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Chemical Analysis of Natural Dyestuff Extracted from Peanut Skin

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Research Article

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ABSTRACT

Most of the peanuts which from the legumes family is the most important agricultural product of Osmaniye province and its surroundings are consumed as snacks. It is also used in the food industry as peanut butter and peanut oil and is also used as an additive. Peanut skin obtained as a by-product of food production in the peanut industry is mostly used as animal feed. Peanut skin, which is rich in protein, fat, carbohydrates and polyphenols, is known to be a powerful antioxidant. Therefore, it is thought that it can be used easily in foods or dietary supplements. In the study, the presence of natural dyestuff in the peanut skin was investigated. Because peanuts are very rich in flavonoids and phenolic acids. For this purpose, as results of the analyzes made in HPLC and UV-Visible Spectrophotometer, it was determined that there are catechin and anthocyanin (Cyanidin) in the peanut skin. In addition, the amount of dyestuff in raw and roasted peanuts was also compared and it was determined that more dyestuff could be extracted from raw peanuts.

Yerfıstığı Tohum Kabuğundan Ekstrakte Edilmiş Doğal Boyarmaddenin Kimyasal Analizi

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ÖZET

Osmaniye ili ve çevresinin en önemli tarım ürünü olan baklagiller familyasından yerfıstığının büyük bir kısmı atıştırmalık olarak tüketilmektedir. Ayrıca fıstık, ezme ve fıstık yağı olarak da gıda sektöründe yer almakta ve katkı maddesi olarak da kullanılmaktadır. Fıstık endüstrisinde gıda üretiminin yan ürünü olarak elde edilen yerfıstığı kabuğu ise çoğunlukla hayvan yemi olarak kullanılmaktadır. Protein, yağ ve karbonhidratlar ile polifenoller açısından zengin olan fıstık kabuğunun güçlü bir antioksidan olduğu bilinmektedir. Bu yüzden gıdalarda veya diyet takviyelerinde rahatlıkla kullanılabileceği düşünülmektedir. Çalışmada, flavonoid ve fenolik asitler açısından zengin olduğu bilinen fıstık kabuğundaki doğal boyar maddenin varlığı araştırılmıştır. Bu amaçla HPLC ve UV-Görünür Spektrofotometrede yapılan analizler sonucunda fıstık kabuğunda kateşin ve antosiyanin (Cyanidin) olduğu belirlenmiştir. Çalışmada ayrıca ham ve kavrulmuş yer fıstığındaki boyar madde miktarları da karşılaştırılmış, ham yerfıstığından daha fazla boyar madde elde edilebileceği ortaya çıkarılmıştır.

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Introduction

Peanut (*Arachis hypogaea* L.), which is among the oilseed plants, is defined as a functional food beneficial for human health because it contains one or more components and nutrients such as oil, protein, carbohydrates and various vitamins/minerals (Özalp and Kürklü, 2020).

Most of the peanut, which is the most important agricultural product of Osmaniye province and its surroundings in terms of its contribution to trade and industry, is consumed as a snack, as well as used in the food industry, especially as an additive in peanut oil, peanut butter, confectionery and confectionery. The plant contains 45-60% oil, 20-30% protein, 18% carbohydrates, vitamins and mineral substances in its seeds (Figure 1). Peanut skin is a conventional valuable plant that can be used in hardboard production, as a feed filler, in mushroom cultivation, as fuel, as a filler in wood production, in charcoal production, as roughage in cattle breeding, as litter and mulch in poultry farming, and to obtain adsorbent by carbonizing the bark (Afrin, 2015; Zeren, 2015).

It is stated that peanut, which is defined as a functional food because of its content of one or more bioactive compounds beneficial for human health, such as resveratrol and phytosterol, as well as antioxidant vitamins and minerals, plays a role in the prevention and treatment of chronic diseases such as obesity, diabetes and cardiovascular diseases. Peanut skin has also gained importance in recent years. It is thought that peanut skin can prevent some diseases in terms of health, due to its high catechin, procyanidin content and its anti-inflammatory effect (Özalp and Kürklü, 2020).

Peanut skin becomes waste in the production of both peanut oil and unshelled peanuts by breaking and separating the skin. After removing the peanut oil, the pulp contains the peanut skin that comprises natural dyestuffs, as well as protein and other substances. In addition, in the production of peanut kernels, a significant amount of peanut skin is obtained as waste during skin cracking and sorting.

Due to the increase in the use of chemically synthesized dyestuffs in the last century, natural dyeing has come to the point of disappearance. However, with the realization that some synthetic dyestuffs and pigments used in textile finishing companies threaten human health and cause environmental pollution due to their toxic and carcinogenic properties, the use of natural dyestuffs has come to the fore in recent years. The use of natural dyestuffs obtained from plants in nature has started to increase due to their biodegradability, non-toxicity and not causing any problems for human health and waste water contaminants. (Osman et al., 2011; Canpolat et al., 2013; Dayıoğlu et al., 2015)

Within the scope of the study, natural dyestuffs were tried to be obtained from peanut skin, since catechins and procyanidins in the peanut skin are in flavonoid structure as functional products. In addition, the effect of roasting applied to peanut on the extracted dyestuff was tried to be determined.

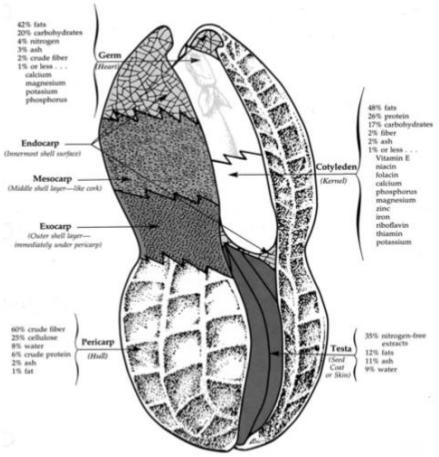


Figure 1. Chemical components of peanuts (Afrin, 2015)

Previous Studies

In review of Özalp and Kürklü (2020), they examined the nutrient content of peanuts and their relationship with diseases, stated that peanut skin has also gained popularity recently and contains high amounts of catechin and procyanidin.

In the article by Constanza et al. (2012), they manufactured spray-dried powders from peanut skin extracts. They determined that these powders contain procyanidin and have high antioxidant activity and create high added value. They also determined that the addition of maltodextrin during production caused the formation of polymeric compounds. Compared to the ground bark, these powders produced higher antioxidant capacity, phenolics and higher solubility. Finally, they proved that peanut skin can be used as a natural source of antioxidants.

In study by Lewis et al. (2013), they determined that peanut skin extracted with acetone or ethanol contained high levels of procyanidins and other phenolic compounds.

In the article by Yu et al. (2005), they investigated the effects of direct peeling, boiling and roasting peeling methods on the total phenolic and total antioxidant activities (TAA) of peanut skin extracts using different solvents. The composition of the extracts was determined by HPLC. As a result, substances such as phenolic acid, flavonoid and stilbene were detected in the peanut skin.

In the article by Yu et al. (2006), peanut skins were extracted according to direct peeling, boiling and roasting methods. Total phenolic (TP), total antioxidant activity (TAA) and free radical scavenging capacity of peanut skin extracts were determined. Peanut skin, a by-product, has been found to be an inexpensive source of antioxidants. It has been said that this product can be used in food or supplements.

In their article by Lee et al. (2009), they were examined the anthocyanins such as “pelargonidin, peonidin and cyanidin” were determined when the HPLC chromatograms of soybeans extract, which come from the same legume family as peanuts.

Wang et al. (2016) used anthocyanin extracts obtained from mulberry fruits to examine the dyeability of cotton fabrics. For anthocyanins, UV-vis spectra and HPLC retention times were examined to determine the chemical structure and it was found to be a cyanidin-derived anthocyanin.

Qin et al. (2010) investigated anthocyanins thought to be found in mulberry pigment. Firstly, fresh mulberry fruits were extracted and LC-MS, HNMR, HPLC-PAD and UV-Visible Spectroscopy instruments were used for the analysis of natural dye pigments. According to the results of the chemical analysis, it was determined that the anthocyanins in the mulberry pigment were cyanide.

Giusti and Wallace (2009) stated in their book chapter "Flavonoids as Natural Pigments" that there is a large consumer demand for natural red colors in the textile finishing industry and that flavonoids can be an alternative to synthetic dyes. Anthocyanins are also found in many fruits (such as strawberries) and vegetables, and they have explained that they give different colors from orange to red and violet.

Materials and Method

Supply of peanut skins and preparation

Virginia type peanuts were supplied through a company in Osmaniye, which produces both snacks and peanut butter. After the peanuts are collected from the field, kept in the sun and ventilated, they are subjected to pre-control and cleaning in the Sorting Machine. At this stage, unheated raw peanut skin (not roasted), which is separated from the machine as waste, can be supplied. During the roasting process at ~140^{o}C for the production of snacks, roasted peanut skin can be obtained from the machine as waste. Roasted and unroasted (raw) peanut skins supplied for use in the experiments were kept in the sun to remove the existing moisture and foreign materials were removed by sifting through a coarse sieve. Afterwards, the skins were passed through a laboratory grinder and sieved again in order to be used in tests such as HPLC and UV-Spectrophotometer and extraction processes.

Extraction

For unroasted and roasted peanut skins, 200 mg of powdered skins were mixed using 100 ml of Methanol in a magnetic stirrer at 1000 rpm for 5 hours on average at 30 ^{o}C and the extract was obtained by filtering on filter papers. Then, the solvent was removed using the evaporator, and it was tried to obtain dry natural dyestuff by using a vacuum pump and a vacuum oven.

Ultraviolet-visible field spectroscopy

Unknown substances can be identified and concentrations of known substances determined using UV-VIS spectroscopy. In order to determine the visible absorption spectra of the peanut skins, spectroscopic analysis with UV/Visible Spectrophotometer was performed in the "Thermo Scientific Evolution 220 UV-Visible Spectrophotometer" test device.

Identification of components by high-performance liquid chromatography (HPLC), Sample preparation

The dyestuff content of organic dyes and pigments can be determined by analyzing them by HPLC (high performance liquid chromatography).

The steps for sample preparation are summarized below;

- ✓ Sample 1, sample weight for peanut seed skin: 14.30 mg, injection volume 100 µL,
- ✓ Sample number 2, sample weight for heat-treated peanut seed skin: 14.40 mg, injection volume is 100 µL.
- ✓ The samples were hydrolyzed 37% (1: 1: 2, v / v / v) in 600 µL of H_2O/MeOH/HCl held at 100 °C for 8 minutes to remove the organic dyestuff. The samples were then evaporated under a gentle stream of nitrogen (65°C). The dry residue was dissolved in 500 µL MeOH / H_2O (2:1, v/v) and the samples were centrifuged. Samples were prepared for HPLC analysis by injecting 100 µL of each sample by further dilution if necessary and then centrifuging at 4000 rpm/25^{o}C/10 minutes (DATU, 2020).

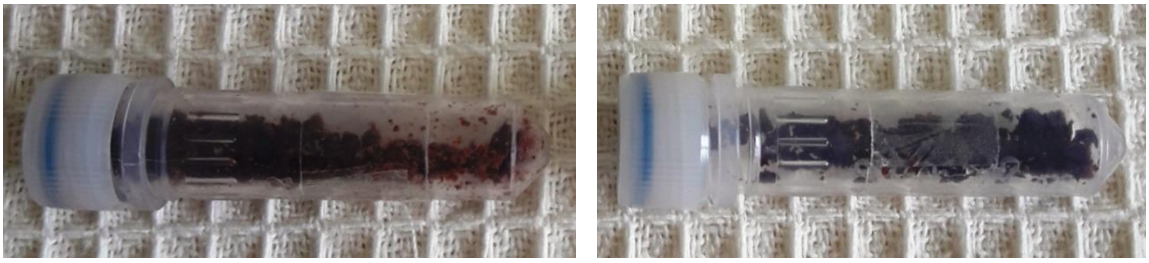
HPLC-DAD Analysis

The technical specifications of the device used for the analysis and the analysis are summarized below;

- ✓ Chromatographic measurements in High Pressure Liquid Chromatography were performed using the Agilent 1200 series system (Agilent Technologies, Hewlett-Packard, Germany) including the G1311A Quat pump, G1322A Degasser, G1329A autosampler, G13166 TCC and G1315D Diode Array Detector.
- ✓ DAD detection was accomplished by scanning from 191 to 799 nm with a resolution of 2 nm.
- ✓ Chromatographic peaks were ascertained at eight different points between 255 and 620 nm.
- ✓ One Nova Pak C18 analytical column (39 × 150 mm, 4 µm, Part No WAT 086344, Waters) was used.
- ✓ Analytical and protective columns were kept at 30 °C and "Agilent Chemstation" data station was used.
- ✓ Two solvents "Solvent A: H_20 - 0.1% TFA and solvent B: CH3CN - 0.1% TFA" were used for the chromatographic separation of the hydrolyzed samples (DATU, 2020).

Results and Discussion

As a result of the extraction process; 0.2750 g dyestuff was obtained from the unroasted (raw) powder skin and 0.1291 g dyestuff was obtained from the roasted powder skin (Figure 2).



Natural Dyestuff 1 Natural Dyestuff 2

Figure 2. Unroasted peanut skin (Natural Dye 1), roasted peanut skin (Natural Dye 2)

As a result of the extractions, it was revealed that more amount natural dyestuffs were obtained from unroasted peanut skin.

As a consequence of the spectroscopic analysis with a UV/Visible Spectrophotometer, the absorption spectrum of the raw peanut skin was found to be 3.54 at 380 nm, 1.25 at 520 nm and 1.10 at 560 nm, as seen in Figure 3. The spectrum results detected at 520 nm wavelength were similar to the HPLC results.

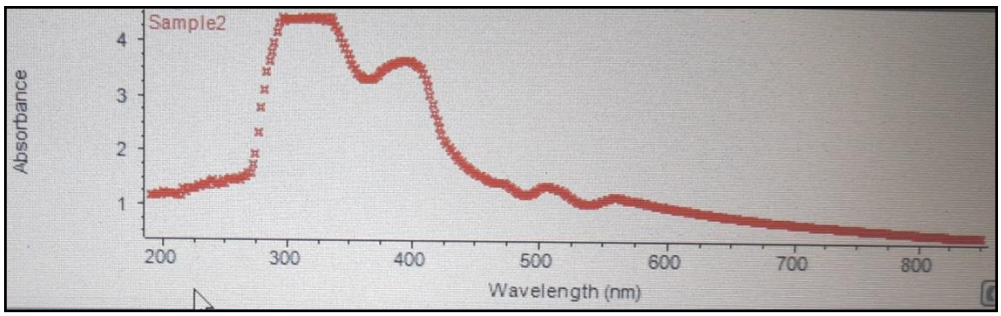


Figure 3. The absorption UV–Vis spectra of peanut skin

The chromatograms and spectra determined as a result of the High Pressure Liquid Chromatography (HPLC-DAD) method are shown in Figure 4-7. It was determined that the dyestuffs were the same in both samples, which were prepared and analyzed under the same experimental conditions (amount, volume and injection volume). However, it was determined that the dyestuffs detected in the raw peanut skin sample had higher peak values (mAU) than the dyestuffs detected in the heat treated (roasted) sample (Figure 7). Qualitative dyestuff analysis results by HPLC are summarized with graphics below;

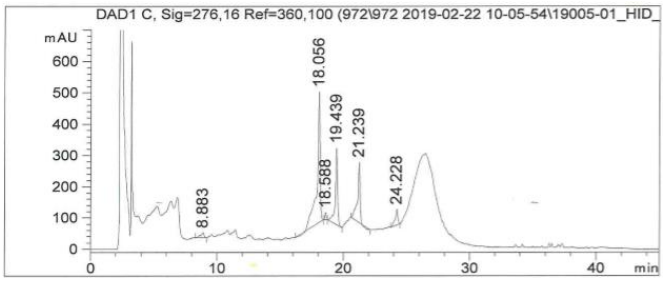


Figure 4. Raw peanut skin chromatogram

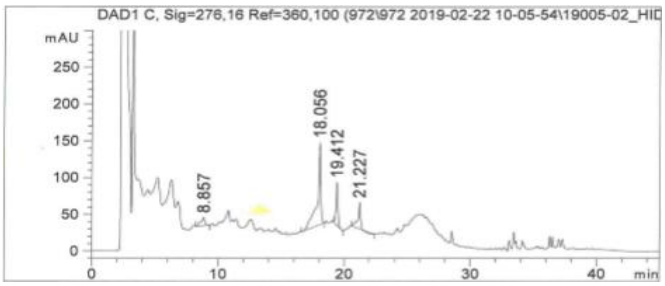


Figure 5. Heat-treated (roasted) peanut skin chromatogram

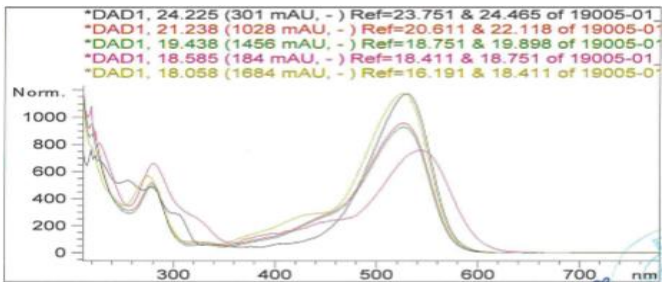


Figure 6. Overlapping of dyestuffs in raw peanut skin

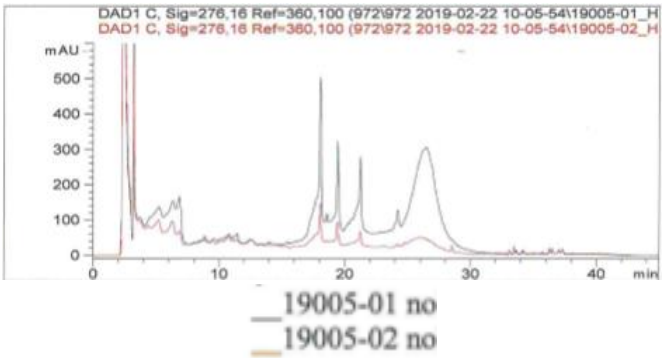


Figure 7. Comparison of chromatograms of raw (01) and heat-treated (roasted) (02) peanut skins

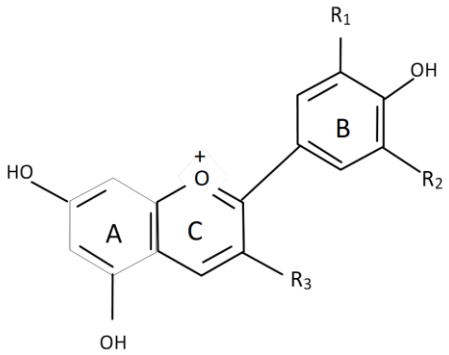
Phenolic acid and anthocyanin groups were determined in both samples subject to analysis. The maximum absorbance (λmax) values of the dyestuffs are shown in Table 1.

Table 1. The maximum absorbance (λmax) values of the dyestuffs

No	Detention Time (minutes)	Maximum absorbance value (nm)	Chemical Group	Dyestuff	References
1	8,883	231, 280, 309	Phenolic acid	Catechin	Constanza et al., (2012)
2	18,056	275, 320sh, 525	Anthocyanin	Cyanidin	Lewis et al., (2013)
3	18,588	281, 543	Anthocyanin	Cyanidin	Yu et al., (2005)
4	19,439	279, 329sh, 527	Anthocyanin	Cyanidin	Lee et al., (2009)
5	21,239	279, 329sh, 526	Anthocyanin	Cyanidin	Wang et al., (2014)
6	24,228	255, 279, 301sh, 529	Anthocyanin	Cyanidin	Qin et al., (2010)
					Giusti and Wallace, (2009)

According to the reference sources (Yu et al., 2005; Giusti and Wallace, 2009; Lee et al., 2009; Qin et al., 2010; Constanza et al., 2012; Lewis et al., 2013; Wang et al., 2014) for the determination of the

dyestuff in Table 1, when the chromatogram absorbance values, retention times, UV-spectra and λmax values were evaluated, results supporting this study were obtained. Especially in studies where HPLC analyzes are carried out to identify the compounds in the peanut skin; It can be said that the detection of phenolic acid and flavonoid determined by Yu et al. (2005), high levels of procyanidin in the studies of Constanza et al. (2012) and of Lewis et al. (2013) are consistent with the results of our study. As a result, it was determined that the natural dyestuff in the peanut skin is Anthocyanin/Cyanidin belonging to the Flavonoid pigment group (Figure 8).



Name	R1	R2	λmax[a](nm)	Color
Pelargonidin	H	H	520	Orange
Cyanidin	OH	H	535	Red
Delphinidin	OH	OH	545	Purple
Peonidin	OCH_3	H	532	Red
Petunidin	OCH_3	OH	543	Purple
Malvidin	OCH_3	OCH_3	542	Purple

a:Absorbance maxima in the visible spectrum in methanol with 0.01 % HCl

Figure 8. Molecular structure of anthocyanins (Giusti and Wallace, 2009)

Conclusion

In our study on the determination of the natural dyestuff in the peanut skin, it has been determined that the natural dyestuff obtained in unroasted (raw) and roasted peanuts is the same with chromatograms and spectra obtained by HPLC-DAD method. As a result of the extraction processes it was revealed that more natural dyestuffs were obtained from unroasted peanut skin. A literature search was made and compared according to the determination of dyestuffs according to the retention time (minutes) and maximum absorbance (nm) values in the chromatograms. The results obtained indicated that the dyestuff in the peanut skin was mainly "Cyanidin" belonging to the "Anthocyanin" group, which is the most important group of "Flavonoid" pigments. It was observed that the spectral results of the raw peanut skin at a wavelength of 520 nm were similar to the HPLC results.

As a result, roasting at high temperatures is the most important process of peanut enterprises in terms of energy cost, taste, consumption and customer satisfaction. However, due to the high temperature processing of peanuts, the anthocyanin compounds in the peanut skin can be damaged and this may adversely affect the quality and efficiency of the natural dyestuff. When anthocyanins are subjected to heat treatment, they undergo hydrolysis of the glycosidic bond which leads to loss of color. In addition, the beneficial effects of peanuts in terms of health may decrease with the damage of beneficial substances such as tannin in the peanut skin with high temperature (Giusti and Wallace, 2009).

Thanks

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Conflict of Interest Statement

The author declares that there is no conflict of interest.

Contribution Rate Statement Summary of Researcher

The author declares that he has contributed 100% to the article.

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