# Health Benefits of Phenolic Compounds from Pigmented Corn

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Pigmented corn is a gramineae food of great biological, cultural and nutritional importance for many Latin American countries, with more than 250 breeds on the American continent. It confers a large number of health benefits due to its diverse and abundant bioactive compounds. Phenolic compounds, among which are anthocyanins are some of the most studied and representative compounds in these grasses, with a wide range of health properties, mainly the reduction of pro-oxidant molecules.

Keywords: Pigmented corn ; *Zea mays* L. ; Fuctional foods ; Nutraceuticals ; Phytochemicals ; Antioxidants ; Phenolic compounds ; Anthocyanins

## 1. Introduction

Phenolic compounds or polyphenols are compounds resulting from the secondary metabolism of plants, with more than 8000 known molecules <sup>[1]</sup>. Although several classifications have been made, the most widely used divides polyphenols into two main families: flavonoids (e.g., chalcones, flavones, flavonols, flavandiols, anthocyanins, condensed tannins and aurones) and non-flavonoids (e.g., free phenols, phenolic acids, polyphenolic ketones, fumarins, chromones, benzofurans, lignans, xanthones, stilbenes and quinones) <sup>[2]</sup>. Phenolic acids are a class of secondary metabolites highly distributed in plants. According to their chemical structure, phenolic acids can be divided into benzoic and cinnamic acids. The main benzoic groups are gallic, pro-tocatechinic and *p*-hydroxybenzoic acids, mainly as conjugates. Cinnamic acids are widely distributed in plants, as esters or amides. The most representative are caffeic, chlorogenic and ferulic acids <sup>[3]</sup>. Phenolic compounds include anthocyanins and anthocyanidins of various types, ferulic acid and phlobaphenes <sup>[4]</sup>.

# 2. Ferulic Acid

Cereals, including corn, are the most important source of ferulic acid, derived from cinnamic acid (intake ranges from 0.092 to 0.32 g) <sup>[5]</sup>. Ferulic acid ([E]-3-[4-hydroxy-3-methoxyphenyl] propa-2-enoic acid) belongs to the phenolic acid group, commonly found in plant tissues <sup>[6]</sup>. Phenolic acids are secondary metabolites of variable chemical structures and biological properties. The antioxidant mechanism of action of ferulic acid is complex, based mainly on the inhibition of the formation of reactive oxygen species (ROS) or nitrogen, but also on the neutralization of free radicals. In addition, this acid is responsible for chelating protonated metal ions, such as Cu(II) or Fe(II) <sup>[Z]</sup>. Ferulic acid is not only a free radical scavenger, but also an inhibitor of enzymes that catalyze the generation of free radicals and an enhancer of the activity of scavenger enzymes. It is directly related to its chemical structure. It has also been shown to have lipid peroxidation inhibitory activity <sup>[8]</sup>. Ferulic acid has low toxicity and possesses many physiological functions, including anti-inflammatory, antimicrobial, anticancer (e.g., lung, breast, colon and skin cancer), antiarrhythmic and antithrombotic activity. It also demonstrated anti-diabetic effects and immunostimulant properties, as well as reduced nerve cell damage, and may help repair damaged cells <sup>[9]</sup>.

Ferulic acid has been shown to have an angiogenesis effect by affecting the activity of the main factors involved, i.e., vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and hypoxia-inducible factor 1 (HIF-1) <sup>[9]</sup>. In research with human umbilical vein endothelial cells, ferulic acid has been shown to enhance VEGF and PDGF expression and increase the amount of hypoxia-induced HIF-1, which generates responses to hypoxia <sup>[10]</sup>. Ferulic acid appears to be an effective substance that promotes the formation of new vessels, as demonstrated in in vivo and in vitro studies <sup>[11]</sup>.

It is important to note that in corn, ferulic acid can be found bound to arabinoxylans, a class of carbohydrates consisting of arabinoses and xyloses, both five-carbon monosaccharides (pentoses) <sup>[Z]</sup>. Several studies have shown that dietary supplementation with cereal-derived arabinoxylans improves the antioxidant capacity of intestinal epithelial cells due to

the production of ferulic acid and short-chain fatty acids (SCFA) from microbial fermentation. Ferulic acid may co-operate with SCFA to regulate intestinal integrity and host immune functions. Peroxisome proliferator-activated receptor y (PPARy) may play an important role in the integration of ferulic acid and SCFA to regulate host health and metabolism <sup>[12]</sup>. In other studies, ferulic acid has been shown to combine with arabinose residues in cereal-derived arabinoxylans, but gut microbiota ferment arabinoxylan to release free ferulic acid, as well as SCFA production <sup>[13]</sup>. It has also shown that as one of the phenolic acids it has a strong antioxidant capacity to scavenge reactive oxygen species (ROS) and enhance anti-oxidant activity through the activation of the Kelch-like ECH-associated protein 1 and nuclear factor E2-related factor 2 (Keap1-Nrf2) signaling pathway <sup>[14]</sup>. Therefore, the pericarp of pigmented corn, rich in ferulic acid, could be metabolized by the intestinal microbiota of humans, generating a release of ferulic acid bound and conjugated into free ferulic acid, in a manner similar to thermal, acidic and alkaline processes.

#### 3. Phlobaphenes

Another phenolic compound found in some pigmented corn, specifically in the red breeds and varieties, is phlobafen. These are condensed tannins of a high molecular weight, coming from the union of several molecules of naringenin and eriodictyol joined by the central ring. They are oxidized, hardly soluble in water—probably due to the abundance of methoxyl groups in their structure—and present a reddish-brown color. There are also phlobaphenes composed of a mixture of polymeric procyanidins, dihydroquercetin, carbohydrate (glucosyl) and methoxyl moieties <sup>[15]</sup>. In the case of red corn, these accumulate in the pericarp of the seed and the glumes of the cob. A study showed that they are related to the thickness of the pericarp of red corn (the higher the amount of phlobafen, the thicker the pericarp) <sup>[16]</sup>. It is speculated that they could have beneficial effects on human health due to their high antioxidant capacity; however, up to this moment there are no clinical trials that confirm this effect. The biological effects of phlobafen are still unknown, so there is a great opportunity for future research to elucidate the effects of these phytochemicals and their biological activity in human physiology.

### 4. Anthocyanins

One of the most important flavonoids are anthocyanins. These are water-soluble pigments, abundant in nature, which can be found in vegetables, fruits, flowers and grains. Chemically, they are glycosides of anthocyanidins, i.e., they consist of an anthocyanidin molecule to which a sugar is attached by a  $\beta$ -glucosidic bond. Anthocyanins can be formed from two metabolic biosynthetic pathways: the shikimate pathway to produce the amino acid phenylalanine and the malonyl-CoA pathway (polyacetates or acetyl-CoA pathway) <sup>[17]</sup>. In purple corn kernels, as in wheat and barley, anthocyanins are found in the pericarp, while in blue varieties they are found in the aleurone layer <sup>[18]</sup>. In black and dark red grains, anthocyanins are found in both the aleurone and pericarp layers <sup>[19]</sup>.

The daily intake of flavonoids and anthocyanins has been reported to be around 200–250 mg/day <sup>[20]</sup>, while the Food and Drug Administration and NHANES (National Health and Nutrition Examination Survey of the United States) have set it at 12.5 mg/day/person <sup>[21]</sup>.

Several in vitro assays, animal and human cell line studies, animal models and human clinical trials indicated that the consumption of anthocyanin-rich foods (among which are pigmented corn), beverages and supplements provides numerous health benefits. In fact, this is due to the easy ability of anthocyanins to scavenge and/or neutralize free radicals and reactive species, chelate metals, control signaling pathways, decrease pro-inflammatory markers and thus reduce the risk of cardiovascular pathologies, cancer and neurodegeneration <sup>[22]</sup>.

Anthocyanins have demonstrated antioxidant potential in both in vitro <sup>[23]</sup> and in vivo studies <sup>[24]</sup> and the consumption of anthocyanin-rich foods has been linked to lower risks of chronic diseases <sup>[25]</sup>. There are several mechanisms of action through which anthocyanins could exert their biological effects on human health, among which is the activation of nuclear factor erythroid 2 (Nrf2). It serves as a transcription factor for the expression, transcription and translation of the antioxidant response element (ARE), which encodes for several antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase, catalase, etc., <sup>[26]</sup>. Another way in which anthocyanins exert their antioxidant power is by donating hydrogenions, thus reducing a large number of pro-oxidant molecules, as well as neutralizing various free radicals. This is due to the hydroxyl groups in anthocyanins, which usually contain between two and three of these. The last mechanism described by which they can exert an antioxidant and thus anti-inflammatory effect is through the chelation of metals and metalloids, mainly transition metals such as iron (Fe), copper (Cu), nickel (Ni), aluminum (Al), cadmium (Cd) and arsenic (As), as well as their respective valence forms <sup>[27]</sup>. The anthocyanins identified in several varieties of peruvian pigmented corns are cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside, cyanidin-3-glucoside, and cyanidin-3-(3",6"-dimalonylglucoside) <sup>[19]</sup>.

Delphinidin, a type of anthocyanidin that can act as a precursor of many anthocyanins, shows the most considerable ability to scavenge superoxide species, followed by petunidin > malvidin = cyanidin > peonidin > pelargonidin, at 1  $\mu$ M. Similar results were obtained for the ability of these compounds, at the same concentration, to scavenge peroxynitrite radicals <sup>[28]</sup>. In addition, cyanidin 3-*O*-glucoside at concentrations between 100 and 200  $\mu$ M showed potential to protect human keratinocyte HaCaT cells against ultraviolet-A radiation, preventing DNA fragmentation and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) release <sup>[29]</sup>. In one study, 12 healthy participants who consumed one of two beverage options high in anthocyanins and anthocyanidins, composed of 165.9 mg/L and 303.8 mg/kg of anthocyanins, respectively, showed increases in their plasma antioxidant capacity by 3-fold and 2.3-fold, respectively <sup>[30]</sup>.

Anthocyanins decrease plasma low-density lipoproteins (LDL), leading to a reduction in their accumulation in the walls of medium and large arteries [31]. Thus, anthocyanins indirectly inhibit LDL-promoted endothelial cell activation/dysfunction. Endothelial damage affects the nitric oxide (NO) release which, together with the increased local degradation of NO by the increased generation of reactive oxygen species (ROS), decreases NO availability. Anthocyanins can increase NO availability by several mechanisms. After activation, the endothelium begins to express cell adhesion molecules on its surface (ICAM-1, intercellular adhesion molecule-1 and VCAM-1, vascular cell adhesion molecule-1) to recruit circulating monocytes to the site of oxidized LDL (oxLDL) accumulation. Anthocyanins downregulate the expression of these adhesion molecules [32]. On the luminal side, anthocyanins decrease chemokines (CK), which also results in decreased myeloid cell recruitment. Anthocyanins counteract ROS on both the luminal and intimal sides, reducing LDL oxidation in the vessel wall [33]. During the progression of atherogenesis, neutrophil-derived granular proteins stimulate macrophage activation to a proinflammatory state that can be inhibited by anthocyanins [34]. Both the antioxidant and anti-inflammatory effects of anthocyanins decrease foam cell formation. Anthocyanins also lower cholesterol by reducing its accumulation in the lipid-rich necrotic core [24]. During the late stages of atherosclerosis, anthocyanins reduce the expression of Toll-like receptor 2 (TLR2) signaling in endothelial cells that regulate the neutrophil stimulation of stress and endothelial cell apoptosis [35]. Regarding anthocyanin-enriched fractions of natural products, extracts of blackberries, blueberries, strawberries, sweet cherries and red raspberries at 10 µM showed the potential to inhibit human LDL oxidation, having been twice as effective as an ascorbic acid control [36]. Blackberry and raspberry fruits also revealed lipid peroxidation inhibitory potential, showing IC<sub>50</sub> values below 50  $\mu$ g/mL <sup>[37]</sup>.

Anthocyanins are also involved in the regulation of the inflammatory status and activation of endogenous antioxidant defenses, as well as in the regulation of the immune system through MAPK-, NF-kB- and JAK-STAT-related signaling pathways <sup>[32]</sup>. The effects of anthocyanins on inflammatory markers are promising and may have the potential to exert anti-inflammatory biological action in vitro and in vivo. Therefore, translating these research findings into clinical practice would effectively contribute to the prolonged maintenance of a healthy state. A review study summarized the results of clinical studies from the last five years in the context of the anti-inflammatory and antioxidant role of anthocyanins in a health state as preventive agents and concluded that there is evidence indicating that anthocyanin supplementation in the regulation of proinflammatory markers among the healthy population is highly functional, although inconsistencies between the outcome of randomized controlled trials (RCTs) and meta-analyses were also noted. Regarding the effects of anthocyanins on inflammatory markers, there is a need for long-term clinical trials with large cohorts that allow the quantifiable progression of inflammation [38]. In another study, different anthocyanin dilutions (concentrations of 100, 150 and 200 µg/mL) showed the ability to reduce the expression levels of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), IL 1β and IL -6 and to suppress AP-1 signaling and nuclear factor kappa B (NF-kB) pathways [39]. It was also verified that, at concentrations of 10, 25 and 50 µg/mL, they can decrease the phosphorylation of IKK, IkBa, p65 and JNK, prevent the nuclear translocation of p65 in RAW 264.7 macrophage cells stimulated with LPS/IFN-y and inhibit lipoxygenase activity [40]. These biological activities demonstrate the direct and indirect antihypertensive, antiinflammatory endothelial vasodilator enhancement and modulation of inflammasome activation, as well as other signal transduction pathways related to the immune response.

In another study, the phenolic profile and associated antioxidant properties of corn samples with different pigmentations were characterized using spectrophotometric and chromatographic techniques and the stability of anthocyanins during gastrointestinal digestion was evaluated. Pigmented varieties showed a significantly higher anthocyanin content compared to common yellow varieties and, as a consequence, higher antioxidant activity. However, although corn is among the cereals mostly used in gluten-free products, it can produce an inflammatory response in some people with gluten intolerance. Therefore, after chemical characterization, the safety of pigmented varieties for patients with gluten intolerance was confirmed by different in vitro models (a cell agglutination test and measurement of transepithelial electrical resistance). Although in vivo studies are necessary, the data collected in the aforementioned study underline that pigmented corn could play a role in reducing oxidative stress at the intestinal level <sup>[41]</sup>. Cellular assays applied in another study confirmed the absence of alterations by pigmented strains in the permeability of the cell monolayer, a key step in the mucosal inflammatory cascade in various intestinal disorders <sup>[42]</sup>. Considering the daily consumption of corn and the high

content of anthocyanins in pigmented corn, these varieties could contribute antioxidant and anti-inflammatory ingredients to the diet of the general population, but in particular, of people with gastroenteric disorders since corn represents one of the most important ingredients among the cereals used in the formulation of gluten-free products <sup>[43]</sup>.

Another trial described some red and blue pigmented maize in terms of their secondary metabolite content and antioxidant and antimutagenic properties. High concentrations of ferulic acid were found for both red and blue corn, while the cyanidin-3-O-glucoside content was prominent for blue corn. Likewise, blue corn samples proved to be good sources of antioxidant and antimutagenic compounds, mainly those belonging to anthocyanins. These pigmented maize can be considered for scaling up production to obtain natural dyes, bioactive extracts for pharmaceutical and cosmetic applications and maize-based products that contribute to human health [44][45]. There is some evidence from in vitro, animal and human studies supporting the beneficial effect of cereal-based anthocyanins on a variety of health outcomes such as obesity, diabetes, aging, cancer and cardiovascular disease. However, more research is needed to determine the true effects of anthocyanins in humans. In addition, most studies used purified extracts to the test health effects. However, this is an unrealistic means of consuming cereal-based anthocyanins. More trials are needed to elucidate the effect of anthocyanin consumption within a matrix of processed cereals, including those made from pigmented corn [27].

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