EVALUATION OF PHYSIO-CHEMICAL CHARACTERISTICS AND ACRYLAMIDE CONTENT IN CRISP FRIED DOUGH WAFERS MADE FROM NIXTAMALIZED PEARL MILLET

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https://doi.org/10.55521/jmbfs.3186

ABSTRACT

Pearl millet (Pennisetum glaucum), a drought tolerant crop belonging to the family Poaceae is an important type of millet. It is a sturdy plant that can sustain adverse growing conditions. It is rich in vitamins, minerals, oil and phytochemicals. The phytates or phytic acid present acts as anti-nutritional factor which bind to the proteins and minerals thereby reducing the digestibility and bioavailability of the proteins, carbohydrates and minerals. In the following study, nixtamalization, a process widely used for the treatment of maize, is being used on the millet to lower the antinutritional properties of the millet. The grain was cooked at 95°C with varying lime concentration (0.5%, 1.0%, 1.5%, 2.0%) while keeping the cooking time (30 min) and steeping time (2 hours) constant. Product (crisp fried dough wafers) was made from the milled nixtamalized flour and different physio-chemical parameters and acrylamide content of the crisp fried dough wafer was analysed. The product made from flour treated with 1.5% lime showed the best results and overall acceptability. This research could be beneficial for increasing the utilization of pearl millet in different parts of the world especially in under developed countries as a health food. Also, the phytochemicals of pearl millet have various health benefits and this study can be used to increase their concentration and thereby promote the use of pearl millet for enrichment of various food products.

Keywords: Nixtamalization, Pearl millet, Tannin, Anti-nutritional factors, Acrylamide, FTIR

INTRODUCTION

Nixtamalization or alkaline cooking is a method where alkaline solutions are used for the treatment of whole grains (Gaytán-Martínez et al. 2017). In Central America and some parts of Mexico this method is widely used for the treatment of corn kernels (Boniface and Gladys, 2011; Rendón-Villabalos et al. 2009). Lime, wood-ash and lye are the common agents used for nixtamalization process. During the process there are physical, chemical and structural changes observed in the whole grains (Owusu-Kwarteng and Akabanda, 2013). The calcium content and the bioavailability of nacim increases with nixtamalization. The treatment helps in the reduction of aflatoxin and improves the protein quality in the final product (Owusu-Kwarteng and Akabanda, 2013; Rajeswari et al. 2015). The process helps in improving the flavour and aroma of the grain (Sefa-Dedeh et al. 2004). The treatment helps in increasing the protein digestibility of the grain, makes the grains softer thereby making it easier to ground into flour (Boniface and Gladys, 2011).

Pearl millet (Pennisetum glaucum) also known as Bajra is one of the most important type of millet. It is a drought tolerant crop belonging to the family Poaceae (Abdel-Hafez et al. 2017; Adebiyi et al. 2016). It is widely cultivated in Indian and South African subcontinents (Sandhu and Siroha, 2017). It can grow well in difficult conditions like low fertile soil, high temperature condition and soil with high salinity. Due to its high tolerance to adverse growing conditions pearl millet can grow in areas where other cereals like wheat, corn, sorghum fail to grow (Shaikh et al. 2017). Compared to other cereal crops it has a higher oil content (Jain and Bal, 1997). It is a rich source of protein, dietary fibre, starch, phytochemicals (tannins, phytic acid, ferulic acid and other phenolic compounds), vitamins like vitamin E, vitamin K and B complex vitamin (Thiamine, Riboflavin, Niacin, Vitamin B6, Folate and Pantothenic acid) (Leslienne et al. 2005; Jain and Bal, 1997). It is a well known source of calcium, iron and zinc and essential amino acids (Leslienne et al. 2007). Pearl millet consists of anti-nutritional factors in the form of condensed tannins, phytaetes etc which bind to the proteins and minerals thereby reducing the digestibility and bioavailability of the proteins, carbohydrates and minerals (Gaytán-Martínez et al. 2017; Leslienne et al. 2007). International Agency for Research on Cancer (IARC) has classified acrylamide as a probable carcinogenic. Acrylamide is chiefly found in fried product such as potato French fries (Abdul Hamid et al. 2018). The formation of acrylamide is associated with Maillard reaction (Salazar et al., 2014). The presence of carbonyl compounds or similar groups which can react with asparagine amino acid and form Schiff base are responsible for acrylamide formation (Salazar et al., 2014; Hidalgo et al., 2009). Various chemical agents such as asparaginase, acids, divalent actions, phospholipids and some amino acids that are used as food additives are found to be reducing the formation of acrylamide in thermally processed foods (Salazar et al., 2014; Kalita and Jayanty, 2013). The purpose of this study were to evaluate the effect of nixtamalization on fried food prepared from nixtamalized pearl millet flour and to analyse the changes associated with the food product in terms of its proximate composition, antioxidant scavenging activity, total polyphenol content, tannin content, colour analysis, TPA and detection of acrylamide in the crisp fried dough wafers.

MATERIAL AND METHODS

Materials

Raw pearl millet was procured from local supermarket. Calcium oxide of analytical grade was purchased from a local dealer. Gallic acid, ascorbic acid, vanillin, Folin Ciocalteu reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sisco Research Laboratory Pvt. Ltd. (SRL). Catechin was purchased from Sigma Aldrich. Reagents like methanol and HCl was purchased from Merck and Co. All the reagents and solvents used were of analytical grade.

Methods

Nixtamalization of the grain

Pearl millet was manually cleaned to remove damaged seeds or any debris. The grains were stored in a clean and dry environment until further use. These grains were cooked in lime solution of concentration 0.5%, 1.0%, 1.5% and 2.0% for a period of 30 min and steeped for 2 hours. The ratio of grain to water used for cooking of all the samples were 1:3 w/v. The cooking temperature were kept constant for all the samples i.e. 95°C. After steeping, the nixtamalized grains were washed thoroughly to remove excess of lime and extraneous pericarp material. Distilled water has been used for cooking and washing the grains throughout the experiment. The grains were dried in a tray drier at a temperature of 65 ± 2°C for 3 hours. Following this, the grains were pulverized using a disc attrition mill and sieved using 0.5mm mesh screen. All the flours were stored in an air tight container

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and were kept away from direct sunlight at 37°C temperature until samples were prepared.

Preparation of crisp fried dough wafers

For preparation of crisp fried dough wafers, nixtamalized pearl millet flour was rehydrated with enough water and mixed with a small amount of clarified butter and salt to make a dough of proper consistency respectively. The dough is shaped into thin discs of 1mm in thickness and having a diameter of 4cm. These discs were deep fried for 1 min on each side in rice bran oil at a temperature of 180°C. After frying the crisp fried dough wafers were cooled on an absorbent paper towel and were tested for proximate composition, antioxidant scavenging activity, total phenolic content, tannin estimation, colour, texture profile analysis and presence of acrylamide. They were stored in clear air tight container until further use.

Proximate analysis

All the samples were analysed for moisture content, ash, calcium content, protein content and total fat content. Total ash content was determined by method 08-03.01 (AOAC, 2000), calcium content was estimated using 983.35 method of (AOAC, 1997), protein content was determined using the method of (Kisan et al., 1973), and total fat was estimated by (AOCC, 2000). All analysis were performed in triplicate.

Extract preparation

Methanolic extracts of the fried samples were prepared using the method reported by (Gaytán-Martínez et al., 2017) with some slight modification. About 1g of fried sample was mixed with 10ml of methanol. The mixture was kept away from direct sunlight and stirred in a magnetic stirrer at an rpm of 450 for 30 min at 25°C. The extracted sample along with the residue was then vortexed and then centrifuged at 3000 RPM for 6 min. The supernatant was collected and stored at -20°C until further use. These extracts were further used for determination of antioxidant activity, total phenolic content and tannin content. All the results were an average of triplicate readings.

Antioxidant scavenging activity using DPPH+

The free radical scavenging activity was measured using a stable radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH) according to the method reported by with some slight modification. A total of 100µL of methanolic extract were mixed with 900 µL of freshly prepared 60 µM DPPH solution. The reaction mixtures were vortexed and placed in dark for 30 min at room temperature. The optical density was measured at 515nm using a UV-Vis spectrophotometer (UV-1700 Pharma Spec, Shimadzu). All the results were an average of triplicate readings. The inhibition percentage was calculated against a reagent blank and the results were expressed.

Estimation of phenolics by Folin-Ciocalteu assay

The total phenolic content of all the methanolic extracts were determined by Folin-Ciocalteu method as mentioned by (Sainshuddin et al., 2015) with some slight modification. The reaction was initiated by oxidizing 100 µL of sample extract with 200µL of freshly prepared Folin Ciocalteu reagent (10%) and 800 µL of sodium carbonate (700 mM). This mixture was kept in dark at room temperature for 30 min. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer (UV-1700 Pharma Spec, Shimadzu). The results were expressed as µg of gallic acid equivalents per gram of sample (µg GAE/g). All the results were an average of triplicate readings.

Estimation of tannins

Tannin content were determined using a method reported by (Gaytán-Martínez et al., 2017). Methanolic extract (200µL) were mixed with 800 µL vanillin, 4% HCl in methanol and kept for 20 min. The absorbance of the mixtures were measured at 492nm using a UV-Vis spectrophotometer (UV-1700 Pharma Spec, Shimadzu). The tannins were expressed as µg (+)- catechin equivalent per gram of sample (µgCAE/g). All the results were an average of triplicate readings.

Colour analysis of crisp fried dough wafers

The colour of all the crisp fried dough wafers were analyzed using a Hunter Lab Colorimeter (ColorFlex EZ, Hunter Lab, Reston USA). L*, a* and b* were the three colour coordinates that were examined, where L* represents the lightness or darkness, a* represents redness or greenness and b* represents yellow or blue. All the results were an average of the triplicate readings.

Texture Profile Analysis (TPA)

Texture profile of all the fried samples are analysed using CT3 texture analyzer (probe: needle probe [T9A, 20mm L]; pre-test speed: 1.00 mm/s, test speed: 0.50 mm/s, post-test speed: 0.50 mm/s; load cell: 10 kg). The resistance of the material to the applied forces is measured by a calibrated load cell and the results were expressed in either grams or Newton. (Rana et al., 2018; Ghosh et al., 2017; Yi et al., 2016). The software used for all the results was Texture Probe CT Software.

Fourier Transmission – Infrared (FTIR) Spectroscopy of crisp fried dough wafers

Attenuated total reflection (ATR) infrared spectra of the samples were obtained using a FTIR Spectrophotometer (FTIR-8400S, Shimadzu). Background spectra of the instrument were collected before mounting the samples (0.1g of each milled sample) on the instrument. All the spectra were recorded with characteristic peak in wave numbers from 500 to 4000 1/cm. All spectra measurement were carried out room temperature.

Statistical analysis

All data were expressed as means ± standard errors of triplicate measurements and analyzed by SPSS for Windows (ver. 16.0). One-way analyses of variance (ANOVA) were carried out to test significant differences (p≤0.05). Mean and standard deviation were computed using Microsoft Excel 2010.

RESULTS AND DISCUSSION

Proximate analysis

Proximate composition of crisp fried dough wafers made from nixtamalized pearl millet flour is shown in Table 1. The ash content for the samples was found to be increasing from 0.5% lime concentration to 2.0% lime concentration. The ash content for the treated sample ranged in 1.86% – 2.86% while the ash content for the untreated sample was 1.56%. It can be said that there was an increase in ash content of the sample. The moisture content of the sample is found to be in the range of 2.95-3.15%. During nixtamalization calcium oxide gets incorporated into the grain thereby increasing the ash content of the flour and ultimately the product made from the flour (Salazar et al., 2014; Villada et al., 2017). The calcium content for the samples were also found to be in an increasing order from 20.24 mg/g for 0.5% lime concentration to 28.09 mg/g for 2.0% lime concentration. The calcium content for untreated samples were a low value of 16.08 mg/g. The increase in the calcium content for the treated sample were a result of Ca²⁺ ions being absorbed into the grain during the lime cooking and steeping (Owusu-Kwarteng and Akabanda, 2013).

The influence of nixtamalization on the protein content was seen with a decrease in the protein content due to starch gelatinization (Obadina et al., 2016). The protein content of the untreated grain was estimated to be 9.73 g/100g and the protein in the treated samples ranged from 7.29 - 9.1 g/100g. The decrease in the protein content is a result of heat treatment of the grain during alkaline cooking which resulted in the changes in the protein structure thereby improving protein digestibility and making it easy for absorption (Gomez et al., 1989). The decrease in the protein content can also be due to high temperature at the time of frying. Further studies need to be done upon the starch structure during nixtamalization.

The fat content of the crisp fried dough wafers is responsible for the mouthfeel of the product and also has an effect on the sensorial characteristic of the product. An increase in the total fat content of the product were observed. The untreated sample shows a lower total fat content of 30.75 g/100g. The total fat content of the treated sample ranged from 31.59 to 36.81 g/100g. Thus there was an increase in the total fat content of the crisp fried dough wafer and this can be due to rapid loss of moisture during cooking. Xu and Kerr, 2012; Salazar et al., 2014 reported similar findings on maize.

Table 1 Proximate composition of crisp fried dough wafers made with nixtamalized pearl millet

<table>
<thead>
<tr>
<th>Concentration of Lime (%)</th>
<th>Moisture content (%</th>
<th>Ash Content (%)</th>
<th>Calcium Content (mg/g)</th>
<th>Protein Content (g/100g)</th>
<th>Total Fat Content (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>3.18 ± 0.04^a</td>
<td>1.56 ± 0.06</td>
<td>16.08 ± 0.05^a</td>
<td>9.73 ± 0.05</td>
<td>30.7 ± 0.05^a</td>
</tr>
<tr>
<td>0.50%</td>
<td>3.02 ± 0.03^b</td>
<td>1.86 ± 0.07</td>
<td>20.24 ± 0.09^b</td>
<td>7.32 ± 0.04^b</td>
<td>31.59 ± 0.06^b</td>
</tr>
<tr>
<td>1.00%</td>
<td>3.07 ± 0.05</td>
<td>2.1 ± 0.02</td>
<td>21.72 ± 0.07^b</td>
<td>9.1 ± 0.08</td>
<td>33.46 ± 0.06</td>
</tr>
<tr>
<td>1.50%</td>
<td>2.95 ± 0.02</td>
<td>2.64 ± 0.03</td>
<td>24.13 ± 0.06^c</td>
<td>8.11 ± 0.08^c</td>
<td>35.36 ± 0.07^c</td>
</tr>
<tr>
<td>2.00%</td>
<td>3.04 ± 0.09</td>
<td>2.86 ± 0.08</td>
<td>28.09 ± 0.04^d</td>
<td>7.29 ± 0.09^d</td>
<td>36.81 ± 0.07^d</td>
</tr>
</tbody>
</table>

^aDifferent small letters following the values in same column indicate differences for each concentration of lime (P<0.05).
Influence on Antioxidant Scavenging Activity of DPPH® Radical

The antioxidant activities of the methanolic extracts of the product was assessed against a basic free radical knowns as 2,2-diphenyl-1-pircrylhydrazyl (DPPH®). The antioxidant scavenging activity of DPPH was evaluated using ascorbic acid as the standard antioxidant. In accordance with Table 2, there is an increase in the antioxidant scavenging activity of the nixtamalized samples. The increase in the activity can be seen from 0.5% lime treated sample exhibiting the antioxidant activity of 59.79% whereas that of 2.0% lime treated sample is 61.99%. The highest antioxidant activity is seen from 1.5% lime treated sample which is 62.89%. The phenolic and tannin content in the grains are responsible for the antioxidant activity exhibited by the grain (Gaytán-Martínez et al., 2017). These phenolic compounds are usually found in a bound form in the grains where the form esterified bonds with the cell wall components of the whole cereal grains. Many physical and chemical processes such as alkali hydrolysis, extrusion cooking, etc has been effective in liberating these bonds and turning the phenolics into a free form which in turn makes them available for the antioxidant activity (Acosta-Estrada et al., 2014). The release of these antioxidant is greatly influenced by higher level of alkaline agent and temperature used during cooking (Oufnac et al., 2007).

Effect on Total Phenolic Content

According to Table 2, significant variation in the total phenolic content can be seen. Decrease in the total phenolic content of the nixtamalized crisp fried dough wafers was observed. The total phenolic content of the sample made from non-nixtamalized pear millet was found to be 42.51 μg GAEE/g whereas the total phenolic content for the treated sample ranged from 6.64 to 40.40 µg GAEE/g. At lime concentration of 0.5% the total phenolic content was 40.04µg GAEE/g. The decrease in the content was observed with an increase in the lime concentration. This effect is because of the lime concentration and the steeping time (Gaytán-Martínez et al., 2017). Pearl millet consists of phenolic compounds situated in the pericarp, which gets removed from the grain at the time of cooking and steeping. This leads to the reduction of total phenols in the treated grains (Adetunji et al., 2015). Rajeswari et al. (2015) observed reduction in free, bound and total phenolic content in foxtail millet after treatment with alkali solution. It was found in this study that these reductions increases with alkaline concentration. In the alkaline environment some molecular structural changes of the phenolic compounds are also observed leading to disruption of these compounds.

Effect on Tannin Content

Tannin content of the nixtamalized crisp fried dough wafers decreases as the lime concentration is increased (Table 2). The tannins in the nixtamalized sample range from 5.08 µg CAE/g to 3.004 µg CAE/g. The process was successful in lowering the tannin content of the final finished product. Tannins in the cereal are polyphenolic in nature and they exhibit antinutritional properties, but many tannins are anti-nutritional factors. They are known to form complex bonds with divalent cations and proteins thereby making them insoluble for digestion. Hence they reduce the availability of divalent cations and proteins in the body by making them escape the intestinal absorption and are excreted (Lestienne et al., 2005). This results in deficiencies. In nixtamalization, the use of high temperature at the time of cooking leads to the removal of pericarp and saturates the tannins content of the treated cereal (Gaytán-Martínez et al., 2017). Scheme, Oludamiola and Gladys (2010) have reported similar studies in which they have observed a significant reduction in tannin content with lime concentration.

### Table 2 Effect of nixtamalization on antioxidant scavenging activity of DPPH® radical, total phenolic content, tannin content of crisp fried dough wafers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Concentration of Lime (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>Antioxidant Scavenging Activity (%)</td>
<td>51.04 ± 0.05a</td>
</tr>
<tr>
<td>Total Phenolic Content (µg GAEE/g)</td>
<td>42.51 ± 0.08a</td>
</tr>
<tr>
<td>Tannin Content (µg CAE/g)</td>
<td>7.44 ± 0.06a</td>
</tr>
</tbody>
</table>

aDifferent small letters following the values in same row indicate differences for each attribute (P < 0.05).

### Table 3 Effect of nixtamalization on the L*, a*, b* values and texture of crisp fried dough wafers

<table>
<thead>
<tr>
<th>Properties</th>
<th>Concentration of Lime %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>L*</td>
<td>46.03 ± 2.43a</td>
</tr>
<tr>
<td>a*</td>
<td>10.58 ± 0.86a</td>
</tr>
<tr>
<td>b*</td>
<td>27.11 ± 2.58a</td>
</tr>
<tr>
<td>Hardness (N)</td>
<td>2.97 ± 0.05a</td>
</tr>
<tr>
<td>Adhesiveness (J)</td>
<td>0°</td>
</tr>
<tr>
<td>Fracturability (N)</td>
<td>3.53 ± 0.35a</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>0°</td>
</tr>
<tr>
<td>Springiness (mm)</td>
<td>1.35 ± 0.09a</td>
</tr>
<tr>
<td>Gumminess (N)</td>
<td>3.48 ± 0.03a</td>
</tr>
<tr>
<td>Chewiness (J)</td>
<td>0°</td>
</tr>
</tbody>
</table>

aDifferent small letters following the values in same row indicate differences for each attribute (P < 0.05).

### Detection of Acrylamide using FTIR

An unsaturated amide commonly known as acrylamide is found in various thermally processed foods. At a high amount, it is considered as a neurotoxin and a potent carcinogenic. Foods containing high content of reducing sugars such as glucose and proteins especially rich in asparagine, amino acid, when heated at high temperature more than 170°C produce acrylamide (Gertz and Klostermann, 2002; Yadav et al., 2018). It consists of -NO group as the functional side chain. The FTIR spectrum for asymmetric N=O is from 1500 – 1600 cm⁻¹ whereas for symmetric N=O the stretch is from 1300 – 1400 cm⁻¹. Also the FTIR stretch for N-H is found to be from 3350 – 3500 cm⁻¹ (Pranamik et al., 2015). It can be observed in Figure 1a, 1c and 1d that all the interferograms showed a peak for either -NO group as the functional side chain. The FTIR spectrum for asymmetric N=O is from 1500 – 1600 cm⁻¹ whereas for symmetric N=O the stretch is from 1300 – 1400 cm⁻¹. Also the FTIR stretch for N-H is found to be from 3350 – 3500 cm⁻¹ (Pranamik et al., 2015).
group or for the N-H stretch. Since none of the interferograms showed peaks for both the stretch it can be concluded that the peaks observed are not because of acrylamide but are due to some protein moieties. In Figure 1b and 1e no peaks were observed for either -NO group or for N-H stretch, this can be due to protein denaturation in the treated samples. Further studies should be done to know better about the protein denaturation. The absence of acrylamide in the treated samples can be due to the increased mineral content of the product, since calcium content of the flour reduces the formation of Schiff’s base responsible for the formation of acrylamide. Similar findings were reported by (Salazar et al., 2014) for maize tortilla. These authors observed a decrease of 52 and 36% in acrylamide content in tortilla chips, when a treatment of nixtamalization at lime concentrations of 1.5 and 2.0 g/100 g respectively was given to corn flour.

CONCLUSION

Based on the study it can be concluded that the nixtamalization treatment was efficient in increasing the ash, calcium content of the product. Protein gelatinization was seen with the reduction in the protein content. Since pearl millet is high in fat, the total fat content of the product showed an increase. There was decrease in the tannin and polyphenolic content of the sample which indicated the reduction in the antinutritional factor of the millet altogether. The increase in antioxidant scavenging activity was associated with the decrease of the antinutritional factors viz., phytic acid in the case of pearl millet. The result obtained for antioxidant scavenging activity, polyphenols and tannins were interdependent. The treatment was done to decrease the antinutritional factor while retaining the maximum antioxidant activity. Considering all the parameters under analysis the product sample made with lime concentration of 1.5% showed the best result. It gave the best overall result and was more acceptable in terms of colour and texture. Thus it can be affirmed that nixtamalization can be used to increase the mineral content of the grains and which in turn helps in lowering the acrylamide of the final fried product. With nixtamalization these problems can be solved, and the bioavailability can be increased. Thus, the amount of condensed tannins in pearl millet can be significantly reduced with increase in the antioxidant activity of the grains. More studies should be conducted on the processing of cereal grains for reduction in their antinutritional property and retention of nutrients. In under developed countries pearl millet can be very effective in eradicating diseases for reduction in their antinutritional property and retention of the grains.

REFERENCES


Figure 1 Effect of various a) 0% b) 0.5% c) 1% d) 1.5% and e) 2% lime concentrations on the acrylamide formation in crisp fried dough wafers

![Figure 1](image-url)


