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Extracting Pigments from Palmyrah Fruit Pulp (*Borassus flabellifer* L.) for the Production of Natural Colorants for Food

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Abstract

Palmyrah (*Borassus flabellifer* L) fruit pulp has an appeasing bright yellow or orange color which has been attributed to the presence of pro-vitamin A carotenoids of medicinal and nutritional value. Non-toxic, natural food colorants are in demand nowadays since the synthetic pigments produce many adverse health effects. This study is aimed at extracting the pigments from Palmyrah fruit pulp in the interest of producing natural food colorants. Here atmospheric liquid extraction with maceration/ solvent extraction was employed and the extraction procedure was optimized. The pigments were extracted using 80% methanol (v/v) and dichloromethane in 2: 1 (v/v) ratio and 10% KOH (w/v) at 35°C for 30min. The optimized method yielded 3ppm β-carotene per g of fruit pulp as determined by UV spectroscopy and thin layer chromatography.

Key words: Carotenoids; Extraction; Natural colorants; Palmyrah fruit pulp.

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1. Introduction

Palmyrah (*Borassus flabellifer* L.) is a tropical palm which belongs to the family Arecaceae and genus *Borassus* [2, 4]. The fruit consists of thick leathery pericarp which encloses 1-3 seeds. Seeds are embedded in fiber enmeshed with yellow to orange fruit pulp [5]. Palmyrah fruit is a good source of fiber, carotenoids, vitamin C, iron, magnesium, phosphorous, calcium and antioxidants [8]. The pulp is used to cure inflammatory reactions. Presence of phytochemicals such as polyphenols, alkaloids, carotenoids, nitrogen containing compounds and oregano sulfur compounds are responsible for tremendous health benefits [6]. Palmyrah Fruit Pulp (PFP) is yellow to orange in colour. This is known to be due to the presence of carotenoids, but the exact nature of carotenoid (eg whether structurally Pro- vit A or not) is unknown. Four types of carotenoids are present in the PFP [2]. As Palmyrah fruit is a seasonal fruit major, fraction of fruit pulp, which is about 10000tons, is wasted annually even though it holds many nutritional and health benefits. Most fruits are wasted due to inadequate technology for the post-harvest processing and preservation [2]. On the other hand, there is currently a growing demand for eco-friendly/non-toxic colorants, specifically for health sensitive applications such as coloration of foods [7]. Therefore an attempt was made to extract the pigments from palmyrah fruit pulp using the atmospheric liquid extraction technique in the interest of producing natural colorant for coloration of food.

2. Material and Methods

2.1 Sample collection

Fresh Palmyrah fruits of same variety (Black-skinned variety), which were free from physical and insect damage were collected from Puttur area in the Northern Province, and stored in the laboratory freezer (-20 ^{o} C) for further use.

2.2 Preparation of Palmyrah fruit pulp

The pulp was extracted manually using water in the ratio of-150 ml water per seed. Then it was filtered through muslin cloth.

2.3 Extraction of carotenoids

Several methods, such as electrical treatment and supercritical fluid extraction, can be employed for carotenoid extraction and recovery [11]. However, only solvent extraction/ atmospheric liquid extraction seems to achieve sufficient levels of efficiency and purity to be considered for scaled-up processes [13]. Therefore, carotenoids were extracted using atmospheric liquid extraction in this investigation. Here the extraction protocol was adapted from [1], and carotenoids were extracted using methanol and dichloromethane (DCM).

2.3.1 Polar solvent optimization with different base

Table 1: Different polar solvents used with different alkaline medium

Serial No.	Polar Solvent Combination	Polar Solvents	Non Polar Solvents	Alkaline strength	Solvent ratio
1	80% Methanol	Methanol, Water	DCM		1:1
2	80% Methanol	Methanol, Water	DCM	10%$NaHCO_3$	1:1
3	80% Methanol	Methanol, Water	DCM	10% KOH	1:1
4	Absolute Methanol	Methanol	DCM		1:1
5	Absolute Methanol	Methanol	DCM	10%$NaHCO_3$	1:1
6	Absolute Methanol	Methanol	DCM	10% KOH	1:1

2.3.2 KOH Strength Optimization

KOH solution was prepared in the concentrations of 10%, 20%, 40%, 60%, and 80%. The prepared KOH solutions at different concentrations were added separately to each different treatment. Three (3) replicates were established for each treatment.

2.3.3 Solvent ratio Optimization

Table 2: Different solvent ratios used in the extraction

Solvent ratio(P:NP)	Polar solvent (P)	Non-Polar solvent (NP)
1:1	80% Methanol	DCM
2:1	80% Methanol	DCM
1:2	80% Methanol	DCM

2.3.4 Temperature optimization

Table 3: Different temperature and time combination for heating in the water bath

Temperature (°C)	Time (minutes)
35	30
35	45
35	60
45	30
55	30

2.4 Studying the effect of different factors on the stability of the extracted pigments

The impact of heat, light and oxygen on the stability of the colorant was analysed using the product from the optimized method and UV spectrophotometer.

2.5 Preparation of standard solution

Standard β- carotene solution was prepared by dissolving 0.1mg of stock β -carotene (Sigma Aldrich) in 10 ml hexane. From this stock solution, β -carotene standard series solutions were prepared.

2.6 Instrument analysis

The DCM extracts were allowed to evaporate at room temperature and the pigments were dissolved in cold processed virgin coconut oil. The absorbance of carotenoids was determined using a spectrophotometer (Jasco V-730), at 450 nm. All analyses were performed in triplicates. The concentrations of the carotenoids in the extracts were determined using the standard curve obtained for the β- carotene solution.

3. Results and discussion

Higher absorption at the wavelength specific for carotenoids which is in the range of 450-500 nm and the least chemical residue in the final product were chosen as the most important criteria in the selection of solvents and other parameters. Carotenoids from plant parts are extracted with non-polar organic solvents facilitated by maceration, mechanic agitation, grinding or refluxing as the non-polar solvent does not reach through the cell membrane to dissolve the carotenoids [3]. It has been reported that carotenoids can be extracted with purified solvents, freshly distilled, chlorinated derivatives (dichloromethane or solvents containing hydrochloric acid) [3, 10]. The use of DCM and chloroform, however, has been restricted due to their carcinogenic nature, volatility and corrosive- nature. Other non-halogenated and less toxic solvents have been proposed as alternatives. The use of the combination of polar alcohols and nonpolar solvents has resulted in the efficient extraction of the desired compounds [14, 15].

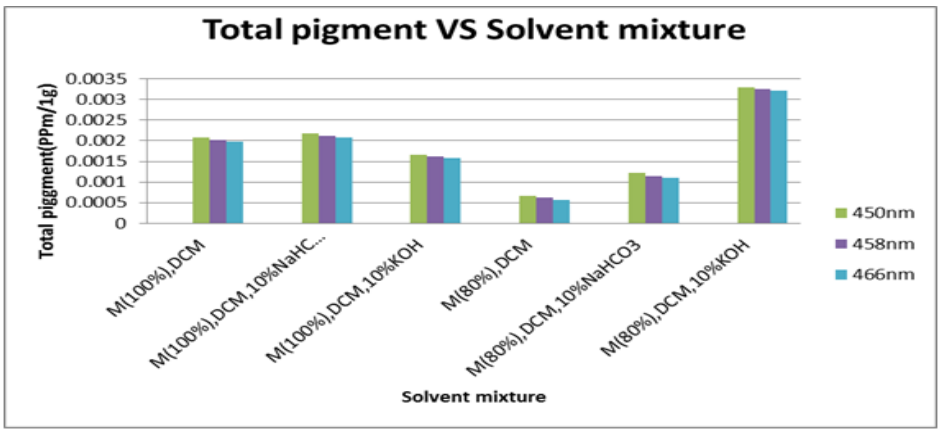

Figure 1: Total pigment amount in ppm from different treatments

The treatment using 80% methanol, DCM and 10% KOH yielded the highest absorbance reading in the 450-500nm range as shown in Figure- 1. Primary carotenoids are mostly associated with the photosynthetic membrane complexed to proteins, so it is necessary to use polar organic solvents capable of forming hydrogen bonds to disrupt the complexes. Secondary carotenoids, however, can be localized in oil bodies. As mentioned

above, it is not possible to recommend a specific solvent or solvent mixture for all samples as it depends on the specific carotenoid composition. In general, the use of a mixture of solvents such as the tricomponent solution used in this study yields good results for a sample containing a mixture of xanthophylls and carotenes. Carotenoids are in free form whereas xanthophylls are usually joined with fatty acids as mono- or diesters[12], so saponification is required to separate them out (and the fatty acids are thus converted into their basic salts or soaps). The data presented below (Table 4) aided in selecting the most appropriate KOH concentration to use in the saponification step for each treatment in terms of total carotenoid extraction.

Table 4: Selection of KOH Strength

KOH %	450nm	458nm	466nm
10%	1.617±0.207[a]	1.597±0.195[b]	1.583±0.203[c]
20%	0.726±0.107[a]	0.714±0.111[b]	0.721±0.102[c]
40%	0.742±0.112[a]	0.739±0.117[b]	0.731±0.115[c]
60%	0.984±0.122[a]	0.974±0.123[b]	0.967±0.123[c]
80%	0.996±0.005[a]	1.006±0.033[c]	1.003±0.035[c]

[a,b,c], values in the same row with different subscripts are significantly different at 0.05 significant level. The highest absorbance readings in the 450-500 nm range were obtained by using 2: 1 polar: non-polar solvent ratio. Table 5 shows the highest absorbance readings were obtained when heating the mixture at 35°C for 30 minutes and that when the exposure time or the processing temperature was increased the absorbance decreased. Many carotenoids are thermolabile (xanthophylls) [9] ; their heating being indicated only when it is absolutely necessary.

Table 5: Optimization of water bath temperature and time

Temperature (°C)	Time(minutes)	450nm	458nm	466nm
35	30	2.545±0.135[a]	2.527±0.117[a]	2.491±0.122[a]
35	45	2.513±0.062[a]	2.51±0.068[b]	2.51±0.067[c]
35	60	2.513±0.015[a]	2.444±0.106[b]	2.408±0.165[c]
45	30	2.458±0.053[a]	2.447±0.057[b]	2.444±0.062[c]
55	30	0	0	0

[a,b,c], values in the same row with different subscripts are significantly different at 0.05 significant level. The exposure to light had a negative impact on the pigments as denoted by absorbance readings (data not shown). The exposure to light (direct sunlight/ Ultraviolet), causes cis–trans photoisomerization, which may lead to their photodestruction [3] However, the differences between the two different treatments were not significant.

The exposure to oxygen also produced a negative impact on the stability of the color (data not shown). Carotenoids may be oxidized in the presence of oxygen or peroxides, because of their sensitivity to oxygen in the adsorbed state. It is necessary to operate in inert conditions (under nitrogen or vacuum). The oxidation

during the extraction and saponification can be minimized if it is carried out in a nitrogen atmosphere [3], as this non-oxidizing gas produces an inert, reducing atmosphere.

4. Conclusion

The extraction procedure was optimized using the organic solvents methanol and dichloromethane as the use of these solvents produced greater abundance of carotenoids as denoted by absorption in the range of 450-500 nm. Here the optimized solvent mixture was composed of 80% methanol and DCM. Strength of KOH was selected to be 10%. The process temperature was optimized as 35°C for a duration of 30minutes. The extracted pigments should be kept in air-tight containers or vacuum packed and stored in the dark, at or below 35 ºC, as exposure to light, heat and oxygen negatively affects the stability of the pigments.

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