HYDROTROPIC EXTRACTION OF THEOBROMINE FROM COCOA BEAN SHELL.

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Cocoa bean shell (CBS) is a waste product from chocolate and cocoa milling industries. CBS is high in nutritive value but it is of limited use in animal feeds because of its toxic theobromine content. Though its theobromine content limited the use of CBS, its amino acid profile of CBS compares favourably with palm kernel cake suggesting that it could be utilized as a medium protein source to substitute grain protein in livestock diets. Theobromine is also possesed many farmacological function such as anti cancer, diuretics, cardiac stimulants, hypocholesterolemic, smooth-muscle relaxants, atshma and coronary vasodilators. Considering that Indonesia is the third largest producer of cocoa in the, the potential usage of both protein and theobromine content of cocoa bean shell, thus it is neccesary to separate theobromine from cocoa bean shell. A new developed separation process is the extraction process using aqueous hydrotropes solution for recovery of naturally occurring secondary metabolites. The solubility of the secondary metabolites extracted are increased sharply and easily recovered. Recently several researches demonstrated that high solubilization capacity and selectivity in solubilization by hydrotropy could be used for extraction of water insoluble bioactive compound such as piperin, limonin, curcuminoids and forskolin. Based on the promising result by hydrotropic extraction of natural product, thus it is a promising method in administering theobromine from cocoa bean shell.

Keywords: *hydrotropic, extraction, theobromine, cocoa bean shell*

Introduction

Cocoa bean shell (CBS) is a waste product from chocolate and cocoa milling industries. It is the thin husk immediately surrounding the cocoa bean. Researchers stated that CBS is high in nutritive value but it is of limited use in animal feeds because of its theobromine content. Theobromine belongs to the same naturally occurring methylated xanthine group as caffeine. When taken in modest quantities, it acts as a stimulant like caffeine but intake of more than 0.0279 kg per body weight is injurious to animals. Though it's theobromine content limited the use of CBS, its amino acid profile of CBS compares favourably with palm kernel cake suggesting that it could be utilized as a medium protein source to substitute grain protein in livestock diets (Olubamiwa et al., 2006).

Though theobromine is considered as a toxic compound, many research stated that it possesed many farmacological function such as anti cancer, diuretics, cardiac stimulants, hypocholesterolemic, smooth-muscle relaxants, atshma and coronary vasodilators (Bispo, 2002).

Considering that Indonesia is the third largest producer of cocoa in the world after Ghana and Cote d'Ivoire, the potential usage of both protein and theobromine content of cocoa bean shell, thus it is neccesary to separate theobromine from cocoa bean shell.

Conventionally, natural products are separated by solvent extraction, whics is often time consuming and require large volume of organic solvent, thus involving additional cost and associated with the purchase and disposal of toxic solvents and dealing with the environmental hazard. Supercritical extraction using CO_2 has been gaining importance for extraction of natural product mainly because of the major advantage of getting product completely free from residual solvent. But the cost of the high pressure equipment needed to obtain supercritical extraction condition, however, becomes prohibitively high and limits the application of of supercritical extraction to only high value and low volume materials. High-pressure steam treatment can also enhance extraction rates by an osmotic shock; however, this technique is relatively slow and consumes a large amount of

steam. Ultrasound treatment has been claimed to increase the yield and mass-transfer rate in several solid liquid extraction processes, but the effect of ultrasound is, however, localized, and its application to a large volume of raw material may be energetically inefficient.

A new developed separation process is the extraction process using aqueous hydrotropes solution for recovery of naturally occurring secondary metabolites. This techniques is usually applied in extraction of slightly water soluble material such as in extraction of piperine (Raman and Gaikar, 2002), curcuminoids (Dandekar and Gaikar, 2003), limonine (Dandeekar, 2008), and forskolin (Mishra and Gaikar, 2009). Those researches stated that the solubility of the secondary metabolites extracted are increased sharply and easily recovered.

Considering that theobromine of cocoa bean shell is slightly soluble in water and hydrotropes solution can enhance solute solubility in water, thus this paper is subjected to review the separation of cocoa bean shell theobromine by hydrotropic extraction.

Literature

Cocoa Bean

Indonesia is the third largest cocoa (Figure 1) producers in the world. In 2007, Indonesian cocoa beans production reached 740,006 tons (or about 15% of world production), 90% of which produced by smallholders. Table 1 depicts Indonesia's cocoa production in terms of land coverage and tonnage.

Figure 1. Cacao L Theobroma

The cacao tree contains several chemical constituents that could be classified as toxic or antinutritious. Examples of such compounds are the methylxanthines theobromine, caffeine, and traces of theophylline, the biogenic amines β-phenethylamine and tyramine, oxalate, cyanogenic compounds, furfural, anandamides, tannin and trypsin inhibitor (Efsa, 2008).

Table 1. Area and Production of Cocoa Plantation in Indonesia

Year	Production (ton)
2003	697.166
2004	691.704
2005	748.828
2006	769.386
2007	740.006

Cocoa Bean Shell (CBS)

Cocoa bean shell (CBS) is a waste product from chocolate and cocoa milling industries. It is the thin husk immediately surrounding the cocoa bean. CBS (Figure 2) is high in nutritive value but it is of limited use in animal feeds because of its theobromine content (Table 2).

Figure 2. Cocoa Bean Shell

Table 2. Proximate Composition of CBS

Component	Percentage
Dry matter	84,95
Crude protein	6,78
Crude fiber	33,00
Ether extract	13,00
Ash	9,00
Nitrogen free extract	23,17
Theobromine	0,55

Theobromine

Theobromine was discovered in extracts from cacao beans (Theobroma cacao) by Woskresensky in 1842 and its chemical structure (Figure 3) determined by Emil Fischer at the end of the 19th century. Theobromine is a colourless and odourless substance (melting point 357^{o}C) with a slightly bitter taste that is naturally present in all parts of the seed and in small quantities in the pod, most likely as a component of the chemical defence mechanism of the cocoa plant. Theobromine, and to some extent caffeine, contributes to the typical bitter taste of cocoa and chocolate.

Though theobromine considered as toxic compound, it is reported possesed many pharmacological activities such as anti cancer, diuretics, cardiac stimulants, hypocholesterolemic, smooth-muscle relaxants, atshma and coronary vasodilators (Bispo, 2002).

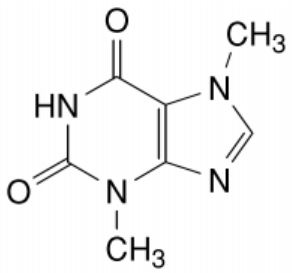

Figure 3. Chemical Structure of Theobromine

Hydrotropy

Hydrotrophy is a solubilisation process whereby addition of a large amount of second solute results in an increase in the aqueous solubility of another solute. Solute consists of alkali metal salts of various organic acids. Hydrotropic agents are ionic organic salts. Additives or salts that increase solubility in given solvent are said to "salt in" the solute and those salts that decrease solubility "salt out" the solute. Several salts with large anions or cations that are themselves very soluble in water result in "salting in" of non electrolytes called "hydrotropic salts" a phenomenon known as "hydrotropism". Hydrotropic solutions do not show colloidal properties and involve a weak interaction between the hydrotropic agent and solute. Hydrotrophy designate the increase in solubility in water due to the presence of large amount of additives.

The mechanism by which it improves solubility is more closely related to complexation involving a weak interaction between the hydrotrophic agents like sodium benzoate, sodium acetate, sodium alginate, urea and the poorly soluble drugs (Vemula, 2010).

Advantages of hydrotropic solubilization technique:

- Hydrotropy is suggested to be superior to other solubilization method, such as miscibility, micellar solubilization, cosolvency and salting in, because the solvent character is independent of pH, has high selectivity and does not require emulsification
- It only requires mixing the drug with the hydrotrope in water.
- It does not require chemical modification of hydrophobic drugs, use of organic solvents, or preparation of emulsion system.

Hydrotropic Extraction

In recent years the phenomenon of hydrotropy for a number of potential applications in process industry has been investigated. One of it was the utilization of hydrotropic solubilization for natural product extraction.

Recently Raman and Gaikar (2002) demonstrated that high solubilization capacity and selectivity in solubilization by hydrotropy could be used for extraction of water in soluble bioactive compound such as piperin from complex bio-matrices. Hydrotropes,such as sodium alkyl benzene sulfonates and sodium butyl monoglycol sulfate, were used for the selective extraction of piperine by cell permeabilization of Pipernigrum fruits. Penetration of the hydrotrope molecules into the cellular structures and subsequent cell permeabilization were hypothesized to explain the enhanced extraction rates of aqueous hydrotrope solutions. Hydrotrope molecules, after adsorption on a cell wall, cause disorder in its structure and in the bilayered cell membrane to facilitate the rapid extraction of piperine. The hydrotrope solution showed selective and rapid extraction of piperine from blackpepper. The recovered piperine was 90% pure and substantially free from oleoresins. The type and nature of the hydrotrope, the hydrotrope concentration, the temperature, and the particle size all had significant effects on the extraction process.

Dandekar and Gaikar (2003) extracted curcuminoids from Curcuma longa by hydrotropic solubilization. The degree of extraction was dependent upon the effect of hydrotrope on the cellular structure and hydrotrope-curcuminoids interaction. Sodium cumene sulfonate was found to be an efficient hydrotrope for the extraction of curcuminoids. The hydrotropic extraction of curcuminoids gave good yields of curcuminoids with good purity.

Dandekar et al. (2008) was also investigated the hydrotropic extraction of limonin from Citrus aurantium seed. The extraction efficiency was dependent on hydrotrope concentration, extraction temperature and percent of raw material loaded. Two hydrotropes such as sodium salicylate (Na-Sal) and sodium cumene sulphonate (Na-CuS) were studied. Both hydrotropes gave maximum limonin yield at 2M concentration, extraction temperature of 45 ^{0}C and 10% solid loading. A maximum limonin yield of 0.65mg/g seeds was obtained using Na-CuS whereas only 0.46mg/g seed was obtained using Na-Sal. Using this process, the use of organic solvents can be reduced dramatically to keep the process environmental friendly for the extraction of bioactive compounds.

CONCLUSION

Considering that i) Indonesia is one of the largest producer of cocoa, ii) cocoa bean shell is a potential source of protein and theobromine that are very potential to be used as animal feed and pharmaceutical product, respectively, and iii) the superiority of hydrotropic extraction method, thus the hydrotropic extraction is a promising method in administering theobromine from cocoa bean shell.

REFERENCES

Bispo, M., Cristina, M., Andrade, J., 2002, "Simultaneous Determination of Caffeine, Theobromine, and Theophylline by High-Performance Liquid Chromatography", Journal of Chromatographic Science, Vol. 40, 46-49

Dandeekar, D., Gaikar, V., 2003," Hydrotropic Extraction of Curcuminoids from Turmeric", Separation Science and Technology, 38, 1185-1215

Dandeekar, D., Jayaprakasha, G.K., Patil, B., 2008, " Hydrotropic Extraction of Bioactive Limonin From Sour Orange Seed", Food Chemistry, 109, 515-520

Efsa, 2008, "Theobromine as undesirable substances in animal feed" The EFSA Journal, 725, 1-66

Jain, P., Goel, A., Parmar, M., 2010, Solubility Enhancement Techniques with Special Emphasis on Hydrotropy", International Journal of Pharma Proffessional's Research, 1, 34-45

Mishra, S., Gailar, V., 2009, " Hydrotropic Extraction Process for Recovery of Forskolin from Coleus forskohlii Roots", Indian Engineering Chemical Research, 48, 8083-8090

Olubamiwa ,O., Ikyo , S.M., Adebowale , B.A., 2006," Effect of Boiling Time on the Utilization of Cocoa Bean Shell in Laying Hen Feeds" International Journal of Poultry Science 5 (12): 1137-1139,

Raman, G., Gaikar, V., 2002, " Extraction of Piperine from Piper nigrum (Blac Pepper) by Hydrotropic Solubilization" Ind. Eng. Chem. Res., 41, 2966-2976

Vemula, V.R., Lagishetty, V., Lingala, S., 2010, " Solubility Enhancement Techniques", International Journal of Pharmaceutical Sciences Review and Research, 5, 41-51