

<Original Article>

## The effect of preparatory conditions of high temperature- and pressure-treated garlic on 5-hydroxymethyl-2-furfural and *S*-allylcysteine formation

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Hidehiko Beppu<sup>1)</sup>, Takashi Higashiguchi<sup>2)</sup> and Shigeru Sonoda<sup>1)</sup>

**Summary** We have previously reported that the feeding of high temperature- and pressure-treated garlic (HTPG) reduced preneoplastic lesions at initiation and post-initiation-stage of chemical induced rat colorectal tumor significantly. The HTPG was prepared by using an autoclave (130°C, 150 min). We confirmed that HTPG contained 5-hydroxymethyl-2-furfural (5-HMF) which was metabolized to genotoxic and mutagenic 5-sulfooxymethylfurfural (SMF). On the other hand, HTPG also contains *S*-allylcysteine (SAC), which has been demonstrated to have chemopreventive effects on a colon cancer model in mice. Therefore, we attempted to prepare HTPG in new condition and examined the production of 5-HMF and SAC. As a result, we showed that the condition for autoclaving at 130°C for 60 min may be better than the previous condition.

**Key words:** High temperature- and pressure-treated garlic (HTPG), 5-hydroxymethyl-2-furfural (5-HMF), *S*-allylcysteine (SAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH)

### 1. Introduction

Recently, Kwon et al.<sup>1)</sup> reported that the antioxidant activity and total both polyphenol and flavonoid contents of garlic juice were increased by high temperature- and pressure-treatment (130°C, 120 min). We tried to make high temperature- and pressure-treated garlic (HTPG) using an autoclave (130°C, 150 min) rapidly and easily. Our attempt showed that antioxidant activity and total polyphenol content were increased<sup>2)</sup>, as well as *S*-allylcysteine (SAC), which

was a water-soluble organosulfur compound with preventive effects on colon cancer induced by 1,2-dimethylhydrazine (DMH) in mice<sup>3)</sup>. We have previously reported that the feeding of HTPG reduced preneoplastic lesions at initiation and post-initiation-stage of chemical induced rat colorectal tumor significantly<sup>4,5)</sup>.

Kwon et al.<sup>1)</sup> reported that the HTPG contained 5-hydroxymethyl-2-furfural (5-HMF) which would be metabolized to genotoxic and mutagenic 5-sulfooxymethylfurfural (SMF). We also confirmed

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that our HTPG contained 5-HMF by HPLC analysis (Chihara et al., unpublished data). 5-HMF is one of the major intermediate in a so called non-enzymatic Maillard reaction, which occurs during the heating of sugars and amino acids<sup>6)</sup>.

The U.S. Food and Drug Administration (FDA) approved the use of 5-HMF for the treatment of sickle cell disease in 2006<sup>7)</sup>. However, the 5-HMF was reported as a carcinogenic substance<sup>8, 9)</sup>. The U.S National Toxicology Program (NTP) showed that high-dose (188 mg/kg and 375 mg/kg) oral administration of 5-HMF increased the incidence of hepatocellular adenomas in only female mice by 2-year gavage studies using mice and rats. But it is suggested that 5-HMF is generally non-carcinogenic at low doses<sup>10)</sup>. The average daily intake of 5-HMF in humans was reported to be in the range of 30-150 mg<sup>11)</sup>. Surh and Tannenbaum reported that 5-sulfooxymethylfurfural (SMF) was metabolically formed from 5-HMF by sulfotransferase in rodent liver cytosols and exhibited genotoxicity and mutagenicity<sup>6)</sup>. However, the studies of 5-HMF metabolism in rodents and humans showed that the SMF was not detected<sup>12, 13)</sup> in the subjects. In 2009, Monien et al.<sup>14)</sup> detected SMF in the plasma of mice after intravenous 5-HMF administration. Therefore, we reexamined preparative conditions of HTPG by analyzing the concentration of 5-HMF, SAC, and assaying radical-scavenging activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH).

## 2. Materials and Methods

### 1. Preparation of HTPG samples

Garlic flakes (Amari Spice Foods Co., Ltd., Kyoto, Japan) were pulverized using an Oster Commercial Blender (Osaka Chemical Co., Ltd., Osaka, Japan). Then 100 g of the pulverized garlic was mixed with 250 ml of boiling Milli-Q water to inactivate alliinase. After that, the mixture was heated and the pressure loaded to it by using an autoclave (Tomy SX-500, Tomy Seiko Co., Ltd., Tokyo, Japan) with different temperature and time. The temperature was examined at 110, 120, and 130°C, and the time in the autoclave ranged from 30 to 180 minutes, with 30-minute intervals at each temperature. After the

autoclave treatment, the mixture was freeze-dried and pulverized using an Oster Power Blender (Osterizer, Osaka Chemical Co., Ltd., Osaka, Japan). Prepared powder was used the experiment as HTPG sample.

### 2. Analysis of 5-HMF concentration in HTPG samples

0.100 g of the prepared HTPG sample was suspended with 5 ml of Milli-Q water, and vortexed vigorously for 1 min. This mixture was centrifuged for 10 min at  $17,400 \times g$ . The 5-HMF concentration of the supernatant was analyzed according to the modified method of Makawi et al.<sup>15)</sup>. The analysis was carried out using a high-performance liquid chromatography (HPLC) system equipped with a Model PU-980 intelligent HPLC pump (Jasco Co., Tokyo, Japan), a Model UV-970 intelligent UV/VIS detector (Jasco Co., Tokyo, Japan) at 275 nm, and an integrator (Chromatocorder 21, System Instruments Co., Ltd., Tokyo, Japan). An analytical column, TSKgel ODS-100V,  $250 \times 4.6$  mm (Tosoh Co., Tokyo, Japan), with the same type of guard column was employed. 5-HMF was eluted with 5% acetic acid/methanol (9:1, v/v) at a flow rate of 0.8 ml/min. The standard sample of 5-HMF for HPLC analysis was purchased from Sigma-Aldrich ( St. Louis, MO, USA).

### 3. Analysis of SAC concentration in HTPG samples

0.500 g of the prepared HTPG sample was suspended with 5 ml of 5% trichloroacetic acid and shaken for 10 min. This mixture was centrifuged for 10 min at  $2,000 \times g$  and the supernatant was transferred to a 25 ml measuring flask. We repeated this procedure three times and the volume of the supernatant was adjusted to 25.0 ml by adding 5% trichloroacetic acid. After that, the mixture was filtrated by a  $0.22 \mu\text{m}$  Omnipore membrane filter, and the SAC concentration was analyzed by the modified method of Imai et al.<sup>16)</sup> which used HPLC with *O*-phthalaldehyde postcolumn derivatization. Briefly, the HPLC system was run at 40°C and equipped with a TSKgel Aminopak,  $120 \times 4.6$  mm (Tosoh Co., Tokyo, Japan). The mobile phase of 50 mM sodium citrate buffer (pH 4.0) was pumped at 0.6 ml/min. The eluted SAC was transformed with 6 mM

*O*-phthalaldehyde reagent running by another pump at a flow rate of 0.4 ml/min and detected with a fluorescence detector at excitation and emission wavelengths of 340 and 455 nm, respectively. The standard sample of SAC for HPLC analysis was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

#### 4. Assay of DPPH radical-scavenging activity

0.010 g of the prepared HTPG sample was mixed with 1 ml of Milli-Q water and shaken for 30 min. The mixture was centrifuged for 10 min at  $17,400 \times g$  and the supernatant used for the assay. DPPH radical-scavenging activity was measured by HPLC using the method of Yamaguchi et al.<sup>17</sup>. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used to make the standard curve. Thus the activity was calibrated as mM Trolox/g powder  $\cdot$  min).

### 3. Results

#### 1. Changes of 5-HMF concentration

5-HMF was not detected by autoclaving at 110°C and 120°C for 150 min. However, the concentration of 5-HMF was increased time dependently by

autoclaving at 130°C. 5-HMF concentration at 130°C were 0.01 mg for 30-min and 2.09 mg/g for 150-min. Thus its concentration for 150-min was about 210-fold higher than that for 30-min (Fig. 1).

#### 2. Changes of SAC concentration

The concentration of SAC by autoclaving at 110°C was 1.76 and 3.48 mg/g powder for 30 and 150-min, respectively. The latter was about twice as much as that after 30-min. The concentration of SAC by autoclaving at 120°C was 2.69 and 4.55 mg/g powder for 30 and 150-min, respectively. The concentration of SAC at 150-min treatment was about 1.7 times greater than that at 30-min. On the other hand, the SAC concentration by autoclaving at 130°C was 3.73 and 4.59 mg/g powder for 30 and 60-min, respectively. The increasing of SAC was only observed until 60 min, the concentration of SAC decreased to 4.57 and 2.45 mg/g powder for 90 and 150-min, respectively (Fig. 2).

#### 3. DPPH radical-scavenging activity

DPPH radical-scavenging activity by autoclaving was increased depending on both treatment time and

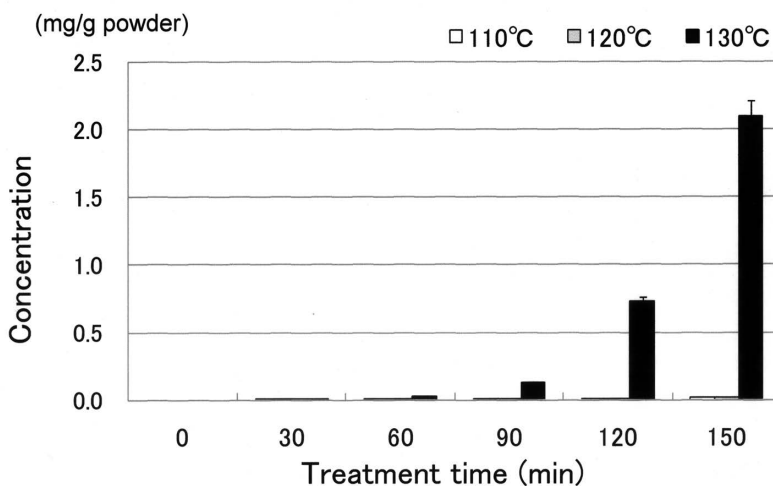


Fig. 1 The effects of time on change of 5-HMF concentration. 5-HMF concentration was almost completely absent by autoclaving at 110°C and 120°C. However, concentrations by autoclaving at 130°C increased depending on the treatment time. Error bars represent standard errors from the mean.

temperature as previously reported<sup>2)</sup>. The activity at 110°C for 150-min was 0.73 mM Trolox/g powder · min, but those at 120°C and 130°C were 1.65 and 7.45 mM Trolox/g powder · min, respectively (Fig. 3).

#### 4. Discussion

We have previously reported that HTPG made

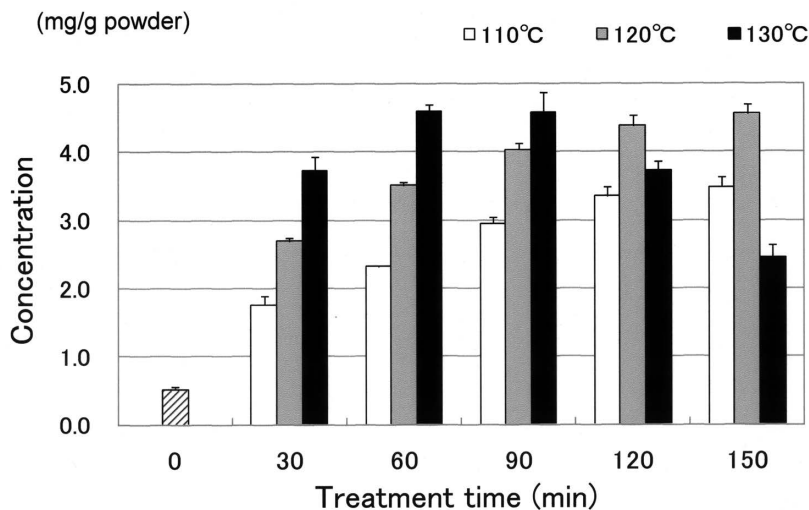


Fig. 2 The effects of time on change of SAC concentration. SAC concentrations were increased by autoclaving at 110°C and 120°C depending on the treatment time. However, concentrations by autoclaving at 130°C reached the maximum at 60 min. Error bars represent standard errors from the mean.

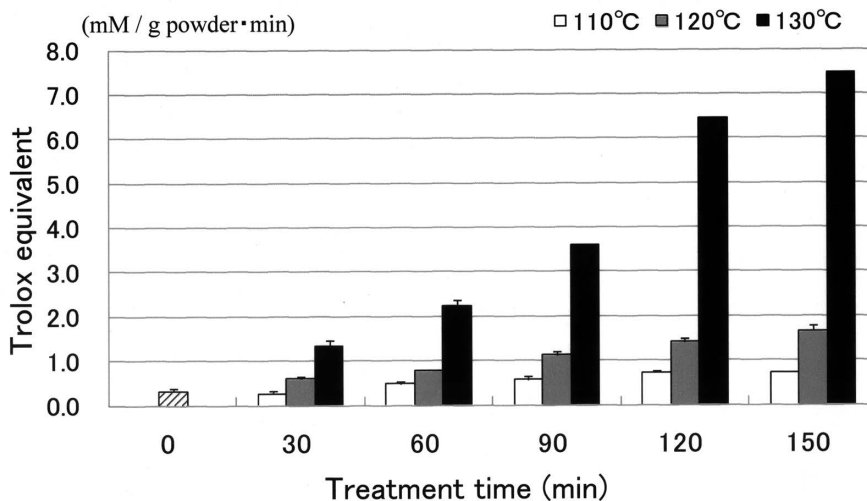


Fig. 3 The effects of time on change of DPPH radical scavenging activity. DPPH radical scavenging activity by autoclaving at 110°C and 120°C was not markedly increased. However, its activity by autoclaving at 130°C increased depending on the treatment time. Error bars represent standard errors from the mean.

with an autoclave (130°C, 150 min) reduced DMH-induced preneoplastic lesions in the rat colorectum<sup>4,5</sup>. In those experiments, we analyzed and discussed only SAC concentrations and DPPH radical-scavenging activity. In the present study, we analyzed 5-HMF which would be metabolized to genotoxic and mutagenic 5-sulfooxymethylfurfural (SMF) and confirmed that the concentration of 5-HMF increased depending on the treating time under the current conditions. We have calculated the daily intake of 5-HMF in rats to 5.79 mg/kg body weight in our previous experiment<sup>5</sup>. Two-year gavage study using mice in the NTP (National Toxicology Program), 5-HMF increased the incidence of hepatocellular adenomas<sup>10</sup>. Daily intakes in the NTP study were carried out with 188 and 375 mg/kg body weight, which was 32 to 64 times greater than the amount of our experiment, respectively. Therefore, daily intake in our previous experiment was able to consider no influence on hepatocellular adenomas, since the daily dosage of 5-HMF in the NTP study was too high. It has become clear that the administration of 5-HMF gave beneficial effects like what antioxidant effects<sup>18</sup> and reducing the risk of life style-related diseases<sup>19</sup>. 5-HMF can protect human hepatocytes against cytotoxicity

induced by hydrogen peroxide<sup>20</sup>. Additionally, 5-HMF suppressed tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )-stimulated vascular cell adhesion molecule-1 (VCAM-1) expression and adhesion of monocytes to the endothelium in human umbilical vein endothelial cells (HUVECs) by inhibiting the activation of redox-sensitive transcription factor NF- $\kappa$ B and reducing the rate of reactive oxygen species formation<sup>21</sup>.

It has been reported that SMF is metabolically formed from 5-HMF by hepatic sulfotransferase and exhibits genotoxicity and mutagenicity<sup>6</sup>. However, SMF was not detected in humans and rodents. On the other hand, Monien et al.<sup>14</sup> reported that SMF was detected in the plasma of 5-HMF-treated mice. It means that considerable quantity of SMF may be formed by the ingestion of foods contained 5-HMF in humans, and reducing the concentration of 5-HMF in HTPG is very important as a health supplement.

SAC concentrations after autoclaving at 130°C reached the maximum at 60 min and the minimum at 150 min. Yamazaki and Okuno<sup>22</sup> have reported that the warming of garlic bulbs at 55°C for 2 weeks resulted in an accumulation of SAC. The concentration reported by their study was nearly equal to that after 60

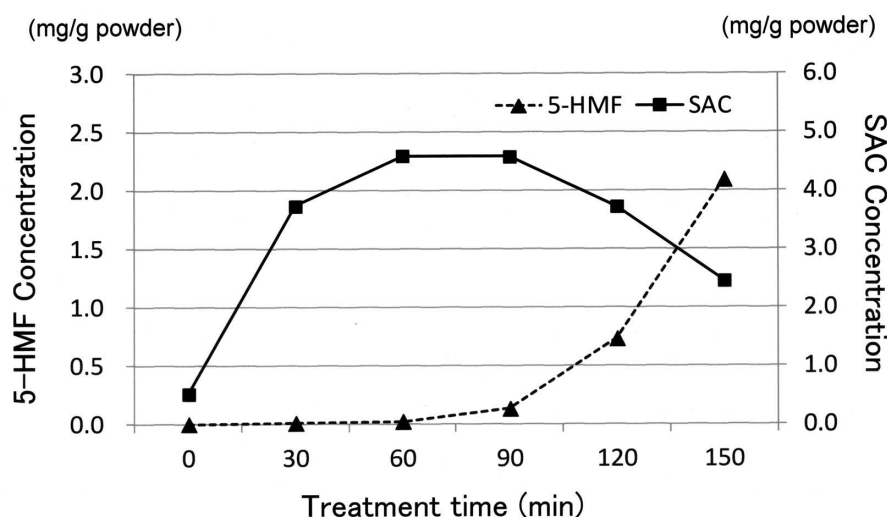


Fig. 4 The effects of time on change of 5-HMF and SAC concentrations by autoclaving at 130°C. Concentrations of 5-HMF did not increase and those of SAC reached the maximum at 60 min.

min at 130°C in our study. It is known that SAC is transformed from  $\gamma$ -glutamyl-S-allylcysteine (GSAC) by the hydrolysis of  $\gamma$ -glutamylpeptidase during aging in water or dilute ethanol<sup>23</sup>. However, it was unclear whether SAC accumulated in the treatment conditions<sup>22</sup>. SAC has been reported to have not only antioxidative activities, but also inhibits the growth of DMH-induced colon cancer in mice<sup>3</sup> as well as the growth of both cultured human neuroblastoma cells<sup>24</sup> and breast carcinoma cells<sup>25</sup>. Considering the SAC concentrations determined in our study at 130°C treatment, 150-min is too long to reach higher concentrations. It may be caused by the progression of the Maillard reaction<sup>22</sup>.

DPPH radical-scavenging activity by autoclaving at 130°C increased time dependently, and reached the maximum at 150 min. However, DPPH radical-scavenging activity by autoclaving at 110°C and 120°C was not markedly increased. In 2007, Hwang et al.<sup>26</sup> identified the antioxidant substance in heated garlic juice (130°C, 120 min) as thiacremonone (2,4-dihydroxy-2,5-dimethyl-thiophene-3-one). Additionally, Ban et al.<sup>27</sup> also reported that thiacremonone inhibited colon cancer cell growth, inducing apoptotic cell death by modulating nuclear transcription factor- $k\beta$ . Thiacremonone may not be increased by autoclaving at 110°C and 120°C and unfortunately, we could not confirm whether HTPG contained this antioxidative substance or not.

In conclusion, the current condition (130°C, 150 min) of HTPG preparation showed that the concentration of 5-HMF increased and that of SAC decreased, although the DPPH radical scavenging activity was enhanced. In our newly presented condition (130°C, 60 min), the concentration of SAC increased (Fig. 4) and DPPH radical scavenging activity slightly enhanced and that the concentration of 5-HMF did not changed. These results suggest that HTPG prepared using the new condition will be more useful as a health supplement than those of previous conditions. However, 5-HMF might be useful for health within an acceptable daily intake. Therefore, we want to examine the inhibitory effects of HTPG prepared in our new condition on preneoplastic lesions in the rat colorectum. This experiment is now

underway in our laboratory.

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#### Disclosures

Conflict of interest and financial disclosure: none.

#### References

- 1) Kwon OC, Woo KS, Kim TM, Kim DJ, Hong JT, Jeong HS: Physicochemical characteristics of garlic (*Allium sativum* L.) on the high temperature and pressure treatment. *Korean J Food Sci Technol*, 38: 331-336, 2006.
- 2) Tomatsu A, Chihara T, Kaneko T, Beppu H, Sonoda S, Shimpo K: Antioxidative effects of high temperature and pressure-treated garlic[Jpn]. *Fujita-Gakuen Igakkaishi (Bull Fujita Med Soc)*, 31: 173-176, 2007.
- 3) Sumiyoshi H and Wargovich MJ: Chemoprevention of 1,2-dimethylhydrazine-induced colon cancer in mice by naturally occurring organosulfur compounds. *Cancer Res*, 50: 5084-5087, 1990.
- 4) Chihara T, Shimpo K, Kaneko T, Beppu H, Tomatsu A, Sonoda S: Inhibitory effects of high temperature- and pressure-treated garlic on formation of 1,2-dimethylhydrazine-induced mucin-depleted foci and O<sup>6</sup>-methylguanine DNA adducts in the rat colorectum. *Asian Pac J Cancer Prev*, 10: 827-831, 2009.
- 5) Chihara T, Shimpo K, Kaneko T, Beppu H, Tomatsu A, Higashiguchi T, Sonoda S: Effects of high temperature- and pressure-treated garlic on 1,2-dimethylhydrazine-induced premalignant lesions in the rat colorectum at the post-initiation stage[Jpn]. *Nippon Shokuhin Kagaku Kogaku Kaishi*, 58: 131-135, 2011.
- 6) Surh YJ and Tannenbaum SR: Activation of the maillard reaction product 5-(hydroxymethyl) furfural to strong mutagens via allylic sulfonation and chlorination. *Chem Res Toxicol*, 7: 313-318, 1994.
- 7) Vogin GD: New FDA Orphan drugs: CellCept, ISIS 301012, 5-HMF. *Medscape Medical News*, Jun 09, 2006. <http://www.medscape.com/viewarticle/535806>.
- 8) Miyakawa Y, Nishi Y, Kato K, Sato H, Takahashi M, Hayashi Y: Initiating activity of eight pyrolysates of carbohydrates in a two-stage mouse skin tumorigenesis model. *Carcinogenesis*, 12: 1169-1173, 1991.
- 9) Zhang XM, Chan CC, Stamp D, Minkin S, Archer MC, Bruce, WR: Initiation and promotion of colonic aberrant crypt foci in rats by 5-hydroxymethyl-2-

- furaldehyde in thermolyzed sucrose. *Carcinogenesis*, 14: 773-775, 1993.
- 10) National Toxicology Program: NTP toxicology and carcinogenesis studies of 5-(hydroxymethyl)-2-furfural (CAS No. 67-47-0) in F344/N rats and B6C3F1 mice (gavage studies). *Natl Toxicol Program Tech Rep Ser*, 554: 7-31, 2010.
  - 11) Janzowski C, Glaab V, Samimi E, Schlatter J, Eisenbrand G: 5-Hydroxymethylfurfural: assessment of mutagenicity, DNA-damaging potential and reactivity towards cellular glutathione. *Food Chem Toxicol*, 38: 801-809, 2000.
  - 12) Godfrey VB, Chen LJ, Griffin RJ, Lebetkin EH, Burka LT: Distribution and metabolism of (5-hydroxymethyl)furfural in male F344 rats and B6C3F1 mice after oral administration. *J Toxicol Environ Health A*, 57: 199-210, 1999.
  - 13) Prior RL, Wu X, Gu L: Identification and urinary excretion of metabolites of 5-(hydroxymethyl)-2-furfural in human subjects following consumption of dried plums or dried plum juice. *J Agric Food Chem*, 54: 3744-3749, 2006.
  - 14) Monien BH, Frank H, Seidel A, Glatt, H: Conversion of the common food constituent 5-hydroxymethylfurfural into a mutagenic and carcinogenic sulfuric acid ester in the mouse in vivo. *Chem Res Toxicol*, 22: 1123-1128, 2009.
  - 15) Makawi SZA, Taha MI, Zakaria BA, Siddig B, Mahmod H, Elhussein ARM, Kariem EAG: Identification and quantification of 5-hydroxymethyl furfural HMF in some sugar-containing food products by HPLC. *Pakistan journal of nutrition*, 8: 1391-1396, 2009.
  - 16) Imai J, Ide N, Nagae S, Moriguchi T, Matsuura H, Itakura Y: Antioxidant and radical scavenging effects of aged garlic extract and its constituents. *Planta Med*, 60: 417-420, 1994.
  - 17) Yamaguchi T, Takamura H, Matoba T, Terao J: HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Biosci Biotechnol Biochem*, 62: 1201-1204, 1998.
  - 18) Yong-Xin Li, Li Y, Qian Z-J, Kim M-M, Kim S-K: In vitro antioxidant activity of 5-HMF isolated from marine red alga *Laurencia undulata* in free radical mediated oxidative systems. *J Microbiol Biotechnol* 19: 1319-1327, 2009.
  - 19) Gato N and Oka K: Impact of 5-HMF as a fragrant micronutrient[Jpn]. *Aroma Res*, 40: 340-346, 2009.
  - 20) Ding X, Wang MY, Yao YX, Li GY, Cai BC: Protective effect of 5-hydroxymethylfurfural derived from processed *Fructus Corni* on human hepatocyte LO2 injured by hydrogen peroxide and its mechanism. *J Ethnopharmacol*, 128: 373-376, 2010.
  - 21) Kim HK, Choi Y-W, Lee EN, Park JK, Kim S-G, Park D-J, Kim B-S, Lim Y-T, Yoon S: 5-Hydroxymethylfurfural from black garlic extract prevents TNF  $\alpha$  -induced monocytic cell adhesion to HUVECs by suppression of vascular cell adhesion molecule-1 expression, reactive oxygen species generation and NF- $\kappa\beta$  activation. *Phytother Res*, 25: 965-974, 2011.
  - 22) Yamazaki Y and Okuno T: Accumulation of S-allyl-L-cysteine in garlic bulbs by warming[Jpn]. *Nippon Shokuhin Kagaku Kogaku Kaishi*, 55: 410-415, 2008.
  - 23) Edited by HP. Koch and LD. Lawson: LD. Lawson: The composition and chemistry of garlic cloves and processed garlic. *Garlic: The science and therapeutic application of *Allium sativum* L. and related species*, 2nd ed. 37-107, Williams & Wilkins, USA (1996)
  - 24) Welch C, Wuarin L, Sidell N: Antiproliferative effect of the garlic compound S-allyl cysteine on human neuroblastoma cells in vitro. *Cancer Lett*, 63: 211-219, 1992.
  - 25) Li G, Qiao CH, Lin RI, Pinto J, Osborne MP, Tiwari RK: Antiproliferative effects of garlic constituents in cultured human breast-cancer cells. *Oncol Rep*, 2: 787-791, 1995.
  - 26) Hwang IG, Woo KS, Kim DJ, Hong JT, Hwang BY, Lee YR, Jeong HS: Isolation and identification of an antioxidant substance from heated garlic (*Allium sativum* L.). *Food Sci Biotechnol*, 16: 963-966, 2007.
  - 27) Ban JO, Yuk DY, Woo KS, Kim TM, Lee US, Jeong H-S, Kim DJ, Chung YB, Hwang BY, Oh KW, Hong JT: Inhibition of cell growth and induction of apoptosis via inactivation of NF- $\kappa\beta$  by a sulfur compound isolated from garlic in human colon cancer cells. *J Pharmacol Sci*, 104: 374-383, 2007.