Note

Distribution and Some Properties of Amylase in Spices

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Amylase activity of 42 kinds of spices was assayed. Capsicum pepper, Cumin seed, Chinese pepper, Fennel seed, Paprika, and Ajowan had a high amylase activity. That of Capsicum pepper and Cumin seed was particularly high due to the starch degrading enzymes α - and β -amylase, as determined from the results of viscosity measurements, reducing sugar assays and TLC-analysis of the hydrolysate. We also examined temperature and pH optima for the amylase activity of Capsicum pepper and Cumin seed.

Keywords: spice, amylase

Their many functional properties have resulted spices having a long history of use in a wide range of foods prepared around the world. They have been used to enhance appetite by adding their characteristic aroma, pungency, or color to food, and have also been used effectively to mask unpleasant odor in fish and meat. The use of spices as food ingredients along with their various properties have attracted the interest of numerous food scientists, and literature on their aromatic components, pungent principles, pigments, antioxidant activity, and antimicrobial activity is abundant.

Among the various enzyme activities in spices reported are myrosinase (Ohtsuru & Kawatani, 1979), known to be involved in the development of the pungent flavor of mustard, horseradish, and wasabi; alliinase (Mazelis & Crews, 1968) plays an essential role in the flavor formation of onion and garlic; phosphatase (Ishii *et al.*, 1979), found in red pepper, thyme and sage, decreases potency of a certain class of flavor enhancers such as 5'-ribonucleotides by hydrolyzation. Protease (Ichikawa *et al.*, 1973) in ginger is known to increase meat tenderness. Amylase activity in spices, however has not been investigated.

In the present study, 42 spices were analyzed for their activity, which may have significant influence on the rheology of starchy foods.

Materials and Methods

Materials All 42 spices used were collected and stored whole. They were ground with dry-ice in a caking machine and then passed through a 710- μ m sieve before use. Commercial preparations of "amylase AD (amano)" (Amano Pharmaceutical Industry, Aichi) and " β -amylase #1500 S" (Nagase Seikagakukougyo Co., Osaka) were used as the enzyme standards. Maltose (G_2), maltotriose (G_3), maltotetraose (G_4), maltopentaose (G_5), maltohexaose (G_6) and maltoheptaose (G_7) were of commercial grade.

Preparation of crude enzyme Fifteen grams of powdered spices were homogenized with 100 ml of 0.04 M acetate buffer, pH 5.6, containing 1 mM $CaCl_2$. The homogenate was centrifuged at 10,000 rpm for 10 min. The supernatant was

made 0.6 saturation with ammonium sulfate, and after pH adjustment to 5.6, kept in an ice bath for 30 min with continuous stirring. The precipitate was collected by centrifugation and dissolved in a small volume of the same buffer. The sample was dialyzed against the same buffer to completely remove ammonium sulfate. The resulting supernatant was used as the crude enzyme solution.

Measurement of amylase activity The reaction mixture comprised of 1 ml of 1% soluble starch and 1 ml of enzyme solution (Okada *et al.*, 1985) was incubated for 20 min at 60°C, and then rapidly heated at 100°C for 10 min to stop the enzyme reaction. The concentration of reducing sugars in the reaction mixture was determined by the method of Somogyi (1952). The same enzyme solution heated at 100°C for 10 min for denaturation was used as the control. One unit of enzyme activity was defined as the amount of enzyme that would yield 1 μ mol of maltose per min under these assay conditions.

Measurement of viscosity The viscosity was measured with a Brabender amylograph. A slurry of wheat starch (9% dry-solid) was prepared with distilled water and heated in the amylograph (rotary speed, 75 rpm; measuring head, 500cp). The starch was gelatinized by heating the slurry from room temperature to 50°C within 7 min, and then to 95°C at a rate of 5°C/min. The slurry was then cooled to 60°C at a rate of 2°C/min before 10 ml of the crude enzyme solution prepared above was added. The enzyme was allowed to act on the gelatinized starch at 60°C for 30 min and the decrease in viscosity was monitored.

Measurement of reducing sugars At intervals, 4 g of the reaction mixture was periodically removed from the amylograph, and immediately heated to 100°C for 10 min to stop the enzyme reaction. Three milliliter of distilled water and 14 ml of acetonitrile were added to 3 g of the reaction mixture to extract reducing sugars formed as a result of enzyme action. After centrifugation at 10,000 rpm for 10 min, the resulting supernatant was evaporated under reduced pressure, and the residue was dissolved in distilled water to make a final volume of 5 ml. The reducing sugars were

determined according to the method of Somogyi.

Isolation and identification of oligosaccharides by thin-layer chromatography Oligosaccharides formed through the enzyme action were extracted as described above. After evaporation, the resulting residue was dissolved in a small amount of distilled water and analyzed by thin-layer chromatography (TLC) using silica gel (Merck) and a solvent system consisting of isopropyl alcohol, acetone and 1 M lactic acid (4:4:2 by v/v/v). The spots on the chromatograms were detected with 50% sulfuric acid.

Results and Discussion

Distribution of amylase activity in various spices Amylase activity of crude enzyme solutions obtained from the 42 spices is summarized in Table 1. Relatively high enzyme activity was found in Capsicum pepper, Cumin seed, Chinese pepper, Fennel seed, Paprika, and Ajowan, all of which belong to one three families; Solanaceae, Rutaceae, or Umbelliferae.

Table 1. Distribution of amylase activity in various spices.

Family	Common name	Harvested country	Amylase activity (units/g of spice)
Labiatae	Savory	Spain	1.25
	Basil	France	0.78
	Marjoram	India	0.73
	Oregano	Greece	0.35
	Sage	Yugoslavia	0.54
	Thyme	Spain	0.56
	Rosemary	France	0.35
	Peppermint	Egypt	0.37
	Spearmint	Egypt	0.43
	Capsicum pepper	China	1.96
Solanaceae	Paprika	Spain	1.49
	White sesame	Guatemala	0.29
Pedaliaceae	Black sesame	Vietnam	0.37
Compositae	Tarragon	U.S.A.	0.37
Piperaceae	White pepper	Malaysia	0.87
	Black pepper	Malaysia	0.29
	Hihatsu	Japan	0.18
Myristicaceae	Nutmeg	Indonesia	0.24
	Mace	Papua New Guinea	0.24
Lauraceae	Laurel	Turkey	0.21
	Cinnamon	Sri Lanka	0.10
	Cassia	China	0.35
Magnoliaceae	Star anise	China	0.26
Cruciferae	Yellow mustard	Canada	0.98
	Horseradish	China	0.54
Rutaceae	Japanese pepper	Japan	0.29
	Mandarin	Japan	0.84
	Chinese pepper	China	1.63
Myrtaceae	Clove	Malagasy	0.21
	Allspice	Mexico	0.18
Umbelliferae	Dill seed	India	0.29
	Celery seed	India	0.78
	Caraway seed	Holland	0.95
	Coriander seed	Morocco	0.56
	Cumin seed	Iran	1.72
	Fennel seed	China	1.50
	Parsley	U.S.A.	0.35
	Anise seed	Spain	1.47
	Ajowan	India	1.30
	Cardamom	India	0.26
Zingiberaceae	Ginger	China	0.24
	Turmeric	China	0.29

It is known that α -amylase plays an important role in seed germination, hydrolysis of raw starch in the seed, as well as maturation of fruits (Sakano, 1989a). In this study, we found high amylase activity in spices that were from the seed or fruit part of the plant.

Naturally, the amylase activity of spices is expected to be greatly influenced by such factors as varietal difference, place and season of harvest, drying methods and storage conditions. Therefore, we were interested in how the amylase activity varied by the varietal difference and the place of harvest, and used Capsicum pepper, which showed the highest enzyme activity of all the spices studied. The results are shown in Table 2. Amylase activity differed depending on the varietal difference, and tentaka, a variety of Capsicum annum, showed the highest activity.

Characterization of amylase in Capsicum pepper and Cumin seed Using the crude enzyme solution from Capsicum pepper and Cumin seed, we studied the time course of the change in viscosity, as well as the increase in reducing sugars, to determine whether the amylase activity was due to α -amylase or β -amylase. As shown in Fig. 1, the enzyme from Capsicum pepper caused a marked decrease in the viscosity during the first 15 min, and a linear increase in reducing sugars throughout the 30 min of reaction time. The enzyme from Cumin seed caused a similar decrease in the viscosity, but the increase of reducing sugars was less pronounced.

We also studied the effect of amylase activity on the viscosity and amount of reducing sugars using a commercial preparation of α -amylase and β -amylase. When gelatinized wheat starch was digested with α -amylase, a rapid decrease in viscosity but only a small increase in reducing sugars were observed. On the contrary, when β -amylase was used, the decrease in viscosity was small, but there was a large and linear increase in reducing sugars.

We next isolated and identified oligosaccharides formed by the action of amylase using TLC. As shown in Fig. 2, amylase from Capsicum pepper and Cumin seed produced oligosaccharides of varying length, including maltose (G_2). As with the enzyme from spices, commercial α -amylase yielded oligosaccharides comprising more than two glucose units, while only maltose could be detected when commercial

Table 2. Comparison of amylase activity in Capsicum peppers by the difference in variety or the harvested country of origin.

Species	Cultivar	Harvested country	Amylase activity (unit/g of spice)
Capsicum annum			
	Unnan-daishyo	China	0.29
	Unnan-shyoshyo	China	0.38
	Boukyou	China	0.71
	Reiryuu	China	0.07
	Seito	China	0.26
	Tentak (1st)	China	1.96
	Tentak (2nd-3rd)	China	1.25
	Tochigi-Tentak	Japan	0.99
	Samman chilluies	India	0.64
Capsicum frutescens			
	Bird' seye	China	0.38

1st; first grade, 2nd-3rd; second-third grade.

Fig. 1. Changes in the viscosity and reducing sugars content of wheat starch solution hydrolyzed by crude amylase of Capsicum pepper and Cumin seed. Wheat starch solution (500 g; 9% solid), gelatinized by heating to 95°C, was hydrolyzed with 10 ml crude enzyme from Capsicum pepper and Cumin seed at 60°C for 30 min in a Brabender amylograph to monitor viscosity change. At intervals, 4 g of mixture was taken from the vessel and analyzed for reducing sugar content as described in Materials and Methods. ● viscosity; ○ reducing sugars.

Fig. 2. Thin-layer chromatogram of oligosaccharides produced by amylases of spices and of commercial source. Oligosaccharides formed through the enzyme action at 60°C for 20 or 30 min in a Brabender amylograph were extracted. Lane 1, commercial α -Amylase; lane 2, commercial β -Amylase; lane 3, crude enzyme from Cumin seed; lane 4, crude enzyme from Capsicum pepper; ST, glucose (G_1)–maltoheptaose (G_7). TLC analysis was performed as described in Materials and Methods.

β -amylase was used. The results of similar analyses performed using HPLC agreed with those of the TLC (data not shown).

Judging from these data obtained, we concluded that the starch degrading enzymes α - and β -amylase were present in Capsicum pepper and Cumin seed.

Properties of crude amylase from Capsicum pepper and Cumin seed We examined temperature and pH optima for the amylase activity of Capsicum pepper and Cumin seed. The highest initial reaction rate of the enzyme was observed at 60°C and 70°C for Capsicum pepper, and at 50°C and 60°C for Cumin seed; the optimum temperature for the amylase activity of Capsicum pepper was thus slightly higher. Both enzymes were stable up to about 70°C. Heating at 90°C for 15 min may be necessary to inactivate the enzyme

and prevent viscosity loss if Capsicum pepper or Cumin seed is to be added to starchy foods.

Optimum pH for the amylase activity, as measured by the increase in reducing sugars, was at pH 5 for Capsicum pepper, and between pH 5 and 6 for Cumin seed. Both enzymes were stable between pH 5 and 7. The optimal pH for α -amylase of plant origin is said to be in the range between 4.5 and 6.0, while that for β -amylase is on the slightly more acidic side and is usually found in the range between 4.0 and 5.6 (Sakano, 1989b). Thus, the optimal pH obtained here for the amylase activity of Capsicum pepper and Cumin seed seemed to be in good agreement with these values.

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