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Tobacco alkaloids reduction by casings added/enzymatic hydrolysis treatments assessed through PLSR analysis

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ABSTRACT

Based on encouraged development of potential reduced-exposure products (PREPs) by the US Institute of Medicine, casings (glucose and peptides) added treatments (CAT) and enzymatic (protease and xylanase) hydrolysis treatments (EHT) were developed to study their effect on alkaloids reduction in tobacco and cigarette mainstream smoke (MS) and further investigate the correlation between sensory attributes and alkaloids. Results showed that the developed treatments reduced nicotine by 14.5% and 24.4% in tobacco and cigarette MS, respectively, indicating that both CAT and EHT are potentially effective for developing lower-risk cigarettes. Sensory and electronic nose analysis confirmed the significant influence of treatments on sensory and cigarette MS components. PLSR analysis demonstrated that tobacco alkaloids were positively correlated to the off-taste, irritation and impact attributes, and negatively correlated to the aroma and softness attributes. Additionally, nicotine and anabasine from tobacco leaves positively contributed to the impact attribute, while they negatively contributed to the aroma attribute ($P < 0.05$). Meanwhile, most alkaloids in cigarette MS positively contributed to the impact and irritation attributes ($P < 0.05$). Hence, this study paved a way to better understand the correlation between tobacco alkaloids and sensory attributes.

1. Introduction

Cigarette has always been a focus of attention due to its high prevalence in nicotine addiction and associated diseases. Since the report of "Clearing the Smoke" issued by the US Institute of Medicine (IoM) in 2001, the development of potential reduced-exposure products (PREPs) has been encouraged to reduce the risks of tobacco use for those smokers who do not quit smoking (Liu et al., 2011; McAdam et al., 2011). Recently, various technologies have been used for the reduction of harmful substances including "filters" (Chen et al., 2006; Coggins and Gaworski, 2008; Marcilla et al., 2015) and "tobacco ingredient" (Liu et al., 2011; McAdam et al., 2011).

Currently, the measures of modifying "tobacco ingredient" mainly focused on blend treated tobacco (BTT) (Liu et al., 2011) and tobacco sheet substitute (TSS) (McAdam et al., 2011). Liu et al. (Liu et al., 2011) made a reconstituted tobacco and subsequently studied the effect of reconstituted cigarette on the reduction of toxicant yields in cigarette smoke. McAdam et al. (McAdam et al., 2011) studied the effect of a novel tobacco-substitute sheet (TSS) used as cigarette component on reducing the tar and potential toxicity of tobacco smoke. Despite the fact that a certain proportion of BTT/TSS used as cigarette component could dilute the tar and nicotine, the overweight proportion of them would give a different smoking feeling compared with conventional cigarettes. This might be the reason why the risk-reduced tobacco products have not been widely used due to the sensory acceptability of the smokers (Crooks

Abbreviations: PREPs, potential reduced-exposure products; PLSR, partial least square regression; MS, mainstream smoke; CAT, casings added treatments; EHT, enzymatic hydrolysis treatments; TBs, treated tobacco samples; DDMP, 2,3-dihydro-3,5-dihydroxy-6-methy-4(H)-pyran-4-one; L-alkaloids, alkaloids from tobacco leaves; MS-alkaloids, alkaloids from cigarette mainstream smoke.

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et al., 2015; Yerger and McCandless, 2011).

Additionally, although numerous researches have been published on cigarette smoke toxicants and/or health risks associated with smoking (Marcilla et al., 2015; Shihadeh et al., 2015; Yerger and McCandless, 2011), there is no scientific consensus over the relationship between specific smoke toxicants and health risks of smoking (Liu et al., 2011). Despite that, it is widely considered that lower nicotine yielding cigarette is an effective approach to reduce health risks of smoking from temporary "smoking reduction" to potentially permanent "smoking cessation" (Hatsukami et al., 2013; Xie et al., 2013). Nicotine is regarded as the most serious health hazardous component in smoke. It is not only the inducer of cigarette addiction behavior, but also the key contributor of smoke associated diseases. For instance, the formation of carcinogenic substance such as tobacco-specific nitrosamines (TSNAs) derives from the nitrosation of nicotine and related alkaloids during tobacco aging, curing and burning (Lewis et al., 2012; Lisko et al., 2013). Furthermore, the related minor alkaloids particularly myosmine, anatabine and cotinine, could also enhance the smoking behavior as evidenced by Clemens et al. (Clemens et al., 2009).

To overcome the above problems, different methods have been developed to reduce tobacco alkaloids including the use of "additives" (Rodgman, 2002a, 2002b) and "enzymatic hydrolysis" (Liu et al., 2011). Additives such as volatile flavors and non-volatile casings are commonly used in tobacco or tobacco products to reduce tobacco alkaloids and their risks (Rodgman, 2002a, 2002b). Talhout et al. (Talhout et al., 2006) described the use of sugar as casings material added to tobacco that significantly reduced the nicotine content. In addition, glucose was used as a humectant and flavoring substance (Rodgman, 2002b; Talhout et al., 2006). Besides sugars, proteins and peptides are naturally present in tobacco (Rodgman, 2002a, 2002b). Proteins were considered as potential toxicant precursors, meanwhile they provided pungent and irritating taste during cigarette smoking (Baker et al., 2004; Torikaiu et al., 2005).

Furthermore, to develop effective methods that could reduce health risks of cigarette while maintaining its sensory quality, by adding and/or removing some precursors is not enough, transforming some precursors into contributors or promoting certain range of chemical conversion, like Maillard reaction (Shihadeh et al., 2015) may be also desirable. It was demonstrated that pungent and irritating taste during cigarette smoking could be inhibited by Maillard reaction between reducing sugars and amino acid/peptides (Rodgman, 2002a, 2002b). For instance, glycoprotein compounds were generally identified as tobacco aroma contributors. Enzymatic hydrolysis is another promising approach to reduce tobacco alkaloids and risks. Liu et al. (Liu et al., 2011) employed water extraction and protease treatment to reduce the protein content, thus lowering the amount of toxicants in cigarette smoke.

Eight alkaloids in tobacco leaves and cigarette mainstream smoke (MS) were identified through GC–MS. The sensory evaluation was performed by well-trained panelists and the results were confirmed through electronic nose analysis. The effects of casings added and enzymatic hydrolysis treatments on tobacco alkaloids and sensory attributes were studied. Furthermore, the correlation between alkaloids and sensory attributes were assessed by partial least squares regression (PLSR) analysis. This study would provide a way to support the development of PREPS and better understand the correlation between tobacco alkaloids and sensory attributes. The cigarettes with reduced levels of nicotine alkaloids might help to decrease the desire for nicotine, and then gain smoking cessation.

2. Materials and methods

2.1. Reagents

The protease (Protex™ 7L enzyme, activity > 1600AU (azo units) g^{-1}) and xylanase (OPTIMASE CX 72L, activity 18000IU mL^{-1}) used in tobacco treatments were purchased from Genencor International, Inc. (Shanghai, China). The glucose was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Peptides were purchased from Qiangwang Seasoning Food Co., Ltd. (Anhui, China). They were produced by the limited enzymatic hydrolysis of soy protein isolate plant protein using Alcalase and Flavourzyme. They are mainly made of small molecule peptide (< 1000Da, 85.35%). Four alkaloids authentic standards (nicotine, nornicotine, anabasine and anatabine), quinoline (99.9%, used as the internal standard (IS)) and n-alkanes (C8 ~ C40) were purchased from Sigma–Aldrich (Shanghai, China). The HPLC grade solvents (dichloromethane, methanol, etc) were acquired from Merck (Darmstadt, Germany).

2.2. Sample preparation

Tobacco used during this study was Flue-cured tobacco of "*Yunyan 87* (2010)". Some treatments including casings, enzymatic hydrolysis and heat treatments were applied during this study. Casings added treatments (CAT): 100.00 g cut leaf tobacco was spread evenly on the table, 10.00 mL casings solution (0.20 g mL^{-1} glucose and 0.01 g mL^{-1} peptides) was added to the tobacco using an atomizer, sample was incubated at 45 °C for 4 h , After that, sample was placed in a drum heating device, and heated either at 110 °C (named TB1), or at 150 °C (named TB2) for 5min. At last, the developed tobacco samples (TB1 and TB2) were placed at 22 °C and 60% relative humidity for later analysis.

Enzymatic hydrolysis treatments (EHT): 100.00 g cut leaf tobacco was spread evenly on the table, 10.00 mL enzyme reaction solution (0.02 g mL^{-1} protease and 0.005 g mL^{-1} xylanase) was added to the tobacco using an atomizer, and then the sample was incubated at 60 °C for 6 h , After that, the samples were placed in a drum heating device, and heated either at 110 °C (named TB3), or at 150 °C (named TB4) for 5min. Finally, the developed tobacco samples (TB3 and TB4) were placed at 22 °C and 60% relative humidity for later analysis.

The concentration of added ingredients was chosen through preliminary trials based on alkaloid content reduction and sensory evaluation. The level of added glucose and peptides was conformed to the regulatory levels. Enzymatic hydrolysis treatment was based on the synergistic effect of protease and xylanase that naturally existed in tobacco during curing process, in which, tobacco protein and polysaccharide are hydrolyzed into amino acids and reducing sugars by protease and xylanase, respectively.

2.3. Sensory evaluation

Before sensory evaluation, cigarettes were conditioned at 22 °C and 60% relative humidity for at least 48 h. Sensory attributes of "impact, irritation, off-taste, aroma, and softness" were studied accordance to the China National Institute of Standardization cigarette sensory profile test (GB5606.4–2005). Sensory evaluation was performed by a well-trained panel made of thirteen specialists (eleven men and two women). The descriptive analysis applied by 9-point scale. The value refers to the intensity of sensory response during smoking. The higher the value is, the greater the intensity is.