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Morning Antioxidants: Molecular Insights on the Chemistry of Coffee Components

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Review

Morning Antioxidants: Molecular Insights on the Chemistry of Coffee Components

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Abstract: Coffee is not only a delicious beverage but also an important dietary source of natural antioxidants. We live in an oxidative world where it is impossible to avoid pollution, stress, food additives, radiation, and other sources of oxidants that eventually lead to severe health disorders. Fortunately, there are chemicals in our diet that counteract the hazards posed by the reactive species that trigger oxidative stress. They are usually referred to as antioxidants; some of them can be versatile compounds that exert such a role in various ways. This review summarizes, from a chemical point of view, the antioxidant effects of relevant molecules found in coffee. Their ways of action and trends in activity are analyzed, considering the data gathered so far from both theory and experiments. The influence of the media and pH in aqueous solution, and structure-activity relationships are discussed. The protective role of the explored compounds is examined. A particular section is devoted to derivatives of some coffee components, and another one to their bioactivity. The data used in the analysis come from theoretical anc computational protocols, which have been proven to be very usefull in this context. Hopefully, the information provided here will promote further investigations into the amazing chemistry contained in our morning cup.

Keywords: free radicals; scavengers; reaction mechanisms; kinetics; trends in activity; coffee components

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1. Introduction

Since ancient times, natural products have been widely appreciated by humankind. The main reason is that they are beneficial for health issues and our general wellbeing. However, only in the last centuries technology and science developments have allowed to pass empiricism and deepened into the knowledge about the bioactive substances found in natural products, as well as on their specific functions and medicinal effects.

Regarding coffee, its origin has been traced to Ethiopia,[1] which is currently the fifth producer worldwide.[2] The legend says that goat herders noticed their animals restless at night after eating the berries of the coffee plant. After trying the fruit, they felt energized and got used to consuming it. Such a stimulating effect is still one of this beverage's appeals, albeit coffee is much better understood and more widely consumed today than twelve centuries ago. In fact, coffee is currently one of the most consumed beverages and the second commodity worldwide.[3]

According to the annual review (2021/2022) of the International Coffee Organization, the *Arabica* variety represents 56% of the coffee production, and *Robusta* the other 44% (Figure 1). The top producers are Brazil, Vietnam and Colombia, in that order, with ~58, 30 and 14 billion bags of 60 kg, respectively. On the other hand, the top consumers are USA, Brazil, Germany, Japan and France (27, 22, 8.7, 7.5 and 6.2 billion bags of 60 kg, respectively).

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Based on the data obtained from the Scopus database (Figure 2), the number of scientific publications on coffee has grown exponentially over the years. The same trend is followed by its antioxidant properties. Today, many of the chemical components of coffee have been identified and a large proportion of them have been investigated. For example, there are 65,825 reports on caffeine, 2,634 of them published last year. The oldest record found in the search for antioxidative properties of coffee dates back to 1940.[4] It dealt with the "antioxygens" produced by roasting and considered several species. Among them, pyrrole, proline, thioglycolic acid, and caffeic acid were identified as those with the highest protection factor against rancidity.

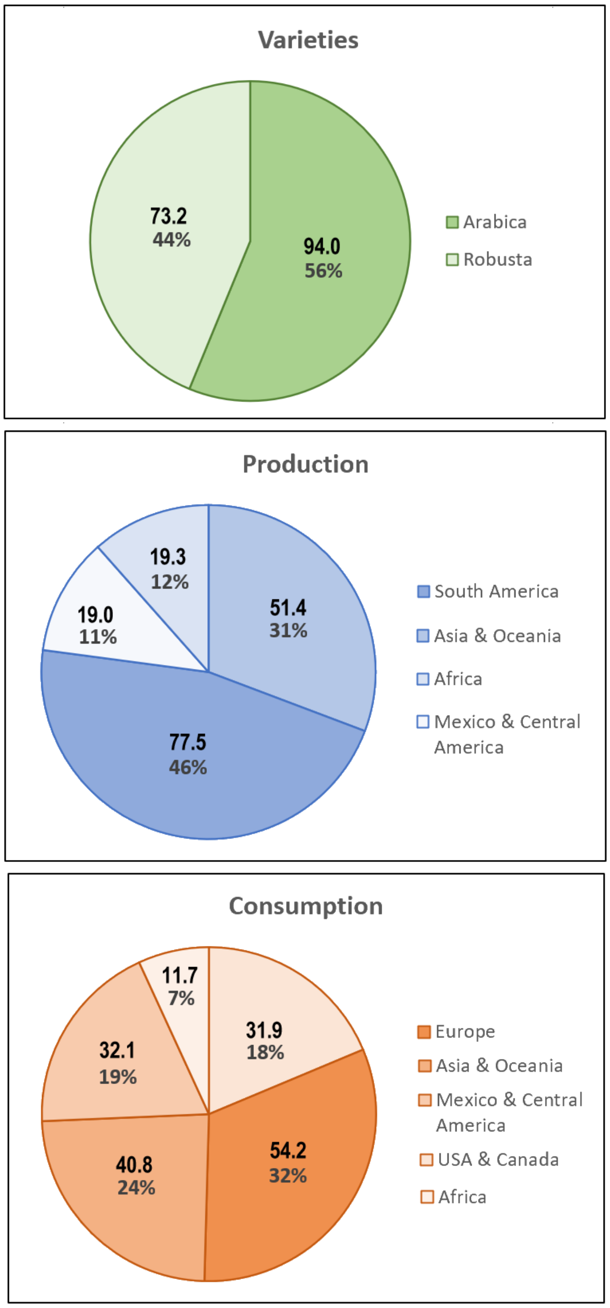

Figure 1. Coffee production and consumption stats (in million bags of 60 kg), according to the annual review (2021/2022) of the International Coffee Organization. https://www.ico.org/documents/cy2022-23/annual-review-2021-2022-e.pdf, accessed March 14th, 2023.

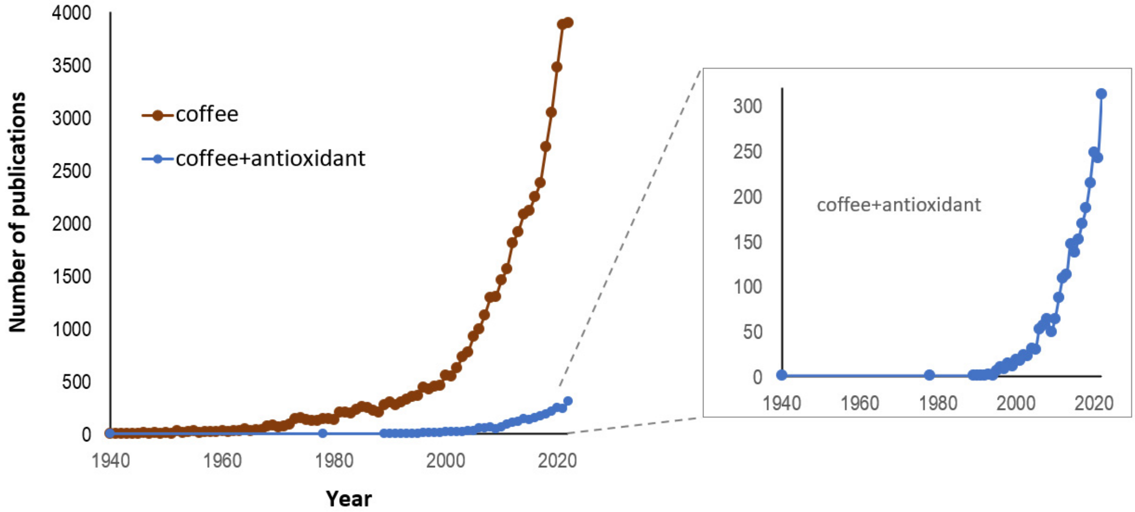

Figure 2. Number of published researches on coffee and coffee+antioxidant, according to Scopus, consulted on February 14, 2023.

Antioxidants, in general, are appealing substances from both scientific and pragmatic points of view. They help counteracting the dangerous effects of oxidative stress (OS),[5–52] which arises from the unbalance between production and consumption of oxidants in living systems. It is considered a chemical stress and has been associated with multiple health issues, including neurodegeneration,[35,39,53–137] cancer,[138–181] cardiovascular diseases,[49,182–228] diabetes,[10,229–252] rheumatoid arthritis,[253–263] renal [264–295] and pulmonary[296–319] failures, ocular disorders,[20,320–334] preeclampsia and fetal development complications.[203,335–368]

Antioxidant protection is one of the many health benefits attributed to coffee,[369–408] and other natural products.[409–436] However, not all its components exhibit such activity and, those that do, have diverse mechanisms of action and efficiency. Phenolic compounds, in general, are recognized as very efficient for counteracting the deleterious effects of OS.[437–455] Phenolic acids, in particular, are among the most potent antioxidants present in coffee.[456–461] Other components that have been identified as efficient antioxidants are melanoidins,[462–465] heterocycles[466,467] Maillard reaction products,[456,466–469] and some volatile compounds.[470–474] Regarding caffeine, some studies suggest that it acts as an antioxidant, [465,475,476] while others indicate that the antioxidant properties of coffee are not directly related to caffeine but to the presence of other components.[401,477,478]

Quantifying antioxidant activity is a challenging task. This is probably because there is no universal assays to do it,[479] and because the available ones depend on the reaction mechanism, which can vary from one antioxidant to another. In fact, they have been classified as electron transfer and hydrogen atom transfer-based assays. In addition, some of these assays are meant to estimate the antioxidant capacity of total phenols. Thus, they are not meant to differentiate among different phenolic compounds. Moreover, it has been reported that conflicting trends may be obtained when using different experimental techniques to evaluate the antioxidant activity of phytochemicals.[480]

When using theoretical and computational chemistry, other difficulties arise. Probably, the most important ones are: (i) the unavoidable use of simplified models for mimicking chemical environments; (ii) the necessary balance between accuracy and computing time that must be taken into account when a particular level of theory is chosen; (iii) the fact that for establishing reliable trends, calculations must be performed using the same methodology and approximations; (iv) the importance of considering all the possible mechanisms and sites of reaction.[481] Therefore, it becomes evident that assessing antioxidant activity is a complex task, regardless of if it is pursued using experimental or theoretical approaches.

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There are previous publications where experimental techniques were used to evaluate the antioxidant activity of coffee and its components *in vitro*, have been thoroughly reviewed.[369,370] Therefore, molecular insights on such activity are the main focus of the analyses and discussion here. The data used to do that derived from computational and theoretical strategies, that have been demonstrated to be usefull and reliable to study the antioxidant chemistry. Several aspects are considered, including structure-activity relationships, the influence of solvent and pH, reaction mechanisms, and the influence of redox metals. Trends in antioxidant activity are proposed for several coffee components and compared with Trolox as a reference. Hopefully, the reviewed data will help improving the current knowledge on the chemical aspects related to the antioxidant effects of coffee and promote further investigations on the chemistry of this fantastic beverage.

2. Chemical overview

Chemical components are responsible for the taste, aroma and bioactivities of coffee. However, its chemical composition is complex and depends on the variety, growing conditions, and processing.[482] Nevertheless, it has been reported that the main components of raw coffee beans are carbohydrates, which account for about 60% of their total weight.[483] They also have significant amounts of cellulose, grease, proteins, amino acids, tannic acid, and starch. In addition, there is a diversity of other minor and trace substances in coffee beans. There are numerous publications providing detailed information on the chemical composition of coffee.[483–488] Thus, it is going to be only briefly summarized here (Table 1).

Table 1. Chemical composition of coffee beans.

Family	Compounds	Ref.
Alkaloids	Caffeine, theobromine, theophyline, trigonelline, nicotinic acid, pyrrolomorpholine spiroketal alkaloids.	[489–494]
Amino acids	Alanine, asparagine, leucine, glycine, aspartic acid, histidine, phenylalanine, serine, lysine, valine	[495–498]
Carotenoids	β-carotene, α-carotene, violaxanthin, neoxanthin.	[499,500]
Fatty acids	Linoleic, palmitic, oleic, stearic, arachic, docosanoic, tetracosanoic, eicosenoic, myristic acid, linolenic acids.	[496,497,501]
Flavonoids	Quercetin, catechin, epicatechin, picatechin gallate, kaempferol, luteolin, fisetin, rutin, myricetin, quercitrin, pigenin.	[502,503]
Organic acids	Acetic, citric, lactic, quinic, oxalic, malic, succinic, formic, tartaric acids.	[491,504–506]
Phenolic acids	Caffeic, chlorogenic, cinnamic, p-hydroxybenzoic acid, ferulic, p-coumaric, vanillic, benzoic, protocatechuic, gentosic, cinapic, caftaric acids.	[483,507–510]
Sugars	Sucrose, fructose, glucose, arabinose.	[511–514]
Terpenes	Ursolic acid, caffruones, caffruenols, tricalysiolides, caffarolides, bengalensol, mascarosides, villanovane, atractyligenin, cafestol, kahweol, dehydrocafestol, cafestal.	[490,502,503,515–519]
Volatiles	aldehydes, esters, ketones, furans, thiophenes, pyrazine, pyrroles, thiazoles, olefins, alcohols, oxazoles, pyridine, geosmin.	[520–526]

3. Bioactivity overview

The versatile bioactivity of coffee has also been thoroughly reviewed.[527–532] Coffee has numerous health benefits from its chemical composition, provided it is moderately consumed. Some of them are summarized in Table 2. However, as it is the case with almost everything, amounts mediate the balance between benefits and harms. It has been pointed out that high consumption of coffee may compromise coronary health, threaten pregnant and postmenopausal women, and cause addiction (withdrawal would trigger muscle fatigue and related problems).[527]

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Table 2. Some health benefits of coffee components.

Benefits	Key components	Ref.
Antibacterial	caffeic acid	[533–535]
	caffeic acid phenethyl ester	[536,537]
	chlorogenic acids	[538]
	eugenol	[539–547]
	ferulic acid	[548–551]
	furaneol	[552]
	guaiacol	[553–555]
	isoeugenol	[547,556–559]
	protocatechuic acid	[560–565]
	scopoletin	[566–571]
	vanillic acid	[561,572,573]
Anticarcinogenic	4-vinylguaiacol	[574,575]
	cafestol and kahweol	[576–579]
	caffeic acid	[580–585]
	caffeic acid phenethyl ester	[586–590]
	chlorogenic acids	[538,591]
	eugenol	[592–608]
	ferulic acid	[609–623]
	quercetin	[624–628]
	mangiferin	[629]
	protocatechuic acid	[630–636]
	tannic acid	[637–646]
	theobromine	[647–649]
	vanillic acid	[650–654]
	vanillin	[655–663]
Antidiabetic	cafestol	[664,665]
	caffeic acid	[666–670]
	caffeol	[483,671]
	chlorogenic acids	[666,672–677]
	isoeugenol	[678]
	scopoletin	[679–683]
	trigonelline	[684]
Antifungal	caffeine	[685]
	eugenol	[686–691]
	furaneol	[552]
	isoeugenol	[692–695]
	vanillin	[696–698]
Antiinflamatory effects	4-ethylguaiacol	[699–701]
	caffeine	[702]
	dicaffeoylquinic acids	[703]
	dihydrocaffeic acid	[704]
	eugenol	[539,705–710]
	ferulic acid	[711,712]
	flavonoids	[629]
	mangiferin	[703]
	phenolic acids and	[629]
	pyrocatechol	[713]
	p-coumaric acid	[714–719]
	rutin	[703]
	theophylline	[720–724]
	vanillic acid	[725–732]
	vanillin	[733–739]
	vanillyl alcohol	[740]
Antiobesity	chlorogenic acids	[672,741–744]
	kahweol	[745–747]

Cardioprotection	caffeic acid	[748–752]
	chlorogenic acids	[591,753–756]
	dihydrocaffeic acid	[757]
	ferulic acid	[758–764]
Cognitive enhancement	paraxanthine	[765,766]
	protocatechuic acid	[767–770]
	theobromine	[771,772]
	vanillic acid	[773,774]
Gastroprotection	chlorogenic acids	[591]
	vanillin	[775,776]
Hepatoprotection	caffeic acid	[777–780]
	chlorogenic acids	[591]
	dihydrocaffeic acid	[453]
	paraxanthine	[781–783]
	theobromine	[784]
	vanillin	[785–788]
Immunoregulation	p-coumaric acid	[718]
	protocatechuic acid	[789,790]
Kidney protection	protocatechuic acid	[791–795]
	theobromine	[796–799]
Neuroprotection	caffeine	[800–821]
	caffeic acid	[483,822–824]
	chlorogenic acids	[483,825–834]
	dihydrocaffeic acid	[835]
	eugenol	[836]
	ferulic acid	[837–847]
	isoeugenol	[836,848]
	paraxanthine	[816,849–852]
	protocatechuic acid	[853–866]
	quercetin	[624,867–876]
	scopoletin	[877–882]
	tannic acid	[883–888]
	theobromine	[889–891]
	trigonelline	[483]
	lipid-Lower	[736,892–896]
	vanillic acid	[897–900]
	vanillyl alcohol	[901]
Lipid-Lowering Effects	caffeic acid	[902]
	chlorogenic acids	[591,671,902,903]

Based on the data in Table 2, it becomes evident that moderate consumption of coffee, i.e., one to four cups a day,[532] may provide beneficial effects. In particular, for inflammation, obesity, diabetes, cancer, cardiovascular diseases, microbial infections, and neurodegeneration. Since antioxidant activity is the main focus of this review, the following section has been entirely devoted to it.

4. Derivatives

Considering the myriad of benefits offered by coffee components, it is not surprising that many investigations have been devoted to designing and synthesizing derivatives based on their molecular frameworks. Many of them keep the bioactivity of the parent molecules, and many others have shown new and improved actions. Albeit a detailed analysis of this point escapes the purpose of this review, it seems worthwhile summarizing (Table 3) some of the great efforts made so far to obtain new molecules from coffee components. Thus, the interested reader can get more comprehensive information on this topic from the provided references.

Table 3. Some previous studies on derivatives based on antioxidants found in coffee.

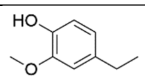
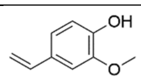
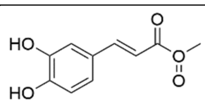
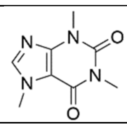
Parent molecule	Ref.
caffeic acid	[752,904–915]
caffeine	[916–924]
chlorogenic acid	[925–932]
eugenol	[933–952]
ferulic acid	[609,611,953–985]
guaiacol	[986–988]
isoeugenol	[694,989–992]
isoferulic acid	[993,994]
p-coumaric acid	[716,995–1001]
protocatechuic acid	[1002–1008]
scopoletin	[683,1009–1020]
theobromine	[1021–1029]
theophylline	[1030–1051]
vanillic acid	[1052–1058]
vanillin	[1059–1085]
xanthine	[1086–1109]

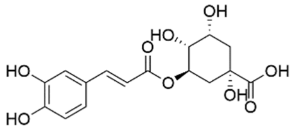
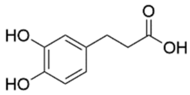
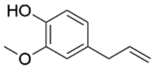
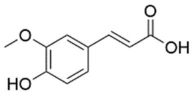
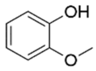
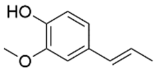
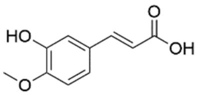
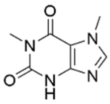
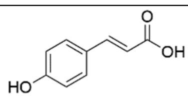
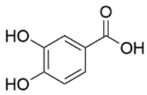
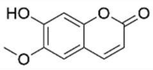
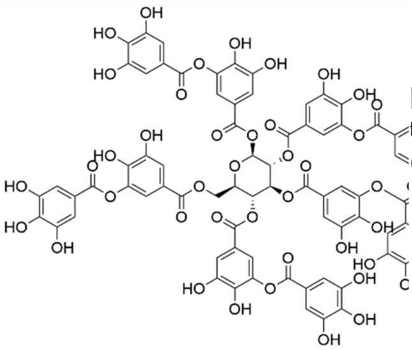
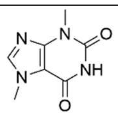
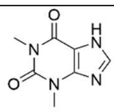
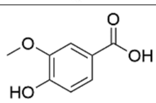
Theoretical and computational chemistry plays an important role in the design of new compounds. There are numerous tools available that allow evaluating important properties of derivatives, particularly when they are meant to be used as medical drugs. Some usefull descriptors in this context are those known as ADME (Absorción, Distribución, Metabolismo y Excreción) properties. They comprise the ctanol/water partition coefficient (logP), molecular weight, number of H bond donors, number of H bond acceptors, molar refractivity, number of non-hydrogen atoms, and polar surface area. There are other important properties to consider such as syntetic accessibility and toxicity. Using computational strategies allows building candidates, sampling the chemical space and evaluating the potential of the new molecules for the intended pupose. In addition, these strategies save time, resources, and even animal testing. Thus, they have become relevant tools for the development of new formulations with health benefits.

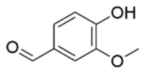
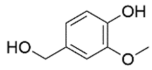
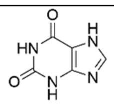
5. Antioxidant activity

Many of the chemicals found in coffee are considered as antioxidants. Twenty of them (Table 4) were selected to illustrate such activity in more detail. The references in this table correspond only to a fraction of the literature supporting their antioxidant activity. Otherwise, they would be too many to be included here. Actually, may of the health benefits of coffee mentioned in the previous section have been attributed to the antioxidant activity of its components.

Table 4. Some antioxidants found in coffee.

Common name	Structure	IUPAC name	Ref.
4-ethylguaiacol		4-ethyl-2-methoxyphenol	[1110–1112]
4-vinylguaiacol		4-ethenyl-2-methoxyphenol	[1113–1115]
caffeic acid		(E)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid	[669,748,1116–1128]
caffeine		1,3,7-trimethylpurine-2,6-dione	[806,1129–1132]

chlorogenic acid		(1S,3R,4R,5R)-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,4,5-trihydroxycyclohexane-1-carboxylic acid	[404,405,748,1116,1133–1143]
dihydrocaffeic acid		3-(3,4-dihydroxyphenyl)propanoic acid	[1144–1146]
eugenol		2-methoxy-4-prop-2-enylphenol	[706,707,934,1147–1160]
ferulic acid		(E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid	[711,712,837,1114,1161–1168]
guaiacol		2-methoxyphenol	[1169,1170]
isoeugenol		2-methoxy-4-[(E)-prop-1-enyl]phenol	[547,559,1171]
isoferulic acid		(E)-3-(3-hydroxy-4-methoxyphenyl)prop-2-enoic acid	[1172–1175]
paraxanthine		1,7-dimethyl-3H-purine-2,6-dione	[1176]
p-coumaric acid		(E)-3-(4-hydroxyphenyl)prop-2-enoic acid	[714,1122,1177–1181]
protocatechuic acid		3,4-dihydroxybenzoic acid	[561,1182–1195]
scopoletin		7-hydroxy-6-methoxychromen-2-one	[1196–1201]
tannic acid		[2,3-dihydroxy-5-[[(2R,3R,4S,5R,6S)-3,4,5,6-tetrakis[[3,4-dihydroxy-5-(3,4,5-trihydroxybenzoyl)oxybenzoyl]oxy]oxan-2-yl]methoxycarbonyl]phenyl] 3,4,5-trihydroxybenzoate	[1202–1212]
theobromine		3,7-dimethylpurine-2,6-dione	[1213,1214]
theophylline		1,3-dimethyl-7H-purine-2,6-dione	[1214–1216]
vanillic acid		4-hydroxy-3-methoxybenzoic acid	[1217–1221]

vanillin		4-hydroxy-3-methoxybenzaldehyde	[788,1112,1218,1222–1226]
vanillyl alcohol		4-(hydroxymethyl)-2-methoxyphenol	[1227,1228]
xanthine		3,7-dihydropurine-2,6-dione	[1176,1213]

Antioxidant activity (AOx) can arise from a variety of processes. This review focuses on chemical one, albeit there are other protection routes that involve enzymatic systems. From a chemical point of view, AOx can be roughly grouped into the following categories.

AOX-I (or primary AOX, or chain braking, or free radical scavenging activity):

It involves the direct reaction with oxidants, mainly free radicals yielding less reactive species or ending the radical chain process. During such a process, the antioxidant acts as a sacrificial target that prevents the oxidation of crucial biomolecules, such as DNA, proteins, and lipids. However, the amounts of these biomolecules in living organisms are significantly higher than those of chemical antioxidants that might be consumed in the diet or as dietary supplements. Consequently, a molecule must react with oxidants faster than the biological target to be efficient as a primary antioxidant. This makes imperative to establish some quantitative thresholds that allow identifying a particular chemical as a primary antioxidant. The rate constants of the $^{\bullet}$OOH damage to polyunsaturated fatty acids have been proposed to that purpose.[481] It ranges from 1.18×10^3 to 3.05×10^3 $M^{-1}s^{-1}$,[1229] at acid pH values, i.e. when the molar fraction of HOO$^{\bullet}$ is ~1. Since lipids are the most easily oxidized among the biomolecules mentioned above, i.e., those reacting the fastest with free radicals, it is expected that any molecule capable of protecting them from oxidation would also be capable of protecting proteins and DNA. An important point arises from this analysis. The first is that kinetics is a key aspect when evaluating free radical scavenging activity. In addition, it is also important to consider that $^{\bullet}$OH is so reactive that it would react with almost any molecule, usually at diffusion-limited rates. In fact, it might be assumed that $^{\bullet}$OH will react with the first molecule it finds near its production site. It has been known for over a decade that peroxyl radicals are among the oxidants likely to be efficiently scavenged to counteract oxidative stress.[1230–1233] This kind of AOX, will be further discussed in the following sections. The other categories are briefly summarized next.

AOX-II (or secondary AOX, or preventing, or OIL behavior):

It may involve diverse chemical routes besides direct free radical scavenging processes. Among them, probably the most relevant one is usually referred to as OH-inactivating ligands (OIL) behavior.[1234,1235] It involves metal chelation and may occur by sequestering metal ions from reductants or by deactivating OH radicals as soon as they are produced via Fenton-like, or Haber-Weiss recombination, processes. The metal chelation step can take place, at least, through two pathways. Namely, by the direct chelation mechanism (DCM) or by the coupled deprotonation-chelation mechanism (CDCM). The latter may become the most important one for antioxidants acid protons.

AOX-III (or tertiary AOX, or fixing AOX, or repairing AOX):

Preventing biomolecules from oxidative damage is not always possible. Therefore, repairing them after the damage occurs is an important way of preserving their chemical integrity. The routes involved in such a process depend on the nature of the damage. Formal hydrogen atom transfer (*f*-HAT) restores allylic hydrogens to lipids. The same mechanism is involved when the most frequent lesions on Cys, Tyr, Leu, Met, and His are fixed, while single electron transfer (SET) repairs oxidized Tyr and Trp. DNA damage, on the other hand, may occur in at least three different ways. One electron loss from guanine, the nucleobase most easily oxidizable; [1236] which is repaired by SET from the antioxidant. One H loss from the deoxyribose units, yielding C-centered radicals; [1237–1240] which is repaired by *f*-HAT from the antioxidant. The formation of the 8-OH-dG adduct by addition of an OH radical, which in turn yields the most abundant DNA lesion, i.e., 8-oxo-7,8-dihydro-2′-deoxyguanosine. [1241]. The latter is considered a biomarker of oxidative stress, [1243,1244] and it has been proposed that such a damage can be fixed via sequential hydrogen atom transfer followed by dehydration (SHATD). [1242]

AOX-IV (or versatile AOX, or multifunctional AOX, or multipurpose AOX):

This would apply to molecules capable of exerting their antioxidant activity through two or more of the above-described mechanisms.

5.1. AOX-I chemical routes

Free radical scavenging processes in living organisms occur in complex chemical environments. Numerous species are present in biological media, which may influence or be involved in competing reactions. In addition, antioxidants' reactivity depends on their chemical nature and may be modulated by the polarity of the environment and pH. Some of the most common chemical routes that may contribute to the observable AOX-I activity are detailed in Table 5.

Table 5. Some of the most common chemical routes that may contribute to the observable AOX-I activity ($H_nAntiox$ and $^•R$ represent the antioxidant and the free radical, respectively).

Single Step Mechanisms	
***Radical Adduct Formation* (RAF)** $\mathbf{H_nAntiox + {}^•R \rightarrow [H_nAntiox\text{-}R]^•}$ **Relevant for antioxidants with multiple bonds and electrophilic free radicals. Viable in polar and non polar media.**	*Examples*: Carotenoids + $^•OOH$,[1243] or benzylperoxyl [1244] or alkyl, alkoxyl, and alkylperoxyl radicals. [1245] $^•OH$ scavenging activity of caffeine, [478] gentisic acid, [1246] hydroxybenzyl alcohols, [1247] edaravone,[1248,1249] melatonin,[1250] and its metabolites,[1251,1252] carnosine, [1253] and rebamipide. [1254]
Single Electron Transfer (SET) $H_nAntiox + {}^•R \rightarrow H_nAntiox^{+•} + R^-$ Relevant for electrophilic free radicals and antioxidants that are good electron donors. Viable in polar media.	*Examples*: For antioxidants curcumin,[1255] and highly galloylated tannin fractions.[1256] Edaravone derivatives + $^•OH$, $^•OCCl_3$ and $CH_3COO^•$.[1257] Resveratrol with oxygen radical.[1258] Catechin analogues with $ROO^•$. [1259] Carotenoids with $CCl_3OO^•$ [1260,1261] and $^•NO_2$ [1262,1263].
Formal Hydrogen Atom Transfer (*f*-HAT) $H_nAntiox + {}^•R \rightarrow H_{n-1}Antiox^• + HR$ Relevant for antioxidants with labile H atoms. Viable in polar and non polar media.	*Examples*: Polyphenols, [1264] chlorogenic acids, [1265] procyanidins, [1266] chalcones,[1267] cynarine, [1265] orientin, [1268] capsaicin, [1269] silybin, [1265] *α*-mangostin, [1270] fisetin, [1271] hydroxychalcones, [1272] baicalein, [1271] ellagic acid, [1273] Lipoic acids, [1274] glutathione, [1275] tryptophan, [1276], *N*-acetylcystein amide.[1277]
Multiple Step Mechanisms	
Sequential Proton Loss Electron Transfer (SPLET) $H_nAntiox \rightarrow H_{n-1}Antiox^- + H^+$ $H_{n-1}Antiox^- + {}^•R \rightarrow H_{n-1}Antiox^• + R^-$ Relevant for antioxidants with acid protons. Viable in polar and protic solvents.	*Examples*: Curcumin, [1278,1279] esculetin, [1280] alizarin, [1281] deoxybenzoins,[1282] hydroxybenzoic acids, [1283–1286] resveratrol, [1287,1288] fraxetin, [1289] piceatannol, [1290] morin, [1291] hydroxychalcones, [1292–1294] xanthones, [1295] flavonoids, [1296] quercetin, [1297] kaempferol, [1298] gallic acid, [1299] Trolox, [1300] isoflavonoids, [1301,1302] baicalein, [1303] purpurin.[1304]
Sequential Electron Proton Transfer (SEPT) $H_nAntiox + {}^•R \rightarrow H_{n-1}Antiox^{•+} + R^-$	*Examples*:

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$H_{n-1}Antiox^{\bullet+}$ → $H_{n-1}Antiox^{\bullet}$ + H^+ Relevant for antioxidants that are good electron donors. Viable in polar and protic solvents.	Baicalein, [1305] astaxanthin, [1306] quercetin, in the presence of bases that have HOMO energies lower than that of the SOMO of its radical cation.[1307] DPPH and galvinoxyl radical scavenging activity of vitamin E models. [1308] The theroxyl radical-scavenging process of α-tocopherol.[1309]
Sequential Proton Loss Hydrogen Atom Transfer (SPLHAT) $H_nAntiox$ → $H_{n-1}Antiox^-$ + H^+ $H_{n-1}Antiox^-$ + $^{\bullet}R$ → $H_{n-2}Antiox^{\bullet-}$ + HR Relevant for antioxidants with acid protons and labile H atoms. Viable in polar and protic solvents.	*Examples*: α-mangostin, [1270] ellagic acid, [1310] propyl gallate, [1311] caffeic and other phenolic acids. [1312] Esculetin + $^{\bullet}OOCH_3$ and $^{\bullet}OOCHCH_2$ radicals. [1280] Gallic acid + $^{\bullet}OH$. [1313]

5.3. Trends in activity

As previously mentioned, kinetics is crucial to assess free radical scavenging activity. Therefore, this analysis will be based on rate constants. However, for trends to be fair, it is essential to consider reactions with the same radical and that the rate constants (k) are estimated with the same methodology and under the same conditions. Those reported in Table 6 correspond to reactions between coffee components and the $HOO^{\bullet}$ radical, in non-polar media that mimic lipid environments. Those reported in Table 7 correspond to the same reactions but in aqueous solution at physiological pH, i.e., pH=7.4. To facilitate comparisons, their log(k) have been plotted in Figure 3. Trolox has been included as a referent antioxidant.

Table 6. Overall, or apparent, rate constants (k) of the reactions between coffee components and $HOO^{\bullet}$, in non-polar environments.

Component	k (M^{-1} s^{-1}, at 298 K)	Ref.
caffeic acid	3.93E+04	[457]
caffeine	3.19E+01	[478]
dihydrocaffeic acid	4.95E+04	[457]
eugenol	2.49E+03	[1314]
ferulic acid	9.13E+03	[457]
guaiacol	1.55E+03	[1314]
p-coumaric acid	4.35E+03	[457]
paraxanthine	1.05E+00	[1315]
protocatechuic acid	5.14E+03	[1316]
theobromine	5.34E+01	[1315]
theophyline	4.21E+00	[1315]
Trolox	3.40E+03	[1300]
vainillin	9.75E+01	[1314]
vanillic acid	1.29E+01	[1314]
vanillyl alcohol	5.67E+03	[1314]

Table 7. Overall, or apparent, rate constants (k) of the reactions between coffee components and $HOO^{\bullet}$, in aqueous solution at physiological pH.

Component	k (M^{-1} s^{-1}, at 298 K, pH=7.4)	Ref.
caffeic acid	2.69E+08	[457]
caffeine	3.29E-01	[478]
dihydrocaffeic acid	1.04E+08	[457]
eugenol	1.55E+06	[1314]
ferulic acid	3.36E+08	[457]

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guaiacol	2.38E+06	[1314]
p-coumaric acid	8.51E+07	[457]
paraxanthine	4.18E+02	[1315]
protocatechuic acid	1.26E+07	[1316]
theobromine	2.76E-01	[1315]
theophyline	3.86E-02	[1315]
Trolox	8.96E+04	[1300]
vainillin	1.54E+05	[1314]
vanillic acid	1.65E+07	[1314]
vanillyl alcohol	4.12E+06	[1314]

The values in Tables 6 and 7 were all computed with the Quantum Mechanics-based Test for Overall Free Radical Scavenging Activity (QM-ORSA).[481] This computational protocol was designed to calculate reliable rate constants and was validated by comparisons with experimental data. The electronic calculations necessary to obtain the rate constants reported in Tables 6 and 7 were carried out with the M05-2X Density Funtional Theory approach, combinned with basis sets 6-31+G(d) to 6-311++G(d,p), and the SMD solvation model to mimic solvent effects. All possible reaction mechanisms and sites were taken into account, and the overall (or apparent) rate constants were estimated as the sum of the rate constants of each thermochemical viable reaction path. More details on this protocol can be found elsewhere.[481]

According to the gathered data dihydrocaffeic acid and ferulic acid are the most efficient $HOO^\bullet$ scavengers in non-polar media and aqueous solution, at pH=7.4, respectively. The trend in non-polar environment was found to be dihydrocaffeic acid > caffeic acid > ferulic acid > vanillyl alcohol > protocatechuic acid > p-coumaric acid > eugenol > guaiacol > vainillin > caffeine > theobromine > vanillic acid > theophylline > p-xanthine. In aqueous solution such a trend changes to ferulic acid > caffeic acid > dihydrocaffeic acid > p-coumaric acid > vanillic acid > protocatechuic acid > vanillyl alcohol > guaiacol > eugenol > vainillin > p-xanthine > caffeine > theobromine > theophylline.

The threshold above-mentioned, i.e. 10^3 $M^{-1}s^{-1}$, corresponds to the reaction of $HOO^\bullet$ with polyunsaturated fatty acids have been used to identify the coffee components that are expected to be efficient as free radical scavengers in biological systems. It has been marked with a red line in Figure 3. According to this criterion, dihydrocaffeic, caffeic, ferulic, protocatechuic, and p-coumaric acids, as well as vanillyl alcohol, eugenol, and guaiacol should be capable of preventing peroxyl damage to biomolecules both in lipid and in aqueous environments. For the latter, vanillin and vanillic acid also seem to be suitable for that purpose.

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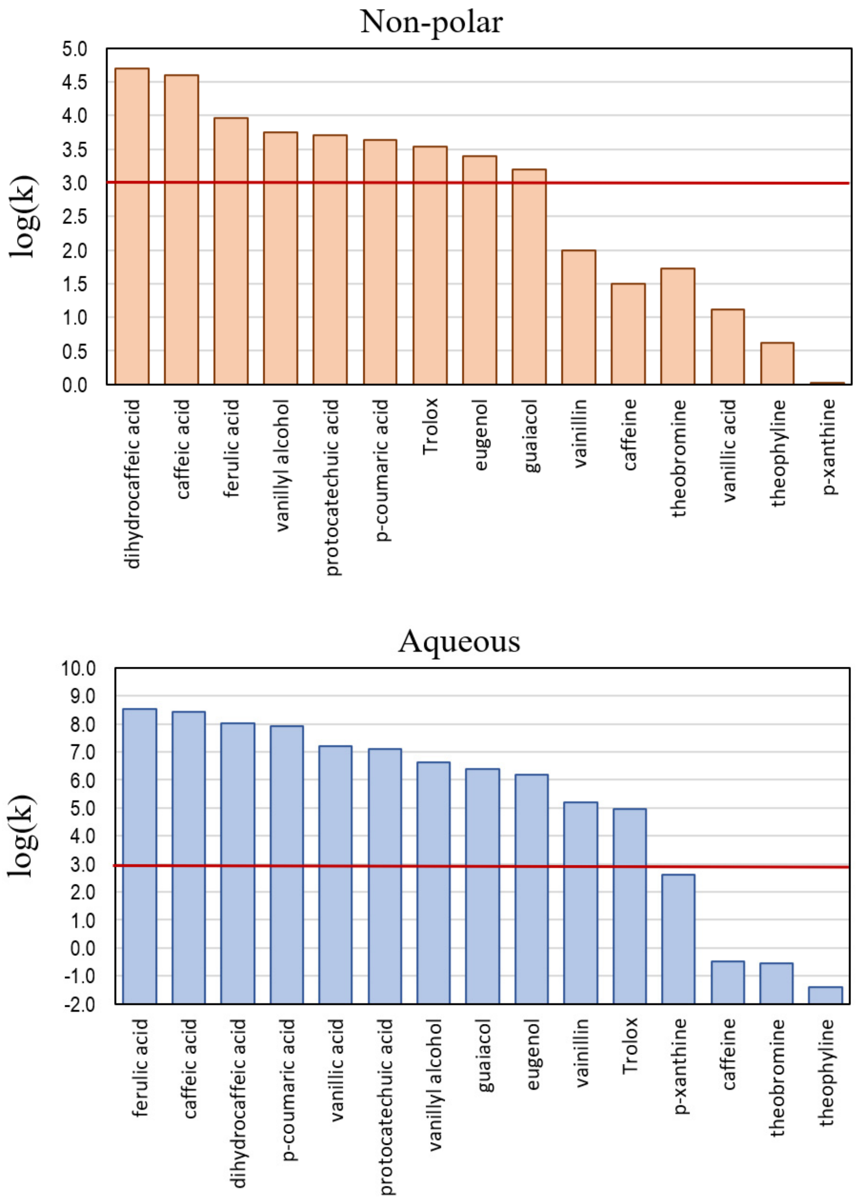


Figure 3. log(k) for the reactions between coffee components with $HOO^{•}$. The red line corresponds to the reaction of $HOO^{•}$ with polyunsaturated fatty acids.

It seems worthwhile mentioning that the reactions of caffeine and its metabolites p-xanthine, theobromine, and theophylline with $HOO^{•}$ are too slow to protect lipids and, therefore, proteins and DNA from the oxidative damage caused by this kind of free radicals. This is in line with previous works. Šeremet et. al. found that the antioxidant properties of coffee brews do not depend on their caffeine content. [401] Miłek et. al. reported that while 'specialty' quality coffees have similar caffeine content as other brands, they significantly surpass them in antioxidant activity. [477] Based on the likeliness of *f*-HAT and SET mechanisms, Petrucci et. al. concluded that caffeine can hardly be considered as an antioxidant. Thus, despite being the most emblematic coffee component, this brew's antioxidant activity arises from its phenolic species, not from caffeine.

5.4. Structure-activity relationships

The reaction mechanism contributing the most to the antioxidant activity of the analyzed coffee components is reported in Tables 8 and 9 for lipid and aqueous environments, respectively. The most reactive site or species are also reported in these tables. It becomes evident that the relatively low reactive of caffeine and its metabolites p-xanthine, theobromine, and theophylline is due to their lack of the phenol moiety. Thus, the main chemical route involved in their reactions with HOO• is the radical adduct formation. They have not labile H atoms to be involved in f-HAT, nor acid protons that favored deprotonation and, consequently, the SPLET mechanisms, i.e., SET from the anions.

Table 8. Main reaction mechanism and most reactive site in the reactions between coffee components and HOO•, in non-polar environments.

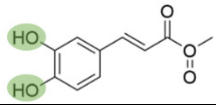
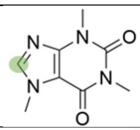
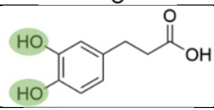
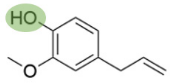
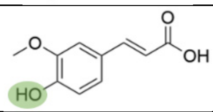
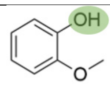
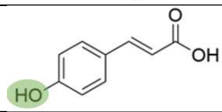
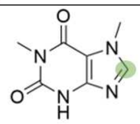
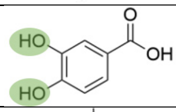
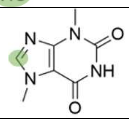
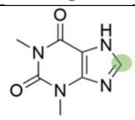
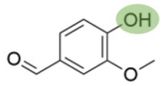
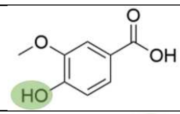
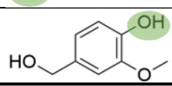
Component	Mechanism	Site	Ref.
caffeic acid	*f*-HAT		[457]
caffeine	RAF		[478]
dihydrocaffeic acid	*f*-HAT		[457]
eugenol	*f*-HAT		[1314]
ferulic acid	*f*-HAT		[457]
guaiacol	*f*-HAT		[1314]
p-coumaric acid	*f*-HAT		[457]
paraxanthine	RAF		[1315]
protocatechuic acid	*f*-HAT		[1316]
theobromine	RAF		[1315]
theophyline	RAF		[1315]
vainillin	*f*-HAT		[1314]
vanillic acid	*f*-HAT		[1314]
vanillyl alcohol	*f*-HAT		[1314]

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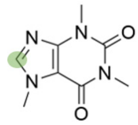
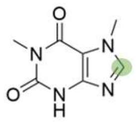
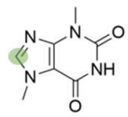
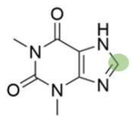
Table 9. Main reaction mechanism and most reactive site or species in the reactions between coffee components and HOO•, in aqueous solution at physiological pH.

Component	Mechanism	Site or species	Ref.
caffeic acid	SPLET	phenolate anion	[457]
caffeine	RAF		[478]
dihydrocaffeic acid	SPLET	phenolate anion	[457]
eugenol	SPLET	phenolate anion	[1314]
ferulic acid	SPLET	phenolate anion	[457]
guaiacol	SPLET	phenolate anion	[1314]
p-coumaric acid	SPLET	phenolate anion	[457]
paraxanthine	RAF		[1315]
protocatechuic acid	SPLET	phenolate anion	[1316]
theobromine	RAF		[1315]
theophyline	RAF		[1315]
vainillin	SPLET	phenolate anion	[1314]
vanillic acid	SPLET	phenolate anion	[1314]
vanillyl alcohol	SPLET	phenolate anion	[1314]

The phenolic structural feature seems to be the key to the high efficiency of coffee components as peroxyl radical scavengers. In lipid media, the OH group acts as H donor leading to AOX-I via *f*-HAT. In aqueous solution, their acid-base equilibria rule reactivity. At physiological pH, there is enough phenolate fraction, which is an excellent electron donor. Thus, under such conditions, the SPLET mechanism becomes the highest contributor to the antioxidant activity of phenolic compounds.

The solvent also plays an important role in this context. The antioxidant + HOO• reactions are faster in aqueous solution, i.e., polar and protic solvent, than in lipid media (Tables 6 and 7, and Figure 3). In addition, the fact that water is a polar and protic solvent promotes the SPLET mechanism, which was proposed by Litwinienko and Ingold, [1278,1317–1319] and it is recognized as most efficient for phenols scavenging free radicals than *f*-HAT, and certainly much more than RAF.

6. Perspectives

Albeit much information has been retrieved from the investigations on coffee, some aspects still deserve further research. Some of the many questions to be answered in more detail are:

- How much does the presence of redox metals modify the chemistry of the coffee components?
- How effective are they as chelating agents?
- Would they act as OH inactivating ligands?
- Are any of them capable of repairing oxidatively damaged biological targets?
- Which of them can be considered multifunctional antioxidants?
- Are their derivatives safe enough to be used as medical drugs?
- What are the metabolites of these derivatives, and what properties do they have?

Nature gave us coffee. Revealing its chemical wonders is up to us.

7. Summary

Many natural products are known for their health benefits, but they comprise a large variety of components. Thus, it is essential to identify their bioactive substances as well as the specific functions and medicinal effects of these substances.

Coffee is a complex mixture containing many chemicals, including alkaloids, amino acids, carbohydrates, carotenoids, fatty acids, flavonoids, organic acids, phenolic acids, sugars, terpenes, and volatile compounds. It is also known to provide many beneficial properties such as antibacterial, anticarcinogenic, antidiabetic, antifungal, anti-inflammatory, antiobesity, cardioprotective, gastroprotective, hepatoprotective, and neuroprotective effects, provided that it is consumed in moderate amounts. The chemicals responsible for such valuable effects have been summarized in this review, as well as numerous investigations devoted to the design and synthesis of their derivatives.

The antioxidative protection of coffee has been related to most of its benefits. Several reaction mechanisms contributing to this protection were overviewed. Namely: radical adduct formation (RAF), single electron transfer (SET), formal hydrogen atom transfer (*f*-HAT), sequential proton loss electron transfer (SPLET), sequential electron proton transfer (SEPT), and sequential proton loss hydrogen atom transfer (SPLHAT). The ones contributing the most to the antioxidant activity of several coffee components were discussed.

The trends in free radical scavenging activity showed that phenolic acids are the ones contributing the most to the antioxidant effects of coffee, while alkaloids are not efficient for that purpose, at least as chemical antioxidants. Thus, despite being the most emblematic coffee component, the antioxidant activity of this brew does not arise from caffeine. In fact, it is not expected to be a good free radical scavenger.

The structure-activity relationships were associated with the main reaction mechanisms and the role of the solvent on the reactivity of the explored compounds. Alkaloids, i.e. caffeine and its metabolites p-xanthine, theobromine, and theophylline, mainly react via RAF, regardless of the solvent nature. Phenolic compounds, on the other hand, mainly react via f-HAT in non-polar media, and via SPLET in aqueous solution, at physiological pH.

Although there are many aspects to explore in the context of coffee chemistry, this review is meant to provide molecular insights on one of its main effects, i.e., antioxidant protection. The data gathered in here demonstrate that computational and theoretical chemistry are very helpful tools to understand the molecular insights of antioxidants, and also for the desing of new compounds that combines this behavior with other health benefits. Hopefully, this review will contribute to a better understanding of the chemistry of our morning cup and promote further investigations on this topic.

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