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DETERMINATION OF INFLUENTIAL FACTORS DURING ENZYMATIC EXTRACTION OF GINGER OIL USING IMMOBILE ISOLATED COW RUMEN ENZYMES

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ABSTRACT

With respect to its multiple uses, such as in cosmetics, foods, aromatherapy and the pharmaceutical industry, ginger oil has a high value in the world market. The ginger oil obtained from conventional extraction usually has low zingiberene content, possibly due to thermal degradation. To overcome this problem, an alternative ginger oil production process by enzymatic extraction using cow rumen enzymes is investigated. The aim of the research is to obtain the optimum conditions for zingiberene-rich ginger oil extraction by using immobile isolated cow rumen enzyme. The experiments were conducted under varying temperatures (40–60^{o}C), enzyme-substrate ratios (0.05–0.2) and extraction times (1–5 days). The microwave assisted distillation was conducted for 90 minute to separate the ginger oil from its mixture. The zingiberene content in the oil was measured by GC analysis. The most influential factor in the enzymatic extraction of ginger oil was determined by experimental design 23. Analysis of the results shows that for the extraction with a rumen ratio of 1:5 at 60^{o}C, the most influential factor was the extraction time, in this case 5 days, and ginger oil was obtained with zingiberene contents of 21.56% and 26.28% at pH 5 and pH 4 respectively. Prolonging the extraction time to 6 days with pH 5 caused a decrease in zingiberene content to 20.76%.

Keywords: Cow rumen; Extraction; Ginger pulp; Zingiberene

1. INTRODUCTION

Ginger oil is known to have multiple uses, such as in the food, aromatherapy, cosmetics and pharmaceutical industries, meaning that this natural product is an important commodity in the world market. Unfortunately, ginger oil is produced in Indonesia by the steam distillation process and therefore contains low levels of zingiberene compared to camphene and curcumene. Typically, the oil has a positive optical rotation value. In contrast, the commercial standard of ginger oil demands a negative optical rotation value, indicating higher zingiberene content than that of curcumene and camphene. The optical rotation value represents the purity of the ginger oil. The zingiberene content was studied by Koroch (2007), who showed that Madagascan ginger oil has a positive rotation value, and contains relatively small amounts of zingiberene, camphene and curcumene.

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The low zingiberene content in ginger oil obtained from the conventional distillation process is usually associated with thermal degradation, as zingiberene is a thermolabile compound (Agarwal, 2001). The conventional distillation process takes between 10–18 hours to produce ginger oil, a process which increases the risk of the thermal degradation of zingiberene. Along with the length of time required for the distillation process, the required energy for heating is also higher, so the process becomes less economical. Other ginger oil extraction processes are generally conducted using ethanol, isopropyl alcohol and petroleum ether as solvents. However, process efficiency is relatively low because it may reach the natural limit (phase equilibrium), so the process rate cannot be further increased (Hu et al., 2011). Although the supercritical fluid extraction method can increase the yield of ginger oil, production costs and the price of its specific equipment are extremely high (Mesomo et al., 2013).

To overcome these problems, alternative developments of the appropriate ginger oil production processes need to be established. Considering that zingiberene is a thermolabile compound, the proposed process should be able to extract ginger oil quickly in order to minimize the use of energy, and with controllable temperatures to prevent thermal degradation. Enzymatic extraction techniques are believed to have many advantages over conventional extraction, such as high yields, high selectivity, and being environmentally friendly (Avilla et al., 2005; Panouile et al., 2007; Ptichkina et al., 2008; Paramita et al., 2015). Although there are several patents for enzymatic extraction processes (US Patents No. 5,952,023, US Patents No. 7,026,152), process efficiency is still less than 80%. This is because about 15–20% of the total production cost is used for the provision of enzymes. The enzymatic extraction technique is a prospective technology because it can shift the phase equilibrium by degrading the cell wall structure of the plant so that the solute can be extracted properly (Panouile & Durant, 2007). The technology is suitable for the collection of thermolabile compounds because it has better temperature control than conventional heating processes (Venkatesh & Raghavan, 2004). Nevertheless, how the application of enzymatic extraction using immobile isolate can increase the yield and zingiberene content in oil production from ginger rhizomes needs to studied. Moreover, Rosenthal et al. (1996) state that in the enzymatic process, the enzyme essentially hydrolyzes the polysaccharide structure, which builds cell walls from seeds and contains oil or proteins that form cells and lipid body membranes.

The fundamental weakness of the enzyme-assisted process approach is that commercial enzymes are relatively expensive and can only be used once (Klein-Marcuschamer et al., 2012). Therefore, to reduce the cost of production when using enzymatic processes, enzymes derived from cow rumen fluid have been isolated and used as the agent (Baba et al., 2013; Li et al., 2017). The isolated enzymes from cow rumen have many advantages over commercial enzymes; for example, they are more stable at high temperatures, have higher specific activity, higher optimum pH, and lower production costs (Heim, 2011). Moreover, in order to use enzymes repeatedly and continuously, they are immobilized in a carrier, such as alginate. The introduction of immobilized enzymes has, in some cases, greatly improved both the technical performance of the industrial processes and their economical aspects (Brena & Batista-Viera, 2006).

Considering the advantages of cow rumen fluid enzyme and the advantages of enzyme immobilization, this research investigates the possibility of the utilization of immobilized cow rumen fluid enzyme in the ginger oil extraction process. The aim of the research is to obtain the optimum conditions for zingiberene-rich ginger oil extraction by using immobile isolated cow rumen enzyme. The experiments were conducted under varying temperatures (40–60^{o}C), enzyme-substrate ratios (0.05–0.2) and extraction times (1–5 days).

2. METHODOLOGY

2.1. Materials

The raw materials used to produce ginger oil by the enzymatic extraction process were the pulp of red ginger rhizomes, cow rumen and alginate.

Table 1 Ginger oil specification

Characteristic	Ginger oil (ISO 7355)	Ginger oil extracted in experiment
Density	0.870–0.890	0.885
Refractive index	1.480–1.490	1.50
Optical rotation	(-20)–(-45)^o	-15^o
Solubility in alcohol 90%	1:4	1:6

Chemical reagents for product analysis, such as phosphate buffer, ethanol, distilled water, sodium hydroxide, hydrochloric acid and phenolphtalein indicator, were purchased from Merc, an authorized chemicals distributor in Semarang-Indonesia.

2.2. Procedure

The main equipment used in the research was an enzymatic extractor, as shown in Figure 1.

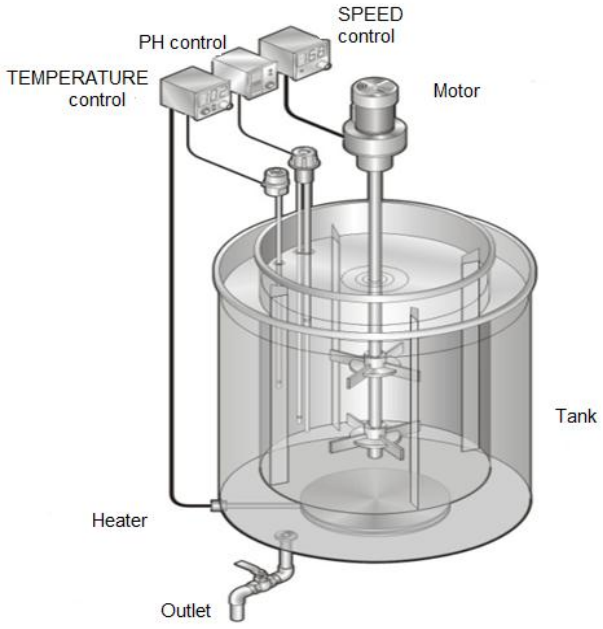

Figure 1 Enzymatic extractor

The operating variables during the study consisted of control variables and independent variables. The experimental research design is shown on Table 2.

2.2.1. Control variables

The control variables consisted of: (i) weight of wet ginger pulp: 400 gr (water content: 73.6%), giving 105.6 gr of dry ginger pulp; (ii) stirring speed of 60 rpm; (iii) water volume of 1000 ml; (iv) pH of 4 and 5; (v) microwave assisted extraction of 150 minutes at 100°C.

2.2.2. Independent variables

A : Ratio of enzyme:material (% w/w) 1:20 (–) &1:5 (+)
B : Temperature extraction (^oC): 40 (–) & 60 (+)
C : Extraction time (days): 1 (–) & 5 (+)

Table 2 Experimental research design

Run	A Ratio	B Temperature ^oC	C Time (days)
1	0.20	60	5
2	0.20	60	1
3	0.20	40	5
4	0.05	60	5
5	0.20	40	1
6	0.05	40	5
7	0.05	60	1
8	0.05	40	1

2.2.3. Enzymatic extraction procedure

The extraction was carried out in an enzymatic extractor (Figure 1) with water as the solvent. Enzymes were added to the ginger pulp of ginger at a certain weight ratio. Distilled water was then added and the pH of the solution was adjusted to the desired value using a phosphate buffer solution. pH affected the enzyme activity as a biocatalisator to shift the equilibrium phase and increase the production rate because of its ability to degrade the cellulose wall of the oil cells. The extraction process was carried out at certain temperatures and periods of time. Before the feed and solvent were introduced into the extractor, it was conditioned set at the desired temperature. The enzymatic extraction was performed according to the targeted time, and the mixture was distilled with microwaves at 100°C for 150 minutes. The ginger oil obtained was measured in volume and the zingiberene content analyzed using GC.

2.2.4. Data interpretation

The research was conducted by experiment and data processing with experimental design 2^3 to obtain useful data in determining the most influential parameters in the production process. Experimental design is one way that is often used compared to other conventional approaches, because it has several advantages, namely: (i) it requires fewer experiments to establish the effects of all the variables; (ii) optimum conditions are obtained more precisely because it includes the interaction factors; and (iii) the conclusion is more certain because it is supported by simple statistical calculation methods.

3. RESULTS AND DISCUSSION

The experiment research was designed and analyzed using an experimental design system, which means a set of experiments was designed to obtain concrete data to prove the hypothesis. In the experimental design, each of the tested variables was determined at several values; in this study with two values for the independent variables. These independent variables were then combined. The combination of independent variables allows data to be obtained which will help reach the conclusions by using statistical methods.

Table 3 Zingiberene content of the enzymatic extraction process

Run	A Ratio	B Temperature °C	C Time (days)	Zingiberene Content (%)	
				pH 4	pH 5
1	0.20	60	5	26.28	21.56
2	0.20	60	1	18.73	10.41
3	0.20	40	5	22.51	19.48
4	0.05	60	5	21.59	18.76
5	0.20	40	1	19.13	9.18
6	0.05	40	5	20.15	12.75
7	0.05	60	1	15.67	15.99
8	0.05	40	1	7.03	6.71

The estimated effect value and the probability value of the experiments conducted at pH 4 are shown in Table 4 and Figure 2.

Table 4 Experimental research analysis at pH 4

Run	Factor Code			Zingiberene Content (%)	Divider	Effect	Result	P = $\left\|(i-0.5)x\frac{100}{7}\right\|$
	A (I_1)	B (I_2)	C (I_3)					
1	+	+	+	26.28	8	Average	18.88625	
2	+	+	-	18.73	4	I_1	-4.0225	64.607
3	+	-	+	22.51	4	I_2	-4.3725	69.607
4	-	+	+	21.59	4	I_{12}	-0.7575	17.964
5	+	-	-	19.13	4	I_3	-6.7825	104.036
6	-	-	+	20.15	4	I_{13}	0.2125	4.107
7	-	+	-	15.67	4	I_{23}	-3.9175	63.107
8	-	-	-	7.03	4	I_{123}	-4.0725	65.321

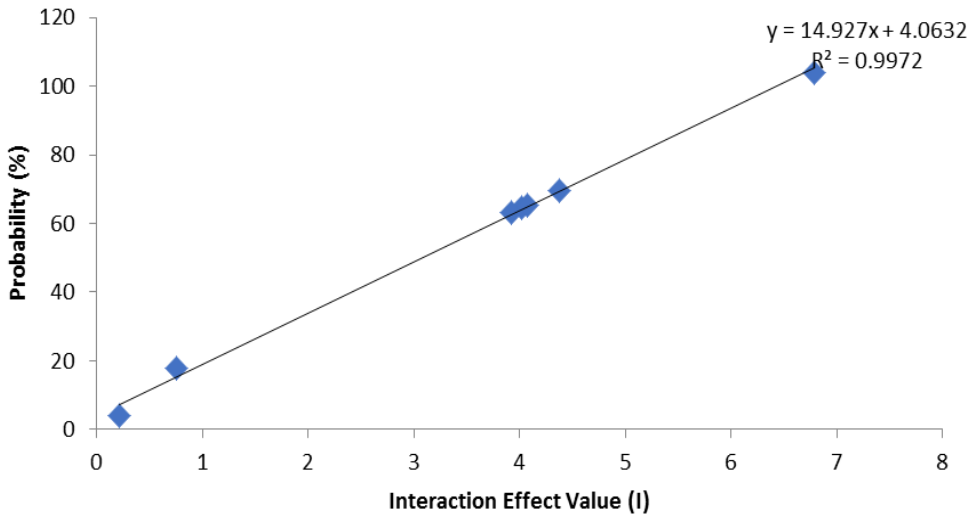

Figure 2 Interaction effect value in experimental research at pH 4

Figure 2 shows that the interaction effect according to equation Y = 14.927X+4.0632 with R^2 = 0.9972, and that the most influential factor is the extraction time.

The estimated effect value and the probability value of the experiments conducted at pH 5 are shown on Table 5 and Figure 3.

Table 5 Experimental research analysis at pH 5

Run	Factor Code A (I_1)	B (I_2)	C (I_3)	Zingiberene Content (%)	Effect	Result	P = $\left\|(i-0.5)x\frac{100}{7}\right\|$
1	+	+	+	21.56	Average	14.355	
2	+	+	-	10.41	I_1	-4.395	69.929
3	+	-	+	19.48	I_2	1.760	18.000
4	-	+	+	18.76	I_{12}	-0.605	15.786
5	+	-	-	9.18	I_3	-6.395	98.500
6	-	-	+	12.75	I_{13}	1.540	14.857
7	-	+	-	15.99	I_{23}	-1.375	26.786
8	-	-	-	6.71	I_{123}	-5.820	90.286

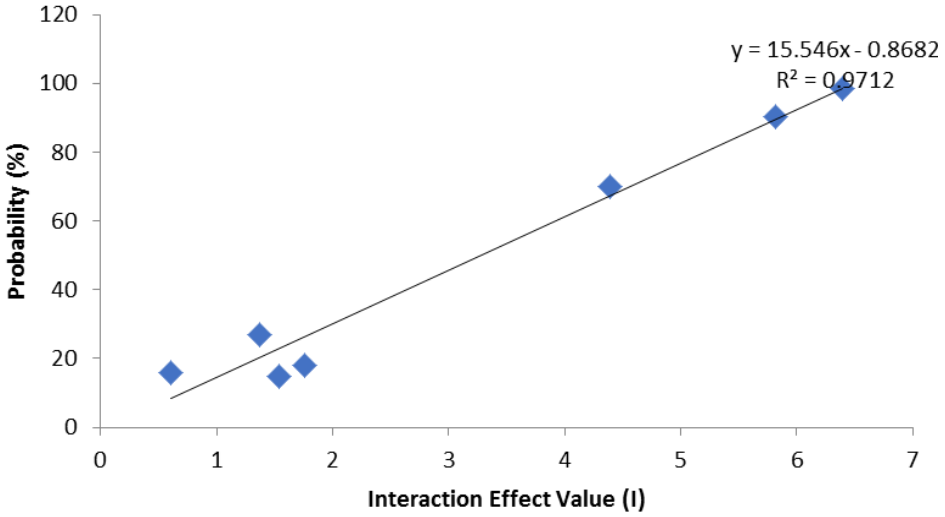

Figure 3 Interaction effect value in experimental research at pH 5

Figure 3 shows that the interaction effect according to equation Y = 15.546X–0.8682 with R^2 = 0.9712, and that the most influential factor is the extraction time.

Figures 2 and 3 show that the regression of the determination of the influencing process variables was appropriate; it is shown that the value of R^2 approaches 1.

4. DISCUSSION

In this research factorial design at two levels was used in the investigation of the enzymatic extraction of ginger oil. In this design, several levels or variations for each variable were selected and the experiment was conducted carefully with all possible variable combinations. From the results, regression analysis was conducted to on the values of the interaction effects and the percentages of probability using the Matlab^R program, so that the influences of the variables on the ginger oil extraction process could be determined. Table 3 shows the results of the zingiberene content of the ginger oil extracts. The estimated effect values and the probability values of the experiments conducted at pH 4 and 5 are shown in Tables 4 and 5, and in Figures 2 and 3.

Figure 2 shows the interaction effect of enzymatic extraction conducted at pH 4. The research shows that the interaction effect according to equation Y = 14.927X+4.0632, with R^2 = 0.9972. The extraction time was also found to be the most influential factor. In the extraction with a rumen ratio of 0.20 at 60°C for 5 days, ginger oil was obtained with a

zingiberene content of 26.28%. Both sets of experiments showed that the factor affecting enzymatic extraction was time, which was optimum at 5 days. Extraction for 6 days resulted in a ginger oil with a lower zingiberene content of 20.76% (Table 3).

Figure 3 shows that the interaction effect according to equation Y = 15.546X–0.8682 with $R^2 = 0.9712$, and that the most influential factor is the extraction time. A rumen ratio of 0.20, extraction temperature of 60°C and extraction period of 5 days gave a ginger oil with a zingiberene content of 21.97%. The research shows that an extraction time of 5 days was the optimum time for the enzymatic extraction, since the zingiberene level decreased when the extraction was conducted for longer. The zingiberene level of the ginger oil was 20.73% when the enzymatic extraction was conducted for 6 days.

The results show that time was the main influencing factor in the enzymatic process. This type of process for extracting oil is considered to be environmentally friendly (Mariano et al., 2009). It has also been found that particle size, water content, time, and the weight ratio of enzyme substrates affect the enzymatic process. Moreover, Rosenthal et al. (1996) report that in this process, the enzyme essentially hydrolyzes the polysacharide structure, which builds cell walls from the seeds and contains oil or proteins that form cells and lipid body membranes. The zingiberene content obtained by this enzymatic process has been found to be higher than from other methods, such as soxhlet extraction. The basis of the enzymatic process is to hydrolyze the polysaccharide structure which builds the ginger oil cell walls. The penetration of cow rumen enzymes causes damage to the polysaccharide structure, hence the ginger oil in its pouch can be extracted. This is due to the move from the oil phase to the aquatic phase. At the same temperature and time, this causes more diluents to cross over into the aquatic phase. Nour et al. (2017) extracted ginger oil by supercritical fluids and soxhlet extraction. They found that the zingiberene content of the ginger oil extracted by the soxhlet (solvent semi-continuous extraction) method for 6 hours with n-hexane was only 13.74%, while supercritical fluid extraction was able to produce ginger oil extract with a zingiberene content as high as 16.98%.

5. CONCLUSION

Evaluation of the effect of the process parameters (temperature, enzyme-substrate ratio and extraction time) on the enzymatic extraction using rumen immobilized enzyme isolates on ginger oil production from ginger pulp has been conducted and it is concluded that extraction time is the most influential factor. At pH 4 and pH 5, optimum conditions were achieved when the rumen enzyme was used at a ratio of 1:5 at 60°C and with a 5 hour extraction time. The ginger oils obtained had zingiberene contents of 21.56% and 26.28% for pH 4 and 5 respectively. Extraction for a longer period resulted in a reduction in the zingiberene content.

6. ACKNOWLEDGMENT

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