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Amylolytic and proteolytic enzyme activities of plant and fungal foods for the flavor enhancement

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Abstract

All fresh food ingredients, including plants and fungi, contain active enzymes. While the enzymes' activity sometimes causes food deterioration, the appropriate control of enzymes can improve the functional and sensory properties of the foods. Shio-koji, a seasoning from rice digested by fungal enzymes, has become popular in Japan recently since it is rich in digestive enzymes and is believed to improve the taste of the foodstuff during cooking. It is expected that both the enzyme activities of the ingredients from plants and shio-koji act complementally during cooking to efficiently change the components in foodstuffs. This article reviews the amylolytic and proteolytic enzyme activities of plant and fungal foods to provide information for establishing the enzyme-based cooking techniques by utilizing the enzymatic activities of the food ingredients themselves.

Keywords: Amylase; Protease; Aminopeptidase; Vegetable; Fruit; Edible fungi; Shio-koji

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Introduction

Plant and fungal foods, such as vegetables, fruits, and edible fungi, are essential sources of numerous biologically active and health-promoting compounds [1,2]. The food's nutritional quality can be improved with proper cooking techniques, such as steaming, roasting, and boiling [3-5]. All food ingredients contain enzymes. Some food enzymes, including oxidases, promote the oxidation of phenolic compounds, undesirable change of color, and destruction of vitamins [6-8]. These undesirable effects can be prevented by inactivating the responsible enzymes by the thermal processes of cooking [9,10].

On the other hand, many digestive enzymes, including amylolytic and proteolytic enzymes, in fresh foods have received much attention for their beneficial roles in cooking or food processing. For instance, some proteases have industrial applications. This review focuses on the properties of the plant and fungal enzymes that contribute to establishing appropriate cooking methods for flavor enhancement.

The Usage of Plant Enzymes in Cooking

The enzymatic properties of pineapple, papaya, kiwifruit, and ginger proteases during meat tenderization processes have been well-studied [11]. It has been established that proteases from kiwi and pineapple act on various substrates in foods, such as soy, milk, rice, egg, fish, and chicken, to produce peptides with specific bioactivities, such as anti-oxidation and ACE inhibition [12]. Therefore, foods with a high protease activity are expected to not only enhance flavor but also provide health benefits. Applying food enzymes to industrial food processing and culinary sciences promotes gastronomy [13]. This review focuses on the properties of the plant and fungal enzymes that contribute to establishing appropriate cooking methods for flavor enhancement.

The Utilization of Microbial Enzymes in Japanese Fermented Seasoning

Microbial enzymes also have been used to produce or prepare food for centuries due to their ability to produce enzymes with diverse activities [14]. For example, filamentous fungi, such as the Penicillium genus, are used for proteolysis and lipolysis during the making of blue cheese and camembert cheese [15]. On the other hand, Koji, a starter culture for traditional Japanese fermented foods, such as sake and miso, is rich in hydrolytic enzymes produced by a fungus, Aspergillus oryzae [16]. The traditional Japanese fermentation technology relies on the hydrolysis of raw food components by koji's enzyme activity. Recent research has investigated the effect of seasoning with salted koji, known as shio-koji, on cooking [17]. Shio-koji contains abundant enzymes, including amylolytic enzymes such as α-amylase, glucoamylase, α-glucosidase, and proteolytic enzymes, such as protein-degrading enzyme and acid carboxypeptidase [17-19].

The commercial shio-koji products have various enzyme activities (**Table 1**) [17]. Shio-koji products with the addition of 2% of alcohol as an additive have higher residual enzyme activities than those without alcohol. Since shio-koji is a seasoning that contains the amylolytic and proteolytic enzyme activities of A. oryzae, it is expected to act on food ingredients and increase their taste and aroma. Marinating pork with shio-koji before cooking doubles the total amount of released amino acid and glutamic acid contents compared to those in the unmarinated pork [19].

Cooking methods and thermal conditions affect the taste of individual vegetables [20]. Enzymes can improve or modify the functional and sensory properties of food and its components [13]. Although enzymes have been broadly applied to industrial food processing and production [13], their involvement in improving the sensory aspects of cooked foods has not been investigated. Characterizing the amylolytic and proteolytic enzymes in plants or fungal food materials will help establish enzyme-based cooking techniques.

Amylolytic and Proteolytic Enzymes of Vegetables, Fruits, and Fungi

Vegetables, fruits, and edible fungi contain enzymes with amylolytic and proteolytic activities (**Tables 2 and 3**) [21]. The Starch Degrading (SD) activity of most vegetables, fruits, and edible fungi is higher at pH 5 than pH 3. Root vegetables exhibit higher SD activity than other vegetables, fruits, and edible fungi. Glucoamylase (GA) activity is not detected in most plant and fungal foods. The proteolytic, or Protein-Degrading (PD), activity is high in fruits and edible fungi; however, it is mostly undetectable in leaf vegetables at pH 3. Tuberous vegetables exhibit high Acid Carboxypeptidase (ACP) activity. Vegetables, fruits, and edible fungi also contain Leucyl-P-nitroanilide hydrolyzing (LAP) and the Glutamyl-P-nitroanilide hydrolyzing (GAP) activities (Table 3). While LAP activity was not particularly high at pH 3 among vegetables, fruits show almost no LAP or GAP activity.

Table 1: The enzyme activities of commercial shio-koji products.

Product	PD (U/g)		ACP	AA	GA	AG
	pH 6	pH 3	(U/g)	(U/g)	(U/g)	(U/g)
Shio-koji (alc-added)*	123.6 ± 37.8	137.7 ± 53.8	0.96 ± 0.34	14.7 ± 3.3	0.25 ± 0.08	0.05 ± 0.01
Shio-koji**	29.2 ± 17.1	21.7 ± 14.2	0.30 ± 0.16	6.5 ± 4.6	0.09 ± 0.06	0.03 ± 0.01

*PD: Protein-Degrading activity, ACP: Acid Carboxypeptidase activity, AA: α-Amylase Activity, GA: Glucoamylase Activity, AG: α-glucosidase activity. **The average of six samples with alcohol as additives. **The average of eight samples without the addition of alcohol is not added. This data is based on the results by Maehashi et al [17].

Table 2: The amylolytic activities of vegetables, fruits, and edible fungi.

Samples	Scientific name	Activity				
		SD (U/g)		AA (mU/g)	AG (mU/g)	GA (mU/g)
		pH 3.0	pH 5.0	pH 5.0	pH 5.0	pH 5.0
Leafy vegetables:						
Coriander	Coriandrum sativum L.	21.47 ± 2.03*	32.73 ± 1.29	3.00 ± 0.00	2.69 ± 0.66	26.88 ± 4.91
Shungiku	Chrysanthemum coronarium	4.64 ± 0.59*	26.04 ± 0.81	nd	15.31 ± 2.32	nd
Spinach	Spinacia oleracea	7.96 ± 1.92	9.14 ± 1.41	26.15 ± 6.15	54.61 ± 2.11	nd
Mizuna	Brassica rapa L. var. nipposinica	<0.00*	33.72 ± 7.03	11.64 ± 3.57	9.70 ± 1.01	nd
Cabbage	Brassica oleracea var. capitata,	5.53 ± 1.20	10.58 ± 2.33	23.04 ± 0.41	5.85 ± 0.53	nd
Stalk and stem vegetables:						
Asparagus	Asparagus officinalis,	0.43 ± 0.11	0.40 ± 0.11	105.30 ± 5.73	20.03 ± 3.05	nd
Flower head vegetables:						
Broccoli	Brassica oleracea var. Italica	0.40 ± 0.05	0.78 ± 0.22	25.05 ± 6.44	13.86 ± 1.01	nd
Sprouts of legume vegetables:						
Pea sprouts	Pisum sativum	0.10 ± 0.01	0.09 ± 0.01	0.14 ± 0.02	0.01 ± 0.00	0.06 ± 0.01
Tuberous vegetables:						
Potato	Solanum tuberosum	6.71 ± 2.14*	20.06 ± 1.13	12.28 ± 5.48	9.06 ± 0.67	nd
Burdock	Ipomoea batatas,	3.32 ± 0.94*	19.05 ± 3.21	86.22 ± 11.67	8.11 ± 0.00	41.43 ± 0.07
Sweet potato	Arctium lappa	70.77 ± 9.07*	95.21 ± 6.51	1192.96 ± 2.32	10.39 ± 0.28	nd
Japanese radish	Raphanus sativus L	199.26 ± 24.41*	987.91 ± 29.35	6.56 ± 1.76	4.91 ± 0.64	nd
Carrot	Daucus carota subsp. sativus	153.50 ± 36.63*	258.65 ± 30.21	6.67 ± 1.50	1.97 ± 0.05	nd

Fruiting vegetables:						
Cucumber	Cucumis sativus	11.81 ± 2.33	22.63 ± 4.57	11.49 ± 1.68	6.48 ± 0.82	nd
Fruits:						
Kiwifruit	Actinidia deliciosa	13.52 ± 2.77*	32.08 ± 8.09	13.07 ± 2.89	2.10 ± 0.39	7.65 ± 3.74
Pineapple	Ananas comosus	28.09 ± 6.31*	46.86 ± 6.16	23.51 ± 1.68	nd	nd
Banana	Musa spp.	23.19 ± 3.93	37.42 ± 5.53	53.46 ± 5.99	8.01 ± 0.38	2.71 ± 2.70
Edible fungi:						
Enokitake	Flammulina velutipes	0.92 ± 0.17*	15.14 ± 0.44	2.53 ± 0.68	91.95 ± 2.64	nd
Shiitake	Lentinula edodes	14.14 ± 1.37*	6.49 ± 0.50	15.29 ± 2.30	203.83 ± 11.51	nd
Maitake	Grifola frondosa	24.77 ± 1.66	34.44 ± 5.86	12.47 ± 3.50	255.46 ± 5.33	nd

*SD: starch-degrading, AA: α-amylase, AG: α-glucosidase, GA: glucoamylase. nd: not detected. Values are mean ± SE (n=3-9). *Superscripts indicate significance at p<0.05 between pH3 and pH5 of SD activity. All values are given as per gram of material. This table is made from the result by Oosone et al. [21]

Table 3: Proteolytic enzyme activities of vegetables, fruits, and edible fungi.

Samples	Activity						
	PD (U/g)		ACP (mU/g)	LAP (mU/g)		GAP (mU/g)	
	pH 3.0	pH 5.0	pH 3.0	pH 3.0	pH 6.0	pH 3.0	pH 6.0
Leafy vegetables:							
Coriander	0.38 ± 0.38	nd	16.00 ± 3.00	5.64 ± 1.04	4.03 ± 0.07	0.89 ± 0.13*	0.01 ± 0.01
Shungiku	nd*	0.95 ± 0.10	17.26 ± 1.78	10.94 ± 1.62*	2.73 ± 0.57	0.78 ± 0.20*	0.21 ± 0.09
Spinach	0.28 ± 0.28*	3.01 ± 0.47	19.21 ± 3.13	9.07 ± 2.55*	365.27 ± 21.69	4.08 ± 0.45*	7.05 ± 0.36
Mizuna	nd*	1.57 ± 0.32	39.31 ± 10.60	3.62 ± 0.24 *	21.01 ± 3.14	3.84 ± 0.39	16.05 ± 5.19
Cabbage	nd	1.85 ± 0.51	101.76 ± 6.30	0.10 ± 0.03*	2.53 ± 0.18	0.08 ± 0.00	0.09 ± 0.04
Stalk and stem vegetables:							
Asparagus	20.59 ± 1.58*	nd	23.28 ± 6.25	0.72 ± 0.21*	26.74 ± 0.56	0.67 ± 0.04*	2.55 ± 0.02
Flower head vegetables:							
Broccoli	15.17 ± 0.44*	nd	794.04 ± 8.00	9.79 ± 0.24*	125.27 ± 2.25	19.13 ± 8.07	6.48 ± 2.78
Sprouts of legume vegetables:							
Pea sprout	nd*	0.83 ± 0.32	50.31 ± 1.00	nd*	863.44 ± 8.54	nd*	23.20 ± 0.33
Tuberous vegetables:							
Potato	0.67 ± 0.20	1.13 ± 0.38	47.69 ± 1.85	9.56 ± 3.42	1.10 ± 0.03	0.75 ± 0.17*	0.43 ± 0.05
Burdock	0.71 ± 0.24	0.92 ± 0.19	39.02 ± 3.13	11.45 ± 2.47	8.15 ± 7.89	0.64 ± 0.28	1.13 ± 0.03
Japanese radish	0.55 ± 0.13	0.93 ± 0.36	43.15 ± 2.18	6.14 ± 4.65*	28.02 ± 0.76	3.19 ± 0.25	2.63 ± 0.03
Carrot	0.71 ± 0.15*	0.07 ± 0.01	64.93 ± 28.48	6.45 ± 1.21*	49.76 ± 0.72	4.76 ± 0.24*	2.13 ± 0.28
Fruiting vegetables:							
Cucumber	nd*	0.30 ± 0.09	<0.00	0.73 ± 0.03*	89.10 ± 1.61	nd*	2.18 ± 0.23
Fruits:							
Kiwifruit	10.26 ± 0.78	10.80 ± 0.38	7.62 ± 2.54	nd	nd	0.04 ± 0.01	0.14 ± 0.04
Pineapple	19.58 ± 1.34*	9.76 ± 0.84	27.33 ± 1.15	0.15 ± 0.02*	0.97 ± 0.02	0.29 ± 0.01*	2.46 ± 0.04
Banana	3.35 ± 0.65	2.21 ± 0.62	1.81 ± 1.71	1.98 ± 0.39*	0.76 ± 0.17	2.84 ± 0.04*	1.10 ± 0.14
Edible fungi:							
Enokitake	0.85 ± 0.20*	nd	2.61 ± 0.83	7.49 ± 0.94*	85.59 ± 0.81	12.06 ± 3.81	18.93 ± 0.77
Shiitake	13.01 ± 1.94*	9.05 ± 2.03	7.71 ± 2.31	1.10 ± 0.25*	78.54 ± 0.17	0.08 ± 0.08*	13.70 ± 1.21
Maitake	9.38 ± 0.07*	3.84 ± 0.16	255.39 ± 4.40	0.01 ± 0.00*	0.04 ± 0.00	6.37 ± 0.38	7.68 ± 0.66

PD: Protein-Degrading, ACP: Acid Carboxypeptidase, LAP: Leucyl-P-nitroanilide-hydrolyzing aminopeptidase, GAP: Glutamyl-P-nitroanilide-hydrolyzing aminopeptidase, nd: not detected.
Values are mean ± SE (n=3 – 9). *Superscripts indicate significance at p<0.05 between pH3 and pH6 of the same enzyme. All values are given as per gram of material. This table is made from the result by Oosone et al. [21]

Proteolytic activities in food ingredient are expected to produce some taste-active compounds such as amino acids and peptides during cooking. In addition, endopeptidases have the potential to produce specific bioactive peptides [22]. It has been established that proteases from kiwi and pineapple act on various protein substrates in foods, producing peptides with specific bioactivities, such as antioxidant and ACE inhibitory effects [23].

The Differential Enzymatic Activities in Vegetables

Leafy vegetables

Coriander: High GA activity is detected. SD activity is relatively high at both pH 3 and 5 among leafy vegetables. Proteolytic activities are not high in any enzymes.

Shungiku: Shungiku (garland) is a popular green vegetable used in sukiyaki and similar dishes in Japan [24]. LAP activity is relatively high at pH 3. GAP activity is low but relatively higher at pH 3 than pH 6.

Spinach: AG activity is relatively high among vegetables and fruits. The highest PD activity at pH 5 among vegetables and the highest LAP activity at pH 6 among leafy vegetables are observed. LAP activity is low up to pH 5, but the highest activity is recorded at pH 6. GAP activity is highest at pH 4 and works well in the acidic region between pH 3 and 6.

Mizuna: ACP activity is relatively high among leafy vegetables. GAP activity is relatively high at pH 6 among vegetables. A recent transcriptome analysis of mizuna revealed a strong positive correlation between sucrose and anthocyanins, suggesting the positive effect of sucrose on anthocyanin biosynthesis [25].

Cabbage: ACP activity is high, but GAP activity is low. Aminopeptidase with phenylalanyl- and leucyl-pNA hydrolyzing activities has been isolated from cabbage and partially characterized. [26].

Stalk and Stem Vegetables

Asparagus: AA activity is remarkably higher than other vegetables. AG activity is also high among vegetables. But SD activity is low. High protease activity is observed at pH 3 in the result of Oosone et al. [21], shown in Table 3. However, Ha et al. [27] reported that asparagus extract exhibited caseinolytic activity over the pH range of 4.5–8.5. The LAP activity is low up to pH 5 but high at pH 6 and above, with the highest activity observed at pH 7–8. [21]. The maximum LAP activity is 1.3-fold higher compared with that at pH 6. GAP activity is high in the neutral region, peaking at pH 7 [21].

Flower Head Vegetable

According to Sun et al. [28] broccoli's PD activity is higher at pH 3 than at pH 7.5. Broccoli reportedly contains acidic metalloproteases, serine proteases, and cysteine proteases [29], but the properties of these proteases are unknown. Broccoli has remarkably high ACP activity and high LAP and GAP activity at pH 6. Its GAP activity is relatively higher among other vegetables at pH 3. Marinova and Tchorbanov [30] also reported higher aminopeptidase activity in broccoli among Brassicaceae vegetables.

Legume Vegetables

Pea sprout exhibits remarkably high LAP and GAP activity. Its GAP activity is relatively high at pH 6. Pea sprout's GAP activity is high in neutral pH, peaking at pH 7.

A study of the partially purified enzymes from pea spout reports that LAP activity is low at pH 5 or lower, but at pH above 9, it increases to a maximum that is 3-fold higher than that at pH 6 [21]. Ellema [31] reported that pea contained three aminopeptidases, one of which exhibits leucyl β-naphtylamide-hydrolyzing activity at the optimal pH of 7. Pyrzyna et al. [32] also reported a pea aminopeptidase with a primary phenyalanyl-β NA hydrolyzing activity and a secondary LAP activity that peaked at pH 7.5.

Tuberous vegetables

Potato: ACP activity is high. LAP activity is relatively high at pH 6. The aminopeptidases specific to the leucyl-residue have been purified from potato, and it exhibits the highest LAP activity at pH 9 [33].

Sweet potato: AA activity is remarkably high, and SD activity is relatively high at pH 3 and 5. PD activity is relatively high, and ACP activity is relatively low among tuberous vegetables [21].

Root vegetables

Burdock: The main carbohydrates of burdock root are fructooligosaccharides, which enzymatically change during storage [34]. GA activity is higher than other vegetables. ACP activity is also high. LAP activity is high at pH 3, although PD activity is low at pH 3.

Japanese radish: SD activity is remarkably high at pH 5 and still high even at pH 3, contrary to low AA activity. There is a report of purification and purification of β-amylase from radish root [35]. ACP activity is relatively high. LAP activity is higher at pH 6 than pH 3.

Carrot: SD activity is remarkably high at pH 5 and even at 3, but AA activity is not high. ACP activity is relatively high. LAP activity is high at pH 6 among root vegetables. GAP activity is high at pH 3 than pH 6.

Fruit vegetables

Cucumber: Like vegetables, fruits have high AG activity. For cucumber, although it has high LAP activity at pH 6, it's PD and GA activities are barely detectable. An alanyl-specific aminopeptidase has been purified from cucumber leaves and identified as a plant aminopeptidase N [36].

The differential enzymatic activities in vegetables fruits

Pineapple: Pineapple is known to have high PD activity due to bromelain, and its the use of bromelain in industry has been established [11,37]. Pineapple's PD activity is high at pH 3, consistent with Sun et al. [28]. Also, an aspartic acid protease gene of pineapple is cloned and characterized [38,39].

Kiwifruits: Kiwifruit's PD activity is high at both pH 3 and 5. Also, kiwifruit's SD activity is higher at pH 6 than at pH 3. Its GA activity is relatively high. In contrast, its LAP activity is not detected, and its GAP activity is weakly detected.

The differential enzymatic activities in edible fungi

Enokitake: LAP and GAP activities are high at pH 6. The LAP activity is also low up to pH 5 but is high at pH 6 and above, with the highest activity observed at pH 7–8. The highest GAP activity is observed at pH 4–6 and works well in the acidic region. GAP shows high activity in the acidic region of pH 4–7.

Shiitake: Shiitake is one of the most popular edible fungi in Japan and can be preserved by drying and is then used as a stock in Japanese cuisine. The enzymes in dried shiitake react with RNA to produce 5'-ribonucleotides, which provide an umami taste upon rehydration [39]. Tatsumi et al. [40] reported that shiitake has polysaccharide-degrading enzymes that produce sugars from boiled potato tuber specimens. AG activity is relatively high among vegetables and edible fungi. Shiitake shows high PD activity and low LAP activity at pH 3, but high LAP activity at pH 6.

Maitake: PD activity is high at pH 3. Since maitake shows high PD activity at pH 3, it is expected to contribute to the tenderization of meat and the release of amino acids in the cooking of high protein foods such as meat and fish using vinegar. ACP activity is the highest among the three edible fungi in Table 3. Aminopeptidases specific to the peptides with leucyl-residue have been purified from maitake [41].

Conclusion

Vegetables, fruits, and edible fungi contain amylolytic and proteolytic enzymes. Although most of the enzymes have much lower activity than shio-koji, several vegetables and edible fungi exhibited higher aminopeptidase activity. When foodstuffs are dipped or marinated in shio-koji, which retains koji's enzyme activities, for cooking, the amylolytic and proteolytic enzymes act to impart a "Japanese-like” flavor profile. It is suggested that the enzymes of food materials and shio-koji act synergistically to produce complex tastes and aromas that enhance food flavor. The information on the enzymatic aspects of food ingredients would help develop enzyme-based cooking techniques that utilize their specific flavor characteristics.

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