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minireview

## Bioactive Compounds from Artichoke and Application Potential

Running head: Artichoke: Compounds and Application

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### SUMMARY

*Cynara cardunculus* L. var. *scolymus* known as artichoke, is originally from the Mediterranean and is currently cultivated in several countries. The artichoke has leaves, stem, and head, also called a floral capitula, covered in green and pointed bracts. It is rich in polyphenols, flavonoids, anthocyanins, phenolic compounds, inulin, coumarins, anthocyanins, terpenes, dietary fiber, enzymes, polysaccharides, minerals, and vitamins, thus having a wide spectrum of applications in food industries, medicine, and biofuels, among others. Several studies have shown that artichokes present properties such as antioxidant, anti-inflammatory, antimicrobial, anticancer, hypocholesterolemic, anti-HIV, cardioprotective, hepatoprotective, and lipid-lowering effects. This research aims to present a literature review on phytochemical composition, bioactivities, and applications, with an emphasis on methods of extraction, purification, and concentration of enzymes present in artichoke.

**Keywords:** enzymes; polyphenols; antioxidant; methods of extraction; purification; bioactivities; applications

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## INTRODUCTION

*Cynara* is a small genus that belongs to the family *Asteraceae*, with eight species and four subspecies, including thistle (*Cynara cardunculus* L.), all native to the Mediterranean region (1,2). Thistle has three botanical varieties: the artichoke (*C. cardunculus* L. var. *scolymus*), the cultivated or leafy thistle (*C. cardunculus* L. var. *altilis*) and the wild thistle (*C. cardunculus* L. var. *sylvestris*) (3–5). According to the report of the United Nations Food and Agriculture Organization (6), the main producing countries are Italy, Egypt, and Spain which produce around 367080, 308844, and 196970 tons per year, respectively (7–9).

*C. cardunculus* L. and their varieties represent a rich source of a wide range of bioactive compounds, in addition, the chemical composition is quite diverse in each class (10,11). Artichoke can be considered as a functional or nutraceutical food because it contains bioactive phyto constituents such as polyphenols (phenolic acids, chlorogenic, caffeic, dicaffeoylquinic, and ferulic acids), flavones (apigenin and luteolin) and their glycosides (apigenin-7-O-glucoside and cinnaroside), anthocyanins (cyanidin 3,5-diglucoside, cyanidin 3-glucoside, cyanidin 3,5-malonyldiglucoside, cyanidin 3-(3"-malonyl) glucoside and cyanidin 3-(6"-malonyl) glucoside), terpenoids (mono-, sesqui, and triterpenes), saturated (palmitic and stearic) and unsaturated (linoleic and oleic acids) fatty acids, carbohydrate polymers (inulin and pectin) (1,12,13), aspartic proteases (EC 3.4.23) (cardosins/cyprosins or cynarase (cardosin A and B, cynarase A, B and C), polyphenol oxidase (EC 1.14.18.1) and peroxidase (EC 1.11.1.7) (3,14–19), minerals and vitamins (vitamin C, folates, biotin, niacin and pyridoxine) (20,21), among others, with potential health benefits (22,23). Thus having a broad spectrum of applications from all parts of the plant (12,24–27). Biological activities mainly include lipid-lowering, antioxidant, anti-inflammatory (28–30), hepatoprotection, cardioprotection, anticancer, antimicrobial, and anti-HIV, among others (3,4).

The present work presents a literature review of the artichoke (*C. cardunculus* L.), its phytochemical composition, bioactivity and industrial applications, making a compilation of extraction methods, purification and concentration of enzymes present in artichoke.

## ARTICHOKE

The artichoke is native to the Mediterranean Basin and adapts to different types of soil and climatic conditions (5). Possibly its origin and domestication were in Sicily (31), in the XV century, it spread to Campania and Tuscany, in Italy. At the beginning of the XVI century, it was cultivated in all Mediterranean countries, Central Europe, and United States (4). At the beginning of the XX century, the artichoke was introduced in Brazil by European immigrants, especially Italians (32).

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The artichoke has a whitish stem, large, lanceolate, fleshy, light green, pubescent leaves, and a scalloped appearance (33), the leaves can reach 50 to 200 cm in length (3) and the plant can reach up to 150 to 180 cm in height (34). The flowers are violet-blue and large, with the appearance of small pinecones, measuring 7 to 10 cm in diameter, being the inflorescence of the inner bracts (17), formed by stylets and stigmas (4,35), the bottom or heart being the edible part of the plant (3,17).

The amount of flowers produced by each plant varies, producing one primary head and 4 to 20 secondary and tertiary flower buds per year (3). The heads or globes produced by the artichoke have different sizes, the primary head is the largest and forms at the apex of the central stem, while the secondary and tertiary heads are smaller and develop on the branches (20).

Harvest is manual, cutting the stem to 20 to 30 cm in length, and the ideal harvest point is when the buds have fleshy and adherent bracts (36). After the end of production, the aerial part of the dry plant is cut at ground level, and in autumn the plant emits new shoots, restarting the cycle (37), the average production time being 6 years (38).

Artichoke heads are eaten as fresh, canned, or frozen vegetables (10,39). A large amount of by-products (80–85 % of the total biomass) is generated by cultivation and industrial processing, which discards leaves, external bracts, stalk, seeds, and roots (5,17,40), and the stem can be separated from this total and used for consumption, if properly prepared (20).

By-products can also be used in the production of food additives and nutraceuticals (17,40), bioactive compounds such as polyphenols and inulin (10), in green chemistry to produce cellulose, biofuels (3) and as fodder (especially stalks and leaves). In addition to containing enzymes (proteases) used in the coagulation of milk (20). Still, the root has a high concentration of sugars, approximately 25 %, where inulin represents 89.4 % (3), and the outer bracts have higher levels of inulin than the stalk. The inulin content varies depending on the composition of the by-product, the artichoke subspecies, its geographical origin, and harvest time (41).

### *Bioactive compounds*

Artichoke heads present around 66.3 % of total carbohydrates, 19.6 % of protein, 2.0 % of crude fat, 8.6 % of ash (20,42) and 3.5 % of fibers, in dry mass (43), 14–19 mg/100 g<sub>fw</sub>- fresh weight Of vitamin C (44), folates, and B-complex vitamins (biotin, niacin, and pyridoxine) (20,21). Stalk, bracts, and roots contain inulin, which is a soluble fiber (5,41,45). In addition, artichoke is also a source of minerals such as potassium, calcium, sodium, magnesium, phosphorus, iron, copper, and manganese (43,46).

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Artichoke parts (stem, leaf, bracts, and flowers) present a range of primary and secondary metabolites, such as dietary fibers, polyphenols, flavonoids, and terpenoids, among others (11). Due to the presence of these compounds, it has beneficial properties for health (3,5,47).

The plant also has sesquiterpene lactones in its composition, compounds responsible for 80 % of the bitter taste of artichoke leaves (48) and other plant tissues (49). Cinnaropicrin is the main sesquiterpene lactone found in *C. cardunculus* (50).

Artichoke by-products (stalk, leaves, and bracts) are a source of pectin, which has several applications in the industry as a gelling agent and as a prebiotic for the proper functioning of the intestinal flora (18).

It also has polysaccharides (50 g/100 g<sub>biomass</sub>), mainly cellulose and hemicellulose (51). The seed is rich in polyunsaturated and monounsaturated fatty acids, such as linoleic acid (51.7 %) and oleic acid (34.2 %), suitable for human consumption (52).

Several factors can interfere with the profile and amount of phytochemical compounds, including biotic and abiotic stress conditions, ultraviolet radiation, light intensity, fertilization, water stress, and plant maturity stage (53).

Artichoke stands out in bioactive compounds, such as phenolics and antocyanins, and the highest concentration is found in the head and in its edible parts (5,54), predominantly moncaffeoylquinic, and dicaffeoylquinic acids, and flavonoids derived from apigenin and luteolin (20). The leaves contain mainly phenolic acids, flavonoids, and sesquiterpene lactones (55).

Phenolic compounds are the result of the secondary metabolism of cells, essential for growth and reproduction, formed during stressful conditions such as UV radiation, injuries, and infections, among others (56). They are necessary for plant growth and reproduction and act as antipathogenic agents, in addition to contributing to pigmentation (57). Phenolic compounds present in their chemical structure an aromatic ring with one or more hydroxyl groups, including the functional groups (58). Their structure is variable, thus, they are multifunctional. Approximately five thousand phenols are known, including flavonoids, simple phenols, phenolic acids, tannins, lignins, coumarins, and tocopherols (57). They range from simple molecules to those with a high degree of polymerization. In vegetables, they are found in their free form or associated with proteins and sugars (glycosides) (59). In the *Cynara*, phenolic compounds belong mainly to the classes of caffeoylquinic acids and flavonoids (60).

Artichoke phenolics present the ability to modulate cellular antioxidants and several important enzymatic pathways (3). Because of this, artichoke has the ability to decrease lipid peroxidation, the creation of reactive oxygen species (ROS), protein oxidation, and glutathione peroxidase activity (61,62).

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The most abundant phenolics in artichoke flowers are those derived from caffeoylquinic acid, especially chlorogenic acid (5-*O*-caffeoylquinic acid), cynarin (1,3-*O*-dicafeoylquinic acid), isochlorogenic acid (3,5-*O*-dicafeoylquinic), flavonoids (apigenin and luteolin), and also caffeoylglycoside and cyanidin derivatives (3,4,63). Fig. S1 shows the chemical structures of caffeoylquinic acid derivatives.

Chlorogenic acid and its isomers, cryptochlorogenic, neochlorogenic, and pseudochlorogenic acids, have several pharmacological activities, such as chemopreventive activity in oncological diseases. In particular, chlorogenic acid can inhibit the formation of mutagenic compounds such as nitrosamines and the development of tumors (53), in addition, phenolic compounds have other health benefits, such as: antithrombotic, antiallergic, anti-inflammatory, cardioprotective, antioxidant, antimicrobial, and vasodilator effects (64).

The content of polyphenolic compounds in artichokes is influenced by environmental factors, plant parts, genotype, agricultural management, post-harvest practices (53,65) and extraction methods. The total phenolic compounds of leaves, bracts, receptacles, flowers, and stalks could vary from 1191 to 3496 mg GAE/100 g<sub>dm</sub>, total flavonoids from 40.1 to 75.5 mg QE/100 g<sub>dm</sub> and anthocyanins from 0.84 to 170.5 mg/100 g<sub>dm</sub> (Table 1).

The antioxidant, antimicrobial, and cytotoxic activities of artichoke extracts are due to the presence of quercetin, catechin, chrysin, rosmarinic acid, apigenin, and protocatechuic acid. Antimicrobial activity may occur due to cytoplasmic membrane permeabilization, destabilization, and/or enzymatic inhibition (71–73). The content of rosmarinic acid in ethanolic extracts of bracts is 1418 mg/100 g<sub>dm</sub> (69).

Noriega-Rodríguez *et al.* (67) reported a total of 2461 mg GAE/100 g<sub>dm</sub> of phenolic compounds for artichoke discards, composed of outer bracts and stalk. The edible parts of the artichoke can present contents of phenolic compounds ranging from 480 to 2980 mg GAE/100 g<sub>dm</sub> (74).

Artichoke by-products, consisting of leaf stalks of residual shoots (branches from the plant's root system) removed during thinning, showed 20835–27051 mg/kg<sub>dm</sub> of phenolic compounds, as moncaffeoylquinic acids, dicafeoylquinic acids, and dicafeoyl succinylquinic acids (66).

According to Giménez *et al.* (75) tertiary artichoke heads have the highest content of phenolic compounds, followed by secondary and main heads. The total polyphenol content identified ranges from 5 to 12 mg/g<sub>fw</sub>, in the edible fraction (internal bracts and receptacle).

Rocchetti *et al.* (65) found 365 compounds in fresh artichoke heads, with the highest amount of flavonoids (144 compounds), 103 phenolic acids, followed by tyrosol, lignans, and stilbenes, 62, 28 and 23 compounds, respectively. Artichoke by-products (stalk, leaves, secondary flowers, and external bracts), as well as the edible part, present phenolic compounds in large quantities, including

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derivatives of caffeic acid and cynarin, flavones (luteolin, apigenin, and their glycoside derivatives) that are related to beneficial effects on health (76).

One important class of natural polyphenols present in artichokes are flavonoids, divided into subclasses such as flavones, flavonols, flavanones, and flavanols. The main flavones (Fig. S2) found are apigenin, luteolin, apigenin-7-O- $\beta$ -D-glucopyranoside, apigenin-7-O-rutinoside, luteolin-7-O-glucoside or cinnaroside, and luteolin-7-rutinoside (77). Flavones and their glycosides are responsible for antioxidant (78), anti-inflammatory properties (79), and on the reduction of total cholesterol (LDL), and triglycerides (80). Ten flavonoids belonging to the flavone family (derived from apigenin and luteolin) were identified and quantified in samples of *Tudela artichoke* (81).

The flavonoids in artichokes are mainly concentrated in the leaves and heads, and are absent in the floral stalk (82). Flavonoids have the ability to protect cells from oxidative damage. These compounds can be used as antiatherosclerotic drugs by decreasing the expression of monocyte chemoattractant protein-1 (MCP-1) (83). Luteolin and cinnarosides are responsible for these activities, as well as increasing the expression of the eNOS promoter (endothelial nitric oxide synthase) and the mRNA of eNOS, leading to the production of nitric oxide and, consequently, antithrombotic and antiatherosclerotic activity (84).

The beneficial and health-promoting effects of artichoke, as well as its use for treating cardiovascular diseases, are a consequence of these activities. Hypolipidemic activity due to the presence of luteolin *via* blocking insulin-dependent HMG-CoA or 3-hydroxy-3-methyl-glutaril-CoA reductase activity, thus inhibiting cholesterol biosynthesis (85). Luteolin is a potential component of skin photoprotective preparations against oxidative damage caused by ultraviolet A (UVA) radiation due to its lipophilic nature and the presence of ortho-dihydroxy groups (86). The total luteolin content is significantly higher in tertiary heads than in secondary or main heads. Genetic variability among cultivars influences the content of luteolin derivatives in floral capitulum orders (87).

Flavonoids may be able to inhibit bacterial growth by altering membrane and cell wall permeability, inhibiting the synthesis of nucleic acids in Gram-positive and Gram-negative bacteria, among others (88,89). The function of catechins as antioxidants is to bind ROS, limit free radicals such as superoxide, peroxy, hydroxyl, and DPPH (90,91), and also stimulate the expression of IL-10 (92). Catechins can increase the concentration of reactive oxygen species in cells and cause endogenous oxidative stress in the bacterium *Escherichia coli*, exerting an antibacterial effect by reducing the antioxidant potential of the bacterium (93). Catechins disrupt the cytoplasmic membrane of bacterial cells to prevent microbial growth against food-borne pathogens (90,94).

Ethanol extracts from artichoke receptacles and bracts can present about 40140 and 75540 mg quercetin equivalents (QE)/100 g<sub>dm</sub>, respectively. The predominant compounds in the extract of

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bracts were catechin (1604 mg/100 g<sub>dm</sub>), and apigenin (526 mg/100 g<sub>dm</sub>) and the ethanolic extract of receptacles presented 327 mg/100 g<sub>dm</sub> of chrysin (69). Another compound phenolic present in the artichoke is anthocyanins (Fig. S3), which include cyanidin and its glycosides, peonidin, and delphinidin glycosides (70). Some of these anthocyanins are non-acylated molecules with strong antioxidant activity (95–97). Acylation, particularly with cinnamic acid derivatives, decreases the antioxidant and anti-inflammatory activities of anthocyanins (95).

Cyanidin has downregulation of the production of the iNOS isomer (inducible nitric oxide synthase), which has pro-inflammatory activity in the vasculature. As eNOS and iNOS have different functions in the vascular system, the former is beneficial while iNOS is harmful. Artichoke flavonoids, mainly cynarin, activate the endothelial expression of NO (eNOS) with a vasoprotective effect, at the same time that anthocyanins decrease the iNOS expression, suggesting synergistic effects of flavonoids. Artichoke presents different effects against different forms of NOS, contributing to its cardioprotective effects (84,98).

Schütz *et al.* (70) performed the detection of anthocyanins in the inner bracts of the artichoke and identified cyanidin 3,5-diglucoside, cyanidin 3-glucoside, cyanidin 3,5-malonyldiglucoside, cyanidin 3-(3"-malonyl) glucoside, and cyanidin 3-(6"-malonyl) glucoside. Cyanidin 3-(6"-malonyl) glucoside was the anthocyanin present in greater quantity in the violet petals. The violet petals of artichoke flowers contain around 0.84 to 170 mg/100 g<sub>dm</sub> of anthocyanins.

According to Zazzali *et al.* (99) stalk had the highest anthocyanin content among artichoke parts, approximately 0.76 mg cyd-3-glu/100 g<sub>fw</sub>, while the bracts also had a relatively high monomeric anthocyanin content (0.19 mg cyd-3-glu/100 g<sub>fw</sub>).

### Enzymes

The artichoke and the thistle (*C. cardunculus*) are similar varieties, and several enzymes from thistle have already been studied, including several aspartic proteases such as cyprosins A, B, and C and cardosins A, B, E, F, G and H, which were isolated, purified, and characterized biochemically (100,101). Aspartic proteases isolated under alkaline conditions are called cynarases or cyprosins (102,103), proteases that are isolated at an acidic pH from fresh stigmas of *C. cardunculus* are called cardosins (14,104,105). These proteolytic enzymes are used as natural coagulants (3).

Cardosin A represents 75–90 % of the total aspartic proteases in *C. cardunculus* while cardosin B represents 10–25 % (106). Cardosins cleave bonds between amino acids with hydrophobic side chains and can accommodate long hydrophobic sequences at the active center (107). Cardosin A, formed by two peptides of 30 and 15 kDa (14), is similar to chymosin, having cleavage specificity (108), cleaving bovine k-casein between Phe105 and Met106 (109).

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Cardosin B, in *C. cardunculus*, is a heterodimeric enzyme with an apparent molecular mass of 34 and 14 kDa and the amino acid sequence shows 73 % similarity with cardosin A (104). It is similar to pepsin in terms of specificity and activity (109). It has greater proteolytic activity compared to cardosin A, but it also has less selective activity, cleaving peptide bonds with at least one amino acid with aliphatic or aromatic side chains and other bonds with amino acids with basic side chains (107). These enzymes have greater stability at pH 5.0 and temperatures below 45 °C, and may be useful as coagulants in the manufacture of some cheeses with a soft and buttery texture and a genuine to slightly spicy aroma (14,110).

From proteases of *C. cardunculus* var. *scolymus* glycoproteins, cynarase A, B and C were isolated and purified (15,111). Cynarase A consists of two subunits of approximately 32.5 and 16.5 kDa, cynarase B has two subunits of 33.5 and 16.5 kDa, and cynarase C is composed of subunits of 35.5 and 13.5 kDa (112,113). Cynarase C has a higher specific activity than cynarases A and B, comparable to that of chymosin. Because of these characteristics, cynarases have proteolytic and milk clotting activity (112), maximum activity at pH around 5.1 using casein as substrate (113) and pH 4.1 using synthetic peptides (112,114).

The artichoke contains an enzyme called polyphenol oxidase (EC 1.10.3.1), which is responsible for browning in plants. The main process responsible for the loss of quality during post-harvest storage is browning, together with peroxidase (EC 1.11.1.7), which is a limiting factor for artichoke processing. Thus, it is possible to consider the artichoke as a useful and cheap source of peroxidase and polyphenol oxidase, of which present industrial interest. To date, only a few papers on the purification and characterization of artichoke peroxidase and polyphenol oxidase have been published (115,116).

Table 2 presents some methods of extracting artichoke (*C. cardunculus*) enzymes from different parts of the plant (leaves, flowers, stigmas, roots, stalk, receptacles, and rhizomes), as well as the corresponding enzyme activities. The methods used for extraction and/or separation of proteases, peroxidases, and polyphenol oxidases involved mechanical agitation, maceration, maceration combined with centrifugation and filtration, an ultrasonic system, and membrane filtration, demonstrating that the enzymatic activity depends on the part of the plant and the method used.

In relation to the protease, the extraction ultrasound method using 40 kHz, 35 °C, 1:3 m/V (sodium citrate 0.1 M, pH=3), and a time of 60 min provides the best enzymatic extraction of leaf, when compared to other methods (mechanical agitation) and extraction operational conditions with other solutions (sodium acetate and Tris-HCl, pH 3, 5 and 7) (117). The flowers and stigmas are sources of cynarase (5 – 6.5 U/mg<sub>protein</sub>) (15, 111), and mechanical agitation and mechanical grinding are methods that can be used for enzyme extraction. The aspartic protease can be obtained from



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different parts of the artichoke, where the highest specific proteolytic activities are found on the flowers, followed by the leaf, receptacle, and stalk of the inflorescence (14). Todaro *et al.* (115) verified how the harvest season affected the polyphenol oxidase activity and observed a decrease in activity in the spring harvest, except for the cultivar Violettodi Provenza, which showed a stable and low level of enzymatic activity in both seasons. However, the Tema 2000 variety, although it showed a decrease in activity during the spring harvest, maintained the highest level of polyphenol oxidase activity. Cardinali *et al.* (116) extracted peroxidase by mechanical agitation and observed that the peroxidase of artichoke leaves ( $10 \text{ U/mg}_{\text{protein}}$ ) was about 20 times higher than that obtained from the heads. This enzyme has been shown to be stable at different temperatures (5 and 65 °C) and pH values (5.5–6.0).

Subsequent steps in the extraction of an enzyme involve recovery, purification, and concentration (118). The degree of purification of the enzyme depends on its application, especially for therapeutic or pharmaceutical use, and it is essential that the enzyme have high purity (119).

Several methods have been applied for the purification and/or concentration of enzymes, including membrane separation processes, which act as a selective barrier, retaining particles that are larger than the pores and allowing smaller molecules to permeate the pores.

The classification of processes is based on the type of force that drives transport across the membrane (121) and is named according to pore size: microfiltration, ultrafiltration, nanofiltration, and reverse osmosis (122). For the purification and concentration of enzymes and proteins, ultrafiltration and nanofiltration are mainly used (123). Sidrach *et al.* (15) used ultrafiltration and nanofiltration on the purification of flower stigmas from artichoke (*C. scolymus* L.), and obtained cynarase A specific activity of  $6.5 \text{ U/mg}_{\text{protein}}$ .

The aqueous two-phase method and precipitation using salts are also used in the separation of enzymes and proteins. Bi-phase aqueous systems, or two-phase aqueous systems, are ternary systems formed by water and two non-volatile water-soluble components (120), which can be polymers, salts, and/or ionic liquids (124). These systems can be used to separate proteins from cell debris or purify proteins from other proteins. Soluble and particulate material will concentrate in the lower, more polar phase, while proteins and enzymes will concentrate in the upper, less polar, and more hydrophobic phase (125).

Precipitation using salts is a very useful and convenient method of purifying proteins. The most commonly used salt in this process is ammonium sulphate, which is added as a solid or a saturated solution to precipitate the proteins of interest (126). The precipitated proteins can be concentrated by removing the remaining ammonium sulphate solution, and then the protein pellet can be resolubilized in standard buffers or at low concentrations of ammonium sulphate (121). Pereira *et al.* (120) in the

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extract of stigmas and stylets of dried flowers from artichoke, performed the precipitation with ammonium sulphate and obtained proteolytic activity of 0.24 and 0.79  $\Delta\text{Abs}/\text{g}_{\text{protein}}/\text{min}$ , and coagulating activity of 90.6 and 74.6 UR/ $\text{g}_{\text{protein}}$ , with 30 and 70 % saturation, respectively.

### *Applications of artichoke*

Parts of the artichoke plant, such as flowers, stems, and leaves, as well as residues from processing the bracts, can be used/reused in the food, chemical, and pharmaceutical industries due to the bioactive compounds of the artichoke, such as: phenolic compounds, enzymes, soluble fibers, fructooligosaccharides, inulin, and others. **Table 3** presents a synthesis of the main applications of the artichoke plant, and below are detailed descriptions of the functions in the different segments.

#### Chemical and food industry

The internal bracts and the heart of the immature inflorescence (3,4,17,35) can be consumed in nature or processed chilled, frozen, or preserved, adding some components to the brine: for example, citric acid is used to reduce the pH and avoid changes in the color of the product, while calcium chloride is used to maintain the proper hardness of the vegetables during heat treatment (145).

The artichoke has applications in the industrial area as it is a potential source of different proteases (127,128). The extract of the artichoke flower is a promising vegetable coagulant, which can add value to the cultivation of this species, as inflorescences not commercialized due to size or appearance (normally discarded) can be used for protease extraction (14,146). The commercial enzymes used in milk coagulation are aspartic proteases (APs; EC 3.4.23), more active at acidic pH and inhibited by pepstatin, and their amino acid sequences have high levels of homology (129–130, 147–148). The artichoke flower also has antioxidant capacity, which allows its use as an additive in the food industry to prevent the oxidation of oils (129).

By-products of artichoke flower processing can be used to extract fructooligosaccharides, inulin (17,18,41,67,76,111,130), polyphenol oxidase, peroxidase (16,131), flavonoids, and phenolic compounds (40,149), as well as isolating fractions rich in soluble fiber (150). Inulin from artichokes was used as a fat substitute in chicken sausage formulations (151) and also in low-fat dairy products such as yogurt (152).

Rodríguez-López, Tudela, García-Cánovas (133) designed and patented a process that uses extracts made from artichoke residues to catalyse the removal of phenols from aqueous solutions.

*C. cardunculus* also has industrial application as an energy crop, reducing the emission of greenhouse gases and ensuring the supply of energy due to its participation in the production of solid

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biofuels by fermentation of lignocellulose and oil from cultivated and wild thistle (153), biogas (bioethanol and biomethane) are good examples (13,134–136). In order to improve the yield of bioethanol production, new techniques were applied, to minimize the levels of fermentation inhibitors. An additional pre-treatment of artichoke residues is carried out during the procedure through autohydrolysis (H<sub>2</sub>O, 121 °C) and acid hydrolysis at low concentrations (0.5 % H<sub>2</sub>SO<sub>4</sub>, 121 °C). These treatments promote a theoretical production of bioethanol per hectare of ~3,900 kg (8).

The cellulose and hemicellulose from the artichoke have potential applications in the production of paper pulp (132). Artichoke by-products have the potential to solve problems in the agri-food industries through the recovery of biological and inedible waste (leaves and stalk) and food additives (27,28,154), thus improving the recycling of waste from the processing of artichoke heads.

Chemically treated raw artichoke leaves can be used as a low adsorbent for the treatment of effluents from textile industries, and may be an alternative to the use of activated carbon (137). The peroxidase enzyme extracted from artichoke can also be involved in the catalytic removal of phenolic and other aromatic compounds from wastewater. This method represents a useful alternative for the treatment of industrial wastewater when conventional methods such as biological treatment are not available (116).

#### Pharmaceutical industry

Artichoke extracts have been utilized in the pharmaceutical industry for their potential medicinal properties. The extracts are typically derived from the leaves of the artichoke plant (*C. scolymus*) and contain various bioactive compounds, including caffeoylquinic acids, flavonoids, and cynaropicrin (47). These compounds are believed to contribute to the potential health benefits associated with artichoke extracts. One of the primary uses of artichoke extracts in the pharmaceutical industry is in the management of digestive disorders. The extracts have been traditionally used as an herbal remedy for conditions such as dyspepsia (indigestion), and research suggests that they may help improve digestion by stimulating bile production and enhancing liver function (64). Artichoke extracts are often included in formulations for digestive health supplements and may be beneficial for individuals with conditions like irritable bowel syndrome or liver diseases.

The use of artichoke leaf extracts has beneficial effects such as decreasing serum LDL (low density lipoprotein), total cholesterol, and triglycerides, without increasing HDL (high density lipoprotein) levels, thus helping in the treatment of diseases (85). Cicero *et al.* (138) and Kraft (139) studied the action of the extract on LDL oxidation and cholesterol metabolism. Artichoke inhibits the biosynthesis of new hepatic cholesterol via inhibition of 3-hydroxy-3-methyl-glutaryl-CoA reductase and intestinal absorption of cholesterol, increasing its excretion via inhibition of acetyl-coenzyme A

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acetyltransferase (ACAT). Furthermore, artichoke extracts have demonstrated potential in supporting cardiovascular health. Studies have indicated that the bioactive compounds in artichoke extracts, particularly cynaropicrin, may help lower cholesterol levels by inhibiting the enzyme involved in cholesterol synthesis. By reducing cholesterol levels, artichoke extracts have the potential for support heart health and reduce the risk of cardiovascular diseases.

The anti-obesity effect is due to several mechanisms, such as inhibition of digestive enzymes (lipase,  $\alpha$ -glucosidase and  $\alpha$ -amylase), while stimulating bile secretion, lipolysis, and lipid metabolism. Inhibition of inflammatory adipokine secretion is also associated with the anti-obesity and prebiotic effects of inulin (140).

There are studies of possible anticancer and chemopreventive activities against different cancer cell lines, including caspase-independent mechanisms in breast cancer inhibition via induction of senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -gal) and up-regulation of tumor suppressor genes (141), apoptosis of the mesothelioma cell line (142), and human hepatoma cells (143).

The antioxidant and anti-inflammatory effects are due to the presence of polyphenols (79), studies of in vitro antioxidant effects are being conducted via DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2-azino-bis-3-ethylbenzothiazolin-6-sulfonic acid), FRAP (ferric reducing antioxidant power), and  $\beta$ -carotene bleaching assays (7,144). In vivo action was determined through oxidative stress biomarkers such as superoxide dismutase-1 (SOD), catalase (CAT), and reduced glutathione (GSH) for antioxidant activity and inhibition of carrageenan-induced edema activities, in addition to histopathological examination (79). In vitro studies, normal cell lines were exposed to inflammatory cytokines, hydrogen peroxide ( $H_2O_2$ ) and ultraviolet B (UVB), confirming the effects of leaves in reducing the production of harmful and destructive reactive oxygen species (ROS) in cells. In vivo studies and meta-analyses confirmed the antioxidant activity of artichoke extract in animals, mediated by the increase of liver-protective enzymes against free radicals (catalase - CAT, superoxide dismutase - SOD, and glutathione peroxidase - GSH-Px), in addition to decreasing levels of malondialdehyde in the liver and plasma (29).

The hepatoprotective effect is due to its antioxidant stress effects and inhibition of the toll-like receptor I (TLR4) inflammatory pathway and the nuclear factor-kappa B (NF- $\kappa$ B) or TLR4/NF- $\kappa$ B (155), regenerating dysfunctional liver cells (156) and curative effects on the regulatory mechanism, allowing repair of DNA damage after hepatotoxicity (157). This effect is demonstrated by the decrease in serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

In addition, artichokes have an enzyme such as peroxidase that has attracted industrial attention because of its purified usefulness as catalysts in clinical biochemistry and enzymatic immunoassays (116).

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It's important to note that while artichoke extracts show promise in these areas, further research is needed to fully understand their mechanisms of action and evaluate their efficacy and safety. As with any pharmaceutical product or supplement, it is essential to consult with healthcare professionals before using artichoke extracts for medicinal purposes.

## CONCLUSIONS

The current review provided information about the phytochemical composition, bioactivities, and applications of artichoke. There is evidence of applications in the food industry using canned and/or frozen bracts; food additives, inulin in the preparation of low-fat foods and as a prebiotic; soluble fiber and fructooligosaccharides as a prebiotic; proteolytic enzymes as natural coagulants, and phenolic compounds with antioxidant activity to prevent the autoxidation of lipids in foods and/or oils. In addition, they can be used in other industrial applications, such as biofuels, pulp production (cellulose and hemicellulose) and others. Due to their bioactive properties (antioxidant, anti-inflammatory, anticancer, hypocholesterolemic, and cardioprotective effects, among others), they can be used as bioactive additives for nutraceutical purposes.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest

## SUPPLEMENTARY MATERIAL

Supplementary materials are available at: [www.ftb.com.hr](http://www.ftb.com.hr).

## AUTHORS' CONTRIBUTION

All authors contributed to the development of the study. The preparation of the material was carried out by E. Valduga, J. Zeni, and J. Steffens and experimental data by T. Feiden.

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**Table 1.** Methods for extraction, operational conditions and quantification of bioactive compounds of artichoke

Parts of artichoke	Extraction method/operational conditions	Compound	Quantity	Reference
Leaf and stalks	<i>Mechanical agitation:</i> Mass:solvent ratio: 1:20 <i>m/V</i> (2 % formic acid in methanol/water 4/1); Homogenization:10 s vortex; Agitation in horizontal shaker: 560 rpm, 15 min; Centrifugation: 2647xg, 3 min).	Phenolic compounds	1191–3496 mg GAE/100 g <sub>dm</sub>	66
Bracts and stalks	<i>Mechanical agitation:</i> Mass:solvent ratio: 1:20 <i>m/V</i> (75 % ethanol); Agitation in horizontal shaker: 200 rpm, 60 °C, 40 min.	Phenolic compounds	2461 mg GAE/100 g <sub>dm</sub>	67
Heads	<i>Mechanical agitation:</i> Mass:solvent ratio: 1:10 <i>m/V</i> (70 % methanol containing 1 mM of butylated hydroxytoluene and 1 mM hesperetin); Agitation: 22 °C, 60 min; Centrifugation: 2000xg, 3 min.	Flavonoids	<i>Total luteolin:</i> Tondo di Paestum, Violetto di Sicilia, Cimiciusa: 2.5 mg/100 g  <i>Total apigenin:</i> Cimiciusa: 747 mg/100 g Tondo di Paestum: 264 mg/100 g Violetto di Sicilia: 266 mg/200 g	68
Bracts and receptacles	<i>Maceration:</i> Mass:solvent ratio: 1:5 <i>m/V</i> , extraction (hexane); Agitation: 3 h; Filtration and Mass dry; Successive extractions: 1:5 <i>m/V</i> (diethyl ether anhydrous, ethanol and water), 3 h.	Flavonoids	Bracts: 75.5 mg QE/100 g <sub>dm</sub> Receptacles: 40.1 mg QE/100 g <sub>dm</sub>	69
Flowers	<i>Mechanical agitation:</i> Mass:solvent ratio: 1:200 <i>m/V</i> (70 % aqueous acetone/0.01 % trifluoroacetic acid-TFA); Agitation: 4 °C, 1 h; Centrifugation: 4000xg, 5 min; Successive extractions: two extractions 1:200 <i>m/V</i> (acetone), 45 min and 30 min; tree extractions 1:100 <i>m/V</i> (TFA/ethyl acetate, pH=1.5); Centrifugation: 4000xg, 5 min.	Anthocyanins	Camus: 170 mg/100 g <sub>dm</sub> Green Globe: 87.8 mg/100 g <sub>dm</sub> Le Castel: 40.5 mg/100 g <sub>dm</sub> Petit Violet: 0.84 mg/100 g <sub>dm</sub> Buette: 104 mg/100 g <sub>dm</sub>	70

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			Poivrade: 2.1 mg/100 g <sub>dm</sub>	
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**Table 2.** Artichoke enzyme extraction methods, operational conditions and enzyme activity

Parts of artichoke	Extraction method/operational conditions	Enzyme	Activity	References
Leaf	<i>Mechanical agitation:</i> Mass:solvent ratio: 1:3 m/V (sodium citrate 0.1 M, pH=3); Time: 3 min.	Protease	Proteolytic: 13.2 U/mL Specific: 9.59 U/mg <sub>protein</sub>	117
	<i>Mechanical agitation:</i> Mass:solvent ratio: 1:3 m/V (sodium acetate 0.1 M, pH=5); Time: 3 min.		Proteolytic: 7.9 U/mL Specific: 6.1 U/mg <sub>protein</sub>	
	<i>Mechanical agitation:</i> Mass:solvent ratio: 1:3 m/V (Tris-HCl 0.1 M, pH=7); Time: 3 min.		Proteolytic: 8.8 U/mL Specific: 6.6 U/mg <sub>protein</sub>	
	<i>Ultrasound:</i> Mass:solvent ratio: 1:3 m/V (sodium citrate 0.1 M, pH=3); Operational conditions: 35 °C, 40 kHz, 60 min.		Proteolytic: 14.4 U/mL Specific: 19.7 U/mg <sub>protein</sub>	
Flowers	<i>Mechanical agitation:</i> Mass:solvent ratio: 1:3 m/V (sodium citrate 0.1 M, pH=3); Centrifugation: 50000xg: 30 min.	Cynarase A, B, C	Crude extract (Specific): 5 U/mg <sub>protein</sub> Cynarase A, B, C: 25, 100 and 20 U/mg <sub>protein</sub>	111
Flowers and stigmas	<i>Mechanical grind:</i> Mass:solvent ratio: 1:8 m/V (sodium citrate 50 mM/1 M NaCl, pH=3); Centrifugation: 24000 rpm, 20 min.	Cynarase	Activity: 17.5 U/mL Specific: 6.5 U/mg <sub>protein</sub>	15
Leaf	<i>Maceration:</i> Mass:solvent ratio: 1:10 m/V (citric acid, 0.1 M, pH=3).	Aspartic protease	Specific proteolytic: 2.4 U <sub>cas</sub> /mg <sub>protein</sub>	14
Receptacle			Specific proteolytic: 1.5 U <sub>cas</sub> /mg <sub>protein</sub>	
Stalk			Specific proteolytic: 1.4 U <sub>cas</sub> /mg <sub>protein</sub>	
Flowers			Specific proteolytic: 5.45 U <sub>cas</sub> /mg <sub>protein</sub>	
Heads	<i>Mechanical grind/Ultra-Turrax, speed 3:</i> Mass:solvent ratio: 1.5:1 m/V (phosphate 0.1 M, pH=3); Time: 60 s; Centrifugation: 15000xg, 30 min, 4 °C; Ultrafiltration: membrane 50 kDa.	Polyphenol oxidase	Violetto di Sicilia: 13.6–20 mmol/min.g <sub>fw</sub>	115
			Violetto di Provenza: 13–14.4 mmol/min.g <sub>fw</sub>	
			Tema 2,000: 19.3–27 mmol/min.g <sub>fw</sub>	



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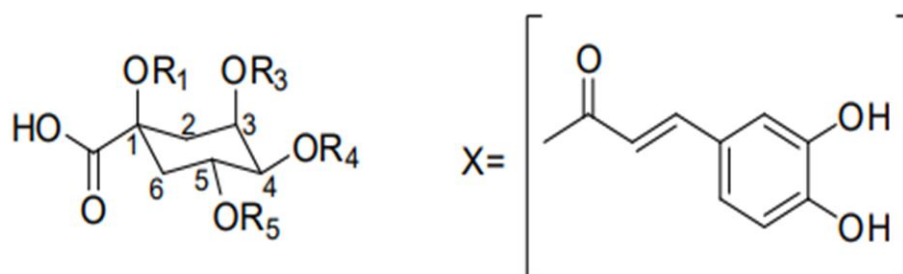
Leaf	<i>Mechanical agitation:</i> Mass:solvent ratio: 1:4 m/V (sodium acetate 0.05 M/2 % of polyvinylpyrrolidone, pH=6); Waring blender: 15000 rpm, 3 min; Centrifugation: 17400xg, 30 min, 4 °C.	Peroxidase	Total activity: 12.1 U/mL Specific: 10 U/mg <sub>protein</sub>	116
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**Table 3.** Applications and functions of artichoke in food, chemical and pharmaceutical industry

Industrial applications	Parts of the artichoke	Compound and functions	References
Food and Chemical	Internal bracts	In natura and/or processed (chilled, frozen or preserved, brine)	6
	Flowers	Vegetable Coagulant - Aspartic proteases (APs; EC 3.4.23), e.g. cheese production)	127,128
		Antioxidant (e.g., oils industry)	129
		Fructooligosaccharides and inulin	130
		Enzymes (polyphenol oxidase; peroxidase)	16,131
	Waste (leaf, flowers, stalks,and others)	Cellulose and hemicellulose (paper pulp)	132
		Removal of phenols of wastewater	133
		Biofuels (bioethanol and biomethane)	134–136
		Adsorbent (e.g., treatment of effluents)	137
	Pharmaceutical	Leaf Flowers Stalks Bracts	Enzymatic immunoassay
Cardiovascular diseases and dyslipidemia (reduction of LDL, total cholesterol and triglycerides)			85,139,140
Anti-obesity			141
Anticancer and chemopreventive			141–143
Antioxidant and anti-inflammatory			79,144

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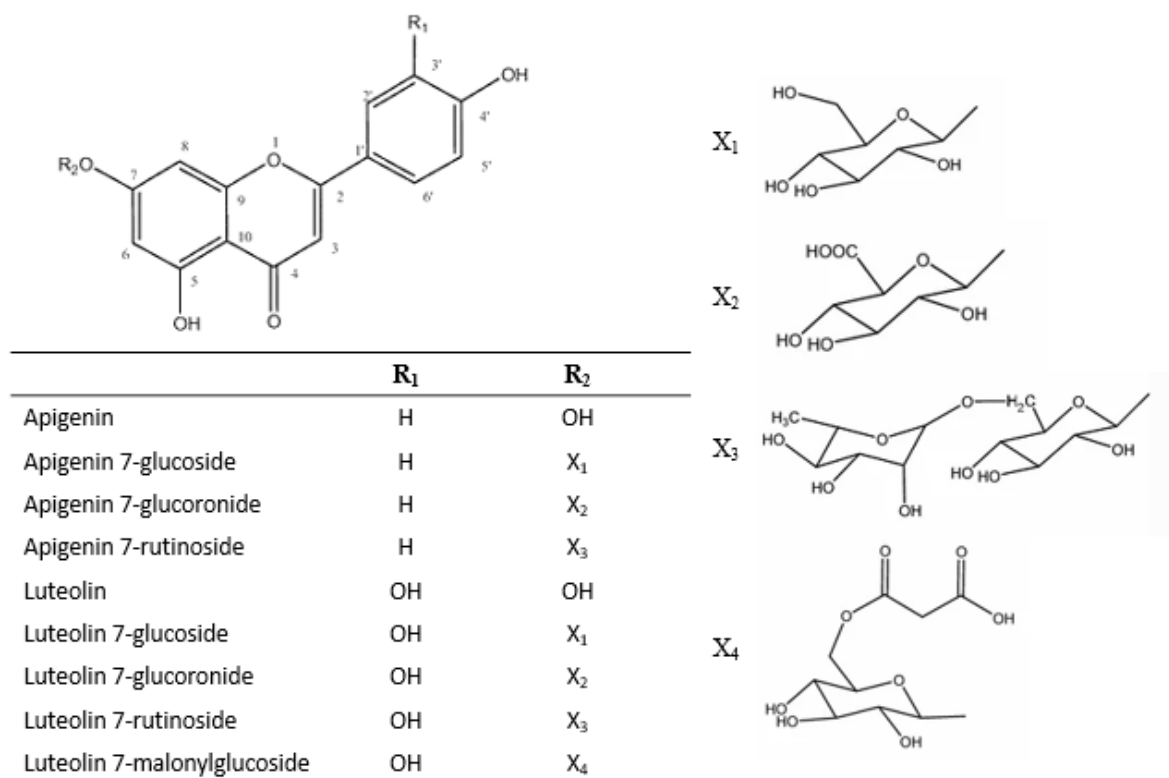
## Supplementary material



- 1-O-Caffeoylquinic acid: R1=X; R3=H; R4=H; R5=H
- 3-O-Caffeoylquinic acid: R1=H; R3=X; R4=H; R5=H
- 4-O-Caffeoylquinic acid: R1=H; R3=H; R4=X; R5=H
- 5-O-Caffeoylquinic acid: R1=H; R3=H; R4=H; R5=X
- 1,3-O-Dicaffeoylquinic acid: R1=X; R3=X; R4=H; R5=H
- 1,4-O-Dicaffeoylquinic acid: R1=X; R3=H; R4=X; R5=H
- 1,5-O-Dicaffeoylquinic acid: R1=X; R3=H; R4=H; R5=X
- 3,4-O-Dicaffeoylquinic acid: R1=H; R3=X; R4=X; R5=H
- 3,5-O-Dicaffeoylquinic acid: R1=H; R3=X; R4=H; R5=X
- 4,5-O-Dicaffeoylquinic acid: R1=H; R3=H; R4=X; R5=X

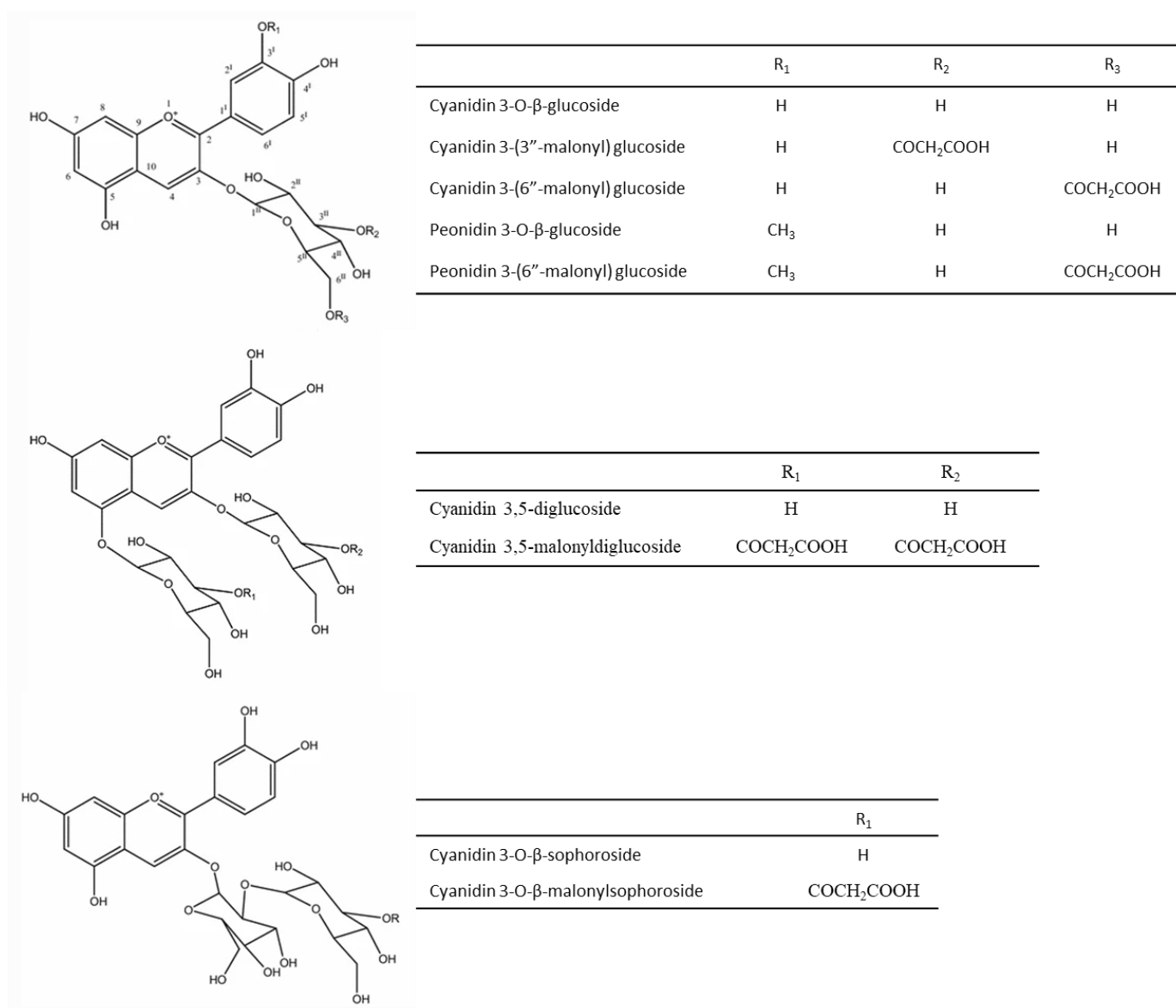
**Fig. S1.** Chemical structures of caffeoylquinic acid derivatives present in artichoke. Font: Adapted from Lattanzio *et al.* (10)

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**Fig. S2.** Chemical structures of the main flavonoids and glycosides present in artichoke. Font: Adapted from Lattanzio *et al.* (10)

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**Fig. S3.** Chemical structure of anthocyanins and glycosides present in artichoke. Font: Adapted from Lattanzio *et al.* (10)