Impact of fermentation time and drying method on the physicochemical parameters of okra (*Abelmoschus esculentus*) consumed in Côte d'Ivoire

ABSTRACT

Aims: The objective of this study is to determine the influence of fermentation time and drying method on ~~certain~~ the physicochemical, mineral, and amino acid profile ~~parameters~~ of okra (*Abelmoschus esculentus*).

Study design: ~~Okra is a vegetable widely consumed in Côte d'Ivoire in various forms. All parts of the plant are used in food and medicine. In particular, its fresh fruits, cut or uncut, sometimes dried and ground into powder, are mainly used in the preparation of sauces. Like other green vegetables, okra is highly perishable. To extend its shelf life, it is often subjected to a pre-cooking process followed by fermentation before drying. The fermentation time and drying method have a significant impact on the quality of the finished product. The variability of these processes leads to considerable variability in the finished product, which calls into question its quality control and limits its value in the food and even technology sectors.~~ Blanched okra was subjected to natural fermentation followed by drying to produce eight distinct samples, enabling a systematic analysis of the processing effects.

Place and Duration of Study: The study was conducted at the Laboratoire de Biocatalyse et des Bioprocédés, Université Nangui Abrogoua, between January and March 2025.

Methodology: ~~To this end, e~~Eight okra samples were prepared based on fermentation duration and drying method, ~~named E0, E1, E3, E5, S0, S1, S3 and S5, were produced using different processes~~. The impact of these processes on physicochemical parameters, mineral composition, and amino acid composition was ~~then~~ determined using standard analytical methods. The data obtained were analysed using ANOVA and Principal Component Analysis (PCA).

Results: ~~The physicochemical analysis of the okra samples reveals significant variations between them. Sample S5 has the most acidic pH (4.01±0.07) and the highest ash content (10.53±0.01%). Sample E1 has the lowest moisture content (12.51±0.01%). Sample S1 has the highest fibre content (2.94±0.02%) and reducing sugar content (5.55%). Samples E3 and S3 show the highest levels (17.30±0.02 and 17.44±0.02 respectively). In terms of minerals, sample E1 has the highest levels of iron (48.66±1.33 mg/100g), sodium (22.20±1.21 mg/100g) and potassium (350.17±2.08). Sample E3, with 126.15±3.15 mg/100g and 100.09±2.55 mg/100g, had the highest calcium and magnesium content. Finally, the most abundant amino acids are histidine, tryptophan, proline and alanine, mainly in samples E1 (1,979±0.001 mg/100g); S3 (2.562±0.002 mg/100g); E3 (2.733±0.004 mg/100g) and E1 (3.621±0.006 mg/100g).~~ The finding showed that fermentation time and drying method has a significant influence on all parameter. A decreasing trend can be observed in pH (minimum value of 4.01), total sugars, and fat content with the increase of fermentation time. Conversely, the ash and specific mineral contents (Fe: 48.66 mg/100g; K: 350.17 mg/100g) increased, peaking at day 1 – 3. Similar to the protein and amino acid (proline: 2.733 mg/100g; alanine: 3.621 mg/100g) were also highest in samples fermented for 1-3 days. It was also demonstrated that oven-dried sample has better retention of most nutrient consistently compared to sun-dried samples.

Conclusion: The study shows that short-term fermentation (1 to 3 days) coupled with oven drying significantly improves the nutritional value of okra by enriching ~~increasing~~ its protein, amino acid and mineral content, while reducing sugar levels and pH. ~~Oven drying preserves~~

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~~nutrients better than sun drying, ensuring greater product stability and quality. These results highlight the benefits of incorporating controlled fermentation and moderate thermal drying into artisanal processes to produce nutritious and stable okra powders.~~ These findings advocate for adopting controlled fermentation and moderate thermal drying in artisanal processing to produce high-quality, stable, and nutritious okra-based food ingredients.

Keywords: okra, Abelmoschus esculentus, fermentation, drying, biochemical parameters.

1. INTRODUCTION

Okra is a plant belonging to the Malvaceae family and the *Abelmoschus* genus. It is cultivated in Southeast Asia, America, and Africa (Tiendrébéogo et al., 2010). Its annual production worldwide is estimated at around 5 to 6 million tons. It is cultivated for its fruits, leaves, and seeds, which are rich in fiber. In West Africa, okra ranks second in vegetable production behind tomatoes (Hamon and Charier, 1997). In Côte d'Ivoire, okra (*Abelmoschus* spp.) is a vegetable that can be consumed in various forms (fresh, fried, cooked and dried) after undergoing different technological processes.
Okra is an exceptional plant particularly due to the fact that all its components (roots, stems, leaves, fruits, and seeds) can be used in food, medicine, crafts and even industrial applications (Njock et al., 2025). Fresh fruit, whether cut or uncut, sometimes dried and processed into powder, is mainly used in the preparation of sauces (Grubben et al., 2004). The fruits are generally harvested at the juvenile stage between the 3rd and 5th day after flowering. Okra is adapted to tropical conditions and is cultivated throughout Côte d'Ivoire (Fondio et al., 2007). Okra is very beneficial for the digestive system due to its high content of polysaccharides and micronutrients (Adelakun et al., 2009 ; N'guessan et al., 2018). In addition, the fruit contains flavonoids, polyphenolic compounds, vitamins E and C, and antioxidants (Atawodi et al., 2009). Like other green vegetables, okra exhibits a high degree of perishability. To extend its shelf life, it is frequently subjected to drying or, in some cases, pre-cooking and fermentation prior to drying. Fermentation is a technology that is readily available to populations and allows for food preservation. It also improves the digestibility of the raw material and enhances the taste and aroma of foods (Motarjemi, 2002).
The microorganisms involved generally come from the raw materials used, the equipment, the production environment, or part of the previous production (Aidoo et al., 1994). However, uncontrolled fermentation makes the quality of the final product variable and compromises its preservation (Nanadourn et al., 2006). Furthermore, the drying method also influences nutrient retention (Silou et al., 2002). The aim of this study is to determine the influence of fermentation time and drying method on certain physicochemical parameters of okra.

2. MATERIAL AND METHODS

2.1. PLANT MATERIAL

The plant material used in this study ~~consisted was~~ ~~of~~ okra (Abelmoschus esculentus). After blanching ~~that had been blanched~~ at 100 °C for three minutes, the okra was subjected to ~~then fermented for~~ different fermentation ~~lengths of~~ times (0, 1, 3 and 5 days) and subsequently dried using one of two methods: oven drying and sun drying. This process resulted in ~~A total of~~ eight samples, ~~which are were prepared,~~ four oven-dried and four sun-dried. The ~~Of the eight samples,~~ two unfermented (0-day) samples served as controls for ~~both drying methods~~their respective drying methods.

2.2 METHODS

2.2.1. Physicochemical Analyses

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The moisture, ash, protein, lipid, crude fibre and pH content was determined in accordance with AOAC (2000) standard methods.

2.2.2. Determination of the content of ethanol-soluble sugars

2.2.2.1. Extraction method

The extraction of ethanol-soluble sugars from the okra samples was carried out according to the method described by Martinez-Herrera et al. (2006). To do this, one gram of each sample was placed in a centrifuge tube, to which 10 mL of 80% ethanol (v/v) was added. The mixture was thoroughly homogenised and then centrifuged at 4200 rpm for 10 minutes using a centrifuge (SIGMA 3-16P, Germany). The supernatant obtained was collected and transferred to a 50 mL Erlenmeyer flask. The residue was then taken up in 10 mL of the same solvent and subjected again to the same extraction conditions. The second supernatant was combined with the first in the Erlenmeyer flask. The ethanol contained in the mixture was then evaporated in a water bath until reduced to approximately one third of the initial volume. Finally, the concentrated extract was adjusted to a final volume of 10 mL, constituting the solution used for the measurement of ethanol-soluble sugars.

2.2.2.2. Reducing sugar analysis

The quantification of reducing sugars present in the various okra samples was carried out using the Bernfeld method (1955), based on the use of 3,5-dinitrosalicylic acid (DNS). A volume of 150 µL of ethanol-soluble extract was placed in a test tube and mixed with 300 µL of DNS reagent. The mixture was heated in a boiling water bath for 5 minutes before being cooled to room temperature for 5 minutes. Subsequently, 2 mL of distilled water was added to complete the reaction medium. The absorbance of the solution was then measured at 540 nm using a spectrophotometer (Model MS-V5100, Spain), in comparison with a control containing 150 µL of distilled water and 300 µL of DNS. The absorbance values obtained were converted into reducing sugar concentrations using a calibration curve established with a 2 mg/mL glucose solution.

2.2.2.3. Total sugars analysis

The total sugar content in the various okra samples was determined using the method described by Dubois et al. (1956), which involves the use of phenol and concentrated sulphuric acid. To do this, 150 µL of ethanol-soluble extract was placed in a test tube, then 1 mL of 5% (w/v) phenol and 1 mL of concentrated sulphuric acid (97%) were added successively. After homogenisation, the reaction mixture was left to cool for 5 minutes at room temperature. The absorbance was then measured at 490 nm using a spectrophotometer (Model MS-V5100, Spain), using a tube containing 150 µL of distilled water instead of the extract as a control. The optical density values obtained were converted into total sugar concentrations using a calibration curve prepared with a 2 mg/mL glucose solution.

2.2.3. Amino acids

The amino acid composition of each okra sample was analysed using an Applied Biosystems high-performance liquid chromatograph (HPLC), model 172 A (Applera Corporation, Foster City, California, USA), equipped with a PTC RP-18 column (2.1 mm × 22 cm). Prior to injection, the proteins in the okra samples were subjected to acid hydrolysis in 6 M HCl containing 1% phenol, carried out at 150°C for 60 minutes in the Pico-Tag system (Waters, Milford, Massachusetts, USA). The released amino acids were then derivatised with phenylisothiocyanates (PITC) and separated by HPLC. Elution was performed using sodium acetate buffers (45 mM, pH 5.9) and (105 mM, pH 4.6, 30%), as well as a mixture of acetonitrile/water (70%).

A calibration chromatogram was first established using standard amino acids. The amino acids present in the okra samples were identified by comparing their retention times with those of the standards. Concentrations were calculated from the average peak areas corresponding to each standard amino acid. The content (EC) (in g/100 g of protein, relative

$$CE = \frac{Area\ E \times CT}{Area\ T}$$

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to dry matter) of each amino acid in the different samples was determined according to the following mathematical relationship:
CE: Concentration of each amino acid in the sample
Area T: Average area of standard amino acid peaks
Area E: Area of the amino acid peak in the sample
CT: Concentration of standard amino acid

2.2.4. Mineral analysis

The quantification of macroelements (K, Ca, Na, Mg and P) and microelements (Fe) was carried out using energy dispersive spectrometry (EDS) coupled with a scanning electron microscope (SEM). To do this, 10 mg of ash residue obtained by incinerating 2 g of okra powder in a muffle furnace was evenly deposited on a pad covered with double-sided carbon adhesive tape. The prepared sample was then attached to the SEM sample holder, connected to an EDS microanalysis platform (Inca Dry Cool, operating without liquid nitrogen). Finally, the sample holder containing the ashes was placed on the stage of the SEM analysis chamber, where qualitative and quantitative measurements of the mineral elements were performed.

2.2.5. Statistical analyses

All physicochemical analyses were performed in three independent replicates. The results obtained are presented as arithmetic means with their corresponding standard deviations. A one-way analysis of variance (ANOVA) was applied to these data to assess the presence of significant differences between the means, at a significance level of 5%. When significant differences were observed, they were specified using Duncan's multiple comparison test, performed with STATISTICA software (version 12, ©StatSoft, Inc., 1984-2014). In addition, a principal component analysis (PCA) was conducted using XLSTAT software (version 2016) to further the multivariate interpretation of the results.

Refer to the following articles and revise the method use:

1. Phytochemical Screening, Nutritional Value, Anti-Diabetic, Anti-Cancer, and Anti-Bacterial Assessment of Aqueous Extract from Abelmoschus esculentus Pods (Khan et al. 2022): **https://doi.org/10.3390/pr10020183**
2. Reducing Sugar, Total Phenolic Content, and Antioxidant Potential of Nepalese Plants (Khatri & Chhetri, 2020): **https://doi.org/10.1155/2020/7296859**
3. Characterization and Phytochemical Property of Okra Fruits (Adekanmi et al. 2020)
4. Effect of the Ethanol Extract of Red Okra Pods (Abelmoschus esculentus (L.) Moench) to Inhibit Cervical Cancer Cells Growth through Cell Cycle-Associated Oncogenes (Nisa et al. 2022): doi: 10.1155/2022/1094771
5. Ethanolic extract of okra has a potential gastroprotective effect on acute gastric lesions in Sprague Dawley rats (Yasin et al. 2020): https://doi.org/10.1002/fsn3.1963

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3. RESULTS AND DISCUSSION

3.1. ~~RESULTS~~

~~3.1.1.~~ PHYSICOCHEMICAL COMPOSITION OF FERMENTED OKRA SAMPLES

The fermentation of okra (Abelmoschus esculentus) resulted in significant changes in its physicochemical properties, which are influenced by the duration of fermentation (1, 3 and 5 days) and the drying method (oven or sun). These parameters are essential for assessing the nutritional quality and stability of the finished product. Data analysis reveals the significant impact of fermentation time and drying method on the biochemical, mineral and amino acid characteristics of fermented okra. The study is based on precise measurements taken on eight samples, divided according to two drying methods (oven and sun) and four fermentation times (0, 1, 3 and 5 days).

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Biochemically, significant differences were observed between samples from one parameter to another (Table 1). ~~Initially, the pH gradually decreased with fermentation time, falling from 4.35 to 4.11 for oven drying and from 4.30 to 4.01 for sun drying.~~ Firstly, the pH showed a gradual decrease as the fermentation time increased. For oven-dried samples, the pH decreased from 4.35 to 4.11 between 0 and 5 days of fermentation, while for sun-dried samples, it decreased from 4.30 to 4.01 from day 0 to day 5 of fermentation. This decrease is attributable to the production of organic acids by fermentative microorganisms, particularly lactobacilli, as confirmed by the work of Di Cagno et al. (2013), Montet et al. (2014) and Wu et al. (2017), who demonstrated that lactic fermentation reduces the pH of vegetables due to the formation of lactic acid. This phenomenon is crucial for product preservation, as a pH below 4.5 inhibits the growth of many pathogens (Caplice & Fitzgerald, 1999; Tayel et al., 2010).

Next, the ash content, representing the total mineral fraction, varied significantly with fermentation time and drying method. Within five days, the ash content was observed to be increasin~~ges~~ with fermentation. For oven-dried samples, it increased from 9.39% to 10.51%, and for sun-dried samples, from 11.27% to 10.53%. This increase may be linked to the concentration of minerals due to the reduction in dry matter through the degradation of organic compounds.

Furthermore, moisture content showed a downward trend during fermentation, particularly for oven-dried okra samples, where it fell from 14.73% to 13.54%. Whereas, for sun-dried samples the reduction was less pronounced with values fluctuating~~, reaching 10.51% for oven drying and 10.53% for sun drying after five days, reflecting a concentration of minerals. In addition, the moisture content decreases slightly, with values ranging from 14.73% to 12.51% for oven drying, and~~ from 14.10% to 13.02%~~ for sun drying~~. The decrease in moisture content is beneficial for product preservation, as it limits enzymatic reactions and microbial proliferation (Syamaladevi et al., 2016; Tapia et al., 2020). In fact, moisture content below 15% is generally considered safe for food powders (Jay et al., 2005; Opaliński et al., 2016; Juarez-Enriquez et al., 2022; Suhag et al., 2024).

In addition, the fibre content decreased significantly with fermentation time and drying method.~~Similarly,~~ the fibre concentration decreases significantly, from 3.17% to 1.90% for oven drying, and from 2.76% to 1.82% for sun drying. This reduction is probably due to the enzymatic action of microorganisms, which hydrolyse complex polysaccharides (Borba et al., 2019). Fermentation is known to degrade insoluble fibre, which can improve digestibility but reduce the beneficial effect of fibre on intestinal health (Swain et al., 2014).

Similarly, total sugar content also decreased during fermentation. For oven-dried samples, it fell from 6.59 mg/100 g DM to 3.08 mg/100 g DM, and for sun-dried samples, from 6.42 to 3.53 mg/100 g DM ~~As for total sugars, their content fell sharply, from 6.59 mg/100 g DM to 3.08 mg/100 g DM for oven drying, and from 6.42 to 3.53 mg/100 g DM for sun drying~~. This decrease is consistent with the use of sugars as an energy substrate by fermentative microorganisms (Trchounian & Trchounian, 2019). Indeed, simple sugars are the first metabolites consumed during fermentation, which explains their rapid decrease (Tamang et al., 2016).

With regard to reducing sugars, oven-dried samples showed a slight increase ~~their levels increase~~ from 0.32 µg/g DM at the start of fermentation, peaking at 0.80 µg/g DM on day 1 (oven drying), ~~to 0.80 µg/g DM at the start of fermentation,~~ before decreasing then stabilise at 0.36 µg/g DM after five days of fermentation~~ for oven-dried samples~~. As ~~F~~for sun-dried samples, this content fluctuates between 0.32 µg/g DM at zero days of fermentation and 0.36 µg/g DM after five days of fermentation. This variation can be explained by the

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enzymatic conversion of polysaccharides into monosaccharides, followed by their consumption (Ambye-Jensen et al., 2014). Indeed, reducing sugars are transient intermediates in fermentation metabolism (Steinkraus, 2004; Krahulec et al., 2010).

The fat content decreased significantly with fermentation from 17.59% to 9.51% for oven drying, and from 16.74% to 10.59% for sun drying. This reduction may be linked to the oxidation of lipids or their use by microorganisms. Indeed, certain fermentation microorganisms possess lipases that break down triglycerides, which could explain this loss (Nout, 2009; Kirana et al., 2016; Shamim et al., 2018). These results confirm that fermentation, by altering pH and biochemical interactions, influences the availability and concentration of minerals. It can be used as a technological lever to improve the nutritional quality of plant products, provided that the duration and drying parameters are controlled.

Finally, protein content showed variable changes. For oven-dried samples, it increased slightly to 17.30% on day 3, before decreasing to 14.34% on day 5. For sun-dried samples, it reached a maximum of 17.44% on day 3. This fluctuation can be attributed to microbial protein synthesis and plant protein degradation (Araújo et al., 2011; Dong et al., 2021). ~~protein concentrations varied little, with a slight decrease after five days of fermentation, reaching 14.34% for oven drying and 14.37% for sun drying.~~

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Table 1. Changes in the biochemical parameters of blanched and dried okra powder as a function of fermentation time

Parameters	Drying methods							
	Oven drying				Solar drying			
	Fermentation time (days)							
	0 day (E0)	1 day (E1)	3 days (E3)	5 days (E5)	0 day (S0)	1 day (S1)	3 days (S3)	5 days (S5)
pH	4.35±0.10^{d}	4.18±0.10^{b}	4.22±0.07^{c}	4.11±0.03^{a}	4.30±0.03^{d}	4.26±0.06^{c}	4.22±0.08^{b}	4.01±0.07^{a}
Ash (%)	9.39±0.01^{a}	10.20±0.01^{c}	10.10±0.01^{b}	10.51±0.01^{d}	11.27±0.13^{d}	9.66±0.03^{a}	9.92±0.01^{b}	10.53±0.01^{c}
Moisture (%)	14.73±0.02^{d}	12.51±0,01^{a}	13.59±0.01^{c}	13.54±0.02^{b}	14.10±0.01^{b}	13.02±0.02^{a}	14.26±0.01^{c}	14.25±0.01^{c}
Fibre (%)	3.17±0.10^{d}	2.68±0.07^{c}	2.11±0.01^{b}	1.90±0.01^{a}	2.76±0.02^{c}	2.94±0.02^{d}	2.07±0.01^{b}	1.82±0.09^{a}
Total sugars (mg/100g MS)	6.59±0.16^{d}	4.60±0.16^{b}	4.85±0.04^{c}	3.08±0.12^{a}	6.42±0,15^{d}	5.55±0.18^{c}	4.12±0.06^{b}	3.53±0.19^{a}
Reducing sugars (µg/g MS)	0.32±0.01^{a}	0.80±0.01^{c}	0.38±0.02^{b}	0.36±0.02^{b}	0.32±0.02^{a}	0.37±0.03^{b}	0.35±0.01^{ab}	0.36±0.02^{ab}
Crude lipid (%)	17.59±0.02^{d}	14.10±0.05^{c}	12.20±0.01^{b}	9.51±0.03^{a}	16.74±0.19^{d}	11.91±0.01^{b}	13.69±0.05^{c}	10.59±0.01^{a}
Protein (%)	16.44±0.98^{bc}	16.87±0.02^{b}	17.30±0.02^{c}	14.34±0.60^{a}	15.37±0.51^{b}	16.51±0.05^{c}	17.44±0.02^{d}	14.37±0.81^{a}

The values in the table are averages of three trials, with standard deviations. Averages marked with the same letter on the same line are not significantly different at the 5% probability threshold according to Duncan's test.

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3.~~1~~2. Discrimination of fermented okra samples based on their physicochemical compositions

The results of the principal component analysis (PCA) was applied to the physicochemical, mineral and amino acid parameters of okra in order to better understand the impact of fermentation time and drying method. It shows that the first two components explain 73.68% of the total variance. The first factor (F1) alone accounts for 49.06%, dominated by the variables pH, fibre, total sugars, fat and protein. The eigenvectors indicate that pH (0.483), fibre (0.451), total sugars (0.472), fat (0.447) and protein (0.295) are the most influential on F1. The cosine squares confirm this dominance, with values of 0.914 for pH, 0.797 for fibre, 0.875 for total sugars, 0.783 for fat and 0.341 for protein. Furthermore, the correlation matrix reveals strong relationships between certain variables. pH is strongly correlated with fibre (r = 0.821), total sugars (r = 0.893), fat (r = 0.812) and protein (r = 0.564), indicating that acidification influences these parameters. Ash is negatively correlated with protein (r = -0.571), suggesting mineral dilution with protein degradation. Moisture is negatively correlated with reducing sugars (r = -0.751), which could reflect a dry phase concentration. Fibre and total sugars are strongly correlated (r = 0.889), as are fat and total sugars (r = 0.857), reflecting a co-evolution of these components. In terms of sample observations, the most significant contributions are those of S0 on F1 (35.86%), E1 on F2 (60.55%), E3 on F7 (41.54%), E5 and S5 on F6 (45.05% and 32.40% respectively), S0 on F3 (51.80%), and S1 and S3 on F4 (40.58% and 30.31% respectively), indicating that these samples are the most influential in the construction of the principal axes.

For the abovementioned results, it reveals that the first two principal components (F1 and F2) alone account for 73.68% of total variability, with F1 representing 49.06% and F2 24.61%. The variables most strongly correlated with F1 are pH (cos^2 = 0.914), total sugars (cos^2 = 0.875), fibre (cos^2 = 0.797) and fat (cos^2 = 0.783). This indicates that F1 is essentially a nutritional quality component, linked to the energy content and structure of the product. F2, on the other hand, is dominated by moisture (cos^2 = 0.782) and reducing sugars (cos^2 = 0.780), suggesting a component linked to microbiological stability and enzymatic processing. These results are consistent with the work of Jolliffe & Cadima (2016), who emphasise that the first components capture the major contrasts in food data.

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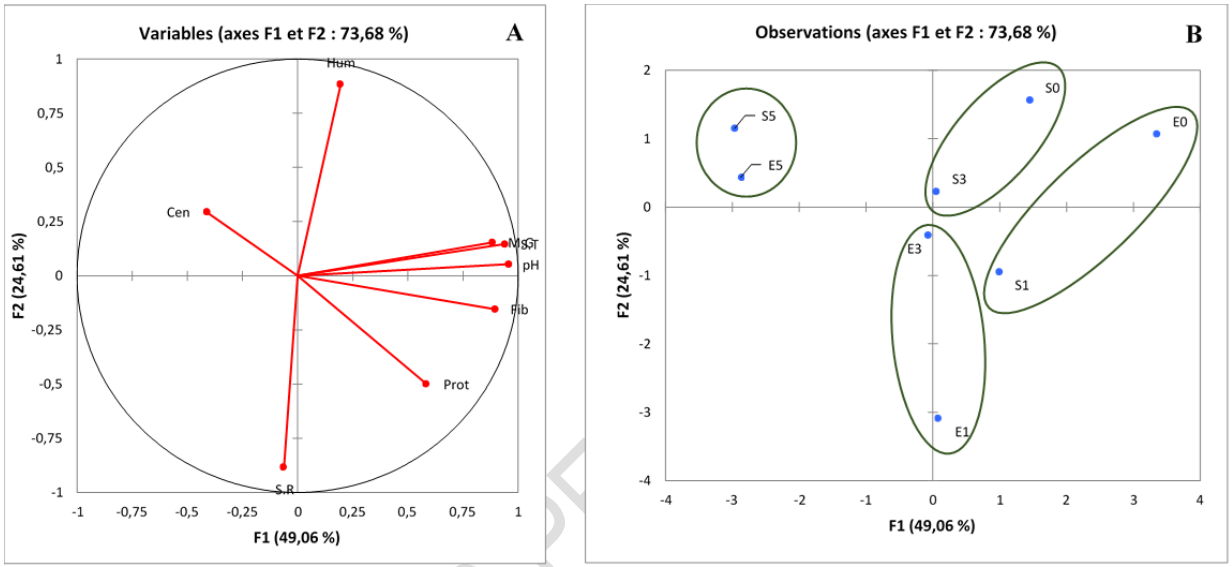

Fig 1. Projection of physicochemical compounds (A) and fermented okra samples (B) in the plane formed by axes F 1 and F 2

Cen: ash; Hum: moisture; Fib: crude fibre; S.T: total sugars; S.R: reducing sugars; M.G: Crude lipid; Prot: protein. E0: control sample bleached and dried in an oven; E1: sample bleached and dried in the study after one day of fermentation; E3: sample bleached and dried in the study after three days of fermentation; E5: sample bleached and dried in the study after five days of fermentation; S0: control sample bleached and dried in the sun; S1: sample bleached and dried in the sun after one day of fermentation; S3: sample bleached and dried in the sun after three days of fermentation; S5: sample bleached and dried in the sun after five days of fermentation.

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3.1.3. Mineral composition of fermented okra samples

The composition of macroelements (calcium, phosphorus, magnesium, potassium and sodium) and trace elements (iron) in the fermented okra samples is shown in Table 2. At a significance level of 5%, differences are observed in each sample and each parameter. The calcium (Ca) content in the fermented okra samples varies significantly depending on the fermentation methods and drying method. Sample S5 has a relatively low concentration (95.32 mg/100 g DM), while sample E3 has the highest concentration of 126.15 mg/100 g DM. Potassium (K) also shows significantly variable concentrations. Sample S5 has the lowest concentration, with a value of 220.55 mg/100 g DM, while sample S1 has the highest concentration, with a value of 350.17 mg/100 g DM. With regard to magnesium (Mg), the values also vary from one sample of fermented okra to another and depending on the fermentation time and drying method. Sample S5 has a concentration of 80.01 mg/100 g DM, and sample E3 has a concentration of 100.09 mg/100 g DM. The phosphorus (P) content varies considerably, with concentrations ranging from 47.57 mg/100 g DM in sample S5 to 63.16 mg/100 g DM in sample E3. The sodium (Na) content also varies depending on the fermented okra samples, the fermentation time and the drying method. Sample S5 has a sodium concentration of 12.06 mg/100 g DM, while E3 has a higher value of 20.12 mg/100 g DM. The highest sodium content is found in sample E1, with a value of 22.20 mg/100 g DM.

With regard to trace elements, the trends observed are similar to those for macroelements. Iron (Fe) is the only representative of this group and shows statistically variable concentrations from one sample of fermented okra to another, depending on the fermentation time and drying method. Its concentrations range from 30.04 mg/100 g DM in sample S5 to 48.66 mg/100 g DM in sample E1.

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Table 2. Mineral composition of fermented okra samples

Minerals (mg/100g)	Drying methods							
	Oven drying				Solar drying			
	Fermentation time (days)							
	0 day (E0)	1 day (E1)	3 days (E3)	5 days (E5)	0 day (S0)	1 day (S1)	3 days (S3)	5 days (S5)
Fe	40.11±2.12^{a}	48.66±1.33^{c}	46.09±1.45^{b}	39.98±0.80^{a}	40.11±1.10^{c}	35.04±1.14^{b}	42.32±1.45^{d}	30.04±2.17^{a}
Ca	120.12±2.67^{a}	122.36±3.15^{a}	126.15±1.17^{b}	119.22±1.88^{a}	120.12±3.00^{d}	110.22±2.80^{c}	100.13±1.20^{b}	95.32±0.90^{a}
P	59.36±0.25^{b}	60.13±1.22^{b}	63.16±1.55^{c}	58.02±0.45^{a}	59.36±1.09^{c}	58.02±1.80^{c}	50.02±1.33^{b}	47.57±1.55^{a}
Mg	91.01±0.56^{a}	99.95±1.33^{b}	100.09±2.55^{b}	90.01±2.83^{a}	91.01±1.89^{d}	88.11±0.75^{c}	87.35±0.45^{b}	80.01±1.22^{a}
K	312.44±1.17^{b}	350.17±2.08^{c}	350.08±2.35^{c}	250.04±3.21^{a}	312.44±1.32^{d}	289.94±2.52^{c}	231.11±2.35^{b}	220.55±2.68^{a}
Na	19.53±1.70^{b}	22.20±1.21^{c}	20.12±2.19^{bc}	18.10±1.32^{a}	19.53±1.39^{d}	17.42±1.45^{c}	15.05±1.77^{b}	12.06±2.17^{a}

The values in the table are averages of three trials, with standard deviations. Averages marked with the same letter on the same row are not significantly different at the 5% probability threshold according to Duncan's test. Fe: iron; Ca: calcium; P: phosphorus; Mg: magnesium; K: potassium; Na: sodium.

3.1.4. Amino acids composition of fermented okra samples

Analysis of the amino acid profile (essential and non-essential) of fermented okra samples reveals significant variations at the 5% threshold depending on the different drying methods (oven or sun) and fermentation time (Table 3). With regard to essential amino acids, lysine concentration varies from one sample of fermented okra to another. Sample E1 has a content of 1.391 mg/100 g, which is much higher than that of sample S5 (0.799 mg/100 g). This trend is also observed in methionine, histidine, tryptophan and tyrosine, where the S0 and E0 samples show concentrations of 1.065 mg/100 g, 1.271 mg/100 g and 0.207 mg/100 g and 0.155 mg/100 g, respectively. These concentrations are significantly lower than those in samples E3 (1.391 mg/100 g and 2.022 mg/100 g) for lysine and histidine, S3 (2.562 mg/100 g) for tryptophan, and S5 (0.560 mg/100 g) for tyrosine.

As for non-essential amino acids, proline shows significant variation between the fermented okra samples. Sample E3 shows a very high concentration of 2.733 mg/100 g, compared to only 1.526 mg/100 g in sample E0, which is the lowest of all the fermented okra samples. As for serine, sample E3 has the highest concentration at 1.592 mg/100 g, compared to only 0.738 mg/100 g in sample E0, which has the lowest concentration. Similarly, alanine follows a comparable trend. Sample E1 shows 3.621 mg/100 g, which is much higher than the 1.364 mg/100 g in sample S0.

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Table 3. Amino acids composition of fermented okra samples

Amino acids (mg/100g)	Drying methods							
	Oven drying				Solar drying			
	Fermentation time (days)							
	0 day (E0)	1 day (E1)	3 days (E3)	5 days (E5)	0 day (S0)	1 day (S1)	3 days (S3)	5 days (S5)
Lysine*	0.847±0.002 a	1.391±0.001 d	1.044±0.001 b	1.065±0.003 c	0.807±0.002 b	0.959±0.001 c	0.980±0.003 d	0.799±0.006 a
Methionine*	1.092±0.003 a	1.345±0.003 b	1.478±0.004 c	1.512±0.002 d	1.065±0.005 a	1.256±0.003 d	1.177±0.001 c	1.103±0,004 b
Histidine*	1.793±0.001 b	1.979±0.001 c	2.022±0.002 d	1.701±0.001 a	1.271±0.003 a	1.540±0.001 c	1.8191±0.003 d	1,458±0.002 b
Tryptophan*	1.848±0.001 a	1.897±0.002 b	2.117±0.003 d	2.058±0.001 c	0.207±0.001 a	2.326±0.002 c	2.562±0.002 d	2.088±0.002 b
Tyrosine*	0.128±0.001 a	0.160±0.004 b	0.161±0.002 b	0.177±0.003 c	0.155±0.004 a	0.192±0.003 b	0.371±0.006 c	0.560±0.001 d
Proline	1.526±0.003 a	2.604±0.001 c	2.733±0.004 d	2.005±0.002 b	1.803±0.006 a	2.140±0.003 b	2.269±0.001 d	2.242±0.001 c
Serine	0.738±0.001 a	1.334±0.001 b	1.592±0.004 d	1.521±0.001 c	0.865±0.003 a	1.154±0.003 d	1.074±0.002 c	1.001±0.003 b
Alanine	2.932±0.003 c	3.621±0.006 d	2.873±0.003 b	2.301±0.001 a	1.364±0.001 a	1.908±0.006 b	2.525±0.005 d	2.408±0.004 c

The values in the table are averages of three trials, with standard deviations. Averages marked with the same letter on the same line are not significantly different at the 5% probability threshold according to Duncan's test.

3.4.5. Discrimination of fermented okra samples based on their amino acid and mineral compositions

The results of the principal component analysis (PCA) show that the first two components explain 80.24% of the total variance. The first factor (F1) alone accounts for 24.95%, dominated by the variables phosphorus (P), magnesium (Mg) and sodium (Na). The eigenvectors indicate that phosphorus (0.304), magnesium (0.352) and sodium (0.322) are the most influential on F1. The cosine squares confirm this dominance, with values of 0.714 for phosphorus, 0.960 for magnesium and 0.805 for sodium. Furthermore, the correlation matrix reveals strong relationships between certain variables. Phosphorus is strongly correlated with calcium (r = 0.970), sodium (r = 0.922), tyrosine (r = 0.936), magnesium (r = 0.864) and potassium (r = 0.890). Magnesium is negatively correlated with tryptophan (r = -0.119) and tyrosine (-0.752), suggesting mineral dilution with protein degradation. Potassium is positively correlated with iron (r = 0.694), calcium (r = 0.857), phosphorus (r = 0.890), magnesium (r = 0.893), sodium (r = 0.908), lysine (r = 0.450), methionine (r = 0.252), histidine (r = 0.414), proline (r = 0.234), serine (r = 0.204) and alanine (r = 0.357). In terms of sample observations, the most significant contributions are those of E0 and E5 on F3 (34.58% and 30.05% respectively), E1 and S5 on F1 (27.91% and 39.35% respectively), E3 on F5 (39.90%), S0 on F2 (55.94%), and S1 and S3 on F7 (45.32% and 18.55% respectively), indicating that these samples are the most influential in the construction of the principal axes.

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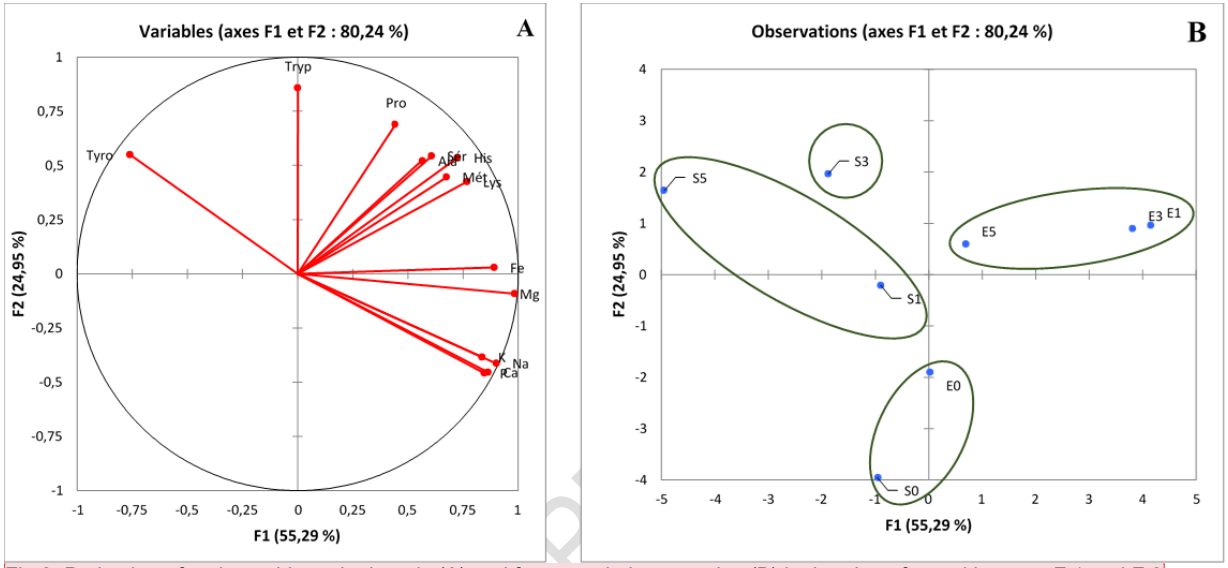

Fig 2. Projection of amino acids and minerals (A) and fermented okra samples (B) in the plane formed by axes F 1 and F 2

Fe: Iron; Ca: Calcium; P: Phosphorus; Mg: Magnesium; K: Potassium; Na: Sodium; Lys: Lysine; Mét: Methionine; His: Histidine; Tryp: Tryptophan; Tyro: Tyrosine; Pro: Proline; Sér: Serine; Ala: Alanine; E0: control sample blanched and dried in an oven; E1: sample bleached and dried under study after one day of fermentation; E3: sample bleached and dried under study after three days of fermentation; E5: sample bleached and dried under study after five days of fermentation; S0: control sample bleached and dried in the sun; S1: sample bleached and dried in the sun after one day of fermentation; S3: sample bleached and dried in the sun after three days of fermentation; S5: sample bleached and dried in the sun after five days of fermentation.

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3.2. DISCUSSION

The fermentation of okra (Abelmoschus esculentus) resulted in significant changes in its physicochemical properties, which are influenced by the duration of fermentation (1, 3 and 5 days) and the drying method (oven or sun). These parameters are essential for assessing the nutritional quality and stability of the finished product.

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Firstly, the pH, a fundamental indicator of acidity, showed a gradual decrease as the fermentation time increased. For oven-dried samples, the pH decreased from 4.35 to 4.11 between 0 and 5 days of fermentation, while for sun-dried samples, it decreased from 4.30 to 4.01 from day 0 to day 5 of fermentation. This decrease is attributable to the production of organic acids by fermentative microorganisms, particularly lactobacilli, as confirmed by the work of Di Cagno et al. (2013), Montet et al. (2014) and Wu et al. (2017), who demonstrated that lactic fermentation reduces the pH of vegetables due to the formation of lactic acid. This phenomenon is crucial for product preservation, as a pH below 4.5 inhibits the growth of many pathogens (Caplice & Fitzgerald, 1999; Tayel et al., 2010).

Next, the ash content, representing the total mineral fraction, varied significantly with fermentation time and drying method. For oven-dried samples, it increased from 9.39% to 10.51%, and for sun-dried samples, from 11.27% to 10.53%. This increase may be linked to the concentration of minerals due to the reduction in dry matter through the degradation of organic compounds. Fermentation can improve the bioavailability of minerals by reducing phytates, which are chelating agents (Blandino et al., 2003; Adebo et al., 2022; Zhang et al., 2022). This improvement is particularly beneficial in contexts of malnutrition where mineral deficiencies are common.

In order to quantify the minerals that make up the okra samples, the mineral profile was studied. The study reveals that fermentation, combined with the drying method, significantly influences the concentrations of essential minerals and trace elements such as calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na) and iron (Fe).

Calcium (Ca), which is essential for bone health, showed a slight increase on day 3 (126.15 mg/100 g) for oven-dried samples, but a gradual decrease for sun-dried samples, reaching 95.32 mg/100 g on day 5. This decrease could be linked to increased solubilisation followed by loss through leaching or complexation with organic acids produced during fermentation. Indeed, fermentation can alter calcium availability depending on pH and the presence of organic ligands (Sulibursk a & Krejpcio, 2014; Dai et al., 2020; Harahap et al., 2023).

With regard to phosphorus (P), which is involved in energy metabolism, its concentration followed a similar trend. For oven-dried samples, it increased from 59.36 to 63.16 mg/100 g, while for sun-dried samples, it decreased to 47.57 mg/100 g. This decrease may be related to the degradation of phospholipids or precipitation in the form of insoluble salts. Fermentation is known to reduce phytates, thereby releasing phosphorus, but this release depends heavily on the type of microorganisms involved (Reddy et al., 1982; Jeyakumar & Lawrence, 2022).

As for magnesium (Mg), a major enzyme cofactor, its content increased in the oven-dried samples, reaching 100.09 mg/100 g on day 3, compared to 91.01 mg/100 g on day 0. In contrast, for sun-dried samples, the content decreased to 80.01 mg/100 g. This difference can be attributed to better magnesium retention under controlled drying conditions (oven) (Huang & Zhang, 2016; Kaur et al., 2019), as suggested by the strong correlation between Mg and Fe ($r = 0.915$), indicating joint mobilisation of these minerals.

Potassium (K), which is essential for osmotic and nervous regulation, showed a significant increase in concentration on day 1 (350.17 mg/100 g) for oven-dried samples, before decreasing to 250.04 mg/100 g on day 5. For sun-dried samples, the decrease was more pronounced, reaching 220.55 mg/100 g. This loss may be related to the high solubility of potassium and its diffusion into the fermentation medium. Indeed, soluble minerals such as potassium are particularly susceptible to losses through diffusion during fermentation (Belitz et al., 2009; Rousseau et al., 2020).

In terms of sodium (Na), there was a gradual decrease in its level in both drying methods. For oven drying, it decreased from 19.53 to 18.10 mg/100 g, and for sun drying, from 19.53 to 12.06 mg/100 g. This reduction can be attributed to sodium consumption by microorganisms or to its loss through diffusion (Lin et al., 2021; Lorén et al., 2023). The negative correlations observed between Na and tyrosine (r = -0.915) suggest an interaction between protein degradation mechanisms and sodium dynamics.

For iron (Fe), a fundamental element in the prevention of anaemia, there was a significant variation in its content. For oven-dried samples, its concentration increased from 40.11 mg/100 g to 48.66 mg/100 g on day 1, before falling back to 39.98 mg/100 g on day 5. For sun-dried samples, the content fluctuated between 40.11 mg/100 g and 30.04 mg/100 g. This dynamic can be attributed to the mobilisation of mineral reserves by microbial enzymes, but also to losses through oxidation or complexation. Indeed, fermentation can improve iron bioavailability by reducing inhibitors such as phytates (Adebo et al., 2022; Nsabimana et al., 2024), which is corroborated by the positive correlations observed between Fe and Mg (r = 0.915) in Pearson's matrix (Hurrell, 2003).

Furthermore, moisture content showed a downward trend during fermentation, particularly for oven-dried okra samples, where it fell from 14.73% to 13.54%. This reduction was less pronounced for sun-dried samples, with values fluctuating around 14%. The decrease in moisture content is beneficial for product preservation, as it limits enzymatic reactions and microbial proliferation (Syamaladevi et al., 2016; Tapia et al., 2020). In fact, moisture content below 15% is generally considered safe for food powders (Jay et al., 2005; Opaliński et al., 2016; Juarez-Enriquez et al., 2022; Suhag et al., 2024).

In addition, the fibre content decreased significantly with fermentation time and drying method. For oven-dried samples, it decreased from 3.17% to 1.90%, and for sun-dried samples, from 2.76% to 1.82%. This reduction is probably due to the enzymatic action of microorganisms, which hydrolyse complex polysaccharides (Borba et al., 2019). Fermentation is known to degrade insoluble fibre, which can improve digestibility but reduce the beneficial effect of fibre on intestinal health (Swain et al., 2014).

Similarly, total sugar content also decreased during fermentation. For oven-dried samples, it fell from 6.59 mg/100 g DM to 3.08 mg/100 g DM, and for sun-dried samples, from 6.42 to 3.53 mg/100 g DM. This decrease is consistent with the use of sugars as an energy substrate by fermentative microorganisms (Trchounian & Trchounian, 2019). Indeed, simple sugars are the first metabolites consumed during fermentation, which explains their rapid decrease (Tamang et al., 2016).

In contrast, reducing sugars showed a slight increase at the start of fermentation, peaking at 0.80 µg/g DM on day 1 (oven drying), before decreasing. This variation can be explained by the enzymatic conversion of polysaccharides into monosaccharides, followed by their consumption (Ambye-Jensen et al., 2014). Indeed, reducing sugars are transient intermediates in fermentation metabolism (Steinkraus, 2004; Krahulec et al., 2010).

The fat content decreased significantly with fermentation. For oven-dried samples, it fell from 17.59% to 9.51%, and for sun-dried samples, from 16.74% to 10.59%. This reduction may be linked to the oxidation of lipids or their use by microorganisms. Indeed, certain fermentation microorganisms possess lipases that break down triglycerides, which could explain this loss (Nout, 2009; Kirana et al., 2016; Shamim et al., 2018). These results confirm that fermentation, by altering pH and biochemical interactions, influences the availability and concentration of minerals. It can be used as a technological lever to improve the nutritional quality of plant products, provided that the duration and drying parameters are controlled.

Protein content showed variable changes. For oven-dried samples, it increased slightly to 17.30% on day 3, before decreasing to 14.34% on day 5. For sun-dried samples, it reached a maximum of 17.44% on day 3. This fluctuation can be attributed to microbial protein synthesis and plant protein degradation (Araújo et al., 2011; Dong et al., 2021). Fermentation can enrich the protein profile through microbial input, but can also lead to

enzymatic degradation depending on the conditions (Singh et al., 2014; Sun et al., 2022; Cao et al., 2024).

Furthermore, analysis of the amino acid composition of the fermented okra samples in this study also shows variations between samples from different fermentation times and drying methods. The study distinguishes between essential amino acids (lysine, methionine, histidine, tryptophan, tyrosine) and non-essential amino acids (proline, serine, alanine), based on their evolution according to fermentation time and drying method.

Lysine, an essential amino acid often deficient in cereal-based diets, showed a notable increase on day 1 of fermentation with oven drying, reaching 1.391 mg/100 g compared to 0.847 mg/100 g on day 0. This improvement could be attributed to microbial biosynthesis during fermentation. For sun drying, lysine fluctuated between 0.807 and 0.980 mg/100 g. Fermentation enriches the lysine profile through the activity of lactic acid bacteria, which improves the protein quality of plant foods (Singh et al., 2014).

Methionine, another essential amino acid, also increased with fermentation. For oven-dried samples, it rose from 1.092 to 1.512 mg/100 g on day 5. This trend was confirmed for sun-dried samples, although the values were slightly lower. As methionine is involved in protein synthesis and liver detoxification, its enrichment is beneficial (Derouiche et al., 2024). Indeed, fermentation improves the methionine content in fermented legumes (Nout et al., 2009), which corroborates the results observed in the present study.

Histidine, which is involved in tissue growth and repair, reached 2.022 mg/100 g on day 3 (oven-dried), compared to 1.793 mg/100 g on day 0. For sun-dried samples, it peaked at 1.819 mg/100 g. This increase is consistent with the mechanisms of proteolysis and microbial biosynthesis. According to the work of Emkani et al. (2022), fermentation can improve the availability of amino acids by releasing bound protein fractions.

As for tryptophan, a precursor to serotonin, it showed a sharp increase, particularly in sun-dried samples, reaching 2.562 mg/100 g on day 3, compared to only 0.207 mg/100 g on day 0. This increase could be linked to the breakdown of complex proteins and the release of free tryptophan. Studies indicate that fermentation can release aromatic amino acids through enzymatic hydrolysis (Blandino et al., 2003; Sun et al., 2025), which is observed in this study.

Tyrosine, meanwhile, plays a role in neurotransmitter synthesis. Its content increased gradually, reaching 0.560 mg/100 g on day 5 (sun drying), compared to 0.128 mg/100 g on day 0 (oven drying). This increase is probably due to the breakdown of proteins by microbial enzymes. Fermentation is known to release aromatic amino acids such as tyrosine, particularly in protein-rich plant matrices (Swain et al., 2014; Feng et al., 2024).

Among the non-essential amino acids, proline showed a significant increase, reaching 2,733 mg/100 g on day 3 (oven-dried), compared to 1,526 mg/100 g on day 0. For sun-dried samples, it peaked at 2,269 mg/100 g. Proline is involved in stress response and protein structure (Ghosh et al., 2022; Renzetti et al., 2024). Its increase is consistent with fermentation mechanisms that promote the release of free amino acids.

With regard to serine, which is involved in cellular metabolism, its content also increased, reaching 1.592 mg/100 g on day 3 (oven-dried), compared to 0.738 mg/100 g on day 0. For sun-dried samples, it reached 1.154 mg/100 g on day 1. This improvement is beneficial for the synthesis of phospholipids and neurotransmitters (Murtas et al., 2020; Holeček, 2022). Fermentation improves the availability of hydrophilic amino acids such as serine (Tamang et al., 2016).

Finally, alanine, a glucogenic amino acid, showed variable changes. For oven-dried samples, it peaked at 3.621 mg/100 g on day 1, before decreasing. For sun-dried samples, it reached 2.525 mg/100 g on day 3. This dynamic could be linked to protein degradation and the metabolic conversion of amino acids (Mitchell et al., 2016; Torres et al., 2023). Fermentation can lead to a transient accumulation of alanine, depending on microbial metabolism (Caplice & Fitzgerald, 1999; Oliphant & Allen-Vercoe, 2019).

These results confirm that fermentation improves the overall amino acid profile of okra, particularly essential amino acids, thereby enhancing its nutritional value. The drying method also plays a role in retaining these compounds, with better preservation observed in oven-dried samples.

Principal component analysis (PCA) is a powerful multivariate statistical method that reduces the dimensionality of data while retaining most of the information. It is particularly useful for identifying discriminating variables and visualising relationships between samples. In this study, PCA was applied to the physicochemical, mineral and amino acid parameters of okra in order to better understand the impact of fermentation time and drying method.

With regard to physicochemical parameters, PCA reveals that the first two principal components (F1 and F2) alone account for 73.68% of total variability, with F1 representing 49.06% and F2 24.61%. The variables most strongly correlated with F1 are pH (cos^2 = 0.914), total sugars (cos^2 = 0.875), fibre (cos^2 = 0.797) and fat (cos^2 = 0.783). This indicates that F1 is essentially a nutritional quality component, linked to the energy content and structure of the product. F2, on the other hand, is dominated by moisture (cos^2 = 0.782) and reducing sugars (cos^2 = 0.780), suggesting a component linked to microbiological stability and enzymatic processing. These results are consistent with the work of Jolliffe & Cadima (2016), who emphasise that the first components capture the major contrasts in food data.

ACP applied to mineral and amino acid data shows a cumulative variability of 80.24% for the first two components, with F1 representing 55.29% and F2 24.95%. F1 is strongly correlated with magnesium (cos^2 = 0.960), sodium (cos^2 = 0.805), iron (cos^2 = 0.788), calcium (cos^2 = 0.742) and phosphorus (cos^2 = 0.714), making it a dominant mineral component. F2 is influenced by tryptophan (cos^2 = 0.736), proline (cos^2 = 0.476) and tyrosine (cos^2 = 0.302), indicating a protein and metabolic component. These results confirm that fermentation modifies both the mineral composition and the protein profile of okra, as suggested by the correlations observed between Fe and Mg (r = 0.915), Lys and Ala (r = 0.689), and Ser and Met (r = 0.966).

Analysis of the observation coordinates shows that samples fermented for 1 to 3 days (E1, E3, S1, S3) are strongly projected onto F1 and F2, indicating nutrient richness and active biochemical transformation. In contrast, samples that were not fermented (E0, S0) or fermented for 5 days (E5, S5) are more dispersed, reflecting a loss or stabilisation of compounds. These observations are consistent with the contributions of the observations, where E1 and S3 have the highest values on F1 and F2, respectively.

Thus, PCA clearly shows the combined effects of fermentation time and drying method on the biochemical quality of okra. It highlights that short fermentation times (1 to 3 days) optimise nutrient concentration, while longer fermentation times can lead to losses or stabilisation. These results are in line with the recommendations of Tamang et al. (2016), who recommend controlled fermentation to maximise nutritional benefits.

4. CONCLUSION

The study on the impact of fermentation time and drying method on the biochemical parameters of okra (Abelmoschus esculentus) reveals that fermentation is an effective technological strategy for improving the nutritional quality of this vegetable, which is widely consumed in Côte d'Ivoire. The results show that short-term fermentation (1 to 3 days) optimises protein, essential amino acid and bioavailable mineral content and reduces total sugars, while lowering the pH, which promotes the microbiological stability of the product. The drying method also influences nutrient retention, with oven drying being superior to sun drying, particularly with regard to the preservation of amino acids and minerals. Principal component analysis enabled okra samples to be differentiated according to their biochemical profile, highlighting that okra samples fermented for 1 to 3 days had the best nutritional characteristics. These results suggest that controlled fermentation, combined with moderate thermal drying, can be integrated into artisanal okra processing practices to produce enriched, stable powders that are suited to the nutritional needs of local populations. With a

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view to sustainable agri-food development, it would be necessary to further document fermented okra through studies on the phytochemical and sensory profiles of fermented okra samples.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The authors hereby declare that generative artificial intelligence (ai) technologies, such as large language models, were not used in the preparation and/or revision of this manuscript.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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