EFFECTS OF THE CONCENTRATION OF *QUERCUS INFECTORIA* GALLS (*MANJAKANI*) EXTRACT ON MOISTURE CONTENT AND QUALITY OF ITS FREEZE-DRIED PRODUCT

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ABSTRACT

Freeze-drying is a drying process based on sublimation under vacuum condition. This process minimizes the degradation of the heat-sensitive herbal product in order to preserve its bioactivity and medicinal properties. This work evaluates the effects of concentration of Quercus infectoria galls extract on the moisture content and quality of its freeze-dried product. Gall of Quercus infectoria, locally named as Manjakani, is a widely used traditional medicine originated from Western Asia and Southern Europe. The galls extract contains Tannic acids and Gallic acids, which are powerful astringents. The moisture content of the freeze-dried products were about 5 %w/w which were below the value required for product stability, 10 %w/w. From the results of High Performance Liquid Chromatography (HPLC) analysis, there was no significant variation between the chemical profiles of the products. Two sharp peaks were detected which indicated the presence of the bioactive compounds. Thus, the bioactivity of the Quercus infectoria galls extract was preserved. The concentration of chemical marker, gallic Acid, was calculated for each product. The energy for sublimation was also analyzed.

Keywords: *Quercus infectoria; freeze-drying; moisture content; gallic acid; chemical profile*

INTRODUCTION

Gall of *Quercus infectoria* (QI), or better known as *Manjakani* (see Fig. 1), is originated from Western Asia and Southern Europe. Galls are irregular plant growth, which is stimulated by the reaction between plant hormones and powerful growth regulating chemicals produced by insects or mites [1]. The QI galls are produced by the insect, *Cynips quercufolii*, for depositing its eggs [2]. Rohana et al. (2004) reported that the QI galls aqueous extract showed high potential in skin whitening and antioxidant properties as the extract inhibited the superoxide and DPPH radical scavenging activities, and tyrosinase activities. The aqueous extract of QI galls was also reported to have high hydrolysable tannin content which inhibits the lethality of the *Naja kaouthia* (Thai cobra) venom [4]. The hydrolysable tannins including tannic acid and gallic acid (see Fig. 2) are powerful astringent that are prescribed in diarrhea. The aqueous extract of QI galls also exhibited high antimicrobial activity against *Excherichia coli* O157:H7 [5]. The scientific studies of aqueous extract of QI galls has revealed its potential to provide an alternative for modern medicinal products as well as cosmetics and skin care products.

The bioactivity and medicinal properties of herbal extracts are dependent on the presence of the chemical compounds, which are mostly heat-sensitive. Freeze-drying or lyophilization is a drying process in which the solvent and/or the suspension medium is crystallized at low temperature and thereafter sublimated from the solid state directly into vapor phase under reduced pressure [6]. Thus, this process is suitable to dry the QI galls extract. The operating cost of this process could be expensive as it require high vacuum for sublimation. Generally, the process time will be shorter if the feed concentration is reduced. Therefore, the optimum feed concentration must be determined in order to minimize the operating cost while preserving the chemical properties of the extract. The objective of this work is to determine the effect of the QI galls extract concentration on the moisture content and quality of its freeze-dried product.

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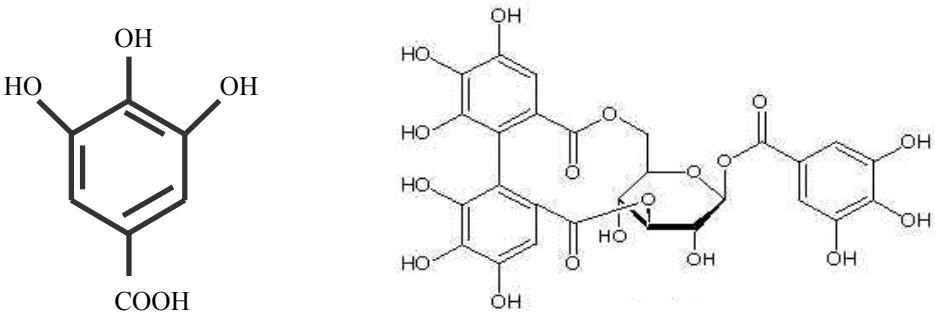
Figure 1: Quercus infectoria Galls (Manjakani).



a) b)

Figure 2: Chemical structure of a) gallic acid; b) tannic acid.

MATERIALS AND METHODS

Aqueous Extract of Quercus infectoria Galls Preparation

The aqueous extracts were prepared in different concentration by dissolving the dried QI galls extract in reverse-osmosis (RO) water. The dried QI galls extract was obtained from Herbal Technology Center, FRIM. The extract concentration studied in this experiment was determined using Digital Abbe Refractometer. The readings were given in Brix scale. This scale is commonly used in sugar refinery industry to measure the percentage by weight of sucrose in a pure solution of this sugar [7]. It is used as a rapid method to measure the extract concentration in this study. The extract with higher concentration shows greater value of Brix. The concentrations used are 10, 15, 25, 30, and 35 Brix.

Freeze-Drying

The freeze-drying process was carried out using a commercial-size Freeze Dryer (Model 35 XL Genesis, Virtis). The freezing and vacuum-drying conditions at various stages were as shown in Table 1. The drying process was carried out for a total of 30 hours. The drying conditions were selected based on the preliminary study of the freeze-drying of other herbal extracts. A total of 1000 ml of aqueous extract was filled in a stainless steel tray (505mm x 255mm). Each batch of process freeze-dried 6 trays of samples. The experiment for each sample was conducted in triplicate.

Table 1: Drying conditions of freeze-drying process

	Temperature (^oC)	Duration (hrs)	Pressure (mmHg)
Freezing	-40.0	4	760.00
Vacuum Drying	-15.0	5	<1.00
Vacuum Drying	0.0	6	<1.00
Vacuum Drying	25.0	15	<1.00

Moisture Content Analysis

The moisture content of the products was determined with halogen drying method using Halogen Moisture Analyzer (Mettler Toledo). 3.0-5.0 g of freeze-dried product was analyzed under rapid drying program for each test. The average of three readings for each sample was taken as the final result.

High Performance Liquid Chromatography (HPLC) Analysis

The quality of the freeze-dried extract was analyzed using HPCL in which the chemical profile and concentration of chemical marker, Gallic acid, were determined. The calibration curve for Gallic acid was obtained using standard pure crystal from Sigma-Aldrich Inc.. The HPLC analysis was carried out using Waters 600E System Controller coupled with Waters 996 Photodiode Array Detector. A Phenomenex Luna C18 100A column (250mm x 4.6mm, 5μm particle size) was used as stationary phase. The mobile phase was in gradient mode by changing the content of 0.1% Orthophosphoric Acid, H_3PO_4 (solvent A) and 100% Acetonitrile, CH_3CN (solvent B). The changes of mobile phase content was shown in Table 2. The flow rate of the mobile phase was 1ml/min and the detection wavelength was 280.0 nm. Each injection contained 10μl of sample.

Table 2: The mobile phase changes in HPLC analysis

Time	Solvent A (%)	Solvent B (%)
0	85	15
12	75	25
20	75	25
22	85	15
25	85	15

RESULTS AND DISCUSSION

Moisture Content

The measured moisture content of the freeze-dried product were as shown in Fig. 3. The moisture content of the product gradually decreased as the extract concentration increased. It was also observed that the maximum difference of moisture content between the products was small, only 0.09 %w/w. This situation might be due to the ineffectiveness of the "bound water" removal during secondary drying. The "bound water" is referred to the unfreezable water of biological system, which binds with polar groups of biopolymers. These bonds are stable at low temperature [8].The vacuum drying temperature must be set higher (>25^oC) if a lower residual moisture content is required. The moisture content of the freeze-dried products were between 5.63 and 5.72 %w/w which were below the targeted value, 10 %w/w.

High Performance Liquid Chromatography (HPLC) Analysis

The HPLC analysis results were summarized in Table 3. Two major peaks were observed in the chromatograms. The data of retention time and wavelength with maximum absorbance of the major peak in the chromatograms indicated the presence of the chemical marker, gallic acid (see Fig. 4). The concentration of gallic acid in the freeze-dried products was determined from the calibration curve based on its percentage of peak area in the chromatogram. There was no significant variation between the chemical profiles of the products (see Fig. 5) which suggested that there is no degradation of chemical properties of the products after the freeze-drying process. This implied that the extract concentration caused no considerable effect on the quality of the freeze-dried product.

Table 3: The results for the major peak in HPLC analysis

Extract Concentration (Brix)	Retention time (min)	Wavelength with maximum absorbance, λ_{max} (nm)	Concentration of Gallic Acid (ppm)
35.0	4.332	215.2	397
30.0	4.223	215.2	348
25.0	4.273	215.2	347
15.0	4.352	215.2	404
10.0	4.317	215.2	364
Gallic acid (Standard)	4.078	215.2	-

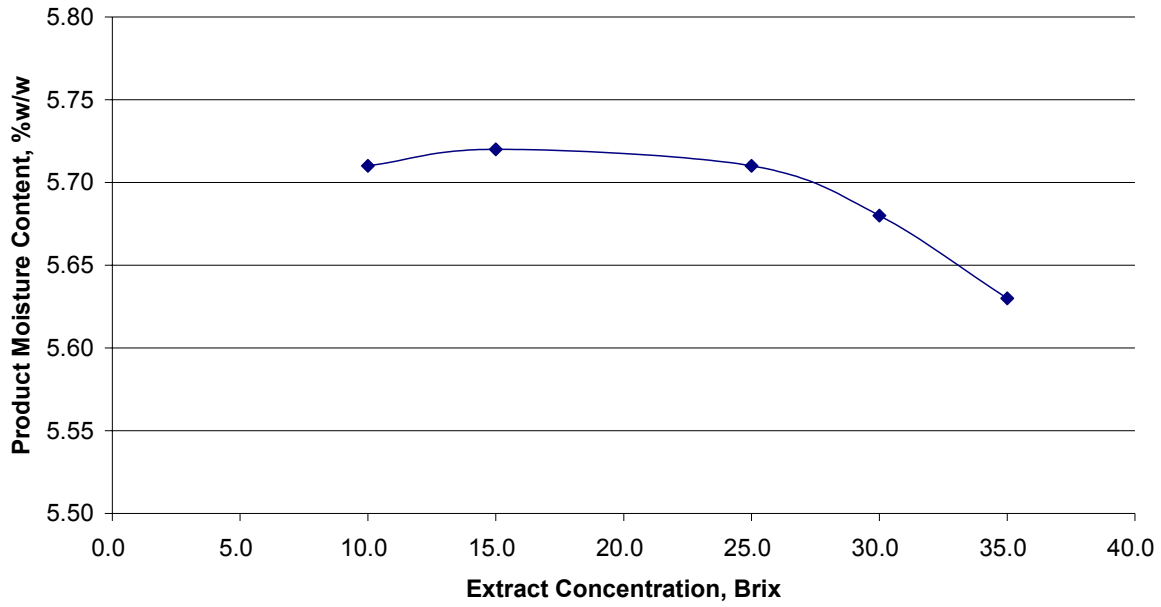


Figure 3: The effect of extract concentration on the product moisture content.

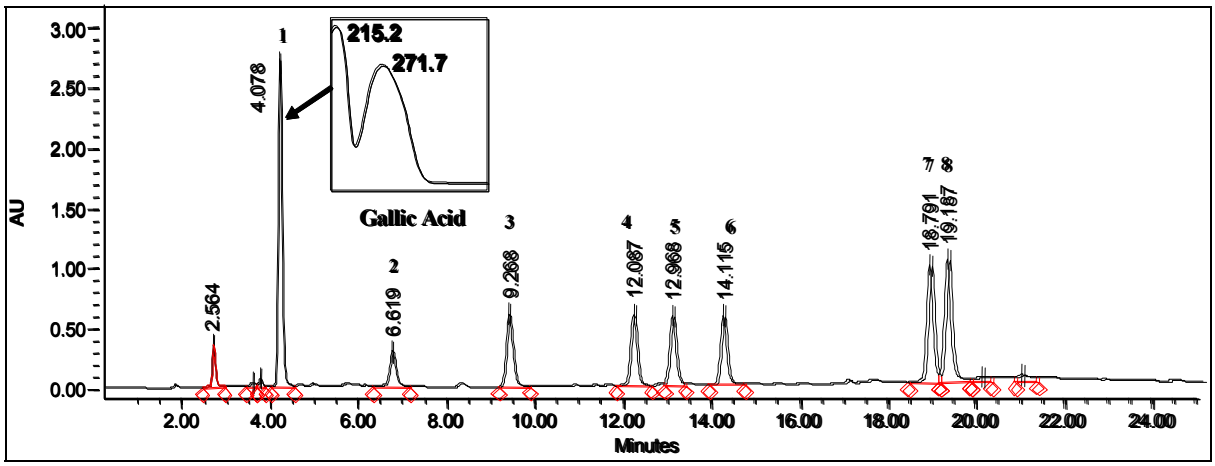


Figure 4: Chromatogram of 8 chemical marker standards monitored at 280.0 nm. The chromatographic conditions are described in text. 1 Gallic acid, 2 Epigallocatechin, 3 Catechin, 4 Epicatechin, 5 Epigallocatechin gallate, 6 Gallocatechin gallate, 7 Epicatechin gallate, 8 Catechin gallate.

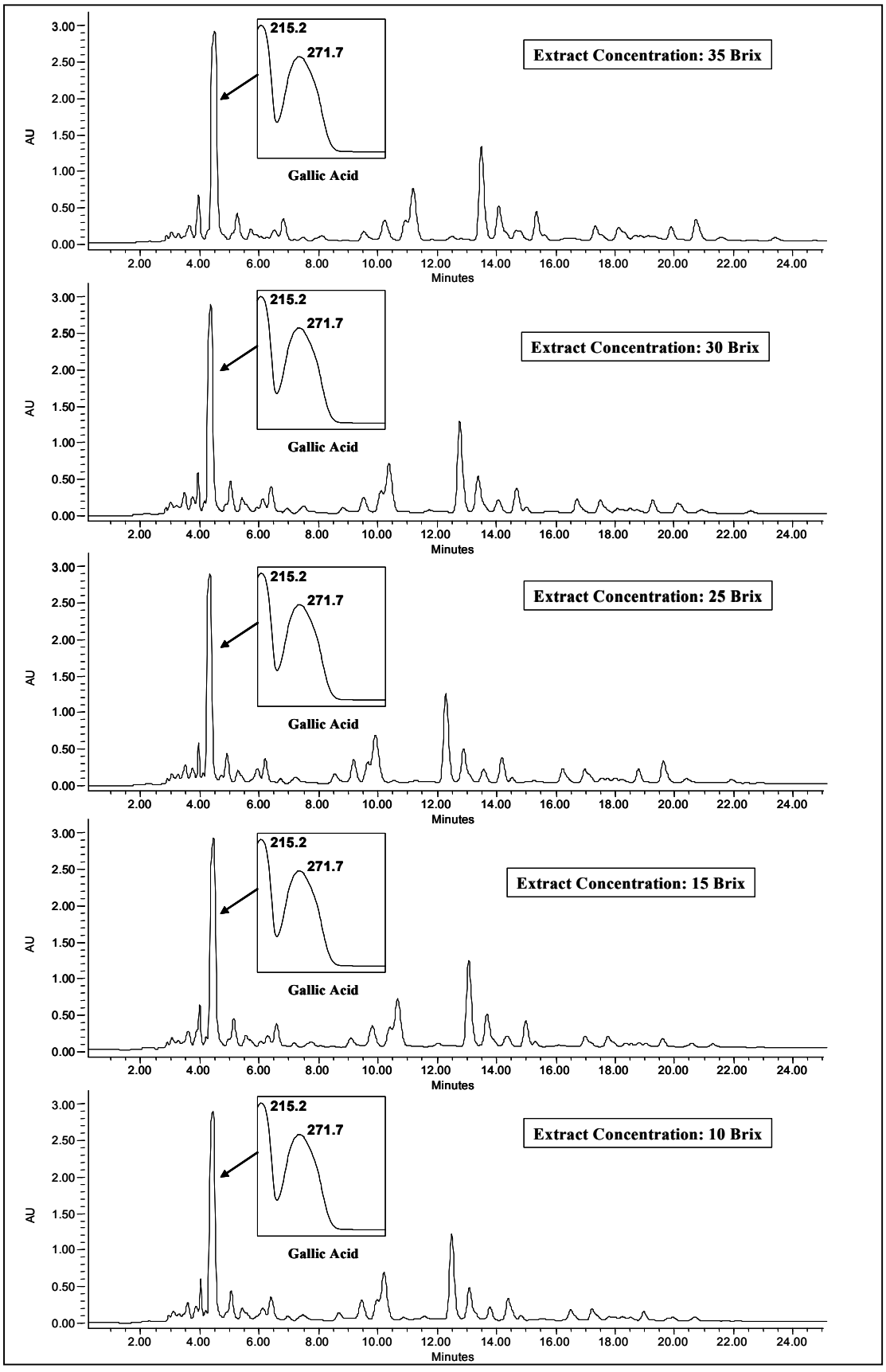


Figure 5: Chromatograms of the freeze-dried products monitored at 280.0 nm.

Energy of Sublimation Analysis

The energy necessary for the freeze-drying process is governed by the energy to transform the ice into water vapor. This energy is referred as the energy of sublimation which depends on the total amount of water sublimated and the latent heat of sublimation, ΔH_s. The value of ΔH_s is estimated from the sum of latent heat of fusion, ΔH_m, and hypothetical latent heat of vaporization, ΔH_v [9].

$$\Delta H_s = \Delta H_m + \Delta H_v \tag{1}$$

which ΔH_m is given as a function of temperature given as:

$$\Delta H_m = \Delta H_{m,T_1} + \int_{T_1}^{T} (C_p^L - C_p^S) dT \tag{2}$$

where

C_p^L = heat capacity of saturated liquid = 4.18 J/g K

C_p^S = heat capacity of solid (ice) = 2.09 J/g K

T_1 = reference temperature = 273 K

$\Delta H_{m,T_1}$ = heat of fusion at T_1 = 333.78 J/g K

It was assumed that the fusion took place at the first step of vacuum-drying at -15^{o}C.

ΔH_v is the difference between the enthalpy of the saturated vapor and saturated liquid at the same temperature. It can be estimated based on the ΔH_v at normal boiling point (T_b) using Watson relation [9]:

$$\Delta H_v = \Delta H_{vb} \left(\frac{1 - T_r}{1 - T_{rb}} \right)^n \tag{3}$$

where

ΔH_{vb} = ΔH_v of water at normal boiling point (100^{o}C)
= 2256.9kJ/kg

T_r, T_{rb} = reduced temperature

n = 0.38

In this study, the hypothetical vaporization temperature was determined by extrapolation of the experimental data given in steam table [10].

The amount of water present in the extract was required in the calculation of the energy of sublimation. The values were calculated based on the solid content which was determined using correlation from Fig. 6:

$$Y = 0.0198\ X^2 + 1.0913\ X \tag{4}$$

where

Y = solid content of QI extract, g/100ml H_2O

X = Brix

The energy for sublimation reduced with the increase of the extract concentration (see Fig. 7). It was found that the energy for sublimation decreased approximately 7% for every increase of 5 Brix. This implies that the time and energy required for the freeze-drying process could be reduced by using a higher concentration feed.

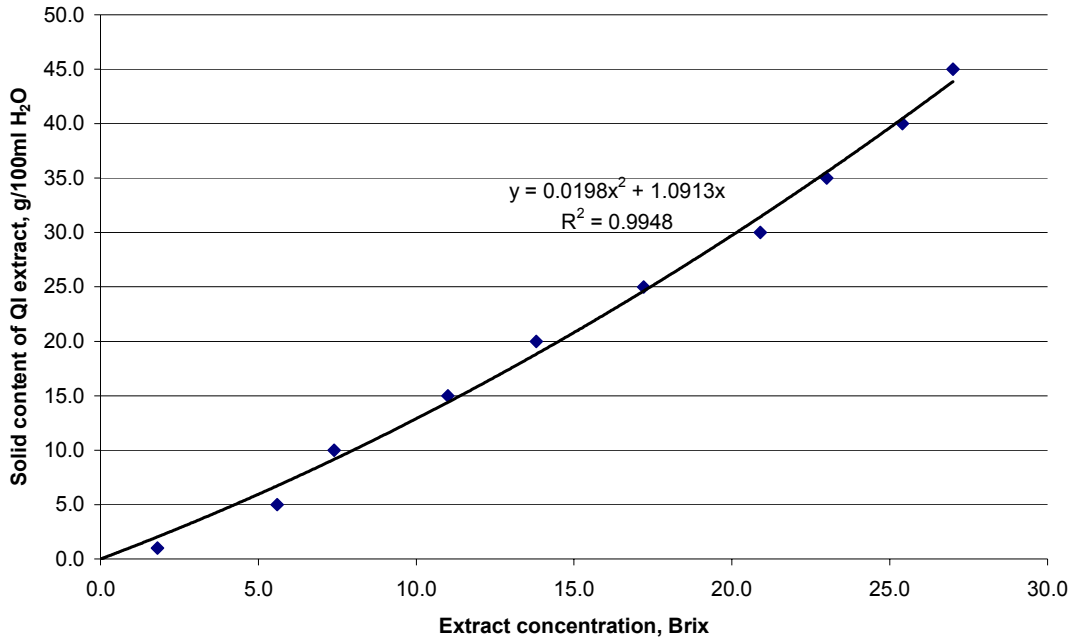


Figure 6: The relationship between the solid content of QI extract and the extract concentration.

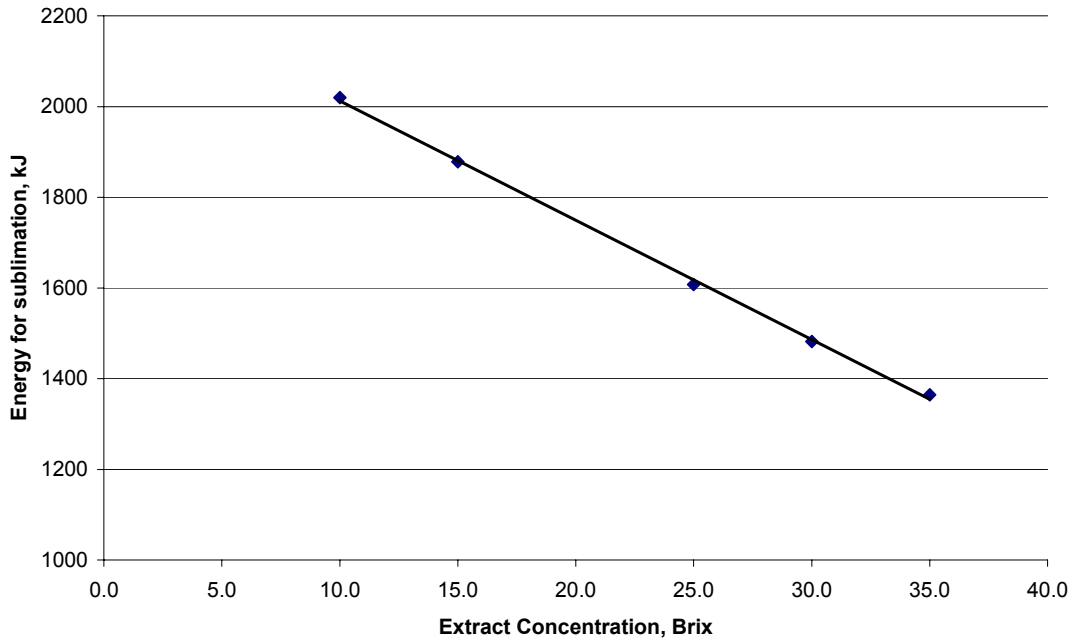


Figure 7: The sublimation energy for different extract concentration.

CONCLUSION

Freeze-drying process is a suitable method to remove the water content of QI galls extract. This drying method is able to preserve the bioactive compounds found in the extract. The sublimation energy analysis showed that the time for freeze-drying could be reduced by using a more concentrated extract. However, more studies and research need to be carried out to determine the optimum freeze-drying conditions including freezing temperature, drying temperature, and operating time.

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