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# Effects of Rice Paddy Processing Methods on the Fatty Acid Content of Local Rice Landraces in Abakaliki, Nigeria

## Onwuchekwa Ogah [a*]

[a] *Department of Biotechnology, Ebonyi State University, Abakaliki, Nigeria.*

***Author's contribution***

*The sole author designed, analyzed, interpreted and prepared the manuscript.*

***Article Information***

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## ABSTRACT

The cooking process is pivotal in rice preparation, and crucial for eliciting its distinctive flavor. This study aimed at evaluating the fatty acid compositions of rice paddy soaked in warm and cold water during rice processing. Applying Gas Chromatography equipped with a flame ionization detector (GC-FID) analysis, the study identified fatty acids including myristic, myristoleic, oleic, linoleic, and vaccenic fatty acids as the predominant components in these samples. Remarkably, the levels of these fatty acids were significantly higher in the rice paddy soaked in warm water than their counterparts soaked in cold water. However, myristoleic and linoleic acid concentrations were consistently low across all rice. Notably, among warm water-soaked rice paddy, 'EBSA3' displayed the highest concentration of oleic acid. In the principal component analysis (PCA) biplot for cold water-soaked rice accounted for an overall variance of 82.7%, while warm water-soaked rice, rose to 89.2%. Additionally, oleic acid exhibited a substantial positive correlation with vaccenic acid in

**Corresponding author: E-mail: chekwas010@gmail.com;*

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both raw (r = 0.95) and parboiled (r = 0.96) rice samples, indicating a high degree of relatedness. This implies that changes in one trait may indeed influence others in a similar direction. The study reveals that the fatty acid composition of warm water-soaked rice paddy was higher than that of cold water-soaked rice samples.

*Keywords: Oryza sativa; Abakaliki rice landraces; fatty acids; nutrient profiling; paddy; minerals; rice variants.*

## 1. INTRODUCTION

Rice (*Oryza sativa*), revered as one of the world's most vital staple foods, serves as a fundamental source of carbohydrates and as such sustenance for over half of the global population [1]. Beyond its role in providing energy, rice boasts a rich nutritional profile, abundant in fiber, vitamins, minerals, and low cholesterol content, making it an indispensable component of a balanced diet [2,3]. Nigeria, a prominent player in the global rice landscape, emerged prominently in 2009, ranking 12th in rice consumption, 17th in production, and leading the ranks in both Africa and West Africa [4]. With an estimated consumption of 6.7 million tonnes, Nigeria's trajectory in rice consumption displays a consistent upward trend, poised to persist into the foreseeable future [5].

Although Rice processing does not have a uniform method of processing after harvesting but most adopted methods of include Cleaning, soaking in warm water, and steaming, drying, and milling. However, of recently, intercepted another method in which the soaking was in cold water for a quite longer time that the usual 12 hours of soaking in warm water. The cold soaking after milling had a different aroma unlike the warm water-soaked rice, hence our motivation to evaluate the fatty acid profiles of warm and cold water-soaked white rice which is largely unexplored within the diverse of rice cultivars of Abakaliki, Nigeria. Essential fatty acids (EFAs), particularly polyunsaturated fatty acids (PUFAs), represent pivotal components of rice, essential for optimal health yet unattainable through endogenous synthesis and thus necessitating dietary intake [6,7]. As dietary patterns evolve, characterized by a growing apprehension towards conditions such as atherosclerosis, obesity, and diabetes, the significance of fatty acid composition in food assumes heightened importance [8,9]. However, while numerous studies have sought to quantify fatty acids across various rice varieties, research specific to cold and warm water-soaked paddy rice variants in the Abakaliki rice landraces remains sparse [10]. This study seeks to bridge this gap by meticulously examining the fatty acid profiles of cold and warm water-soaked paddy rice varieties cultivated in Abakaliki, Nigeria. This will shed light on an aspect crucial for both nutritional understanding and consumer preferences.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

A total of fifteen samples of Abakaliki rice landraces (Table 1) were meticulously collected from diverse farm sites scattered across Ebonyi State, Nigeria. These samples were directly procured from local farmers, ensuring authenticity, and representing the regional agricultural landscape comprehensively.

### 2.2 Sample Preparation

The experiment was carried out in the research and teaching farm of the Department of Crop Production and Landscape Management, Ebonyi State University, Abakaliki, Nigeria, during the dry season of January 2023. A total of 10 kilograms each of 15 distinct rice landrace cultivars were procured directly from local farmers in Ebonyi State, Nigeria. Each cultivar was meticulously divided into two equal portions of 5 kilograms each. The first portion underwent conventional parboiling processes, including soaking in warm water for 12 hours, draining, steaming at 70 degrees Celsius, and sun drying until the moisture content of 15% was achieved. Subsequently, the fully dried grains were carefully sealed in waterproof bags and stored at 70% relative humidity. Later, these grains were milled using a specialized testing rice miller. The second portion of each cultivar was cleaned, soaked in cold water for 5 days, drained, steamed at 70 degrees Celsius, and sun drying until the moisture content of 15% was achieved and milled using the same milling machine. Both portions were then ground into fine powder using a blender and sieved separately through a 100-mesh sieve to facilitate the laboratory analysis of fatty acid content.

**Table 1.** ***Oryza sativa*** **L. accessions used in the analysis**

| S/N | Code | Sources | Status | Picture |
|---|---|---|---|---|
| 1 | 'EBSA1' | Izza | Landrace |  |
| 2 | 'EBSA2' | Ikwo | Landrace |  |
| 3 | 'EBSA3' | Ezzamgbo | Landrace |  |
| 4 | 'EBSA4' | Ikwo | Landrace |  |
| 5 | 'EBSA5' | Abaomege | Landrace |  |
| 6 | 'EBSA6' | Izzi | Landrace |  |
| 7 | 'EBSA7' | Ikwo | Landrace |  |
| 8 | 'EBSA8' | Ikwo | Landrace |  |
| 9 | 'EBSA9' | Onueke | Landrace |  |
| 10 | 'EBSA10' | Abakaliki | Landrace |  |
| 11 | 'EBSA11' | Ikwo | Landrace |  |
| 12 | 'EBSA12' | Ikwo | Landrace |  |
| 13 | 'EBSA13' | Ikwo | Landrace |  |
| 14 | 'EBSA14' | Ezza | Landrace |  |
| 15 | 'EBSA15' | Ikwo | Landrace |  |

### 2.3 Extraction and Determination of Fatty Acid Profile

The fatty acids in the cold water-soaked and warm water-soaked paddy rice samples were determined using gas chromatography equipped with a flame ionization detector (GC-FID) as described by Hu et al. [11]. Exactly 2.0g of the rice flour was extracted using 5.0 mL toluene and 6.0 mL 10% acetyl chloride (in methanol solution) in a sealed glass tube for two hours at a temperature of 80 ± 0.5 ◦C. The mixture was shaken intermittently every 30 minutes for a complete two hours. The mixture was then cooled to room temperature and transferred together with 3mL of $Na_2CO_3$ solution, (0.5 mol/L) in a 50 ml centrifuge tube. The whole mixture was centrifuged at 2795 × g for exactly five minutes before collecting the supernatant and filtering it using 0.45 µm filter film. Exactly 1.0 µL of the filtrate was injected into the GCFID equipped with an HP-88 column (100 m × 0.25 mm × 0.2 µm) and Nitrogen gas at a flow rate of 1.2 mL/min. The injection port temperature was maintained at 260 ◦C with a split ratio of 30:1. The column temperature was adjusted to increase from 120 ◦C to 170 ◦C at a rate of 30 ◦C/min for two minutes, followed by an increase to 200 ◦C at 6 ◦C/min for another 2 minutes. Subsequently, the temperature was raised to 220 ◦C at 20 ◦C/min and further to 230 ◦C at 2 ◦C/min, where it was held for five minutes. Then, the temperature was increased to 232 ◦C at 1 ◦C/min and maintained for 2 minutes before reaching 240 ◦C at 3 ◦C/min and held for five minutes. All experiments were conducted using completely randomized design in triplicate to increase precision of the experiment and ensure the reliability of the results. The laboratory analysis was carried out at the National Root Crops Research Institute (NRCRI) central molecular and biology laboratory, Umudike, Abia State.

### 2.4 Data Collection

Data were collected from the cold water-soaked and warm water-soaked paddy rice samples alike on oleic, linoleic, myristic, myristoleic, and vaccenic acids.

### 2.5 Statistical Analysis

Statistical analyses including the descriptive bar chart, principal component, cluster, and correlation were conducted using R (version 4.0.2), with statistical significance set at a p-value of less than 0.05.

## 3. RESULTS AND DISCUSSION

The findings from our study revealed that all samples analyzed contained the fatty acids examined, namely Myristics, myristoleic, oleic, vaccenic, and linoleic acids. This aligns with the results by Zhou et al. [12] and Hu et al. [11] who also identified these fatty acids in rice cultivars (Figs 1 and 2). Notably, 'EBSA3' whether warm or cold water soaked exhibited significantly higher levels of oleic acid compared to other cultivars, while vaccenic acid predominated in 'EBSA15', 'EBSA11', 'EBSA10', 'EBSA9', and 'EBSA3'. Moreover, all cultivars displayed relatively low levels of myristoleic and linoleic acids in the two rice portions. Particularly, 'EBSA3' among warm water-soaked rice cultivars exhibited the highest concentration of oleic acid when the two rice portions (warm and cold-soaked) were compared.

Interestingly, the overall concentration of all fatty acids was found to be higher in warm water-soaked rice compared to cold water-soaked rice samples in our investigation (Fig. 3). This phenomenon could be attributed to the leaching and rupture of oil globules induced by elevated temperature during the 12hrs soaked in warm water process, as suggested by Chukwu and Oseh [13]. Similarly, Hu et al. [11] and Zhou et al. [14] proposed that cooking enhances the activities of lipase and lipoxygenase, thus facilitating lipid oxidation. Furthermore, several studies have indicated that parboiling rice may enhance grain quality and nutritional content, albeit with the caveat that this process should be conducted promptly [15,16,17].

It's noteworthy, as highlighted by Wardlaw and Kessel [18] and Siri-Tarino et al. [19] that excessive consumption of saturated fats is a key dietary factor contributing to elevated cholesterol levels and potential weight gain. Kromhout et al. [20] and Saraswathi et al. [21] also observed that ingestion of myristic fatty acid, a common saturated fatty acid correlates with increased blood cholesterol levels, potentially exacerbating coronary heart disease risk. However, in comparison to myristic fatty acid, which represents the sole saturated fatty acid in our samples, the dietary fatty acid compositions of our study samples contain a higher proportion of unsaturated fatty acids. This suggests a potentially favorable nutritional profile in terms of lipid intake.

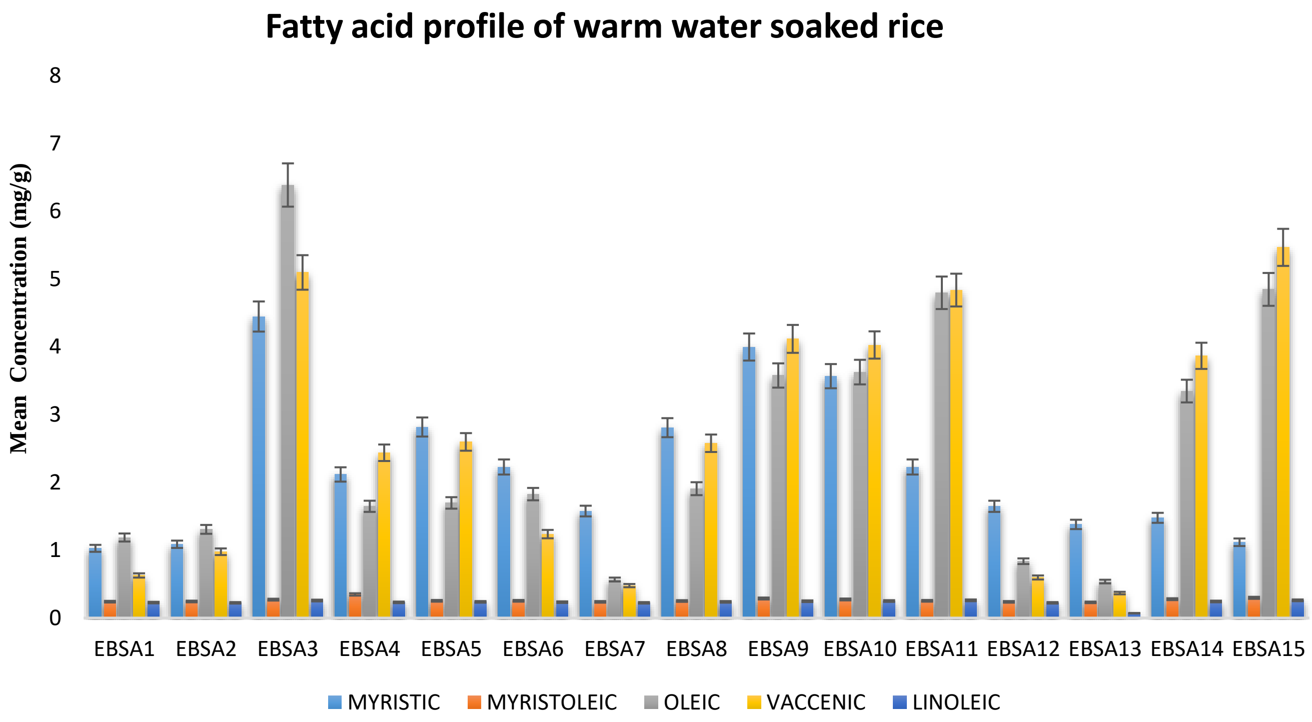


**Fig. 1. Fatty acid composition of the Abakaliki warm water-soaked rice samples**

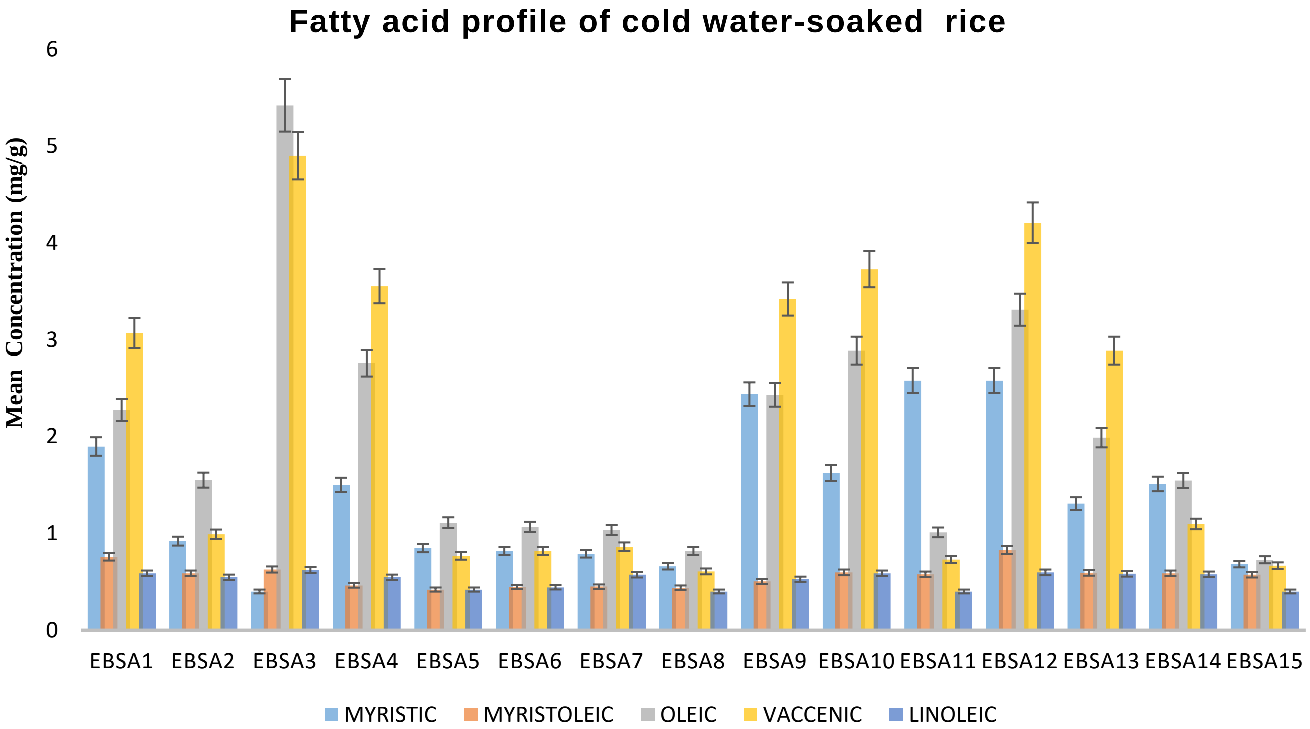


**Fig. 2. Fatty acid composition of the Abakaliki cold water-soaked rice samples**

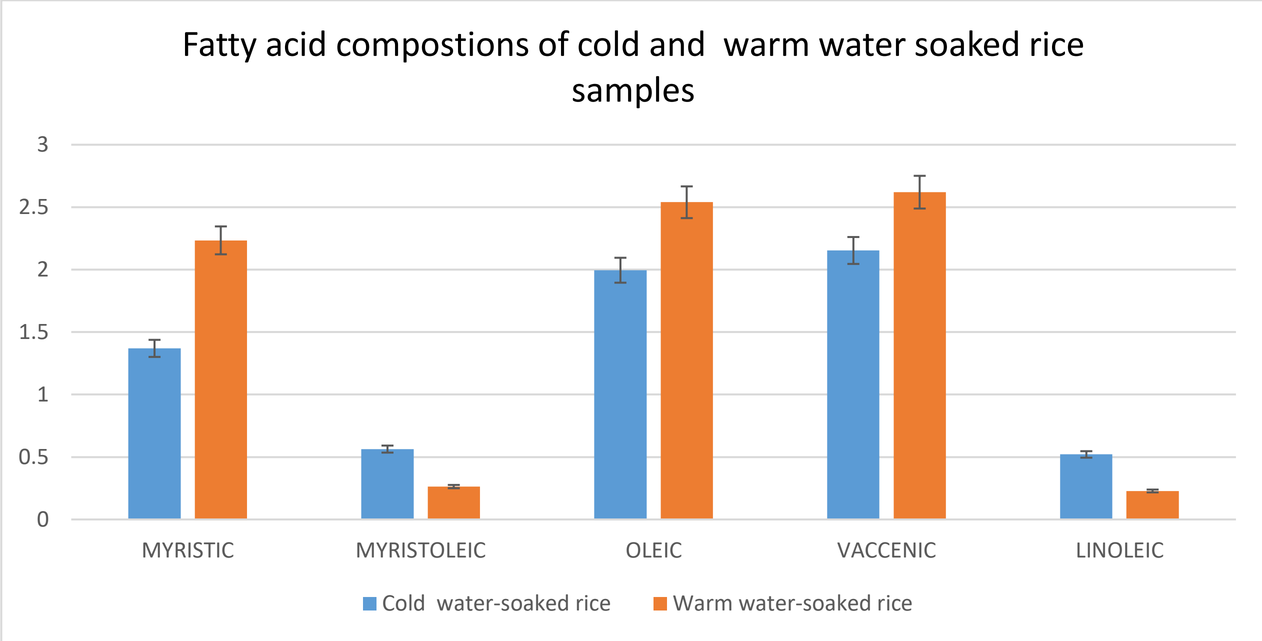


**Fig. 3. Fatty acid compositions of cold and warm water-soaked Abakaliki rice samples**

Verma and Srivastav [22] reported oleic and linoleic acids as major and of course unsaturated fatty acids which is tipped as being desirable from nutritional and health points of view as their consumption will not lead to heart-related issues [23]. Whereas myristic on the other hand was reported as minor and saturated fatty acids which can pose health risk such as atherosclerosis, a disease condition associated with heart attack [24]. By and large, the fatty acid profile of rice whether cold water-soaked or warm water-soaked rice, the aromatic and non-aromatic rice accessions have shown that it can be good for consumption if it is well refined [22].

The comprehensive analysis of fatty acid compositions in both cold water-soaked and warm water-soaked rice landraces revealed significant positive correlations across all fatty acids, as determined by Pearson's correlation analysis. Notably, oleic acid exhibited a substantial positive correlation with vaccenic acid in both cold water-soaked (r = 0.95) and warm water-soaked (r = 0.96) rice samples. Additionally, strong positive correlations were observed between oleic and linoleic acid (r = 0.90), as well as between myristic and mystrileic acid (r = 0.81), for cold water-soaked and warm water-soaked rice samples, as illustrated in Figs 4 and 5. These robust and significant correlations among traits suggest a high degree of relatedness, indicating that changes in one trait may influence others in the same direction. However, Zaplin et al. [25] reported a strong negative linear relationship between the oleic acid and linoleic acid contents for tested rice grains. The positive significant correlation observed in the present study showed that one of the traits could be enough selection criteria. Although the traits identified as being predominant in the present study were small in number, however, a major advantage of correlation among traits is its ability to identify excessive traits, select fewer traits, and reduce costs in traits analysis, recording and management without undermining experiment precision [26].

In the biplot of the principal component analysis (PCA) for cold water-soaked rice, an overall variance of 82.7% was revealed for dimensions 1 and 2, while for warm water-soaked rice, the overall variance was 89.2%. PC1 accounted for the highest variation at 61% and 65.6% respectively for cold water-soaked and warm water-soaked, while PC2 explained 21.7% and 23.6% for cold and warm water-soaked rice, respectively (Figs 6 and 7).

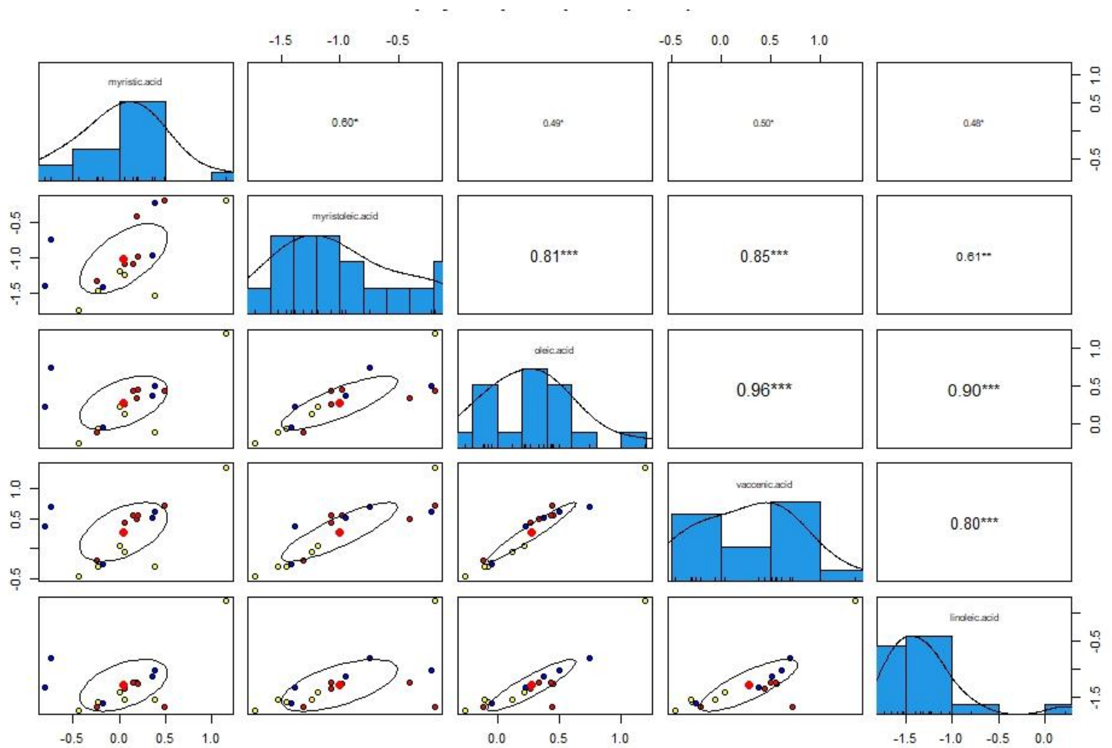


**Fig. 4. Pearson correlation plot for warm water-soaked rice samples**

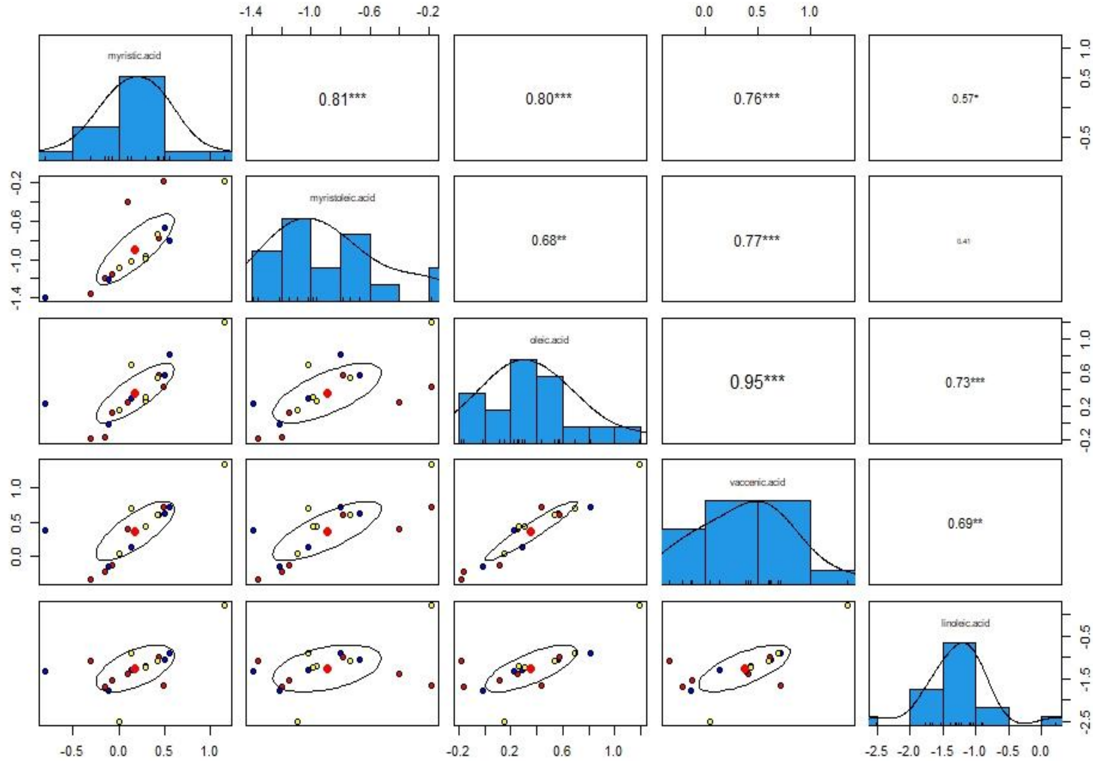


**Fig. 5. Pearson correlation plot for cold water-soaked rice samples**

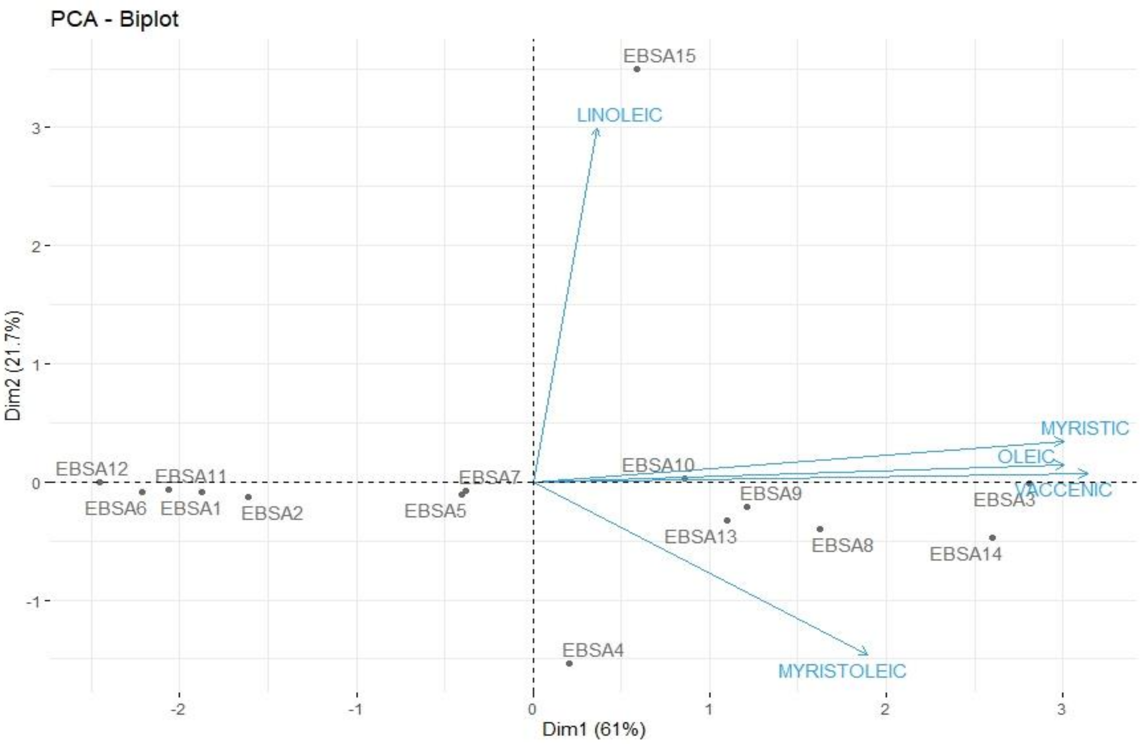


**Fig. 6. Principal component analysis for fatty acid composition in cold water-soaked rice samples**

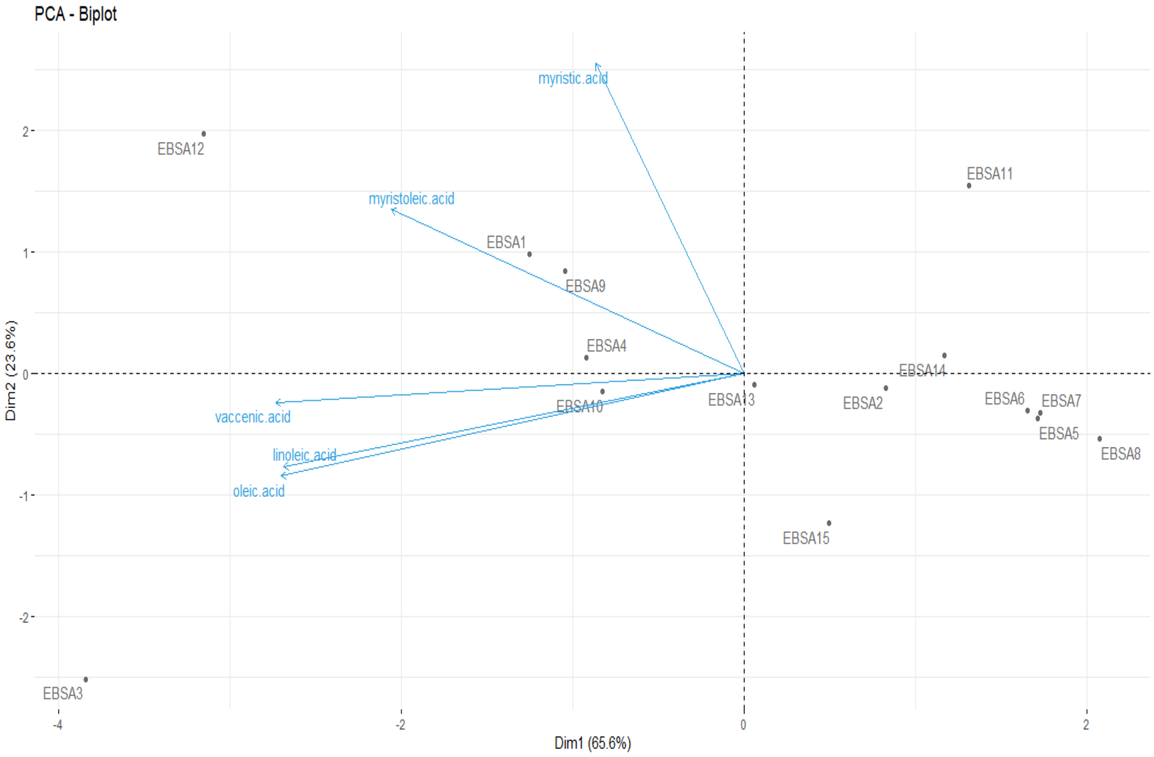


**Fig. 7. Principal component analysis for fatty acid composition in warm water-soaked rice samples**

Based on the factor loadings in cold water-soaked rice, all the fatty acids studied contributed to the variation observed in PC1 indicating a positive correlation among them which is higher among some, with myristoleic acid contributing the most to the variation observed in PC1. Notably, the PCA biplot clearly demonstrated that accessions loading in PC1, particularly 'EBSA3', displayed higher relationship with myristic, oleic and vaccenic acids, while 'EBSA15' showed higher association with linoleic acid content compared to other cultivars loadings in both PC1 and PC2 for the cold water-soaked rice. For the warm water-soaked rice, myristoleic acid was positive with higher factor loading comparable also contributing to the most variation explained but this time in PC2, and mostly associated with 'EBSA12'. Additionally, the plot depicting different dimensions for cold water-soaked rice illustrated that dimensions 4 and 3 exhibited the highest concentrations of myristic and myristoleic acids, respectively while Dim 2, 3 and 5 had higher concentrations of myristic, myristoleic and oleic acids, respectively were illustrated for warm water-soaked rice (Figs 8 and 9). While the present study is limited in terms of the rice samples used and the number of fatty acids examined; our findings are consistent with those of Hu et al. [27,11] suggesting an increase in fatty acid content due to an increase in temperature. This is based on the higher cumulative variation recorded in warm water-soaked rice over cold water-soaked rice samples.

**The results** of the hierarchical clustering analysis using the pvclust cluster method with AU/BPs P-values in percentages and employing bootstrapping of 10,000 iterations are presented in Figs 10 and 11 for warm water-soaked rice and cold water-soaked rice, respectively. The cluster dendrogram for both cold water-soaked and warm water-soaked rice categorizes the genotypes into two distinct groups, labeled as A and B, based on the similarity of their fatty acid composition. Specifically, Cluster A comprises five cultivars for cold water-soaked rice and six cultivars for warm water-soaked rice, while Cluster B has 10 cultivars for cold water-soaked rice and 9 cultivars for warm water-soaked rice. This analysis yields two types of p-values: AU (Approximately Unbiased) p-value and BP (Bootstrap Probability) value. The AU p-value, obtained through multiscale bootstrap resampling, is considered a more accurate approximation of the unbiased p-value compared to the BP value, which is derived from normal bootstrap resampling. As indicated by Suzuki and Shimodaira [28] and de Croos and Pálsson [29] AU p-values exceeding 95% indicate statistically significant clusters [30-34].

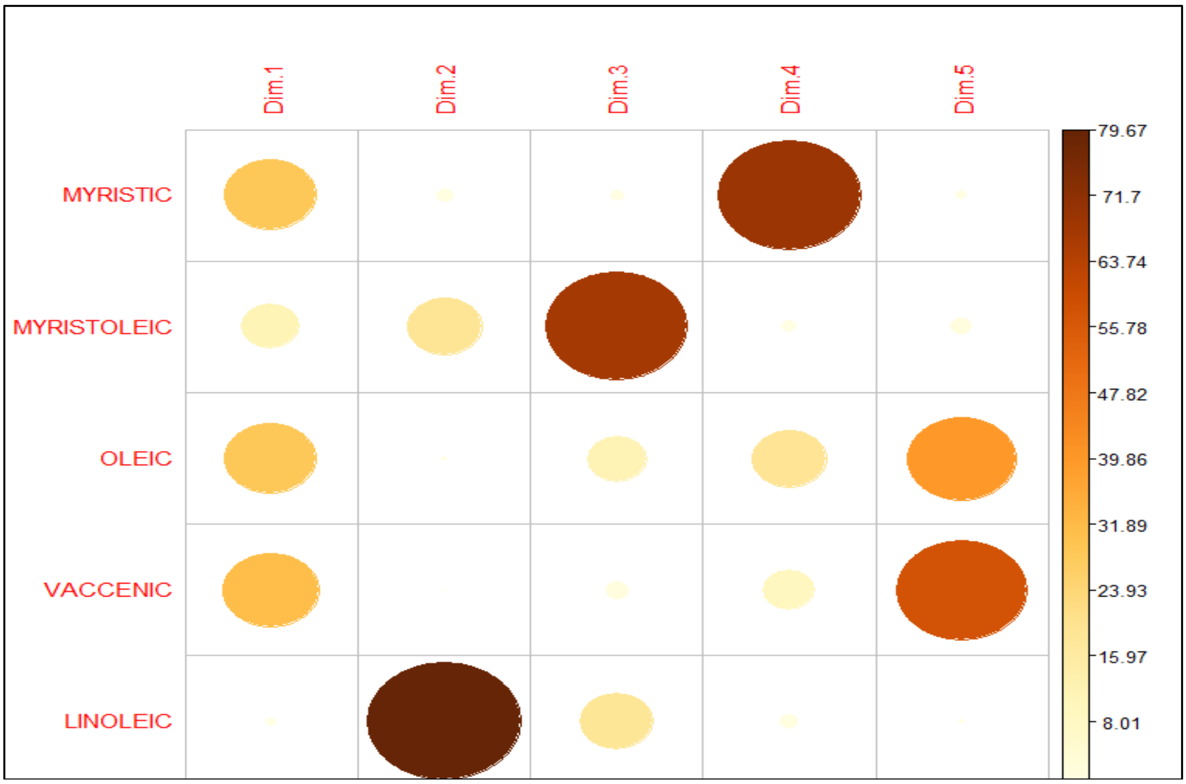


**Fig. 8. Dimension plots analysis for fatty acid composition in cold water-soaked rice samples**

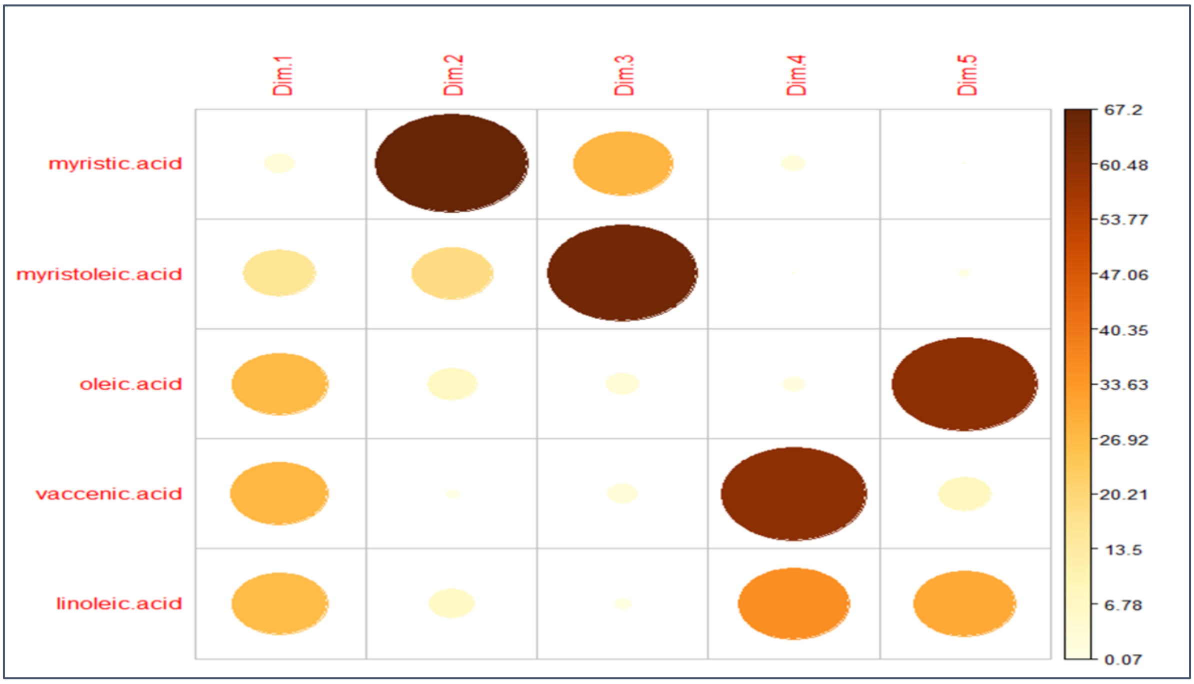


**Fig. 9. Dimension plots analysis for fatty acid composition in warm water-soaked rice samples**

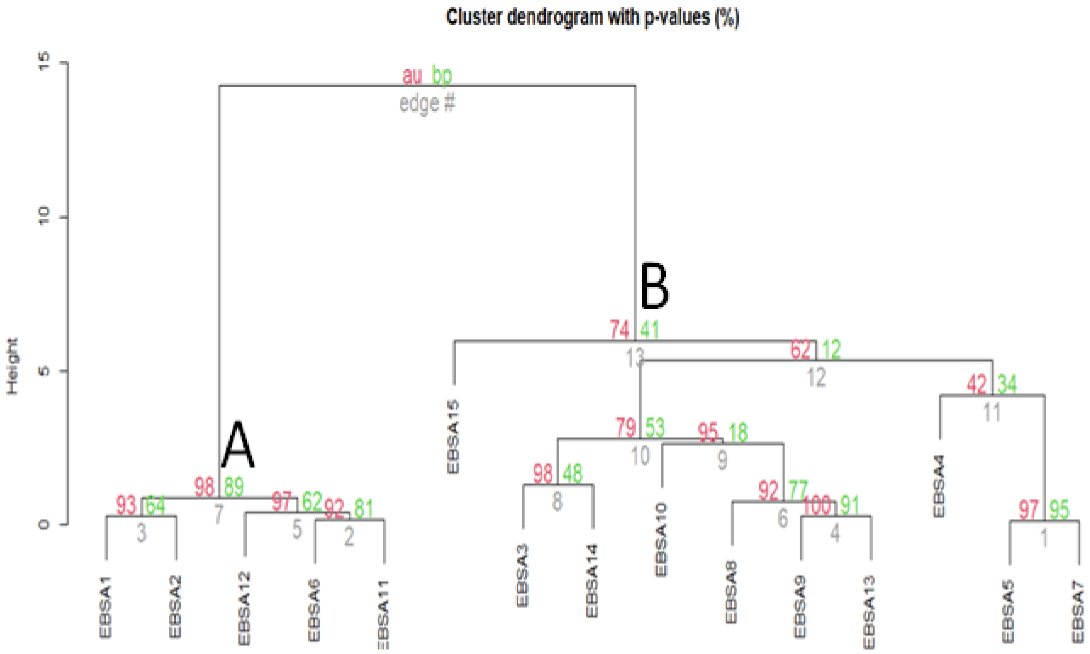


**Fig. 10. Cluster dendrogram with au/bp values (%) based on fatty acid compositions in warm water-soaked rice samples The values at the edges of the cluster are P-values (%) calculated over a multiscale bootstrap with 1000 resamples. Values on the left in red = au (approximate unbiased) P-values, and values on the right in green = bp (bootstrap probability) values. Clusters with au above 95% are highlighted in blocks suggesting high relatedness**

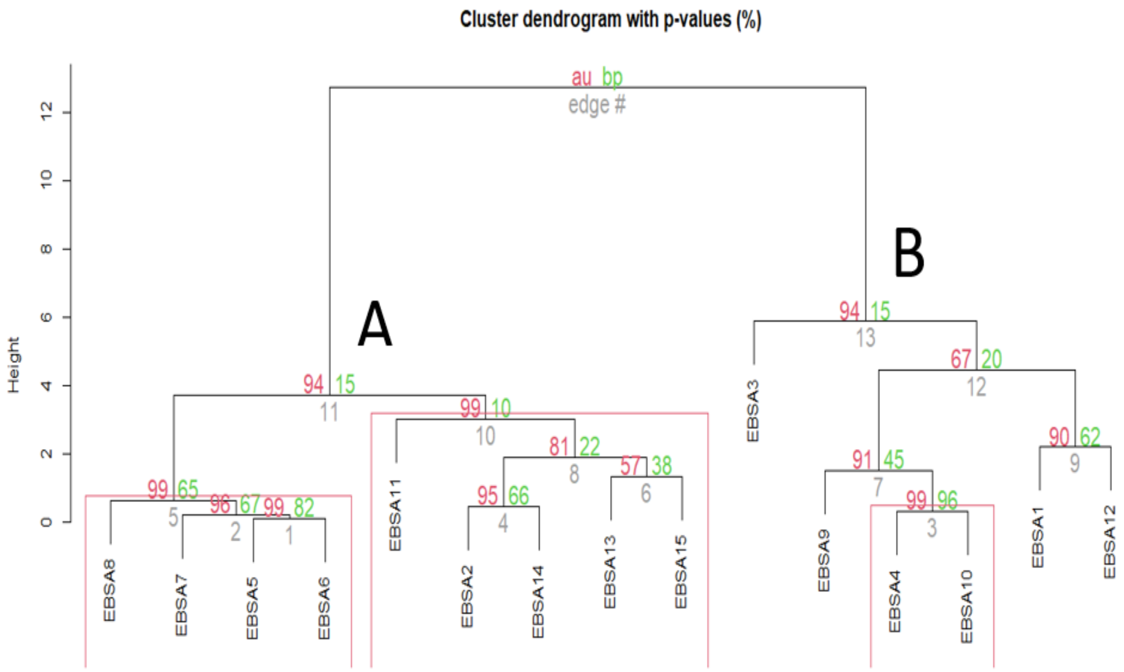


**Fig. 11. Cluster dendrogram with au/bp values (%) based on fatty acid compositions of cold water-soaked rice samples. the values at the edges of the cluster are p-values (%) calculated over a multiscale bootstrap with 1000 resamples. values on the left in red = au (approximate unbiased) p-values, and values on the right in green = bp (bootstrap probability) values. clusters with au above 95% are highlighted in blocks suggesting high relatedness**

## 4. CONCLUSION

This study analyzed the fatty acid composition of the local rice varieties of Abakaliki in both cold water-soaked and warm water-soaked samples and concluded that the fatty acids composition of warm water-soaked rice was higher than that of cold water-soaked rice in all samples. Therefore, warm water-soaking of paddy rice during rice processing is necessary for understanding the nutritional value and consumer preferences. The highest concentration of oleic acid witnessed in 'EBSA3' among warm water-soaked rice cultivars showed that heat could have been responsible for the trait being very noticeable compared to its counterpart among the cold water-soaked rice cultivars.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

## DATA AVAILABILITY STATEMENT

All data set generated during and/or analyzed to support the findings of this study are phenotypic data and can be made available from the corresponding author on request.

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## COMPETING INTERESTS

Author has declared that no competing interests exist.

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