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不同晾制湿度下雪茄烟叶变黄期非结构糖类 及质体色素的动态变化

汪薇¹, 谭永浩^{2,3}, 卓毅锋¹, 全益华¹, 刘欣瑶¹, 时雅琪⁴, 符昌武¹, 王振华¹

1. 湖南省烟草公司张家界市公司, 张家界 427000; 2. 河南农业大学烟草行业烟草栽培重点实验室, 郑州 450046; 3. 四川中烟工业有限责任公司, 成都 610017; 4. 安徽中烟工业公司技术中心雪茄所, 合肥 230000

摘要 为提升烟叶的晾制品质, 以云雪2号作为试验材料, 探究在环境温度为28℃, 环境湿度分别为90%(高湿)、80%(中湿)、70%(低湿)的条件下雪茄烟叶晾制变黄期烟叶内在化学成分的变化。结果显示: (1) 不同晾制湿度下, 烟叶晾制期间质体色素、淀粉含量整体呈下降趋势, 葡萄糖、果糖、蔗糖整体呈上升趋势。(2) 高湿处理(变黄湿度为90%)烟叶叶绿素含量降解速率缓慢, 但降解周期长, 而低湿与中湿处理在变黄96 h之后含量便趋于较低水平, 变化不明显。(3) 低湿(变黄湿度为70%)处理葡萄糖与果糖含量在变黄前期积累量明显增加, 在变黄144 h之后增量较小, 而中湿与高湿含量变化趋势与低湿相反, 在变黄前期增量不明显, 而在变黄144 h之后显著增加; 不同处理下淀粉与蔗糖含量变化趋势几近相同。(4) 相关性分析结果显示, 晾制过程中质体色素含量与非结构性糖类含量变化均达到了显著或极显著水平。综上, 低湿(变黄湿度为70%)烟叶变黄速率较快, 非结构性糖类转化迅速, 烟叶提前结束变黄; 高湿(变黄湿度为90%)烟叶在变黄前期各项指标变化较小, 144 h之后非结构性糖类转化速率较快, 一定程度上延长了变黄期时长。中湿(变黄湿度为80%)烟叶的质体色素与非结构性糖类的降解转化程度处于低湿与高湿之间。以上结果表明, 高湿晾制为烟叶变黄提供了较为合适的环境、时间条件, 促进了烟叶内含物质的转化, 因此在实际生产中, 在晾制变黄期为烟叶提供高湿的晾制环境更有利于高品质烟叶的形成。

关键词 雪茄烟叶; 晾制; 质体色素; 单糖; 低聚糖; 淀粉

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近年来, 随着我国雪茄烟生产规模的扩大, 烟叶质量提升显得愈发重要, 而目前雪茄烟叶含青(青斑、青筋和浮青)是影响品质的主要问题, 其主要原因是烟叶晾制过程中环境温湿度调控不当引起的。雪茄烟的调制是烟叶的理化性状固定、质量风格呈现的动态调控过程。晾制温度决定了烟叶晾制速度, 合适的温度有利于促进烟叶碳水化合物及质体色素类物质的降解^[1-2], 而提高温度有利于加快叶片内氮素的转化, 降低硝酸盐类物质的含量^[3]。晾制湿度决定了烟叶的晾制程度, 晾制过程中合理的温湿度调控有助于烟叶内多酚类物质转化、提高碳氮代谢能力^[4]; 已有研究表明, 中湿晾制有利于淀粉向还原糖转化^[5], 而高湿晾制则更能提高多酚氧化酶、过氧化物酶的活性^[6-7], 促进烟叶内质体色素的降解, 减少烟叶“青斑”的形

成^[8]。非结构性糖类(葡萄糖、果糖、蔗糖、淀粉等)是烟叶内各项生命活动的能量来源, 也是信号分子、渗透调节物质^[9]。前人虽然对于晾制过程中的糖类有了初步的研究, 但对于非结构性糖类的降解情况以及与质体色素之间的关系研究较少, 并且主要集中在烤烟的烘烤方面^[10-11]。本研究根据当地晾制工艺设置不同的晾制湿度, 分析晾制湿度对雪茄烟叶晾制过程中烟叶变黄期的非结构糖类及质体色素含量变化的影响, 旨在为探明非结构糖类及质体色素含量的变化规律, 并为优质雪茄烟叶生产提供科学依据。

1 材料与方法

1.1 试验地点与材料

试验于2022年在湖南省张家界市永定区教字垭

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汪薇, E-mail: 55543718@qq.com

通信作者: 王振华, E-mail: 11815401@qq.com

镇进行(29°28'86"N, 110°33'30"E), 试验田土壤质地类型为黏土, 试验地前茬作物为水稻, 土壤pH为5.96, 有机质含量40.1 g/kg、全氮2.24 g/kg, 全磷1.01 g/kg, 全钾32.13 g/kg, 有效磷28.4 mg/kg, 速效钾353.2 mg/kg、碱解氮163 mg/kg, 供试品种为当地主栽茄衣品种之一—云雪2号^[12-13], 种质来源为云南省烟草农业科学研究院。

1.2 试验设计

选取云雪2号种子进行漂浮育苗, 移栽前整地并开沟条施基肥, 所用肥料为烟草专用复合肥(N-P₂O₅-K₂O=8-14-8)、钙镁磷肥(P₂O₅-CaO-MgO=14-45-12)、提苗肥(N-P₂O₅=20-9)、腐熟菜籽饼肥(N-P₂O₅-K₂O=27-6-11)、硝酸钾、硫酸钾。保证N-P₂O₅-K₂O=1-1.5-2, 基肥、追肥比例为6:4, 全部有机肥、钙镁磷肥和60%的氮肥及钾肥于整地时开沟条施, 40%的氮肥和钾肥作为追肥, 分2次施入, 追肥时间为移栽后10 d和30 d。移栽期为2022年4月24日, 单垄单行种植, 按照行距120 cm, 株距40 cm, 20 835株/hm²移栽。烟株初花打顶, 同时抹去3~4片底脚叶。田间管理按当地雪茄烟生产技术规范进行。试验选择云雪2号中部叶(自下而上11~12叶位)作为试验材料, 待烟叶成熟后进行采收, 成熟标准为: 叶色淡黄, 叶尖叶缘黄绿偏黄, 主脉2/3发白, 支脉2/3发白, 茎叶夹角75°左右, 采收声音清脆, 断口平整。烟叶采收后统一置于智能化晾房中进行晾制, 记烟叶编烟上架后为0 d, 调制第3天选取颜色、大小相对一致的烟叶各80片转入温度28℃、湿度分别为70%(H1)、80%(H2)、90%(H3)的恒温恒湿箱中进行调制, 调制时长为8 d。分别在置于恒温恒湿箱中的第2、4、6、8天进行取样, 每次取样20片, 用锡箔纸包裹后放置液氮中暂时保存, 用于后续质体色

素、碳水化合物以及相关酶活的测定。

1.3 测定项目及方法

1) 质体色素含量的测定。采用分光光度法^[14]测定烟叶内叶绿素、类胡萝卜素含量。

2) 非结构性糖类含量的测定。烟叶淀粉、葡萄糖、果糖、蔗糖等非结构性糖类含量的测定参照苏州科铭生物技术有限公司提供的试剂盒(型号分别为: DF-1-Y、PT-1-Y、GT-1-Y、ZHT-1-Y)说明书。

3) 相关酶活性的测定。烟叶蔗糖酶、中性转化酶、淀粉酶的活性检测, 参照苏州科铭生物技术有限公司提供的酶试剂盒(型号分别为: ZTM-1-Y、NI-1-Y、DFMA-1-Y)说明书。

4) 数据的处理与分析。采用Microsoft Excel 2019进行数据整理、Origin2021进行相关性分析及图表的绘制。

2 结果与分析

2.1 雪茄烟叶变黄期叶绿素含量的动态变化

变黄期是烟叶颜色由绿变黄的重要时期。图1为不同晾制湿度下雪茄烟叶晾制变黄期叶绿素随晾制时间的变化情况, 由图1可见, 不同处理下叶绿素含量变化趋势相同, 均呈现出随晾制时间的推进逐渐降低的趋势, 表明不同晾制湿度在一定程度上影响雪茄烟叶变黄进程, 但未改变烟叶的褪绿趋势。由图1A可知, 烟叶的叶绿素a含量变化主要发生在变黄48~144 h, 各处理分别降低了90.2%、88.2%、54.4%, 但H3处理在144 h时叶片内仍含有较高的叶绿素a, H1和H2处理在变黄144~192 h叶绿素a含量变化甚微, 而H3处理在此期间降低了20.4%, 由此可见持续高湿影响了烟叶叶绿素a的降解速率, 导致烟叶褪绿进程缓慢。图1B反映了雪茄烟叶晾制

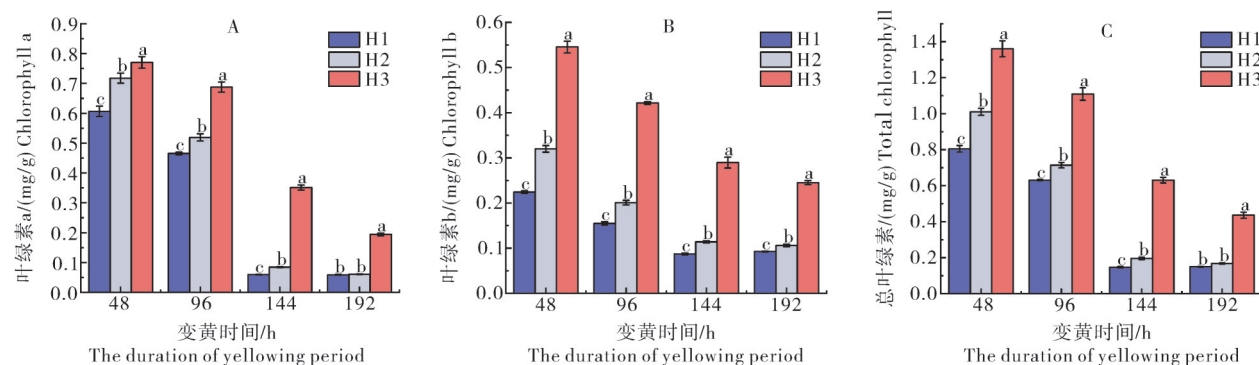


图1 不同晾制湿度下雪茄烟叶叶绿素a(A)、叶绿素b(B)及总叶绿素(C)含量

Fig. 1 Chlorophyll a (A), Chlorophyll b (B) and total chlorophyll (C) content of cigar tobacco leaves under different air-curing humidity

过程中叶绿素b含量变化趋势,从图中可以看出虽然晾制湿度不同但叶绿素b含量变化趋势基本一致,降解速率均呈现出“前期快,后期慢”的特点,在各个阶段不同处理下叶片内叶绿素b含量始终保持 $H3 > H2 > H1$,且差异达到显著水平($P < 0.05$)。而从图1C中可以看出不同晾制湿度下,叶片内叶绿素总量变化趋势与叶绿素a的变化趋势相似,说明在雪茄烟叶晾制过程中叶绿素的降解由叶绿素a主导。这些结果表明叶片在变黄期处于高湿状态下不利于烟叶褪绿显黄。

2.2 雪茄烟叶变黄期类胡萝卜素含量的动态变化

烟叶在变黄期的类胡萝卜素含量如图2所示,不同湿度处理下雪茄烟叶变黄期类胡萝卜素含量变化趋势一致,即呈持续降低的趋势,且降解幅度较均匀。变黄初期,H2处理类胡萝卜素显著高于H1和H3,且呈显著差异($P < 0.05$),H1与H3之间差异不

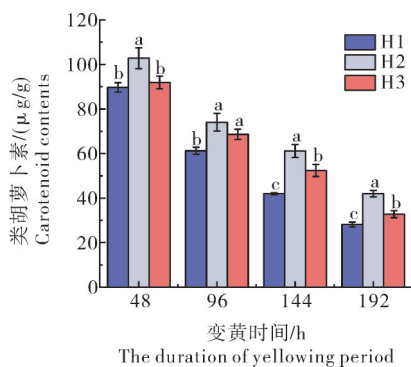


图2 不同晾制湿度下雪茄烟叶类胡萝卜素含量

Fig. 2 Carotenoid content in cigar tobacco leaves under different air-curing humidity

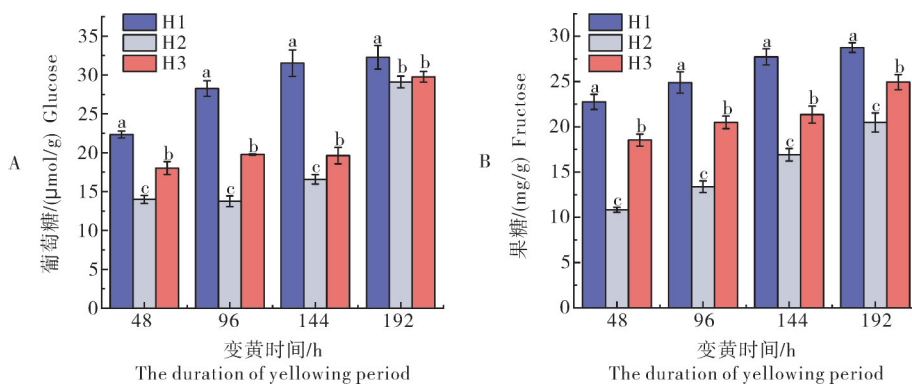


图3 不同晾制湿度下雪茄烟叶葡萄糖(A)与果糖(B)含量

Fig.3 The content of glucose (A) and fructose (B) in cigar tobacco leaves under different air-curing humidity

2.4 雪茄烟叶变黄期蔗糖与淀粉含量动态变化

蔗糖是植物体内重要的能源和碳源物质,在烟草体内蔗糖也是糖类运输的主要形式。淀粉是由多个D-葡萄糖通过苷键结合而成的多糖,是烟叶各项

生命活动的重要供能物质。随着晾制过程的进行各处理类胡萝卜素含量表现为 $H2 > H3 > H1$,变黄144 h与192 h时,各处理间差异显著。变黄后期与变黄前期相比,各处理下降幅度分别为68.6% (H1)、59.2% (H2)、64.3% (H3)。表明不同湿度可以影响变黄期烟叶类胡萝卜素的降解速率与降解程度。

2.3 雪茄烟叶变黄期单糖含量动态变化

葡萄糖、果糖属单糖类化合物,在烟叶内可直接代谢,参与糖酵解过程,为烟叶的各项生命活动提供能量。试验测定不同晾制湿度下雪茄烟叶葡萄糖、果糖含量变化的动态情况如图3所示。从图3A可以看出,各处理葡萄糖含量随变黄期的进行,逐渐积累增加,但增加速率略有不同,H1处理在变黄前中期稳步增长,至144 h时增幅为41.1%,144 h之后烟叶葡萄糖含量虽有增长,但增幅较小,仅有2.4%;H2处理在变黄48~96 h期间烟叶葡萄糖含量基本不变,在144 h时略有增加,但增长幅度较小,增幅为20.4%,而在144~192 h烟叶葡萄糖含量明显增多,增幅为75.6%;H3处理在前期葡萄糖含量基本不变,在变黄144~192 h增长速率较快(增幅为35.1%)。图3B为各处理烟叶果糖含量变化情况,在整个变黄阶段叶片内果糖含量始终保持 $H1 > H3 > H2$,且差异显著($P < 0.05$)。变黄前后各处理果糖含量分别增加了26.3% (H1)、89.1% (H2)、34.6% (H3)。整体来看,变黄期间叶片的葡萄糖积累量要大于果糖积累量,可能是由于叶片对于果糖的消耗量大于葡萄糖。

黄48 h时叶片内蔗糖含量基本一致,差异不显著($P>0.05$);随晾制时间的推进,各处理间差异程度逐渐显著。变黄144 h时各处理蔗糖含量增幅分别为60.3%(H1)、21.2%(H2)、42.5%(H3);变黄至192 h,H1、H2、H3增幅分别达到了176.1%、90.6%、133.1%。变黄中后期叶片内蔗糖含量始终以H1处理含量最高,H3处理次之,H2处理最低。如图4B可

知各处理变黄期淀粉含量均随晾制时间逐渐降低。从图4B中可以看出,在变黄前期,H2处理烟叶淀粉含量略低于其他处理;而在变黄96 h之后,H1与H3淀粉含量降解缓慢,进入平台期;在变黄144~192 h时,H2处理淀粉仍有降解,且含量要低于其他2个处理。变黄前后相比,各处理淀粉含量分别降低了53.6%(H1)、44.5%(H2)、42.5%(H3)。

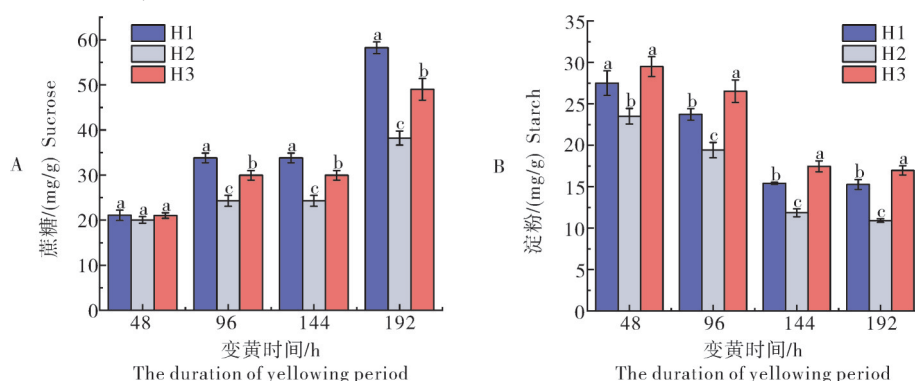


图4 不同晾制湿度下雪茄烟叶蔗糖(A)与淀粉(B)含量

Fig.4 Contents of sucrose (A) and starch (B) in cigar leaves under different air-curing humidity

2.5 雪茄烟叶变黄期脂氧合酶活性动态变化

脂氧合酶既是类胡萝卜素降解的关键酶,又和叶绿素降解密切相关。图5为不同晾制湿度下雪茄烟叶变黄期脂氧合酶的活性变化。可以看出,变黄期烟叶内脂氧合酶活性变化整体呈现出“先升高,后降低”的单峰变化趋势,均在变黄96 h左右达到峰值。不同湿度晾制过程中,各处理脂氧合酶活性变化趋势一致,活性高低始终保持H1>H2>H3,但各处理的所达到的峰值有所不同,H1、H2、H3峰值分别为93.40、69.87、45.76 U/g,增幅分别为290.0%、378.3%、199.3%。各处理脂氧合酶活性在达到峰值

后开始下降,但下降速率略有差别,变黄96 h至结束,H2处理下降速率最快(降幅为52.5%),其次为H1处理(降幅为39.6%),H3处理脂氧合酶活性变化幅度最小(降幅为29.4%)。由此可见,叶片内脂氧合酶的活性变化受晾制湿度影响较大。

2.6 雪茄烟叶变黄期蔗糖酶、中性转化酶和淀粉酶活性变化

蔗糖酶、中性转化酶和淀粉酶是植株碳代谢强度的重要指标,可将大分子碳水化合物分解为单糖等小分子化合物。不同晾制湿度下雪茄烟叶变黄期蔗糖酶、中性转化酶以及淀粉酶活性动态变化情况如图6所示。图6A为蔗糖酶活性变化情况,从图中可以看出不同湿度下蔗糖酶活性整体呈右偏单峰变化趋势,变黄前期时H1与H2蔗糖酶活性差距不大,但显著高于H3处理;变黄96 h之后,各处理蔗糖酶活性差距逐渐拉开,至144 h时,H1蔗糖酶活性增长了85.9%,H2涨幅为103.6%,H3涨幅为47.1%。随后各处理蔗糖酶活性逐渐趋于稳定直至变黄结束。由图6B可知,不同湿度下雪茄烟叶变黄期中性转化酶活性均呈持续上升的趋势,但各处理随晾制时间的推进增长幅度有所不同。从图6B中可以看出H2处理中性转化酶活性增长速率随时间的推进逐渐增大,其活性在整个变黄期始终高于H1、H3处理。H1与H3处理中性转化酶活性变化基本一致,均呈现出

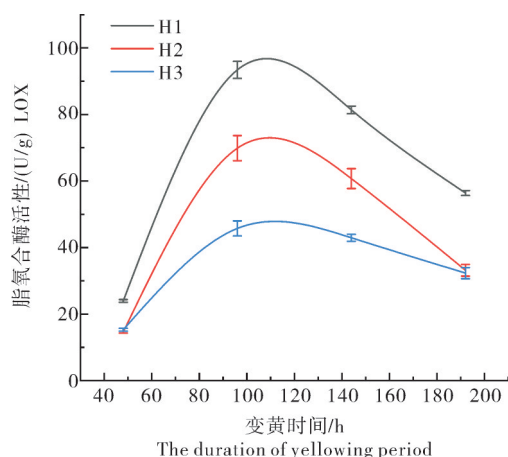


图5 不同晾制湿度下雪茄烟叶变黄期脂氧合酶活性

Fig.5 Lipoxigenase activity of cigar tobacco leaves at yellowing stage under different air-curing humidity

前期增长快,中后期增长慢的特点。变黄期结束,各处理中性转化酶活性分别增加了92.9%(H1)、92.0%(H2)、82.0%(H3)。图6C反映了变黄期各处

理淀粉酶活性变化情况,可见,不同处理下淀粉酶活性均保持在一个较高的水平上,变黄144 h时活性稍有提升,随后又缓慢下降。

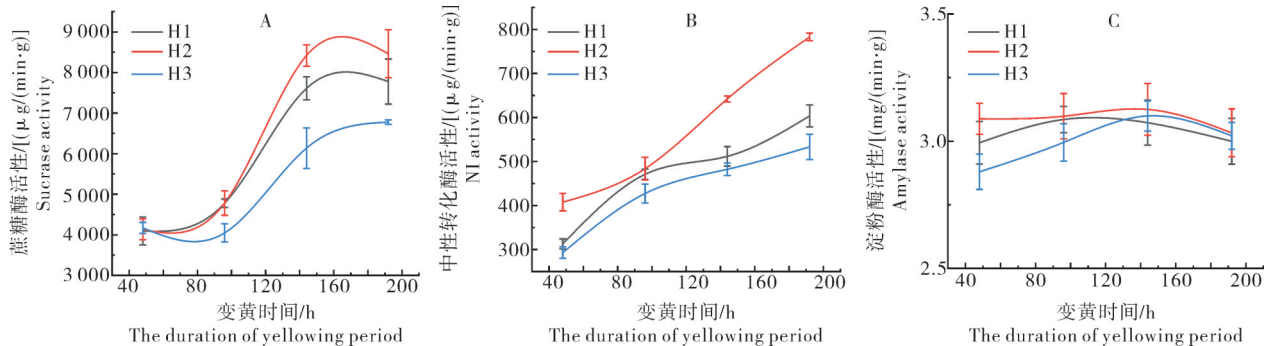


图6 不同晾制湿度下雪茄烟叶变黄期蔗糖酶(A)、中性转化酶(B)和淀粉酶(C)活性

Fig.6 Activities of sucrase (A), neutral invertase (B) and amylase (C) in cigar tobacco leaves at yellowing stage under different air-curing humidity

2.7 雪茄烟叶变黄期非结构性糖类、质体色素与相关酶活相关性分析

如图7所示,3种处理湿度下的烟叶质体色素含量变化与葡萄糖、果糖、蔗糖含量呈显著负相关,而与淀粉含量呈显著正相关。表明晾制过程中淀粉转化为小分子的同时影响着质体色素的降解速率与程度。蔗糖酶与中性转化酶在晾制过程中与葡萄糖、果糖、蔗糖呈显著正相关,而与淀粉含量呈显著负相关,与此同时,中性转化酶和蔗糖酶还侧面影响了质体色素的降解,从图7中可以看出,上述2种酶与叶绿素a、叶绿素b、叶绿素总量以及类胡萝卜素呈显著负相关。再次印证了雪茄烟叶晾制过程中质体色素的降解与非结构性糖类的转化息息相关。H2处理下淀粉酶活性与质体色素类物质呈正相关,与单糖以及蔗糖呈负相关;而H1、H3处理淀粉酶活性与质体色素类物质呈负相关,与单糖及蔗糖呈正相关。

3 讨论

变黄期是雪茄烟叶品质形成的重要环节,在此过程中由于烟叶内各种色素含量占比不断变化,烟叶颜色由采收时的嫩绿色逐渐转变为黄中带绿^[15]。本研究对不同湿度下变黄期烟叶内质体色素类物质含量变化进行了分析,结果显示,3种湿度下烟叶内叶绿素a均呈逐渐下降的趋势,降解速率均为“慢-快”,但降解程度有所不同,这可能是由于高湿状态下叶片与周围环境的湿度差较小,叶片始终保持较高的含水率,细胞内与细胞间水分散失量较少,而较高的水分限制了脂氧合酶等相关酶的活性^[4],进而影响了质体色素的代谢;同时高湿环境还导致叶片吸

水饱和,表皮细胞含水量较高,挤压保卫细胞导致叶片的气孔导度减小或关闭,进而叶片与外界气体交换程度降低,呼吸作用减弱,碳水化合物对于叶片内各项生命活动的供能减少,故而质体色素降解速率缓慢,烟叶颜色褪绿较慢^[16-17]。低湿环境晾制过程除了呼吸作用增强外,叶片失水速率较快还会造成细胞间与细胞内水分的亏缺,而水分亏缺会损伤植物的叶绿体,导致叶绿体基粒的紊乱,在此情况下,基粒紊乱扰乱了叶绿体内正常的蛋白质代谢,进而加速了叶绿素的降解^[18];同时,由于叶片离体后长期处于水分亏缺状态,叶片细胞内与细胞间的水分已无法满足正常生理代谢的条件,叶肉细胞死亡速度加快,各项生命代谢活动不得不提前终止,叶绿素的降解受到抑制以至于无法降解,可能在叶片上形成“青斑”,更甚者形成“浮青”;又因叶片水分散失较快,基于水势差,叶脉的维管束等组织的水分向叶片迁移速率加快,导致叶脉的叶绿素也不能充分降解造成“青筋”^[19]。总的来说,低湿环境下虽然在变黄前中期可以加速叶绿体的降解,但是由于细胞水分不足,叶绿素的降解可能并不充分,易导致烟叶出现含青情况进而对烟叶外观及香吃味存在较大的影响。

碳水化合物是晾制过程中维持烟叶生命活动以及品质形成的重要物质。本研究中各处理烟叶在变黄期碳水化合物的含量、转化速率以及相关酶活性不尽相同。低湿环境下的叶片小分子糖类含量始终最高,而淀粉含量变化幅度在144 h前明显高于其他处理,而在144 h之后变化幅度又低于其他处理。一方面是由于在水分亏缺的状态下,低湿度晾制的烟

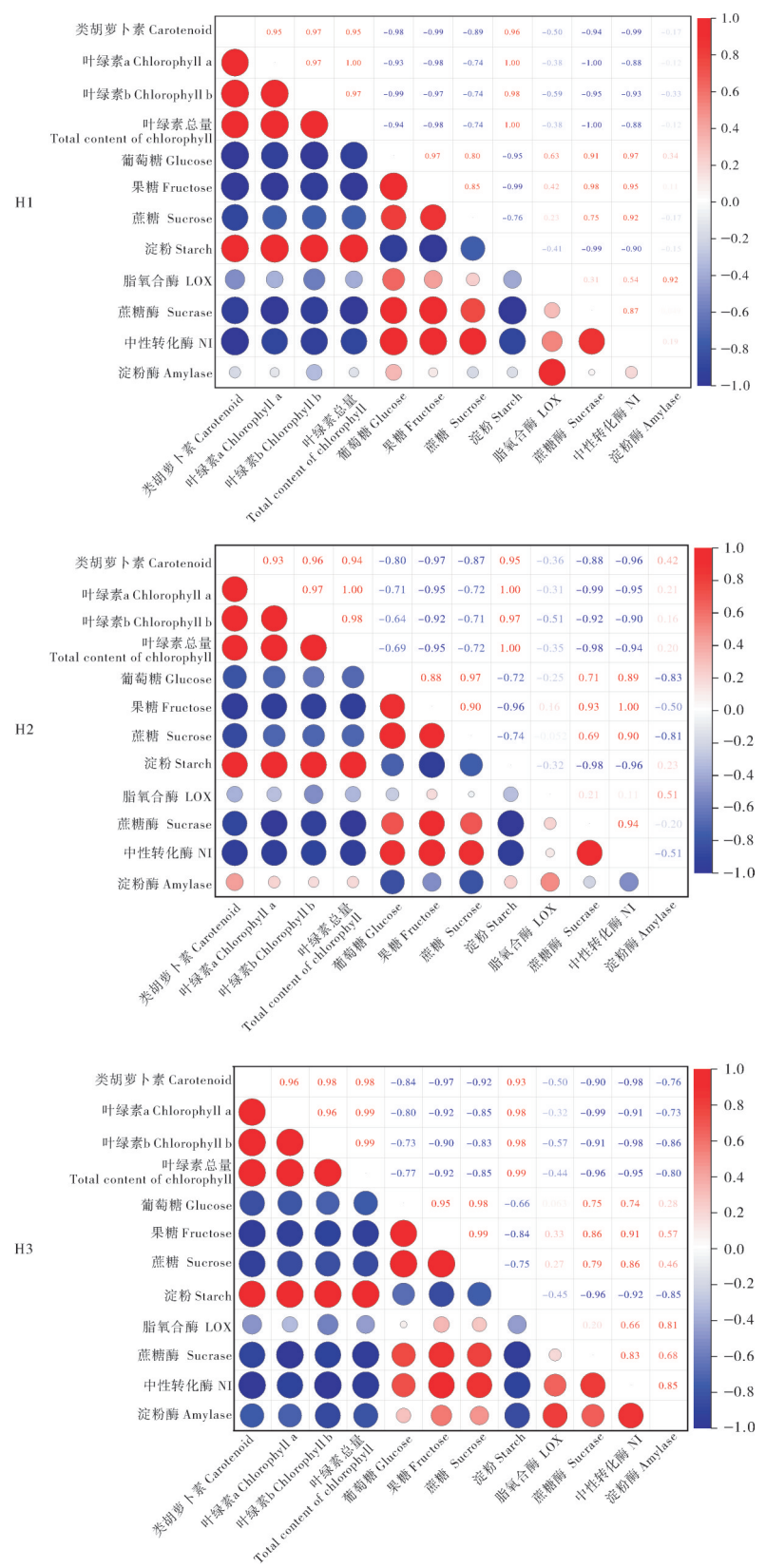


图 7 不同晾制湿度下烟叶变黄期非结构性糖类、质体色素与相关酶活相关性分析

Fig.7 Correlation analysis of non-structural sugars, plastid pigments and related enzyme activities in yellowing stage of tobacco leaves with different air-curing humidity

叶所受的逆境胁迫更为严重,叶片与环境湿度存在较大的湿度差,叶片失水量较大,而细胞为调节内外渗透压,迫使淀粉等多糖迅速降解为小分子糖类用于维持烟叶内外渗透压的平衡^[20-21];另一方面是因为叶片气孔导度的打开促使了气体交换,呼吸强度得到了提升,暗呼吸对于淀粉等多糖消耗量增加,故而低湿条件下变黄前期叶片淀粉含量变化幅度大,单糖、低聚糖含量稍高,叶片变黄进程较快^[22]。随着晾制时间的推进,低湿状态下叶片中的水分已不足以维持细胞的各项生命活动,叶肉细胞活性降低,烟叶细胞生命活性相对较弱,细胞器和细胞质膜逐渐溶解,膜选择通透性丧失,叶片细胞的内部环境已不适于碳水化合物降解,故而低湿处理的烟叶淀粉过早的进入平台期,降解缓慢^[23-24]。

通过分析不同晾制湿度下雪茄烟叶质体色素降解与非结构性糖类代谢及其相关酶活性的相关性可知,不同湿度下质体色素的降解与非结构性糖类的转化关系密切,究其原因可能是因为在晾制过程中,烟叶淀粉等不溶性多糖的降解生成了大量的葡萄糖等小分子糖类,在此过程中,不仅细胞内氢离子增多,并且由于细胞的呼吸作用,葡萄糖发生的糖酵解、三羧酸循环等分解代谢,为质体色素的降解提供大量的能量,促使其分解与转化,进而影响烟叶颜色的变化^[23,25-26]。

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Dynamic changes in non-structural sugars and plastid pigments during yellowing stage of cigar tobacco leaves under different air-curing humidities

WANG Wei¹, TAN Yonghao^{2,3}, ZHUO Yifeng¹, QUAN Yihua¹,
LIU Xinyao¹, SHI Yaqi⁴, FU Changwu¹, WANG Zhenhua¹

1.Zhangjiatie City Branch Company of Hunan Tobacco Company, Zhangjiatie 427000, China;

2.Henan Agricultural University Key Laboratory of Tobacco Cultivation in Tobacco Industry, Zhengzhou 450046, China; 3.Sichuan China Tobacco Industry Co., Ltd., Chengdu 610017, China;

4.Institute of Cigar at the Technology Center of Anhui Tobacco Industrial Company, Hefei 230000, China

Abstract In order to improve the air-curing quality of tobacco leaves, Yunxue No.2 was used to study the changes and correlation between non-structural sugars and plastid pigments during the yellowing stage of cigar tobacco leaves under different air-curing humidities. The changes in the intrinsic chemical composition of cigar tobacco leaves during the yellowing stage under conditions of an ambient temperature of 28°C and ambient humidity of 90% (high humidity), 80% (medium humidity), and 70% (low humidity). The results showed that the content of plastid pigments and starch in tobacco leaves had an overall decreasing trend, while the content of glucose, fructose, and sucrose had an overall increasing trend Under different air-curing humidities. The degradation rate of the content of chlorophyll in tobacco leaves treated with high humidity (yellowing humidity of 90%) was slow, but the cycle of degradation was long, while the content of chlorophyll in tobacco leaves treated with high and medium humidity after 96 hours of yellowing tended to a lower level, and the change was not significant. Low humidity (yellowing humidity of 70%) treatment significantly increased the accumulation in the content of glucose and fructose at the early stage of yellowing, and the increase was relatively small after 144 hours of yellowing. The trend of changes in the content of glucose and fructose treated with medium and high humidity was opposite to that with low humidity, with no significant increase at the early stage of yellowing, but a significant increase after 144 hours of yellowing. The trend of changes in the content of starch and sucrose under different treatments was almost the same. The results of correlation analyses showed that the changes in the content of plastid pigments and non-structural carbohydrate during the air-curing process reached significant or extremely significant levels. It is indicated that the rate of yellowing in tobacco leaves under the low humidity (yellowing humidity of 70%) was relatively fast, and the conversion of non-structural sugars was rapid, resulting in the early yellowing of tobacco leaves. Tobacco leaves under the high humidity (yellowing humidity of 90%) had relatively small changes in various indexes at the early stage of yellowing, but the conversion rate of non-structural sugars after 144 hours was faster, which prolonged the yellowing period to some extent. The degree of degradation and transformation of plastid pigments and non-structural sugars in tobacco leaves under the moderate humidity (yellowing humidity of 80%) was between that under the low humidity and the high humidity. The above results show that the high humidity air-curing provides a more suitable environment and time conditions for the yellowing of tobacco leaves, and promotes the transformation of the substances contained in tobacco leaves. Therefore, in the actual production, providing a high humidity air-curing environment for tobacco leaves during the yellowing period is more conducive to the formation of high-quality tobacco leaves.

Keywords cigar tobacco leaves; air-curing; plastid pigments; monosaccharide; oligosaccharides; starch

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