JSc-EUSL(2001) **Vol.2 No.1**, p 42-50

ISSN 1391-586X; ©2001 Published by Eastern University, Sri Lanka

FERMENTATION RATES AND EFFICIENCY OF FRUIT PULPS FROM PALMYRAH CONTAINING DIFFERENT FLABELLIFERIN PROFILES

Dilupi P Vandebona[a], Errol R Jansz[a],
S Chandrani Wijeyaratne[b] and Nalaka Ileperuma[a]

[a] Department of Biochemistry, [b]Department of Botany, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka.

(Received 16 June 2001; Accepted 12 February 2002)

Abstract

The potential use of Palmyrah (Borassus flabellifer L.) fruit pulp (PFP) which is largely a waste owing to its bitterness, was studied for alcoholic fermentation. The rate and efficiency of alcoholic fermentation of eight selected fruit pulps containing different flabelliferin profiles were studied. This showed that with one exception, the fruit pulps were suitable media for utilization in this way. Seven of the fruit pulps had fermentation efficiencies of more than eighty percent. Although rates of fermentation frequently slower than the control (sucrose in synthetic medium). The fruit pulp showing inhibitory properties contained more than ten flabelliferins (steroidal saponins) one of which, the previously identified anti-microbial flabelliferin (F_B), was the dominant peak on a TLC densitometric scan (>50mg dl^{-1} PFP).

keywords :Palmyrah Fruit Pulp, Alcoholic Fermentation, Flabelliferin Profile

1 Introduction

The Palmyrah (*Borassus flabellifer* L.) grows in the arid zones of Sri Lanka. Palmyrah is a tall palm mainly cultivated for its starchy tuberous shoot and influoresence sap. Palmyrah has many morphologically distinct fruit types [1].

Fruits are globose, 300-1000 g in weight and vary in colour and markings. More than 15 Kt of fruit pulp is available annually [2]. This fruit pulp usually contains 10-18% fermentable sugar [3]. While some fruit pulp is used to make traditional confectionery most of the fruit pulp goes waste on account of the its bitter taste [2]. Bitterness is due to a steroidal saponin (flabelliferin) a tetraglycoside of spirost-5en-3β0l (MW, 1041) containing 2 rhamnoses and two glucoses with a rhamnose terminus [4]. Although this tetraglycoside is mildly inhibitory to yeast and bacteria, the potent inhibitor is a saponin triglycoside (MW, 868) termed flabelliferin B (F_B)[5] which contains 2 glucoses and a rhamnose terminus. Other flabelliferins identified are F_C (MW, 868) and F_D (MW, 722) which have no inhibitory action toward yeast and bacteria [5]. F_B, F_C and F_D are not bitter.

There are several fruit types in Palmyrah. These differ in colour, size and markings. Attempts to correlate morphology with flabelliferin profile did not yield positive results[6]. Since it had become clear that alcoholic fermentation would be the best end-use, it was deemed important to try to correlate the flabelliferin profile with efficiency of alcoholic fermentation. Samples from different locations were selected because unpublished work had indicated that location of collection had a bearing on the flabelliferin profile.

Since the fermented pulp was bitter when the commonly occurring F - II was present and since the bitter principle was non-volatile, the objective was to prepare a distilled beverage. Therefore, bitterness is absent in distillates as the bitter principle is not volatile. The undistilled ferment however has the bitterness.

2 MATERIALS AND METHODS

2.1 Morphological types of fruits

There are 4 main morphological types of fruit. All types are globose with a prominent perianth and tough pericarp.

Type I	Size, medium to big, 600-900 g; Colour, black; Pericarp, rough with longitudinal striations (this is the major type).
Type II	Size, medium, 500-700 g; Colour, black; Pericarp smooth, no striations
Type III	Size, big, > 800 g; Colour, black with orange Pericarp, longitudinal stripes, no striations.
Type IV	Size small, <400 g; Colour, orange; Pericarp, smooth.

2.2 Location of Types

Type I	Polonnaruwa, Amparai, Vavuniya, Trincomalee, Mannar.
Type II	Kalpitiya
Type III	Anamaduwa
Type IV	Anuradhapura

2.3 Selection of fruits

Fruits were selected from each location on the basis of ripeness which is determined by a strong odour (mango-like) with the pericarp getting slightly depressed on pressing with a thumb. Fruits were transported to the laboratory in Colombo within 24 *hours* and the pulp extracted from the fruit. Fruits were collected in Mannar and Vavuniya in the North of Sri Lanka, Anuradhapura and Polonnaruwa in the North Central, Anamaduwa and Kalpitiya in the North west and Amparai and Trincomalee in the East.

2.4 Extraction of Pulp

The pericarp was immediately removed using a knife thus exposing the flesh. The flesh comprised the fruit pulp embedded in fibres surrounding 2-3 seeds. The pulp was extracted manually using water in the ratio (v/v) of 1:1. This was done by manually extracting the pulp with a measured minimum quantity of water, blending, filtering through muslin cloth and determining the pulp extracted from residue (by volume) and adding water to make up to 1:1. The extracted pulp (300 - 400 g) was stored at -20^0C in polythene bags.

2.5 Analysis

Palmyrah fruit pulp (PFP) before and after fermentation was analyzed for sugar by the 3′5′ dinitrosalicylic acid method [7] after incubation for 2 *hours* at 37^0C and pH 6.0 with invertase (50 *units*) purchased from Sigma, USA. Corrections were made for volume changes after fermentation and distillation. A standard curve (r^2=0.9993) was used for calculations. After fermentation, and distillation, alcohol content was determined by the AOAC specific gravity method[8].

2.6 Extraction of Crude Flabelliferins

Crude flabelliferins were extracted by the method developed by Nikawala *et. al*[4]. This was carried out on fresh samples. (autoclaving and fermentation does not affect the flabelliferin profile).
This involved extraction into methanol and cleaning with petroleum ether (bp 60 - 80^0C) to remove carotenoids. The methanol layer was rotor evaporated at 40-50^0C to yield a syrup from which the flabelliferins were extracted into acetone. Any sugar remaining was removed by dry cellulose chromatography [4]. These crude extracts were subjected to high performance thin layer chromatography (HPTLC) on pre-prepared silica gel G_{60} TLC plates (100μm) obtained from Merck, Germany. Butanol: ethanol: amonium hydroxide (sp.gr. 0.88) in the ratio of 7:3:4 was used as a solvent system. Anisaldehyde spray was used to visualize the flabelliferin spots[4]. Densitometry was carried out on a Sharp scanner, model JX 330 at 500 nm.

A Compaq Deskpro computer, with image Master ID Elite software version 2.0 was used for obtaining densitograms. (Pharmacia Biotech Inc. San Francisco, California, USA).
Pure F-II, F_B, F_C and F_D were used as standards with peak enrichment to identify the flabelliferins. As there were many other flabelliferins present which were not identified, densitometry was used only to obtain an idea of the relative ratio of flabelliferins after obtaining scans. Total flabelliferins was of the order of 275-325 $mg.dl^{-1}$ in each sample.

2.7 Fermentation

The ripe fruits of palmyrah are enclosed in a tough pericarp and are free from contamination until opening the fruit in the laboratory for extraction of fruit pulp. As it was desirable for the sake of comparison to conduct fermentation with a pure culture it was important that the PFP was sterilized. This was needed to destroy adventitious microbes introduced in the laboratory during the extraction of pulp. PFP is a viscous pectinaceous pulp with small particles. Therefore, sterilization by filtration was not possible. Autoclaving was performed for 15 *min* at 10 *psi* (115^0C). There was no visual sign of caramalization and this was confirmed by the high efficiency of fermentation observed with specimens of PFP.
The *Saccharomyces cervisiae* strain (S11 F3) had been isolated from fermenting coconut toddy (sap from cut infloresence) by the Botany Department, University of Sri Jayewardenepura. It was selected due to its characteristics of high rates and efficiencies of fermentation on fruit sap media at pH 6.0. The strain has been deposited in the Norwich University, UK, yeast culture collection.
PFP (100 g) original pH (4.5) contained in Erlenmeyer flasks was adjusted with 0.1 M NaOH to pH 6.0 (pH optimum of the yeast used) and sterilized by autoclaving (10 *psi*). The *Saccharomyces cervesiae* strain S_{11} F_3 in the log phase of growth (1.2×10^7 cells) was inoculated to the PFP under sterile conditions and fermentation was carried out for 144 *hours* at 30^0C and pH 6.0. The CO_2 evolved was measured daily from loss of weight of the system by the method developed by Flour and Hyashida[9]. As the PFP varieties contained different initial sugar contents, control experiments using 8%, 10%, 12%, 14% and 16% sucrose were conducted using the synthetic medium of Hyashida[10] under the same conditions as PFP. All fermentations were done in duplicate.
The comparisons to the synthetic medium were made by a graphical method, plotting a curve of CO_2 evolved (g) at 24 and 48 *hours* against concentration of initial sugar in the synthetic medium and comparing g CO_2 evolved by PFP types at the same sugar concentration. Data for 24 and 48 *hours* evolved in the synthetic medium are shown in Table 1.

Table 1: Synthetic Medium - CO_2 Evolved at 24 and 48 *hours* with % Sucrose

Sucrose($g.dl^{-1}$)	CO_2 Evolved at 24 *hour*(g)	CO_2 Evolved at 48 *hour*(g)
8	2.3	3.2
10	2.2	4.4
12	2.1	5.2
14	1.8	5.3
16	1.8	4.8

Data are a mean of duplicate fermentations.

2.8 Colour and taste of pulp.

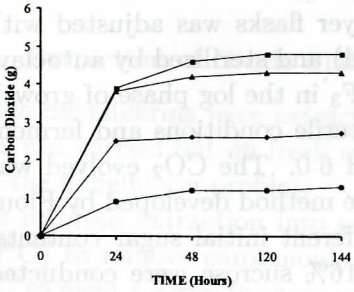
Colour was estimated by visual comparison. There were marked differences in colour that were clearly visible to the naked eye. This was confirmed by extracting the only pigment (carotenoids) into petroleum ether 60-80^0C and measuring U.V spectra at 300-500 *nm* [6].

Taste was evaluated by placing a small amount of pulp on the middle of the distal point of the tongue to evaluate bitterness using 4-5 researchers as tasters. Sweetness was detected by tasting with the tip of the tongue.

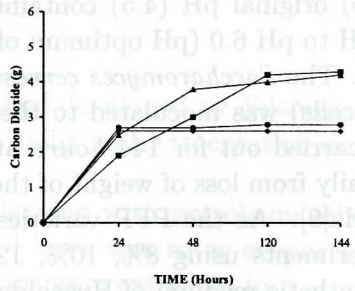
3 RESULTS

3.1 Rates of Fermentation

The fermentation rates of PFP from Anamaduwa, Anuradhapua and Mannar were



(a) ♦-Amparai ■-Mannar ▲-Anuradhapura •-Polannaruwa



(b) ♦-Trincomalee ■-Vavuniya ▲-Anamaduwa •-Kalpitya

Figure 1: Carbon dioxide evolved (g) with time (*hours*)using PFP; Each point is a mean of duplicate fermentation

faster than the synthetic medium (Table 1), particularly at the initial reading of 24 *hour*. (Figure-1(a) Figure-1(b)) Similar fermentation rates to the synthetic medium were shown by the Amparai, Kalpitiya and Trincomalee. The sample comparisons were made at the same sugar concentration. The slow fermentors were the Vavuniya and Polonnaruwa samples.

3.2 Fermentation Efficiency

Fermentation efficiency was calculated from alcohol produced and the sugar utilized relative to the theoretical alcohol yield.

Table 2: Fermentation Characteristics of selected Palmyrah Fruit pulps

Location	Colour	Initial Sugar ($g.dl^{-1}$)	Residual Sugar ($g.dl^{-1}$)	Alcohol W/V ($g.dl^{-1}$)	Efficiency %
Anamaduwa	Yellowish orange	11.7	0.1	5.6	95.2
Vavuniya	Dark orange	12.9	0.1	6.2	94.7
Anuradhapura	Yellowish orange	15.9	0.1	7.3	90.9
Amparai	Dark orange	10.2	ND	4.5	87.5
Trincomalee	Dark orange	10.4	0.1	4.5	85.6
Mannar	Orange	14.3	0.1	6.2	85.1
Kalpitiya	Dark orange	8.8	0.1	3.7	83.1
Polonnaruwa	Orange	8.3	0.4	2.2	53.6

ND- Not Detected; Visual colour was recorded in order to show that there is no correlation between colour pulp and fermentation efficiency.

Results (Table 2) showed that with the exception of the Polonnaruwa sample (53.5% efficiency), all other PFP samples gave 83-95% efficiency. The Polonnaruwa sample which was a slow fermentor showed the presence of 0.4% residual sugar even after 6 days showing that fermentation activity had virtually stopped (Figure-1a).The same yeast gave over 90% efficiency for the synthetic medium [5]. There was no relationship between bitterness (presence of F – II, Table 3) and rate or efficiency of fermentation since the bitter principle is only a mild fermentation inhibitor. This result is in accordance with previous studies [5].

3.3 Flabelliferin Profiles

The flabelliferins detected in each sample are given in Table 3. It was clear that variations among the samples were great. The inhibitory flabelliferin F_B was high

Table 3: Taste and R_f values of flabelliferins from selected palmyrah fruits

Location	Taste	R_f Value	Identity
Anamaduwa	Sweet	0.51	F-II
		0.57	F_C
		0.65	NI
Vavuniya	Bitter	0.51	F-II
		0.57	F_C
		0.64	NI
Anuradhapura	Bitter	0.51	F-II
		0.57	F_C
		0.59*	F_D
		0.61	NI
		0.64	NI
Amparai	Sweet	0.56	NI
		0.57	F_C
		0.59	F_D
Trincomalee	Mild Bitter	0.54	F_B
		0.56	F_C
		0.59*	F_D
		0.61	NI
		0.64	NI
		0.69	NI
Mannar	Mild Bitter	0.51	F-II
		0.57	F_C
		0.64	NI
Kalpitiya	Very Bitter	0.50	F-II
		0.54	F_B
		0.58	F_C
		0.65	NI
Polonnaruwa	Very Bitter	0.47	NI
		0.48	NI
		0.51*	F-II
		0.54*	F_B
		0.57*	F_C
		0.59*	F_D
		0.61	NI
		0.64	NI

* Very dominant on TLC (major peaks, on densitometric scan)
NI Not Identified
F-II Bitter flabelliferin- Sapogenin tetraglycoside
F_B Anti-microbial flabelliferin-Sapogenin triglycoside
F_C Non-bioactive flabelliferin -Sapogenin triglycoside
F_D Non-bioactive flabelliferin -Sapogenin diglycoside

(760 $mg.dl^{-1}$) in the poor fermenting PFP from Polonnaruwa. However, the confirmed presence of F_B alone did not explain the variation in fermentation rates although the 3 best fermentors did not contain F_B.
The known flabelliferins were identified by R_f and peak enrichment. Densitometry was used to get a better idea of relative peak areas to detect which flabelliferins were most abundant in each sample. Quantification could not be done as it would have required isolation, purification and plotting of standard curves for each new flabellilferin detected.

4 Discussion

The purpose of the study was to determine whether there is a relationship between the flabelliferin profile and rate and efficiency of fermentation. The studies showed that the palmyrah fruit type had no relation to the flabelliferin profile. Eight samples collected from different locations had different flabelliferin profiles.
Early studies [5] had shown that F_B inhibits fermentation and growth of yeast. These studies showed that the presence of F_B does not necessarily rule out the use of PFP for fermentation. Although rate of fermentation may be lower, efficiencies are still acceptable. F-II a mild fermentation inhibitor[5] did not appear to affect efficiency of fermentation but the presence of residual bitterness precludes the use of such samples for non- distilled products like wines.
Only one of the samples (from Polonnaruwa) showed poor fermentation characteristics. In this sample the flabelliferins were dominated by F_B. However this sample had more than 10 flabelliferins and one could not exclude the possibility of a yet unknown flabelliferin contributing to fermentation inhibition.

5 Conclusion

Most palmyrah samples can be fermented efficiently (> 80% efficiency). However, the end product must be a distilled spirit in order to remove bitterness resident in the majority of PFP. The bitter principle being non-volatile would be present only in non-distilled beverages like wines. Therefore, PFP from all parts of the country, with the exception of some Polonnaruwa pulps, could be considered to be a suitable base for fermentation.

Acknowledgement

The authors thank the International Program in Chemical Sciences (IPICS) Uppsala, Sweden for the grant SRI : 07 and the National Science Foundation, Sri Lanka for the grant RG/99/C/03.

References

[1] K. Theivendirarajah, (1992). Fruit based varietal selection of Palmyrah. *Proceedings of the seminar: New developments and Exploitation of Palmyrah. Palmyrah Development Board, Sri Lanka.* June 16th 1992, Colombo, pp 1-2.

[2] K. Theivendirarajah, (1992). Palmyrah fruit products and processing. *Palmyrah Development Board Bulletin,* Oct, pp 1-20.

[3] J.K. Nikawala and E.R. Jansz, (1994). The effect of naringinase on sugar utilization by yeast on Palmyrah fruit pulp. *Chemistry in Sri Lanka*, **11**, 4-5.

[4] J.K. Nikawala, A.M. Abeysekera and E.R. Jansz, (1998). Flabelliferins –Steroidal saponins from Palmyrah (*Borasus flabellifer* L) fruit pulp I. Isolation by flash chromatography, quantification, and saponin related activity. *Journal of National Science Council* (Sri Lanka) **26**, 9-14.

[5] J.K. Nikawala, S.C. Wijeyaratna, E.R. Jansz and A.M. Abeysekera, (1998). Flabelliferins –Steroidal saponins from Palmyrah (*Borassus flabellifer* L) fruit pulp II.. Preliminary investigations on yeast and selected bacteria. *Journal of National Science Council* (Sri Lanka), **31**, 141-150.

[6] D.D. Ariyasena, E.R. Jansz and A.M. Abeysekara (2001). Some studies directed at increasing the potential use of palmyrah (*Borassus flabelifer* L.) fruit pulp. *Journal of Science of Food and Agriculture* **81** : 1347 –1352.

[7] G.L. Miller, (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry* **31**, 426-428.

[8] AOAC (1980). *Association of Official Analytical Chemists. Official methods of analysis,* 15th edition, Beverages: Distilled Liquors, pp 147-148.

[9] P.E. Flor and S. Hyashida, (1983). Saccharomyces uvacea inulyticus var nov. A new high concentration ethanol tolerant yeast from rice wine. *Europion Journal of Applied Microbiology and Biotechnology*, **18**, 148-152.

[10] S. Hyashida, D.D. Feng and M. Hongo, (1974). Function of the high alcohol producing factor. *Agricultural Biological Chemistry* **38**, 2001-2006.