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Extraction from Agricultural Waste, *Ipomoea batatas L.* Lam Leaves as a Cheap Source of Natural Dye

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Abstract The negative effects of yellow synthetic dyes on human health and environment were extensively studied and various methods have been proposed to overcome these problems. In the current study, we proposed a simple method of extraction via solvent-solvent extraction from Ipomoea batatas L. lam leaves, a common agriculture waste. Through this method, the dye was found to be completely devoid of any pesticides and herbicides and thus safe for consumption. Besides, the extracted dye was also found to be stable up to 2 years of storage. This method is simple and can be easily adapted by small industries as a form of supplement income in developing countries.

Keywords: *sweet potato, carotenoids extraction, organic dye, green technology*

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1. Introduction

In the recent years, the production and application of synthetic dyes has released vast amount of waste and unfixed colourants that causes serious health hazards to human and the environment [1]. The usage of synthetic dye such as tartrazine (food Yellow no.4) has brought negative effects to human health such as skin rashes, hay fever, breathing problems, anxiety, migraines, depression, general weakness, heat waves and sleep disturbance [2]. Hence, natural source of yellow dye was seen as an ultimate solution to solve these problems.

Currently, the production of natural yellow dye available in the market is from African marigold (*Tageteserecta*) [3]. However, the main drawback of the African marigold is their seasonal nature, which deterred constant supply of these raw materials throughout the year to the natural dye industry. This is further coupled with the opening of new lands for cultivation and a high labour costs for maintenance, thus contributing to the high cost of production. Hence, the exploitation of traditional crops grown with low inputs (especially in developing countries) would be a step towards better resource utilisation for growing consumer demands.

Ipomoea batatas or commonly known as sweetpotato plant was described by Villareal (1982) [4] as the world most under-rated crop and best kept secret in the agriculture field. *I. batatas* ranked seventh in the food crop production and one of the two major tuber crops in Asia, the Pacific and Ocenia region [5]. Currently approximately 95 %–98 % of the *I. batatas* leaves are discarded after the roots are harvested thus making thema suitable alternative source of natural yellow dye. A previous study has demonstrated that the *I. batatas* leaves contained a comparable level of lutein (yellow colour pigments) with vegetables such as spinach and kale, both currently ranked highest in their lutein contents [6]. However, one of the major concerns of using agricultural waste is the presence of pesticides residues which may have adverse effects on human health.

A good dye should meet the three main criteria which are: cheap, abundance and safe. In this study, *Ipomoea batatas L.* lam leaves collected during the harvesting season were used as natural source of yellow dye performed via solvent-solvent extraction. The compositions of the yellow dye were characterised using liquid chromatography mass spectrometry / mass spectrometry (LCMS / MS). Besides, the extracted dye was subjected to gas chromatography for the detection of herbicides and insecticides residues on the leaves. The stability of the dye was then study in a period of two years under various storage conditions.

To our information, this is the first paper that reported the extraction of yellow dye from *Ipomoea batatas* leaves using a simplified solvent-solvent extraction method. This method is cheap and easily reproducible in small scale industry and is beneficial to improve farmers' income especially in developing countries.

2. Materials and Methods

2.1. Plant Material

Ipomoea batatas L. lam leaves used were collected from the *I. batatas* farm in Tanjung Sepat, Kuala Langat, Selangor, Malaysiaacross three harvesting seasons.

2.2. Extraction

Three sets of experiments were performed to optimise the extraction protocolsfor the yellow dye which include extraction solvents, potassium hydroxide concentrations (% KOH) used in saponification and saponification durations.

The extraction process of *I. batatas* leaves were performed under low light condition. Theleaves were ground using liquid nitrogen and extracted in acetoneovernight. The extract was filtered with Whatman No.1 filter paper and butylated hydroxy toluene (BHT) was added. The extraction steps were repeated using methanol, tetrahydrofuran and petroleum ether and tetrahydrofuran mixture (4:1) to replace acetone.

The solvent in the filtrate was removed using a rotary evaporator (Butchi). Then, 40 % of aqueous potassium hydroxide (KOH) was added into the extract and the mixture was left to saponify at 45°C. Acetone was then added to the saponified sample and two distinct layers were observed. The bottom layer was discarded and the upper layer was collected into an evaporatory flask. The extract was evaporated using a rotary evaporator leaving a layer of oleoresin. Petroleum ether and tetrahydrofuran mixture (4:1) was added to the oleoresin layer and a layer of green lipid was form at the base of the tube. The upper yellow layer was removed and used for subsequent analysis. In the second set of experiment, the different concentrations of potassium hydroxide (KOH) used were 10 % KOH, 40 % KOH and 60 % KOH following the above mentioned methods. In the third set of experiment, different saponification durations (30 min, 2 hours, 4 hours and overnight) were implemented following the above mentioned protocols.

2.3. Analysis

The yellow dye compositions were detected usinga LCMS / MS system (Applied Biosystems 3200Q Trap LCMS / MS). Shimadzu Ultra Performance Liquid Chromatography system was used for the separation of carotenoids while identification was done using a mass spectrometry. Separation was achieved using a C_{18} column (Phenomenex Aqua, 50 mm x 2.0 mm x 5 µM) and the buffer methanol: acetonitrile (40:60) was added with 0.1 % of formic acid and 0.1 % of ammonium formate. Analysis was performed at 0.5 mL/min flow rate under isocratic run with (Multiple Reaction Monitoring) MRM run time of 5 min. The β-carotene (Cal Bio Chem, Merck) and lutein (Sigma) standards were used to determine the presence of β-carotene and lutein in the yellow dye extract.

Screening of active carotenoids in the dye was performed using LCMS / MS system (Applied Biosystems 3200Q Trap LCMS / MS). The analysis was conducted using electrospray mass spectrometry (EMS) and the data was collected using the MS / MS system. Two different ionisation modes (positive and negative) were used for scanning. The mass spectrum obtained from the analysis was used to identify the compound by comparing the mass-to-charge (m / z) ratio values with the database provided by the National Institute of Standards and Technology.

Pesticides screening was performed to detect the presence of organophosphorus insecticides, organochlorinated insecticides and herbicides residues in the yellow dye using gas chromatography-mass spectrometry (GCMS) method. The analysis was performed at Consolidated Laboratory (M) Sdn. Bhd.

2.4. Stability

The stability of the yellow dye was determined under different storage conditions: (a) different storage temperatures (-20°C, 4°C, 25°C and 40°C) (b) presence or absence of light and (c) different matrices (acetone and soybean oil). The yellow dye was kept in clear vials under selected conditions for two years and pigments analysis was conducted from time to time. Statistical analyses were performed on data using SPSS.

3. Results and Discussion

The different types of solvents used often plays a role in the extraction efficiency on different plant tissues and are likely to be dependable on the resistancy of the plant cell to the solvent. In the first set of experiment, yellow coloured sample was observed from the extraction using acetone while pale yellow samples were obtained when methanol and petroleum ether and tetrahydrofuran mixture (4:1) were used. Extraction using tetrahydrofuran on the other hand produced green coloured sample. Highest concentration for both the β-carotene and lutein was observed with the usage of acetone as the extraction solvent while petroleum ether and tetrahydrofuran (4:1) mixture shown lower concentration of both pigments. Acetone was found to be the best extraction solvent due to its ability to be both polar and non-polar.

The degree and efficiency of saponification process in an extract is dependable on the concentrations of KOH used which could adversely affect the colour and composition of the extracted product. Saponification is an important step in the carotenoid extraction process to remove chlorophyll pigments from leaf extracts, to remove unwanted lipids and to hydrolized carotenoid esters [7]. Saponification using 10 % potassium hydroxide (KOH) was unsuccessful in removing chlorophyll from the leaf extracts and hence the extract was deemed unsuitable for subsequent experiment. A yellow-coloured sample was observed when 40 % KOH was used in saponification while a brown-coloured sample was observed when 60 % KOH was used. Hence, 40 % KOH was chosen as the best concentration of KOH used in saponification. Saponification process in vegetables and leaves is slightly different from other sources since carotenoids are embedded in the complex vegetables matrices. To maximise carotenoids yield, the inclusion of different antioxidants (nitrogen, ascorbic acid or BHT) into the saponification mixture andminimization of the exposure to light during saponification have shown improvement in the overall carotenoids yield [8,9]. Result from the third set of experiment indicated that 2 hours and 4 hours saponification durations did not show statistically significant differences in terms of the concentration of the

extracted pigments and hence the formal was choosen as the best saponification duration. Contrastly, 30 min and overnight saponification yielded much lower amounts of lutein than expected.

Previous studies on the extraction of carotenoids from green leaves extracts employed the usage of chromatography instruments such as HPLC and LC to separate the carotenoids from the chlorophylls. However, these processes are often expensive and requires skilful technicians to conduct [10] and hence, liquid-liquid extraction method introduced in this paper is a suitable alternative to the chromatography methods. Analysis conducted via LCMS / MS confirmed the presence of β-carotene pigment in the dye and shown as a single peak at 537.5 amuand at retention time of 2.45 min. Besides, a single peak was also observed for lutein at m / z value 569.5 amu and 0.5 min retention time. The concentrations of the pigments were calculated from the area below the peak. β-carotene concentration was found to be 5.4 mg / L while the concentration of lutein pigments was approximately 5.0 mg / ml. The final concentrations of β-carotene and lutein in the dyefrom *I. batatas* leaves were 10.8 mg / 100g FW and 10.0 mg / 100g respectively. Comparatively, these values were higher compared to *Amaranthusudinis* (12.6 mg / 100g), *Mangiferaindica* (2.21 mg / 100g), *Ashirantesaspera* (2.67 mg / 100g) and *Carica papaya* (2.7 mg / 100g) [11,12]. Hence, this makes the *I. batatas*leaves a cheap and abundant source of natural yellow dye.

Screening of active carotenoids in the dye conducted in the negative ionisation mode did not yield any result. Positive ionisation mode however showed the presence of other compounds besides β-carotene and lutein (main contributor to dye colour). The highest peak detected was 2-monolinoleoylglycerol trimethylsilyl ether, followed by malonic acid, decanoic acid and fumaric acid, which are the commonly found organic acids in plants. Besides that, beta cryptoxanthin and 4-ketozeaxanthin (an isomer lutein) was also detected in the extracted sample. The results of the analysis were summarised in Figure 1.

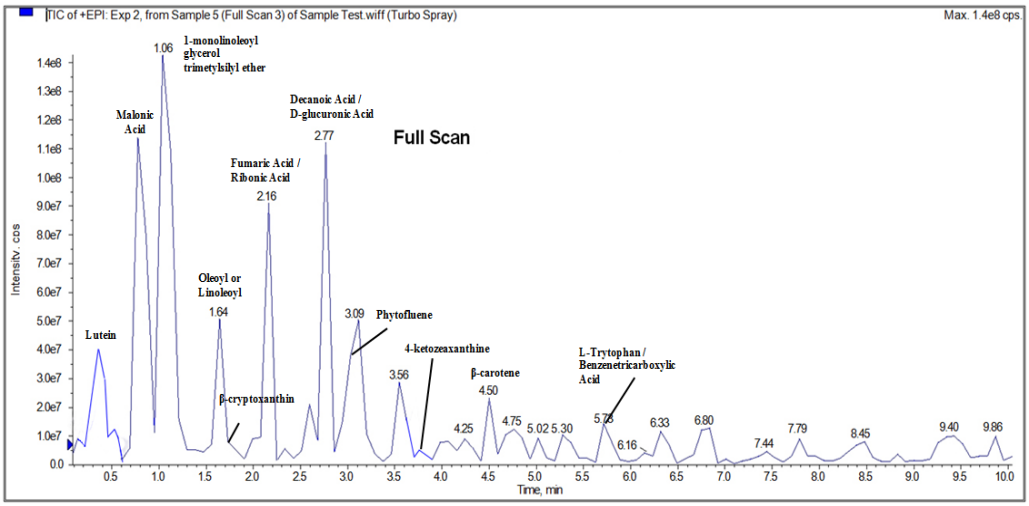


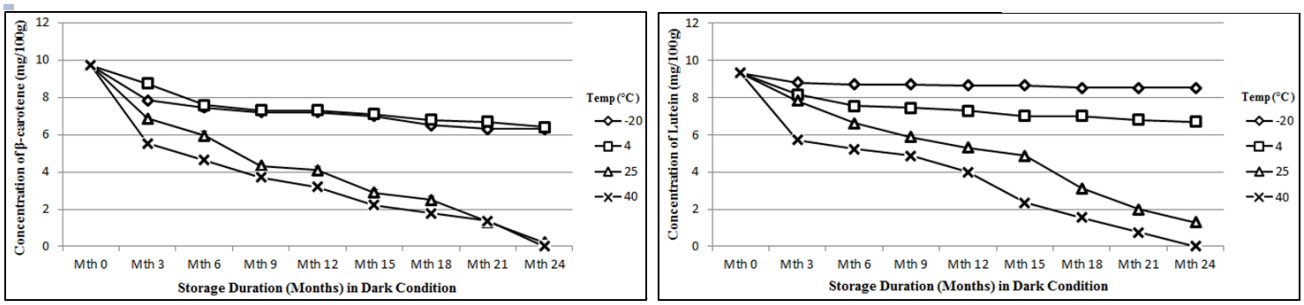
Figure 1. Composition of yellow dye extracted from *Ipomoea batatas* leaves detected using LCMS / MS

Beta cryptoxanthin and 4-ketozeaxanthin were detected in lower concentrations in the dye besides the main carotenoid such as β-carotene and lutein. 4-ketozeaxanthin is an isomer of the lutein pigment that has undergone degradation or isomerization which was probably contributed by several factors such as exposure to light, oxygen and high temperature during extraction and processing. Phytofluene on the other hand is a carotenoid precursor involved in the initial step of the carotenoids biosynthesis pathways. Common plant organic acids such as malonic acid, decanoic acid, fumaric acid and benzenemetricarboxylic acid were also detected in the dye. This group of organic acids are intermediates in the tricarboxylic acid cycle involved in the fatty acids production in plants. The accumulation of organic acids in plant tissue is mostly related to their vital functions in the transportation of micronutrients, the production of photosynthetic intermediates and the adaptation to stress by the plant [13,14,15]. Plant oils such as oleoyl and linoleoyl were also detected and might possibly play an important role in maintaining the stability of pigments in the dye.

The main concern of using leaf as source of extraction is the remaining pesticides residues in the final product after processing. The ultimate goal especially in the food industry is a pesticide free final product after the extraction and manufacturing process. Pesticides are used in the *I. batatas* farming to ensure the healthy growth of leaves and storage roots. In this study, gas chromatography coupled with mass spectrometry method (GCMS) was used to screen for the presence of most commonly used organophosphorus insecticides (136), organochlorine insecticides (52) and herbicides (251) residues in the yellow dye. Pesticides screening on the dye showed the absence ofthe 136 organophosphorus insecticide, 52 organochlorine insecticide and 251 herbicide residueswith the detection limit of 1 ppm. This may possibly contributed by the liquid-liquid partitioning process in removing pesticide residues from the yellow dye. This showed that the extraction method used in this study is sufficient to remove pesticides residues from the final extract.

The study on dye stability was conducted at the different storage temperatures (-20°C, 4°C, 25°C and 40°C), in presence or absence of light and storage in different matrices (acetone and soybean oil). In this part of our study, we focussed on quantifying only the major carotenoids in the dye which are β-carotene and lutein (Figure 2a. and Figure 2b.). From our results, lutein pigments were found to attain higher stability in -20°C

compared to β-carotene regardless of the matrix systems. Overall, soybean oil was found to be a better storage matrix compared to acetone. Total degradation of β-carotene and lutein in acetone at 25°C was observed only after 4 months and 2 months of storage respectively while degradation was observed after a month of storage at 40°C. Exclusion of light could also bring about greater stability to the yellow dye. Hence, without the usage of any preservatives, the yellow dye we have extracted from *Ipomoea batatas* L. lam leaves was stable in soybean matrix at 4°C and -20°C (absence of light) for up to 2 years. Study conducted by Jia*et al*. (2007) [16] concluded that the addition of soybean oil to the β-carotene pigments hasbeen shown to increase the β-carotene stability. Soybean oil was also used extensively worldwide andaccounted up to 75 % of the total vegetable oil consumption in the United States with a total production exceeding tenfolds compared to cottonseed oil production. Its low saturated fat and no-cholesterol features make it a suitable and healthy choice to store the extracted yellow dye.



a b

Figure 2. a. Concentration of β-carotene in Soybean Oil Stored at Different Temperatures under Dark Condition. **b.** Concentration of Lutein in Soybean Oil Stored at Different Temperatures under Dark Condition

4. Conclusion

The natural dye industry has been viewed as an upcoming and lucrative industry given the right source and technology for commercial dye production. Agriculture waste has been targeted as an alternative source to replace the usage of synthetic dye. The solvent-solvent extraction method proposed in this study was able to extract yellow dye from Ipomoea batatas L. lam leaves, which is a common agricultural waste. A yellow dye, which devoid of pesticides and is stable up to 2 years can been successfully reckoned as the next alternative to natural yellow dye. This may also help small scale industries and farmers in developing countries.

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Competing Interest

The authors have no competing interest.

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