Biosci. Biotechnol. Biochem., **68** (12), 2619–2622, 2004

Note

Presence of Higher Alcohols as Ferulates in Potato Pulp and Its Radical-Scavenging Activity

Keita YUNOKI,[1,2] Renaguli MUSA,[1,2] Mikio KINOSHITA,[1] Hiroyuki TAZAKI,[1,2] Yuji ODA,[3] and Masao OHNISHI[1,2,†]

[1]*Department of Bioresource Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan*
[2]*The United Graduate School of Agricultural Science, Iwate University, Morioka, Iwate 020-8550, Japan*
[3]*Department of Upland Agriculture, National Agricultural Research Center for Hokkaido Region, Memuro, Kasai, Hokkaido 082-0071, Japan*

Received July 3, 2004; Accepted September 19, 2004

Higher alcohols with a carbon length ranging from 16 to 30 found in the lipophilic fraction from potato pulp were shown to be present as ferulate and in a free form, but not as wax. Thin-layer chromatography of the neutral lipids in potato pulp indicated a few spots with scavenging activity toward the 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable radical, the major active component being characterized as alkyl ferulate which showed almost the same level of activity as γ-oryzanol.

Key words: potato pulp; alkyl ferulate; octacosanol; radical-scavenging activity; γ-oryzanol

Some 100 thousand tons of potato pulp are produced annually in Hokkaido (northern Japan) as a by-product of starch production. Since this potato pulp is not effectively utilized despite its relatively high nutritional value, the authors established a method of lactic acid fermentation of this potato pulp with the *Rhizopus oryzae* fungus in order to use the pulp as cattle feed.[1)] In addition, fermented potato pulp can be utilized as a foodstuff for improving the quality of bread and noodles.[2)] We have previously described that higher alcohols, which improve lipid metabolism and enhance muscular ability,[3)] were present in the lipophilic fraction from potato pulp and that the concentration of these alcohols was increased by lactic acid fermentation.[4)] Higher alcohols have also been shown to leave the periderm of processed potato tubers and enter potato pulp during starch production, but their form has remained unclear.

A determination of the distribution and form of higher alcohols in lipid classes extracted from potato pulp in this study indicated the presence of alkyl ferulate as the principal compound combined with the higher alcohols. This compound had radical-scavenging activity comparable to that of other natural antioxidants such as γ-oryzanol.

The total lipids in potato pulp were extracted and separated as described previously.[4)] To determine the distribution of aliphatic alcohols, the neutral lipid fraction obtained by silicic acid column chromatography was applied to TLC with a solvent system of hexane–diethyl ether–acetic acid (80:30:1, v/v), and the silica gel zones corresponding to individual spots were scraped off. The alcohol components liberated by methanolysis of the lipophilic components were examined by GC, using the internal standard (nonadecanol) reported previously.[4)] Alternatively, the neutral lipids were fractionated by further silicic acid column chromatography, using stepwise elution with mixtures of hexane and diethyl ether.[5)] Each lipid fraction was also converted to its TMS ether derivative and determined by GC–MS, using a capillary column of ULBON HR-1 (15 m × 0.2 mm, GL Science, Tokyo, Japan).[4)] The column temperature was programmed from 80 °C to 200 °C at 15 °C/min and then increased to 260 °C at 7 °C/min and subsequently at 2 °C/min to a final temperature of 320 °C. All data are expressed as the average from at least two independent experiments.

The radical-scavenging activity was determined by using the DPPH stable radical.[6,7)] Fifty, 100 or 150 μl of a 10 mM ethanol solution of a lipid sample was added to a mixture of 2 ml of a Tris–HCl buffer (100 mM, pH 7.4) and 2 ml of a 0.5 mM DPPH (Sigma) ethanol solution. After stirring, the reaction mixture (4.05 ml, 4.10 ml and 4.15 ml final volume, respectively) was kept for 15 min in the dark, before the absorbance was spectrophotometrically measured at 517 nm. For comparison, commercial γ-oryzanol and α-tocopherol were also examined for their activity. The radical-scavenging activity is expressed as the ratio of the decrease (%) in absorbance

† To whom correspondence should be addressed. Fax: +81-155-49-5549; E-mail: mohnishi@obihiro.ac.jp
Abbreviations: TLC, thin-layer chromatography; TMS, trimethylsilyl; DPPH, 1,1-diphenyl-2-picrylhydrazyl

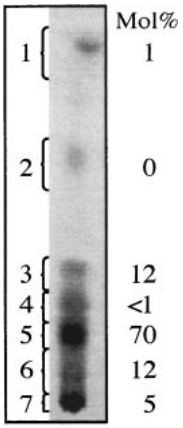

Fig. 1. Thin-Layer Chromatogram of the Neutral Lipid Fraction from Potato Pulp and Distribution (mol%) of Higher Alcohols in Individual Spots (1–7) Determined by GC.

Solvent system, hexane-diethyl ether-acetic acid (80:30:1, v/v). Detection, 50% H_2SO_4.

compared to the control value.[6)]

Total lipids in the potato pulp were present in a yield of 1.2%, the neutral and polar lipid fractions being obtained in the ratio of 9:1. Figure 1 summarizes the distribution of higher alcohols in seven spots (nos. 1–7) of the neutral lipids separated by TLC. Seventy percent of the total higher alcohols was found in spot 5 and 12% in spots 3 and 6. However, hardly any higher alcohols were found in spot 1, which corresponded to wax.[8)] The substances included in spot 5 gave the same *Rf* value as standard free higher alcohol on the TLC plate, but showed a pink reaction to 50% sulfuric acid spraying with subsequent brief heating, similar to γ-oryzanol. A GC–MS analysis of the hexane–diethyl ether extract from the TMS-converted mixture of methanolyzated spot 5 indicated the presence of ferulic acid as well as higher alcohols. Spots 3, 4 and 6 gave the same color reaction as that of spot 5, and their methanolyzates contained ferulic acid and vanillic acid. These results strongly suggest that spots 3 to 6 contained ferulic acid or its ester derivatives which would easily afford the methyl ester of ferulic acid during methanolysis. However, these spots could not be determined in detail due to their small amounts and difficulty for separating into single components. Further direct trimethylsilylation of spot 5 revealed the details.

An analysis of spot 5 as a TMS derivative by GC–MS indicated the mass fragment of m/z 75, originating from the TMS ether of a primary alcohol, with carbon length in the range of 16–30 with a retention time of 25 min (Fig. 2A). However, total ion monitoring gave other peaks with longer retention times. In the mass spectrum of peak *a* in Fig. 2A, a characteristic ion derived from the ferulic acid residue was found at m/z 249 (Fig. 2B).[9,10)] Taken together with ions showing molecular weight at m/z 658 (M^+) and 643 (M − 15), peak *a* was characterized as octacosanyl ferulate.[11)] Other peaks in the mass fragment of m/z 249 were also found to be alkyl ferulates of higher alcohols ranging in carbon length from 16 to 30. The compositions of the higher alcohols present in the free form and ferulate are specified in Fig. 2C. Octacosanol was usually the main component, although somewhat different in alcohol composition between the two lipid classes. The free form had high proportions of higher alcohols with very long chains like octacosanol, while alkyl ferulate was composed mainly of relatively short-chain alcohols. This may indicate different substrate selectivity of the enzymes involved in the biosynthesis or degradation of alkyl ferulates.

When the DPPH solution was sprayed on TLC of the neutral lipid fraction to evaluate the radical-scavenging

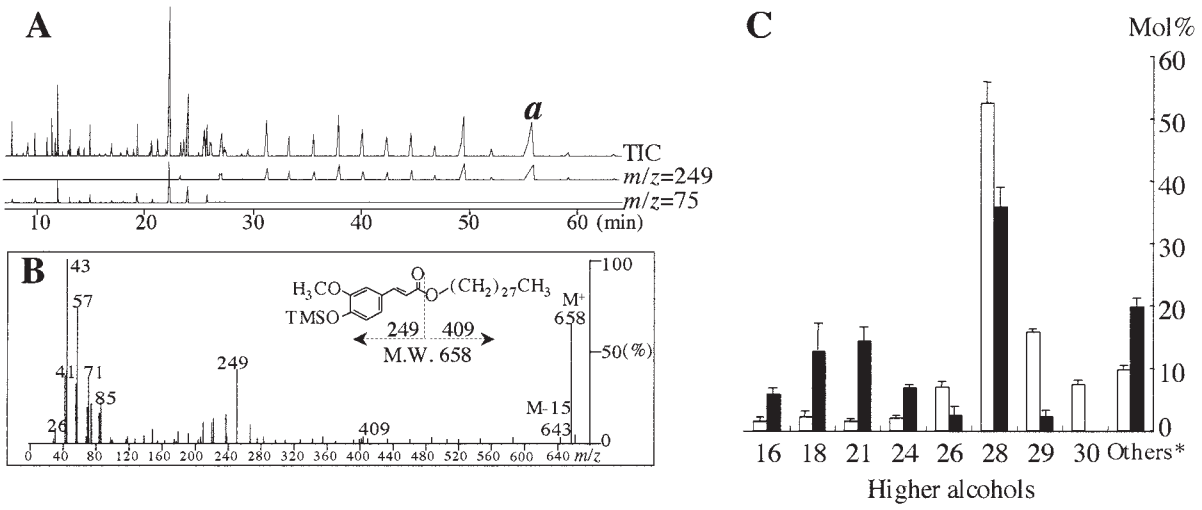

Fig. 2. GC–MS Analysis of TMS Ether Derivatives of Spot 5 Separated by TLC.

(A) TIC, total ion chromatograms. $m/z = 249$ and $m/z = 75$ respectively indicate the selected ion chromatograms of TMS ether derivatives of alkyl ferulates and free alcohols. (B) Mass spectrum of peak *a*. (C) Higher alcohol composition of free type (□) and ferulate (■) in spot 5 (see Fig. 1). *Others contain higher alcohols with carbon length of 20, 22, 23, 25 and 27.

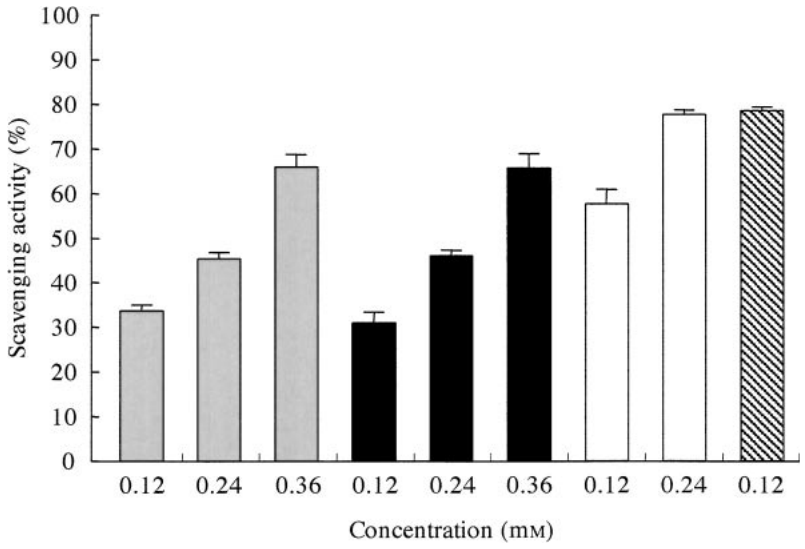

Fig. 3. Evaluation of the Radical-Scavenging Activity of Alkyl Ferulates Separated from the Neutral Lipids in Potato Pulp.
□ alkyl ferulate; ■ γ-oryzanol; □ ferulic acid; ▨ α-tocopherol. Data show the mean ± s.e.m. of three independent experiments.

activity, five spots (corresponding to spots 3, 4, 5, 6 and 7 in Fig. 1) indicated a positive reaction against the radical-scavenging activity. Each fraction prepared from the neutral lipid fraction by silicic acid column chromatography was examined for its radical-scavenging activity. The fraction eluted with 70:30 (v/v) hexane–diethyl ether, in which ferulic acid esters of higher alcohols were included, accounted for 31% of the total amount and showed the highest activity (data not shown).

The alkyl ferulate fraction in spot 5 including free higher alcohols was separated by preparative TLC, and its radical-scavenging activity was compared at the levels of 0.12, 0.24 and 0.36 mM of alkyl ferulate, and γ-oryzanol was estimated as the ferulic acid equivalent (Fig. 3). The radical-scavenging activity of the alkyl ferulate fraction was found to be almost the same as that of commercial γ-oryzanol, a ferulic acid-containing lipid with antioxidative activity,[12] and about 40% and 60% of that of α-tocopherol and ferulic acid, respectively, at 0.12 mM. Since the free higher alcohols present with alkyl ferulate in the fraction did not show any radical-scavenging activity in this assay, the active principal must have been alkyl ferulate.

Bernard and Lewis have reported that alkyl ferulate, present only in the periderm, began to accumulate 3–7 days after wounding the potato tuber.[9] Alkyl ferulate was also found in partial degradation products of suberin obtained by methanolysis with $Ca(OH)_2$ from the potato periderm.[10] Since suberin, which is a complex heteropolymer with both aromatic and lipophilic domains, could not be rendered soluble by organic solvents used for lipid extraction,[13] it is assumed that alkyl ferulate in potato pulp would have been derived from the precursor or degradation products of suberin in the potato periderm.[4] Five ferulic acid derivatives (ferulic acid, ethyl ferulate, isoeugenol, coniferyl alcohol, and coniferyl aldehyde) have recently been comparatively examined for their antioxidative activity, of which ethyl ferulate was similar in its scavenging activity toward the DPPH radical to ferulic acid.[14] However, to our knowledge, there is no report describing the radical-scavenging activity of alkyl ferulate with long-chain fatty alcohols.

We have described here that octacosanol with physiological functions was present mainly as alkyl ferulate which showed antioxidative activity. It is, therefore, expected that fermented potato pulp could be effectively utilized as a functional foodstuff.

Acknowledgments

This work was supported in part by Special Coordination Funds for Promoting Science and Technology (Leading Research Utilization Potential of Regional Science and Technology) of Japanese Ministry of Education, Culture, Sport, Science and Technology.

References

1) Oda, Y., Saito, K., Yamauchi, H., and Mori, M., Lactic acid fermentation of potato pulp by the fungus *Rhizopus oryzae*. *Curr. Microbiol.*, **45**, 1–4 (2002).
2) Yamauchi, H., Noda, T., Matsuura-Endo, C., Takigawa, S., Saito, K., Oda, Y., Funatsuki, W., and Hashimoto, N., Improving the utility of potato pulp for bread-making by fermentation with *Rhizopus oryzae*. *Food Sci. Technol. Res.*, in press.
3) Taylor, J. C., Rapport, L., and Lockwood, G. B., Octacosanol in human health. *Nutrition*, **19**, 192–195 (2003).
4) Musa, R., Yunoki, K., Kinoshita, M., Oda, Y., and Ohnishi, M., Increased levels of policosanol and very long-chain fatty acids in potato pulp fermented with *Rhizopus oryzae*. *Biosci. Biotechnol. Biochem.*, **68**,

2401–2404 (2004).
5) Ohnishi, M., and Fujino, Y., Chemical composition of acyl neutral lipid classes in *Aspergillus oryzae*. *Nippon Nōgeikagaku Kaishi* (in Japanese), **52**, 525–531 (1978).
6) von Gadow, A., Joubert, E., and Hansmann, C. F., Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*), α-tocopherol, BHT and BHA. *J. Agric. Food Chem.*, **45**, 632–638 (1997).
7) Yen, G. C., and Duh, P. D., Scavenging effect of methanolic extracts of peanut hulls on free and active-oxygen species. *J. Agric. Food Chem.*, **42**, 629–632 (1994).
8) Ohnishi, M., Obata, S., and Fujino, Y., Composition and molecular species of waxy lipids in wheat grain. *Cereal Chem.*, **63**, 193–196 (1986).
9) Bernards, M., and Lewis, N. G., Alkyl ferulates in wound healing potato tubers. *Phytochemistry*, **31**, 3409–3412 (1992).
10) Graca, J., and Pereira, H., Suberin structure in potato periderm: glycerol, long-chain monomers, and glyceryl and feruloyl dimers. *J. Agric. Food Chem.*, **48**, 5476–5483 (2000).
11) Malde, A., Claeys, M., Pieters, L. A., Wray, V., and Vlietinck, A. J., Ferulic acid esters from stem bark of *Pavetta owariensis*. *Phytochemistry*, **30**, 1024–1026 (1991).
12) Moldenhauer, K. A., Champagne, E. T., McCaskill, D. R., and Guraya, H., Functional products from rice. In "Functional Foods: Biochemical & Processing Aspects", ed. Mazza, G., Technomic Publishing, Lancaster, pp. 71–89 (1998).
13) Holloway, P. J., Some variations in the composition of suberin from the cork layers of higher plants. *Phytochemistry*, **22**, 495–502 (1983).
14) Nenadis, N., Zhang, H.-Y., and Tsimidou, M. Z., Structure-antioxidant activity relationship of ferulic acid derivatives: effect of carbon side chain characteristic groups. *J. Agric. Food Chem.*, **51**, 1874–1879 (2003).