Vidyodaya J. of Sci, (2002) Vol. 11, pp 79-85

Studies on the effect of the heat on the neurotoxic factor of palmyrah flour

A. A. P. Keerthi[1], R. van der Hoevan[1], J. K. Nikawala[2] and E. R. Jansz[1]

[1] *Department of Bio Chemistry, University of Sri Jayewardenapura, Gangodawila, Nugegoda.*

[2] *Palmyrah Development Board, Galle Road, Colombo.*

Received on 12.14.03
Accepted on 12.22.03

Abstract

Previous reports state that the flour of palmyrah *(Borassus flabellfer L),* called odiyal, on heating at 80°C for 45 minutes caused removal of hepatotoxic and neurotoxic factors effectively, and that odiyal flour originated from Kalpitiya was more toxic to Wistar rats than odiyal flour from Jaffna. These studies show that the heating can cause loss of volatiles but there is apparently no toxic factor in the volatiles. GC-MS analysis shows that the decanoic acid (capric acid), undecanoic acid (hendecanoic acid), 2-butenedioic acid and their esters as main volatile compounds. No amines were present.

Heating of Kalpitiya odiyal flour shows a decline in ninhydrin positives prominently those from the MPLC fractions from ethyl acetate to methanol. Kalpitiya odiyal flour unheat-treated had higher total amine concentration than that of Jaffna odiyal. Circumstancial evidence shows that the increased toxicity of Kalpitiya odiyal flour compared to Jaffna odiyal flour may be due to the kottakilangu (dried shoot of palmyrah) milling temperature (58-60°C) for Kalpitiya flour and >80°C for Jaffna flour.

Keywords: Palmyrah flour, Odiyal, neurotoxicity, effect of heat, volatiles, amine content.

1. Introduction

The flour of palmyrah (odiyal) is from the dried seed shoot of the plant. It is reported to have many toxicities. (Arsekuleratne *et al.,* 1971; Greig *et al.,* 1981; Revesz *et al.,* 1999 and Anderson P.H. & Poulsen E.,1985) Recent studies of our group focused on the hepatotoxic and neurotoxic characters and one main finding was that both toxicities are destroyed by dry heating the flour at 80°C for 45 min. Boiling, steaming and other wet heat and along

washing does not eliminate toxicity (Sumuduni, 2002). The neurotoxicity is judged by symptoms such as ruffled coat, muscle in-coordination, characteristic fits, coordinated spasms, falling over backwards, immobility of hind limbs and finally death.

The objective of this study was to determine if the toxic compound/s are volatile or destroyed by heat.

2. Materials and Methods

Palmyrah flour

Odiyal was obtained from the Palmyrah Development Board. The kottakilangu, dried seed shoot, had originated from Kalpitiya and milled in a rice mill in Colombo at 58 - 60^{0}C. Flour from Jaffna milled in a chilli mill at Jaffna was also used in these studies.

Collection of volatiles

The volatiles were collected after heating at 80^{0}C in an oven for 50 min in an apparatus shown in Figure 1 by blowing N_2 gas in rate of 2 - 10 ml/min for 15 min through the packed Kalpitiya palmyrah flour (45g) column and the volatiles trapped in a cold finger.

Animal experiments

In an animal experiment volatiles from a total 90g Kalpitiya odiyal flour was separated in a cold finger and orally administrated into rats (n = 3) via the oesophagus using a sondi needle. So that the each dose calculated to be 2.5g Odiyal flour per day for 6 days, which is the amount consumed for neurotoxic symptoms to develop.

In another animal study, rats were placed in the assembly show in figure 2. In an environment of N_2: 02 (80:20) with a system absorb to CO_2 evolved. At the beginning of each day volatiles from *45g* odiyal were flushed in to the system for 15 minutes twice over a period of *5* days.

GC-MS Analysis

The trapped volatiles were analysed using GC-MS for compound's, both methanol soluble and water soluble. The latter was re-dissolved in methanol after freeze drying. AGILENT, GC- 6890 PLUS& MSD 5973N together with database Nist 98.1 was employed. GC column LC DABS and temperature gradient of 5^{0}C/min for 10 min then 1^{0}C/min for 30 min. under auto-operation mode was used in compound analysis and identification.

The effect of source of odiyal flour and effect of heat

Odiyal was subjected to MPLC (Ariyasena *et al.*, 2002) samples from Jaffna and Kalpitiya (heated and non heated) The ethyl acetate, methanol and fractions were collected. These were subjected to TLC (Silica gel G_{60}) developed by n-butanol: ethanol: ammonia (NH_4OH Sp.Gr 0.88) ratio 7:3:4 and sprayed separately with ninhydrin in acetone, phosphomolybidic reagent and anisaldehyde reagent. (Jansz *et al.*, 1994) Quantification of ninhydrin positives were carried out spectrophotometrically by the measuring the concentration of isolates in 70% acetone at 570 nm using the ninhydrin positive scrapings of the TLC plates.

3. Results

Studies on volatiles

GC profile of volatiles in Kalpitiya odiyal is shown in figure 3. MS analysis shows the presence of decanoic acid (capric acid), M+/z 186, 155, 143, 87, 74, 55.undecanoic acid (hendecanoic acid), (methyl ester) M~/z 214, 183,143, 129, 87, 74, 67. 2- butenedioic acid (dibutyl ester) M~/z 173, 155,117, 99, 57 and their derivative esters. No evidence could be found in the literature that these are toxic to rats.

The total volatiles did not indicate that neurotoxic effect manifests either when the rats were dosed with the volatiles for 6 days by introducing into oesophagus using sondi needle or by keeping the rats in an environment containing volatiles of heated flour. A criticism may be that only n=3 rats were used. But it is felt that this is not serious as the neurotoxic effect when exhibited, is shown by all rats. i.e. it is an all or none effect.

Flabelliferin profiles

Jaffna and Kalpitiya odiyal (heat treated and non heat treated) showed no marked difference in flabelliferin profiles (anisaldehyde spray) phosphomolybidic acid spray reagents (reducing substances) was did not show significant differences between the Kalpitiya heated and non heat treated but both had spot at 0.38 that was not present in Jaffna odiyal sample.

The ninhydrin positive purple (primary amine profile) showed marked variation especially in the ethyl acetate and methanol fractions. (The content is shown in Table 1). TLC spots (ninhydrin positive) not found at Rf. 0.36 and 0.60 in the Kalpitiya heat-treated samples the spot at 0.36 was not found in Jaffna sample.

4. Discussion

Sumuduni (2002) made two crucial observations. Firstly heating at 80^{0}C for 45 min destroyed both neurotoxicity and hepatotoxicity. Secondly, the Kalpitiya odiyal flour samples were much more neurotoxic than the Jaffna odiyal samples.

Considering the effect of heat, there was a possibility of heat volatalisation of toxic compounds. This does not seem to be the case as: (I) The main volatiles are known non- toxins and seen by experimentally to be non-toxic. (II) There is no evidence of any amine being volatile. Amines appear to be the key for toxicity from the findings of Jansz *et al.,* 2002.

The another alternative is that 80^{0}C heat destroys a fraction needed for toxicity. Amines (ninhydrin positive) become a candidate as the more toxic Kalpitiya flour has more amines than the Jaffna flour and Kalpitiya flour lost amines on heat treatment. So that the circumstantial evidence is that amines may be involved in toxicity as previously postulated (Jansz *et al.,* 2002) This is also lead us to believe that the reduced toxicity of Jaffna flour is due to milling in Chilli mill (temperature> 80^{0}C) as opposed to Kalpitiya flour (milled at < 60^{0}C according to J.K. Nikawala, unpublished results). Thus the factor involved in relative toxicity is probably milling temperature and not the cultivars or environment.

5. Conclusions

Heating of odiyal flour causes loss of toxicity most likely due to destruction of toxic factors and not loss of volatiles.

6. Acknowledgements

The authors thank IPICS for grant Sri: 07, and the Palmyrah Development Board for palmyrah flour from Jaffna and Kalpitiya, and ITI for GC-MS analysis.

7. References

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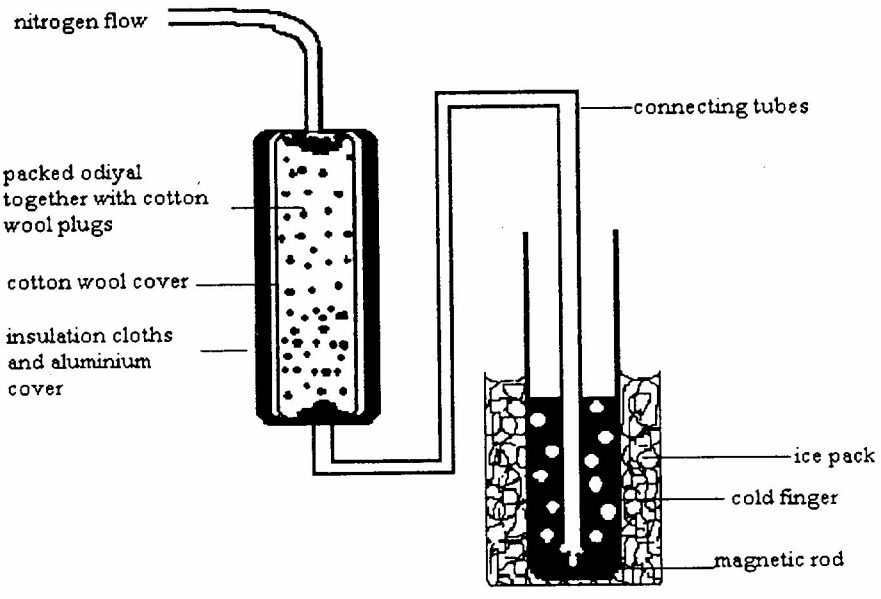

Figure 1. System for generating volatiles maintained at 80^{0}C

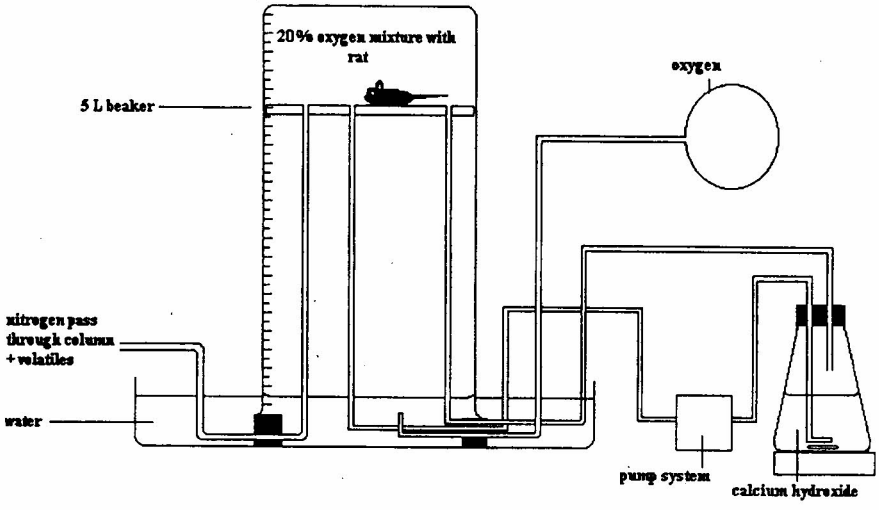

Figure 2. Chamber to expose rats to odiyal volatiles generated at 80^0C.

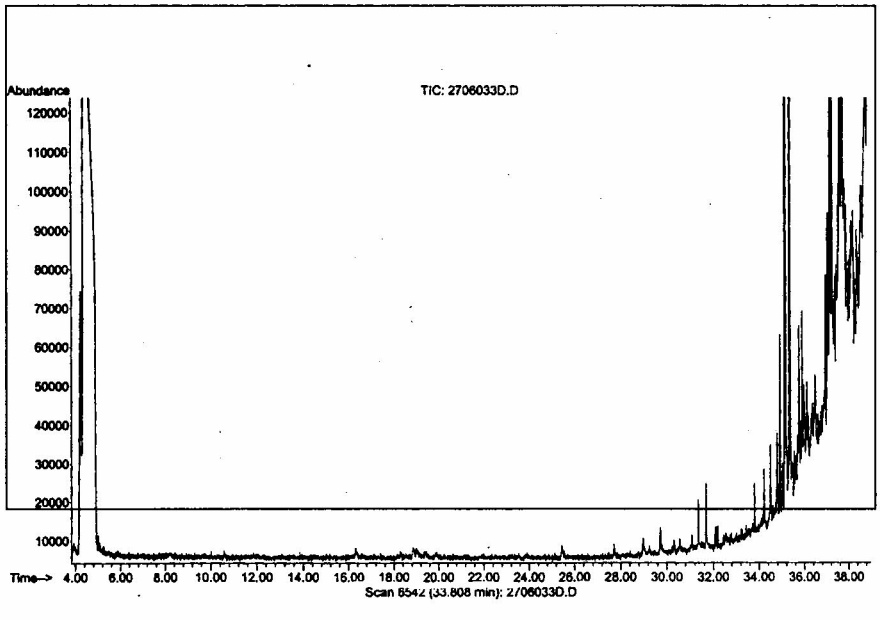

Figure 3. Gas Chromatogram of Kalpitiya odiyal volatiles

Table 1. Ninhydrin positive (Primary amines) contents of odiyal

Sample	Amine(m molL^{-1})	
	Ethyl acetate to Methanol*	water*
Jaffna	600	418
Kalpitiya	837	437
Kalpitiya heat treated	419	319

* MPLC fractions eluents

Method - TLC UV method