Nixtamalization application to shelf life of corn flour

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Abstract. Corn is very potential to be developed as a source of flour and starch for the raw material of the food industry and others, but the functional properties of corn flour are less favored in its application to processed products because the lack of texture and the durability of flour is lack due to the fat content of corn. One way to improve it is by the process of nixtamalization. The purpose of this study was to evaluate the effect of nixtamalization on aflatoxin content and shelf life of corn flour. The treatments used were long immersion in water or without nixtamalization (W1), and soaking time in a solution of Ca(OH)2 consisting of 8 hours (W2), 16 hours (W3), 24 hours (W4), and 32 hours (W5), washed. and ground to form a smooth dough (mass). The experiments were arranged with a single treatment, with variation in the duration of immersion in lime solution (control, 8, 16, 24, and 32 hours). This treatment was repeated 3 times. The results showed a decrease in level of type B1 aflatoxin with increasing immersion time, namely 85 µg/kg (0 hours), 69 µg/kg (8 hours), 62 µg/kg (16 hours), 55 µg/kg (24 hours), and 52 µg/kg (32 hours). The shelf life of corn flour through observation of critical moisture content is 426 days (0 hours), 430 days (8 hours), 489 days (16 hours), 408 days (24 hours), and 462 days (32 hours).

1. Introduction
Corn is one of the strategic commodities and has economic value as well as having opportunities to be developed because of its position as the main source of carbohydrates and protein which substitutes rice [1]. Indonesia's corn production in 2018 is more than 28.61 million tons and the growth of Indonesia's corn production is predicted to be a surplus until 2021, which is 32.65 million tons. While corn consumption in 2018 is predicted to reach 20.35 million tons. The amount consists of animal feed consumption of 14.27 million tons and household consumption and the needs of the food industry weighing 6.08 million tons [2]. Therefore, increasing production must also increase by using corn by the community.

Corn has long been used as a staple food in several regions in Indonesia, and processed and consumed as snacks. Corn can also be stored or processed into corn flour. However, corn flour when
used as a raw material in food processing cannot produce good rheological quality, especially in the texture. In addition, so far the consumption of corn as a staple food is still identified with a low standard of living. Corn is reported to contain carotenoids both function as pro-vitamin A and non-pro-vitamin A, both of which are functional because they have an important beneficial role in maintaining health [3].

In addition to the importance of conducting studies on the potential of processed corn products as a functional food, another important thing that must be considered is the food safety problem of the processed corn products. Corn is one of cereals products which is easily infected by mycotoxins, especially those producing aflatoxin [4]. Publications on techniques to improve the quality of corn flour, aflatoxin content and techniques to reduce the aflatoxin content of corn-based food products are still rarely obtained.

In order to increase the potential of corn commodity, related to the planned diversification and national food security program based on local commodities, it is necessary to study more deeply about the processing of products based on corn starch, such as corn noodles, high-calorie fibrous biscuits, and tortilla chips.

Corn is very potential to be developed as a source of starch for the basic ingredients of the noodle industry and high-fiber biscuits, but the process of extracting corn starch requires a lot of water availability, requires heat energy for drying, therefore utilization as corn flour can be used as an alternative for corn utilization with a simpler process to be developed commercially at the household or medium industry level. However, the functional properties of natural corn meal are less favored in its application to processed products because the resulting texture is not good, as well as the durability of flour which is not durable due to the fat content of corn.

To produce a good texture requires starch that has low viscosity. One way to reduce viscosity is by the process of nixtamalization. According to Mendez-Montalvo [5], nixtamalization process, namely boiling corn grains in lime water followed by soaking for several hours, washed and milled to form a dough (mass). Corn processing through the process of nixtamalization reportedly very beneficial because it can help provide a safe and quality food. These advantages include increasing the product crispness, increasing the availability of niacin and protein digestibility and reducing the content of pathogenic bacteria [6,7,8]. It was further reported that with or without spontaneous fermentation, nixtamalization could be used to improve the functional properties of corn which could ultimately improve the rheological properties of corn flour.

Some researchers report that corn is one of cereals products which is easily infected by mycotoxins [9,10]. The dominant mycotoxin in maize is aflatoxin [4]. Aflatoxin has long been proven to be toxic and carcinogenic to humans [11]. The aflatoxin content in cereal-based foods can be reduced through cooking under alkaline pH conditions [12] and the fermentation process [13,14]. This study was aimed at evaluating the effect of nixtamalization on shelf life and aflatoxin content of corn flour.

2. Material and methods

2.1. Materials and equipments

The main raw material used was dried corn grain DK hybrid variety, yod solution, Ca(OH)2, dimethyl sulfoxide (DMSO), ion free water, aquades, methylene chloride, diethyl ether, methanol, NaOH (aw 0.06), MgCl2 (aw 0.32), K2CO3 (aw 0.44), KI (aw 0.69), NaCl (aw 0.75), KCl (aw 0.84), BaCl2 (aw 0.90), K2Cr2O7 (aw 0.98), selenium, H2SO4, Zn, and 67% NaOH solution, H3BO3, phenolptalin blue and red 1%.

Equipment used were pans, stoves, blander, scalecs, grinding machines, sieves, ovens, slide glass, hemacytometers, test tubes, vortices, volumetric tubes, and other supporting tools.

2.2. Research method

This research was conducted by identifying the shelf life and aflatoxin content of corn flour made by nixtamalization. The treatments used were long immersion in water or without nixtamalization (W1),
and soaking time in a solution of Ca(OH)$_2$ consisting of 8 hours (W2), 16 hours (W3), 24 hours (W4), and 32 hours (W5). The study was conducted with 3 replications. The samples were observed for shelf life and aflatoxin levels. Then the data were analyzed descriptively and the data were presented in tables and graphs.

Figure 1. Production of corn flour without soaking in Ca(OH)$_2$ solution [15].

Figure 2. Production of corn flour with nixtamalization process[16].
2.2.1. Nixtamalization process. The process of nixtamalization was carried out according to Rooney and Serma-Saldívar (2003) with slight modifications to the number of samples and the steps involved. A total of 2 kg of dry shelled corn was put into a pot containing 6 liters of water and 20 g of Ca(OH)$_2$ to boil for 30 minutes. Then immersed in boiling water for 8 hours (W2), 16 hours (W3), 24 hours (W4), and 32 hours (W5). Next, the corn was washed with water to remove the remaining Ca(OH)$_2$ contained in the corn kernels. Meanwhile, for control, flour was processed by means of corn in water for 4 hours, then washed and drained. Washed corn was then ground until crushed with a grinding machine. The corn which had been distilled and ground was then dried to be made into flour. Flour drying could be done at a temperature of 55°C to a moisture content of about 5%. Then the flour was mashed and sieved in an 80 mesh filter. The flow chart of the process of making corn flour without nixtamalization and by means of nixtamalization can be seen in figure 1 and figure 2.

2.3. Observation

2.3.1. Shelf life analysis [17]. The shelf life of corn flour was determined by the Accelerated Shelf Life Testing (ASLT) method with a critical water content model approach. The main principle of this model is to determine the equilibrium moisture content of cornmeal stored at various RH.

\[
\theta_{gain} = \ln\left(\frac{me - mc}{me - mi}\right) - \frac{k A P_o}{x Ws B}
\]

Information:
- \(\theta_{gain}\) = Estimated time of shelf life (days)
- \(me\) = equilibrium moisture content (% bk)
- \(mi\) = initial moisture content (% bk)
- \(mc\) = critical moisture content (% bk)
- \(Ws\) = Dry weight of material (g)
- \(A\) = The surface area of the package (m$^2$)
- \(k/x\) = Bottled water vapor permeability (g/m$^2$.hari.mmHg)
- \(P_o\) = saturated water vapor pressure (mmHg)

a. Initial moisture measurement. The initial moisture content is the percentage of the original moisture content in a material. The initial moisture content of the corn starch product was determined at the beginning of storage. Dry clean empty cup in a oven at approx. 105°C. Cooled in a desiccator for about 15 minutes and weighed. A total of 2 grams of sample in a cup was put in an oven at 105°C for 6 hours until it reached a constant weight. The plate containing the sample was cooled in a desiccator and then weighed. The initial moisture content could be calculated using the formula:

\[
Kadar air = \frac{A - B}{C} \times 100\%
\]

Information:
- \(A\) = Plate and sample weight before drying (g)
- \(B\) = Weight of the plates and samples after drying (g)
- \(C\) = Weight of the sample before drying (g)

b. Measurement of critical water content. Critical water content is the amount of critical water content possessed by a product in its critical condition. The critical condition itself is defined as a condition where the product is at the limit of consumer acceptance, in other words it has begun to be rejected...
The critical water content was obtained by storing the sample at room temperature with RH conditions of 76% using saturated NaCl solution. Periodically (every 24 hours) an organoleptic acceptance test was carried out on the appearance of the product. Every day the average acceptance score was calculated, until it reached a value of 2 (dislike), it was determined that the product was in a critical condition. The measurement of critical water content was carried out using the gravimetric method as was done in the initial water content measurement.

c. Determination of the isothermic sorption curve. Isothermic sorption curve is a curve that illustrates the relationship between water content in foodstuffs and water activity (aw) or relative humidity (RH) of storage in the storage room. Isothermic sorption curve obtained by storing corn samples into a humidity chamber which has a certain aw. Furthermore, an amount of water was added and stirred until saturated. The humidity chamber was closed and left for 24 hours at a temperature of 30°C. The packaged product (as much as 5 grams) was hung in the humidity chamber containing a saturated salt solution. Samples were weighed periodically (every 24) hours until a constant weight obtained (meaning that the equilibrium water content has been reached). Samples that had reached constant weight were measured for moisture using the oven method and expressed on a dry basis as in the initial water content measurement. Isothermic sorption curves were created by plotting water content and balance water activity. Water activity (aw) was calculated by dividing the RH value of each humidity chamber by 100.

d. Determination of supporting parameters. The supporting parameter is a variable that is considered to influence the process of estimating shelf life. Parameters must be known because they are very influential in the process of estimating shelf life. The supporting parameters include:
- Packaging permeability value (P), obtained from literature references
- The value of saturated vapor pressure (Po) at 30°C is obtained from the Labuza table
- The value of b (slope of the curve) is obtained from the gradient of the selected isothermic sorption equation model
- The value of the cross-sectional area (A) is obtained by multiplying the dimensions of the package
- The total solids value (Ws) is obtained by correcting for the total weight of the sample minus the initial moisture content.

2.3.2. Analysis of aflatoxin content. Analysis of aflatoxin levels B1, B2, G1, G2 was carried out on all treatments of nixtamalized corn flour. using the TLC Desitometer (Thin Layer Chromatography Desitometer) method. Densitometric TLC is a method analysis which is still being used up to this time. Aflatoxin analysis was performed using the TLC plate stationary phase silica gel 60 F_{254} size 20 x 10 cm with chloroform-ethyl acetate as mobile phase (7: 3). Detection and quantitation were carried out using a TLC scanner densitometry, fluorescence detector, at maximum excitation wavelength 354 nm and 400 nm of emissions.

This method has a detection limit (LOD) for aflatoxins B1 is 9.62 pg and for aflatoxin G1 amounting to 10.9 pg. Meanwhile, limits quantitation (LOQ) for aflatoxin B1 and G1 respectively amounted to 32.08 pg and 36.41 pg [18].

3. Results and discussion

3.1. Shelf life analysis
The results of the observations on the variables needed in estimating the shelf life of corn starch that were stabilized with different soaking times are presented in table 1. In table 1, it can be seen that there was a change in the value of the estimating factors for the shelf life of corn flour due to the difference in immersion time in the Ca(OH)$_2$ solution, namely an increase in the initial water content, critical water content and equilibrium water content. During storage in various RH conditions there would be interactions between the product and its environment. Water vapor would move from the
environment to the product or vice versa until equilibrium was reached. This water vapor transfer occurred as a result of differences in RH environment and product, where water vapor would move from high RH to low RH.

Table 1. Value of the factors in the treatment.

| Faktor-faktor                          | Nilai  |
|----------------------------------------|--------|
|                                        | W1     | W2     | W3     | W4     | W5     |
| Initial moisture content (%)           | 6,9986 | 7,0134 | 7,0602 | 8,1712 | 8,3217 |
| Critical moisture content (%)          | 12,1415| 11,6292| 11,5738| 11,4917| 11,9250|
| Balanced moisture content (%)          | 12,9598| 12,2288| 11,9646| 11,9047| 12,2518|
| Isothermic curve slope                 | 14,67  | 13,64  | 13,23  | 12,84  | 12,91  |
| Packaging permeability (H2O/m².hari.mmHg) | 0,5    | 0,5    | 0,5    | 0,5    | 0,5    |
| Packing area (m²)                      | 0,012  | 0,012  | 0,012  | 0,012  | 0,012  |
| Dry weight (g)                         | 2,7900 | 2,7895 | 2,7881 | 2,7548 | 2,7503 |
| Saturated vapor pressure 30°C (mmHg)   | 31,824 | 31,824 | 31,824 | 31,824 | 31,824 |
| Estimated shelf life (days)            | 426    | 430    | 489    | 408    | 462    |

The achievement of equilibrium conditions between the sample and the environment was indicated by a constant sample weight. According to [19], the water content of equilibrium in a food material will be achieved, which is indicated by the constant weight of the material. The weight of the material is said to be constant if the difference in weight between three consecutive weights is not more than 2 mg/g for RH conditions ≤ 90% and not more than 10 mg/g for RH conditions> 90%.

In this study, the samples stored at RH 7 had a tendency to decrease the weight of the material. While the samples stored at RH 7, 32, 69.76, 84, 90, and 98 tended to increase the weight of the material. This was because at RH 7 the sample underwent a desorption process while on other RH the sample underwent absorption. The desorption process is the process of releasing material moisture into the environment and absorption is the process of absorbing water vapor by materials from the environment. The equilibrium moisture content obtained from each experiment is plotted with the aw or RH value of the environment, thus forming a curve called an isothermic sorption curve.

Table 1 also shows that the higher the slope value of the isotherm curve, the dry weight of the sample, the difference between the equilibrium moisture content value and the initial moisture content, the longer the shelf life of a product. Meanwhile, the higher the difference between the value of equilibrium water content and critical water content causes a decrease in shelf life. Because of the same packaging permeability, package area, and saturated vapor pressure, the most influential factors are the interaction between the initial moisture content, the equilibrium water content, the critical water content, the sample weight, and the isothermic curve slope. According to [20], the gradient or slope of the curve (b) is determined from the straight line formed on the curve of the isothermic sorption equation model that is formed.

Other supporting parameters that must be known are packaging permeability \( K \), packaging surface area (A), and pure vapor pressure on storage. Each type of packaging has a different water vapor permeability. Packaging permeability is the speed of water vapor transmission through a unit area of material with a certain thickness due to differences in water vapor pressure between the product and the environment at a certain temperature and humidity [21].

In table 1, it can be seen that the shelf life of maize flour with treatment W1 to W5 was 426 days, 430 days, 489 days, 408 days, and 462 days, respectively. The shelf life of the coating flour based on corn flour was 209 days, calculated through the critical water content approach [22]. When compared
with the research of [22], the shelf life of corn flour with a critical moisture content approach, this study had a slightly longer shelf life. This might be due to differences in initial moisture content, packaging permeability, and packaging area.

Based on these data, it can be seen that although the nixtamalization process was able to reduce the fat content of corn flour [23], the fat content did not affect the estimated shelf life of corn flour from each treatment using the critical water content approach method. This is because in the process of determining the shelf life of corn flour through the critical water content approach, the determining factor is the ability of the product to absorb water from the environment. According to [17], models of critical moisture content are usually used for food products that are relatively easily damaged due to absorption of moisture from the environment. Arrhenius method may be more suitable to see the shelf life of corn flour which is influenced by the fat content contained in corn. Arrhenius model is generally used to estimate the shelf life of food products that are sensitive to changes in temperature, including food products that are prone to rancidity (due to fat oxidation), changes in color by browning reaction, or damage to vitamin C. Examples of products that can be determined its shelf life with the Arrhenius model is flour, nuts, and other products that contain high fat or contain reducing sugars and proteins that enable fat oxidation or browning reactions [26].

3.2. Aflatoxin content

The results of the analysis of aflatoxin content of corn flour (table 2) showed a decrease in aflatoxin content with increasing immersion time. In corn flour W1 (control) showed the highest content of aflatoxin, then decreased in the soaking time W2 (8 hours), W3 (16 hours), W4 (24 hours), and W5 (32 hours). This shows that the process of nixtamalization can reduce aflatoxin content in corn flour.

Table 2 also shows that the aflatoxin content of corn flour from the five treatments were quite high, exceeding the minimum content required by SNI, which was a maximum of 50 ppb for aflatoxin B1. Content of aflatoxin B2 (< 1), G1 (< 4), and G2 (< 1) were also positive, although relatively low. This situation was caused because the corn samples used were no longer good and has been stored for a long time, so that the process of nixtamalization could not eliminate the aflatoxin. According to [25], aflatoxin has a stable nature of physical, chemical, heat-stable treatment, the melting point is quite high, namely above 237°C.

| Aflatoxin | B1 (µg/kg) | B2 (µg/kg) | G1 (µg/kg) | G2 (µg/kg) |
|-----------|------------|------------|------------|------------|
| W1        | 85         | < 1        | < 4        | < 1        |
| W2        | 69         | < 1        | < 4        | < 1        |
| W3        | 62         | < 1        | < 4        | < 1        |
| W4        | 55         | < 1        | < 4        | < 1        |
| W5        | 52         | < 1        | < 4        | < 1        |

Corn quality requirements in SNI 4483: 2013, states that the maximum aflatoxin content for corn as animal feed is Quality I 100 ppb and Quality II is 150 ppb. If referring to the BPOM RI Regulation on the maximum limit of aflatoxin in food, the level exceeds the maximum level required, which is 20 ppb for aflatoxin B1 and total aflatoxin 35 ppb in seeds, flour and corn starch, as well as corn flakes. WHO, FAO and UNICEF set a limit on the aflatoxin content of carbohydrate sources of food no more than 30 ppb, while the European Commission set a maximum limit of 4 ppb total aflatoxin for cereal products [26].

Aflatoxins are secondary metabolites of Aspergillus flavus and Aspergillus parasiticus which are found in corn, peanuts, soybeans, rice and other agricultural commodities starting from planting, harvesting, storing in storage or processing. The climatic conditions in Indonesia strongly support the growth of Aspergillus sp and the formation of aflatoxins. There are at least 13 types, the most important are B1, B2, G1, G2, M1, and M2. The type of aflatoxin is based on its intensity with UV light, namely B1 and B2 (which gives blue color).
Aflatoxin G1 and G2 are only produced by A. Parasiticus, which gives a green color. The four aflatoxins are found together in varying proportions in corn kernels. Aflatoxin B1 is produced by both species, the most toxic compounds, potentially stimulating cancer, especially liver cancer. The mildest toxin attack is mild abrasions due to tissue death (necrosis). Exposure to high levels can cause cirrhosis, carcinoma of the liver, and digestive disorders, absorption of food ingredients, and nutrient metabolism. This toxin in the liver will be reacted to epoxide which is very reactive to the compounds in the cell. Carcinogenic effects occur because the base N guanine on DNA will be bound and disrupt the work of genes.

Aflatoxin M1 and M2 are the metabolic products of aflatoxin B1 and B2 detected in cow's milk, whose feed contains aflatoxin B1 and B2, whose danger levels are lower than their initial form. The negative impact of aflatoxin on body health is quite significant, various reports mention that aflatoxin is carcinogenic and teratogenic, and consumption of food contaminated with low concentrations of aflatoxin that can continuously damage the liver and reduce the immune system in the body.

4. Conclusions
The results showed a decrease in level of type B1 aflatoxin with increasing immersion time, namely 85 µg/kg (0 hours), 69 µg/kg (8 hours), 62 µg/kg (16 hours), 55 µg/kg (24 hours), and 52 µg/kg (32 hours). The shelf life of corn flour through observation of critical moisture content was 426 days (0 hours), 430 days (8 hours), 489 days (16 hours), 408 days (24 hours), and 462 days (32 hours).

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