

Authenticating peanut butter and yoghurt in the Kumasi Metropolis of Ghana using near infrared spectroscopy

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ABSTRACT

Peanut butter and yoghurt are targeted for adulteration intended at consumer deception. This study aimed to fingerprint and detect peanut butter and yoghurt adulteration with cassava flour and starch using Near Infrared Spectroscopy (NIRS) in a quasi-experimental approach. Ingredients for laboratory sample preparation were obtained from the Kumasi Metropolis. Peanut butter was adulterated at 1, 3, 5, 10, 15, 20% w/w and yoghurt at 0.25, 0.5, 1, 3, 5, 10, 15, 20, 25, 45, 50% w/w. Selected concentrations mimicked practices on the market. Marketed products were randomly sampled from six markets in the Kumasi Metropolis to validate the study models. Samples were scanned with a hand-held NIRS in triplicates. Chemometric (Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA) and Partial Least Square Regression (PLSR) models) statistical methods were employed to develop classification and prediction models. Peaks with spectral bands such as 1050, 1200 and 1450 nm were observed for peanut butter and 990–1100 nm, 1100–1200 nm and 1300–1408 nm were observed for yoghurt in the NIR spectrum. Some yoghurt brands were suspected of containing cassava starch, while Peanut butter from the different markets differed based on classification models. Cassava flour and starch concentrations were quantitatively predicted by PLSR with an R^2_{CV} of 0.98 and an error of 0.9 g/100 g (low error).

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KEYWORDS

chemometrics, adulteration, cassava starch, cassava flour

INTRODUCTION

Most diets consist of at least one protein portion and a variety of main meals and desserts being proteinaceous. Peanut butter and yogurt have featured in various meals in Ghanaian cuisine, and are regarded very beneficial for various health concerns (Mattes et al., 2008). Their ability to feature as part of a main meal, as a snack or even as a comfort food makes them highly patronized by many (Adeboye et al., 2018). Adding to this, their versatility has also led to a rapid increase in several peanut butter and yoghurt-producing start-ups. The high cost of milk poses a challenge for yogurt start-ups and industries to produce pure products, creating an avenue for economically motivated adulteration of this nutritious food. A survey done by Jolly et al. (2008) found that about 32.0% of Ghanaian respondents are ingesting peanut butter and/or 80% of Ghanaians are consuming peanut butter in a week, and the study by Meng et al. (2017) found that roughly 40.0% of respondents in urban Ghana consumed peanut butter in a comparable survey, indicating that a large population of the country patronizes peanut butter. It is, however, worthy to note that in a country where the purchase of commercial thickeners may be expensive, these yogurt and peanut butter start-ups use less expensive thickeners such as starches from natural sources; cassava starch being the least expensive and most readily available, to maximize their profits from an already booming market.

Adding peanut butter to one's diet does not result in weight gain or an increase in body weight. People's appetites are restrained via peanut butter consumption owing to its fullness effect (Schwartz et al., 2008). Furthermore, according to Schwartz et al. (2008), peanut butter, which is commonly referred to in Ghana and the majority of Sub-Saharan Africa and the rest of the world, is rich in monounsaturated fats and B-sitosterol, both of these helps to prevent cancer in humans. As a source of protein, it is consumed by most people all around the globe. Food adulteration refers to the act of lowering the quality of food supplied for sale, either by the addition of inferior components or the removal of a valued element (Kar et al., 2019). According to MacArthur et al. (2020), most foods are adulterated for financial benefit, to make food more palatable, to improve organoleptic features, and to protect nutritional properties. According to Oti (2021), food adulteration has several serious health implications, including liver damage, cancer, and respiratory tract infections, as well as shortened lifespan and food allergies.

The thickeners are highly susceptible to being used as adulterants due to their ability to improve the semi-solid and viscous nature of yoghurt and peanut butter. A study by Oti (2021) declared peanut butter as one of the most adulterated food commodities in Ghana, with additions of vegetable oil, water, corn flour, cassava flour, and roasted maize meal being used as adulterants. This fact is corroborated by Essuman et al. (2022) in a similar survey conducted to alert Ghanaians about this act. Based on the Government of Ghana Food and Drugs Law, 1992, PNDCL, 305B, all dairy products containing melamine, including baby formula, are banned in Ghana. This ban does its part in making sure that dairy products do not contain melamine. However, a subtle adulteration of our dairy products still goes on, with the addition of substances such as water, sugar, and flour, usually in unacceptable quantities, to make up for a lack



or deficit in an important ingredient, milk. With a lot of literature recording the adulteration of milk with substances such as vegetable oil, urea, water, detergent, salt, and sugar, there is a need for other dairy products like yoghurt to be checked to ensure their safety and quality thereof. The value of milk is solely dependent on its protein content, and most of the protein determination methods are dependent on nitrogen assays. Though adulteration may pose adverse health effects on consumers (especially when high-value ingredients are compromised with cheaper and more commercially available ingredients), such practices may also affect the growth of the market and undermine consumer confidence (Alamprese et al., 2013).

NIRS is a non-destructive analytical device that utilizes light absorption patterns at wavelengths ranging from 750 to 2,500 nm and is used in conjunction with chemometrics to determine food chemical fingerprints. Food raw ingredients, process intermediates, and end products are frequently analysed using NIRS to provide a compositional, functional, and sensory overview (Zinia Zaukuu et al., 2020). NIRS has the amazing benefit of requiring little to no sample preparation. Because NIRS requires little or no sample preparation it means that the analysis is quick (from less than a second to several minutes), safe (for both the user and the analyte), and may be done online (Zinia Zaukuu et al., 2020). The detection of new wheat breeds, proximate analysis on meat, non-destructive fruit ripeness assessment, moisture determination in confectionery, sample authenticity, and transgenic product identification in comparison to conventional techniques are just a few of the food application fields where NIR spectroscopy has been applied (Qu et al., 2015).

NIR spectroscopy with chemometric techniques have recorded some success in the authentication of yogurt and peanut. For example, in a study by Paixao Teixeira et al. (2021), NIR spectroscopy was employed for the detection of protein adulterants in yogurt and cheese made from goat milk. The chemometric technique; PLS-DA was most helpful in the detection recording sensitivity and specificity of 100% in calibration and cross validation.

Although the exploration of NIR in detecting peanut butter adulterated is limited, there have been reports of detection and quantification of peanut contamination in garlic powder using NIR sensors and machine learning (Schmitt et al., 2020). With issues of adulteration and the high demand for quality by consumers, there arises a need to find more efficient methods in terms of ease, accuracy, and quickness in the analysis of authenticity and quality of food products; semi-solid products especially due to their high tendency of them being adulterated.

This study sought to fingerprint and detect cassava flour and starch adulteration in peanut butter and yogurt, and to assess the applicability of using NIR spectroscopy.

MATERIALS AND METHODS

Sample collection

Authentic peanut kernels, milk powder, and adulterants (cassava flour known as 'konkonte' and cassava starch) were purchased from a reliable source from identified and defined individuals selected randomly for this study (Zaukuu et al., 2019). They were kept in ziplock bags and were then taken to the laboratory for processing. The milk powder was used to make yogurt from scratch with culture added after sterilization of the milk. Three peanut butter and yoghurt samples each were sampled from the seven selected markets: *Suame, Kejetia, Bantama*,



Ayeduse, Tech, Ayigya, and Kumasi City Mall. All raw materials were transported aseptically to the laboratory to be analysed.

Sample preparation

Laboratory preparation of yogurt. For yogurt samples, the adulterated concentrations were 0, 0.25, 0.5, 1, 3, and 5% w/w starch. Adulteration levels started at 0.25% because that is the lower limit of the industrially accepted range for adding starches to yogurt. The yogurts were produced according to the method of [Ribeiro et al. \(2014\)](#). The method required that milk powder be solubilised in 100 mL distilled water before being heat treated (85 °C/30 min) and brought to cool to 4 °C, with the addition of 2% w/w of the lyophilised culture of *S. thermophilus* and *L. bulgaricus* (YC-X11-CHANSERV). Incubation was maintained at 45 °C, and pH was controlled during the entire procedure. Fermentation was only interrupted when pH reached 4.9 by rapid cooling of the samples in an ice bath. The samples were kept at a refrigeration temperature of 4 °C. To suit economically motivated adulteration purposes, concentrations of 0, 0.25, 1, 5, 10, 15, 20, 25, 45, and 50% starch/milk powder were further prepared. Since samples were prepared in triplicate, there was a total sample size of 42 (30 laboratory samples and 12 market samples). The market samples were collected based on their availability. Ten grams of each sample was placed in ziplock bags for NIR spectroscopy measurements.

Laboratory preparation of peanut butter. The peanut butter was prepared according to [Agbenorhevi \(2017\)](#). A top pan balance was used to weigh the peanut kernels which were cleaned and sorted to remove dirt, bad nuts (based on red color), and sand. They were then roasted at 400 °C for thirty minutes using a Memmert oven (Ghent, Belgium). Finally, they were cooled dehulled, and ground into a fine butter using a Kimatsu blender (Maharashtra, India) at a speed of 8 rpm for forty minutes. This served as the control. Peanut butter was measured using an analytical balance with a series of adulteration using cassava flour. The adulterated concentrations of cassava flour were 0, 1, 3, 5, 10, 15, and 20% w/w ([Zaukuu et al., 2019](#)). The samples were prepared in triplicates. A total of 36 (21 laboratory and 15 market) samples were obtained. Ten grams of each sample was placed in ziplock bags for NIR spectroscopy measurements.

Near infrared spectroscopy measurement

Each triplicate sample from both experimental setups (yogurt and peanut butter adulteration) was scanned using the DLP NIRScanNano instrument (Texas Instruments, Dallas, TX, USA). Spectral measurements were taken directly from the samples in ziplock bags, with the sample pushed to the bottom of one side of the bag with the help of a rubber band ([Fig. 1](#)). Each sample was scanned 5 times and the spectral data were then collected for further analysis. In total there were 210 recorded spectra for yogurt samples and 180 for peanut butter samples. Yogurt and peanut butter made with 0% starch served as the positive control. The light-emitting screen of the NIR device was in proximity to the external layer of the samples throughout each capture to guarantee that the sensor window was completely obscured ([Fig. 1](#)). The spectral data were obtained in the wavelength region of 950–1700 nm with a 3 nm spectral resolution by the diffuse reflectance model.



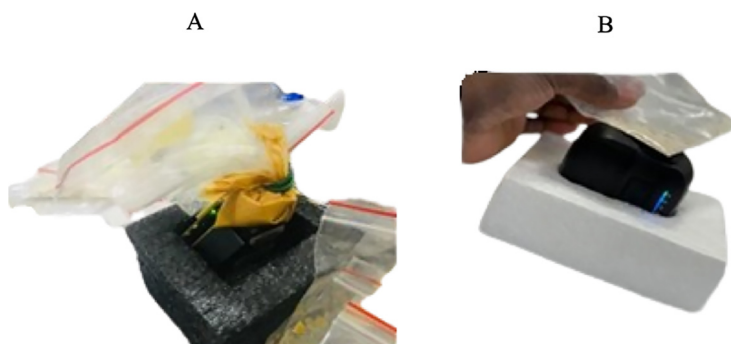


Fig. 1. NIR scanning of peanut butter (A) and yogurt (B) samples using the hand-held spectrophotometer

Spectral pre-processing. Savitsky Golay smoothing (filter 21, no derivation) was applied in combination with standard normal variate (SNV), multiplicative scatter correction and detrend. Only results of the best pre-processing techniques were presented and discussed in this study. Savitsky Golay smoothing is often applied to remove peak overlap and linear and constant baseline shifts between samples (Gallagher, 2020) that is why it was used in combination with other pre-processing techniques. All spectra analysis was done using R-project at the wavelength range of 950–1,650 after truncating the extremities of the spectra.

Multivariate data analysis

Principal components analysis (PCA), principal components analysis-linear discriminant analysis (PCA-LDA), and partial least regression models (PLSR) were built to visualize and quantify yogurt and peanut butter adulteration.

PCA. PCA, which is an unsupervised qualitative linear transformation approach, was first used to visualise pattern recognition in data and remove outliers in multivariate data analysis and chemometric analysis.

PCA-LDA. The supervised linear transformation approach (PCA-LDA) was utilised to generate a multi-class classification of the different samples. The optimal number of PCs was chosen based on the ability to provide the best accuracy of validation and to minimise any differences between the accuracies of training and validation (Aouadi, 2023). Three-fold cross-validation (CV) was utilised to assess the predictive value of the LDA results. To achieve this, the datasets were divided into two groups: a training set and a validation set. The first and second replicates' spectra served as the training sets. The third replicate's spectra served as the validation sets. To be sure that every replicate had been replaced in the training and validation sets, the data splitting process was carried out three times. The statistical measures utilised to evaluate the LDA models' performance were the average recognition accuracy for training and the average prediction accuracy for validation (Zaukuu et al., 2023).

PLSR. PLS regression models were developed to predict the concentration of cassava starch in yogurt and cassava flour in peanut butter. To predict the adulteration of peanut butter with



cassava flour, partial least squares (PLS) regression models were utilised. Three-fold cross-validation (CV) was utilised to assess the predictive value of the regression in PLSR. To achieve this, the datasets were divided into two groups: a training set and a validation set. The first and second replicates' spectra served as the training sets. The third replicate's spectra served as the validation sets. To be sure that every replicate had been replaced in the training and validation sets, the data splitting process was carried out three times. The performance of the PLSR models was evaluated statistically using the Root Mean Square Error, the coefficient of determination in calibration (RMSEC and R2C), and cross-validation (RMSECV and R2CV), respectively. The minimum RMSECV value was used to calculate the ideal number of latent variables.

RESULTS AND DISCUSSION

NIR raw spectra

NIR raw spectra for yogurt. Figure 2 shows the raw spectra for yogurt prepared in the laboratory indicating the various levels of starch in milk powder adulteration. i.e., 0–5% w/w. Spectral analysis revealed the presence of four distinct spots with significant bands. Considering the spectra of the yogurt, the bands were observed in region 1(R1) (990–1,100 nm), which can be related to the 2nd overtone of O–H stretch. Region R2 (1,100–1,200 nm) can be related to the 2nd overtone of CH stretch while region R3 (1,300–1,408 nm) and R4 (1,488–1,450 nm) are characteristic of pure water (Raypah et al., 2022). Wavelengths associated with starch are seen present at the R1 and R2 regions with the C–H and O–H bonds.

NIR raw spectra for peanut butter. Figure 3 shows spectra of peanut butter samples prepared in the laboratory (containing 0–20% w/w cassava flour) and also those from the market samples with 0% w/w serving as the control. A study by Wu and Sun (2013) indicated that the spectral band 1,050 nm is attributed to the H–O–H third overtone. The second peak can also be observed

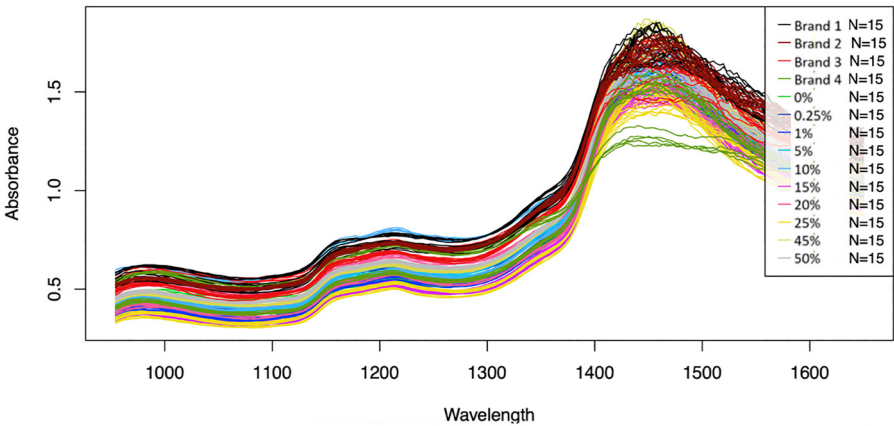


Fig. 2. NIR spectra of unadulterated yogurt and adulterated yogurt sample



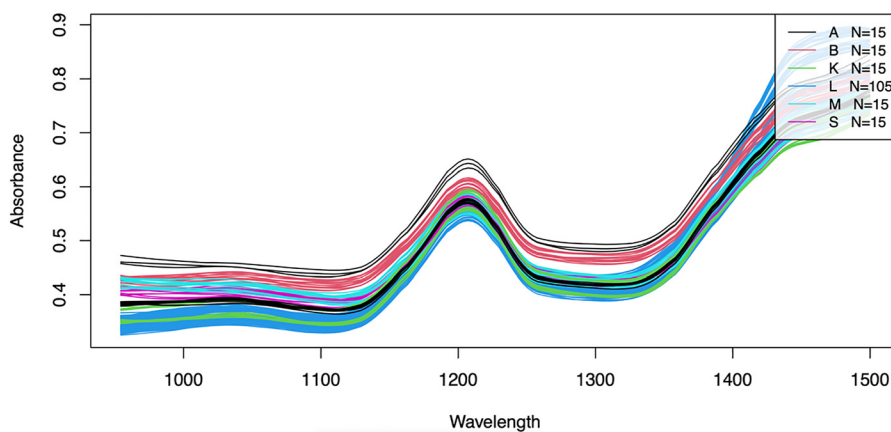


Fig. 3. NIR spectra of artificially adulterated peanut butter and peanut butter samples from markets at a wavelength range of 950–1,650 nm

at region 2 (R2) (1,200 nm). [Dos Santos Augusto et al. \(2017\)](#) revealed that the spectral band 1,200 nm is related to the C–H stretching second overtone, second and first overtone regions of N–H bonds. The absorption of lipids and carbohydrates could be attributed to the wavelength of 1,050 nm and 1,200 nm spectral bands, respectively, while the second and first overtone regions of N–H bonds are characteristic of their high protein content. Furthermore, the highest and last peak was recorded at region 3 (R3), which is the 1,450 nm band which indicates the first overtone vibration of water and the first overtone vibration of starch. The absorption of the adulterant (cassava flour) is attributed to the wavelength of 1,450 nm in the NIR spectrum ([Song et al., 2018](#)). The peanut butter containing the highest cassava flour (20% w/w) showed the most absorbance while the control (0% w/w) showed the least absorbance. From [Fig. 3](#), peanut butter from the different markets and laboratories (containing 1–20% w/w cassava flour) showed the same wavelength path and the same peaks or spectral bands in the respective regions. It can therefore be hypothesised that peanut butter sold in Kumasi Metropolitan assembly may contain cassava flour as shown in [Figs 4 and 6](#) from the survey conducted.

Principal component analysis and linear discriminant analysis

Principal component analysis and linear discriminant analysis of yogurt samples. No observable patterns were seen in the PCA plot ([Fig. 4](#)) containing artificially adulterated yogurt samples, therefore further chemometric analysis was required.

[Figure 5](#) shows the classification plot for artificially adulterated yogurt samples (A) and also comparing the artificially adulterated samples to the market samples. Samples with different adulterant concentrations could be correctly classified in [Fig. 5A](#). There were few overlapping samples in [Fig. 5B](#) suggesting some similarities. For example, yogurt brand 4 from the market overlapped with the sample containing 45% w/w cassava starch. However, it can be observed that market samples labelled Brand 1, 2 and 3 could be clearly distinguished as not containing starch.



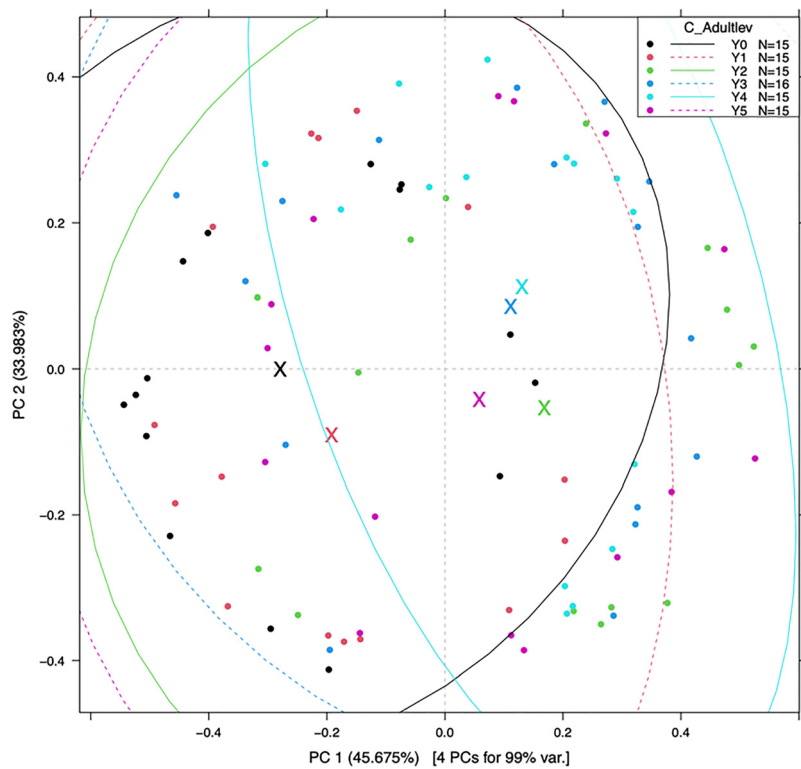


Fig. 4. PCA plot of laboratory samples at the wavelength range of 950–1,650 nm

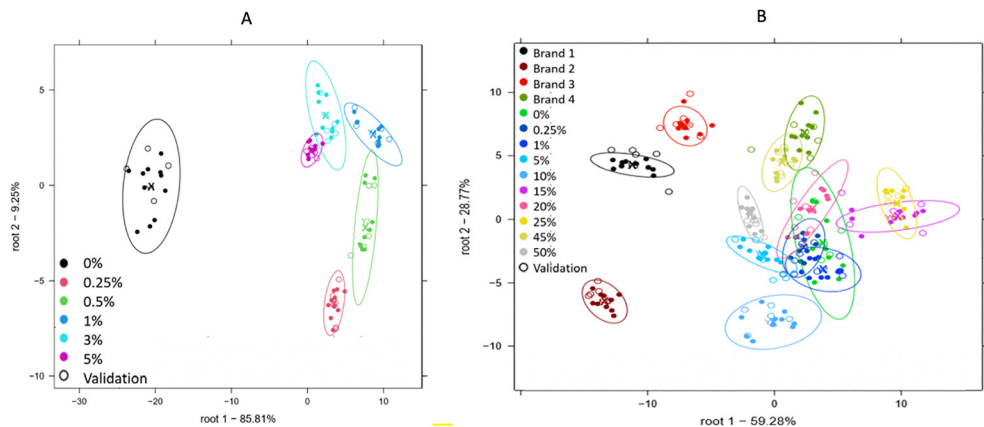


Fig. 5. LDA plot of yogurt adulterated with cassava starch at six different concentrations (A) and market and laboratory samples (B) at a wavelength of 950–1,650 nm



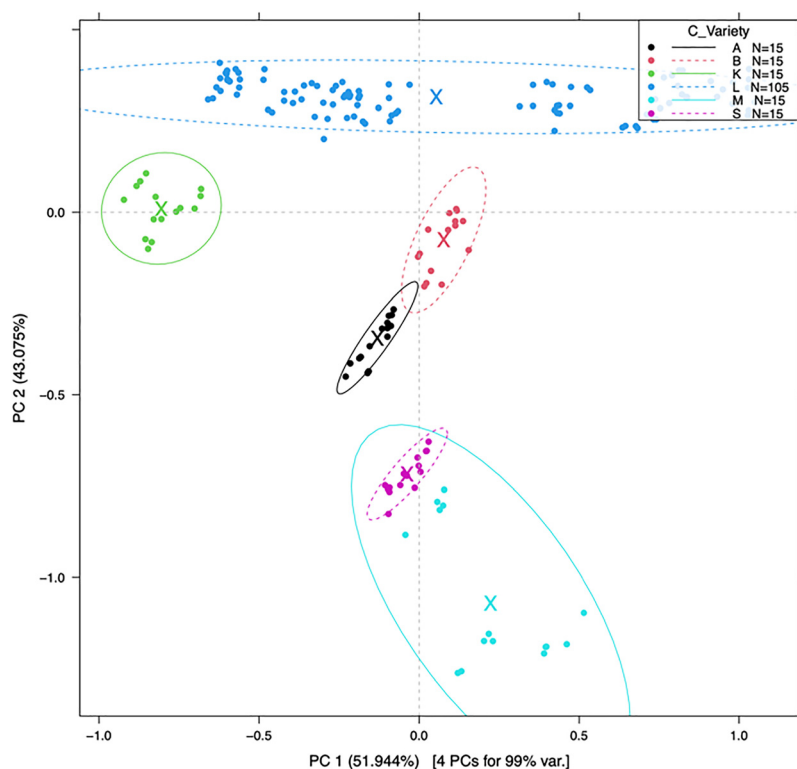


Fig. 6. PCA plot of laboratory and market samples at the wavelength range of 950–1,650 nm

There was 100% average classification accuracy in prediction when models were developed to classify artificially adulterated yogurt samples. The average prediction accuracy was 97.84% average prediction when concentration levels and brands of yogurt were used as class variables.

Principal component analysis and linear discriminant analysis of peanut butter samples. Figure 6 shows the PCA plot for the visualisation of peanut butter samples. It can be observed from the plot PC1 and PC2 contributed to most of the variance between the samples. All the market samples from the different location could be visually discriminated in the plot. This could imply a variation in groundnut paste processing methods in the different markets.

From Fig. 7A it appears that there was an increasing pattern of adulteration from 0 to 20% corn flour. There was a distinct pattern of separation based on the selected adulteration concentration, which ranged from 1 to 20% w/w cassava flour even in the lesser amounts of 1, 3, and 5% cassava flour. This suggests successful categorisation, as seen by the average recognition accuracy of 100% and prediction accuracy of 100%. As a result, pure peanut butter and all other concentration levels could be successfully separated from one another using concentration levels as the class variable.

In Fig. 7B, there was a distinct pattern of separation between concentration levels (laboratory samples) and markets (100% classification). Thus, all the other markets were classified separately



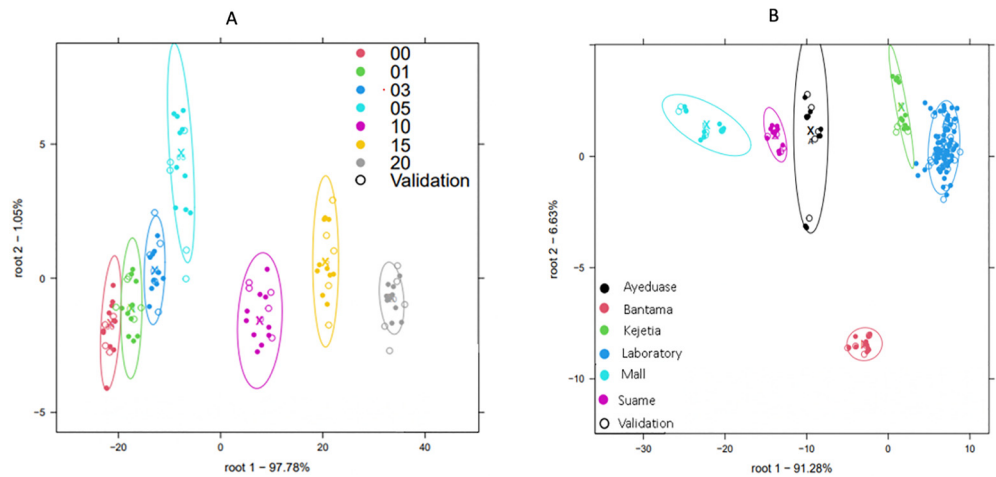


Fig. 7. LDA plot of peanut butter Adulterated with cassava flour at six different concentrations (A) and in comparison, to market samples (B)

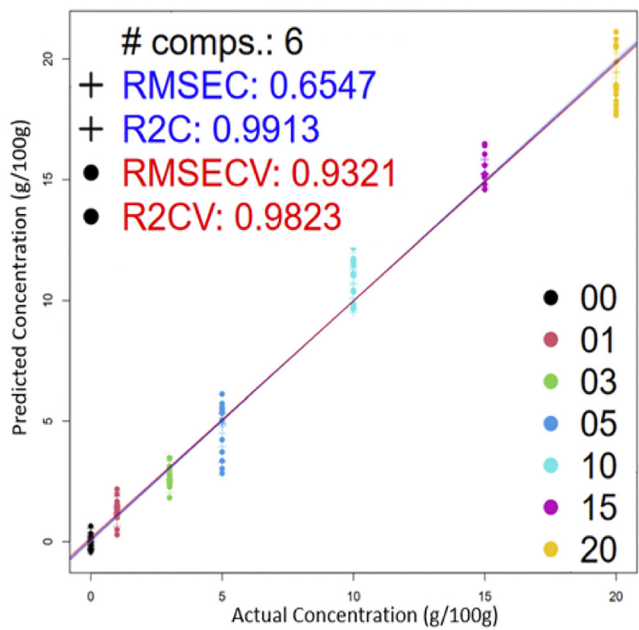


Fig. 8. PLSR for the quantification of cassava flour in peanut butter samples at a wavelength range of 950–1,650 nm

from each other. Peanut butter from the kejetia market is relatively close to laboratory samples containing 1–20% w/w cassava flour (Fig. 7B). This means that peanut butter from the kejetia



market is suspected to contain cassava flour. Good classification results indicate that by using distinct markets and concentration levels as the class variables, concentration levels can be successfully separated from the various markets when using NIRS as a rapid technique.

Partial least squares regression results

Figure 8 shows the predictive PLSR models developed to predict cassava flour concentration in groundnut paste using handheld NIR spectroscopy. The model proved to be very sensitive to cassava flour adulteration, thus with an R^2C and R^2CV (high accuracy) of 0.99 and 0.98 respectively. The PLSR model for the experiment successfully predicted the adulteration of groundnut paste with cassava flour. The model had an RMSECV and an RMSEC (low error) of 0.7 g/100°g and 0.9 g/100°g respectively after cross-validation. An ideal prediction condition is an R^2 value below 1. RMSEs should always be acquired much closer to 0, and R^2 should always be closer to 1. However, R^2 of over 0.90 indicates outstanding performance, and less than 0.82 indicates subpar performance, according to Su and Sun (2017). This indicates that our model has good accuracy and low error in predicting 20, 15, 10, 5, 3, and 1% adulteration of cassava flour in peanut butter. Weak model parameters were obtained for the prediction of cassava starch in yogurt. This may be due to the low concentration of adulterants.

CONCLUSION

The NIR spectroscopy could be used to detect cassava flour and starch adulteration in yogurt respectively with excellent accuracy. When NIR spectroscopy is coupled with chemometric analytical tools and used to cross-validate market samples, the prediction accuracy recorded is very high, making it suitable and recommended for usage and adoption by regulatory bodies to be employed for rapid and easier detection of other forms of adulteration not yet reported in yogurt and peanut butter and also for further detection of adulteration in other food commodities with a high probability of being adulterated and those popularly adulterated but literature not providing elaborate knowledge on.

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