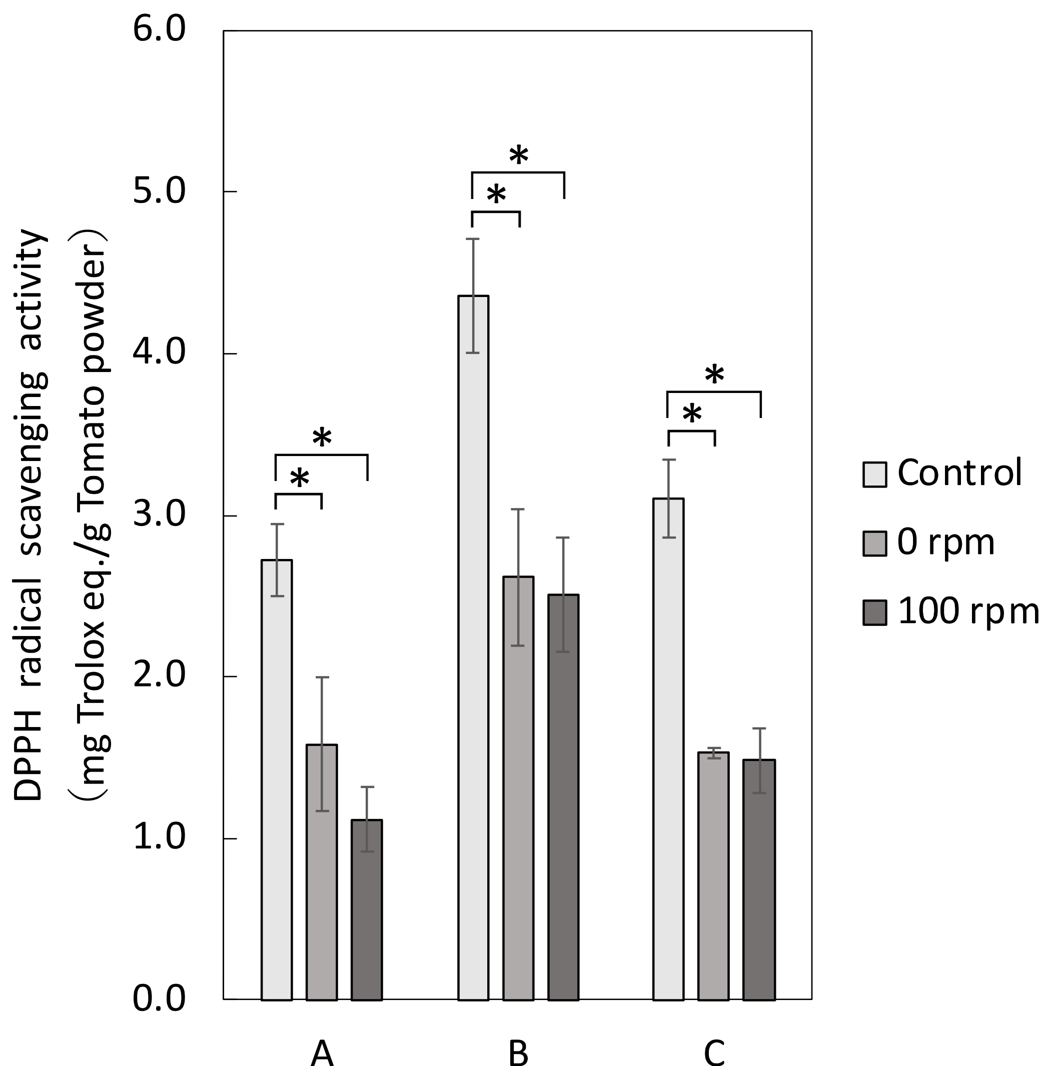

Figure 6. Effects of shaking reaction on DPPH radical scavenging activity of tomato powder. The same analysis was performed three times, and the average value is shown. Error bars mean standard deviation (SD). Calculated as mg Trolox equivalent per g of tomato powder. Control indicates the DPPH radical scavenging activity before the reaction. Significant difference (p < 0.05) determined by T-test are indicated by ＊.

compared to before (10.30 mg). It also increased 1.32-fold (13.6 mg) under standing conditions. The highest increase in inhibitory activity of lipid peroxidation was observed for enzyme A, which increased 1.96-fold (25.6 mg) under the standing condition and 1.94-fold (25.4 mg) under the shaking condition, compared to before saccharification (13.06 mg). The results of the DPPH radical scavenging activity showed a significant (p < 0.05) reduction in antioxidant activity in all enzyme preparations compared to before saccharification (Figure 6). There were no significant differences in the results in the presence or absence of shaking. It was enzyme B that was able to inhibit the decline in antioxidant activity. The maintenance of antioxidant activity per gram of tomato powder was 60.1% (2.62 mg) under static conditions and 57.6% (2.51 mg) under shaking conditions compared to before saccharification (4.36 mg).

When saccharified with enzymes A and C, the antioxidant activity was lower than that of enzyme B, with 58.1 and 49.4% (1.58 mg and 1.53 mg) maintained under static conditions compared to before saccharification (2.72 mg and 3.10 mg) for enzymes A and C, respectively. On the other hand, shaking resulted in a 41.2 and 47.7% (1.12 and 1.48 mg) retention rate, respectively.

DISCUSSION

Vegetable fruits (VF) contain a variety of useful components, with sugar being one of them. VF are also

rich in cellulose, and the hydrolysis of cellulose usually involves a synergistic effect of endoglucanase (EG, EC 3.2.1.4), exoglucanase (EC 3.2.1.91, also known as cellobiohydrolase: CBH), and β-glucosidase (BGL, EC. 3.2). EG hydrolyses β-1,4 glycosidic bonds in cellulose randomly. CBH releases cellobiose from the cellulose end, and BGL hydrolyses the β-1,4 bonds of cellobiose to produce glucose (Singhania et al., 2013) (Teugjas and Väljamäe, 2013). The main plant cell wall of VF is composed of hemicellulose and pectin in addition to cellulose, which is reported to be degraded by enzymes produced by Aspergillus sp. and Trichoderma sp., releasing oligosaccharides (Sabater et al., 2020). Therefore, it was necessary to use these enzymes to efficiently recover sugars from VF. For example, it has been reported that galacturonic acid, glucose, arabinose and galactose are released from apple pomace by combining commercially available enzyme preparations to enhance polysaccharification (Gama et al., 2015).

However, the polysaccharide content of each VF was different, and the saccharification enzymes and conditions had to be configured for each VF (Nawirska and Kwaśniewska, 2005). Until now, few studies have been conducted on enzymatic hydrolysis of tomato fruit using commercial enzyme preparations.

We, therefore, investigated saccharification time as one of the conditions for enzymatic hydrolysis of tomatoes and reported on the optimal saccharification time of tomatoes (Hirata et al., 2024). Although cellulases, including BGL, were reported to be less active in saccharification of cellulose by shaking (Ganesh et al., 2000; Gunjikar et al., 2001), there are few reported similar experiments on VF. Therefore, in this paper, tomatoes were used among VF with commercial enzyme preparations with cellulase and BGL activities, and the effects of cellulase and BGL activities on the shaking saccharification of tomatoes were evaluated. Furthermore, the possibility of creating a highly soluble tomato powder by efficiently saccharifying insoluble cellulose contained in tomato powder was studied. In the study, the release of glucose and reducing sugars was inhibited in saccharification by shaking of Avicel compared to standing conditions, which was suggested to be due to the inactivation of cellulase activity and β-glucosidase (BGL), which have already been reported. As the effect of shaking was more pronounced in the 48-h saccharification, tomatoes were saccharified under similar conditions. The results showed no difference in the release of sugars with and without shaking, indicating that inactivation of cellulase and BGL activities did not occur during saccharification of tomatoes by shaking. It has been reported that the addition of a surfactant (Tween 20) protects cellulase inactivation in saccharification of cellulose by shaking (Bhagia et al., 2019). It is also known that plants contain natural surfactants called saponins (Sochacki and Vogt, 2022), and tomato saponins are present as α-tomatin and esculeoside A (Pareja-Jaime et al., 2008), while α-tomatin is found in mature abundant in mature blue fruits, but decreases with maturity and is converted to esculeoside A (Yamanaka et al., 2009; Ngo et al., 2022). It has also been reported that mature tomatoes contain about four times as much esculeoside A as lycopene (Manabe et al., 2011), and in the study, the inactivation of cellulase and BGL was suppressed by saccharification of tomatoes by shaking, which was presumably due to the protection of cellulase and BGL by the effect of esculeoside A contained in the tomatoes. It was also shown that the sedimentation of the saccharified solution was reduced by saccharification due to the release of water-insoluble cellulose as soluble glucose and cellooligosaccharides. However, the cellulase and BGL activities were not inactivated by saccharification with shaking, so the sedimentation in the saccharification with shaking was also reduced compared to that before saccharification, and the sedimentation was also reduced in the shaking saccharification compared to before saccharification. Furthermore, when the saccharified and untreated powders were dissolved in water, the saccharified powder was more soluble, indicating that the polysaccharides in the tomatoes were actually degraded, thereby improving solubility. Furthermore, tomato cell walls are rich in vitamin C, caffeic acid, and chlorogenic acid, especially in the peel rather than in the pulp (George et al., 2004). We previously reported that saccharification released these phenolic compounds and that these phenolic compounds were even stable at 45 and 50°C (Hirata et al., 2024).

However, when the inhibitory activity of lipid peroxidation after saccharification was measured, a correlation was found between the increase in total polyphenols and the inhibitory activity of lipid peroxidation. This indicates that the released polyphenols are thermally stable and are also polyphenols with inhibitory activity of lipid peroxidation. Furthermore, it has been reported that decomposition of tomato cell walls increases the content of polyphenols and lycopene (Podsędek et al., 2003), and it was highly possible that lycopene released with polyphenols from the decomposition of tomato cell walls by saccharification could be detected as an inhibitory activity of lipid peroxidation. On the other hand, vitamin E is also heat unstable (Abushita et al., 2000), so it was expected that it was not detected in this inhibitory activity of lipid peroxidation.

DPPH radical scavenging activity was reduced for all enzymes and conditions, but since vitamin C, as well as vitamin E, is thermally unstable (Navarro et al., 2011), it was inferred to be reduced due to prolonged saccharification at 45 and 50°C. In addition, the DPPH radical scavenging activity decreased with shaking, especially for enzyme A. It has been reported that vitamin C is unstable to air (Oyetade et al., 2012), and this may be due to the oxidation of vitamin C caused by the increased surface area exposed to air by shaking. Enzyme A saccharified at 45°C, indicating that heat was less likely to cause a decrease in vitamin C activity than enzymes B and C.

The fact that heat-induced loss of vitamin C activity occurred less frequently than enzymes B and C indicated that the effect of shaking was more likely to be observed. In this study, the effects of saccharification on the release of sugars and phenolic compounds and antioxidant activity in shaking tomatoes were investigated. As a result, the saccharification under the shaking condition did not inhibit cellulase activity or BGL activity of enzymes A, B, and C used in this study, and the same amount of sugar was released as under the standing condition. Enzymes A and B released more sugars than enzyme C, and enzyme A released more polyphenols than enzyme C, even under standing conditions, and was able to increase the inhibitory activity of lipid peroxidation and maintain DPPH radical scavenging activity better than shaking. Enzyme B significantly released polyphenols and increased inhibitory activity of lipid peroxidation by shaking. Also, the DPPH radical scavenging activity was the best maintained among all enzyme preparations. These results indicate that tomatoes have the potential to be used as a new food material. In the production of highly soluble tomato powder, enzyme A was shown to be the best choice from the point of energy economics, as it can release sugars at low temperatures and in a standing condition, thereby increasing and maintaining its functionality. For the production of highly soluble tomato powder with higher polyphenol and inhibitory activity of lipid peroxidation, the use of enzyme B and saccharification by shaking were shown to be the best.

Conclusion

This paper demonstrates that sufficient sugar and phenols can be recovered in the saccharification of tomatoes by shaking without being affected by cellulase inactivation. In the case of saccharification by shaking, enzyme B was also found to be suitable for saccharification of tomatoes, in addition to enzyme A, which was previously reported among the enzyme preparations used in this study. These results also suggest the possibility of improving the solubility of tomato powder. Although the results of this study were obtained for one tomato variety, further studies on other tomato varieties are warranted. The findings indicate the potential for reducing food waste and contributing to the Sustainable Development Goals (SDGs) through the effective use of unused plant resources. While this study showed that the release of phenolic compounds by saccharification of tomatoes increased the inhibitory activity of lipid peroxidation, future research should focus on investigating the bioactivity of the released phenolic compounds. 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 concern. As sugar and polyphenols were sufficiently recovered even under standing conditions in this study, further investigation is needed to determine how much antioxidant activity can be maintained by shortening the saccharification time or lowering the saccharification temperature under standing conditions. It is believed that by employing saccharification by shaking under lower saccharification temperature conditions, polyphenols can also be effectively released. Moreover, if antioxidant activity is maintained and highly soluble tomato powder can be developed, storage conditions for highly soluble tomato powder related 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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