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RESEARCH PROGRESS ON THE ROLE OF EXOGENOUS ADDITIVES IN THE FERMENTATION OF CIGAR TOBACCO LEAVES

Abstract: *Cigars are a pure natural tobacco product, with an increasing market share in the Chinese economy. At present, compared with high-quality cigars from abroad, there is still significant room for improvement in the raw materials of Chinese cigars. High quality cigar tobacco leaves need to go through multiple steps such as cultivation, preparation, fermentation, aging, rolling, and maintenance. Among them, fermentation is the main link that affects the improvement of cigar tobacco quality. Therefore, improving fermentation quality and efficiency has increasingly become a focus of research in the field of cigar tobacco. Multiple studies have shown that adding exogenous additives plays an important role in promoting the fermentation of cigar tobacco leaves, which can effectively improve the quality and usability of cigar tobacco leaves. This article reviews the research progress on the effects of adding different types of exogenous additives on cigar fermentation, and looks forward to the research direction and importance of exogenous additives.*

Key words: *cigars, fermentation, exogenous additives, effects, tobacco quality.*

1. Introduction

With the development of the world economy and the integration of culture, the Chinese cigar market is showing a significant trend of activity. Cigars are pure natural tobacco products, and compared to other tobacco products such as cigarettes, the raw materials of cigars largely determine the characteristics of cigar products [1]. High quality cigar leaves are the basis for producing high-quality cigars. However, high-quality cigar raw materials are mostly produced in countries such as the Dominican Republic, Cuba, and Nicaragua. Relatively speaking, the shortage of domestic cigar

raw materials has become one of the main reasons restricting the development of Chinese cigars. The production of cigars mainly includes multiple processes such as cultivation, air drying, fermentation, rolling, and maintenance. During the fermentation stage, the physical and chemical properties of cigar leaves will undergo significant changes. Fermentation is a natural continuation process of cigar tobacco leaves after cultivation and preparation in the field. Once fermented, the color of cigar tobacco leaves deepens, the green impurities decrease, the aroma is revealed, the taste is gentle, the irritation is reduced, and the ability to resist mold infections is enhanced [2]. The fermentation process is a key manufacturing process that determines the quality of cigar products. Research has shown that fermentation has a certain impact on the appearance quality, physical properties, chemical composition, and smoking quality of cigar tobacco leaves.

Tobacco fermentation can be divided into primary fermentation and secondary fermentation. The primary fermentation mainly promotes the rapid transformation of intrinsic components in tobacco, while the secondary fermentation mainly compensates for the shortcomings in the initial fermentation, making the chemical composition of tobacco more refined, and improving the quality of tobacco. Previous studies have shown that adding microbial agents, enzyme preparations, or other fermentation media during the fermentation process of cigar tobacco can improve the quality of tobacco leaves and enhance their industrial availability.

2. Mechanism of Cigar Leaf Fermentation

At present, there are three hypotheses about the fermentation mechanism of tobacco:

(1) Oxidation: first proposed in 1867 by Nessler and Schlepg from Russia, who both believe that the inorganic catalysts (iron, magnesium) contained in tobacco are the main reasons for promoting tobacco fermentation [3]. This process roughly involves a catalytic reaction between oxygen in the air and the aforementioned elements, resulting in a chemical reaction. As a result, the moisture content of the tobacco leaves after modulation is low, the color of the tobacco leaves turns yellow, most cells lose activity, most enzymes are inactive, and the remaining enzymes have little effect on aging and fermentation.

(2) Enzymatic catalysis: This effect is mainly caused by enzymes such as oxidase, catalase, and peroxidase. Studies have shown that the biochemical process during fermentation is related to the enzymes present in tobacco leaves. There are many enzymes in the cells of tobacco leaves, and their effects are the main factors affecting tobacco fermentation. Enzymatic action is the fundamental driving force for accelerating tobacco fermentation, and the combined action of multiple enzymes forms tobacco fermentation [4].

(3) Microbial action: Xiao Shileputa believes that the activity of microorganisms that take effect at the beginning of fermentation causes fermentation, and the action of inorganic catalysts only begins and occurs in the later stages of fermentation. Reid who conducted cigar fermentation for the first time, found that microbial activity is particularly active during the fermentation process, and the process of microbial activity is closely related to the fermentation process and fermentation quality. Numerous studies have shown that Bacillus and Clostridium are the dominant bacteria in tobacco fermentation.

3. External auxiliary materials

3.1 Overview of auxiliary materials

Exogenous excipients are artificially added excipients that have a beneficial effect on tobacco fermentation. These excipients are diverse, some of which are anti-corrosion and moisture-proof, including lubricants, flame retardants, mold inhibitors, etc; There are also plant materials, including plant additives, tea extracts and rice paste water, as well as chemical additives such as essence, spices and nanomaterials. It is mostly an economical and environmentally friendly material, so it is widely used in fermentation.

3.2 The role of exogenous excipients in cigar fermentation

Research has shown that using natural plant additives can not only effectively reduce the content of harmful components such as nicotine in tobacco leaves, but also make the aroma of tobacco leaves richer, the taste more mellow and delicate, and improve the quality and usability of tobacco leaves [5]. Liu Shaohua et al. added some plants with medicinal value, such as perilla, astragalus, honeysuckle, sea cucumber, saffron, wormwood leaves, siraitia grosvenorii, cloves and cinnamon leaves, into tobacco fermentation as tobacco flavor. The results showed that these plant additives had effects on improving the quality and flavor of tobacco [6]. Li Dan [7] added apple, plum,

maple leaf, jasmine and tobacco extracts as well as chicory and sour horn extracts into essence to tobacco fermentation to improve the quality and flavor of tobacco [8]. Zhang Bingfeng et al. found that when tobacco leaves were fermented with different degrees of fried rice paste water, the total sugar, reducing sugar, and starch content in the leaves significantly increased after fermentation, while the nicotine content slowly decreased. The total amount of aroma components significantly increased, and the overall sensory quality improved [9]. Li Shilin found that rice paste juice has a very significant inhibitory effect on aerobic and anaerobic bacteria in tobacco leaves during fermentation. The more rice paste juice is used, the more obvious the inhibitory effect [10]. Xu Shijie added chrysanthemum, water, green tea extracts, and rice wine to tobacco fermentation, resulting in an increase in total sugar content, a decrease in nicotine and alkaloid content, an improvement in tobacco combustion, and an increase in neutral aroma compounds, which is beneficial for improving tobacco aroma [11]. Yin Quanyu et al. accelerated the degradation and transformation of internal substances, increased the content of reducing sugars, enhanced the sweetness of smoke, reduced irritation, and improved the quality of tobacco leaves through the fermentation of polyethylene pyrrolidone excipients. Adding six acidic substances for fermentation resulted in an increase in the content of volatile and semi volatile aroma components and a decrease in nicotine content after fermentation [12]. Therefore, exogenous excipients play an important role in coordinating chemical composition, improving aroma quality, reducing irritation, and improving taste.

4. Exogenous enzymes

4.1 Introduction to Exogenous Enzymes

Enzymes are catalysts of the same type of substance, mainly catalyzing the same type of reaction, with substrate specificity, high catalytic reaction efficiency, and mild reaction conditions. Therefore, in recent years, adding biological enzymes to improve the intrinsic chemical composition of tobacco leaves has been a hot topic in the tobacco industry. Enzymes are involved in the degradation of many substances in tobacco during the modulation and alcoholization process. The content of various endogenous conversion enzymes and other enzymes in tobacco is relatively low, so it is necessary to add exogenous enzymes in tobacco fermentation to improve the quality and flavor formation of tobacco.

4.2 The role of exogenous enzymes in cigar fermentation

During the fermentation process of cigar tobacco, it is necessary to convert a large amount of macromolecular substances in the tobacco into small molecule substances, which requires the participation of a large number of enzymes. Some of these small molecule substances are precursors of aroma substances, with different types and contents, which can reduce the impurities of the tobacco itself and give it unique aroma characteristics. After modulation, the enzyme content of the tobacco itself is reduced, so adding exogenous enzymes is necessary.Research has found that enzymes play an important role in the fermentation process of tobacco leaves. Enzymes can catalyze the decomposition or synthesis of certain substances in tobacco leaves, thereby improving the coordination of tobacco chemical components and improving the smoking quality of tobacco leaves. The experimental results of Pu Yuanzhu et al. showed that the protein content of tobacco leaves treated with different types of proteases was reduced to varying degrees. At the same time, the aroma quality of tobacco leaves was improved, the taste was more mellow, and the irritation to the oral cavity was also reduced [13]. The research results of Shi Zhifa and others show that using α- The sensory and suction quality of B3F leaves treated with amylase, glycosylase, and protease were improved. Li Jigang et al. found that the content of module starch and protein in tobacco leaves significantly decreased after being treated with a composite enzyme composed of amylase, saccharifying enzyme, and flavor protease in a certain proportion, resulting in an increase in aroma quality and a significant improvement in smoking quality. Zheng Linlin et al. added four enzyme preparations of different concentrations of phytase, cellulase, pectinase, and neutral protease to tobacco leaves for secondary fermentation experiments, which can improve the coordination of chemical components and sensory quality of tobacco leaves. Ruan Xiangwen et al. found that using cellulase and protease treatment can shorten the fermentation cycle of tobacco leaves, increase the total sugar content of tobacco leaves, and significantly increase the aroma. Xia Bingle found that the total nitrogen and nicotine content of tobacco leaves treated with composite enzyme preparations decreased, the total amount of aroma substances increased, and the sensory quality significantly

improved. Adding exogenous enzymes can significantly improve the aroma and flavor of tobacco leaves, enhance their combustibility, and harmonize the content and ratio of 3 chemical components.Therefore, enzymatic preparations play an important role in balancing chemical components, improving aroma quality, and reducing impurities in tobacco fermentation.

5. Microbial diversity

There are many types and huge quantities of microorganisms in tobacco leaves, and research on tobacco leaf microorganisms is becoming increasingly extensive and in-depth both domestically and internationally. The types and methods of identifying microorganisms vary. Research has found that each gram of tobacco contains approximately 50-80000 microorganisms, which can be divided into four categories: bacteria, fungi, actinomycetes, and yeast. Among them, the number of bacteria is the highest, with Bacillus being the most abundant and yeast being the least abundant.

According to Table 1, the microorganisms in tobacco leaves include 14 types of bacteria, 10 types of molds, 4 types of actinomycetes, and 2 types of yeast. After research and identification by domestic and foreign scholars on tobacco leaf microorganisms, the common result is that the number and type of bacteria have an absolute advantage in tobacco leaf species. Due to differences in geographical location and environment, the quantity of various microorganisms varies among different varieties of tobacco from different regions.

Table 1 – Statistical table of microbial species of tobacco

Bacteria	Mould	Actinomyce	Yeast
Bacillus	Asperillus	Streptomyces	Saccharomyces
Clostridium	Pencillium	Micromonospora	pichia pastoris
Sporolactobacillus	Mucor	Kineosporia	-
Xanthomonas	Rhizopus	Micrococcaceae	-
Micrococcus	Cephalosporium	-	-
Corynebacterium	Syzygites	-	-
Erwinia	Coremium	-	-
Sporosarcina	Fusarium sp.	-	-
Paenibacillus sp.	Tichoderma	-	-
Enterobacter sp.	-	-	-
Citrobacter sp.	-	-	-
Pantoea sp.	-	-	-
Pseudomonas	-	-	-
Lactobacillus	--	-	-

Therefore, the reasons for the differences in the results of microbial identification of various tobacco leaves by domestic and foreign scholars using various methods can be roughly attributed to differences in variety, place of origin, climate environment, identification methods, fermentation time, and conditions.

5.2 The role of microorganisms in tobacco fermentation

5.2.1 Accelerate the fermentation process of tobacco leaves

After the tobacco leaves are modulated, the next step is fermentation, which can be divided into agricultural fermentation and industrial fermentation. Adding exogenous substances is generally used in agricultural fermentation. Cigars are divided into primary fermentation and secondary fermentation, with the first fermentation lasting more than 60 days and the second fermentation lasting around 30 days. Studies have shown that applying beneficial microorganisms on the surface of tobacco leaves can significantly shorten the fermentation and aging time of tobacco leaves. In the mid-19th century, Koller first attempted to add yeast to the fermentation of cigar tobacco leaves and found that yeast can greatly shorten the fermentation time of cigar tobacco and improve its taste performance. Subsequently, Xie pointed out that adding Bacillus subtilis to the fermentation process of tobacco can shorten the artificial fermentation time to 8 days. Later, some scholars found that the quality of tobacco leaves fermented by microorganisms for 8 days and then aged for 90 days was very close to that of natural fermentation for 20 days and then aged for 730 d-1095 days. Therefore, the application and development of microbial fermentation and aging of tobacco leaves can shorten inventory time, thereby reducing fermentation and aging costs, and has enormous economic potential.

5.2.2 Improving the quality of tobacco leaves

5.2.2.1 Regulating the content of chemical components in tobacco leaves

The main chemical components in tobacco are carbon and nitrogen, with nicotine being the most inorganic nitrogen. Studies have shown that:Chen et al. screened a pseudomonas strain Nic22 that can effectively degrade nicotine from tobacco leaves and tobacco growing soil. The strain can reduce approximately 73.1% of nicotine under pH 6.5 and temperature range of 30-34℃; Ruan et al. isolated a strain of Pseudomonas HF-1 from soil contaminated with tobacco waste, which can degrade 99.6% of nicotine in the culture medium after 25 hours of cultivation under optimal conditions; utilized the isolated Pseudomonas aeruginosa ZUTSKD to degrade 97% of nicotine in the culture medium within 12 hours under certain conditions. In domestic research, Li Jue et al. screened a strain of Agrobacterium tumefaciens or Actinobacterium tumefaciens that can use nicotine as the sole carbon source from tobacco growing soil in Zhangjiajie. After 48 hours of cultivation in a nicotine containing medium, the strain can degrade about 71% of nicotine; Wu Liangwei further screened the strain EA-17 with the strongest nicotine reducing ability from 81 strains of nicotine degrading microorganisms, with a nicotine reducing rate of over 80%. Numerous studies by scholars have shown that Bacillus subtilis strain 11L140, rhizobia strain 5-28, Bacillus thuringiensis strain GZUIFR-YC02, maltophilic oligotrophic bacteria, and Bacillus parahaemolyticus all have extremely strong ability to reduce nicotine. Simply inoculating these microorganisms before tobacco fermentation can increase the organic acid content in tobacco leaves and significantly reduce nicotine content, laying a good foundation for nicotine reduction.From the perspective of carbon sources, studies have shown that the use of microbial agents can promote starch degradation, increase sugar content in tobacco leaves, and thus improve tobacco quality. Within the same aging time, the better the quality of tobacco, the greater the number and variety of leaf microorganisms.

5.2.2.2 Reducing TSNA content in tobacco leaves

Tobacco specific nitrosamines (TSNA) are a harmful substance unique to tobacco. The use of microorganisms to reduce the content of TSNA in tobacco leaves and smoke and improve the safety of tobacco products is an emerging research hotspot. The microorganisms that have been studied for the degradation of nicotine are mainly bacteria [33]. The use of efficient denitrifying bacteria can reduce nitrate to nitrite, further reducing it to gas, thereby achieving the goal of reducing TSNA content. Spraying the fermentation broth of the foul- smelling Pseudomonas T1-2 and T2-2 directly onto the surface of the upper tobacco leaves, the nitrate degradation rate was highest after 14 days of aging at 28℃ and 45% relative humidity, reaching 2.25% and 8.05%, respectively. The nitrite reduction rate was highest after 21 days of aging, reaching 19.90% and 20.99%, respectively [34].

5.2.2.3 Increase the content of aroma compounds in tobacco leaves

The aroma substances and types of tobacco are the most important indicators for evaluating the sensory quality of tobacco products during smoking, and they are also one of the most important factors that consumers value. Therefore, increasing the content of aroma compounds in tobacco leaves is particularly important.In 1953, Tamayo et al. first conducted an experiment on microbial inoculation to enhance the aroma of tobacco. The study confirmed that Bacillus and Streptococcus can improve the aroma of tobacco. Since then, many tobacco aroma producing microorganisms have been reported. For example, it has been proven that microorganisms belonging to the pan bacterial genus can degrade carotenoids to produce important aroma compounds in tobacco, and only some actinomycetes have the ability to catalyze the production of vanillin from ferulic acid. For tobacco leaves, after inoculation with Klebsiella pneumoniae, Bacillus thuringiensis strain V16, or Bacillus thermophilus on their leaves, sensory evaluation shows that the tobacco leaves quickly produce a pleasant aroma; Through the analysis of the chemical components of tobacco leaves, it was found that the content of aromatic components such as aldehydes, ketones, phenols, alcohols, acids, and heterocycles in tobacco leaves has increased to varying degrees. In addition, the overall aroma quality of reconstituted tobacco leaves by papermaking method was significantly improved by coating them with microbial fermentation enhancing concentrate, and then adding aroma evaluation by rolling and smoking. In summary, it is feasible and extremely important to add microorganisms to enhance the aroma of tobacco leaves.

5.3 Inhibition of tobacco leaf mold

Due to the long fermentation and aging time of tobacco leaves, a slight negligence can lead to tobacco leaf mold, and the annual losses caused by mold are enormous. Researchers isolated and screened five dominant microorganisms (M1, M2, M3, M4, M5) from tobacco, and found that they all had varying degrees of inhibitory effects on the growth and reproduction of mold on the culture medium, tobacco cut, and tobacco leaf surface. Among them, the M1 strain had the most significant inhibitory effect on mold growth. Han Jinfeng isolated and identified the microorganisms on the surface of tobacco leaves during artificial fermentation, and compared the dynamic changes of microorganisms during different fermentation processes. The results showed that the number of microorganisms on the surface of unfermented tobacco leaves was the highest. With natural aging and artificial fermentation, the number of microorganisms on the surface of tobacco leaves gradually decreased, while the number of fungi gradually decreased.During the artificial fermentation process, the number of molds has increased. A biological preparation (TFA) was prepared by mixing and screening several dominant strains of bacteria for tobacco fermentation. The results showed that TFA can accelerate tobacco fermentation, improve the quality of fermented tobacco leaves, and also have the effect of inhibiting tobacco mold.

6. Expectation

High quality cigar tobacco leaves need to go through multiple steps such as cultivation, preparation, fermentation, rolling, and maintenance, among which fermentation is the main link that affects the improvement of cigar tobacco quality. In recent years, there has been an increasing amount of research on the addition of exogenous substances in cigar fermentation, which has become a focus of research in the tobacco industry. The author believes that the role and prospects of exogenous additives in cigar tobacco fermentation in the future are mainly reflected in the following three aspects: firstly, excipients, biological enzymes, and microorganisms have certain beneficial effects on cigar fermentation, and some aspects have similar effects. Secondly, it can be considered to continue exploring whether the combination of different types of exogenous additives that have not been used for cigar fermentation will have a 1+1>2 effect, and further investigate the mechanism of its occurrence. Thirdly, the addition of different exogenous substances can have different effects and achieve different results. Therefore, exogenous additives can be added to cigars according to the desired target quality to reduce costs and improve quality.

Conclusion

At present, there is a lack of in-depth research on the fermentation mechanism of cigars, and the metabolic mechanism of cigar fermentation is not yet clear. Especially after adding exogenous substances, it will affect the fermentation process of cigars to varying degrees, thereby affecting the quality of cigars. We should deepen our research on the metabolic mechanism of cigar fermentation, clarify the process of substance conversion, and provide theoretical basis for the cigar fermentation process, accurately control cigar fermentation, and improvethe quality of tobacco leaves.

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ТЕМЕКІ ЖАПЫРАҚТАРЫН АШЫТУДАҒЫ ЭКЗОГЕНДІК ҚОСПАЛАРДЫҢ РӨЛІН ЗЕРТТЕУ БАРЫСЫ

Сигаралар – бұл Қытай экономикасындағы нарық үлесі артып келе жатқан таза табиғи темекі өнімі. Қазіргі уақытта шетелден келген жоғары сапалы сигаралармен салыстырғанда Қытай сигараларын өндіру үшін шикізат сапасын жақсартудың әлі де айтарлықтай әлеуеті бар. Жоғары сапалы темекі жапырақтары өсіру, дайындау, ашыту, қартаю, бұралу және күтім сияқты көптеген кезеңдерден өтуі керек. Олардың ішінде ашыту темекі сапасының жақсаруына әсер ететін негізгі буын болып табылады. Сондықтан ашытудың сапасы мен тиімділігін арттыру темекі өндірісі саласындағы зерттеулердің тақырыбына айналуда. Көптеген зерттеулер экзогендік қоспаларды қосу темекі жапырақтарының ашытуын ынталандыруда маңызды рөл атқаратынын көрсетті, бұл темекі жапырақтарының сапасы мен ыңғайлылығын тиімді жақсарта алады. Бұл мақалада экзогендік қоспалардың әртүрлі түрлерін қосудың сигараны ашытуға әсері туралы зерттеу барысы қарастырылады, сонымен қатар зерттеу бағыты мен экзогендік қоспалардың маңыздылығы қарастырылады.

Түйін сөздер: *сигаралар, ашыту, экзогендік қоспалар, әсерлер, темекі сапасы.*

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ХОД ИССЛЕДОВАНИЙ РОЛИ ЭКЗОГЕННЫХ ДОБАВОК В ФЕРМЕНТАЦИИ ЛИСТЬЕВ СИГАРНОГО ТАБАКА

Сигары – это чистый натуральный табачный продукт, доля рынка которого в экономике Китая растет. В настоящее время, по сравнению с высококачественными сигарами из-за рубежа, все еще существует значительный потенциал для улучшения качества сырья для производства китайских сигар. Высококачественные листья сигарного табака должны пройти множество

этапов, таких как выращивание, подготовка, ферментация, выдержка, скручивание и уход. Среди них ферментация является основным звеном, влияющим на улучшение качества сигарного табака. Поэтому повышение качества и эффективности ферментации все чаще становится предметом исследований в области производства сигарного табака. Многочисленные исследования показали, что добавление экзогенных добавок играет важную роль в стимулировании ферментации листьев сигарного табака, что может эффективно улучшить качество и удобство использования листьев сигарного табака. В этой статье рассматривается ход исследований влияния добавления различных типов экзогенных добавок на ферментацию сигар, а также рассматривается направление исследований и важность экзогенных добавок.

Ключевые слова: *сигары, ферментация, экзогенные добавки, эффекты, качество табака.*

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SCREENING OF XYLANASE PRODUCING STRAINS AND ITS APPLICATION IN FLUE-CURED TOBACCO LEAF FERMENTATION

Abstract: *In this experimental study, a strain producing xylanase extracted from tobacco leaf was developed. Through the basic strain screening method and xylanase activity determination, strain M416 with the highest enzyme activity was preliminarily identified and M416 was A. niger, the enzyme has biological safety and can be used in the fermentation process of flue-cured tobacco. The fermentation conditions were optimized by shaking the flask culture, in which the best formulation of xylanase production from A. niger and the optimal culture temperature, pH, inoculum amount and culture time were obtained. Under the optimal fermentation and aroma production conditions, the strain are evenly sprayed to the surface of tobacco leaf to*