EVALUATION OF THE LEVEL OF AFLATOXINS IN RAW PEANUTS AND REDUCTION LEVELS OF AFLATOXINS IN PEANUTS THROUGH NIXTAMALIZATION (LIME TREATMENT) AND CALCIUM ENRICHMENT

ABSTRACT

Nixtamalization has been widely used as a method of reducing aflatoxin contamination in peanuts as well as in calcium enrichment. The aim of this study is to investigate the effects of nixtamalization on aflatoxin and nutrient content in peanuts among wholesalers and retailers at Nyamakima Market in Nairobi, Kenya. A representative sample was drawn from a population of 70 market vendors using a systematic random sampling technique, resulting in 35 raw peanut samples for analysis via ELISA to determine initial aflatoxin levels. These samples were then subjected to nixtamalization treatments of 4, 8, and 16 minutes.The findings revealed that the average concentration of total aflatoxins in peanuts initially exceeded the accepted safety limit, with 71% of the samples failing to meet the standards. However, nixtamalization led to a substantial reduction in aflatoxin levels by 49%, 48%, and 50% after treatment durations of 4, 8, and 16 minutes, respectively. Statistical analysis, including ANOVA and Two-sample T-tests, confirmed significant distinctions between initial and final mean aflatoxin levels post-treatment, as well as variations between treatments.The study also observed a significant variation in calcium levels between the different time treatments, supported by linear regression analysis, which demonstrated a robust correlation between time treatments and calcium levels (R^2=0.83, P=0.011). Furthermore, the analysis revealed significant differences in mean moisture contents across various treatments (P=0.000), and a linear relationship between mean moisture content and exposure time (P=0.02). **T**he results emphasize the positive impact of nixtamalization in reducing aflatoxin levels and enhancing calcium content in peanuts. However, it is crucial to note the negative effect on moisture content, which exceeded stipulated limits, potentially raising concerns about product safety and quality.

Keywords: Nixtamalization, aflatoxin, ELISA, safety limits, calcium, wholesalers, retailers, ANOVA, linear regression

4.1. INTRODUCTION

Peanut (*Arachis hypogaea L*.) is an important and valuable crop in Africa, particularly in Kenya, however the country faces challenges in meeting its domestic demand for the product. The data from the Kenya National Bureau of Statistics (2023) economic report reveals a stark contrast between the volume of peanuts imported and the quantity produced domestically. Despite being a crucial and valuable crop, Kenya's peanut production falls short of its requirements, leading to a substantial reliance on imports, primarily from Malawi. This reliance on external sources for peanuts is driven by the inability of Kenya's own peanut production to sustain the demands of industries, particularly those involved in peanut butter processing. The report further states that Kenya imported a substantial 49,000 Metric Tonnes of peanuts, while its own production stood at a comparatively meager 3,000 Metric Tonnes (KNBS 2023). This trade imbalance underscores the challenges Kenya faces in achieving self-sufficiency in peanut production, which has implications for its economy, food security, and industrial processes.

However, the issue doesn't end with production and trade dynamics alone. The quality and safety of the peanuts being sold in local markets also emerge as a significant concern. Peanut vendors in these markets appear to operate without sufficient consideration for food safety practices, particularly concerning the contamination of peanuts with aflatoxins (Koraish*et al*., 2014). Aflatoxins are naturally occurring toxins produced by certain molds, primarily Aspergillus species, that can contaminate various crops, including peanuts. Aflatoxin contamination in peanuts poses serious health risks to consumers, as aflatoxins are potent carcinogens and can have detrimental effects on human health when ingested over time (W.H.O., 2018). The limit for aflatoxins in raw and roasted peanuts is set at 15ppb (KS EAS 888:2018) while that of peanut butter is set at 10ppb (KS EAS 67:2000).

The lack of awareness and knowledge among peanut vendors about aflatoxin contamination and food safety practices is evident (Pandey *et al*., 2019). This knowledge gap contributes to inadequate implementation of best practices during the handling and sale of peanuts. Aflatoxin contamination is a complex issue that requires attention at various points along the supply chain, from cultivation and harvest to storage and distribution. Therefore, the practices of peanut vendors have implications not only for consumer health but also for the overall food safety ecosystem. According to Masaka *et al*. (2022) among the various types of aflatoxins, aflatoxin

B1 emerges as a particular concern, given its prevalence in peanuts and peanut-derived products such as peanut butter. Aflatoxin B1 is known for its carcinogenic properties and adverse impact on human health, making its presence in peanuts a critical issue that needs to be addressed urgently (Dhanshetty*et al*., 2021).

Efforts to enhance the value of corn led to the adoption of nixtamalization, a process used to create masa dough, tortillas, and corn flours originally in Mexico (Ramírez-Araujo *et al*., 2019). Traditional nixtamalization involved soaking the grains in wood ash to remove the pericarp. However, lime became a preferred alternative due to its higher alkalinity, which expedited pericarp removal (Escalante *et al*., 2020). Lime shortened processing time, reduced waste, and conserved water. Nixtamalization involves treating corn with 0.2% Calcium Hydroxide (Lime), then drying and grinding it to create a powder used for making tortillas (Escalante *et al*., 2020). The maize is combined with lime and water, steeped for 12 hours, rinsed to remove excess lime, milled, and then utilized in producing corn-based products.

Aflatoxin contamination poses a significant threat in the peanut value chain. Losses suffered by stakeholders within the value chain have become increasingly unbearable over time. These losses are primarily attributed to inadequate post-harvest handling and a lack of knowledge regarding proper food safety practices during handling. Therefore, innovative methods are essential to combat this impending issue and enhance food safety. The application of Calcium Hydroxide is recommended for peanuts, given its successful track record in reducing aflatoxin B1 and M1 levels in maize (Elias-Orozco *et al*., 2002). This method could potentially be adapted to eliminate aflatoxins in peanuts and diminish toxin content.

Enzyme-Linked Immunosorbent Assay (ELISA) is a widely employed method for detecting aflatoxins in peanuts, a class of highly toxic mycotoxins produced by certain molds. In this procedure, a sample of peanuts is first ground into a fine powder and then mixed with a suitable solvent to extract the aflatoxins. The extracted sample is then incubated in a microtiter plate, which has been pre-coated with antibodies that specifically bind to aflatoxins. After the incubation, the plate is washed to remove any unbound substances. Next, an enzyme-linked secondary antibody is added, which binds to the primary antibodies. This secondary antibody is conjugated to an enzyme, usually horseradish peroxidase, which catalyzes a color-producing reaction. The intensity of the color change is directly proportional to the concentration of

aflatoxins in the sample. By measuring the absorbance of this colored solution at a specific wavelength, one can accurately quantify the amount of aflatoxins present in the peanuts. ELISA is valued for its sensitivity, specificity, and efficiency in testing for aflatoxins, making it a crucial tool in ensuring the safety and quality of peanut products.

ELISA, a cost-effective and straightforward method, is accessible to small enterprises for testing aflatoxins in peanuts. This technique involves binding aflatoxins with specific antibodies on a pre-coated plate, utilizing an enzyme-linked secondary antibody to create a color change. The intensity of this color corresponds to aflatoxin concentration. Due to its simplicity and affordability, ELISA serves as a practical choice for small businesses, enabling them to ensure the safety and quality of their peanut products.

Furthermore, nixtamalization has been shown to enhance the bioavailability of riboflavin and niacin (Suri, 2016). However, this process has some drawbacks, including the loss of dry matter as the grains shed phenolics and free flavonoids during processing (Jiménez *et al*., 2019). To address these negative impacts, alternative, gentler methods of carrying out nixtamalization could be embraced to preserve the nutritional content of oil seeds (Jiménez & Castro, 2021).

On top of nixtamalization, addressing the challenges at hand necessitates a comprehensive approach that encompasses various strategies. These initiatives include making targeted investments in research, crop management techniques, and disease prevention tactics to enhance the productivity of domestic peanut cultivation. Additionally, it is imperative to raise awareness among both peanut vendors and consumers about the risks associated with aflatoxin contamination, along with promoting best practices for the safe handling and consumption of peanuts. Regulatory measures should be put in place to establish and uphold standards for aflatoxin levels in peanuts and peanut-derived products, accompanied by consistent monitoring and testing procedures. The objective of the research is to evaluate the level of aflatoxins in raw peanuts and determine the reduction levels and enrichment with calcium after calcium hydroxide treatment.

4.2. MATERIALS AND METHODS.

4.2.1. STUDY AREA

The study area was as in sections 3.

4.2.2. RESEARCH DESIGN

The research utilized a mixed research approach that incorporated elements of both a cross-sectional study and an experimental design. In the cross-sectional component, peanuts were sampled from various vendors at the Nyamakima Trade Centre using a systematic random sampling method. Meanwhile, in the experimental phase, different time intervals (Independent variables) were manipulated and observed in relation to the final aflatoxin concentration and the calcium content (Dependent variables). Nixtamalization was done according to the methods used by Wanjiru *et al*. 2020.The ELISA test was carried out with reference to the method used by Marete *et* al. 2020. Table 1 shows the various time treatments administered on peanuts during the experiment at a constant lime concentration with the control dipped in 0.4% calcium hydroxide for 2 seconds which is indicated as 0 minutes in the study.

Table 1: Variation of time treatments on peanut samples at 0.04% lime concentration

Treatment	Weight (Kg)	Time (Minutes)	Lime Concentration
1 (Control)	500g	0 minutes	0.04%
2	500g	4 minutes	0.04%
3	500g	8 minutes	0.04%
4	500g	16 minutes	0.04%

4.2.3. STUDY POPULATION

Purposive sampling was used to specifically target and identify peanut vendors. Nyamakima Trade Centre, being the primary peanut market in Nairobi, hosts a significant population of peanut vendors. The sample size was determined using the Fischer's formula, as detailed in section 4.2.3.1 of this dissertation.

4.2.3.1 SAMPLE SIZE DETERMINATION FOR THE VENDORS'

The sample size was determined through a two-step process. Initially, with the Fischer formula, and subsequently, the sample interval was calculated using the systematic sampling interval calculation method to arrive at the final sample size.

Population calculation for vendors was as follows:

The sample size was determined using the Fischer formula as follows:

$$n = \frac{Z^2 P(1-P)}{I^2}$$

where:

n= "Sample size [For population> 10,000]"

Z= "Normal deviation at the desired confidence interval at 95% it will be 1.96".

P= "Proportion of the population with the desired characteristic"

Q = "Proportion of the population without the desired characteristic"

I^2 = "Degree of precision; will be taken to be 5%"

*" Since the proportion of the population with the characteristic is unknown, then 50% will be used"

Therefore

$$n = \frac{1.96^2 * 0.5(0.5)}{0.05^2}$$

=384

Since the population of vendors in totals to 80, sample size is adjusted as follows for a population less than 10000:

$$nf = \frac{n}{1 + \frac{n}{N}}$$

Where:

Nf=" desired sample size"

n=" calculated sample size 384"

N=total population 80 vendors

$$nf = \frac{384}{1 + \frac{384}{54}}$$

Desired sample size=66

With an attrition rate of 5% the sample size is adjusted to:

66*100/95=70

Population is 70

To compute the sampling interval (K) we use the following formula: K= N/n

Where,

K=Sampling interval

N=Population

n= Desired sample size

Thus N=70 and n=35

K=70/35=2

K=2.

Therefore, samples will be selected form 70 vendors at every 2nd interval until the sample size of 35 is complete

4.3.3. SAMPLING

Systematic random sampling was carried out at every 2nd interval. 35 raw peanuts (1.5 Kg each) were purchased from the vendors packed in kraft paper bags. The 35 samples were weighed and duplicated into three 500g samples. One of the triplicates was transported immediately to the Kenya Bureau of Standards (KEBS) for aflatoxin analysis. The other two 500g samples was retained for nixtamalization analysis.

4.3.4. ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) TEST

4.3.4.1. SAMPLE PREPARATION

The 500g peanut samples, received at the lab in kraft paper bags, underwent a preliminary cleaning process by passing them through a 20-mesh (0.9mm) screen to eliminate any debris or foreign particles. The 500g samples were each carefully ground and blended to a fine powder and weighed into 60g subsamples. The peanuts were precisely measured using an electronic balance calibrated to an accuracy of 0.01g.

For the extraction process, a solution of 70% methanol was prepared by combining 300ml of distilled water with 700ml of 100% methanol. Each of the 60g peanut samples, in duplicate, were added to 100ml of the 70% methanol solution in a boiling tube. To ensure thorough extraction, the samples were agitated on a vortex mixer for a duration of 30 minutes. Subsequently, the samples were filtered through Whatman filter paper no. 1. The resulting clarified solutions were analyzed to detect the presence of aflatoxins.

4.3.4.2. AFLATOXIN ANALYSIS- ELISA TEST

The reagents were brought to room temperature from their storage condition of 2 to 8℃. Each sample and the standard to be tested were allocated to separate dilution micro wells and placed on a holder. An equal number of microtitre wells coated with antibodies were arranged on another holder. A solution containing Aflatoxin-HRP Conjugate, made up of peroxidase in a buffered solution with preservatives, was dispensed into each mixing well at a volume of 200µL. Subsequently, 100 µL of both the samples and standards were added to the respective mixing wells containing the conjugate, using a new pipette tip for each addition.

The solution was thoroughly mixed by pipetting and shaking at least three times. Then, 100 µL of the mixed solution from each mixing well was transferred to corresponding antibody-coated microtitre wells. These were then left to incubate at room temperature for 15 minutes. After incubation, the contents were removed, and each micro well was meticulously washed with a PBS-Tween wash buffer solution (with a pH of approximately 6.8-7.0), repeated five times. The micro wells were tapped upside down onto an absorbent towel to drain off excess buffer.

Subsequently, 100 µL of a substrate solution (containing tetramethylbenzidine) was added to each micro well using a pipette. The wells were then covered with aluminium foil to shield them from direct light and incubated for 5 minutes. To stop the reaction, 100 µL of an acidic stop solution was added to each well in the same order as the substrate solution.

The optical density (OD) of each well was measured using a microtiter plate reader set at a wavelength of 450nm, and the resulting OD values were recorded. A dose-response curve was generated using these values, expressed as a percentage (%B/Bo) of the OD of the zero standard against the Aflatoxin content of the standard. The concentration of unknown samples was determined by interpolating from the standard curve.

The samples were diluted in a ratio of 5:1 in 70% methanol, so the Aflatoxin levels indicated by the standards were multiplied by 5 to provide the Aflatoxin content in parts per billion (ppb).

4.3.5. NIXTAMALIZATION OF PEANUTS

4.3.5.1. SAMPLING

A subsample of the 35 samples obtained from the market by systematic random sampling at every 2nd interval to obtain 12 sub-samples that would be used in nixtamalization. Two 500g samples were obtained from each selected group to serve as the treatment and control respectively.

4.3.5.2. CALCIUM HYDROXIDE (LIME) TREATMENT (NITXAMALIZATION).

A 0.04% lime concentration was prepared by weighing 4.1g of 96% purity Ca(OH)2 in 10L of distilled water chilled to 4℃ to increase solubility. The solution was blended with an immersion blender to dissolve it completely.

500g portions of peanuts were weighed on an electronic balance and placed in a 2 Litre steel bucket. 1 litre of 0.4% lime was added to each of the samples to ensure all the seeds were completely covered in the solution. Three treatments were administered on the peanuts as shown in table 2 Each treatment received a different time variation, 4 minutes, 8 minutes and 16 minutes. The control for each treatment was soaked for 0 minutes in 0.04% solution. The treatments were replicated

The peanuts were then removed from the water and placed on a 20-mesh (0.9mm) screen to drain off excess lime for 5 minutes. The peanuts were then transferred to a batch peanut roaster and roasted for 30 Minutes. After roasting, the peanuts were ground into peanut butter on a electrical grinder. The calcium content was determined using the atomic absorption spectrophotometry method (ISO 6869: 2000) and the AOAC method, using inductively coupled plasma-optic emission spectrometry. Moisture content was determined using the AOAC Method 925.10. The final aflatoxin content was then determined using the ELISA test as shown in section **4.3.4.2 .**

4.3.6. STATISTICAL ANALYSIS.

SPSS and R were used to analyse the data. The data was summarized using the mean, standard deviation and the common variance. A One-way ANOVA test, was carried out to analyse the differences between means of the treatments. Means within treatments were analysed for aflatoxins at different exposure times as well as the moisture content and calcium levels. A linear regression analysis was used to predict the time versus calcium and moisture relationship.

4.4. RESULTS

4.4.1. AFLATOXIN CONTAMINATION LEVELS

Upon evaluating the results of aflatoxin analysis from the 35 samples, each weighing 500 grams and collected at Nyamakima market, it was evident that the average total contamination level amounted to 25 parts per billion (ppb). This figure exceeded the permissible limit of 15 ppb for aflatoxin presence in raw peanuts. None of the sampled peanuts met the criteria for aflatoxin safety. The results are as shown in table 2 whereby 28.5% of the sample had aflatoxin levels above 15ppb while 71.5% were below 15ppb.

Table 2: Aflatoxin levels in the samples

Aflatoxin Level	Frequency	Percentage	Interpretation
<15ppb	10	28.5	Acceptable
>15ppb	25	71.5	Not acceptable

N=35 Mean ± SD =25.58±7.6839

4.4.2. CALCIUM HYDROXIDE EFFICACY

In this experiment, the focus was on assessing the effects of a calcium hydroxide soaking solution on the reduction of aflatoxin levels in peanuts. The mean aflatoxin levels within treatments before and after soaking in calcium hydroxide are as shown in table 2 and 3. Initially, the peanuts were tested for their aflatoxin content, which exhibited a range of concentrations between 19 and 51 parts per billion (ppb), with an average of 31.27 ppb. Subsequently, the peanuts were subjected to varying exposure times in the calcium hydroxide solution, specifically 4 minutes for, 8 minutes for, and 16 minutes for.

Upon completion of the soaking process, distinct trends emerged within each group. The first group, treated with calcium hydroxide for 4 minutes, experienced a large reduction in aflatoxin content by an average of 16 ppb, corresponding to a 49% reduction. The variability within this reduction was measured at 9.86 ppb. After a 8-minute soak, the peanuts demonstrated a similar reduction, with an average of 15 ppb (48%) and a variability of 7.86 ppb. Notably, the third group, soaked for 16 minutes, showcased the most substantial reduction, averaging 17 ppb (50.4%), with a variability of 9.98 ppb. In contrast, the control group displayed a contrary pattern, witnessing a decrease only 3ppb in mean aflatoxin levels.

Employing a One-way Analysis of Variance (ANOVA) test, a significant distinction emerged between the initial and final mean aflatoxin levels post-treatment ($p=0.0025$). A Two-sample T-test aimed to elucidate potential variations among the treatments resulted in a P-value of 0.8433,

at a significance level of 0.05. This outcome suggested that statistically significant distinctions with respect to the aflatoxin contents among the three treatments A, B and C were not detected.

Table 3: **Initial and final mean levels of aflatoxins within treatments.**

Treatment	Initial mean ±SD	Final mean ± SD
0 minutes	25.6 ± 7.7	25.6 ± 7.7
4 Minutes	33.9± 10.4	17.2 ± 9.9
8 minutes	31.8 ± 9.5	16.5 ± 7.9
16 minutes	33.8 ± 10.5	16.8 ± 10.0

4.4.3. CALCIUM ENRICHMENT.

In this experiment, the peanut butter produced from peanuts treated with 0.4% calcium hydroxide registered high calcium content that increased gradually with increase in the period of exposure as shown in Table 4 . The control group had 0.431mg/g of calcium while the other treatments at 4 minutes, 8 minutes and 16 minutes had 0.44614 mg/g, 0.4518mg/g and 0.66657mg/g respectively.

Table 4: Mean calcium levels in peanuts after soaking.

TREATMENT	Mean Calcium ± SD (mg/g)
0	0.431 ± 0.359
4	0. 4461 ± 0.846
8	0.4518 ± 3.05
16	0.6657 ± 33.874

RDA=1000-1200mg

A ANOVA test done proved that there was a significant variation in calcium levels between the different time treatments P=0.4294. A linear regression analysis done on the experiment showed that there is a strong correlation between the time treatments and the calcium levels R^2=0.83. Since P=0.011, this proves that there exists a significant linear relationship between time treatments and the calcium levels as shown in the figure 1.

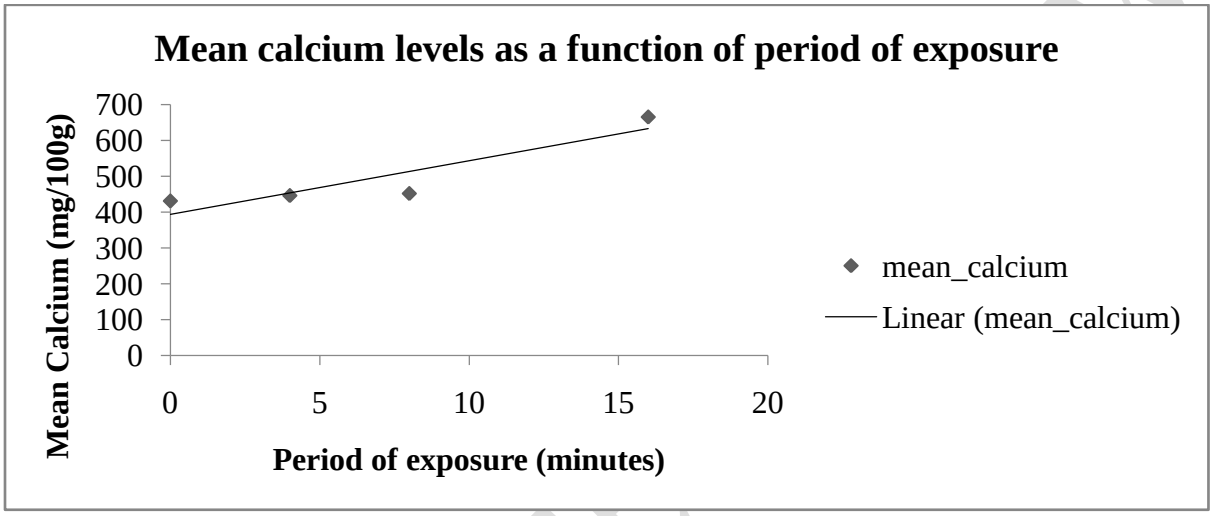

Figure 1: Mean calcium levels versus the time of exposure (soaking) in lime

(Y=14.913X+394.26; R^2=0.83)

4.4.4. EFFECT ON THE MOISTURE CONTENT

In this experiment, the moisture content of the peanut was tested against the standard moisture content of 2% in roasted peanuts. The results are as shown on table.5 with the highest moisture content being recorded after 16 minutes of soaking with the limit set at 2%. A ANOVA test done on the experiment shows that there is a significant difference in the mean moisture contents between treatments (P=0.000).

Table 5: Mean moisture levels per treatment

TREATMENT	Mean Moisture ± SD (%)	Interpretation
0	5.28 ± 0.76	Not Acceptable
4	7.22 ± 0.48	Not Acceptable

8	8.06 ± 0.23	Not Acceptable
16	10.89 ± 1.22	Not acceptable

A linear regression model of mean moisture plotted against time proved that there is a significant linear correlation between the two variables, moisture and period of exposure with P=0.02. This is as illustrated in figure 2 below.

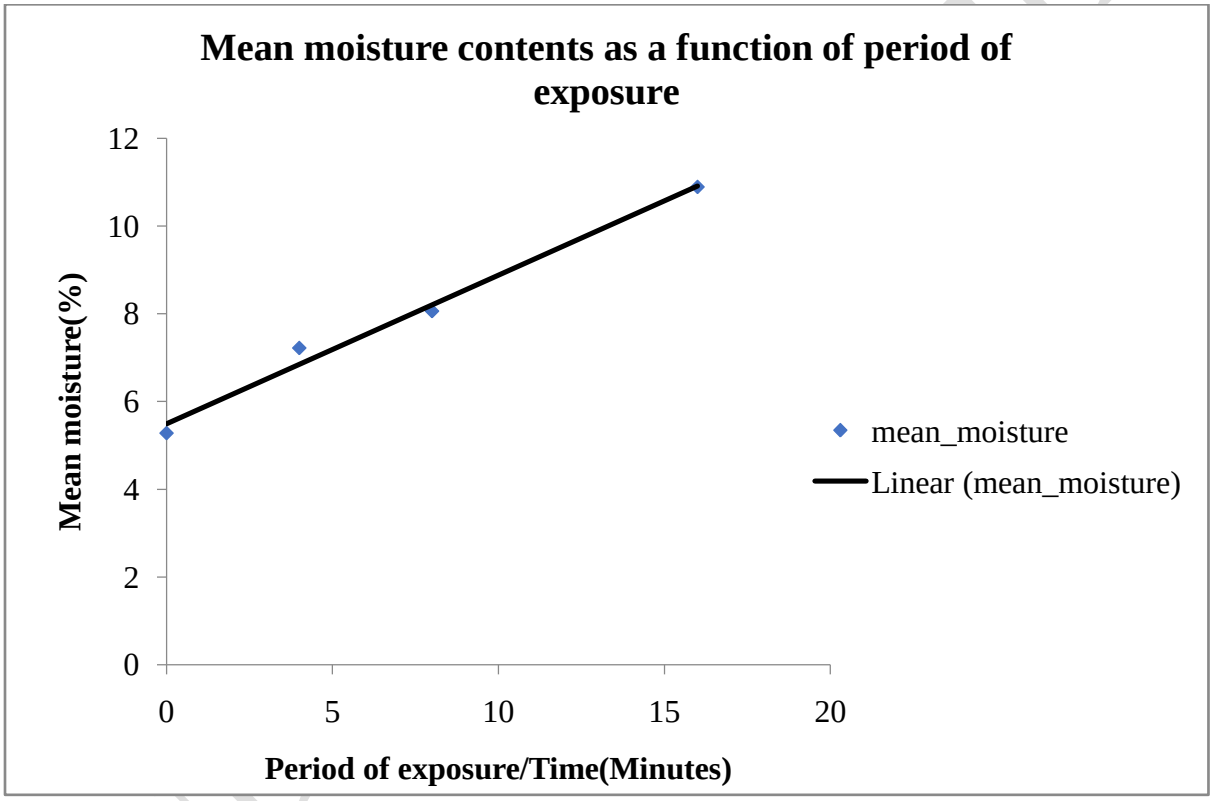

Figure 2: Mean moisture contents versus period of exposure (soaking) in lime

4.5. DISCUSSION

4.5.1. AFLATOXIN CONTAMINATION LEVELS

The findings from the aflatoxin analysis of the samples collected at Nyamakima market raise significant concerns regarding food safety, particularly in relation to raw peanuts. Aflatoxins are potent toxins with a complex chemical structure that are highly carcinogenic and can pose serious health risks to humans upon consumption (Nazhand*et al*., 2020).

The average total contamination level in the collected samples surpasses the acceptable limit of 15ppb for aflatoxins in peanuts intended for further processing, as set by CODEX food safety standards for contaminants and toxins in food (CODEX STAN 193-1995). Some countries like Thailand however permit aflatoxin levels in peanuts up to 20ppb (Standard TA, 2014) This overage indicates a potential lack of proper storage conditions, handling practices, or quality control measures in place at Nyamakima market, which may have contributed to the elevated aflatoxin levels Norlia*et at*., 2019).

The fact that a small portion of the samples passed the aflatoxin test highlights a widespread issue of aflatoxin contamination in the market. The results concur with the findings of Masaka *et al*. 2022 whereby 60% of the raw peanut samples tested at an informal market failed to pass the set limit for total aflatoxins. In a study conducted by Kamika *et al*. (2014) all the samples collected at a market in Pretoria were contaminated with aflatoxin producing fungi. This situation is particularly alarming, as the consumption of products containing aflatoxin levels above the acceptable limit can lead to both acute and chronic health problems, including liver damage and an increased risk of developing liver cancer (Škrinjar &Baltić, 2010).

The graphical representation of the distribution of aflatoxin concentrations emphasizes the variability of contamination levels among the samples, with a wide variability between peak and minimum levels thus further underscoring the inconsistency in the quality and safety of the available peanuts (Akullo *et al*., 2023).

4.5.2. CALCIUM HYDROXIDE EFFICACY ON AFLATOXINS

In this experiment, peanuts were initially tested for aflatoxin levels and failed to pass the set limit. This coincides with the findings of Nyirahakizimana*et al* (2013) whereby market samples from formal and informal markets in Eldoret and Kericho towns failed to meet the threshold for compliance. High aflatoxin levels in peanuts can be attributed to poor storage conditions and specifically high moisture content (Norlia*et al*. 2019). This necessitates the need to create

awareness on the effects of aflatoxins on human health and the importance of food safety. The peanuts were immersed in a calcium hydroxide solution for varying durations to assess the optimal time-treatment for aflatoxin reduction, as it is well-established that different durations of exposure can significantly affect the effectiveness of an intervention (De Alencar*et al*., 2012)

Following the soaking process, each group exhibited distinctive trends. When subjected to a 16-minute soak, it exhibited the most substantial reduction. Intriguingly, the control group diverged from this pattern, as the mean aflatoxin levels decreased by 3 ppb. According to Emadi*et al*. (2021), roasting dry peanuts alone as an intervention reduced aflatoxin level by at least 26%. The control group was however dipped in water first and as such the increased moisture content hindered rapid heating and aflatoxin destruction (Norlia*et al*. 2019).

The ANOVA test unveiled a noteworthy distinction between the initial and final mean aflatoxin levels post-treatment (P=0.0025). The Two-sample T-test was executed to explore potential distinctions among the treatments. The calculated p-value of 0.8433, at a significance level of 0.05, led to the rejection of the null hypothesis. This suggests that statistically significant differences among the three treatments were not observed. No significant variations were discerned among the three treatment groups. This suggest that varying time treatments had no statistically different effect on the aflatoxin content

The experiment's results reveal differential impacts of calcium hydroxide soaking on aflatoxin reduction in peanuts. While at 4 minutes the peanuts displayed a significant reduction compared to the control, no marked disparities were found between treatments at 4,8 and 16 minutes. These findings underscore the complexity of the treatment's effects on aflatoxin levels. The experiment demonstrated that soaking peanuts in a calcium hydroxide solution led to varying degrees of aflatoxin reduction. While the control group and the three treatments displayed noteworthy differences, the analysis did not find significant distinctions between the treatments at 4,8, and 16 minutes.

4.5.3. EFFECT ON CALCIUM CONTENT.

The experiment focused on the impact of treating peanuts with 0.4% calcium hydroxide on the calcium content of peanut butter. The results revealed that the peanut butter produced from treated peanuts exhibited a notable increase in calcium content as the exposure time to calcium

hydroxide increased. The findings coincide with findings of Rojas-Molina *et al*. (2009) whereby temperature and steeping times had a positive effect on the level of calcium on corn flours.

An ANOVA test was conducted, indicating a significant variation in calcium levels among the different time treatments (P=0.4294), suggesting that exposure time to calcium hydroxide indeed had a significant effect on calcium content. The difference in calcium contents between treatments was very significant. Furthermore, a linear regression analysis demonstrated a strong correlation (R^2=0.83) between time treatments and calcium levels (Serna-Saldivar, 2021). This suggests that 83% of the variation in calcium content can be accounted for by the variation in period of exposure. According to Argun, & Doğan, (2017) varying conditions during nixtamalization has a significant effect on the nutritional content of the final product. The low P-value of 0.011 in this analysis reinforces the presence of a significant linear relationship between the exposure time and calcium levels. The data suggests that longer exposure to calcium hydroxide leads to a greater increase in calcium content in the peanut butter, as illustrated in the accompanying figure (Ramírez-Jiménez & Castro-Muñoz, 2021). These findings are valuable for understanding the potential enhancement of calcium levels in peanut products through controlled treatment processes.

4.5.3. EFFECT ON MOISTURE CONTENT.

The experiment aimed to assess the moisture content of peanuts in comparison to the standard moisture content of 2% typically acceptable for roasted peanuts. The results, as depicted in the table, indicate that the highest moisture content was recorded after 16 minutes of soaking, surpassing the established limit. The moisture content of the peanuts greatly depends on the cooking time and this concurs with the findings of Argun, (2020), that proved that steeping times during nixtamalization processes had a major effect on the moisture content of the final product. An ANOVA test conducted on the experiment clearly demonstrates a significant difference in the mean moisture contents across the various treatments (P=0.000). This implies that the duration of exposure to moisture significantly affects the moisture content in the peanuts.

Furthermore, a linear regression model depicting the relationship between mean moisture content and exposure time illustrates a significant linear correlation between these two variables (P=0.02). This means that as the time of exposure to moisture increases, the moisture content also rises, which, in this context, exceeds the safe limit of 2%. These results are indicative of a

critical issue, as the moisture content surpassing KEBS standards for peanut butter renders the product unsafe for consumption, potentially leading to issues related to quality and safety.

4.5. CONCLUSION

- Nixtamalization has proven to be effective in reducing the aflatoxin contamination levels of peanuts.
- The method has a positive effect on the calcium content as it significantly increases with time of exposure.
- The moisture content however goes above the set limit for peanut butter.

4.6. RECOMMENDATIONS

- Robust measures should be implemented, encompassing strict storage and handling practices for peanuts and enhancing quality control procedures at Nyamakima market. risks associated with aflatoxin contamination and the paramount importance of adhering to food safety guidelines.
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- It is recommended to support research initiatives aimed at developing aflatoxin-resistant peanut varieties and the implementation of innovative post-harvest technologies to effectively reduce aflatoxin levels.