



Bioactive Compounds and Industrial Peeling Applications of Inner and Outer Shells of Chestnuts (*Castanea* spp.)

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ABSTRACT

The aim of this review is to provide information concerning the types of chestnut shells (inner and outer), their compositions and bioactive compounds, as well as to mention industrial peeling applications. These shells are comprised of high-valued natural active compounds, such as polyphenols (phenolic acids, flavonoids, tannins, hydroxycoumarins -scopoletin, scoparone-), pigments (melanin) and minor compounds (minerals, dietary fiber, vitamin C and E, essential amino acids and fatty acids). The total phenolic acids and flavonoid content of *C. sativa* shell were ranged between 119.17-223.62 mg/kg db and 330 – 503 mg CE/g. It is also a good source of vitamin C with reported levels of 15.57 and 28.97 mg AA/100 mg db in water and ethanol extracts, respectively. The shells are used as food additives due to their colorant, antioxidant and antimicrobial properties. The shells are exposed by the peeling process applied to obtain the fruit without the shell which is mainly used. The most frequently used technique in chestnut peeling is the Brulage peeling method. However, in this technique, used peeling mechanism is insufficient to obtain both inner and outer shells separately at the same time. Moreover, further research is needed to obtain the shells individually, to analyse each shell in detail, and to increase the industrial use of shells.

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Introduction

Chestnut is the fruit of *Castanea* spp. which belongs to the Fagaceae family. The genus *Castanea* is made up of deciduous trees and shrubs and they primarily grow in the northern hemisphere regions (Nelson et al., 2014). Table 1 displays the ten widely known species of chestnuts. These species are divided in two groups based on the number of nuts in a bur: a) one nut per bur (chinquapins in *Castanea* spp.) and b) three nuts per bur (usually three or more) (Anagnostakis, 2010). The European chestnut (*Castanea sativa* Mill.) originated in the Eastern Mediterranean region during the middle Cretaceous period, and later spread to Europe during the Cenozoic period. Chestnut plantations were begun in the Caucasus Mountains between 900 and 700 BC. It is considered that the name of “Castanea” derives from the city of Kastanea, located near these mountains which was a part of the Pontus Empire. Following the Romans conquest of the Pontus Empire, the cultivation of chestnut throughout Europe (Anagnostakis, 2010; de Vasconcelos et al., 2010a).

According to the Food and Agriculture Organization (2023), among the nuts production worldwide, the chestnut has been ranked number six. I checked the FAO chestnut production data and realised some changes in the data. Global chestnut production was 2,134,872 tonnes in 2021, with China contributing approximately 74% (1,570,224 t) of total production. Other significant chestnut producing countries include Spain (187,680 t), Bolivia (83,327 t), Türkiye (77,792 t), the Republic of Korea (52,502t) and Italy (43,000 t). Following these countries, Portugal and Greece recorded production amounts exceeding 30,000 t.

Chestnut fruits have high nutritional value as they contain health-beneficial compounds. The main composition of the dried and peeled European chestnut (*C. sativa*) is reported as carbohydrate (78.43%), water (9.00%), protein (5.01%), fat (3.91%) and ash (3.64%) by the United States Department of Agriculture (2019a). Raw chestnuts can be consumed after boiling or roasting. Additionally, chestnut puree and chestnut candy (marron-glacé) are common chestnut derived products.

Table 1. The classification of *Castanea* spp. based on the number of nuts per bur (Anagnostakis, 2010)

Three nuts per bur		One nut per bur	
Latin name	Common name	Latin name	Common name
<i>Castanea sativa</i> (Miller)	European chestnut	<i>Castanea pumila</i> (Miller)	Allegheny chinquapin
<i>Castanea dentata</i> (Marshall) Borkhausen	American chestnut	<i>Castanea ozarkensis</i> (Ashe)	Ozark chinquapin
<i>Castanea crenata</i> (Siebold and Zuccarini)	Japanese chestnut	<i>Castanea floridana</i> (Sargent) Ashe	Florida chinquapin
<i>Castanea mollissima</i> (Blume)	Chinese chestnut	<i>Castanea alnifolia</i> (Nuttal)	Trailing chinquapin
<i>Castanea seguinii</i> (Dode)	Dwarf Chinese chestnut	<i>Castanea henryi</i> (Skan) Rehder and Wilson	Chinese chinquapin

Chestnut flour by containing over 50% starch is generally used in pastry after dehydrating and milling of the flesh. Gluten-free and high starchy chestnut flour can be utilised in the production of bread (Demirkesen et al., 2010), gel (Torres et al., 2014), cake ((Yildiz & Dogan, 2014) and chip (Di Monaco et al., 2010), serving as an alternative raw material for celiac patients (Squillaci et al., 2018; Zhu, 2017). Vitamins, minerals, fiber, organic acids, carotenoids and polyphenols (e.g., tannins) are minor components found in chestnuts. Many of these minor compounds possesses antioxidant, anti-carcinogenic, anti-tumor, anti-toxic, anti-inflammatory, anti-microbial and anti-malarial activity (Barreira et al., 2009; de Vasconcelos et al., 2010a; de Vasconcelos et al., 2010b; Goncalves et al., 2010; United States Department of Agriculture, 2019a). Additionally, chestnuts are a good source of dietary fiber due to the presence of indigestible components in the shell (Blaiotta et al., 2013). With these properties, chestnut shell promotes the proliferation of beneficial bacteria, while inhibiting pathogenic bacteria (Xie et al. 2023).

Surrounding the edible part of the chestnut, there are two-layers: a shell coating the flesh and a bur, which are separated as during the peeling process. Other by-products of chestnut processing include leaves, flowers and the tree trunk. Chestnut by-products have various applications in different industries, such as fuel, natural colorant in the food industry, natural antioxidant source in the food, cosmetic, and pharmaceutical industries, as well as in the formulations of wood adhesives and leather tanning (Aires et al., 2016; Cruz-Lopes et al., 2020; Echegaray et al., 2018). The economic value of chestnut by-products is increasing due to their food and non-food applications (Echegaray et al., 2018). Among the various by-products, the shell, which constitutes 10-15% of the chestnut, exhibits the highest antioxidant activity (Gullón et al., 2018; Shen et al., 2023). Rodrigues et al. (2015) analysed the nutritional composition of the *C. sativa* shell from three different production regions in Portugal (Minho, Trás-os-Montes and Beira-Alta). The main components of the shell were carbohydrates (56.51-74.06%), followed by water (21.29-38.61%), protein (2.77-3.13%), ash (1.08-1.60%) and fat (0.15-0.52%). The shell consists of two layers: the inner shell (also known as inner skin, integument, pellicle, seed coat or testa) and the outer shell (also known as outer skin, pericarp, husk, fruit coat or hull). The inner shell is adherent to the fruit flesh, while the outer shell is harder and forms the outermost layer of the fruit (Barreira et al., 2008; de Vasconcelos et al., 2010c; Hwang et al., 2001; Yao et al., 2016; Zamuz et al., 2018).

Industrial Peeling Applications

The economic value of chestnuts is increasing due to their nutritional value and beneficial effects on health. Peeling the chestnuts is necessary before consumption, resulting in a significant amount of inedible chestnut shells being produced. The shell accounts for approximately 10-15% of the chestnut fruit on dry basis (db) (Shen et al., 2023). Assuming the FAO data on the chestnut production in the world, over 230,000 tonnes (db) of chestnut shells are expected to emerge from the processes. These shells are of great significance as they contain high levels of antioxidant polyphenols, and pigments, minerals, vitamins, dietary fiber, essential fatty acids, and essential amino acids (Squillaci et al., 2018; United States Department of Agriculture, 2019a; Yao & Qi, 2016). These by-products can be utilized as fuel in factories and in various industries such as leather tanning, bioenergy, painting, cosmetic, nutraceutical and pharmaceutical industries. Additionally, in the food industry, these by-products are used as food additives due to their colorant, antioxidant and antimicrobial properties (Aires et al., 2016; Chen et al., 2018; Echegaray et al., 2018; Shen et al., 2023; Zhu et al., 2022). Moreover, in a recent study, it was stated that the inner and outer shells of chestnuts have a high potential in obtaining the products required for bioenergy production by pyrolysis, and even the pyrolysis products of the inner shell consist of more gaseous products (Shen et al., 2023). Since the two-layered shell has different compositions and distinct areas of use, it is important to separate shells from the flesh, and from each other. de Vasconcelos et al. (2010c) reported that four Portuguese chestnut cultivars (*C. sativa*) produce inner shells ranging from 6.33-10.10% and outer shell ranging from 8.96-13.54% of the fresh weight of the whole fruit.

Since there is a stronger interest in the inner shell, it becomes important to obtain the shells individually. It should be noted that separating the flesh from the inner and outer shells is challenging due to their adhesiveness. Tanaka et al. (1981) reported that tannins were relatively more abundant in the inner shell and reacted with proteins or polysaccharides to form complexes. These complexes may contribute to the adhesion of the inner shell. The findings of Hara et al. (1995) supported this inference, as they discovered that tannin accumulation in the inner shell led to the bonding of the shell to the flesh. The results of the study conducted by Hwang et al. (2001) are consistent with these findings. They analysed 14 chestnut varieties (*C. crenata*) and found a significant negative correlation

between the tannin content of the inner shell and the peeling ratio, as well as a positive relationship between the tannin content of the outer shell and the peeling ratio.

The initial peeling process of the shells was done by hand. However, to reduce labour requirements and shorten processing time, researchers began searching for new peeling techniques that combine physical and chemical treatments (Kim et al., 1997; Oh et al., 1985). Hwang et al. (2001) developed a machine that integrate high temperature and mechanical scraping to peel off both shells, but it was not sufficient for practical applications. Industrially, the most known and preferred peeling method worldwide is called Brulage peeling (Figure 1). This process results in two types of shell residues: a blend of inner and outer shells, and the inner shell itself. The Brulage peeling process consists of four parts. Firstly, chestnuts with the shell intact are fed into a burner. They are then conveyed through an oven using a screw auger cage to make the peel brittle. Next, they are passed into a thrasher containing rubber-ended paddles that move against steel rods, breaking away the outer shell and, in some cases, parts of the inner shell. The chestnuts are then conveyed to a steamer, which is a closed screw conveyor partially filled with water and heated with steam to 70-80°C (158-176°F) to loosen any remaining inner shell. Finally, the chestnuts are moved onto a brusher/washer, where loose inner shell is removed using counter-rotating pairs of rollers, followed by a cleaning rinse (Squillaci et al., 2018; Yen, 2006).

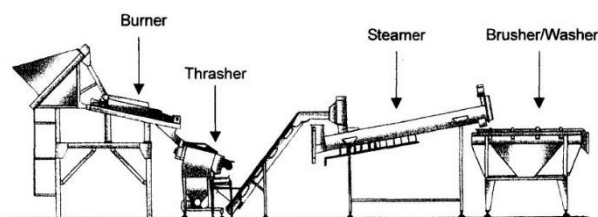


Figure 1. Schematic of process steps of the Brulage peeling line (Yen, 2006)

Bioactive Compounds

Antioxidant Polyphenols

There have been several studies demonstrating that chestnut (*C. sativa*) fruit and by-products contain a considerable amount of phenolic compounds (Barreira et al., 2008; Vella et al., 2018; Živković et al., 2009). Barreira et al. (2008) investigated the total phenolic content (TPC) of different parts of the chestnut plant and reported that the shells had the highest TPC. Conversely, Vella et al. (2018) and Živković et al. (2009) determined the highest TPCs for leaves (90.35 mg GAE/g db) and flowers (33 mg GAE/g dry extract (de)), respectively. Vazquez et al. (2008) stated that TPC of chestnut (*C. sativa*) shell extracts ranged between 26.6 and 59.7 g GAE/100 g extract. The total phenolic content of chestnut shells reported by different authors is presented in Table 2.

The phenolic extract of the shells consists of different groups of compounds such as *ortho*-diphenols, flavonoids and tannins (Squillaci et al., 2018). The number of included active groups (OH or NH₂) and their positions affect the antioxidant capacity of phenolics. The *ortho*- position is more active than *para*- and *meta*- positions of compounds.

The antioxidant properties of *o*-diphenols can be attributed to hydrogen donation, i.e., their ability to improve radical stability by forming an intramolecular hydrogen bond between free hydrogens of their hydroxyl group and their phenoxyl radicals (Bendary et al., 2013; Visioli & Galli, 1998). Squillaci et al. (2018) analysed the *o*-diphenols contents of the inner and a combination of the inner and outer shells of chestnut (*C. sativa*) (Table 2). The amounts of *o*-diphenols of the inner and the combined shells were 19.55 and 98.06 mg CAE/g de, respectively. As can be understood from this result, when compared with the inner shell, outer one has much more *o*-diphenols content.

Additionally, it was observed that there was a direct relationship between the TPC and antioxidant activity (AA) of chestnut shells (Vazquez et al., 2008; Vella et al., 2018). In a study carried out conducted by Barreira et al. (2008), the AA of different parts of chestnut (leaf, flower, fruit, inner and outer shells) were analysed using five different biochemical assays including DPPH radicals scavenging activity, reducing power, inhibition of β -carotene bleaching, hemolysis inhibition and inhibition of lipid peroxidation. Among all assays, chestnut shells, especially the outer shell, exhibited the highest AA. The results of other studies as regards antioxidant activity of shells are given in Table 3.

Seo et al. (2016) conducted an investigation on the DNA protection and antioxidant potential of chestnut (*C. crenata*) shell extracts. They determined the radical-scavenging activity using an Electron Spin Resonance spectrometer and evaluated the protection against oxidative DNA damage. The assays applied included DPPH, ABTS, nitrite, hydroxyl, superoxide, reducing power, inhibition of linoleic acid oxidation, and prevention of oxidative DNA damage (Table 3). The extracts showed significantly antioxidant activity.

Sorice et al. (2016) conducted research to determine the potential anti-cancer effects of polyphenols extracted from chestnut (*C. sativa*) shells testing them on six different human cell lines (A375, H460, HT29, MCF7, HepG2, and HaCaT). After 48 h of treatment with extracted polyphenols, only HepG2 cells showed inhibition relative to EC₅₀, along with increased apoptosis and mitochondrial depolarization. The evaluation of the cytokinome before and after treatment revealed a decrease in vascular endothelial growth factor and the tumor necrosis factor, suggesting potential anti-angiogenic and anti-inflammatory effects of the extract. In conclusion, it was observed that polyphenols had significant effects on biomolecules related to cell proliferation, apoptosis, cell cycle and mitochondrial depolarization, as well as on cytokinomes and metabolomics profiles. It was also considered that bioactive compounds of chestnut shell were very resistance to *in vitro* digestion conditions. With these properties, chestnut shells were evaluated as a promising ingredient for the delivery of polyphenols in nutraceutical studies (Pinto et al. 2023).

Noh et al. (2010a) demonstrated the antioxidant effects of chestnut (*C. crenata*) inner shell extract in cell line and oxidative stress-induced animal models. The study showed that inner shell stimulation increased antioxidant enzyme activities while simultaneously decreasing lipid peroxidation in *tert*-butylhydroperoxide-treated HepG2 cells.

Table 2. Polyphenol content of chestnut shells

Latin name	Outer shell					References	
	TP	<i>o</i> -d	F	T	CT		
<i>C. sativa</i>	4.18-5.95 mg GAE/g db	2.68-3.71mg CAE/g db	2.34-2.80mg CE/g db	0.70-1.44mg GAE/g db		(Vella et al., 2019)	
<i>C. sativa</i>	510mg GAE/g extract		503mg CE/g extract			(Barreira et al., 2008)	
<i>C. sativa</i>	12mg GAE/g de		6.5mg CE/g de			(Zivkovic et al., 2009)	
<i>C. sativa</i>	2.22-105.66mg GAE/g wb					(de Vasconcelos et al., 2010c)	
<i>C. sativa</i>	45.01mg GAE/g db					(Mustafa et al., 2021)	
<i>C. crenata</i>					0.31-2.04% (db)	(Hwang et al., 2001)	
<i>C. sativa</i> × <i>C. crenata</i>	3.62mg GAE/g db	2.32mg CAE/g db	1.74mg CE/g db	1.11mg GAE/g db		(Vella et al., 2019)	
Latin name	Inner shell					References	
	TP	<i>o</i> -d	F	T	CT		HT
<i>C. sativa</i>	212.82- 251.76mg GAE/g db	101.99- 116.17mg CAE/g db	82.84- 103.15mg CE/g db	31.00- 36.46mg GAE/g db			(Vella et al., 2019)
<i>C. sativa</i>	475mg GAE/g extract		330mg CE/g extract				(Barreira et al., 2008)
<i>C. sativa</i>	3.37- 136.35mg GAE/g wb						(de Vasconcelos et al., 2010c)
<i>C. sativa</i>	54.04mg GAE/g db						(Mustafa et al., 2021)
<i>C. crenata</i>					7.83-71.42% (db)		(Hwang et al., 2001)
<i>C. sativa</i> × <i>C. crenata</i>	337.33mg GAE/g db	191.47mg CAE/g db	129.14mg CE/g db	107.91mg GAE/g db			(Vella et al., 2019)
<i>C. sativa</i>	43.69mg GAE/g de	19.55mg CAE/g de	7.94mg CE/g de		25.84mg GAE/g de	2.02mg GAE/g de	(Squillaci et al., 2018)
<i>C. crenata</i>	264.10- 558.12mg GAE/g extract		47.41- 166.28mg CE/g extract		85.13- 244.63mg CE/g extract		(Ham et al., 2015)
Latin name	Shell					References	
	TP	<i>o</i> -d	F	T	CT		HT
<i>C. sativa</i>	205.99mg GAE/g de	98.06mg CAE/g de	40.98mg CE/g de		162.49mg GAE/g de	12.94mg GAE/g de	(Squillaci et al., 2018)
<i>C. sativa</i>	190.12- 312.44mg GAE/g de	73.90- 148.72mg CAE/g de	47.75- 62.18mg CE/g de	118.97- 205.99mg GAE/g de			(Cacciola et al., 2019)
<i>C. sativa</i>	2.38-17.68mg GAE/g db	1.11-8.29mg CAE/g db	7.36mg CE/g db	3.48mg GAE/g db			(Vella et al., 2018)
<i>C. sativa</i>	533.81- 805.74mg GAE/g extract		49.92- 146.08mg CE/g extract				(Barreira et al., 2010)
<i>C. sativa</i>	143.00- 796.80mg GAE/g db		31.38- 43.33mg CE/g db				(Rodrigues et al., 2015)
<i>C. sativa</i>	33.32-49.14g GAE/100 g extract						(Nazzaro et al., 2012)
<i>C. sativa</i>	590.2g GAE/kg de						(Sorice et al., 2016)
<i>C. crenata</i>	136.12- 353.92mg GAE/100 mg db		367.43- 459.09mg CE/100 mg db				(Seo et al., 2016)

TP, Total Phenols; *o*-d, *ortho*-diphenols; F, Flavonoids; T, Tannins; CT, Condensed Tannins; HT, Hydrolysable Tannins; GAE, Gallic Acid Equivalents; CAE, Caffeic Acid Equivalents; CE, Catechin Equivalents; db, dry basis; wb, wet basis; de, dry extract

Table 3. Antioxidant activity of shells

Latin name	AB	DPPH radical	RP	β	FRAP	Unit	References
Outer shell							
<i>C. sativa</i>		39.7 27.1-	55.1	133		$\mu\text{g/ml}$ (EC_{50})	(Barreira et al., 2008)
<i>C. sativa</i>		79.2($\mu\text{g/ml}$ (EC_{50}))			2.25-5.11(mg AAE/g db)		(Vella et al., 2019)
<i>C. sativa</i>		21.4				% (0.2 mg extract/ml solution)	(Zivkovic et al., 2009)
<i>C. sativa</i>		5.04				mg TE/g db	(Mustafa et al., 2021)
<i>C. sativa</i> \times <i>C. crenata</i>		77.9($\mu\text{g/ml}$ (EC_{50}))			1.82(mg AAE/g db)		(Vella et al., 2019)
Inner shell							
<i>C. sativa</i>		32.7 32.9-	68.7	164		$\mu\text{g/ml}$ (EC_{50})	(Barreira et al., 2008)
<i>C. sativa</i>		35.3($\mu\text{g/ml}$ (EC_{50}))			113.63-149.27(mg AAE/g db)		(Vella et al., 2019)
<i>C. sativa</i> <i>C. crenata</i>	270.73	5.25 23.81	187.78	93.03 (LPI %)		mg TE/g db $\mu\text{g/ml}$ (EC_{50}) free phenolic extract	(Mustafa et al., 2021) (Tuyen et al., 2017)
<i>C. crenata</i>	538.70	28.41	209.56	93.35 (LPI %)		$\mu\text{g/ml}$ (EC_{50}) bound phenolic extract	(Tuyen et al., 2017)
<i>C. crenata</i> <i>C. sativa</i> \times <i>C. crenata</i>		159.79-174.61				mM TE/g extract	(Ham et al., 2015)
<i>C. sativa</i> \times <i>C. crenata</i>		35.8($\mu\text{g/ml}$ (EC_{50}))			113.25(mg AAE/g db)		(Vella et al., 2019)
Shell							
<i>C. sativa</i>					475-3808	nmol AAE/mg extract	(Vazquez et al., 2008)
<i>C. sativa</i>					624-3555	nmol AAE/mg extract	(Vazquez et al., 2009)
<i>C. sativa</i>					21.65-105.62	mmol AAE/ 100 g db	(Leclercq et al., 2010)
<i>C. sativa</i>					2268-3779	nmol AAE/mg extract	(Nazzaro et al., 2012)
<i>C. sativa</i>					13.62	mg AAE/g db	(Vella et al., 2018)
<i>C. sativa</i>		78.5				% inhibition	(Sorice et al., 2016)
<i>C. sativa</i>		82.41-159.99	79.25- 117.58	74.62- 151.27		$\mu\text{g/ml}$ (EC_{50})	(Barreira et al., 2010)
<i>C. sativa</i>		31.80-37.61 $\mu\text{g/ml}$ (EC_{50})			6008.70- 8083.50 μmol ferrous sulphate/g db		(Rodrigues et al., 2015)
<i>C. crenata</i>	98.60- 99.59	92.85-96.13				% (2 mg extract/ml solution)	(Seo et al., 2016)
Latin name	HI	Hydroxyl radical	TBARS	NR	Unit	References	
Outer shell							
<i>C. sativa</i>	91.4		7.87		$\mu\text{g/ml}$ (EC_{50})	(Barreira et al., 2008)	
<i>C. sativa</i>						(Vella et al., 2019)	
<i>C. sativa</i>		21.8			% (0.2 mg extract/ml solution)	(Zivkovic et al., 2009)	
<i>C. sativa</i>					mg TE/g db	(Mustafa et al., 2021)	
Inner shell							
<i>C. sativa</i>	47.5		11.5		$\mu\text{g/ml}$ (EC_{50})	(Barreira et al., 2008)	
<i>C. sativa</i>						(Vella et al., 2019)	
Shell							
<i>C. sativa</i>			27.29- 49.07		$\mu\text{g/ml}$ (EC_{50})	(Barreira et al., 2010)	
<i>C. crenata</i>				28.55-32.85	% (2 mg extract/ml solution)	(Seo et al., 2016)	

AB: ABTS radical; HI: Hemolysis inhibition; RP: Reducing power; TBARS: TBARS inhibition (lipid peroxidation); β : β -carotene bleaching inhibition; NR: Nitrite radical; LPI, Lipid Peroxidation Inhibition; TE, Trolox Equivalent; AAE, Ascorbic Acid Equivalent; db, dry basis

The inner shell extract also inhibited lipid peroxidation in the livers of mice fed a high-fat diet and treated with CCl₄. The researchers attributed this finding to scoparone and scopoletin. In addition, in an oxidative stress-induced *in vitro* system, the antioxidant capacity of scopoletin was reported to be relatively higher than that of scoparone. With a such high antioxidant capacity of chestnut shell, it is suggested as an effective antioxidant for future *in vivo* research and drug application (Hu et al. 2021).

Phenolic Acids and Flavonoids

Two subgroups of phenolic compounds are phenolic acids and flavonoids. Phenolic acids consist of hydroxycinnamic acids with a phenylpropane (C₆-C₃) structure, and hydroxybenzoic acids with a phenylmethane (C₆-C₁) structure (Acar & Gökmen, 2014). Flavonoids, on the other hand, are composed of flavan-3-ols, flavonols, flavones, isoflavones, flavanones and anthocyanidins, with their composition depending on the oxidation state of the central C ring (Dai & Mumper, 2010; Gan et al., 2019). These compounds are present not only in chestnuts but also in other fruits and vegetables. Barreira et al. (2008) reported that the by-product parts of *C. sativa* contain a significant amount of flavonoids. They noted that the outer shells of chestnuts (503 mg CE/g) had a higher flavonoid content than the inner ones (330 mg CE/g). Similar findings were reported by Squillaci et al. (2018) (Table 2). In another study, which reported lower values than the aforementioned research, Ham et al. (2015) examined the inner shell of chestnut (*C. crenata*) and determined its total flavonoid content to be 47.41-166.28 mg CE/g. The differences in results could be attributed to species diversity, ecological factors and extraction conditions. Liu et al. (2020) found that rutin and quercetin were the main flavonoids in chestnut shell.

In various studies conducted to determine phenolics profile of the chestnut shells (Table 4), gallic acid was reported as the most abundant phenolic compound (Nazzaro et al., 2012; Sorice et al., 2016; Squillaci et al., 2018; Rodrigues et al., 2023). Moreover, considerable amounts of protocatechuic and ellagic acids were also reported. Minor phenolic acids of chestnut shells were chlorogenic, syringic, *p*-coumaric and ferulic acids. Only Sorice et al. (2016) determined syringic acid in the shell of *C. sativa*, and provided an approximate result due to interference from condensed tannins. The total content of phenolic acids (12 phenolic acids) was reported as 223.62 and 119.17 mg/kg db for the inner and outer shells of *C. sativa*, respectively (Mustafa et al., 2021).

Catechins and their derivatives, involved in the flavan-3-ols group of flavonoids, are classified based on their structures as catechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate. Among the identified catechin derivatives in the chestnut shells so far are catechin, epicatechin and epigallocatechin (Table 4). Nazzaro et al. (2012), Aires et al. (2016) and Squillaci et al. (2018) detected catechin, epicatechin and epigallocatechin in the chestnut (*C. sativa*) shells by HPLC (RP-HPLC, HPLC-DAD/VIS-MS, HPLC/UV, respectively). Epigallocatechin was also reported in the shells by Aires et al. (2016). Additionally, Sorice et al. (2016) qualitatively determined the presence of epicatechin and epigallocatechin in the shell extracts.

Squillaci et al. (2018) observed that the inner shell and the combined inner and outer shells contained catechin in amount of 0.30 and 0.70 mg/g de, respectively, and epicatechin in amount of 0.32 and 0.71 mg/g de, respectively. The results showed that the contents of catechin derivatives in the outer shell were higher than those in the inner shell. The study of Mustafa et al. (2021) declared the differences in the amount of catechin and epicatechin between the inner and outer shells. In addition, for both shells, while dihydrocalcones (phloridzin and phloretin) were detected, flavanones (hesperidin and naringin) were not found in either shell. Despite their small amounts, rutin and quercetin from flavonols were identified in the chestnut shells (Nazzaro et al., 2012; Sorice et al., 2016). Along with these compounds, quercitrin, isoquercitrin, hyperoside, myricetin, isorhamnetin, kaempferol and kaempferol-3-glucoside from other flavonol compounds were also observed in both shell structures (Mustafa et al., 2021). It should also be noted that the content of these compounds varied with species diversity, origin, ecological factors, storage and selected extraction conditions (Cerulli et al. 2020).

Tannins

Characteristically, tannins have a tendency to bind and precipitate proteins. The ability of tannins to remove gelatin and other proteins in raw juices, as well as collagen proteins in animal hides, plays a crucial role in the juice and tanning industries, respectively. Tannins also cause sensations of astringency, which is characterized by feeling of dryness and puckering in the mouth. This occurs when tannins bind to salivary proteins (Crozier et al., 2006; Lee & Lawless, 1991; McRae & Kennedy, 2011). Tannins show various beneficial aspects, including antioxidant, anti-cancer, antimicrobial, anti-nutritional, anti-diabetic, anti-obesity and cardio-protective effects.

Tannins can be classified into two groups based on their structure: hydrolysable and condensed tannins. Hydrolysable tannins can be further divided into gallotannins, which are esters of glucose (most often β-D-glucose) or another polyol with gallic acid) and ellagitannins (with hexahydroxydiphenic acid) (Mammela et al., 2000; Salminen et al., 1999). The term "ellagitannin" is derived from ellagic acid, which is formed spontaneously from hexahydroxydiphenic acid in an aqueous solution through an intra-molecular esterification reaction (Vermerris & Nicholson, 2006). The main ellagitannins identified in chestnut include vescalagin, castalagin, vescalin, castalin, chestanin, acutissimin A and acutissimin B (de Vasconcelos et al., 2010c; Esposito et al., 2019; Mannelli et al., 2019; Martinez & Stagljar, 2003). Additionally, condensed tannins are oligomers and polymers of flavan-3-ol (catechin and its derivatives) units. They are referred to as catechin tannins, procyanidins, and proanthocyanidins (Anonymous, 2003; Karonen et al., 2004).

Tannin content of chestnut shell was reported as higher than that of phenolic acids and flavonoids (Pinto et al. 2021). Vella et al. (2018) reported the tannin content of chestnut shells as 3.48 mg GAE/g db. Rodrigues et al. (2023) determined total hydrolysable and condensed tannin content of chestnut shell as ranged between 1.00-3.33 and 1.02-3.58 mg/g, respectively.

Table 4. Phenolic acids, flavonoids and tannins profile of chestnut (*C. sativa*) shells

Phenolic acids							Unit	References
Gal	Pro	Ell	Chl	Syr	Cou	Fer		
Outer shell								
69.20		35.49	0.06	0.79	0.25	2.37	mg/kg db	(Mustafa et al., 2021)
0.14-0.36		0.14-0.19					mg/g wb	(de Vasconcelos et al., 2010c)
0.60-1.08		0.24-0.90					mg/g db	(Vella et al., 2019)
		0.74-5.98					mg/g db	(Vekiari et al., 2008)
Inner shell								
118.85		86.35	0.07	0.96	1.27	4.82	mg/kg db	(Mustafa et al., 2021)
29.62	3.43	0.63			0.32		mg/g de	(Squillaci et al., 2018)
0.22-0.35		0.03-0.07					mg/g wb	(de Vasconcelos et al., 2010c)
3.37-6.67		0.80-1.38					mg/g db	(Vella et al., 2019)
		0.54-0.79					mg/g db	(Vekiari et al., 2008)
Shell								
86.97-150.09	11.20-21.57	0.58-1.09	0.67-1.18	0.14-0.21	0.22-0.52	0.03-0.31	mg/g de	(Cacciola et al., 2019)
63.51	11.24	0.81			0.22		mg/g de	(Squillaci et al., 2018)
7.9-584.9		47.6-3542.6					µg/g db	(Aires et al., 2016)
4.53-9.31		0.04-1.05	0.02-0.54		0.16-0.23	0.01-0.20	µg/mg extract	(Nazzaro et al., 2012)
2.12		1.05		0.50			g/kg db	(Sorice et al., 2016)
Flavonoids							Unit	References
Cat	Epic	Epig	Rut	Que				
Outer shell								
91.34	7.94		2.45		0.79		mg/kg db	(Mustafa et al., 2021)
					n.d.		mg/g db	(Vella et al., 2019)
Inner shell								
110.93	6.30		3.48		0.53		mg/kg db	(Mustafa et al., 2021)
0.30	0.32				n.d.		mg/g de	(Squillaci et al., 2018)
							mg/g db	(Vella et al., 2019)
Shell								
0.70	0.71-1.28						mg/g de	(Cacciola et al., 2019)
15.1-295.9	0.71						mg/g de	(Squillaci et al., 2018)
0.38-1.33	9.0-91.6	13.6-213.4					µg/g db	(Aires et al., 2016)
	0.07-0.95		0.05				µg/mg extract	(Nazzaro et al., 2012)
			0.059		0.081		g/kg db	(Sorice et al., 2016)
Tannins				Hydroxy-coumarins			Unit	References
Ves	Cas	AcuA	AcuB	Sco				
Outer shell								
0.05-0.13	0.39-0.85	0.05-0.08	0.41-0.52				mg/g wb	(de Vasconcelos et al., 2010c)
Inner shell								
0.04-0.08	0.07-0.21	0.03-0.05	0.04-0.09		0.41		mg/g de	(Squillaci et al., 2018)
							mg/g wb	(de Vasconcelos et al., 2010c)
Shell								
					0.11-0.41		mg/g de	(Cacciola et al., 2019)
67.5-109.4	49.6-100.4	n.d.	n.d.		0.11		mg/g de	(Squillaci et al., 2018)
							µg/g db	(Aires et al., 2016)

(Gal, Gallic acid; Pro, Protocatechuic acid; Ell, Ellagic acid; Chl, Chlorogenic acid; Syr, Syringic acid; Cou, *p*-Coumaric acid; Fer, Ferulic acid; Cat, Catechin; Epic, Epicatechin; Epig, Epigallocatechin; Rut, Rutin; Que, Quercetin; Ves, Vescalagin; Cas, Castalagin; AcuA, Acutissimin A; AcuB, Acutissimin B; Sco, Scopoletin; n.d., not detected; wb, wet basis; db, dry basis; de, dry extract)

The contents of condensed and hydrolysable tannins related to the chestnut shell structure were provided in Table 4. In relation to these contents, Squillaci et al. (2018) conducted a study on the inner and combined shells of *C. sativa*. The amount of condensed tannins in both the inner shell (25.84 mg GAE/g de) and shell combination (162.49 mg GAE/g de) was higher than that of hydrolysable tannins (2.02 mg GAE/g de and 12.94 mg GAE/g de, respectively). Hence, the outer shells had higher contents of both condensed and hydrolysable tannins compared to the inner shells. However, in *C. crenata* chestnut variety, Hwang et

al. (2001) determined that the condensed tannins content of the inner shell was higher than that of the outer shell. The total procyanidins (condensed tannins) of the inner and outer shells of chestnut were reported as 6.28-110.35 and 3.14-35.11 mg/g wb by de Vasconcelos et al. (2010c). Ellagitannins, including vescalagin, castalagin, acutissimin A and acutissimin B were analysed in the chestnut shells (Aires et al., 2016; de Vasconcelos et al., 2010c). All these compounds were found in both shells according to de Vasconcelos et al. (2010c), but; in the study by Aires et al. (2016), acutissimin A and acutissimin B were not detected.

Table 5. Minor compounds of unpeeled and peeled European chestnut (*C. sativa*) on wet and dry bases (United States Department of Agriculture, 2019a, 2019b, 2019c, 2019d)

	Unity (per 100 g)	Wet Basis			Dry Basis		
		unpeeled	peeled	shell	unpeeled	peeled	shell
Minerals							
Ca	mg	27	19	8	67	64	3
Fe	mg	1.01	0.94	0.07	2.38	2.39	
Mg	mg	32	30	2	74	74	0
P	mg	93	38	57	175	137	38
K	mg	518	484	34	986	991	
Na	mg	3	2	1	37	37	0
Zn	mg	0.52	0.49	0.03	0.35	0.35	0
Cu	mg	0.447	0.418	0.029	0.65	0.653	
Mn	mg	0.952	0.336	0.616	1.3	1.183	0.117
Vitamins							
Vitamin C, total ascorbic acid	mg	43	40.2	2.8	15	15.1	
Thiamin	mg	0.238	0.144	0.094	0.295	0.354	
Riboflavin	mg	0.168	0.016	0.152	0.36	0.054	0.306
Niacin	mg	1.179	1.102	0.077	0.85	0.854	
Panhotenic acid	mg	0.509	0.476	0.033	0.897	0.901	
Vitamin B-6	mg	0.376	0.352	0.024	0.663	0.666	
Folate, total	µg	62	58	4	109	110	
Folic acid	µg	0	0	0	0	0	0
Folate, food	µg	62	58	4	109	110	
Folate, DFE	µg	62	58	4	109	110	
Vitamin B-12	µg	0	0	0	0	0	0
Vitamin A, RAE	µg	1	1	0	0	0	0
Retinol	µg	0	0	0	0	0	0
Vitamin A, IU	IU	28	26	2	0	0	0
Vitamin D (D2+D3)	µg	0	0	0	0	0	0
Vitamin D	IU	0	0	0	0	0	0
Fatty acids, total saturated	g	0.425	0.235	0.190	0.837	0.736	0.101
14:0	g	0.01	0.005	0.005	0.019	0.017	0.002
16:0	g	0.384	0.212	0.172	0.755	0.664	0.091
18:0	g	0.021	0.012	0.009	0.042	0.037	0.005
Fatty acids, total monounsaturated	g	0.78	0.43	0.350	1.535	1.349	0.186
16:1	g	0.021	0.012	0.009	0.042	0.037	0.005
18:1	g	0.749	0.413	0.336	1.473	1.296	0.177
20:1	g	0.01	0.005	0.005	0.019	0.017	0.002
Fatty acids, total polyunsaturated	g	0.894	0.493	0.401	1.758	1.546	0.212
18:2	g	0.798	0.44	0.358	1.57	1.381	0.189
18:3	g	0.095	0.053	0.042	0.188	0.165	0.023
Aminoacids, essential							
Arginine	g	0.173	0.116	0.057	0.457	0.359	0.098
Histidine	g	0.067	0.045	0.022	0.177	0.139	0.038
Threonine	g	0.086	0.058	0.028	0.228	0.179	0.049
Isoleucine	g	0.095	0.064	0.031	0.252	0.198	0.054
Leucine	g	0.143	0.096	0.047	0.378	0.297	0.081
Lysine	g	0.143	0.096	0.047	0.378	0.297	0.081
Methionine	g	0.057	0.038	0.019	0.151	0.118	0.033
Phenylalanine	g	0.102	0.069	0.033	0.27	0.212	0.058
Valine	g	0.135	0.091	0.044	0.357	0.280	0.077
Tryptophan	g	0.027	0.018	0.009	0.071	0.056	0.015
Amino acids, non-essential							
Cystine	g	0.077	0.052	0.025	0.202	0.159	0.043
Alanine	g	0.161	0.109	0.052	0.427	0.335	0.092
Tyrosine	g	0.067	0.045	0.022	0.177	0.139	0.038
Aspartic acid	g	0.417	0.281	0.136	1.103	0.886	0.217
Glutamic acid	g	0.312	0.21	0.102	0.824	0.647	0.177
Glycine	g	0.124	0.084	0.040	0.329	0.258	0.071
Proline	g	0.127	0.086	0.041	0.336	0.264	0.072
Serine	g	0.121	0.081	0.040	0.319	0.251	0.068

(The values in the column for shells, not included in the original data, were obtained by subtracting the values of peeled chestnuts from those of unpeeled one.)

Hydroxycoumarins

Hydroxycoumarins are a group of phenolic compounds similar to phenolic acids, flavonoids, tannins, etc. Scopoletin and scoparone, which are coumarins, have been found in chestnut shells by various researchers (Jung et al., 2019; Noh et al., 2010a; Noh et al., 2011; Noh et al., 2010b; Squillaci et al., 2018). In the study by Squillaci et al. (2018), it was determined that scopoletin content was 0.41 mg/g de in the inner shell and 0.11 mg/g de in the combined shells of *C. sativa*. As the result suggests, inner shell is the main source of scopoletin for chestnut. From the inner shell extract of *C. crenata*, Noh et al. (2010a) and Noh et al. (2011) isolated two compounds, scoparone and scopoletin, as the main components using repeated column chromatography. Noh et al. (2010a) investigated the effect of scoparone and scopoletin on *t*-BHP treated HepG2 cells under oxidative stress conditions, focusing on intracellular ROS generation and antioxidant enzyme activities. They confirmed that both compounds have antioxidant effects, with scopoletin demonstrating relatively higher antioxidant capacity than scoparone in an in vitro oxidative stress-induced system. The findings of Noh et al. (2011) suggest that scoparone provides a stronger antioxidant effect than scopoletin under oxidative stress induced by ethanol in an in vitro system. These studies revealed that the inner shell extract of chestnut has an inhibitory effect on lipid accumulation and a preservative effect on antioxidant potential in the liver. Furthermore, chestnut inner shell showed protective effects against ethanol-induced oxidative damage, potentially due to its inhibition of lipid accumulation, peroxidation and the enhancement of the antioxidant defense system in the liver. Scopoletin, scoparone and quercetin have been referred to as “natural pesticide” by Sanzani et al. (2014) due to their antifungal characteristics and antioxidant properties.

Pigments

Chestnut shells, which are by-products of chestnut processing, contain natural brown pigments. Due to their strong coloring power, antioxidant activity and bacteriostatic effects, they can be evaluated as a food additive (Chen et al., 2018; Gao et al., 2019; Li & Song, 2004; Yao & Qi, 2016). The pigments in chestnut shells associated with lignin and cellulose in cell wall components (Zhu et al., 2022). Chestnut shells primarily consist of Klason lignin, α -cellulose and hemicellulose structures. The high lignin content makes them suitable for use in the adhesive sector (Cruz-Lopes et al., 2020; Gullón et al., 2018). Furthermore, positive findings have been observed in hair coloring due to colorant properties of chestnut shells (Rose et al., 2018; Zhu et al., 2022). These natural pigments have been reported to contain 15% melanin (Yao & Qi, 2016; Yao et al., 2016; Yao et al., 2012). Melanin has also isolated from various food sources as *Osmanthus fragrans*' seeds (Wang et al., 2006), black cumin (*Nigella sativa* L.) seeds (Al-Mufarrej et al., 2006), silky fowl (Chen et al., 2008) and Atlantic salmon (Leclercq et al., 2010). Melanins produced by bacteria, fungi, animals and plants, and their structures are enigmatic, complex and variable. It is worth noting that melanins are large, extended, amorphous, irregular, heterogeneous, highly crosslinked and hydrophilic (Enochs et al., 1993; Łopusiewicz, 2016).

Melanins are categorized into three groups according to the type of polymerization: eumelanins, pheomelanins and allomelanins. Eumelanins are generated by polymerization of a nitrogenous melanogen. Pheomelanins are generated by the polymerization of a sulphurated melanogen. Additionally, allomelanins are also generated by polyphenols (Velisek et al., 2007). Allomelanins, which are a heterogeneous group and commonly found in some fungi, higher plants. These are pigments that show color range from dark brown to black comprising polymers of simple phenols and their quinones (Hendry, 1995).

Yao and Qi (2016) reported a relationship between melanin and antioxidant activity, citing the investigations of Sava et al. (2001) and Huang et al. (2011). The antioxidant activity of melanins depends on their oxidative state and chemical structures. The oxidation of melanins decreases the antioxidant capacity, leading to the formation of quinone groups from phenolic ones. Consequently, the phenolic content serves as an indicator to evaluate the oxidative degree of melanins (Hung et al., 2002; Yao & Qi, 2016). Additionally, melanins have been reported to have other beneficial effects, including immune-stimulating properties (Sava et al., 2001), anti-HIV (Montefiori & Zhou, 1991), anti-venin (Hung et al., 2004), anti-tumor (Kamei et al., 1997) and radioprotection (Hill et al., 1987) activities.

Other Bioactive Phytochemicals

In Table 5 (United States Department of Agriculture, 2019a, 2019b, 2019c, 2019d), minerals, vitamins, essential fatty acids and essential amino acids contents of unpeeled, peeled and shell of fresh and dried European chestnuts (*C. sativa*) are provided. The difference in values between unpeeled and peeled chestnuts provides information about the properties of the shell. Some minerals such as phosphorus (38 mg/100 g), calcium (3 mg) and manganese (0.117 mg), as well as some vitamins like riboflavin (0.306 mg), can be found in the shell structure. The presence of vitamin C in chestnut (*C. crenata*) shell extracts was determined by Seo et al. (2016) who determined vitamin C levels to be 15.57 and 28.97 mg AA/100 mg db in water and ethanol extracts, respectively. Rodrigues et al. (2015) examined the shell of three European chestnut varieties for total vitamin E content and found that the total vitamin E content of the shells ranged from 481 to 962 mg/100 g sample. The author also declared that γ -tocopherol was the precursor of E vitamin in shells. On the other hand, de Vasconcelos et al. (2010c) analysed α -, γ - and δ -tocopherols in the inner and the outer shells of *C. sativa*, but did not detect any of them. The differences between studies could be related to the growing conditions and ecological practices.

Table 5 indicates that the quantities of total fatty acids in the shells of fresh and dried European chestnuts are 0.941% and 0.499%, respectively. The rate of essential fatty acids (linoleic and linolenic acids) in the shells is 42.5% for each sample.

Chestnut shell was found to be a rich source of amino acid. Rodrigues et al. (2015) also investigated the amino acid content of chestnut shells, and detected 17 different amino acids, including non-essential (alanine, serine, aspartic acid, glutamic acid, glycine, proline, tyrosine and ornithine) and essential (histidine, isoleucine, leucine, lysine, phenylalanine, threonine, arginine, valine and

methionine). Among these amino acids, arginine was the dominant one, with levels ranging from 355 to 721 mg/100 g db, while ornithine had the lowest concentration in the shell ranging from 1 to 9 mg/100g db.

Conclusion

This review summarizes the peeling applications of chestnut (*Castanea* spp.) and highlights the bioactive compounds of shells. Evaluating these shells is important from both environmental and health perspectives. These by-products have significant economic and nutritional value due to their high content of bioactive compounds such as phenolic acids, flavonoids, tannins, hydroxycoumarins, pigments and certain vitamins. Over time, the unknown properties of the inner and outer shells have emerged, and the assessment of both shells can differ. Therefore, there is a need for peeling machines that minimise loss and maximise efficiency, allowing to the separate collections of shells.

In addition to their anti-wrinkle, anti-aging, antioxidant, antibacterial and anticancer effects, the inner shell of the chestnut gains more importance in the pharmaceutical industry due to its anti-asthmatic properties. Moreover, it has gained attention in the cosmetic industry for its high deodorizing effect and potential as a natural source for hair dyeing. Chestnut shells can also be utilised as colorant, antioxidant and antimicrobial agents in the food, food supplement and nutraceutical industries.

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Conflict of interest

The author declares no conflict of interest.

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