Analysis of Lutein in Mugwort (Artemisia princeps Pamp.) Paste and Evaluation of Manufacturing Processes


1. INTRODUCTION

Previous studies have shown that oxidized lipids such as phospholipid hydroperoxide (PLOOH) accumulate to abnormally high levels in the red blood cells (RBCs) of patients with Alzheimer’s disease (AD)\(^1,2\). It has been suggested that RBCs with high levels of oxidized lipids (e.g., PLOOH) may have a decreased ability to transport oxygen to the brain, thus, impairing blood rheology and facilitating AD\(^3\)\(^-\)\(^6\).

Any compound that can minimize such an accumulation of RBC PLOOH could therefore be used therapeutically as an effective drug or functional food to prevent AD. We have previously shown that when humans orally ingest lutein, a type of polar carotenoid (xanthophyll), the lutein is absorbed, distributed, and accumulated in the RBC\(^7\)\(^-\)\(^8\). In addition, lutein supplementation reduced RBC PLOOH concentrations, indicating that lutein transported in RBC attenuated phospholipid peroxidation of the RBC membrane\(^8\). We thus considered that lutein could potentially act as an important antioxidant in RBC, and that dietary lutein supplementation may therefore contribute to the prevention of AD. In addition to the effect on AD, scientific interest into xanthophylls including lutein has increased because of their anti-obesity and anti-inflammatory properties\(^9\)\(^-\)\(^11\), characteristic to xanthophylls and not seen in other carotenoids.

These aforementioned results and studies focused our attention on the search for a food source containing lutein. Preliminary screening of various foods showed that Japanese mugwort (Artemisia princeps Pamp.) contains high amounts of lutein\(^12\). The level was roughly comparable to other foods that also contain high amounts of lutein\(^12\). In this study, our aim was to confirm the lutein...
concentration in a processed Japanese mugwort product (mugwort paste) using high-performance liquid chromatography (HPLC) coupled with either visible light detection (Vis) or mass spectrometry (MS). In addition, we investigated the effect of processing methods and the timing of the harvest on the lutein content in mugwort paste. To the best of our knowledge, this is the first study of mugwort lutein for nutraceutical purposes.

2 EXPERIMENTAL

2.1 Chemicals

Lutein, zeaxanthin, and β-cryptoxanthin were purchased from Extrasynthese (Genay, France). α-Carotene and methyl tert-butyl ether (MTBE) were obtained from Wako Pure Chemical Industries (Osaka, Japan). β-Carotene and lycopene were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of the highest grade available.

2.2 Preparation of mugwort paste

Mugwort paste was produced according to the following procedures (Fig. 1). Branches (including the leaves and stems; approximately 15 cm in length) were collected from mugwort (Artemisia princeps Pamp.) at local farms in Zao-machi, Miyagi, Japan during May, June, July, and August, 2015. After washing with water, the mugwort branches were heated in boiling water (containing about 0.3% sodium hydrogen carbonate) for 5 min. The sample was then washed with water, dehydrated by centrifuging in a spin dryer, cut into 3.5 mm, packed in a nylon bag, and sterilized in boiling water for 30 min. After sterilization, the resultant paste was cooled and frozen at −25°C.

2.3 Analysis of lutein and other carotenoids in mugwort paste

Extraction of carotenoids (including lutein) from the mugwort paste was performed according to a published method13 with a slight modification. Commercially sold mugwort paste (Zao Mochikusa, Fukuichi Co., Ltd., Yokohama, Japan) and mugwort paste prepared according to the method described above were first subjected to freeze-drying. A portion of the resultant powder (60 mg) was suspended in 3 mL of 1 mM aqueous ethylenediaminetetraacetic acid and sonicated for 30 min. A portion of the solution (500 µL) was mixed with 2.4 mL of ethanol, 200 µL of 6 M ethanolic pyrogallol, and 150 µL of 11.5 M aqueous potassium hydroxide, and then saponified at 80°C for 2 h. After the addition of 2 mL of water, the sample was further mixed with 3 mL of hexane/diethyl ether (3:1, v/v) containing 0.02% butylated hydroxytoluene. The mixture was centrifuged at 1000 × g for 10 min, and the upper layer (carotenoid fraction) was collected. This extraction was repeated twice and all extraction procedures were conducted at 4°C. The upper layers were combined, evaporated, and dissolved in 500 µL of methanol/MTBE (3:2, v/v). A portion of the extract (10 µL) was subjected to HPLC-Vis or HPLC-MS7. The HPLC system consisted of a Shimadzu LC system, including a vacuum degasser, a quaternary pump, and an autosampler (Shimadzu, Kyoto, Japan). A C30 column (YMC Carotenoid 5 µm, 250 × 4.6 mm I.D., YMC, Kyoto, Japan) was eluted with a binary gradient consisting of the following HPLC solvents: (A) methanol/MTBE/water (83:15:2, v/v/v) containing 3.9 mM ammonium acetate, and (B) methanol/MTBE/water (8:90:2, v/v/v) containing 2.6 mM...
ammonium acetate. The gradient profile was as follows: 0 to 12 min, 10 to 45% B linear; 12 to 24 min, 45 to 100% B linear; 24 to 30 min, 100% B. The flow rate was adjusted to 1 mL/min and the column temperature was maintained at 20°C. The column eluent was sent to either a Shimadzu UV/Vis detector for the detection of carotenoids at 463 nm or a quadrupole-time-of-flight mass spectrometer (microOTOF-QII, Bruker Daltonics, MA, USA) for identification of the carotenoid species. Atmospheric pressure chemical ionization was used as the ion source with the following experimental parameters: capillary, 4500 V; collision energy, 8.0 eV; N₂ nebulizer gas pressure, 1.6 bar; N₂ dry gas flow rate, 8.0 mL/min; N₂ dry gas temperature, 200°C. The collision cell parameters were as follows: collision RF, 700 Vpp; transfer time, 40 µs; pre-pulse storage, 12 µs. Based on the HPLC-Vis data, the concentrations of mugwort carotenoids were calculated using their respective standard curves.

2.4 Effects of process methods on lutein content in mugwort paste

Samples were collected at different timings in the manufacturing process of mugwort paste as shown in Fig. 1 (sample A: between washing and boiling; sample B: between washing and dehydration; sample C: between packing and sterilization; sample D: after freezing). The lutein content in the collected samples was analyzed using the method described above. Analysis was performed on mugwort harvested during May, June, July, and August 2015.

2.5 Statistical analysis

Results are expressed as a mean ± standard deviation (SD). The data were analyzed using one-way ANOVA, and all differences were analyzed using Tukey-Kramer test. Differences were considered to be significant at $p < 0.05$.

3 RESULTS AND DISCUSSION

3.1 Analysis of lutein and other carotenoids in mugwort paste

It is well known that the major carotenoids in vegetables and fruits are xanthophylls (lutein, zeaxanthin, and β-cryptoxanthin) and hydrocarbon carotenoids (α-carotene, β-carotene, and lycopene). In this study, we focused on these carotenoids (especially lutein) because their presence was assumed in mugwort. However, in plants, lutein is not only found in its free form but is also present as fatty acid esters. In order to detect lutein in its free form, in this study, we saponified mugwort paste samples with potassium hydroxide prior to analysis. HPLC analysis using C18 reversed-phase columns have frequently been used for carotenoid analysis; however, the separation of lutein and zeaxanthin cannot be achieved with C18 columns. By contrast, C30 columns offer a stationary phase that enable the separation of lutein and zeaxanthin. A C30 column, therefore, was used to allow for the separation of standard carotenoids evaluated in this study (Fig. 2A). Detection limits of carotenoids with HPLC-Vis were in the range of 0.5–1.5 pmol.

Carotenoids in mugwort paste were analyzed using the HPLC-Vis method. On the HPLC-Vis chromatogram (Fig. 2B), two main peaks were detected, one corresponding to lutein (6.7 min) and the other to β-carotene (15.3 min). The identities of the peaks were determined based on their MS profile and retention times when compared with those of the carotenoid standard. For instance, the peak observed at the retention time of 6.3 min identified $[M - H_2O + H]^+$ at m/z 551.4252 and a small peak of $[M + H]^+$ at m/z 569.4330, which were identical to those of the lutein standard (Fig. 3). In addition, the peak at 14.4 min identified the ion $[M + H]^+$ at m/z 537.4460, which was identical to that of the β-carotene standard. Zeaxanthin, β-cryptoxanthin, α-carotene, and lycopene were all below the detection limits. On the other hand, there were a few peaks on the HPLC-Vis chromatogram that did not match the expected profiles.

HPLC-Vis chromatogram of standard carotenoids (A) and mugwort paste extract (B). Detailed conditions are described in the EXPERIMENTAL section. UK, unknown.
with the carotenoid standards (Fig. 2B). Based on HPLC-MS analysis (data not shown), the m/z value of UK1 corresponded to [C_{40}H_{56}O_4 + H]^+ , which suggests that this peak represents epoxycarotenoids such as neoxanthin. Similarly, the peaks UK2 and UK3 might represent β-carotene isomers including their cis-isomers. However, further evaluation using the respective standards will be necessary to identify these peaks.

Although β-carotene in mugwort has been studied\(^\text{17}\), to the best of our knowledge, this is the first study to provide evidence of the presence of lutein in mugwort. The level of lutein in a commercially sold mugwort paste product was identified as 37.9 mg/100 g dry weight (Table 1). Previous studies have shown that spinach, one of the best natural food sources of lutein, contains about 60–80 mg/100 g dry weight or 5–7 mg/100 g fresh weight\(^\text{12, 18, 19}\) of lutein. Although the methods to determine lutein contents differ between previous studies and the current study, based on these results, mugwort could be a potentially valuable natural food source of lutein. Moreover, greater amounts of lutein than β-carotene were found (Table 1), suggesting that lutein is the predominant carotenoid in mugwort paste. The β-carotene concentration was roughly comparable to that in previous studies\(^\text{17}\).

Physiological properties of carotenoids such as their antioxidant action and protective effect against lipid peroxidation in biological membranes has been identified in previous studies. In addition, xanthophylls including lutein have recently become a focus of scientific interest because of their characteristic functions\(^\text{9, 11}\). Furthermore, we previously identified that lutein supplementation reduced RBC

![Fig. 3](image)

**Fig. 3** HPLC-MS analysis of mugwort paste. Extracted ion chromatograms (XIC) corresponding to lutein (m/z 551.4252) and β-carotene (m/z 537.4460) (A), and mass spectra at the retention times corresponding to lutein (6.3 min) and β-carotene (14.4 min) (B). Detailed conditions are described in the EXPERIMENTAL section.

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>mg/100 g dry weight</th>
<th>mg/100 g fresh weight</th>
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<tr>
<td>Lutein</td>
<td>37.9 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>ND</td>
<td></td>
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<tr>
<td>β-Cryptoxanthin</td>
<td>ND</td>
<td></td>
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<tr>
<td>α-Carotene</td>
<td>ND</td>
<td></td>
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<tr>
<td>β-Carotene</td>
<td>20.3 ± 5.1</td>
<td></td>
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<tr>
<td>Lycopene</td>
<td>ND</td>
<td></td>
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Values given are means ± SD (n = 3).

ND, not detected.

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ND, not detected.
PLOOH concentrations in humans. The present findings (Fig. 2 and Table 1) along with the above-mentioned studies indicate that mugwort paste could be used as a new and effective dietary source of lutein for nutraceutical purposes.

3.2 Effects of process methods on lutein content in mugwort paste

The lutein content in mugwort paste was evaluated in samples collected at different timings during the manufacturing process (Fig. 1, samples A–D). As a result, it was revealed that the lutein content in mugwort was high in samples C (collected after boiling and washing in water) and D (collected after dehydrating, cutting, and packing) (Fig. 4). This is presumably because the water-soluble components in mugwort (e.g., proteins) were removed, especially during the boiling and dehydrating processes, and as a consequence, the lutein was condensed. In other words, lutein was not decomposed during the manufacturing process, even though the washing and boiling processes often reduce the contents of functional ingredients such as carotenoids and polyphenols. The persistence of lutein after boiling and heat processes was further confirmed by the fact that the sterilizing process did not reduce the lutein content in mugwort paste (Fig. 4, samples C and D). A similar trend was noted with β-carotene as well (data not shown).

Mugwort is generally harvested between June and August, and we assumed that the lutein content in mugwort paste would be higher during these times. This was somewhat confirmed in this study, since the mugwort paste manufactured from mugwort harvested in June and August had high lutein content (Fig. 4). However, the effect of harvest timing on lutein content was less than what was anticipated, and further studies are necessary to evaluate this hypothesis.

It is also worth mentioning that the mugwort paste analyzed above was produced from the leaves and stems of mugwort. In order to evaluate the part of the plant that contains more lutein, we individually analyzed the lutein content in the leaves and stems. The leaves and stems were collected in June 2015, and freeze-dried prior to extraction. The lutein content in the stem was 1 mg/100 g dry weight, whereas that in the leaf was 15 mg/100 g dry weight. Therefore, by using mugwort leaves as the ingredient, rather than the stems, the lutein content in mugwort paste can supposedly be increased. Mugwort paste, such as that explained above, rich in lutein, may serve as an effective nutraceutical.

Acknowledgements

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Conflict of interest

None of the authors have any conflicts of interest.

REFERENCES


