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## By – Products of Mango as an Alternative for the Food Industry

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### ABSTRACT

Besides knowing the benefits brought by the agro-industrial by-products, it is necessary to know the behavior after incorporation in different dietary matrices, evaluating processing effects and stability of their antioxidant properties. In this study, the mango powder from mango peels was the raw material that replaced the flour mixture in 25% for the preparation of cookies, evaluating the phenolic compounds (FC) and antioxidant activity (AA) after the processing and during eight weeks in the storage. The antioxidant activity was evaluated by ABTS, DPPH and FRAP methods, while the FC were determined by HPLC and Folin-Ciocalteu assays. The food was baked at 170°C for 10 minutes and it was observed that the heat treatment did not decrease the FC and AA, on the contrary, the baking led to an increase, especially in 1,2,3,4,6-penta-O-galloryl-β-D-glucose with 143.42%; quercetin 36%, mangiferin with 14% and the DPPH with 19%. Mango peels is a rich source of antioxidants due it the cookies made with this by-product increased their FC and AA by almost four times with respect to the control. The main FC found in by-products and cookies were mangiferin, 1,2,3,4,6-penta-O-galloryl-β-D-glucose and quercetin. The cookies were fortified with those compounds which were not present in control.

**KEYWORDS:** Mango by-products, antioxidant activity, total phenolic content, cookies, retention of antioxidants

### INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most popular tropical fruits in the world. Approximately 20% of fruits are processed for products, such as puree, pulp, juice, leather, chutney, pickles, slices and nectar [1, 2]. From the industrialization of mango, 35 to 60% of the total weight is obtained as by-products depending on the mango variety [3], 7-24%

corresponding to the shell and 10-24% to the seeds [4–6]. Several researches confirm that mango by-products such as stem, bark, leaves, peels and seeds present antioxidant [7, 8], anti-inflammatory [9], anti-proliferative [10] and anti-cancer activities [11], due to their content in bioactive compounds such as pectin, polyphenols, carotenoids, tannins, flavonols, xanthenes and anthocyanin [3, 8, 12].

The utilization of the by-products is feasible from the nutritional, technological, functional and environmental point of view [13]. In the food industry, the use of by-products has shown positive results in nutritional quality and sensory acceptance when they are incorporated into different dietary matrices, especially in the baking and pastry industry. For example, Ajila *et al.* [4], incorporated mango peels in the preparation of macaroni, determining that the content of dietary fibre, phenolic compounds and carotenoids gradually increasing according to the added concentration of the by-products. Also Ramírez-Maganda *et al.* [14], partially replaced wheat flour and sugarcane with mango peels (20 and 39%) for the production of muffins, determining that the soluble total polyphenols content increased and that the substitution did not affect the consumer preference.

It is evident that by-products of the fruits are currently no longer considered as industrial waste, since they are products with high added value due to chemical composition which compounds are considered as potential additives or functional ingredients. For this reason, this research aims to provide antioxidants to food matrices such as cookies, using the mango by-product as an ingredient, and to evaluate the stability of these compounds in front of the baking process and storage of the food.

## MATERIALS & METHODS

### Chemicals and reagents

Acetone, acetic acid, sodium bicarbonate, Folin-Ciocalteu reagent, hydrochloric acid, sodium acetate trihydrate were purchased from Merck. Methanol, gallic acid, Iron (III) chloride hexahydrate ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ), 2, 4, 6 – Tripyridyl-s-Triazine (TPTZ), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, 2,2'-Azino-bis (3-ethylbenthiiazoline-6-sulfonic acid) (ABTS), potassium persulfate, trolox (6-hidroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

### Mango by-products

Mango fruits (*Mangifera indica* L. cv. Tommy Atkins and Haden) were purchased in a super market of Loja (Ecuador). The fruits were washed and then the peels, seed and pulp were separated manually. Mango by-products corresponding to peels were dehydrated at 60°C

until a final humidity of about 10% before being ground to a particle size minor of 250  $\mu\text{m}$ , because of the similarity in the texture of flour.

### Functional food preparation

The cookies were fortified with mango by – product. In the matrix considered (cookies) 25 % of mango by–product was added as an ingredient per batch of food (CS), as a consequence, by – product and other ingredients (Table 1) were exposed to a baking process. Additionally, the same amount of by – product was added to the finished food samples prepared without by – product (CSS) in order to generate reference samples containing by – products that had not been subjected to the processing steps. A control cookie (CC) was prepared, which has no by - product in the elaboration, this sample was made in order to verify the amount of antioxidants provided by the by - product.

**Table 1. Ingredients for the elaboration of cookies**

INGREDIENTS	CS (%)	CC (%)
“7 Harinas”	11.0	22.0
Cornmeal	2.0	4.0
Wheat flour	4.0	8.0
By-product of mango	25.0	-
Oats flakes	8.0	16.0
Almonds	5.0	5.0
Olive Oil / Sunflower (1:1)	13.0	13.0
Honey bee	25.0	25.0
Baking powder	1.8	1.8
Salt	0.2	0.2
Water	5.0	5.0

**CS:** cookie with by-product of mango; **CC:** control (cookie without by-product); **“7 harinas”:** ancestral Ecuadorian product that corresponds to the mixture of seven flours.

The samples was placed in a plastic cylinder and milled with a blender for 10 min prior to the addition of by–product. The fortified foods were then homogenized by stirring and were stored in a closed container at 20 °C. The baking was for 10 minutes at 170 ° C [15].

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**Extracts preparation for antioxidant analysis and total phenolic content**

Extracts were obtained from by-products and cookies (CC, CS and CSS). The procedure developed by Pérez-Jiménez y Saura-Calixto [16] was employed and is briefly described as follows: samples (dry by-products and cookies) were weighted with relation sample-solvent 1:20. The first extraction was performed with methanol-water (50:50 v/v), pH 2 adjusted with HCl and the second extraction with acetone - water (70:30 v/v). The extracts were centrifuged 30 minutes at 2800 rpm and the supernatant was stored for further analysis.

**Antioxidant capacity**

Antioxidant capacity was determined by three methods, the ABTS<sup>+</sup> assay according to Arnao *et al.* [17] with slightly modifications, the DPPH free radical-scavenging done under the procedure of Brand-Williams *et al.* [18] with some modifications described by Thaipong *et al.* [19] and FRAP was determined according Benzie y Starin [20] with some modifications described by Thaipong *et al.* [19], in all cases Trolox, a well-known synthetic antioxidant, was used as standard reference.

**Free radical-scavenging by DPPH assay**

The change in absorbance produced by reducing DPPH radical was used to measure the radical-scavenging activity. This assay was performed based on the technique of Brand-Williams *et al.* [18] with some modifications described by Thaipong *et al.* [19]. Briefly, to prepare the radical stock solution, 24 mg of DPPH was dissolved in 100 mL of methanol. The working solution was prepared by diluting 10 mL of the radical stock solution with 45 mL of methanol to obtain a reading of  $1.1 \pm 0.02$  absorbance units at a wavelength of 515 nm in a UV spectrophotometer. The working solution was prepared fresh daily. For quantification of antioxidant capacity, a standard curve of Trolox was prepared at different concentrations between 15 to 550  $\mu\text{M}$  Trolox. All determinations were performed by triplicate.

From each extract, 150  $\mu\text{L}$  were mixed with 2850  $\mu\text{L}$  of DPPH working solution in an amber vial, then the mixture solution was shaken for 3 minutes and allowed to react during 24 hours at room temperature protected from light. The final absorbance was measured in UV

spectrophotometer at a wavelength of 515 nm. The results are expressed as micromoles of Trolox equivalent per milligram of plant extract ( $\mu\text{M TE/mg extract}$ ).

**Free radical-scavenging by ABTS<sup>+</sup> assay**

The ABTS<sup>+</sup> assay was performed using the procedure described by Arnao *et al.* [17] with some modifications by Thaipong *et al.* [19]. Two stock solutions were prepared: 7.4  $\mu\text{M}$  ABTS and 2.6  $\mu\text{M}$  potassium persulfate. ABTS<sup>+</sup> radical cation was produced after mix both solutions in equal quantities and allowed to react protected from light during 12 hours to obtain the ABTS standard solution. For the study of extracts, 1 mL of the ABTS standard solution was diluted with 60 mL of methanol until obtain an absorbance of  $1.1 \pm 0.02$  in a UV spectrophotometer at 734 nm. A standard curve of Trolox was made with concentrations between 15 to 550  $\mu\text{M}$  Trolox. From each concentration as well with each extract 150  $\mu\text{L}$  were added to 2850  $\mu\text{L}$  of the ABTS working solution, then were allowed to react by 2 hours protected from light, after this the absorbance was measured at 734 nm. The results are expressed as micromoles of Trolox equivalents per milligram of extract ( $\mu\text{M TE/mg extract}$ ).

**Ferric reducing antioxidant power (FRAP) assay**

The antioxidant capacity of each sample was estimated by FRAP assay, following the procedure described by Benzie & Strain, [20] with modifications described by Thaipong *et al.* [19]. Three stocks solutions were prepared: 300 mM acetate buffer pH 3.6 in acetic acid, 10 mM TPTZ solution in 40 mM hydrochloric acid and 20 mM ferric chloride solution. The working solution was prepared adding 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL chloride ferric solution, the mixed was warmed at 37 °C. A standard curve of Trolox was made with concentrations between 15 to 550  $\mu\text{M}$  Trolox. From each concentration as well with each extract 150  $\mu\text{L}$  were added to 2850  $\mu\text{L}$  of the ABTS working solution, then every solution was allowed to react during 30 minutes protected from light, after this the absorbance was measured at 593 nm. The results are expressed as micromoles of Trolox equivalents per milligram of extract ( $\mu\text{M TE/mg extract}$ ).

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### Total phenolic determination

Total phenolic content was measured using the Folin-Ciocalteu colorimetric method with some modifications described by Kong & Lee, [21] and Thaipong *et al.* [19]. Different solutions were prepared, Folin-Ciocalteu (0.25 N), sodium carbonate (1N), and gallic acid solutions between 0 to 1 mg GA/mL. 150  $\mu$ L of the extracts were mixed with 150  $\mu$ L of Folin-Ciocalteu reagent and 2400  $\mu$ L of water, the mixture was stirred by two minutes and allowed to react by 3 minutes; then 300 mL of sodium carbonate were added and allowed to react for 2 hours protected from light. Finally, the absorbance was measured at 725 nm. The total phenolic content of extract was calculated from the standard curve of gallic acid, the results are expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract).

### Phenolic HPLC analysis

The separation and identification of phenolic compounds in the by-product and CS was carried out on an Agilent HPLC series 1100 system (Agilent, Germany) coupled to a UV/vis detector and controlled by ChemStation® HP software. A reverse phase Synergi Hydro-RP C18 column (150 mm  $\times$  3 mm i.d., 4  $\mu$ m) from Phenomenex (USA) with a 4.0 mm  $\times$  2.0 mm i.d. C18 ODS guard column was used.

A gradient elution program was used with two phases: A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). The applied gradient was as follows (time, % B): 0 min, 0%; 0.2 min, 0%; 0.3 min, 7%; 14.7 min, 8.5%; 40 min, 19%; 45 min, 33%; 148 min, 50%; 50 min, 95%; 57 min, 0%; 63 min, 0%. The flow rate was set at 1 mL/min. Compounds were detected at 278 nm according to the retention time and using the calibration curves for the different standards. The injection volume was set at 20  $\mu$ L [22]. The main antioxidants compounds, namely methyl gallate, mangiferin, quercetin 3-D-galactoside, quercetin 3- $\beta$ -D-glucoside, quercetin 3-O- $\alpha$ -L-arabinopyranoside, penta-O-galloyl glucose and quercetin aglycone, were quantified according to their respective calibration curves. Iriflophenone 3-C- $\beta$ -D-glucosid, iriflophenone 3-C-(2-O-p-hydroxybenzoyl)- $\beta$ -D-glucoside and iriflophenone-3-C-(2-O-galloyl)- $\beta$ -D-glucoside were quantified according mangiferin curve, while quercetin-3-O-xyloside was quantified according quercetin curve. The correlation

coefficient R was greater than 0.99 for all calibration curves. All analyses were carried out in triplicate and the standard deviation (SD) was calculated in each case.

### Retention of antioxidants compounds

The incorporation of by-product of mango into the cookies was performed in two ways: using the by-product as ingredient at the beginning of food preparation and adding at the end with the final product by mixing, in this way the stability of the antioxidants against the baking process was evaluated in the cookies. The antioxidant retention were obtained using the expression: (content after processing/content without processing) $\times$ 100, so that 100% indicates the absence of any loss and 0% the total loss of antioxidant activity, while a value above 100% would refer to gains over on the initial [23].

### Statistical analyses

All determinations were performed in triplicate, and all results were calculated as mean and standard deviation (SD) using Minitab 6.0. All standard curves had good correlation coefficients,  $R^2 \geq 0.99$ .

## RESULTS AND DISCUSSION

### Mango by-products characterization

It can be observed in Table 2, that mango by-product presented an intermediate content of phenol compounds  $3520 \pm 103$  mg EAG/100 g BS when compared with others by-products as grape, passion fruit, pineapple, guava, cocoa, tuna cladode and bambaran. The antioxidants content depend on several factors such as variety, place of origin, genotype, climatic or seasonal factors, harvest and post-harvest and also of the degree of maturity; the same one that is linked to the degradation of chlorophyll pigments and the simultaneous increase of carotenoid pigments during the maturation stage [24].

On the other hand, to evaluate antioxidants, one method is not sufficient to accurately reflect all sources of free radicals or all of the antioxidants present in one system, because of multiple reaction mechanisms.

Antioxidants disable radicals by two reaction mechanisms: electron transfer (SET) or

hydrogen atom transfer (HAT) reaction [25]. The HAT mechanism quantifies the ability to yield hydrogen atoms, whereas SET based assays measure the reducing capacity of antioxidants

[26]. The commonly used methods for determining antioxidant capacity are: ABTS, DPPH, FRAP.

**Table 2. Antioxidant activity and total phenolic of by-products of fruits**

By – product	ABTS	DPPH	FRAP	FT
Mango	489.23±4.02	523.90±17.05	325.41±6.36	3520±130
Mango <sup>a</sup>	38.0±1.90	47.1±2.89	19.1 ± 0.15	-
Passion Fruit <sup>a</sup>	5.5±0.58	5.1±0.36	6.9±0.05	160
Pineapple <sup>a</sup>	7.7±0.90	4.8±0.10	6.2±0.26	130
Guava <sup>a</sup>	20.9±1.25	15.4±1.93	11.1±0.23	240
White grape pomace <sup>b</sup>	284±24	-	466±43	-
Cocoa <sup>c</sup>	-	134.1	-	154.43
Cladode (Atlixco) <sup>d</sup>	52.37±2.00	-	52.22±1.07	2690±0.85
Red tuna <sup>d</sup>	65.76±2.02	-	47.35±2.10	-
Green tuna <sup>d</sup>	66.33±2.61	-	40.39±1.62	-
Bambaran <sup>f</sup>	-	-	-	9830±0.12
Manto negro grape <sup>g</sup>	-	-	-	2630±0.04

FT: mg gallic acid equivalent/100 g; ABTS, DPPH, FRAP:  $\mu$ M Trolox equivalent/100 g; <sup>a</sup>[7], <sup>b</sup>[49], <sup>c</sup>[27], <sup>d</sup>[50], <sup>f</sup>[51], <sup>g</sup>[52]

The ABTS test is based on the reduction produced by the oxidation of the ABTS cation radical caused by the antioxidant present in the sample. It is widely used to measure the antioxidant activity of hydrophilic compounds. The antioxidant activity measured by ABTS was 489.23  $\mu$ M Trolox equivalent/ 100 g sample. The DPPH assay measured the ability of the antioxidants to capture the 2,2-difenil-1-picrihidracil (DPPH<sup>•</sup>). This method is highly reproducible, selective and comparable. In the DPPH assay the analysed mango by-product showed a values 523.90  $\mu$ M Trolox equivalent/ 100 g sample. While, the FRAP method is based on the power of antioxidants so that at low pH, the ferric complex 2,4,6-tripiridil-s-triazina (TPTZ-Fe<sup>+3</sup>) is reduced to is ferrous form (TPTZ-Fe<sup>+2</sup>). The antioxidant capacity measured by FRAP was 325.41  $\mu$ M Trolox equivalent/ 100 g sample. These values of antioxidant capacity in by-products of mango were superior that other by-products as can be seen in Table 2.

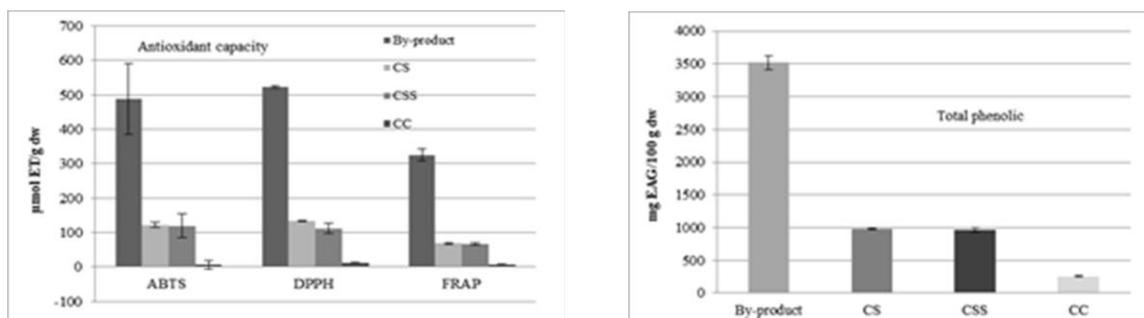
The mango by - product presented a higher value than other by-products reported in literature such as guava, cocoa, mango, but inferior to passion fruit, pineapple and bambaran [7, 27, 28]. In general, the by-product has good technological properties, for this reason this could be used as a technological tool for food product development.

## Functional food

### Total phenolic and antioxidant activity

Incorporation of mango by-product as an ingredient in cookies formulation, significantly improved the content of total phenolic and the antioxidant activity. The amount of phenolic compounds increased approximately 74%, while the increase in antioxidant activity was 95% for ABTS, 90% for DPPH and 88% for FRAP, in comparison with cookies control (CC), as shown in Graph 1.

**Graph 1: Comparison of total phenols and antioxidant capacity by ABTS, DPPH and FRAP of the cookies with by-product of mango and the control cookies**

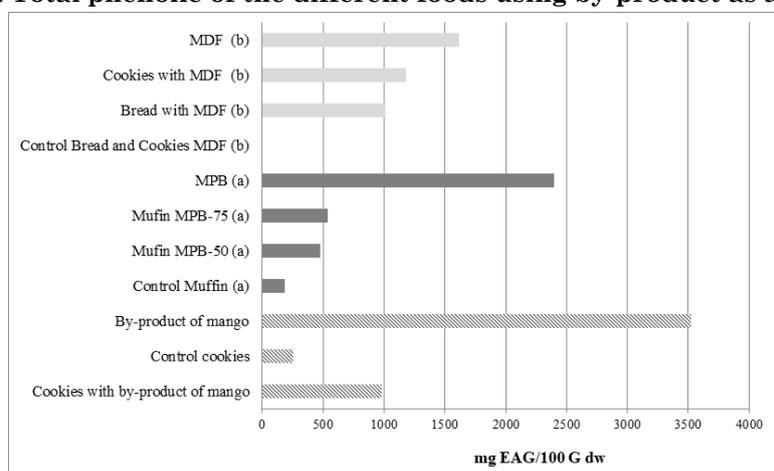


CS: cookie with by-product of mango (by-product with baking process); CC: control (cookie without by-product); CSS: cookie with by-product of mango (by-product without baking process)

The content of total phenolic in mango by-product, mango fibre (MDF), mango paste (MPB) and processed products from the baking and pastry industry such as cookies, bread and muffins, is shown in Graph 2. The cookies (1180 mg EAG / 100 g dw), bread with MDF (1010 mg EAG / 100 g dw) [29] and muffins with MPB (477 - 536 EAG / 100 g dw) [14] had higher values of total phenols, when compared with their respective control. In our case, the cookies (CS) ( $983 \pm 7$  mg EAG / 100 g dw) after the incorporation of the by-product ( $3521$  mg EAG /

100 g dw), increased in phenolic compounds content by approximately three times with respect to the control (CC) ( $254$  mg EAG / 100 g dw), as shown in Graph 1 and 2. The cookies with incorporation of mango by-product could become a food source of antioxidants since compared with other sources with such denomination as red wine ( $160$  mg EAG / 100 mL) or fruits ( $538$  mg EAG / 100 g BS) [30]. This product (cookies) provides a greater quantity of phenolic compounds.

**Graph 2: Total phenolic of the different foods using by-product as an ingredient**



(a) Ramírez-Maganda et al. [14], (b) Vergara-Valencia et al. [29]

On the other hand, in the Graph 1 can be observed that, the antioxidant capacity measured by ABTS ( $121.51 \pm 1.84$  µmol ET/g dw) in our product is superior to some commercial foods as dark chocolate  $78.8$  µmol ET/g dw [31], raw fucus powder  $119.7$  µmol ET/g dw and fucus commercial antioxidant extract  $81.2$  µmol ET/g dw [32], but inferior of the whole walnut  $165.18$

µmol ET/g dw [33] and grape antioxidant dietary fibre  $124.41$  µmol ET/g dw [34]. With respect of the FRAP results the cookies with mango by-product ( $68.77 \pm 1.72$  µmol ET/g dw) is superior than other foods highly participatory in diets of different cultures such as raw rice  $1.57$  µmol ET/g dw, wheat bran  $4.41$  µmol ET/g dw, french bread  $2.86$  µmol ET/g dw [35] and nuts  $44.8$

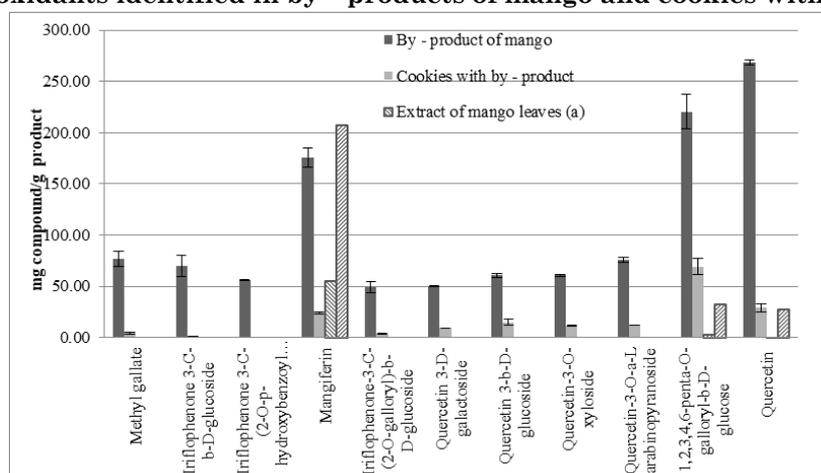
$\mu\text{mol ET/g dw}$  [36]. The analysis of DPPH resulted in a value of  $122.99 \mu\text{mol ET/g dw}$ , a value higher than the pulp of fruits considered with high antioxidant activity as greater than certain foods such as andean blackberry ( $41 \mu\text{mol ET/g}$ ), capulí cherry peel ( $76.99 \mu\text{mol ET/g}$ ), banana passion fruit ( $70.99 \mu\text{mol ET/g BS}$ ), and strawberry ( $11 \mu\text{mol ET/g}$ ) [37]. Thus the use of by-product of mango as an ingredient is a profitable and advantageous option for the preparation of healthy foods with high antioxidant activity.

### Identification phenolic compounds

Benzophenones derivatives, xanthones, phenolic acids and flavonoids as methyl gallate,

iriflophenone 3-C- $\beta$ -D-glucoside, iriflophenone 3-C-(2-O-p-hydroxybenzoyl)- $\beta$ -D-glucoside, mangiferin, iriflophenone-3-C-(2-O-galloryl)- $\beta$ -D-glucoside, quercetin 3-D-galactoside, quercetin 3- $\beta$ -D-glucoside, quercetin-3-O-xyloside, quercetin-3-O- $\alpha$ -L arabinopyranoside, 1,2,3,4,6-penta-O-galloryl- $\beta$ -D-glucose and quercetin were identified in by-product and cookies. As can be seen in Graph 3, 1,2,3,4,6-penta-O-galloryl- $\beta$ -D-glucose ( $220.47 - 69.24 \text{ mg compound/g product}$ ), quercetin ( $268.32 - 28.95 \text{ mg compound/g product}$ ) and mangiferin ( $175.79 - 24.20 \text{ mg compound/g cookies}$ ) are the major compounds in both the by-product and the cookies respectively.

**Graph 3: Antioxidants identified in by – products of mango and cookies with by – products**

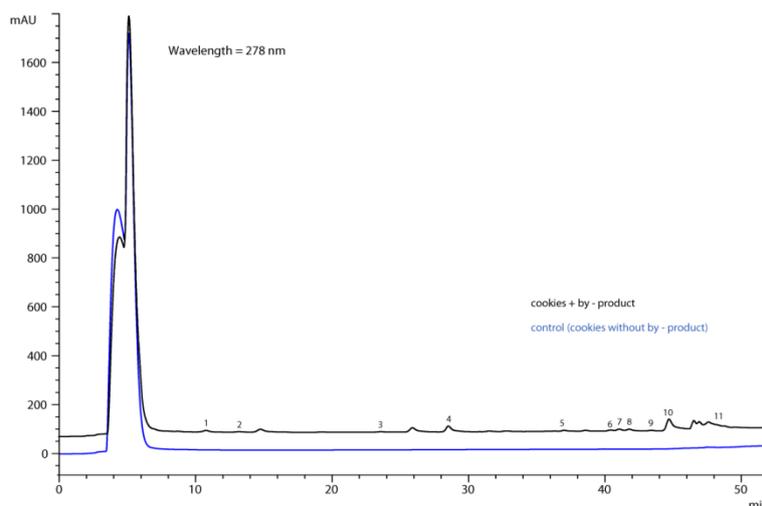


(a) Guamán-Balcázar, M. et al. [38]

The compounds identified in the cookies (CS) were not identified in the control (CC), therefore the addition of mango by-product to the matrix, fortified the food in phenolic compounds such as mangiferin, quercetin and 1,2,3,4,6-penta-O-galloryl- $\beta$ -D-glucose in greater quantity. In Figure 1, can be observed the chromatograms obtained in the mango by-product, cookies with by-product (CS) and control (CC).

Some compounds obtained in the samples analysed in the present investigation were also quantified in another research, in which Guamán-Balcázar et al. [38] worked with extract and micro particles of mango leaves. In Graph 3, it can be observed that compounds such as

quercetin and 1,2,3,4,6-penta-O-galloryl- $\beta$ -D-glucose are present in greater quantity in the mango by-product than in the other products (extract, micro particles and cookies), but the same does not happen with the mangiferin that is higher in the micro particles; on the other hand, when comparing the cookies with the micro particles of mango leaves, it can be observed that quercetin and 1,2,3,4,6-penta-O-galloryl- $\beta$ -D-glucose are found in greater quantities. Both the extract and the mango leaf particles are considered products with high antioxidant capacity, consequently we can corroborate that our food has a high antioxidant potential.



**Fig. 1:** HPLC chromatograms of the control and cookies with by-product of mango. Peaks: 1. methyl gallate, 2. iriflophenone 3-C-  $\beta$  -D-glucoside, 3. iriflophenone 3-C-(2-O-p-hydroxybenzoyl)-  $\beta$  -D-glucoside, 4. mangiferin, 5. iriflophenone-3-C-(2-O-galloryl)-  $\beta$  -D-glucoside, 6. quercetin 3-D-galactoside, 7. quercetin 3-  $\beta$  -D-glucoside, 8. quercetin-3-O-xyloside, 9. quercetin-3-O- $\alpha$ -L arabinopyranoside, 10. 1,2,3,4,6-penta-O-galloryl-  $\beta$  -D-glucose, 11. quercetin

Compounds as mangiferin, quercetin, quercetin monoglycosides obtaining in the present investigation were reported by Schieber *et al.* [12] and Meneses *et al.* [39] in mango peel, while the other compounds were reported in Fernández-Ponce *et al.* [22] and Guamán-Balcázar *et al.* [38]. Some research mentioned that mangiferin have been biological activities suggested, including antidiabetic and anti-inflammatory abilities. According to García-Rivera, *et al.* [40], essential compound of the mango bark extract as mangiferin and indanone gallic acid present significant anti-tumour effect in the highly aggressive and metastatic breast cancer cell type MDA-MB231. Quercetin, major compound in mango by – product and cookies with by product is a flavonoid that has shown pharmacological properties like antioxidant and anti-inflammatory properties as well as capability to prevent bone loss [41].

#### Retention of phenolic compounds

The retention of antioxidant compounds was evaluated with the aim of determining how the baking process (170°C during 10 min) affects the stability of the phenolic compounds and their antioxidant capacity. Some research indicate that

the majority of phenolic compounds are sensitive to light, oxygen and high temperatures [42, 43], which limits the use of antioxidant source products in the food industry, since processes are generally used in which food is fed to high temperatures as is the case of pasteurization, sterilization, cooking and baking. In our case the opposite happened, the combination of temperature and time of baking slightly increased the amount of phenolic compounds and antioxidant capacity as can be seen in Table 3. The increase could be due to the destruction of the cellular walls and sub-cellular compartments of the by - product [23], isomerization of the trans to the cis form of the carotenoids [44] or the formation of phenolic compounds with greater antioxidant capacity (Maillard reaction) from sugars and amino acids [45–47]. Reddy, U. *et al.* [48] used as a source of natural antioxidants amla (*Emblica officinalis*), moringa leaves (*Moringa oleiferal*) and raisins (*Vitis vinifera*) against the use of synthetic antioxidants for the preparation and storage of cookies, baked at a temperature of 160 to 180°C, and observed that the temperature did not affect the antioxidant activity and that there was no decrease of these compounds during a period of 6 weeks of storage.

**Table 3: Retention of phenolic compounds and antioxidant activity of cookies with by – product of mango after baking process**

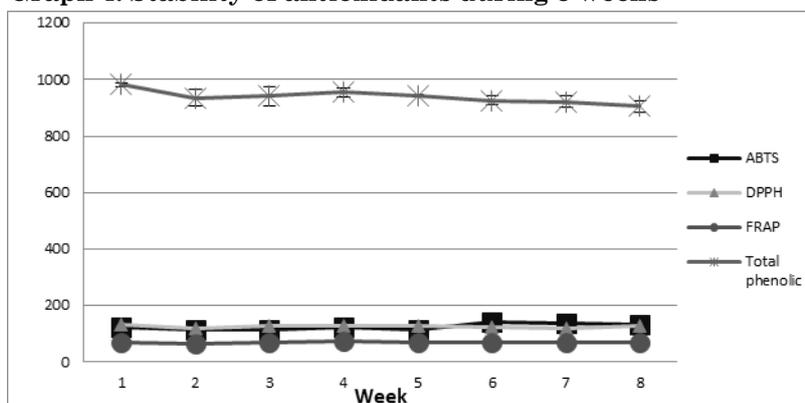
Samples	Cookies <sub>AP</sub>	Cookies <sub>WP</sub>	% Retention
Total phenolic	982.83 ± 7.35	971.27 ± 34.75	101.29
ABTS	121.51 ± 1.84	119.69 ± 15.59	102.56
DPPH	133.55 ± 2.62	112.55 ± 4.40	118.76
FRAP	68.77 ± 1.72	66,47 ± 3.49	103.71
Mangiferin <sup>a</sup>	24.20±0.84	21.19±1.16	114.19
1,2,3,4,6-penta-O-galloryl-β-D-glucose <sup>a</sup>	69.24±8.07	28.45±0.82	243.42
Quercetin <sup>a</sup>	28.95±3.84	21.37±1.54	135.45

Cookies<sub>AP</sub>: content after processing; Cookies<sub>WP</sub>: content without processing; Total phenolic: mg Gallic acid equivalents /100 g dw; DDPH, ABTS and FRAP: μmol Trolox equivalent /g dw; <sup>a</sup> mg compound/g cookies.

### Stability of antioxidants

Throughout the 8 weeks the cookies were stored at a temperature of 20 °C in aluminium containers in order to determine if the storage time affects the amount of total phenols and antioxidant capacity. In Graph 4, it can be observed that the cookies with mango by-product showed a decrease in phenolic compounds, but without significant difference between week 1 and week 8 ( $p>0.05$ ). On the other hand, the antioxidant capacity (Graph 4)

does not show a significant difference ( $p>0.05$ ) between the first and the last week of analysis. With respect to compounds such as mangiferin (24.20 to 27.68 mg compound/g cookies.) and 1,2,3,4,6-penta-O-galloryl-β-D-glucose (69.24 to 51.56mg compound/g cookies.), the amounts of these compounds remained similar between week 1 and week 8, while in the case of quercetin the amount decreased from 28.95 to 1.54 mg compound/g cookies.

**Graph 4: Stability of antioxidants during 8 weeks**

ABTS, DPPH, FRAP: μM Trolox equivalent/ 100 g; Total phenolic: mg gallic acid equivalent /100 g

### CONCLUSION

The incorporation of mango skin for the preparation of cookies improves the content of phenolic compounds and the antioxidant capacity, increasing from 254 to 983 mg EAG / 100 g in the phenolic content, from 6 to 122 μmol ET / g in the methods of antioxidant activity, but could not only be incorporated with the functional and natural purpose as it has been

shown in this research, but also attribute the technological scope since it could increase shelf life by preventing the oxidation of fats and the proliferation of microorganisms, achieving a favourable impact economically, environmentally and industrially.

11 compounds were identified in the cookies, but these compounds were not identified in the control cookies, therefore adding the product was

able to fortify the product with these phenolic compounds.

Contrary to what is indicated in some references, where they report that antioxidants are sensitive to temperature, baking at 170°C for 10 minutes did not cause degradation. However the amount of antioxidants in all methods, showing a retention of 101% in total phenols, 103% in ABTS, 104% in FRAP, 119% in DPPH and 114% was increased in compounds such as mangiferin, 243% in 1,2,3,4,6-penta-O-galloryl-β-D- glucose and 135% in quercetin.

The by-products of fruits such as mango can be a promising ingredient for use in the food industry as a replacement for flour in baking, pastry and biscuits, both for their technological and functional characteristics.

#### CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest in this research article.

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