

Phytonutrient and antioxidant Activity of Nixtamalized Brown top Millet Flour fortified with *Nelumbo nucifera* flower powder

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Abstract

Nelumbo nucifera Gaertn (Nymphaeaceae), a perennial aquatic plant, has been used as a medicinal herb in China and India. It has been recorded in the most famous medicinal book in China for more than 400 years. Different part of plant (leaves, seeds, flower, and rhizome) can be used in traditional system of medicine. The pharmacological studies have shown that *N. nucifera* possesses various notable pharmacological activities like anti-ischemic, antioxidant, anticancer, antiviral, antiobesity, lipolytic, hypocholesteremic, antipyretic, hepatoprotective, hypoglycaemic, antidiarrhoeal, antifungal, antibacterial, antiinflammatory and diuretic activities. The objectives of the current work was to study the physiochemical properties of Nixtamalized Brown top Millet flour enriched with *Nelumbo nucifera* at 2,6,8 and 10%. Proximate analysis, thermal, viscosity, textural and acceptability analysis were done. It was found that the *Nelumbo* a new sierra is rich in protein phytochemicals, antioxidants, phenolic compounds and that inductive rated coupled plasma mass spectrometry analysis showed the presence of important minerals. The overall the results revealed that the tested cooking ingredients were effective reducing the target mycotoxins. The ANOVA analysis revealed that the treatment of calcium concentrations and the length of boiling time, influence of water content, ash, protein, fat, calcium content of nixtamal fortified *Nelumbo nucifera* Brown top Millet masa. In this study water content was 11.26 to 12.54%, ash content 1.28 to 1.39% fat content 3.39 to 3.48 %, protein content 8.27 to 9.32 %, calcium 10.08 to 10.62 % antioxidants and phenolic compounds were done in this process. The present work gives a concise idea regarding the physiochemical properties of Nixtamalized Brown top Millet flour enriched with *Nelumbo nucifera*.

Keyword: *Nelumbo nucifera*, Nixtamalization, Mycotoxins, ANOVA, masa.

Introduction

Nixtamalization or lime treatment refers to a process of preparing Mostly in maize in which the grains are cooked and soaked in alkaline solution, usually lime solution. The term 'nixtamalization' also refers to the removal of the pericarp from any grain using an alkaline process. The basic process begins by cooking whole grains in water with lime and steeping the cooked grains for 12-16h. The steeped grains called 'nixtamal' (Sahai *et al*, 2000). Grains subjected to nixtamalization process have several benefits over unprocessed grains for food preparation. They are more easily ground; nutrient values increase; flavor and aroma are

improved and mycotoxins are reduced (Sefa Dedeh *et al.*, 2004), calcium content increases and there is reduction of aflatoxin (Bressani *et al.*, 1990). These benefits make nixtamalization a crucial preliminary step for further processing of grains into food products.

Nixtamalization is defined as the alkaline cooking of Brown top millet. It is an ancestral process originated in Mesoamerica to transform the grains into different products. This process is used to produce tortillas, flours, snacks, among others (Cornejo-Villegas *et al.*, 2010). Masa are the primary source of calories for Mexicans, and nowadays, its consumption goes from Mexico to America and some European and Asian countries. For this reason, this food is an excellent vector for the enrichment of human diets (Dunn *et al.*, 2008). Waliszewski *et al.*, 2002) analyzed *Nelumbo nucifera* supplementation on specific physicochemical and sensorial properties of the Masa. *Nelumbo nucifera* was added at 5, 10, 15, 20, and 25% w/w to Nixtamalized Brown top millet flour.

Nelumbo nucifera Gaertn., commonly known as lotus, sacred lotus, Indian lotus or Chinese water lily, is a well-known dietary and medicinal plant. It is a large, perennial aquatic plant that belongs to the family Nelumbonaceae and consists of a sole genus *Nelumbo* with two species, *N. nucifera* and *N. lutea*, which are called Asian lotus and American lotus, respectively. Almost all parts of the lotus plant have been used as food and medicine for more than 2000 years in Asia. The rhizome (modified stem), perianth (non-reproductive part of the flower) and seeds of lotus are popular food ingredients and are used in various food products due to their delicious taste and nutritional value. Lotus is a useful flower in Thailand, having taken advantage of lotuses in the past. Most of them are used as the components in medicine. The lotus flower has a mild and cool aroma. It has a comfortable sleep effect (Ketsara *et al.*, 2012). It smells like hyacinth, with a little banana aroma, which has the properties to calm the mind. The cool aroma of the lotus flower also helps to nourish the heart, and it can relieve the heat from fever, as the late lotus is known as a cool ingress that helps to quench the thirst and cool off.

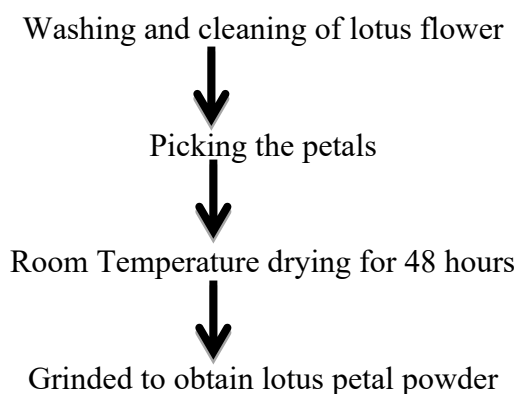
Lotus petals from the lotus flower were picked at blooming. Separate the pollen and dry them. Dry petals have a pink color fine aroma and astringent. Dry petals composed of flavonoids and alkaloids (Ketsara *et al.*, 2012). Moreover, lotus scent is commonly added to many foods, beverages as well as confectionery products.

Nutrition analysis refers to the process of determining the nutritional content of foods and food products. The process can be performed through a variety of certified methods. An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule. The characteristic feature of an antioxidant is ability to scavenge the free radicals due to their redox hydrogen donors and singlet oxygen quencher. The free radicals can be scavenged by the natural (plants) and synthetic (butylated hydroxyl toluene, butylated hydroxyl anisol and tetra butyl hydro quinone) antioxidants. But the usages of these synthetic antioxidants are now replaced because the natural antioxidants could be considered as safer without any side effect. In recent decades, many researchers are interested in medicinal plants for evaluation of antioxidant phytochemicals such as phenols, flavonoids and tannins which have received more attention for their potential role in prevention of human diseases.

Materials and Methods

Lotus flower (*N. nucifera*) was collected (in the morning), from Kuttampuli, Thoothukudi District. It was soaked in the water, and removed the petals by hand. Then the obtained sample was washed 3 times with water to make sure the completed defect elimination. Then the dried flower was grinded for further analysis and stored in airtight containers.

Preparation of lotus petals powder



Nixtamalized Brown Top Millet (NBT) preparation

The nixtamalization process was carried out using the methodology proposed by Gutiérrez *et al.*, 2007), using a computer-controlled system. The sample was prepared by cooking 1 kg of Brown top millet in a solution of 1.5 L of distilled water and 10.5gm of calcium hydroxide (1%, w/w) (reagent powder, Fermont, Monterrey, NL, México). The Millet were added to the container and heated until they reached 92°C for 40 min and achieved 36% moisture content. Cooked grains (nixtamal) were steeped for 8 h. The nejayote was drained off. The nixtamalized sample was rinsed twice with water using a 2:1 (v/w) ratio by stirring the kernels in the rinsing water for 1 min. The milling process of nixtamal was carried out using stones, at 1260 Hz (Fumasa-Mexico), nixtamal was converted to masa, and dried in an oven for 8 h (<12% moisture content). The masa was dried and milled to obtain the Nixtamalized Brown top millet flour (NBT). The NBT was ground in a coffee grinder and sieved in mesh 60 (<250 µm), and then it was added with Lotus petal powder (NBL) at three different concentrations (2, 6, 10% w/w) these samples were labeled as 2% NBL, 6% NBL and 10% NBL respectively. The formulations were mixed in a bowl for 10 min to their homogenization. Each sample was hydrated by the method of water absorption capacity (Gaytán Martínez *et al.*, 2000).

The tortillas were formed with a manual press with 20 cm of diameter and 2 mm of thickness. The disks were placed on a heating plate at 220°C, and then, they were cooked for 1 min for one side, 30 s for the second side, and finally, tortillas were turned over to the first side for 20 s until they inflated (Rojas-Molina *et al.*, 2020). The tortillas were rest in a blanket to achieve a temperature of 30°C for their textural evaluation.

Chemical proximate analysis

The chemical composition of Nixtamalized Brown top millet and Fortified *Nelumbo nucifera* petals were obtained according to AOAC, (2005) methodology.

Determination of functional properties

Moisture content of the sample was estimated by hot air oven method, protein was determined by available nitrogen in the sample by Micro Kjeldhal method, fat was estimated by soxhlet extraction and the ash content was estimated by dry ashing method whereas fibre content was estimated by acid and alkali method (AOAC, 1995). The minerals viz. iron, copper and zinc were estimated using atomic absorption spectrophotometer and calcium by titration method (AOAC, 1995). The data obtained from experiments were subjected to statistical analysis to find out the impact of nixtamalization (lime treatment) on physical, functional and nutritional properties of quality protein Brown top millet.

Polyphenolic and Antioxidant properties

Antioxidant activity of different Brown top millet was determined by free radical scavenging activity and ferric reducing power. The antioxidant activity of the extracts of brown top millet and of the extracts of the samples was determined by using the technique of Fan *et al.*, 2012) with slight modification.

Total phenolic content

Total phenolic content (TPC) of samples were determined by using Folin–Ciocalteu reagent, as described by Salar and Purewal, (2017). The absorbance of the extracts was recorded at 765 nm against a blank, and the results were expressed as mg Gallic acid equivalent/100 g (mg GAE/100 g) of the extract on dry weight (DW) basis obtained from the standard calibration curve.

Viscosity profile

The viscosity profiles of grains and the mixes (2, 6, and 10% NBL) were measured using a starch cell of an Anton Paar Rheometer model MCR 102 (Austria). 3.00 g of flour were added with 18 mL of distilled water. Each sample was heated from 50 to 90°C and held at a constant temperature of 90°C for 5.3 min. After this, the samples were cooled down to 50°C. All tests were carried out in duplicate at a constant frequency of 193 rpm (Acosta-Osorio *et al.*, 2011).

Results and discussion

Proximal analysis and mineral content

The chemical composition for Nixtamalization of Brown top millet and the mixes (2% NBL, 6% NBL, and 10% NBL) Significant differences were found in the composition of materials ($p < .05$) in the protein, lipids, and carbohydrates content. The NBL showed higher protein content (9.32%) compared with the NIXTAMALIZED BROWN TOP MILLET (7.89%). The protein content for the NBL used in this study was in levels of this range. The addition of NBL to the NBT increased the protein content significantly. Ash content was similar in both raw materials NBT and NBL and did not show ($p > .05$) significant differences between

treatments. However, the mineral content of raw materials (NBT and NBL) shows significant differences and the presence of some different minerals in both cases. The NBT has a high value of mineral content.

Carlos Martín Enríquez-Castro *et al.*, 2020, compared the physicochemical, rheological, and morphological characteristics of corn, nixtamalized flour, masa, and tortillas from the traditional nixtamalization process (TNP) and the extrusion nixtamalization process (ENP) and their relationship with starch. The traditional and extrusion processes were carried out using the same variety of corn.

Table.1. Proximate Analysis of Nixtamalized Brown Top Millet and the mixes with 2, 6, 10% NBL

S.No	Sample Name	Moisture (%)	Protein (%)	Fat (gm)	Ash Level (%)	Calcium (mg)
1.	BTM	10.36	6.4±0.5	1.5±0.1	13.3±0.5	8.52±0.5
2.	NBT	18.4	7.2±0.8	1.5±0.04	12.6±0.2	9.34±0.6
3.	NBL (2%)	18.6	8.7±0.7	1.3±0.07	11.9±0.9	10.35±0.3
4.	NBL (6%)	18.4	8.8±0.4	1.3±0.09	11.4±0.5	10.48±0.5
5.	NBL (10%)	18.2	8.8±0.8	1.3±0.09	11.01±0.6	10.52±0.3

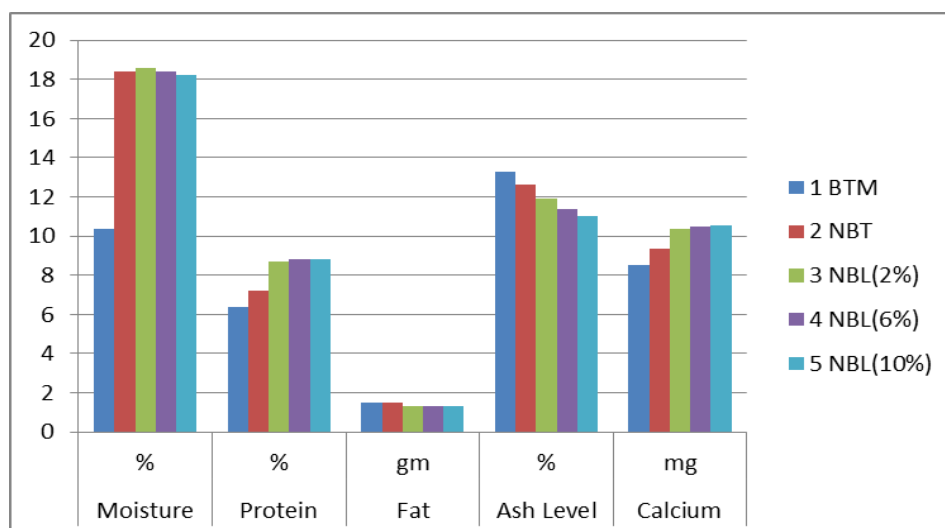
BTM- Brown Top Millet

NBT- Nixtamalized Brown top millet

NBL- Nixtamalized flour fortified with lotus powder

The above table was represented, that the moisture level was high in the NBL sample like 18.2 to 18.6%, low in the BTM (10.36%). The Protein content was high in the NBL (6%) and NBL (10%) of sample low in BTM. Fat level was higher in the BTM in the range of 1.5gm and was found lower in NBL sample. Ash level was also higher in BTM sample and lower in the NBL sample. Calcium level was higher in NBL (10%) range of 10.52 and low in the BTM range 8.52mg. Table.1. and Figure.1, illustrated the proximate analysis of Nixtamalized Brown Top Millet and the mixes with 2, 6, 10% NBL.

Figure.1. Proximate Analysis of Nixtamalized Brown Top Millet and the mixes with 2, 6, 10% NBL



Phenolic and Antioxidant property of Nixtamalized products

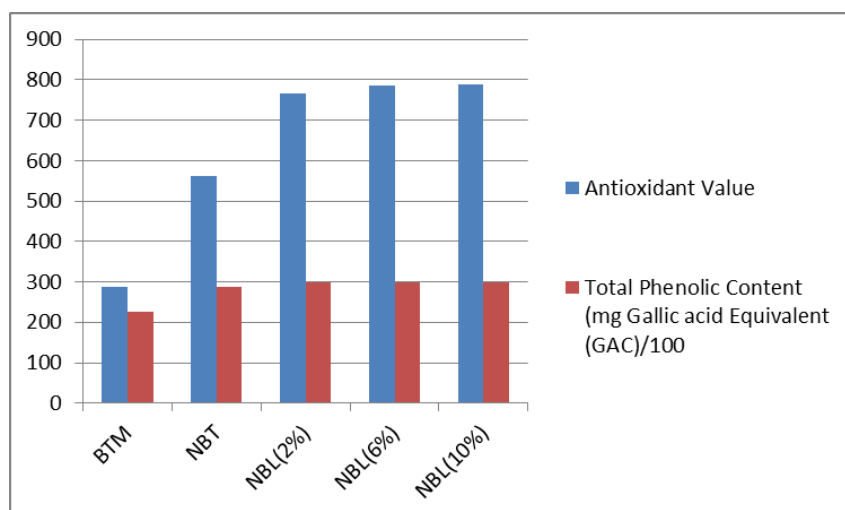
The DPPH method was used to evaluate the ability of the samples to remove free radicals. The total antioxidant values of the extracts from samples varied from 287.05 to 788.23. NBL (10%) had the highest amount of antioxidants (788.23 ± 0.2), followed by NBL (6%) and BTM had the least antioxidant property (287.05 ± 0.8).

Phenolic compounds are important plant constituents with redox properties responsible for antioxidant activity. The highest level of phenolic level of 298.8 was found in the sample of NBL (2%, 6%, 10%) and low in the BTM (226.04) respectively. Columba de la Parra *et al.*, (2007) revealed the phytochemical profiles and antioxidant activities of five types of corn (white, yellow, high carotenoid, blue, and red) processed into masa, tortillas, and tortilla chips. The nixtamalization process significantly ($p < 0.05$) reduced total phenolics and antioxidant activities when compared to raw grains. Nixtamalized grains exhibited higher concentration of free phenolics and soluble conjugated ferulic acid and had lower concentrations of bound phenolics and ferulic acid than unprocessed grains. Among processed products, there was a little variation in the phytochemical contents and antioxidant properties.

Table.2. Phenolic and Antioxidant property of Nixtamalized products

S.No	Sample Name	Antioxidant Value	Total Phenolic Content (mg Gallic acid Equivalent (GAC)/100
1.	BTM	287.05 ± 0.8	226.5 ± 0.4
2.	NBT	563.03 ± 0.4	287.2 ± 0.6
3.	NBL (2%)	765.25 ± 0.7	298.7 ± 0.3
4.	NBL (6%)	784.76 ± 0.3	298.8 ± 0.4
5.	NBL (10%)	788.23 ± 0.2	298.8 ± 0.9

Figure.2. Phenolic and Antioxidant property of Nixtamalized products



Viscosity profiles

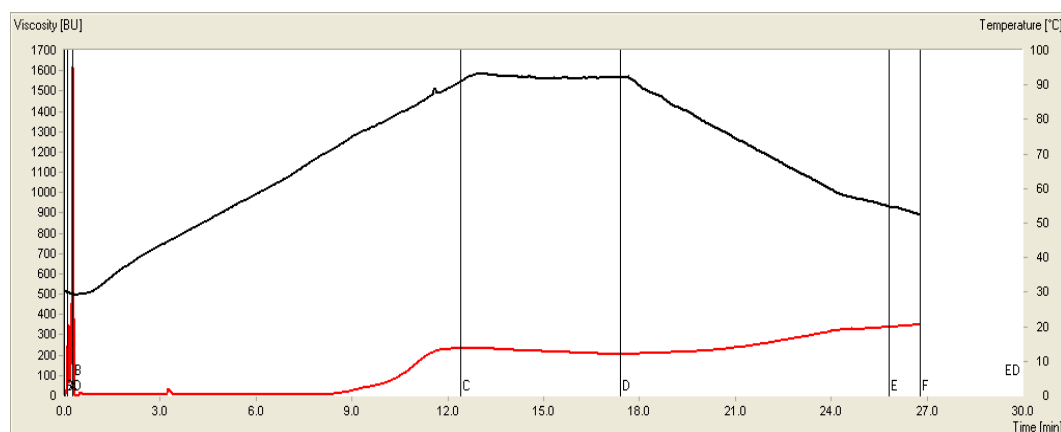
Viscosity is a fundamental parameter evaluated in the nixtamalized Brown top millet flours because it affects tortillas textural behavior. The viscosity for nixtamalized brown top millet was higher than the mixes, and the viscosity of the samples enriched with NBL showed an inversely proportional behavior with the concentration of lotus petals flour. The NBL was high in protein and fat, producing a decrease in the mixes viscosity. During the Fasting profile, the starch granules are hydrated, stirred, and heated, producing changes in the viscosity by the gelatinization phenomenon, giving rise to a gel. However, the addition of other compounds in the formulation like gums, fiber, or proteins can affect this property (Rojas-Molina *et al.*, 2020). The system of compounds in the NBL does not contribute to the gel formation. The apparent viscosity for NBL was 19mPa.s. It was significantly lower ($p < .05$) than the nixtamalized brown top millet and the mixes. Valderrama-Bravo *et al.*, 2018 analyzed Maize grains treated with electromagnetic fields and nixtamalized with nejayote and demonstrated their effect of such treatments on the microbiological and viscoelastic properties of masa, and on sensory tests of tortilla. The results indicated that the application of electromagnetic fields modifies the viscoelastic modulus of masa. Figure. 3 and Table.3 showed the viscosity profile for nixtamalized brown top millet and the mixes with 2, 6, 10% NBL.

Table.3. Viscosity profile of Nixtamalized Brown Top Millet and the mixes with 2, 6, 10% NBL

Point	Evaluation point	Time [min]	Viscosity [BU]	Temperature [°C]	SRC
A	Beginning of gelatinization	0.1	244	29.8	D
B	Maximum viscosity	0.27	1617	29.3	D
C	Start of holding period	12.4	232	91	T
D	Start of cooling period	17.4	203	92.1	T
E	End of cooling period	25.8	337	54.7	T
F	End of final holding period	26.8	349	52.3	T
BD	Breakdown	0	1414	0	D

ED	Setback	0	134	0	D
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Figure. 3. Viscosity profile of Nixtamalized Brown Top Millet and the mixes with 2, 6, 10% NBL



Conclusion

The formulation of Nixtamalized Brown top millet flour with lotus petals flour has nutritional potential due to its supplementation increases the protein content. Also, Nixtamalization brown top lotus powder has a Highest Antioxidant value and phenolic value compare to the native brown top millet. The addition of *Nelumbo nucifera* petal to NBT affected some physicochemical properties such as viscosity, hardness in masa, and it produces a reduction of tension and cut forces in tortillas. Biscuits samples prepared by incorporating increasing levels of NBL (10%) resulted in increased TPC, and DPPH inhibition as compared to the control. Hence we may conclude that since NBL is a good source of bioactive components such as the Protein and polyphenols with high antioxidant activity, Brown top millet and Nixtamalization process with *Nelumbo nucifera* products will improve the health benefits for the consumers. Furthermore, the present formulation may be considered to enhance the utilization of NBL in the food industry for cost reduction, nutritional enhancement, and utilization of a food industry waste for commercialization.

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