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Food Sustainability Study in Ecuador: Using PCA Biplot and GGE Biplot

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Abstract: Agriculture is one of the main sectors of Ecuador's economy, and the principal agricultural product for exportation is cocoa. Flour samples of two mixtures were taken: a total of 50 samples of 85% cocoa bean shell (harvested from a farm) mixed with 15% soy flour (Mixture 1) and 50 samples of 75% cocoa bean shell (harvested from a farm) mixed with 25% soy flour (Mixture 2). The parameters evaluated were: moisture, protein, fat, carbohydrates, ash, total dietary fiber (TDF), and biological activity. Multivariate statistical techniques, such as PCA biplots and GGE biplots, were used to present each parameter (vector) measured. The biplot techniques suggested that the flour samples corresponding to Mixture 1 indicated the most significant values of nutritional and commercial properties. The results suggest that the use of mixtures of cocoa bean shell flour with soy flour can be used as ingredients to produce new foods.

Keywords: new foods; nutritional and commercial parameters; GGE biplot; PCA biplot

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Citation: Valenzuela-Cobos, J.D.; Guevara-Viejó, F.; Vicente-Galindo, P.; Galindo-Villardón, P. Food Sustainability Study in Ecuador: Using PCA Biplot and GGE Biplot. *Sustainability* **2022**, *14*, 13033. https://doi.org/10.3390/su142013033

Academic Editors: Abdo Hassoun, Monica Trif, María Carpena Rodríguez and Begüm Önal

Received: 14 September 2022
Accepted: 6 October 2022
Published: 12 October 2022

1. Introduction

In recent decades, people have modified ecosystems more rapidly and extensively than in other periods of history, increasing the demands for food, fresh water, timber, fiber, and fuel [1]. Sustainability involves a high degree of cohesion and balance between the market, government, and civil society in designing participatory planning processes, which are understood as interactions between the agents involved in these processes, such as sustainable agriculture and water management. The five pillars of sustainability include productivity, stability and security, the protection of natural resources, viability, and acceptability. Important advances have been made in some aspects of environmental sustainability: the total area of protected areas has grown steadily in the last decade; the consumption of substances that deplete the ozone layer has decreased considerably; and the region has made progress in expanding the coverage of drinking water and sanitation services [2]. Agriculture is part of the second and twelfth sustainable development goals to achieve the eradication of hunger and meet the global demand for food in an environmentally sustainable way [3]. Studies have shown that the application of synthetic chemicals, such as pesticides, increase agricultural production while causing harmful effects on human health, the environment, biological diversity, and food production. The main challenge of food sustainability is to produce food for the consumption of people of different socioeconomic classes while protecting the environment and human health [4].

Agriculture is one of the most important activities of the Ecuadorian economy; it maintains an economic dynamic that supplies raw materials to the industry and guarantees the food security and sovereignty of the country [5]. The principal Ecuadorian agricultural products for exporting are: bananas, shrimp, and cocoa, with Ecuador being the main leader in the production of the fine aroma cocoa called Cacao Arriba, which is internationally recognized [6]. Ecuador is the main country in the export and commercialization of the Arriba Nacional variety worldwide; this cocoa is known for its intense aroma [7]. Cocoa in Ecuador is characterized by a floral profile, of blackcurrant and spices, due to the phytochemical composition, which is influenced by the botanical origin, the place of growth, the time of sun and rain, the nutrients in the soil, the maturation, and the harvest [8,9]. The climate and geology of different regions of the country are important for the growth of this cocoa [10]. In addition, cocoa waste (cocoa bean shell, "CBS") has been mainly employed to elaborate different food products, such as biscuits, gluten-free bread, functional beverages, pork sausages, cookies, chocolate muffins, cooked beef, extra virgin olive oil, jam, dairy drinks, yogurt drinks, extruded snacks, and pound cake [11,12]. Agriculture has been an obvious target for applying multivariate statistical techniques for big data to achieve practical and effective solutions [13].

Multivariate statistical techniques allow the handling of big data in order to analyze information that cannot be studied using conventional statistical methods. Currently, large databases are analyzed using data mining [14]. Multivariate statistical methods identify similarities between large databases [15]. For studies in agriculture, techniques such as PCA biplot and GGE biplot have been designed; these divide the data into different groups depending on the similarities they present. The present study is based on identifying groups that are generated from databases that are very useful in different areas, such as science, technology, engineering, and mathematics [16]. Additionally, the efficient use of these techniques is related to a probabilistic and well-founded model [17]. Different authors have indicated that clustering methods are a derivation of heuristic techniques [18]. For this reason, it is important to compare these two multivariate statistical techniques to define the best algorithm for the agricultural sector.

The objective of this research was to use multivariate statistical techniques, such as PCA biplot and GGE biplot, to identify the use of mixtures of cocoa bean shell flour and soy flour as ingredients in the production of new by-products.

2. Materials and Methods

2.1. Experimental Design

Cocoa bean shells were manually harvested from 50 farms located in the Province of Guayas, and 5 bags of 10 kg of soybeans (VARGAS brand) were purchased at the Central de Abasto "Montebello" in Guayaquil, Ecuador. The cocoa bean shells and soybeans were exposed directly to sunlight until they were dried, then ground into powder in a knife mill for 30 s and passed through to a mesh of 417 μm [19].

The edaphoclimatic characteristics of the Province of Guayas are: maximum temperature of up to 35 °C, minimum temperature of 16 °C, relative humidity of 89%, and annual rainfall of 810.2 mm.

2.2. Elaboration of Mixtures

The mixtures were made using the following compositions:

- Mixture 1 (M1): 85% cocoa bean shell (flour) harvested from a farm, mixed with 15% soy flour;
- Mixture 2 (M2): 75% cocoa bean shell (flour) harvested from a farm, mixed with 25% soy flour.

The proportions of cocoa bean shells (75% and 85%) were used in this research because authors have found high organoleptic properties when the content of cocoa bean shells increased [20].

2.3. Nutritional Composition of Mixtures

The nutritional composition of the mixtures was analyzed using AOAC procedures, analyzing protein, fat, carbohydrates, ash, and total dietary fiber (TDF). The protein content (N $\times$ 6.25) was estimated using the Kjeldahl method.

The fat content was determined using a Soxhlet technique; a flask with hexane was adapted to the cartridge holder (3 g) and condenser, and the extraction was realized for 4 h. Once the extraction was finished, the solvent was removed by evaporation in a rotary evaporator. The fat was cooled in a desiccator and weighed.

To determine the ash content, the sample (0.5 g) was weighed in a porcelain crucible. Subsequently, the sample was heated in a burner until completely calcined. Next, the crucible with the sample was placed in a muffle at a temperature of 600 °C for 30 min.

Carbohydrates (%C) were determined using Equation (1) [21]:

$$C(\%) = 100 - (\%moisture + \%protein + \%fat + \%ash\ contents) \tag{1}$$

Equation (1). Carbohydrates of mixtures.

2.4. Antioxidant Activity

The antioxidant activity values were obtained using a DPPH assay. Firstly, 30 μL of the extract and 270 μL of methanol (DPPH radicals) were mixed in a 96-well plate. The reaction was incubated in the dark for 30 min, and the absorption was measured at 515 nm [22]. The DPPH assay was calculated according to a percentage of DPPH discoloration using Equation (2):

$$RSA(\%) = \frac{ADPPH - AS}{ADPPH} \times 100 \tag{2}$$

Equation (2). DPPH Assay.

2.5. Antimicrobial Activity

The evaluation of this activity was realized using the bacteria *Staphylococcus aureus* (ABP 784).

Bacterial suspensions were adjusted with sterile saline to a concentration of 1.0×10^6 CFU/mL. The extracts of the mixtures were dissolved in 30% ethanol and mixed with nutrient media for bacteria with 1.0×10^5 CFU per well of Tryptic Soy Broth in a final volume of 100 μL [23].

2.6. Multivariate Statistical Analysis

Multivariate statistical techniques, such as PCA biplots and GGE biplots, were realized using R, software version 4.1.1. (R Core Team, Vienna, Austria).

PCA Biplot

Substituting $\mathbf{Z} = \mathbf{D_o V_{[r]}}$ gives [24–26]:

$$\hat{\mathbf{D}}_{\mathbf{o}[\mathbf{r}]} = \mathbf{Z V}_{[\mathbf{r}]}^{\mathbf{T}}$$

$$\mathbf{Z V}_{[\mathbf{r}]}^{\mathbf{T}} = \mathbf{D_o V}_{[\mathbf{r}]} \mathbf{V}_{[\mathbf{r}]}^{\mathbf{T}} = \mathbf{D_o V}_{[\mathbf{r}]} \left(\mathbf{V}_{[\mathbf{r}]}^{\mathbf{T}} \mathbf{V}_{[\mathbf{r}]} \right)^{-1} \mathbf{V}_{[\mathbf{r}]}^{\mathbf{T}}$$

The representation of the sample projected on the biplot plane is given by:

$$\mathbf{d}_{\mathbf{oproj}}^{\mathbf{T}} = \mathbf{d}_{\mathbf{o}}^{\mathbf{T}} \mathbf{V}_{[\mathbf{r}]} \left(\mathbf{V}_{[\mathbf{r}]}^{\mathbf{T}} \mathbf{V}_{[\mathbf{r}]} \right)^{-1} \mathbf{V}_{[\mathbf{r}]}^{\mathbf{T}} = \mathbf{z}^{\mathbf{T}} \mathbf{V}_{[\mathbf{r}]}^{\mathbf{T}}$$

The coordinates make projections on the biplot plane, which are given by $\mathbf{z}^{\mathrm{T}}$. That is, the sample is interpolated in the biplot plane by:

$$\mathbf{z}^{\mathbf{T}} = \mathbf{d}_{\mathbf{o}}^{\mathbf{T}} \mathbf{V}_{[\mathbf{r}]}$$

GGE Biplot

$$Y_{ijk} = \mu + t_i + l_k + (k) + t_{lik} + \epsilon_{ijk}$$

Y_{ijk} refers to the observation obtained in the i-th genotype, evaluated at the j-th repetition, at the k-th location [27];
μ is the mean general;
t_i refers to the fixed effect of the i-th genotype used in the trials, with $i = 1, 2, \ldots$, twenty;
l_k is the random effect of the k-th locality, with $k = 1, 2, \ldots, 7$;
(k) is the effect random of the j-th repetition within the k-th locality, with $j = 1, 2, 3$;
t_{lik} is the random effect of the interaction between the i-th genotype with the k-th locality;
ϵ_{ijk} is the error associated with the observation Y_{ijk}.

3. Results and Discussion

The main objective of the study was to present the use of two mixtures of cocoa bean shell with soy flour for the production of new foods by analyzing different nutritional and commercial properties, such as moisture, protein, fat, carbohydrate, ash, total dietary fiber (TDF), and biological activity.

The samples of the cocoa bean shell and soy flour mixtures were made using the following distribution:

1–50: Flour samples corresponding to Mixture 1 (85% cocoa bean shell harvested from a farm, mixed with 15% soy flour);
51–100: Flour samples corresponding to Mixture 2 (75% cocoa bean shell harvested from a farm, mixed with 25% soy flour).

3.1. Multivariate Statistical Techniques for Nutritional Composition of Mixtures

The nutritional parameters of the mixtures of cocoa bean shells and soy flour allow us to show the importance of the health benefits of by-products. Figure 1 presents the plane 1-2 (PCA biplot); Graphic (a) indicates the accumulated inertia amounts to 53.8%, whereas Graphic (b) shows the accumulated inertia amounts to 54%. These figures present 50 flour samples corresponding to Mixture 1 or Mixture 2, with six variables (moisture, protein, lipid, carbohydrate, ashes, and total dietary fiber "TDF"), each one using the software RStudio.

Figure 1a shows three groups corresponding to the nutritional values of the flour samples of Mixture 1, whereas in Figure 1b, the three groups presented correspond to the nutritional parameters of the flour samples of Mixture 2. The group method allows the identification and location of similar samples in the data reported in small areal units. Statistical distribution using groups indicates the statistical significance between the individuals. [28].

The size of each group is related to the number of data points. For Figure 1a: group 1 (blue points) was 27 samples, group 2 (green points) was 12 samples, and group 3 (red points) was 11 samples. The samples belonging to Mixture 1 (group 1) presented the highest values of nutritional parameters. Furthermore, for Figure 1b: group 1 (blue points) was 14 samples, group 2 (green points) was 19 samples, and group 3 (red points) was 17 samples. The samples belonging to Mixture 2 (group 1) presented the highest nutritional values. The data points present normal distribution, and the groups vary in size with the number of data points. Flour samples corresponding to Mixture 1 presented the highest nutritional parameters in comparison with the flour samples corresponding to Mixture 2.

Figure 2 indicates the graph formed by the plane 1-2 (GGE biplot); Figure 2a presents the accumulated inertia amounts to 80.2%, whereas Figure 2b displays the accumulated inertia amounts to 70.1%. The groups were formed using six variables and were calculated using the GGE biplot coordinates.

Figure 2a shows the presence of 27 flour samples from Mixture 1 and the highest nutritional values, such as moisture, protein, fat, ash, and total dietary fiber ("TDF"); it also shows significant differences in 10 flour samples which only showed the highest carbohydrate values and 13 flour samples that did not present significant nutritional values. Moreover, Figure 2b also presents the presence of 15 flour samples of Mixture 2 and the highest nutritional values, such as moisture, protein, fat, ash, and total dietary fiber

("TDF"); 17 flour samples only showed the highest carbohydrates values, and 18 flour samples presented the lowest nutritional parameters.

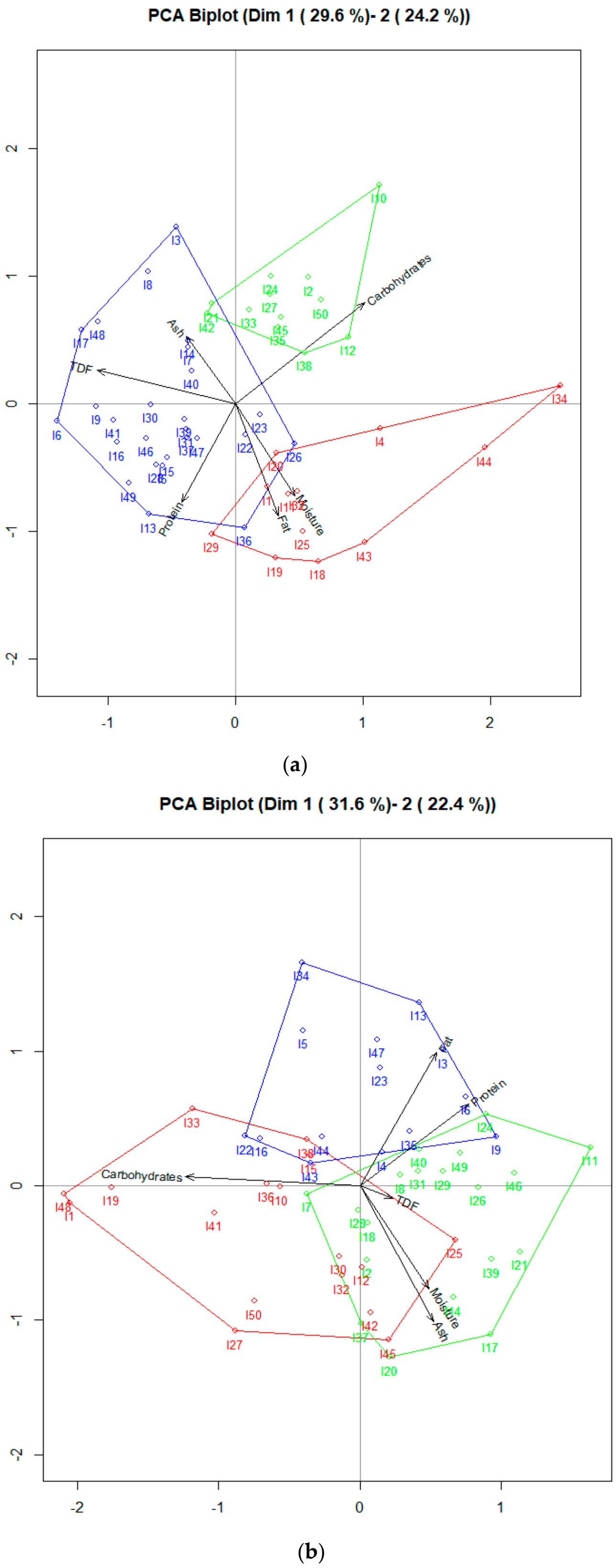
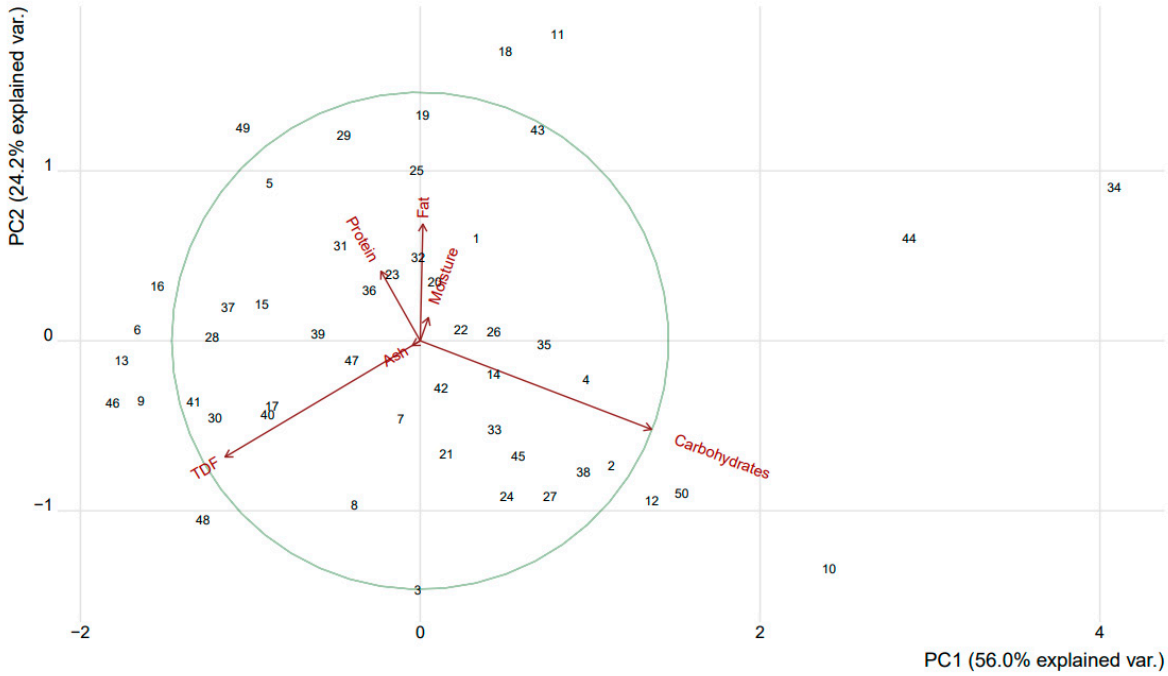
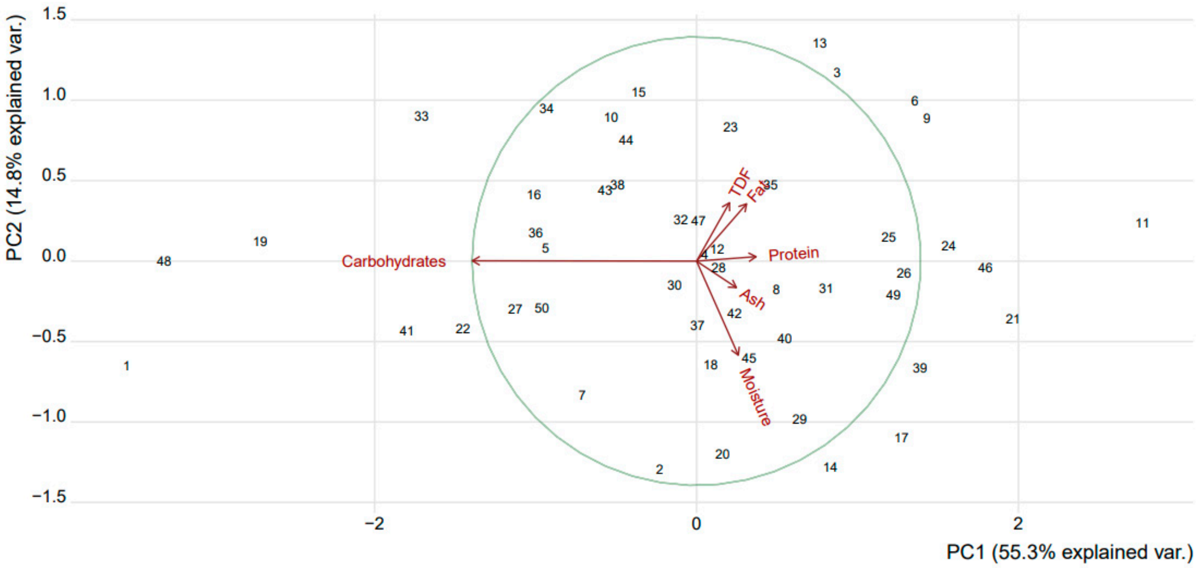


Figure 1. (**a**) PCA biplot of flour samples of Mixture 1 corresponding to nutritional values; (**b**) PCA biplot to flour samples of Mixture 2 corresponding to nutritional values.



(**a**)



(**b**)

Figure 2. (**a**) GGE biplot of flour samples of Mixture 1 corresponding to nutritional values; (**b**) GGE biplot of flour samples of Mixture 2 corresponding to nutritional values.

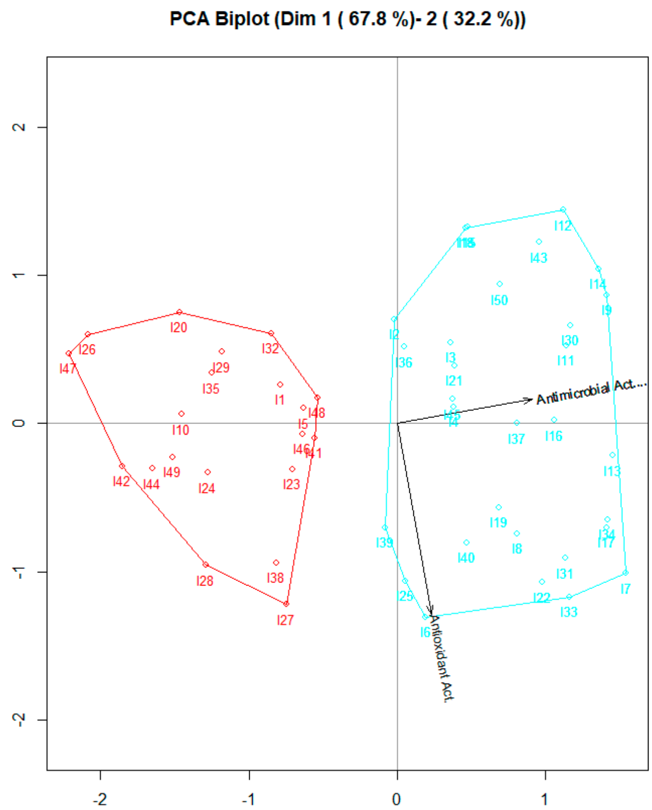
Protein is one of the most important nutrients for daily intake; fat has a great influence on the organoleptic properties of the product, such as the color, aroma, and flavor; and dietary fiber promotes the digestibility of food, helping to reduce the risk of diseases, such as cholesterol and diabetes [29–31]. The values of TDF and protein indicate the importance of the mixtures to be used as ingredients in the elaboration of by-products. The values of the nutritional parameters allow us to indicate that the two mixtures can be a substitute for meat.

3.2. Multivariate Statistical Techniques for Commercial Properties of Mixtures

The biological activities indicate the elimination of pathogenic microorganisms. Figure 3 shows the application of the PCA-biplot algorithm to 50 objects with two variables (antioxidant and antimicrobial activities), each one using the software RStudio. Figure 3 presents the factorial graph of the plane 1-2 (PCA biplot); Figure 3a indicates the accumulated inertia amounts to 100%, whereas Figure 3b points out the accumulated inertia amounts to 100%.

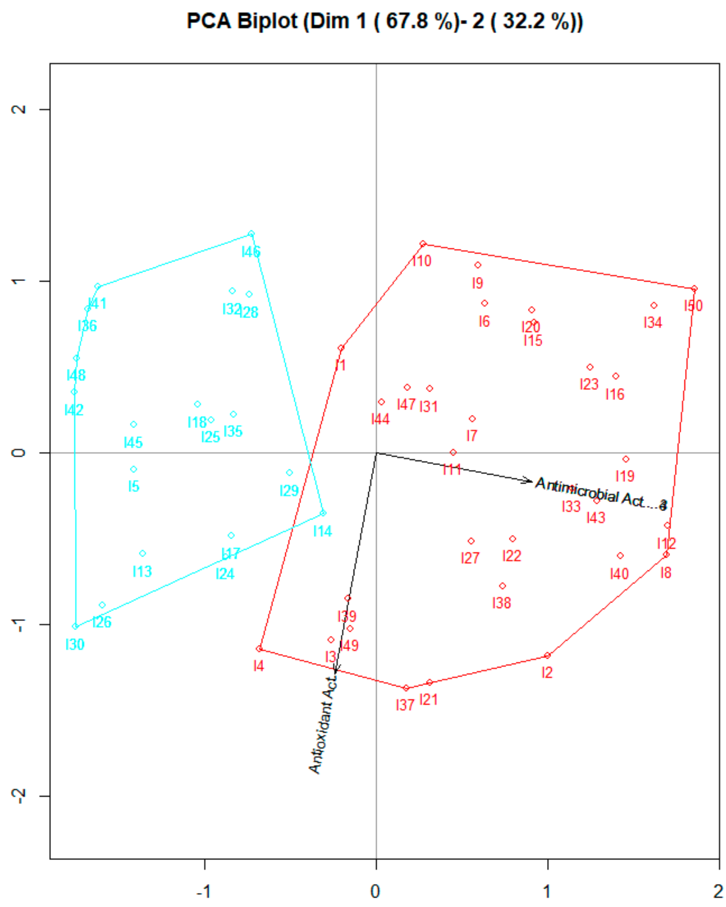
Figure 3a presented two groups corresponding to the commercial properties of flour samples of Mixture 1, whereas Figure 3b showed two groups corresponding to the commercial properties of flour samples of Mixture 2. In Figure 3a: group 1 (red points) was 21 samples, and group 2 (blue points) was 29 samples. Samples belonging to group 2 presented the highest values of antioxidant and antimicrobial activities. For Figure 3b: group 1 (blue points) was 19 samples, and group 2 (red points) was 31 samples. Samples belonging to group 2 highlighted the highest values of antioxidant and antimicrobial activities. Flour samples from Mixture 1 indicated the most significant commercial features in comparison with the flour samples from Mixture 2.

Figure 4 indicates the plane 1–2 (GGE biplot); Figure 4a presents the accumulated inertia amounts to 100%, and Figure 4b depicts the accumulated inertia amounts to 100%. Groups were formed using two variables, and they were calculated using GGE-biplot coordinates.



(**a**)

Figure 3. *Cont.*

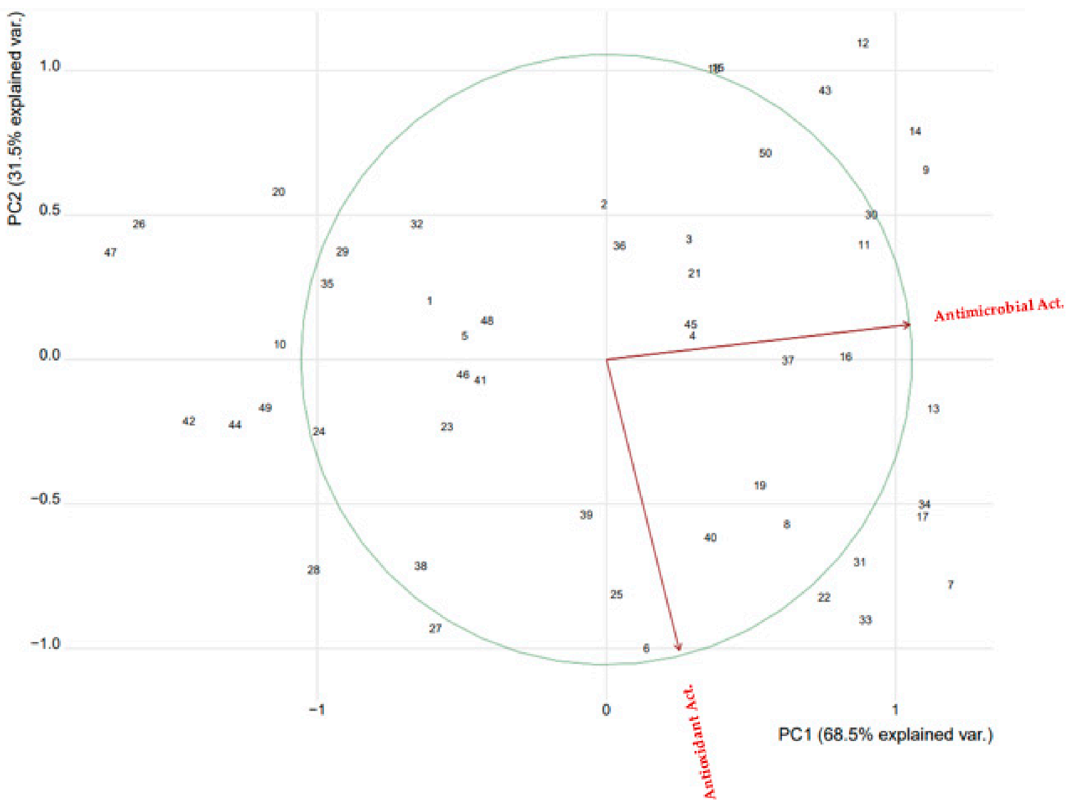


(**b**)

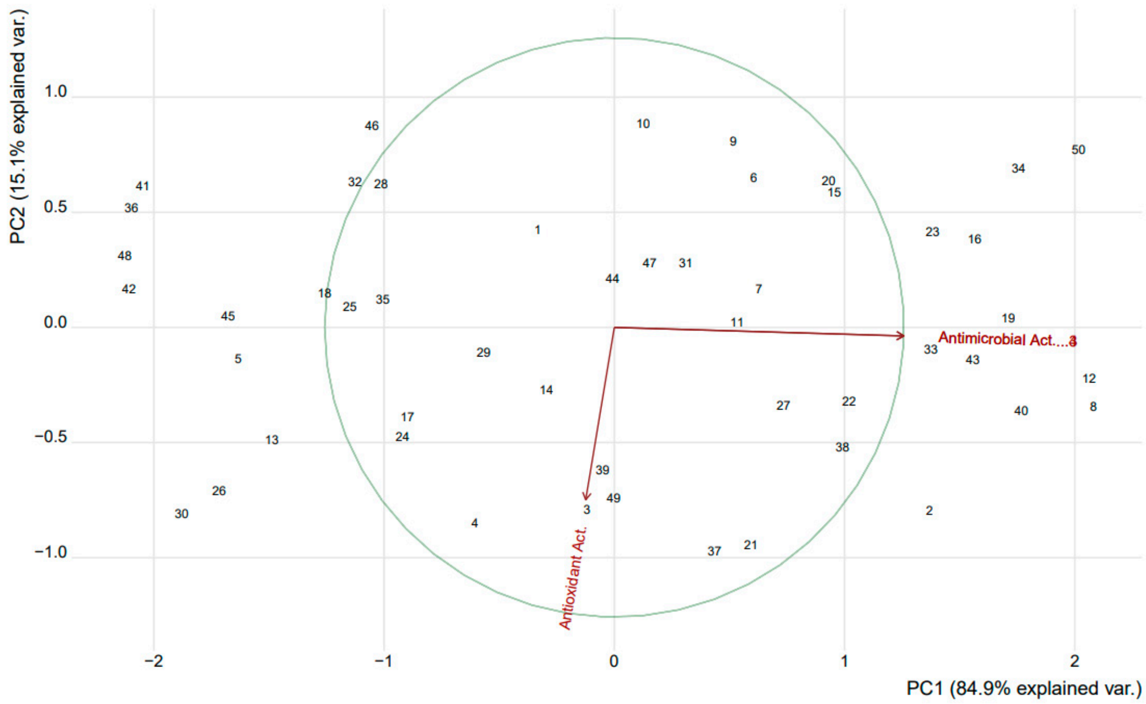
Figure 3. (**a**) PCA biplot of flour samples of Mixture 1 corresponding to commercial properties; (**b**) PCA biplot of flour samples of Mixture 2 corresponding to commercial properties.

Figure 4a indicates 15 flour samples from Mixture 1 with the highest commercial properties values, such as antioxidant and antimicrobial activities, and shows a significant difference in 35 flour samples that did not present significant antioxidant and antimicrobial activities. Figure 4b presents 13 flour samples from Mixture 2 with the highest commercial properties values, such as antioxidant and antimicrobial activities, and shows an important difference with 37 flour samples that present the lowest antioxidant and antimicrobial activities.

The DPPH potential is a significant predictor of the antioxidant activity value of a food product [32]. Cocoa shells and soybeans have been shown to be important sources of phenolic compounds, and they also have presented high values of antioxidant activity [33]. The finer particles of the products are related to the highest values of antioxidant activity because they have a greater release of bioactive compounds. [19]. Cocoa "Arriba Nacional", cultivated in the Amazonian region of Ecuador, showed higher values of avonoids, anthocyanins, and stilbenes (trans-resveratrol) compared to cocoa obtained from the coastal region [34]. Cocoa waste (cocoa shells) is used as an ingredient in the production of food bioproducts due to its high content of polyphenols and fiber, or it is used as an antimicrobial agent. [35]. The inhibitory properties are influenced by the bacteria strains, the manner in which the extract was obtained, and whether or not the raw material was subjected to heat treatment [36]. The consumption of cocoa products or by-products provides important health benefits [37].



(**a**)



(**b**)

Figure 4. (**a**) GGE biplot of flour samples of Mixture 1, corresponding to commercial properties; (**b**) GGE biplot of flour samples of Mixture 2, corresponding to commercial properties.

PCA biplot transforms correlated variables into uncorrelated variables called principal components in order to reduce the number of variables and show independence. On the other hand, the GGE-biplot logarithm is used in the field of agriculture to identify the characteristics of the environment [27,38]. The use of multivariate statistical techniques, such as PCA biplot and GGE biplot, described a real visualization of the flour samples, corresponding to Mixture 1 and Mixture 2, with the highest commercial parameters. The antioxidant and antimicrobial activity values are a good indicator of the possibility of using the mixtures in vivo studies.

4. Conclusions

The mixtures of cocoa bean shell flour and soy flour can be used as ingredients to produce new foods with higher nutritional values and commercial parameters.

Biplot techniques presented specific flour samples corresponding to Mixture 1, which showed the highest nutritional values, such as moisture, protein, fat, carbohydrate, ash, and TDF, and the commercial parameters, such as antioxidant and antimicrobial activities.

Multivariate statistical techniques can be used to describe improved combinations of ingredients (mixtures of flour), improving the production of new foods with important health benefits.

This research has shown the valorization of mixtures of cocoa shell flour and soybean flour as a biofunctional by-product, which can be a substitute for meat.

Author Contributions: Conceptualization, F.G.-V. and J.D.V.-C.; formal analysis, J.D.V.-C.; investigation, F.G.-V.; methodology, F.G.-V. and J.D.V.-C.; supervision, P.G.-V. and P.V.-G.; writing—original draft, F.G.-V., J.D.V.-C., P.G.-V. and P.V.-G.; writing—review and editing, P.G.-V. and P.V.-G. All authors have read and agreed to the published version of the manuscript.

Funding: This work is funded by Universidad Estatal de Milagro (UNEMI) Scholarship.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are grateful to Facultad de Ciencias e Ingeniería de la Universidad Estatal de Milagro (UNEMI) and Ecuahidrolizados Industry.

Conflicts of Interest: The authors declare no conflict of interest.

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