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DIFFERENT EXTRACTION METHODS TO OBTAIN THE HIGHEST PHYTIC ACID YIELD FROM PEANUT SEEDS

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ABSTRACT

In this study, phytic acid is prepared from peanut seeds by normal extraction, enzymatic, ultrasonic and combined method. Normal extraction conditions are optimized by the use of response surface methodology (RSM) based on four-variable central composite design (CCD) to obtain highest extraction yield. Estimated optimum conditions are as follows: HCl concentration 0.61M, solvent/material ratio 22.50 mL/g, extraction time 3.75 hours, and extraction temperature 43.46^{o}C with phytic acid extraction yield 0.98%. Enzymatic and ultrasonic conditions are optimized by the use of response surface methodology (RSM) based on two-variable central composite design (CCD) to obtain highest extraction yield. In the ultrasound-assisted extraction, estimated optimum conditions are as follows: treatment time 4.17 minutes, sonication power 9.42 W/g and phytic acid extraction yield 1.19%. In the enzyme-assisted extraction, the results show that the extraction yield could be reached up to 1.17% compared with normal extraction when using the following optimum conditions: treatment time 33.51 minutes and enzyme concentration 5.62 U/g. Moreover, we improve phytic acid extraction yield to 1.31% and 1.42% by the way of treating defatted peanut powder suspension in the order of ultrasound-enzyme and enzyme-ultrasound, respectively

Keywords: Peanut Seed, Phytic Acid, Extraction Method, Ultrasonic, Enzymatic, Response Surface Methodology

INTRODUCTION

The peanut.or groundnut *(Arachis hypogaea)*, is a species in the legume or bean" family (Fabaceae). The peanut was probably first domesticated and cultivated in the valleys of Paraguay. It is an annual herbaceous plant growing 30 to 50 cm (1.0 to 1.6 ft) tall. The

leaves are opposite, pinnate with four leaflets (two opposite pairs; no terminal leaflet); each leaflet is 1 to 7 cm (⅜ to 2¾ in) long and 1 to 3 cm (⅜ to 1 inch) across **(Table 1; Figure 1)**.

Phytic acid

Phytic acid (known as inositol hexakisphosphate, IP6, or phytate when in salt form) is an organic acid extracted from rice bran and other beans. Phytic acid is used as an acidulant for pH adjustment. Phytic acid binds to metals strongly because of strong chelating effect. Moreover, phytic acid shows antioxidant action and prevention of color degradation. The most outstanding feature of phytic acid is its strong metal chelate function, allowing metal ions such as ferrum (Fe) which often adversely affect the production or storage of food in various forms to be removed or deactivated **(Figure 2)**.

Ultrasonic and Enzymatic Tissue Disruption

The use of sound waves in fluids can disrupt cells. The operation starts with normal electrical current (50Hz or 60Hz) being transformed to 20,000 Hz. This electrical signal is fed to a piezo-electric crystal causing it to oscillation at this high frequency. The vibrations move a titanium metal HORN about 5–15 microns. The shape of the horn amplifies this motion to 100–150 microns per cycle. By placing the horn's end-the tip-into fluid, the tip moves the liquid forward (away) and then retracts (back) quicker than the liquid can return. During the return stroke, the pressure in the system drops below the vapor pressure of the liquid so boiling occurs ("cavitation"). As the liquid flows back, the bubbles collapse. This bubble collapsing imparts the energy needed to disrupt the tissues.

The use of enzymatic methods to remove cell walls is well-established for preparing cells for disruption, or for preparation of protoplasts (cells without cell walls) for other uses such as introducing cloned DNA or subcellular organelle isolation. The enzymes are generally commercially available and, in most cases, were originally isolated from biological sources (e.g. snail gut for yeast or lysozyme from hen egg white). The enzymes commonly used include lysozyme, lysostaphin, zymolase, ce llulase, mutanolysin, glycanases, proteases, mannase etc.

Phytic Acid Extraction

Secil Turksoy *et al*., 2010, [1], verified the effect of wheat variety and flour extraction rate on phytic acid content of bread. Effects of wheat variety and flour extraction rates on phytic acid content of bread made from six different wheat varieties (Bezostaya, Gun-91, Dagdas-94, Gerek-79, Kirgiz-95, Ikizce) at four different flour extraction rates (65, 75, 85 and 100%) were evaluated. The contents of phytic acid and total

phosphorus of flour and bread samples were determined. The loss of phytic acid content during bread making was also determined. Wheat variety and flour extraction rate significantly affected the phytic acid content ($p<0.05$) of bread. As the flour extraction rate was increased, the phytic acid and total phosphorus contents of flour samples ranged from 71.8 (Kirgiz-95) to 1054.9 mg/100 g (Gerek-79) and from 66.2 mg/100 g (Ikizce) to 431.0 mg/100 g (Dagdas-94), respectively. For bread samples, the phytic acid and total phosphorus values ranged from 36.8 mg/100 g (Kirgiz-95) to 855.5 mg/100 g (Gerek-79) and from 89.4 mg/100 g (Ikizce) to 451.0 mg/100 g (Dagdas-94), respectively. The phytic acid and total phosphorus contents increased with the increase in the extraction rate of flour and bread samples obtained from each wheat variety. During bread-making process, destruction of the phytic acid decreased as the extraction rate increased. The mean of phytic acid reduction of the bread samples made from flour with 65, 75, 85 and 100% extraction rates were 48.5, 39.4, 31.3 and 21.2%, respectively.

Hongkuan Kang *et al.*, 2011, **[2]**, investigated the extraction of phytic acid from HCl extract of rapeseed meal using Alamine 336/n-octanol/sulfonated kerosene. The results showed that the addition of n-octanol can significantly enhance the extraction of phytic acid by Alamine 336/sulfonated kerosene, indicating the synergetic effect between Alamine 336 and n-octanol. The key affecting factors of the extraction include pH and the concentration of the extractants, whose effects on the distribution ratio were quantitatively analyzed. Phytic acid extracted into the organic solutions can be well strip extracted back by using a dilute NaOH solution. At the optimum operating conditions, the extraction efficiency from a 0.03 mol/L phytic acid solution can reach 85.4%, and the corresponding single strip-extraction efficiency is 74.6%, while the total strip-extraction efficiency after three strip extractions can reach 96.4%.

Canan Cristiane *et al.*, 2011, [3], studied the extraction and purification of phytic acid from rice bran. For the extraction of PA, a complete 2^4 factorial design with triplicates at the central point was used, and the effects of concentration of rice bran and HCl, time and temperature were investigated. During purification, different pH values were tested with addition of 1.5 M Na_2CO_3 or 4.0 M NaOH. The results obtained by the statistical analysis of the factorial design showed that temperature, time and HCl concentration influenced the PA extraction technique significantly ($p \leq 0.05$), whereas the concentration of rice bran had no influence. The content of PA was evaluated

in all the stages of purification and it was possible to establish an improved methodology of extraction and purification with high purity and yields.

Yuwei Luo *et al*., 2013, [4] evaluated the impact of processing on phytic acid, in vitro soluble zinc and Phy/Zn molar ratio of faba bean (Vicia faba L.). Influence of soaking, germination and fermentation with expectation of increasing its bioavailability was investigated. Fermentation treatments were most effective in decreasing phytic acid (48-84%), followed by soaking at 10°C after preheating (36-51%). Steeping of faba beans for 24 h at 25°C had the least effect on phytic acid removal (9-24%). With increased germination time at 30°C, phytic acid progressively decreased from 9 to 69%. Most wet processing procedures, except soaking after wet preheating, caused losses of dry matter and zinc (8-22%). In vitro zinc solubility, as a percentage of total zinc in soaked faba bean after dry preheating, was significantly higher than in raw faba bean ($P < 0.05$). Probably complex association between dietary fiber and zinc is the reason for the poor bioavailability of zinc in faba bean.

In our study, phytic acid was prepared from peanut seeds by normal extraction, enzymatic, ultrasonic and combined method.

MATERIAL AND METHODS

Raw Material

Peanut Seed

In our research, we choose peanut seeds belonging to varietyTB-25 (Thai Binh province, Vietnam).

Alcalase Enzyme

We use enzyme Alcalase 2.4 LFG (Novozymes), an endopeptidase enzyme having pH optimal 7.0 and temperature optimal 55^{o}C, activity 2.4 AU/g (1 AU = 550 U). This enzyme is inactivated at 90 ^{o}C in 10 minutes.

Equipment

Ultrasonic Equipment

We utilize the ultrasonic equipment in bar shape Sonicator ®, Mode VC 500, frequency 20 kHz (Misonix, USA). Maximum capacity 500W, sonication cone 12cm.

Ion Exchange Resin

Ion exchange resin is cationite acid, Purolite C-100 (Purolite, England). It's composed from Styrene-DVB with primariry ion H+(R-SO_3H) and exchanging volume 2 meq/mL.

Research Protocol

Research proposal has been given in **Figure 3**.

Phytic Acid Extraction

Normal Extraction

Experiment #1: Effect of HCl concentration

Experimental parameter:

HCl concentration 0.1, 0.2, 0.3, 0.4, 0.5 M.

Fixed parameter:

- Ratio of solvent/ material: 6 mL/g dry matter defatted
- Time of extraction: 4h
- Temperature of extraction: 40^{o}C.

Target parameter:

- Efficiency of phytic acid extraction.

Experiment # 2: Effect of solvent/material (mL/g dry matter defatted)

Experimental parameter:

Ratio of solvent/ material 2, 4, 6, 8, 10 mL/g dry matter.

Fixed parameter:

- HCl concentration: optimal value in experiment #1
- Time of extraction: 4h
- Temperature of extraction: 40^{o}C.

Target parameter:

- Efficiency of phytic acid extraction.

Experiment # 3: Effect of extraction time (h)

Experimental parameter:

Extraction time 1, 2, 3, 4, 5h.

Fixed parameter:

- HCl concentration: optimal value in experiment # 1
- Ratio of solvent/ material: optimal value in experiment #2
- Temperature of extraction: 40^{o}C.

Target parameter:

- Efficiency of phytic acid extraction.

Experiment # 4: Effect of extraction temperature (^{o}C)

Experimental parameter:

Extraction temperature 20, 30, 40, 50, 60^{o}C.

Fixed parameter:

- HCl concentration: optimal value in experiment # 1
- Ratio of solvent/ material: optimal value in experiment #2
- Extraction time: optimal value in experiment #3.

Target parameter:

- Efficiency of phytic acid extraction.

All experiments are triplicated to show the significant difference by ANOVA. With the purpose of finding the optimal normal phytic acid extraction, we conduct the experimental plan having matrix: two rotating cores and four factors with target function: phytic acid extraction efficiency. We conduct 31 tests including 16 tests at master matrix, 8 tests at star matrix and 7 tests at center with $\alpha = 2$. After receiving the regression equation, we continue apply the experimental plan by climbing method.

Ultrasonic extraction

Experiment # 5: Effect of sonication power (W/g)

Experimental parameter

Sonication power 0, 4.8, 6.4, 8.0, 9.6, 11.2. 12.8 W/g.

Fixed parameter:

HCl concentration: optimal value in experiment # 1

Ratio of solvent/ material: optimal value in experiment #2

Extraction time: optimal value in experiment #3

Extraction temperature: optimal value in experiment #4

Treatment time: 2 minutes

Target parameter:

- Efficiency of phytic acid extraction.

Experiment # 6: Effect of sonication treatment time (minutes)

Experimental parameter

Sonication treatment time 0, 2, 4, 6, 8, 10, 12, 14 minutes.

Fixed parameter:

HCl concentration: optimal value in experiment # 1

Ratio of solvent/ material: optimal value in experiment #2

Extraction time: optimal value in experiment #3

Extraction temperature: optimal value in experiment #4

Sonication power: optimal value in experiment #5

Target parameter:

- Efficiency of phytic acid extraction.

All experiments are triplicated to show the significant difference by ANOVA. With the purpose of finding the optimal sonication phytic acid extraction, we conduct the experimental plan having matrix: two rotating cores and two factors with target function: phytic acid extraction efficiency. We conduct 13 tests including 4 tests at master matrix, 4 tests at star matrix and 5 tests at center with $\alpha = 1.14$. After receiving the regression equation, we continue apply the experimental plan by climbing method.

Enzymatic extraction

Experiment # 7: Effect of enzymatic activity/ material (U/g dry matter defatted)

Experimental parameter:

Enzymatic activity/ material 0, 2.28, 4.55, 6.83, 9.11, 11.38, 13.66 U/g dry matter defatted.

Fixed parameter:

- HCl concentration: optimal value in experiment # 1
- Ratio of solvent/ material: optimal value in experiment #2

Extraction time: optimal value in experiment #3

Extraction temperature: optimal value in experiment #4

Enzymatic treatment: 30 minutes

Target parameter:

- Efficiency of phytic acid extraction.

Experiment # 8: Effect of enzymatic treatment (minutes)

Experimental parameter:

Enzymatic treatment 0, 10, 20, 30, 40, 50, 60 minutes

Fixed parameter:

- HCl concentration: optimal value in experiment # 1

- Ratio of solvent/ material: optimal value in experiment #2

Extraction time: optimal value in experiment #3

Extraction temperature: optimal value in experiment #4

Enzymatic activity/ material: optimal value in experiment # 7

Target parameter:

Efficiency of phytic acid extraction

All experiments are triplicated to show the significant difference by ANOVA. With the purpose of finding the optimal sonication phytic acid extraction, we conduct the experimental plan having matrix: two rotating cores and two factors with target function: phytic acid extraction efficiency. We conduct 13 tests including 4 tests at master matrix, 4 tests at star matrix and 5 tests at center with α= 1.14. After receiving the regression equation, we continue apply the experimental plan by climbing method.

Ultrasonic + enzymatic extraction

After finding optimal processing conditions by ultrasonic and enzymatic methods, we combine these methods as enzyme – ultrasonic and ultrasonic– enzyme in phytic acid extraction. After getting the product, we compare different treatment methods (enzyme, ultrasonic, enzyme – ultrasonic, ultrasonic – enzyme) to control.

Comparison of the Antioxidant Capacity Between Phytic Acid and Other Anti-Oxidants

Compare the chelating capacity of free radical DPPH and phytic acid – ascorbic acid, gallic acid at the same concentration.

Analysing Method

Determine moisture content by oven drying at 105 ^{o}C.

Determine total ash by burning at 600^{o}C.

Determine total lipid by Soxhlet method.

Determine soluble protein (**Lowry, 1951) [5]**.

Determine protease activity (**Anson, 1937) [6]**.

Determine acid phytic content (**Gao *et al*., 2007) [7**].

Determine chelating with ion Fe^{2+} (**Dinis *et al*., 1994) [8**].

Determine anti-oxidant activity by radical capture DPPH (1,1-diphenyl-2-picrylhydrazyl) (**Brand-Williams *et al*., 1995) [8**].

Data analysis by ANOVA software.

RESULTS AND DISCUSSION

Phytic Acid Extraction

Peanu seeds are grindered into powder (moisture 5.82%) and then removed lipid by sohxlet (initial lipid content in peanut seed 32.50% dry matter). High lipid content will create backward effect to phytic acid extraction. So samples defatted are used for experiments.

Effect of HCL to Phytic Acid Extraction

During phytic acid extraction, solvent polarity plays a key role. Although phytic acid can be extracted by water, efficiency will not high owing to its low polarity. Phytic acid is a organic substance having high polarity because it contains many phosphate groups; so solvent having high polarity will enhance the extraction, **Wu *et al*., 2009, [10]**. In this experiment, we select HCl as solvent and investigate its concentration to get the best phytic acid extraction efficiency. During extraction, apart from phytic acid, solvent also solubilizes other substances, especially soluble protein. Phytic acid and these substances are likely increasing while increasing solvent concentration. Over 0.60M, there is no significant difference.

In peanut seeds, phytic acid exists under salt form with metal ion or linked with protein. **Herrero *et al*., 2005**, **[11]**, basis of phytic acid extraction is separation and dissolving of phytate in acidic solution. In acidity, phytate will be dissolved and diffuse into solvent strongly, increase the extraction efficiency. **Makower, 1970**, **[12]**, conducted the phytic acid extraction in some beans and found that HCl 0.5N gave the highest phytic acid extraction efficiency. So we choose HCl 0.60M to extract phytic acid for further experiments **(Figure 4)**.

Effect of Solvent/ Material to Extraction

Phytic acid extraction is a process of solid-liquid extraction. Speed of this extraction depends mostly on various factors: shape, size, and structure of particles; physic-chemical characteristics of solvent, extraction method; ratio of solid: liquid. In this experiment, we examine the effect of solvent/ material to the phytic acid extraction efficiency. At ratio 0 mL/g, the extraction efficiency is maximum at 1.11% and slightly increasing if it's over 20 mL/g. Capillarity, molecular diffusion, and gradient are dynamics of extraction. To speed up the extraction, it should be extracted with more solvent. The more solvent uses, the higher extraction efficiency gets. In other extraction, using more solvent will create a dilution. However, in this research we primarily collect phytate condensation so dilution is not important. Ratio of solvent/ material 20 mL/g dry matter is selected for further experiments **(Figure 5)**.

Effect of Extraction Time to the Extraction Efficiency

Extraction time is also a crucial factor. The longer of time, the more phytic acid we receive. If it's over 3h, there is not significant difference in phytic acid efficiency. At 3h, phytic acid is nearly balance so we choose 3h for further experiments **(Figure 6)**.

Effect of Extraction Temperature to Phytic Acdic Extraction Efficiency

Temperature affects to the dissolving velocity of soluble into solvent. When temperature increases to 40^{o}C, phytic acid extraction efficieny is maximum. Solubility and diffusion of phytate into solvent increase consently to temperature. In peanut seeds, there is always a small amount of phytase. This enzyme belongs to phosphatase having ability to decompose phytic acid, release inorganic phosphor **[13**]. It well activates in acidity, so its operation is a reason of phytic acid degradation **[14, 15].** According to **Gonnety *et al.*, 2007**, **[15]**, phosphatase in peanut seeds has the optimal temperature 55^{o}C so phytic acid reduced dramatically. Finding the appropriate solvent and temperature will create favorable condition for interaction between solvent and material; proceed to dissolve of soluble particles into solvent. 40^{o}C is the adequate temperature **(Figure 7)**.

Optimization the Phytic Acid Extraction

In order to optimize the phytic acid extraction, we decide to use the experimental plan 2 rotating cores Box-Wilson with 4 independent varians: Z1–HCl concentration (M), Z2– ratio of solvent/ material (mL/g), Z3– extraction time (h) and Z4– extraction temperature (^{o}C). To facilitate the optimization, we modify varians so that each independent varian change to three levels -1, 0, 1 by following formula **(Figure 8-13)**:

$$X_i = \frac{Z_i - Z_0}{\Delta Z}$$

Where:
Xi: coded varian
Zi: uncoded varian
Zo: value at core of varian
ΔZ: jump step

After optimizing by climbing method, we choose various ultrasonic conditions: HCl concentration 0.61 M, solvent/ material 22.50 mL/g, extraction time 3.57h and extraction temperature 43.46 ^{o}C. Maximum phytic acid extraction efficiency is calculated from regression equation (1.01%). When comparing to experimental value 0.98%, we don't see any significant difference, high reliability (R^2= 0.994).

Phytic Acid Extraction Under Ultrasonic Support

Effect of Ultrasonic Power to Phytic Acid Extraction

In this experiment, we survey the effect of ultrasonic power to phytic acid extraction efficiency. The increment of ultrasonic power 0÷9.6 W/g will enhance its extraction efficiency and maximum at 9.6 W/g. In comparison, maxium efficiency is 1.17%, increase 19% compared to control (0.98%). Interaction between ultrasonic and solvent will create bubble. This phenomenon will form high shear force and facilitate mass transfer for extractable particles **[16**]. Bubble cavitation create stirring, speed up

particle interaction, spiral and internal diffusion.

This cavitation appears at outer surface of solid-liquid, liquid move through solid surface, erosion and disruption, new surface layer formation with liquid and mass transfer increment. When increasing the ultrasonic power, extraction efficiency increases respectively owing to more bubble cavitation and tissue disruption. However, if ultrasonic power is too high, bubble formation is also too much and power transfer will be reduced **[17**]. So when we increase the ultrasonic power from 9.6 W/g to 12.8 W/g, extraction efficiency is nearly stable. At ultrasonic power 12.8 W/g, phytic acid extraction efficiency is in downward trend. Probably this appearance is owing to temperature accumulation, favorable for phytase activation **(Figure 14)**.

Effect of Ultrasonic Treatment Time to Phytic Acid Extraction Efficiency

At 4 minutes, phytic acid extraction efficiency is optimal, from 0.98% to 1.20% (22% increment compared to control) **(Figure 15)**.

Optimization the Phytic Acid Extraction Under Ultrasonic

In order to optimize the phytic acid extraction, we decide to use the experimental plan 2 rotating cores Box-Wilson with 2 independent varians: Z1– Ultrasonic power (W/g), Z2– ultrasonic treatment time (minutes). To facilitate the optimization, we modify varians so that each independent varian change to three levels -1, 0, 1 by following formula:

$$X_i = \frac{Z_i - Z_0}{\Delta Z}$$

Where:

Xi: coded varian
Zi: uncoded varian
Zo: value at core of varian
ΔZ: jump step

After optimizing by climbing method, we choose various ultrasonic treatment conditions: treatment time 4.17 minutes, ultrasonic power 9.42 W/g. Maximum phytic acid extraction efficiency is calculated from regression equation (1.21%). When comparing to experimental value 1.19%, we don't see any significant difference, high reliability (R^2= 0.994) **(Figure 16)**.

Phytic Acid Extraction Under Enzymatic Support of Alcalase

In the following experiments, we investigate the phytic acid extraction efficiency under enzymatic method using Alcalase (1457 U/mL).

Effect of Enzymatic Activity to Phytic Acid Extraction

Treat peanut powder with Alcalase, phytic acid extraction efficiency increases to the enzymatic activity/ material, maximum at 4.55 U/g. At this point, phytic acid efficiency increases (1.27%) approximately 30% compared to control (0.98%). This

increment is explained by Alcalase acting as protein hydrolization. According to **Kumar *et al*., 2010**, **[18]** inside peanut cells, phytic acid exists in form of linkage with intracellular protein. So Alcalase (one type of endopeptidase) can hydrolyze linkage between phytic acid and protein, release this substance out off cell. However if we continue increasing enzymatic activity/ material, total phytic acid doesn't obtain respectively **(Figure 17)**.

Effect of Enzymatic Treatment Time to Phytic Acid Extraction Efficiency

In this experiment, we verify the effect of enzymatic treatment time to phytic acid extraction efficiency. Enzymatic activity/material is fixed at 4.55 U/g. Phytic acid extraction efficiency increases when increasing the enzymatic treatment time and maximum at 1.19% in 30 minutes. At this maxium value, enzymatic extraction efficiency is higher than control (0.98%) approximately 18%. After 30 minutes, there is not significant difference. This reason can be explained that, treatment time prolongs, enzymatic activity center is overload or substrate become shortage. So enzymactic reaction will also be ceased, phytic acid extraction gets stable **(Figure 18)**.

Optimization the Phytic Acid Extraction Under

In order to optimize the phytic acid extraction, we decide to use the experimental plan 2 rotating cores Box-Wilson with 2 independent varians: Z1– Enzymatic activity/ material (U/g), Z2– Enzymatic treatment time (minutes) **(Figure 19)**. To facilitate the optimization, we modify varians so that each independent varian change to three levels -1, 0, 1 by following formula:

$$X_i = \frac{Z_i - Z_0}{\Delta Z}$$

Where: Xi: coded varian ; Zi: uncoded varian; Zo: value at core of varian
ΔZ: jump step

After optimizing by climbing method, we choose various enzymatic treatment conditions: enzymatic treatment time 33.51 minutes, enzymatic activity/ material 5.62 U/g. Maximum phytic acid extraction efficiency is calculated from regression equation (1.20%). When comparing to experimental value 1.17%, we don't see any significant difference, high reliability (R^2= 0.98).

Phytic Acid Extraction Under Combination of Ultrasonic And Enzyme

After finding the optimum conditions of ultrasonic and enzyme, we combine these methods: ultrasonic-enzyme, and enzyme-ultrasonic.

Ultrasonic treatment is a non-selective method because its wave can penetrate to all corners of samples. Bubble cavitation facilitates the disruption and diffusion **[19**]. However, linkage between phytic acid and protein will hinder the extraction.

Meawhile, enzymatic treatment is a selective method. Alcalse breaks the linkage of peptides inside protein of peanut cell wall, loose intracellular linkages as well as phytic acid-protein.

Our results show that there is no significant diffirence between enzymatic and ultrasonic method (1.17% and 1.19%, respectively). Control sample has extraction efficiency 0.98%. However when we combine two methods, we clearly see the improvement. In order, enzyme-ultrasonic (1.42%), ultrasonic-enzyme (1.31%). Peanut power treated first by ultrasonic, cell wall become loose, facilitate for ultrasonic action, phytic acid releases more compare to method ultrasonic-enzyme **(Figure 20 and Table 2)**.

We realize high content of soluble protein and ash in product. Ash amount is mainly in oxidable form of phosphate.

Comparison of Anti-Oxidant Activity of Product With Other Anti-Oxidants

Phytic acid is a natural antioxidant. Phytic acid forms a chelate with iron, thereby preventing the radical formation and oxidative damage. It blocks the formation of hydroxyl radicals and suppresses lipid peroxidation. In fruits and vegetables, phytic acid helps to prevent oxidative browning by inhibiting polyphenol oxidase. Phytic acid may be used as a safe preservative and antioxidant in food products.

Gallic acid and vitamin C are two strong anti-oxidants **[20**]. Free radical scavenge DPPH of these substances are nearly the same and higher than phytic acid (8 times). However, antioxidant activity of phytic acid depends on chelating with metal ions in solution.

Phytic acid is extracted under enzyme-ultrasonic 2457 mg/L. By chelating of phytic acid to inhibit multivalent metal ions Fe, Cu acting as electron transfer agent in oxidation. Complex of Fe-phytic acid inhibits free hydroxyl (-$OH^{\cdot}$) and cease peroxy lipid **[18**]. Chelating of phytic acid is superior to other anti-oxidants **[21, 22].**

CONCLUSION

In our research, we optimize the phytic acid extraction from peanut seeds in different conditions: with and without ultrasonic, enzyme. Moreover, we also examine the anti-oxidant activity of phytic acid with other anti-oxidants such as ascorbic and gallic acid. Mechanism of anti-oxidant capacity from phytic acid is demonstrated by chelating with multivalent metal ions such as Fe and Cu.

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Figure 1: Peanut Seed

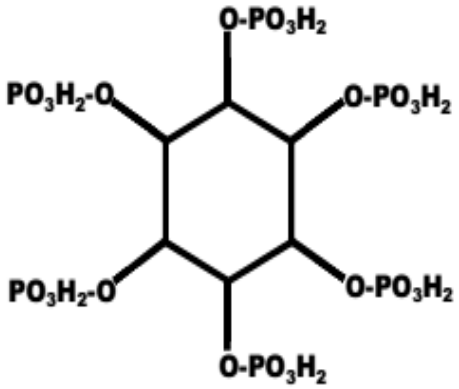
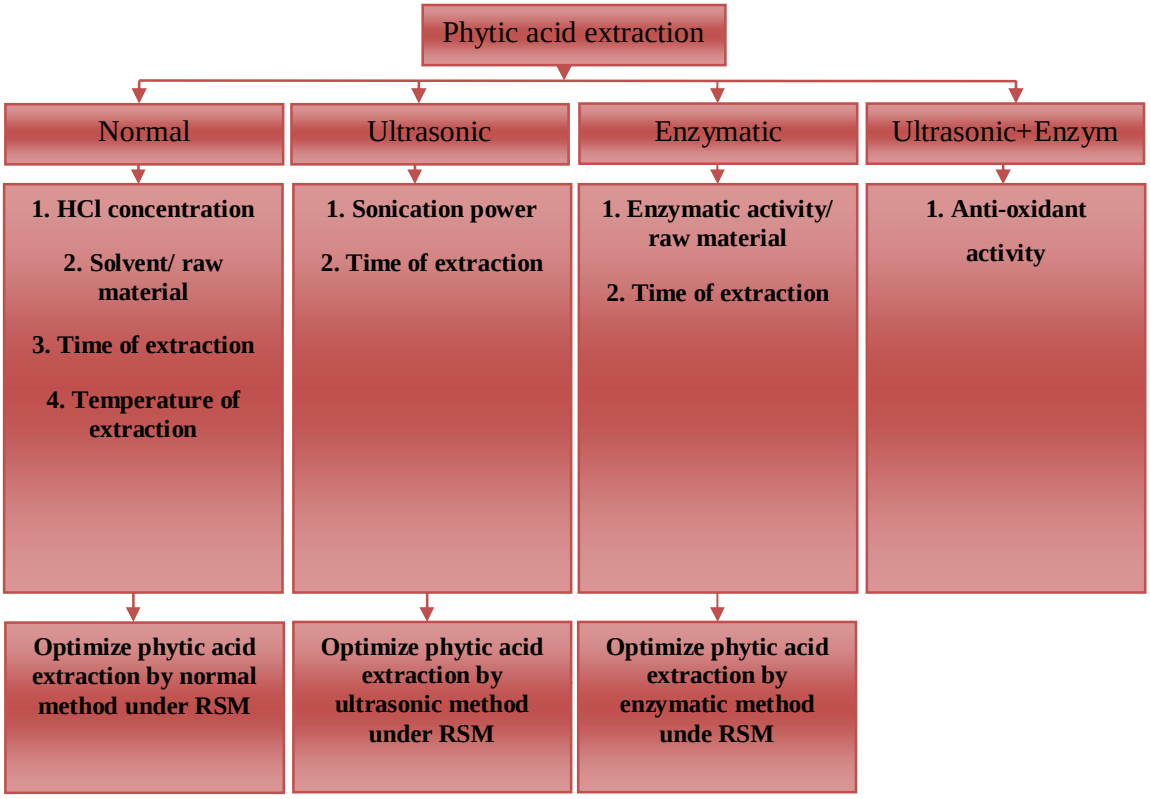


Figure 2: Structure of Phytic Acid



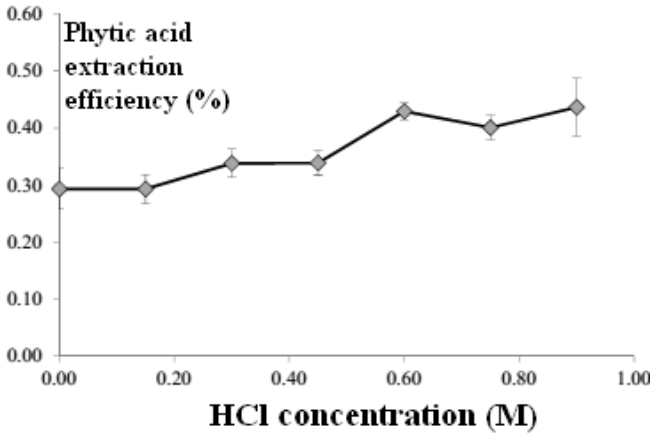


Figure 4: Effect of HCl Concentration (M) to Phytic Acid Extraction (%)

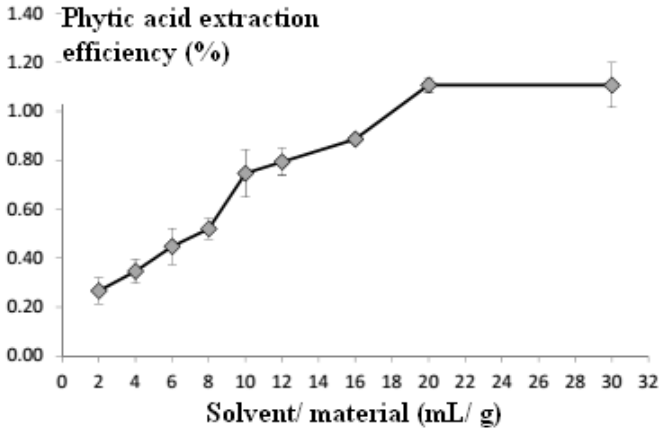


Figure 5: Effect of Solvent/ Material (mL/g Dry Matter Defatted) to Phytic Acid Extraction (%)

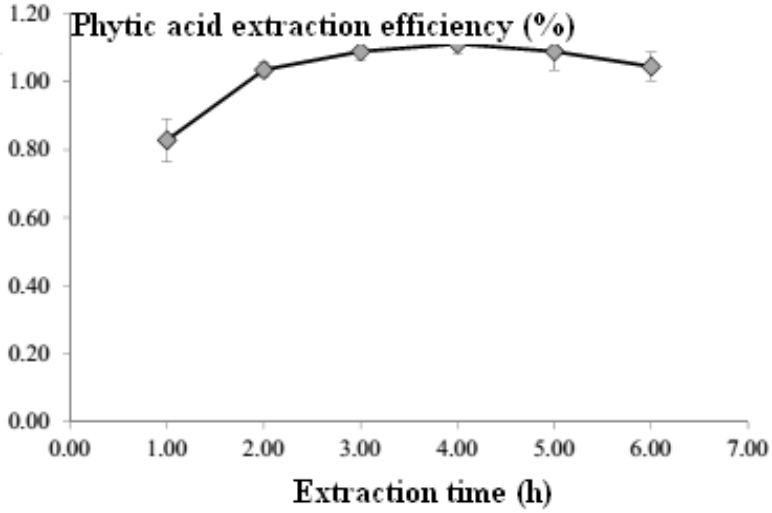


Figure 6: Effect of Extraction Time to Phytic Acid Extraction Efficiency (%)

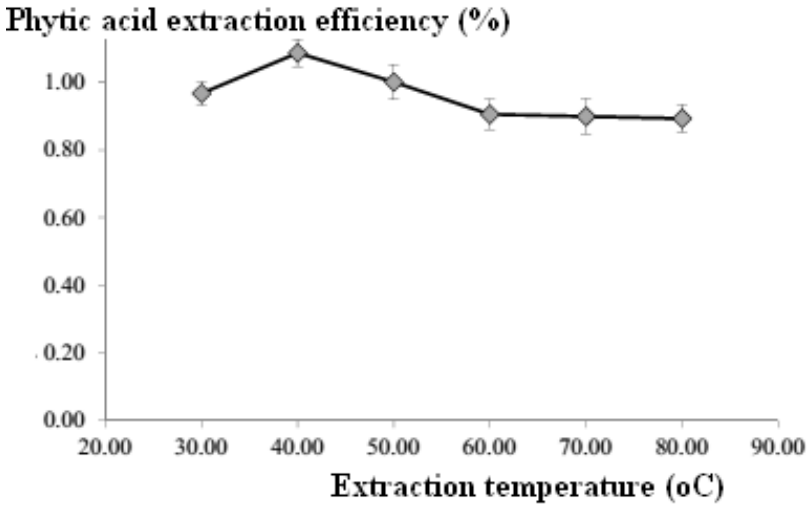


Figure 7: Effect of Extraction Temperature (^{o}C) to Phytic Acid Extraction Efficiency (%)

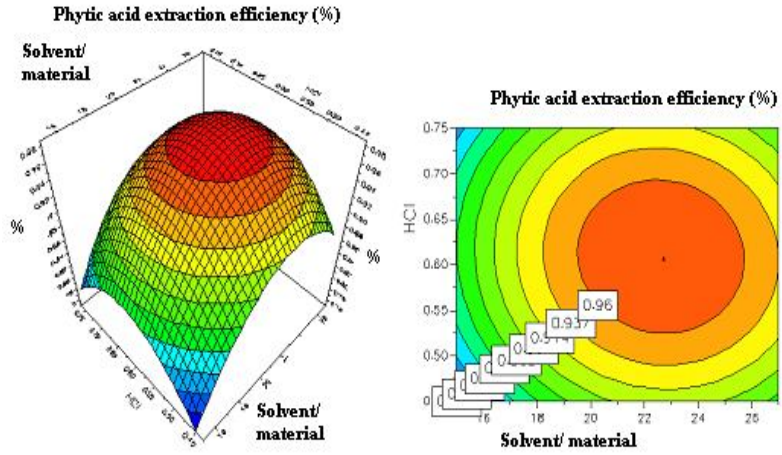


Figure 8: Effect of HCL Concentration and Ratio of Solvent/ Material to Phytic Acid Extraction Efficiency

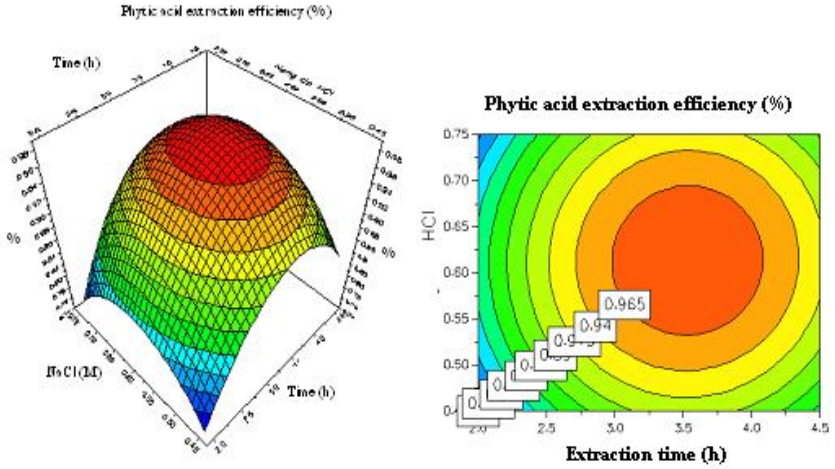


Figure 9: Effect of HCl Concentration and Extraction Time to Phytic Acid Extraction Efficiency

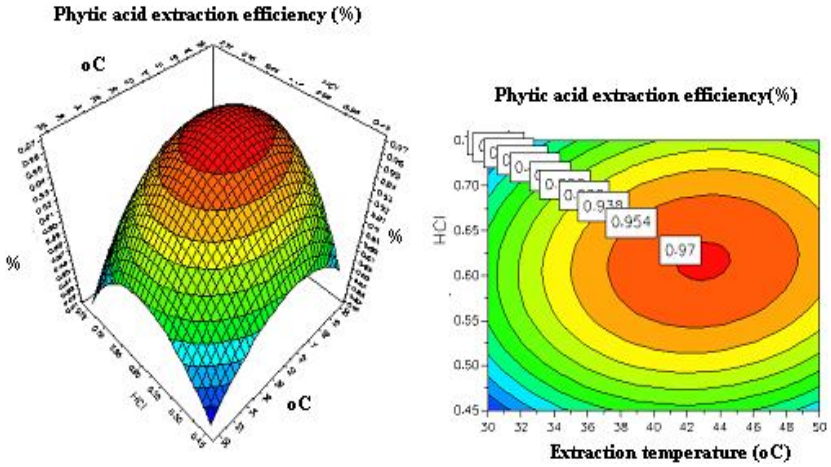


Figure 10: Effect of HCl Concentration and Extraction Temperature to Phytic Acid Extraction Efficiency

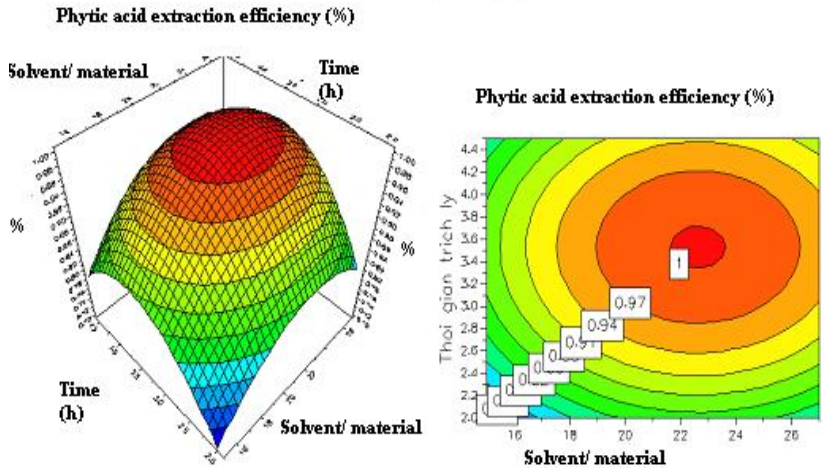


Figure 11: Effect of Solvent/ Material and Extraction Time to Phytic Acid Extraction Efficiency

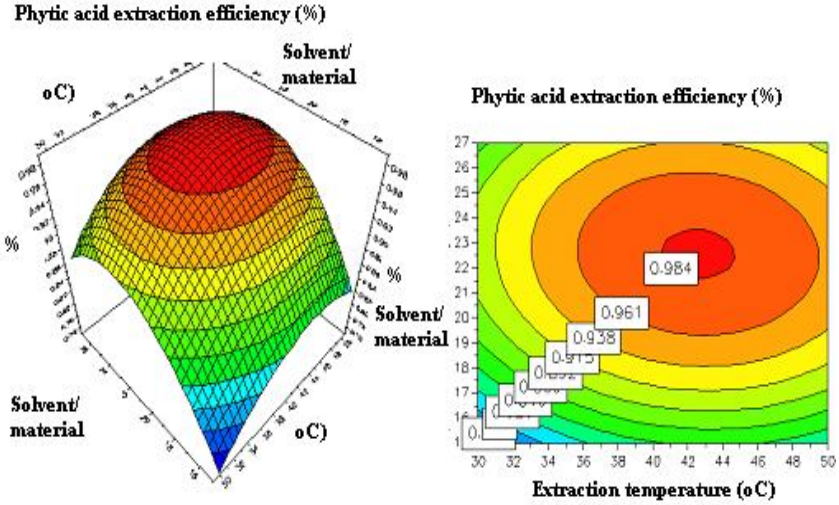


Figure 12: Effect of Solvent/ Material and Extraction Temperature to Phytic Acid Extraction Efficiency

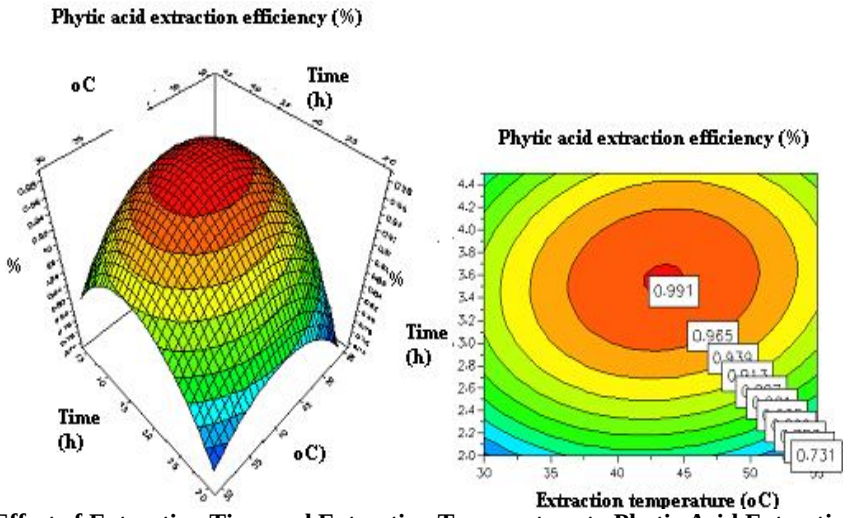


Figure 13: Effect of Extraction Time and Extraction Temperature to Phytic Acid Extraction Efficiency

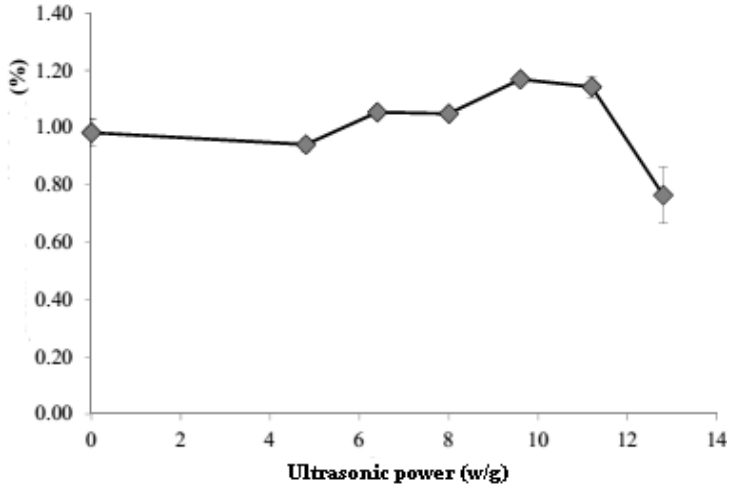


Figure 14: Effect of Ultrasonic Power (W/g) to Phytic Acid Extraction Efficiency (%)

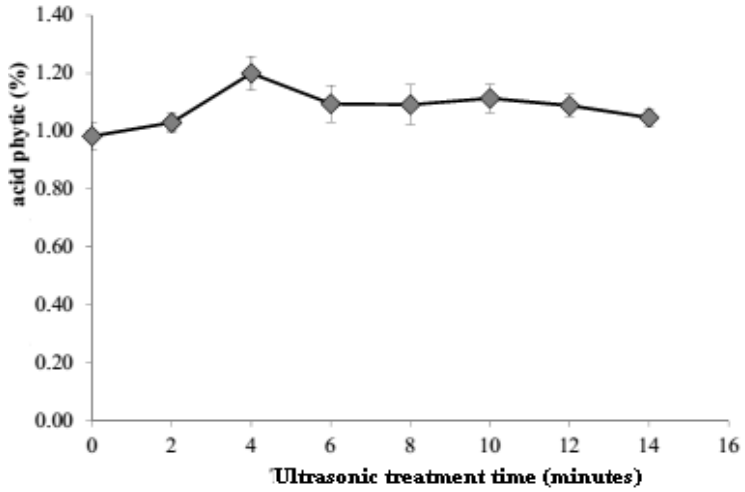


Figure 15: Effect of Ultrasonic Treatment Time (Minutes) to Phytic Acid Extraction Efficiency (%)

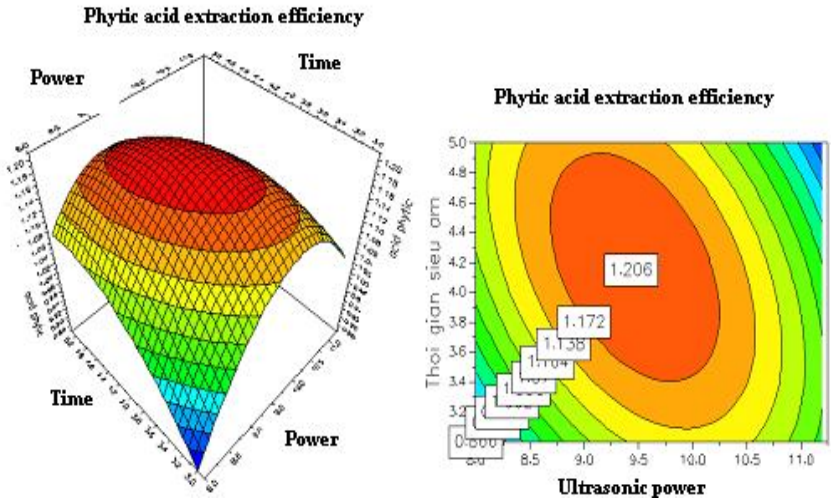


Figure 16: Phytic Acid Extraction Efficiency Under Ultrasonic

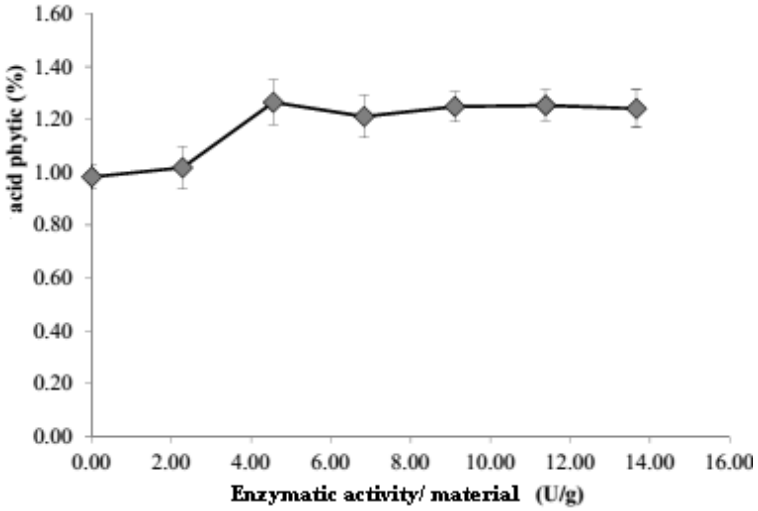


Figure 17: Effect of Enzymatic Activity (U/G) to Phytic Acid Extraction Efficiency (%)

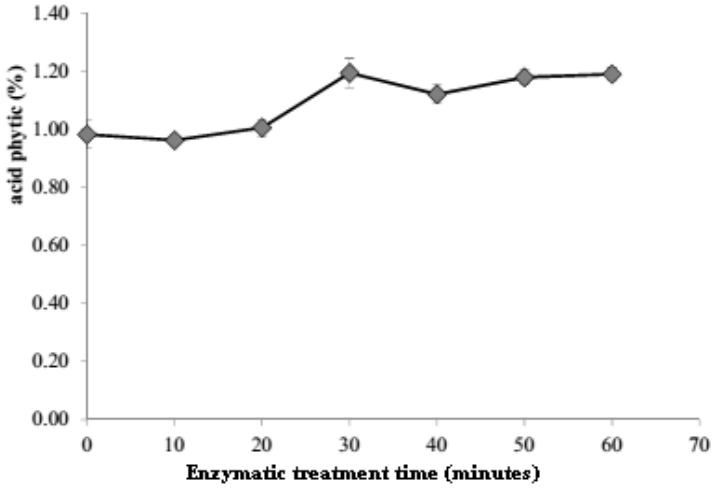


Figure 18: Effect of Enzymatic Treatment Time (Minutes) to Phytic Acid Extraction Efficiency (%)

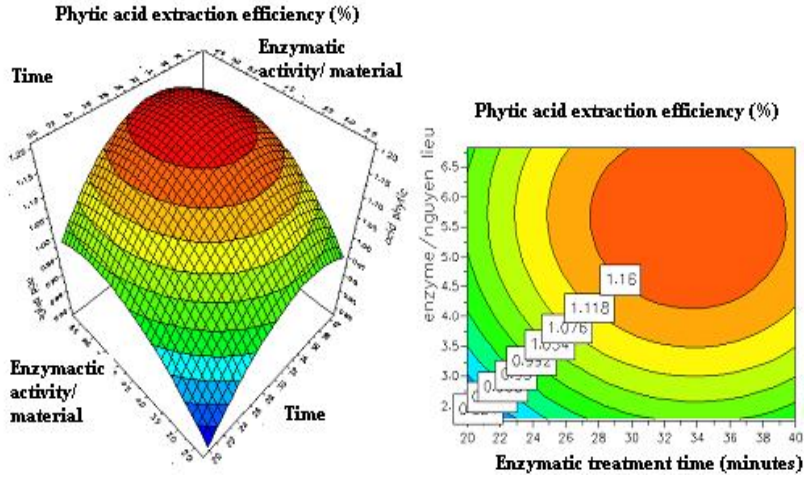


Figure 19: Effect of Enzymatic Treatment to Phytic Acid Extraction Efficiency (%)

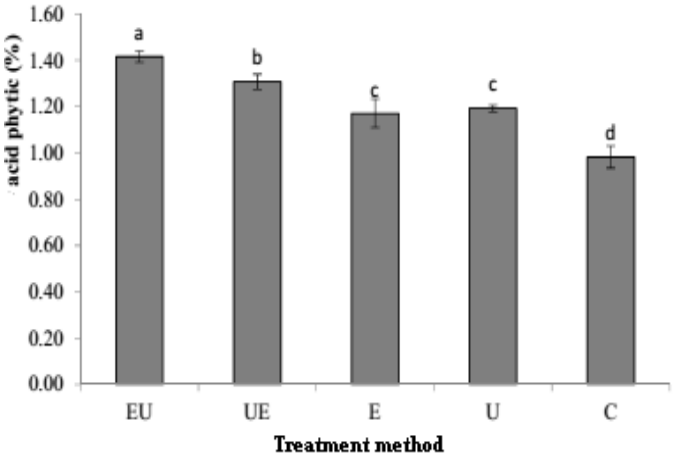


Figure 20: Different Treatment Methods to Phytic Acid Extraction Efficiency: EU_Enzyme-Ultrasonic, UE_Ultrasonic-Enzyme, E_Enzyme, U_Ultrasonic, C_Control

Table 1: Peanut Classification

Kingdom:	**Plantae**
(unranked):	**Angiosperms**
(unranked):	**Eudicots**
(unranked):	**Rosids**
Order:	**Fabales**
Family:	**Fabaceae**
Subfamily:	**Faboideae**
Tribe:	**Aeschynomeneae**
Genus:	***Arachis***
Species:	***A. hypogaea***

Table 2: Composition of Acid Phytic Product

	Phytic acid (mg/L)	Soluble protein (mg/L)	Ash (%)
Enzyme-ultrasonic	**2,457±48[a]**	**146.46±15.99[a]**	**0.65±0.03[a]**
Ultrasonic-Enzyme	**2,270±64[b]**	**145.61±30.79[a]**	**0.64±0.02[a]**
Enzyme	**2,032±125[c]**	**135.43±15.59[a]**	**0.51±0.01[b]**
Ultrasonic	**2,070±35[c]**	**149.28±6.40[a]**	**0.53±0.04[c]**
Control	**1,645±79[d]**	**90.47±4.40[b]**	**0.24±0.02[d]**

Table 3: Anti-Oxidant Activity of Phytic Acid and Other Anti-Oxidants

	Free radical scavenge DPPH (mmol TEAC/L)	Chelating (mmol EDTA/L)
Vitamin C	**817.70±3.20[a]**	**0.24±0.03[a]**
Galic acid	**812.20±1.83[a]**	**1.69±0.05[b]**
Phytic acid	**109.75±0.74[b]**	**22.34±0.24[c]**