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茶叶中黄烷醇类化合物在加工过程中的变化及其机理

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摘　要:黄烷醇类化合物是一类常见的植物多酚类化合物,广泛存在于植物的茎、叶和果实中。近年来,许多研究证明了黄烷醇具有多种健康功效,使其成为当今研发的热点。本文针对茶叶中黄烷醇类化合物的天然结构、加工稳定性以及酶促氧化产物的研究进行了阐述,并对黄烷醇类化合物的开发前景提出了一些建议,以期为黄烷醇化合物的开发应用提供一定的参考和依据。

关键词:黄烷醇;加工稳定性;酶促氧化;茶黄素;茶红素

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Review on Chemical Variations and Mechanism of Tea Flavanol Compounds During Processing

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Abstract: Flavanol compounds are the most abundant phenolic subclass of plant phytochemicals known as flavonoids, which are commonly found in the stems, leaves and fruits of plants. Both epidemiological and biological evidences suggest a health protective role for dietary flavanols, leading to increasing interest in the composition and bioavailability of these compounds from foods. The purpose of this paper is to provide an overview of variation in flavanol composition from tea, which are common types of tea products (green tea, oolong tea, black tea *etc*). Key researches describing the effect of processing on flavanol composition were summarized, including its chemical change, stability, enzymatic oxidation and bioactivity. In addition, some suggestions for the future study of flavanols were put forward in order to provide reference for the development and application of flavanols.

Key words: flavanols; processing stability; enzymatic oxidation; theaflavins; thearubigins

类黄酮化合物(Flavonoids)是具有黄酮化合物典型骨架,以苯基-苯并吡喃结构(C6-C3-C6)为基本母核的系列化合物,共分为六大类[1]:黄烷醇、黄酮、黄酮醇、黄烷酮、黄烷二醇、花青素。其中黄烷醇类化合物约占植物类黄酮化合物的77% ~83%[2],是人体摄入的类黄酮化合物中最多的一类。越来越多的研究发现,黄烷醇能够预防一些慢性和退行性疾病,如癌症[3]、心脑血管疾病[4]、肥胖[5]、糖尿病[6]以及神经退行性疾病[7],这使得黄烷醇化合物成为天然活性成分的研究热点。

黄烷醇广泛存在于植物的茎叶和果实中,如茶叶、可可、葡萄、苹果、蓝莓等,均含有不同形式和含量的黄烷醇[8],其中尤以茶叶中单体黄烷醇含量最高[2]。2015年全国干茶叶年产量约227.8万吨,可提供世界人均消费50L茶饮料[9]。根据加工方法不同,茶主要有三种类型[10]:绿茶(非发酵茶),乌龙茶(半发酵茶)和红茶(发酵茶)。绿茶经过的加工工艺较少,其中黄烷醇化合物保留量较多,占总黄酮的85%[11]。绿茶中的黄烷醇化合物主要是以单体儿茶素、及其单体异构体或衍生物的形式存在(表1),也存在少量的二聚体。由于品种、产地、生长条件的不同,儿茶素的含量也存在差异,茶叶中儿茶素总含量大约为4 ~140 mg/g(湿重)[12]。

乌龙茶和红茶与绿茶的制作材料相同,但加工过程中的发酵工艺使叶片中的天然氧化酶(多酚氧化酶、过氧化物酶等)作用于酚类底物,进而改变了茶叶中儿茶素的形态与含量[13],进一步氧化会产生大量结构复杂的聚合物,如茶黄素、聚酯型儿茶素、

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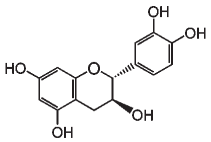
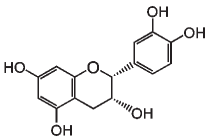
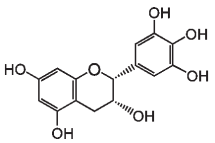
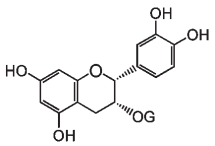
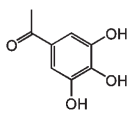
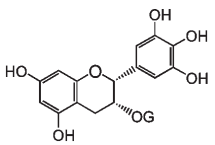
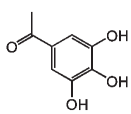
茶红素、茶褐素等[14]，这些氧化产物赋予茶叶不同的色泽与风味。本文主要对茶叶中黄烷醇化合物种类、加工稳定性，及其加工过程中酶促反应产物的研究进展进行阐述，并对黄烷醇类化合物的开发前景提出了一些建议，以期为黄烷醇化合物的在食品色素、天然药物的开发应用提供一定的参考和依据。

表 1 绿茶中五种常见黄烷醇的单体结构式和含量

Table 1 Five structures and contents of monomeric flavanol in fresh tea leaves

结构式 Structure	名称 Compound name	缩写 Abbreviation	C-3 配基 Substitution	含量(mg/g 湿重) Content (mg/g FW)
	儿茶素 Catechin	C	–	0 ~ 8[15]
	表儿茶素 Epicatechin	EC	–	0.1 ~ 17[10,16]
	表没食子儿茶素 Epigallocatechin	EGC	–	0 ~ 55[10,16]
	表儿茶素没食子酸酯 Epicatechin gallate	ECG		1 ~ 40.5[10,16]
	表没食子儿茶素没食子酸酯 Epigallocatechin gallate	EGCG		7 ~ 74[10,16]

1 茶叶中黄烷醇在加工过程中的化学稳定性

1.1 热稳定性

随着对黄烷醇生理活性尤其是构效关系研究的深入，越来越多的研究集中在其加工过程中的稳定性方面。食品体系在加工过程中通常会进行热处理，因此，热加工条件下黄烷醇化合物的化学稳定性尤其重要。

有研究表明：黄烷醇在暴露于热条件下的主要反应是异构化和自氧化[17,18]。从热力学能量上看，加热会促进表儿茶素向其非表型结构的异构化，非表型儿茶素的形成热(ΔH_f)比表型结构的热量低 1-2 kcal/mol，该能级差异在加热过程中会引起聚酯化[19]。实际加工中，经常出现热诱导异构化现象，新鲜茶叶中的儿茶素类化合物主要是表没食子儿茶素没食子酸酯(EGCG)[20]，加工成茶叶后主要的儿茶素是没食子儿茶素没食子酸酯(GCG)[21]，同时焙烤过程也会导致表儿茶素(EC)向儿茶素的差向异构化[22]。

据报道[23]，几种儿茶素的热稳定性依次是 ECG > EGC > EC > EGCG。但常常伴随着自动氧化，尤其是煮茶过程中，水体系中掺杂的少量金属离子和氧气，使热导致异构化与氧化异构化变得难以区分。Wang 等[24]对比分析了自来水与纯净水所煮茶汤中儿茶素的种类，发现自来水(含有少量金属离子和 1.1 mg/L O_2)煮制茶叶的体系中含有较多的没食子儿茶素(GC)和 GCG，而表没食子儿茶素(EGC)和 EGCG 则相对较少。

1.2　氧化稳定性

黄烷醇的氧化稳定性高度取决于 pH[25],酸性条件下,黄烷醇比较稳定,随 pH 值升高(pH > 5.5),几种儿茶素的稳定性依次为 EC > ECG > EGCG ≥ EGC[26,27]。碱性条件会加速黄烷醇的降解,例如可可豆制作中,碱处理加深了可可豆的褐变度,但也导致成品中儿茶素、EC 和原花青素的含量降低 3-8 倍[28]。pH 引起的 EGCG 和 EGC 氧化降解常被认为是其作为供氢体淬灭氧自由基来实现的[29]。与 EC 和 ECG 相比,B 环结构为连苯三酚结构的 EGCG 和 EGC 更容易被氧化[30]。EGCG 在 pH 7.2 ~ 7.4 条件下的半衰期为 30 ~ 120 min[31],肠液(pH 8.5)中 EGCG 在 30 min 内降解大约 85%[32]。较高 pH (>5.5)条件下活性氧(ROS)引起的 EGCG 氧化导致自氧化,产生多种氧化产物,以二聚体为主,如聚酯型儿茶素(Theasinensin,THSN)和其他结构更为复杂的二聚体(B 环开环聚合物 P-2)[32](见图 1)。

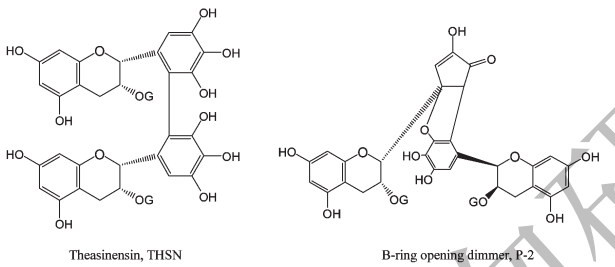


图 1　聚酯型儿茶素和二聚体 P-2 结构式(EGCG 同源二聚体)

Fig. 1　Structures of theasinensin and dimer P-2 (EGCG homodimer)

大多数有关 pH 引起儿茶素自氧化的研究集中在 EGCG 上,而关于 EGC 的较少。EGC 是绿茶中含量较丰富的儿茶素之一,氧化条件下高度不稳定,在茶加工[33]和溶液体系[40]中易产生氧化二聚体。此外,黄烷-3-醇混合物的自氧化会导致各种儿茶素之间的异源二聚化,如 EGCG 和 EGC 混合物的模拟体统中自氧化会形成结构类似于 THSN 和 P-2 的同源或异源二聚体[34]。食品体系及人体消化环境中(如小肠液、血浆)普遍存在自氧化的有利条件,如较高的 pH(≥5.5)、溶解的 O_2 和 ROS[35],所以这几种儿茶素和聚合产物的性质变化及其在人体中发挥的功效活性仍有待进一步确定。

2　茶叶中黄烷醇在加工过程中的酶促产物

在红茶、乌龙茶制作过程中不仅会受到温度的作用,揉捻、发酵等关键制作步骤对茶中黄烷醇也有较大的影响。揉捻导致茶叶液泡破裂,细胞结构受损,黄烷醇与细胞膜上的多酚氧化酶(PPO)和过氧化物酶(POD)接触发生酶促氧化;发酵则是酶促黄烷醇氧化聚合的关键步骤。在此过程中,黄烷醇会发生复杂的化学变化,生成茶黄素(Theaflavins,TFs)、茶红素(Thearubigins,TRs)和茶褐素(Theabrownins,TBs)等多种黄烷醇色素,赋予产品多种健康作用(表 2),同时也会引起茶叶色泽和风味上的改变。

表 2　茶色素的功效活性

Table 2　Functional activity of tea pigments

功效活性 Functional properties	功效物质 Functional compounds	活性机理 Active mechanisms
体外抗氧化活性	茶黄素	酚羟基含量高,提供质子能力强[36]
	茶红素	TFDG > TF-3-G ≈ TF-3'-G > TF > EGCG[37,38]
	茶褐素	
体内抗氧化活性	茶黄素	清除自由基[39]
	茶红素	螯合金属离子、抑制氧化酶系的酶活[40]
	茶褐素	TF > EGCG > TR > TB[41,42]
抗癌活性	茶黄素	诱导氧化应激,影响癌细胞稳态[43,44,45]
	茶红素	抑制细胞增殖信号通路[46,47,48,49,50]
		诱导细胞凋亡[49,51,52,53]
降血脂活性	茶黄素	减少肠道对胆固醇的吸收[54]
	茶褐素	降低血清血脂[55]
		减少胆固醇的生物合成[56]
抗心脑血管疾病	茶黄素	调节血管内外渗透压[57]
		抑制血管紧张素转移酶[58]
抗炎活性	茶黄素	下调炎症介质、炎症细胞因子的释放[59,60]
	茶红素	抑制免疫通路 NF-κB 的激活[61,62]

续表 2(Continued Tab. 2)

功效活性 Functional properties	功效物质 Functional compounds	活性机理 Active mechanisms
抗病毒活性	茶黄素 茶红素	抑制病毒对细胞的吸附[63] 抑制病毒复制过程所需须酶的酶活[64,65]
调节神经功能	茶黄素	基于抗氧化性,抑制细胞凋亡和神经退行病变,如阿尔兹海默症、帕金森病[66,67]
改善消化系统	茶红素 茶褐素	减少肠道结构损伤,改善肠道微生物环境[68,69]

2.1 茶黄素形成机理及可能的结构

Roberts 等[70]首先报道在发酵红茶中含有橙黄色中性色素和红棕色酸性色素,并分别命名为茶黄素和茶红素,他们发现茶黄素是 EGC 和 EGCG 的氧化聚合产物,具有苯并环庚二烯酚酮的结构。Takino 等[71]在随后的研究中发现茶黄素是 EC 和 EGC 的氧化聚合产物,是通过成对的儿茶素单体酶促氧化聚合而形成苯并环庚酚酮的结构,并且推测其它种类的黄烷醇也是这样的结合方式。形成茶黄素聚合物的主要底物是含有连苯三酚结构(B 环 3、4、5 位)与含有邻苯二酚结构(B 环 3、4 位)的儿茶素,在氧化酶或氧化剂的作用下各自氧化成邻醌,再由邻醌间配对进行聚合反应形成苯并环庚二烯酚酮[72](见图 2)。邻苯二酚或连苯三酚以及没食子酸等物质也可氧化生成邻醌,也能够与没食子儿茶素或儿茶素发生聚合反应。

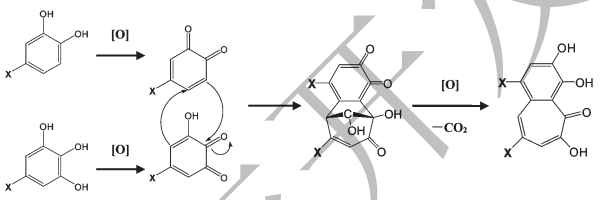


图 2 茶黄素中苯并环庚二烯酚酮结构形成的可能机理

Fig. 2 Possible mechanism for the formation of benzotropolone structures in theaflavins

Bryce 等[73]从红茶中分离出四类茶黄素化合物,并鉴定其母体结构为黄烷醇:TF1 的单体组成与 Roberts 等人报道的茶黄素相同[70];TF2 为 TF2A 和 TF2B 的混合物,是分别通过从 EGCG 与 EC(TF2A)聚合和 EGC 与 ECG (TF2B)聚合产生;TF3 是从 EGCG 和 ECG 混合的铁氰化物氧化得到的化合物;TF4 是一种由没食子酸和非表型儿茶素氧化聚合的产物。Collier 等[74]在红茶中发现除了上述四种茶黄素外,还存在表茶黄酸和表茶黄酸没食子酸酯,它们分别是 EC 与没食子酸聚合以及 ECG 与没食子酸聚合的产物。Coxon 等人[75]报道红茶中也存在异茶黄素,是 EGC 和非表型儿茶素的氧化产物。

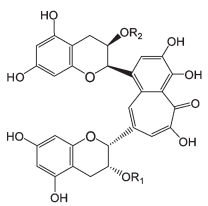


图 3 茶黄素的骨架结构式

Fig. 3 Skeletal structure of theaflavins

表 3 四种常见的茶黄素结构

Table 3 Structures of four common theaflavins

名称 Compound name	缩写 Abbreviation	前体 Precursors	R_1	R_2
茶黄素	TF	EGC + EC	H	H
茶黄素-3-没食子酸酯	TF3G	EGCG + EC	Gallate	H
茶黄素-3'-没食子酸酯	TF3'G	EGC + ECG	H	Gallate
茶黄素-3,3'-二没食子酸酯	TFDG	EGCG + ECG	Gallate	Gallate

茶叶中常见的茶黄素化合物有四种(见图 3、表 3):茶黄素(18%)、茶黄素-3-没食子酸酯(18%)、茶黄素-3′-没食子酸酯(20%)和茶黄素-3,3′-二没食子酸酯(40%),与上述四种茶黄素相比,茶叶中

表茶黄酸、茶黄酸没食子酸酯产生的量相对较少(4%)[76]。在近几年有关茶黄素的研究报道中,几种稀有的儿茶素寡聚体也陆续被研究人员发现(见图 4),比如 Theacitrin[77]、Theanaphthoquinone[78]、Olongintheanin[79]和 Theadibenzotropolone[80]。

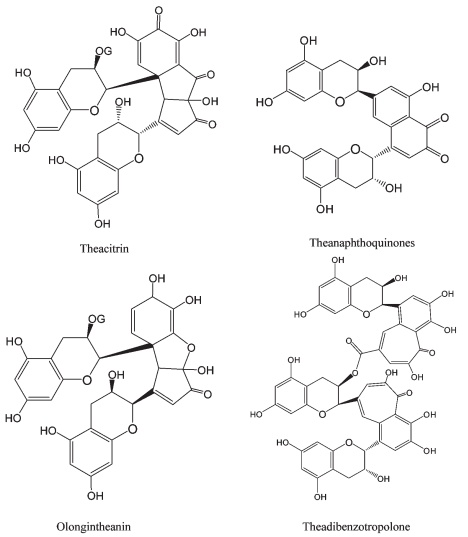


图 4　几种不常见的儿茶素寡聚体结构式

Fig. 4　Several unusual catechin oligomer structures

2.2　茶红素形成机理及可能的结构

目前,儿茶素以及茶黄素的形成机理研究较为广泛,而茶红素的生成机理与结构仍不清楚。综合目前的研究报道,茶红素形成的可能途径大致为以下三个方面:1)简单儿茶素或聚酯型儿茶素的直接酶促氧化;2)茶黄素形成过程中中间产物的氧化;3)茶黄素本身的自氧化或偶联氧化[81]。

Roberts 发现茶黄素后,于 1962 年又发现了茶红素并命名为 Thearubigins[82],他们认为茶红素是一类在茶黄素氧化降解等化学变化的基础上形成红棕色酸性色素聚合物的总称。Brown 等[83]进一步研究发现,茶红素是一种原花色素高聚合物,经过降解可得到单体儿茶素、没食子酸和原花色素,可能的形成机制是在酶促反应后生成了 C-C 键。Berkowitz 等[84]将 EC 加入到含有茶黄酸的茶发酵体系中后,发现两种化合物的单体迅速消失,而茶红素的含量明显增加,而当体系中只有茶黄酸时,却没有这种现象。

儿茶素类为多羟基苯并吡喃结构,除其 B 环上的邻二羟基极为活泼,存在一定酸性、较强的还原性以外,C-2′、C-4、C-6、C-8 和 C-3 氧合的没食子基团的羟基均具有较强的活性,易发生聚合反应形成二聚体[85]。如果存在 PPO 和 POD 的情况下,则更容易发生多级聚合反应,儿茶素、原花青素、茶黄素、酚酸等酚类化合物均可作为中间反应物发生氧化聚合反应,生成茶红素[86]。茶红素是一类分子质量分布范围广的多酚聚合物,约为 700 ~ 40000 Da,含量约占红茶干重 9% ~ 19% 及红茶多酚类物质 70%

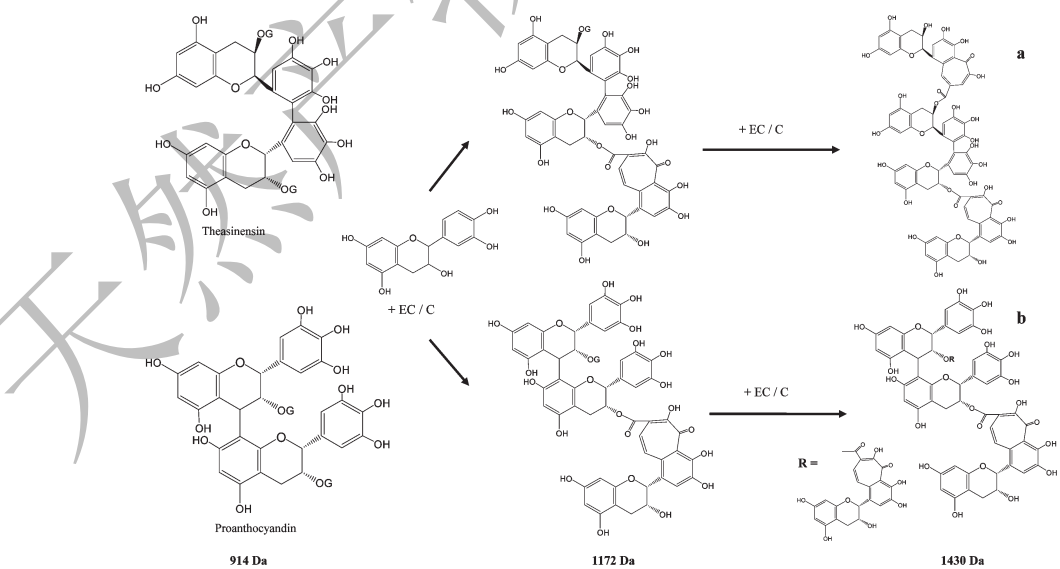


图 5　两种茶红素生成的可能途径和结构

Fig. 5　Proposed structures and formation pathway of two thearubigins

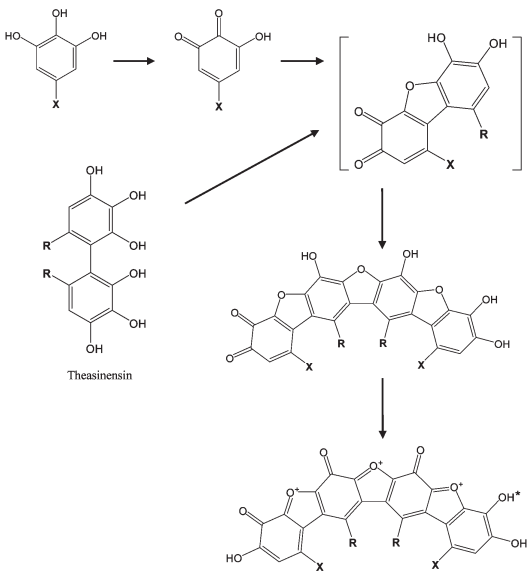


图 6 一种茶红素可能的聚合结构示意图

Fig. 6 A diagrammatic sketch of proposed thearubigin structure

左右[87]。

Menet 等[88]从红茶提取物中去除茶黄素和小分子多酚之后提纯了茶红素,并对其性质进行鉴定,得到两种儿茶素的四聚体(图 5 a & b),均为典型茶黄素的苯并环庚二烯酚酮结构,聚合后的儿茶素骨架呈“S”型。Haslam[89]提出了一种以聚酯型儿茶素为关键底物的茶红素聚合方式(图 6),另一底物可以是任何具有三酚羟基的酚类物质,在酚类底物被氧化成邻醌之后与聚酯型儿茶素发生酶促聚合;其聚合基团是由儿茶素前体的 B 环聚合而成,骨架大致呈平面线形,该基团为主要的发色基团,呈现棕色;其酸性是由酚羟基在不同 pH 和氧化态环境下的解离实现。该推测与 Roberts 首次发现茶红素时确定其为酸性聚合物的研究一致。

2.3 茶褐素的形成机理

茶褐素是一类水溶性非透析性高聚合的深褐色物质,普遍存在于发酵茶中[90]。目前国际上尚未有此类物质的统一定义,笼统称为“多酚氧化的高聚合产物”[89]。一般将其认为是茶色素与体系中的酚酸、多糖、蛋白、核酸等物质进一步聚合形成,以普洱茶为例,普洱茶在渥堆发酵过程中,酚酸类物质、儿茶素、黄酮类物质、茶红素和水溶性寡糖大幅度下降:其中,酚酸类物质下降约 60%,儿茶素类下降 80%,黄酮类下降 55%,茶红素下降 90%,水溶性寡糖下降 65%,水浸出物下降 25%,而茶褐素类物质则增加了 4.5 倍[91]。

李连喜等[92]研究认为茶褐素除含有儿茶素类的氧化聚合产物外,还含有氨基酸、含氮物质、糖类、没食子酸、咖啡酸等结合物,但是其化学组成及结构尚未完全探明。谭超等[93]对普洱茶中的茶褐素进行不同分子量的分级后发现,茶褐素的分子量最小约为 3500,最大范围尚无法测量,活性羟基和羧基随分子量的增加而增加;不同分子量茶褐素粒子形貌并不均一,单分子呈岛屿状态或颗粒状聚集物结构,该结构聚合了多糖和蛋白质残基,当粒子聚集较多时,呈线状链接且有较多分支或形成网状结构。

茶叶中天然氧化酶种类较多,而多酚物质及其他成分组成也十分复杂,因此在生成茶褐素的酶促反应中的机理与产物尚不明确。在前人研究的基础上,Wang 等[94]提出了茶褐素合成的可能途径(图 7)。

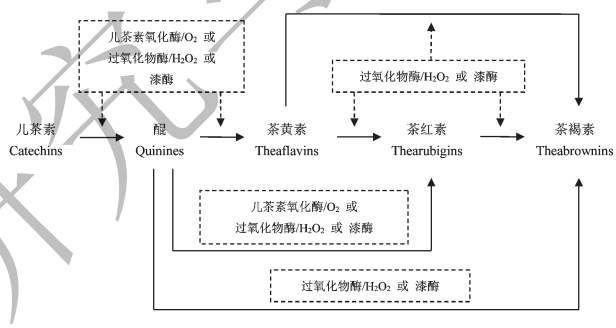


图 7 茶褐素形成的可能途径

Fig. 7 Proposed pathway of Theabrownins formation

3 结语

黄烷醇类化合物广泛存在于日常饮食中,具有广谱的生物活性。黄烷醇既是理想的天然抗氧剂,又是具有开发价值的天然药物原料。茶黄素和茶红素是黄烷醇化合物氧化聚合而成,是一类良好的功能性食用色素。茶色素在药理学方面的研究已取得重大进展,现已作为抗癌、降血脂和预防心脑血管疾病等的药物原料被成功应用开发。然而,茶色素的研究多集中在茶黄素,由于茶红素和茶褐素的聚合前体来源广泛,聚合基团和聚合方式复杂多样,尚未有对其单体化合物进行分离鉴定的有效方法。因此,未来茶色素的研究重点仍需集中在以下几个方面:(1)在茶色素分离鉴定方面,改进提取方法和条件提高提取率、发现有效色谱技术分离纯化出更高纯度的茶色素都是首要难题;(2)在茶色素制备方面,促进黄烷醇氧化聚合的氧化酶系(PPO、POD)的

作用机理与反应条件并不十分明确,其分离纯化的制备方法也不完善,通过酶工程制备茶色素的研究尚需要大量系统的工作;(3)在茶色素生物活性研究方面,现阶段活性功能明确的茶色素多为黄烷醇低聚体,因多聚体对食品感官有消极影响(如茶褐素造成红茶茶汤发暗、无收敛性),其生物活性多被人们所忽视,对多聚体的形成机理、结构特点及其构效关系都需要进一步的探索。

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