

Effect of Pectolytic Enzyme Preparations on the Phenolic Composition and Antioxidant Activity of Asparagus Juice

TING SUN,[†] JUMING TANG,[§] AND JOSEPH R. POWERS^{*,†}

Department of Food Science and Human Nutrition, Washington State University,
 Pullman, Washington 99164-6376, and Department of Biological Systems Engineering,
 Washington State University, Pullman, Washington 99164-6120

Commercial pectolytic enzymes were investigated for their influence on phenolics and antioxidant activities of asparagus juice. The antioxidant activity of asparagus juice was analyzed according to 2,2'-diphenyl-1-picrylhydrazyl and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) methods. The enzymes, with the exception of pectinase from *Rhizopus* sp., contained rutinase, which hydrolyzed rutin to quercetin. Asparagus juice treated with Viscozyme had the highest quercetin content without exhibiting a significant increase in the antioxidant activity. For a pectinase from *Aspergillus niger*, the antioxidant activity of asparagus juice was markedly reduced. Caution should be paid in the selection of pectolytic enzyme preparations for production of antioxidant activity-rich juice.

KEYWORDS: Asparagus juice; pectolytic enzyme preparation; rutinase; antioxidant activity; rutin; quercetin

INTRODUCTION

Asparagus (*Asparagus officinalis* L.) is a nutritious and healthy vegetable containing ascorbic acid, vitamin B₆, folic acid, rutin, saponin, and glutathione (1). The antioxidant activity of asparagus was the greatest among 23 vegetables analyzed according to the method of inhibition of low-density lipoprotein oxidation and one of the greatest among 43 vegetables tested by using the β -carotene bleaching method (2, 3). Antioxidants in asparagus can protect human beings against diseases, such as cancer and cardio- and cerebrovascular diseases. The major antioxidant of asparagus has been reported to be rutin (3).

Asparagus lignifies and deteriorates quickly after harvest. To extend the shelf life of asparagus and maintain its nutrients, fresh asparagus can be frozen, canned, or pickled. A significant amount of wholesome asparagus is rejected due to cosmetic defects. One of the major needs for the asparagus industry at the present time is the development of new processed asparagus products to utilize the waste asparagus. One utilization of such asparagus could be in the beverage industry. A portion of asparagus waste could be made into juice and marketed as an antioxidant-rich product. Asparagus juice is a relatively new product in the United States, although it has been a commercial product in Asia. To produce juices, pectolytic enzymes with the ability to degrade pectic substances in plants are commonly used to increase the yield and improve the liquefaction, clarification and filterability of juice (4). During liquefaction, cell walls of fruits or vegetables are degraded to release the

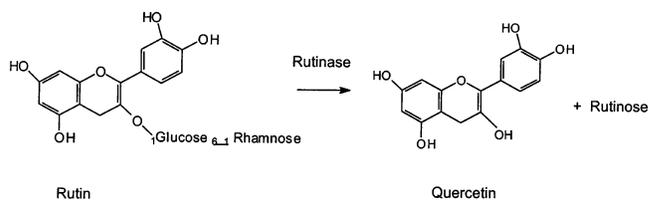


Figure 1. Cleavage of glycoside from rutin to form quercetin by rutinase.

cell contents. Pectolytic enzymes are crude fungal preparation containing impurities, such as extraneous enzymes, mucilage, and melanoidins (5, 6). Side activities have been found in these crude enzymes, such as hemicellulases (arabinases, arabinogalactanases, and cellulases), glucosidase (β -glucosidase, β -1,2-glucosidase, α -L-arabinofuranosidase, and α -L-rhamnopyranosidase), and esterases (tannase and chlorogenase) (7). β -Glucosidase in commercial pectolytic enzyme preparations can hydrolyze the glycoside of anthocyanins and cause pigment loss of strawberry juice (8). These enzymes also contained glucuronidase-type activity, which can hydrolyze quercetin glucuronide, leaving the quercetin aglycon. Some enzymes have an esterase activity with the ability to hydrolyze the tartaric ester of caffeic acid, rendering free caffeic acid (9). The effect of crude pectolytic enzyme preparations on the phenolics of asparagus juice has not been reported. Rutinase has been purified from tartary buckwheat seeds (10, 11), which can cleave the glycoside of rutin to form quercetin (Figure 1). Rutin showed lower antioxidant activity than quercetin as measured by various methods (12–14). The main objective of our research work was to investigate the presence of rutinase in commercially available pectolytic enzymes and their effects on the phenolic composition and antioxidant activity of asparagus juice.

* Corresponding author [e-mail powersjr@wsu.edu; telephone (509) 335-5634; fax (509) 3335-4815].

[†] Department of Food Science and Human Nutrition.

[§] Department of Biological Systems Engineering.

Table 1. Source and Polygalacturonase Activity (PG) of the Pectolytic Enzyme Preparations ($n = 3$)

enzyme	source	PG activity ^a (units/mL)	PG activity ^a (units/mg of protein)
Crystalzyme	Valley Research Inc.	124.2 ± 3.8c	2.62 ± 0.08f
high-strength pectinase	InterSpex	88.7 ± 6.3e	2.66 ± 0.19f
super-blend pectinase	InterSpex	97.5 ± 8.0de	3.34 ± 0.27e
Rhozyme	InterSpex	30.0 ± 2.6g	5.49 ± 0.48c
Viscozyme	Novo Nordisk	172.5 ± 7.2a	4.43 ± 0.18d
pectinase from <i>A. aculeatus</i>	Sigma	155.0 ± 11.0b	8.60 ± 0.61b
pectinase from <i>A. niger</i>	Sigma	115.0 ± 2.8d	4.87 ± 0.12cd
pectinase from <i>Rhizopus</i> sp.	Sigma	51.0 ± 2.0f	20.33 ± 0.80a

^a ANOVA to compare data in each column: data sharing the same letter were not significantly different ($P = 0.05$). One unit of activity means 1 μ mol of reducing groups produced per minute. Rhozyme and pectinase from *Rhizopus* sp. were powder and prepared in 10% (w/v) solution.

MATERIALS AND METHODS

Chemicals. Rutin, quercetin, ABTS, DPPH, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), potassium persulfate, polygalacturonic acid, and 2-cyanoacetamide were of analytical grade and supplied by Sigma-Aldrich (St. Louis, MO). Sodium acetate (analytical grade), acetonitrile, and acetic acid (HPLC grade) were purchased from Fisher Scientific (Springfield, NJ). Coomassie Brilliant Blue concentrate was purchased from Bio-Rad Laboratories (Hercules, CA). Pectolytic enzyme preparations were purchased from different sources as listed in Table 1.

Preparation of Asparagus Juice. Asparagus (*A. officinalis* L.) was purchased from a local supermarket and blanched in 10 volumes of water (w/v) in a water bath (Precision Scientific Co., Chicago, IL) at 90 °C for 2 min to inactivate enzymes. Blanched asparagus was macerated to produce juice using a model 1738 domestic juicer (Braun). Crude juice was centrifuged using a Beckman J2-HS centrifuge (Beckman, Palo Alto, CA) at 26712g for 15 min, and the clear supernatant was stored at -20 °C for future experiments.

Polygalacturonase (PG) Activity Analysis. The PG activity of the pectolytic enzyme preparations was analyzed (15). The reaction solution (2 mL) with 37.5 mM sodium acetate (pH 4.4) and 0.2% polygalacturonic acid was mixed with 25 μ L of enzyme solution and incubated at 30 °C for up to 30 min. One milliliter of cold 100 mM borate buffer (pH 9.0) was added to the solution to terminate the reaction. To quantify the released reducing groups, 0.2 mL of 1% 2-cyanoacetamide was added and the mixture was incubated in boiling water for 10 min (15). After the solution had cooled to 25 °C, the absorbance of the solution was determined at 276 nm with an Ultrospec 4000 UV-visible spectrophotometer (Pharmacia Biotech, Cambridge, U.K.). Galacturonic acid (10–100 μ M) was used to prepare a standard curve. One unit of PG activity was defined as 1 μ mol of reducing groups produced per minute. The protein content of the pectolytic enzyme preparations was determined (16). Coomassie Brilliant Blue concentrate was diluted by 4 times with water, 1 mL of which was mixed with 20 μ L of enzyme solution. The absorbance of the solution was measured at 595 nm after 10 min of incubation. Bovine serum albumin in 0.15 M NaCl solution was used to construct a standard curve. The PG activity of pectolytic enzyme preparations was calculated on the basis of the protein content.

Determination of Rutinase Activity of Pectolytic Enzyme Preparations. Aliquots of enzyme solution equivalent to 3.4 units of PG activity were added to 2 mL of 160 μ M rutin solution with 0.4% sodium acetate and 20% methanol at pH 5.0. The mixture was incubated at 37 °C for 5, 10, or 20 min to determine the produced quercetin content and evaluate its rutinase activity. One unit of rutinase activity was defined as 1 μ mol of quercetin produced per minute under standard assay conditions (10).

Incubation of Pectolytic Enzyme Preparations with Asparagus Juice. Pectolytic enzyme preparations were added at 6.9 units of PG activity to 4 mL of asparagus juice, which was incubated in capped glass vials in a water bath shaker at 37 °C. At 0.5, 1, 2, 4, 8, 16, and 22 h, 0.4 mL of asparagus juice was removed and added with 1.2 mL of methanol to inactivate the added enzyme. Control was asparagus juice incubated at the same condition without the added pectolytic enzyme.

HPLC Analysis. Concentrations of rutin and quercetin in asparagus juice were determined using an Agilent 1100 HPLC (Palo Alto, CA) equipped with a quaternary pump, vacuum degasser, thermostatic column compartment, and diode array detector. A Vydac 201TP C18 (50 mm × 4.6 mm i.d., 5 μ m particle size) guard column (Columbia, MD) and a Vydac 202TP54 C18 column (250 mm × 4.6 mm i.d., 5 μ m particle size) (Columbia, MD) were used and maintained at 40 °C during analysis. External standards of rutin and quercetin (0.01–1 mg/mL) were used to prepare the standard curves. Isocratic elution with 5% acetic acid, 24% acetonitrile, and 71% water was used. The running time of analysis for each sample was 25 min. The sample injection volume was 25 μ L, the flow rate of HPLC was 0.5 mL/min, and the detector was set at 280 and 360 nm.

Measurement of Antioxidant Activity of Asparagus Juice. For the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay, aliquots of diluted asparagus juice were added to 1 mL of DPPH solution and the absorbance of the DPPH solution was determined at 515 nm after 30 min of incubation (17). Trolox was used as a reference to compare the antioxidant activity of asparagus juice. The inhibition percentage of the absorbance of DPPH solution added with sample was calculated using the following equation:

$$\text{inhibition \%} = (\text{Abs}_{t=0} - \text{Abs}_{t=30\text{min}}) / \text{Abs}_{t=0} \times 100$$

$\text{Abs}_{t=0\text{min}}$ was the absorbance of DPPH at time 0, and $\text{Abs}_{t=30\text{min}}$ was the absorbance of DPPH after 30 min of incubation.

The reduction of the absorbance was plotted against the amount of sample tested to draw a regression line. The ratio between the sample and Trolox's regression line was calculated and expressed as the Trolox equivalent antioxidant activity (TEAC). For the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, the blue/green ABTS⁺ free radical was produced from the reaction of ABTS and potassium persulfate (18). Aliquots of diluted asparagus juice were added to 1 mL of ABTS solution, and absorbance was determined at 734 nm after 10 min of incubation. The inhibition percentage of absorbance and the TEAC of asparagus juice were calculated in the same way as described for the DPPH method.

The antioxidant activities of pure rutin and quercetin were analyzed. Rutin (330 μ M) and quercetin (590 μ M) were prepared in methanol. Aliquots of the standard solutions were added to 1 mL of DPPH or ABTS solution to determine their antioxidant activity as described previously. TEAC was the concentration of antioxidant giving the same reduction of absorbance of the free radical as 1 mM Trolox.

Analysis of Total Phenolic Content. Total phenolic content of asparagus juice was analyzed according to the Folin-Ciocalteu method (19). Concentrated Folin-Ciocalteu reagent was diluted by 10 times with water. Asparagus juice (0.1 mL) was mixed with 0.75 mL of diluted Folin-Ciocalteu reagent and incubated for 5 min at room temperature, and 0.75 mL of 2% sodium carbonate solution was added. After incubation for 15 min at room temperature, the absorbance of the solution was determined at 750 nm. Catechin was used to prepare the standard curve.

Statistical Analysis. Enzyme analysis, such as PG activity and rutinase activity, and the experiments on Viscozyme and pectinase from *Aspergillus niger*, were performed in triplicate. The average values and

Table 2. Rutinase Activity of Pectolytic Enzyme Preparations Analyzed in Rutin Buffer ($n = 3$)

enzyme	rutinase activity ^a (units/mL)	rutinase activity ^a (units/mg of protein)
Crystalzyme	0.018 ± 0.003e	0.005 ± 0.001e
high-strength pectinase	0.023 ± 0.000d	0.004 ± 0.000f
super-blend pectinase	0.065 ± 0.006c	0.013 ± 0.001c
Rhozyme	0.006 ± 0.000f	0.010 ± 0.000d
Viscozyme	0.226 ± 0.015a	0.081 ± 0.005a
pectinase from <i>A. aculeatus</i>	0.081 ± 0.005b	0.026 ± 0.002b
pectinase from <i>A. niger</i>	0.020 ± 0.001e	0.005 ± 0.000d
pectinase from <i>Rhizopus</i> sp.	nd	nd

^a ANOVA to compare data ($P = 0.05$): data sharing the same letter were not significantly different. One unit of rutinase activity is defined as 1 μ mol of quercetin produced per minute under standard assay conditions. Rhozyme and Pectinase from *Rhizopus* sp. were powder and prepared in 10% (w/v) solution.

standard deviations were calculated using Excel (Microsoft Inc., Redmond, WA). The data were analyzed by one-way ANOVA and multiple comparisons (Fisher's least-significant-difference test) to evaluate the significant difference at $P < 0.05$ (20). The statistical calculations were done using SYSTAT (Systat Software Inc., Point Richmond, CA).

RESULTS AND DISCUSSION

PG Activity. The pectolytic enzyme preparations showed various PG activities (Table 1). The variability in the activities was due to the source of the enzyme and the purity as supplied by the manufacturers. The rank of the PG activities of the enzyme preparations differed when compared on the basis of volume and protein content. Pectinase from *Rhizopus* sp. showed the largest PG activity, 20.3 units/mg of protein. On the basis of volume, Viscozyme showed the greatest PG activity, 172.5 ± 7.2 units/mL.

Rutinase Activity. The pectolytic enzyme preparation with the exception of pectinase from *Rhizopus* sp. showed rutinase activity. Our finding is the first report of rutinase activity in pectolytic enzyme preparations. The rutinase activity of the enzyme preparations ranged from 0.006 ± 0.000 to 0.226 ± 0.015 unit/mL or from 0.005 ± 0.000 to 0.081 ± 0.005 unit/mg of protein. On the basis of both volume and protein content, Viscozyme showed the greatest rutinase activity, 3 times greater than that of the second greatest, pectinase from *Aspergillus aculeatus* (Table 2).

Effect of Pectolytic Enzyme Preparations on Rutin and Quercetin Contents of Asparagus Juice. Asparagus juice contained 460 μ M rutin and undetectable quercetin, which agrees with previous research (21). The amount of quercetin in the enzyme-treated juices varied with time of incubation. Rutin content did not change apparently with time for the control (Figure 2). Viscozyme, super-blend pectinase, and pectinase from *A. aculeatus* showed a trend of increasing concentration of quercetin (230–390 μ M). These three pectolytic enzyme preparations also demonstrated the greatest rutinase activity when tested in the rutin buffer (Table 2). Rhozyme, Crystalzyme, and high-strength pectinase showed a moderate ability to produce quercetin (90–150 μ M). Pectinase from *A. niger* showed little rutinase activity, and pectinase from *Rhizopus* sp. showed no detectable rutinase activity. Viscozyme showed the greatest rutinase activity, and the loss of rutin and formation of quercetin was rapid with 220 μ M rutin and 180 μ M quercetin at the fourth hour. After 22 h, the rutin content decreased to 80 μ M and the quercetin content increased to 370 μ M. The rates of rutin loss and quercetin formation were similar for super-

Table 3. Rutin and Quercetin Content of Asparagus Juice with Time for Control, Viscozyme, or Pectinase from *A. niger* Incubated at 37 °C ($n = 3$)

time (h)	control	Viscozyme		pectinase from <i>A. niger</i>	
	rutin ^a (μ M)	rutin ^a (μ M)	quercetin ^a (μ M)	rutin (μ M)	quercetin ^a (μ M)
0	257 ± 3b	257 ± 3a	0f	257 ± 3a	nd
0.5	265 ± 5ab,x	217 ± 3b,z	30 ± 1e	230 ± 3b,y	nd
1	256 ± 8b,x	181 ± 2c,y	60 ± 2d	105 ± 6c,z	nd
2	253 ± 7b,x	130 ± 1d,y	144 ± 5c	5 ± 1d,z	nd
4	273 ± 4a,x	98 ± 1e,y	191 ± 5b	0e,z	nd
8	268 ± 3a,x	42 ± 1f,y	261 ± 10a	0e,z	nd

^a ANOVA to compare data ($P = 0.05$): data sharing the same letter in columns (a–f) and rows (x–z) were not significantly different. nd, not detected.

blend pectinase, Viscozyme, and pectinase from *A. aculeatus*, suggesting that rutin was mainly hydrolyzed to quercetin. For high-strength pectinase, the rate of rutin loss was 3 times greater than that of quercetin formation, indicating that the high-strength pectinase preparation may have two or more distinct activities with different rates on rutin and quercetin. Pectinase from *Rhizopus* sp. did not change the rutin content in asparagus juice without rutinase activity. Most of the rutin in asparagus juice was degraded by pectinase from *A. niger* with a small amount of quercetin observed only in the first 2 h of incubation. Viscozyme and pectinase from *A. niger* treated asparagus juice showed interesting results on the change of rutin concentration, and the experiments on the two enzymes were repeated again to confirm the preliminary results (Table 3). For the control, the rutin content in asparagus juice did not change significantly. Viscozyme decreased the rutin in asparagus juice by 50% after 2 h and by 85% after 8 h of incubation; quercetin content was significantly increased by a similar rate. For asparagus juice treated with pectinase from *A. niger*, the rutin content was decreased to zero mostly in the first 2 h.

Antioxidant Activity of Asparagus Juice Affected by Pectolytic Enzyme Preparations. Taking Trolox as a reference, the relative antioxidant activities of quercetin and rutin standards were 3.27 ± 0.12 and 1.61 ± 0.10 , respectively, as measured by the DPPH method, and 3.93 ± 0.09 and 1.39 ± 0.16 , respectively, as analyzed by the ABTS method. That the antioxidant activity of quercetin is greater than that of rutin is in agreement with others' work (12, 13).

Enzyme treatment showed various effects on the antioxidant activity of asparagus juice. Measured according to the DPPH method, the antioxidant activity of asparagus juice did not show apparent change with time for Crystalzyme, super-blend pectinase, Viscozyme, and pectinase from *A. aculeatus* (Figure 3). For these enzymes, the rates of rutin loss and quercetin formation in asparagus juice were similar. However, for high-strength pectinase, Rhozyme, and pectinase from *A. niger*, asparagus juice showed an apparent decrease of antioxidant activity compared to control. For these enzymes, the rate of rutin loss in asparagus juice was higher than that of quercetin formation, which could be one reason for the decrease of antioxidant activity in asparagus juice. Similar results for the antioxidant activity of asparagus juice were obtained using the ABTS assay, except for pectinase from *Rhizopus* sp. treated asparagus juice, which showed no apparent change of antioxidant activity by the ABTS method but an apparent decrease of antioxidant activity after 8 h by the DPPH method (Figures 3 and 4). Although quercetin has a higher antioxidant activity than rutin, transferring rutin to quercetin by the pectolytic enzyme preparations did not effectively increase the antioxidant activity of

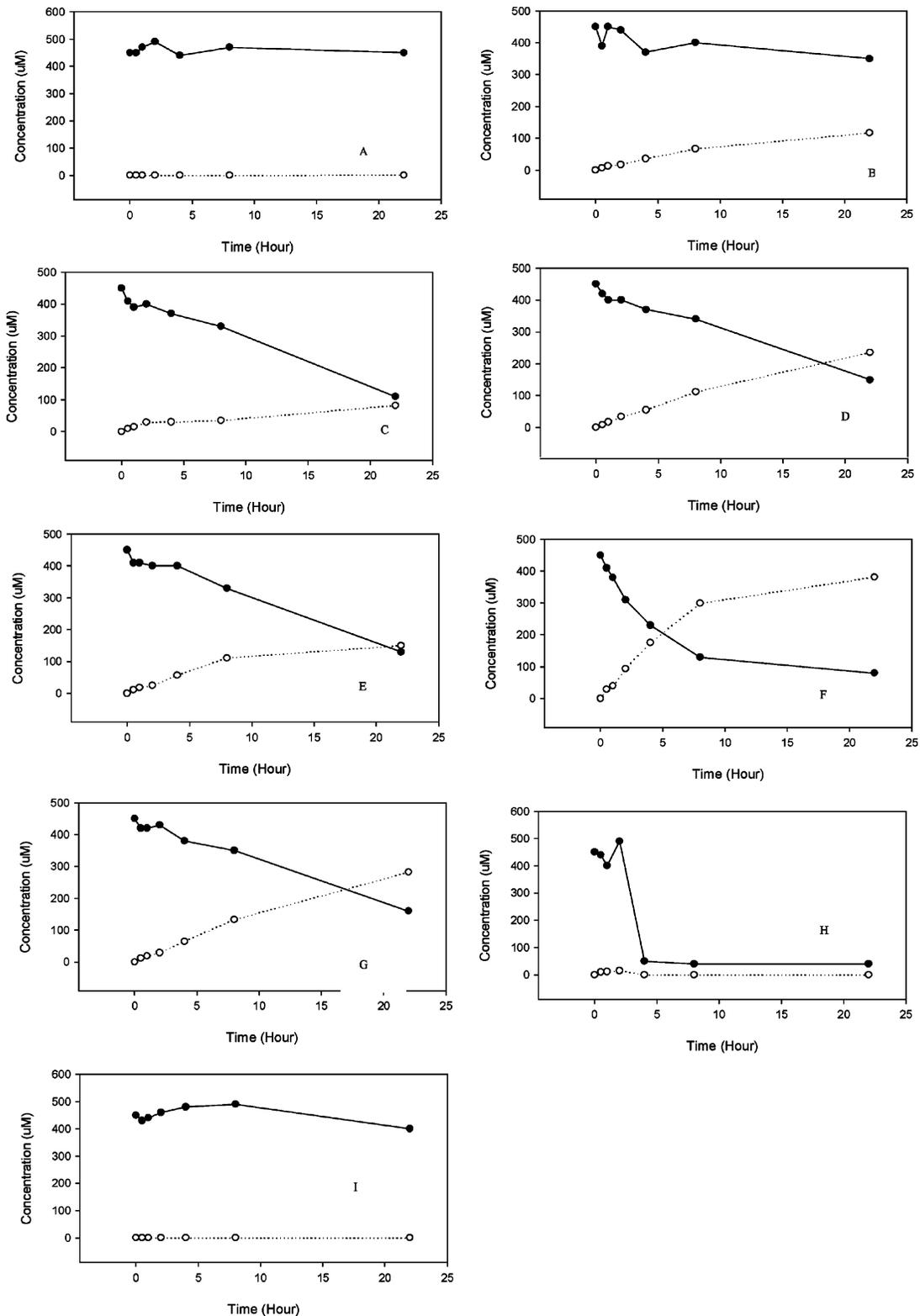


Figure 2. Rutin (●) and quercetin (○) content of asparagus juice incubated with pectolytic enzymes at 37 °C with time: (A) control; (B) Crystalzyme; (C) high-strength pectinase; (D) super-blend pectinase; (E) Rhozyme; (F) Viscozyme; (G) pectinase from *A. aculeatus*; (H) pectinase from *A. niger*; (I) pectinase from *Rhizopus* sp.

asparagus juice as determined by both ABTS and DPPH methods. This phenomenon could be explained as follows: asparagus juice contained other antioxidants in addition to rutin, and the contribution of rutin to the total antioxidant activity of asparagus juice may not be great enough to show a significant increase of antioxidant activity when rutin is converted to quercetin; the synergism among the antioxidants in asparagus

juice make the antioxidant activity dependent on the concentration of antioxidant, the structure and interaction among the antioxidants, making the difference in the antioxidant activity of quercetin and rutin less important in the total antioxidant activity. The antioxidant activity of treated asparagus juice was examined (Tables 4 and 5). Except at the second hour, there was no significant change of antioxidant activity in control

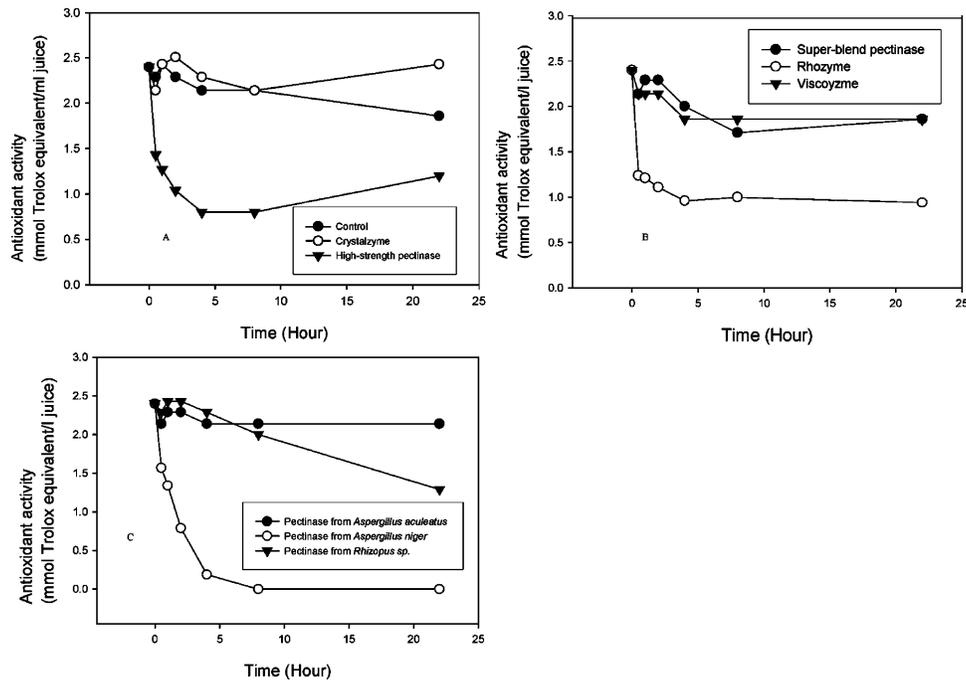


Figure 3. Antioxidant activity (mmol of Trolox equiv/L of juice) of asparagus juice incubated with pectolytic enzymes at 37 °C with time analyzed by DPPH method: (A) control, Crystalzyme, and high-strength pectinase; (B) super-blend pectinase, Rhozyme, and Viscozyme; (C) pectinase from *A. aculeatus*, pectinase from *A. niger*, and pectinase from *Rhizopus sp.*

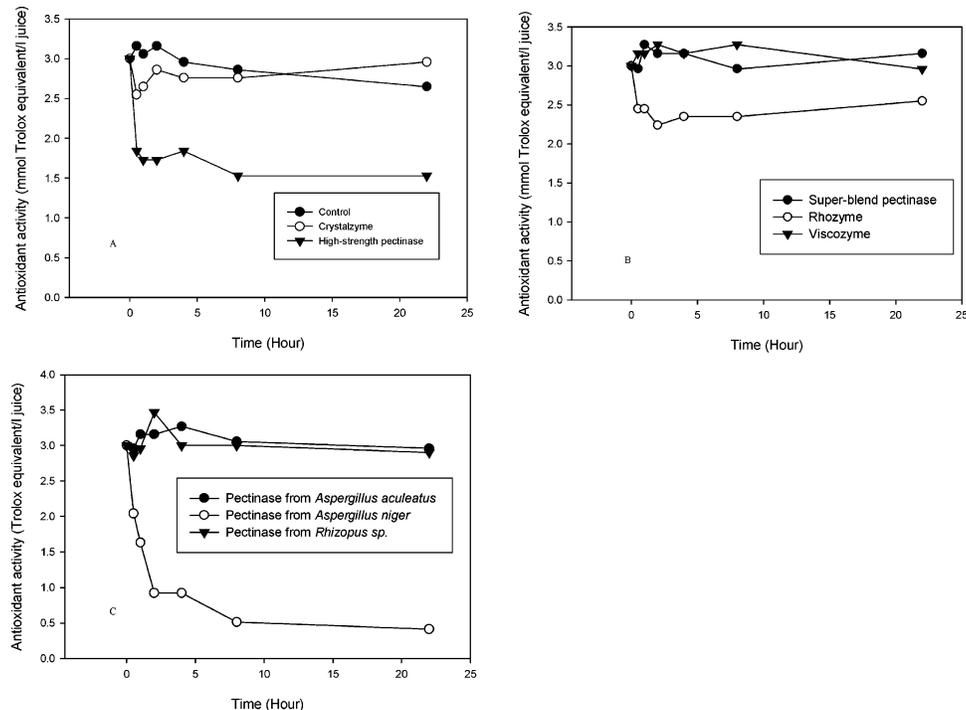


Figure 4. Antioxidant activity (mmol of Trolox equiv/L of juice) of asparagus juice incubated with pectolytic enzymes at 37 °C versus time analyzed by ABTS method: (A) control, Crystalzyme, and high-strength pectinase; (B) super-blend pectinase, Rhozyme, and Viscozyme; (C) pectinase from *A. aculeatus*, pectinase from *A. niger*, and pectinase from *Rhizopus sp.*

asparagus juice measured by both DPPH and ABTS methods. The antioxidant activity of Viscozyme-treated juice was significantly different only at the second hour as measured by the DPPH method. When the Viscozyme-treated and control juices were compared at the same sampling times, the Viscozyme-treated juice had a greater antioxidant activity than the control at the eighth hour (DPPH method) and at the second hour (ABTS method) (Tables 4 and 5). Regardless of the differences in antioxidant activity at these two sampling times, Viscozyme-

treated juice did not produce consistently higher antioxidant activity than control, which confirmed the observation in Figure 3B. When pectinase from *A. niger* was totally inactivated by heating at 90 °C, it did not cause rapid loss of rutin and antioxidant activity of asparagus juice, indicating that the rutin loss was catalyzed by a side enzyme activity, not metals.

The dosage of enzymes used may influence their effect on the phenolic composition of asparagus juice. To test the effect of β -glucosidase on anthocyanin in apple juice, pectolytic

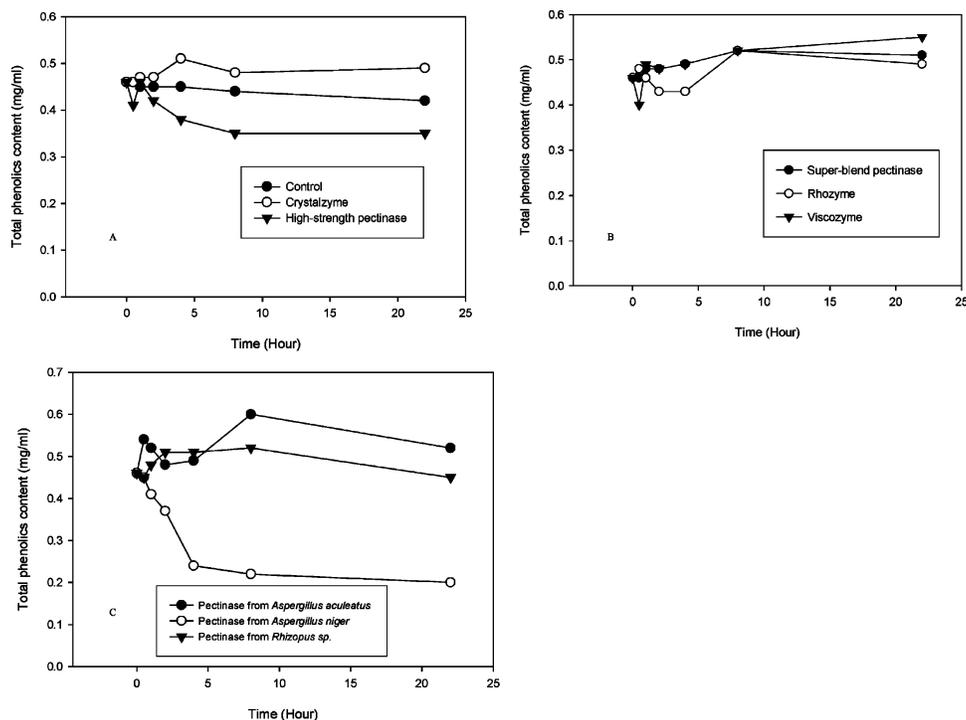


Figure 5. Total phenolics of asparagus juice incubated with pectolytic enzymes at 37 °C versus time: (A) control, Crystalzyme, and high-strength pectinase; (B) super-blend pectinase, Rhozyme, and Viscozyme; (C) pectinase from *A. aculeatus*, pectinase from *A. niger*, and pectinase from *Rhizopus sp.*

Table 4. Antioxidant Activity (Millimoles of Trolox Equivalent per Liter of Juice) of Asparagus Juice at Different Times at 37 °C for Control, Viscozyme, and Pectinase from *A. niger* Evaluated by DPPH Method ($n = 3$)

time (h)	control ^a	Viscozyme ^a	pectinase from <i>A. niger</i> ^a
0	1.70 ± 0.15b	1.70 ± 0.15b	1.70 ± 0.15a
0.5	1.75 ± 0.15b,x	1.77 ± 0.05b,x	1.36 ± 0.04b,y
1	1.70 ± 0.02b,x	1.72 ± 0.04b,x	0.75 ± 0.08c,y
2	1.92 ± 0.04a,x	1.90 ± 0.05a,x	0.27 ± 0.01d,y
4	1.68 ± 0.09b,x	1.69 ± 0.04b,x	0.14 ± 0.00f,y
8	1.58 ± 0.04b,y	1.75 ± 0.09b,x	0.20 ± 0.02e,z

^a ANOVA to compare data ($P = 0.05$): data sharing the same letter in columns (a–d) or rows (x–z) were not significantly different.

Table 5. Antioxidant Activity (Millimoles of Trolox Equivalent per Liter of Juice) of Viscozyme, Pectinase from *A. niger* Treated Asparagus Juice, or Control at Different Incubation Times at 37 °C Evaluated by ABTS Method ($n = 3$)

time (h)	control ^a	Viscozyme ^a	pectinase from <i>A. niger</i> ^a
0	3.75 ± 0.00a	3.75 ± 0.00a	3.75 ± 0.00a
0.5	3.60 ± 0.15a,x	3.90 ± 0.06a,x	3.40 ± 0.02b,y
1	3.80 ± 0.10a,x	4.00 ± 0.26a,x	3.16 ± 0.12c,y
2	3.40 ± 0.19b,y	3.80 ± 0.12a,x	2.22 ± 0.22d,z
4	3.51 ± 0.39ab,x	3.60 ± 0.06a,x	1.60 ± 0.12e,y
8	3.80 ± 0.10a,x	3.90 ± 0.27a,x	1.05 ± 0.14f,y

^a ANOVA to compare data ($P = 0.05$): data sharing the same letter in columns (a–f) or rows (x–y) were not significantly different.

enzyme preparations have been added to juice at 0.1% (w/v) (5) up to 2% (w/v) (22) in previous research. The recommended dose of Viscozyme for maceration of plant material or clarification of juice is 1 mg of enzyme per 100 mg of plant cells with reaction time of 2–20 h. In our research, Viscozyme was added

to asparagus juice at 6.9 units of PG activity, which is 1% (v/v), approximately the recommended dosage. For industry-scale juice clarification, attention should be paid that rutin content in asparagus juice may be decreased by Viscozyme. Pectinase from *A. niger* significantly decreased the rutin content (Table 3) and antioxidant activity of asparagus juice (Tables 4 and 5); therefore, it is not recommended for juice clarification with long incubation time.

Total Phenolic Content of Asparagus Juice Affected by Pectolytic Enzyme Preparations. Pectinase from *A. niger* greatly decreased total phenolics in asparagus juice due to the loss of rutin. For most enzymes, total phenolics were not markedly different from that of control (Figure 5). However, total phenolics of asparagus juice treated by high-strength pectinase or pectinase from *A. niger*-treated juice were greatly decreased, and antioxidant activity also decreased. This result agreed with Velioglu et al.'s work that the correlation coefficient between total phenolics and antioxidant activities was statistically significant (19).

Conclusion. Rutinase activity was found in six of the eight crude pectolytic enzyme preparations without significantly increasing the antioxidant activity of asparagus juice. When compared by the same PG activity, Viscozyme showed the greatest rutinase activity. Crude pectinase from *A. niger* significantly degraded rutin and decreased the antioxidant activity of asparagus juice. Pectolytic enzyme preparations need to be closely examined in application as they may alter the phenolic composition of asparagus juice. Further work needs to be done to investigate the mechanisms of these enzymes on the antioxidant activity of asparagus juice.

ACKNOWLEDGMENT

We thank Karen Weller in the Department of Food Science and Human Nutrition at Washington State University for technical assistance in HPLC analysis.

LITERATURE CITED

- (1) Shao, Y.; Chin, C. K.; Ho, C. T.; Ma, W.; Garrison, S. A.; Huang, M. T. Anti-tumor activity of the crude saponins obtained from asparagus. *Cancer Lett.* **1996**, *104*, 31–36.
- (2) Vinson, J. A.; Hao, Y.; Su, X. H.; Zubik, L. Phenol antioxidant quantity and quality in foods: vegetables. *J. Agric. Food Chem.* **1998**, *46*, 3630–3634.
- (3) Tsushida, T.; Suzuki, M.; Kurogi, M. Evaluation of antioxidant activity of vegetable extracts and determination of some active compounds. *J. Jpn. Soc. Food Sci. Technol.* **1994**, *41*, 611–618.
- (4) Pilnik, W.; Voragen, A. G. J. Pectic enzymes in fruit and vegetable juice manufacture. In *Enzymes in food processing*; T. Nagodawithana and G. Reed, Eds.; Academic Press, Inc.: London, 1993; pp 363–399.
- (5) Wightman, J. D.; Wrolstad, R. E. β -Glucosidase activity in juice-processing enzymes based on anthocyanin analysis. *J. Food Sci.* **1996**, *61*, 544–547, 552.
- (6) Wrolstad, R. E.; Wightman, J. D.; Durst, R. W. Glycosidase activity of enzyme preparations used in fruit juice processing. *Food Technol.* **1994**, *48*, 90–98.
- (7) Barbe, C.; Dubourdieu, D. Characterisation and purification of a cinnamate esterase from *Aspergillus niger* industrial pectinase preparation. *J. Sci. Food Agric.* **1998**, *78*, 471–478.
- (8) Versari, A.; Biesenbruch, S.; Barbanti, D.; Farnell, P. J.; Galassi, S. Effects of pectolytic enzymes on selected phenolic compounds in strawberry and raspberry juices. *Food Res. Int.* **1997**, *30*, 811–817.
- (9) Wightman, J. D.; Price, S. F.; Watson, B. T.; Wrolstad, R. E. Some effects of processing enzymes on anthocyanins and phenolics in Pinot noir and Cabernet Sauvignon wines. *Am. J. Enol. Vitic.* **1997**, *48*, 39–48.
- (10) Suzuki, T.; Honda, Y.; Funatsuki, W.; Nakatsuka, K. Purification and characterization of flavonol 3-glucosidase, and its activity during ripening in tartary buckwheat seeds. *Plant Sci.* **2002**, *163*, 417–423.
- (11) Yasuda, T.; Nakagawa, H. Purification and characterization of the rutin-degrading enzymes in tartary buckwheat seeds. *Phytochemistry* **1994**, *37*, 133–136.
- (12) Oszmianski, J.; Lee, C. Y. Inhibitory effect of phenolics on carotene bleaching in vegetables. *J. Agric. Food Chem.* **1990**, *38*, 688–690.
- (13) Sanchez-Moreno, C.; Larrauri, J. A.; Saura-Calixto, F. A procedure to measure the antiradical efficiency of polyphenols. *J. Sci. Food Agric.* **1998**, *76*, 270–276.
- (14) Frankel, E. N.; Meyer, A. S. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *J. Sci. Food Agric.* **2000**, *80*, 1925–1941.
- (15) Gross, K. C. A rapid and sensitive spectrophotometric method for assaying polygalacturonase using 2-cyanoacetamide. *Hort-Science* **1982**, *17*, 933–934.
- (16) Bradford, M. M. Rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.
- (17) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free-radical method to evaluate antioxidant activity. *Food Sci. Technol.* **1995**, *28*, 25–30.
- (18) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231–1237.
- (19) Velioglu, Y. S.; Mazza, G.; Gao, L.; Oomah, B. D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* **1998**, *46*, 4113–4117.
- (20) Zar, J. H. *Biostatistical Analysis*, 3rd ed.; Prentice Hall: Upper Saddle River, NJ, 1996; p 662.
- (21) Makris, D. P.; Rossiter, J. T. Domestic processing of onion bulbs (*Allium cepa*) and asparagus spears (*Asparagus officinalis*): effect on flavonol content and antioxidant status. *J. Agric. Food Chem.* **2001**, *49*, 3216–3222.
- (22) Jiang, J.; Paterson, A.; Piggott, J. R. Effects of pectolytic enzyme treatments on anthocyanins in raspberry juice. *Int. J. Food Sci. Technol.* **1990**, *25*, 596–600.

Received for review May 28, 2004. Revised manuscript received October 1, 2004. Accepted October 5, 2004. We thank the USDA Cooperative State Research, Education and Extension Service (CSREES) for providing the grant without which this research would not have been conducted.

JF0491299