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Analytical Methods in Proximate Composition of Ten Commonly Used Seasonings in Nigeria

[1] Udochukwu Victor Echebiri , [2]Christogonus Ifeanyichukwu Ugoh, [3]Francis Chukwuemeka Eze, [3] Ifunanya Lydia, Omeje [4] Besiru Momoh

[1]Department of Statistics, Faculty of Physical Science, University of Benin, Benin, Nigeria
[2,3]Department of Statistics, Faculty of Physical Science, Nnamdi Azikiwe University, Awka, Nigeria
[4]Department of Statistics, Federal Polytechnic, Auchi, Nigeria

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ABSTRACT: *This paper examines the proximate composition of ten commonly used seasonings (named A, B, C, D, E, F, G, H, I and J) in Nigeria. The data for this study were collected from Eke-Awka market in Anambra State as a representative of Nigeria using the simple random sampling method. The techniques of Single-factor Analysis of Variance and Kruskal-Wallis Test (One-Way ANOVA on Ranks) were employed in this study; Proximate compositions across the seasonings were tested for constant variance and normality. The findings of this study showed that the proximate compositions across the seasonings lack evidence of equality in variance and also lacks normality. Furthermore, the results from the Kruskal-Wallis test, as an alternative nonparametric to Single-factor ANOVA, showed that there is statistically significant difference between the proximate compositions across the seasonings. The Dunn Kruskal-Wallis Multiple Comparison test showed an evidence of no significant difference in the mean percentage composition of the following pairs of the proximate compositions: Ash and Fat, Carbohydrate and Fat, Ash and Moisture, Carbohydrate and Moisture, and Moisture and Protein. Thus, this study shows that there is significant difference in the proximate compositions of seasonings in Nigeria.*

KEYWORDS: Proximate compositions, Seasonings, Single-Factor ANOVA, Kruskal-Wallis, Dunn's Test, Nigeria

INTRODUCTION

Examining numerous treatment means and figuring out which one, if any, produces a superior result is one of the major problems in science and research [1]. An important tool which helps the researchers to carry out studies on more than one experimental group and control group is the Analysis of Variance (ANOVA). Analysis of variance is a technique for partitioning the total variation of a set of data into several components. It also makes it possible to ascertain the proportion of the total variation attributable to each source of variation in the data set [2]. The analysis of variance (ANOVA) is used to determine whether the groups under study are significantly different, but it fails to tell us which of the groups are significantly different. However, the Multiple Comparison test (using Tukey's Honestly Significant Difference method) will help us to get the solution to which of the groups differ significantly. However, in some scenarios where the assumptions of normality and constant variance are violated, an alternative nonparametric test such as Kruskal-Wallis rank sum test is used instead. In this case, Dunn (1964) Kruskal-Wallis Multiple Comparison will help us to get the groups that are significantly different. This study will employ the techniques of Single-factor Analysis of Variance (also known as One-way Analysis of Variance) and Kruskal-Wallis Rank Sum Test (also known as One-Way ANOVA on Ranks) to investigate the proximate percentage compositions of the five standard proximate in ten commonly used seasonings in Nigeria.

Single-Factor Analysis of Variance (One-way Analysis of Variance) aims at investigating the effects of the levels of a single independent variable (factor) on a dependent variable under study. However, before a Single-factor Analysis of Variance is applied on a set of data, the assumptions underlying it are first of all diagnosed. These assumptions are: Normality (this means that the distribution of the independent variable in the population from which the samples are drawn must be normally distributed with mean zero and variance σ_e^2); Homoscedasticity (this means that the variances in the population from which the samples are drawn are equal) [2].

Kruskal-Wallis test (also known as One-Way ANOVA on ranks) is a nonparametric method used for testing whether samples originate from the same distribution [3,4]. It does not assume a normal distribution of the residuals. A significant Kruskal-Wallis test indicates that at least one of the groups stochastically dominates others. The Kruskal-Wallis only shows that there is significant difference in the groups but it does not tell us which of the groups differ significantly. The Dunn's test (or Dunn's Kruskal-Wallis Multiple Comparison) helps us to get the statistically significant difference of the groups.

Proximate analysis is the method which determines the values of the macronutrients in food samples; the values of the macronutrients are usually declared as nutritional facts which are most often shown on the labels of the final food products, and are also determined during the production process. Proximate analysis is of key commercial concern as food-manufacturing companies (especially the food additives manufacturing companies) need to ensure that their products meet the appropriate laws and legal declaration requirements as well as the safety aspects of the end products when released to the end consumer. To meet the industry-desired standards and be competitive on the market, food-manufacturing companies need to find and utilize in their workflow reliable analytics techniques to analyze the production alongside with the ongoing manufacturing process.

Proximate is used in the analysis of biological materials as a decomposition of a human-consumable good into its major constituents. They are good approximation of the contents of packaged comestible goods and serve as a cost-effective and easy verification of nutritional panels. Ash, Moisture, Proteins, Fat and Carbohydrates are the five standard proximate. Analytically, four of the five constituents are obtained via chemical reactions and experiments. The constituent, carbohydrates, are obtained based on the determination of the four others. According to [5,6], Proximate accounts for 100% of a food product; and any deviation from 100% leads to a chemical test, and thus smaller variations in the way each test is performed accumulate or overlap the compositional make-up.

Seasonings are ingredients which are added to foods to enhance their flavour. Examples of such ingredients are salt, herbs (like mint, thyme, and spices), condiments (like mustard, vinegar). However, some seasonings also contain health and medical benefits [7]. Other types of seasonings include cinnamon, curry, onion, rosemary, parsley and sesame seeds. Hence, as food, seasonings have been shown to be sources of nutrients [8-11].

[12] carried out proximate analysis on fish sausages, and the results of their findings showed that Moisture above 40% is not favorable as it could promote the microbial growth in fish sausages. By increasing the incorporation of BBE from 0 to 0.75%, the moisture level decreased from 47.79 to 40.33%. Increment in pollen concentration also decreased the moisture content when added to meatballs and gluten-free bread. One of the possible factors of decreasing moisture content is due to the substitution of sago starch to make fish sausage with BBE. Sago starch contains high amylopectin, which has high water absorbing capacity. Hence, lowering sago starch content reduces fish sausages' moisture level. Carbohydrate content significantly increased (p-value less than 0.05) from 28.93 to 37.41% in 0.75% BBE fish sausage compared to the negative control.

In a research carried out by [13] on proximate analysis of poultry-mix formed feed using maize bran as a base, the findings of their results shows that moisture ranged from 1.18% to 1.54%, unrefined lipids ranged from 0.99% to 3.08%, total carbohydrate ranged from 57% to 72%, ash content ranged from 38.48% to 38.92%, unrefined protein ranged from 18.38% to 22.53%, and unrefined fiber ranged from 2.0% to 4.65%. Their findings also showed that all the feed samples showed a substantial variation.

MATERIALS AND METHODS

This paper adopts the techniques of Single-Factor Analysis of Variance (One-Way ANOVA) and Kruskal-Wallis Test (also known as One-Way ANOVA on Ranks) as a Non-Parametric alternative to One-Way ANOVA.

Single-Factor Analysis of Variance (One-Way ANOVA)

In applying the techniques of Analysis of Variance in a set of observations, the following steps are employed

Step 1: Specifying the Model

The model for Single-Factor Analysis of Variance (One-Way Analysis of Variance) according to the aim of this study is defined as

$$x_{ij} = \mu + \alpha_i + e_{ij}\,, i = 1, \cdots, p, j = 1, \cdots, q \qquad (1)$$

where x_{ij} is the proximate percentage compositions from the seasonings; α_i is the mean effect of the ith proximate; e_{ij} is the error associated with the proximate percentage compositions, and μ is a constant

Step 2: Testing the Assumptions Underlying the Data Set

(a) Testing for Normality using Shapiro-Wilk normality test (it is used to verify whether the population from which the samples are drawn are normally distributed) [14]

$$W = \frac{\left(\sum_{i=1}^{n} a_i x_{(i)}\right)^2}{\sum_{i=1}^{n}(x_i - \bar{x})^2} \tag{2}$$

$$\bar{x} = \frac{x_1 + \cdots + x_n}{n} \tag{3}$$

where $x_{(i)}$ is the ith ordered random sample values (that is the smallest number in the sample), the coefficient a_i are constants obtained from the covariance, variances, and means of the sample (size n) from a normally distributed sample.

(b) Testing for Constant Variances using Bartlett's Test (it is used to verify whether variances between several groups are equal)

$$B = \frac{(n-k)ln\left(S_p^2\right) - \sum_{i=1}^{k}(n_i - 1)\, ln(S_i^2)}{1 + \frac{1}{3(k-1)}\left[\sum_{i=1}^{k}\left(\frac{1}{n_i - 1}\right) - \frac{1}{n-k}\right]} \tag{4}$$

where S_p^2 is the pooled estimate for the variance and S_i^2 is the variance of i group; k is the total number of groups; n total observations across all groups; n_i is the number of observations in group i

$$n = \sum_{i=1}^{k} n_i\,;\;\; S_p^2 = \frac{1}{n-k}\sum_{i}(n_i - 1)\, S_i^2;\;\; S_i^2 = \sum_{i=1}^{n_i}\frac{(x_i - \bar{x})^2}{n_i - 1} \tag{5}$$

If Step 2 is met (that is the assumptions of normality and constant variance are met), then the process is moved to Step 3. And if Step 2 is not met, then, we analyze the data using a nonparametric alternative to Single-Factor Analysis of Variance (One-Way ANOVA) which is Kruskal-Wallis Test (One-Way ANOVA on ranks).

Step 3: Estimating the Parameters in the Model in equation (1)

The estimates of μ, α_i and e_{ij} are given as

$$\hat{\mu} = \frac{\sum_{j=1}^{q}\sum_{i=1}^{p} x_{ij}}{pq} = \frac{\sum_{j=1}^{q} T_{.j}}{pq} \;;\quad \hat{\alpha}_i = \frac{\sum_j x_{ij}}{q} - \hat{\mu} \;;\quad \hat{e}_{ij} = x_{ij} - \frac{\sum_j x_{ij}}{q} \tag{6}$$

Step 4: Estimating the Sum of Squares, Mean Squares and the F-Ratio
The sum of squares are estimated as

$$SS\mu = \frac{\left(\sum_{j=1}^{q}\sum_{i=1}^{p} x_{ij}\right)^2}{pq} = \frac{T_{..}^2}{pq} \tag{7}$$

$$SS\alpha = \frac{\sum_{j=1}^{q}\left(\sum_{i=1}^{p} x_{ij}\right)^2}{q} - SS\mu \;\Rightarrow SS\alpha = \frac{\sum_{j=1}^{q} T_{.j}^2}{q} - \frac{T_{..}^2}{pq} \tag{8}$$

$$SSe = \sum_{j=1}^{q}\sum_{i=1}^{p} x_{ij}^2 - \frac{\sum_{j=1}^{q}\left(\sum_{i=1}^{p} x_{ij}\right)^2}{q} \Rightarrow \; SSe = \sum_{j=1}^{q}\sum_{i=1}^{p} x_{ij}^2 - \frac{\sum_{j=1}^{q} T_{.j}^2}{q} \tag{9}$$

The mean squares and the F-Ratio are estimated as

$$MS\alpha = \frac{SS\alpha}{q-1}; \quad MSe = \frac{SSe}{pq-q} = \frac{SSe}{q(p-1)} \quad ; F = \frac{MS\alpha}{MSe} \tag{10}$$

We move to Step 5 if we conclude that there is a significant difference in the proximate percentage composition across the seasonings

Step 5: Tukey's Multiple Comparison Test
Tukey's Honestly Significant Difference is employed for the multiple comparison test

$$T_s = \frac{\bar{x}_A - \bar{x}_B}{S\sqrt{\frac{2}{q}}} \;; \text{where } \; S = \hat{\sigma}_e = \sqrt{MSe} \tag{12}$$

where se is the standard error; $\bar{x}_A$is the larger of the two means being compared; $\bar{x}_B$is the smaller of the two means being compared

Kruskal-Wallis Test (One-Way ANOVA on Ranks)
If there is/are ties in the data, the Kruskal-Wallis is obtained using

$$H = (N-1)\frac{\sum_{i=1}^{k} n_i(\bar{r}_{i.} - \bar{r})^2}{\sum_{i=1}^{k}\sum_{j=1}^{n_i}\left(r_{ij} - \bar{r}\right)^2} \tag{13}$$

where N is the total number of observations across all groups; k is the number of groups; n_i is the number of observations in group i; r_{ij} is the rank of observation j from group i; $\bar{r}_{i.}$is the average rank of all observations in group i, and $\bar{r}$ is the average of all the r_{ij}

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If there are no ties in the data, then the Kruskal-Wallis is obtained using

$$H = \frac{12}{N(N+1)} \sum_{i=1}^{k} n_i \left(\bar{r}_{i.} - \frac{N+1}{2} \right)^2 \tag{14}$$

$$\bar{r}_{i.} = \frac{\sum_{j=1}^{n_i} r_{ij}}{n_i}; \quad \bar{r} = \frac{1}{2}(N+1) \tag{15}$$

Dunn's Kruskal-Wallis Multiple Comparison
The pairwise comparison between two independent groups i and j performed by Dunn's Test is given as

$$D = \frac{\bar{R}_i - \bar{R}_j}{\sqrt{\frac{N(N+1)}{12}\left[\frac{1}{n_i} + \frac{1}{n_j}\right]}} \quad ;\text{where } \bar{R}_i = \frac{R_i}{n_i} \tag{16}$$

Bonferroni Adjustment of the Original P-value
The Bonferroni adjustment is used to control the family-wise error rate by adjusting the p-value, which occurs as a result of the multiple comparisons conducted. It is defined as

$$\text{Adjusted p} - \text{value} = p \times m \tag{17}$$

Where p is the original p-value, m is the total number of comparisons being made
Estimation of Percentage Carbohydrate
This is done in accordance to the official methods of [15], and is defined as

$\text{Carbohydrate} = 100 - (\%\ \text{Moisture} + \%\ \text{Crude Protein} + \%\ \text{Crude Fat} + \%\ \text{Ash Content})$
(18)

RESULTS/FINDINGS

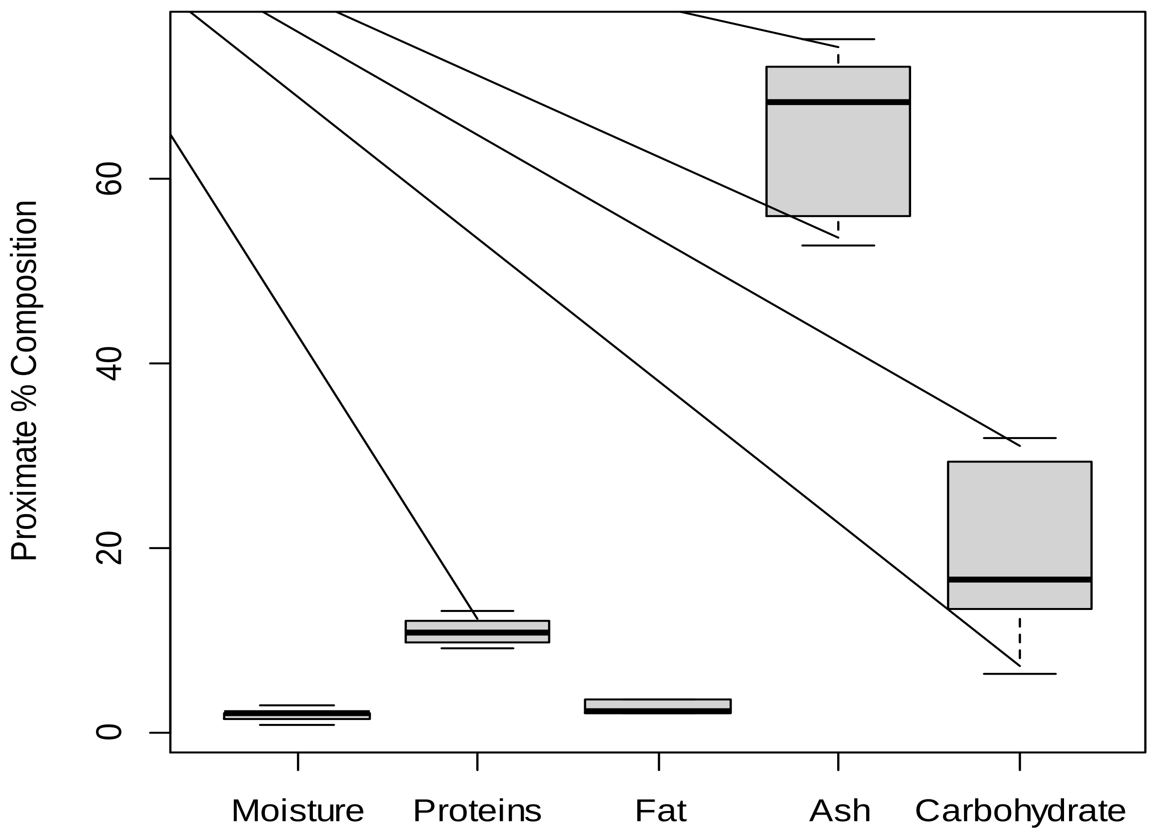

Figure 1. Boxplot Showing the Percentage Composition of the Proximate

In Figure 1, the boxplot for the proximate Moisture and Protein show a slight variation in their percentage composition across the seasonings; the proximate Fat show no variation across the seasonings; the proximates Ash and Carbohydrate show variation in their both percentage compositions across the seasonings. There is a significant difference between the percentage composition of Protein and Moisture and Fat, while Fat and Moisture are even in their distribution. Ash shows the highest percentage composition, followed by Carbohydrate. Furthermore, the distribution of Ash and Carbohydrate is skewed.

Table 1. Descriptive Statistics of the Proximate Composition

Proximate	Mean	Median	Standard Deviation	1st Quartile	3rd Quartile
Moisture	1.888	2.015	0.638	1.560	2.087
Proteins	10.97	10.90	1.430	9.880	11.950
Fat	2.628	2.345	0.661	2.110	3.322
Ash	64.79	68.17	8.354	56.73	71.62
Carbohydrate	19.73	16.65	9.633	13.74	28.97

In Table 1, the average composition of the proximate Moisture across the seasonings is 1.888% with standard deviation of 0.638%, and at 25% and 75% quantile, the composition of Moisture across the seasonings is 1.560% and 2.087% respectively; the average composition of Protein across the seasonings is 10.97% with a standard deviation of 1.430%, and at 25% and 75% quantile, the composition of Protein across the seasonings is 9.880% and 11.950% respectively; the average composition of Fat is 2.628% with a standard deviation of 0.661%, at 25% and 75% quantile, the composition of Fat across the seasonings is 2.11% and 3.322% respectively; the average composition of Ash is 64.79% with a standard deviation of 8.354%, and at 25% and 75%, the composition of Ash is 56.73% and 71.62% respectively; the average composition of Carbohydrate is 19.73% with a standard deviation of 9.633%, and at 25% and 75% quantile, the composition of Carbohydrate is 13.74% and 28.97% respectively.

Table 2. Test for Normality and Constant Variance

Shapiro-Wilk normality test W = 0.91994, p-value = 0.002347
Bartlett test of homogeneity of variances Bartlett's K-squared = 83.878, df = 4, p-value < 2.2e-16

The Shapiro-Wilk normality test in Table 2 is 0.91994, and the p-value is 0.002347 (which is less than 0.05). However, this indicates that the percentage composition of the proximate across all the seasonings are not normally distributed. Furthermore, Bartlett's K-squared is 83.878 and the p-value is < 2.2e-16 (which is less than 0.05), there is strong evidence that the variances across the seasonings are not the same.

The Normal Q-Q plot in Figure 2 shows that the residuals are not normally distributed (the points are spread far from the straight line. The Residual Vs Fitted plot show lack of constant variance. However, this study needs to advance to Kruskal-Wallis test (as a non-parametric alternative to One-Way ANOVA).

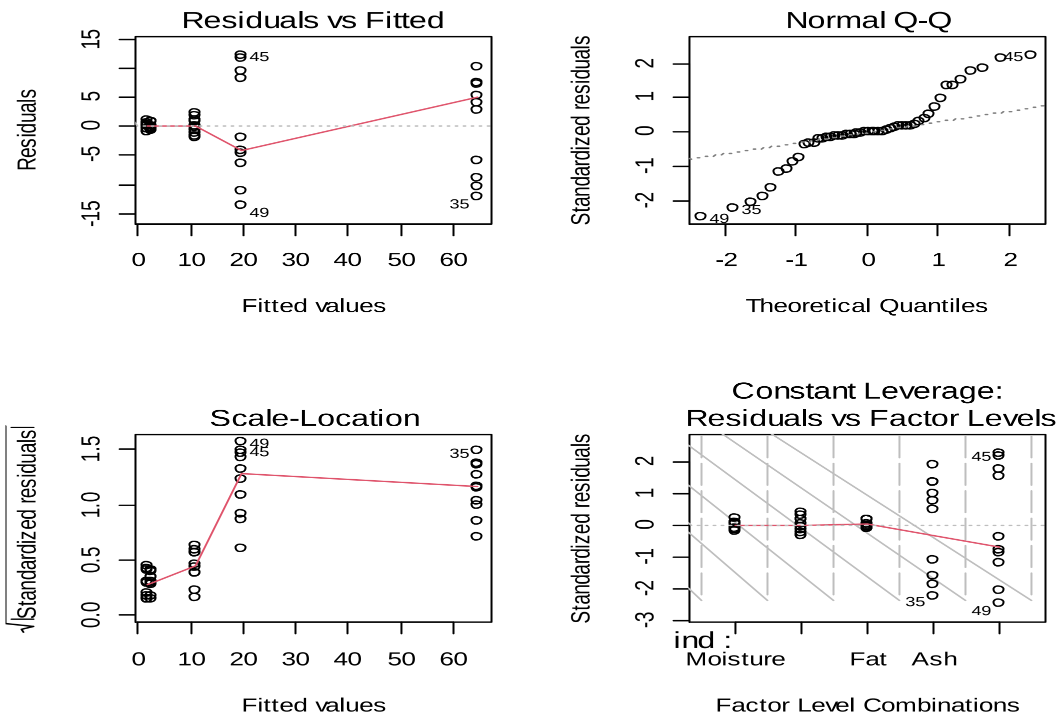

Figure 2. Residual Diagnostic for Normality and Constant Variance

Table 3. Kruskal-Wallis Rank Sum Test for Significance

Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 43.918, df = 4, p-value = 6.672e-09

Kruskal-Wallis chi-squared in Table 3 is 43.918 and the p-value is 6.672e-09 (which is less than 0.05). This implies that there is a statistically significant difference between the proximate percentage composition across the seasonings. Thus, there is a need to perform Dunn Kruskal-Wallis multiple comparison test to determine which of the groups (the proximate(s)) differ significantly.

Table 4. The Dunn Kruskal-Wallis Multiple Comparison Test

Dunn (1964) Kruskal-Wallis multiple comparison p-values adjusted with the Bonferroni method.

Comparison	Z	P.unadj	P.adj
Ash - Carbohydrate	1.8408486	6.564375e-02	6.564375e-01
Ash – Fat	4.9472805	7.525748e-07	7.525748e-06
Carbohydrate - Fat	3.1064320	1.893599e-03	1.893599e-02
Ash - Moisture	5.7910028	6.996741e-09	6.996741e-08
Carbohydrate - Moisture	3.9501542	7.810085e-05	7.810085e-04
Fat - Moisture	0.8437223	3.988246e-01	1.000000e+00
Ash - Protein	2.7612729	5.757655e-03	5.757655e-02
Carbohydrate - Protein	0.9204243	3.573511e-01	1.000000e+00
Fat - Protein	-2.1860077	2.881504e-02	2.881504e-01
Moisture - Protein	-3.0297299	2.447725e-03	2.447725e-02

In Table 4, the adjusted p-value (P.adj) for the mean differences of the following pairs of proximate compositions: Ash and Carbohydrate, Fat and Moisture, Ash and Protein, Carbohydrate and Protein, and Fat and Protein are 6.564375e-01, 1.000000e+00, 5.757655e-02, 1.000000e+00, and 2.881504e-01 respectively (which are greater than 0.05), showing an evidence of no significant difference in the mean percentage composition of the pairs of the proximate compositions; furthermore, the adjusted p-value (P.adj) for the mean differences of the pairs: Ash and Fat, Carbohydrate and Fat, Ash and Moisture, Carbohydrate and Moisture, and Moisture and Protein are 7.525748e-06, 1.893599e-02, 6.996741e-08, 7.810085e-04, and 2.447725e-02 respectively (which are less than 0.05), showing an evidence of significant difference in the mean percentage composition of the pairs of the proximate compositions.

CONCLUSION

This paper examines the proximate compositions of ten commonly used seasonings in Nigeria using the applications of some analytical techniques. The results of the findings revealed that Single-Factor Analysis of Variance is not the best techniques to analyzing the proximate compositions across the ten commonly use seasonings selected at random, rather, Kruskal-Wallis an alternative nonparametric test to Single-Factor Analysis of Variance is use instead. The five standard proximate (Moisture, Protein, Fat, Ash, and carbohydrate) base on the results of the findings of this study showed evidence of statistically significant difference in their mean compositions.

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