MATHEMATICAL MODEL OF GAULTHERASE INACTIVATION KINETICS FOR THE GAULTHERIN PRODUCTION FROM WINTERGREEN (*Gaultheria fragantissima*)

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Abstract

Wintergreen (Gaultheria fragantissima) is the source of the essential oil of wintergreen which is comprised predominantly of methyl salicylic. During this time wintergreen is not yet economically developed because of the lack of the cultivation technology. One of effort that is needed to be studied beside the cultivation technology and the wintergreen oil quality development is the product diversification. Wintergreen comprised by far the highest concentration of both free salicylic and total salicylic acid which is expected of a plant known for the formation of the wintergreen oil, an essential oil consisting primarily of methyl salicylic. The concentration of total salicylate found in wintergreen is over 20-fold greater than the total salicylic concentration (salicylic and any derivates combined) found in Filipendula, and 100 fold greater than that found in Lemon Thyme. The active form of salycilic acid in wintergreen is Gaultherin. Gaultherin has many properties related to human health. Gaultherin consists of methyl salicylic conjugated to the disaccharide, primeverose. When plant tissues are disrupted, the endogenous gaultherin is rapidly lost, presumably by enzymatic hydrolysis with the release of methyl salicylic. The problem is until this time there is not yet available an effective method for the gaultherin extraction. The difficulties faced on the gaultherin production are the fact that along the gaultherin extraction, and along with the disruption of the wintergreen tissue, the gaultherin is hydrolyzed into its individual components which are methyl salicylic and disaccharide. The alternative process for the gaultherin production from wintergreen is the gaultherin production process by enzyme inactivation and extraction process in an inactivation extractor using a n organic solvent (alcoholic solvent extraction). This paper is intended to study the enzyme inactivation rate which is play an important role in the dimension determination of the process equipments. Enzyme inactivation can be describes as first orde reaction. The enzyme inactivation constant k_p is the temperature, pressure, and water concentration function. The dependency model of the temperature based on Arhenius type while the pressure dependency is based on Eyring equation.

Keywords: *gaultherin, inactivation, kinetics*

Introduction

Wintergreen (gaultheria fragantissima), also known as checkerberry or teaberry, is a small ericaceous plant found growing in the understory of dense temperate zone forest. It has shinny coriaceous leaves, white bell shaped flowers and bright red berries. Wintergreen is cultivated for use in the landscape industry and is the source of the essential oil of wintergreen which is comprised predominantly of methyl salicylic.

Wintergreen is commonly found on dry slopes at 1300-3300 meters elevation (Hernani, 2004). During this time wintergreen

is not yet economically developed because of the lack of the cultivation technology. During this time the leaves of the wintergreen are harvested from the wintergreen plant found in the mountain area such as in Gunung Lawu, tawangmangu and in Wonosobo, dieng. One of wintergreen oil industry in Wonosobo is the Rukun Farmer Group, located in Sikunang Village, Wonosobo.

The methyl salicylic level of the wintergreen oil is up to 93-98%. The methyl salicylic level of the wintergreen oil yielded by local farmer is only up to 82.23 % (Mauludi, 2003). Nowadays Indonesia still imports synthetic wintergreen oil from China in order to fulfill the pharmacy industry demand (Manurung, 2002).

Methyl salicylic, also known as oil of wintergreen, is responsible for the smell and taste of wintergreen. Although methyl salicylic can be toxic when ingested at concentrations used for topical application, this ester has been shown to have decreased ulcerogenic activity when compared with an equal dose of salicylic acid, as measured by the respective salicylic contents.

One of effort that is needed to be studied beside the cultivation technology and the wintergreen oil quality development is the product diversification. Wintergreen comprised by far the highest concentration of both free salicylic and total salicylic acid which is expected of a plant known for the formation of the wintergreen oil, an essential oil consisting primarily of methyl salicylic. Level of free and total salicylic acid in the other species were generally much lower then reported in literature, which utilize less precise analytical method.

Wintergreen, or *Gaultheria fragantissima*, contains a very high concentration of salicylic derivatives, reaching concentrations exceeding 10 mg per gram fresh weight of tissue. This concentration is over 20-fold greater than the total salicylic concentration (salicylic and any derivates combined) found in *Filipendula*, and 100 fold greater than that found in Lemon Thyme, the plant from which salicylic acid was first isolated. Reports disclosing the presence of gaultherin (a salicylic derivative described below) in *Filipendula* tissues, but not gaultherin recovery yields (Barnaulov et al., *Rastit. Resur* 1977) and (Yeo etal., *Saengyak Hakhoechi* , 1992). Amount of free and total salicylic acid are shown in Table 1.

Table 1. Amount of free and total salicylic acid

Plant	Free Salicylate	Total Salicylate
English	0.81	31.63
Tyme	1.55	42.32
Lemon	0.33	13.26
Thyme	0.28	6.14
French	0.58	3.84
Thyme	**19.0**	**5770**
Lavender	1.4	9.5
Rosemary		
Wintergreen		
Rice		

The active form of salicylic acid in wintergreen is Gaultherin. Gaultherin consists of methyl salicylic conjugated to the disaccharide, primeverose. When plant tissues are disrupted, the endogenous gaultherin is rapidly lost, presumably by enzymatic hydrolysis with the release of methyl salicylic. This process presumably occurs as a protective mechanism for the plant.

In 1844, Proctor defined gaultherin as a conjugate of methyl salicylic with glucose but claimed that it did not exist within the plant for which it was named. Interest in such conjugates did not recur until nearly 60 years later. Bridel et.al described that the sugars of these conjugates and defined monotropidoside as a conjugate of methyl salicylic with primeverose, which is a disaccharide of xylose and glucose. Those studies conducted by proctor and Bridel were performed with various species, and led to the occurrence of excessive terminology.

In 1844, gaultherin was described as a conjugate of methyl salicylic with glucose but claimed that it did not exist within the plant for which it was named, presumably due to its instability. Interest in gaultherin did not reoccur until nearly 60 years later. The

conjugates and defined monotropidoside as a conjugate of methyl salicylic with primeverose, which is a disaccharide of xylose and glucose. These studies were performed in various species and lead to the development of excessive terminology, most of which seemed, in retrospect, to describe the same conjugate and enzyme activity. Wintergreen was not examined, however, until 1928, when it was determined that monotropidoside was identical to gaultherin and that it could only be extracted from *Gaultheria* with boiling water giving low yields. These combined observations confirmed the nature of gaultherin as a conjugate of methyl salicylic with a disaccharide of xylose and glucose. These scientific publications also defined the enzymatic activity leading to the hydrolysis of gaultherin to methyl salicylic as gaultherase. Any current literature that includes the terms gaultherin or gaultherase, refers to these original sources. These terms have been perpetuated by the literature until now without any further confirmation, and despite the development of much more sophisticated analytical methods and equipment. In addition, the putative gaultherase enzyme was never characterized as more than an activity.

Aspirin was invented in 1899 as a safe and effective substitute for salicylic acid that was commonly used to treat chronic conditions such as rheumatism. In contrast to salicylic acid, it caused much less gastric upset. Since the addition of the acetyl group to salicylic acid proved very effective as a means of decreasing its gastro-intestinal irritation, further conjugation could provide even greater safety and efficacy. In fact, research examining the effect of aspirin derivation on its efficacy and contraindications is ongoing. A derivative of aspirin conjugated to isopropylantipyrene, for example, showed all the effectiveness of aspirin, but with less gastric ulcerogenic activity. Other studies produced similar results with triglyceride derivatives of aspirin as well as methyl, ethyl and phenyl esters of aspirin. In addition, methyl salicylic itself causes lower incidence of gastric ulceration than aspirin. Methyl salicylic is released from gaultherin after hydrolysis of the disaccharide side chain by ubiquitous hydrolases in humans (or by gaultherase in plants), much in the same way as acetyl salicylic acid is released from glucopyranose pro-drug derivatives of aspirin.40 The conversion of methyl salicylic to salicylic acid then occurs readily in the blood plasma and takes one hour as shown in studies with orally administered methyl salicylic.41 Thus it is likely that gaultherin, a highly conjugated form of salicylic acid, will cause less side effects by providing, in essence, a time release form of pharmacologically active salicylic that can pass the gastrointestinal barrier before being converted to salicylic acid.

As describe above, gaultherin seems to have properties that make gaultherin as the best candidate as natural aspirin, anti cancer, anti inflammatory and cardiopulmonary (Ribnicky, 2003). As a natural aspirin, gaultherin has the same healing power but with less negative effect than synthetic aspirin. Nowadays, aspirin (Acetylsalicylic acid) is the world most consumable drug because of its function as the anti pyretic, anti inflammatory and analgesic. Based on the estimation, the world demand of aspirin is up to 20-50 million pounds per year (Barat, 1998), so it is estimated that the pharmacy industry demand of gaultherin is increasing.

The problem is until this time there is not yet available an effective method for the gaultherin extraction. The difficulties faced on the gaultherin production are the fact that along the gaultherin extraction, and along with the disruption of the wintergreen tissue, the gaultherin is hydrolyzed into its individual components which are methyl salicylic and disaccharide. The hydrolysis process is believed catalyzed by enzyme on the wintergreen plant, gaultherase (Waters, 1931).

In order to solve that problem, it is need to be developed a new method for the gaultherin extraction from wintergreen in a condition where the activity of the gaultherase enzyme is minimum or even at zero activity level of the gaultherase.

Method for the gaultherin extraction performed by several researchers such as in 1928, it was revealed that gaultherin from wintergreen only can be extract by using hot water and the addition of carbonate calcium, followed by several steps of solvent extraction. The solvent used in the extraction is acetic ester hydrate at 100^{0}C. The process was yielded 4 g/kg fresh tissue (Bridel and Gillon, 1928). The small yield predominantly caused by the gaultherase activity in hydrolyzing the gaultherin.

Poiev et al 1998 stated that tha gaultherase activity can be hindered by the addition of polar solvent. It is believed that alcohol can hinder the gaultherase activity. Several chemical compounds also give the same effect as alcohol. The chemical compounds are methylene cloride, acetonitril or boiling water.

The alternative process for the gaultherin production from wintergreen is the gaultherin production process by enzyme inactivation and extraction process in an inactivation extractor using a n organic solvent (*alcoholic solvent extraction)*. The polar solvent will as an inactivation agent and as an extraction solvent. The polar solvent that is planned to be used is ethanol. The enzyme inactivation by using alcoholic solvent (ethanol) has several benefits such as: the process is summarized two process in a row, which are the enzyme inactivation and the gaultherin extraction, the process yield is higher because gaultherin is hopefully not converted into methyl salicylic, and the ethanol choice is ingestible for the nutra-ceutical products so gaultherin can be used in the form of tablet, pill or capsule.

Design of the gaultherin production process as one of diversification effort and the development of the drug plant is not yet available. Although from many literatures it can be understand that the gaultherin production is hindered by the enzymatic hydrolysis, until this time there is no effort in the development for gaultherin production by hindered the hydrolysis process. Hydrolisis can be hindered if the gaultherase enzyme can be inactivied effectively. The problems are how is the process design and the processing system by using alcoholic solvent extraction and the process condition of the gaultherase inactivation on the gaultherin production. So, it is need to be studied, the development of the process design and the processing system by using alcoholic solvent extraction and the determination of the optimum process condition of the gaultherase inactivation on the gaultherin production. Moreover the achievement of those benefits is depend on the rate of the enzimatic inactivation, enzimatic extractor design and the optimum operation condition on the extractor. This paper is intended to study the enzyme inactivation rate which is play an important role in the dimension determination of the process equipments.

Mathematical Model of The Gaultherase Inactivation Kinetics

The most simple model, an active enzyme molecule (Ea) undergo a chemical changing or an irreversible chemical change into an active form (Ei), (Bailey dan Ollis, 1986; Martens *et al.*, 2001).

$$E_a \longrightarrow E_i$$

Enzyme inactivation can be describes as first orde reaction (Kerkhof and Schober, 1974; Wijlhhuizen *et al.*, 1979; Liou, 1982; Luyben *et al.*, 1982; Saguy, 1983; Bailey and Ollis, 1986; Ganthavorn *et al.*, 1991; Meerdink and Riet, 1991; Yamamoto and Sano, 1992; Lievense *et al.*, 1992; Owusu and Makhzoum, 1992; Bhirud dan Sosulski, 1993; Meerdink, 1993; Nunes *et al.*, 1993; Owusu, 1993; Campos *et al.*, 1996; Ariahu, 1997; Kieviet, 1997; Busto *et al.*, 1999; Cunha and Oliveira, 2000; Sriwatanapongse *et al.*, 2000; and Martens *et al.*, 2001). The enzyme inactivation process with thermal treatment can be describe with the equation below:

$$\frac{dE_p}{dt} = -k_p.E_p \qquad (1)$$

By using the integration form and variable distinction, and consider the boundary condition $E_p(t)$ to $E_p(0)$ at $t = 0$, then Equation 1 can be solved into:

$$\int_{E_p(0)}^{E_p(t)} \frac{dE_p}{E_p} = -\int_{t_o}^{t} k_p.dt \quad (2)$$

$$\ln\left[\frac{E_{P(t)}}{E_{P(0)}}\right] = -k_p t \quad (3)$$

Where, E_p is the enzyme activity, t is the time of reaction, and k_p is the inactivation constant.

The enzyme inactivation constant k_p is the temperature, pressure, and water concentration function (Kerkhof and Schober, 1974; Wijlhhuizen *et al.*, 1979; Luyben *et al.*, 1982; Liou, 1982; Bailey and Ollis, 1986; Ganthavorn *et al.*, 1991; Meerdink and Riet, 1991; Yamamotoand Sano, 1992; Lievense *et al.*, 1992; Owus and Makhzoum, 1992; Bhirud and Sosulski, 1993; Meerdink, 1993; Nunes *et al.*, 1993; Owusu, 1993; Campos *et al.*, 1996; Ariahu, 1997; Kieviet, 1997; Busto *et al.*, 1999; Cunha and Oliveira, 2000; Erkmen, 2000; Sriwatanapongse *et al.*, 2000; Indrawati *et al.*, 2001; and Martens *et al.*, 2001). In research papers, several models are used to describe the dependency. The dependency model of the temperature based on Arhenius type (Equation 4) while the pressure dependency is based on Eyring equation (Equation 5).

$$k_p = k_\infty \exp\left(-\frac{E_a}{R_t T}\right) \quad (4)$$

$$k_p = k_\infty \exp\left(\frac{V_a P}{R_p T}\right) \quad (5)$$

where, k_∞ is the frequency factor, E_a ais the activation energy, V_a is the activation volume, $R_{t,p}$ is the universal gas constant, T is the absolute temperature and P is pressure.

Arrhenius and Eyring equation can be transformed into a shaped that is more understandable (Saguy, 1983; Yamamoto and Sano, 1992; Nunes *et al.*, 1993; Cunha and Oliveira, 2000; Indrawati *et al.*, 2001; and Martens *et al.*, 2001).

$$k_p = k_{p,\,Tref} \exp\left[-\left(\frac{E_a}{R}\right)\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right]$$

(6)

$$k_p = k_{p,Pref} \exp\left[\left(\frac{V_a}{R_p T}\right)(P - P_{ref})\right]$$

(7)

where, $k_{p,Tref}$ is the rate constant on a reference temperature that is prior determinated, $k_{p,Pref}$ is the rate constant on a reference pressure, , T_{ref} is the reference temperature and P_{ref} is the reference pressure.

The rate constant k_p for the enzyme inactivation can be estimated from a logaritmic plot of enzyme activity percentage toward time. The value of E_a or $\Delta E^{\#}$ and k_∞ can be achieved by linier regression of ln k_p toward one per absolut temperatur ($1/T$). Transition condition parameters such as activation entalphy ($\Delta H^{\#}$) is determiantes based on equation below (Owusu *et al.*, 1992; Owusu and Berthalon, 1993; Ariahu *et al.*, 1997; and Busto *et al.*, 1999):

$$\Delta H^{\#} = \Delta E^{\#} - RT \quad (8)$$

where, T is the absolute temperature. Activation free energy of Gibbs will be determinate based on the relation:

$$\Delta G^{\#} = -RT \ln\left(\frac{kh}{KT}\right) \quad (9)$$

where, h is Planck constant (=6.6262 x 10^{-34} Js), K is the Boltzmann constant (=1.3806 x 10^{-23} JK^{-1}). From Eq. 8 and 9 the activation entropy ($\Delta S^{\#}$) for the enzyme inactivation can be calculated by using equation 10

$$\Delta S^{\#} = \frac{(\Delta H^{\#} - \Delta G^{\#})}{T}$$

(10)

In order to describe the combination of the temperature-pressure dependency from the rate constant of the gaultherase inactivation, a model based on a modified thermodynamics equation (Hawley, 1971) is developed as shown below:

$$\ln k_p = \ln k_o - \frac{\Delta V_o^{\#}}{R_T T}(P-P_o) + \frac{\Delta S_o^{\#}}{R_T T}(T-T_o) - \frac{1}{2}\frac{\Delta \kappa^{\#}}{R_T T}(P-P_o)^2$$

$$\frac{\Delta \zeta^{\#}}{R_T T}(P-P_o)(T-T_o) + \frac{\Delta C_p^{\#}}{R_T T}\left[T\left(\ln\frac{T}{T_o}-1\right)+T_o\right]$$

1)

A linier regression analysis, including an iterative numeric procedure based on the smallest quadratic procedure is being used to estimate the model parameters (k_o, ΔV_o^1, ΔS_o^1, Δk_o^1, Δz_o^1, and ΔCp^1). The model accuracy is evaluated by correction calculation r^2, deviation standard, and residue error standard (SE) for each estimated model parameters.

$$\text{Correction } r^2 = \left[1 - \frac{(m-1)\left(1-\frac{SSQ_{regresion}}{SSQ_{total}}\right)}{(m-j)}\right]$$

(12)

$$\text{Deviation} = \sqrt[2]{\frac{SSQ_{residual}}{(m-j)}} \quad (13)$$

$$RSE(\%) = \frac{\text{asimtot (SE)}}{\text{estimated value}} x100$$

(14)

Arrangement procedure of the mathematical model of gaultherase enzyme inactivation kinetics is shown on Fig 1. The measurable data are uses as input in the model development in form of empirical equation by using Matlab programme.

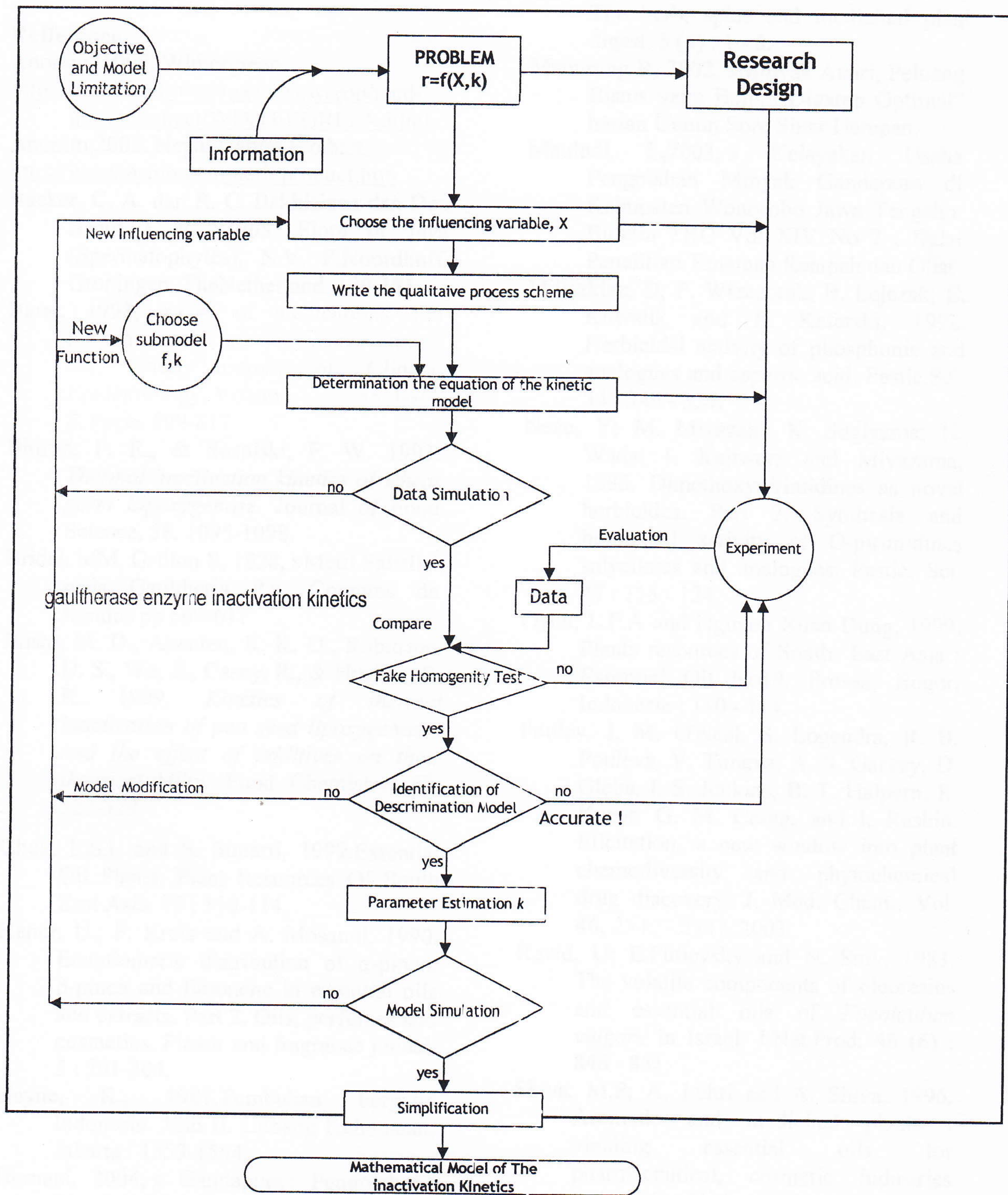

Fig 1. Arrangement procedure of the mathematical model of gaultherase enzyme inactivation kinetics

Conclusion

Rate of gaultherase inactivation for gaultherion production from wintergreen using alcoholic solvent can represent on the determination of the enzymatical inactivation bioreactor dimension. The study of the mass transfer is needed to complete the technical data of the equipment design.

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