Wageningen Academic Publishers

Effect of heating on the content and composition of ginkgolic acids in ginkgo seeds

G.-J. Fan[1,2], X. Wang[1,2], C.-E. Wu[1,2*], H.-M. Pan[1,2], J.-T. Yang[1,2], T.-T. Li[1,2], Z.-X. Tang[1,2] and F.-L. Cao[1]

[1]Co-Innovation Centre for Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing 210037, China P.R.; [2]College of Light Industry Science and Engineering, Nanjing Forestry University, Nanjing 210037, China P.R.; wucaie@njfu.edu.cn

Received: 22 December 2015 / Accepted: 3 July 2016

RESEARCH ARTICLE

Abstract

Ginkgolic acids (GAs) are generally identified as the allergen in ginkgo seeds. The effect of steaming and microwave treatment on GAs in ginkgo seeds was investigated in this paper. GAs were extracted with ethanol/water (85:15, v/v) and detected by high performance liquid chromatography (HPLC). The results indicated that GAs contents decreased significantly after heating treatments compared to the control. After steaming (115 °C, 75 min) and microwave treatment (1000 W, 6 min), GAs contents reduced with 54.5 and 27.0%, respectively. Three main GAs were detected by HPLC, and the alkyl side chains of which were $C_{13:0}$, $C_{15:1}$ and $C_{17:1}$, respectively. Steaming and microwave treatment could effectively decrease GAs contents in ginkgo seeds, which could be used in the industrial production of ginkgo foods.

Keywords: ginkgo seeds, ginkgolic acids, steaming, microwave

1. Introduction

Ginkgo seeds called 'baiguo' in Chinese, are eaten in China, Japan and Korea. They have high edible and medical values, which are rich in protein, amino acid, fat, sugar, vitamin C and riboflavin, as well as other functional components (Singh *et al.*, 2008; Zhang *et al.*, 2007a,b).

According to the practice experience and clinical reports, people have toxipathic allergic reaction when ingesting unbaked ginkgo seeds. Up to 2004, more than 100 cases on the poisoning of ginkgo seeds were reported in the world (Tian *et al.*, 2006). The poisoning of ginkgo seeds occurred frequently in children under six years of age, which account for about three-quarters of patients (Deng *et al.*, 2011; Kobayashi *et al.*, 2011). The clinical toxic behaviours after ingesting ginkgo seeds represent nausea, vomit, bellyache, diarrhoea, coma, twitch, decompensation, mydriasis, and death (Van Beek, 2005; Van Beek and Montoro, 2009). Kobayashi *et al.* (2011) reported that 4-O-methylpyridoxine in ginkgo seeds was the main poisonous content. But many others thought that ginkgolic acids (GAs) in ginkgo seeds were also the allergic substances.

GAs are series of structurally related n-alkyl phenolic acid compounds and extensively exist in leaves, nuts and external seed coat of *Ginkgo biloba* L. The alkyl side chain varies from 13 to 17 carbons in length with 0-2 double bonds (Van Beek, 2005; Van Beek and Montoro, 2009). GAs are strong allergens that can cause severe allergic reactions (Sun *et al.*, 2012; Zhang *et al.*, 2011). The international standard of Ginkgo extract (EGB761) stipulated that the extract contains 24% flavonoid glycosides, 6.0% terpene lactones, and less than 5 mg/kg GAs (Donfrancesco and Ferrante, 2007). Other limiting rules on GAs contents in ginkgo products have not been established. In recent years, GAs have been studied widely. However, the present studies are focused on the determination, separation, purification and biological activities evaluation of GAs. The content and composition of GAs in ginkgo seeds have not been reported.

Steaming and microwave treatment are conventional heating methods in food industries. During heating, the enzyme and bacterium may be destroyed under high temperature, meanwhile the physical and chemical characteristics of foods, such as colour and tissue state, can be changed under these conditions. As well, the nutrition ingredients can be improved, and the needless or harmful

components also can be eliminated. To our knowledge, there is no information on the effect of heating treatment on the content and composition of GAs. Therefore, in this paper, ginkgo seeds were heated by steaming and microwave methods. The effect of both heating methods on GAs content and compositions in ginkgo seeds were mainly studied.

2. Materials and methods

Materials

Fresh gingko seeds were harvested in Taixing, China. GAs standard sample with purity exceeding 98.5% was purchased from Shanghai Shunbo Biological Engineering Co. Ltd (Shanghai, China). Acetonitrile, methanol and trifluoroacetic acid are of HPLC-grade from Nanjing Chemical Agent Co. Ltd (Nanjing, China). All other reagents are of analytical grade.

Steaming treatment

Thirty grams of fresh gingko seeds were put in automatic vertical electro-thermal pressure steam steriliser (YXQ-LS-Sa; Shanghai Boxun Industry & Commerce Co. Ltd., Shanghai, China), processed under different temperatures (100-120°C) and different time (15-90 min).

Microwave treatment

Thirty gram of fresh gingko seeds was put in microwave oven (Shanghai Panasonic Microwave Oven Co. Ltd, Shanghai, China), processed under different microwave powers (300-1000 W) and different time (2-10 min).

Ginkgolic acids extraction

After heating, gingko seeds were dried with electric drying oven with forced convection (60 °C), and then pulverised by the disintegrator (FSD-100A; Aipu Instrument Co. Ltd, Hangzhou, China). 1.0 g of dried powder was extracted with 10.0 ml of ethanol/water (85:15, v/v) for 12 h, followed by vacuum filtration and rotary evaporation to vaporise ethanol. Afterwards, the extractum was extracted with petroleum ether, vaporised the petroleum ether, dissolved with 10.0 ml methanol. Finally, the content and composition of GAs were detected by UV-spectrophotometric method and HPLC, respectively.

Ginkgolic acids content

The content of GAs was detected by UV-spectrophotometric method (UV mini; Shimaduz, Kyoto, Japan). The standard sample of GAs was dissolved with methanol to prepare the solution with the concentrations 0.0153, 0.02295, 0.0306, 0.0459, 0.0612, 0.0765, 0.0918 and 0.1071 mg/ml. The detecting wavelength was 310 nm. The linear regression equation of the relation between the peak area and the concentrations of GAs is $A = 11.731C + 0.0075$, $R^2=0.9995$; A is the absorbency at 310 nm; C is the concentrations of GAs; R^2 is the correlation coefficient.

HPLC of ginkgolic acids

GAs were also determined with HPLC (Agilent 1200; Agilent Technology Company, Santa Clara, CA, USA). The chromatographic column was TC-C18. The detector was VWD. The detecting wavelength was 310 nm (Van Beek and Wintermans, 2001; Xia *et al.*, 2010). The mobile phase was acetonitrile/0.050% trifluoroacetic acid (85:15, v/v). The elution speed was 1.0 ml/min. The temperature of the column was 35 °C.

Statistical analysis

All experiments were performed in triplicate. The analysis of results was implemented with DPS version 3.0 (Zheda, Hangzhou, China). Analysis of variance and Duncan's multiple-range tests were used for statistical analysis. All the results are expressed as mean ± standard deviation of the three determinations. Differences between means were considered significant when $P<0.05$.

3. Results and discussion

Effect of steaming treatment on the content of ginkgolic acids

Steaming is one of the common food processing methods. The effect of steaming on the content of GAs is shown in Figure 1. The content of GAs in gingko seeds decreased when the temperature increased from 100 to 120 °C.

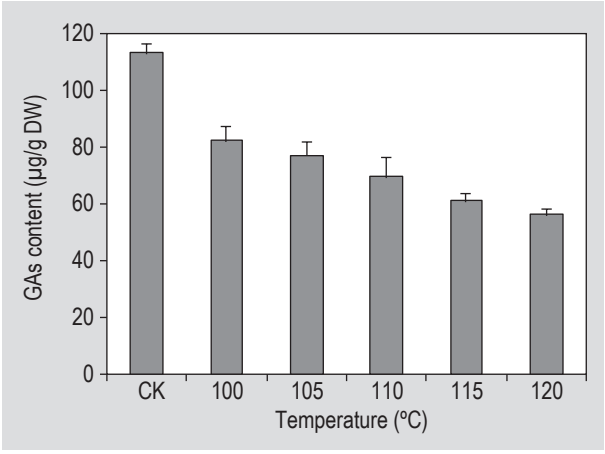

Figure 1. Effect of steam heating temperature on the content of ginkgolic acids (GAs). CK = control.

Steaming could significantly affect the content of GAs compared to the control. The minimum content of GAs was obtained under 115 °C. There was no significant difference between 115 and 120 °C. Steaming time also significantly affected the content of GAs. As shown in Figure 2, the content of GAs was reduced when steaming time increased from 15 to 90 min. The results suggested that GAs may be unstable to thermal treatment. GAs were stable under temperature 5 °C, but their stability decreased with the increase of the heating temperature and time (Yang *et al.*, 2007). Van Beek and Montoro (2009) also pointed out that the alkyl phenol acids in ginkgo seeds could be removed by frying treatment. These agreed with our studies.

Effect of microwave treatment on the content of ginkgolic acids

Microwave is a kind of electromagnetic wave. The microwave equipment produces high frequency vibration (2,450 MHz) which makes water, fat and other polar molecules vibrate excessively. It will lead to the molecules friction and collision. And, as a result some molecules' structure will be disintegrated by microwave treatment. Microwave may cause the change of food quality (Fan *et al.*, 2012; Kaur *et al.*, 2012). The larger the power was, the lower the content of GAs was detected. When the microwave power increased to 1000 W, compared to control, the content of GAs decreased with 27.4% (Figure 3). As microwave time was extended from 2 to 10 min, the content of GAs decreased slowly (Figure 4). Under the power of 1000 W, no significant differences were found between different times from 6 to 10 min. The energy of microwave is very powerful, which can damage the structure of starch and affect other properties (Arocas *et al.*, 2011). Choi *et al.* (2009) reported that guaiacol solution and hyaluronic acid powder could be degraded by microwave radiation. In our present study, the structure of GAs may be affected by microwave. The content of GAs in ginkgo seeds significantly decreased after microwave compared to control.

HPLC characterisation of ginkgolic acids

HPLC chromatograms of GAs extracted from the ginkgo seeds are showed in Figure 5. Three main peaks appeared in the chromatograms. Peaks 1, 2 and 3 were accounting for 67.9, 6.5 and 25.6%, of the total GAs, respectively. Eluted time was 8.17, 8.75 and 12.1 min, respectively. After steam heating, three main peaks were accounting for 67.3, 7.3 and 25.4% of the total GAs, respectively. For the sample treated by microwave heating, three main peaks were accounting for 66.1, 6.9 and 27.0% of the total GAs, respectively. Compared to the standard sample, the side-chains of these three monomers were $C_{13:0}$, $C_{15:1}$ and $C_{17:1}$. Four GAs are successfully separated from leaves from *G. biloba* L. (Sun, *et al.*, 2012; Tian *et al.*, 2006; Van Beek and Montoro, 2009). The side-chains of these four monomers were $C_{15:1}$, $C_{13:0}$, $C_{17:1}$ and $C_{15:0}$.

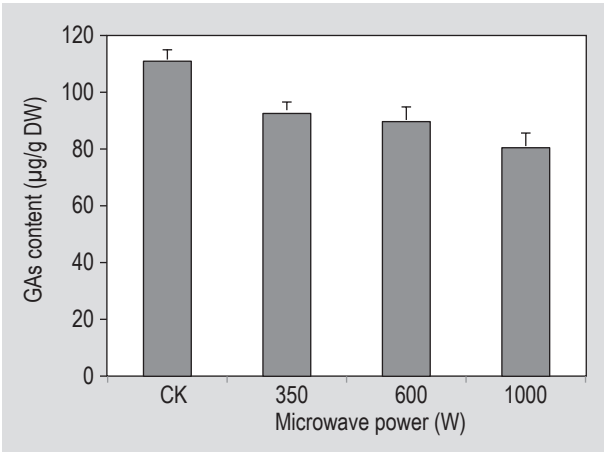

Figure 3. Effect of microwave heating powder on the content of ginkgolic acids (GAs). CK = control.

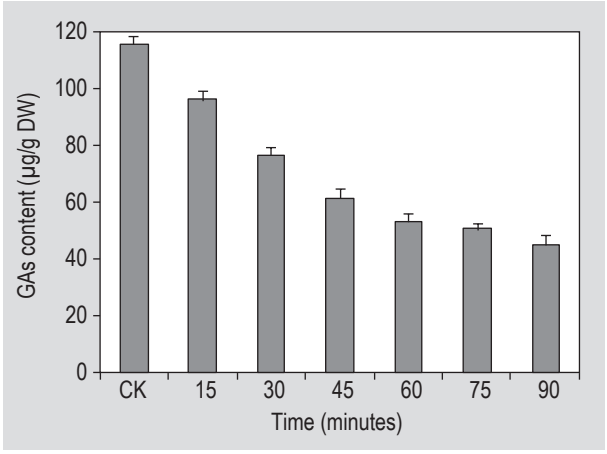

Figure 2. Effect of steam heating time on the content of ginkgolic acids (GAs). CK = control.

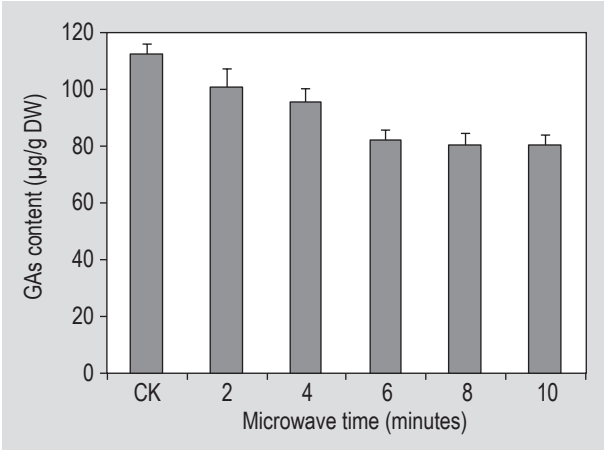

Figure 4. Effect of microwave heating time on the content of ginkgolic acids (GAs). CK = control.

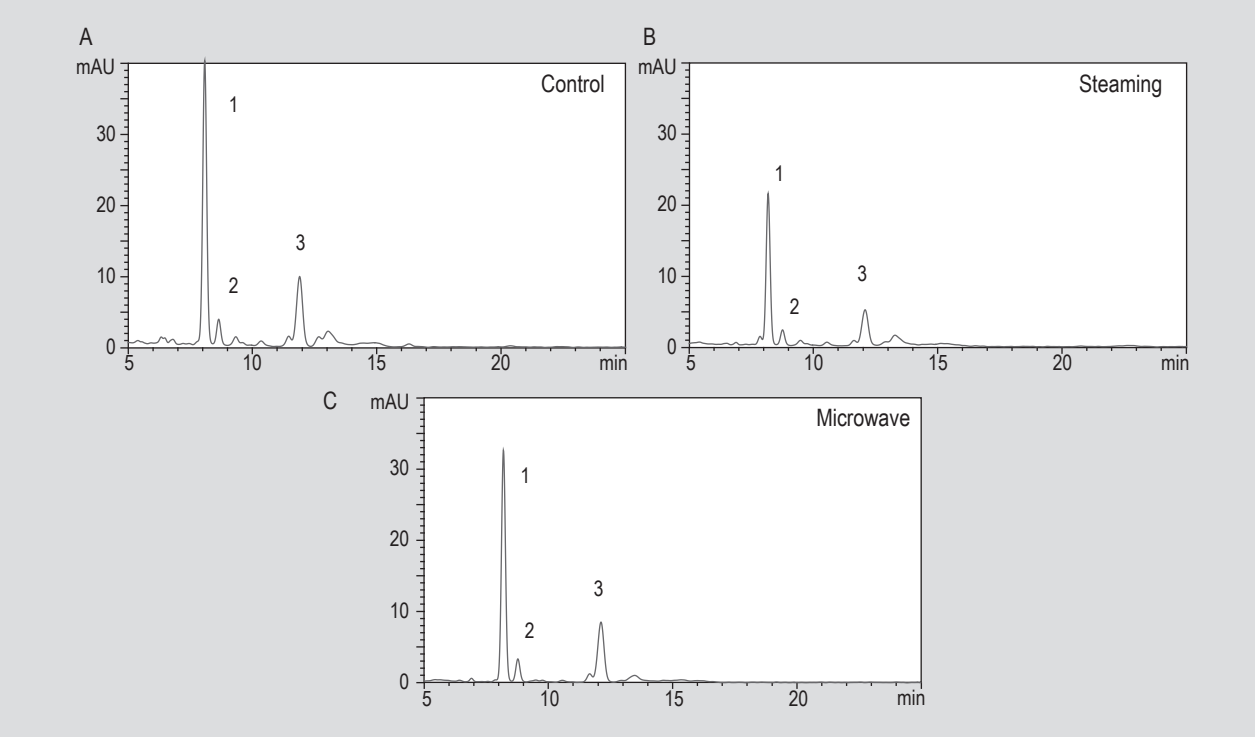

Figure 5. HPLC chromatogram maps of ginkgolic acids.

4. Conclusions

Three kinds of GAs were detected in ginkgo seeds. According to the GAs standard, the alkyl side chains of GAs were $C_{13:0}$, $C_{15:1}$ and $C_{17:1}$, respectively. Steaming and microwave treatment can decrease the content of GAs. These heating methods can be used in ginkgo processing industry. Further studies elucidating the toxicodynamic and toxicokinetic properties of GAs in the human body, are highly needed.

Acknowledgements

It is a great pleasure to thank research fund for key projects in the National Science & Technology Pillar Program (2012BAD21B04), National College Students' Innovative Training Program of Jiangsu (201510298032Z), and the science and technology project of Jiangsu province (BE2015315). The authors also would like to acknowledge the support of the project funded by the Priority Academic Program Development (PAPD) of Jiangsu higher education institutions.

References

Arocas, A., Sanz, T., Hernando, M. and Fiszman, S., 2011. Comparing microwave- and water bath-thawed starch-based sauces: infrared thermography, rheology and microstructure. Food Hydrocolloids 25: 1554-1562.

Choi, J., Jeong, S., Ku, C., Sathishkumar, M., Lee, J., Mun, S. and Kim, S., 2009. Antibacterial activity of hydroxyalkenyl salicylic acids from sarcotesta of *Ginkgo biloba* against vancomycin-resistant *Enterococcus*. Fitoterapia 80: 18-20.

Deng, Q., Wang, L., Wei, F., Xie, B., Huang, F., Huang, W., Shi, J., Huang, Q., Tian, B. and Xue, S., 2011. Functional properties of protein isolates, globulin and albumin extracted from *Ginkgo biloba* seeds. Food Chemistry 124: 1458-1465.

Donfrancesco, R. and Ferrante, L., 2007. *Ginkgo biloba* in dyslexia: a pilot study. Phytomedicine 14: 367-370.

Fan, L., Ding, S., Liu, Y. and Ai, L., 2012. Dehydration of crude protein from *Ginkgo biloba* L. by microwave freeze drying. International Journal of Biological Macromolecules 50: 1008-1010.

Kaur, P., Chaudhary, A., Singh, B. and Gopichand, 2012. An efficient microwave assisted extraction of phenolic compounds and antioxidant potential of *Ginkgo biloba*. Natural Product Communications 7: 203-206.

Kobayashi, D., Yoshimura, T., Johno, A., Sasaki, K. and Wada, K., 2011. Toxicity of 4'-O-methylpyridoxine-5 '-glucoside in *Ginkgo biloba* seeds. Food Chemistry 126: 1198-1202.

Singh, B., Kaur, P., Singh, G. and Ahuja, P., 2008. Biology and chemistry of *Ginkgo biloba*. Fitoterapia 79: 401-418.

Sun, Y., Tang, C., Wu, X., Pan, Z. and Wang, L., 2012. Characterization of alkylphenol components in *Ginkgo biloba* Sarcotesta by thermochemolysis-gas chromatography/mass spectrometry in the presence of trimethylsulfonium hydroxide. Chromatographia 75: 387-395.

Tian, Y., You, W. and Li, Y., 2006. Determination of total ginkgolic acids by fluorescence spectrophotometry. Chinese Journal of Analytical Chemistry 34: S155-S157.

Van Beek, T. and Wintermans, M.S., 2001. Preparative isolation and dual column high-performance liquid chromatography of ginkgolic acids from *Ginkgo biloba*. Journal of Chromatography A 930(1-2):109-117.

Van Beek, T., 2005. Ginkgolides and bilobalide: their physical, chromatographic and spectroscopic properties. Bioorganic and Medicinal Chemistry 13: 5001-5012.

Van Beek, T. and Montoro, P., 2009. Chemical analysis and quality control of *Ginkgo biloba* leaves, extracts, and phytopharmaceuticals. Journal of Chromatography A 1216: 2002-2032.

Xia, H., Wang, X., Li, L., Wang, S., Guo, C., Liu, Y., Yu, L., Jiang, H. and Zeng, S., 2010. Development of high performance liquid chromatography/electrospray ionization mass spectrometry for assay of ginkgolic acid (15:1) in rat plasma and its application to pharmacokinetics study. Journal of Chromatography B 878(28): 2701-2706.

Yang, Y., Su, Y., Ng, M., Chao, P., Tung, L. and Lu, K., 2007. Extract of *Ginkgo biloba* EGb761 facilitates extinction of conditioned fear measured by fear-potentiated startle. Neuropsychopharmacology 32: 332-342.

Zhang, H., Wang, Z. and Xu, S., 2007a. Optimization of processing parameters for cloudy ginkgo (*Ginkgo biloba* Linn.) juice. Journal of Food Engineering 80: 1226-1232.

Zhang, H., Wang, Z., Yang, R. and Xu, S., 2007b. Inactivation of microorganisms in cloudy Ginkgo (*Ginkgo biloba* Linn.) juice by pulsed electric fields. Food Science and Technology International 13: 83-90.

Zhang, P., Pan, J., Duan, W., Li, X., Zhang, Y., Zhou, Y., Jiang, Q., Mao, Z. and Yu, P., 2011. Synthesis of Ginkgolic acid analogues and evaluation of their molluscicidal activity. Molecules 16: 4059-4069.