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Effects of Microwave Vacuum Pressure on Drying Curve and Bioactive Compounds of African Night Crawler (*Eudrilus eugeniae* Kinberg)

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ABSTRACT

African Night Crawler (Eudrilus eugeniae Kinberg) was dried by applying constant power (204 W) at different vacuum levels (17, 34, 51 kPa). The drying kinetics, proximate composition, and anticoagulant were determined. Experimental moisture loss data versus drying time were fitted to nine thin-layer models. The Midilli model is the most suitable in describing drying kinetics for all drying conditions. The 17 kPa vacuum exhibited shorter drying time, higher drying rates and lower power consumption. Proximate analysis showed that dried earthworms have high protein content ranging from 61 to 65 percent of its total dry weight. Proximate composition of dried Eudrilus eugeniae is relatively similar to other earthworm species like Eisenia fetida. Microwave-vacuum dried earthworm displayed stronger anticoagulant activity though weaker than the fresh sample. This study suggests that microwave-vacuum dried earthworm has anticoagulant property but not as potent as fresh sample due to the effects of temperature and pressure.

Keywords: drying kinetics, African night crawler, microwave-vacuum, anticoagulant, thin-layer

INTRODUCTION

Thrombolysis or breaking down of a blood clot is one of the current research concerns. Dried earthworms have been intensively used as thrombolytic agents and its thrombolytic mechanism has been studied by the modern pharmacological researchers. However, the drying kinetics and its effects on the anticoagulant activity were rarely investigated.

Several studies on drying of earthworms using different drying methods were recently reported. Fu *et al*. (2013) used sun drying and freeze-drying in processing the earthworm (*Eisenia fetida*). Earthworms were sun-dried at 35-40^{o}C and freeze-dried at -35°C for 1 h and pulverized into pulp thereafter. Based on the study, it was observed that thrombolytic activities of freeze-dried earthworms were greater than those of the sun-dried. Blood clots lysis is high in the groups of a freeze-dried and sundried earthworm. Results show that 71.77% and 63.13% of the blood clot were lysed in the group of freeze-dried and sun-dried earthworms, respectively as compared to 52.83% of the positive control group. The data suggest that freeze drying is a better processing means for earthworm as the thrombolytic therapy.

Bercansil and Belgado (2015) used freeze-drying in processing the African Night Crawler (*Eudrilus eugeniae*). The existence of the protein of the proteoglycan in the freeze-dried earthworm was determined using the bradford assay. The PG1, PG2, PG3 were the three proteoglycans (anticoagulant) fractions isolated from African Night Crawler. The mixture of these proteoglycans, not individual fractions proved to have blood anticoagulant properties but not as potent as heparin.

Another known drying method for earthworms is the microwave-vacuum drying method. Because of its advantages like shortening drying time and improving product quality, the pharmaceutical industry has become very interested in microwave-vacuum drying particularly in the manufacture of tablet granulations (Poska, 1991). These microwave systems are gaining use and may combine mixing, granulating, lubrication, and dry sizing in a single step. Systems as large as 1200 L, employing 36 kW of microwave power, are in use. They demonstrate advantages in operator safety, cleaning, pollution control, and energy savings at costs often comparable to conventional systems (Filkova and Mujumdar, 1995).

For successful drying operation, the choice of adequate technique, particularly for high-value foods including fruits and vegetables becomes the requisite. There are many conventional drying methods used in postharvest technology including solar drying (El-sebaii & Shalaby, 2012), osmotic dehydration (Luchese, Gurak, & Marczak, 2015), vacuum drying (Nadi, Rahimi, Younsi, Tavakoli, and Hamidi-Esfahani, 2012), hot-air drying (Onwude *et al*., 2016), fluidized bed drying (Sagar & Suresh Kumar, 2010), and freeze-drying (Ciurzynska & Lenart, 2011).

Microwave drying has the advantages of shortening drying times and improving product quality, resulting in high nutritional and sensory quality products (Zhang *et al*., 2006). The energy adsorption by the wet material depends on its moisture distribution, which causes selective heating of its interior parts, protecting low moisture parts, e.g. material surface, from overheating (Chandrasekaran *et al*., 2013). Moreover, microwave heating causes volumetric heating so vapor is generally inside the product, developing internal pressure gradients that cause water flow from the interior to the surface of the material (Zhang *et al*., 2006).

Vacuum drying is particularly suitable for products that are sensitive to heat, such as fruits with high sugar content and certain vegetables with high added value (Zhang *et al*., 2006). Microwave heating under vacuum improves the efficiency of drying and prevents or reduces oxidation, preserving product color, texture, and flavor, leading to the products with the quality compared to freeze-dried products (Gunasekaran, 1999; Lin *et al*., 1998).

The study aimed to determine the most suitable thin-layer drying models for microwave-vacuum drying conditions and determine if the drying methods would not alter the anticoagulant activity.

In this study, only vacuum pressure was varied due to the constraint in temperature. Since the sample was heat-sensitive, the power output was constant (204 W) which gave 43°C. The anticoagulant activity is due to the high protein content of the sample. When the drying temperature is beyond 43°C there will be protein denaturation which affect the anticoagulant activity of the earthworm.

MATERIALS AND METHODS

Fabrication of drying equipment

A domestic microwave oven (Whirlpool, Model:MWX 201 XEB) with 700W rated microwave power output, 1200W power consumption, 2450 MHz operation frequency , 20L internal space capacity, and a built-in turntable (helped in the uniformity of the microwave distribution) was used as a dryer.

A ¼ inch diameter pyrex glass tube, which is a nontoxic and good heat resistant material, was connected to the copper tube of the vacuum pump (1/3 hp) using a silicon tube. This was done to avoid the sparkling caused by the incompatibility of the dielectric properties of the copper tube during microwave heating. The pyrex glass tube was placed inside the oven through the top center portion. Figure 1 shows the fabricated microwave vacuum dryer.

Figure 1. The fabricated microwave vacuum dryer set-up.

Preparation of Sample Materials

Cultured African Night Crawler (*Eudrilus eugeniae*) was purchased from vermiculture farm located at Brgy. Putho, Los Baños, Laguna. It was washed with running tap water to remove dirt such as sand/ soil particles and decomposed agricultural waste from the body surface. The earthworms were soaked in distilled water for 8 to 10 hours to release the contents of their guts.

Drying Procedure

The drying procedure was done under 204 W power level and three vacuum levels (17, 34, 51 kPa). The 204 W is the lowest power level of the domestic microwave oven which can attain the 43°C temperature (highest acceptable temperature that will not cause protein denaturation in the sample). About 60-g fresh earthworms were used for each drying setup; the weight of the sample was measured and recorded every 15 minutes and the moisture loss was computed. The samples were dried to approximately 10% (d.b.) final moisture content.

Mathematical Modelling of Drying Data

The moisture loss data were fitted to nine models (Table 1) usually used for the drying curves. The moisture ratio (MR) and drying rates were calculated using Eq. 1 and 2, respectively where M_t = the moisture content (g water.g dry solid-1) at time t (min), M_{t+dt} = moisture content (g water. g dry solid-1) at time t+dt (min); M_0 = initial moisture content, M_e = equilibrium moisture content. The best model describing the thin-layer drying characteristics of earthworms was chosen as the one with the lowest reduced root mean square error (RMSE) and the highest coefficient of determination

$$MR=\frac{M_t-M_e}{M_0-M_e} \quad (1)$$

$$\text{Drying Rate}=\frac{M_{t+dt}-M_t}{dt} \quad (2)$$

(R^2) values (Menge *et al.,* 2005; Goyal *et al.,* 2006; Abalone *et al.,* 2006; Ozbek and Dadali, 2007).

Proximate Analysis

The proximate composition of microwave-vacuum-dried earthworm was evaluated based on the Official Methods of Analysis of the AOAC (1993). Five grams from each sample was used for ash, fat, protein, fiber, and moisture content determination.

Anticoagulant Assay

Anticoagulation activity was measured by assimilating the clotting time of the dried earthworms using three drying methods, fresh earthworm sample, heparin as a positive control, water as a negative control, and $CaCl_2$ as the clotting factor. Three-point eight percent (3.8%) sodium citrate solution was utilized as the anticoagulant during blood extraction. Three Fresh blood samples (50 mL each) were drawn from three different pigs. Blood was collected after ante-mortem inspection and prior to stunning and sticking. Blood collection was done using blood drawing needle and Vacutainer tube with sodium citrate solution. Blood plasma was separated from other components using a refrigerated centrifuge (2400Xg, 20 mins). The coagulation was triggered by adding 0.2 mL 1% $CaCl_2$ to 0.5 mL citrated plasma. The absorbance of the solution was read at 700 nm for every 10 s until the absorbance reaches 0.8 (coagulation) (Adopted from Modified Activated Partial Thromboplastin Time (APTT) Test with some revisions). This study used 1000 IU dose of heparin (commonly available commercial anticoagulant).

Table 1. Thin Layer drying models used to fit moisture loss vs. time data for earthworms dried using three drying methods

NO	MODEL NAME	MODEL	REFERENCES
1	Henderson and Pabis	$MR = a \exp(-kt)$	Westerman *et al*. (1973)
2	Newton	$MR = \exp(-kt)$	Ayensu (1997)
3	Page	$MR = \exp(-kt^n)$	Agrawal and Singh (1978)
4	Logarithmic	$MR = a \exp(-kt)+c$	Yaldiz *et al*. (2001)
5	Two Term	$MR = a \exp(k_o t)+b \exp(-k_1 t)$	Madamba *et al*. (1996)
6	Two Term Exponential	$MR = a \exp(-kt)+(1-a)\exp(-kat)$	Sharaf-Eldeen *et al*. (1980)
7	Wang and Singh	$MR = 1+at+bt^2$	Wang and Singh (1978)
8	Diffusion Approach	$MR = a \exp(-kt)+(1-a) \exp(-kbt)$	Kassem (1998)
9	Midilli	$MR = a \exp(-kt^n)+bt$	Midilli *et al*. (2002)

Table 2. Experimental Design in determining the Anticoagulant activity

GROUP	DOSE	CLOTTING TIME (s)
Negative control		
Heparin	1000 IU	
Microwave-vacuum dried		
16.93 kPa, 204 W	200 mg/ml	
33.86 kPa, 204 W	200 mg/ml	
50.8 kPa, 204 W	200 mg/ml	

RESULTS AND DISCUSSIONS

Drying Kinetics

The moisture loss data for microwave-vacuum experimental conditions are presented in Figures 1, 2, and 3. At a given microwave power level, results showed that drying time was shorter in the setup with 17 kPa vacuum pressure (840 mins) compared to setups at 34 kPa (870 mins) and 51 kPa (900 mins). Hu *et al*. (2006) and Orsat *et al*. (2007) stated that vacuum pressure has important effects on drying time. Increasing the vacuum level produced substantial increase in the drying time at the same microwave power level. These results are similar to those described by Izli and Gunasekaran (2014) for microwave-vacuum drying of carrots.

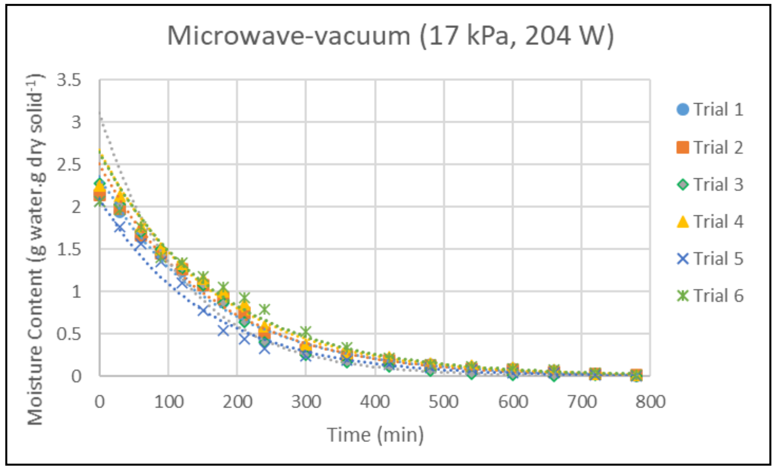

Figure 1. Moisture Content vs. time at 17 kPa pressure and 204 W microwave power output

Figures 4, 5, and 6 described the drying rate data versus the moisture content data at different drying conditions. The drying rate of the dried samples at 204 W-17kPa, 204 W-34 kPa, and 204 W-51 kPa ranged from 0.00002 to 0.07098, 0.00001 to 0.07084, and 0.00001 to 0.07172, respectively. A study by Giri and Prasad (2007) stated that microwave-vacuum drying resulted in almost three-to-five-fold increase in drying rate compared with hot-air drying. Based on the results, higher drying rates were obtained at lower vacuum levels. Similar results were reported by Izli and Gunasekaran (2014) for microwave-vacuum drying of carrots. Some researchers have stated the combined advantages of both microwave drying and vacuum drying with higher drying rates compared with other drying methods (Yongsawatdigul and Gunasekaran 1996; Durance and Wang 2002; Cui *et al*., 2008).

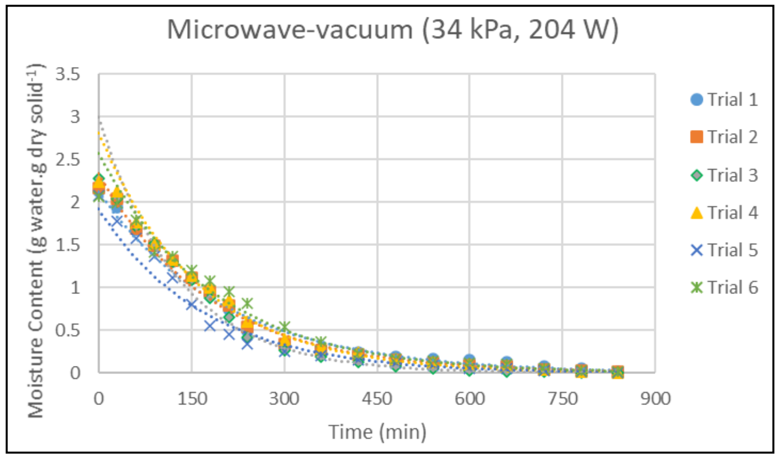

Figure 2. Moisture Content vs. time at 34 kPa pressure and 204 W microwave power output

Fitting of Thin Layer Drying Models

Tables 3, 4, 5 show the model constants and quality of fit in terms of R^2 and RMSE values for nine thin-layer drying models fitted to the moisture loss data. Good fit results were observed from the values of R^2 and RMSE for all models which ranged from 0.9142 to 0.99747, 0.01619 to 0.150312, respectively. Generated values of R^2 and RMSE were used as the selection criteria to determine the best model. The best model describing the thin-layer drying characteristics of earthworms

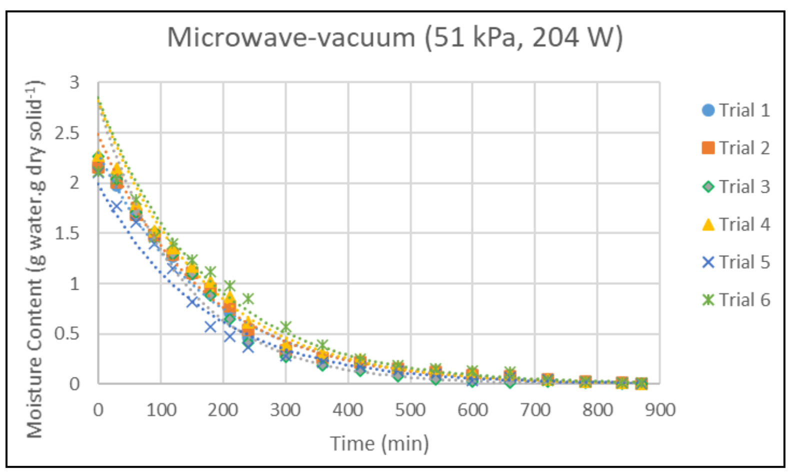

Figure 3. Moisture Content vs. time at 51 kPa pressure and 204 W microwave power output

was chosen as the one with the lowest reduced root mean square error (RMSE) and the highest coefficient of determination (R^2) values. Results showed that Midilli model was the most fitting for all drying conditions tested. Madilli exhibited highest R^2 value and lowest decreased RMSE.

Comparison between the experimental and the predicted values from Midilli model are presented in Figures 7, 8, 9. Although these models slightly over-predicted or under-predicted the experimental data, they were still satisfactory in describing the thin-layer drying behavior of earthworms under the microwave-vacuum drying conditions. The results of nine drying models are also in good agreement with other reported drying models including the results presented by Izli and Gunasekaran (2014).

Proximate Composition

In general, the proximate contents of microwave-vacuum dried earthworms are numerally similar to each other as shown in Table 6. The dry matter content of earthworms has been found to be between 17 and 21 percent of the fresh weight. Dry matter content of earthworms contains 60-63 % protein, 9% fat, 7% ash, and 1-2 % fiber. Sharma *et al.* (2005) obtained comparable results of earthworm dry matter between 20 and 25 percent of the fresh weight and contained around 60 % protein, 7-10 % fat, and 8-10 % ash. Results showed that microwave-vacuum drying methods maintained the high protein content of *Eudrilus eugeniae*. The results showed further that the protein content of *Eudrilus eugeniae* is comparable with a reported protein content of earthworm species like *Eisenia fetida*. Because of its high protein content, it has been observed to be economically feasible to culture earthworms. The isolated active matter from earthworms can be developed into vermiceutical or pharmaceutical products as medication for certain human diseases. It has been established that the presence of anti-

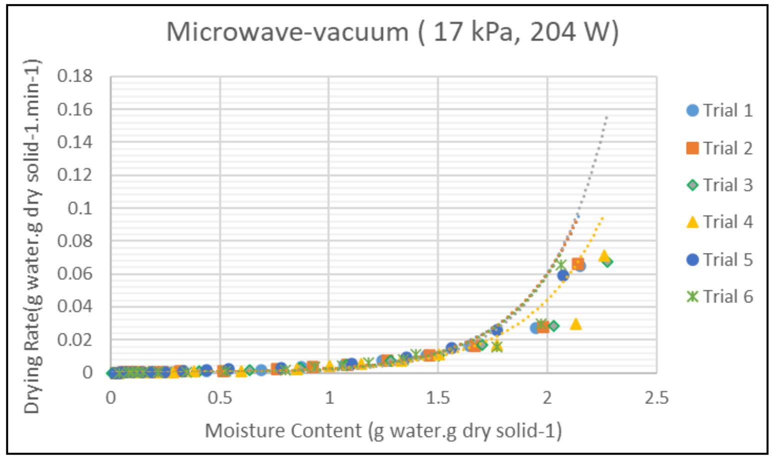

Figure 4. Drying Rate vs. Moisture Content at 17 kPa pressure and 204 W microwave power output

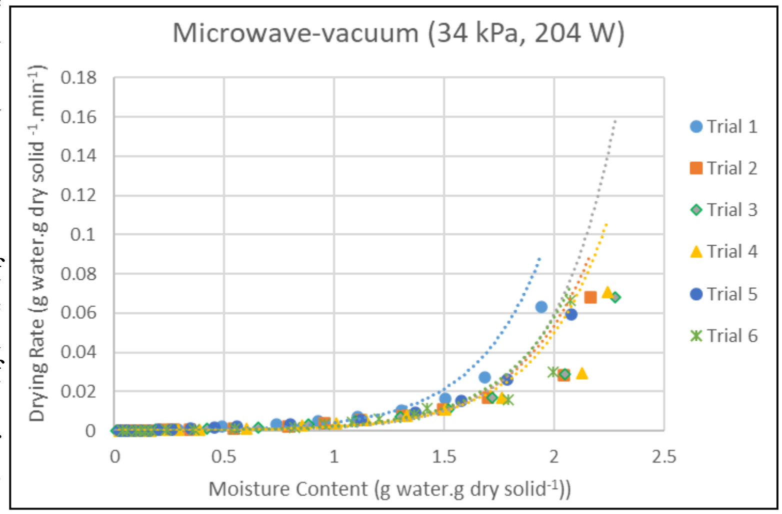

Figure 5. Drying Rate vs. Moisture Content at 34 kPa pressure and 204 W microwave power output

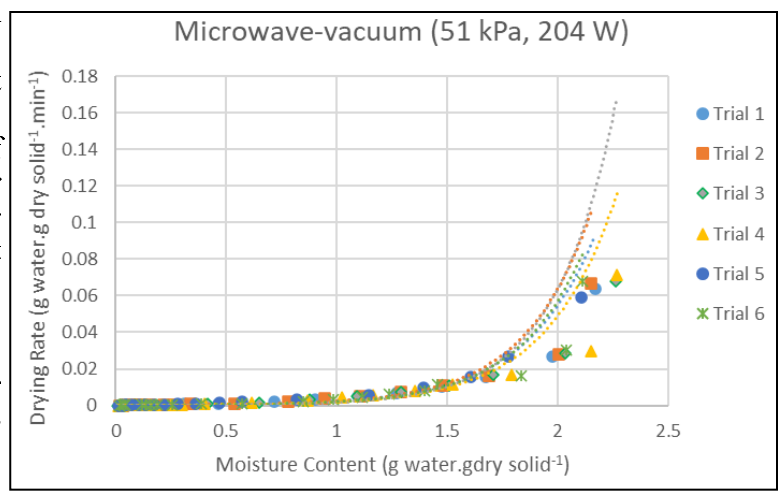

Figure 6. Drying Rate vs. Moisture Content at 51 kPa pressure and 204 W microwave power output

blood clotting action of a crude extract from earthworms used by indigenous people to thin the blood in the elderly (Syariah *et al*., 2013).

Anticoagulant Activity

The fresh earthworm sample and microwave-vacuum dried were subjected to anticoagulant activity evaluation. The estimated clotting times are shown in Table 7 based on the change in absorption of the test solution at 700 nm. Microwave-vacuum method reduced the anticoagulant activity of *Eudrilus eugeniae* compared with fresh earthworm. The decrease in the anticoagulant activity of dried earthworms is due to the inactivation of biologically active enzymes during drying. Fu *et al*. (2013) reported that the active ingredients like fibrinolytic enzymes may inactivate during the drying process.

The change in the absorbance of the test solutions at 700nm for every 10-second interval is depicted in Figure 10. A sharp change in the slope indicates the start of the coagulation process. It could be noticed that there is no significant increase in the slope of heparin-treated and fresh-earthworm-treated plasma, and showing no signs of coagulation. This indicates that the biologically active and naturally present compounds in the fresh *Eudrilus eugeniae* are comparable with heparin.

CONCLUSION

In this study, the effects of microwave-vacuum drying on drying kinetics, proximate composition and anticoagulant activity were evaluated under different drying conditions. The results showed that 17 kPa gave more promising drying rate and drying time and had relatively low operational cost compared with 34 kPa and 51 kPa. Of the nine mathematical drying models tested, the Midilli model was considered the best model in Microwave-vacuum drying conditions. Dried earthworms used in this study had a high crude protein

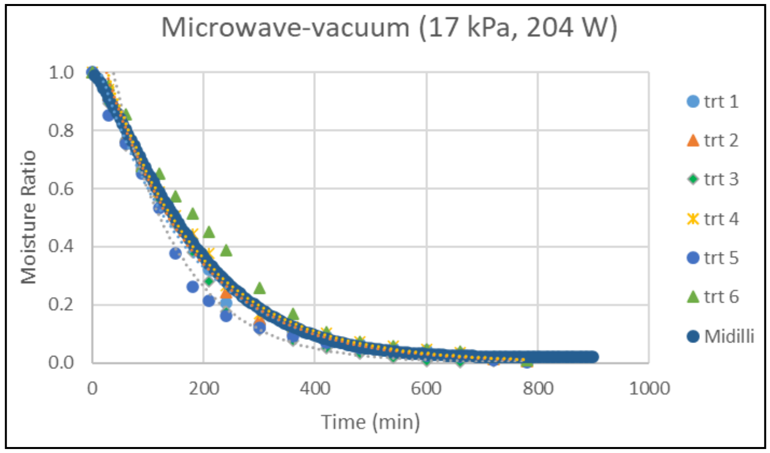

Figure 7. Experimental and predicted (Midilli model) moisture ratio vs. drying time at 17 kPa, 204 W

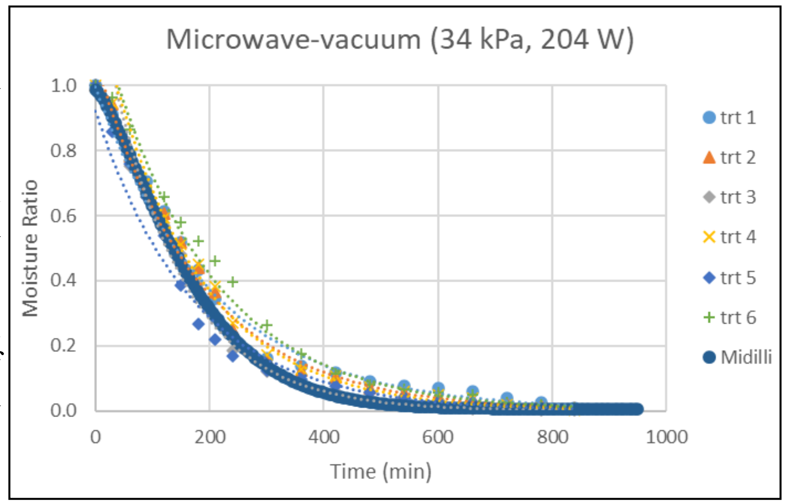

Figure 8. Experimental and predicted (Midilli model) moisture ratio vs. drying time at 34 kPa, 204 W

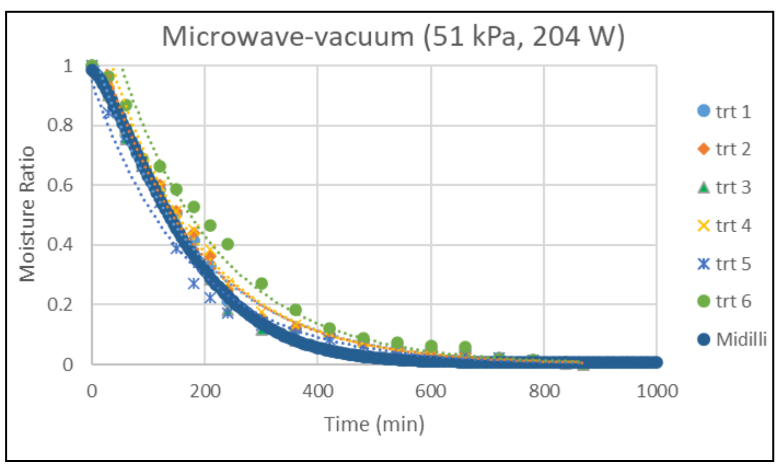

Figure 9. Experimental and predicted (Midilli model) moisture ratio vs. drying time at 51 kPa, 204 W)

Table 3. Values of coefficients and statistical analysis obtained from different thin-layer drying models for drying of earthworm using microwave-vacuum method (17 kPa, 204 W).

NO	MODEL	MODEL CO-EFFICIENTS	R^2	RMSE
1	MR = a exp(-kt)	a = 1.051147 k=0.005166	0.99174	0.123793
2	MR = exp(-kt)	k = 0.005075	0.99126 9	0.124052
3	MR = exp(-kt^n)	k = 0.001889 n = -1.21169	0.994226	0.083279
4	MR = a exp(-kt) +c	a = 1.060318 c = -0.01003 k = 0.004956	0.99397 8	0.02425 9
5	MR = a exp(k_ot) +b exp(-k_1t)	a = -0.46828 b = 1.469266 k_o = 0.016114 k_1 = 0.006729	0.997149	0.016898
6	MR = a exp(-kt)+ (1-a)exp (-kat)	a = 1.846766 k = 0.008394	0.996736	0.018161
7	\MR = 1+at+bt^2	a = -0.00303 b = 0.00000229	0.91424 5	0.091874
8	MR = a exp(-kt)+ (1-a) exp(-kbt)	a = 1.467095 b = 2.407601 k = 0.006727	0.997148	0.0169
9	MR = a exp(-kt^n) +bt	a = 1.000185 b = 0.000022 k = 0.001229 n = 1.26899	0.997381	0.016196

Table 4. Values of coefficients and statistical analysis obtained from different thin-layer drying models for drying of earthworm using microwave-vacuum method (34 kPa, 204 W).

NO	MODEL	MODEL CO-EFFICIENTS	R^2	RMSE
1	MR = a exp(-kt)	a = 1.315163 k = 0.007739	0.99456	0.150312
2	MR = exp(-kt)	k = 0.005378	0.991031	0.134278
3	MR = exp(-kt^n)	k = 0.001704 n = -1.22764	0.996298	0.067495
4	MR = a exp(-kt) +c	a = 1.073218 c = -0.01952 k = 0.004937	0.993635	0.02525
5	MR = a exp(k_ot) +b exp(-k_1t)	a = -6.50607 b = 7.497511 k_o = 0.011343 k_1 = 0.010141	0.997234	0.016761
6	MR = a exp(-kt) +(1-a)exp (-kat)	a = 1.85336 k = 0.008316	0.996924	0.017676
7	MR = 1+at+bt^2	a = -0.00302 b = 0.00000228	0.989921	0.033514
8	MR = a exp(-kt) +(1-a) exp(-kbt)	a = 15.9006 b = 1.050881 k = 0.010368	0.997197	0.016872
9	MR = a exp(-kt^n) +bt	a = 0.986571 b = 0.00000703 k = 0.000898 n = 1.350427	0.997334	0.016455

based on the proximate analysis. Proximate composition of dried *Eudrilus eugeniae* is comparable with the reported proximate composition of different earthworm species like *Eisenia fetida*. The presence of anticoagulant property of fresh earthworm *Eudrilus eugeniae* was substantiated and results showed that it is comparable with commercially available anticoagulant like heparin. It was demonstrated that microwave-vacuum drying method altered the anticoagulant activity of earthworms but still exhibited a relatively high anticoagulant activity. The

Table 5. Values of coefficients and statistical analysis obtained from different thin-layer drying models for drying of earthworm using microwave-vacuum method (51 kPa, 204 W).

NO	MODEL	MODEL COEFFICIENTS	R^2	RMSE
1	MR = a exp(-kt)	a = 1.011124 k = 0.004832	0.993554	0.120313
2	MR = exp(-kt)	k = 0.004814	0.993533	0.117455
3	MR = exp(-kt^n)	k = 0.002153 n = -1.17782	0.992761	0.100556
4	MR = a exp(-kt)+c	a = 1.05547 c = -0.00253 k = 0.004948	0.994265	0.026079
5	MR = a exp(k_ot)+b exp(-k_1t)	a = -0.10565 b = 1.105647 k_o = 0.335516 k_1 = 0.005266	0.996902	0.019721
6	MR = a exp(-kt)+(1- a)exp (-kat)	a = 1.852855 k = 0.008292	0.997076	0.018304
7	MR = 1+at+bt^2	a = -0.00285 b = 0.00000202	0.98659	0.040109
8	MR = a exp(-kt)+(1- a) exp(-kbt)	a = 14.00226 b = 1.05818 k = 0.010303	0.997294	0.017162
9	MR = a exp(-kt^n)+bt	a = 0.986929 b = 0.00000861 k = 0.000901 n = 1.349542	0.997467	0.016603

Table 6. Proximate composition of the total dry weight of *Eudrilus eugeniae*

DRYING CONDITIONS	% MOISTURE	% ASH	% CP	% Cfi	% Cfa
17 kPa,204 W	10	7	63	2	9
34 kPa,204 W	10	7	61	1	9
51 kPa,204 W	10	7	60	1	9

results herein revealed that microwave-vacuum drying is feasible alternatives for drying heat-sensitive sample like earthworms.

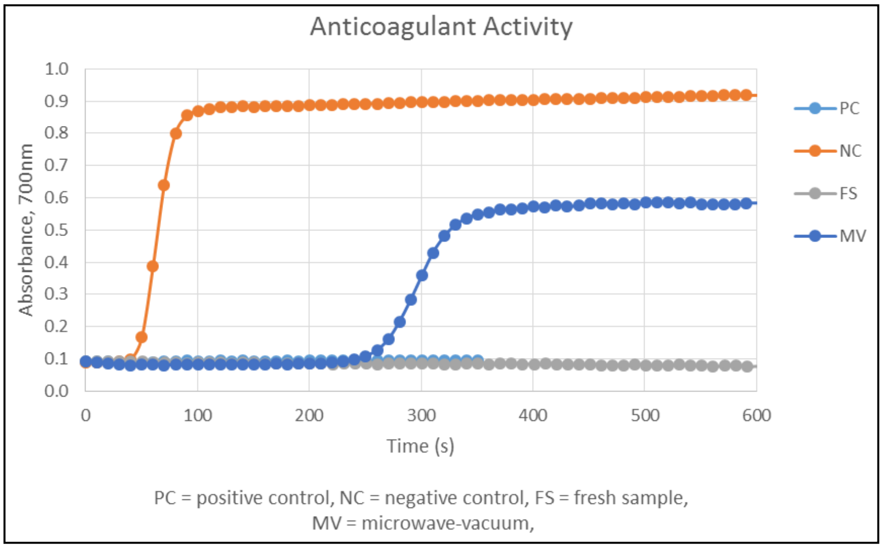

Figure 10. Absorbance at 700 nm of test solutions for anticoagulant assay

RECOMMENDATIONS

The following recommendations are provided as a result of the study: (1) Bacteriological test should be done to determine if the sample is contaminated during the drying; (2) processes; (3) Other biological activity assessments such as anti-inflammatory, anti-bacterial, and anti-ulcer tests should also be conducted; (4) Microwave-vacuum drying is feasible alternatives for drying heat-sensitive sample like earthworms.

Table 7. Clotting time of blood plasma and other test solutions

TREATMENT	CLOTTING TIME, s
Heparin (positive)	No clotting
Blood plasma (negative)	40
Fresh earthworm sample	No clotting
Microwave-vacuum dried	250

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