Accepted manuscript

Arrazola, G., N. Grane, and F. Dicenta. 2021. Quantification of cyanogenic compounds, amygdalin, prunasin, and hydrocyanic acid in almonds (*Prunus dulcis* Miller) for industrial uses. Revista Colombiana de Ciencias Hortícolas 15(3), 13049. Doi: https://doi.org/10.17584/rcch.2021v15i3.13049

Quantification of cyanogenic compounds, amygdalin, prunasin, and hydrocyanic acid in almonds (*Prunus dulcis* Miller) for industrial uses

Cuantificación de compuestos cianogénicos, amigdalina, prunasina y ácido cianhídrico en almendras (*Prunus dulcis* Miller) para usos industriales

GUILLERMO ARRÁZOLA[1,4]
NURIA GRANE[2]
FEDERICO DICENTA[3]

[1] Universidad de Cordoba, Faculty of Engineering, Food Engineering Program, Vegetables Processes and Agroindustry Research Group, Monteria (Colombia). ORCID Arrázola, G.: 0000-0001-6878-0806

[2] Universidad de Alicante, Faculty of Sciences, Department of Analytical Chemistry, Nutrition and Food Science, Alicante (Spain). ORCID, Grané, N.: 0000-0001-5882-6444

[3] Centro de Edafología y Biología Aplicada del Segura (CEBAS), Consejo Superior de Investigaciones Científicas (CSIC), Group of Genetic Improvement of Fruit Trees, Murcia (Spain). ORCID, Dicenta, F.: 0000-0002-9593-7351

[4] Author corresponding. gsarrazola@correo.unicordoba.edu.co

Fruits, cultivation, seeds and flowers of almond tree.
Photo: F. Dicenta

Last name: ARRAZOLA / GRANE / DICENTA

Accepted manuscript

Arrazola, G., N. Grane, and F. Dicenta. 2021. Quantification of cyanogenic compounds, amygdalin, prunasin, and hydrocyanic acid in almonds (*Prunus dulcis* Miller) for industrial uses. Revista Colombiana de Ciencias Hortícolas 15(3), 13049. Doi: https://doi.org/10.17584/rcch.2021v15i3.13049

Short title: LEVELS OF TOXICITY IN ALMOND VARIETIES

Doi: https://doi.org/10.17584/rcch.2021v15i3.13049

Received: 28-06-2021 Accepted: 25-08-2021 Published: 21-10-2021

ABSTRACT

The objective of this research was to quantify the concentration levels of the cyanogenic compounds, amygdalin and prunasin present in some varieties of almonds, considering their conversion to hydrocyanic acid, and their possible consumption in addition to other industrial uses, seeds of 29 commercial varieties were used of almond (*Prunus dulcis* Miller), evaluating its concentration and toxicity levels, taking into account the minimum degree of theoretical intake both for human consumption and for animals, through feed, this in terms of by-products. In addition, thermophysical properties thermophysical properties (thermal conductivity, thermal diffusivity, specific heat and density) and industrial uses were determined. The concentration was determined by chromatographic techniques (HPLC) and colorimetry (microdiffusion). The results obtained showed low levels of amygdalin from "not detected" to 375.40 mg/100 g of sample, depending on the sweet, slightly bitter and bitter varieties. The results indicate its possibility of commercialization, uses and applications in the food and pharmaceutical industry.

Additional key words: toxicity cyanogenic compounds; candies; nutrition; industrial uses; *Cp* almond.

RESUMEN

El objetivo de esta investigación fue cuantificar los niveles de concentración de los compuestos cianogénicos, amigdalina y prunasina presentes en algunas variedades de almendras, considerando su conversión a ácido cianhídrico, y su posible consumo además de otros usos industriales, se utilizaron semillas de 29 variedades comerciales de almendro (*Prunus dulcis* Miller), evaluando su concentración y niveles de toxicidad, teniendo en cuenta el grado mínimo de ingesta teórica tanto para el consumo humano como para los animales, a través de los piensos,

Arrazola, G., N. Grane, and F. Dicenta. 2021. Quantification of cyanogenic compounds, amygdalin, prunasin, and hydrocyanic acid in almonds (*Prunus dulcis* Miller) for industrial uses. Revista Colombiana de Ciencias Hortícolas 15(3), 13049. Doi: https://doi.org/10.17584/rcch.2021v15i3.13049

esto en términos de subproductos. Además, se determinaron propiedades termofísicas (conductividad térmica, difusividad térmica, calor específico y densidad) y usos industriales. La concentración se determinó mediante técnicas cromatograficas (HPLC) y colorimetría (microdifusión). Los resultados obtenidos mostraron niveles bajos de amigdalina desde "no detectado" hasta 375.40 mg/100 g de muestra, dependiendo de las variedades dulce, ligeramente amarga y amarga. Los resultados indican su posibilidad de comercialización, usos y aplicaciones en la industria alimentaria y farmacéutica.

Palabras clave adicionales: toxicidad compuestos cianogénicos; golosinas; nutrición; usos industriales; *Cp* almendra.

INTRODUCTION

The almond tree, (*Prunus dulcis* Miller), is a species with great genetic variability, the fruit is a drupe, with its outer part formed by the pericarp and the mesocarp. *Prunus amygdalus* is now recognized as the result of natural hybridizations involving several wild species (*Prunus bucharica* and *Prunus kuramica*) that still exist in central and south-western Asia (Grasselly and Crossa-Raynaud, 1980). The almond is rich in vitamins and minerals, being considered a good source of vitamin E (tocopherols), riboflavin, calcium, magnesium, phosphorus, potassium, zinc, copper and manganese (Rodushkin *et al.,* 2008; Ballhorn, 2011). Almonds also contain a wide variety of phenolic compounds, mainly proanthocyanidins, flavonoids and phenolic acids (Hayes *et al.,* 2016) that are predominantly found in the skin and are responsible for their antioxidant properties (Mandalari *et al.,* 2010). Phytosterols are also found in significant quantities (~270 mg/100 g) in sunflower-almond grains, the predominant type being β-sitosterol (Fernández-Cuesta *et al.,* 2012; Alasalvar and Bolling, 2015; Forcada *et al.,* 2015). Other compounds, with an important characteristic in bitter almonds, is the presence of the glucoside amygdalin, responsible for the bitter taste, whose proportion in the grain is 2 to 4% (Ibar, 1985).

The main use of sweet almonds is for human consumption, either alone or as part of other products (Bainbridge, 1996). In other uses, flour for pastry and / or pastries, fatty acids to produce margarine and edible oil, obtained from bitter almonds after eliminating hydrocyanic acid (Abd Aal *et al.,* 1987). Almonds are also used as ingredients in ice cream creams and in

Arrazola, G., N. Grane, and F. Dicenta. 2021. Quantification of cyanogenic compounds, amygdalin, prunasin, and hydrocyanic acid in almonds (*Prunus dulcis* Miller) for industrial uses. Revista Colombiana de Ciencias Hortícolas 15(3), 13049. Doi: https://doi.org/10.17584/rcch.2021v15i3.13049

various cooking recipes. The fatty acids in almonds serve as caking preventives and external preservatives in extruded snacks (Kobayashi and Hisamatsu, 1978).

Taking into account that the bitter taste of the almond tree seed is due to the glucoside amygdalin (McCarty *et al.,* 1952; Conn, 1980; Polesello and Rizzolo, 1989; Frehner *et al.,* 1990; Sánchez-Pérez *et al.,* 2008; Sánchez-Pérez *et al.,* 2019), nowadays, studies have shown that the sweet or bitter taste of almonds is a monogenic character, the bitter being the homozygous recessive (Heppner 1923; Heppner 1926; Dicenta and García 1993; Vargas *et al.,* 2001; Thodberg, *et al.,* 2018). However, it has been shown that the sweet or sour taste of the almond is a characteristic of the variety and is not influenced by the type of pollen that pollinated the flower (Dicenta *et al.,* 2002). Cyanogenic compounds could produce hydrocyanic acid under certain conditions and in the presence of specific enzymes, today, the cyanogenic glycosides known to date are structurally very similar, in some cases changing the position of the radicals.

The toxicity of cyanogenic glycosides and their derivatives depends on the release of hydrogen cyanide, (FAO and WHO, 2012). The primary action of hydrocyanic acid in a person's body is to inhibit cytochrome oxidase, which blocks cellular respiration. The lethal dose of hydrocyanic acid in humans is 0.5 to 3.5 mg kg^{-1} of body weight in a single dose (Blum, 2010; Borron and Baud, 2012). Regarding the symptoms, a person with mild cyanide poisoning suffers headache, nausea and weakness, due to oxygen deprivation, the above is conditioned by the concentration of the compound and the time of exposure to it, now when it is ingested small amounts of cyanogenic compounds, the most common route for detoxification is the conversion of hydrocyanic acid to thiocyanates in the liver and kidneys, which is subsequently excreted in the urine (Chaouali *et al*., 2013, Abraham, *et al.,* 2016), where it is important to know that the cyanide concentration is higher in erythrocytes than in plasma. Studies show that the cyanide level in different human tissues in a fatal case of HCN poisoning is 0.03 gastric content, 0.50 blood, 0.03 liver, 0.11 kidney, 0.07 brain, and 0.20 urine (mg/100 g) (EPA, 1990); yes, toxic levels of cyanogenic glycosides are estimated based on the amount of free cyanide generated after hydrolysis (EFSA, 2007). Although the level of cyanide up to (10 mg L^{-1}) was reported as safe for cassava flour (FAO and WHO, 2012). The lack of quantitative toxicological tests and epidemiological information makes it difficult to establish a safe level of intake of cyanogenic glycosides in many foods. The objective of this research was an evaluation of the toxicity levels

Arrazola, G., N. Grane, and F. Dicenta. 2021. Quantification of cyanogenic compounds, amygdalin, prunasin, and hydrocyanic acid in almonds (*Prunus dulcis* Miller) for industrial uses. Revista Colombiana de Ciencias Hortícolas 15(3), 13049. Doi: https://doi.org/10.17584/rcch.2021v15i3.13049

of some cyanogenic compounds, considering their conversion to cyanhydric acid, in almonds for consumption and industrial uses.

MATERIALS AND METHODS

Vegetal material

Table 1, almond varieties were taken in the experimental farm "Tres Caminos" of the Center for Soil Science and Safe Applied Biology (CEBAS), of the Higher Council for Scientific Research (CSIC). The farm is in Santomera (Murcia), at 130 msnm, with very hot summers and mild winters and with minimum temperatures that do not usually drop below (0-4°C). The trees, of different ages, are found with localized irrigation and at different planting frames depending on the case.

Collection, conditioning and conservation of the sample

The almonds were taken from the tree in full maturity when the mesocarp was completely open, each sample is made up of 50 almonds that were taken randomly. The almonds were stripped of the mesocarp and placed in mesh bags, duly labeled, and transported to the laboratory. The samples were lyophilized (Telstar 2000 lyophilizer), at a pressure of $4 \cdot 10^{-2}$ mbar and a temperature between -79 and -82°C, then they were kept at -18°C until subsequent analyzes, were evaluated two years in a row.

Table 1. Almond variety studied and theoretical flavor in each harvest year.

Sweet	'Desmayo´ ´Largueta', 'Del Cid', 'Atocha', 'Ferragnès', 'Peraleja', 'Primorskii', 'Marcona', 'Ramillete', 'Ferraduel', 'Achaak', 'Planeta', 'Bonita', 'Colorada', 'Carretas', 'La Mona', 'Tioga', 'Titan', 'CEBAS', 'Pajarera', 'Rumbeta'
Slightly bitter	'Garrigues', 'Genco', 'Tuono'
Bitter	'S3060', 'S3062', 'S3076', 'S3108', 'S3112', 'S3126'

Arrazola, G., N. Grane, and F. Dicenta. 2021. Quantification of cyanogenic compounds, amygdalin, prunasin, and hydrocyanic acid in almonds (*Prunus dulcis* Miller) for industrial uses. Revista Colombiana de Ciencias Hortícolas 15(3), 13049. Doi: https://doi.org/10.17584/rcch.2021v15i3.13049

Bromatological characterization

The manual extraction of the seed was carried out. The characterization of the almond was carried out using the techniques of proximal analysis: humidity, method 930.15 (AOAC, 1990); determination of ether extract, method 920.39 (AOAC, 1990); ash determination, method 942.04 (AOAC, 1990); crude fiber determination, method 962.09 (AOAC, 1990); crude protein determination, method 955.04 (AOAC, 1990); and fat determination, method Colombian technical standard – NTC 336 (INCONTEC, 2002) (EFSA, 2007).

Thermophysical analysis

The thermophysical properties of the almond seed determined were thermal conductivity, thermal diffusivity, specific heat, and density. These were estimated according to the bromatological composition, using mathematical models based on temperature, in a range from -40ºC to 150ºC (Choi and Okos, 1986). The models are presented in table 2. The results obtained were compared with those of the Deproter® v. 2 software (2012).

Table 2. Models used to determine thermophysical properties in almonds.

Thermal conductivity (k)	$K = \sum K_i X_i^v$
Density (ρ)	$\rho = \frac{1}{\sum X_i^w / \rho_i}$
Thermal diffusivity (α)	$\alpha = \sum \alpha_i X_i^v$
Specific heat (C_p)	$C_p = \sum Cp_i X_i^w$

X_i^w : weight fraction of component *i*, ρi is the density of pure component *i* (protein, fat) and X_i^v is the estimated volume fraction for component *i* (adapted from Choi and Okos, 1986).

Chemical analysis of cyaniogenic compounds

The cyaniogenic compounds of the almonds were determined by microdiffusion and High-performance liquid chromatography (HPLC) (Fig. 1). The chromatographic conditions selected have been: Symmetry C18 column, eluent acetonitrile: water (80: 20), flow rate 1.3 mL / min and

Arrazola, G., N. Grane, and F. Dicenta. 2021. Quantification of cyanogenic compounds, amygdalin, prunasin, and hydrocyanic acid in almonds (*Prunus dulcis* Miller) for industrial uses. Revista Colombiana de Ciencias Hortícolas 15(3), 13049. Doi: https://doi.org/10.17584/rcch.2021v15i3.13049

photometric detector at 218 nm. The coefficient of variation obtained in chromatography, as a measure of the reproducibility of the method, was 2.3% at concentration levels 100 times above the detection limit and 22% at concentration levels close to the detection limit. The detection limit for amygdalin is 0.387 mg/100 g and for prunasin 0.136 mg/100 g.

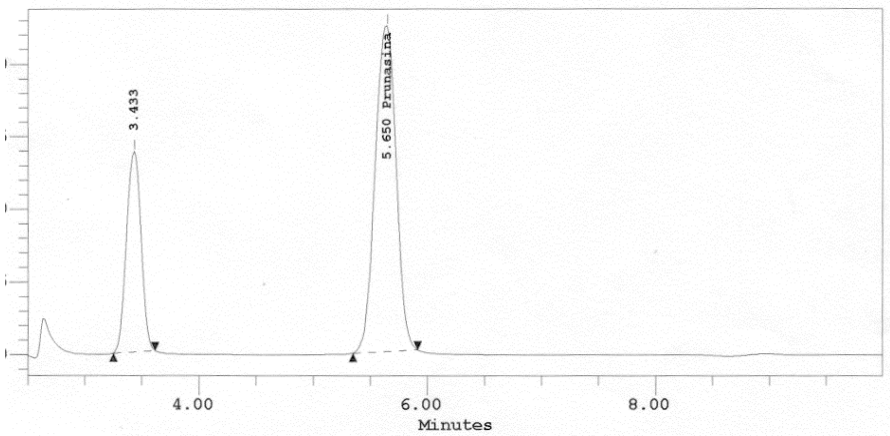

Figure 1. Chromatogram of a standard mixture of amygdalin (3.4 min) and prunasin (5.7 min) obtained using a Symmetry C18 column. Source: Arrázola *et al.* (2015).

Microdiffusion method

The hydrolysis of the glucoside (first stage) was carried out as follows: generation of hydrocyanic acid by acid hydrolysis (Haque and Bradbury, 2002), using autoenzymatic methods, but controlling and slowing down the process of generating hydrocyanic acid by adding reagents that inhibit hydrolysis. Once the hydrocyanic acid is generated by hydrolysis, it diffuses into the microdiffusion cell designed for this purpose and is absorbed in a concentrated 0.2N NaOH solution placed inside the device where the hydrolysis is carried out. For quantitative analysis, concentrations greater than 10 mgL^{-1}, were valued by gravimetry with silver nitrate, using a rhodanine solution as an indicator (Bark and Higson, 1963). For the determination with picrate, a container like the one in Figure 2 (Egan *et al.,* 1998) was used, where a support was placed that contains at one end the enzyme (own) necessary for the hydrolysis of the cyanogenic compound and in the the other end is a strip of paper impregnated with sodium picrate (yellow in color), which turns orange when cyanide is released. This method has also been used for quantitative determination, extracting the compound formed by the Guignard reaction from the paper strip and measuring the absorbance of the extract at 520 nm (Lucas and Sotelo, 1984). Egan *et al.*

Arrazola, G., N. Grane, and F. Dicenta. 2021. Quantification of cyanogenic compounds, amygdalin, prunasin, and hydrocyanic acid in almonds (*Prunus dulcis* Miller) for industrial uses. Revista Colombiana de Ciencias Hortícolas 15(3), 13049. Doi: https://doi.org/10.17584/rcch.2021v15i3.13049

(1998) have developed a technique that makes it possible to measure absorbance in the solid state, that is, in the strip of paper itself.

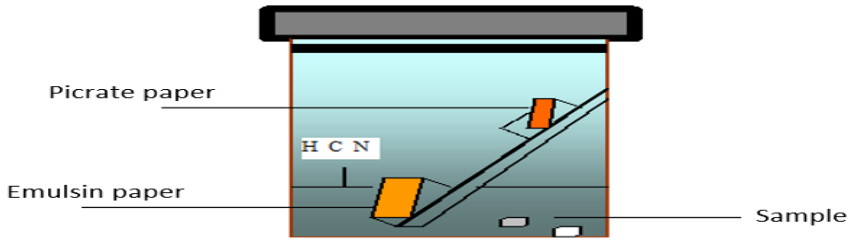

Figure 2. Device for measuring with picrate paper. Source: Arrazola *et al.* (2015)

Hydrolysis and microdiffusion of cyanide in almonds

0.2-0.4 (± 0.0001) g are placed in the reactor of freeze-dried bitter or slightly bitter almond weighed exactly. In the collector vial placed inside the reactor that is detailed in figure 2, 1.0 mL of 0.2 M NaOH solution is available and finally 4 mL of phosphate buffer pH = 5.5 is added to the sample to adjust pH and close the reactor immediately. It is kept in a water bath at 35ºC for 24 h, after which time the reactor is removed from the bath and allowed to cool, then the reactor is opened, and the cyanide is collected from the collecting vial for its determination either by colorimetry or titration gravimetric, according to its concentration. The extraction performance was evaluated as follows: A sample was prepared with 5 mL of CN^- standard with 1.0 mL of 0.2M NaOH (1 mg L^{-1}) in the collecting vial and 0.7 mL of phosphoric acid, to adjust the pH to 5.5. The microdiffusion procedure is applied followed by the colorimetric determination and the yield is determined, which turned out to be 94%.

Colorimetric determination of total cyanide, (low concentrations of CN^-)

The alkaline solution from the diffusion collector was quantitatively transferred to a 25 mL flask, with a dropper, washing the collector with 4 mL of NaOH (measured with a pipette and poured into a 50 mL beaker) in three portions, which were added to the flask. 5 mL of the KH_2PO_4 solution was added and then immediately 0.5 mL of chloramine. It was allowed to stand for 1 min. Barbituric acid was added 1 mL after 20 min later, the absorbance was measured at 580 nm, against a blank prepared with 5 mL 0.2 M NaOH + 5.0 mL KH_2PO_2 + 0.5 mL of

Arrazola, G., N. Grane, and F. Dicenta. 2021. Quantification of cyanogenic compounds, amygdalin, prunasin, and hydrocyanic acid in almonds (*Prunus dulcis* Miller) for industrial uses. Revista Colombiana de Ciencias Hortícolas 15(3), 13049. Doi: https://doi.org/10.17584/rcch.2021v15i3.13049

chloramine + 1.0 mL of barbiturate. The determination was made by comparison with the absorbance of a standard prepared from 5 mL of CN in 0.2 M NaOH (1 mg L^{-1}) + 5.0 mL KH_2PO_4 + 0.5 mL chloramine + 1.0 mL barbituric acid, also brought to 25.0 mL that should give an absorbance versus the white of (0.810-0.850). It should be considered, if the absorbance of a test sample is <0.020; considering that the cyanide concentration is below the detection limit, it must be calculated with the following equation (1)

$$C_M = \frac{A_M \times C_p \times Vx_f}{A_p \times P_i} \times 100 \quad (1)$$

where, C_M was sample concentration, V_f final volume, P_i sample weight (g), A_M sample absorbance, A_p standard absorbance, C_p = Cyanide standard concentration (mg L^{-1}) f = Factor due to 94% recovery.

The cyanide concentration was calculated in mg/100g of dry sample by comparing the absorbance of the sample (A_M) with the absorbance of the standard (Ap) of 1 mg L^{-1} of cyanide from which 5 mL were taken and taken to a volume of 25 mL, with which the concentration of the standard (Cp) is 0.2 mg L^{-1}, thus: $Cp = \frac{1.0}{25} = 0.2$, so we have:

$$C_M = \frac{\frac{A_M}{A_p} \times \frac{0.2mg}{L} * 25X10^{-3}L \times 1.06}{P_i} \times 100 \quad (2)$$

The extraction yield is 94% which indicates that the factor: $f = \frac{100}{96} = 1.06$

Gravimetric titration of cyanides (for high concentrations of CN^-)

The alkaline solution of CN^- from the collector is transferred quantitatively to a 100 mL beaker, with a dropper, washing it and combining the washings with the first transfer, with (3) three portions of H_2O of 1-2 mL It was diluted to 50 mL with water and 1 drop of indicator was added. $AgNO_3$ titrant reagent was added slowly (especially near the turn) with magnetic stirring, until it turned from yellow to reddish. The grams of $AgNO_3$ spent in the titration were calculated

Arrazola, G., N. Grane, and F. Dicenta. 2021. Quantification of cyanogenic compounds, amygdalin, prunasin, and hydrocyanic acid in almonds (*Prunus dulcis* Miller) for industrial uses. Revista Colombiana de Ciencias Hortícolas 15(3), 13049. Doi: https://doi.org/10.17584/rcch.2021v15i3.13049

by weight difference of the flask containing said solution before starting the titration and after the end point, the reaction is as follows:

$$Ag^{+} + 2CN^{-} \rightarrow Ag\,(CN)_2$$

A blank was made with 1 mL 0.2 M NaOH + H_2O up to 50 mL + 1 drops of indicator.

$$mg\ de\ CN^{-}/100g\ Sample = \frac{g_{ndis} \times C_{dis} \times f}{P_i} \times 100 \quad (3)$$

g_{ndis} was net grams of $AgNO_3$ dissolution (the net grams are those spent in the titration minus those of a blank, it is usually between 0.03 and 0.05 g), C_{dis} the concentration of the $AgNO_3$ solution (mg of CN^- gram of solution), P_i the weight of the sample, f = the factor due to 94% recovery.(1.06).

Flour elaboration

The almond flour elaboration process was carried out to use them in the different analyzes in this investigation to quantify the level of toxicity and products to be obtained, the necessary unit operations were carried out, such as seed suitability, peeling, grinding, classification by particle size, 50 whole units were left to obtain candied almonds according to Arrázola *et al.,* (2015).

Manufacture of candied almonds

Although the consumption of dry products including almonds is growing, in this work it was considered to elaborate candied almonds from the seed of the almond tree, for which the manufacturer's grading drum was used (Fedeacero, model WQA 2011, Bogota D.C.). The kernels or kernels were precoated with powdered sucrose syrup and gelatin. The ratio of the precoat to the cores was 5:1 p/w. The procedure consisted of adding the kernels (almond seeds) to the crushing drum, adjusting the drum inclination to 45° and the revolutions to 30 rpm (Andréo *et al.,* 2007). Then, the precoating solution (syrup) previously prepared was slowly added with the consequent drying of the almonds with hot air (±45°C). Coating solution was added gradually with subsequent drying by air supply. Finally, the coating powder (sugar and starch) was added, and the cores were dried at the previous temperature. Then the thickening and smoothing of the nuclei was carried out, the first was carried out with a solution of sucrose with dyes in a ratio of 0.9: 1 p/p, times the weight of the nuclei and the smoothing of the nuclei was carried out using

Arrazola, G., N. Grane, and F. Dicenta. 2021. Quantification of cyanogenic compounds, amygdalin, prunasin, and hydrocyanic acid in almonds (*Prunus dulcis* Miller) for industrial uses. Revista Colombiana de Ciencias Hortícolas 15(3), 13049. Doi: https://doi.org/10.17584/rcch.2021v15i3.13049

USP syrup with powdered sugar dissolved in a ratio of 1.9: 1 p/w, times the weight of the cores. Both stages were carried out under the same procedure described above (pre-coating), except for the application of coating powders and a higher grading drum inclination (Arrázola *et al.,* 2015).

Statistical analysis

An analysis of variance (ANOVA) and Tukey's HSD test were performed, with a significance level of 5%, to the results obtained in the quantification of amygdalin, prunasin and hydrocyanic acid, to determine the influence of the levels of cyanogenic compounds, converted to hydrocyanic acid. In addition, a correlation was made from the R-Pearson test between each of the thermophysical parameters. The correlation was considered highly significant at the 0.01 level (two-sided). The data were processed using the statistical program Minitab Inc. ® version 16.0.

RESULTS AND DISCUSSION

Industrial uses and components

An alternative for handling and conservation for almonds is their confit with very thin films whose coating affects about 12 months guaranteeing their chemical and nutritional composition. In obtaining flour, the particle diameter of almonds presented sizes between 500 and 250 µm, with 70% sieve passing through the 250 µm. The fat and moisture content are factors to consider in the treatment of almonds potentially destined for industrial processing, because they are parameters that affect the conservation of the raw material and play a role in the technological use of the almond, although it was not objective in this investigation. However, likewise, the high percentage of crude fat obtained, with percentages of 51%, offers the opportunity to extract the oil, to take advantage of it in the preparation of pharmaceutical and cosmetic products. These results are similar to industrially obtained products, used as raw materials (Arrázola *et al.,* 2013; Delgado-Tobon *et al.,* 2018). In table 3, bromatological results can be observed:

Arrazola, G., N. Grane, and F. Dicenta. 2021. Quantification of cyanogenic compounds, amygdalin, prunasin, and hydrocyanic acid in almonds (*Prunus dulcis* Miller) for industrial uses. Revista Colombiana de Ciencias Hortícolas 15(3), 13049. Doi: https://doi.org/10.17584/rcch.2021v15i3.13049

Table 3. Compositional analysis of almonds (mixtures) (*Prunus dulcis* Miller).

Composition	Percentage (%)
Moisture	17.18±02
Fat	51.00±01
Ash	5.00±03
Protein Fiber Carbohydrate	15.60±05 9.50±01 1.72±0.02

Source: Arrázola *et al.* (2013)

For the candied almonds in their elaboration, the almonds were toasted and coated with syrups and food additives (color). Candied almonds coated in various colors were obtained, where the thermophysical characteristics of the almond were determined as one of the objectives of the work, given the need-to-know thermophysical values.

The diffusivity, thermal conductivity, specific heat and density of fresh almonds was $1.13 \cdot 10^{-7}$ m^2 s^{-1}, 0.32 W $m^{-1}C^{-1}$, 2.65 kJ kg^{-1} °C^{-1} and 1138.6 kg m^{-3}, respectively.

The results of the thermophysical properties of the almond are observed in table 4. Consequently, the results of the thermophysical properties show that the almond has characteristics for the easy removal of water in roasting, drying, and heating processes in unit operations, that are used to obtain flour or candied products, where the most used mathematical model to know the thermophysical properties is the one developed by Choi and Okos (1986) based on the temperature, in a range from -40 to 150ºC, and the composition that has the food in moisture, protein, fat, fiber, carbohydrates and ash. Was compared with the Deproter software, to determine thermophysical properties.

Table 4. Thermophysical properties of almonds for industrial uses.

Property	Thermal conductivity	Specific heat	Diffusivity	Density
Units	K (W m^{-1} C^{-1})	C_p (KJ kg^{-1} °C^{-1})	α (m^2s^{-1})	ρ (kg m^{-3})
Dried almond	0.32±01	2.65±03	$1.13 \cdot 10^{-7}$±002	1138.6±004

Compared to other products that are used in heat processing operations, such as coffee, with specific heat between 1.442 kJ kg^{-1} K^{-1} to 3.298 kJ kg^{-1} K^{-1}, conductivity between 0.117 Wm^{-1} K^{-1} to 0.204 W m^{-1} K^{-1} and average diffusivity of $1.671 \cdot 10^{-7}$ m^2 s^{-1} (Casanova *et al*., 2013);

Arrazola, G., N. Grane, and F. Dicenta. 2021. Quantification of cyanogenic compounds, amygdalin, prunasin, and hydrocyanic acid in almonds (*Prunus dulcis* Miller) for industrial uses. Revista Colombiana de Ciencias Hortícolas 15(3), 13049. Doi: https://doi.org/10.17584/rcch.2021v15i3.13049

the almond has adequate characteristics for heating and its use in transformation operations. In this order of ideas, compared to some varieties of cocoa such as *Theobroma grandiflorum* that has 0.51$Wm^{-1}C^{-1}$, specific heat of 2.86 KJ $kg^{-1}K^{-1}$, diffusivity of $9.94 \cdot 10^{-10}$ to $6.29 \cdot 10^{-10}$ m^2 s^{-1} (Cunha *et al*., 2021). The almond is a product that allows a good distribution of heat in its structure, now taking into account the number of Luikov (1966) referenced by Ferreira and Costa (2009), from the thermophysical properties of the almond it can be affirmed that the internal transfer of matter dominates the simultaneous transfer of heat and matter. During the confit process, temperatures of 80-90°C are used to dry the syrup and color used, to form the protective shell of the almond. The results obtained during this work in relation to the thermophical properties, will help to control the indicated temperatures, in order not to alter the nutritional composition of the final product. For candied almonds, statistical analysis shows that there are statistically significant differences between treatments (P<0.05) with respect to the appearance and taste of candied almonds.

Concentration levels of cyanogen and hydrocyanic acid found in the different samples

In the present work, a reliable and simple method of determination was applied, which also allowed to separately quantify the cyanogenic compounds that were present in the almond seed, using high-performance liquid chromatography (HPLC), since it allows the quantification of the glycosides separately, and microdiffusion was chosen as the reference method. Regarding the need to add or not β-glucosidase, there is controversy, while some authors (Armstrong *et al*., 1908) defend that the almond contains enough enzyme Emulsin (EC 3.2.1.21) to achieve hydrolysis without the need for the addition of external enzyme, others suggest adding β-glucosidase in their microdiffusion assays. In table 5, the mean concentrations of the cyanogenic compounds present in the analyzed almonds are listed, by HPLC. Figure 1 shows a chromatogram of a standard mixture of amygdalin and prunasin obtained using a Symmetry C18 column, depending on the concentration levels of these glycosides, sweet, slightly bitter and bitter almonds were determined, with a direct correlation between concentration and degree of cyanide present. Considering the results using HPLC, to determine the cyanogenic compound amygdalin, it is necessary that among the sweets the 'Atocha', presents a concentration of 7.65

Arrazola, G., N. Grane, and F. Dicenta. 2021. Quantification of cyanogenic compounds, amygdalin, prunasin, and hydrocyanic acid in almonds (*Prunus dulcis* Miller) for industrial uses. Revista Colombiana de Ciencias Hortícolas 15(3), 13049. Doi: https://doi.org/10.17584/rcch.2021v15i3.13049

mg/100 g, while for the case of 'Ferraduel' (sweet) it presents a concentration of 23.37 mg/100 g, almost the same amount of a slightly bitter as the 'Garrigues' with 23.37 mg/100 g.

Table 5. Mean values (mg/100 g sample) of amygdalin content obtained for each variety studied in each harvest year.

Sample	Variety	Taste	Year 1		Year 2		Total	
			Mean	SD	Mean	SD	Mean	SD
1	'Desmayo L.'	Sweet	8.11 d	0.09	6.86 d	0.10	7.49 d	0.88
2	'Del Cid'	Sweet	-		2.15 b	0.04	2.15 b	0.04
3	'Atocha'	Sweet	7.28 d	0.04	8.02 d	0.13	7.65 d	0.06
4	'Ferragnès'	Sweet	5.16 c	0.10	5.5 c	0.16	5.33 c	0.04
5	'Peraleja'	Sweet	1.76 a	0.20	2.56 a	0.37	2.16 a	0.12
6	'Primorskii'	Sweet	nd	-	nd	-	nd	-
7	'Marcona'	Sweet	1.87 a	0.10	1.87 a	0.27	1.87 a	0.12
8	'Ramillete'	Sweet	nd	-	nd	-	nd	-
9	'Ferraduel'	Sweet	21.96 h	0.53	23.36 h	0.53	22.66 h	0.00
10	'Achaak'	Sweet	10.22 e	0.03	11.25 e	0.37	10.74 e	0.24
11	'Planeta'	Sweet	3.71 c	0.16	4.65 c	0.09	4.18 c	0.05
12	'Bonita'	Sweet	nd	-	nd	-	nd	-
13	'Colorada'	Sweet	2.41 b	0.22	2.72 b	0.07	2.56 b	0.10
14	'Carretas'	Sweet	2.49 b	0.32	2.65 b	0.19	2.57 b	0.09
15	'La Mona'	Sweet	4.63 c	0.31	6.10 c	0.10	5.37 c	0.15
16	'Tioga'	Sweet	0.34 a	0.04	0.52 a	0.02	0.43 a	0.01
17	'Titan'	Sweet	0.43 a	0.04	0.62 a	0.02	0.53 a	0.01
18	'CEBAS'	Sweet	nd	-	nd	-.	nd	-
19	'Pajarera'	Sweet	-	-	27.26 f	1.14	27.26 f	1.14
20	'Rumbeta'	Sweet	-	-	5.39 c	0.10	5.39 c	0.10
21	'Garrigues'	Slightly bitter	23.81 f	0.54	22.93 f	0.44	23.37 f	0.07
22	'Genco'	Slightly bitter	18.74 f	0.09	17.34 f	0.10	18.04 f	0.01
23	'Tuono'	Slightly bitter	25.05 f	0.44	25.81 f	0.27	25.43 f	0.12
24	'S3060'	Bitter	4915 i	35.2	5036 i	57.73	4976 i	5.93
25	'S3062'	Bitter	3870 h	86.24	3784 h	66.88	3827 h	3.69
26	'S3076'	Bitter	5894 g	84.48	6028 g	19.01	5961 g	6.29
27	'S3108'	Bitter	3799 h	21.72	3810 h	24.64	3805 h	2.07
28	'S3112'	Bitter	5206 g	55.49	5011 g	62.94	5109 g	5.27
29	'S3126'	Bitter	2439 f	46.66	2360 f	131.09	2400 f	9.70

Means with different letters indicate significant difference according to the HSD Tukey test ($P \leq 0.05$). SD: Standard deviation.

For the concentrations found in the studied almonds, this does not mean that they are toxic, since the conversion of cyanogen to hydrocyanic acid is close to 12-14% (Arrázola *et al.,* 2013).

Arrazola, G., N. Grane, and F. Dicenta. 2021. Quantification of cyanogenic compounds, amygdalin, prunasin, and hydrocyanic acid in almonds (*Prunus dulcis* Miller) for industrial uses. Revista Colombiana de Ciencias Hortícolas 15(3), 13049. Doi: https://doi.org/10.17584/rcch.2021v15i3.13049

Many communications describe suicide attempts by ingestion of cyanide compounds, but generally do not state the doses. The average lethal dose per ingestion in humans is estimated to be 200 mg of CNK or CNNa (Egekeze and Oehme, 1980; Ansell and Lewis, 1970). The sweet almond contains small amounts (~ 0.2 to 16 mg/100 g of almond) of amygdalin, while the bitter almond has a high level of this glucoside (2400 to 5970 mg/100 g) precursor of hydrocyanic acid (Arrázola *et al.,* 2015). Authors like Briggs and Yuen (1978), describe how the effect of soaking on cyanogenic glycosides, for example, in apricot seeds decreases their total cyanide content by 13-52% after 24 h, 73-75% after 48 h and 90% after 72 h (Tuncel *et al.,* 1995), describes that endogenous β-glucosidase activity induces a significant degradation of amygdalin in apricot seeds ground and soaked at 20°C. That is, there are physical means by means of temperature and heat to control the final concentration of hydrocyanic acid in a product to be consumed either for people or animals. Table 6 presents the results from the analysis by HPLC and microdiffusion, these results equivalent to total cyanide contributed by the cyanogenic compounds analyzed, were obtained from samples taken on the Julian calendar from day 83 to on day 240 where it was presented and was the maximum concentration obtained as cyanide with an average of 375.40 mg/100 g cyanide. The importance of the processing temperatures must be considered in each use of the different components of sweet, slightly sweet, and bitter almonds for the control of hydrocyanic acid, given its volatility.

Table 6. Mean values of amygdalin and prunasin content, total cyanide obtained by HPLC (amygdalin and prunasin equivalent) and total cyanide by microdiffusion, all expressed in mg of cyanide/100 g sample.

		HPLC				Microdiffusion	
		Cianide (amygdalin)	Cyanide (prunasin)	Total cyanide (amygdalin+prunasin)		Total cyanide	
Sample	Julian day	Mean	Mean	Mean	SD	Mean	SD
'S3067'	83	2.10	17.30	19.40	0.14	8.20	1.13
	118	15.20	32.45	47.65	1.70	43.48	2.05
	146	97.98	44.25	142.20	0.80	138.30	1.90
	180	322.65	38.50	361.20	2.70	352.50	2.03
	210	374.25	nd	374.30	2.30	372.00	4.90
	240	381.80	nd	381.80	4.00	375.40	5.30
'S3056'	83	0.77	7.43	8.20	0.14	10.40	1.98
	118	5.20	14.70	19.90	3.88	22.20	4.20
	146	43.90	25.95	69.85	0.45	74.20	1.79
	180	151.20	20.05	171.25	8.20	170.55	1.00
	210	186.40	nd	186.40	5.52	186.50	2.80

Arrazola, G., N. Grane, and F. Dicenta. 2021. Quantification of cyanogenic compounds, amygdalin, prunasin, and hydrocyanic acid in almonds (*Prunus dulcis* Miller) for industrial uses. Revista Colombiana de Ciencias Hortícolas 15(3), 13049. Doi: https://doi.org/10.17584/rcch.2021v15i3.13049

	240	190.20	nd	190.20	2.44	189.38	8.30
'Genco'	83	nd	0.05	0.05	nd	0.05	0.01
	118	0.04	0.08	0.11	0.02	0.14	0.04
	146	0.11	0.12	0.23	0.06	0.28	0.10
	180	0.75	0.10	0.85	0.02	0.81	0.07
	210	0.89	nd	0.89	0.10	0.91	0.09
	240	0.97	nd	0.97	0.06	0.95	0.03
'Marcona'	83	0.01	nd	0.01	0.01	nd	-
	118	0.01	nd	0.01	<0.01	0.01	<0.01
	146	0.03	nd	0.03	<0.00	0.03	<0.01
	180	0.10	nd	0.10	<0.01	0.10	<0.01
	210	0.11	nd	0.11	<0.01	0.11	<0.01
	240	0.11	nd	0.11	<0.01	0.10	<0.01

Source: Arrázola *et al.* (2013).

In other fruits of the same family of almonds, the level of cyanide, for example, raw or improperly processed apricots can cause serious acute problems that can lead to death (Haque and Bradbury, 2002). Other studies have shown that apricot kernels contain a cyanide (CN) content of 1450 mg kg^{-1}, approximately 0.5 mg g^{-1} (Mandenius *et al.,* 1983). This value is similar to the toxic dose of cyanide (0.5 mg kg^{-1} body weight) indicated by WHO (2004).

Table 7. Mean values of cyanide content (mg/100 g of dry sample) and standard deviations. Pearson's correlation coefficients and their significance.

	Sweet samples and slightly bitter		**Bitter samples**	
	HPLC	**Microdiffusion colorimetry**	**HPLC**	**Microdiffusion gravimetric titration**
Average	4.72	4.53	265.78	262.95
Standard deviation	6.54	6.27	74.85	71.5
Correlation coefficient	0.980		0.993	
Correlation significance	< 0.001		< 0.001	

Table 7 shows the mean cyanide values for the samples with high and low cyanide contents, they do not differ practically between the two methods used, the standard deviation values

Arrazola, G., N. Grane, and F. Dicenta. 2021. Quantification of cyanogenic compounds, amygdalin, prunasin, and hydrocyanic acid in almonds (*Prunus dulcis* Miller) for industrial uses. Revista Colombiana de Ciencias Hortícolas 15(3), 13049. Doi: https://doi.org/10.17584/rcch.2021v15i3.13049

obtained are also very similar. On the other hand, it is worth highlighting the fact that the correlation obtained between the data corresponding to the two methods is high and significant for both sets of samples, where it is important for the industry that uses almonds as raw material for its various derivatives, that when making food from cyanogenic plants and stored at room temperature (35±2°C), the cyanide content of the food volatilizes due to its low evaporation temperature (26°C) described by Onabolu *et al*. (2002), for a 50-64% decrease in the cyanide content of a cassava product (Gari) stored for 4 weeks at room temperature. The values obtained through the analyzes for bitter almonds (265.78-262.95 mg/100 g dry sample) show a great difference between the concentrations with sweet and slightly bitter ones, but they can still be used in industry as raw material or as a final product if they are subjected to treatment by value to guarantee their volatilization as cyanhydric acid.

CONCLUSIONS

The results obtained show that the varieties of almonds studied, especially the bitter fruits, do not offer danger of intoxication due to their direct consumption. Where the maximum concentration found (375.40 mg/100 g) in flour obtained used in the different products studied does not reach harmful levels to produce immediate intoxication. It is important to consider that constant consumption could be cumulative and then it would be necessary to evaluate the degree of affectation at the physiological level. On the other hand, during processing, especially when the compounds are subjected to temperatures higher than 26°C, hydrocyanic acid volatilizes. Regarding the thermo-physical characteristics, it was determined that the internal transfer of matter dominates the simultaneous transfer of heat and matter, allowing the almond an adequate conduction of heat in transformation operations. Industrially almonds today are a trend in the consumption of a healthy diet, including candied almonds.

Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

Arrazola, G., N. Grane, and F. Dicenta. 2021. Quantification of cyanogenic compounds, amygdalin, prunasin, and hydrocyanic acid in almonds (*Prunus dulcis* Miller) for industrial uses. Revista Colombiana de Ciencias Hortícolas 15(3), 13049. Doi: https://doi.org/10.17584/rcch.2021v15i3.13049

BIBLIOGRAPHIC REFERENCES

Abd Aal, M.H.E., E.G. Gomoa, and H.A. Karara. 1987. Bitter almond, plum and mango kernels as sources of lipids. Fett/Lipid 89(8), 304-306. Doi: 10.1002/lipi.19870890803

Abraham, K., T. Buhrke, and A. Lampen. 2016. Bioavailability of cyanide after consumption of a single meal of foods containing high levels of cyanogenic glycosides: A crossover study in humans. Arch. Toxicol. 90, 559-574. Doi: 10.1007/s00204-015-1479-8

Alasalvar, C. and B.W. Bolling. 2015. Review of nut phytochemicals, fat-soluble bioactives, antioxidant components and health effects. Br. J. Nutr. 113(Suppl 2), 68-78. Doi: 10.1017/S0007114514003729

Andréo Filho, N., L. Pessole, M. Yang, M. Issa, and H. Ferraz. 2007. Aplicación de recubrimiento gastro-resistente en núcleos comprimidos conteniendo didanosina utilizando diferentes equipos: Bombo grageador convencional, tambor perforado y lecho fluido. Lat. Am. J. Pharm. 26(5), 669-676.

Ansell, M. and F.A. Lewis. 1970. A review of cyanide concentrations found in human organs. A survey of literature concerning cyanide metabolism, 'normal', non-fatal, and fatal body cyanide levels. J. Forensic. Med. 17(4), 148-155.

AOAC, Association of Official Agricultural Chemists. 1990. Método 930.15 – determinación extracto etéreo; método 920.39 – determinación cenizas; método 942.04 - determinación fibra cruda; método 962.09 – determinación proteína cruda; método 955.04 – determinación de grasa. Washington, DC.

Armstrong, H.E., E.F. Armstrong, and E. Horton. 1908. Studies on enzyme action. XII.-The enzymes of emulsin. Proc. Royal Soc. B: Biol. Sci. 80, (540), 321-331. Doi: 10.1098/rspb.1908.0031

Arrázola, G., F. Dicenta, and N. Grané. 2015. Evolution of the amygdalin and prunasin content during the development of almond (*Prunus dulcis* Miller). Rev. Fac. Agron. (LUZ) 32, 63-81.

Arrázola, G., N. Grane, M.L. Martin, and F. Dicenta. 2013. Determination of cyanogenic compound amygdalin and prunasin in almond kernels (*Prunus dulcis* L.) by using liquid chromatography. Rev. Colomb. Quim. 42(1), 13-21.

Bainbridge, M. 1996. In methods for assessing quality characteristics of non-grain starch staples. Part 2: Field methods. Natural resources Institute, Chatham, UK. pp. 27-29.

Ballhorn, D.J. 2011. Cyanogenic glycosides in nuts and seeds. pp. 129-136. In: Preedy, V.R., R.R. Watson, and V.B. Patel (eds.). Nuts and seeds in health and disease prevention. Academic Press, London. Doi: 10.1016/B978-0-12-375688-6.10014-3

Bark, L.S. and H.G. Higson. 1963. A review of the methods available for the detection and determination of small amount of cyanide. Analyst 1051, 751-760. Doi: 10.1039/an9638800751

Bernat, N., M. Cháfer, A. Chiralt, and C. González-Martínez. 2015. Development of a non-dairy probiotic fermented product based on almond *milk* and inulin. Food Sci. Technol. Int. 21(6), 440-453. Doi: 10.1177/1082013214543705

Blum, D. 2010. The Poisoner's handbook: Murder and the birth of forensic medicine in Jazz Age New York. Penguin Press, New york, NY.

Borron, S.W. and F.J. Baud. 2012. Antidotes for acute cyanide poisoning. Curr. Pharm. Biotechnol. 13(10), 1940-1948. Doi: 10.2174/138920112802273182

Briggs, D.R. and D. Yuen. 1978. Determination of cyanide in apricot kernels. Proc. Nutr. Soc. Austral. 3(1), 103-104.

Casanova, P., P.C. Corrêa, K. Solís, and J.C.C. Campos. 2013. Thermal properties of Conilon coffee fruits. IOSR J. Eng. 3(11), 29-35. Doi: 10.9790/3021-031152935

Chaouali, N., I. Gana, A. Dorra, F. Khelifi, A. Nouioui, W. Masri, I. Belwaer H. Ghorbel, and A. Hedhili. 2013. Potential toxic levels of cyanide in almonds (*Prunus amygdalus*), apricot kernels (*Prunus armeniaca*), and almond syrup. Int. Sch. Res. Notices 2013, 610648. Doi: 10.1155/2013/610648

Choi, Y. and M.R. Okos. 1986. Effects of temperature and composition on the thermal properties of foods. pp. 93-101. In: Maguer, L.M. and P. Jelen (Eds.), Food engineering and process applications. Vol. 1: Transport phenomena. Elsevier, New York, NY.

Conn, E.E. 1980. Cyanogenic compound. Ann. Rev. Plant Physiol. 31, 433-451. Doi: 10.1146/annurev.pp.31.060180.002245

Cunha, E.S., G.P.A. Lima, J.H.O. Sales, and E.A. Oliveira. 2021. Determinação de propriedades termofísicas de amêndoas secas de cupuaçu (*Theobroma grandiflorum*). J. Eng. Exact Sci. 7(2), 11955-01. Doi: 10.18540/jcecvl7iss2pp11955-01-12e

Davis, R.H. 1991. Cyanogens. pp. 202-225. In: Felix D'Mello, J.P., C.M. Duffus, and J.H. Duffus (eds.). Toxic substances in crop plants. Woodhead Publishing, Cambridge, UK. Doi: 10.1533/9781845698454.202

Delgado-Tobón, A.E., W.A. Aperador-Chaparro, and R.G. García-Cáceres. 2018. Evaluation of the lubricating power of sweet almond oil without additives. DYNA 85(205), 179-183. Doi: 10.15446/dyna.v85n205.68033

Dicenta, F. and J.E. García. 1993. Inheritance of the kernel flavour in almond. Heredity 70, 308-312. Doi: 10.1038/hdy.1993.44

Dicenta, F, P. Martínez-Gómez, N. Grané, M.L. Martín, A. León, J.A. Cánovas, and V. Berenguer. 2002. Relationship between cyanogenic compounds in kernels, leaves, and roots of sweet and bitter kernelled almonds. J. Agric. Food Chem. 50(7), 2149-2152. Doi: 10.1021/jf0113070

Du, L., M. Bokanga, B.L. Möller, and B.A. Halkier. 1995. The biosynthesis of cyanogenic glucosides in roots cassava. Phytochemistry 39(2), 323-326. Doi: 10.1016/0031-9422(94)00878-W

EFSA, European Food Safety Authority. 2007. Opinion of the scientific panel on contaminants in the food chain [CONTAM] related to cyanogenic compounds as undesirable substances in animal feed. EFSA J. 434. Doi: 10.2903/j.efsa.2007.434

Egan, S.V., H.H. Yeoh, and J.H. Bradbury. 1998. Simple picrate paper kit for determination of the cyanogenic potential of cassava flour. J. Sci. Food Agric. 76(1), 39-48. Doi: 10.1002/(SICI)1097-0010(199801)76:1<39::AID-JSFA947>3.0.CO;2-M

Egekeze, J.O. and F.W. Oehme. 1980. Cyanides and their toxicity: A literature review. Vet. Q. 2(2), 104-114. Doi: 10.1080/01652176.1980.9693766

EPA. 1990. Summary review of health effects associated with hydrogen cyanide. Health issue assessment environmental criteria and assessment office. EPA/600/8-90/002F. Office of Health and Environmental Assessment, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC.

Evans-Johnson, J.A., J.A. Garlick, E.J. Johnson, X.D. Wang, and C.Y. Oliver Chen. 2013. A pilot study of the photoprotective effect of almond phytochemicals in a 3D human skin equivalent. J. Photochem. Photobiol. 126, 17-25. Doi: 10.1016/j.jphotobiol.2013.07.006

FAO, Food and Agricultural Organization; WHO, World Health Organization. 2012. Safety evaluation of certain food additives and contaminants: prepared by the seventy-fourth meeting of the joint FAO/WHO expert committee on food additives. WHO Food Additives Series 65, Genova.

Fernández-Cuesta, A., M.R. Aguirre-González, M.V. Ruiz-Méndez, and L. Velasco. 2012. Validation of a method for the analysis of phytosterols in sunflower seeds. Eur. J. Lipid Sci. Technol. 114(3), 325-331. Doi: 10.1002/ejlt.201100138

Ferreira, S.R. and A.R.S, Costa. 2009. Parámetros de transferencia de materia en el secado de frutas. Información Tecnológica 20(2), 89-104.

Forcada, C., L. Velasco, R. Socias i Company, and A. Fernández i Marti. 2015. Association mapping for kernel phytosterol content in almond. Front. Plant Sci. 6, 530. Doi: 10.3389/fpls.2015.00530

Frehner, M., M. Scalet, and E.E. Conn. 1990. Pattern of the cyanide-potential in developing fruits: Implications for Plants Accumulating Cyanogenic Monoglucosides (*Phaseolus lunatus*) or Cyanogenic Diglucosides in Their Seeds (*Linum usitatissimum, Prunus amygdalus*). Plant. Physiol. 94(1), 28-34. Doi: 10.1104/pp.94.1.28

García-Pascual, P., P. Mateos, M. Carbonell, and D.M. Salazar. 2003. Influence of storage conditions on the quality of shelled and roasted almonds. Biosyst. Eng. 84, 201-209. Doi: 10.1016/S1537-5110(02)00262-3

Garrido, I., M. Monagas, C. Gómez-Cordoves and B. Bartolomé. 2008. Polyphenols and antioxidant properties of almond skins: influence of industrial processing. J. Food Sci. 73(2), 106-115. Doi: 10.1111/j.1750-3841.2007.00637.x

Gleadow, R.M. and B.L. Møller. 2014. Cyanogenic glycosides: synthesis, physiology, and phenotypic plasticity. Annu. Rev. Plant Biol. 65, 155-185. Doi: 10.1146/annurev-arplant-050213-040027

Grasselly, C. and P. Crossa-Raynaud. 1980. L´amandier, techniques agricoles et productions méditerraneennes. G. P. Maisonneuve & Larose, Paris.

Halkier, B.A. and B.L. Møller. 1989. Biosynthesis of the cyanogenic glucoside dhurrin in seedlings of *Sorghum bicolor* (L.) Moench and partial purification of the enzyme system involved. Plant Physiol. 90(4), 1552-1559. Doi: 10.1104/pp.90.4.1552

Haque, M.R. and J.H. Bradbury. 2002. Total cyanide determination of plants and foods using the picrate and acid hydrolysis methods. Food Chem. 77(1), 107-114. Doi: 10.1016/S0308-8146(01)00313-2

Hayes, D., M. Angove, J. Tucci, and C. Dennis. 2016. Walnuts (*Juglans regia*) Chemical Composition and Research in Human Health. Crit. Rev. Food Sci. Nutr. 56(8), 1231-1241. Doi: 10.1080/10408398.2012.760516

Heppner, M.J. 1923. The factor for bitterness in the sweet almond. Genetics 8, 390-392. Doi: 10.1093/genetics/8.4.390

Heppner, M.J. 1926. Further evidence on the factor for bitterness in the sweet almond. Genetics 11, 605-606. Doi: 10.1093/genetics/11.6.605

Ibar, L. 1985. Cultivo moderno del almendro. Editores Aedos, Barcelona, Spain.

Jensen, N., M. Zagrobelny, K. Hjernø, C.E. Olsen, J. Houghton-Larsen, J. Borch, B.L. Møller, and S. Bak. 2011. Convergent evolution in biosynthesis of cyanogenic defence compounds in plants and insects. Nat. Commun. 2, 273. Doi: 10.1038/ncomms1271

Kobayashi, K. and K. Hisamatsu. 1978. Coating agent for roasted nut and grins. Patent 77 38, 107 Chem. Abstr. 88, 361-362.

Lucas, B. and A. Sotelo. 1984. Simplified test for the quantitation of cyanogenic glucosides in wild and cultivated seeds. Nutr. Rep. Int. 29, 711-719.

Mandalari G., A. Tomaino, T. Arcorasi, and M. Martorana. 2010. Characterization of polyphenols, lipids and dietary fibre from almond skins (*Amygdalus communis* L.). J. Food Compos. Anal. 23(2), 166-174

Mandenius, C., L. Büelow, and B. Danielsson. 1983. Determination of amygdalin and cyanide in industrial food samples using enzymic methods. Acta Chem. Scand. 37(8), 739-742. Doi: 10.3891/acta.chem.scand.37b-0739

Martins, I.M., Q. Chen, and C.Y. Oliver Chen. 2016. Emerging functional foods derived from almonds. In: Ferreira, I.C.F.R., P. Morales and L. Barros (eds.). Wild plants, mushrooms and nuts: Functional Food Properties and Applications. John Wiley & Sons, Chichester, UK.

McCarty, C.D., J.W. Leslie, and H.B. Frost. 1952. Bitterness of kernels of almond x peach hybrids and their parents. Proc. Am. Soc. Hort. Sci. 59, 254-258.

Møller, B.L. and D.S. Seigler. 1991. Biosynthesis of cyanogenic glycosides, cyanolipids and related compounds. 563-609. In: Singh, B.K. (ed.). Plant amino acids: Biochemistry and biotechnology. Marcel Dekker, New York, NY.

Monagas, M., I. Garrido, R. Lebron-Aguilar, B. Bartolomé, and C. Gómez-Cordovés. 2007. Almond (*Prunus dulcis* (Mill.) D.A. Webb) skins as a potential source of bioactive polyphenols. J. Agric. Food Chem. 55(21), 8498-8507. Doi: 10.1021/jf071780z

ICONTEC, Instituto Colombiano de Normas Técnicas y Certificación. 2002. Norma Tecnica Colombiana, NTC 336, grasas y aceites animales y vegetales. Método de la determinación de la densidad (masa por volumen convencional). Bogota.

Oliveira, I., A.S. Meyer, S. Afonso, A. Sequeira, A. Vilela, P. Goufo, H. Trindade, and B. Gonçalves. 2020. Effects of different processing treatments on almond (*Prunus dulcis*) bioactive compounds, antioxidant activities, fatty acids, and sensorial characteristics. Plants (Basel) 9(11), 1627. Doi: 10.3390/plants9111627

Onabolu, A.O., O.S.A. Oluwole, H. Rosling, and M. Bokanga. 2002. Processing factors affecting the level of residual cyanohydrins in *gari*. J. Sci. Food Agric. 82(9), 966-969. Doi: 10.1002/jsfa.1131

Polesello, A. and A. Rizzolo. 1989. Caratteristiche nutrizionali e utilizzazione industriale delle mandorle. Frutticoltura 51(4), 43-50.

Poulton, J. and C.P. Li. 1994. Tissue level compartmentation of (R)-amygdalin and amygdalin hydrolase prevents large-scale cyanogen in undamaged *Prunus* seeds. Plant Physiol. 104, 29-35. Doi: 10.1104/pp.104.1.29

Rodushkin, I, E. Engström, D. Sörlin, and D. Baxter. 2008. Levels of inorganic constituents in raw nuts and seeds on the Swedish market. Sci. Total Environ. 392(2-3), 290-304. Doi: 10.1016/j.scitotenv.2007.11.024

Sánchez-Pérez, R., K. Jørgensen, C.E. Olsen, F. Dicenta, and B. Møller. 2008. Bitterness in almonds. Plant Physiol. 146(3), 1040-1052. Doi: 10.1104/pp.107.112979

Sánchez-Pérez, R., S. Pavan, R. Mazzeo, C. Moldovan, R.A. Cigliano, J. Del Cueto, F. Ricciardi, C. Lotti, L. Ricciardi, F. Dicenta, R.L. López-Marques, and B.L. Møller. 2019.

Mutation of a bHLH transcription factor allowed almond domestication. Science 364(6445), 1095-1098. Doi: 10.1126/science.aav8197

Sibbesen, O., B. Koch, B.A. Halkier, and B.L. Møller. 1994. Isolation of the heme-thiolate enzyme cytochrome P-450TYR, which catalyzes the committed step in the biosynthesis of the cyanogenic glucoside dhurrin in *Sorghum bicolor* (L.) Moench. Proc. Natl. Acad. Sci. USA 91(21), 9740-9744. Doi: 10.1073/pnas.91.21.9740

Thodberg, S., J. Del Cueto, R. Mazzeo, S. Pavan, C. Lotti, F. Dicenta, E.H.J. Neilson, B.L. Møller, and R. Sánchez-Pérez. 2018. Elucidation of the amygdalin pathway reveals the metabolic basis of bitter and sweet almonds (*Prunus dulcis*). Plant Physiol. 178(3), 1096-1111. Doi: 10.1104/pp.18.00922

Tuncel, G., M.J.R. Nout, and L. Brimer. 1995. The effects of grinding, soaking and cooking on the degradation of amygdalin of bitter apricot seeds. Food Chem. 53(4), 447-451. Doi: 10.1016/0308-8146(95)99841-M

Vargas, F.J., M.A. Romero, and I. Batlle. 2001. Kernel taste inheritance in almond. Options Méditerr. 56, 129-134..

WHO, World Health Organization. 2004. Hydrogen cyanide and cyanides: Human health aspects. Concise International Chemical Assessment Document 61. Geneva.

Zacheo, G., M.S. Cappello, A. Gallo, A. Santino, and A.R. Cappello. 2000. Changes associated with post-harvest ageing in almond seeds. LWT Food Sci. Technol. 33(6), 415-423. Doi: 10.1006/fstl.2000.0679