Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 91 (2005) 255–259
Food Chemistry
www.elsevier.com/locate/foodchem

Supercritical carbon dioxide extraction of 2-acetyl-1-pyrroline from *Pandanus amaryllifolius* Roxb

Paramita Bhattacharjee, Amol Kshirsagar, Rekha S. Singhal *

Food and Fermentation Technology Department, Institute of Chemical Technology, University of Mumbai, Matunga, Mumbai 400 019, India

Received 29 October 2003; received in revised form 12 January 2004; accepted 12 January 2004

Abstract

A comparative evaluation of the extraction of 2-acetyl pyrroline (2-AP) from *Pandanus amaryllifolius* Roxb. using either solvent extraction (3:1 chloroform:methanol), Likens–Nickerson apparatus or supercritical fluid extraction (SFE) with carbon dioxide extraction was carried out. SFE at 450 bar pressure for 3 h at 60 °C, at a constant flow rate of 0.1 l min^{-1} of CO_2, could extract 2-AP from *P. amaryllifolius* Roxb. in yields greater than those obtained by solvent extraction or Likens–Nickerson extraction. This extract could find novel applications in food flavouring.

Keywords: Pandan leaves; Likens–Nickerson extraction; Supercritical fluid extraction; 2-Acetyl pyrroline

1. Introduction

Plants belonging to the genus *Pandanus* (Pandanaceae) are palm-like evergreen trees or shrubs, widely distributed in the moist tropics from Africa to the Pacific Islands. Among the 36 species that have been recorded in India, *Pandanus odoratissimus* Linn. and *Pandanus amaryllifolius* Roxb. are of commercial interest to the flavour industry. In *P. odoratissimus,* the flowers are the scented part of the plant, while, in *P. amaryllifolius*, the leaves are scented (Zaheer et al., 1966). In south–east Asia, the leaves of *P. amaryllifolius* Roxb. are widely used for flavouring foods such as rice, jellies, or sweets. Juices extracted from the leaves are used as an essence in cake making and the entire leaf, along with coconut water and rice, is used to make *nasi-temak* and *nasi-kuning* in Indonesia. In India and the Phillipines, pandan leaves are traditionally used while cooking common non-aromatic rice to impart a resemblance of the leaf aroma to the cooked rice. Pandan leaves are sometimes added to iced drinks prepared from the water of unripe coconuts and also to sweet puddings and custards prepared from sticky, glutinous rice.

The flavour components of *Pandanus* leaves are not very well known. Teng, Shen, and Goh (1979) isolated the scent by chloroform–methanol extraction. Spectroscopic analysis of the extract revealed that the flavour was an oxidative degradation product of a yellow carotenoid pigment that develops only when the plant withers; the fresh, intact plants hardly have this odour. The leaves yield traces of essential oil up on distillation and the flavour component, 2-acetyl-1-pyrroline (2-AP) (popcorn-like aroma, as described by non-orientals, and pandan-like aroma, as described by orientals) was identified as a major component of the volatile oil of freeze-dried pandan leaves (Buttery, Ling, & Mon, 1986; Paule & Powers, 1989). This is also the principal aroma component of aromatic rice varieties such as Basmati and Jasmine. It is present in the rice endosperm at ten times greater concentration in scented rice than in non-aromatic rice (Buttery, Juliano, & Ling, 1983; Buttery, Turnbaugh, & Ling, 1988).

There is a controversy regarding the amount of 2-AP present in pandan leaves. Continuous steam-distillation-extraction of freeze-dried fresh leaves of *Pandanus* yields 12 ppm (based on dry weight of leaves) of steam-volatile oil. Gas chromatography-mass spectrometry (GC-MS) analysis showed 1 ppm of 2-AP in the volatile oil. The concentration of 2-AP in pandan leaves is reportedly 10

* Corresponding author. Fax: +91-022-5414-5614.
E-mail address: rekha@foodbio.udct.ernet.in (R.S. Singhal).

0308-8146/$ - see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2004.01.062

times greater than that found in milled, scented rice varieties, and 100 times greater than that found in common non-aromatic milled rice, where it is present at 0.04–0.06 ppm (Buttery et al., 1986). Extraction of 2-AP by solvent extraction and simultaneous Likens–Nickerson steam-distillation-solvent extraction has been previously reported (Laksanalamai & Ilangantileke, 1993; Ramarathnam & Kulkarni, 1984). However, these authors were unable to quantify 2-AP in the pandan oil extract by GC-MS. The major flavour impact compound of pandan leaves, 2-AP, is also the flavour impact compound of 'baguette-type' wheat white-bread crust, cooked meat and popcorn. It is also associated with the mousy off-flavour in wine.

In the present work, an attempt has been made to extract the flavour compound, 2-AP, from the scented leaves of *P. amaryllifolius* Roxb., either by conventional solvent extraction, or Likens–Nickerson simultaneous solvent-extraction-steam-distillation method, or by supercritical carbon dioxide. A new densitometric method was developed for quantitative determination of 2-AP in the extracts. Optimization of the parameters of supercritical extraction was carried out by the densitometric analysis of 2-AP in the flavour extracts.

2. Materials and methods

2.1. Materials

GR grade solvents and silica-gel plates, and 60 F_{254}(TLC aluminium sheets 20×20 cm, particle size 4–6 nm) were purchased from M/S E. Merck India Ltd, Mumbai, and vacuum grease and silicone oil (as an antifoaming agent) were from M/S Himedia Lab Pvt. Ltd, Mumbai. 2-AP was procured from T. Hasegawa Co. Ltd, Tokyo, Japan. All reagents used for analysis were of AR Grade.

2.2. Sample preparation

Fresh leaves of young plants of *P. amaryllifolius* were plucked, washed free of dirt, wiped with a cloth and sliced into small portions. The sliced leaves were then mashed in an electrically operated mixer-grinder for solvent extraction and Likens–Nickerson extraction; but for supercritical extraction, they were very coarsely ground.

2.3. Extraction and isolation of 2-AP

2.3.1. Solvent extraction

130 g of mashed leaves and 870 ml of solvent mixture ($CHCl_3$:MeOH = 3:1), enough to moisten the whole mass, were placed in a 1 l stoppered conical flask and kept at room temperature for 4 h in accordance with the method described earlier (Teng et al., 1979). The mixture was filtered through a nylon bolting cloth to remove the leaf residues. The filtrate formed two layers in a separating funnel – a muddy orange coloured upper layer and a dark bluish-green coloured lower layer. Both the layers were separated and analyzed for the presence of 2-AP after concentration.

Both the orange and bluish-green layers, obtained by solvent extraction, were separately concentrated on a rotavac system (40–45 °C and 200 mm Hg for 10–15 min) to a volume of ~10–12 ml. The concentrated solutions were transferred separately to two screw-capped glass vials and concentrated further by slowly purging nitrogen so as to completely remove the solvent. When not analyzed, the vials were kept in a deep freeze at −18 to −20 °C. The yield of the extract was gravimetrically estimated and both the layers were analyzed separately by densitometry.

2.3.2. Likens–Nickerson extraction

Likens–Nickerson concurrent steam-distillation-solvent extraction was used to extract 2-AP from pandan leaves. The procedure was similar to that developed by Laksanalamai and Ilangantileke (1993). First, 4 l of distilled water and 4 ml of antifoaming agent (silicone oil) were placed in a 5 l round-bottom flask, and boiled to obtain a volatile-free mixture. The boiling was continued until the volume of the mixture was reduced by 500 ml. 250 g of fresh pandan leaves were blended and mixed with 800 ml distilled water, and then filtered to remove the blended leaf residues. The filtrate was made up to 1000 ml with distilled water and the resulting pandan solution was added gradually to the volatile-free mixture in the round-bottom flask. The mixture was steam-distilled and the flavour extract was collected in a 250 ml round-bottom flask containing 80 ml of distilled water, 2 ml of dilute sulphuric acid and 120 ml of diethyl ether; maintained at 50 °C.

After 2 h of continuous steam-distillation and solvent extraction in diethyl ether, the solvent flask was removed and the sulphuric acid layer was transferred into a 250 ml Erlenmeyer flask, using a 250 ml separating funnel. The solution was neutralized by adding solid sodium bicarbonate, and then poured into a clean 250 ml separating funnel containing 120 ml of fresh diethyl ether. The separating funnel was shaken vigorously until the diethyl ether (upper layer) was clearly separated from the neutral solution (lower layer). The upper layer was poured into a clean 250 ml Erlenmeyer flask and the lower layer was discarded. Anhydrous sodium sulphate was added to the flask to remove dissolved water. Filtering through a non-absorbent cotton bed of anhydrous sodium sulphate further dried the ether extract. The dry ether extract was then filtered through a Whatman No. 1 filter paper into a 200 ml stoppered conical flask.

The above extract was concentrated to ~2 ml using a Vigreux fractional distillation column. The concentrated

solution was transferred to a screw-capped glass vial and concentrated further by slowly purging nitrogen so as to completely remove the solvent. When not analyzed, the extract in the vial was stored at −18 to −20 °C. The amount of the extract was gravimetrically estimated and diluted in *n*-hexane to an appropriate volume, prior to densitometric assay.

2.3.3. Supercritical carbon dioxide extraction (SFE)

Though the extraction time reported for SFE of flavour and fragrance compounds is 20–60 min (Sass-Kiss, Gao, Simandi, Boross, & Vamos-Falusi, 1998; Sugiyama & Saito, 1988; Temelli, Chen, & Braddock, 1988), preliminary trials at low extraction time did not yield 2-AP in quantities sufficient for densitometric estimation. Further, these trials indicated a 1 h static time and 1–2 h dynamic time for an appreciable extractability of 2-AP and hence were maintained in this work.

For SFE, a SPEED-SFE model of Applied Separations, Allentown, USA, was used. An attempt was made to optimize the yield of 2-AP. The extraction parameters, such as temperature and pressure, were optimized to selectively extract 2-AP. Around 300 g leaves were used in all the extractions. Each of these parameters was varied at various levels; pressure at two levels: 120–125 and 450–455 bar; temperature at three levels: 40, 60 and 80 °C and the dynamic time of extraction at two levels: 1 and 2 h. The flow rate of CO_2 in the dynamic extraction phase was kept with in the narrow range 0.1–0.2 l min^{-1}, as at higher flow rates, the flavour volatiles would escape, along with CO_2, without undergoing condensation (Hawthorne, 1990). The extracts were collected in ~50 ml *n*-hexane in a collection vial kept in a cooling bath containing a water–ice–methanol (1:3:2) freezing mixture to condense the flavour volatiles from CO_2 (Manninen & Kallio, 1997). The temperature of the freezing bath was maintained at −2 to −5 °C throughout the dynamic extraction phase by continuously adding crushed ice and rock salt. Cooling baths providing temperatures lower than this were not used, to avoid condensation of CO_2 in the trap.

For analysis, the extracts were filtered through a bed of anhydrous sodium sulphate and then concentrated on a rotavac system (28 °C and 200 mm Hg for 5–10 min) to a volume of ~5 ml. The extracts were transferred to screw-capped glass vials and the solvent was then completely removed from them by a gentle stream of nitrogen. When not analyzed, the extracts in the vials were stored in a deep freeze at −18 to −20 °C. The yields of the extracts were gravimetrically estimated and the solutions diluted in *n*-hexane, prior to densitometric assay.

2.4. Densitometric estimation of 2-AP

In this work, a rapid densitometric assay method was developed for the quantification of 2-AP in the extracts. For densitometric assay, several solvent systems such as *n*-hexane, chloroform, benzene, ethyl acetate, chloroform/ethyl acetate, hexane/ethyl acetate and methanol/chloroform, were tried. The developing solvent system was standardized with the extract obtained by solvent extraction. Both ethyl acetate and 10% methanol in chloroform gave the best resolution of the solvent-extracted aroma concentrate. Six bands were obtained, with both the solvent systems, and 2-AP recorded R_f value of 0.38 ± 0.01. The extracts were spotted on the aluminium-coated silica gel 60 (F_{254}) plates by use of Camag Linomat IV. The extracts dissolved in *n*-hexane, were applied to the plates in the form of bands, each 6 mm wide, spacings between consecutive bands being 8 mm. Nitrogen gas was used at a low flow rate, at 4 bar, for spotting. The plates were developed at room temperature (23 ± 2 °C) in a glass chamber containing the developing solvent. Spectrum scanning of the spots after development was carried out in a Camag HPTLC unit (TLC scanner II). 2-AP recorded a λ_{max} value of 370 nm. Densitometric studies were performed at 370 nm and the area under the curve for 2-AP was recorded. A standard curve was plotted for the pure standard 2-AP at 370 nm (area under the curve vs. μg of pure 2-AP spotted) and concentration of 2-AP in the extracts were evaluated from the slope of the curve.

3. Results and discussion

The solvent-extracted flavour extract was bilayered – a muddy orange coloured upper layer and a bluish-green coloured lower layer. TLC analysis of the orange layer did not show any band, while the bluish-green layer was resolved into 6 prominent bands, which could be observed under visible light and UV (370 nm), using ethyl acetate or 10% MeOH in $CHCl_3$, as developing solvents (Table 1). 2-AP could be detected as a pale yellow band at R_f 0.38 only in the bluish-green layer.

The Likens–Nickerson extract of pandan leaves showed only 2 bands, in which 2-AP had a R_f of 0.39 (Table 1). The extract was homogeneously pale yellow in

Table 1
TLC analysis of the solvent-extracted and Likens–Nickerson flavour extract

Band number	Appearance of band	R_f value
A. Solvent-extracted		
1	Pale yellow	0.31
2	Pale yellow	0.38
3	Light yellow	0.48
4	Dark yellow	0.60
5	Dark yellow	0.89
6	Green	0.99
B. Likens–Nickerson-extracted		
1	Pale yellow	0.39
2	Dark yellow	0.89

diethyl ether, with less pigment (chlorophyll and other pigments) co-extraction.

Table 2 gives the densitometric assay of 2-AP in the extracts obtained by solvent extraction, Likens–Nickerson extraction and under different conditions of SFE. The yield of 2-AP, by solvent extraction and Likens–Nickerson extraction, was ~1 ppm.

An increase in yield of 2-AP with increasing pressure and temperature was observed (Table 2) in the case if SFE. A temperature range of 40–60 °C has been reported to be the best for extraction of flavour and fragrance compounds such as limonene, from lemon peel (Calame & Steiner, 1982), essential oil from orange peel (Mira, Blasco, & Subirats, 1996) and from hops (Grimmett, 1981), such as angelica (Doneanu & Anitescu, 1998). While the yield of 2-AP was higher, at 450 bar, when temperature was increased from 40 to 60 °C, the yield decreased with increase of temperature at 125 bar. This was in accordance with the retrograde phenomenon of decreased solubility effect observed commonly in supercritical systems (Palmer & Ting, 1995). Maximum yield of 2-AP was observed at higher pressure (450 bar) and temperature (60 °C). Under these conditions, there was also a significant co-extraction of chlorophyll, and possibly other compounds.

The extract obtained at 125 bar and 40 °C was very pale yellow in colour. This extract, in physical appearance, was closest to the pure standard sample of 2-AP. Densitometric analysis of the extract showed the presence of only 2 bands – a yellow band at R_f 0.89 and 2-AP at R_f 0.38. It was opined that the yellow band was due to the oxidation product of carotenoid pigments (Teng et al., 1979) since carotenoids are soluble in SC-CO_2 (Polak, Balaban, Peplow, & Phlips, 1989; Yamaguchi et al., 1986). At 125 bar, 60 °C, there was a decrease in yield of 2-AP. The extract obtained under these conditions was distinctly yellow in colour and analysis revealed increased amount of the carotenoid oxidation product, corresponding to R_f 0.89. At 450 bar and 40 °C, the yield of 2-AP was significantly higher (~4 ppm) than that obtained at 125 bar (40 and 60 °C). The extract was bright yellow in colour and only 2 bands appeared on TLC. The band patterns were similar to those obtained at 125 bar and 60 °C, but the amount of the carotenoid derivative was higher, as was indicated by the colour of the extract and the corresponding peak area for the compound. At 450 bar and 60 °C, a yellowish-green coloured extract was obtained which was resolved into 3 bands – a green band with R_f 0.99–1.00, of chlorophyll; a yellow band of R_f 0.87, of the carotenoid oxidation compound; and a pale yellow band of R_f 0.38, of 2-AP. Though these extraction conditions gave maximum yields of 2-AP, there was also an appreciable co-extraction of pigments. At low pressure, there was no extraction of chlorophyll. This was in agreement with previous reports that chlorophyll is al-

Table 2
Densitometric assay of 2-acetyl-1-pyrroline from Pandanus leaves

Type of extract			µg of extract applied on the plate	Y1[a]	Y2[a]	X1[b] = (Y1/m)	X2[b] = (Y2/m)	Mean	SD ($n-1$, $n=2$)	% RSD = (SD/mean) × 100	µg of 2AP in the extract	µg of 2AP found/µg extract put on plate	mg of 2AP/kg leaves (ppm of 2AP in *Pandanus*)
Solvent extract			125.2	603.4	610.5	0.595	0.602	0.599	3.5E−3	0.584	300	2.312	0.965
Likens–Nickerson extract			127.3	608.7	616.2	0.600	0.608	0.604	4E−3	0.662	295	2.314	0.982
SFE extracts													
Pressure (bar)	Temperature (°C)	Time[c] (h)											
125	40	2	874.5	1679.1	1680.2	1.66	1.66	1.657	5E−4	0.030	0.276	0.095	0.276
125	40	3	1030	1867.7	1868.2	1.842	1.843	1.843	5E−4	0.027	0.307	0.089	0.370
125	60	2	999.7	603.0	610.0	0.595	0.602	0.599	3.5E−3	0.584	0.200	0.060	0.200
125	60	3	1194	663.7	666.4	0.655	0.657	0.656	1E−3	0.152	0.219	0.055	0.219
450	40	2	98.8	1145.2	1150.1	1.13	1.134	1.132	2.5E−3	0.221	3.333	10.120	3.333
450	40	3	108.9	1266.3	1272.3	1.25	1.26	1.252	3E−3	0.239	4.163	11.5	4.163
450	60	2	119.6	2016.5	2022.6	1.99	2.00	1.992	3E−3	0.151	6.630	16.622	6.630
450	60	3	123.60	2177.5	2179.3	2.15	2.15	2.149	5E−4	0.023	7.163	17.34	7.163

[a] Y1 and Y2 are areas under the curves for 2AP when the extract is spotted in replica in one TLC plate.
[b] X1 and X2 are amount of 2AP in µg in the extract, evaluated from a standard curve of pure 2AP with slope, $m = 1013.8$, $R_2 = 0.9611$.
[c] Total time of extraction (static + dynamic).

most insoluble in SC-CO_2 (Moyler, 1983). Further, chlorophyll is not extractable up to a pressure of 340 bar, as has been reported for algal lipid extraction (Polak et al., 1989). In the present work, at 450 bar and above, extraction of chlorophyll occurred.

At 450 bar and 80 °C, the yield of 2-AP was ~4 ppm, which was lower than that obtained at 450 bar and 60 °C. An additional band, corresponding to R_f 0.53, was observed. There was a significant extraction of chlorophyll. The extract was distinctly green in colour. Chlorophyll leaching may have impeded dynamic extraction beyond 1h under these conditions, which decreased the yield of 2-AP. However, at 125 bar and 80 °C, there was no chlorophyll extraction, nor was there a band corresponding to the compound with R_f 0.53. Possibly this compound was deeply embedded in the leaf tissues and penetrability of SC-CO_2 under low-pressure conditions may not have been sufficient for its extraction.

Thus, SFE proved to be a better and efficient technique for the extraction of 2-AP from *Pandanus* leaves. Though a single band of 2-AP did not appear, even under these optimized SFE conditions, the difference in polarity of the impurity band ($R_f = 0.89$) from that of 2-AP ($R_f = 0.38$) was significant and hence chromatographic separations to obtain pure 2-AP could be effected.

Previously reported solvent and Likens–Nickerson extractions have isolated 1–14 ppm of 2-AP from freeze-dried Pandan leaves but there is no report of its supercritical extraction. Thus, there is no firm database on the exact amount of 2-AP present in the pandan leaves for comparison of efficacies of the extractions in the present work.

4. Conclusion

Supercritical carbon dioxide extraction could extract 2-AP from *P. amaryllifolius* Roxb., under conditions of 450 bar, 60 °C and a 3 h extraction gave a yield of 7.16 ppm 2-AP that was higher than those obtained by solvent extraction or Likens–Nickerson extraction. This extract could find novel applications in food flavouring.

References

Buttery, R. G., Juliano, B. O., & Ling, L. C. (1983). Identification of rice aroma compound 2-acetyl-1-pyrroline in pandan leaves. *Chemistry and Industry*, 478.

Buttery, R. G., Ling, L. C., & Mon, T. R. (1986). Quantitative analysis of 2-acetyl-1-pyrroline in rice. *Journal of Agricultural and Food Chemistry, 34*, 112–114.

Buttery, R. G., Turnbaugh, J. G., & Ling, L. C. (1988). Contribution of volatiles to rice aroma. *Journal of Agricultural and Food Chemistry, 36*, 1006–1009.

Calame, J. P., & Steiner, R. (1982). CO_2 extraction in the flavour and perfumery industries. *Chemistry and Industry*, 399–402.

Doneanu, C., & Anitescu, G. (1998). Supercritical carbon dioxide extraction of *Angelica archangelica* L. root oil. *Journal of Supercritical Fluids, 12*, 59–67.

Grimmett, C. (1981). The use of liquid carbon dioxide for extracting natural products. *Chemistry and Industry*, 359–362.

Hawthorne, B. S. (1990). Analytical-scale supercritical fluid extraction. *Analytical Chemistry, 62*, 633A–642A.

Laksanalamai, V., & Ilangantileke, S. (1993). Comparison of aroma compound (2-acetyl-1-pyrroline) in leaves from pandan (*Pandanus amaryllifolius*) and Thai fragrant rice (Khao Dawk Mali-105). *Cereal Chemistry, 70*, 381–384.

Manninen, P., & Kallio, H. (1997). Supercritical fluid chromatography-gas chromatography of volatiles in cloudberry (*Rubus chamaemorus*) oil extracted with supercritical carbon dioxide. *Journal of Chromatography A, 787*, 276–282.

Mira, B., Blasco, M., & Subirats, S. (1996). Supercritical CO_2 extraction of essential oils from orange peel. *Journal of Supercritical Fluids, 9*, 238–243.

Moyler, D. A. (1983). Extraction of flavours and fragrance. In M. B. King & T. R. Bott (Eds.), *Extraction of natural products using near-critical solvents*. Blackie Academic and Professional, an imprint of Chapman and Hall Glasgow.

Palmer, M. V., & Ting, S. S. (1995). Applications for supercritical fluid technology in food processing. *Food Chemistry, 52*, 345–352.

Paule, C. M., & Powers, J. J. (1989). Sensory and chemical examination of aromatic and non-aromatic rices. *Journal of Food Science, 54*, 343–346.

Polak, J. T., Balaban, M., Peplow, A., & Phlips, A. J. (1989). Supercritical carbon dioxide extraction of lipids from algae. In K. P. Johnston & J. M. L. Penninger (Eds.), *ACS Symposium Series: 406. Supercritical fluid science and technology* (pp. 449–467). Washington DC: American Chemical Society.

Ramarathnam, N., & Kulkarni, P. R. (1984). Volatiles of scented Ambemohar rice and leaves of *Pandanus odoratissimus*. *Naturwissenschaften, 71*, S215.

Sass-Kiss, A., Gao, Y., Simandi, B., Boross, F., & Vamos-Falusi, Z. (1998). Study on the pilot-scale extraction of onion oleoresin using supercritical CO_2. *Journal of the Science of Food and Agriculture, 76*, 320–326.

Sugiyama, K., & Saito, M. (1988). Simple microscale supercritical fluid extraction system and its application to gas-chromatography-mass spectrometry of lemon peel oil. *Journal of Chromatography, 422*, 121–131.

Temelli, F., Chen, C. S., & Braddock, R. J. (1988). Supercritical fluid extraction in citrus oil processing. *Food Technology, 42*, 145–150.

Teng, L. C., Shen, T. C., & Goh, S. H. (1979). The flavouring compound of the leaves of Pandanus amaryllifolius. *Economic Botany, 33*, 72–74.

Yamaguchi, K., Murakami, M., Nakano, H., Konosu, S., Kokura, T., Yamamoto, H., Kosaka, M., & Hata, K. (1986). Supercritical carbon dioxide extraction of oils from Antarctic krill. *Journal of Agricultural and Food Chemistry, 34*, 904–907.

Zaheer, S. H., Prasad, B., Chopra, R. N., Krishnan, M. S., Santapau, R. H., & Deshaprabhu, S. B. (1966). *The wealth of India: Raw materials* (Vol. 7, pp. 216–221). New Delhi: Publications and Information Directorate, CSIR.

Further reading

Yang, C., Xu, Y. R., & Yao, W. X. (2002). Extraction of pharmaceutical components from *Ginkgo biloba* leaves using supercritical carbon dioxide. *Journal of Agricultural and Food Chemistry, 50*, 846–849.