International Journal of Chemical, Materials and Biomolecular Sciences
ISSN: 2415-6620
Vol:8, No:5, 2014

Mechanisms of Ginger Bioactive Compounds Extract Using Soxhlet and Accelerated Water Extraction

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***Abstract*—**The mechanism for extraction bioactive compounds from plant matrix is essential for optimizing the extraction process. As a benchmark technique, a soxhlet extraction has been utilized for discussing the mechanism and compared with an accelerated water extraction. The trends of both techniques show that the process involves extraction and degradation. The highest yields of 6-, 8-, 10-gingerols and 6-shogaol in soxhlet extraction were 13.948, 7.12, 10.312 and 2.306 mg/g, respectively. The optimum 6-, 8-, 10-gingerols and 6-shogaol extracted by the accelerated water extraction at 140°C were 68.97±3.95 mg/g at 3min, 18.98±3.04 mg/g at 5min, 5.167±2.35 mg/g at 3min and 14.57±6.27 mg/g at 3min, respectively. The effect of temperature at 3mins shows that the concentration of 6-shogaol increased rapidly as decreasing the recovery of 6-gingerol.

***Keywords*—**Mechanism, bioactive compounds, soxhlet extraction, accelerated water extraction.

I. Introduction

GINGER, a common natural herb and widely used as medicinal and food beverage purpose. The presence of pharmacological activities such as anti-inflammatory, anti-oxidatives and anti-cancer from the extraction of ginger mainly is due to the existence of gingerols and shogaols[1], [2].During thermal processing or storage, the gingerols may be modified to a series of homologous compounds of shogaols such as 6-, 8-, and 10-shogaol[3], [4].

Various techniques had been studied on extracting these bioactive compounds [5], [6]. Previously, the extraction of ginger by subcritical water extraction had been studied intensively [7]. The subcritical water extraction applied water as bulk solvent and operates above boiling point (100°C) and below critical point (374.5°C) of water with saturation pressure to ensure water in liquid state. Operating at these conditions, the relative permittivity, ε of water can be almost similar to those of organic solvents, i.e. 53 and 36.5 at 110°C and 190°C, respectively for water compared to the relative permittivity of methanol of 32.6 at 25°C [8]. Therefore, subcritical water extraction can be selective technique since polarity of water as solvent may be varied from polar to non-polar conditions.

However, the challenges of using this extraction technique are dealing with thermal labile compounds such as ginger bioactives. The results showed that the recovery of bioactive compounds for 6-gingerol and 6-shogaol were 20.7 and 85.0%, respectively [7]. The percentage of recovery could be increased by optimizing the extraction process through a mathematical modeling.

The mathematical modeling facilitates optimization, scaling up and designing the process. Indeed, the mathematical modeling also describes the fundamental and mechanism studies on the extraction of bioactive compounds from a plant matrix [9]. The initial or maximum amount of bioactive compounds is determined through soxhlet extraction in the development of the models [9], [10]. In developing the model, the mechanism of extracting ginger bioactive compounds has to be understood. In this work, the mechanisms and the models using solvent extraction are compared with the proposed accelerated water extraction (ASE) method using subcritical water as solvent.

Since the work using ASE is operated at elevated temperatures, the temperature effect on the degradation of ginger bioactive compounds is studied. The effect of prolonged extraction time at moderate temperature by soxhlet extraction on the degradation of the compounds is also investigated.

II. Mechanism of Solute Extract from Plant Matrix

The mechanism of solutes (ginger bioactive compounds) extract is illustrated in Fig. 1. The extraction of solutes from a plant matrix (solid) involves the diffusion of bulk solvent (liquid) into the matrix through stagnant film layer. The transportation of solutes within plant matrix to bulk solvent is due to the concentration gradient of the solutes. The transportation is described by an internal diffusion. Theoretically, the internal diffusion of solute is explained by Fick's law as in (1) [11].

$$\frac{\partial C_s}{\partial t} = D\frac{\partial^2 C_s}{\partial r^2} \tag{1}$$

where D is the diffusion coefficient (m^2/s), C_s (mg/g) is the concentration of solute at time, t(s) in plant matrix and r is the radial distance (m) of the solid matrix.

Meanwhile the external diffusion involves the transportation of solute across the stagnant film surrounding the solid matrix. The external mass transfer is a convection process in which bulk solvent plays an important role as in (2):-

$$\frac{dC_f}{dt} = 3\frac{k_f}{R}\frac{m}{\rho V}(C_{r=R} - C_s) \tag{2}$$

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where k_f is the external mass transfer coefficient (m/s), R is the radius of solid matrix (m). $C_{r=R}$ is the concentration of solute at the boundary stagnant film (mg/g). ρ, V and m are the density of bulk solvent (g/cm^3), the volume of solvent (m^3) and the mass of solid matrix (g), respectively.

The degradation is described by first order rate reaction with concentration of degradation, C_{deg} (mg/g) is respect to degradation time (t) as follows[12]:

$$\frac{dC_{\deg}}{dt} = -k_{\deg} t \quad (3)$$

where the degradation coefficient is given by k_{deg}. Thus, the mechanism of bioactive compounds extract can be simplified as:

Plant matrix $\xrightarrow{k_{ext}}$ Bulk Solvent $\xrightarrow{k_{deg}}$ Degradation

The extraction of solute into bulk solvent may consist of either internal or external diffusions or both. This step is described by the rate constant, k_{ext}. Simultaneously during the extraction of ginger bioactive compounds, degradation occurs during prolonged extraction time and elevated temperature. The rate constant of degradation is given by k_{deg}.

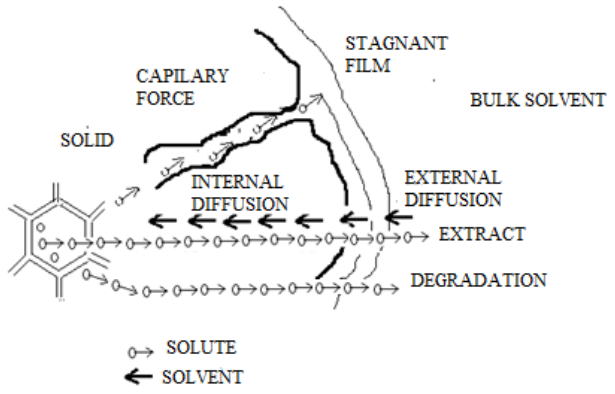

Fig. 1 Mechanisms of solute extract for typical solvent

III. Material and Methods

A. Materials and Chemicals

Dried ginger (*Zingiber officinale* Roscoe) was supplied from local supplier (Sabah, Malaysia). All samples were stored in a refrigerator at 4°C for maintaining the freshness.

HPLC grade methanol (99.9%, ACS, Houston, USA), acetonitrile (99.99%, Fisher, Loughborough, UK) and ultrapure water with resistivity > 18.2 MΩ cm (Barnstead, USA) were used as mobile phase in HPLC analysis. 6-Gingerol and 6-shogaol standards were purchased from ChromaDex (Irvine, CA).

B. Soxhlet Extraction

The initial concentrations of bioactive compounds presented in the ginger matrix were identified through the soxhlet extraction. 20.0g of dried and ground ginger with <1.18mm of particle size were weighed and extracted with 200mL of ethanol (96% AR grade, QReC). The extraction temperature was constant at the normal boiling point of ethanol (78.1°C) and monitored using an infrared laser thermometer (AR300, China). The experiments were conducted for 12 h in triplicate and the standard deviations were calculated (<5%).

C. Accelerated Water Extraction (ASE)

Accelerated water extraction was performed using an accelerated solvent extractor (ASE) as shown in Fig. 2. The ASE 350 Dionex (Thermo Fisher Scientific, US) was operated at constant pressure and temperature of 1500 psi and 140°C, respectively with 200s of purging time in one cycle and 60% of flush volume. The extraction times were varied from 1 to 30 min with 2min increment for the first 15min after which the increment was 15min. 5.0g of ground ginger was mixed with 2.0g of diatomaceous earth (Thermo Fisher Scientific, P/N 062819). A cellulose filter (Dionex, P/N 056780) was placed in the bottom of extraction cell before the sample was loaded into it.

Once an oven was reached to the set temperature and extraction cell was automatically loaded into the oven and filled with solvent. At this static stage, the content of the cell was heated to the set temperature before the extraction was started at the set time. When the extraction was completed, the 135 mL of extract was transferred into a collection bottle. The experiments were conducted in duplicate with acceptable standard deviation of < 5%.

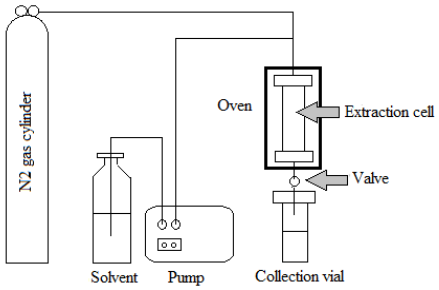

Fig. 2 Schematic diagram of ASE 350

D. HPLC Analysis

The extracted sample was dissolved with 0.5 dilution factor in methanol. The mixtures were then filtered through 0.45µm membrane filter (Nylon, Waters Corporation). The analysis was performed by HPLC (e2695, Waters, USA) with a photo diode array detector (PDA). The compounds were separated on C18 column (symmetry®) 5.0µm particle size, (150mm × 4.6 mm) and detected at 282nm. The gradient mobile phase consisted of (A) water, 50%: methanol, 50% and (B) acetonitrile at a flow rate of 0.8 ml/min. The elution of binary solvent was conducted in gradient fashion, using the following profile: 0-8min, 45% B; 8-10min, 65% B; 10-11min, 55% B; 11-20min, 55% B and column temperature was set at 30°C ± 5°C. 20µl of sample extracts were filtered using syringe filter

(nylon, 0.45μm, Whatman, USA) before injected into the HPLC.

IV. Results and Discussion

A. Mechanism of Extraction Using Soxhlet Extraction

The concentrations of ginger bioactive compounds extract with time by soxhlet extraction using ethanol are shown in Fig. 3. Soxhlet extraction is a percolation method in which fresh solvent flows through the solid matrix. It can be observed that during the first 2h, the concentrations of bioactives were relatively low. This is attributed to the diffusion of the solvent which saturate the ginger matrix. This is due to the internal diffusion in which solutes diffuse into the solvent within the solid matrix.

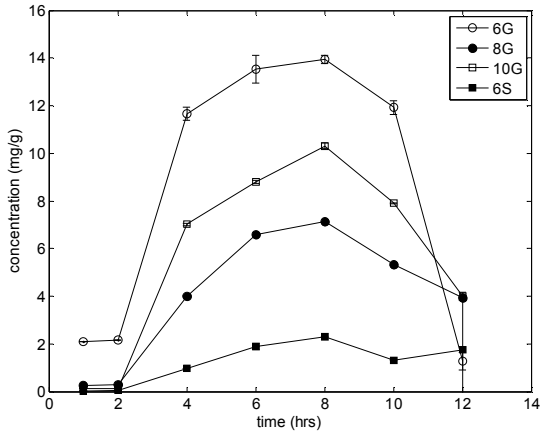

Fig. 3 Concentrations of ginger bioactives with varied time using Soxhlet extraction (at 78°C)

Once the ginger matrix is saturated with the solvent, the bioactive compounds easily diffuse into the bulk solvent and the rate of extraction increases for the next 6 hrs. At this stage, external diffusion plays a role in which the solute diffuse out of the solid matrix into the bulk solvent as indicated by the rapid increment of the concentration for each solute. All bioactive compounds (6-, 8-, 10-gingerols and 6-shogaol) showed the highest concentration at 8 hrs. The highest bioactive compound present in the extract is 6-gingerol (14 mg/g) because of its availability within the ginger matrix.6-Gingerol also is the most polar compound among the four bioactives and has the lowest molar volume which facilitates the extraction process.

The maximum concentrations which is due to its availability in the ginger matrix of 10-gingerol is 10mg/g followed by 8-gingerol at 7mg/g and 6-shogaol at 2.5mg/g. Ethanol has been proven to be the best solvent compared to other organic solvents and is therefore used to give the initial concentrations of ginger bioactives in this work [6].

TABLE I
Optimum Recovery of Bioactive Compounds in ASE

Bioactive compounds	Concentration (mg/g)	Recovery (% w/w)
6-gingerol	10.508	68.97±3.95
8-gingerol	0.524	18.98±3.04
10-gingerol	0.065	5.167±2.35
6-shogaol	0.353	14.57±6.27

Recovery= concentration of bioactive compounds/ initial concentration of bioactive compounds using soxhlet extraction

B. Mechanism of Extraction Using Accelerated Water Extraction

The effect of temperatures with time varied on extraction of 6-, 8-, 10-gingerols and 6-shogaol by ASE is shown in Fig. 4. Rapid increased of concentrations is observed for all bioactive compounds at the earlier stage of extraction within 3 min for 6-, and 8-gingerols and 5 mins for 10-gingerol and 6-shogaol. For ASE, bulk of the extraction occurs during this stage indicating mass transfer from ginger matrix to the bulk solvent. Indeed, the extraction operates at elevated temperature and pressure which increases the diffusivity rates, disrupt the solute-matrix interaction through reduced viscosity of solvent at these conditions [9]. The attractive forces of O-H bonds will be weaken at elevated temperature thus increasing the solubility through improve interaction of solute-solvent [10]. In ASE, the stagnant film that surrounds the solid matrix will be eliminated due to reduced surface tension at elevated temperature thus enhances mass transfer of solute to solvent.

The concentration of 6-gingerol is the highest compared to the other bioactive compounds as observed in both ASE (10.5 mg/g) and soxhlet extraction. The higher 6-gingerol concentration is attributed to the high initial concentration (14 mg/g) indicating abundant availability of the ginger bioactive compound. 6-Gingerol is also polar in nature compared to the other bioactives. 8-Gingerol and 6-shogaol using ASE are at the concentrations of 0.5 mg/g which are 14 and 12 times, respectively lower than its initial concentration. Using ASE 10-gingerol at 0.18mg/g is very much lower than its initial concentration of 10 mg/g.

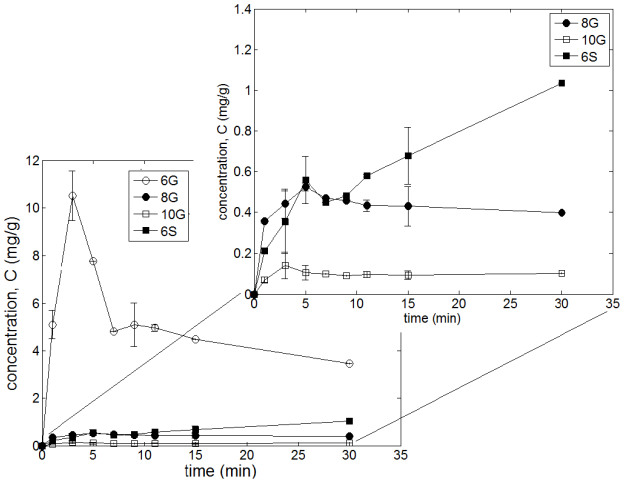

Fig. 4 Concentration of ginger bioactives extracted in accelerated water extraction at 140°C

The recovery of each bioactive compound based on its initial concentration using soxhlet extraction was determined and summarized in Table I. By using water as solvent, the recovery of 6-gingerol could be achieved up to 68.97% w/w, followed by 8-gingerol (19% w/w), 6-shogaol (15% w/w) and 10-gingerol at 5.17% w/w. The optimum concentration of each bioactive compound could be achieved within 3-5 min before it starts to degrade.

C. Degradation of Ginger Bioactive Compounds

It can be observed that soxhlet extraction after 8 h gives decreasing concentrations of the four bioactive compounds which indicates degradation is taking place. However, for 6-shogaol after 10 h of extraction the concentration increases.

This indicates that soxhlet extraction using ethanol should be used to find initial concentration at 8 h of extraction. Extraction above 8 h causes degradation for the three bioactives (6-, 8-, and 10-gingerols). The concentration of 6-shogaol increases over prolonged extraction beyond 10h suggesting dehydration reaction which involves the other bioactives as an example 6-gingerol converted to 6-shogaol as shown in Fig. 5.

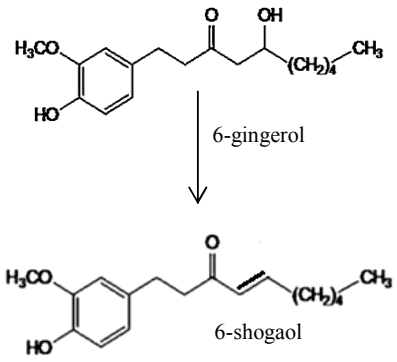

Fig. 5 Schematic diagram of degradation 6-gingerol to 6-shogaol

Once the concentration of each bioactive compound reaches the optimum concentration, rapid degradation was observed in ASE. The degradation is due to the extraction process operated at elevated temperature and pressure. Moreover, ginger bioactives are thermal labile in nature in which degradation was observed even at moderate temperature at over prolonged extraction time. [13] The extraction and degradation could occur simultaneously.

This study suggests that it is possible degradation occurs after prolonged extraction time at moderate temperature but degradation and extraction may occur simultaneously at elevated temperatures. However, further studies are needed to be done to confirm this.

V. Conclusion

In conclusion, the mechanisms of extraction for bioactive compounds from ginger matrix are different for different extraction methods. In a typical solvent extraction (soxhlet extraction), the stagnant film surrounding the solid matrix act as a resistance which reduces diffusivity thus longer time is needed. However, in ASE rapid extraction was observed within 3 min of extraction time since the stagnant film is eliminated. The elimination of stagnant film ease the transportation of bioactive compounds to the bulk solvent.

Further work should be done on developing mathematical modeling that considering the extraction and degradation of the bioactive compounds.

Acknowledgment

The financial support provided for this project by Malaysia-Japan International Institute of Technology (MJIIT), Universiti Teknologi Malaysia is gratefully acknowledged.

References

[1] H-Y. Yeh, C-H. Chuang, H-C. Chen, C-J. Wan, T-L. Chen, L-Y. Lin, "Bioactive components analysis of two various gingers (*Zingiber officinale* Roscoe)and antioxidant effect of ginger extracts," *LWT - Food Science and Technology*, 2014, pp.

[2] Harliansyah, M. Noor Azian, W.N. Wan Zurinah, M.Y. YasminAnum, "Antiproliferative, antioxidant and apoptosis effects of zingiberofficinale and 6-gingerol on HepG2 cells,"*Asian Journal of Biochemistry*, 2007, vol. 2, pp. 421-426.

[3] S.Sang, J. Hong, H.Wu, J.Liu, C.S.Yang, M-S. Pan, V. Badmaev, C-T. Ho, "Increased growth inhibitory effects on human cancer cells and anti-inflammatory potency of shogaols from *Zingiber officinale* relative to gingerols,"*J Agric Food Chem*, 2009, vol.57, pp. 10645-10650.

[4] X. Shao, L. Lv, T.Parks, H. Wu, C-T. Ho, S.Sang, "Quantitative analysis of ginger components in commercial products using liquid chromatography with electrochemicalarray detection," *Journal of Agricultural and Food Chemistry*, 2010, vol. 58, pp. 12608-12614.

[5] S. Balachandran, S.E. Kentish, R.Mawson, "The effects of both preparation method and season on the supercritical extraction of ginger," *Separation and Purification Technology*, 2006, vol. 48, pp. 94-105.

[6] J.Hu, Z.Guo, M.Glasius, K.Kristensen, L. Xiao, X.Xu, "Pressurized liquid extraction of ginger (Zingiberofficinale Roscoe) with bioethanol: an efficient and sustainable approach,"*J Chromatogr A*, 2011, vol.1218, pp. 5765-5773.

[7] M.S.MdSarip, N. A.Morad, N. A.Mohamad Ali, Y. A.MohdYusof, M.A.CheYunus, "The kinetics of extraction of the medicinal ginger bioactive compounds using hot compressed water,"*Separation and Purification Technology*, 2014, vol. 124, pp. 141-147.

[8] M. G. Bogdanov, I. Svinyarov, "Ionic liquid-supported solid–liquid extraction of bioactive alkaloids. II. Kinetics, modeling and mechanism of glaucine extraction from *Glaucium flavum* Cr. (*Papa veraceae*)," *Separation and Purification Technology*, 2013, vol. 10, pp. 279-288.

[9] E. V. Petersson, J. Liu, P.J.R. Sjöberg, R. Danielsson, C. Turner, "Pressurized hot water extraction of anthocyanins from red onion: A study onextraction and degradation rates,"*AnalyticaChimicaActa,* 2010, vol. 663, pp. 27-32.

[10] L.Wang, C.L.Weller,"Recent advances in extraction of nutraceuticals from plants," *Trends in Food Science & Technology*, 2006. vol.17,pp. 300-312.

[11] E.L., Cussler, "*Diffusion: Mass transfer in fluid system*,"New York: Cambridge University Press, 1984.

[12] X. Xiao, W.Song, J. Wang, G. Li, "Microwave-assisted extraction performed in low temperature and in vacuo forthe extraction of labile compounds in food samples,"*Analytica ChimicaActa*, 2012, vol. 712: pp. 85-93.

[13] M.Co,C.Zettersten, L. Nyholm, P.J.R. Sjöberg, C. Turner, "Degradation effects in the extraction of antioxidants from birch bark usingwater at elevated temperature and pressure,"*AnalyticaChimicaActa*, 2012. vol. 716, pp. 40-48.

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