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Extraction Condition of *Phaseolus vulgaris*

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***Abstract*—**Theoptimal extraction condition of dried *Phaseolus vulgaris* powderwas studied. The three independent variables are raw material concentration, shaking and centrifugaltime. The dependent variables are both yield percentage of crude extract and alpha-amylase enzyme inhibition activity. The experimental design was based on box-behnkendesign. Highest yield percentage of crude extract could get from extraction condition at concentration of 1, 0,1, concentration of 0.15 M ,extraction time for 2hour, and separationtime for60 min**.** Moreover, the crude extract with highest alpha-amylase enzyme inhibition activityoccurred by extraction condition at concentration of 0.10 M, extraction time for 2 min, and separation time for 45 min

***Keywords*—**Extraction time, Optimal condition, Alpha-amylase enzymeinhibition activity

I. Introduction

WHITE kidney beans (*Phaseolus vulgaris*) is a member of the Leguminosae, tribe Phaseoleae, subfamily Papilionoideae. Cultivated forms are herbaceous annuals, which are determinate or indeterminate in growth habit. *Phaseolus vulgaris* L. was originally a crop of the New World, but is now grown extensively in all major continental areas. Its production spans from 52°N to 32°S latitude. It is a major source of dietary protein throughout both Latin America and Eastern Africa, but per capita consumption is declining as population increases outdistance production[1]. Archeological investigations showed that *Phaseolus vulgaris* originated on the American Continent, specifically in southern United States, Mexico, Central America, and the northern part of South America. In particular, the species *P. vulgaris* was introduced into Europe in the sixteenth century and since then it has become a very important crop in many regions of the world [2].

The presence of α-amylase inhibitors in white kidney beans originally was first reported by Bowman (1945) [3]. Alpha-amylase inhibitor (αAI) is present in embryonic axes and cotyledons, but not in other organs of the plant [4].In white kidney beans (*Phaseolusvulgaris*), proteinaceous inhibitor of α-amylase names Phaseolamin [5]. Phaseolamin is a glycoprotein (about 15% carbohydrate) that inhibits the activity of mammalian and insect α-amylases, but not of plant α-amylases. The protein is synthesized during the same time period that phaseolin and phytohemagglutinin are made and also accumulates in the protein storage vacuoles (protein bodies). Its native molecular weight has been estimated to be 43 to 50 kD by gel filtration experiments. The inhibitor is composed of subunits of Mr 15,000 to 18,000, and it has been proposed that it is either a trimer or a tetramer of identical polypeptides or different polypeptides. The inhibitor binds to animal α-amylases at a pH optimum of 5.6, forming a stable 1:1 (molar ratio) complex [4].More attention is now being paid to these phaseolamins for two main reasons. First, they widely occur in both human and cattle diets and could therefore diminish the digestibility of starch and starch-derived products by inhibiting the α-amylase enzymes [6].The use, in the early 1980s, of crude extracts of kidney bean as starch blockers to control human non-insulin-dependent diabetes mellitus and obesity, was hampered by their very low inhibitor content and the presence of potentially harmful lectins (PHA) and trypsin inhibitors [7]. Nevertheless, further investigations on humans showed that purified α-AI perfused into the duodenum significantly inhibited intraluminal amylase activity while ingested with dietary starch, it significantly reduced the postprandial increase in glucose of both normal and diabetic patients [8]. Second, their use as insecticidal proteins to prevent the attack of predatory insects to susceptible seeds.[9] Because α-AI inhibits the development of bruchid beetles, its gene is considered as potentially useful for crop protection via plant genetic engineering.

Optimization is the method that applied to get the optimal condition for varieties of food processing such as applying genetic algorithm in natural cheese products process, brewery fermenting process, active ingredient extraction from medicinal plants, including biomass power plant [10-16].

This study focuses on the optimal extraction condition for phaseolamin preparation at which to acquire highest α-amylase inhibitory activity, published.

II. Materials and Method

A. Sample preparation

White kidney beans (*Phaseolus vulgaris*) cutivarPangda 2, obtained from Royal Project Foundation, Thailand, were dried and grounded to powder, sieve by mesh size 2 mm and kept in vacuum package at 4° C until used.

B. Proximate analysis

Dry white kidney beans powder were analysed for moisture, ash, sand, fat, crude fiber and protein by AOAC (2005) as the following; moisture (AOAC 925.10), ash (AOAC 900.02A), sand (AOAC 900.02D), protein (AOAC method 928.08), fat (AOAC 963.15), crude fiber (AOAC 978.10).

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C. Extraction method

White kidney beans powder were extracted by stirred 1, 2, 3 hour with 0.05M, 0.10M, 0.15M (1:6w/v) at room temperature(25°C). The homogenate was centrifuged at 10,000 g for 30, 45, 60 min at 4 □C. The supernatant was filtered through cotton wool and centrifuged at 10,000 g for 30, 45, 60 min at 4 °C again. The clear supernatants were collected, filtrated and stored at -20°C.

D. Experimental Design

One-Way ANOVA was applied to identify optimum levels of three variables of the concentration of solvent (M), extraction time (hour) and separation time (min) regarding of two responses extract yieldsand alpha-amylase enzyme inhibitory activity in the *Phaseolus vulgaris*extracts. The design independent and dependent variables are list inTable I. Concentration of solvent (X_1), extractiontime (X_2) and separation time (X_3). The experiments were designed according to the box-behnken design as shown in Table I. The order of the experiments has been fully randomized. Data were analyzed by One-Way ANOVA.

III. Results

A. Proximate analysis

The dried *Phaseolus vulgaris* powder composed of moisture 11.07 ± 0.01 %, ash4.10 ± 0.01 %, sand0.01 ± 0.00 % , protein 20.28 ± 0.01%, fat 1.80 ± 0.02 %, and crude fiber 31.73 ± 0.01%.

TABLE I
UNCODED AND CODED LEVELS OF INDEPENDENT VARIABLES USED IN THE EXPERIMENTAL DESIGN.

		Coded levels		
Symbols	Independent variables	– 1	0	1
X_1	Concentration of solvent (M)	0.05	0.10	0.15
X_2	Extraction time (hour)	1	2	3
X_3	Separation time(min)	30	45	60

B. Extraction condition

The yields of crude extract (Y_1), alpha-amylase inhibition activity (Y_2) inwhite kidney beans (*Phaseolus vulgaris*) crude extracts obtained from all the experiments are listed in Table II. The regression coefficients and results of ANOVA show in Table III and IV for response of yield percentage and alpha-amylase inhibition activityrespectively.

IV. Statistical Analysis

Data from Table II was analyzed by One-Way ANOVA to determine the correlation between independent variable including get optimal extraction condition for highest yield and alpha-amylase inhibition activityas shown in Table III.

From Table II, the highest yield had shown at the condition of solvent concentration at 0.15M, extraction timefor 2 hour, and separation time for60 min. In addition, at what time data was analyzed for correlation between independent variable and yield percentage, the statically analysis showed no significant at p value higher than 0.01. In the studied of optimal condition to get highest inhibitory activity to alpha amylase enzyme, theresults showed that at the solvent concentration at 0.10M, extraction timefor2 hour, and separation time for45 minas well correlated with statistically significant at p value less than 0.01.

Fig 1 White bean (*Phaseolus Vulgaris*)[17]

TABLE II
EXPERIMENTAL DESIGN AND RESPONSES OF THE DEPENDENT VARIABLES TO THE EXTRACT PARAMETERS

Exp. No[a].	Independent variables				
	Concentration of solvent (M) X_1	Extraction time (hour) X_2	Separationtime (min) X_3	Yield (%) (Y_1)	Inhibition activity (%) (Y_2)
1	0.1 (0)	1 (-1)	30 (-1)	17.9663	88.5439
2	0.1 (0)	3 (1)	30 (-1)	13.2607	88.7280
3	0.1 (0)	1 (-1)	60 (1)	17.7100	85.1077
4	0.1 (0)	3 (1)	60 (1)	14.6083	87.1326
5	0.05 (-1)	1 (-1)	45 (0)	13.2540	86.4576
6	0.05 (-1)	3 (1)	45 (0)	13.5990	84.0032
7	0.1 (1)	1 (-1)	45 (0)	16.7897	80.3829
8	0.1 (1)	3 (1)	45 (0)	22.8807	80.7511
9	0.05 (-1)	2 (0)	30 (-1)	12.5397	84.4941
10	0.05 (-1)	2 (0)	60 (1)	12.5287	85.0463
11	0.1 (1)	2 (0)	30 (-1)	22.2117	82.2237
12	0.1 (1)	2 (0)	60 (1)	23.3187	79.7079
13	0.1 (0)	2 (0)	45 (0)	14.3050	91.3665
14	0.1 (0)	2 (0)	45 (0)	14.1750	91.4279
15	0.1 (0)	2 (0)	45 (0)	14.9897	91.3665

[a] Experiments were conducted in a random order.
+1 = High level, 0 = Medium level,-1 = Lowlevel

TABLE III
THE ONE-WAY ANOVA FOR YIELD RESPONSE

ANOVA

Model	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	599.152	14	42.797	9399.764**	0.00
Within Groups	0.137	30	0.005		
Total	599.288	44			

**: significant at p> 0.01

TABLE IV
THE ONE-WAY ANOVA FOR ENZYME INHIBITORY ACTIVITY RESPONSE

ANOVA

Model	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	668.015	14	47.715	102.364**	0.00
Within Groups	13.984	30	0.466		
Total	681.999	44			

**: significant at p > 0.01

V. CONCLUSION

The optimal extraction condition to prepare white kidney beans (*Phaseolus vulgaris*)extract with highest yield and alpha-amylase inhibition activity was determined using Box-Behnken experimental design and data was used for statistically analysis by One-Way ANOVA. The percentage yield of white kidney beans (*Phaseolus vulgaris*)extract is between 12.5287-23.3187%. Still, future research for totalphaseolamin and active glycoprotein by high performance liquid chromatography should be applied for quantification of active glycoprotein in white kidney beans (*Phaseolus vulgaris*)and get specific optimal extraction condition.

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