

# Use of peanut's (*Arachis hypogaea L.*) industrial waste rich in polyphenols, to develop a functional food kind of “marzipan”

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## Abstract.

Mexican agribusiness generates approximately 96,000 tons of peanut waste per year, most of which are unused and often become environmental pollution problem. Several studies have shown that these byproducts contain phytochemicals, such as polyphenols, whose concentrations depend on the variety, maturity and crop conditions. The aim of this work was to extract the phytochemical compounds present in the pericarp and skins of the peanut (*Arachis hypogaea L.*) and its incorporation in a functional food. Extracts of peanut skins had a higher content of polyphenols and antioxidant capacity compared to those obtained in the pericarp. It was found that this component contains 56.0 g of dietary fiber / 100 g of sample, total phenols of  $72.11 \pm 7.81$  mg of gallic acid equivalents / g and its antioxidant capacity determined by the FRAP reducing power method was  $491.4 \pm 54.2$   $\mu$ mol of trolox / g. In this study, 1.25, 2.5, 3.75 and 5% skins was incorporated on the marzipan formulation to increase the polyphenol and fiber content. The substitution of 2.5% of skins had greater sensorial acceptability.

**Keywords:** peanut, waste, polyphenols, marzipan, skin.

## 1. Introduction

In recent years food industry has increased its interest by the use of agro-industrial wastes to develop functional foods; because of they are natural sources of polyphenols and dietary fibre which can be used as nutraceutical to improve the nutritional quality of foods.

Peanuts are an important crop in many parts of the world. Recent data suggest that production of peanuts in the United States is about 2 million tons (USDA, 2011). Peanut skins (testae or seed coat), comprising about 3.0% (w/w) of a peanut seed, are low-value, residue materials resulting from peanut blanching and roasting. Removal of the skin is normally done in preparation for the production of products such as peanut butter. Approximately 60000 tons of peanut skins are accumulated annually in the United States as a result of peanut processing. Peanut skin

use is generally limited to animal feeds (Zhao *et al.*, 2012). The potential exists for value added use of this material to improve antioxidant capacity and shelf-life of lipid-containing foods (Nepote *et al.*, 2002).

Mexican agribusiness generates approximately 96,000 tons of peanut waste per year (SIAP, 2015); most of them become an environmental pollution problem. The skins are one of the peanut's residues; this component contains polyphenols that could be used as ingredients in the production of low-cost functional foods.

Phenolic compounds or polyphenols are the most extensive group of non-energetic substances present in foods from plants (Quiñones *et al.*, 2012). These molecules are secondary plant's metabolites that contribute to the sensory qualities as taste and color. Also, have influence in the foods nutritional value; possess pharmacological and toxic properties, as well as provide a defense against pathogen, insect and herbivore attacks (Krzyzanowska *et al.*, 2010). The chemical structure of the phenols has one or more aromatic rings, with one or more hydroxyl groups. Its free radical scavenging activity consists of the ability to directly inactivate reactive oxygen species (ROS) or pro-oxidant metal ions (Quiñones *et al.*, 2012).

Although studies on polyphenols composition and antioxidant properties began by 1990s, nowadays there are few reports related with their use as ingredients in foods. High content of polyphenols in foods could be beneficial for human health; however, products could become bitter and astringent. For this reason, it is necessary conduct research in order to find the correct amount of ingredients to increase the antioxidant properties of foods, without affecting the level of sensory acceptance (Camargo *et al.*, 2014).

Researches in China, have reported flavonoids in the peanut pericarp (*Arachis hypogaea L.*) ranging from 0.25 to 1.42 mg / g. Flavonoids are powerful antioxidants that eliminate free radicals and have chelating action, in addition, inhibit the lipid peroxidation and have physiological activities like antihypertensive and anti-inflammatory. It has also been reported that they may

exhibit antibacterial and antiviral activity, so flavonoids from the peanut pericarp (*Arachis hypogaea L.*) can be used in the food industry for the benefit of human health (Bi *et al.*, 2011).

Some phytochemicals of the peanut pericarp (*Arachis hypogaea L.*) with antioxidant properties are: luteolin, gallic acid, catechin, rutin, chlorogenic acid, eriodictiol, 5, 7-dihydroxycromone and quercetin (Xing and Chen, 2015). Luteolin is an important natural flavonoid, with anti-inflammatory and vasodilator effect, which can usually be obtained via organic extraction directly from plants such as *Resada luteola* and *Angelica keiskei*; however, these herbs used in traditional medicine have a high market price compared to the peanut pericarp (Ge *et al.*, 2014).

Peanut skins, it is also rich in procyanidins, which are monomeric derivatives of flavan-3-ols (catechin and epicatechin) linked together to form oligo and polymer compounds. Procyanidins exhibit antioxidant, anti-inflammatory, cardiovascular, antimicrobial and anticancer properties (Constanza *et al.*, 2012). Also, phenolic acids such as protocatechuic, p-coumaric, gallic, caffeic, ferulic, sinapinic and ellagic have been identified in this skins (Camargo *et al.*, 2015).

Francisco and Resurreccion (2009) and Ma *et al.*, (2014) have formulated different food products mainly peanut butter and butters incorporating the peanut skins as an ingredient; however, there are few reports on the preparation of confectionery products, especially marzipan. This is a kind of candy traditionally consumed in Mexico, who mainly provides proteins, vitamins and minerals as well as fatty acids such as oleic, linoleic and palmitic acid (Ozcan, 2010). Peanut skins can be incorporated as a source of polyphenols and fiber in the development of a nutraceutical food.

The aim of this work was to extract the phytochemical compounds present in the pericarp and skins of the peanut (*Arachis hypogaea L.*) and to incorporate them in marzipan to obtain a functional food.

## 2. Materials and Methods

Peanut (*Arachis hypogaea L.*), Virginia variety was collected from Xomor agroindustry, located in the Central of Abastos of Mexico City. The area of peanut cultivation was in the Morelos State, Mexico. The peanut vegetative cycle was approximately 130 days, it was sun dried for five days, and it was husked by machine. The skins were obtained by peeling peanuts by hand. Skins were ground with a domestic blender until mesh 40. Samples were stored in polyethylene bags at 4°C until use.

### 2.1. Marzipan preparation

The marzipan formulation was developed following the different substitutions shown in table 1.

**Table 1.** Marzipan formulations

Ingredients	Formulation (%)		
	wet basis		
	0	2.5	5.0
Kernel (g)	100	97.5	95.00
Sugar (g)	35	35	35
Skin (g)	0	2.5	3.75

The process of making the marzipan was carried out in several steps. Dry bleaching was performed on the bare grain (175 ° C for 5 minutes), the bare peanut grain was ground, the other ingredients were added and mixed until obtain a homogeny mixture.

#### a. Aqueous extraction of polyphenols

Prior to the extraction, the samples were degreased with petroleum ether using a soxhlet kit. The sample was then heated at 90 ° C for 5 minutes in a 2.450 MHz microwave oven of the LG model MS1744XT. Subsequently, in 50 mL tubes, 0.5 grams of sample plus 30 mL of distilled water were added. They were then brought to 90 ° C in a water bath for 5 minutes without stirring. After heating time the samples were maintained at room temperature. Then samples were centrifuged at 12,000 rpm for 10 minutes at 4 ° C.

Finally, the aqueous extracts were stored at 4 ° C in amber vials.

#### b. Polyphenols quantification

The content of total polyphenols was quantified using the technique proposed by (Osorio *et al.*, 2011). Folin-Ciocalteu reagent (1:10, 900 µl) was mixed in amber tubes with 100 µl of aqueous extract. The reaction was allowed to stand for 5 minutes and 750 µl of 7% sodium bicarbonate was added. The solutions were shaken for 30 seconds using a vortex, and then allowed to reach room temperature for 90 minutes. Finally, the absorbance was measured using the UV-Vis spectrophotometer at 725 nm and compared to a calibration curve with gallic acid. The results were expressed in mg equivalents of gallic acid (GAE) / g dry sample. All measurements were made in triplicate and their standard deviation was calculated.

#### c. Flavonoids quantification

The flavonoid content was determined following the technique proposed by Osorio *et al.*, (2011) 250 µL of aqueous extract plus 1,250 µL of distilled water and 75 µL of 5% NaNO<sub>2</sub> were placed in a 5 mL micro tube; the reaction was vigorously shaken for 30 seconds and then allowed to stand for 6 minutes. Then 150 µl of 10% AlCl<sub>3</sub> was added and the mixture was allowed to stand 5 minutes at room temperature. To conclude, 500 µL of 1 M NaOH plus 275 µL of distilled water were added. Absorbance was measured using the UV spectrophotometer at 510 nm. The flavonoid content was calculated using a catechin (CA) calibration curve.

#### d. Antioxidant capacity

##### i. DPPH (2,2-diphenyl-1-picrylhydrazyl)

The DPPH radical method was carried out as described by Brand *et al.*, (1995) with some modifications. Aqueous extract (50 µL) plus 1,950 µL of 60 µM DPPH solution were placed in a 2 mL micro tube. The mixture was stirred for 30 seconds and allowed to stand for 120 minutes. Absorbance was measured using a UV-Vis light spectrophotometer at a wavelength of 515 nm. The antioxidant capacity was calculated using a trolox (acid-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic) calibration curve using µmol trolox equivalent units (µmol ET).

##### ii. Ferric reducing power (FRAP)

FRAP was determined by the technique proposed by Benzie *et al.*, (1996) and Berker (2007). For this purpose, FRAP reagent was prepared, mixing 1,500 µL of reagent plus 50 µL of aqueous extract and 150 µL of distilled water. The reaction was vigorously shaken for 30 seconds, incubated at 37 °C for 20 minutes and then allowed to stand for 6 minutes and immediately the absorbance was measured at 595 nm. To calculate the antioxidant capacity, a calibration curve was made using trolox standard.

##### e. Iron ion chelating capacity (IICC)

The chelating capacity of the aqueous extracts was determined by the method implemented by Dinis, *et al.*, (1994) and developed by Wang (2008). 100 µL of aqueous extract, 100 µL of 0.2 mM FeCl<sub>2</sub> · 4H<sub>2</sub>O and 100 µL of distilled water were deposited in 5 mL micro tubes. The mixture was stored at room temperature (28 °C ± 2) for 30 seconds. To the reaction was added 100 µL of 5 mM ferrozine, stirred for 30 seconds and stored, at room temperature for 10 minutes. Subsequently, absorbance changes of the Fe<sup>2+</sup>-ferrozine complex were measured using a UV-Vis spectrophotometer at a wavelength of 562 nm, curve of EDTA-Na<sub>2</sub> (disodium ethylene diamine). The chelating capacity was calculated using a calibration tetraacetic acid.

##### f. Sensory evaluation

An affective test for marzipan in its different substitutions of skins was elaborated using a hedonic scale of 5 points to determine the marzipan with greater acceptability, for this was requested the participation of 100 people. To measure acceptability, grades were assigned to each level of enjoyment on a maximum scale of 10 points.

##### g. Identification of Polyphenols by Liquid Chromatography (HPLC)

It was performed in an Agilent 1100 system, equipped with a quaternary pump G1311, a degasser G1379A and an automatic dispenser G1329A. For separation, a C-18 column was prepared with a mobile phase elution a) 0.1% formic acid and b) 0.1% formic acid in acetonitrile at a

flow rate of 0.5 mL / minute. Standards, p-coumaric acid, caffeic acid and catechin were prepared with a concentration of 1 mg / mL. 0.02 mL; prepared and standard samples were injected into the column (Camargo *et al.*, 2015).

### 3. Results and discussion

#### 3.1 Polyphenols and antioxidant activity (FRAP) of the skins and pericarp

In Table 2 are shown the skins and pericarp polyphenol content and FRAP antioxidant capacity. As expected skins contained higher amount of polyphenols and antioxidant activity than pericarp.

**Table 2.** Polyphenols and antioxidant capacity (FRAP) in the peanut skin and pericarp

Sample	Polyphenols (GAE/g)	FRAP (µmol TE / g)
Tegument	72.11 ± 7.81	491.4 ± 54.2
Pericarp	4.26 ± 0.19	76.08 ± 4.91

The polyphenol value obtained for peanut skin in this research of 72 mg GAE/g sample is comparable to the value obtained by Francisco and Resurreccion (2012) in peanut skin of 64 mg GAE/g for Virginia varieties of peanuts. Gutfinger (1981) discovered that high polyphenol content was associated with high resistance to oxidation of olive oils. Francisco and Resurreccion (2009) reported that a high total phenolic content in peanut skins was associated with a high antioxidant activity. These investigations suggest that total phenolic compounds are closely related to antioxidant activity.

Due to the higher polyphenol content skins was used for marzipan enrichment.

##### a. Polyphenols and flavonoids content, antioxidant capacity and chelating capacity of marzipans

The addition of 2.5% and 5% of skins in marzipan, showed an increase in polyphenol content up to 5.4 and 6.3 mg eq GAE/g respectively (Table 3). These values are low comparing with values obtained for other byproducts as: Venezuelan cocoa cuticle 249. ± 0.06 mg EAG /g sample (Sangronis *et al.*, 2014) and mango peels (11.66 mg EAG /g sample) (Rojas *et al.*, 2015). However, the concentration of polyphenols and flavonoids was increased significantly compared with original marzipan polyphenols content. Antioxidant activity measured by DPPH, FRAP and chelating capacity were according with polyphenol and flavonoid increases (Table 3).

**Table 3.** Total Polyphenols Content (TPC), Flavonoids Content (FC) and antioxidant capacity (DPPH, FRAP and IICC) in marzipan with additions of 0, 2.5 and 5% of peanut skin

Sample	TPC (mg GAE/g)	FC (mg CAE/g)	DPPH ( $\mu\text{mol}$ TE/g)	FRAP ( $\mu\text{mol}$ TE/g)	IICC ( $\mu\text{g}$ EDTA/g)
Tegument	72.11 $\pm 7.81^a$	44.43 $\pm 1.55^a$	208.95 $\pm 7.28^a$	491.4 $\pm 54.2^a$	10.60 $\pm 1.71^a$
Marzipan (control)	2.81 $\pm 0.13^b$	0.24 $\pm 0.10^b$	6.53 $\pm 0.17^b$	10.44 $\pm 0.61^b$	3.69 $\pm 0.42^b$
Marzipan (2.5% tegument)	5.44 $\pm 0.21^c$	1.24 $\pm 0.03^c$	16.75 $\pm 1.00^c$	28.24 $\pm 1.31^c$	5.46 $\pm 0.11^c$
Marzipan (5% tegument)	6.34 $\pm 0.32^d$	2.84 $\pm 0.24^d$	24.06 $\pm 0.50^d$	40.54 $\pm 1.29^d$	8.16 $\pm 0.30^d$

Means followed by the different letters within a column are significantly ( $p > 0.05$ ) different.

### b. Sensory evaluation

The results of sensory evaluation (Table 4) showed a higher level of acceptance of marzipan with skin added compared with marzipan added with 2.5 and 5% of peanut skin ( $p > 0.05$ ). These results could be attributed to the astringent flavor of the phenolic compounds in the skin. However, products with a substitution of 2.5 and 5% still presented acceptable level of panelist acceptance.

**Table 4.** Sensory acceptance level of marzipans with additions of 0, 2.5 and 5% peanut skin

Five point hedonic scale	Score	Score by additions		
		0	2.5	5
I like it very much	10	600	300	340
I like it moderately	8	264	352	328
Neither I like it nor I dislike it	6	36	126	102
I dislike it moderately	4	4	16	28
I dislike like it very much	2	0	2	2
Levels of pleasure (10 max.)		9.04 <sup>a</sup>	7.96 <sup>b</sup>	8.00 <sup>b</sup>

### 3.4 Identification of Polyphenols by Liquid Chromatography (HPLC).

Acids cafeic, p-coummaric and flavonoids as catechin were identified in both skin and marzipan.

## 4. Conclusion

Between the peanut parts tested, peanut skin contained relatively higher amount of phenolic compounds and also exhibited superior antioxidant activity. The tested peanut components possessed varying but meaningful antioxidant activity which was correlated well to their total phenolic contents. Therefore, further studies are recommended to optimize formulatios and percentage of skin substitution for standardizing and maximizing the antioxidant attributes of. In conclusion, peanuts processing by-products namely peanut skin, being inexpensive source of natural antioxidants, could be explored as valuable ingredients for functional foods.

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