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The use of a novel tobacco treatment process to reduce toxicant yields in cigarette smoke

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ABSTRACT

The US Institute of Medicine has encouraged the pursuit and development of potential reduced-exposure products (PREPs) – tobacco products that substantially reduce exposure to one or more tobacco toxicants and can reasonably be expected to reduce the risk of one or more specific diseases or other adverse health effects. One potential approach is to reduce levels of some smoke toxicant precursors, such as proteins and polyphenols, in tobacco. We describe a treatment process involving aqueous tobacco extraction and treatment with protease; filtration of the extract to remove peptides, amino acids and polyphenols, and recombination of extract and treated tobacco. The process reduced levels of protein nitrogen (59%), polyphenols (33–78%) and nicotine (12%) while sugars increased 16%. ISO mainstream smoke yields of 43 toxicants were measured from cigarettes containing treated tobaccos; lower yields of tar, nicotine, carbon monoxide (16–20%), acrylonitrile, ammonia, aromatic amines, pyridine, quinolene and hydrogen cyanide (33–51%), tobacco specific nitrosamines (25–32%); phenolics (24–56%), benzene (16%), toluene (25%) and cadmium (34%) were obtained. There were significantly increased yields of formaldehyde (49%) and isoprene (17%). Reductions in sidestream yields of nitrogenous smoke toxicants and increases in sidestream yields of several carbonyls, benzo(a)pyrene and isoprene were also observed.

1. Introduction

In 2001 the US Institute of Medicine (IoM) issued a report, Clearing the Smoke, that encouraged the development of potential reduced-exposure products (PREPS) as a possible way to reduce the harm caused by tobacco use. The IoM defined a PREP as "A product that (1) results in the substantial reduction in exposure to one or more tobacco toxicants and (2) can reasonably be expected to reduce the risk of one or more specific diseases or other adverse health effects" (Stratton et al., 2001).

Of the over 5000 identified constituents of tobacco smoke (Rodgman and Perfetti, 2008), approximately 150 are considered to be toxicants (Rodgman and Green, 2003; Fowles and Dybing, 2003). It is not yet known which of these toxicants are the most important in relation to the diseases caused by smoking, though several researchers have attempted to develop risk assessment models to try and set priorities (Fowles and Dybing, 2003; Meredith et al., 2008).

Overall reductions in smoking machine measured toxicant yields can be achieved by diluting the smoke using filter ventilation or using cigarette papers with high permeability, and, in the case of toxicants that are associated with the particulate phase of smoke, by increasing the filtration efficiency of the filter. For many years, governments and public health authorities in various parts of the world considered lower ISO tar yielding cigarettes as a way to reduce the health risks of smoking for those smokers who did not quit smoking. However, this product modification approach has more recently been highly criticised by various bodies, including the US National Cancer Institute (US National Cancer Institute, 2001). The Study Group on Tobacco Product Regulation (TobReg) of the World Health Organization (WHO, 2008; Burns et al., 2008) has proposed a regulatory approach that would limit the yields of a selected group of specific toxicants. This group also recommended that the yields of toxicants should be limited on the basis of their yields measured with an intense smoking machine regime and determined per mg of nicotine.

Approaches to selectively reducing specific smoke toxicants relative to machine-measured tar and nicotine yields are very dependent upon the physiochemical nature of the individual toxicant. Conventional cigarette design parameters offer limited scope for

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relative reductions in the smoke toxicants. For example, by increasing the filter efficiency of a conventional cellulose acetate filter, the particulate phase constituents are reduced with the tar and nicotine and no selective reduction occurs. And, since cellulose acetate filters have little or no effect on volatile toxicants, increasing filtration efficiency increases the ratios of their yields relative to tar and nicotine.

Increasing filter ventilation has varied effects on the toxicants. The absolute yields of all the smoke toxicants are reduced, but, relative to tar or nicotine, yields of most of the particulate phase toxicants are unchanged. The yields of some of the volatile toxicants, such as ammonia and carbon monoxide, are reduced relative to both tar and nicotine, while the relative yields of some of the semi-volatile toxicants such as the aromatic amines and phenols are increased (Norman, 1999).

Many of the volatile vapour phase components, such as the volatile aldehydes and hydrogen cyanide may be selectively reduced using adsorbent materials in the filter such as activated charcoal or certain resins (Horsewell, 1975; Branton et al., 2009). However, permanent gases, such as carbon monoxide and nitric oxide, are not amenable to adsorption at room temperature, and toxicants in the particulate phase cannot be selectively reduced by filtration since they are largely bound into the aerosol particles.

The most promising approach to achieving substantial specific reductions in particulate toxicants from a conventionally structured cigarette is to modify the tobacco. Substitution of different tobacco varieties into the blend can have an impact on yields of several smoke toxicants. For example there are higher yields of the nitrogen containing smoke toxicants from burley tobacco than from flue-cured or oriental, and higher yields of formaldehyde and catechol from flue-cured tobaccos (Baker, 1999). However, decreases in one toxicant or set of toxicants are often offset by increases in other toxicants. To avoid this it would be useful to be able to identify and remove precursors to smoke toxicants from the tobacco leaf.

With the exception of the metallic toxicants (chromium, nickel, arsenic, selenium, cadmium, mercury and lead) and several of the tobacco specific nitrosamines (such as NAT and NAB) which are transferred directly from the leaf, the majority of the smoke toxicants are formed by pyrosynthesis from the leaf components. Thus, the major precursors for the volatile carbonyls, benzo(a)pyrene, carbon monoxide, benzene and toluene are the structural carbohydrates such as pectin and cellulose as well as the sugars (Baker, 1999).

The nitrogenous smoke toxicants are formed from nitrogenous precursors in the leaf, and there is considerable evidence that protein and amino acid combustion contributes to the generation of several nitrogen containing smoke toxicants on the Health Canada list (shown in Table 4). Proteins and amino acids have been reported to be precursors for hydrogen cyanide (Johnson and Kang, 1971; Tso et al., 1982), pyridine and quinoline (Higman et al., 1970; Schmeltz et al., 1972), 2-aminonaphthalene and 4-aminobiphenyl (Torikaiu et al., 2005). Tobacco protein is also strongly correlated with the formation of mutagenic heterocyclic amines and the resulting mutagenicity of smoke condensate in the TA98 Ames assay (Mizusaki et al., 1977; Yoshida and Matsumoto, 1980; Clapp et al., 1999).

The polyphenols in tobacco are major precursors for phenolic smoke compounds. Chlorogenic acid, the most abundant polyphenol in flue-cured tobacco, is a major precursor for phenol, catechol and the substituted catechols (Zane and Wender, 1963; Sakuma et al., 1982; Schlotzhauer et al., 1982; Sharma et al., 2002; Wooten et al., 2006; Torikaiu et al., 2005), while hydroquinone has also been reported as a chlorogenic acid pyrolysis product (Wooten et al., 2006; Sakuma et al., 1982; Torikaiu et al., 2005). Rutin and caffeic acid also generate catechol and substituted catechols on pyrolysis (Wooten et al., 2006; Schlotzhauer et al., 1982) but because of their low concentrations in tobacco and because of their lower pyrolytic yields their contributions to catechol in flue-cured tobacco smoke are much less than chlorogenic acid. Resorcinol is known to be a major product from pyrolysis of rutin (Zane and Wender, 1963).

There have been reports of processes for removal of protein from tobacco. Kung and Tso (1978) described a process for aqueous protein extraction from uncured tobacco leaf, mainly as a way of using tobacco as a protein source but also as a means of reducing the potential of the leaf to generate some of the smoke toxicants. The process required the leaf to be homogenised before extraction and cured in a slurry process (Tso et al., 1975) prior to conversion to a reconstituted sheet material. Pyrolysis of the low protein tobacco sheet showed some reductions in generation of tobacco specific nitrosamines and phenols and increases in volatile nitrosamines and benzo(a)pyrene (Woodlief et al., 1984).

Clapp et al. (1999) described a process for extracting protein from cured tobacco, using water extraction followed by protease digestion. The extracted tobacco, in the form of pulp, together with the water solubles, was made into reconstituted sheet and used to make cigarettes. Although toxicants in the smoke were not reported, the activity of the tar in the Ames TA98 mutagenicity assay was determined and significant reductions in specific activity compared with an untreated control were reported.

In this paper the effects of protein and polyphenol removal from tobacco on smoke toxicant yields have been investigated. The tobacco treatment was carried out on cut, flue-cured tobacco, and involved extraction of the tobacco with water followed by treatment with an aqueous protease enzyme solution. After treatment of the tobacco extract with adsorbents and concentration, the solubles were re-applied to the extracted tobacco. The treated tobacco retained the structure of the original tobacco and was made into cigarettes using conventional cigarette making equipment, without the need for reconstitution into a sheet material.

This paper describes the treatment process and its effect on tobacco chemistry, including quantification of residual enzyme on the tobacco, the yields of mainstream and sidestream smoke toxicants from cigarettes made with this tobacco when smoked under ISO smoking conditions, and estimation of the potential transfer of the applied enzyme to smoke.

2. Experimental

2.1. Tobacco treatment

2.1.1. Materials

Tobacco: The tobacco used for treatment was an all-lamina flue-cured tobacco blend, cut at 35 cuts per inch (approximately 0.7 mm in width).

Novozym 80001: A protease preparation, currently identified according to the enzyme classification system as EC 3.4.21.62, but previously included in EC 3.4.21.14. It is a bacterial serine protease of the subtilisin family with broad specificity for peptide bonds (Outtrup and Boyce, 1990). It was produced with food grade raw materials and under food grade quality conditions, and supplied by Novozymes Inc. (Denmark); it is hereafter referred to as the protease, or applied enzyme. The pure enzyme is a globular protein composed of 269 amino acids in a single chain with a molecular weight of 27.6 kD. The enzyme was supplied in a concentrated aqueous solution with a protein concentration of 40 g/L. The protease solution used to treat the tobacco consisted of 0.1 L of concentrated enzyme solution and 0.05 L of 1.05 M calcium chloride solution in 65 L of de-ionised water. After mixing, the pH was adjusted to 10.2 with 8 M sodium hydroxide solution just before use.