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Physicochemical, Antioxidant and Beta-Carotene Evaluation of Flours Obtained from Squash Pulp (Bermellon, Pepo and Macre)

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Abstract | In the quest for new food options for consumers, there has been a proposal to add value to a raw material that is underutilized by the Ecuadorian industry, such as squash. This was accomplished by evaluating its properties and conducting physicochemical, antioxidant, and beta-carotene analyses on the flours obtained from the pulp of three squash varieties: vermilion squash, pepo and macre. For the research project, the squash samples were subjected to a process of selection, classification, sectioning process, dehydration at 60°C for 12 hours, and grinding at 210 mm. The physicochemical evaluation was determined by: fat NTE INEN-ISO11085AOAC 2003.06, ash NTE INEN-ISO 2171, moisture NTE INEN-ISO 712, protein NTE INEN-ISO 20483, fiber NTE INEN 542, carbohydrates; antioxidant capacity by DPPH and carotenoids by HPLC. The results showed that the flour extracted from macer squash reached better averages in terms of fat 8.35 %; ash content of13.45 %; moisture 24.39 % and protein 17.70 %; the flour from vermilion squash reached better results in terms of FDN 19.76 % and FDA 8.16 %, the best carbohydrate content was for the flour obtained from pepo squash with a content of 33.85 %. All three varieties of flour showed antioxidant capacity, reaching a higher average for the flour of vermilion squash with a value of 8327 mg/L with an inhibition of 58.90%; the highest content of carotenoids was obtained by the flour extracted from mace squash with a content of 3387.68 µg/100g of sample.

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Introduction

In Ecuador, squash production is high; Manabí is the province with the highest production, with the most productive areas being Rocafuerte, Tosagua, Portoviejo and Bolívar, where the largest planting area of 1,693 hectares is concentrated (MAGAP, 2012). Economic and social factors are considered to be the factors that impede the development of squash processing and industrialization. Currently, a large percentage of squash is used directly as cattle and pig feed without adding value to the product. Satisfying the demand for food products in today's market with foods that have excellent nutritional properties has become a challenge for professionals in the agri-food field.

Squash offers important indispensable nutrients that can be used for the preparation of countless different foods such as desserts, pastas, cakes, compotes, flour, soups, ice cream, creams, salads, etc. (Morales, 2013; Arroyo, 2018). Squash is a food of nutritional value, because it has high amounts of vitamins A, C, B, B2 and B5 and minerals such as iron, phosphorus and calcium (Castro, 2013). Everything can be obtained from squash, which is always suitable for people of all ages because it is very easy to digest (Guananga *et al*., 2007; Tenorio, 2007). Among the most noteworthy properties of squash, is its antioxidant action stands out because of the presence of substances such as vitamins, carotenes, coumarins, lycopene and other components capable of neutralizing free radicals (Guillot, 2010; Melendez *et al*., 2007).

Since ancient times, traditional flours have been an indispensable part of human nutrition, since they balance the food supply (AgpNutrición, 2009). Turning squash pulp into flour becomes the possibility of an innovative product that allows using the nutritional benefits of squash. For its subsequent use in the preparation of foods that can improve people's nutrition and quality of life.

Creating products that satisfy nutritional requirements for human nutrition is considered a challenge nowadays. The objective of this research was to obtain squash flour and to evaluate the physicochemical, antioxidant and beta-carotene quality of the flours obtained from the squash pulp of three varieties (vermilion, pepo and macre).

Materials and Methods

The research was carried out at the Technical University of Manabí, specifically at the Faculty of Agrosciences, Chone extension. The laboratory utilized for the study was the Laboratory of Agroindustrial Processes in the area of vegetables. The faculty is geographically located in the Chone canton, Km 2½ via Boyacá, Ánima site, at 0°41′ and 17″ South latitude and 80° 7′ 25.60″ West longitude.

A completely randomized design (CRD) with one factor was employed, with the variety of squash (vermilion squash, pepo, and macre) being the focus of the study. Each variety, particularly those most cultivated in the city of Rocafuerte, the canton with the highest squash production in Manabí, was sampled in triplicate to ensure robust results, as depicted in Table 1. The data processing was carried out using the statistical software InfoStad version 2019. An ANOVA was applied using Tukey's test with a confidence interval of $p<0.05$.

Table 1: *Treatments under study.*

Treatment	Code	Squash varieties	Repetitions
1	V1	Vermilion	3
2	V2	Pepo	3
3	V3	Macre	3

Physicochemical analysis

Moisture: maximum 14.5% NTE INEN-ISO 712; Proteins: minimum 9 %. NTE INEN-ISO 20483; Ash: maximum 0.8 % NTE INEN-ISO 2171; Fat: maximum 2%. NTE INEN-ISO11085AOAC 2003.06; Fiber: NTE INEN 542 method; Carbohydrates: Total carbohydrate content was determined by indirect calculation using the equation proposed by Abadía *et al*. (2002).

$$\%CT = 100 - (\%H + \%G + \%C + \%F + \%Pc)$$

Where, % CT = percent total carbohydrate; %H = percent moisture content of the sample; %G = percent fat; %C = percent ash; %F = percent crude fiber; and %Pc = percent crude protein.

Antioxidant capacity

To evaluate the antioxidant activity of specific compounds or extracts, these should react with the stable radical in methanol solution. For the measurement of the antioxidant capacity, 1 ml of DPPH solution was added to 1 mL of the sample, the mixture was left to stand for 30 minutes at room temperature and in the dark, and then the absorbance was measured with the spectrophotometer (Genesys 180 UV/VIS) at a wavelength of 517 nm. The results were expressed as percent inhibition of the DPPH radical, using the following equation:

$$\%Inh = \frac{Control\ Absorbance - Aample\ Absorbance}{Control\ Absorbance} * 100$$

Where; Abs. control= is the absorbance of the DPPH solution without sample; Abs. sample = is the absorbance of the DPPH solution and the sample; Antioxidant capacity was performed on the squash flour of the three varieties studied without repeats.

Total carotenoids
The analysis of total carotenoids was performed by high performance liquid chromatography (HPLC), the concentrations of carotenoids were calculated using the following equation:

$$Carotenoids = \frac{Absorption\ x\ volume\ (mL)}{A_{1cm}^{1\%}x100xWeightxSample\ weight\ (g)}$$

It was taken 5 g of flour with which an acetone extraction was made, the acetone helped to extract the carotenoid components, after making the extraction it was passed to a filtrate using a separation funnel remaining the necessary amount, then it is washed with hexane. The hexane has the closest polarity to the carotenoids contained in the flours; it was carried out in two phases for containing different densities, leaving the hexane in the upper part and the carotenoids in the lower part.

Results and Discussion

Physicochemical quality of squash flours
Table 2 details the results obtained for the variables fat, ash, moisture, NDF, FDA, protein and carbohydrate of the squash flours, where an ANOVA with Tukey's rank comparison was applied, with a confidence interval of ($p<0.05$), indicating that there was statistical significance in the three varieties of flour.

Fat
Table 2 illustrates that the treatments were categorized into three ranges (a, b, and c), where V3 (Macre squash) attained a higher average with a value of 8.35% fat, while V2 (Pepo squash) achieved a lower average with a value of 1.49%. These values vary according to the variety of squash. They were higher than those reported by Ramos and Hernández (2011), who prepared squash flour (*Cucurbita maxima*) and obtained fat values of 5.99%. Armijo (2013) elaborated squash flour (*Cucurbita maxima*) fortified with soybean flour (*Glycine max*) for food use, reaching an average fat content of 13.17%, which is higher than those reported in the research.

The INEN Standard 616: Requirements for wheat flour indicates that flour should have a maximum fat content of up to 2%. The results of the research exceed the limits established by the standard; only the variety of squash, pepo, complied with this range, with an average fat content of 1.49%.

Ash
Table 2 indicates that the treatments were divided into three ranges (a, b, and c), showing significant differences among them. V3 (Macre squash) reached a higher average with a value of 13.45% ash, while V1 (Vermilion squash) achieved a lower average with a value of 9.33%. The ash results were higher than those reported by Armijo (2013), who obtained ash averages ranging between 5.24% and 5.36%.

The INEN Standard 616: Requirements for Wheat Flour indicates that wheat flour should have a maximum ash content of up to 0.8%. The results of the research exceed the limits established by the standard.

Humidity
Table 2 indicates that the treatments were divided into three ranges (a, b, and c), where V3 (Macre squash) reached a higher average with a value of 24.39% moisture, while V2 (Pepo squash) achieved a

Table 2: *Physicochemical quality of squash flours.*

Treat-ment	Fat % X ± S.D.	Ash % Ẋ ± S.D.	Humidity % Ẋ ± S.D.	NDF % Ẋ ± S.D.	ADF % Ẋ ± S.D.	Protein % Ẋ ± S.D.	Carbohydrate % Ẋ ± S.D.
V1	5.21 ± 0.28 b	9.33 ± 0.25 a	23.92 ± 0.04 b	19.76 ± 0.68 b	8.16 ± 0.15 b	6.41 ± 0.51 a	27.20 ± 0.81b
V2	1.49 ± 0.19 a	10.20 ± 0.36 b	18.38 ± 0.25 a	15.55 ± 0.27 a	8.09 ± 0.13 b	12.44 ± 0.68 b	33.85 ± 0.65 c
V3	8.35 ± 0.27 c	13.45 ± 0.09 c	24.39 ± 0.09 c	19.35 ± 0.42 b	7.68 ± 0.15 a	17.70 ± 0.29 c	9.07 ± 0.21 a
P-value	0.0001**	0.0001**	0.0001**	0.0001**	0.0117*	0.0001**	0.0001**
V. C	4.91	2.36	0.70	2.67	1.77	4.26	2.61

Means with a common letter are not significantly different ($p > 0.05$).

lower average with a value of 18.38%. This percentage varies due to the characteristics of the squashes used. The moisture results were higher than those reported by Ramos and Hernández (2011), who obtained moisture values of 8.78%. Álava (2007) characterized squash flour and by product formulation, reaching a moisture content of 14%, which is lower than the results reported in this research.

The INEN 616: Requirements for wheat flour indicates that the moisture percentage should be within a maximum range of up to 14.5% in flours for all uses. The results of the research exceed the limits set by the standard because this standard is for cereals. The high moisture values reported in the research are due to the variety of crop, storage conditions to which the squashes are subjected, among other factors. Additionally, fresh tubers have a moisture content of 85% (Barrera *et al*., 2003).

NDF (neutral detergent fiber)

In Table 2, it indicates that the treatments were divided into two ranges (a and b), where V1 (Vermilion squash) reached a higher average with a value of 19.76%, and V2 (Pepo squash) reached a lower average with a value of 15.55% NDF. The NDF results were higher than those reported by Albán (2013), who replaced wheat flour with squash flour in the preparation of gourmet desserts and assessed their acceptability, obtaining a fiber value of 1.01%.

ADF (Acid detergent fiber)

In Table 2, it indicates that the treatments were divided into two ranges (a and b), where V1 (Vermilion squash) reached a higher average with a value of 8.16%, and V3 (Macre squash) reached a lower average with a value of 7.68% of FDA. The FDA results were lower than those reported by Bastidas (2011), who prepared an instant soup from the pulp of zambo (*Cucurbita ficifolia*), squash (*Cucurbita maxima*), leaves, and stems of the zambo plant with three formulations and two types of flavorings (chicken and pork), reaching an average fiber content of 8.27%.

Protein

In Table 2, it indicates that the treatments were divided into two ranges (a and b), where V3 (*Macre squash*) reached a higher average with a value of 17.70% protein, and V1 (*Vermilion squash*) reached a lower average with a value of 6.41%. The results obtained were lower than those reported by Armijo (2013) and Ramos and Hernandez (2011), who obtained values of 4.16% and 6.6% protein, respectively, in squash flour. These results are lower than those reported in the research.

Carbohydrate

Table 2 shows that the treatments were divided into three ranges (a, b, and c), where V2 (*Pepo squash*) reached a higher average with a value of 33.85% carbohydrate, and V3 (*Macre squash*) reached a lower average with a value of 9.07%. The carbohydrate results were lower than those reported by Álava (2007), who obtained a carbohydrate value of 76% in the squash flour prepared.

Antioxidant capacity by the DPPH method of squash flours

Bermellón: The antioxidant activity of vermilion squash flour, determined by the DPPH radical method (Figure 1), resulted in an IC50 value of 8.327 g/L, with a maximum inhibition of 58.90% observed at a concentration of 0.03 g/L.

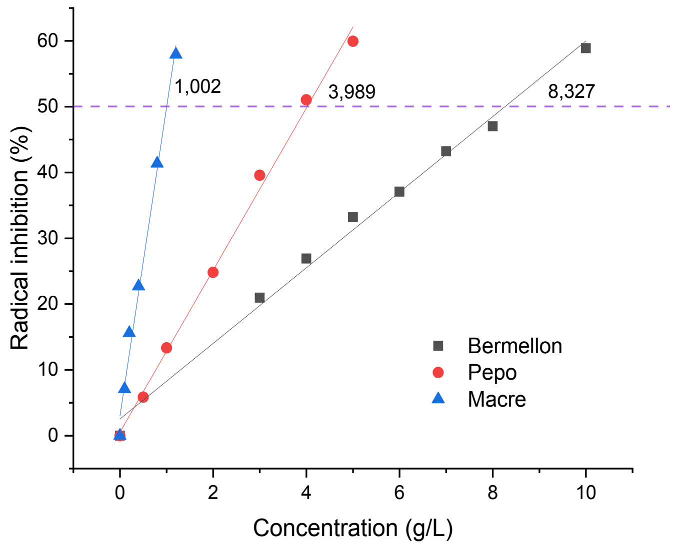

Figure 1: *IC50 of squash flours.*

Pepo

The antioxidant activity of pepo variety squash flours, determined by the DPPH radical method (Figure 1), resulted in an IC50 value of 3.989 g/L, with a maximum inhibition of 59.95% observed at a concentration of 0.03 g/L.

Macre

The antioxidant activity of macre variety squash flours, determined by the DPPH radical method (Figure 1), resulted in an IC50 value of 1.002 g/L, with a maximum inhibition of 57.92% observed at a concentration of 0.03 g/L.

According to the results obtained on the antioxidant capacity of the squash varieties under study, V1 (vermilion squash) exhibited the highest antioxidant value, with a value of 8.327 g/L.

The results obtained demonstrate that the three varieties of squash studied exhibit antioxidant activity, suggesting that they can be considered as a source of natural antioxidants, potentially contributing to health benefits. These findings are consistent with those expressed by Valenzuela *et al.* (2014) in a study investigating the relationship between antioxidant activity and total phenolic and flavonoid content in seeds of Cucurbita spp, indicating a correlation between phenolic and flavonoid content and antioxidant activity.

Carotenoids

The total carotenoids in the flour obtained from the three varieties of squash were characterized, the results are detailed below:

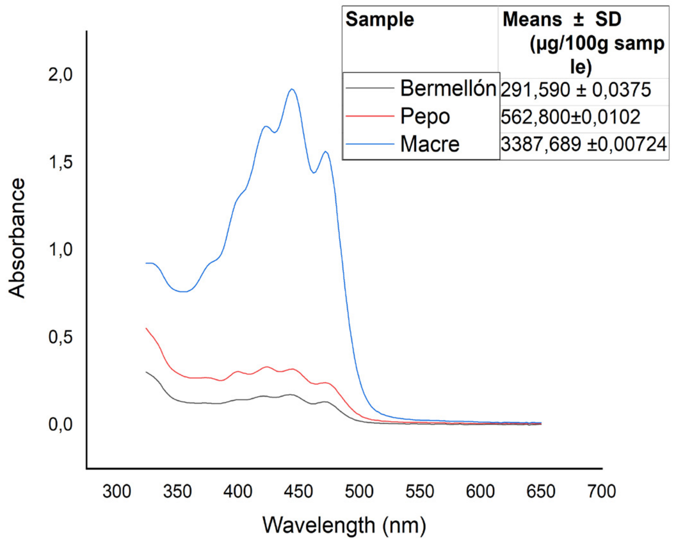

Figure 2: *Comparison of absorption spectra of the squash varieties.*

Figure 2 compares the absorption spectra of the squash varieties concerning carotenoids. It is evident that the macre variety exhibited the highest concentration, reaching 3387.680 µg/100 g of carotenoid samples, surpassing the other varieties studied. In squash, the yellow and orange flesh is attributed to the presence of carotenoids located in the chromo-amyloplasts, which impart the characteristic color of the variety (Zaccari *et al.*, 2007; Zhang *et al.*, 2014; Nisar *et al.*, 2015; Stanger, 2016). However, Techeira *et al.* (2014) noted that carotenoids and polyphenols in flours lead to a decrease in the whiteness index in tuber flours. Similarly, Carvalho *et al.* (2015) observed a high variation in carotene content for squash (Cucurbita moschata), ranging from 10.7 to 65.5 mg per 100 g-1 of fresh weight in boiled and steamed pulp.

Conclusions and Recommendations

The flour obtained from the three varieties of squash produced in Rocafuerte canton, showed statistical significance in the parameters evaluated, the flour extracted from macre squash obtained better results in terms of fat, ash, moisture and protein; the flour from common squash reached a better result in terms of NDF and ADF and the pepo squash reached a better carbohydrate content.

The highest content of antioxidant capacity was presented in the common squash flour and the highest content of carotenoids was obtained by the flour extracted from macaque squash.

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Novelty Statement

In the absence of recorded data on the chemical composition, antioxidant capacity and carotenoid content of squash flour, it becomes an alternative to be consumed in the human diet thanks to the characteristic nutrients it contains.

Author's Contribution

Wagner Antonio Gorozabel Muñoz: Revision and correction of the work.
Mayra Jossenka Loor Solórzano: Search for information.
Josselyn Gema Palacio Intriago: Manuscript writing.
Virginia Vanessa Andrade Andrade: Laboratory experimentation.
Carlos Alfredo Cedeño-Palacios: Tabulation and interpretation of results.

Conflict of interest

The authors have declared no conflict of interest.

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