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Enzymatic Release of Ferulic Acid from *Ipomoea batatas* L. (Sweet Potato) Stem

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Abstract Ferulic acid is a phenolic compound that serves as a major biosynthetic precursor of vanillin in higher plants. We investigated the ability of the 3 commercial enzymes – Ultraflo L, Viscozyme L, and α-Amylase – to induce the release ferulic acid from the *Ipomoea batatas* L. (sweet potato) stem. The rate of release for ferulic acid was optimal when Ultraflo L (1.0%) was used compared with the other enzymes, whereas Viscozyme L was most effective for the release of vanillic acid and vanillin. Thus, these enzymes may be useful for the large-scale production of ferulic acid and other phenolic compounds from sweet potato stem.

Keywords: ferulic acid, enzymatic treatment, sweet potato

Ferulic acid, the main phenolic compound in plants, serves as a major biosynthetic precursor of vanillin [1] and is a component of Chinese herbs such as *Angelica sinensis*, *Cimicifuga heracleifolia*, and *Lignsticum chuangxiong* [2], as well as rice, wheat, barley, and other cereal grains [3-5]. Ferulic acid has a powerful antioxidant effect and is much less affected by pH changes than other phenolic compounds such as chlorogenic acid, caffeic acid, and gallic acid [6,7]. Its biological activity includes an antimicrobial effect [3] and chemoprevention of coronary heart disease, thrombosis, carcinogenesis, and mutagenesis [8]. It is also a potential precursor to natural vanillin and a prerequisite to the production of vanillin using various biotechnological methods [9].

Ferulic acid is usually extracted by hydrolysis under very alkaline or acidic conditions [10], which may modify other components of the plant, including the cell wall. Enzymatic hydrolysis is a gentler and more product-specific method of releasing ferulic acid from plants [11,12] while increasing the yield and triggering fewer filtration problems, greater industrial throughput, less waste production, and a better quality for the final product.

Ferulic acid forms cross-linkages between lignin and cell-wall polysaccharides; the release of ferulic acid from the plant requires enzymatic digestion of the structures with which it is linked to the cell wall. We used 3 commercially available enzymes that have been studied previously for their ability to induce the release of ferulic acid from the cell wall [13-15]: Ultraflo L, which has demonstrated high levels of feruloyl esterase activity [13]; Viscozyme, which has been shown to induced the release of ferulic acid from pectin-type agricultural residues such as sugar beet pulp [16,17]; and α-Amylase, which has been used to hydrolyze starch in the cell wall and, thus, would be expected to enhance the release of ferulic acid in the cell wall [14].

A variety of agricultural byproducts are potential sources of ferulic acid, which constitutes approximately 0.14 to 3.1% of plant materials based on dry weight. *Ipomoea batatas* L. (sweet potato) is a particularly rich source of ferulic acid. The tuber contains 0.54% per g dry weight; the non-tuber portion of the plant, which is usually thrown away after the harvest, contains more than >3.0% per g by dry weight. Sweet potato is one of the most important tuber crops in the world and the seventh most important crop in South Korea, with an annual production of 345,239 tones. The tuber is used for livestock feed and for the production of starch and alcohol. However, sweet potato vines have not been put to any commercial use yet. This paper reports the findings of our search for the optimal conditions for releasing ferulic acid from the edible portion of the sweet potato stem using commercially available enzymes.

Sweet potato stems were collected from the Park's farm (Jinju, Korea), and both fresh and dry samples were prepared. Ultraflo L (liquid phase) was purchased from Novozymes A/S (Bagsvaerd, Denmark); Viscozyme L (liquid phase) and α-Amylase were purchased from the Sigma-Aldrich Company (St. Louis, MO, USA). All other reagents used in this study were extra-pure grade.

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Enzymatic hydrolysis of fresh and dried samples was carried out using a method described by Bartolome and Gomez-Cordoves [14], with modification. Enzymatic release of ferulic acid from sweet potato stems was determined in 100 mM 3-(*N*-morpholino)-propane sulphonic acid (MOPS) buffer (pH 6.0) at 37°C on a rotary incubator (12 rpm). Ultraflo L, Viscozyme L (0.01 to 1.0%, v/v, each) and α-Amylase (5 to 100 mU) were each mixed with 100 mM MOPS buffer, pH 6.0 (ICN Biomedicals Inc., Aurora, OH, USA) to a final volume of 2.75 mL. Fresh or dried sweet potato stem samples (50 mg each) were then incubated with various amounts each enzyme mixture for 1 to 12 h. At the end of the incubation period, 150 μL of glacial acetic acid was added. The samples then underwent centrifugation at 6,000 rpm for 10 min and were filtered through a 0.2-μm Milllipore filter. After filtration, 20-μL samples were analyzed by high-performance liquid chromatography (HPLC). Each sample was analyzed in 3 separate experiments. One unit (U) of enzyme activity was defined as the amount of enzyme that induced the release of 1 μmol ferulic acid/min at pH 6 and 37°C.

HPLC analysis was performed using a method described by Kang *et al.* [18], with modification. The filtered samples were loaded into an HPLC system (Gilson, France) equipped with a 5-μm TSK gel ODS-80Ts column (4.6 mm × 25 cm; Tosoh, Tokyo, Japan) and an ultraviolet light detector operating at a 280 nm using water:glacial acetic acid 95:5 (v/v) (solvent A) and methanol (solvent B) for the mobile phase with a solvent flow rate of 0.6 mL/min. The elution sequence was as follows: 0 to 10 min, 90% A and 10% B; 18 to 25 min, 25% A and 75% B; finally 30 to 40 min, 90% A and 10% B. The sample retention times were calculated by comparison with the results of a concurrent HPLC carried out with standard solutions, which were used to calibrate the HPLC system.

Data are expressed as the mean ± SD of at least 3 separate experiments. The error bars indicate the range of SDs from all experiments. The statistical significance of enzymatic exposure was calculated using Duncan's multiple range test ($P = 0.05$).

The amount of ferulic acid released from both fresh and dried samples of sweet potato stems was observed. The amount of ferulic acid released from fresh stems was much higher than from dried stems. Ultraflo L (0.5%) catalyzed the release of 3.41 mg/g ferulic acid after 1 h of incubation with fresh stems compare with release of 1.27 mg/g ferulic acid in dried stems at same conditions. The amount of ferulic acid released from the samples also varied with the type of enzyme used; α-Amylase was not effective in releasing ferulic acid from either fresh or dried samples. The release of more ferulic acid from fresh samples than from dried samples may be due to the fact that the enzyme can be adsorbed onto the stem more readily and therefore has greater catalytic efficiency in fresh stems. The release of less ferulic acid from dried stems may be attributed to the extreme resistance of dry tissue to enzyme activity and to the fact that dried plants are highly cross-linked with heteroxylans through diferulic bridges. Thus, the state of the sweet potato stems and type of enzyme used must be considered to ensure the maximal release of ferulic acid. It was also noted that the level of ferulic acid in sweet potato stems was higher than that of various types of barley (0.32%) [19].

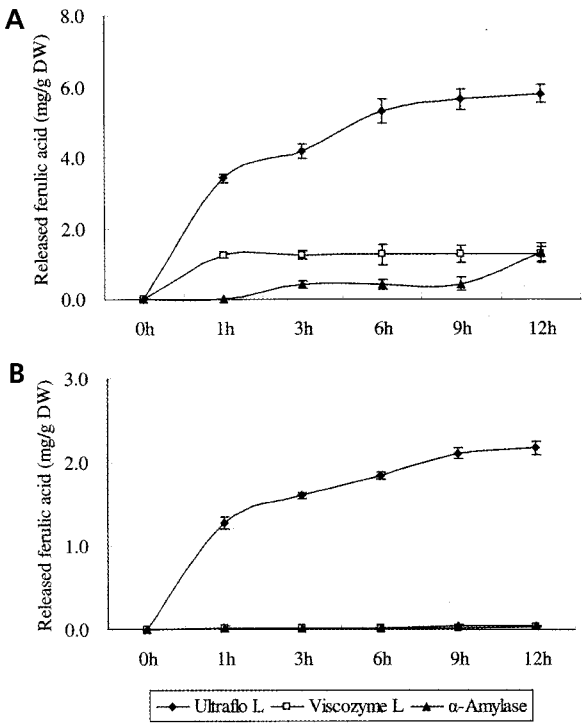

Fig. 1. Amount of ferulic acid released in the presence of 3 enzymes during various incubation periods. Each sample was incubated with 0.5% Ultraflo L, 0.5% Viscozyme L, or 50 mU of α-Amylase at 37°C. (A) fresh stems; (B) dried stems.

Enzymatic activity enhanced the release of ferulic acid from both fresh and dried sweet potato stems (Fig. 1). Ultraflo L was the most efficient at inducing the release of ferulic acid. Produced by *Humicola insolens*, this enzyme is known to induce the release of maximal amounts of arabinose from both soluble and insoluble arabinoxylan and ferulic acid from insoluble arabinoxylan [20]. Sorensen *et al.* [15] have indicated that Ultraflo L is the best enzyme for catalyzing the release of arabinose. The hydrolytic reaction mediated by Ultraflo L solubilized more than half of the biomass and released all of the ferulic acid in its free form [20]. After 1 h of incubation with Ultraflo L, 3.42 mg/g of ferulic acid was released from fresh samples compared with 1.27 mg/g from dried samples. Under the same conditions, Viscozyme L and α-Amylase released 1.23 and 0.01 mg/g, respectively, of ferulic acid from fresh samples (Fig. 1A). Therefore, we can infer that 1 h of incubation with Ultraflo L results in the release of optimal amount of ferulic acid from sweet potato stems (Figs. 1A and 1B). Similar results have been reported for most enzyme reactions, with product formation observed within 3 h of incubation, which is a relatively short period of time [14,19]. In this study, the mechanisms involved in the release of ferulic acid in the

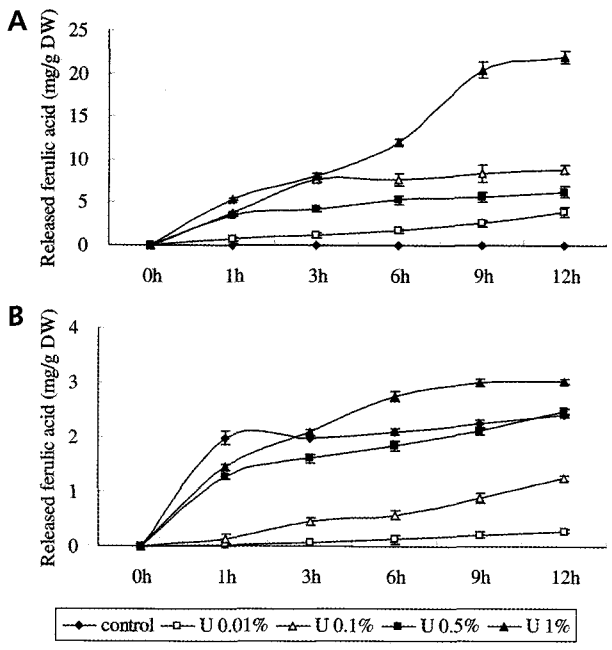

Fig. 2. Influence of various concentrations of Ultraflo L on the release of ferulic acid from sweet potato stems. (A) Fresh stems; (B) dried stems.

presence of these enzymes were not clear. The plant cell wall contains non-starch polysaccharides, such as xylanases and feruloyl esterases. Almost all the ferulic acid observed in our study was released in the presence of commercial preparations of feruloyl esterases that had been combined with other cell-wall glycosyl hydrolases [13]. The findings of this study suggest that commercial enzyme preparations may be effective in releasing relatively large amount of ferulic acid and other phenolic compounds.

We determined the amount of ferulic acid released from sweet potato stems in the presence of various concentrations of Ultraflo L (0.01~1.0%) (Fig. 2). We found that the release of ferulic acid was influenced by the enzyme concentration and that 1.0% Ultraflo L was the most effective. In a previous study, Ultraflo catalyzed the release of increased amounts of mono- and dimeric ferulate [13], as well as the release of 65% of the available ferulic acid from Brewer's spent grain, together with 3 forms of diferulates.

We also investigated the effect of the 3 commercial enzymes on the release of the other phenolic compounds – vanillic acid, vanillin, and cinnamic acid – from sweet potato stems. The amount of each phenolic compound that was released varied with the enzyme concentration. The amount of vanillic acid and vanillin released was 11.04 and 14.69 mg/g, respectively, when incubated with 1.0% Viscozyme L for 1 h (Table 1), compared with only 7.47 and 8.30 mg/g, respectively, when incubated for an hour with 1.0% Ultraflo L. The amount of cinnamic acid released from sweet potato stems was negligible and could not be detected by HPLC.

When we investigated the relationship between the release of phenolic compounds and the reaction time, we found that the rate of release for vanillic acid, vanillin, and cinnamic acid was prolonged compared with that of ferulic acid in the presence of Ultraflo L (0.5%). A total of 6.98 mg/g of vanillic acid and 5.85 mg/g of vanillin were released after 6 h of incubation with this enzyme;

Table 1. Effect of Commercially available enzymes on the release of 3 phenolic compounds from sweet potato stems

Enzyme	Concentration	Amount of phenolic compounds released (mg/g DW)		
		Vanillic acid	Vanillin	Cinnamic acid
Ultraflo L	0.00%	<0.20	<0.20	0.55 ± 0.09^{a}
	0.01%	$0.81 \pm 0.07^{j*}$	0.71 ± 0.03^{e}	0.52 ± 0.04^{a}
	0.10%	0.75 ± 0.05^{j}	0.66 ± 0.02^{e}	<0.20
	0.50%	5.18 ± 0.13^{d}	1.91 ± 0.08^{d}	<0.20
	1.00%	8.30 ± 0.19^{c}	7.47 ± 0.13^{b}	<0.20
Viscozyme L	0.00%	<0.20	<0.20	0.54 ± 0.05^{a}
	0.01%	<0.20	<0.20	<0.20
	0.10%	1.68 ± 0.06^{g}	0.33 ± 0.01^{f}	<0.20
	0.50%	8.60 ± 0.12^{b}	5.32 ± 0.15^{c}	<0.20
	1.00%	11.04 ± 0.14^{a}	14.69 ± 0.23^{a}	<0.20
α-Amylase	0.00 mU	<0.20	<0.20	0.52 ± 0.06^{a}
	5.00 mU	1.21 ± 0.06^{i}	<0.20	<0.20
	10.00 mU	2.57 ± 0.08^{e}	<0.20	<0.20
	50.00 mU	1.37 ± 0.05^{h}	<0.20	<0.20
	100.00 mU	1.90 ± 0.04^{f}	<0.20	<0.20

*The statistical significance of the experimental results was assessed by Duncan's multiple range test ($P = 0.05$).

cinnamic acid was not detected in any of these experiments. These findings are important for biotechnological applications, because vanillic acid is used as the starting material for the chemical synthesis of oxygenated aromatic chemicals, such as vanillin (one of the most important flavorings in the food industry) [21,22], and it is a prominent intermediate in the degradation of ferulic acid by many bacteria [23].

We found that the order of efficiency for the enzyme preparations used to induce the release of ferulic acid from sweet potato stems was Ultraflo L > Viscozyme L > α-Amylase. The amount of ferulic acid released in the presence of Ultraflo L was greater than that released in the presence of pure *Aspegillus niger* FAE-III in combination with xylanases [19]. This means that Ultraflo L preparations could be used effectively to release ferulic acid from sweet potato stems. Wheat and sugar beets are also excellent sources of ferulic acid [24,25]. However, the sweet potato stem may be an even more important source, because until now it has been considered an agricultural waste products; thus, its use as a source of ferulic acid would not reduce the availability of an edible agricultural crop. We detected ferulic acid and 3 phenolic compounds – vanillic acid, vanillin, and cinnamic acid – in the sweet potato stem by means of enzymatic hydrolysis. It is known that the processing of cereal cell walls may restrict enzymatic activity, possibly through compression of the cell wall [13]. The use of enzymes may be more effective in releasing ferulic acid, which can then be converted into highly valued products, such as vanillin [19].

The optimal release of ferulic acid was achieved through 12 h of incubation with 1.0% Ultraflo L (yield: >2.0% per g dry weight). This resulted in the release of more than 45% of the ferulic acid content of the sweet potato stem, compared with 16% of the vanillic acid content and 29% of the vanillin content released under the same conditions.

We conclude that the release of the ferulic acid – as well as vanillic acid, vanillin, and cinnamic acid – from sweet potato stems can be achieved through enzymatic hydrolysis. Among the 3 commercially available enzymes tested in this study, Ultraflo L was most effective, and its effect was greater in fresh stems than in dried stems. The release of ferulic acid was also influenced by the enzyme concentration and reaction time; the rate of release of two of the phenolic compounds (vanillic acid and vanillin) correlated with the enzyme concentration.

The applications of ferulic acid and the other phenolic compounds investigated in this study are many and varied. Sweet potato stems are edible, but are considered agricultural wastes products. Their used to produce ferulic acid is facilitated by the use of a simple pre-treatment procedure and inexpensive enzyme, a small amount of which can be used for the mass production of ferulic acid and useful phenolic compounds. Enzymatic hydrolysis may also be an attractive alternative method of producing ferulic acid, because it has the potential to serve as an environmentally friendly mechanism for the production of biological active compounds.

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