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PREPARATION AND THE CHEMISTRY OF NATURAL FOOD COLOURS FROM ANNATTO PLANT

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RINGKASAN

Pewarna annatto yang larut minyak telah diekstrak dari biji annatto dengan menggunakan minyak jagung atau propilena glikol, manakala warna annatto yang larut air pula telah diekstrak dengan menggunakan 0.1 M kalium hidroksida. Analisa ke atas ekstrak tersebut menunjukkan pewarna annatto dalam minyak jagung mengandungi 0.06% jumlah pigmen dan enam bintik pada kromatografi lapisan nipis. Ekstrak dalam propilena glikol pula mengandungi 0.05% jumlah pigmen dan enam komponen apabila dianalisa dengan kromatografi lapisan nipis. Hasil kajian juga menunjukkan ekstrak pewarna annatto dalam kalium hidroksida mengandungi 0.17% jumlah pigmen dan juga enam komponen dari kajian kromatografi lapisan nipis.

Dalam pengeluaran serbuk pigmen kasar, ekstrak beralkali didapati lebih cekap dari ekstrak berair. Pewarna annatto dalam minyak jagung yang diekstrak dari serbuk pigmen kasar mengandungi 0.06% jumlah pigmen, manakala ekstrak dalam kalium hidroksida pula mengandungi 0.58% jumlah pigmen. Ekstrak pewarna dalam minyak jagung dan propilena glikol stabil sekurang-kurangnya selama enam bulan, manakala pewarna annatto dalam kalium hidroksida didapati stabil selama tiga bulan.

INTRODUCTION

Annatto food colours are natural alternatives to the synthetic yellow and orange colours. They are natural carotenoid colorants extracted from the seeds of the annatto tree, *Bixa orellana*. The annatto tree which is native to tropical America, is now grown in most tropical countries. In the food industry, annatto colours are mainly used for colouring butter, margarine, cheese, ice-cream and confectionery. In Latin America, annatto-coloured oil is used as a colorant in cooking.

The pericarp of the seed is rich in pigment, consisting of more than 80% cis-bixin (MCKEOWN, 1961). Cis-bixin is orange in colour and insoluble in vegetable oil. It is partially converted on heating into the more stable isomer, trans-bixin, which is red and soluble in oil, together with some degradation products (PRESTON and RICKARD, 1980). Bixin has the basic structure of carotene with both a free and an esterified carboxyl group as end groups *(Figure 1)*. Upon saponification, the methylester group is split off and the resulting diacid is norbixin, which is water-soluble.

Annatto colour extracts are obtained by extracting the pigment from annatto seeds. Manufacturers of annatto colours regard precise details of their processes as secrets. Annatto is usually sold in solution, although it is also marketed in the form of powder or paste. Like all carotenes, annatto food colours in solution will fade on prolonged exposure to strong light. Annatto pigment is heat stable up to 100°C and fairly heat stable up to 125° Centigrade. At temperatures higher than 125°C, rapid thermal decomposition occurred (ANON, 1977). Thus for foods that need high heat treatment, the amount of colour used may be increased or added only after heat processing. Annatto food colours are stable when applied to non-acidic food products (pH>4) as bixin will precipitate at lower pH values.

MATERIALS AND METHODS

Materials

Ripe annatto fruit capsules or pods were obtained from MARDI Station, Jalan Kebun and then sun dried. When completely dried, the pods will split open to release the seeds.

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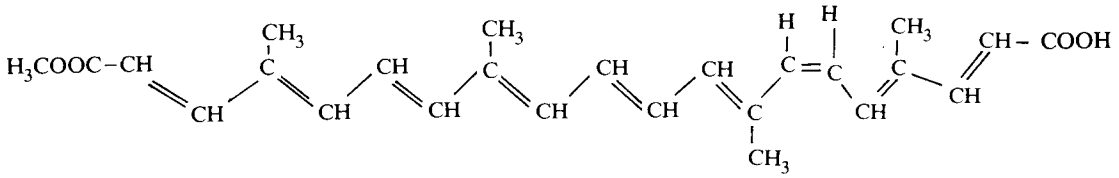

Figure 1. The structural formula of cis-bixin $C_{25}H_{30}O_4$

Preparation of oil-soluble annatto colour

Annatto seeds were immersed in corn oil or propylene glycol (seed : oil = 1 : 4) at temperatures not exceeding 70°C and mechanically stirred for three hours. For pigment stabilization, 0.4% lecithin was added to corn oil and 0.5% Tween 80 was added to propylene glycol. The slurry of annatto in oil or propylene glycol was then heated under vacuum at 100°C for one hour. The solution was filtered through muslin cloth to remove the seeds, centrifuged at 3 000 rpm for 30 minutes and then filtered through a glass fiber paper (Toyo GC 90). The product is a clear, viscous, orange-red solution of oil-soluble annatto colour.

Preparation of water-soluble annatto colour

Annatto seeds were mechanically stirred for one hour in 0.1 M potassium hydroxide (seed : KOH = 1 : 20) at temperatures not exceeding 70° Centrigrade. For pigment stabilization, 0.5% Tween 80 was added during the extraction. The slurry was filtered through muslin cloth, centrifuged at 3 000 rpm for 30 minutes and then filtered through a glass fiber paper (Toyo GC 90). The product is a clear, dark orange-red solution of water-soluble annatto colour.

Preparation of dry crude pigment

There are two methods for preparing dry crude annatto pigment powder from the seeds, *viz:* a) aqueous extraction
b) alkali extraction

a) Aqueous extraction: Annatto seeds were mechanically stirred in water (seed : water = 1 : 3) for two hours and then filtered through a coarse sieve (Mesh No. 32). The turbid filtrate was allowed to settle and the lower layer was separated and centrifuged at 3 000 rpm for 30 minutes. The wet pigment mass obtained was dried in an air-oven at temperatures less than 70°C and then ground to powder.

b) Alkali extraction: Annatto seeds were mechanically stirred in 0.1 M potassium hydroxide (seed : KOH = 1 : 5) for two hours and then filtered through a fine sieve (Mesh No. 60). The pigment was precipitated from the alkaline solution by addition of excess 1 M hydrochloric acid. The slurry was then centrifuged at 3 000 rpm for 30 minutes. The wet pigment mass obtained was dried in an air-oven at temperatures less than 70°C and then ground to powder.

Preparation of annatto colour from crude pigment powder

a) Oil-soluble colour: Crude pigment powder obtained from aqueous extraction of seeds was stirred mechanically with corn oil (powder : oil = 1 : 100) for three hours at temperatures less than 70° Centigrade. For pigment stabilization, 0.4% of lecithin was added to corn oil during the extraction. The slurry of pigment in oil was then heated under vacuum at 100°C for one hour, followed by centrifugation at 3 000 rpm for 30 minutes. Finally, the colour extract was filtered through glass fiber paper (Toyo GC 90) to give a clear, viscous, orange-red solution of annatto colour.

b) Water-soluble colour: Crude pigment powder obtained from either aqueous or alkali extraction of the seeds was stirred mechanically with 0.1 M potassium hydroxide (powder : KOH = 1 : 50) for one hour at temperatures less than 70° Centigrade. For pigment stabilization, 0.5% Tween 80 was added during the extraction. The slurry of pigment in potassium hydroxide was then centrifuged at 3 000 rpm for 30 minutes, followed by filtration through glass fiber paper(Toyo GC 90) to give a dark, orange-red solution of annatto colour.

Analysis of extracts

a) Determination of annatto pigment content: Different methods were used for the determination of total pigment content as different extraction media had been used for annatto colour extraction. However, all methods described below determine annatto total pigment content.

i) Annatto seeds: The total pigment content and bixin content were determined by the rapid method described by FRANCIS (1981, Unpublished).

ii) Oil-soluble annatto colour: The total pigment content was determined from the absorption at 500 nm and 404 nm in chloroform according to MCKEOWN and MARK (1962).

iii) Water-soluble annatto colour: The total pigment content was determined as α-norbixin from the absorption at 482 nm in 0.1 M NaOH according to REITH and GIELEN (1971).

iv) Annatto crude pigment powder: The total pigment content of crude powder obtained by aqueous extraction of seeds was determined according to the method described by MCKEOWN and MARK (1962). However, the method described by REITH and GIELEN (1971) was used for the determination of total pigment content in crude powder obtained by alkali extraction of the seeds.

b) Spectra analysis: Scanning of oil-soluble annatto extract in chloroform was carried out from 400 to 600 nm using a one-cm cell in Unicam Sp 800 spectrophotometer. For water-soluble annatto extract, scanning was carried out in 0.1 M potassium hydroxide.

c) Thin-layer chromatography (TLC): The method described by PRESTON and RICKARD (1980), using precoated silica gel G plates (Merck) and petroleum ether (bp 40°C–60°C)/diethyl ether/ glacial acetic acid (85 + 15 +2.5) as developing solvent was used for all thin-layer chromatography studies. The section of the silica gel containing the orange spots was carefully scraped off the plate. The colour was then extracted from the silica gel with a little chloroform, filtered through sintered glass funnel and the absorbance measured. According to REITH and GIELEN (1971), the absorption maxima of cis-bixin dissolved in chloroform are 501 nm and 470 nm, trans-bixin at 507 nm and 476 nm, cis-norbixin at 499 nm and 468 nm, and trans-norbixin at 506 nm and 475 nanometre.

Stability Studies

Annatto colour extracts in corn oil, propylene glycol, and potassium hydroxide were kept for at least six months at room temperature in air-tight containers. The total pigment content and thin-layer chromatography pattern were determined every two weeks. A rapid decrease in total pigment content, precipitation of pigment and the presence of breakdown products in thin-layer chromatography studies signified breakdown of annatto colour extracts on storage.

Application in food

Oil-soluble annatto colour in corn oil was added to cake preparations at concentrations of 0.5%, 1.0%, 2.0%, 3.0% and 4.0% (v/w). After baking, the colour for each preparation was compared with the control, which has no colour added.

Water-soluble annatto colour in potassium hydroxide was added to ice-cream preparations at concentrations of 0.2%, 0.5%, 1.0%, 1.5% and 2.0% (v/w). The colour for each preparation was compared with the control, which has no colour added.

RESULTS AND DISCUSSION

The annatto seeds used were found to have a total pigment content of 2.6%–3.4% (w/w) and the bixin component constituted 90%–91% (w/w) of the total pigment content. Generally, bixin should constitute well over 70% of the total pigment content except in the case of artefacts (DENDY, 1966). Chloroform extract of the seeds showed three distinct absorption peaks at 436, 460 and 488 nm *(Figure 2)* and five spots on TLC studies *(Table 1)*. Spot No. 1 could be orellin (yellow), which is a minor colouring matter in annatto, whereas spots No. 4 and 5 are likely to be the degradation products of cis-bixin.

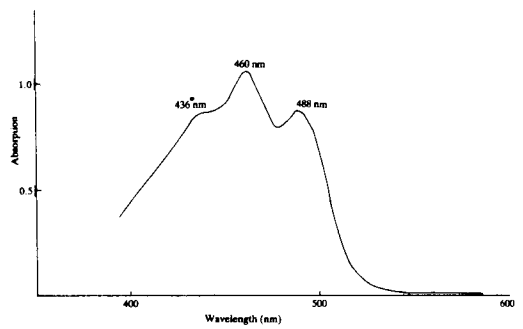

Figure 2. Absorption spectrum of annatto colour extracted in chloroform.

Table 2 shows the total pigment content of annatto extract in corn oil, propylene glycol and potassium hydroxide. The difference in recoveries is due to the poor solubility of the colour in corn oil and propylene glycol, as compared to potassium hydroxide. *Figure 3* shows the absorption spectrum of annatto extracts in corn oil, propylene glycol and potassium hydroxide. The absorption maxima of the various annatto colour extracts are as follows: corn oil 436, 458, 488 nm; propylene glycol 436, 458, 488 nm; potassium hydroxide 426, 446, 473 nanometre. Thin layer chromatography of annatto colour extracts in corn oil, propylene glycol and potassium hydroxide are shown in *Tables 3, 4* and *5* respectively, each showing the presence of six colour spots. The R_f values of cis-bixin and trans-bixin are variable in *Tables 1, 3* and *4* probably due to the presence of traces of extraction media in the TLC studies. The presence of an extra spot as compared with the TLC obtained in *Table 1* is due to the conversion of cis-bixin to the more stable trans-isomer during the extraction process.

The recoveries and total pigment content of crude annatto pigment powder obtained by both aqueous and alkaline extraction of the seeds are shown in *Table 6*. For alkaline extraction process, it is important that the extract is acid-free before drying, as acids cause the degradation of pigment during drying at elevated temperature (DENDY, 1966). The weight of crude pigment powder obtained by alkaline extrac-

Table 1. Thin layer chromatography of annatto colour extracted in chloroform

Spot no.	R_f	Colour	Pigment size	Identity
1	0.04	Pale yellow	Minor	–
2	0.12	Pale yellow	Minor	–
3	0.35	Orange	Major	Cis-bixin
4	0.50	Pale yellow	Minor	–
5	0.54	Pale yellow	Minor	–

Table 2. Total pigment content and recovery of annatto colour in various medium of extraction

Medium of extraction	Total pigment content % (w/w)	Specific gravity	Recovery of pigment % (w/w)	Colour
Corn oil	0.06	0.90	8	Orange-red
Propylene glycol	0.05	1.05	7	Reddish-brown
Potassium hydroxide	0.17	1.01	98	Dark orange-red

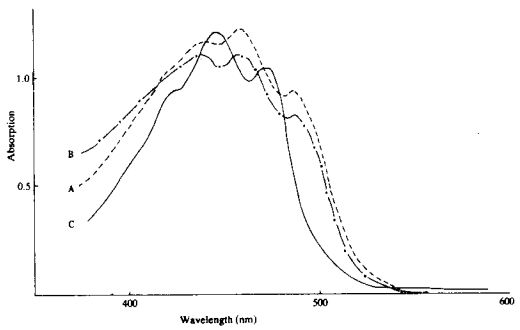

Figure 3. Absorption spectra of annatto colour extracts in corn oil (A), propylene glycol (B) and potassium hydroxide (C).

tion is nearly twice that obtained by aqueous extraction process. Therefore, alkaline extraction is more efficient than aqueous extraction in producing annatto crude pigment powder, even though the total pigment content of powder obtained by alkaline extraction is slightly less than that obtained by aqueous extraction.

Table 7 shows the results of analysis on annatto colour extracts prepared from the crude pigment powder. It was found that the total pigment content of annatto extract in corn oil prepared from crude pigment powder is similar to that prepared from the seeds, indicating that bixin is poorly soluble in corn oil. On the other hand, the total pigment content of annatto extract in potassium hydroxide prepared from crude pigment powder is nearly three times higher than that prepared from seeds, showing that

Table 3. Thin-layer chromatography of annatto colour extract in corn oil

Spot no.	R_f	Colour	Pigment size	Identity
1	0.04	Pale yellow	Minor	–
2	0.10	Pale orange	Minor	–
3	0.18	Orange	Major	Trans-bixin
4	0.21	Orange	Major	Cis-bixin
5	0.26	Pale orange	Minor	–
6	0.28	Pale yellow	Minor	–

Table 4. Thin-layer chromatography of annatto colour extract in propylene glycol

Spot no.	R_f	Colour	Pigment size	Identity
1	0.03	Pale yellow	Minor	–
2	0.12	Pale orange	Minor	–
3	0.25	Orange	Major	Trans-bixin
4	0.31	Orange	Major	Cis-bixin
5	0.44	Pale orange	Minor	–
6	0.48	Pale yellow	Minor	–

Table 5. Thin-layer chromatography of annatto colour extract in potassium hydroxide

Spot no.	R_f	Colour	Pigment size	Identity
1	0.01	Pale yellow	Minor	–
2	0.19	Pale orange	Minor	–
3	0.32	Orange	Major	Trans–norbixin
4	0.35	Orange	Major	Cis-norbixin
5	0.48	Pale orange	Minor	–
6	0.57	Pale yellow	Minor	–

Table 6. Yield and total pigment content of the crude pigment powder extracted from seeds

Extraction process	% yield by weight of the seeds	Total pigment content % (w/w)
Water	3.3	40.0 (as bixin)
Potassium hydroxide	6.3	37.0 (as norbixin)

Table 7. Total pigment content and recovery of annatto colours prepared from crude powder

Medium of extraction	Total pigment content % (w/w)	Recovery of pigment % (w/w)
Corn oil	0.06	14
Potassium hydroxide	0.58	98

norbixin is very soluble in potassium hydroxide. Corn oil is a better extraction media for oil-soluble colour as propylene glycol has a characteristic odour that could be unpleasant when added to food. Furthermore, corn oil extracted slightly more colour from annatto seeds compared with propylene glycol *(Table 2)*. Potassium hydroxide (0.1 M) is a suitable extraction media for preparation of water-soluble annatto colour as the colour is very soluble in alkali and either the seeds or the crude pigment powder can be used.

The oil-soluble annatto food colours developed were stable for six months, whereas water-soluble annatto colour was found to be stable for three months. Precipitation of pigment and decrease in total pigment content were observed following the breakdown of annatto colour extracts. At 1% and 2% concentrations, annatto colour imparted a light yellowish-orange shade to cake. However, the colour imparted was too light at 0.5% and too strong at 3%–4% concentrations. At 0.2% and 0.5% concentrations, annatto colour gave ice-cream a light yellowish shade. However, at 1%–2% concentrations of annatto colour, the yellow colour of ice-cream was too strong.

CONCLUSION

Annatto food colours are approved under the Food and Drug Act (U.S.A.) and do not require certification. It is also permitted under the Sale of Food and Drugs Ordinance and Regulations (1952) in Malaysia. Annatto food colours can be used in any amount consistent with good manufacturing practices in any food where colour additives are permitted.

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SUMMARY

Oil-soluble annatto colours were developed by extracting the annatto seeds with corn oil or propylene glycol, whereas water-soluble annatto colour was developed by extracting with 0.1 M potassium hydroxide. Analysis of the annatto colour extracts showed that annatto colour in corn oil has 0.06% total pigment content and six spots on TLC studies. Annatto colour in propylene glycol has a total pigment content of 0.05% and also six spots on TLC studies. Results also showed that annatto colour in potassium hydroxide has 0.17% total pigment content and six spots on TLC studies.

Alkaline extraction was found to be more efficient than aqueous extraction in the production of crude pigment powder. Annatto colour in corn oil prepared from crude pigment powder has 0.06% total pigment content. On the other hand, annatto colour in potassium hydroxide prepared from crude pigment powder has 0.58% total pigment content. Annatto colour in corn oil and propylene glycol developed were stable for at least six months, whereas annatto colour in potassium hydroxide was found to be stable for at least three months.

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