Reduction of Nitrogenous and Phenolic Cigarette Smoke Toxicant Yields Through The Use of a Tobacco Treatment Process

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Background, Objectives and Approach

The 2001 Institute of Medicine (IOM) report "Clearing the Smoke" discussed the development of products which might result in substantial reduction in exposure to one or more tobacco toxicants and can reasonably be expected to reduce the risk of one or more specific diseases or other health effects.

There have been a number of attempts to reduce toxicant exposure, from use of filter adsorbents targeted at vapour phase smoke toxicants, modified curing methods to limit TSNA formation in tobacco, to the use of Tobacco Substitutes to provide general reduction in smoke yields. However, none of these approaches have demonstrated the characteristics called for by the IOM report at the same time as demonstrating consumer acceptability on a commercial scale.

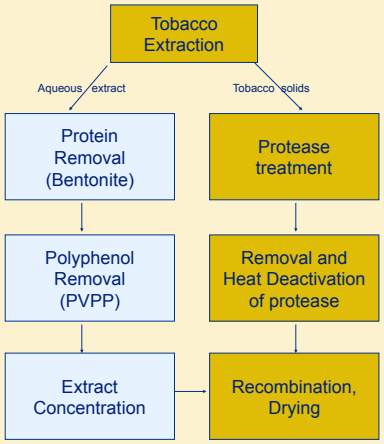
Combustion and smoke science researchers have demonstrated that constituents of cigarette smoke may be generated within burning cigarettes by either pyrolytic volatilisation of toxicants present in tobacco (e.g. metals) or pyrosynthesis of tobacco constituents (e.g. carbon monoxide from carbonaceous materials). Some species, e.g. tobacco specific nitrosamines (TSNAs) are found in smoke through the combined action of both routes.

Of the approximately 5600 constituents identified in smoke approximately 150 are considered to be toxicants. A number of these species are nitrogenous, e.g. the IARC Group 1 carcinogens 2-aminonaphthalene and 4-aminobiphenyl, and the respiratory and cardiovascular toxicant hydrogen cyanide. There is considerable evidence that protein and amino acids are precursors for these smoke constituents; tobacco protein is also strongly correlated with the formation of mutagenic heterocyclic amines and the mutagenicity of smoke condensate in the TA98 Ames assay.

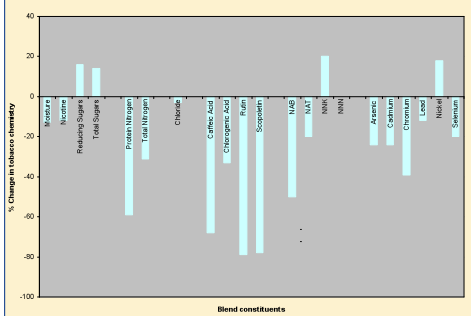
Tobacco polyphenols such as chlorogenic acid, rutin and caffeic acid are major precursors for phenolic smoke compounds such as phenol, cresols, hydroquinone, resorcinol and catechol.

The objective of this work was to develop a tobacco treatment process which could reduce nitrogenous and phenolic smoke toxicant yields from cigarettes, by removal of substantial quantities of tobacco protein and polyphenols; and to characterise the chemical and *in vitro* toxicology properties of smoke from cigarettes containing this tobacco.

Process Schematic



Changes in Tobacco Chemistries following treatment



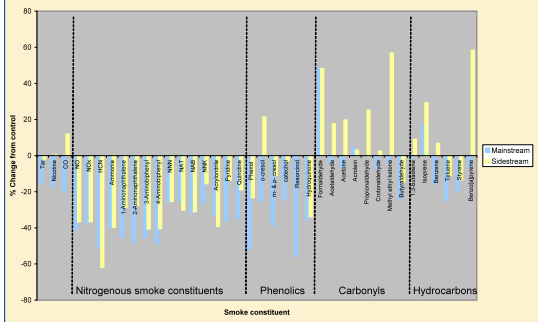
Statistical significance of these changes are discussed in Liu et al (2011).

Enzyme Residues

Approximately 30ppm savinase was left on the tobacco after washing. The upper limit for savinase transfer to smoke has been calculated as <0.009% with a 15mg ISO tar cigarette. This equates to maximum smoke yields of protease from 1 (ISO) to 4ng (Canadian Intense)/cigarette; values below the TLV for savinase.

In addition, the heat treatment process deactivates savinase such that <30ppb of active savinase is present on tobacco after the treatment; thus further reducing smoker exposure to the active enzyme to a level several orders of magnitude below the TLV.

Mainstream and Sidestream Smoke Chemistries



Statistical significance of these changes are discussed in Liu et al (2011).

In vitro toxicity assays

The *in vitro* cytotoxicity and genotoxicity of particulate matter (PM) from cigarettes containing untreated and treated tobaccos were examined in the Ames test (strains TA98, TA100, TA102, TA1535 and TA1537; with and without S9); Neutral Red Uptake assay and *in vitro* micronucleus test (V70 Chinese hamster lung cells), and mouse lymphoma assay (L5178Y cells).

Cigarettes were constructed using 80% of untreated flue-cured lamina or treated BT tobacco, and filters containing activated carbon and an amine functional resin,CR20, to counteract increases in volatile smoke toxicants described above. Two reference products, an internal BAT reference product (M4A) with flue-cured tobacco and a cellulose acetate (CA) filter, and a US-blend product with a CA filter (3R4F).

The mutagenic potencies (mean revertants per microgram NFDPM), as tested in TA98 with S9, of smoke from the reference and treated tobacco cigarettes are presented below (* different from control, p<0.05):

PM Source	Filter type	MEAN REVERTANTS/µg NFDPM			
Tobacco Blend		*EXPT A*	*EXPT B*	*EXPT C*	*EXPT D*
80% Flue-cured lamina / 20% Stem	CA with 40mg CR20 / 20mg Charcoal	1.36	1.55	1.32	1.04
80% BT Tobacco / 20% Stem	CA with 40mg CR20/ 20mg Charcoal	1.22*	1.17*	0.85*	0.72*

In general, there were no changes in response in the other assays investigated:

PM Source		*In vitro* test							
		Ames			MN		MLA		NRU
		-S9	+S9		-S9	+S9	-S9	+S9	-S9
		All strains	TA102, 1535	TA98, 100, 1537					
Tobacco Blend	Filter type								
80% FC Lamina /20% Stem	CA with 40mg CR20/ 20mg Charcoal	-	-	+	+	+	+	+	+
80% BT FC Lamina /20% Stem	CA with 40mg CR20 / 20mg Charcoal	-	-	+	+	+	+	+	+
M4A Reference FC blend	CA	-	-	+	+	+	+	+	NT
3R4F USB Reference blend	CA	-	-	+	+	+	+	+	NT

MN = micronucleus test; MLA = Mouse lymphoma assay; NRU = Neutral Red Uptake test; - = inactive; + = active; NT = not tested

Conclusions

A tobacco treatment process has been described which removes substantial quantities of proteins and polyphenols from tobacco while maintaining the physical integrity of the tobacco.

Cigarettes made containing tobacco treated in this way provide reductions in the mainstream and sidestream yields of nitrogenous smoke constituents such as aromatic amines and HCN, and phenolics such as cresols, hydroquinone, and phenol. However, there were also increases in the mainstream yields of carbonyl species, particularly formaldehyde, and also sidestream benzo[a]pyrene yields. The use of an effective filter adsorbent such as activated charcoal or an amine functional resin such as CR20 may provide a means of alleviating the increases in mainstream smoke toxicant yields.

In vitro assays showed decreased activity in the TA98+S9 Ames assay with particulate matter from these cigarettes (consistent with a reduction in tobacco protein levels) and no changes in activity in the other investigated assays.

References

All chemical methodology and references from this poster can be found in the Open Access article Liu., C. et al., Food Chem. Toxicol. (2011), doi:10.1016/j.fct.2011.02.015; a manuscript discussing the *in vitro* assays reported here is in preparation but details of the methodology can be found in the Open Access article McAdam K.G. et al. "The use of a novel tobacco-substitute sheet and smoke dilution to reduce toxicant yields in cigarette smoke". Food Chem. Toxicol. (2011), doi: 10.1016/ j.fct.2011.04.002

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