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Evaluation of Some Specific Components Existences in Okra (*Abelmoschus Esculentus L. (Moench)*) Cultivated from Different Areas

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Abstract Studies on the chemical composition of okra pods (*Abelmoschus esculentus Moench*) were carried out. Mean concentrations of moisture contents were about 84.67 - 87.65%, crude protein 2.367 - 3.41 %, fat 4.343 - 4.523% ash 10.314 - 12.197%, carbohydrate 67.857 - 82.261 % and fiber 6.781 - 8.314 % were reported. Sugars (fructose, glucose and sucrose) were determined by high performance liquid chromatography (HPLC). Water-soluble polysaccharide values were ranged from 11.22 to 17.35 g/100g. After ashing, the mineral constituents (Ca, Mg, Cu, Zn, Fe, pb, As, Se, Cr, Cd, and Mn) were separately analyzed (using Atomic Absorption Spectrometer) and recorded which met the recommended dietary allowance.

Keywords: *abelmoschus esculentus, sugars, polysaccharide, minerals element*

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1. Introduction

Okra pods *(Abelmoschus esculentus)*, which known as lady's finger or gumbo, are tropical vegetables and are belonging to the mallow family. Its immature pods could be consumed as a source of carbohydrates, minerals and vitamins and dietary medicines [1]. Various reports recorded that okra possessed pharmacological properties like antidabetic, nootropic, eye and heart disease as well as neurological disorders etc., [2]. Moreover, it also found that phytochemicals which may have antioxidant, antibacterial, antifungal, antiviral and anticarcinogenic properties were detectable. Therefore, its immature form may also use in the folk medicine as a diuretic agent and in dental disease [3,4].

Okra seeds could be considered as alternative sources of proteins, fats, fibers and sugars [5]. On the other hand, okra is reported to show hypolipidemic effects, i.e., decreasing absorption of cholesterol from diets [6,7]. A successful impact of the okra polysaccharides could be detected in the lowering of body weight and glucose levels, improving the glucose tolerance, and decreasing the serum total cholesterol (TC) levels [8].

The water extract of okra contains polysaccharides, which are thick slimy, acidic and consists of galactose, rhamnose and galacturonic acid [9]. Therefore, okra polysaccharides are able to use in thickening soups, stews and gums and could be used in chocolate bar cookies and chocolate frozen dairy dessert preparation as egg white and fat substitutes [10].

Minerals, in general, assist in fluid balance regulations, contractions of muscles and impulses of the nerves. Such components, also, are absorbed through the intestine and the body usually regulates mineral stores to keep them in balance. Interfered drugs with such balance lead to medical illnesses and dehydration and may result in deficiencies, toxicity and sometimes death may happen [11]. The principal elements in pods were K, Na, Mg and Ca and represented about 17% of seeds and Fe, Zn, Mn and Ni existence were, also, reported [12].

The objectives of this study are to throw the light on some okra characteristics and their nutritional values.

2. Materials and Methods

2.1. Chemical and Reagents

All the used solvents in the current study were of reagent grade without any further purification. Acetonitrile reagent was obtained from Lab-Scan (Tedia,

MO, USA). Glucose and sucrose were purchased from (Benchmark, MO, China). Fructose standard, phenol and other reagent and acids were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Phosphorus was from (Tianjin Yongda, MO, China). Absolute ethanol was from Tianli, MO, China. The water used in High-Performance Liquid Chromatography (HPLC) and sampling, preparation and analysis was prepared by a Millipore Simplicity Deionizer (Millipore S.A.S. 67120, Molsheim, France).

2.2. Plant Material

Okra pods were harvested from four geographical regions in Egypt, i.e., S pod (Suez) beside the Desert, M pod (Mansoura) beside the River Nile, K pod (Kafr El-Sheikh) beside the Mediterranean Sea, D pod (Dakahlia) beside a lake. Table 1 lists the geographic regions of the okra pods samples.

Table 1. Geographic location of okra varieties

City	Code	Latitude	Longitude
Dakahlia	D	31.053103	31.580615
Mansoura	M	31.042536	31.380014
Kafer Elshaikh	K	31.347304	30.80246
Suez	S	29.984721	32.524309

2.3. Preparation of Samples

The sun drying method was achieved by weighting fresh okra pods and place them under direct sunlight in the dry season with an overall maximum daytime air temperature of about 37°C and a night temperature of about 20°C over 20 days drying cycle. The relative humidity (RH) during nights must be lower than 78% with no rains. The pods were weighed at various intervals over the whole drying period up to a constant weight [13]. Samples then were milled to pass through a 0.5 mm screen by a Cycotec mill and the resulted powder was stored in poly bags at room temperature until used.

2.4. Chemical Analysis

Standard procedures [14] were done to determine the chemical analysis (moisture, crude protein (N*6.25), crude fat, fiber, and ash contents) in the resulted powder while the carbohydrate content of the tested samples was calculated by differences [14].

2.5. Sugar Analysis (HPLC)

The reducing and non-reducing sugars from okra were triplicate determined by HPLC. Prior to injection into the HPLC system. An aliquite of dried sample were refluxed with 100 mL ultrapure water – ethanol (80/20, v/v), boiling for 30 min. It was centrifuged at 6000 rpm for 20 min and filtrated over a 0.45 µm membrane filter.

Liquid chromatography separation was carried out at 35°C on carbohydrate column, 5 µm particle size and 250X4.6 mm i.d. from Knauer (Germany). The separated solvents were filtered over a 0.45 µm membrane filter and then degazzed for 15 min in an ultrasonic bath (Ultrasonic Cleaner Device, Model HZSH, CSF-1A, Shanghai, China). The mobile phase was acetonitrile and ultrapure water (75: 25% v/v) with a flow rate of 1.0 mL/min. The injection volume were adjusted to 10 µL. The detector was RI, K-230l, Knauer, Germany. The resulted peaks was calibrated with external standards of specific concentrations of glucose, fructose and sucrose.

2.6. Polysaccharide Analysis

A modified method of phenol-sulfuric colorimetric method was used to determine total polysaccharides by using glucose as a standard [15]. A glucose standard solution was prepared at 0.04 mg/mL concentration. Specific amounts (0.2 up to 1.6 mL, with 0.2 mL intervals) of the standard solution was transferred to a test tube and completed to 2 mL with deionized water. The blank solution was deionized water. Then, 1 mL of phenol (6%) solution (Sigma-Aldrich, St. Louis, MO) was added into each test tube, then, 5 mL sulfuric acid (98%) was added. Each tube was well mixed and placed at room temperature for 30 min. The optimum absorbance of the reacted solution was measured at 490 nm using a Spectrum spectrophotometer, 754 PC, Shanghai, China.

2.7. Mineral Analysis

The ashed sample was dissolved in 100 mL of HNO_3 (2%). The final volume was achieved with pure water. The mineral constituents (Ca, Mg, Cu, Zn, Fe, Pb, As, Se, Cr, Cd, and Mn) present in the date ashes were analyzed separately, using Atomic Absorption Spectrometer (PerkinElmer, AAnalyst 800). Phosphorus content was determined by a Spectrum spectrophotometer, 754 PC, Shanghai, China at the wavelength of 440 nm absorbance.

2.8. Statistical Analysis

Analysis of Variance (ANOVA) was used to statistical analysis the data of the tested samples by using the SPSS 16.0 for Windows. Significant differences were determined by Duncan's Multiple Range Test ($P < 0.05$). The principal component analysis (PCA) was used to estimate the correlation among all the studied parameters through XLSTAT software.

3. Results and Discussions

3.1. Chemical Compositions

A highly variation was observed in chemical composition among different okra pods under investigation. Table 2 shows high mean moisture contents of 84.67% - 87.65% for K pod and M pod samples, respectively. These values were within the reported range of okra and mean that these vegetables contain a low storage capacity and are easily perishable, highlighting the problem of conservation in the warm climatic condition. In spite of that, the high water content in vegetables be useful in enhancing food digestion and help peristaltic movement on consumption [16]. M pod has the highest protein content (3.41 g/100 g).

It was found that protein content was in agreement with Adenipekun and Oyetunji, [3]. The fat content showed a nonsignificantly variation among okra pods, it ranged from 4.34 g/100 g for M pod to 4.52 g/100g for S pod. All okra pods have higher fat content than those values reported in that found by Adenipekun and Oyetunji, [3].

Ash content were significantly among different okra pods, being 10.31 g/100 g (in K pod and 12.20 g/100 g in M pod. As shown in Table 2, there was a significant variation among okra pods in their fiber content, being 6.78 g/100 g in M pod and 8.31 g/100 g in D pod samples. Results were in agreement with the previous report of Mohsen, [17] which is an important quality attribute where higher fiber content of okra pods is related to progress in age. The carbohydrate content of different pods was, also, significantly varied; where K pod has the highest carbohydrate content (75.22 g/100 g) followed by D pod (74.06 g/100 g). On the other hand, M pod found to have the lowest carbohydrate content (73.27 g/100 g) as compared with other okra pods. All samples have lower carbohydrate content than those values reported by Mohsen, [17].

3.2. Sugar Compositions

The presence of the reducing sugars in the studied pods is an important factor, where it is a benefit factor for the human health. Reducing sugars are the important constitutes, since they immediately bring some energizing calories available. Table 3 shows fructose, glucose and sucrose as main sugars.

Figure 1 shows the typical HPLC standard chromatogram of soluble sugars for sample.

Table 2. Chemical composition of different tested okra pod samples

Varieties	S	K	M	D
Moisture, %	86.69^{AB}±0.81	84.67^{C} ±0.74	87.65^{A} ±0.92	85.83^{BC} ±1.79
Protein, g/100g DW	2.75^{B} ± 0.63	2.90^{B} ± 0.17	3.41^{A} ± 0.73	2.37^{B} ±0.115
Ash, g/100g DW	11.16^{B} ±0.23	10.31^{D} ±0.19	12.20^{A} ±0.38	10.81^{C} ±0.42
Fiber, g/100g DW	7.88^{B} ±0.57	8.03^{AB} ±0.48	6.78^{C} ±0.55	8.31^{A} ±1.25
Crude fat, g/100g DW	4.52^{A} ±0.02	4.44^{B} ±0.02	4.34^{C} ±0.01	4.45^{B} ±0.01
Carbohydrate, g/100g DW	73.69^{B} ±0.45	75.22^{A} ±0.06	73.27^{C} ±0.65	74.06^{B}±0.20A

Each value presented as the mean ± standard deviation (n=3). Data with different superscript letters in the same row of variety indicate significant difference (P<0.05) analyzed by Duncan's multiple range test. Carbohydrate was calculated by difference, Carbohydrates % = [100 − (protein% + crude fat% + ash %)].

Table 3. Sugar profile and polysaccharide compositions (in g/100g DW)

	Fructose	Glucose	Sucrose	Total sugar	Polysaccharide
S	0.69^{A} ±0.09	0.26^{A} ±0.07	1.57^{B} ±0.19	2.52^{B} ±0.22	15.17^{B} ±0.83
K	0.28^{C} ±0.09	0.11^{B} ±0.10	3.34^{A} ±0.80	3.73^{A} ±0.99	17.35^{A} ±0.50
M	0.51^{B} ±0.15	0.18^{B} ±0.05	1.94^{B} ±0.26	2.63^{B} ±0.37	11.22^{C} ±0.53
D	0.46^{B} ±0.07	0.14^{AB}±0.10	1.44^{B} ±0.25	2.04^{B} ±0.22	12.27^{C} ±0.81

Total sugars are the sum of glucose, fructose and sucrose. Each value presented as the mean ± standard deviation (n=3). Data with different superscript letters in the same column of variety indicate significant difference (P<0.05) analyzed by Duncan's multiple range test.

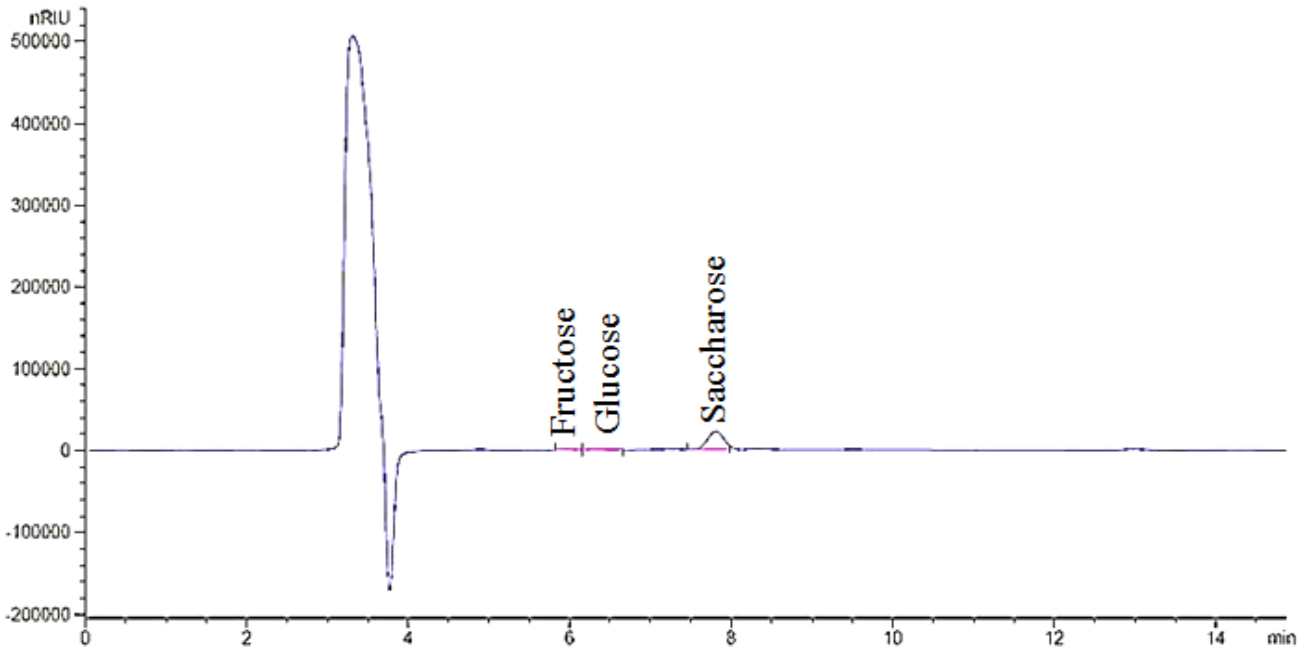

Figure 1. Typical HPLC chromatogram of soluble fraction

The present study described the sugars composition in these okra pods. For S pod (0.69 g/100g) and M pod (0.51 g/100g) fructose was the most abundant sugar, while sucrose was predominated in K pod (3.34 g/100g). Otherwise, D pod showed the lowest levels in total sugars (2.04 g/100g). Results are higher than (0.14, 0.10 and 0.11 g/100g) for fructose, glucose and sucrose, respectively reported by Adenipekun and Oyetunji, [3]. Also, results are higher than (13.19 mg/100g) reported by Sabreen *et al*., [18] and Osunde and Makama, [19].

Starch is an important polysaccharide component. It is a storage form of carbohydrates in plants and somtimes abundantly found in roots, tubers, stems, leaves, fruits and cereals. Starch, is a glucose molecules condensation and consists of a mixture of two type of components namely amylose and amylopectin. Starch that hydrolysed into single glucose molecules can be colorimetrically measured. Polysaccharide content (starch) was determined from the standard curve of glucose. Results indicated that polysaccharide accumulation occurs in pods, the maximum value is 17.35 g/100g in K pods, the minimum value was 11.22 g/100g in M pods. Pectic polysaccharide was not detected using them-phenylphenol procedure with preliminary dissolution in concentrated sulphuric acid as reported by Avallone *et al*., [20].

3.3. Mineral Compositions

In this study, the existences of twelve elements were determined in all okra pods (Ca, Mg, Cu, Zn, Fe, P, pb, As, Se, Cr, Cd, and Mn), as shown in Table 4. Okra contained a significant amount of such important minerals. Magnesium was the highest concentration and was within the 897.92 - 1459.58 mg/100 g range, followed in descending order by phosphorus (594.64 - 700.35 mg/100 g), calcium (112.50 - 345.83 mg/100 g), manganese (6.05 - 7.55 mg/100 g), copper (528.05 - 651.67 µg/100 g), lead (ND - 58.95 µg/100g), chromium(5.05 - 42.16 µg/100g), cadmium (7.90 - 23.46 µg/100g), iron (11.38 - 16.62 µg/100g), selenium (4.50 - 7.43 µg/100g), zinc (1.68 - 2.45 µg/100g) and arsenic not detected.

Magnesium has the highest concentrations. Statistically ($P<0.05$), it maintains a healthy bone densities, helps the heart electrical conduction, reduces the asthma attacks severity through relaxing muscles and respiratory airways, lowering blood pressure, possessed a good role against cardiovascular diseases and may be benificial in the prevention and complications of diabetes. D and S pods presented the highest content (1459.58 and 1247.92 mg/100 g, respectively), followed by K and M pods. This high content can be used to classify okra as natural resources for magnesium.

Calcium and phosphorus, often deficient in current food, are found with relatively important quantities in the studied pods. The daily contribution of these two elements, suitable for good nutritional balance. Calcium essential for bone structure and function, plays a significant role in photosynthesis, carbohydrate metabolism and nucleic acids. Also, the highest contents of calcium is found in M pods and the lowest is found in D pods (345.83 - 112.50 mg/100 g). Calcium value was higher than (1330 mg/100g) reported by [21].

Phosphorus is highly essencial for and cell growth, kidney functions and plays a role in maintaining the body's acid-alkaline balance [22]. The highest amount was detected in D pods and the lowest is K pods (700.35 - 594.64 mg/100 g). In addition, the same Table shows that manganese has a less value of calcium and phosphorus (6.05 - 7.55 mg/100 g) in M and S pods, respectively. The mineral composition of the studied varieties also showed levels of iron, selenium and zinc, as well as relatively high values of copper. These micro-minerals could be intervened were in several therapeutic aspects such as normal functioning of immune system (zinc) and nausea and headache diseases treatments (Iron) [23,24]. These results observed by [20] were higher in iron and zinc (56.6 mg/100g) and (6.10 mg/100g), respectively.

Arsenic was no detectable in all the tested samples, lead just appears in M pods (58.95 µg/100g). For the levels of chromium and cadmium, the highest value in chromium was K pods (42.16 µg/100g), and the highest in cadmium was M pods (23.46 µg/100g), the lowest in chromium and cadmium was S pods (5.05 µg/100g) and (7.90 µg/100g). These results were observed by Effiong *et al*., [16] who found that the sample was higher in phosphorus (583.91 mg/100g) and calcium (155 mg/100g) but lower in manganese, magnesium, iron, copper and zinc (21.77, 52.20, 76.20, 0.91 and 18.81 mg/100g), respectively.

Table 4. Mineral composition (on DW)

	S	K	M	D
Ca*	212.50^{B}±12.50	241.67^{AB} ±32.07	345.83^{A} ±131.15	112.50^{B} ±12.50
Mn*	7.55^{A} ±0.06	6.84^{B} ±0.34	6.05^{C} ±0.64	6.09^{C} ±0.13
Mg*	1247.92^{A} ±20.09	977.08^{A} ±126.30	897.92^{A} ±134.68	1459.58^{A} ±706.09
Cu**	651.67^{A} ±26.82	528.05^{B} ±58.05	617.50^{A} ±43.12	620.83^{A} ±8.02
Fe**	13.28^{A} ±0.60	12.32^{A} ±1.13	11.38^{A} ±6.83	16.62^{A} ±1.53
Zn**	2.03^{AB} ±0.24	1.68^{B} ±0.03	2.23^{AB} ±0.23	2.45^{A} ±0.65
Se**	5.77^{AB} ±1.32	4.50^{B} ±0.63	7.43^{A} ±1.03	5.35^{B} ±0.98
P*	681.32^{A} ±122.85	594.64^{A} ±56.41	597.81^{A} ±18.31	700.35^{A} ±12.82
CD**	7.90^{B} ±3.69	9.22^{B} ±4.82	23.46^{A} ±2.21	7.94^{B} ±4.11
CR**	5.05^{B} ±4.50	42.16^{A} ±7.14	34.37^{A} ±14.83	8.65^{B} ±2.63
AS**	ND	ND	ND	ND
Pb**	ND	ND	58.95±5.99	ND

Each value presented as the mean ± standard deviation (n=3). Data with different superscript letters in the same row of variety indicate significant difference ($P<0.05$) analyzed by Duncan's multiple range test. *= mg/100g, **= µg/100g, ND= Not detected.

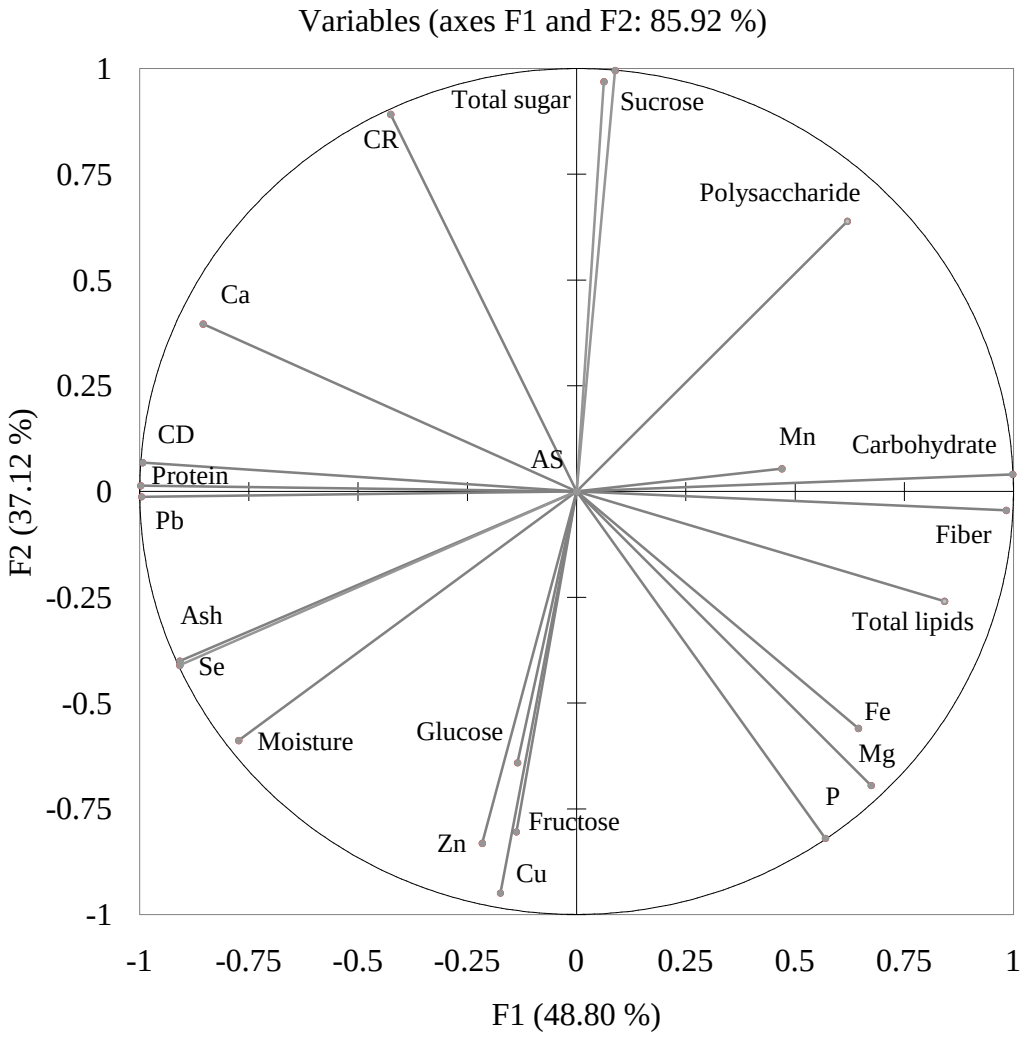

Figure 2. Plots of the scores for chemical properties

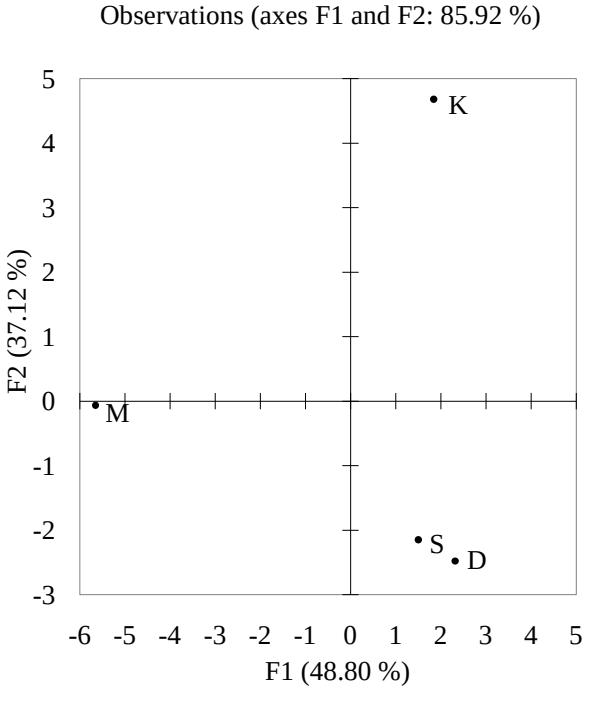

Figure 3. Plots of the x- Loudings for chemical properties

Table 5. Discriminate variables factors of principal components analysis

	F1	F2
Eigen value	10.74	8.17
Variability (%)	48.80	37.12
Cumulative (%)	48.80	85.92
Moisture	-5.58	-
Protein	-9.27	-
Ash	-7.69	-
Fiber	+9.01	-
Total lipids	+6.61	-
Carbohydrate	+9.28	-
Fructose	-	-7.93
Glucose	-	-5.04
Sucrose	-	+12.14
Total sugar	-	+11.50
Polysaccharide	-	+5.00
Ca	-6.81	-
Mn	+2.06	-
Mg	-	-5.92
Cu	-	-11.04
Fe	+3.88	-
Zn	-	-8.47
Se	-7.69	-
P	-	-8.24
CD	-9.20	-
CR	-	+9.74
AS	0	0
Pb	-9.24	-

3.4. Principal Component Analysis

Specific chemical properties of okra pods had been submitted to Principal Component Analysis (PCA). Figure 2 and Figure 3 present the plots of their scores and correlation loadinds, respectively. The scores plot of PCA illustrates a large variability of the tested four okra samples (S, M, K and D). Inertia percentage and correlated variables with axes 1 and 2 are displayed in Table 5. Axes 1 explained 48.80% of the total inertia 85.92%. An ax 2 explained 37.12% of the inertia and was made positively by sucrose, total sugar and chromium. The inertia was negative by fructose, copper, zinc and phosphorus. Plots of the scores in Figure 2, indicating that the data cloud was mainly bi-dimensional. With regards to the explanatory variables, (Figure 3) showed two individualized clusters of such samples. The first cluster included D and S pod samples and the second cluster included K and M pod samples.

4. Conclusion

Okra has high crude protein content, low fat, dietary minerals, fiber and carbohydrates contents. Okra sugars can be used, as a carbon source, to produce metabolites (alcohol, acetic acid, citric acid, lactic acid, oxytetracyclin) and biomasses (yeasts and lactic bacteria) via biological transformations. Grafting of polyacrylamide with okra mucilage, a vegetable origin polysaccharide component, offer new polymeric materials with properties that can be industrially exploited. In addition to this energizing potentiality, it seemed an acceptable wealth in macro-minerals and micro-minerals with a dominance of magnesium. So, it is recommended for regular consumption as a supplement for other important minerals. Consequently, okra could be considered a suitable foodstuff due to their nutritional and therapeutic values.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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