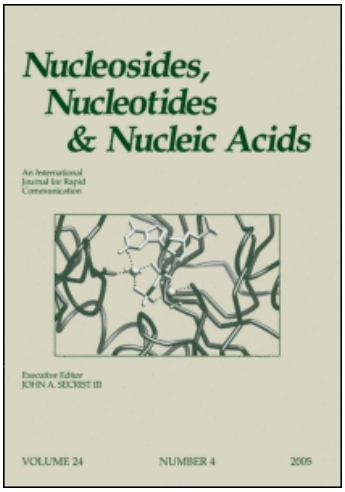

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ANALYSIS OF PURINE IN PURINE-RICH CAULIFLOWER

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□ *Purine is a general term for purine nucleotides, nucleosides, bases, and nucleic acid. The amount of purine nucleotides, nucleosides, and bases in purine-rich cauliflower was determined with the use of LC-MS and HPLC, and the ratio of these molecules were compared with in raw and in heated condition. Total purine content of raw and heated cauliflower was 42.6 and 43.2 mg/100 g, respectively. Nucleotide content was increased from 0.02 to 50.8 μmol/100 g, and nucleoside content was decreased from 12.4 to 7.7 μmol/100 g, by heating.*

Keywords Purine; nucleotides; nucleosides; cauliflower; LC-MS; HPLC

INTRODUCTION

Purine compounds, including nucleotides, nucleosides, and bases, are converted to uric acid by the purine metabolism pathway. It has been reported that purine nucleotide, nucleoside, and base have different effect on the elevation of serum uric acid level.[1] These molecules are considered to be different in their absorption, utilization, metabolism, and bioavailability.

Hyperuricemia is related to daily lifestyle. The serum uric acid level in individuals who usually consume many purine-rich foods has been reported to be higher than those who consume less of these foods.[2]

In order to investigate the effect of purine-rich foods on the elevation of serum uric acid level, it is useful to examine each amount of nucleoside, nucleotide, base, and nucleic acid, in food. In this study, purine nucleotides, nucleosides, and bases were determined using liquid chromatography–mass spectrometry (LC-MS) and high performance liquid

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chromatography (HPLC), and the purine content was compared with in raw and heated cauliflower.

MATERIALS AND METHODS

Cauliflower purchased at local stores was used as a purine-rich vegetable. After homogenizing cauliflower, the samples were centrifuged using a filter with a 30-kDa cutoff.

Analysis of purine nucleosides and nucleotides was performed by LC-MS. The analytical conditions were as follows: instrument, Waters UPLC-ZQ; column, HSS T3 2.1 mm ID and 100 mm length; mobile phase, 1.25 mM dihexylammonium acetate in 10 mM HCOOH–$HCOONH_4$ (pH 5.0) and 5–50% of acetonitrile; flow rate, 0.3 mL/min; column temperature, 10°C; detector wavelength, 260 nm; ionization, electrospray ionization (ESI); selected ion monitoring, positive ions for nucleosides (adenosine, *m/z* 268; inosine, *m/z* 269; guanosine, *m/z* 284; and xanthosine, *m/z* 285), and negative ions for nucleotides (AMP, *m/z* 346; IMP, *m/z* 347; GMP, *m/z* 362; XMP, *m/z* 363; ADP, *m/z* 426; IDP, *m/z* 427; GDP, *m/z* 442; ATP, *m/z* 506; GTP, *m/z* 522); software, Empower 2.

Purine bases and total purine content were quantitatively determined with HPLC as previously reported.[3] The analytical conditions were as follows: instrument, Shimadzu LC10A HPLC system with the SIL-10AD auto-injector; column, Shodex Asahi Pak HQ-310 (7.5 mm ID and 300 mm length); mobile phase, 150 mM sodium phosphate buffer (pH 2.5); flow rate, 0.6 mL/min; column temperature, 35°C; and detector wavelength, 260 nm. Total content was calculated by the sum of four purine bases: adenine, guanine, hypoxanthine, and xanthine. Free purine bases were determined by the same method without hydrolysis with 70% perchloric acid. Amounts of purines are shown as the mean of two measurements in triplicate experiments.

RESULTS

The purine nucleosides (inosine, guanosine, adenosine, and xanthosine) and nucleotides (GMP, IMP, AMP, XMP, IDP, GDP, ADP, GTP, and ATP) were well separated by LC-MS. The retention time of nucleosides were 8.3, 5.8, 6.1, and 9.3 minutes for adenosine, inosine, guanosine, and xanthosine, respectively; those of nucleotides were 24.9, 24.1, 23.7, 31.2, 33.5, 32.9, 32.8, 40.4, 39.9 minutes for AMP, IMP, GMP, XMP, ADP, IDP, GDP, ATP, and GTP, respectively.

The amount of nucleosides and nucleotides is shown in Table 1. Raw cauliflower contained 12.4 μmol/100 g purine nucleosides and 0.02 μmol/100 g purine nucleotides, respectively. Cauliflower heated with

TABLE 1 Amount of purine nucleosides and nucleotides in cauliflower

Nucleosides	Adenosine	Guanosine	Inosine	Xanthosine	(μmol/100 g) Total
Raw	0.30	12.1	n.d.	n.d.	12.4
Heated	6.40	1.31	n.d.	n.d.	7.7
Nucleotides	**AMP**	**GMP**	**IMP**	**XMP**	(μmol/100 g) ADP
Raw	n.d.	0.02	n.d.	n.d.	n.d.
Heated	14.8	0.74	n.d.	n.d.	15.1
	GDP	IDP	ATP	GTP	Total
Raw	n.d.	n.d.	n.d.	n.d.	0.02
Heated	2.56	n.d.	14.0	3.60	50.8

n.d.: not detected

a microwave contained 7.7 μmol/100 g purine nucleosides and 50.8 μmol/100 g purine nucleotides. Nucleotide content was increased but nucleoside content was decreased, by heating.

The purine content is summarized in Table 2. The content of total purine bases of raw and heated cauliflower was 42.6 and 43.2 mg/100 g, respectively. These values were similar as expected. The quantity of free bases decreased after heating. The purine content derived from DNA and RNA was calculated as 34.7 and 34.9 mg/100g in raw and heated cauliflower, respectively.

DISCUSSION

The purine content, derived from different kinds of purine molecules, was successfully determined by LC-MS and HPLC, indicating these methods

TABLE 2 Purine content

Cauliflower (raw)[a]	Adenine	Guanine	Hypoxanthine	Xanthine	(mg/100 g) Total
After $HClO_4$ treatment (total purines)	17.9	21.9	2.6	0.2	42.6
Detected as free bases	3.4	1.9	0.6	0.03	5.9
Detected as nucleosides and nucleotides	0.04	1.8	0.00	0.00	1.9
Cauliflower (heated)[b]	**Adenine**	**Guanine**	**Hypoxanthine**	**Xanthine**	(mg/100 g) Total
After $HClO_4$ treatment (total purines)	18.2	21.7	3.2	0.1	43.2
Detected as free bases	0.06	0.08	0.02	0.03	0.2
Detected as nucleosides and nucleotides	6.8	1.2	0.00	0.00	8.0

[a]Dervived from DNA and RNA: 34.7 mg/100 g.
[b]Derived from DNA and RNA: 34.9 mg/100g.

to be very useful. Using these methods, change in ratio of purine nucleosides, nucleotides, and bases by heating was determined in cauliflower. As the effects of these molecules on serum uric acid level are different from each other, future studies should focus on examining the ratio of these purine molecules in various foods.

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