Effect of Enzymatic Macerate Treatment on Rutin Content, Antioxidant Activity, Yield, and Physical Properties of Asparagus Juice

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ABSTRACT: Asparagus is a vegetable with high antioxidant activity. In this research, asparagus juice was produced from fresh asparagus macerate treated with a carbohydrases mixture (Viscozyme) at 37 °C for up to 8 h. Rutin content, antioxidant activity, yield, soluble solid content, and color of the produced asparagus juice were determined. The results showed that Viscozyme significantly increased the yield of asparagus juice, especially in the 1st hour, which was higher than control (without Viscozyme treatment), but the juice had significantly less rutin content than control and had higher antioxidant activity than control only in the 1st 2 h. Juice with Viscozyme treatment had significantly higher soluble solid content than control. The greenness of asparagus juice deteriorates quickly for both the Viscozyme group and control. Viscozyme had advantage in producing juice with high yield, antioxidant activity, and soluble solid content in shorter time (2 h) of treatment compared to control.

Keywords: antioxidant activity, asparagus juice, soluble solid content, Viscozyme, yield

Introduction

Asparagus (*Asparagus officinalis* L.) is a healthy green vegetable, which contains antioxidants, such as rutin, ascorbic acid, and glutathione, and so on (Fuleki 1999; Saito and others 2000). Antioxidant activity of asparagus has been reported to be one of the greatest among the commonly consumed vegetables (Vinson and others 1998). In recent years, more and more researches have shown that consumption of vegetables can prevent cardiovascular disease, several common cancers, and other chronic diseases in human (Lampe 1999), and people are paying more attention to the health benefit of vegetables. Accordingly, several vegetable juice products have been available in the market. One advantage of vegetable juice is that that some antioxidants could be more bioavailable in juice form than in raw or cooked vegetables (McEligot and others 1999). Because asparagus lignifies and deteriorates quickly after harvest, fresh asparagus is hard to store and has a very short shelf life. One of the major needs of the asparagus industry is to develop new processed asparagus product to utilize asparagus (Bakker 2004). One such utilization could be asparagus juice, which is a relatively new product that has been commercially available only in a few countries and has not been sold worldwide.

Modern processes of fruit and vegetable juice production often use enzymes as important processing aids to obtain juice with higher yield and higher soluble solid contents. Several researches have found that pectinase and cellulose enzyme treatment can significantly increase juice yield and soluble solid content (Demir and others 2001; Essa 2002; Kyamuhangire and others 2002). Viscozyme is a commercial multienzyme preparation from *Aspergillus aculeatus*, and contains a wide range of carbohydrases, such as arabanase, cellulase, hemicellulase, and xylanase (NCBE 2006). Viscozyme is often used to process cereals and vegetables in food industry. Viscozyme can degrade the nonstarch polysaccharides and branched pectin-like substances in plant cell wall, which can decrease the viscosity of plant extract and increase the extraction yields of juice. In our previous research, we found that Viscozyme contained small amount of rutinase that can change rutin to quercetin in asparagus juice (Sun and others 2005). So far, little research has been reported on effects of pectolyic enzyme treatment of macerate on phenolic composition and antioxidant activity of the produced asparagus juice.

The objective of the current research was to produce asparagus juice from fresh asparagus using commercially available Viscozyme and investigate the effect of Viscozyme on rutin content, antioxidant activity, yield, color, and soluble solid contents of asparagus juice, and to find the optimum conditions to produce asparagus juice using Viscozyme.

Materials and Methods

Chemicals

2,2′-Diphenyl-1-picrylhydrazyl (DPPH) radical, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), rutin, and quercetin were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Viscozyme was purchased from Novozymes (Franklinton, N.C., U.S.A.). Methanol and acetic acid were HPLC grade and purchased from Fisher Scientific (Springfield, N.J., U.S.A.).

Preparation of asparagus macerate

Fresh asparagus was harvested from a local farm in Washington State, hydro-cooled immediately, shipped to the lab overnight on ice, and stored at 4 °C for 1 d before experiment. Asparagus spears were trimmed at the basal end to a length of 18 to 20 cm, and the diameter of the trimmed asparagus was in the range of 1.0 to 1.5 cm. Asparagus was then cut into small pieces of 1 to 3 cm^3, and 500 g of asparagus was macerated for 30 s at high speed in a food processor (Hamilton Beach/Proctor-Silex, Inc., Southern Pines, N.C., U.S.A.).

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Enzymatic treatment of asparagus macerate

Asparagus macerate (50 g) was mixed with 1% (v/w) Viscozyme solution. Then asparagus macerate was transferred to a 200-mL glass bottle, capped, and incubated at 37 °C in a water bath. Three bottles of asparagus macerate were taken out at 0.5, 1, 2, 4, 6, and 8 h, respectively. Then asparagus macerate was wrapped by 2 layers of cotton cloth, and pressed to produce juice using a hand press. The obtained asparagus juice was weighed and the yield of juice was calculated based on the fresh weight of asparagus macerate. Control was asparagus macerate incubated without Viscozyme at the same conditions. Asparagus juice (0.2 mL) was mixed with 0.8 mL methanol to inactivate Viscozyme or the endogenous enzymes of asparagus, and centrifuged at 11872 × *g* at 25 °C for 10 min using a bench-top Marathon centrifuge (Fisher Scientific, Pittsburgh, Pa., U.S.A.). The clear supernatant of the solution was used for analysis.

Determination of antioxidant activity of asparagus juice by DPPH radical method

Aliquots of asparagus juice (10, 20, and 40 μL) were added to 1 mL DPPH-free radical solution (0.75×10^{-4} M) in ethanol and kept at 25 °C for 30 min in the dark (Brand-Williams and others 1995; Sun and others 2005). The absorbance of DPPH radical solution was determined at 515 nm by an Ultrospec 4000 UV/visible spectrophotometer (Pharmacia Biotech, Cambridge, England). The inhibition percentage of the absorbance of DPPH radical solution was calculated by the following equation:

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

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

The inhibition percentage of the absorbance was plotted against the amount of asparagus juice added to DPPH radical solution to obtain a linear regression curve. Trolox was added to DPPH radical solution and the slope was obtained from the regression line of inhibition percentage compared with amount of Trolox added to DPPH radical. The ratio between the value of slopes of sample and Trolox was defined as the Trolox equivalent antioxidant activity (TEAC) (mmol Trolox equivalent/L juice).

Determination of rutin content of asparagus juice by HPLC

Rutin content in asparagus juice was determined by an Agilent 1100 HPLC (Palo Alto, Calif., U.S.A.) (Sun and others 2005). The HPLC system included a quaternary pump, a vacuum degasser, a thermostatic column compartment, and a diode array detector. The columns are composed of a Vydac 201TP (50 mm × 4.6 mm i.d, 5 μm particle size) guard column (Columbia, Md., U.S.A.) and an Agilent Eclipse XDB-C8 column (150 mm × 4.6 mm i.d., 5 μm particle size). The mobile phase contained 5% acetic acid, 25% methanol, and 70% water at a flow rate of 0.8 mL/min. The injection volume was 10 μL and the detector was set at 360 nm for analysis. Rutin content was quantified using external standard (0.01 to 1.0 mg/mL in methanol). Quercetin content of asparagus juice was analyzed by HPLC using the same conditions as rutin.

Analysis of the soluble solid contents of asparagus juice

Soluble solid contents of asparagus juice were measured using an ABBE refractometer (MISCO, Cleveland, Ohio, U.S.A.) at 25 °C. The value of the soluble solid contents was read from the soluble solid scale. Water was used to calibrate the instrument.

Instrumental color measurements

A petri dish with a depth of 0.8 cm was put on a white paper and filled with asparagus juice. A portable Minolta colorimeter (Minolta Spectrophotometer CM-2002, Minolta Camera, Osaka, Japan) was hold and positioned on the lid of the petri dish to determine the *L*, *a*, and *b* values of asparagus juice color Hue (H^0) and chroma (C) were calculated by the following equations (McGuire 1992; Voss 1992):

$$H^0 = tan^{-1}(b^*/a^*) \text{ when } a^* > 0 \text{ and } b^* \geq 0, \text{ and } H^0 = 180 + tan^{-1}(b^*/a^*) \text{ when } a^* < 0. \text{Chroma C} = (a^{*2}+b^{*2})^{1/2}$$

Statistical analysis

All the experiments were performed in triplicate. One-way analysis of variance (ANOVA) and multiple comparisons (Fisher's least-significant-difference test) were used to evaluate the significant difference of the data at $P < 0.05$ (Zar 1996). Data were expressed as means ± standard deviation.

Results and Discussion

Yield of asparagus juice

The yield of asparagus juice with Viscozyme treatment was significantly higher than control (Figure 1), which was increased mostly in the 1st hour from 38.3% to 68.6% and did not change significantly between 1 and 4 h but increased a little between 4 and 8 h. Viscozyme contains several carbohydrases that can degrade pectin and cellulose in cell walls, leading to the release of cell contents, which may be recovered in high yield. The juice yield for control was increased gradually in the 1st 6 h from 34.7% to 64.9% (Figure 1). This result showed that fresh asparagus may contain some hydrolases released from asparagus cells during maceration, which can hydrolyze pectin and cellulose in asparagus cell wall and increase juice yield. For example, galactosidase in asparagus that can hydrolyze pectin has been found (O'Donoghue and others 1998). When asparagus

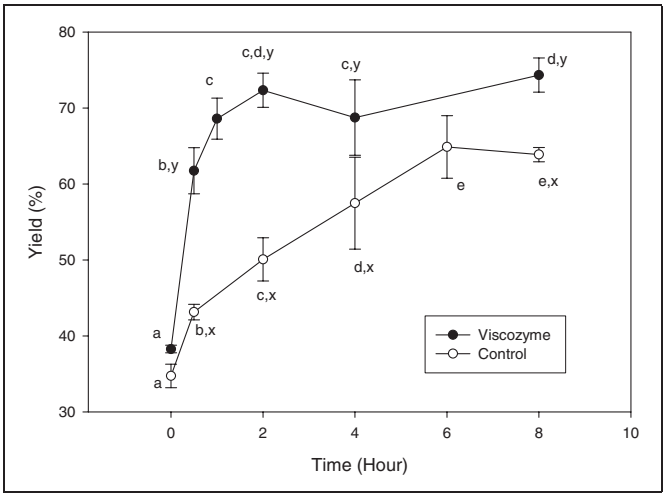

Figure 1 — Yield of asparagus juice produced from asparagus macerate with or without Viscozyme treatment at 37°C. Data are means ± SD (n = 3). Data with different letters (a to e) represent a significant difference at $P < 0.05$ with incubation time and data with different letters (x, y) at a given time represent a significant difference at $P < 0.05$ between enzyme treatment and control.

was blanched to inactivate its endogenous enzymes, frozen during storage, and thawed to prepare the macerate, the yield of juice was not significantly increased (Sun 2005), which was 49.3% to 52.3% at 37°C (Sun 2005). Fresh asparagus showed advantage in producing high yield of juice comparable to that with Viscozyme treatment, but it may take longer time to get the maximum yield if not using the aid of Viscozyme.

Concentration of rutin and antioxidant activity of asparagus juice

For control, rutin content of the produced juice was not increased significantly in the 1st 2 h, but between 2 and 8 h, which was increased by 2 times after 8 h (Figure 2). The increase of rutin content in control juice occurred in parallel with the increase of juice yield. The carbohydrase of fresh asparagus may degrade the cell wall to release more rutin into juice. No quercetin was detected in control juice. But quercetin was detected in juice of the Viscozyme group, because Viscozyme contains rutinase that can change rutin to quercetin (Sun and others 2005). For the Viscozyme group, rutin content of asparagus juice was decreased and quercetin content was increased, but the total content of rutin and quercetin was not increased significantly, which was lower than rutin content of control juice (Figure 2).

The antioxidant activity of control juice was not increased significantly in the 1st 2 h, but between 2 and 6 h (Figure 3). The antioxidant activity of juice of the Viscozyme group was only increased between 1 and 2 h and was not changed significantly between 0 and 1 h or 2 and 8 h. The antioxidant activity of control juice was significantly lower than the Viscozyme group between 0 and 2 h possibly due to quercetin in the Viscozyme group that has higher antioxidant activity than rutin as determined by DPPH-free radical method; the antioxidant activity of control juice was significantly higher than the Viscozyme group between 4 and 8 h possibly because of much higher of rutin content in control juice than the Viscozyme group. Compared to that at time zero, the antioxidant activity of juice of control and the Viscozyme group after 8 h was increased by 6 and 3 times, respectively (Figure 3). For control juice, the antioxidant activity and rutin content were increased at the same time.

Soluble solid contents of asparagus juice

Soluble solids are compounds truly soluble in the juice, such as fructose, sucrose, and glucose, which make up the predominant soluble solids. For control juice, soluble solid contents decreased significantly by 40% after 4 h. For the Viscozyme group, the soluble solid content of asparagus juice produced was not changed significantly, and was higher than that of control juice (Figure 4). After 2 h, juice of the Viscozyme group contained twice the amount of soluble solids as that of control group when taking account of yield in calculation. The decreased soluble solid content in control group could be due to the dilution effect of the increased juice yield or volume, although more soluble solids may be released into juice by the endogenous hydrolase of asparagus. Viscozyme can cause greater degree of tissue breakdown, releasing more components that contribute to soluble

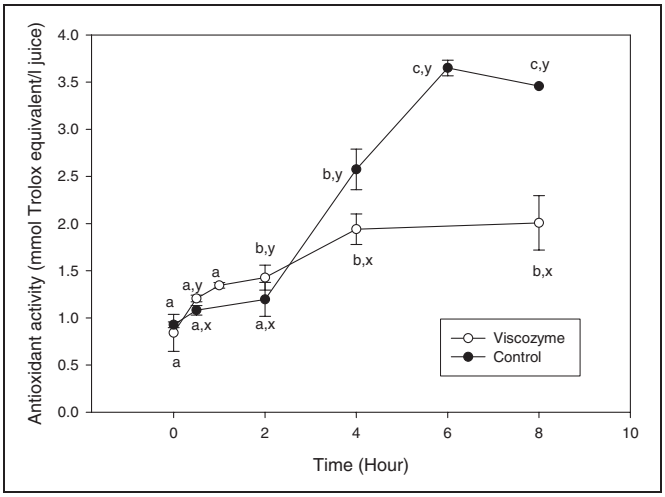

Figure 3—Antioxidant activity of asparagus juice produced from asparagus macerate with or without Viscozyme treatment and determined by DPPH radical method. Data are means ± SD ($n = 3$). Data with different letters (a to c) in the same curve represent a significant difference with time and data with different letters (x, y) at a given time represent a significant difference with enzyme treatment at $P < 0.05$.

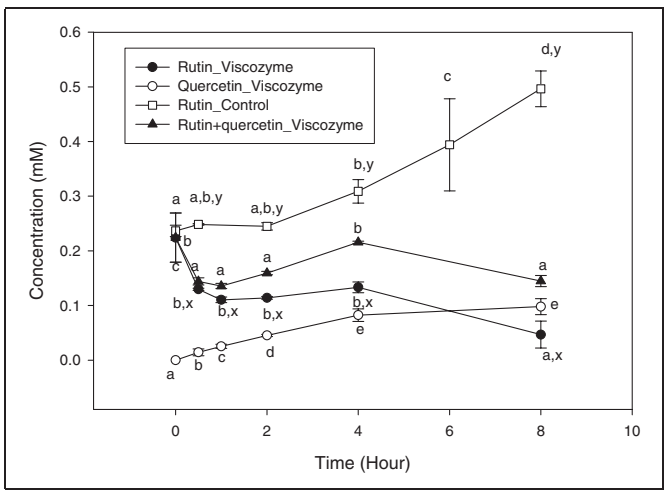

Figure 2—Rutin and quercetin content of asparagus juice produced from asparagus macerate with or without Viscozyme treatment at 37 °C. Data are means ± SD ($n = 3$). Data with different letters (a to e) in the same curve represent a significant difference with time and data with different letters (x, y) represent a significant difference at $P < 0.05$ between enzyme treatment and control at a given time.

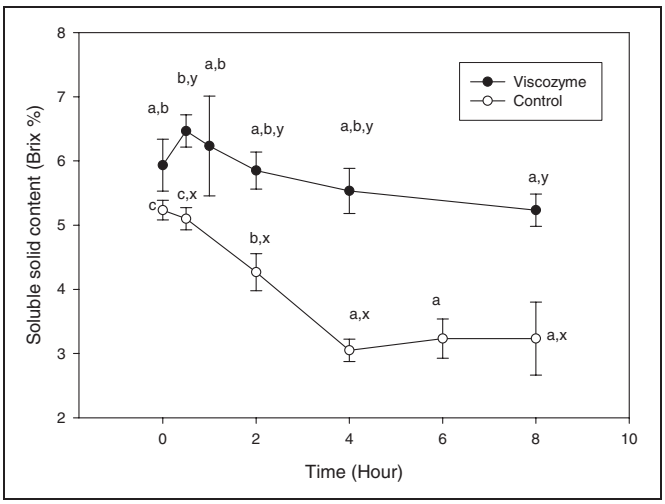

Figure 4—Soluble solid content of asparagus juice produced from asparagus macerate with or without Viscozyme treatment at 37 °C. Data are means ± SD ($n = 3$). Data with different letters (a to c) in the same curve represent a significant difference and data with different letters (x, y) represent a significant difference with enzyme treatment at $P < 0.05$.

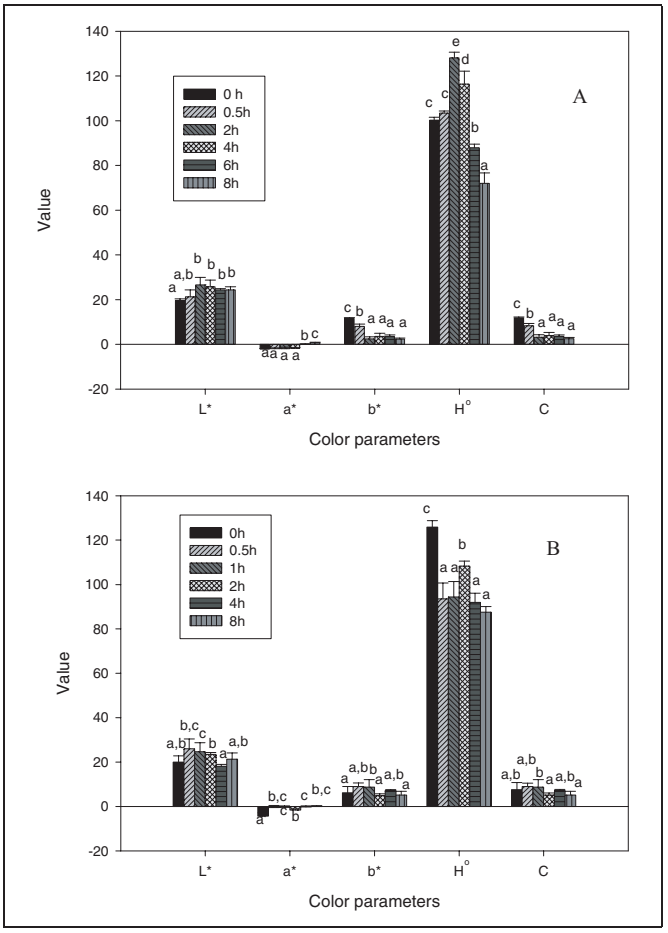

Figure 5—Color parameters (*L, *a**, *b**, H⁰, and C) of asparagus juice produced from asparagus macerate with incubated at 37 °C without (A) and with (B) Viscozyme treatment. Data are means ± SD (*n* = 3). ANOVA to compare data with time, data sharing the same letter (a to d) were not significantly different for each parameter (*P* > 0.05).**

solids of juice. Our result agreed with previous researches that banana juice (Kyamuhangire and others 2002) or prickly pear juice (Essa 2002) extracted with cellulose or pectinase contained more soluble solids than untreated juice.

Color of asparagus juice

For control juice, L^* was increased significantly after 2 h and was not significantly changed between 2 and 8 h; a^* was increased significantly to positive after 6 h; b^* and C were decreased significantly between 0 and 2 h and did not change after 2 h. H^0 was increased significantly after 2 h and was decreased significantly between 2 and 8 h (Figure 5A). It was reported that Hue (H^0) correlates significantly with chlorophyll content and can be used to evaluate the color of asparagus (Lau and others 2000). For asparagus juice of the Viscozyme group, L^*, b^*, and C did not change apparently, but a^* was increased and H^0 was decreased significantly (Figure 5B). Both group lost their greenness and gained redness after 8 h, but juice of the Viscozyme group was yellowier than control juice (Figure 5B). Control juice had higher H^0 value or better greenness than juice of the Viscozyme group at 2 h. The pigments of asparagus juice include anthocyanin, carotenoids, and chlorophyll, which are responsible for the red, yellow, and green color of the juice, respectively. Chlorophylls and carotenoids are important pigments of asparagus that can affect the color quality of asparagus juice. Pectolytic enzymes can hydrolyze plant cell walls, which may release more carotenoids and anthocyanins from plant cells (Essa 2002). Chlorophyll of asparagus juice seemed to be degraded by the natural enzymes in fresh asparagus to colorless end product. For example, it was reported that chlorophyllase can catalyze the cleavage of phytol from chlorophylls and its Mg-free derivatives (pheophytins) (Fennema 1996). But the mechanism of color loss of asparagus juice in this research needs to be investigated further.

To compare juices with or without Viscozyme treatment, the following calculations are made based on 1 kg fresh asparagus. For control and Viscozyme group after 8 h, the yield of juice was 64% and 74%, respectively; the antioxidant activity of juice was 3.4 and 2.0 mmol Trolox equivalent/L juice, respectively; and the soluble solid content of juice was 3.2 and 5.2 Brix%, respectively. Thus, the produced juice of control and Viscozyme group after 8 h contained 2.2 and 1.5 mmol Trolox equivalent of antioxidants, as well as 20.5 and 38.5 Brix of soluble solid, respectively. Juice of Viscozyme group had 1.9 times of soluble solids and 0.7 times of antioxidants compared to control juice after 8 h. As the greenness of juice was destroyed after 8 h, to protect the color of juice, as well as save time, shorter time (2 h) can be used to produce juice. For control and Viscozyme group after 2 h, the yield of juice was 50% and 72%, respectively; the antioxidant activity of juice was 1.2 and 1.4 mmol Trolox equivalent/L juice, respectively; and the soluble solid content of juice was 4.3 and 5.8 Brix%, respectively. Therefore, 0.6 and 1.0 mmol Trolox equivalent of antioxidants, or 21.5 and 41.8 Brix of soluble solids, can be obtained for juice of control and Viscozyme group, respectively. After 2 h, juice of Viscozyme group had more soluble solid and higher amount of antioxidants than control juice.

Conclusions

Using unblanched fresh asparagus to produce juice, high yield, high rutin content, and high antioxidant activity of juice can be obtained only after long time of incubation (6 to 8 h) compared to Viscozyme treatment. One percent of Viscozyme can be used to increase the yield, antioxidant activity, and soluble solid content of asparagus juice in a short time (2 h). Viscozyme also decreased the rutin content and increased quercetin content of the produced juice. The greenness of asparagus juice was decreased for both control and Viscozyme group with incubation time but juice after 2 h can still retain some greenness.

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