

Research Article

Effects of sodium ascorbate and drying temperature on active protease of dried ginger

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Abstract

Ginger is normally used in food as a spice or condiment and it is also used for medicinal purposes. In addition to its flavour and taste, ginger also contains a cystein protease known as Zingibain. To maintain the activity of ginger protease in dried ginger, the drying conditions were studied. Fresh sliced ginger was treated in 0 – 0.2% sodium ascorbate for 5 minutes and then dried in an hot air oven at 40–70°C to gain a moisture content of about 10 % (Wb). The results showed that sodium ascorbate of 0.1 and 0.2% preserved protease activities of 1.26 ± 0.19 and 1.28 ± 0.18 unit/mg protein respectively, compared to untreated 1.00 ± 0.25 unit/mg protein ($p < 0.05$). At the different drying temperatures of 40, 50, 60 and 70°C, the protease activities remaining were 1.05 ± 0.29 , 1.35 ± 0.11 , 1.36 ± 0.14 and 0.99 ± 0.09 unit/mg protein respectively ($p < 0.05$). After storing the dried ginger powder (through 18 mesh sieve) under vacuum conditions at room temperature and 4°C for 6 months, the protease activity was preserved approximately 67.5 and 77.3 %. The optimal condition to prepare dried ginger was by adding 0.1% ascorbate to sliced ginger and drying in a hot air dryer at 50°C.

Keywords: *Zingiber officinale*, protein, protease, proteolysis, Zingibain, Thailand

Introduction

Ginger (*Zingiber officinale* Roscoe) is a monocotyledon belonging to the family Zingiberaceae. Ginger cultivation is one of economical importance and it is now grown in all regions of Thailand. The rhizome is the part that is normally utilized as a spice or herb for food and medicinal purposes. Both fresh and dried ginger (powder) is widely used for culinary purposes, such as in gingerbread, biscuits, cakes, puddings, soups and pickles. It is also a standard constituent of curry powder or spices in Asian cooking. For medicinal purposes it is known as a carminative stimulator, counter-irritant and digestive aid [1, 2]. Ginger rhizome also contains some proteolytic enzymes known as Zingibain or ginger protease [3, 4, 5, 6].

Many of the proteolytic enzymes of plant origin such as papain, bromelain, cucumin and ficin have been most extensively studied [7, 8]. However, this study with ginger rhizome investigated a new source of plant proteolytic enzymes that is of importance to food products. The ginger protease is a thiol protease with an optimum temperature of 60°C. Rapid denaturation of the enzyme occurs at 70°C [6], and the optimum pH of activity is 5.0-7.0 [4, 5].

The objective of this work is to study the effect of ascorbate and drying temperature on dried ginger powder containing active protease.

Materials and Methods

Fresh ginger (*Zingiber officinale* Roscoe) was purchased from a local market in Songkhla province. All chemicals used were analytical grade, unless otherwise stated.

Optimal process for producing dried ginger powder containing active protease

Ginger was weighed, washed, peeled and sliced (2 mm thickness) then sodium ascorbate was added at 0, 0.1 and 0.2%, respectively. Sliced ginger was dried with a tray drier at different temperatures of 40, 50, 60 and 70°C until moisture content was less than 10% (wet basis). Ground dried sliced ginger was reduced to powder through 18 mesh sieve. Moisture content, activity and specific activity of protease was determined according to Arima *et al.* [9]. Protein content was determined by a modified Bradford's method [10]. The optimal process was chosen by consideration between activity and specific activity of protease.

Protease activity changes in dried ginger powder containing active protease during storage

Dried ginger powder was packed and stored in nylon/polyethylene bags under vacuum conditions at different temperatures, room temperature and 4°C. During storage, moisture content and water activity were determined. Activity and specific activity of protease and protein content were determined using methods from previous experimental work [3].

Statistical analysis

The experiment was designed as a factorial (3x4) in completely randomized design and completely randomized block design. All data were analyzed by using repeated measurement of the ANOVA procedure. Means were separated by Duncan's New Multiple Range Tests.

Results and Discussion

Optimal process conditions

Figure 1 shows the relationship between temperature and ascorbate dosage in the dried ginger process on specific activity of protease and protein content. The results indicate that different temperature and ascorbate dosage affects the specific activity of protease. With increasing temperature, specific activity of protease also tends to increase. The results showed that the addition of sodium ascorbate of 0.1 and 0.2% preserved protease activities of 1.26 ± 0.19 and 1.28 ± 0.18 unit/mg protein, respectively, compared to untreated ginger of 1.00 ± 0.25 unit/mg protein ($p < 0.05$), as shown in Table 1. At the different drying temperatures of 40, 50, 60 and 70°C, the remaining protease activity was 1.05 ± 0.29 , 1.35 ± 0.11 , 1.36 ± 0.14 and 0.99 ± 0.09 unit/mg protein, respectively ($p < 0.05$). However, at 60°C, the results showed a decrease in protein content and specific activity, due to the denaturation of protein at higher temperature. With the addition of ascorbate, the specific activity of protease did not differ significantly ($p > 0.05$). However, both conditions were also significantly higher than dried ginger powder process without ascorbate added. These results are similar to the findings of Adulyatham, [3], who reported that the protease activity of ginger extract with ascorbate (0.1-1.0% solution) was more than the control (no ascorbate added).

The enzymatic browning process starts with the initial enzymatic oxidation of phenols to quinones by the polyphenoloxidase (PPO) in the presence of oxygen. The quinones are subjected to further reactions, leading to the formation of brown pigment [11]. In addition, protease activity decreases because of the enzyme-phenol reaction [12, 13]. Ascorbate is an effective reducing compound most often used to inhibit the PPO activity [14]. It reduces the O-quinone to limit the browning through a process known as reaction deactivation [15]. Furthermore, ascorbate can reduce O_2 , ascorbate is oxidized to dehydroascorbate, preventing PPO activity. As a result, ascorbate was demonstrated to be most effective both as a preservative and as an enhancer of ginger protease activity. When considering activity and specific activity of enzyme protease of dried ginger powder process, a combination of 50°C temperature and the addition of 0.1% ascorbate, seems to offer the best potential alternative for further study.

Table 1. Effect of sodium ascorbate and drying temperature on protease activities in dried ginger powder.

% Sodium ascorbate	Drying Temp. (°C)	Moisture Content (% Dry basis)	Protein content (mg/g dry basis)	Total activity (unit/g dry basis)	Specific activity (unit/mg protein)
0.0		10.28±0.11	71.75±1.01	72.06±18.48	1.00±0.25 a
0.1		10.40±0.11	71.64±1.49	90.16±13.74	1.26±0.19 b
0.2		10.40±0.16	71.41±0.66	91.77±12.99	1.28±0.18 b
	40	10.41±0.16	71.29±1.01	74.80±21.58	1.05±0.29 x
	50	10.49±0.06	71.02±0.99	95.57±6.61	1.35±0.11 y
	60	10.24±0.05	72.60±0.74	97.8±9.77	1.36±0.14 y
	70	10.27±0.08	71.47±0.97	70.47±6.07	0.99±0.09 x

The different superscripts in the same column (a,b and x,y) denote significant difference ($p < 0.05$)

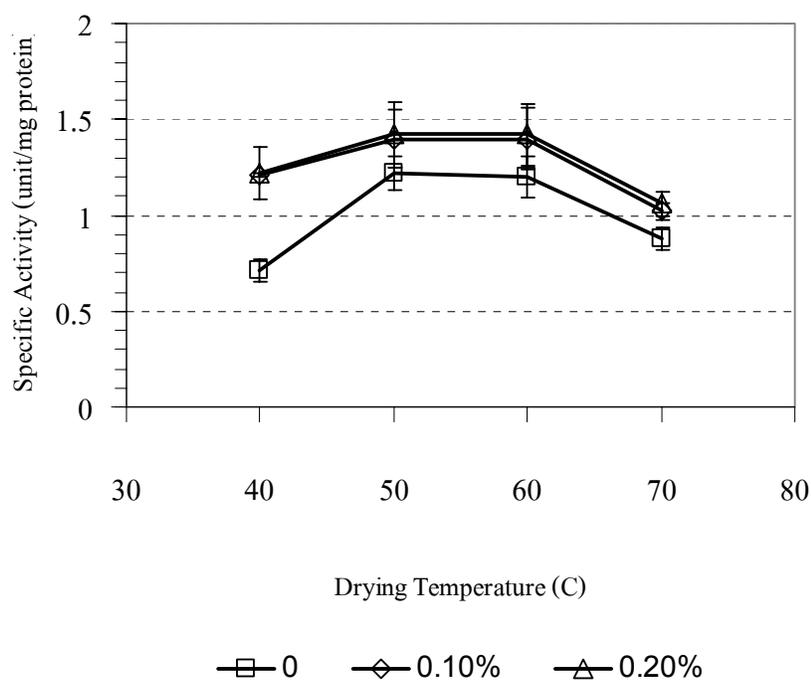


Figure 1. Temperature-specific activity profile for dried ginger powder with the addition of 0, 0.1 and 0.2% ascorbate.

Protease activity changes during storage

Figure 2 shows the relationship between storage time and A_w in dried ginger powder. The results indicate that A_w did not differ significantly ($p > 0.05$) with increasing storage time. The A_w of ginger powder at both storage conditions was about 0.42-0.47. At this A_w range, most of the microorganism growth is suppressed. Figure 3 shows the relationship between storage time and protease specific activity in dried ginger powder. The results indicate that storage time does have an effect on protease specific activity. With increasing storage time, protease specific activity tended to decrease. The protease specific activity of dried ginger powder was investigated for up to 24 weeks storage at room temperature and at 4°C. The protease specific activity decreased with about 67.5% and 77.3% remaining at end of a 24 week period under storage at room temperature and 4°C respectively. Estimation for a 50% remainder was about 32 and 45 weeks storage at room temperature and 4°C, respectively (Figure 3). These results are similar to the findings of Adulyatham, [3], who reported that the protease activity of ginger acetone powder was investigated for up to 15 weeks storage at 5°C and the activity was about 80% remaining.

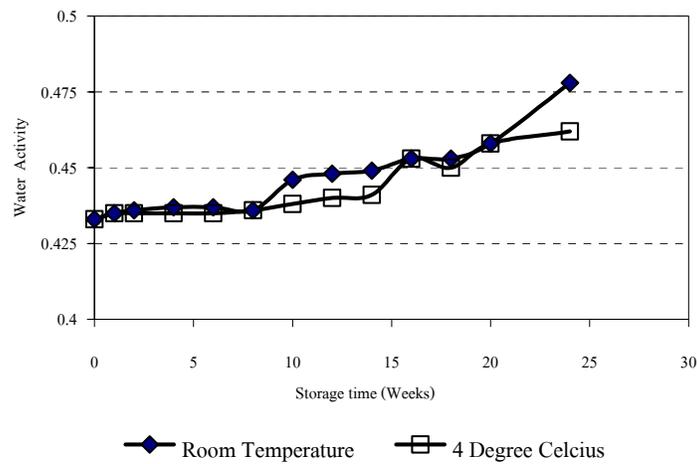


Figure 2. Aw of dried ginger powder stored at different levels of temperature.
Letters show significant differences of mean ($p < 0.05$)

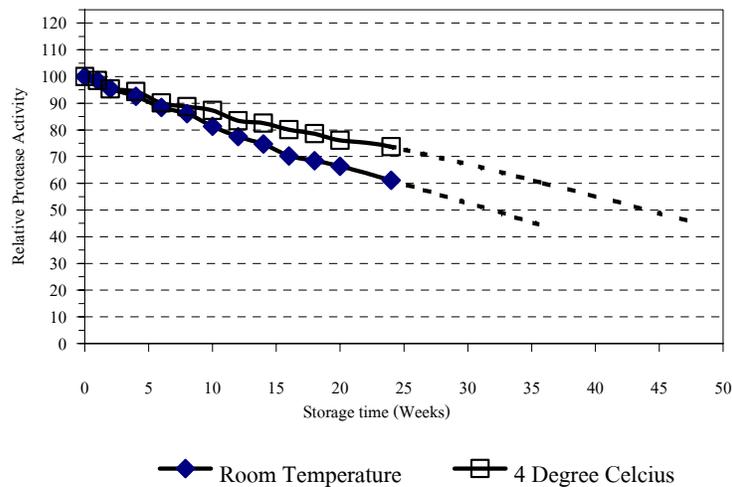


Figure 3. Protease specific activity of dried ginger powder stored at different levels of temperature.

Conclusions

Technical preparation of dried ginger powder containing active protease was studied. Dried ginger powder using mature ginger was prepared after water washed fresh mature ginger was peeled, sliced into 2 mm. slices, added with 0.1% ascorbate, and dried at 50⁰C for 5 hours. During storage of dried ginger powder at room temperature and 4⁰C for 24 weeks, the protease activity and specific activity of dried ginger powder were investigated. The protease specific activity decreased to about 66.5 and 77.3% after storage at room temperature and 4⁰C respectively.

The dried ginger powder used in this study can be applied in food processing such as meat tenderization or directly used as a ginger tea. The possible use as an additive to liquid protein food such as milk or soup should be further studied.

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