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Chemical composition of volatiles from coconut sap (*neera*) and effect of processing

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Abstract

Volatiles from (i) fresh, (ii) clarified and (iii) fermented coconut sap *neera* were isolated by a simultaneous distillation and solvent extraction method using a Likens-Nikerson apparatus and subjected to GC–MS analysis for identification of chemical constituents. Twenty-one compounds (5.33 ppm), constituting more than 98% of the volatiles from fresh *neera*, were characterised. Typical major flavour compounds found in volatiles of fresh *neera* were ethyl lactate, phenyl ethyl alcohol, ethyl lactate, 3-hydroxy-2-pentanone, farnesol, 2-methyl tetrahydrofuran, and tetradecanone. Clarified *neera* contained lower quantities of volatiles, in which 13 compounds (1.31 ppm), constituting more than 97%, were identified. However, the typical flavour components retained were ethyl lactate, phenyl ethyl alcohol, 1-hexanol, 2-methyl tetrahydrofuran, 3-hydroxy-2-pentanone and 2-hydroxy-3-pentanone. Fermented *neera* contained a greater quantity of volatiles, in which 12 compounds (37.4 ppm), representing more than 95% of the volatiles, were characterised. Ethyl lactate, phenyl ethyl alcohol and farnesol were among the seven compounds retained from fresh *neera*. The astringency and harsh note of the fermented *neera* could be due to the increased amounts of acids (19.0 mg/l), such as palmitoleic acid and dodecanoic acid, along with higher concentrations of ethyl alcohol and ethyl esters.

Keywords: *Cocos nucifera* L.; Arecaceae; *Neera*; Fresh; Fermentation; Clarification; Volatiles; GC–MS

1. Introduction

Coconut palm, botanically known as *Cocos nucifera* L., belongs to the family of Arecaceae (Palmae), which is an important member of the monocotyledons. Coconut is a unique tree, where every part is useful in one way or another.

Coconut sap (*neera*) is obtained by tapping the unopened spadix of the coconut palm. *Neera* is traditionally tapped from the coconut tree in an organized manner, and consumed largely by the rural population (Baliga & Ivy, 1961; Nathanael, 1966). It is reported to be highly nutritive and a good digestive agent (Devdas, Sundari, & Susheela, 1969; Lata & Kamala, 1966).

In the recent past, coconut trees grown in the southern part of India have been largely affected by mite (*Acerai guerreronis)* attack and the repercussion of the mite attack is worse than the attack of other pests and debilitating diseases, such as root wilt, gonoderma wilt, Thanjavur wilt and tatipaka disease. This has tremendously affected the yield and thereby the lives of coconut farmers and farm workers. To sustain the loss of revenue, the farmers have resorted to tapping the trees for collection of coconut sap (*Neera*) and selling it to realize some revenue for their livelihood.

Neera contains sugar and the fresh *neera* is sweet, oyster-white and translucent, with a nearly neutral pH (Gupta, Jain, & Shanker, 1980). However, it is highly susceptible to spontaneous fermentation, initially alcoholic, followed by acidic fermentation (Iwuoha & Eke, 1996; Odunfa, 1985). This process is reported to be rapid under sunlight and the fermented *neera* is known as toddy. Sources of the

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fermenting organisms are the gourds, tapping implements and air (Odunfa, 1985). The fermenting organisms are dominated by yeasts, particularly *Saccharomyces cerevisiae* (Okafor, 1974; Sanni, 1993). *Neera* is rich in sugar (10–15%) and, unless it is collected under hygienic conditions, rapidly ferments and gets converted to toddy as the sugar is transformed to alcohol (5–8%) during the fermentation (Iwuoha & Eke, 1996). Further, the toddy, through a process of acetic fermentation, yields 'coconut vinegar' containing 4–7% acetic acid (Gupta et al., 1980; Onyekwere & Koleoso, 1978). The composition and quality of *neera* is found to vary with the place, time and duration of tapping. This poses a preservation problem, leading to harsh aroma and taste, because of the highly fermentable nature of the sap. This has prompted developments in processing of coconut sap for improvement of its shelf-life. Processed or clarified *neera* is suitable for storage (Raghavan, Ramalakshmi, Ramesh, Borse, & Prakash, 2003). The chemical composition of volatiles from fresh, clarified and fermented *neera* was determined in the present study, to identify the compounds which could be responsible for harsh notes.

Samarajeewa, Adams, and Robinson (1981) reported the chemical composition of major volatiles of Sri Lankan arrack (a palm wine distillate). In the study, several commercial samples were analysed and it was found that the major volatile compounds were similar with differences in concentration. Ethyl lactate was found in all samples and has been reported to be the characteristic feature of lactic-alcoholic fermentation.

Neera, when tapped fresh, possesses a tolerable odour which turns harsh on fermentation and makes it unpalatable, despite being nutritious. With a view to determine the components responsible for the astringency and harsh odour of fermented *neera*, work was undertaken to isolate and characterise the chemical composition of volatiles directly from (i) fresh, (ii) clarified and (iii) fermented *neera*. This is the first report on chemical composition of volatiles directly from *neera*.

The results of the present study will help the consuming population to understand the differences between three different forms of *neera*.

2. Materials and methods

2.1. Materials

Sap (*neera*) is collected by tapping the unopened spadix of the palm of the *Cocos nucifera* L. (Tiptur tall cultivar) in a farm in Mandakalli, near Mysore (Karnataka state, India). The species and cultivar were identified and a voucher specimen was deposited at the Horticulture Department, Mysore District, Karnataka, India.

2.2. Hygienic collection and processing of coconut sap

A farm with healthy coconut trees (*Cocos nucifera* L.; Tiptur tall cultivar) was identified as a suitable source to collect fresh *neera* samples as needed. *Neera* was collected in earthen (mud) pots under hygienic conditions. During the collection of *neera*, nylon covering nets were used on pots to avoid the fall/entry of ants, insects and spiders. High molecular high density polyethylene (HMHDPE) carboys, of 10 l capacity, were thoroughly washed with boiled water and drained completely and capped immediately as a precautionary measure to avoid microbial contamination. The *neera* collected between 6 p.m. and 6 a.m. was used for processing and preservation. *Neera* sample, immediately after tapping from the tree, was filled in HMHDPE carboys after filtration, using a strainer. Each carboy was immediately placed in a wide-mouth drum filled with ice cubes to keep the *neera* below 5 °C. The containers were transported to the laboratory and stored at the same temperature, prior to the commencement of processing. Part of the sap was treated with clarifying agents such as hyflosupercel and activated granular carbon and thermally sterilised (Raghavan et al., 2003). It was ensured that the processing was completed within 2 h. Another part of the sap was allowed to undergo auto fermentation for about 24 h at 30 ± 2 °C and then used for flavour analysis as fermented neera.

2.3. Flavour isolation from coconut sap

Fresh *neera* (1 L) was placed in a 2 l round-bottom flask, along with an internal standard (ethyl caproate). In another 250 ml round-bottom flask, dichloromethane (100 ml) was placed. These two round-bottom flasks were attached to the two arms of Likens-Nickerson simultaneous distillation-cum-solvent extraction apparatus, equipped with a condenser and having a cryogenic liquid for circulation. The temperature of coolant was maintained at 15 °C and the extraction carried out for 3 h by heating both of the flasks. After the extraction, the solvent was removed using a Vigreux column on a water bath with the chilled water circulation. After removing the solvent, 5 ml of extract was collected and further concentrated to 0.05 ml by flushing with nitrogen. The experiment was repeated for clarified *neera* and fermented *neera*. Volatiles of fresh, clarified and fermented *neera* were isolated and preserved at 4 °C for further processing.

2.4. Gas chromatographic–mass spectrometric analysis

The volatile concentrates were also analyzed, using a Shimadzu 17A-GC (Kyoto, Japan) equipped with a QP-5000 (quadrupole) mass spectrometer, fitted with a fused silica column SPB-1 (30 m × 0.32 mm, film thickness 0.25 μm, Supelco, USA), coated with polydimethyl siloxane. Helium was the carrier gas at a flow rate of 1 ml/min. The injector port temperature was 220 °C, the detector temperature was 220 °C and the oven temperature was maintained at 35 °C for 2 min and then increased to 90 °C at the rate of 1 °C/min and further increased to 220 °C at the rate of 3 °C/min. One microlitre of the sample

was injected by splitless injection mode and the ionization voltage was 70 eV. Retention indices for all the compounds were determined according to the Kovats method, using *n*-alkanes as standards (Jennings & Shibamoto, 1980). Essential oil constituents were identified by comparing retention times of the GC peaks with those of reference compounds run under identical conditions and by comparison of retention indices with literature data (Adams, 2001; Davies, 1990; Jennings & Shibamoto, 1980), and fragmentation patterns in mass spectra were matched with those of the NIST62-LIB library and published mass spectra (Adams, 2001; Ten Noever de Bravw, Bovwman, Gramberg, & La Vos, 1988). Compounds were quantified using the internal standard method. Ethyl caproate was used as the internal standard.

3. Results and discussion

Fresh *neera* was collected under hygienic conditions during the night, to avoid exposure to sunlight, transported at low temperatures (<5 °C) to prevent fermentation and processed immediately to obtain clarified *neera*. Volatiles from the fresh, clarified and fermented *neera* were isolated by the SDE (simultaneous distillation and solvent extraction) method using Likens-Nikerson's apparatus. Volatiles were subjected to GC–MS analysis for the identification of chemical constituents. Twenty-one compounds (5.33 ppm) from the fresh *neera* were characterised, which constitutes more than 98% of the volatiles (Table 1). The prominent volatile components may be classified as ester (one), aromatic hydrocarbons (three), aliphatic ketones (five), alcohols (four) and the remaining one was a heterocyclic compound. In addition to this, fatty acids (seven) and aliphatic hydrocarbons (three) were also found. Quantitatively, acids are the major compounds (3.3 mg/l), which may not contribute towards aroma. Ethyl lactate was the next major compound and may be the flavour characteristic compound of *neera*. Four alcohols, namely, phenyl ethyl alcohol, 1-hexanol, two other sesquiterpene alcohols – nerolidol and farnesol-along with four ketones, namely, 3-hydroxy-2-pentanone, 2-hydroxy-3-pentanone, tetradecanone and hexadecanone, may contribute to the overall flavour of fresh *neera*. Palmitic acid and palmitoleic acid were the major fatty acids. Aliphatic hydrocarbons were simple straight chain hydrocarbons. The quantity of the ethyl alcohol was found to be 0.07% in fresh *neera* as by the procedure of AOAC (1999).

Thirteen compounds (1.31 ppm), which constitute more than 97% of the volatiles from clarified *neera*, were identified. The quantities of all these compounds were found to be reduced during the clarification process. Among the typical flavour components, the following were present: ethyl lactate, phenyl ethyl alcohol, 1-hexanol, 2-methyl tetrahydrofuran, 3-hydroxy-2-pentanone and 2-hydroxy-3-pentanone. The total quantity of the acids was considerably lower in the clarified *neera*. The amount of acids found in fresh *neera* was around 3.3 mg/l, whereas it was 0.5 mg/l in clarified *neera*. This may be the reason for its improved

Table 1
Chemical composition of the volatile oils from fresh, clarified and fermented *neera*

Sl. No.	RT (min)	Compound	Quantity (μg)/1000 ml			RI	Identification
			Fresh	Clarified	Fermented		
1	3.72	2-Methyl tetrahydrofuran	105	45.4	–	760	RI, MS
2	3.88	3-Hydroxy-2-pentanone	236	75.9	–	768	RI, MS
3	4.07	2-Hydroxy-3-pentanone	45.6	14.0	–	777	RI, MS
4	4.10	Isoamylalcohol	–	–	7467	780	RI, MS, CoI
5	4.55	Ethyl lactate	560	300	4636	797	RI, MS, CoI
6	6.90	1-Hexanol	27.3	24.8	–	872	RI, MS
7	11.65	Hexanoic acid	49.8	54.7	–	958	RI, MS
8	25.82	Phenyl ethyl alcohol	357	195	4189	1095	RI, MS, CoI
9	38.97	Ethyl caprylate	–	–	503	1200	RI, MS
10	39.97	Dodecane	74.3	30.5	–	1203	RI, MS, CoI
11	44.78	Nonanoic acid	84.8	–	–	1239	RI, MS
12	62.47	Ethyl caprate	–	–	797	1380	RI, MS
13	63.18	Tetradecane	167	46.9	–	1399	RI, MS, CoI
14	68.00	Tridecanone	24.5	–	–	1467	RI, MS
15	69.45	Pentadecane	48.4	21.8	–	1492	RI, MS, CoI
16	71.57	Nerolidol	44.9	–	–	1530	RI, MS
17	72.28	Dodecanoic acid	52.6	–	1084	1556	RI, MS
18	73.90	Ethyldodecanoate	–	–	709	1582	RI, MS
19	74.35	Hexadecane	37.2	16.4	–	1594	RI, MS
20	77.65	Tetradecanone	104.5	–	–	1664	RI, MS
21	78.42	Farnesol	125.5	–	224	1682	RI, MS
22	81.12	Tetradecanoic acid	94.0	–	597	1750	RI, MS
23	85.27	Hexadecanone	25.9	–	–	1870	RI, MS
24	87.62	Palmitoleic acid	1042	141	14,603	1947	RI, MS
25	88.68	Palmitic acid	2024	342	2421	1981	RI, MS

RI: retention indices; MS: mass spectra, CoI: co-injection.

shelf-life. The quantity of ethyl alcohol was also marginally reduced to 0.06%.

Twelve compounds (37.4 ppm) from fermented *neera* were identified, which comprised more than 95% of the volatiles. Of these, only eight were found in fresh *neera*. The important aroma-contributing compounds were ethyl lactate, phenyl ethyl alcohol and farnesol. The quantities of ethyl lactate and, phenyl ethyl alcohol improved several-fold, while the amount of farnesol was increased marginally. The remaining five compounds were fatty acids (namely, decanoic acid, dodecanoic acid, tetradecanoic acid, palmitoleic acid and palmitic acids). Total acids increased from 3.3 mg/l in fresh *neera* to 18.7 mg/l in fermented *neera*. Except for palmitic acid, all the other acids increased several-fold. The newly detected compounds were mainly ethyl esters of fatty acids, along with isoamyl alcohol, which might have been produced during the fermentation. Presence of ethyl esters was expected as the ethanol content increased (2.56%) during the fermentation. Astringency and the harsh note of the fermented *neera* could be due to the increased amounts of acids, along with the produced ethyl alcohol and ethyl esters. The fermented odour observed could be due to the presence of isoamyl alcohol (Jackson & Linskens, 2002).

Except for ethyl lactate, all the other compounds identified in this study were different from those reported for palm wine distillates, i.e., Sri Lankan arrack (Samarajeewa et al., 1981). The reason for this difference could be the method of collection of *neera* and isolation of volatiles. In our study, the fresh *neera* was collected hygienically, avoiding exposure to direct sunlight, extraneous matter and organisms. Volatiles were isolated using the SDE method, directly, from different forms of *neera* (which was again collected hygienically, avoiding sunlight and other organisms).

4. Conclusion

GC–MS analysis of the fresh, clarified and fermented *neera* revealed certain interesting facts. Compounds responsible for the fermented odour of coconut sap could be isoamyl alcohol, along with other compounds, such as ethyl lactate, phenyl ethyl alcohol, ethyl caprate, ethyl caprylate, dodecanoic acid and palmitoleic acid. In addition, the ethyl alcohol contents of fresh *neera*, clarified *neera* and fermented *neera* were 0.07%, 0.06% and 2.56% by volume, respectively.

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