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A review on starch changes in tobacco leaves during flue-curing

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Abstract This article discusses a range of studies on starch changes in tobacco leaves during flue-curing. Important effects resulting from using different flue-curing equipment and techniques on starch content are presented. It concludes that future researches should be focused on the structure and physico-chemical properties of tobacco starch during the curing process.

Keywords tobacco, flue-curing, starch

1 Introduction

Starch is an important carbohydrate accumulated in tobacco leaves during the field production period, and its content in fresh tobacco leaves in certain cropping areas is as high as 40 percent (Wang et al., 2005). The synthesis, accumulation, degradation and conversion of starch in the cells of tobacco leaves may determine the balance of chemical constituents in the post-cured tobacco leaves (Gong et al., 1994). After cure and fermentation, most starch will be converted into small-molecule carbohydrates which have numerous effects on the pure and mild aroma of the smoke by adjusting the acid base balance (Yan et al., 2003). The starch content in flue-cured tobacco leaves is an important indicator of the intrinsic properties and appearance. Residual starch in the flue-cured tobacco leaves has adverse effects on the color, odor and flavor of flue-cured tobacco. On one hand, the residual starch affects the combustion rate and complete combustibility; on the other hand it has a bitter and irritating taste when smoking,

together with negative effects on the smoke (Weeks et al., 1985). Therefore, further studies should be conducted on the metabolism of starch in tobacco leaves and factors influencing the starch content in flue-cured tobacco leaves as well as changes of the starch fine structure and physico-chemical properties during the curing process (Wang, 2002, 2003), which is significant to improve the quality of flue-cured tobacco in China and narrow down the gap with imported tobacco leaves.

2 Curing techniques

The degradation and conversion of starch during flue-curing may determine the intrinsic and appearance properties of the flue-cured tobacco (Matheson, 1996; Yuan and Gong, 2001). It has been found that the residual starch has negative effects on the color, odor and flavor of tobacco leaves as well as on the intrinsic and appearance qualities of the flue-cured tobacco (Weeks et al., 1985). Therefore, whether the starch degrades completely or not reflects the tobacco quality. Many studies show that starch and other biological macromolecules in tobacco leaves tend to degrade rapidly, whereas reducing sugars and other small molecule compounds tend to increase rapidly (Bacon and Wener, 1977; Lovetr and May, 1978). Most starch degrades during the first 72 h, with the 48–72 h period as the prime time, and less starch degrades after that (Zhao et al., 1996; Gong et al., 1998; Yang et al., 2001). The amyolytic enzyme plays a major role in the conversion of starch in tobacco leaves. The activity of this enzyme tends to increase with temperature ranging from 20°C to a maximum of 60°C; when higher it tends to decrease and disappear completely at 70°C. Moreover, the amyolytic enzyme is more sensitive to humidity and low humidity is more likely to deprive the amyolytic enzyme of its activity (Zong et al., 2001). Thus, the crux of scientific curing is how to regulate the temperature and humidity in accordance with the physiological and bio-chemical changes in tobacco leaves, making it advantageous to

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improve the quality of flue-cured tobacco (Wang et al., 2004). In addition, the amylolysis is sensitive to the environmental temperature. During the yellowing stage of tobacco flue-curing, the starch, under low-temperature conditions, begins to degrade rapidly 24 h after the beginning of curing, with a longer duration and more thorough degradation; in contrast, rapid starch degradation under high-temperature conditions begins as soon as the curing starts, with a shorter duration, and the starch content in tobacco leaves is high after curing (Gong et al., 1998). Dong et al. (2000) confirmed that low temperature treatment tended to result in better starch degradation than high temperature treatment during the yellowing stage of curing; a rapid increase in temperature tended to result in less starch degradation than a slow increase in temperature during the color fixing stage, and the treatment with increasing temperature during the yellowing stage tended to result in more starch degradation than in the color fixing stage. To conclude, low temperature curing can enhance the activity of amylase and accelerate the rate of degradation and conversion of starch largely into reducing sugars, which is advantageous to smoking and the improvement of flue-cured tobacco qualities.

The relative humidity of the flue-curing environment and the leaf moisture constitute the premise for the amylase to maintain a certain activity. Table 1 reveals that the limit value of starch degradation ranges from 65 to 70 percent humidity during flue-curing (Gong et al., 2003). Yuan et al. (2003) suggested that during the initial yellowing stage the rates of decrease in starch degradation and leaf moisture in tobacco leaves were the highest with low humidity treatment (at 79 percent relative humidity), and were lowest with maximum humidity treatment (at 93 percent relative humidity); the degradation duration in low humidity treatment was longer than that in CK treatment (at 86 percent relative humidity). Also, the residue starch content in tobacco leaves in high humidity treatments at the yellowing stage was higher than that in low humidity treatments, which was itself higher than that in CK. The effects of additive amylase on starch degradation (Wang et al., 2004, 2005) were the most obvious at the latter yellowing stage and the initial color fixing stage, indicating that adding amylase was an effective way to facilitate the degradation of starch during tobacco flue-curing. Namely,

the starch degradation could be strengthened with the increase in the amount of additive amylase. Different treatments showed significant differences in starch contents in the flue-cured tobacco leaves.

3 Curing equipment

Tobacco Leaf Flue-curing equipment and accompanied curing techniques are the key to improve the curing quality. The conversion of starch can be expedited by creating an environment with appropriate temperature and humidity in an advantageous way for the demands of tobacco quality (Deng et al., 2004, 2005). Deng et al. (2004) studied the effects of the curing equipment and accompanied curing techniques on the starch content in flue-cured tobacco leaves, introducing two different types of curing equipment and accompanied curing techniques and choosing Chuxiong, Nanhua and Yao'an (Yunnan Province, China) as the experimental sites. The results showed that the mean value of starch content in flue-cured tobacco was 6.83% in the case of the heated air circulating flue-curing barn; whereas, the mean value of starch content was 7.73%, much higher, when the ordinary vertical flue-curing barn was used. The statistical analysis revealed a significant level of difference in the effects of different curing equipment and accompanied curing techniques on the starch content in flue-cured tobacco leaves. Gong et al. (2002) studied the changes of starch, amylase and starch-isoenzyme in tobacco leaves during flue-curing by using the electric-heated flue-curing barn designed and made by Henan Agricultural University. The temperature and humidity of the barn were controlled automatically. The results indicated that the content of starch decreased rapidly and leveled off 48 h after curing. The activity of amylase increased with the progress of curing and reached a maximum around 36 h after curing and then decreased. At 40–45 percent leaf moisture and more than 70 percent relative humidity, starch degradation showed a dynamic positive correlation with the amylase activity, and the higher the activity of amylase was the faster the starch degraded. However, even when the leaf moisture decreased to 10 percent, the amylase activity still remained at a high level whereas the starch degradation was low and

Table 1 Effects of different changes of humidity on starch degradation (NC89, Xuchang, 2001)

treatments		0 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h	108 h	dry
starch	A	33.70	24.70	14.70	13.30	10.19	9.42	9.35	9.42	9.14	9.21	9.06
	B	33.70	25.00	19.47	17.77	15.85	10.45	7.34	7.40	7.21	7.32	7.12
	C	33.70	25.20	21.31	20.01	17.17	10.06	8.56	8.60	8.54	8.46	8.52
humidity	A	92.00	85.00	72.00	72.00	65.00	61.00	64.00	54.00	48.00	39.00	15.00
	B	92.00	88.00	78.00	78.00	75.00	74.00	64.00	60.00	51.00	38.00	15.00
	C	92.00	88.00	86.00	86.00	86.00	77.00	70.00	65.00	47.00	39.00	15.00

Note: A, B and C represent high speed decrease of humidity, medium speed decrease of humidity and low speed decrease of humidity, respectively.

starch content leveled off. Three amylase bands A, B and C could be identified on the starch-isoenzyme electrophoresis sheet, namely α -amylase, β -amylase and R-amylase, among which the activity of α -amylase was the highest. The activity of the isoenzyme was in accordance with that of physiological biochemical enzymes. In short, the amyllum degradation in tobacco leaf was actively completed in the process of relevant enzyme hydrolyzation at certain temperatures and humidity. Therefore, the advanced curing equipment and accompanied curing techniques play an important role in the starch degradation during flue-curing. Starch is an important carbohydrate accumulated in tobacco leaves during their growth period, which is inevitable and necessary. However, the accumulated starch should be degraded or converted to its reducing sugars during flue-curing to achieve the balance between reducing sugars and nitrogenous compounds, which is advantageous for the improvement of the aroma and taste of tobacco products. The low temperature treatment, natural aging, selection of dominant micro-organisms, the use of enzymolysis, and other advanced curing equipment and techniques should be applied to converting starch and other biological macromolecules in tobacco leaves into small molecular compounds and hence improve tobacco quality.

4 Conclusions and discussion

4.1 The latest research on tobacco starch

The research on indexes for tobacco starch content should be furthered and deepened. Neither too little nor too large amounts of starch in fresh tobacco leaves are good and the chemical constituents should be in overall balance. In recent years some tobacco organizations and experts in China have proposed that starch content should be lower than 3.5 percent (Liu, 2003), which is hard to achieve in actual tobacco production. From seven cropping areas in Yunnan Province, Deng et al. (2004) chose 42 representative tobacco samples with 6.93% starch content on the average, almost twice as high as the proposed 3.5%. They managed to achieve the goal of 3.5% starch content by reducing fertilizer application, cultivating medium-height tobacco plants, increasing tobacco curing humidity, properly raising curing temperature and taking other measures to actively promote the degradation and conversion of starch. The starch content was finally reduced to 3.6%, however the commercial value and usability of tobacco was almost close to zero. The quality of tobacco products is ultimately determined based on the smoking taste and the acceptance among consumers. The aroma and flavor of tobacco products are affected in an integrated manner by a number of chemical constituents. Excessive emphasis or consideration of certain chemical constituents may result in neglecting other constituents, inevitably

affecting the overall balance of the chemical constituents in tobacco leaves, and even result in a loss of the inherent characteristics of indigenous tobacco.

4.2 The dynamics of study abroad

The metabolism of tobacco starch is considered to have great effects on tobacco quality, especially on the aromatic components in tobacco manufacturing, therefore the research on tobacco starch in China should be strengthened and deepened. Actually, the USA and some other tobacco manufacturing countries have already been persisting in the research on tobacco starch without interruption. As early as in the 1970s–80s, the USA illustrated the process of tobacco starch metabolism from the physiological standpoint, ascertaining the conversion of sugar and starch, the relation of starch with sucrose phosphate synthetase and other key enzymes, and other factors influencing their conversion such as ADPG (adenosine diphosphate glucose). In China, research on various aspects including the metabolism and regulation of tobacco starch and tobacco cultivation and curing techniques have already been conducted to confirm the key factors influencing starch accumulation, and research has also been done to determine the relationships between starch and tobacco aroma and taste, and improve the quality of flue-cured tobacco.

4.3 The direction of future research

All the changes in starch during flue-curing may affect the traits of flue-cured tobacco, including starch content, starch fine structure, starch physico-chemical properties, size of starch granules, and the ratio of amylose to amylopectin, by regulating and controlling the temperature and humidity in curing barns to reduce the current starch content. Therefore, a priority ought to focus on the following aspects in future research:

(1) Plant cell structure analysis method can be used to determine the fine structure of tobacco starch. Previous studies indicated that starch granules can be obviously divided into big pellet starch (A-starch) and small pellet starch (B-starch or tail starch) in terms of size. Their inner structures are significantly different due to their different positions in the seed granule and different lengths of their growth periods. B starch contains the more linear type fragments, with shorter average chain lengths. The varying gelatinization degrees of A-starch and B-starch will surely affect the products. A-starch in wheat, for instance, is the most decisive factor that determines and enhances starch paste viscosity and swelling starch properties of noodles. B starch can increase the viscosity of noodles. The content ratio of A-starch and B-starch can affect the quality of noodles, too. Therefore, it can be deduced that the fine structure of tobacco starch is closely related with the quality of flue-cured tobacco. The scanning electron microscope (SEM) and the polarized light microscope

can be used to ascertain the fine structure of tobacco starch, specifically its shape and size, its crystal characteristic property and the composition and molecular structure.

(2) Chemical analysis methods can be adopted to test the content ratio of amylose to amylopectin and the chain length distribution of tobacco debranched amylopectin. The amylose content and amylopectin content and their ratio have long been regarded as key indicators of starch quality (Vandeputtea et al., 2003; Li et al., 2007). In general, iodine-blue spectrophotometry is used to determine the amylose content in starch. In fact not only the amylose but also part of the amylopectin (amylopectin in the long B-chain) has been found to react with the iodine solution. Iodine-blue spectrophotometry can show the limitations in the determination of amylose content. In the case of rice, different species of rice with close amounts of apparent amylose content vary significantly in quality, particularly in their taste. Many reports reveal that this difference is caused by the fine structure of amylopectin such as the difference in the fine structure of the branch, the degree of polymerization, chain length distribution, the average chain length, and others. Most relevant researches in the past were done using rice and corn as the objects. In the case of wheat, protein was the key indicator for quality evaluation, while there are no specific requirements for starch. Therefore, taking different tobacco species as study objects, future research should focus on the iodine absorption capacity of amylose and amylopectin and the difference in the distribution of amylopectin chain length, simultaneously analyzing the relationships between amylopectin chain length distribution and starch viscosity and crystal properties for the purpose of clarifying the effects of amylopectin chain length distribution on the physicochemical characteristics of starch and providing a theoretical basis for high-quality tobacco cultivation and control. Moreover, dual-wavelength tests of phenol and sulfate-phenol ($\lambda = 490$ nm measured sugar) and Pack-Johnson (measured reduction value) can be used to measure the ratio of amylose content to amylopectin content and the ratio of polymerization; alcohol repeated crystallization can be used to purify amylose and amylopectin; Han's method (the average degree of polymerization components) can be used to measure tobacco amylopectin chain length distribution, specifically in average chain length, the average length of the outside chain and, the average length of the inside chain.

(3) Modern equipment can be used to test the viscosity of tobacco starch and gelatinization properties. Gelatinization properties are the most important characteristics of starch. The starch tends to gelatinize when made into food products and has remarkable effects on the quality of foods. In the case of wheat, its viscosity parameter and starch swelling have a greater impact on the quality of appearance and eating quality of noodles; the amylose content adversely affects the noodle quality, and the gelatinization temperature has an impact on the processing

of noodles. A high correlation co-efficient of the viscosity characteristics and swelling volume with the evaluation of noodles has been documented in many research reports (Sun and Sun, 2008). They may affect not only the appearance quality of noodles (color and appearance) but the viscoelasticity and smoothness and taste of the noodles as well. Starch gelatinization happens under high-temperature and high-humidity conditions, as it does during the tobacco flue-curing. Therefore, it is of theoretical and practical significance to study the starch gelatinization properties in tobacco. The viscosity and gelatinization properties of tobacco starch can be determined by using a Rotary viscometer (test instrument) and Ukrainian-viscosity (test instrument), the Brabender sticky baked-(BV) (test instrument) and the Australian CSIRO's Rapid Viscosity Analyzer (RVA) (test instrument).

We suggest that a whole new research framework for tobacco starch should be established to study the changes in starch fine structure and its relationship with morphological features of starch granules during flue-curing. Comparison of the starch fine structures of different geographical tobacco species and determination of the amylose-amylopectin ratio, chain length distribution of tobacco debranched amylopectin, starch viscosity and gelatinization properties should also be done. In so doing, the formation mechanism of tobacco quality and style can be better explained by exploring their relationship with the quality of tobacco, thus providing a biochemical basis for the improvement of the smoking quality during the process of flue-curing. These studies are of great theoretical and practical significance to reveal the mechanism of tobacco aroma and flavor formation.

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