Indonesian Journal of Biotechnology

VOLUME 28(3), 2023, 137-142 | RESEARCH ARTICLE

Effect of fluidised bed drying on ginsenoside content in hairy root cultures of *Panax ginseng* C.A. Meyer

James Setiabudi[1], Komang Mega Oka Sri Bintang[1], Stella Stacia Gani[1], Pissa Christanti[2], Evanie Noer Putri[2], Se Chan Kang[3], Johan Sukweenadhi[1,*]

[1]Faculty of Biotechnology, University of Surabaya, Raya Kalirungkut, Surabaya 60293, Indonesia
[2]PT. Bintang Toedjoe, Pulomas, Jakarta 13210, Indonesia
[3]Graduate School of Biotechnology, College of Life Sciences, Kyung Hee University, Yongin 17104, Republic of Korea
*Corresponding author: sukwee@staff.ubaya.ac.id

SUBMITTED 12 December 2022 **REVISED** 16 February 2023 **ACCEPTED** 17 May 2023

ABSTRACT Korean Ginseng (*Panax ginseng* C.A. Meyer) is a high-value herb with many pharmacological benefits due to its primary active compound, ginsenosides. The most ginsenosides are known to be thermolabile and susceptible to degradation at high-temperature processing. Our previous studies revealed that the optimum parameters related to the *P. ginseng* tissue culture protocol, particularly for hairy root propagation of Cultured Roots of Mountain Ginseng (CRMG)-88, was using a lab-scale bioreactor. The next stage involves screening for a suitable post-harvest treatment, i.e., drying, will be production of the best quality ginsenoside content. This study therefore aimed to examine the ginsenoside content by using a fluidised bed dryer (FBD) on the ginseng roots. Our results showed that FBD produced a significantly higher of total ginsenoside content (5.386 ± 1.167%), compared to control (3.750 ± 0.641%). FBD-dried CRMG-88 also appeared lighter in colour and more voluminous with a Loss on Drying (LOD) of 6.448 ± 1.900%. This study concluded that fluidised bed drying is superior in retaining ginsenoside content and has the potential for large-scale application.

KEYWORDS Ginseng; Ginsenosides; Thermal decomposition; Tissue culture.

1. Introduction

Ginseng is widely distributed in dozens of countries and is in great demand on the world market. The trend of increasing ginseng consumption is due to growing awareness of healthy food cultures. Several attempt to produce high quality of ginseng are expanding towards conventional products, such as improvement of root culture using bioreactor (Dannis Yuda Kusuma et al. 2023). Processed ginseng products are becoming an alternative of consumers due to their greater practicality and enhanced functional value (Vol et al. 2013; Baeg 2022).

The main active compound of ginseng is ginsenosides, which are known as triterpenoid saponins, has many medical benefits, including anti-inflammatory activity (Kim et al. 2017), mental health promotion (St-Laurent and Hammami 2022), anti-cancer activity (Hu et al. 2019; Zhang et al. 2019a), memory loss improvement (An et al. 2019), neuroprotective ability (Zhang et al. 2016), and anti-fatigue activity (Shin et al. 2019). Aside from genetic and environmental factors (Chen et al. 2019; Lee et al. 2019; Zhang et al. 2019b), processing can change the ginsenoside content in ginseng, which in turn can affect its functional properties (Koh et al. 2015; Jang et al. 2017; Zheng et al. 2017; Jang et al. 2018).

Food drying is essential in safeguarding product safety, as ingredients with a high water content are always susceptible to contamination (Nazghelichi et al. 2010). Nevertheless, a balanced drying technique focuses not only on suppressing spoilage but also on conserving the vital elements in a product. Raw ginseng root has a rich moisture content and often heat-dried to be more efficient. It is indisputable that ginsenosides are thermolabile compounds and susceptible to random conversion to their derivatives at high temperatures (Kim et al. 2020). This creates inconsistency in product composition, leading to a decrease in quality.

Our research group has worked intensely with ginseng to build an optimal *in vitro* system from cultivation to post-harvest. The development of suitable drying methods is imperative to ensure stable product composition. The existing oven-drying process relies on heat convection, which tends to dry slowly and can potentially induce the degradation of heat-susceptible contents. Several research findings have indicated a loss of phytochemical content attributable to oven treatment. The carotenoid levels of celery leaves were reported to be dramatically reduced when drying at 70 °C for 4 h (Kamel et al. 2013). In another

case, the total phenolic and antioxidant content of *Cosmos caudatus* showed a remarkable decrease after heating at 44.5 °C for 4 h (Mediani et al. 2014).

A fluidised bed dryer (FBD) offers an alternative drying method. The working principle is fluidisation, where solids interact with hot gas at high pressure to become partially suspended in gas form (Doymaz and Ismail 2010). Several studies have demonstrated the effectiveness of FBD on beetroot (Kumar Y 2015) and mint leaves (Motevali et al. 2016). However, to our knowledge, the literature contains only limited information regarding the effect of FBD on *Panax ginseng*, especially regarding its thermolabile ginsenoside.

Based on its potential, the current study intends to examine the effect of FBD on the ginsenoside composition of ginseng tissue culture. It is expected that a shorter contact time between the roots and hot gas will minimise the decomposition of ginsenosides.

2. Materials and Methods

The plant material used was a hairy root culture of *Panax ginseng* C.A. Meyer, initially provided by Hanbang Bio Laboratory (Kyung Hee University, South Korea). The cell line used was a developmental collection called Cultured Roots of Mountain Ginseng (CRMG)-88. The research was conducted at Kalbe UBAYA-Hanbang Bio Laboratory (Surabaya, Indonesia).

2.1. Production of CRMG-88

Production was carried out in an 18 L bioreactor system (BR-BIO180, Hanbang Bio Inc., South Korea). The growth medium was prepared through modifications from previous studies (Chandra et al. 2021). The previously optimised "B" formula, which comprises a combination of modified Schenk and Hildebrandt basal medium and auxin hormones, was applied to the concentrated media. However, the dilution level of the concentrated media was increased to reach a final volume of 15 L. Furthermore, sterilization using an autoclave (Hankuk, HK-AC200P, Korea) was modified with the temperature of 121 °C for 72 min at a pressure of 1.5 atm. Meanwhile, seeding and incubation proceeded without modification. The 150 g of fresh roots were used as inoculum seeds and the growing conditions were maintained at 21 °C to 25 °C, 500 lux of light intensity with 10/14 h photoperiod (day/night), for 7 weeks at 40 to 50% humidity.

2.2. Harvest and drying of CRMG-88

After the incubation period, the roots were harvested and rinsed three times using tap water, and then once with reverse osmosis water. The draining efficiency was elevated using a washing machine (Mito, WM1, Indonesia) for 2 × 5 min. The samples used were taken from three separate month harvest periods. In this study, separate fluidised bed- and oven-drying treatments were applied for each sample. In the FBD, fresh CRMG-88 was placed evenly on the porous plate of an FBD (made locally, 585 watts, two $m \cdot s^{-1}$ airflows) and set at 55 °C. The CRMG-88 was turned over periodically during the drying process to ensure every part of the roots was dry. The FBD is generally able to dry 500 g of fresh roots in 3–4 h in a single run. Drying using a universal oven (Memmert, UF45, German) was conducted at 60 °C, 60% fan, and 60% flap (Chandra et al. 2021). One tray containing approximately 1.5 kg of fresh roots will dry within 9–10 h of oven drying. Both drying treatments were performed until the CRMG-88 reached a Loss on Drying (LOD) value of 5–10%. LOD was measured using a moisture analyser (Sartorius, MA150, German).

2.3. Extraction and sample preparation of CRMG-88

The dried roots were extracted gradually based on an earlier procedure (Chandra et al. 2021). The first step utilised the Soxhlet method to extract 5 g of dried root in 300 mL of 80% methanol for 2 h. The resulting extract was dried with a rotary evaporator (Buchi, R-300, German) and reconstituted in 20 mL of reverse osmosis water. The second step was liquid–liquid extraction using water-saturated butanol at a ratio of 1:1. The organic phase was collected by centrifugation at 8000 rpm (1 rpm = 1/60 Hz) for 15 min. The second step was then repeated with fresh solvent until a clear organic phase was obtained. The resulting extract was evaporated again.

2.4. Ginsenosides analysis

Ginsenoside levels were analysed using high-performance liquid chromatography (HPLC) (Agilent, 1260 Infinity II, USA). Samples were dissolved in 50 mL HPLC-grade methanol and filtered with a 0.2 µm PTFE filter. A Symmetry Shield RP18 column 3.5 µm with a size of 4.6 × 150 mm was used, while the eluent was water pH 2.0/acetonitrile (A). The elution gradient was set as follows: 8 min at 80% A (isocratic); 8 min to 40 min from 80% to 60% A; 40 min to 45 min from 60% to 40% A; 45 min to 47 min from 40] to 0% A; 47 min to 52 min at 0% A (isocratic); 52 min to 55 min from 0% to 80% A. The injected sample volume was 20 L, and the absorbance was measured using a Diode Array Detector at a wavelength of 205 nm. Quantitative interpretations were made by comparing the peak areas of 14 types of ginsenosides with their corresponding reference standards, which are listed in Table 2.

The data obtained were analysed by IBM SPSS v. 25 software using an independent t-test for normal distribution. The significance of the difference between the data is indicated by a p-value < 0.05 (Huang et al. 2023).

3. Results and Discussion

Recent studies have indicated a divergence in the abundance of ginsenoside types from the two drying treatments. Quantitative variations of ginsenosides are represented by differences in peaks in the ginsenoside profiles of FBD and oven drying, as shown in Figure 1. A significant difference (p = 0.033) was observed in the total ginsenoside

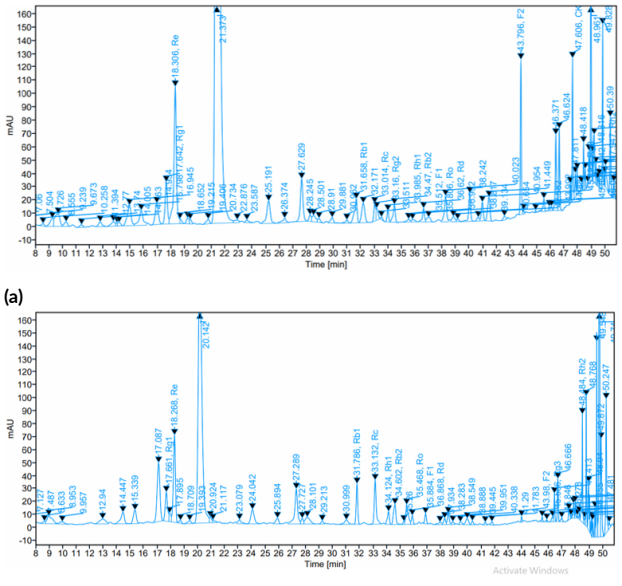

FIGURE 1 HPLC chromatograms showing the ginsenoside profiles of CRMG-88 prepared under different conditions. a) Fluidised bed drying. b) Oven drying.

content among two drying treatments, with FBD yielding 40% higher ginsenoside content compared to oven drying, as shown in Table 1. Fluidised bed drying produced higher total ginsenoside levels (5.386 ± 1.167%) than oven drying (3.750 ± 0.641%). At the individual level, there were a significant decrease in protopanaxatriol (PPT) groups, such as Re, in the oven-drying treatments compared to fluidised bed drying, as shown in Table 2. A similar finding was reported whereby Re decreased gradually during the first four days of heating with a temperature of 80 °C (Kim et al. 2020). Ginsenoside Re has been studied for its potential health-promoting properties, including anti-inflammatory, antioxidant, and neuroprotective effects. It is also believed to contribute to the adaptogenic properties of ginseng, which may help the body adapt to stress and improve overall well-being (Jang et al. 2018). Other research has reported that in the steaming process at 100 °C, Re undergoes a series of hydrolysis starting from Re → Rg1 or Rg2 → F1 → PPT (Yao et al. 2021). Otherwise, the reaction can run from Re → Rh1 → PPT, as shown in Figure 2.

TABLE 1 Effect of fluidised bed drying and oven drying on total ginsenoside percentage.

Treatment	Average of total ginsenoside (%) (*)	*P*-value
FBD	5.218 ± 1.040	0.033(**)
Oven	3.749 ± 0.641	

(*) Independent T-test with α = 5%.
(**) $p < 0.05$ shows a significant difference.

TABLE 2 Content change of ginsenosides in fluidised bed drying and oven drying.

Compound Name	Ginsenoside Content (%)	
	FBD	Oven
Rg1	0.402 ± 0.241	0.329 ± 0.179
Re	2.921 ± 0.294	2.270 ± 0.685
Rb1	0.336 ± 0.076	0.212 ± 0.068
Rc	0.379 ± 0.110	0.321 ± 0.080
Rh1	0.068 ± 0.042	0.054 ± 0.036
Rg2	0.035 ± 0.057	0.009 ± 0.017
Rb2	0.222 ± 0.102	0.178 ± 0.092
Ro	0.206 ± 0.094	0.109 ± 0.077
F1	0.050 ± 0.022	0.061 ± 0.053
Rd	0.176 ± 0.051	0.081 ± 0.054
F2	0.259 ± 0.464	0.021 ± 0.012
Rg3	0.051 ± 0.019	0.024 ± 0.014
CK	0.069 ± 0.098	0.025 ± 0.016
Rh2	0.044 ± 0.051	0.054 ± 0.059

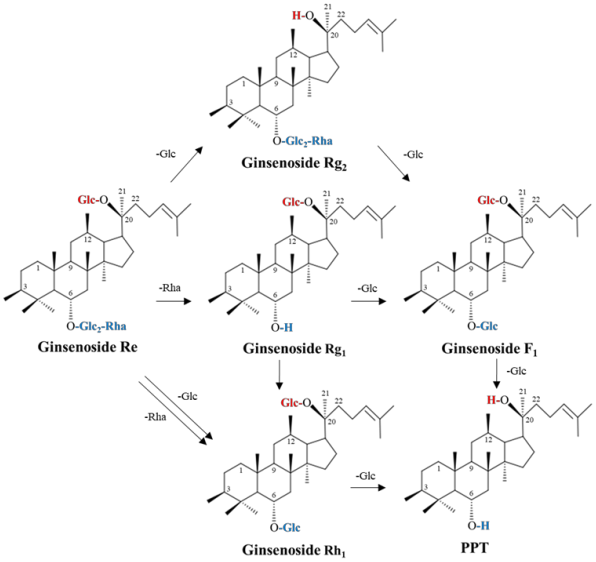

FIGURE 2 Presumed conversion mechanism of protopanaxatriol-type ginsenosides.

A significant decrease was also observed in the protopanaxadiol (PPD) group, namely Rb1, F2, and CK, of oven-dried CRMG-88. This effect could be explained by the conversion mechanism described previously (Yao et al. 2021). In this case, the hydrolysis continuously proceeds from Rb1, Rb2, and Rc → Rd → F2 → CK or Rh2 → PPD, as shown in Figure 3. The number of upstream molecules will eventually decline as molecular breakdown continues toward the pathway. This mechanism is justified by the evidence that the related ginsenosides were also reduced in this current study, except for Rh2. Thus, the detected ginsenoside change phenomenon may refer to the finding that oven drying at 60 °C sufficiently induces thermal decomposition, which converted the related ginsenosides into their simplest form (PPT/PPD) or possibly other novel ginsenosides that were not included in the measurement standards in the current research. Both possibilities may have contributed significantly to the total ginsenoside per-

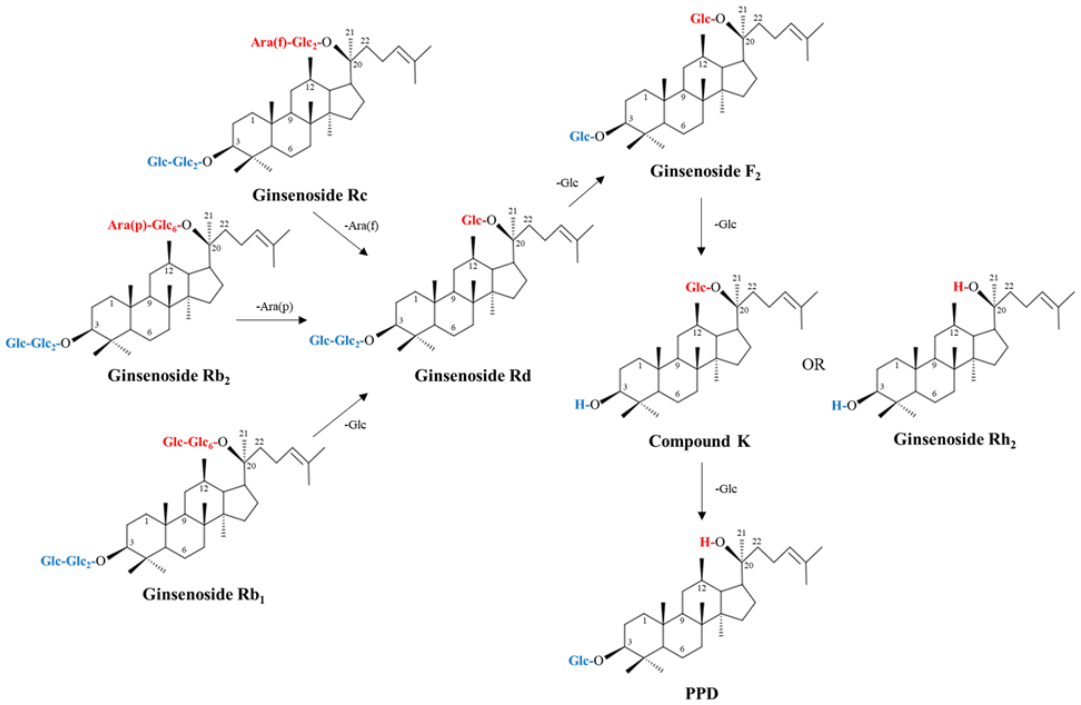

FIGURE 3 Presumed conversion mechanism of protopanaxadiol-type ginsenosides.

centage.

FBD-dried CRMG-88 was found to give a LOD of 6.448 ± 1.900% in drying for 224 ± 26 min and produced a yield of 4.350 ± 0.372%, as shown in Table 3. Our optimisation showed that a fresh weight of 450 g is the maximum capacity of the FBD. A higher weight tends to cause uneven heat distribution. However, this small-scale FBD is capable of delivering the desired LOD in a short time.

TABLE 3 Drying parameters of FBD and oven-dried CRMG-88.

Treatment	Drying time (min)	LOD (%)	Yield (%)
FBD	224 ± 26	6.448 ± 1.900	4.350 ± 0.372
Oven	543 ± 57	4.986 ± 1.403	5.428 ± 0.393

(a) (b)

FIGURE 4 (a) Fluidised bed drying of CRMG-88. (b) Oven drying of CRMG-88.

Oven drying presented a LOD and yield of 4.986 ± 1.403% and 5.428 ± 0.393%, respectively, while the drying time was 543 ± 57 min. The oven has a 6 kg fresh weight capacity in a single run with four trays. The appearance of the two dryers can be seen in Figure 4. The condition of the roots often determines the degree of dryness attained. If the roots are dense, they may not dry properly.

In terms of physical appearance, the CRMG-88 resulting from FBD was a light golden yellow colour (Figure 5a), while oven drying produced a darker brown colour (Figure 5b). This can be understood based on the fact that heat transfers slowly in an oven and can lead to a burning effect on the drying material to produce a dark colour over time. Meanwhile, in fluidised bed drying, based on the shorter drying contact time (faster heat transfer), the natural colour of the material is maintained during the process. In addition, the CRMG-88 subjected to fluidised bed drying had a voluminous look, while it tended to deflate and

(a) (b)

FIGURE 5 (a) Fresh ginseng from FBD-drying. (b) Fresh ginseng from oven-drying.

harden with oven drying.

Fluidised bed drying resulted in a better total ginsenoside content and physical appearance compared to oven drying. As such, drying in an FBD would be more suitable for large-scale continuous production as it is also superior in terms of maintenance cost, temperature requirement (lower), and drying rate.

4. Conclusions

Based on the results of our research, it can be concluded that drying using an FBD is preferable to oven drying based on consideration of the total ginsenoside content and the physical appearance of the drying results. However, efficiency tests and validation of large-scale FBD operations in the KUH Lab are yet to be conducted. Moreover, comprehensive future studies regarding the heat transformation of ginsenosides are required.

Acknowledgments

This work was supported by the Ministry of Education, Culture, Research and Technology, Republic of Indonesia (155/E1.1/KS.06.02/2022) and PT. Bintang Toedjoe.

Authors' contributions

PC, EN, and J Sukweenadhi designed the study. J Setiabudi, KMOSB, SSG, and PC carried out the laboratory work. J Setiabudi, PC performed data acquisition and data analysis. J Setiabudi wrote the manuscript. SCK, J Sukweenadhi elaborated on the intellectual content and manuscript review. All authors read and approved the final version of the manuscript.

Competing interests

The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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