

## Quantification of Karanjin, Tannin and Trypsin Inhibitors in Raw and Detoxified Expeller and Solvent Extracted Karanj (*Pongamia glabra*) Cake

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**ABSTRACT :** Despite being a rich source of protein (28-34%), karanj (*Pongamia glabra*) cake is found to be bitter in taste and toxic in nature owing to the presence of flavonoid (karanjin), tannin and trypsin inhibitor, thereby restricting its safe inclusion in poultry rations. Feeding of karanj cake at higher levels (>10%) adversely affected the growth performance of poultry due to the presence of these toxic factors. Therefore, efforts were made to detoxify karanj cake by various physico-chemical methods such as dry heat, water washing, pressure cooking, alkali and acid treatments and microbiological treatment with *Saccharomyces cerevisiae* (strain S-49). The level of residual karanjin in raw and variously processed cake was quantified by high performance liquid chromatography and tannin and trypsin inhibitor was quantified by titrametric and colorimetric methods, respectively. The karanjin, tannin and trypsin inhibitor levels in such solvent and expeller pressed karanj cake were 0.132, 3.766 and 6.550 and 0.324, 3.172 and 8.513%, respectively. Pressure-cooking of solvent extracted karanj cake (SKC) substantially reduced the karanjin content at a cake:water ratio of 1:0.5 with 30-minute cooking. Among chemical methods, 1.5% (w/w) NaOH was very effective in reducing the karanjin content. Ca (OH)<sub>2</sub> treatment was also equally effective in karanjin reduction, but at a higher concentration of 3.0% (w/w). A similar trend was noticed with respect to treatment of expeller pressed karanj cake (EKC). Pressure cooking of EKC was effective in reducing the karanjin level of the cake. Among chemical methods alkali treatment [2% (w/w) NaOH] substantially reduced the karanjin levels of the cake. Other methods such as water washing, dry heat, HCl, glacial acetic acid, urea-ammoniation, combined acid and alkali, and microbiological treatments marginally reduced the karanjin concentration of SKC and EKC. Treatment of both SKC and EKC with 1.5% and 2.0% NaOH (w/w) was the most effective method in reducing the tannin content. Among the various methods of detoxification, dry heat, pressure cooking and microbiological treatment with *Saccharomyces cerevisiae* were substantially effective in reducing the trypsin inhibitor activity in both SKC and EKC. Based on reduction in karanjin, in addition to tannin and trypsin inhibitor activity, detoxification of SKC with either 1.5% NaOH or 3% Ca (OH)<sub>2</sub>, w/w and with 2% NaOH were more effective. Despite the effectiveness of pressure cooking in reducing the karanjin content, it could not be recommended for detoxification because of the practical difficulties in adopting the technology as well as for economic considerations. (**Key Words :** Karanjin, Tannin, Trypsin Inhibitor, Quantification, Karanj Cake)

### INTRODUCTION

Poultry production in India has gained momentum during the last three decades, which on today assumed the shape of an industry. It, in turn, has enhanced the need of high quality feed ingredients to meet ever-growing poultry population. At present, the poultry industry in India is being challenged by the escalating prices of feed ingredients and shortage of conventional feed resources for increased productivity. Constant efforts are, therefore, being made for the search of newer and alternate feed resources through their evaluation for optimum inclusion in the poultry ration. The poultry nutritionists thus, has compelled to explore the

possibilities of feeding non competitive unconventional agro-forest based industrial byproducts. One such feed is the karanj cake.

*Pongamia glabra*, popularly known as karanj, belongs to the family *Leguminosae*, and is a medium sized glabrous tree capable of growing under wide range of agro-climatic conditions (Pamar et al., 1976). In India, the availability of the seed has been estimated to be around 130,000 tonne per year (Ministry of Agriculture, 1992). The seeds contain on an average 28.30% oil. Depending upon type of cake and method of oil extraction, crude protein (CP) varies from 24.30 to 27.20 and 30.00 to 33.99% in expeller and solvent extracted cake, respectively. Karanj seed cake (KSC), the residue left after oil extraction, however, could not be utilized for poultry feeding even at lower levels due to the presence of toxic principle, karanjin, a furanoflavonoid

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Received April 11, 2005; Accepted September 28, 2005

(Limaye, 1925; Roy et al., 1977). The expeller and solvent extracted cake also contain trypsin inhibitor upto 8.2% and 8.7% of protein and tannins to the extent of 3.16 and 3.41% respectively (Natanam et al., 1989b).

No detailed studies are available on the detoxification of karanj cake for poultry feeding except physical treatment with hot water extraction (Mandal and Banerjee, 1974) and oven drying, autoclaving and cold water extraction (Natanam et al., 1989a). Efforts could therefore be made to convert karanj cake into a wholesome poultry feed after suitable processing and quantifying the residual toxins left back in the processed cake, which could be easily adopted by farmers and feed compounding industry. Keeping the above facts in view, the present investigations were undertaken to detoxify karanj cake by adopting various physicochemical methods like solvent extraction, water washing, pressure cooking, alkali and acid treatments and microbiological methods and to estimate residual karanj, tannin and trypsin inhibitors left back in the treated cakes.

## MATERIALS AND METHODS

Various processing methods such as physical, chemical and microbiological methods were tried at laboratory scale to detoxify the solvent extracted and expeller pressed karanj cake, which were obtained from the local market.

### Physical treatments

**Solvent extraction** : About 500 g of expeller cake was subjected for extraction of residual oil in the cake using petroleum ether (60-80°C) as solvent by Soxhlet apparatus for 10-12 h.

**Water washing** : The supernatant of water soaked and intermittently stirred cake (1:4, w/v) in a plastic trough was siphoned off after 24 h. With the same quantity of water, washing was repeated for another two times at 45 min. interval and was sun dried.

**Pressure cooking** : The cake was pressure cooked (115 lb pressure) with water in the ratio of 1:0.5 (w/v) and 1:1 (w/v) each for 30 and 60 min., respectively from the time of development of pressure and was sun dried.

**Oven drying** : About 500 g of karanj cake was subjected to oven drying at 100°C each for 12 and 24 h.

### Chemical treatments

**Sodium hydroxide treatment** : Exactly 100 g of ground karanj cake was soaked in 100 ml of water (1:1, w/v) giving an effective concentration of 0.5, 1.0, 1.5, 2.0 and 2.5% NaOH (w/w). The homogenously mixed samples left in air-tight beakers were sun dried after 24 h.

**Calcium hydroxide treatment** : 100 g of ground karanj cake was soaked in 100 ml of water (1:1, w/v) giving an

effective concentration of 1, 2, 3, 4 and 5% Ca(OH)<sub>2</sub> (w/w). The homogenously mixed samples kept in air-tight beakers were sun dried after 24 h.

**Urea ammoniation** : Urea ammoniated karanj cake was prepared by ensiling the cake in water (1:1, w/v) containing fertilizer grade urea at 1, 2, 3, 4 and 5% of cake (w/w) for 5 d and was sun dried.

**Acid treatment** : The cake was soaked in water (1:1, w/v) containing hydrochloric acid (HCl) at 0.5, 1.0 and 1.5% (w/v) for 24 h and sun dried. Similarly, the cake was soaked in water (1:1, w/v) containing glacial acetic acid at 0.5, 1 and 2% (w/v) for 24 h and sun dried.

**Combined acid and alkali treatment** : The cake was soaked in water (1:1, w/v) containing acid (HCl) and alkali (NaOH) at 1.0 and 0.5% and 1.0 and 1.0% (w/v; w/w), respectively for 24 h and sun dried.

**Microbiological treatment** : Exactly to 100 g of cake with required quantity of water to maintain 60% moisture in the cake was inoculated with *Saccaromyces cerevisiae* strain -49 (broth culture) at 1% of cake and incubated at 39°C for 24, 48, 72, 96 and 120 h and sun dried.

All the samples under each treatment were analyzed in triplicates. Such physically, chemically and microbiologically treated, sun dried, solvent extracted and expeller pressed karanj cakes were stored after grinding for further analysis.

**Estimation of karanj, tannin and trypsin inhibitor** : To assess the effectiveness of various methods of detoxification, the levels of karanj and tannin and trypsin inhibitor activities were estimated in raw as well as processed karanj cake in order to suggest the best method (s) of detoxification.

### Karanjin

**Extraction of karanj** : Exactly 8 g of ground and thoroughly mixed sample was weighed and transferred into the thimble and extracted for 12 h using 60 ml of freshly distilled methanol as solvent. Methanol extract was then cooled and filtered into a preweighed round bottom flask using Whatman No.1 filter paper. The excess methanol was distilled off under vacuum. The flask containing the extract was weighed again to obtain the weight of extract by subtracting the empty flask's weight. Sufficient amount of moisture free extract was transferred into vials for further analysis.

**HPLC analysis of karanj in raw and processed karanj cake** : The residual karanj content in raw and processed karanj cake was estimated under the following HPLC system information and conditions.

**HPLC system information** :

Model:	Shimadzu HPLC
Column oven:	CTO-10 As vp
On line degasser:	DGU-14A
Flow control valve:	FCV-10AL vp
Solvent delivery module:	LC-10 AT vp
Detector:	SPD-M 10A vp
LC work station:	Class Vp version 6.12
Cloumn:	C18 reverse phase column
Mobile phase flow rate:	1.0 ml/min
Detection:	250 nm UV detector
Sample size:	20 µl

The mobile phase consisted of methanol:water (80:20). Standard karanjin solution was prepared by dissolving 10 mg of karanjin in 10 ml of methanol (working standard- 1 mg/ml). Around 2 ml of standard karanjin solution was filtered through a 0.2 µm mille- HV filter. The 20 µl injector loop was then flushed and filled with filtered sample. The same was injected into LC with a total run time of 10 min. The same procedure was followed for all the extracted samples of raw and processed cakes. The weight of pure karanjin used and peak areas determined with standard karanjin were then utilized to calculate the karanjin content in the extracts under the following HPLC conditions.

The detector was UV detector set at 250 nm  
 Column was silica gel pre-packed analytical column (RP-C18)  
 The mobile phase consisted of methanol and water (80:20)  
 The oven temperature: 40°C  
 Flow rate: 1 ml/minute  
 Working standard: 1 mg/ml

#### Calculation of karanjin content

Area points were considered to calculate the karanjin levels and the following formulae were used to express the karanjin content as mg/ml and in percentage.

$$\text{Karanjin (mg/ml)} = \frac{\text{Area point of test}}{\text{Area point of standard}}$$

$$\text{Karanjin (\% in treated cake)} = \frac{A \times B \times 100}{W \times 100}$$

Where

A = Karanjn in mg/ml

B = Methanol extract

W = Weight of the sample taken

#### Tannin

*Extraction of tannin from raw and processed cake :*

Exactly 5 g of fat free, dried and powdered sample was refluxed in 300 ml distilled water for 1 h and the filtrate was collected by decantation. Again, the residue was refluxed with another 200 ml of distilled water for 15 minutes and the filtrate was collected and cooled. Subsequently the volume was made up to 500 ml and was filtered through Whatman filter paper No. 1.

*Estimation of tannin :* To assess the effectiveness of various methods of detoxification, the level of tannin was estimated in raw as well as processed karanj cake (AOAC, 1967) as quercitannic acid by titrating against potassium permanganate in the presence of indigo carmine (1 ml of 0.1 KMnO<sub>4</sub> = 0.006235 g tannic acid).

*Calculation of tannin content :* The following formula was used to express the tannin content in percentage.

$$\text{Tannin (\%)} = \frac{(V-V_0) \times 0.006235 \times 500 \times 100}{W}$$

Where,

V = volume of 0.1 N KMnO<sub>4</sub> used in case of sample

V<sub>0</sub> = volume of 0.1 N KMnO<sub>4</sub> for blank and

W = dried weight of sample refluxed

#### Trypsin inhibitor

*Extraction of trypsin from raw and processed cake :* Exactly 4 g of ground defatted cake was treated with 40 ml of 0.05 M sodium phosphate buffer and 40 ml of distilled water. The sample suspension was shaken for 3 h and then centrifuged at 700 g for 30 minutes, subsequently the supernatant was taken for analysis.

*Estimation of trypsin inhibitor :* Trypsin inhibitor in the feed was measured using casein as a substrate for assaying the activity of trypsin enzyme. The inhibition of the trypsin enzyme activity was measured in the extract obtained from the defatted material (Roy and Rao, 1971). From the supernatant 0.1 ml of extract was taken and to it 0.5 ml of trypsin solution (0.005%), 1 ml of 0.1 M sodium phosphate buffer, 0.4 ml of HCl (0.001 M) was added and incubated at 37°C for 20 minutes. Subsequently, 6.0 ml of 5% trichloro acetic acid solution was added to stop the reaction. Similarly, corresponding blank was prepared. Then the absorbance was recorded at 280 nm. One trypsin unit was defined as an increase of 0.01 absorbance unit at 280 nm in 20 minutes for 10 ml reaction mixture and the trypsin inhibitory activity as number of trypsin units inhibited and subsequently expressed as percent basis.

#### Statistical analysis

The data were subjected to one way analysis of variance as per the methods of Snedecor and Cochran (1989). The means in the different treatment were tested for statistical significance using Duncan's multiple range test (Duncan,

**Table 1.** Karanjin, tannin and trypsin inhibitor in solvent extracted and expeller pressed karanj cake

Cake	%		
	Karanjin	Tannin	Trypsin inhibitor
SKC	0.132 <sup>b</sup>	3.766 <sup>a</sup>	6.550 <sup>b</sup>
EKC	0.324 <sup>a</sup>	3.172 <sup>b</sup>	8.513 <sup>a</sup>
SEM	0.04	0.14	0.45

<sup>a, b</sup> Means with different superscripts in a column differs significantly ( $p < 0.05$ ).

SKC: solvent extracted karanj cake, EKC: expeller pressed karanj cake, SEM: standard error of mean.

1955) and considered at  $p \leq 0.05$  level.

## RESULTS AND DISCUSSION

The concentration of karanjin and tannin and trypsin

inhibitor activity in raw and variously processed solvent extracted (SKC) and expeller pressed karanj cake (EKC) at laboratory scale are presented in Table 1 to 3. The karanjin, tannin and trypsin inhibitor levels in as such solvent and expeller pressed karanj cake was found to be 0.132, 3.766 and 6.550 and 0.324, 3.172 and 8.513% respectively (Table 1). The concentration of karanjin and trypsin inhibitor was significantly lower and that of tannin was significantly higher in SKC as compared to that of EKC. Detoxification of both SKC and EKC by different methods substantially reduced the concentration of above anti-nutritional factors.

Contrary to the findings of the present study, Prabhu et al. (2002) reported lower karanjin content of 0.19 and 0.01% in raw expeller and solvent extracted karanj cake, respectively. Concomitant to the findings of the present study, Natanam et al. (1989b) reported similar tannin

**Table 2.** Karanjin, tannin and trypsin inhibitor content in raw and processed solvent extracted karanj cake

Methods of detoxification	Concentration-duration of treatment	%		
		Karanjin	Tannin	Trypsin inhibitor
Raw (without detoxification)		0.132 <sup>a</sup>	3.766 <sup>a</sup>	6.550 <sup>a</sup>
Physical methods				
Water washing		0.132 <sup>a</sup>	2.597 <sup>c</sup>	6.010 <sup>bc</sup>
Dry heat	12 h	0.129 <sup>a</sup>	3.193 <sup>b</sup>	3.183 <sup>k</sup>
	24 h	0.125 <sup>b</sup>	3.062 <sup>b</sup>	3.176 <sup>k</sup>
Pressure cooking				
	1:1-1:2 h	0.056 <sup>opq</sup>	1.552 <sup>ghi</sup>	3.363 <sup>k</sup>
	1:1-1 h	0.066 <sup>nppq</sup>	1.558 <sup>ghi</sup>	3.020 <sup>k</sup>
	1:0.5-1:2 h	0.052 <sup>q</sup>	1.362 <sup>ghu</sup>	3.287 <sup>k</sup>
	1:0.5-1 h	0.061 <sup>pq</sup>	1.442 <sup>ghii</sup>	3.076 <sup>k</sup>
Chemical methods				
Acid treatment				
Hydrochloric acid	0.5%	0.101 <sup>fg</sup>	2.598 <sup>c</sup>	6.010 <sup>bc</sup>
	1.0%	0.101 <sup>fg</sup>	1.818 <sup>ef</sup>	5.740 <sup>cde</sup>
	1.5%	0.104 <sup>fg</sup>	1.428 <sup>ghu</sup>	5.650 <sup>cde</sup>
Glacial acetic acid	0.5%	0.103 <sup>efg</sup>	2.338 <sup>cd</sup>	6.190 <sup>b</sup>
	1.0%	0.107 <sup>defg</sup>	2.078 <sup>de</sup>	5.920 <sup>bcd</sup>
	1.5%	0.102 <sup>efg</sup>	1.688 <sup>efg</sup>	5.740 <sup>cde</sup>
Alkali treatment				
Sodium hydroxide	0.5%	0.090 <sup>hi</sup>	1.818 <sup>efg</sup>	5.740 <sup>cde</sup>
	1.0%	0.072 <sup>lm</sup>	1.558 <sup>ghi</sup>	5.470 <sup>ef</sup>
	1.5%	0.062 <sup>nppq</sup>	0.785 <sup>kl</sup>	4.300 <sup>j</sup>
	2.0%	0.065 <sup>mno</sup>	0.755 <sup>l</sup>	4.300 <sup>j</sup>
	2.5%	0.065 <sup>mnu</sup>	0.755 <sup>l</sup>	4.270 <sup>j</sup>
Calcium hydroxide	1.0%	0.088 <sup>ij</sup>	1.818 <sup>ef</sup>	5.740 <sup>cde</sup>
	2.0%	0.079 <sup>jkl</sup>	1.558 <sup>gh</sup>	5.470 <sup>ef</sup>
	3.0%	0.082 <sup>ijk</sup>	1.298 <sup>ghii</sup>	5.380 <sup>ef</sup>
	4.0%	0.087 <sup>ijk</sup>	1.039 <sup>ijkl</sup>	4.660 <sup>ghii</sup>
	5.0%	0.078 <sup>kl</sup>	1.242 <sup>ghijk</sup>	4.480 <sup>ij</sup>
Urea (fertilizer grade)	1.0%	0.103 <sup>efg</sup>	2.467 <sup>cd</sup>	5.920 <sup>bcd</sup>
	2.0%	0.098 <sup>gh</sup>	1.818 <sup>ef</sup>	5.650 <sup>c</sup>
	3.0%	0.109 <sup>def</sup>	1.688 <sup>efg</sup>	4.840 <sup>gh</sup>
	4.0%	0.088 <sup>ij</sup>	1.298 <sup>ghu</sup>	4.570 <sup>hu</sup>
	5.0%	0.102 <sup>efg</sup>	0.909 <sup>ijkl</sup>	4.530 <sup>j</sup>
Combined acid and alkali				
0.5% NaOH and 1.0% HCl		0.089 <sup>hi</sup>	1.429 <sup>ghu</sup>	5.560 <sup>def</sup>
1.0% NaOH and 1.0% HCL		0.069 <sup>mno</sup>	1.459 <sup>ghu</sup>	5.266 <sup>f</sup>
Microbiological method				
	24 h	0.119 <sup>bc</sup>	1.501 <sup>ghi</sup>	3.133 <sup>k</sup>
	48 h	0.107 <sup>defg</sup>	1.501 <sup>ghu</sup>	3.176 <sup>k</sup>
	72 h	0.111 <sup>cde</sup>	1.154 <sup>huk</sup>	3.133 <sup>k</sup>
	96 h	0.105 <sup>defg</sup>	1.392 <sup>ghi</sup>	3.160 <sup>k</sup>
	120 h	0.113 <sup>cd</sup>	1.469 <sup>ghu</sup>	3.143 <sup>k</sup>
SEM		0.003	0.068	0.113

<sup>opq</sup> Means with different superscripts in a column differs significantly ( $p < 0.05$ ).

**Table 3.** Karanjin, tannin and trypsin inhibitor in raw and processed expeller pressed karanj cake

Methods of detoxification	Concentration/ duration of treatment	%		
		Karanjin	Tannin	Trypsin inhibitor
Raw (with out detoxification)		0.324 <sup>a</sup>	3.172 <sup>a</sup>	8.513 <sup>a</sup>
<b>A. Physical methods</b>				
Water washing		0.267 <sup>c</sup>	1.571 <sup>efg</sup>	7.836 <sup>bc</sup>
Dry heat	12 h	0.307 <sup>ab</sup>	2.572 <sup>b</sup>	3.540 <sup>lmn</sup>
	24 h	0.299 <sup>b</sup>	2.239 <sup>c</sup>	3.670 <sup>lm</sup>
Pressure cooking	1:1-1/2 h	0.168 <sup>lmn</sup>	1.016 <sup>kl</sup>	3.727 <sup>l</sup>
	1:1-1 h	0.170 <sup>lmn</sup>	0.986 <sup>kl</sup>	3.206 <sup>ll</sup>
	1:0.5-1/2 h	0.162 <sup>mn</sup>	0.968 <sup>kl</sup>	3.257 <sup>mn</sup>
	1:0.5-1 h	0.161 <sup>n</sup>	0.976 <sup>kl</sup>	3.103 <sup>n</sup>
<b>B. Chemical methods</b>				
<b>Acid treatment</b>				
Hydrochloric acid	0.5%	0.233 <sup>efgh</sup>	1.190 <sup>hu</sup>	7.836 <sup>bc</sup>
	1.0%	0.233 <sup>efgh</sup>	1.060 <sup>ijkl</sup>	7.500 <sup>cde</sup>
	1.5%	0.229 <sup>fgh</sup>	0.977 <sup>ijkl</sup>	7.390 <sup>cde</sup>
Glacial acetic acid	0.5%	0.256 <sup>cde</sup>	1.593 <sup>fgh</sup>	8.063 <sup>b</sup>
	1.0%	0.246 <sup>cdef</sup>	1.487 <sup>fg</sup>	7.723 <sup>bcd</sup>
	1.5%	0.223 <sup>fgh</sup>	1.152 <sup>ijk</sup>	7.500 <sup>cde</sup>
<b>Alkali treatment</b>				
Sodium hydroxide	0.5%	0.219 <sup>ghu</sup>	1.115 <sup>uk</sup>	7.500 <sup>cde</sup>
	1.0%	0.178 <sup>klmn</sup>	0.986 <sup>jk</sup>	7.166 <sup>e</sup>
	1.5%	0.170 <sup>lmn</sup>	0.816 <sup>lmn</sup>	5.710 <sup>k</sup>
	2.0%	0.163 <sup>mn</sup>	0.751 <sup>mn</sup>	5.710 <sup>k</sup>
	2.5%	0.171 <sup>lmn</sup>	0.745 <sup>n</sup>	5.603 <sup>k</sup>
Calcium hydroxide	1.0%	0.218 <sup>ghi</sup>	1.830 <sup>d</sup>	7.500 <sup>cde</sup>
	2.0%	0.182 <sup>klmn</sup>	1.731 <sup>de</sup>	7.167 <sup>e</sup>
	3.0%	0.176 <sup>klmn</sup>	1.664 <sup>def</sup>	7.053 <sup>ef</sup>
	4.0%	0.179 <sup>klmn</sup>	0.990 <sup>ijkl</sup>	6.156 <sup>ijk</sup>
	5.0%	0.180 <sup>klmn</sup>	1.010 <sup>ijkl</sup>	5.930 <sup>k</sup>
Urea (fertilizer grade)	1.0%	0.211 <sup>hu</sup>	1.661 <sup>def</sup>	7.723 <sup>bcd</sup>
	2.0%	0.199 <sup>jk</sup>	1.562 <sup>efg</sup>	7.390 <sup>cde</sup>
	3.0%	0.199 <sup>ijk</sup>	1.077 <sup>ijk</sup>	6.383 <sup>hij</sup>
	4.0%	0.188 <sup>jkl</sup>	0.992 <sup>jkl</sup>	6.043 <sup>jk</sup>
	5.0%	0.189 <sup>jkl</sup>	0.950 <sup>klmn</sup>	6.490 <sup>hi</sup>
<b>Combined acid and alkali</b>				
0.5% NaOH and 1.0% HCl		0.246 <sup>ijk</sup>	0.976 <sup>ijkl</sup>	7.280 <sup>de</sup>
1.0% NaOH and 1.0% HCL		0.242 <sup>defg</sup>	0.958 <sup>klm</sup>	6.660 <sup>fgh</sup>
<b>C. Microbiological method</b>				
	24 h	0.181 <sup>klmn</sup>	1.376 <sup>gh</sup>	3.306 <sup>lmn</sup>
	48 h	0.186 <sup>klm</sup>	1.506 <sup>fg</sup>	3.217 <sup>mn</sup>
	72 h	0.255 <sup>cde</sup>	1.270 <sup>hu</sup>	3.200 <sup>n</sup>
	96 h	0.261 <sup>cd</sup>	1.501 <sup>fg</sup>	3.150 <sup>ll</sup>
	120 h	0.260 <sup>cd</sup>	1.509 <sup>fg</sup>	3.187 <sup>ll</sup>
SEM		0.004	0.050	0.178

<sup>ab</sup> Means with different superscripts in a column differs significantly ( $p < 0.05$ ).

contents of 3.16 and 3.41% in raw expeller and solvent extracted karanj cake, respectively. However, the same workers reported higher activity of trypsin inhibitor (8.2%) in SKC as compared to that (6.55%) of the findings of the present study. This difference in the karanjin, tannin and trypsin inhibitor contents could be attributed to the variety, soil, stages of harvest and other factors (Davis and Hosney, 1979; Montgomery et al., 1986).

### Karanjin

The karanjin concentration of raw and residual karanjin content of variously processed SKC and EKC was quantified through HPLC. Under the present chromatographic conditions, the retention time of standard

karanjin was found to be about 4.224 minutes (Figure 1). The retention time of karanjin in SKC and EKC, however, was 4.459 and 4.480 minutes (Figures 2 and 3), respectively and other constituents extracted, if any, did not interfere as they were eluted either before or after the peak of interest. However, Prabhu et al. (2002) reported higher retention time of 10.09 min. for karanjin. This difference could be attributed to the chromatographic conditions applied for quantification of a particular substance during that time, under High Performance Liquid Chromatography.

Pressure-cooking of SKC substantially reduced the karanjin content at cake and water ratio of 1:0.5 with 30-minute cooking (Table 2). Among chemical methods 1.5% (w/w) NaOH was found to be much effective in reducing

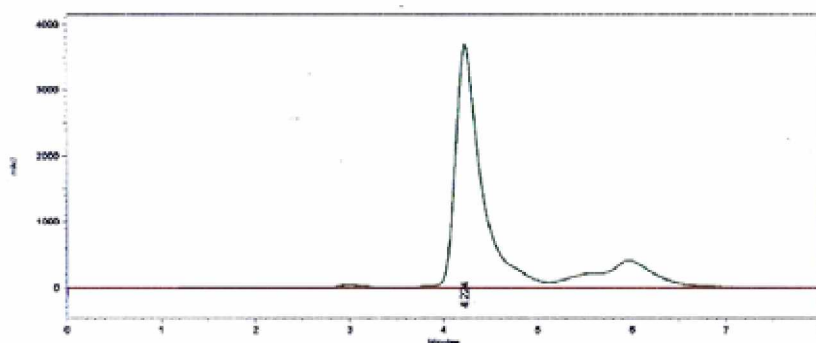


Figure 1. HPLC chromatogram of karanjin standard.

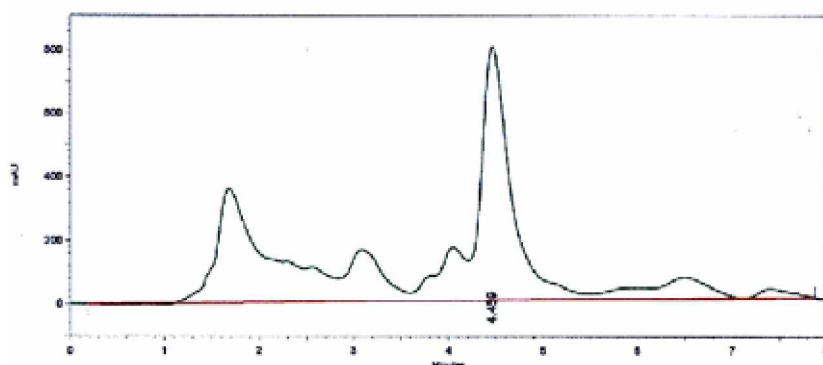


Figure 2. HPLC chromatogram of karanjin in solvent extracted karanjin cake.

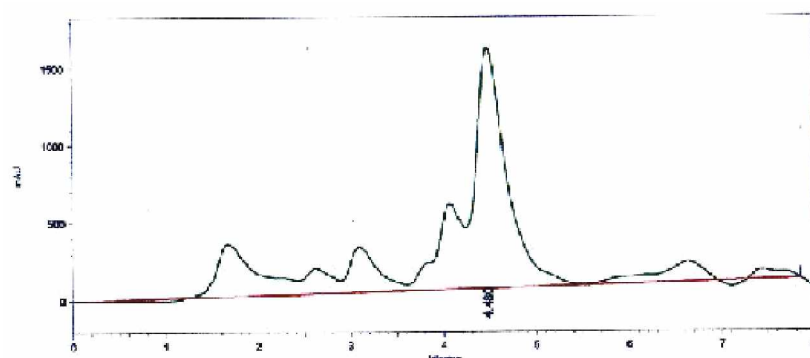


Figure 3. HPLC chromatogram of karanjin in expeller pressed karanjin cake.

the karanjin content (Figure 4). On the other hand, lime  $\text{Ca}(\text{OH})_2$  treatment was also found to be equally effective in karanjin reduction but at higher concentration of 3.0% (w/w) (Figure 5). No extra advantage was, however, found by enhancing the concentration of NaOH and  $\text{Ca}(\text{OH})_2$  beyond 1.5% and 3.0%, respectively.

Similar trend was noticed with respect to treatment of EKC (Table 3). Pressure cooking of EKC was found to be effective in reducing the karanjin level of the cake. Though there was not much difference in the reduction of karanjin levels due to variation in the ratio of cake and water or cooking time, 30 minutes cooking time with 1:0.5 cake and water ratio was found to be more effective in karanjin

reduction. Among chemical methods alkali treatment 2% (w/w) NaOH substantially reduced the karanjin levels of the cake (Figure 6).

Other methods like water washing, dry heat, HCl, glacial acetic acid, urea -ammoniation, combined acid and alkali, and microbiological treatments marginally reduced the karanjin concentration of SKC and EKC.

Similarly, Prabhu et al. (2002) reported a 50% reduction in the karanjin level by pressure cooking of EKC. They also reported that soaking of cake for 24 h with NaOH but at a lesser concentration of 1.0% was adequate to remove most of the karanjin as further increase in the dose of alkali did not improve the detoxification.

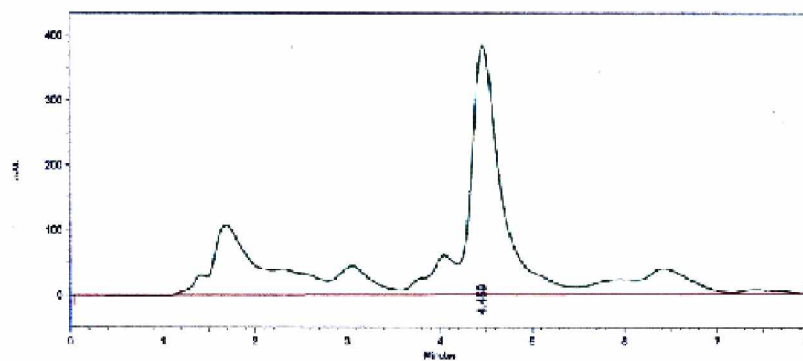


Figure 4. HPLC chromatogram of karanjin in sodium hydroxide (1.5%) treated solvent extracted karanj cake.

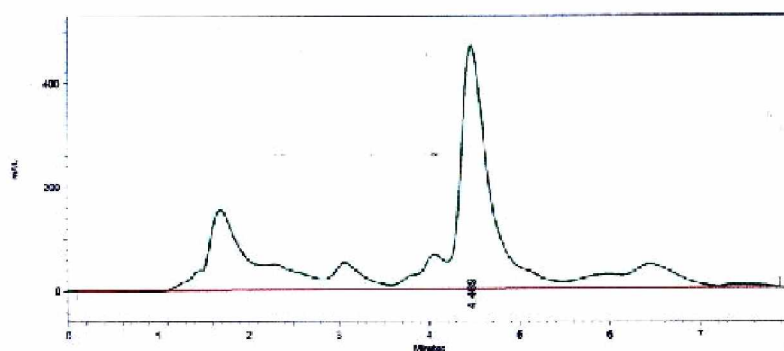


Figure 5. HPLC chromatogram of karanjin in calcium hydroxide (3.0%) treated solvent extracted karanj cake.

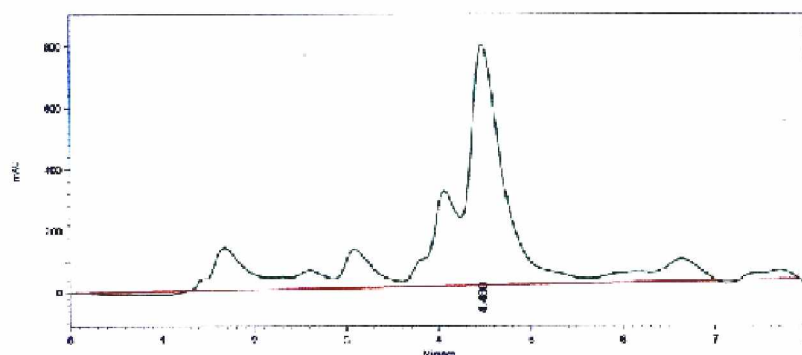


Figure 6. HPLC chromatogram of karanjin in sodium hydroxide (2.0%) treated expeller pressed karanj cake.

The same workers also reported that solvent extraction is the best method of detoxification and no further advantage could be noticed by detoxification after solvent extraction of karanj cake. However, the findings of the present study indicated that further detoxification of expeller karanj cake after solvent extraction reduced the concentration of karanjin substantially. Treatment of SKC with 1.5% (w/w) NaOH for 24 h was found to reduce karanjin content by 53%.

#### Tannins

To assess the effectiveness of various methods of

detoxification, the level of tannin was estimated in raw as well as processed karanj cake as quercitannic acid by titrating against potassium permanganate in the presence of indigo carmine. The tannin content was found to be higher in SKC (Table 1) as compared to EKC (3.766 vs. 3.172).

Treatment of sal seed meal with 0.1 N NaOH at cake and water ratio of 1:12 for 24 h reduced the tannin content by 64% (Singh and Arora, 1978). A similar reduction in the tannin content of sorghum grain by treating with 0.5 M NaOH (30°C) for 24 h was reported by Chavan et al. (1979). In the present study, the tannin content of SKC and EKC was also reduced by 79% and 76% when treated with 1.5%

and 2.0% NaOH (w/w), respectively. Wah et al. (1977) found 100% reduction in the tannin present in sal seed meal when treated with 5% Ca(OH)<sub>2</sub> for 24 h. However, in the present study NaOH treatment was found to be superior than Ca(OH)<sub>2</sub> treatment for detannification of karanj cake.

### Trypsin inhibitor

Trypsin inhibitor in the raw and processed SKC and EKC (Table 1) was measured using casein as a substrate for assaying the activity of trypsin inhibitor for assessing the best methods of detoxification. The trypsin inhibitor activity of EKC (8.513%) was found to be some what higher than that of SKC (6.550%). Among the various methods of detoxification, dry heat, pressure cooking and microbiological treatment with *Saccharomyces cerevisiae* were found to be substantially effective in reducing the trypsin inhibitor activity in both SKC and EKC. All these three methods were found to be equally effective in reducing the trypsin inhibitor activity of SKC as evidenced by similar residual inhibitor activity after detoxification by these methods. However, pressure-cooking of EKC for 1 h with cake, water ratio of 1:0.5 and 1:1 (W:V) was found to be more effective than cooking for 1/2 h with cake, water ratio of 1:1. No beneficial effect was noticed in the reduction of trypsin inhibitor activity of EKC by treating with *Saccharomyces cerevisiae* beyond 72 h. Other methods of detoxification like water washing, alkali and acid treatment were not found promising in reducing the trypsin inhibitor activity.

Reduction in the trypsin inhibitor activity of defatted seed meals of akashmoni (*Acacia auriculaeformis*) and karanj (*Pongamia glabra*) by autoclaving was observed by Mandal et al. (1985). Autoclaving of toasted guar meal (278 units/g) reduced the trypsin inhibitor activities to 94 units/g which was further lowered to 24 units/g through solid substrate fermentation using *Aspergillus niger* (Nagra et al., 1994). Concomitant to the above findings, pressure-cooking of EKC for 1 h with cake, water ratio of 1:0.5 and 1:1 (W:V) was found to be effective in reducing the trypsin inhibitor activity by 62% in the present study. A similar reduction in the trypsin inhibitor activity by 62% in the EKC treated with *Saccharomyces cerevisiae* through solid substrate fermentation for 72 h was recorded in our study.

Based on reduction in karanjin, in addition to tannin and trypsin inhibitor activity, detoxification of SKC with either 1.5% NaOH or 3% Ca (OH)<sub>2</sub>, (w/w) and with 2% NaOH were found to be more effective. Despite the effectiveness of pressure cooking in reducing the karanjin content, it could not be recommended for detoxification because of the practical difficulties in adopting the technology as well as for economical considerations.

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