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Alkali Pre-soaking Effects on Acridity, Colour Parameters and Oxalate Content of Elephant Foot Yam

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Abstract

Acridity, an itchy sensation felt in mouth and throat, is a major problem felt upon consumption of elephant foot yam. It is believed to be due to the presence of needle-like crystals (raphides) of calcium oxalate. As a means to overcome this problem presoaking of elephant foot yam (*Amorphophallus paeoniifolius*) in an alkaline solution was studied. The effect of time (1-3 h) and temperature (40-50°C) of alkali pre-soaking (0.1% sodium hydroxide solution) on sensory acridity, Hunter colour parameters, oxalate and total solids contents of yam was assessed using response surface methodology. Sensory acridity score was not significantly influenced by the soaking treatment, but the pH, oxalate content and colour co-ordinates and solid losses in soak water were significantly influenced. The linear regression models ($p \leq 0.01$) were valid for pH, soak water total solids and soluble oxalate content of elephant foot yam, whereas the quadratic model was valid for the total oxalate content ($p \leq 0.01$). The soaking time and temperature had a generally darkening effect on yam as indicated by Hunter L^*, a^* and b^* values as well as the whiteness index derived therefrom, the relevant regression equations representing quadratic models for a^* value and whiteness index ($p \leq 0.01$) and linear models for the remaining parameters ($p \leq 0.01$).

Key words: Elephant foot yam, alkali soaking, acridity, colour, oxalate

Introduction

Elephant foot yam (*Amorphophallus paeoniifolius* (Dennst.) Nicolson) is one of the most nutritious, but underutilized tuber crop. It belongs to the family Araceae along with taro, giant taro, swamp taro and cocoyam, and the family is known for the supply of famine foods (Sankaran and Palaniswami, 2008a). Because of its higher yield potential, culinary properties, medicinal utility and therapeutic values, it is referred to as 'King of Tuber Crops' (Sengupta et al., 2008). It contains a wide range of phytochemicals viz. alkaloids, phenols, flavonoids, glycosides, saponins, steroids and tannins (Ramalingam et al., 2010). In Sanskrit, it is known as *Arsoghna* because it cures piles (Dey, 1896). It also has hepatoprotective, antioxidative and uterus stimulating effect (Singh et al., 2011).

The major problem associated with the consumption of elephant foot yam is its acridity and/or oxalate content. Acridity is experienced as a severe itching, stinging or burning sensation in the mouth and throat, followed by swelling or as a less severe itching of external skin on hands. Crystals of calcium oxalate perform various functions in plants, including herbivory deterrence (Hudgins et al., 2003), calcium regulation (Volk et al., 2002), heavy metal tolerance (Franceschi and Nakata, 2005), structural strength and insect repulsion (Lane, 1994; Horner and Wagner, 1995). In sufficient quantity these calcium oxalate crystals cause mechanical abrasion of the mucous membranes, which results in irritation and burning sensation in the mouth and throat (Lebot, 2009). Bradbury and Nixon (1998) coined the term "Nature's poisoned spear" for the action of raphide/ irritant complex on the soft skin tissue that is presumably responsible for acridity. Besides acridity, the consumption of oxalate foods can cause severe health problems as mammals are unable to degrade oxalate. Accumulation of oxalate (hyperoxaluria) in body can have undesirable effects including increased urinary oxalate excretion (Brinkley et al., 1990).

Various detoxification techniques are used for plant foods to render them edible for humans. The change in pH can alter the solubility of various compounds and can help in hydrolysis of undesirable substances (Johns and Kubo, 1988). In North-Eastern India, elephant foot yam is traditionally boiled in bamboo shoot-ash water for removing acridity (Sankaran et al., 2008). The mitigation of acridity and/or oxalate in the elephant foot yam by suitable processing intervention can be effective in enhancing the consumption of this tuber crop and utilizing its functional benefits. Alkali pre-soaking is one such treatment. Thus, the present study was designed to assess the effect of alkali soaking treatment on the properties of elephant foot yam.

Materials and Methods

Raw materials

Elephant foot yam (var. Gajendra) was purchased from Navsari Agricultural University, Navsari. The yam was washed thoroughly with water to remove external soil. The corm was then peeled and diced into 2 cm cubes. The cubes were immediately dipped in 0.1% potassium metabisulphite solution for 5 min to prevent browning. The cubes were blanched in boiling water (cubes:water::1:6) for 10 min and then cooled immediately with water. The blanched cubes were allowed to surface dry, packed in nylon pouches (Hitkari Industries Ltd., Parwanoo) and stored in freezer (-20 ± 2°C) till further study.

Design of experiment

A Response Surface Methodology (RSM) experiment was designed incorporating the soaking time and temperature ranging from 1-3 hours and 40-50°C, respectively as the process variables (factors). A total of 13 different combinations were worked out using Central Composite Rotatable Design (CCRD) to investigate the effect of aforementioned factors on the response variables viz., oxalate content (total and soluble), sensory acridity score, hunter colour parameters, whiteness index and total solids in soak water. The coded and actual values of the process variables in the RSM experiment are given in Table1.

Pre-soaking treatment

The frozen elephant foot yam cubes were thawed in a microwave oven (60% power level for 14-15 min). The thawed yam cubes were soaked in sodium hydroxide solution (0.1%) for varying periods at different temperatures as given by CCRD. At the end of the pre-soaking period, the cubes were allowed to drain and then stored in beakers at 5°C till further analysis. The soak water was collected separately for analysis. The pre-soaked elephant foot yam cubes were made into paste using food processor (Inalsa appliances, Gautam Budh Nagar, Uttar Pradesh) and subjected to analysis. The yam paste for oxalate analysis was stored at -20°C until use.

Physico-chemical analysis

Hunter L*, a*, b* values of elephant foot yam paste was measured employing spectrocolorimeter, Colorflex® Model No. 45/0 (Hunter lab, Reston, USA). Whiteness index (WI) was calculated according to Sheen (1990) as shown below:

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}$$

where L, a and b are Hunter L, a and b values. Total

Table 1. Coded and actual values of the process variables in the RSM experiment

Independent variable	Levels				
	Axial point	Factorial point	Centre co-ordinate	Factorial point	Axial point
	-1.413	-1	0	+1	+1.413
A: Temperature (°C)	37.929	40	45	50	52.071
B: Time (h)	0.586	1	2	3	3.414

solid content of soak water was determined by the gravimetric method as described by AOAC (2000). Oxalate was extracted as per the procedure described by Okombo and Liebman (2010) with minor modifications. The extracted samples were then analyzed for oxalate using the oxalate kit (Trinity Biotech Co., Wicklow, Ireland). This method is based on oxidation of oxalate by oxalate oxidase, followed by detection of hydrogen peroxide (H_2O_2) produced during the reaction by a peroxidase-catalyzed reaction. Yam paste was evaluated for sensory acridity by a panel of judges (5) selected from the Division using a 5 point semi-structured acridity evaluation card. The panel members were asked to judge the acridity after applying the yam paste on the soft part (inside) of the forearm for 3-4 min for feeling the itchy sensation if any, and make a "vertical mark" along giving the relevant sample numbers to indicate the intensity of acridity (0-no acridity, 4-extreme acridity). The pH of yam paste was determined using digital pH meter (pH Tutor, Eutech Instruments, Malaysia).

Statistical analysis

The RSM data obtained were analyzed using the Design Expert software version 8.0.5.2. The responses were related with the coded factors using either a response surface quadratic model:

$Y = b_0 + b_1A + b_2B + b_{11}A^2 + b_{22}B^2 + b_{12}AB + \varepsilon$ or, response surface linear model:

$$Y = b_0 + b_1A + b_2B + \varepsilon$$

The coefficients of the polynomial were represented by b_0 (constant term), b_1, b_2 (linear terms), b_{11}, b_{22} (quadratic terms), b_{12} (interactive terms) and ε (random error).

Results and Discussion

Effect of process variables on the soluble oxalate content of elephant foot yam

The soluble oxalate (water soluble) exists in plants as crystals of sodium, potassium and ammonium salts (Holloway et al., 1989). The amount of soluble oxalate is important in food because its bioavailability is more than insoluble oxalate (Chai and Liebman, 2004). The soluble oxalate content of elephant foot yam paste ranged between 7.20-12.75 mg $100g^{-1}$. The minimum and maximum levels were observed for pre-soaking conditions of 50°C/3h and 37.92°C/2h treatment combination, respectively. The partial coefficients of regression model (Table 2) indicated that at the linear level, both pre-soaking time and temperature had a negative ($p \leq 0.01$) effect on the soluble oxalate content (Fig.1 (i)). Thus, with the increasing level of time or temperature soluble oxalate content declined. The soluble oxalate content decreased linearly with increasing soaking time, but the decrease was greater at higher temperatures. Also the temperature had little effect at lower soaking time but had significant effect with longer soaking time. The decline in soluble oxalate content could apparently have happened via leaching in soaking water (Osisiogu et al., 1974). The temperature could have contributed to the leaching phenomenon by softening of the elephant foot yam cube, thereby hastening the process. Hang and Preston (2010) observed a reduction in oxalate content of water soaked taro leaves after three hours. Similarly, Iwuoha and Kalu (1995) noticed 43.3% reduction in the oxalate content of taro after steeping in water for 24 h. Further, the statistical analysis indicated that the model fitted the observed data well, the model F value being 10.42 ($p \leq 0.01$) (Table. 2). The soluble oxalate content could be predicted by using the following linear model:

Soluble oxalate = + 23.09446 -0.21405 * temperature -1.69852 * time

Effect of process variables on the total oxalate content of elephant foot yam

Oxalic acid chelates the metal ions and exists as crystals of water insoluble salt of calcium, iron or magnesium (Noonan and Savage, 1999). Together with water soluble salts and free acid, these salts constitute the total oxalate content of the food material. High oxalate concentrations in the leaves and corms of plants consumed daily are of concern because of the associated harmful health effects especially mineral bioavailability.

The total oxalate content of elephant foot yam paste ranged between 31.36-52.20 mg$100g^{-1}$. The minimum and maximum levels were observed for 50°C/3h and 45°C/0.59h treatment combinations, respectively. The quadratic model fitted the observed data well, the model F- value being 42.75 ($p \leq 0.01$) (Table 2). The partial coefficients of the regression model indicated that at the linear level both pre-soaking time and temperature had a negative ($p \leq 0.01$) effect on total oxalate content.

Thus, with the increasing level of time and temperature, the total oxalate content generally tended to decline (Fig. 1(ii)). Insoluble oxalates i.e calcium oxalate are hydrothermally labile (Iwuoha and Kalu, 1995). Although, the soaking temperature was relatively low, a longer soaking duration might have contributed to the degradation and leaching of the oxalates by rendering the tissue flaccid and causing the attenuation. A significant depletion has been observed in spinach upon blanching (spinach) (Kim et al., 1993; Park et al., 1994) and in different vegetables upon boiling (Wanasundera and Ravindran, 1992; Judprasong et al., 2006), although, these processes are much more severe in heat intensity than the process used in present study. The total oxalate content could be predicted by the following quadratic model:

$$\text{Total oxalate} = -167.78447 + 10.23609 * \text{temperature} + 5.04654 * \text{time} - 0.10745\ \text{temperature} * \text{time} - 0.11921 * \text{temperature}^2 - 1.47108 * \text{time}^2$$

Effect of process variables on sensory acridity score

The sensory acridity score of soaked elephant foot yam paste as perceived by the sensory panellists ranged between 0.00-0.81 (i.e. from "nil" to "slight"). The minimum and maximum levels were observed at 45°C/0.59h and 45°C/2h treatment combinations, respectively. The model F-value was found to be statistically non-significant (Table 2) and the results followed an irregular pattern. Even though the panellists were familiar with the acridity sensation, lack of specialized training associated with acridity itself might have contributed to the peculiar trend. Sensory adaptation i.e. decrease in responsiveness to conditions of more or less constant stimulation (Lawless and Heymann, 2010) might also have impacted the results. Kilcast (1996) recommended using a large sensory panel for flavour testing and also relaxing the significant level to 20% compared with the traditional 1% or 5%.

Effect of process variables on Hunter colour parameters and whiteness index (WI)

Colour is the perception in the brain that results from the detection of light after it has interacted with an object. A consumer often assesses the initial

Table 2. Regression coefficients and ANOVA of fitted quadratic model for Hunter colour parameters (L*, a*, b*), whiteness index, total oxalate, soluble oxalate, acridity score and linear model for soluble oxalate, pH and total solids in soak water of pre-soaked elephant foot yam

Parameters/	L* value	a* value	b* value	Whiteness index	Total oxalate	Soluble oxalate	Acridity score	Total solids (soak water)	pH
Partial Coefficients									
Intercept	38.86	20.70	22.10	31.77	45.97	10.07	0.32	1.12	9.63
Temperature (A)	-1.37^{NS}	0.75**	-0.057^{NS}	-1.44*	-3.54**	-1.07**	-0.031^{NS}	0.17**	-3.195E-003^{NS}
Time (B)	-5.32**	2.13**	-1.31**	-4.96**	-5.67**	-1.70**	-0.014^{NS}	0.26**	0.38**
A^2	0.36^{NS}	-0.12^{NS}	-0.21^{NS}	0.41^{NS}	-2.98**	-	-0.047^{NS}	-	-
B^2	0.76^{NS}	-0.70**	-5.167E-003^{NS}	0.82^{NS}	-1.47*	-	-0.11^{NS}	-	-
AB	-0.79^{NS}	-0.62*	-0.37^{NS}	-0.41^{NS}	-0.54^{NS}	-	0.085^{NS}	-	-
R^2	0.9107	0.9709	0.6934	0.9289	0.9683	0.8705	0.2625^{NS}	0.8339	0.7841
Model F value	14.28**	46.76**	3.17^{NS}	18.30**	42.75**	33.60**	0.50^{NS}	25.11**	18.16**
Adequate Precision	12.158	20.058	5.808	13.532	20.035	16.642	1.659	14.395	12.546
Lack of Fit	S	NS	NS	NS	NS	NS	NS	NS	NS

** Highly significant ($p \leq 0.01$); * Significant ($p \leq 0.05$); NS: Non-significant ($p > 0.05$); S: Significant quality of product by its colour and appearance. In Colorflex spectrocolorimeter, colour is read in terms of Hunter L*, a* and b* values based upon 'opponents colour theory' and all three values are required to completely describe an object's colour.

The pre-soaked elephant foot yam was analyzed for its colour co-ordinates viz. Hunter L*, a* and b* values and whiteness index (derived from aforementioned parameters) as influenced by pre-soaking temperature and time. The non-significant lack of fit and significant F value for all parameters except L* value (Table 2), suggested that the respective quadratic models could be used to predict the effect of the soaking time and temperature on the colour parameters of elephant foot yam. The L* value, indicating 'lightness' (opposite of 'darkness') of the product varied from 31.52 to 47.42. The lowest L* value was obtained with temperature-time combination of 50°C/3h and maximum with 45°C/ 0.59h. However, as the lack of fit was significant, the model cannot be used to predict the effect of process variable on L value. The a* value (positive), which indicates the redness varied from 16.12 to 22.51. The lowest a* value was obtained with temperature-time combination of 40°C/1h and maximum with 45°C/ 3.41h. At the linear level, both time and temperature positively affected ($p \leq 0.01$) the a* value which means that increasing either of the parameter linearly increased the redness possibly due to formation of reddish-brown

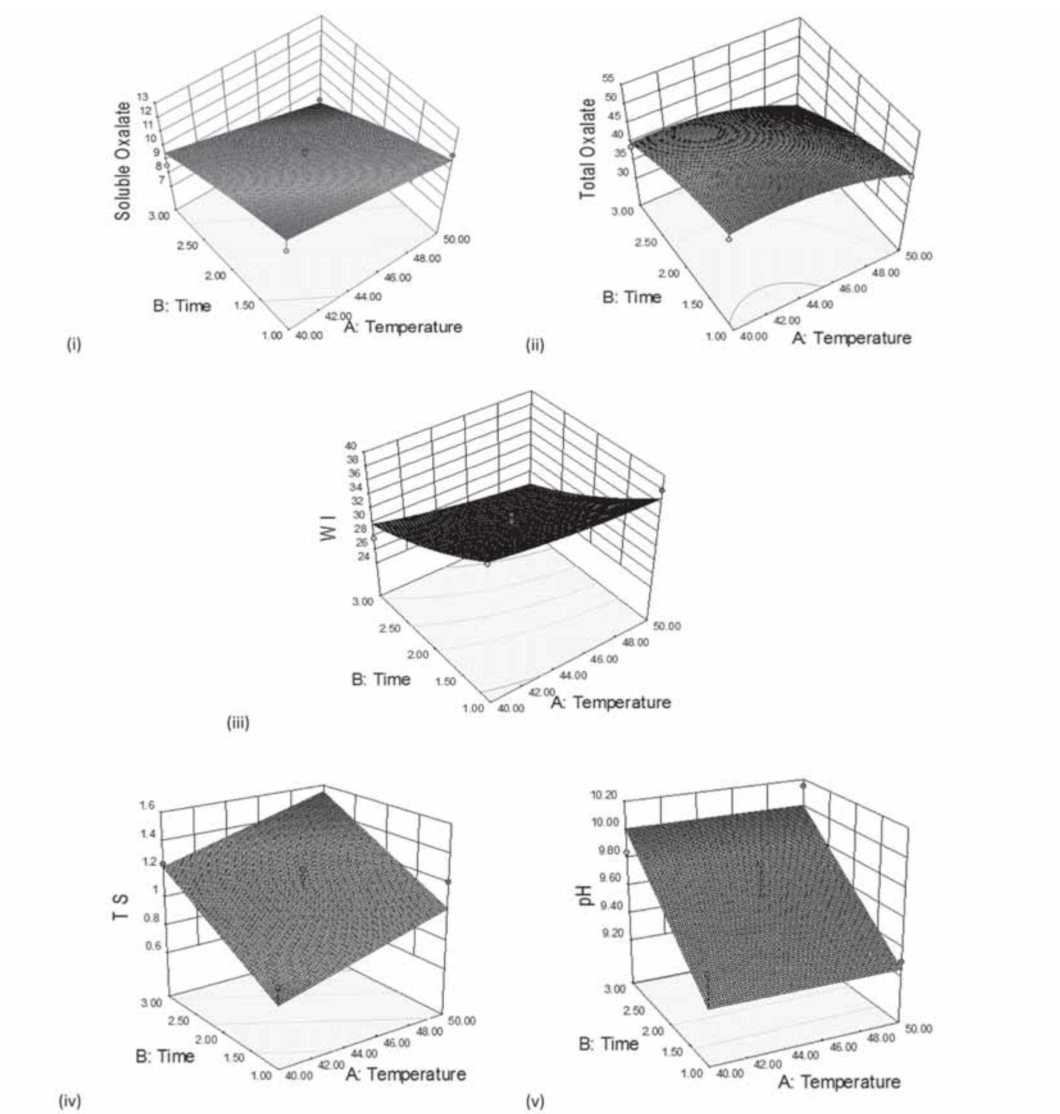

Fig.1. 3D response surface plots of (i) soluble oxalate content ($mg100g^{-1}$) (ii) total oxalate content ($mg100g^{-1}$) (iii) whiteness index (iv) total solids in soak water and (v) pH; as function of time (h) and temperature (°C)

components through browning after the pre-soaking treatment. The quadratic effect of time ($p \leq 0.01$) as well as interaction effect of time-temperature ($p \leq 0.05$) was found to be negative with respect to a* value. The b* value (positive), which indicates the yellowness varied from 19.88 to 24.48. The lowest b* value was obtained with temperature-time combination of 40°C/3h and maximum with 45°C/0.59h. The time had a negative effect ($p \leq 0.01$) on yellowness at linear level i.e increasing soaking time decreased the yellowness of the product.

Whiteness is defined as a measure of how closely a surface matches the properties of a perfect reflecting diffuser. It may indicate the extent of discoloration/ fading of colour during the processing condition. Whiteness worked out as whiteness index (WI) of pre-soaked elephant foot yam varied from 25.35 to 39.69. The lowest WI was obtained with temperature-time combination of 50°C/3h and highest with 45°C/0.59h. Both temperature ($p \leq 0.05$) and time ($p \leq 0.01$) had a negative influence on WI (Fig.1 (iii)) implying that with an increase in either of the process variables, whiteness decreased suggesting browning or discolouration of the product. Alkaline pH greatly enhances the non- enzymatic browning. The rate of Maillard reaction has been reported to increase with increased pH having maxima at pH 9-10 (Ashoor and Zent, 1984; Pokorny et al., 1988). Alkali catalyzes the initial steps of reaction between carbonyl and amine group by removing the proton from nucleophile and thereby increasing its nucleophilicity. Higher pH favours the formation of reductones instead of furfural, from Amadori product, leading to colour development (Bates et al., 1998). The whiteness index could be predicted by the following quadratic model:

$$WI = +83.67029 - 1.59396 * \text{temperature} - 4.59465 * \text{time} - 0.081322 * \text{temperature} * \text{time} + 0.016320 * \text{temperature}^2 + 0.82314 * \text{time}^2$$

Effect of process variables on total solids in soak water

Total solid loss is an important factor in soaking or cooking process if the product has to be consumed after draining the soak or cook water. This can lead to decreased nutritional and economical value of the food product (Guzel and Sayar, 2012) or it may increase the nutritional value by detoxifying the anti-nutritional factors present in the food system. The content of total solids of soak water varied from 0.53 to 1.56%. The maximum total solid loss was observed with 45°C/3.41h and minimum at 45°C/0.59h. At linear level both time ($p \leq 0.05$) and temperature ($p \leq 0.01$) positively affected the total solid loss in the soak water (Fig. 1(iv)). Any increase in pre-soaking time and temperature increased the total solid loss in soak water. Increase in any of the process variables obviously caused flaccidness and softening of the elephant foot yam cubes which presumably contributed to increased leaching of total solids. The total solid loss in soak water can be predicted by linear model equation:

$$TS = -0.89681 + 0.033238 * \text{temperature} + 0.26208 * \text{time}$$

Effect of process variables on pH of elephant foot yam dispersion

Alkali soaking of elephant foot yam is expected to increase its pH. The pH of 10% dispersion of yam increased steadily with increased soaking time or temperature. The pH of the elephant foot yam dispersion varied from 8.86 to 10.15. The minimum pH was noticed at treatment combination of 45°C/0.59h and maximum at 50°C/3h. The time directly affected ($p \leq 0.05$) the pH indicating that increasing the pre-soaking duration resulted in increased alkalinity of elephant foot yam (Fig.1(v)). The pH can be predicted by linear model equation:

$$pH = +8.90725 - 6.39087E{-}004 * \text{temperature} + 0.37576 * \text{time}$$

Conclusion

Alkali pre-soaking of elephant foot yam resulted in appreciable discolouration/darkening of the product although it had no significant effect on the sensory acridity score. Importantly, the pre-soaking treatment appeared to be an effective strategy for combating the high oxalate content of foods as significant reductions were observed in soluble as well as total oxalate content after the pre-soaking treatment.

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