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Changes in Protease Activity of Ginger Rhizome during Postharvest Storage

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Abstract

It has been reported that ripe ginger rhizomes contain proteolytic enzymes. In this study, we investigated the fluctuations in protease activity from ginger rhizomes stored after harvest for the utilization of proteases. The juice from ginger rhizomes that had been stored for 4 months after harvest exhibited the highest specific protease activity. Additionally, juices with high specific protease activity could form soymilk and cow milk gels. Electrophoresis experiments revealed that the protein bands in ginger rhizome juice were degraded by the proteases contained in the juice. This degradation was prevented by adding the cysteine protease inhibitor E-64.

Keywords: ginger rhizome, protease activity, postharvest storage, gel formation

1. Introduction

Plant proteases have been used in manufacturing processes such as cheesemaking, meat tenderization, the brewing industry, and peptide production for decades. Recently, these enzymes have shown great potential for valorizing industrial waste and converting it into high value products through cost-effective processes (Troncoso, Sánchez, & Ferreira, 2022). The reduced availability of calf rennet, combined with the growing global demand for cheese and increasing vegetarianism of some consumers, has led to the exploration of alternative enzymes capable of replacing traditional rennet in cheese-making. Currently, several plant-derived milk-clotting enzymes are employed in cheese-making technology (Shah, Mir, & Paray, 2014; Nicosia, Puglisi, Pino, Caggia, & Randazzo, 2022).

The rhizome of ginger (*Zingiber officinale Roscoe*) contains zingibain (EC 3.4.22.67), a protease with high proteolytic activity (Thompson, Wolf, & Allen, 1973; Ohtsuki, Taguchi, Sato, & Kawabata, 1995; Choi & Laursen, 2000). Ginger protease has a high affinity for collagen and other connective tissue proteins, demonstrating excellent meat tenderization and milk-clotting activities (Su, Huang, & Wang, 2009; Hashim, Mingsheng, Iqbal, & Xiaohong, 2011; Huang, Chen, Luo, Guo, & Ren, 2011).

A traditional Chinese desert, *jiang zhi zhuang nai*, is made by curdling heated milk using ginger rhizome juice (Hung, 2001; Nishimura & Goto, 2010). This food is believed to consist of a gel formed from protein aggregates through the action of proteases in the ginger rhizome juice. With the acceleration in societal aging, there is an increasing demand for foods with controlled fluidity among people with swallowing difficulties. Therefore, gel-based foods using ginger rhizome juice are thought to contribute to the development of foods that address new social challenges. However, ginger milk pudding sometimes fails despite following previously successful attempts. We speculated that the constituents of milk and ginger may be responsible for this phenomenon. Although the influence of cow milk type on the ginger milk pudding production has been previously investigated (Yamada, 2015), the impact of different types of ginger is unknown.

In Japan, ginger rhizomes ripened for over two months after harvest are called “Hine-Syoga” while those used immediately after harvest are called “Sin-Syoga”. Although the medicinal components (gingerol, shogaol, etc.) in ginger rhizomes stored after harvest have been investigated (Bailey-Shaw, Williams, Junor, Green, Hibbert, Salmon, & Smith, 2008; Ghasemzadeh, Jaafar, & Rahmat, 2016), there have been few reports on proteases (Ichikawa, 1988; Paull, Chen, & Goo, 1988). Therefore, the variations in protease activity of ripe ginger rhizomes and the role of proteases involved in ginger rhizome juice-induced gel formation has not yet been fully

explored. In this study, we investigated the fluctuations of protease activity and gel forming ability in the juice of ginger rhizomes stored after harvest.

2. Materials and Methods

2.1 Materials

Ginger rhizomes were purchased from Narita Shokuryo Co., Ltd. (Chiba Prefecture). Ginger rhizomes harvested in November 2022 were stored according to the producer's procedures (13°C, 70-80% relative humidity). Preserved samples from each month, ranging from 2 to 10 months after harvest, were purchased and used in the experiments.

2.2 Preparation of Ginger Juices

Ginger rhizomes were washed and cut into fine pieces, and juiced using a slow juicer (HEALSIO Juicepresso; Sharp Corp., Osaka, Japan). The juice samples were centrifuged at 2150 × g for 20 min at 4 °C, and the supernatants were filtered using a filter paper (No. 2, Advantec, Tokyo, Japan). To stabilize the enzymes, 0.2 % L-ascorbic acid and 10 mM L-cysteine hydrochloride were added (Adulyatham & Owusu-Apenten, 2005; Yamada et al., 2020). The prepared ginger rhizome juices were stored at -80 °C until further use.

2.3 Evaluation of Specific Protease Activity in Ginger Rhizome Juices

The protease activity was measured using the following method: The substrate solution was prepared by dissolving 1 % (w/v) of casein in 0.1 M potassium phosphate buffer, pH 7.0. The assay involved incubating 500 μL of the substrate solution with 10 μL of the ginger rhizome juice, at 60 °C for 30 min. the enzymatic reaction was terminated by adding 500 μL of 0.4 M trichloroacetic acid (TCA) to the mixture. A blank sample was prepared by adding the TCA solution to the substrate solution before adding the ginger juice. After 30 min of incubation at room temperature, the precipitated protein was removed by centrifugation at 20,000 × g for 15 min. The amount of degraded protein in the supernatant was assessed by measuring the increase in spectrophotometric absorbance at 280 nm (GeneQuant 1300, GE Healthcare, Illinois, USA). One unit of protease activity was defined as the concentration of the protease required to raise the absorbance value to 1.00 per minute. Protein concentration of the ginger rhizome juice was determined using a BCA assay kit (Takara Bio, Shiga, Japan). The specific protease activity per protein mass (mg) in ginger rhizome juice was calculated as units/mg.

2.4 Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using the Laemmli method under reduced conditions. One volume of SDS-PAGE sample loading buffer (EzApply; ATTO Co., Ltd., Tokyo, Japan) was mixed with one volume of ginger rhizome juice sample were mixed and heated at 100 °C for 5 min. When a protease inhibitor was used, the ginger rhizome juice sample was mixed with cysteine protease inhibitor E-64 (Peptide Institute, Osaka, Japan) at a concentration of 1.8 mM, and then combined with the SDS-PAGE sample loading buffer at a 1:1 ratio, and heated at 100 °C for 5 min. Treated samples of 10 μL were loaded into each well of a precast polyacrylamide gel (12.5 %, Hybrid Gel; Kishida Chemical Co., Ltd., Osaka, Japan). A protein molecular weight marker (EzStandard II; ATTO Co. Ltd., Tokyo, Japan) was used to estimate the molecular weight of the proteins. Electrophoresis was performed at a constant current of 30 mA for 60 min. After electrophoresis, the proteins were stained with Coomassie Brilliant Blue (EzStain AQua; ATTO Co., Ltd., Tokyo, Japan).

2.5 Evaluation of Gel Forming Ability

Gels were prepared by incubating the mixture of 50 mL jersey cow's milk (Jersey Milk Premium 5.0; Hiruzen Dairy Agricultural Cooperatives, Okayama, Japan) or soymilk (Shinozakiya Inc., Saitama, Japan) with 1.5 mL of ginger rhizome juice in a cylindrical container (48 mm diameter) at 60 °C for 60 min. Gel breaking stress was measured using a creep meter (RE2-33005S, Yamaden Co., Tokyo, Japan) with a 20 N load cell. Gels were compressed using a cylindrical plunger (diameter: 16 mm) at 1 mm/s, with clearance set at 50 % of the gel height. Gel breaking stress was determined as the peak force during the first compression.

2.6 Statistical Analysis

The experiments were performed in at least triplicates and the data are presented as mean ± standard deviation. Analysis of variance and Tukey’s tests were performed using R statistical software. Statistical significance for the comparative tests were defined as $p < 0.05$.

3. Results and Discussion

3.1 Specific Protease Activities of the Ginger Rhizome Juices

Figure 1 shows the specific protease activity of juice extracted from ginger rhizomes stored for 2 to 10 months after harvesting. The highest specific protease activity was observed in the ginger rhizomes stored for 4 months after harvest. After 5 months, a decrease in specific protease activity was observed in stored ginger rhizomes. Paull et al. reported no apparent trend in protease activity during storage for up to 32 weeks after harvesting (Paull et al., 1988). In contrast, protease activity was not detected in ginger rhizomes after harvest, but increased one to two months after harvest (Ichikawa, 1988). In addition, Ichikawa suggested that these fluctuations in activity may be influenced by endogenous protease inhibitors, but further detailed research is lacking. The present study suggests that variations in specific protease activity are greatly affected by the storage of ginger rhizomes. An increase in protease activity has also been reported during the postharvest storage period has also been observed in other plant proteases such as kiwifruit actinidine (Afshar-Mohammadian, Rahimi-Koldeh, & Sajedi, 2011; Nam, 2016). Additional biochemical studies are needed to determine the expression of proteases during storage of ginger rhizomes.

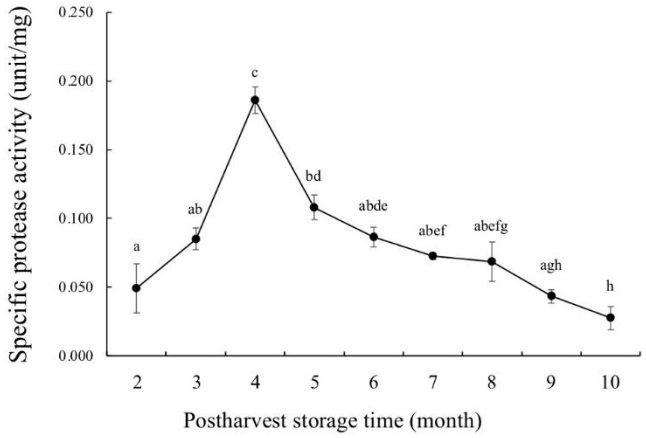

Figure 1. Specific protease activity of juice from postharvest stored ginger rhizomes

Data are expressed as the mean ± SD (n = 3). Different letters express significant differences ($p < 0.05$).

3.2 Protein Profile of the Ginger Rhizome Juices

Figure 2 shows the SDS-PAGE of ginger rhizome juice extracted from stored ginger rhizomes for 2, 4, and 9 months after harvest. Ginger rhizome juice, which contains protease activity, was degraded during SDS-PAGE experiments, which was thought to be due to autolysis by proteases (Fig. 2). However, when the cysteine protease inhibitor E-64 was added, degradation was prevented. When samples containing proteases are subjected to electrophoresis, autolysis can occur during the heat denaturation treatment of the sample with SDS, affecting the accuracy of the results (Itoh, Sato, Moriyama, & Sasaki, 1990). E-64 inhibits the proteolytic activity of the extracts and significantly improves the electrophoretic pattern. Furthermore, juices with higher specific protease activity depicted a thick band around 36 kDa. This protein band was thought to be ginger protease (Adulyatham & Owusu-Apenten, 2005; Hashim et al., 2011).

3.3 Gel Forming Ability of the Ginger Rhizome Juices

Juices squeezed from stored ginger rhizomes were used to investigate the gel forming ability of cow milk and soymilk. Gel formation was observed at 3–5 months of postharvest storage for cow milk and at 4 months for soymilk (Fig. 3). For both cow milk and soymilk gels, the hardest gel formation was obtained from rhizome samples stored for 4 months. This result strongly suggests that gel forming ability is correlated with the degree of specific protease activity in ginger rhizome juice. The hardness of cow milk gel is affected by the type of milk used (Yamada, 2015), and decomposition of glycinin fraction in soy protein is important for soymilk gel (Yamada, Kokean, & Tsumura, 2019).

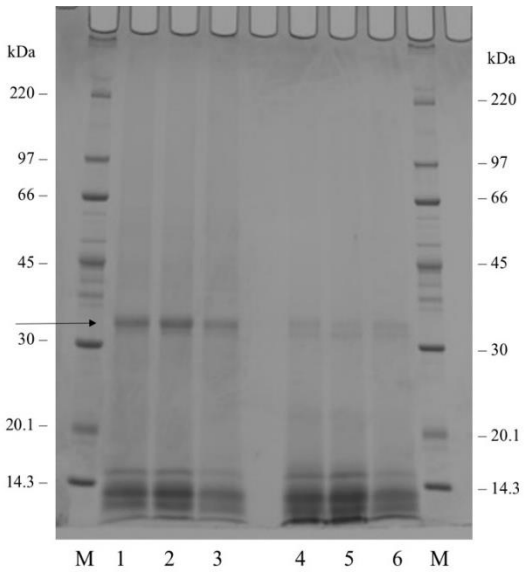

Figure 2. SDS-PAGE of juice from postharvest stored ginger rhizomes

M: molecular weight marker; 1: ginger rhizome juice from 2 months storage mixed with E-64; 2: ginger rhizome juice from 4 months storage mixed with E-64; 3: ginger rhizome juice from 9 months mixed with E-64; 4: ginger rhizome juice from 2 months storage; 5: ginger rhizome juice from 4 months storage; 6: ginger rhizome juice from 9 months storage. The bands indicated by arrow are considered as ginger protease.

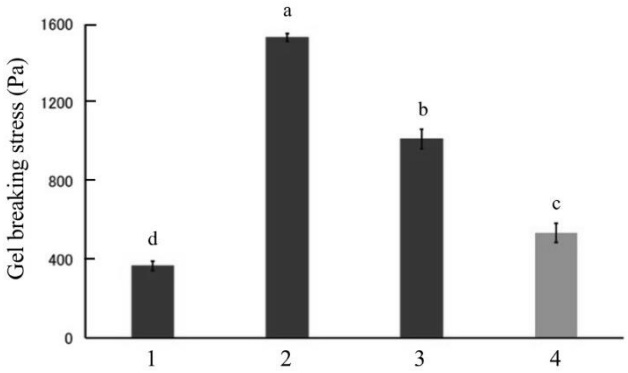

Figure 3. Gel breaking stress of the cow milk gel and soymilk gel induced by juice from postharvest stored ginger rhizomes

1: cow milk gel using juice for 3 months stored ginger rhizome; 2: cow milk gel using juice for 4 months stored ginger rhizome; 3: cow milk gel using juice for 5 months stored ginger rhizome; 4: soymilk gel using juice for 4 months stored ginger rhizome.

Data are expressed as the mean ± SD (n = 5). Different letters express significant differences ($p < 0.05$).

4. Conclusion

The results of this study demonstrate that postharvest storage of ginger rhizomes affect their specific protease activity and gel forming ability. During postharvest storage, ginger rhizomes showed increased specific protease activity in their juices. Juices with high specific protease activity were found to have gel forming ability for cow milk and soymilk. Further research is needed to determine the effect of different types of ginger on gel formation. These results provide information about the industrial application of ginger protease as a clotting enzyme that can be used to prepare plant-based cheeses.

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Authors contributions

Kazunobu Tsumura: Conceptualization, experimentation, data analysis, original draft preparation, and

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Norihiro Yamada: Conceptualization, experimentation, data analysis, and review and editing.

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Competing interests

The authors declare no conflict of interest.

Informed consent

Obtained.

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

References

Adulyatham, P., & Owusu-Apenten, R. (2005). Stabilization and partial purification of a protease from ginger rhizome (Zingiber offinale Roscoe). *Journal of Food Science, 70*(3), C231-C234. https://doi.org/10.1111/j.1365-2621.2005.tb07130.x

Afshar-Mohammadian, M., Rahimi-Koldeh, J., & Sajedi, R. H. (2011). The comparison of protease activity and total protein in three cultivars of kiwifruit of Northern Iran during fruit development. *Acta physiologiae plantarum, 33*, 343-348. https://doi.org/10.1007/s11738-010-0553-3

Bailey-Shaw, Y. A., Williams, L. A., Junor, G. A. O., Green, C. E., Hibbert, S. L., Salmon, C. N., & Smith, A. M. (2008). Changes in the contents of oleoresin and pungent bioactive principles of Jamaican ginger (Zingiber officinale Roscoe.) during maturation. *Journal of agricultural and food chemistry, 56*(14), 5564-5571. https://doi.org/10.1021/jf072782m

Choi, K. H. & Laursen, R. A. (2000). Amino-acid sequence and glycan structures of cysteine proteases with proline specificity from ginger rhizome Zingiber officinale. *European Journal of Biochemistry, 267*(5), 1516-1526. https://doi.org/10.1046/j.1432-1327.2000.01152.x

Ghasemzadeh, A., Jaafar, H. Z., & Rahmat, A. (2016). Changes in antioxidant and antibacterial activities as well as phytochemical constituents associated with ginger storage and polyphenol oxidase activity. *BMC Complementary and Alternative Medicine, 16*, 1-11. https://doi.org/10.1186/s12906-016-1352-1

Hashim, M. M., Mingsheng, D., Iqbal, M. F. & Xiaohong, C. (2011). Ginger rhizome as a potential source of milk coagulating cysteine protease. *Phytochemistry, 72*(6), 458-464. https://doi.org/10.1016/j.phytochem.2010.12.002

Huang, X. W., Chen, L. J., Luo, Y. B., Guo, H. Y. & Ren, F. Z. (2011). Purification, characterization, and milk coagulating properties of ginger proteases. *Journal of Dairy Science, 94*(5), 2259-2269. https://doi.org/10.3168/jds.2010-4024

Hung, D. (2001). Hong Kong sweet by Mrs. Daisy. Tokyo: BUNKA PUBLISHING BUREAU, p.38 (in

Japanese).

Ichikawa, Y. (1988). Nature of proteolytic activity in ginger root–changes in proteolytic activity in different root segments and in varying stages of root maturation. *Seikatsukagakuronsou (Review Liv. Sci.), 20*, 69-78.

Itoh, M., Sato, S., Moriyama, A., & Sasaki, M. (1990). Effective pretreatment by cysteine proteinase inhibitor for improved analysis of protein components of trematodes on SDS-PAGE. *Journal of helminthology, 64*(3), 175-179. https://doi.org/10.1017/S0022149X00012128

Nam, S. H. (2016). Changes in the physicochemical quality, functional properties, and actinidin content of kiwifruit (Actinidia chinensis) during postharvest storage. *Korean Journal of Food Preservation, 23*(3), 291-300. https://doi.org/10.11002/kjfp.2016.23.3.291

Nicosia, F. D., Puglisi, I., Pino, A., Caggia, C., & Randazzo, C. L. (2022). Plant milk-clotting enzymes for cheesemaking. *Foods, 11*(6), 871. https://doi.org/10.3390/foods11060871

Nishimura, K. & Goto, M. (2010). Identification of the protease involved in and the effects of vitamin C on gel formation in ginger milk pudding (Jiang Zhi Zhuang Nai). *Journal of Home Economics of Japan, 61*(8), 463-471. https://doi.org/10.1016/j.compind.2010.01.001

Ohtsuki, K., Taguchi, K., Sato, K. & Kawabata, M. (1995). Purification of ginger proteases by DEAE-Sepharose and isoelectric focusing. *Biochimica et Biophysica Acta (BBA)-General Subjects, 1243*(2), 181-184. https://doi.org/10.1016/0304-4165(94)00145-N

Paull, R. E., Chen, N. J., & Goo, T. T. (1988). Compositional changes in ginger rhizomes during storage. *Journal of the American Society for Horticultural Science, 113*(4), 584-588. https://doi.org/10.21273/JASHS.113.4.584

Shah, M. A., Mir, S. A., & Paray, M. A. (2014). Plant proteases as milk-clotting enzymes in cheesemaking: a review. *Dairy Science & Technology, 94*, 5-16. https://doi.org/10.1007/s13594-013-0144-3

Su, H. P., Huang, M. J. & Wang, H. T. (2009). Characterization of ginger proteases and their potential as a rennin replacement. *Journal of the Science of Food and Agriculture, 89*(7), 1178-1185. https://doi.org/10.1002/jsfa.3572

Thompson, E. H., Wolf, I. D. & Allen, C. E. (1973). Ginger rhizome: A new source of proteolytic enzyme. *Journal of Food Science, 38*(4), 652-655. https://doi.org/10.1111/j.1365-2621.1973.tb02836.x

Troncoso, F. D., Sánchez, D. A., & Ferreira, M. L. (2022). Production of plant proteases and new biotechnological applications: an updated review. *ChemistryOpen, 11*, e202200017. https://doi.org/10.1002/open.202200017

Yamada, N. (2015). Effects of the source and components of milk on the properties of milk gels prepared by the action of ginger juice. *Journal for the Integrated Study of Dietary Habits 26*(2), 79-84. https://doi.org/10.2740/jisdh.26.79

Yamada, N., Kokean, Y., & Tsumura, K. (2019). Novel insights into the formation of soymilk gel induced by ginger rhizome juice. *Food Science and Technology Research, 25*(5), 751-754. https://doi.org/10.3136/fstr.25.751

Yamada, N., Kokean, Y., Umetani, K., Shimizu, J., Kurita, O., & Tsumura, K. (2020). Protease activity and gel-forming ability of ginger rhizome juice. *Journal of Food Research, 9*(2), 30-35. https://doi.org/10.5539/jfr.v9n2p30