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不同条件对冠突散囊菌发酵夏秋茶品质的影响

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摘要 为提升夏秋季茶树鲜叶资源利用率、改善夏秋茶品质, 以夏秋季茶叶为原料, 通过人工接种冠突散囊菌(*Eurotium cristatum*)定向发酵, 探究不同条件(含水量、接种量、温度、时间)对冠突散囊菌发酵茶感官与理化品质的影响。结果显示: 当冠突散囊菌接种量为 1.5×10^7 CFU、揉捻叶含水量为 30%、发酵温度为 28℃、发酵时间为 8 d 时, 冠突散囊菌发酵茶感官最优, 具体表现为“金花”生长茂盛, 菌香浓郁, 纯正协调, 汤色黄橙尚亮, 滋味醇厚回甘; 与未发酵样品相比, 发酵夏秋茶中茶多酚、水浸出物、可溶性糖、游离氨基酸及茶黄素的含量均出现不同程度下降, 降幅分别为 23.38%、17.42%、47.18%、30.41% 和 9.30%, 茶红素和茶褐素含量分别显著升高 31.53%、21.10%。可见, 冠突散囊菌发酵能明显改善夏秋茶品质, 有助于开发利用夏秋茶资源。

关键词 冠突散囊菌; 茯砖茶; 夏秋茶; 发酵; 感官品质; 理化成分

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黑茶属于中国传统六大茶类的一种, 具有独特的风味品质, 在茶产业中占据重要地位^[1]。茯砖茶作为黑茶代表性品类, 其品质核心依赖于冠突散囊菌(*Eurotium cristatum*, 俗称金花菌)主导的“发花”工艺^[2]。研究表明, 冠突散囊菌不仅赋予茯砖茶特殊风味, 还具有调节代谢、抗氧化^[3]及改善肠道菌群^[4]等功效。传统茯砖茶的制作主要依赖自然发花, 该模式存在显著缺陷: 生产周期冗长、品质稳定性不足、人力成本较高, 严重制约黑茶产业规模化与标准化发展。另外, 夏秋茶因苦涩味较重, 鲜爽度不足, 市场接受度低, 导致我国夏秋季茶树鲜叶资源利用率不足 30%, 严重影响整个茶产业的经济效益^[5]。针对上述问题, 本研究利用夏秋茶高多酚、低氨基酸这一特点, 将其作为原料, 通过人工接种冠突散囊菌定向发酵开发新产品, 探究揉捻叶含水量、接种量、发酵温度和发酵时间对冠突散囊菌发酵茶感官与理化品质的影响, 旨在揭示人工调控发酵过程中茶叶的品质变化规律, 解决传统茯砖茶品质稳定性不足这一缺陷, 为夏秋茶资源高效利用提供技术支撑。

1 材料与方法

1.1 材料

冠突散囊菌茶原料来自华中农业大学茶园的夏秋季鲜叶, 采摘标准为 1 芽 3~4 叶。菌株为笔者所在课题组从湖南益阳茯砖茶上筛选保存的冠突散囊菌(*Eurotium cristatum*)。本研究所用福林酚、甲醇、碳酸钠、磷酸二氢钾、茚三酮、浓硫酸、萘酚、磷酸氢二钠等化学试剂, 均购自国药集团化学试剂有限公司, 纯度级别为分析纯^[6]。

1.2 菌种液制备

为获得纯种冠突散囊菌, 首先将菌种在 PDA 平板上进行活化培养, 培养条件为 28℃。6~8 d 后, 挑取典型菌落再次于 PDA 平板上划线分离, 以确保菌种的纯度。随后, 刮取培养 7 d 的菌丝体, 悬浮于含无菌玻璃珠的无菌水中, 经 1 500 r/min 离心 4 min 后, 取上清液利用无菌脱脂棉进行过滤, 通过形态学初步鉴定, 确定其为纯种冠突散囊菌。将冠突散囊菌孢子悬液进行梯度稀释, 使其浓度保持在 1×10^7 CFU/mL, 即可得到发酵所用种子液。

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1.3 不同条件下发酵冠突散囊菌茶

采摘1芽3~4叶夏秋茶,摊放厚度3~5 cm,温度22~26℃,湿度65%~70%,摊放8 h左右,进行250℃滚筒杀青,时间2~3 min,然后揉捻10 min,将揉捻叶进行40℃低温烘干失水处理,控制最初含水率为50%。揉捻叶分装50 g/瓶,于121℃灭菌20 min,冷却后接入冠突散囊菌孢子悬浮液,置于恒温培养箱培养,取出于80℃干燥100 min,至含水量低于6%。在此基础上,设置揉捻叶含水量(20%、30%、40%、50%)、发酵时间(0、2、4、6、8、10、12、14 d)、接种量(分别接种0.5、1.0、1.5 mL,接种量分别为 0.5×10^7 、 1.0×10^7 、 1.5×10^7 CFU)、发酵温度(20、28、37℃)4个因素进行单因素试验(固定因素水平为:揉捻叶含水量40%、发酵时间8 d、接种量 1.0×10^7 CFU、发酵温度28℃)。单因素试验所获样品烘干后置于-20℃冰箱保存。

1.4 冠突散囊菌茶发酵条件优化与验证

根据单因素试验结果,确定28℃为适宜发酵温度,并选取对茶叶品质具关键影响的3个因素:揉捻叶含水量(A)、接种量(B)和发酵时间(C),进行三因素三水平的正交试验设计(因素水平编码见表1),从而确定最优发酵工艺参数^[7]。

表1 正交试验条件因素水平

Table 1 Factors and levels of orthogonal design

水平 Levels	含水量(A)/%	接种量(B)/CFU	发酵时间(C)/d Fermentation time
1	50	0.5×10^7	5
2	40	1.0×10^7	8
3	30	1.5×10^7	11

在单因素和正交试验基础上,利用获得的较优条件发酵夏秋茶,同时随机选取条件1(含水量40%、接种量 1.0×10^7 CFU、发酵5 d),条件2(含水量50%、接种量 0.5×10^7 CFU、发酵11 d)与传统茯砖茶进行感官与理化品质比较,编号为YZ-1、YZ-2、YZ-3、YZ-4,验证最优发酵条件^[7]。

1.5 茶叶感官审评方法

感官审评于华中农业大学园艺林学学院茶叶审评室进行。审评小组由6位审评员组成(3男3女,平均年龄25岁),均有2 a以上审评经验。感官审评参照GB/T 23776—2018《茶叶感官审评方法》中的黑茶(散茶)审评方法,结合冠突散囊菌发酵茶特有的品质,审评其外形、汤色、香气、滋味和叶底^[8]。

1.6 茶叶理化成分测定方法

水分测定:直接干燥法(GB 5009.3—2016《食品

安全国家标准 食品中水分的测定》);水浸出物测定:减压抽滤法(GB/T 8305—2013《茶水浸出物测定》);茶多酚测定:福林酚比色法(GB/T 8313—2018《茶叶中茶多酚和儿茶素类含量的检测方法》);游离氨基酸测定:茚三酮比色法^[8];可溶性糖测定:蒽酮比色法^[9];茶色素测定:系统分析法^[10]。

1.7 数据处理与分析

所有试验均重复3次,采用Excel 2019进行数据处理,采用IBM SPSS Statistics 26进行方差分析和显著性检验。

2 结果与分析

2.1 揉捻叶含水量对冠突散囊菌发酵茶品质的影响

1)揉捻叶含水量对发酵茶感官品质的影响。对不同含水量揉捻叶制成的发酵茶进行感官审评,见表2。随着揉捻叶含水量增加,发酵茶感官评分先高后低。当含水量为30%时,汤色黄橙尚亮,滋味表现为浓醇回甘,且菌香较浓,发酵香明显,感官评分最高,为88.00分。当含水量在20%与50%时,汤色黄褐尚亮,滋味醇和;含水量为40%时,滋味表现为醇厚回甘。故当揉捻叶含水量为30%时,冠突散囊菌发酵茶的感官品质最好。

2)揉捻叶含水量对发酵茶理化品质的影响。对不同含水量揉捻叶制备的发酵茶进行理化成分分析,结果(图1)显示,随着含水量增加,可溶性糖、水浸出物和茶褐素含量增加;游离氨基酸和茶黄素含量减少;茶红素含量先升后降。水浸出物含量与茶汤的品质呈正相关,含量越高茶汤滋味越丰富,含量越低茶汤滋味越寡淡^[11],其含量升高,可能是由于冠突散囊菌在生长过程中分泌蛋白酶、纤维素酶,将茶叶中的蛋白质、纤维素等成分降解,生成了氨基酸、多酚等物质^[12]。茶红素在抗氧化、抗癌、改善消化系统等方面有较好的生物活性,其抗氧化机制主要表现在抑制自由基的产生和螯合过渡金属离子^[13]。茶红素含量在含水量为30%时最高,且与其他含水量水平存在极显著差异($P < 0.01$)。经过发酵,茶黄素和茶红素含量减少,茶褐素含量增加,原因可能是冠突散囊菌在生长过程中分泌的胞外酶催化多酚类物质,使茶黄素、茶红素氧化络合形成茶褐素^[14]。因此揉捻叶含水量为30%时有利于发酵茶理化品质的形成。综合感官品质,揉捻叶的含水量在30%时有助于冠突散囊菌发酵茶品质的形成。

2.2 接种量对冠突散囊菌发酵茶品质的影响

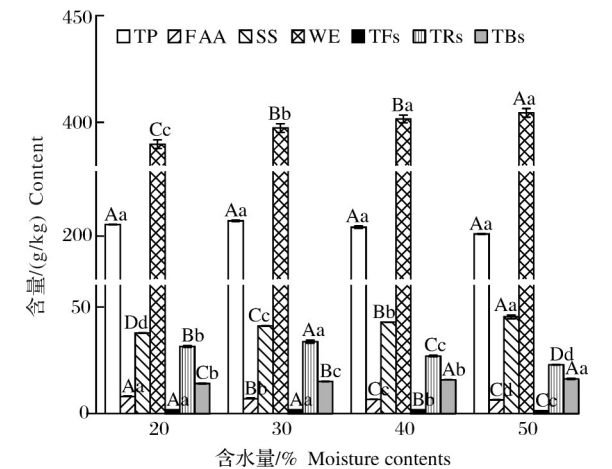
1)接种量对发酵茶感官品质的影响。对不同梯

表2 不同含水量的冠突散囊菌发酵茶感官品质

Table 2 Sensory qualities of *E. cristatum* tea fermented by different moisture contents

含水量/% Moisture contents	外形(20%) Appearance	汤色(15%) Liquor color	香气(25%) Aroma	滋味(30%) Taste	叶底(10%) Infused leaf	总分 Total score
20	金花茂盛、暗黄 Lush goldenrod fungi, dull yellow	黄褐、尚亮 Yellow-brown, little bright	菌香较浓、发酵香明显 Stronger mushroom aroma, pronounced fermented aroma	醇和 Mellow	褐黑、尚匀 Black-brown, little uniform	78.67± 1.29Bc
30	金花茂盛、褐 Lush goldenrod fungi, brown	黄橙、尚亮 Yellow-orange, little bright	菌香较浓、发酵香明显 Stronger mushroom aroma, pro- nounced fermented aroma	浓醇回甘 Rich and sweet	褐黑、尚匀 Black-brown, little uniform	88.00± 1.14Aa
40	金花茂盛、褐 Lush goldenrod fungi, brown	黄橙、尚亮 Yellow-orange, little bright	菌香较浓、发酵香明显 Stronger mushroom aroma, pronounced fermented aroma	醇厚回甘 Heavy and mellow, sweet after taste	褐黑、尚匀 Black-brown, little uniform	86.67± 2.08Aa
50	金花较盛、黄褐 Slightly lush goldenrod fungi, yellow-brown	黄褐、尚亮 Yellow-brown, little bright	菌香较浓、发酵香明显 Stronger mushroom aroma, pronounced fermented aroma	醇和 Mellow	褐黑、尚匀 Black-brown, little uniform	82.33± 0.62Bb

注：表中不同大小写字母分别表示在0.01与0.05水平上的差异显著性。下同。Note: Different uppercase and lowercase letters in the table indicate significant differences at the 0.01 and 0.05 levels, respectively. Values in the same column followed by the same letter are not significantly different. The same as below.



TP、FAA、SS、WE、TFs、TRs、TBs 分别表示茶多酚、游离氨基酸、可溶性糖、水浸出物、茶黄素、茶红素、茶褐素。下同。In the figure, TP, FAA, SS, WE, TFs, TRs, and TBs represent tea polyphenols, free amino acids, soluble sugars, water extract, theaflavins, thearubigins and theabrownins, respectively. The same as below.

图1 不同含水量的冠突散囊菌发酵茶理化成分

Fig.1 Physicochemical components of *E. cristatum* tea fermented by different moisture contents

度接种量条件下制备的样品开展感官品质评定,结果(表3)显示,在冠突散囊菌接种量为 1.5×10^7 CFU时,香气和滋味最佳,表现为菌香浓郁、纯正和浓醇回甘,其感官审评得分也最高,为86.33分。接种量为 2.0×10^7 CFU时,菌香明显但闷味也较明显;滋味表现为醇和。冠突散囊菌是好氧性微生物,出现闷味可能是因为接种量过大,导致生长过程中缺氧,生

长受抑制,而一些厌氧或兼性厌氧的杂菌开始大量繁殖,产生酸、醛、醇等物质,形成“闷味”^[15]。因此,冠突散囊菌的接种量为 1.5×10^7 CFU时,冠突散囊菌发酵茶的感官品质最好。

2)接种量对发酵茶理化品质的影响。对不同梯度接种量条件下制备的茶样品进行理化品质分析,结果(图2)显示,接种量为 1.5×10^7 CFU时,游离氨基酸、茶黄素的含量最高;茶红素含量先升后降,在 1.0×10^7 CFU时最高;茶褐素含量随接种量的增加而增加;茶多酚含量显著降低,可能是因为随着接种量的增加,冠突散囊菌会分泌过氧化酶、多酚氧化酶等物质,使茶多酚降解,导致其含量降低^[16]。发酵过程中冠突散囊菌或许将可溶性糖作为直接营养源来维持自身生长代谢,导致其含量下降^[17]。由此得出,接种量为 1.0×10^7 CFU和 1.5×10^7 CFU更有利于冠突散囊菌发酵茶理化品质的形成。结合感官品质分析,接种量以 1.5×10^7 CFU更有利于冠突散囊菌茶品质的形成。

2.3 发酵温度对冠突散囊菌发酵茶品质的影响

1)发酵温度对发酵茶感官品质的影响。对不同温度下发酵的茶叶进行感官审评,结果(表4)显示,随着发酵温度的升高,感官评分先高后低。当发酵温度为28℃时,感官评分最高,为93.33分,具体表现为金花生长茂盛且色褐;汤色黄橙尚亮;菌香浓郁且纯正;滋味浓醇回甘。这可能是因为冠突散囊菌“发花”,使得茶多酚含量减少,降低了茶汤的苦涩味,使回甘更明显^[18]。20℃时,茶叶表面存在少量金花,色

表 3 不同接种量的冠突散囊菌发酵茶感官品质

Table 3 Sensory qualities of <i>E. cristatum</i> tea fermented by different inoculation amounts						
接种量/ CFU Inoculation amount	外形(20%) Appearance	汤色(15%) Liquor color	香气(25%) Aroma	滋味(30%) Taste	叶底(10%) Infused leaf	总分 Total score
0.5×10 ⁷	金花较少、黄褐 Less goldenrod fungi, yellow-brown	褐、尚亮 Brown, little bright	菌香较淡、发酵香较淡 Weaker mushroom aroma, weaker fermented aroma	醇和 Mellow	褐黑、尚匀 Black-brown, little uniform	76.67± 1.13Bc
1.0×10 ⁷	金花茂盛、黄褐 Lush goldenrod fungi, yellow-brown	黄褐、尚亮 Yellow-brown, little bright	菌香较浓、发酵香明显 Stronger mushroom aroma, pronounced fermented aroma	醇厚 Heavy and mel- low	褐黑、尚匀 Black-brown, little uniform	83.67± 1.58Ab
1.5×10 ⁷	金花茂盛、褐 Lush goldenrod fungi, brown	黄橙、尚亮 Yellow-orange, little bright	菌香浓郁、纯正 Strong mushroom aroma, pure	浓醇回甘 Rich and sweet	褐黑、尚匀 Black-brown, little uniform	86.33± 1.25Aa
2.0×10 ⁷	金花茂盛、褐 Lush goldenrod fungi, brown	黄橙、尚亮 Yellow-orange, little bright	菌香明显、闷味明显 Pronounced mushroom aro- ma, pronounced stuffy flavor	醇和 Mellow	褐黑、尚匀 Black-brown, little uniform	74.33± 0.48Bc

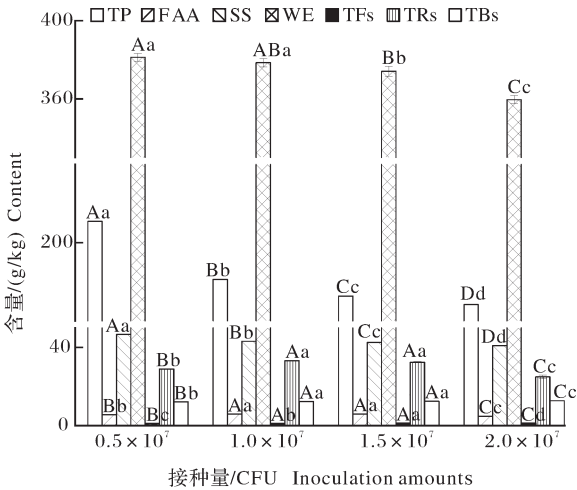


图 2 不同接种量的冠突散囊菌发酵茶理化成分

Fig.2 Physicochemical components of *E. cristatum* tea fermented by different temperatures

泽暗黄；汤色为褐色尚亮，菌香较淡，滋味醇和。37℃时，金花生长较盛，汤色为黄褐；菌香较浓但有明显的闷味。可能因为发酵温度过高，超出冠突散

囊菌最适生长温度使其生长放缓，利于其他杂菌生长以至于出现闷味。根据感官审评结果，发酵温度为 28℃时，冠突散囊菌发酵茶的感官品质表现最好。

2) 发酵温度对发酵茶理化品质的影响。对不同温度条件下制备的发酵茶样品开展理化品质分析，结果(图 3)显示，随着温度的升高，茶多酚、游离氨基酸和水浸出物的含量先下降后上升，且在 28℃时，含量最低；可溶性糖含量持续降低；茶褐素含量一直升高；茶黄素和茶红素的含量在 28℃时最高。茶多酚是茶汤中主要的苦味物质^[19]，28℃时，冠突散囊菌生长最旺盛，能分泌大量的胞外酶如多酚氧化酶、淀粉酶等，对茶多酚、儿茶素等具有转化作用^[20]，茶多酚在酶的催化下被氧化聚合，生成茶色素，因此茶多酚含量减少，茶红素和茶褐素含量增加^[14]。温度从 20℃上升至 28℃，游离氨基酸和可溶性糖含量显著下降，可能是因其作为冠突散囊菌生长所需的主

表 4 不同发酵温度的冠突散囊菌发酵茶感官品质

Table 4 Sensory qualities of <i>E. cristatum</i> tea fermented by different fermentation temperatures						
发酵温度/℃ Fermentation temperature	外形(20%) Appearance	汤色(15%) Liquor color	香气(25%) Aroma	滋味(30%) Taste	叶底(10%) Infused leaf	总分 Total score
20	金花较少、暗黄 Less goldenrod fungi, dull yellow	褐、尚亮 Brown, little bright	菌香较淡、发酵香较淡 Weaker mushroom aroma, weaker fermented aroma	醇和 Mellow	褐黑、尚匀 Black-brown, little uniform	76.67± 1.64Cc
28	金花茂盛、褐 Lush goldenrod fungi, brown	黄橙、尚亮 Yellow-orange, little bright	菌香浓郁、纯正 Rich mushroom aroma, pure	浓醇回甘 Rich and sweet	褐黑、尚匀 Black-brown, little uniform	93.33± 0.58Aa
37	金花较盛、黄褐 Slightly lush goldenrod fungi, yellow-brown	黄褐、尚亮 Yellow-brown, little bright	菌香较浓、闷味较重 Stronger mushroom aroma, stronger stuffy flavor	醇厚 Heavy and mellow	褐黑、尚匀 Black-brown, little uniform	81.33± 0.38Bb

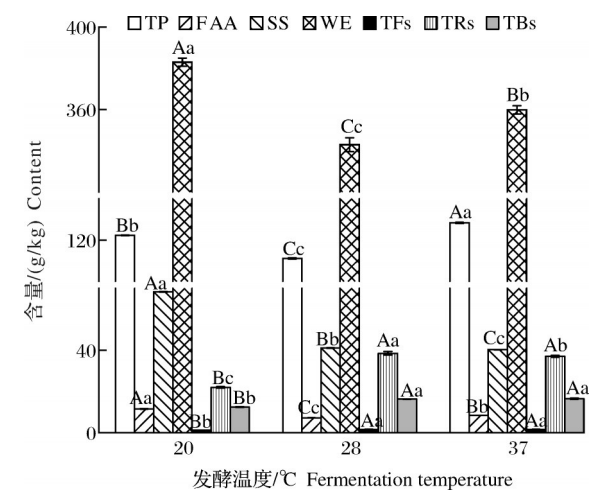


图3 不同发酵温度的冠突散囊菌发酵茶理化成分
Fig.3 Physicochemical components of *E. cristatum* tea fermented by different temperatures

要碳源与氮源物质而被利用消耗^[21]。结合感官品质,发酵温度为28℃时,冠突散囊菌的生长状况良好,且对茶叶中的理化成分影响显著($P<0.05$),利于发酵茶香气、汤色和滋味达到较好的水平。因此,发酵温度为28℃更有利于冠突散囊菌发酵茶品质的

形成。
2.4 发酵时间对冠突散囊菌发酵茶品质的影响

1)发酵时间对发酵茶感官品质的影响。对不同发酵时间的茶叶进行感官审评,结果(表5)显示,随着发酵时间的增加,感官得分先高后低。第8天时,金花生长旺盛、色褐、汤色黄橙尚亮,菌香浓郁且纯正,滋味浓醇回甘,各项指标表现优异,感官得分最高,为92.26分。可能是由于冠突散囊菌促进糖苷水解和类胡萝卜素降解,形成 β -芳樟醇、 β -紫罗兰酮等关键香气化合物,赋予茶叶特殊的“金花菌香气”^[22]。0~8 d时,金花生长逐渐茂盛,汤色由褐转变为黄褐再表现为黄橙,菌香逐渐变浓,滋味也由醇和转变为浓醇回甘。可能是因为茶叶中的苦涩味物质被冠突散囊菌利用代谢,茶汤滋味得到改善。8~14 d时,茶叶逐渐出现闷味,滋味由浓醇回甘变为醇和。表5还显示,冠突散囊菌茶在发酵前、中、后期的感官得分具有极显著差异($P<0.01$),表明发酵时间对发酵茶风味品质具有重要影响。发酵时间过短或过长均不利于感官品质的形成,因此,确定最佳发酵时间为8 d,此时茶叶的感官品质表现最优。

表5 不同发酵时间的冠突散囊菌发酵茶感官品质 Table 5 Sensory qualities of <i>E. cristatum</i> tea fermented by different fermentation times						
发酵时间/d Fermentation time	外形(20%) Appearance	汤色(15%) Liquor color	香气(25%) Aroma	滋味(30%) Taste	叶底(10%) Infused leaf	总分 Total score
0	无金花、褐 Void goldenrod fungi, brown	褐、尚亮 Brown, little bright	无菌香 Void mushroom aroma	略带苦涩 Slightly bitter	褐黑、尚匀 Black-brown, sittle uniform	75.33±1.09Ef
2	金花显露、暗黄 Goldenrod fungi revealed, dull yellow	褐、尚亮 Brown, little bright	菌香较淡 Weaker mushroom aroma	尚醇和,略苦涩 Still mellow, slightly bitter	褐黑、尚匀 Black-brown, sittle uniform	79.67±1.46De
4	金花较盛、黄褐 Slightly lush goldenrod fungi, yellow-brown	黄褐、尚亮 Yellow-brown, little bright	菌香较淡、发酵香较淡 Weaker mushroom aroma, weaker fermented aroma	醇和 Mellow	褐黑、尚匀 Black-brown, little uniform	83.00±0.86Cd
6	金花茂盛、黄褐 Lush goldenrod fungi, yellow-brown	黄褐、尚亮 Yellow-brown, little bright	菌香较浓、发酵香较浓 Stronger mushroom aroma, stronger fermented aroma	醇厚回甘 Heavy and mellow, sweet after taste	褐黑、尚匀 Black-brown, little uniform	87.67±1.18Bb
8	金花旺盛、褐 Exuberant goldenrod fungi, brown	黄橙、尚亮 Yellow-orange, little bright	菌香浓郁、纯正 Rich mushroom aroma, pure	浓醇回甘 Rich and sweet	褐黑、尚匀 Black-brown, little uniform	92.26±0.46Aa
10	金花茂盛、褐 Lush goldenrod fungi, brown	黄橙、尚亮 Yellow-orange, little bright	菌香明显、略带闷味 Pronounced mushroom aro-ma, slightly stuffy flavor	醇厚回甘 Heavy and mellow, sweet after taste	褐黑、尚匀 Black-brown, little uniform	88.33±1.23Bb
12	金花较盛、褐 Slightly lush goldenrod fungi, brown	黄褐、尚亮 Yellow-brown, little bright	菌香明显、闷味较重 Pronounced mushroom aroma, stronger stuffy flavor	醇厚 Heavy and mellow	褐黑、尚匀 Black-brown, little uniform	85.33±0.68Cc
14	金花较盛、褐 Slightly lush goldenrod fungi, brown	黄褐、尚亮 Yellow-brown, little bright	菌香明显、闷味明显 Pronounced mushroom aro-ma, pronounced stuffy flavor	醇和 Mellow	褐黑、尚匀 Black-brown, little uniform	83.00±1.05Cd

2) 发酵时间对发酵茶理化品质的影响。由图4可知,在发酵过程中茶多酚、水浸出物、可溶性糖含量下降,游离氨基酸、茶黄素、茶红素含量先升后降,而茶褐素含量一直上升。图4还显示,不同发酵时间发酵茶理化成分的含量差异显著($P<0.05$),茶红素含量在发酵第8天时达到最大值,为43.07 g/kg,且与其他发酵时间存在极显著差异($P<0.01$)。随着发酵时间的延长,茶多酚、可溶性糖和游离氨基酸被消耗过多,导致茶汤滋味淡薄^[21];茶

黄素与茶红素经过氧化聚合生成茶褐素,因此茶褐素含量升高^[14],导致汤色表现为黄褐尚亮。在发酵后期,冠突散囊菌生长速度减缓,出现一些杂菌如黑曲霉、青霉等,致使茶叶出现闷味^[15]。整体来看,8 d时,除茶褐素外的理化成分含量与其他时间均存在极显著差异($P<0.01$)。由此得出,发酵8 d更有利于冠突散囊菌茶理化品质的形成。结合感官品质考虑,发酵时间为8 d更有利于冠突散囊菌发酵茶品质的形成。

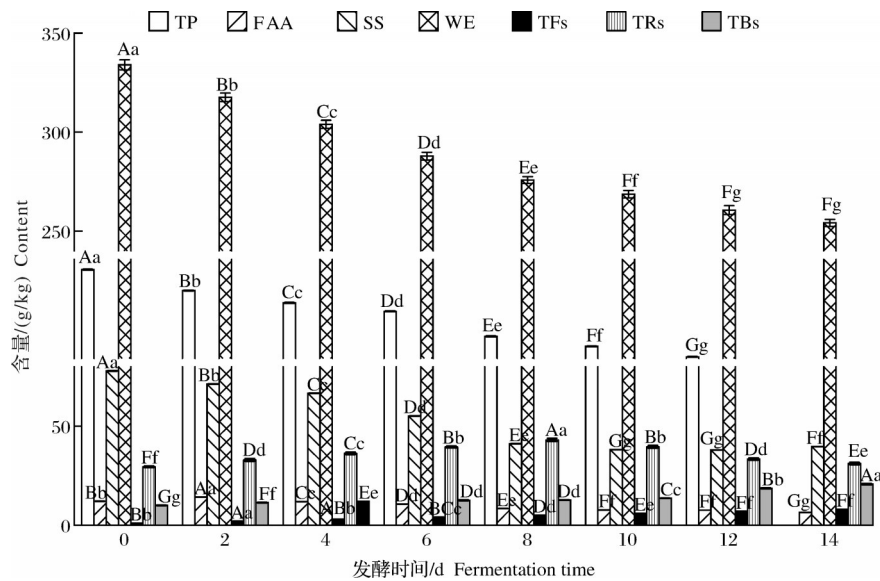


图4 不同发酵时间的冠突散囊菌发酵茶理化成分

Fig.4 Physicochemical components of *E. cristatum* tea fermented by different fermentation times

2.5 正交试验条件对冠突散囊菌发酵茶品质的影响

1) 正交试验条件下发酵茶感官品质。对正交试验发酵的茶叶进行感官分析,结果(表6)显示,处理9(含水量30%、接种量 1.5×10^7 CFU、发酵8 d)的感官审评得分最高,为92.67分;其次为处理2(含水量50%、接种量 1.0×10^7 CFU、发酵8 d)、处理4(含水量40%、接种量 0.5×10^7 CFU、发酵8 d)和处理6(含水量40%、接种量 1.5×10^7 CFU、发酵5 d),均在80分以上,其余处理分数偏低。4个处理中有3个的发酵时间为8 d,接种量在 1.0×10^7 CFU及以上,说明发酵8 d、接种量至少为 1.0×10^7 CFU,有利于茶叶感官品质形成,而含水量在30%~50%均可。表7还显示,不同发酵时间发酵茶感官品质差异极显著($P<0.01$),不同接种量处理下感官品质差异显著($P<0.05$),但失水率差异不显著。基于此,冠突散囊菌发酵茶感官品质形成的较优发酵条件组合为A1/2/3B2/3C2。

2) 正交试验条件下发酵茶理化品质。对不同正

交试验条件下制备的发酵茶样品进行理化分析,结果如图5所示,方差分析结果汇总于表7。结果显示,揉捻叶含水量对茶多酚含量影响不显著,而对其他理化成分影响均极显著($P<0.01$);接种量对各理化成分的影响程度不同:接种量极显著($P<0.01$)影响游离氨基酸、茶红素和茶褐素含量,显著影响可溶性糖与茶黄素含量($P<0.05$),而对茶多酚和水浸出物含量的影响不显著($P>0.05$)。此外,发酵时间对全部被测理化成分影响均极显著($P<0.01$)。茶多酚和水浸出物是影响茶汤品质的重要因素,含量低会导致茶汤滋味寡淡,而茶红素是构成茶汤红色基调和汤色亮度的核心物质,也是影响茶汤浓厚的主要物质^[23]。经综合比较单因素和正交试验数据,以感官得分高及茶多酚、水浸出物、茶红素等关键指标含量较优为依据,筛选最终优选的发酵工艺为A3B3C2,具体参数为揉捻叶含水量30%、接种量 1.5×10^7 CFU、发酵时间8 d。

表 6 正交试验条件下制备的冠突散囊菌发酵茶感官品质

Table 6 Sensory qualities of *E. cristatum* tea fermented by orthogonal experiments

正交试验 Orthogonal experiments	外形(20%) Appearance	汤色(15%) Liquor color	香气(25%) Aroma	滋味(30%) Taste	叶底(10%) Infused leaf	总分 Total score
1	金花较少、暗黄 Less goldenrod fungi, dull yellow	黄褐 Yellow-brown	菌香较淡,发酵香较淡 Weaker mushroom aroma, weaker fermented aroma	醇和 Mellow	褐黑、匀整 Black-brown, uniform	78.00± 2.21Cd
2	金花茂盛,褐 Lush goldenrod fungi, brown	黄橙尚亮 Yellow-orange, little bright	菌香浓郁、发酵香浓郁 Rich mushroom aroma, rich fermented aroma	醇厚回甘 Heavy and mellow, sweet after taste	褐黑、尚匀 Black-brown, little uniform	90.33± 0.48AaBb
3	金花较盛,黄褐 Slightly lush goldenrod fungi, yellow-brown	黄褐 Yellow-brown	菌香明显、闷味较重 Pronounced mushroom aro- ma, stronger stuffy flavor	醇和 Mellow	暗黑、粗老 Dark and dull, coarse and old	68.67± 2.62De
4	金花较盛,黄褐 Slightly lush goldenrod fungi, yellow-brown	黄褐尚亮 Yellow-brown, little bright	菌香较浓、发酵香较浓 Stronger mushroom aroma, stronger fermented aroma	醇厚回甘 Heavy and mellow, sweet after taste	褐黑、尚匀 Black-brown, little uniform	86.33± 2.36AbBcC
5	金花茂盛,褐 Lush goldenrod fungi, brown	黄褐尚亮 Yellow-brown, little bright	菌香明显、有闷味 Pronounced mushroom aro- ma, slightly stuffy flavor	醇和 Mellow	暗黑、粗老 Dark and dull, coarse and old	68.67± 2.04De
6	金花茂盛,褐 Lush goldenrod fungi, brown	黄褐 Yellow-brown	菌香较浓、发酵香较浓 Stronger mushroom aroma, stronger fermented aroma	醇和 Mellow	褐黑、尚匀 Black-brown, little uniform	83.67± 1.21BCcd
7	金花较盛,褐 Slightly lush goldenrod fungi, brown	黄褐尚亮 Yellow-brown, little bright	菌香明显、闷味明显 Pronounced mushroom aro- ma, pronounced stuffy flavor	醇和 Mellow	暗黑、尚匀 Dark and dull, little uniform	66.67± 2.51De
8	金花较盛,黄褐 Slightly lush goldenrod fungi, yellow-brown	黄褐 Yellow-brown	菌香较浓、发酵香较浓 Stronger mushroom aroma, stronger fermented aroma	醇和回甘 Mellow, sweet after taste	褐黑、尚匀 Black-brown, little uniform	79.67± 1.72Ccd
9	金花茂盛,褐 Lush goldenrod fungi, brown	黄橙尚亮 Yellow-orange, little bright	菌香浓郁、发酵香浓郁、纯正 Rich mushroom aroma, rich fermented aroma, pure	浓醇回甘 Rich and sweet	褐黑、匀整 Black-brown, uni- form	92.67± 1.15Aa

注:正交试验 1~9 分别为 A1B1C1、A1B2C2、A1B3C3、A2B1C2、A2B2C3、A2B3C1、A3B1C3、A3B2C1、A3B3C2;因素 A、B、C 详见表 1。
Note: Orthogonal experiments were A1B1C1, A1B2C2, A1B3C3, A2B1C2, A2B2C3, A2B3C1, A3B1C3, A3B2C1, A3B3C2, respectively.
Factors A, B, C are shown in Table 1.

表 7 正交试验条件下制备的冠突散囊菌发酵茶理化成分及感官得分的方差分析

Table 7 Analysis variance of the physicochemical components and the sensory scorces of *E. cristatum* tea fermented by orthogonal experiments

因素 Factors	自由度 df	TP	FAA	SS	WE	TFs	TRs	TBs	感官品质 Sensory qualities
含水量 Moisture contents	2	0.803	95.629**	6.946**	12.240**	15.425**	17.113**	29.220**	0.214
接种量 Inoculation amount	2	0.103	102.667**	5.108*	1.896	3.280*	19.720**	25.469**	4.738*
发酵时间 Fermentation time	2	28.755**	1 409.293**	593.313**	11.339**	15.688**	77.577**	156.886**	102.288**

注: $F(2, 20)_{0.05} = 3.49$, $F(2, 20)_{0.01} = 5.85$ 。“*”和“**”分别表示差异显著和极显著。Note: $F(2, 20)_{0.05} = 3.49$, $F(2, 20)_{0.01} = 5.85$.
“*” and “**” indicate a significant difference at the 0.05 and 0.01 levels, respectively.

2.6 冠突散囊菌茶发酵工艺技术的验证

1)感官品质验证分析。YZ-1 是正交试验中的最优工艺参数, YZ-2 和 YZ-3 是随机选取的工艺参数, YZ-4 是传统益阳茯砖茶。对 4 个验证样品感官审评结果(表 8)显示, YZ-1 获得最高感官评分, 为 92.13

分, 和其他对照样品相比具有极显著差异($P < 0.01$)。YZ-4 金花较盛, 汤色黄褐尚亮, 菌香较淡有陈味, 滋味醇和略有苦涩味。与之对比, YZ-1 的金花更加茂盛, 汤色为黄橙, 菌香也更浓郁, 滋味醇厚回甘, 感官品质更好。

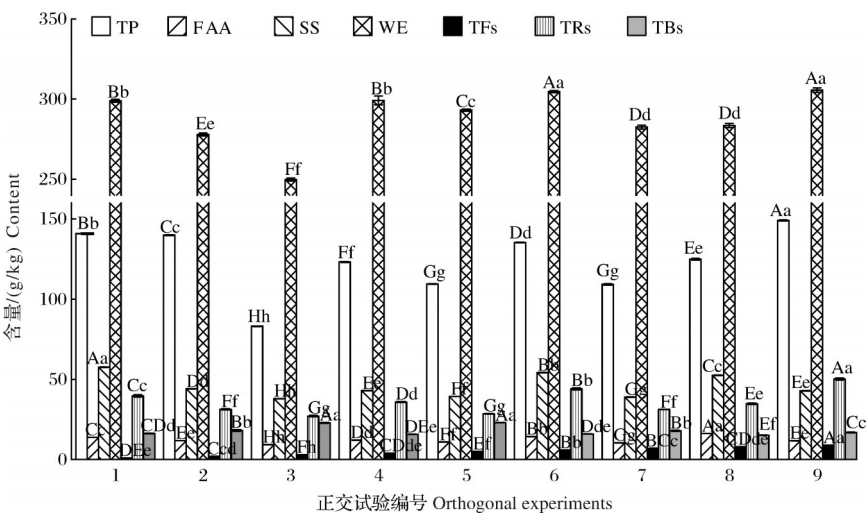


图 5 正交试验的冠突散囊菌发酵茶理化成分

Fig.5 Physicochemical components of *E. cristatum* tea fermented by orthogonal experiments

表 8 验证试验样品的感官品质

Table 8 Sensory qualities of validation test samples

茶样 Tea samples	外形(20%) Appearance	汤色(15%) Liquor color	香气(25%) Aroma	滋味(30%) Taste	叶底(10%) Infused leaf	总分 Total score
YZ-1	金花茂盛、褐 Lush goldenrod fungi, rown	黄橙尚亮 Yellow-orange, little bright	菌香浓郁、发酵香浓、纯正 Rich mushroom aroma, rich fermented aroma, pure	醇厚回甘 Heavy and mellow, sweet after taste	褐黑、匀整 Black-brown, uniform	92.13± 2.08Aa
YZ-2	金花较盛、黄褐 Slightly lush goldenrod fungi, ellow-brown	黄橙尚亮 Yellow-orange, little bright	菌香较浓、发酵香较浓 Stronger mushroom aroma, stronger fermented aroma	醇厚 Heavy and mellow	褐黑、匀整 Black-brown, uniform	85.67± 1.35Bb
YZ-3	金花茂盛、黄褐 Lush goldenrod fungi, ellow-brown	黄褐尚亮 Yellow-brown, little bright	菌香明显、略带闷味 Pronounced mushroom aro- ma, slightly stuffy flavor	醇厚 Heavy and mellow	褐黑、匀整 Black-brown, uniform	83.00± 2.65Bbc
YZ-4	金花较盛、黄褐 Lush goldenrod fungi, yellow-brown	黄褐尚亮 Yellow-brown, little bright	菌香较淡、陈味 Weaker mushroom aroma, aged aroma	醇和、略苦涩 Mellow, slightly bitter and astringent	褐黑、匀整 Black-brown, uniform	81.67± 1.24Bc

2)理化品质验证分析。对4个验证样品进行理化成分分析,结果(图6)显示,与传统茯砖茶相比, YZ-1的茶多酚含量较低,可能是因为冠突散囊菌大量繁殖时,产生的胞外酶会氧化分解多酚类化合物,使其含量下降^[16]。YZ-2的游离氨基酸、可溶性糖和水浸出物含量最高, YZ-3最低, YZ-1和YZ-4居中, 两两差异极显著($P<0.01$);这是因为冠突散囊菌在生长过程中会消耗碳源、氮源等物质,但若消耗太多,会导致茶汤滋味淡薄,影响感官品质^[21]。YZ-1的茶黄素和茶红素含量最高,与其他三者存在极显著差异($P<0.01$);YZ-4的茶褐素含量较高, YZ-2含量最低, YZ-1居中。茶褐素含量高会导致茶汤色泽暗淡,黄褐为主;茶红素和茶黄素比例协调,茶叶汤色为黄橙较亮。综合感官品质,选择YZ-1即揉捻叶含水量为30%、接种量 1.5×10^7 CFU、发酵8 d为最优发酵条件。

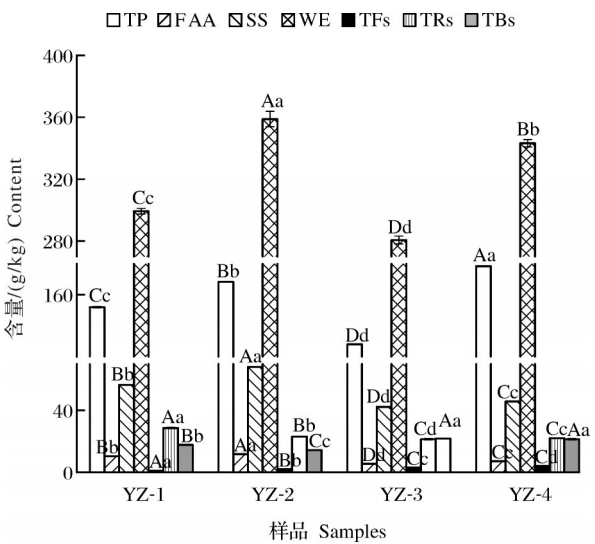


图 6 验证试验样品的理化成分

Fig.6 Physicochemical components of validation test samples

3 讨论

夏秋季茶鲜叶是我国茶叶资源的重要组成部分,其产量在全年总产量中占比60%以上^[24]。夏秋茶中多酚类物质含量较春茶高,而氨基酸和芳香类物质含量偏低,这导致夏秋茶鲜爽度不足、苦涩味重^[25]。通过冠突散囊菌对夏秋茶的定向发酵,可实现基于酶促代谢调控的品质提升。其核心机制在于菌体分泌的胞外酶系精准驱动了滋味与香气物质的协同转化。在滋味方面,多酚氧化酶与酯酶协同作用,催化酯型儿茶素发生水解及氧化聚合,将其转化为茶黄素(贡献鲜爽度)、茶红素(形成红浓汤色)及茶褐素(提升醇厚口感)^[14],并伴随没食子酸等呈味物质的生成,共同奠定茶汤“浓醇回甘”的化学基础^[26],本质上是苦涩前体被定向转化为风味产物的过程。在香气方面,糖苷水解酶释放 β -芳樟醇等萜烯醇类,类胡萝卜素降解则产生 β -紫罗兰酮等酮类物质,二者共同构成典型的“菌花香”^[22]。该转化过程依赖于适温(28℃)、好氧的发酵条件;若控制失当,出现高温缺氧条件,将促使杂菌代谢产生低阈值醛类,如己醛等物质,导致“闷味”生成^[27]。黑茶发酵的本质是:在微生物发酵、酶促反应和湿热环境的综合作用下,使茶叶内含物质发生深刻转化,形成黑茶特有的色、香、味品质^[28]。通过调控微生物代谢流,可以助力夏秋茶从“苦涩低闷”到“醇厚菌香”的品质提升,实现其高值化利用。

本研究结果表明,通过精准调控微生物代谢可以实现夏秋茶品质定向改良。当冠突散囊菌接种量为 1.5×10^7 CFU、揉捻叶含水量为30%、发酵温度为28℃、发酵时间为8 d时,在此条件下发酵的夏秋茶综合品质最好。宋林瑶等^[2]通过响应面试验表明,利用贵州绿茶制备发酵茶的最佳工艺条件为:温度28℃、含水量40%、冠突散囊菌接种量1‰、发酵时间8 d。本研究等到的最佳发酵温度和时间与此一致,表明冠突散囊菌的最佳生长温度为28℃,在茶叶中的最优发酵时间为8 d。含水量不同可能是因为本研究是用的揉捻叶,而宋林瑶采用的是成品绿茶。郑选东等^[29]通过单因素和正交试验得出的最佳发酵工艺为夏秋茶含水量30%、冠突散囊菌接种量15%、发酵时间9 d,发酵温度30℃。接种量不同可能是因为冠突散囊菌来源不同且操作细节存在差异。

本研究在无菌条件下通过人工接种冠突散囊菌进行定向发酵,验证了精准调控微生物代谢以提升夏秋茶品质的可行性。为推进该技术的工业化应

用,未来研究需着力于解析微生物代谢机制,通过实时跟踪多酚氧化酶等关键酶活性变化动态,结合代谢组学量化儿茶素、氨基酸等底物的转化通量,绘制精准的“代谢图谱”,实现对品质形成的定向引导。同时,应鉴定冠突散囊菌代谢产生的特征风味物质,建立快速品质预测模型。面对工业化开放体系的复杂性,需重点研究接种菌株与环境微生物的互作机制,通过构建以功能菌为核心的稳定群落,解决杂菌竞争问题。未来需进行中试规模试验,建成标准化工艺路线,推动该技术从实验室纯种发酵迈向工业化可控发酵,为夏秋茶高效利用提供技术支撑。与传统茯砖茶相比,冠突散囊菌发酵茶的金花更多,能够产生对人体有益的活性代谢产物^[30],促进人体消化,具有降“三高”、抗氧化和抗衰老等保健功能,适宜三高患者、肥胖人群^[20]。因此,利用夏秋季鲜叶发酵制成的冠突散囊菌茶不仅丰富了茶叶种类,还有潜在的消费需求和广阔的市场前景。

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Effects of different conditions on quality of summer-autumn tea fermented with *Eurotium cristatum*

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Abstract Fresh leaves from summer-autumn tea were directionally fermented through artificial inoculation with *Eurotium cristatum* to increase the utilization efficiency of fresh leaves from summer-autumn tea trees and improve the quality of summer-autumn tea. The effects of different conditions including the content of water, the amount, temperature, and time of inoculation on the sensory quality and physicochemical components of tea fermented by *Eurotium cristatum* were studied. The results showed that the sensory quality of tea fermented by *Eurotium cristatum* was optimal with an inoculation amount of 1.5×10^7 CFU, water content of 30% in rolled leaves, fermentation temperature of 28 °C, and fermentation time of 8 days. The tea produced under these conditions exhibited vigorous growth of “golden flowers”, a rich and pure fungal aroma, a yellowish-orange and bright infusion, and a mellow and sweet-aftertaste flavor. The content of tea polyphenols, water extracts, soluble sugars, free amino acids, and theaflavins in fermented summer-autumn tea decreased to varying degrees, with reductions of 23.38%, 17.42%, 47.18%, 30.41%, and 9.30% compared with that in unfermented summer-autumn tea. The content of thearubigins and theaflavins significantly increased by 31.53% and 21.10%, respectively. It is indicated that the fermentation with *Eurotium cristatum* can significantly improve the quality of summer-autumn tea, which is helpful for the development and utilization of summer-autumn tea resources.

Keywords *Eurotium cristatum*; Fuzhuan brick tea; summer-autumn tea; fermentation; sensory qualities; physicochemical components

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