烘烤过程中变黄条件对烤烟淀粉代谢的影响

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[摘 要] 【目的】探讨烘烤过程中变黄条件对烤烟淀粉代谢的影响。【方法】采用河南农业大学设计制造的电热式温、湿度自控烤烟箱对供试烟叶进行烘烤,设置不同的变黄温度、变黄时间和凋萎时间,研究烘烤过程中变黄条件对烤烟淀粉代谢的影响。【结果】随着烘烤的进行,淀粉酶活性迅速升高,变黄时间延长比正常烘烤处理淀粉酶活性达到高峰的时间推迟了 12 h,且烤后烟叶淀粉含量低于正常烘烤处理;较高的变黄温度能促使烟叶中的淀粉在烘烤前期快速降解,但后期淀粉降解停滞的时间较早,最终淀粉含量较高;在 42 ℃延长凋萎时间,烤后烟叶淀粉残留量低。淀粉同工酶电泳胶板上明显可见有 3 条酶带,分别为 α-淀粉酶、β-淀粉酶、R-淀粉酶,其中 β-淀粉酶活性最高。电泳图谱显示,各同工酶活性 0 h 时没有差异,24 h 时高温处理的活性稍高于低温,36 h 时高温变黄比低温变黄酶活性稍低。【结论】烘烤过程中在 35～38 ℃变黄,并在烟叶变黄后延长 12 h 和在 42 ℃条件下凋萎 12 h,有利于淀粉降解和烟叶品质改善。

[关键词] 烟叶烘烤;淀粉代谢;淀粉酶;淀粉酶同工酶 ;变黄温度;变黄时间

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Effect of yellowing conditions on starch metabolism during curing process of tobacco leaf

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Abstract: 【Objective】 The effect of yellowing condition on starch metabolism during curing process of tobacco leaf was studied. 【Method】 The electric-heated flue-curing barn designed and made by Henan Agriculture University was used by setting different yellowing time, wilting time and yellowing temperature to study the effect of yellowing condition on starch metabolism during curing process. 【Result】 The activity of amylase increased with the proceeding of curing. The time of the amylase activity to reach a peak of the lengthening of yellowing time treatment was postponed 12 h than normal curing treatment, and the starch content after curing was lower than normal curing. A higher yellowing temperature could impel the toabacco leaf starch degraded completely in the earlier curing stage, but in the later stage the residence time that the starch degraded was earlier, the starch content was higher in the final. Residue starch content was low after 42 ℃ wilting curing. There were three amylase-isoenzyme bands in electrophoresis plate, α-AMY, β-AMY and R-AMY, and the β-AMY activity was the highest. The electrophoretogram showed that there was no significant difference at 0 h, high temperature enzyme activity treatment was higher than that in low temperature at 24 h, but lower at 36 h. 【Conclusion】 The low temperature yellowing(35-38 ℃ yellowing)

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prolonged yellowing time(prolonger 12 h after 35-38 ℃ yellowing) and prolonged wilting time(42 ℃ wilting 24 h) during the curing process, which was beneficial to starch degraded and tobacco quality improvement.

Key words: tobacco leaf flue-curing; starch metabolism; amylase; amylase-isoenzyme; yellowing temperature; yellowing time

淀粉是烤烟叶片大田期积累的重要碳水化合物,成熟的鲜烟叶中淀粉含量高达 40%左右[1-2]。与其他作物不同,淀粉只是烟草发育过程中碳水化合物的暂存形式,调制过程中淀粉的分解和转化状况,对烤后烟叶内部各种化学成分之间的协调程度[3]及烟气醇和性与芳香性[4]有重要影响。烘烤过程中淀粉的降解实质上是在一定温度、湿度和烟叶水分含量条件下,由相关酶水解来完成的[5-6]。目前,淀粉含量偏高是影响我国烤烟质量的一个重要问题,降低烤烟淀粉含量已成为烤烟研究的热点之一[7]。如何利用淀粉酶来降低淀粉含量,是降低烤烟淀粉含量研究的一个重要方面[8-9]。以往的研究主要侧重于烘烤过程中淀粉酶活性和淀粉含量本身的变化[10-12],而对影响淀粉降解的淀粉酶活性作用的有效条件研究较少,其中有关淀粉酶同工酶的研究更少 。本试验研究了烘烤过程中变黄条件对烤烟淀粉代谢的影响,以期为提高烟叶烘烤质量提供理论和技术支持。

1 材料与方法

1.1 试验材料

供试烟叶取自河南许昌,为中部烟叶,品种为 NC89。试验烟田土壤肥力中等,规范化栽培管理,烟叶适时成熟采收。

1.2 试验设计

试验于 2005～2006 年进行。供试烟叶采用河南农业大学设计制造的电热式温、湿度自控烤烟箱烘烤,烘烤过程中每隔 12 h 取一次,样品去除叶尖和叶基各 1/3 区域,分成 3 份:一份立即用于淀粉酶活性测定;一份杀青烘干,测定水分含量并留样作常规化学成分分析;一份置于－20 ℃低温冰箱 ,用于淀粉酶同工酶分析。试验设以下处理:(1)变黄时间。A. 35～38 ℃变黄,然后转为正常烘烤;B. 35～38 ℃变黄后延长 12 h,再转为正常烘烤。(2)凋萎时间。A. 35～38 ℃变黄,在 42 ℃进行凋萎 12 h,然后以 1 ℃每 2 h 的速度升温定色;B. 35～38 ℃变黄后,在 42 ℃进行凋萎 24 h,然后以 1 ℃每 2 h 的速度升温定色。(3)变黄温度。A. 35～38 ℃变黄,然后转为正常烘烤;B. 40～41 ℃变黄,然后转为正常烘烤。

1.3 测定项目与方法

淀粉酶活性采用 3,5-二硝基水杨酸比色法[13-14]测定;淀粉含量采用酸解法[13]测定;淀粉酶同工酶分析参照文献[15-16]的方法。

1.4 数据统计分析

实验数据采用 spss 统计软件进行处理。

2 结果与分析

2.1 变黄时间对烟叶淀粉含量和淀粉酶活性的影响

烘烤过程中变黄时间对烟叶淀粉含量和淀粉酶活性的影响结果见图 1。

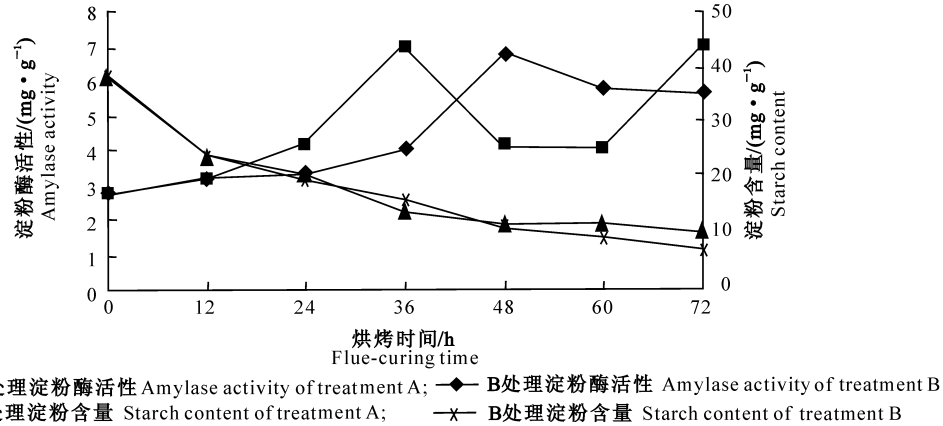

图 1 烘烤过程中变黄时间对烟叶淀粉含量和淀粉酶活性的影响

Fig. 1 Effect of yellowing time on the activity of amylase and content of starch during curing process

由图 1 可以看出,正常烘烤(处理 A)和变黄后延长 12 h(处理 B),烟叶淀粉酶活性均随着烘烤时间的延长而升高,达到一个高峰后酶活性有所下降,但正常烘烤于烘烤后期淀粉酶活性又有所升高;变黄后延长 12 h 比正常烘烤淀粉酶活性达高峰的时间推迟了 12 h,并在高峰之后缓慢下降。淀粉含量在烘烤变黄阶段(0～24 h)急剧下降,烘烤的前 36 h 降解量最大,36 h 后降解缓慢,变黄后延长 12 h 处理的淀粉含量显著低于正常烘烤处理。在烘烤中后期(定色和干筋阶段),淀粉酶活性的升高与淀粉的降解不同步。

2.2　凋萎时间对烟叶淀粉含量的影响

由图 2 可见,A 处理和 B 处理淀粉含量在烘烤的前 60 h 变化规律一致,60 h 之后 B 处理淀粉含量进一步降低,与 A 处理差异显著。

2.3　变黄温度对烟叶淀粉含量的影响

从图 3 可以看出,B 处理淀粉含量在烘烤前期(0～12 h)快速下降,但到后期淀粉降解停滞的时间比较早,因而最后淀粉残留量较高,显著高于 A 处理。

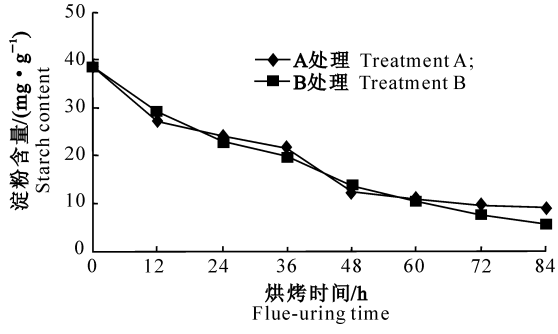

图 2　烘烤过程中凋萎时间对烟叶淀粉含量的影响

Fig. 2　Wilting time on the content of starch of tobacco leaves during curing process

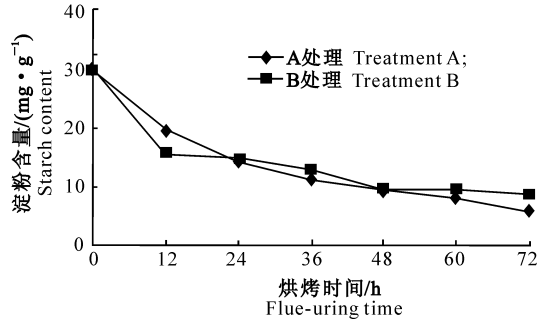

图 3　烘烤过程中变黄温度对烟叶淀粉含量的影响

Fig. 3　Yellowing temperature on the content of starch of tobacco leaves during curing process

2.4　变黄温度对淀粉酶同工酶活性的影响

由图 4 可以看出,烘烤过程中烟叶电泳胶板上主要有 3 条酶带,从上到下依次为 R-淀粉酶、β-淀粉酶、α-淀粉酶,其中 β-淀粉酶活性最强。不同变黄温度处理结果表明,3 种同工酶活性变化规律总体趋势一致,从图谱上看高温变黄和低温变黄,0 h 时没有大的差异,24 h 时高温处理的酶活性稍高于低温处理,36 h 时低温处理的酶活性稍高于高温处理。

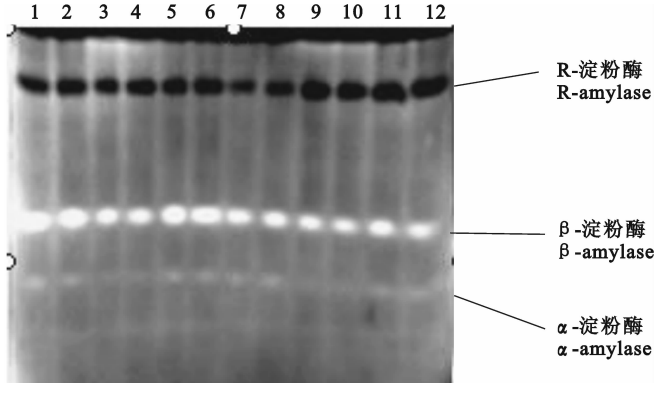

图 4　不同变黄温度对烟叶淀粉酶同工酶活性的影响

1,2. B 处理 36 h;3,4. B 处理 24 h;5,6. A 处理 36 h;7,8. A 处理 24 h;9,10. A 处理 0 h;11,12. B 处理 0 h

Fig. 4　Effects of different yellowing temperature on amylase-isoenzyme of tobacco leaves

1,2. B treatment 36 h;3,4. B treatment 24 h;5,6. A treatment 36 h;
7,8. A treatment 24 h;9,10. A treatment 0 h;11,12. B treatment 0 h

3　结论与讨论

烘烤对烟叶的化学成分和内在质量具有重要影响,改变烘烤条件,合理调控烟叶淀粉含量,对提高烟叶质量具有重要作用[4]。本试验发现,延长变黄时间能有效地延长烟叶的淀粉酶活性、降低淀粉含量,这与前人[6]的研究结果一致,表明推迟淀粉酶到达活性高峰的时间,延长其有效活性的维持时间有

利于淀粉充分降解;正常变黄处理虽能促使淀粉在烘烤前期快速降解,但后期淀粉停止降解的时间比较早,因而其淀粉残留量较高。另外本研究还发现,与不延长凋萎时间相比,在 42 ℃延长凋萎时间可明显降低烤后烟叶淀粉残留量。

烟叶中淀粉的降解实质上是在一定温度、湿度和烟叶水分含量条件下,由相关酶水解来完成的,最终淀粉是否彻底降解取决于一定条件下淀粉酶的有效活性[17],但对淀粉同工酶的研究报道在烟草上尚不见多。冉梦莲等[18]在对水稻叶片淀粉酶同工酶的研究中发现,电泳结果主要有 A,B,C 3 条酶带,其中 B 为 β-淀粉酶,A、C 为 α-淀粉酶。杨煜峰等[15]在对水稻淀粉酶同工酶的研究中也得到了同样的 3 条酶带。本研究观察到的酶带与上述研究结果不尽一致,在淀粉酶同工酶图谱第 3 条酶带下面还隐约显示一条亮带,但此亮带并不是在每个处理上都存在,对此还需进一步研究。另外,研究结果还显示,高温变黄和低温变黄对淀粉酶同工酶图谱影响不大,只是在 36 h 时低温变黄处理的酶活性稍高于高温变黄处理;正常变黄处理烘烤中,后期淀粉酶活性又呈上升趋势,但淀粉的降解几乎停止,这可能与定色中后期环境湿度和烟叶自身水分含量有关[5]。

综合分析认为,烟叶在烘烤过程中,淀粉的降解主要发生在变黄阶段,淀粉酶和淀粉同工酶对淀粉降解具有重要作用。根据鲜烟叶素质适当延长变黄时间,提高变黄程度,能够使淀粉得到充分降解,并转化为对烟叶质量有正向贡献的糖,对提高烟叶品质具有积极的意义。

[参考文献]

[1] 尹启生,蔡宪杰,王信民,等. 大田中后期烤烟淀粉酶活性及淀粉含量的变化 [J]. 烟草科技,2006(9):55-58.
Yin Q S,Cai X J,Wang X M,etal. Changes of amylase activity and starch content in flue-cured tobacco during middle to late growing stage [J]. Tobacco Science and Technology,2006(9):55-58. (in Chinese)

[2] 许自成,黄平俊,卢秀萍,等. 不同覆盖措施对大田烤烟叶片淀粉积累的影响 [J]. 西北农林科技大学学报:自然科学版,2006,34(5):77-82.
Xu Z C,Huang J P,Lu X P,etal. Effects of different mulching measures on starch accumulation in flue-cured tobacco leaves during field growth [J]. Journal of Northwest A&F University:Natural Science Edition,2006,34(5):77-82. (in Chinese)

[3] 宫长荣,刘 霞,郭 瑞,等. 淀粉代谢及影响烤烟淀粉含量的因素 [J]. 云南农业大学学报,2006,21(6):742-748.
Gong C R,Liu X,Guo R,et al. Starch metabolism and the influential factors of starch content in flue-cured tobacco leaves [J]. Journal of Yunnan Agricultural University,2006,21(6):742-748. (in Chinese)

[4] 宫长荣,王能如,汪耀富. 烟叶烘烤原理 [M]. 北京:科学出版社,1994.
Gong C R,Wang N R,Wang Y F,et al. Theory for curing of flue-cured tobacco [M]. Beijing: Technology Press,1994. (in Chinese)

[5] 宫长荣,袁红涛,陈江华. 烤烟烘烤过程中烟叶淀粉酶活性变化及色素降解规律的研究 [J]. 中国烟草学报,2002,8(2):16-20.
Gong C R,Yuan H T,Chen J H,et al. Studies on activity of amylase and degradation of starch and pigment in tobacco leaf during the process of curing [J]. China Tobacco Journal,2002(2):16-20. (in Chinese)

[6] 王怀珠,杨焕文,郭红英. 烘烤条件对烤烟淀粉降解及相关酶活性的影响 [J]. 中国烟草学报,2005,11(5):35-39.
Wang H Z,Yang H W,Guo H Y. Effect of curing condition on starch degradation and related enzyme activity of flue-cured tobacco [J]. China Tobacco Journal,2005,11(5):35-39. (in Chinese)

[7] 王怀珠,杨焕文,郭红英,等. 淀粉类酶降解鲜烟叶中淀粉的研究 [J]. 中国烟草科学,2005(2):37-39.
Wang H Z,Yang H W,Guo H Y,et al. Effects of additional amylase on degradation of starch in fresh tobacco leaves during flue-curing [J]. China Tobacco Science,2005(2):37-39. (in Chinese)

[8] 李富强,宋朝鹏,宫长荣,等. 烤烟烘烤环境条件对烟叶品质影响研究进展 [J]. 中国烟草学报,2007,13(4):70-74.
Li F Q,Song C P,Gong C R,etal. Research advances in improving flue-cured tobacco quality by changing curing conditions [J]. China Tobacco Journal,2007,13(4):70-74. (in Chinese)

[9] 牛燕丽,张 鹏,宋朝鹏,等. 酶法降解河南烤烟烟叶 B2F、C3F 和 X2L 淀粉的初步试验 [J]. 烟草科技,2005(3):26-28.
Niu Y L,Zhang P,Song C P,et al. The primary experiment of using Enzyme to degrate the starch of B2F、C3F and X2L of flue-cured tobacco from Henan [J]. Tobacco Science and Technology,2005(3):26-28. (in Chinese)

[10] 杨焕文,丁金玲,耿宗泽,等. 烘烤过程中淀粉酶及淀粉含量的变化 [J]. 云南农业大学学报,2003,18(4):385-388.
Yang H W,Ding J L,Geng Z Z,etal. Variations of activity of amylase and starch content in tobacco leaves during flue-curing process [J]. Journal of Yunnan Agricultural University,2003,18(4):385-388. (in Chinese)

[11] 杨立钧,宫长荣,陈江华,等. 烘烤过程中烟叶淀粉含量及烤后化学成分分析 [J]. 河南农业大学学报,2001,35(2):152-155.
Yang L J,Gong C R,Chen J H,et al. The analysis of starch content during flue-curing process and chemistry components after flue-curing in tobacco leaves [J]. Journal of Henan Agricultural University,2001,35(2):152-155. (in Chinese)

[12] 蔡宪杰,尹启生,王信民,等. 烘烤过程中温湿度对烤烟淀粉酶活性的影响 [J]. 烟草科技,2006(12):43-45.
Cai X J,Yin Q S,Wang X M,et al. Effects of temperature and humidity on amylase activity in flue-cured tobacco during Cu-

ring [J]. Tobacco Science and Technology, 2006(12): 43-45. (in Chinese)

[13] 王瑞新. 烟草化学 [M]. 北京:中国农业出版社,2003.
Wang R X. Tobacco chemistry [M]. Beijing: Chinese Agricultural Press, 2003. (in Chinese)

[14] 白宝璋,史芝文. 植物生理学 [M]. 北京:中国科学技术出版社,1990.
Bai B Z, Shi Z W. Plant Physiology [M]. Beijing: Chinese Technology Press, 1990. (in Chinese)

[15] 杨煜峰,毛华菊,陆定志,等. 杂交水稻及其亲本三系种子萌发过程中淀粉酶同工酶分析 [J]. 杂交水稻,1986(4):32-34.
Yang Y F, Mao H J, Lu D Z, et al. 1986, (4): 32-34. The analysis of amylase-isoenzyme of hybrid rice and three parent lines during seed germination [J]. Hybrid Rice, 1986(4): 32-34. (in Chinese)

[16] 何忠效,张树政. 电泳 [M]. 2 版. 北京:科学出版社,1999.
He Z X, Zhang S Z. electrophoresis [M]. Second Edition. Beijing: Technology Press, 1999. (in Chinese)

[17] 宫长荣,周义和,杨焕文. 烤烟三段式烘烤导论 [M]. 北京:科学出版社,2005.
Gong C R, Zhou Y H, Yang H W. Introduction for three stage curing of flue-cured tobacco [M]. Beijing: Technology Press, 2005. (in Chinese)

[18] 冉梦莲,陈友荣,肖敬平. 乙草胺对水稻淀粉酶同工酶的影响 [J]. 中国农业科学,1999,32(1):93-95.
Ran M L, Chen Y R, Xiao J P. Effects of acetochlor on amylase-isoenzyme in rice [J]. China Agricultural Science, 1999, 32(1): 93-95. (in Chinese)

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[37] Wu L M, Ni Z F, Meng F R, et al. Cloning and characterization of leaf cDNAs that are differentially expressed between wheat hybrids and their parents [J]. Mol Genet Genomics, 2003, 270: 281-286.

[38] 于澄宇,金平安,薛玮红,等. 候选基因策略在植物遗传学中的应用 [J]. 生物信息学,2004,2(1):25-31.
Yu C Y, Jin P A, Xue W H, et al. Application of the candidate gene strategy to plant genetics [J]. Bioinformatics, 2004, 2(1): 25-31. (in Chinese)

[39] Finlay M, Dale B, Bradshaw J E. Progress in improving processing attributes in potato [J]. Trends Plant Sci, 2003, 8: 310-312.

[40] Lang C, Whittaker J C. On prediction of genetic values in marker-assisted selection [J]. Genetics, 2001, 159: 1375-1381.

[41] Huang N, Angeles E R, Domingo J, et al. Pyramiding of bacterial blight resistance genes in rice: marker assisted selection using RFLP and PCR [J]. Theor Appl Genet, 1997, 95: 313-320.

[42] Singh S, Sidhu J S, Huang N, et al. Pyramiding three bacterial blight resistance genes (*xa*5, *xa*13 and *Xa*21) using marker-assisted selection into indica rice cultivar PR106 [J]. Theor Appl Genet, 2001, 102: 1011-1015.

[43] Hansen M, Hallden C, Nilsson N O, et al. Marker-assisted selection of restored male-fertile *Brassica napus* plants using a set of dominant RAPD markers [J]. Mol Breed, 1997, 3: 449-456.

[44] Kelly J D, Geptsb P, Miklasc P N, et al. Tagging and mapping of genes and QTL and molecular marker-assisted selection for traits of economic importance in bean and cowpea [J]. Field Crops Research, 2003, 82: 135-154.