

DOI: 10.13652/j.spjx.1003.5788.2022.90147

发酵条件对红茶茶色素形成的影响研究进展

Research progress on the influence of black tea fermentation conditions on the tea pigment formation

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摘要:综述了发酵温度、发酵湿度、发酵时间和外源添加物等发酵因子对红茶茶色素形成的影响,并对红茶茶色素形成的未来研发趋势进行了展望。

关键词:发酵;红茶;茶色素;形成

Abstract: The research progress of the influence of fermentation temperature, fermentation humidity, fermentation time and exogenous additives on the formation of black tea pigment were reviewed. The future research and development trend of black tea pigment formation was prospected.

Keywords: fermentation; black tea; tea pigment; formation

红茶属全发酵茶,是以茶树新鲜芽叶为原料,通过萎凋、揉捻(切)、发酵、干燥等工艺制成,其中发酵是红茶风味形成的关键工序^[1-2]。红茶发酵过程的实质是揉捻茶叶多酚发生酶促反应,形成茶黄素、茶红素、茶褐素等风味物质。茶黄素、茶红素、茶褐素统称为茶色素^[3-6]。红茶加工过程中的发酵温度、发酵湿度、发酵时间、外源添

加物等对茶色素的形成均具有重要影响,进而影响红茶的风味品质^[7-10]。

Sharma 等^[11-12]证实了茶多酚氧化聚合可形成红茶茶色素。茶色素是红茶中的一类酚性色素,其茶黄素、茶红素、茶褐素的含量和比例不仅对红茶的汤色、滋味、叶底等具有决定性作用^[13-15],还具有抗氧化、降血脂、降血压、降血糖、抗应激等多种生物活性^[16-18]。茶黄素(TFs)是茶叶多酚类物质氧化聚合形成的具有苯骞卓酚酮结构的色素,占红茶干重的0.2%~3.0%,包括茶黄素、茶黄素-3-单没食子酸酯、茶黄素-3'-单没食子酸酯、茶黄素-3,3'-双没食子酸酯、茶黄酸、异茶黄素、新茶黄素等近30种物质,是构成红茶茶汤“金圈”的主要物质,也是构成茶汤亮度、滋味强度的重要因子。茶红素(TRs)是红茶中分子差异极大的异质性红色或褐红色的酚性物质,占红茶干重的6%~15%,其相对分子量为700~40 000 Da,由儿茶素、茶黄素等发生氧化聚合反应形成,也是构成红茶汤色红艳、滋味浓度和醇度的综合因子之一^[19]。茶褐素(TB)是红茶中一类酚性氧化聚合物的异质类群,目前已知的组分包括茶多酚、儿茶素低聚合物、茶多糖、蛋白质、核酸等,占红茶干重的4%~9%,是红茶茶汤色暗的主要原因之一。

茶色素在红茶发酵过程中形成的途径相对比较复杂(见图1)。茶色素的前体物质除儿茶素外还有原花青素、黄酮苷、酚酸等酚类化合物,甚至还有氨基酸、蛋白质等非酚类物质。茶黄素仅是复杂儿茶素二聚物中一类苯骞卓酚酮结构的化合物,不是茶红素形成的必须中间体。

基金项目:浙江省茶叶产业技术团队项目(编号:2020-2022);浙江省农业(茶树)新品种选育重大科技专项(编号:2021C02067-5);浙江省公益技术研究计划(编号:LGN21C160009)

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收稿日期:2021-11-18 **改回日期:**2022-03-21

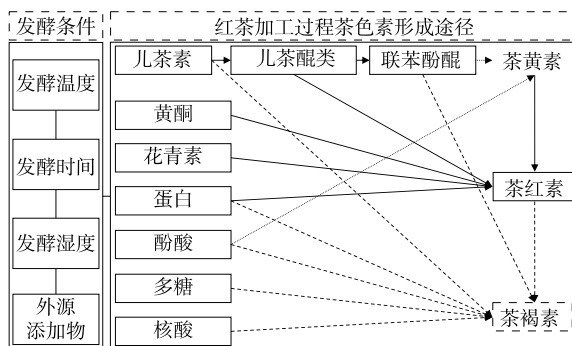


图 1 红茶发酵过程中茶色素形成的影响因素

Figure 1 Formation pathway and influencing factors of tea pigment

茶红素是以儿茶素二聚物和儿茶素低聚物为主体,而茶褐素是一类由多酚类、儿茶素氧化聚合物、多糖、蛋白质、核酸等组成的非透析性高聚物。从现有茶色素形成机制研究的现状来看,红茶发酵过程中的温度、湿度、时间、外源添加物等主要条件,对茶黄素、茶红素、茶褐素形成的影响有差异。研究拟对近 40 年来发酵条件对红茶茶色素形成的影响研究进展进行综述,以期对红茶风味品质提升提供依据。

1 发酵温度对红茶茶色素形成的影响

发酵温度包括环境温度和叶温。叶温对发酵过程中茶色素形成具有直接影响,并受环境温度、摊叶厚度等因素制约。目前,叶温的控制和监测手段包括空调、高温蒸汽、炭火、电加热等。发酵温度通过影响氧化还原酶活性,进而影响茶色素形成。茶色素是茶多酚类物质酶促氧化形成的产物,包括多酚氧化酶和过氧化物酶。多酚氧化酶和过氧化物酶催化茶色素形成,需要适宜的温度和 pH 值。茶叶加工过程中很难调整 pH 值,因此,调整适宜温度激发多酚氧化酶和过氧化物酶活性成为重要途径。多酚氧化酶较佳的催化温度与过氧化物酶的不同,温度过高或过低均不利于其发挥催化作用,需要针对不同的鲜叶原料,控制适宜的发酵温度,或者找到平衡点的固定温度发酵,亦或采用变温发酵方式,适时调整发酵温度^[20]。Cloughley^[21]认为低温(15℃)发酵能获得更高的茶黄素含量和提高马拉维红茶品质。彭继光等^[22-24]也认为低温发酵有利于茶黄素的形成和品质提高。

不同的发酵温度的控制策略,对茶黄素、茶红素、茶褐素等不同类别色素影响程度不同。马梦君等^[25-27]采用提高发酵温度的方式提高红茶品质,调控茶黄素、茶红素、茶褐素的形成量和比例。陈以义等^[28-29]采用“先高温后低温”的变温发酵方式,提高了红茶品质和茶黄素含量。Qwuor 等^[30-31]发现茶黄素在发酵温度低于 20℃时含量较高,而茶红素在 30℃以上时含量较高。王振康等^[32-33]也认为发酵温度 30℃有利于红茶品质的形成和

茶红素的积累。方世辉等^[34]研究表明,发酵温度 22℃时的茶黄素含量高于 28℃的,发酵温度 22℃时的茶红素含量低于 28℃的。滑金杰等^[35]研究发现,当发酵温度为 25,30,35,40℃时,茶黄素和茶红素形成量均是先增加后下降,且茶红素形成量峰值点出现时间晚于茶黄素,即发酵温度 25℃有利于茶黄素的形成和累积,而发酵温度 30℃有利于茶红素累积,且茶黄素含量与 L_c 值、 b 值、 L_b 值呈极显著正相关,与 L_c 、 L_a 值呈显著正相关,TRs 仅与 a 值和 L_b 值呈显著正相关。Qu 等^[36]研究发现随着发酵温度的升高,茶黄素、茶红素形成量逐渐下降,最高含量分别为 0.78%,5.84%,茶褐素则呈先上升后下降的趋势,最高含量为 8.94%。由此看来,合理的变温发酵策略,对于改变茶黄素、茶红素、茶褐素的比例和形成量具有实际意义,目前有关此方面的研究还有待加强。

2 发酵湿度对红茶茶色素形成的影响

红茶发酵需要一定的湿度来维持茶多酚类物质向茶色素转化^[37-38],进而形成独特的红茶风味^[39]。如果发酵湿度过低,揉捻叶内部的多酚类物质与氧化还原酶类不易溶出,进而酶促和非酶促反应不充分,不仅茶黄素、茶红素、茶褐素等红茶关键成分形成量减少,而且发酵时间延长,影响发酵进程,造成红茶发酵不充分,干茶色泽不乌润,滋味偏青涩,香味淡薄,因此需要合理控制发酵湿度。常用的技术手段包括加湿器、撒水、喷雾、覆盖湿布等^[40]。

此外,发酵叶湿度也不宜过高,过高易导致发酵叶内部通气性能变差,氧气供应不足,不利于叶片中多酚氧化酶、过氧化物酶等氧化还原酶类发挥酶促作用,进而影响儿茶素、酚酸类物质等转化形成茶黄素、茶红素、茶褐素类物质,造成干茶色泽发暗、滋味和香气发闷^[41]。综上,90%以上的高湿条件下有利于风味形成。而且,湿度增加可以有效降低花青暗条率,当发酵环境湿度低于 83%时,发酵叶的花青暗条率达 25%以上,当湿度提高至 90%以上,花青暗条率降为 18%以下^[42]。因此,从酶促氧化反应的本质来说,发酵湿度的高低对茶多酚氧化聚合形成茶色素具有重要影响。

3 发酵时间对红茶茶色素形成的影响

茶多酚在发酵过程的不同阶段逐渐形成茶黄素、茶红素、茶褐素,因此发酵时间的控制对于茶色素形成和红茶风味品质形成非常重要^[43]。发酵时间较短的情况下,茶红素、茶褐素类物质形成量较少,红茶成茶色泽淡、滋味不浓醇、带青气,相反,发酵时间过长易造成茶多酚过度氧化聚合,形成较多茶褐素等高聚物,易造成红茶成茶色泽暗、香气不纯^[44]。Muthumani 等^[45]认为发酵过程中茶黄素含量呈先升高后缓慢下降的变化趋势,主要归因于前期发酵叶内部氧化聚合反应催生茶黄素逐渐形成,当底物茶多酚类物质的酶促氧化聚合反应达到动态平衡

后,茶黄素不再继续生成,逐渐形成更高聚合态的茶红素、茶褐素等物质。

红茶实际生产中较多通过闻气味、看叶色、开汤审评等感官方法判断发酵适度。尹杰等^[33]以福鼎大白一芽一叶为原料,发现 3.5 h 条件下,发酵叶青气逐渐减弱,花香或果香凸显,叶色向黄红色转变,茶红素增加 0.48%,茶黄素和茶褐素不再发生显著变化,即为发酵适度。刘玉芳等^[46-47]探索了一种新的发酵适度判断方法——分光光度法,将发酵不同时间的样品冲泡,通过 460 nm 处吸光值的变化确定发酵适度。Nabarun 等^[48]将电子鼻技术和感官审评法结合,通过研究不同发酵时间点样品的香气变化规律来明确发酵适度。Hsien 等^[49]采用毛细管色谱法,通过不同发酵时间点的茶多酚类物质、茶黄素类物质含量变化,分析红茶发酵适度。

4 外源添加物对红茶茶色素形成的影响

研究^[50-51]表明,在红茶发酵的关键加工工序添加外源酶、天然提取物、无机盐、无机酸等外源添加物,不仅可以针对性地改善红茶茶色素类物质的含量和比例,提升红茶风味品质,还可以改善红茶发酵进程。不同种类外源添加物对红茶茶色素变化率的影响见表 1。目前,红茶加工中外源添加的酶类包括单宁酶、纤维素酶、木聚糖酶、木瓜蛋白酶、多糖水解酶、胰蛋白酶等水解酶,以及多酚氧化酶、过氧化物酶等氧化还原酶类^[52-55]。全佳音等^[56]发现添加纤维素酶、多酚氧化酶后茶黄素含量分别比对照提高了 4.76%, 7.83%, 而茶红素含量分别比对照降低了 7.40%, 0.32%。罗晶晶等^[57]研究发现,纤维素酶、木瓜蛋白酶有助于提高红茶的茶黄素、茶红素含量,而木聚糖酶处理后红茶茶黄素含量比对照提高了 0.01%, 茶红素含量较对照下降了 3.97%。林剑峰等^[58]发现添加单宁酶后茶黄素含量比对照降低了 0.22%, 添加纤维素酶后茶黄素含量比对照提高了 38.89%, 添加蛋白酶后茶黄素含量比对照提高了 5.56%, 而且通过“蛋白酶+单宁酶”“蛋白酶+纤维素酶”“单宁酶+纤维素酶”复配后,红茶茶黄素含量均比对照有所提高。叶飞等^[59-61]在红茶加工中添加金水 1 号、丰水、黄花和鄂梨 2 号砂梨品种等外源梨多酚氧化酶,发现红茶亮度显著上升($P < 0.01$),茶汤色相值提高了 12.00%, 茶黄素含量比对照提高了 16.22% ($P < 0.01$), 茶红素含量比对照提高了 12.65% ($P < 0.01$), 而且还改善了红茶风味品质。

毛清黎等^[62-65]将外源多糖水解酶添加到红碎茶加工工艺过程中,发现外源多糖水解酶水解茶叶细胞壁的不溶性多糖,带来破壁效应,促使发酵茶胚酸化,进而提高茶叶多酚氧化酶活性,即通过外源多糖水解酶和茶叶内源酶的协同作用,红碎茶中茶黄素含量比对照提高了 59.68%, 进而提高红碎茶品质。刘仲华等^[66]将 80 $\mu\text{g/mL}$ 的胰蛋白酶溶液分别喷洒在揉捻叶和发酵叶

上,茶叶多酚氧化酶活性分别比对照提高了 67.38%, 75.93%, 茶黄素含量较对照分别提高了 42.68%, 48.72%, 茶红素含量较对照分别提高了 11.17%, 10.86%, 茶褐素含量较对照分别降低了 19.14%, 19.08%, 而且红茶的红色色度(a)、橙黄色度(b)、亮度(L)、红色色度与橙黄色度之比(Hab)明显提高,从而使茶汤和叶底的色调偏向红色,说明添加胰蛋白酶可减轻中小叶种红茶发酵不匀、汤色深暗、叶底花朵的现象。在发酵过程中喷施硫酸铜、柠檬酸、亚油酸溶液后,茶叶多酚氧化酶活性提高,茶叶过氧化酶活性得到了显著抑制,茶黄素和茶红素含量显著提高,茶褐素得到显著降低,效果为亚油酸>硫酸铜>柠檬酸。

程谦伟等^[67]研究发现,添加罗汉果提取物、银杏叶提取物处理的茶黄素含量分别比对照提高了 25.63%, 9.38%, 茶红素含量分别比对照提高了 10.61%, 4.55%, 茶褐素含量分别比对照提高了 23.33%, 17.22%。马中华等^[68]研究发现,乙酸钠处理既可提高茶黄素、茶红素含量,与对照相比分别提高了 6.36%, 14.59%, 茶褐素含量比对照降低了 9.00%;氯化钠处理后茶黄素、茶红素、茶褐素含量分别比对照降低了 33.33%, 4.04%, 6.29%;柠檬酸钠处理后茶黄素、茶红素、茶褐素含量分别比对照降低了 41.90%, 4.65%, 8.39%;硫酸铵处理后茶黄素、茶红素、茶褐素含量分别比对照降低了 23.81%, 31.06%, 21.56%。综上,不同种类的酶类、天然提取物、无机酸类、盐类等添加剂对红茶茶黄素、茶红素、茶褐素形成量变化的影响不同。

5 展望

发酵是红茶茶色素形成的关键环节,不同发酵因子的影响作用差异较大。发酵温度、发酵湿度、发酵时间、外源添加物等发酵因子的影响,既有单因子影响效应,也有交互影响,其交互作用特点和互作机理研究仍比较薄弱,可能是未来红茶发酵的研究热点和难点之一。茶黄素是儿茶素及其他酚类物质的低聚物,分子结构、理化特性相对比较明晰,可以进行定性、定量分析^[69]。茶红素、茶褐素是聚合度更高、聚合关系更复杂的混合物^[70-73],其具体的分子结构、理化特性、形成机理等均不明晰,虽然有较多的研究和报道^[74-78],但目前还未形成统一的结论,茶红素、茶褐素的分离纯化、风味特征、生物活性等有待深入研究。

经典红茶发酵工艺,通常采用前后一致的发酵温度,无论温度高还是温度低,都无法兼顾发酵速度提升、酶活性发挥、目标内含物转化充分等多个因素水平。红茶发酵动力学研究^[79]表明,发酵过程中,茶多酚类物质首先氧化成邻醌,酚醌聚合形成茶黄素类物质的活化能要高于聚合形成茶红素、茶褐素的;高温促进茶黄素的形成,但不利于茶黄素的持续积累,低温促进茶黄素的积累,但由

于茶黄素形成量较低,茶红素、茶褐素的形成量也较低。因此,采用发酵前期高温处理、中后期低温处理的变温发酵方式,可能有利于茶黄素、茶红素、茶褐素类物质的协调形成,有关变温发酵的关键控制技术、作用机理等方面研究比较薄弱,也是红茶发酵研究的热点和难点之一。

近年来,国内外学者围绕红茶发酵适度开展了广泛且深入的研究,主要集中在红茶发酵适度判定方法、红茶发酵适度调控技术等^[80],感官评价和定量评价结合将是红茶发酵度判别的主要方向。此外,外源酶、天然提取物、无机盐、无机酸等外源添加物的引入,可以改变红茶茶黄素、茶红素、茶褐素的含量和比例,可以用来加工特色风味的红茶新产品,或用于加工适宜茶叶深加工的原料茶,不仅可以拓宽利用途径,而且可以提高茶资源的高值化、多元化利用水平。

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