



The generation of formaldehyde in cigarettes—Overview and recent experiments

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Abstract

In recent years much effort has been devoted to assessing the influence of tobacco ingredients on the chemistry and toxicity of cigarette mainstream smoke. All of the studies have indicated that commonly used tobacco ingredients do not change the toxicity of smoke as measured in specified assays. Also, the ingredients have little effect on the levels of most smoke constituents that may be relevant to smoking-related diseases. One exception to this generalisation is formaldehyde, which is generated from saccharides used as tobacco ingredients. However, the past studies have generally used mixtures of ingredients added to the tobacco so that the exact effect of each saccharide in turn could not be precisely determined. This is addressed in the present study.

Many diverse studies over the last 30 years have examined particular aspects of formaldehyde in smoke and its generation although no attempt has been made to draw the various aspects together. This has also been addressed in the present paper and an overview is developed on the subject. The experimental results of the present study are rationalised within the framework of this previous knowledge. In the present experimental study, several individual saccharides commonly used as tobacco ingredients have been added to cigarettes, the cigarettes have been machine-smoked and the yields of formaldehyde in the resultant smoke have been compared to those from a control (no ingredient) cigarette. Using four series of cigarettes made on different occasions, the results indicate that all tested sugars added to tobacco increase the yield of formaldehyde in mainstream cigarette smoke under ISO standard smoking machine conditions. Increases up to 60% are observed at maximum sugar levels used on cigarettes. The increases are mostly statistically significant although their magnitudes are variable. These results with formaldehyde are consistent with all previously published studies on the subject.

The increases in mainstream formaldehyde are also observed using smoking machine conditions that are more intense than the standard ISO conditions. Different sugars increase mainstream formaldehyde to different extents, which may be due at least partially to the presence of varying amounts of amino compounds in some of the sugars, such as honey and maple syrup. The presence of such compounds has been shown to inhibit the generation of formaldehyde from sugars. In general, the first puff of the cigarette generates abnormally high yields of formaldehyde, and this effect has been shown to persist in the presence of added sugars. In contrast to the situation with mainstream smoke, the levels of formaldehyde in sidestream smoke are not affected by the presence of sugars.

The addition of the various saccharides to tobacco also produced some statistically significant effects in the cigarette mainstream yields of six other carbonyl smoke constituents that were analysed at the same time as formaldehyde. These effects were generally small, less than 16%, were not consistent amongst the various cigarette series and lost their significance when the long-term analytical variability was taken into account.

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Abbreviations: CORESTA, Co-operation Centre for Research Relative to Tobacco, Paris, France; DAP, diammonium hydrogen phosphate; dwb, dry-weight basis; ETS, environmental tobacco smoke; HPLC, high performance liquid chromatography; IARC, International Agency for Research on Cancer; ISO, International Standards Organisation; *n*, number of replicate measurements; nd, not determined; NFDPM, nicotine-free dry particulate matter (commonly called 'tar'); 1R4F, a reference cigarette distributed by the University of Kentucky, Lexington, KY, USA; PAH, polynuclear aromatic hydrocarbon; sd, standard deviation; TPM, total particulate matter; uv, ultra violet.

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1. Introduction

The general concept of cigarette composition, including ingredients, and the study of the ensuing effects on smoke toxicity, is a very active area of research. Cigarette smoke constituents and potency in various bioassays vary with tobacco composition (e.g. Röper et al., 2004). Thus, the composition of tobacco, including ingredients, as influential on smoke chemistry and toxicity is an important subject. A recurring question over many years has been whether or not the use of ingredients increases the toxicity of cigarette smoke. In their classic 1967 book on tobacco smoke, Wynder and Hoffmann (1967) hypothesised that the use of tobacco ingredients might potentially either increase or decrease the toxicity of tobacco smoke. Since the 1950s much research has been undertaken on the influence of tobacco ingredients on the chemical and biological properties of smoke. This work has recently been summarised in five general reviews on ingredients (Paschke et al., 2002; Rodgman, 2002a,b, 2004; Baker and Smith, 2003). In addition, large studies have recently been published on the influence of ingredients on the properties of smoke. This work includes pyrolysis of ingredients and the influence of ingredients on 44 “Hoffmann analytes” in smoke, smoke particulate matter genotoxicity and cytotoxicity, inhalation toxicity of smoke and skin painting activity (Carmines, 2002; Rustemeier et al., 2002; Roemer et al., 2002; Vanscheeuwijck et al., 2002; Carmines et al., 2005; Carmines and Gaworski, 2005; Gaworski et al., 1997, 1998, 1999; Heck et al., 2002; Baker et al., 2004a,b,c, 2005; Baker and Bishop, 2004, 2005; Stavanja et al., 2003). In this context, the “Hoffmann analytes” are those 44 substances in tobacco smoke that regulatory authorities in Canada, the USA, Brazil and elsewhere consider to be relevant to smoking-related diseases. These include, *inter alia*, some volatile carbonyl compounds, tobacco-specific *N*-nitrosamines, aromatic amines, phenols, volatile alkenes, benzo[*a*]pyrene and metals. The lists of analytes have been drawn-up by Dietrich Hoffmann and co-workers of the former American Health Foundation in New York since the mid 1980s (e.g. Hoffmann and Hoffmann, 1998, 2001; Hoffmann et al., 2001). This list of 44 substances is a core, but not exhaustive, list of hazardous smoke constituents. The full compilation by Hoffmann and colleagues in fact lists 82 substances. Those substances on the list of “Hoffmann analytes” are not necessarily the only cigarette smoke constituents that have toxicological properties. Rodgman and Green (2003), and independently Fowles and Dybing (2003), have listed up to 149 substances, including more extensive lists of polynuclear aromatic hydrocarbons, *N*-nitrosamines, *N*-heterocyclic amines and other organic molecules. In addition, free radicals may also be important (e.g. Church and Pryor, 1985).

The biological assays used to assess the toxicity of cigarette smoke in the work conducted in the 1950s and 1960s used mouse skin painting techniques. These techniques have been superseded in more recent years by a broader range of bioassays: rodent sub-chronic inhalation toxicity and *in vitro* genotoxicity and cytotoxicity of smoke particulate matter. Individually, each of the *in vitro* biological assessments may have some limitations, for example Röper et al. (2004) have recently shown that the vapour phase of smoke is an important contributor to the *in vitro* bioassay responses, in addition to the smoke particulate phase. Whole smoke assessment techniques for use with *in vitro* bioassays are under development (e.g. Massey, 2002; Phillips et al., 2005) but are not currently available for use in systematic studies. The assays used in assessing the effects of tobacco ingredients on smoke toxicity are the tests that are recommended in the UK. Guidelines for the assessment of new tobacco ingredients (Report of the Scientific Committee on Tobacco and Health, 1998). Collectively, the bioassays mirror recommendations covering toxicological assessment of a large range on consumer products and provide broad information on the potential effects of tobacco ingredients on the toxicological profile of cigarette smoke. Within this toxicological framework there is no evidence to support the hypothesis that common cigarette ingredients play a major role in influencing cigarette smoke toxicity. Also, the ingredients have no effects on the levels of most smoke constituents that may be relevant to smoking-related diseases. One exception to this generalisation is formaldehyde, which is generated from saccharide tobacco ingredients (Rustemeier et al., 2002; Paschke et al., 2002; Baker et al., 2004a,b,c, 2005). However, these studies on the effects of saccharide ingredients on smoke chemistry have generally used several ingredients added to the tobacco at the same time, and the exact effect of each saccharide in turn cannot be precisely determined.

Formaldehyde has recently been re-classified by IARC as a Group 1 carcinogen, i.e. ‘it is carcinogenic to humans’ (IARC, 2004). It is present in both mainstream and sidestream cigarette smoke. In mainstream smoke it is distributed between the vapour and particulate phases of mainstream smoke, with typically about 30% being in the particulate phase (Sakuma et al., 1978).

Formaldehyde is known to be generated from saccharide materials in tobacco, such as sugars and cellulose, which are naturally present and also added as ingredients in some countries for taste reasons (Rustemeier et al., 2002; Paschke et al., 2002; Baker et al., 2004a,b,c, 2005).

Many studies in recent years have examined particular aspects of formaldehyde in smoke and its generation although no attempt has been made to draw the various aspects together. In addition, a number of uncertainties

exist on the exact role of saccharide ingredients in formaldehyde levels in cigarette smoke. The present paper addresses both of these issues. The objectives of the paper are (1) to review what is known about the generation of formaldehyde by a burning cigarette, and (2) to examine the effect of particular saccharides on the generation of formaldehyde in cigarette smoke.

It is, of course, recognised that the mechanisms for the adverse effects of smoking are complex and many are not known. The overall toxicity of smoke could depend on combinations of effects and chemicals. Simply reducing the levels of one component, such as formaldehyde, may not have a beneficial effect. However, the selective reduction of such substances would seem to be sensible, where possible, pro-

vided that the means of selective reduction did not give rise to other harmful smoke constituents or harmful effects.

2. Formaldehyde in cigarette smoke

Yields of formaldehyde in cigarette mainstream smoke have been reported in the range 1.3 μg in filter cigarettes to 283 μg in unfiltered cigarettes, although 60 μg is a more typical upper limit for modern filter cigarettes smoked under standard ISO smoking machine conditions (Smith et al., 2000; Miyake and Shibamoto, 1995; Schaller et al., 1989; Hoffmann and Hoffmann, 1998, 2001; Hoffmann et al., 2001). Various studies have recently determined, *inter alia*, the yields of mainstream formaldehyde in various

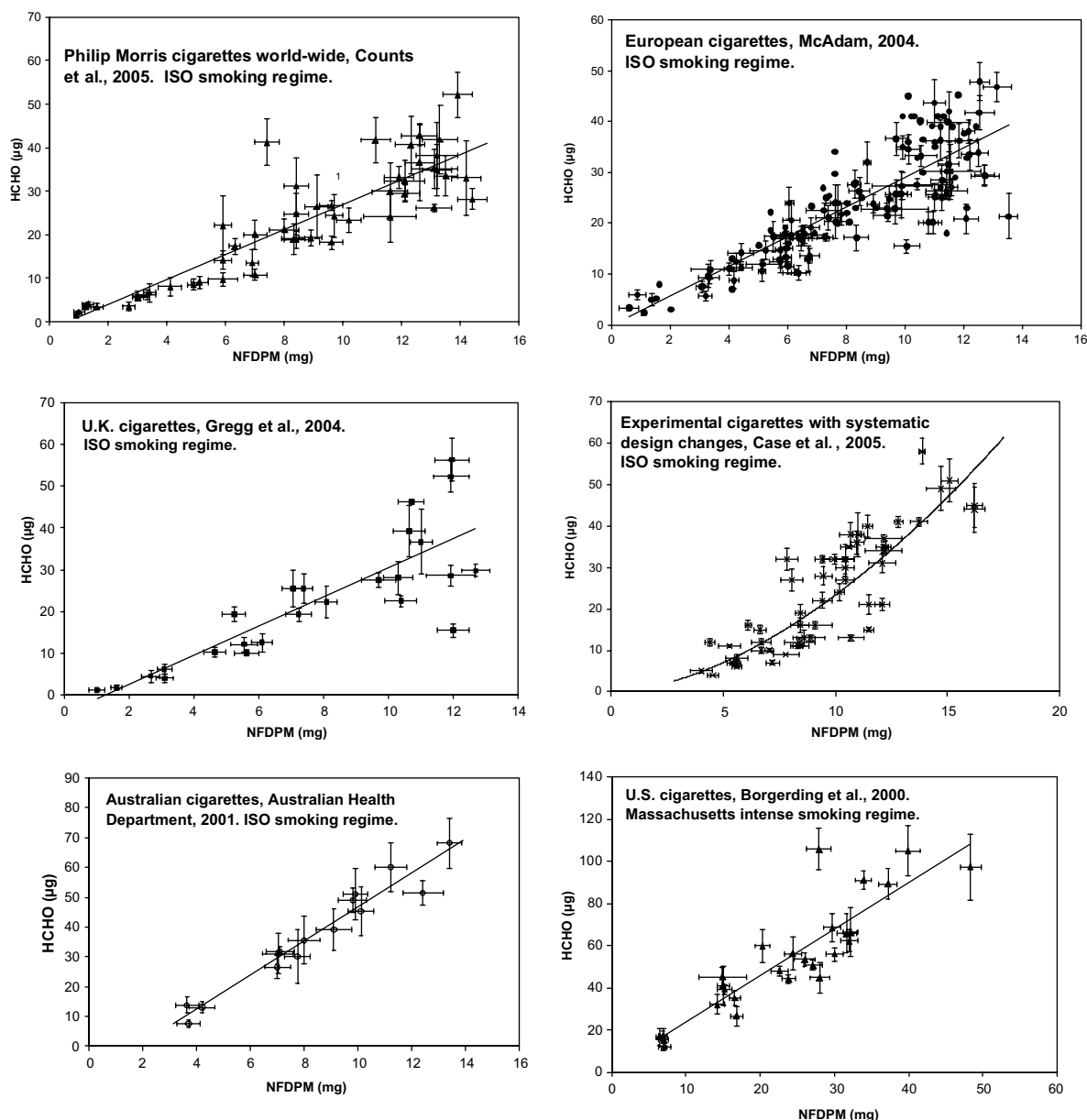


Fig. 1. Mainstream formaldehyde as a function of NFDPM yield in various studies and two machine smoking regimes.

series of cigarettes and the results from six studies are summarised in Fig. 1, in which formaldehyde is plotted as a function of the nicotine-free dry particulate matter (NFDPM, or 'tar') yield of the cigarette. In five of the studies the cigarettes were smoked under ISO standard smoking machine conditions, *viz* a puff of 35 ml volume and 2 s duration, taken once per minute (ISO Standard 4387, 2000). In the sixth study (Borgerding et al., 2000a,b,c) the cigarettes were smoked using the 'Massachusetts intense' smoking regime, *viz* a puff of 45 ml volume and 2 s duration, taken every 30 s with 50% of the filter ventilation zone blocked. These six studies cover world-wide Philip Morris cigarette brands (Counts et al., 2005), cigarette brands across eight European countries (McAdam, 2004), cigarette brands from the UK (Gregg et al., 2004), experimental cigarettes in which the design parameters and tobacco type were systematically changed (Case et al., 2005), cigarette brands from Australia (Australian Health Department, 2001) and cigarette brands from the USA (Borgerding et al., 2000a,b,c). In a given study both the within-laboratory and inter-laboratory analytical variability will be large, as has been discussed previously (Baker et al., 2004a; Gregg et al., 2004). Nevertheless, all six studies depicted in Fig. 1 show a consistent increase in formaldehyde yield as NFDPM yield increases, with the average yield being in the range 25–45 µg for an NFDPM yield of 10 mg (ISO smoking machine conditions), which is the upper limit of NFDPM yields allowed in the European Union.

Formaldehyde is also present in sidestream smoke. The sidestream/mainstream ratio for formaldehyde is quoted as 36.5 for the University of Kentucky 1R4F reference non-filter cigarette (Jenkins et al., 2000). The sidestream formaldehyde yields of fifteen Australian cigarette brands smoked by taking one 35 ml puff of 2 s duration every minute were in the range 326–385 µg per cigarette, independent of mainstream yields, which were in the range 7.6–68 µg per cigarette with the equivalent 'tar' yields in the range 3.7–12.4 mg per cigarette (Australian Health Department, 2001). This suggests that the reactions occurring during the smoulder period of the cigarette that generate formaldehyde are occurring independently of the reactions during the puff. However, in the concentrated sidestream plume that leaves the cigarette burning zone during smoulder, ammonia and formaldehyde react almost instantaneously to produce hexamethylenetetramine, which resides in the particulate phase of smoke (Borgerding et al., 1984; González and Sarabia, 1992; Baker, 1999). Because of the excess of ammonia, there is very little formaldehyde actually present in the sidestream smoke plume. However, during the collection and work-up procedures for the analysis of sidestream smoke, the hexamethylenetetramine decomposes into ammonia and formaldehyde, resulting in the sidestream smoke measurement including the formaldehyde liberated from the hexamethylenetetramine as well as the free formaldehyde. In contrast, in mainstream smoke ammonia is present in low levels and so very little hexa-

methylenetetramine is formed. In the presence of water vapour, formaldehyde will exist as a hydrated form in mainstream smoke, $\text{CH}_2(\text{OH})_2$. As the sidestream plume, and exhaled mainstream smoke, diffuses into the room to form environmental tobacco smoke (ETS), they are both greatly diluted and the hexamethylenetetramine decomposes to release formaldehyde and ammonia. In ETS, formaldehyde is present in the free form or possibly the hydrated form. Thus formaldehyde levels that are quoted in ETS are correct.

Most commercial cigarettes have mainstream formaldehyde yields of about 20–60 µg (ISO machine-smoking conditions) and sidestream yields of 350–450 µg. However, environmental tobacco smoke is only a relatively minor contributor to indoor air concentrations of formaldehyde compared with other sources (Jenkins et al., 2000; based on a review of 19 field studies). The commonly observed excess of formaldehyde in indoor air as compared to outdoor air is most frequently found to result from formaldehyde being emitted by building materials, furnishings and consumer products. These include urea-formaldehyde resins such as urea-formaldehyde foam insulation, pressed-wood products such as plywood and fibreboard, and common household items such as cleaning supplies, varnishes and adhesives. Combustion sources such as gas ranges and space heaters can also emit formaldehyde at 20–40 mg/h. Consequently, it has been difficult to demonstrate consistently elevated levels of formaldehyde in indoor environments due to ETS, although this has been done in some cases where room ventilation is minimal (Jenkins et al., 2000; a review of 19 field studies). However, Nazaroff and Singer (2004) have, more recently, reported that ETS is an important source of formaldehyde in indoor environments.

3. Overview of studies on the generation of formaldehyde from tobacco

3.1. Pyrolysis of tobacco, tobacco components and ingredients

Studies have been reported on the generation of formaldehyde during the pyrolysis of tobacco and tobacco components over the last 30 years. In such pyrolysis experiments, two types of atmosphere can be used: an inert atmosphere (usually helium or nitrogen) and an atmosphere containing oxygen (usually between 5% and 21% by volume). During pyrolysis in the inert atmosphere, the reaction products are generated by thermal decomposition of the tobacco or tobacco component, while during pyrolysis in the atmosphere containing oxygen, product formation by combustion of the tobacco/component is also included. These are useful means of separating two of the major mechanisms of formation of smoke constituents that occur inside the burning cigarette. Several studies have shown that formaldehyde is generated during the pyrolysis of flue-cured, burley and oriental tobacco in both inert

atmospheres and air between 200 and about 500 °C (Burton and Childs, 1975, 1977; Bassilakis et al., 2001; Torikai et al., 2002, 2004). Torikai et al. (2002, 2004) have shown that the presence of oxygen in the pyrolysis atmosphere had little effect on formaldehyde generation at a heating rate of 17 °C s⁻¹, whereas Burton and Childs Jr. have reported a doubling of the formaldehyde yield when the tobacco was pyrolysed in air at a heating rate of 0.01 °C s⁻¹ (Burton and Childs, 1977). The formaldehyde pyrolysis profiles of Burton and Childs (1977) indicate that there is one major region of formaldehyde formation during the pyrolysis in helium, which is 200–400 °C, and two regions of formation during the pyrolysis in air, 150–300 and 380–500 °C. The lower temperature profile is similar in shape to the profile obtained in helium, but displaced by 50–100 °C to lower temperatures. This suggests that in air the lower temperature formaldehyde profile is formed essentially by the thermal decomposition of tobacco, while the higher temperature profile is formed by the incomplete combustion of carbonaceous residue. Torikai et al. have shown that the tobacco pH (strictly, the pH of aqueous extracts of tobacco, range 2.9–7.1) and linear gas flow rate over the pyrolysing tobacco (range 4–33 cm s⁻¹) did not affect the yield of formaldehyde generated by pyrolysis in nitrogen at a heating rate of 17 °C s⁻¹ up to 800 °C (Torikai et al., 2002, 2004).

Several studies have observed formaldehyde formation during the pyrolysis of saccharides found as tobacco components or used as tobacco ingredients. Formaldehyde has been observed in several studies from the pyrolysis of glucose, fructose, sucrose, starch and cellulose in inert atmospheres (Burton, 1976; Banyasz et al., 2001a,b; Bassilakis et al., 2001; Tarora et al., 2003). Baker et al. (2005) observed the formation of formaldehyde during the pyrolysis in 10% oxygen in nitrogen, at a heating rate of 0.5 °C s⁻¹ to 300 °C followed by 2.9 °C s⁻¹ to 900 °C, of the following saccharides: brown sugar, white sugar, cane sugar, invert sugar, molasses, cellulose, hydroxylpropyl cellulose, starch, acacia gum and xanthum gum. Further studies by the same authors at a heating rate of 1.5 °C s⁻¹ confirmed these findings and also observed formaldehyde generation from the pyrolysis of fructose, glucose, honey, maple syrup and corn syrup (Baker et al., 2006). Burton (1976) reported formaldehyde evolution from cellulose pyrolysed in helium, in one formation peak over the temperature range 270–330 °C at a heating rate of a few degrees per minute. Banyasz et al. (2001a,b) reported formaldehyde evolution from cellulose pyrolysed in nitrogen, in one formation peak over the temperature range 250–490 °C at exponential heating rates varying from 2 to 60 °C s⁻¹. These are in reasonable agreement with the results of Baker et al. (2005) in at atmosphere of 10% oxygen in nitrogen and a variable heating rate of 0.5–3 °C s⁻¹, where formaldehyde was evolved from cellulose in one peak over the temperature range 300–550 °C. Banyasz et al. (2001a,b) undertook kinetic studies on the pyrolysis of cellulose and concluded that formaldehyde was a secondary product

derived from the thermal decomposition of the pyrolytic tars. Bassilakis et al. (2001) observed the generation of formaldehyde in various temperature regions below about 400 °C during the pyrolysis of glucose, xylan and chlorogenic acid in helium at a heating rate of 0.5 °C s⁻¹.

Burton (1976) has also compared the effect of the pyrolysis atmosphere on the total amount of products formed from the pyrolysis of saccharides. He has shown that the atmosphere has a large effect, as indicated by the results depicted in Table 1. Thus, the presence of oxygen in the pyrolysis atmosphere greatly increases the total yield of formaldehyde from cellulose pyrolysis, and greatly reduces the yield from sucrose and fructose pyrolysis.

Burton and Childs (1975) have shown that extraction of tobacco with solvents (hexane, chloroform, acetone, acetonitrile and methanol) did not influence the temperature of maximum formation of formaldehyde from pyrolysis in helium but did increase the amount formed, more than doubling it. Similar results were observed for the pyrolytic formation of acetaldehyde. Extraction with these solvents removed less than 40% of the dry weight of tobacco. Thus the soluble leaf constituents have an inhibiting influence on formaldehyde and acetaldehyde formation. This suggests that soluble leaf constituents interact with formaldehyde and acetaldehyde, or their precursors such as carbohydrates. Burton and Childs Jr. pointed out that these pyrolytic observations were consistent with cigarette studies which had shown that the level of acetaldehyde in the gas phase of smoke from tobacco extracted with either ether or methanol was higher than the level of acetaldehyde in the smoke from the non-extracted tobacco (Kaburaki et al., 1969).

In a paper presented at the Tobacco Science Research Conference in 2003, Tarora and co-workers examined the pyrolysis of saccharides on the generation of carbonyl compounds and compared the yields to the yield from the pyrolysis of flue-cured tobacco (Tarora et al., 2003). The materials were pyrolysed at 17 °C s⁻¹ up to 800 °C. They reported that glucose, fructose, sucrose, cellulose, pectin, starch and rutin all generated formaldehyde, acetaldehyde, acetone and acrolein. They found that for formaldehyde and acrolein, the pyrolytic yield from tobacco was substantially greater than the yield estimated from summing the yields of the individual saccharides; the reverse was the case for acetaldehyde and acetone yields. They subsequently water-extracted the tobacco and pyrolysed the residue

Table 1
Ratio of quantities of aldehydes generated during pyrolysis of saccharides in air versus helium, from Burton (1976)

Saccharide	Yield in air/yield in helium		
	Formaldehyde	Acetaldehyde	Acrolein
Cellulose	5.4	4.3	nd
Starch	1.8	2.2	1.2
Fructose	0.02	1.8	nd
Sucrose	0.01	1.4	3.9

nd = not detected.

tobacco and found that the yield of formaldehyde increased, consistent with the results of Burton and Childs (1975), discussed above. Water-extraction had removed nitrogen-containing material from the tobacco, including amino acids, ammonium salts, nitrates, proteins and alkaloids, as well as sodium and magnesium ions and sugars. Tarora et al. concluded that the nitrogenous compounds in tobacco inhibit the generation of formaldehyde during saccharide pyrolysis in nitrogen. In confirmation of this conclusion, in further studies the authors showed that adding ammonium salts, amino acids, protein (albumine), pyrrolidine and nicotine to the tobacco at levels up to 10% by weight did decrease the pyrolytic yield of formaldehyde (Tarora et al., 2003; Yoshida et al., 2003). In a parallel study, the authors also reported that the addition of up to 10% by weight of oxidisers (KClO_4 , BiNaO_3 , CuO , KMnO_4 and K_2CrO_4) to flue-cured tobacco significantly increased the pyrolytic yield of formaldehyde. Radical scavengers and inducers had no effect, while some carbonates (Na_2CO_3 , MgCO_3 but not K_2CO_3 , CaCO_3) increase formaldehyde yields (Torikai et al., 2003). It should be noted that all these results were for pyrolysis in nitrogen.

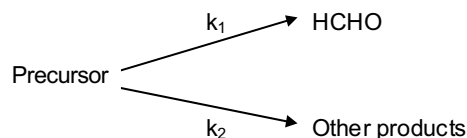
In a similar study published in 2005, Torikai et al. added various tobacco components to flue-cured and burley tobacco at 0.5- and 1.0-fold the amounts naturally present in the tobaccos (Torikai et al., 2005). Following pyrolysis in nitrogen at $17\text{ }^\circ\text{C s}^{-1}$ up to $800\text{ }^\circ\text{C}$, they again showed that the yields of formaldehyde increased significantly with the amount cellulose and sugars added to the tobacco, and decreased with the amount of protein (added to flue-cured but not burley tobacco), and magnesium and calcium ions (for burley but not flue-cured tobaccos). Torikai et al. (2005) showed that a 1.0-fold increase in cellulose increased the pyrolytic formaldehyde yield by 76% with flue-cured tobacco but only 41% with burley tobacco. They attributed the lower increase with burley tobacco to the larger amounts of nitrogenous components in burley tobacco, which would suppress the generation of formaldehyde. In contrast to their earlier studies (Tarora et al., 2003; Yoshida et al., 2003), the latest study of Torikai et al. (2005) found that the addition of starch, pectin, amino acids, ammonia and nicotine had no significant effects on the pyrolytic generation of formaldehyde.

3.2. Studies on generation of formaldehyde in a burning cigarette

The yields of most smoke components increase on a puff-by-puff basis due to a gradual reduction in tobacco rod length as the cigarette is consumed. This results in a decrease in filtration by the tobacco rod for products in the particulate phase of smoke, or a decrease in air dilution and outward gaseous diffusion for products in the gaseous phase (Baker and Crellin, 1977; Baker and Robinson, 1990). In contrast, formaldehyde is somewhat unusual in that its yield in the first puff is abnormally high, being up to six times higher than the yield in the second puff, under

standard ISO machine-smoking conditions (Parrish and Harward, 2000; Plunkett et al., 2001; Li et al., 2002a; Wagner et al., 2005). The formaldehyde yield in the first puff contributes 15–44% of the total yield, depending on cigarette type. Although the first puff in a cigarette can be atypical because of its potentially incompletely formed burning zone, these very high formaldehyde yields in the first puff are fairly unique. They are not completely unique because similar but smaller increases have been reported in the first puff for ethylene and other unsaturated hydrocarbons, and, to a lesser extent, for benzene and some carbonyl smoke constituents (Plunkett et al., 2001; Shi et al., 2003; Mitschke et al., 2005; Adam et al., in press; Wagner et al., 2005). Similarly, higher yields of benzo[*a*]pyrene and other polynuclear aromatic hydrocarbons (PAHs) have also been reported in the first puff when the cigarette was lit by a match or the yellow flame of a conventional butane cigarette lighter but not when lit by a blue flame from a butane torch lighter, or an electric heater (Li et al., 2002b; Li et al., 2003). Li et al. (2002b, 2003) deduced that the excess benzo[*a*]pyrene and other PAHs in the first puff was from soot particles covered in polynuclear aromatic hydrocarbons, produced in the incomplete combustion of the yellow lighting flame.

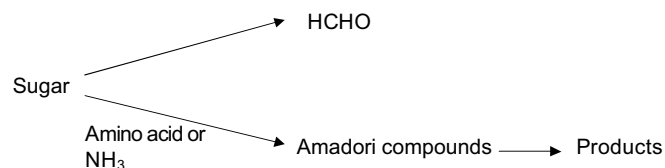
In contrast to the situation with benzo[*a*]pyrene, the high levels of formaldehyde in the first puff were not associated with the method of lighting the cigarette (Parrish and Harward, 2000). Parrish and Harward (2000) and Li et al. (2002a) undertook two series of experiments in two studies, in which cigarettes were extinguished, the burning zone and various lengths of the tobacco rod removed and the remaining cigarette re-lit. They also varied the flow velocity during the puff, the interpuff smoulder period and level of filter ventilation, and pre-heated the tip of the tobacco rod to various temperatures. They deduced that the high levels of formaldehyde in the first puff were due predominantly (approximately 90%) to the tobacco rod in the region of about 4 mm downstream of the burning zone not having been pre-heated in the previous puff. The pre-heating needed to be done to at least $250\text{ }^\circ\text{C}$ in order to eliminate the high levels of formaldehyde in the first puff. A minor contribution (approximately 10%) was due to particulate matter condensed in the tobacco rod behind the burning zone acting as a filtering medium for formaldehyde by somehow binding to it. Li et al. (2002a) went on to postulate that formaldehyde in cigarette smoke is formed from one or more precursors that can also competitively form other products, as shown in Scheme 1.



Scheme 1.

The authors have discussed this type of mechanism in detail in the formation of formaldehyde during the pyrolysis of cellulose in nitrogen (Banyasz et al., 2001a,b; Li et al., 2001). In a burning cigarette situation, they postulated that formaldehyde generation predominates at high temperatures, i.e. the reaction rate constant $k_1 \gg k_2$ at high temperatures (Li et al., 2002a). They also postulated that the competing reactions predominate at low temperatures, i.e. $k_1 \ll k_2$ at low temperatures. Thus, during the low-temperature pre-heating of the tobacco in the smoulder period preceding the puff, significant quantities of the precursor(s) are converted to the competing products, leaving less available to generate formaldehyde when the temperature is raised in the subsequent puff. For the first puff, there is no preceding smoulder period and the tobacco has not been pre-heated. Consequently, during the first puff the tobacco passes rapidly through the low-temperature region to the high-temperature region, where sufficient precursor(s) are available to generate large quantities of formaldehyde. Li et al. (2002a) gave the cross-over point of the two competing mechanisms, where $k_1 = k_2$, as about 500 °C for the pyrolysis of cellulose in nitrogen, presumably similar in the burning cigarette situation. As discussed in the preceding section, the generation of formaldehyde from the pyrolysis of tobacco and saccharides occurred at temperatures between about 200 and 500 or 550 °C when the material was heated at the relatively slow heating rates of a few °C s⁻¹ (Burton, 1976; Burton and Childs, 1975, 1977; Banyasz et al., 2001a,b; Bassilakis et al., 2001; Torikai et al., 2002, 2004; Baker et al., 2005). However, at the peak heating rates of a few hundred °C s⁻¹ that occur in a puff, the formation zone will move to higher temperatures, possibly by 300 °C (Baker, 1979). Consequently, the estimated 500 °C for the cross-over point, where $k_1 = k_2$, could be a reasonable estimate for a burning cigarette during a puff.

Parrish and Harward (2000) have determined the mainstream formaldehyde yields with cigarettes containing different types of tobacco. Their results suggest that tobaccos high in total reducing sugars tend to give high formaldehyde yields while tobaccos with high soluble ammonia content tend to give low formaldehyde yields. These results are consistent with the pyrolysis results outlined in the above section as well as cigarette smoke chemistry studies in which sugars and cellulose were added to the cigarette (Rustemeier et al., 2002; Paschke et al., 2002; Baker et al., 2004b). It is well known that ammonia as well as amino acids can react with sugars to form adducts (Maillard reactions) that then re-arrange to form non-volatile sugar-amino compounds called Amadori compounds (Nursten, 2005). These products include fructosazine and deoxyfructosazine from the reaction of ammonia with fructose and glucose. Such reactions occur in tobacco as it is stored and ages over several months (Leffingwell, 1976, 1999; Green, 1977a,b). Pyrolysis of these Amadori compounds yields a variety of decomposition products, including pyrazines (Heckman



Scheme 2.

et al., 1981). Moens et al. (2004) have shown that these reactions can occur at temperatures in the range 170–325 °C when amino acids react with glucose. It is likely that such reactions will occur during the burning of tobacco in a puff, so for sugar components there is a competitive reaction inside the cigarette burning zone, as shown in Scheme 2.

It is postulated that these two types of reaction of sugars have similar reaction rate constants at the temperatures in the cigarette burning zone. Consequently, when ammonium compounds are present in the burning tobacco, they will react with the sugars, leaving less sugar to decompose into formaldehyde. In addition, it has been shown that ammonia and formaldehyde react almost instantaneously in the sidestream plume of a smouldering cigarette to produce hexamethylenetetramine, which resides almost exclusively in the particulate phase of sidestream smoke (Borgerding et al., 1984; González and Sarabia, 1992). Ammonia generated inside the burning zone of the cigarette could also react with the formaldehyde generated from the decomposition of sugars, which would be a further mechanism by which ammonium compounds decrease the yields of formaldehyde. If hexamethylenetetramine were to be formed in mainstream smoke, the level of free formaldehyde would be reduced. It may be speculated that once the hexamethylenetetramine (in the particulate phase) was inhaled into the lungs it would be diluted and decompose to liberate free formaldehyde. Thus the effect of hexamethylenetetramine formation in mainstream smoke would probably not decrease the exposure of formaldehyde once the smoke is inside the lung.

In an internal British American Tobacco study, Irwin (1989) examined the effect of cigarette circumference on the mainstream yields of a range of smoke constituents, including formaldehyde, when the cigarettes were smoked under standard ISO smoking machine conditions. He undertook the study using six experimental non-filter cigarettes containing a US tobacco blend and with circumferences in the range 13–29 mm. He found that the mainstream yields of all smoke constituents decreased as the cigarette circumference decreased, due to progressively smaller quantities of tobacco burnt. However, Irwin also found that the yield of formaldehyde per gram of tobacco burnt also increased systematically as the circumference was decreased, as did the formaldehyde yield/tar yield ratio. Some other carbonyls (acrolein and 2-dimethylfuran) also showed this effect although to a lesser extent, while acetaldehyde and various vapour phase constituents (e.g.

isoprene, catechol and saturated hydrocarbons) did not. In addition, Irwin also found that the mainstream CO/CO₂ ratio increased as the circumference decreased, as observed previously by Yamamoto et al. (1985). Irwin pointed out that as the cigarette circumference was decreased the circumference/cross-sectional area ratio increased, allowing a greater proportion of the tobacco to be in contact with the incoming air during a puff. This would allow a larger proportion of oxidation reactions as the circumference was systematically decreased. The increasing CO/CO₂ ratio could be an effect of higher combustion temperatures at the lower circumference—greater oxidation condition, allowing a greater proportion of the carbonaceous reduction of CO₂ to CO (Yamamoto et al., 1985). Case et al. (2005) have very recently confirmed that slim cigarettes (17 mm) produce larger formaldehyde yield/'tar' yield ratios than the equivalent conventional circumference (25 mm) cigarette. None of the other 'Hoffmann analytes' showed this effect of circumference.

In order to confirm the view that formaldehyde would be produced in a more oxidising environment, Irwin (1989) smoked a standard circumference (25 mm) cigarette in three atmospheres, containing 17%, 21% and 25% oxygen v/v in nitrogen. He found that the yield of 'tar' and nicotine decreased sharply as the level of oxygen in the atmosphere increased, due to a much faster burning of the cigarette and a reduced puff count. He also found that the ratio of formaldehyde yield/'tar' yield increased systematically as the level of oxygen increased, supporting his postulation that mainstream formaldehyde yields are related to the relative amount of oxidation occurring. All of Irwin's results are consistent with the pyrolysis results of Torikai et al. (2003), discussed in Section 3.1. These demonstrated that the addition of oxidisers such as KClO₄ and KMnO₄ to tobacco significantly increased the pyrolytic formation of formaldehyde. It would thus seem that in Schemes 1 and 2, an oxidation step should also be included, see Scheme 3.

The results of Burton (1976), who pyrolysed various saccharides in both an inert atmosphere and air, discussed in Section 3.1 above and depicted in Table 1, are relevant to Scheme 3. Generation of formaldehyde from cellulose and starch would occur by both thermal decomposition and oxidation of the saccharide whereas generation from fructose and sucrose will occur predominantly by thermal decomposition of the saccharide.

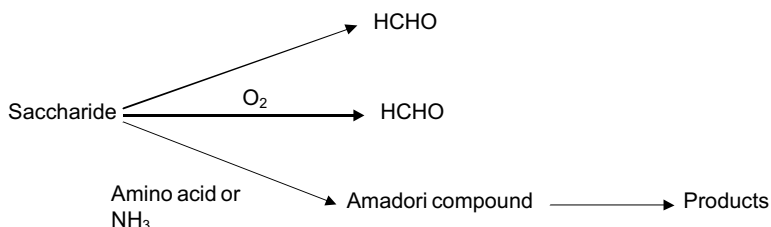
3.3. Conclusions on the formation of formaldehyde in cigarettes from information in the scientific literature

Although there are some conflicting results in the various studies reviewed above, the following conclusions can be drawn:

1. Saccharides such as sugars and cellulose are precursors in the burning cigarette to the generation of formaldehyde in smoke.
2. At temperatures below a certain value, of the order of 500 °C in the burning cigarette during a puff, the precursors decompose to give products other than formaldehyde.
3. Some ammonium compounds present in tobacco react with sugars and so inhibit the generation of formaldehyde from decomposition of the sugars. Ammonia also reacts with formaldehyde in the sidestream smoke plume, to form a complex, further suppressing the yield of formaldehyde. (This complex subsequently decomposes back to free formaldehyde and ammonia as the sidestream plume is diluted and forms ETS.)
4. The yield of formaldehyde in the first puff is abnormally high, up to six times higher than the yield in the second puff, under standard ISO machine-smoking conditions.
5. Pre-heating tobacco to the order of 250 °C, as in the smoulder period preceding the puff, decreases the formaldehyde generated in the puff.
6. A more oxidising environment in the cigarette burning zone leads to the generation of higher levels of formaldehyde. Reducing the cigarette circumference, or the presence of oxidising compounds in the tobacco, produces such an environment.
7. The generation of formaldehyde from cellulose and starch is by both combustion and thermal decomposition; its generation from fructose and glucose is predominantly by their thermal decomposition.

4. Experimental

Various series of experimental cigarettes have been made in this study, all using a typical American blend of tobacco with the exception that they did not contain any non-tobacco additives or sheet material. As-received food grade samples of saccharides were used with no further



Scheme 3.

preparation. These were similar to the grade of the ingredient used commercially when added to tobacco as an ingredient. Some were obtained as solid materials instead of aqueous syrups. The samples were dissolved in water and added to the tobacco using a direct casing and conditioning cylinder in the normal manufacturing technique. The resultant cased tobaccos were made into various series of cigarettes with identical design features. These included a tobacco filler weight of 710 mg, total length of 83.5 mm, diameter of 8 mm, a standard cellulose acetate filter 25 mm in length with no filter ventilation, and cigarette paper of permeability 80 CORESTA units ($\text{cm min}^{-1} \text{kPa}^{-1}$). For each cigarette series there was a control cigarette in which the ingredient had not been added to the tobacco but was otherwise identical.

The cigarettes were generally stored at 22 °C and 60% relative humidity and were always kept at these conditions for at least 48 h prior to smoking. The cigarettes were machine smoked under the standard ISO machine smoking regime of one 35 ml puff of 2 s duration taken every minute to a butt length of (filter tipping overwrap + 3 mm), under ambient conditions of 22 °C and 60% relative humidity (ISO Standards 3308 (2000) and 3402 (1999)). TPM, NFDPM (nicotine-free dry particulate matter, or 'tar'), nicotine and carbon monoxide in the mainstream smoke were all determined by the relevant ISO standard method (ISO Standards 4387 (2000), 10315 (2000), 10362-1 (1999) and 8454 (1995)). TPM was determined gravimetrically from smoke collection on a glass fibre (Cambridge) filter pad. Nicotine was determined by capillary gas chromatography with flame ionisation detection from a propan-2-ol extract of the TPM collected on the Cambridge filter. Carbon monoxide was determined by non-dispersive infra-red spectrophotometry.

There are no internationally recognised standard methods for the determination of the other analytes in smoke. The analytical methodology used in the present study is briefly described below. For the analysis of formaldehyde and other volatile carbonyl compounds (acetaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, methyl ethyl ketone and *n*-butyraldehyde), whole smoke was passed through acidified 2,4-dinitrophenylhydrazine. This converted the carbonyls to involatile hydrazones. The solution was stabilised with pyridine and analysed by HPLC with ultra violet (uv) detection at 362 nm. For ammonia determination, smoke was drawn into two glass impingers containing dilute sulphuric acid, which trapped ammonia as the ammonium ion. After appropriate dilution the combined solutions passed through a Dionex CS12A cation exchange chromatography column and the ammonium ion was determined conductometrically. For both carbonyl and ammonia determinations, three replicate machine smoking/measurements were made. For some cigarette samples the full range of Hoffmann analytes were determined, using analytical procedures described previously (Baker et al., 2004a).

Puff-by-puff analyses of mainstream carbonyl constituents in smoke, and sidestream carbonyl constituents, were

determined by Arista Laboratories in Richmond, Virginia, USA. The puff-by-puff methodology has recently been published (Wagner et al., 2005). It involved smoking the cigarette on a Borgwaldt 20-port rotary smoking machine and analysing the smoke from each puff in turn, in a series of experiments. The actual carbonyl analysis was similar to that described above for the whole puff analysis. Three replicate smokings were undertaken for each puff determination. For the sidestream analyses, the sidestream smoke from one cigarette was collected through a fishtail chimney (Proctor et al., 1988), followed by two impingers containing 2,4-dinitrophenylhydrazine and perchloric acid in acetonitrile. The impinger solutions were then combined and analysed as described above. Three replicate smokings were undertaken for each sidestream determination. Both the puff-by-puff and sidestream determinations were undertaken using the standard machine smoking regime of one 35 ml puff of 2 s duration taken every minute.

The total sugar contents of the sugar samples were determined by first reacting an aqueous solution of the sample with invertase, which hydrolysed any sucrose present to fructose and glucose. The total levels of fructose and glucose were then measured by reducing the cupric chelate of neocuproine in alkaline solution to the highly coloured cuprous form, and determining its optical density at 460 nm. Individual sugars in some of the sugar samples were also determined in a separate experiment by taking an aqueous solution of the sugar and separating the individual sugars by HPLC followed by refractive index detection.

5. Results and discussion

5.1. Analysis of sugar samples

Results of analyses of the sugar content of the samples are given in Table 2, including information given by the suppliers. The white and brown sugar samples were obtained as crystalline solids and were at least 98% sucrose. The other samples were all obtained as syrups whose actual sugar contents were in the range 17–76%. The fructose syrup sample was essentially 76% fructose and the rest was water. It is clear that many of the commercial sugar samples were actually mixtures of sugars. Invert sugar is obtained by hydrolysing an aqueous sugar solution with tartaric acid to prevent crystallisation, is a roughly equimolar mixture of fructose and glucose (total 42%); it also contains of the order of 30% sucrose (Table 2). The sugar cane syrup, molasses and corn syrup samples all contained some maltose as well as fructose and glucose. The glucose syrup contained only 17% glucose, a roughly equivalent amount of maltose, and also maltotriose and higher saccharides. Five of the sugar samples (glucose syrup, corn syrup, cane syrup, maple syrup and molasses) were also analysed by gas chromatography–mass spectrometry in full scan mode in order to determine the identity of any significant impurities, in a series of

Table 2
Analysis of sugars

Sugar	State used	Reducing sugars ^a (% sample)	Total sugar ^b (% sample)	Individual sugar content ^c (% dwb)	Moisture ^d (% sample)	Supplier	Supplier's analysis
Fructose	Syrup	75.0	75.5	nd	28.6	Ragus Sugars, UK	Fructose: 99.5% dwb Glucose: 0.5%
Glucose	Syrup	17.0	17.1	Glucose: 11.0 ± 0.3 Maltose: 12.0 ± 1.8	nd ^e	Ragus Sugars, UK	Glucose: 17% dwb Maltose: 13% Maltotriose: 11% Higher saccharides: 59%
Invert sugar	Syrup	42.0	72.5	nd	20.6	Ragus Sugars, UK	Invert sugar: 42.5–45.5% sample Sucrose: 32.5–35.5% Moisture: 21.7–22.0%
White sugar	Solid	4.0	99.0	nd	0.3	Ragus Sugars, UK	Moisture: 0.4% sample max Reducing sugars: 0.4% sample max
Demerara (brown) sugar	Solid	3.0	98.0	nd	0.0	Ragus Sugars, UK	Sucrose: 98.0–99.2% sample Invert sugar: 0.25–0.4% Moisture: 0.2–0.3% Organic non-sugars: 0.3–0.5%
Sugar cane syrup	Syrup	42.0	71.0	Fructose: 21.4 ± 0.2 Glucose: 25.4 ± 0.4 Maltose: 26.7 ± 0.5	10.9	Ragus Sugars, UK	Sucrose: 33.1–36.1% sample Invert sugar: 43.3–46.3% Moisture: 18.2–18.5% Organic non-sugars: 1.35% max
Molasses	Syrup	15.0	54.0	Fructose: 7.9 ± 0.2 Glucose: 8.8 ± 0.6 Maltose: 34.1 ± 0.5	16.8	Ragus Sugars, UK	Sucrose: 31–41% sample Invert sugar: 15–25% Moisture: 8–14% Organic non-sugar: 8–14%
Corn syrup	Syrup	33.0	33.5	Glucose: 28.1 ± 0.8 Maltose: 17.7 ± 1.3	43.6	Corn Products, Canada	Glucose: 35% dwb Maltose: 30% Maltotriose: 13% Higher saccharides: 22%
Maple syrup	Syrup	5.0	62.0	Sucrose: 59.2 ± 1.5	21.9	Givaudan, USA	nd
Honey	Syrup	67.0	70.5	nd	17.3	Cotswold, UK	Sucrose: 19–21% sample Moisture: up to 5% Bee protein: trace

dwb: dry-weight basis.

nd: not determined.

^a The reducing sugars analysed are fructose and glucose. Determined by a method involving the reduction of the cupric chelate of neocuproine in alkaline solution to the highly coloured cuprous form, and determining its optical density—see text.

^b Total sugars are fructose, glucose and sucrose. Determined by first reacting an aqueous solution of the sample with invertase, which hydrolysed any sucrose present to fructose and glucose, and then as in footnote a.

^c Determined by dissolving the sugar in methanol, followed by near infra-red determination.

^d Determined by taking an aqueous solution of the sugar and separating the individual sugars by HPLC followed by refractive index detection.

^e The glucose syrup did not dissolve in the methanol used in the near infra-red moisture determination. Therefore the moisture level could not be determined.

experiments in which the syrup had been dissolved in methanol, acetone, ethyl acetate and dichloromethane. In all of the analyses there were no significant impurities in any of the syrup solutions.

In the following sections it should be borne in mind that only the white and brown sugar samples, and the cellulose sample, were near 100% of the stated saccharide. For all of the other sugar samples, the sample contained water and sometimes other saccharides. The stated levels of the saccharide added to the tobacco are the level of the sample added. The added level of the actual saccharide will generally be lower than this.

5.2. Effect of sugars on mainstream smoke yields

Three series of cigarettes with various added sugars at various levels were made in 2003, 2004 and 2005. These are designated cigarette series D, E and F respectively, and the designation follows systematically from the cigarette series A, B and C used in the earlier ingredient studies (Baker et al., 2004a,b,c). It should be noted that the ingredients are added at experimental levels; in many cases the added levels of ingredients were higher than the maximum levels used on British American Tobacco cigarette brands. This was to exaggerate any effects of the sugars so that they

would be more readily observed. The results of the mainstream smoke analyses are given in Tables 3–5 for the three series of cigarettes, all smoked under the ISO standard smoking machine regime. Although the tobaccos used in each year were a typical American blend of tobacco except that they did not contain any non-tobacco additives or sheet material, the tobacco blends were not identical. Thus, it is seen that the yields of carbonyl smoke constituents from the control (tobacco only) cigarettes were up to 37% higher in series E (Table 4) than in series D and F (Tables 3 and 5). This was due to natural differences in the tobacco from year to year.

In Tables 3–5 it is indicated where there is a statistically significant difference at the 95% confidence level between the means of the control cigarette in a given series, e.g. D1, which has no added sugar, and the test cigarettes in the series containing added sugars, e.g. D2–D13. This was done using a Student's *t*-test with unknown and unequal variances. Since it was not known in which direction the differences might lie, a two-tailed test was used. As discussed previously (Baker et al., 2004a), for those pairs of test and control cigarettes where there was a statistically significant difference, the significance was calculated again, taking into account the long-term variability of the analytical methodology. This was done using the *pro rata* standard deviations obtained from the measurements of the reference 1R4F cigarette obtained over the 12-month period in which this study was undertaken. Significant differences obtained using this wider analyte level variation are also indicated in the tables. Consequently, significant differences between the test and control cigarettes are indicated for (1) the measurements made at one point in time, and (2) when the long-term variability of the measured levels over a few months is taken into account.

The total filler weight (tobacco + added ingredients) was kept constant in a given cigarette series, of the order of 710 mg. The test cigarettes were generally loaded with 2.5–8% of the ingredients (Tables 3–5). Consequently, the amounts of actual tobacco in the test cigarettes were up to 57 mg lower than in the control cigarette.

The presence of the saccharides either had no statistically significant effect on the puff count or produced small significant decreases of up to about half a puff. This reflects the effect of the added saccharides on producing small increases in the overall combustibility of the filler relative to pure tobacco.

The presence of the saccharides also had either no statistically significant effect, or produced small decreases in the mainstream yields of TPM (up to 20% decrease), NFDPM (up to 8.4% decrease), nicotine (up to 17% decrease) and carbon monoxide (up to 23% decrease). The decreases will be due partly to the dilution effect of the presence of the ingredients, and partly to effects on the balance of combustion and distillation processes occurring in the burning zone of the cigarette. An exception to this generalisation is the presence of 7% fructose in cigarette F3, which produced a 7% increase in NFDPM yield (Table 5). This

increase was, however, not significant when the long-term analytical variability was taken into account, nor was there a significant change in NFDPM yield in cigarette E6, which contained 6.2% fructose, relative to its control E1 (Table 4). Consequently, this one observed increase in NFDPM yield may have been a short-term analytical variability artefact.

The smoke constituent that is the focus of the present paper is formaldehyde. For all formaldehyde results in Tables 3–5, an increase in the mainstream yield was observed when the cigarette had an added saccharide. Although some of the increases with added saccharides were small and not statistically different to the control (no ingredient) cigarette, most of the increases were statistically significant compared to the control (no ingredient) cigarette at the 95% confidence level and were up to 63% for a single saccharide. Many of the increases remained statistically significant even when the long-term analytical variability was taken into account. The coefficient of variation for the analytical method for formaldehyde analysis was 11% for over the time that these analyses were being undertaken, (Baker et al., 2004a). This is smaller than that found by Rickert and Wright (2002), who have reported a coefficient of variation of 18.5% for formaldehyde measured over an eleven month period using the same analytical methodology in the same laboratory with the University of Kentucky 1R4 F reference cigarette. A summary of the formaldehyde increases is given in Table 6. The magnitude of the increases was variable in the different cigarette series. For example, 6.2–7.0% brown sugar gave formaldehyde increases of 16, 11 and 5.2 μg in Series D, E and F respectively. This must be due, at least partially, to the variability of the analytical data. All of these increases were statistically significant.

The increases generally became larger as the level of a given ingredient was increased, as shown when systematic increases were made to the levels of added white sugar (2.5–10.5%), brown sugar (2.1–8.2%) and cellulose (0.8–2.4%). Furthermore, increases were larger in the presence of other ingredients, *albeit* only one example is given in the present study. Thus, 10.5% white sugar increased the formaldehyde level by 12 μg (40%) and by 27 μg (90%) when 0.9% acetic acid was also present.

It is also observed from the summary in Table 6 that relatively small increases in formaldehyde were obtained with 3.1% honey in Series E (1.0 μg increase, not statistically significant), and with 7.0% maple syrup in Series F (2.4 μg increase, not statistically significant). However, this was variable and larger increases were obtained with different samples of these ingredients in the other cigarette series. It is known that both honey and maple syrup contain small amounts of amino acids and proteins, and that their levels are variable between different samples (e.g. White, 1978; Gilbert et al., 1981; Hortvet, 1904). As discussed in Section 3 above, from studies on the pyrolysis of tobacco samples in nitrogen Tarora et al. concluded that the nitrogenous compounds in tobacco inhibit the generation of formaldehyde during pyrolysis in nitrogen (Tarora et al., 2003;

Table 3
Experimental cigarettes with added saccharides (2003): mainstream smoke yields, ISO smoking machine regime

Cigarette	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13
Saccharide	–	White sugar	White sugar	White sugar	White sugar	White sugar	White sugar	Invert sugar	Molasses	Brown sugar	Cellulose	Cellulose	Cellulose
Level added (%)	–	2.5	5.0	7.5	10.5	5.0	10.5	7.0	5.3	6.2	0.8	1.6	2.4
Second additive	–	–	–	–	–	Acetic acid	Acetic acid	–	–	–	–	–	–
Level added (5)	–	–	–	–	–	0.9	0.9	–	–	–	–	–	–
Puff count	7.5	7.4	7.4	7.2	7.2 ^a	7.6	7.3	7.3	7.1 ^{a,b}	7.2 ^a	7.0 ^{a,b}	7.2 ^a	7.4
sd (<i>n</i> = 5)	0.2	0.2	0.3	0.3	0.2	0.2	0.1	0.3	0.1	0.2	0.1	0.2	0.3
TPM (mg/cig)	17.1	16.8	16.7	17.2	16.2	16.7	16.6	15.7	15.6 ^{a,b}	16.2	16.9	16.1	16.3
sd (<i>n</i> = 5)	1.14	0.91	1.05	0.45	0.95	0.64	0.95	1.47	0.29	0.72	0.24	0.65	0.95
NFDPM (mg/cig)	13.3	13.0	12.7	12.9	12.5	12.9	12.8	12.4	12.2 ^{a,b}	12.6	12.9	12.4 ^a	12.8
sd (<i>n</i> = 5)	0.67	0.40	0.52	0.32	0.60	0.26	0.47	0.84	0.10	0.48	0.21	0.43	0.54
Nicotine (mg/cig)	1.17	1.12	1.08 ^a	1.06 ^{a,b}	1.05 ^{a,b}	1.08 ^a	1.02 ^{a,b}	1.07 ^a	1.09 ^a	1.13	1.18	1.12	1.12
sd (<i>n</i> = 5)	0.04	0.06	0.04	0.05	0.05	0.02	0.03	0.07	0.02	0.04	0.03	0.05	0.05
CO (mg/cig)	12.4	9.8 ^{a,b}	9.8 ^{a,b}	9.5 ^{a,b}	11.6 ^a	12.4	11.7 ^a	11.1 ^{a,b}	11.4 ^a	11.3 ^{a,b}	12.0	11.3 ^{a,b}	11.5 ^a
sd (<i>n</i> = 5)	0.48	0.80	0.59	1.0	0.55	0.68	0.36	0.64	0.25	0.45	0.19	0.41	0.50
<i>Carbonyls (µg/cig)</i>													
Formaldehyde	30	36	39 ^a	44 ^{a,b}	42 ^{a,b}	41 ^{a,b}	57 ^{a,b}	49 ^{a,b}	38 ^a	46 ^{a,b}	36	39 ^{a,b}	38 ^a
sd (<i>n</i> = 3)	3.2	2.9	1.0	2.0	1.0	0.9	6.2	2.1	1.5	5.4	2.4	4.4	2.4
Acetaldehyde	535	562	546	544	532	542	558	574	526	562	591	600 ^a	595 ^a
sd (<i>n</i> = 3)	19.5	16.6	32.9	20.9	11.1	34.9	36.5	18.0	7.0	27.3	44.0	22.2	24.1
Acetone	315	324	325	328	333	323	338	334	306	326	331	329	338
sd (<i>n</i> = 3)	12.9	12.1	25.8	13.2	6.8	22.6	15.8	6.6	3.8	16.2	22.7	13.1	11.5
Acrolein	67	76 ^a	69	75 ^a	64	74 ^a	77	78 ^a	67	74	73	78 ^a	73
sd (<i>n</i> = 3)	0.8	1.1	2.8	2.4	0.1	1.6	6.3	4.1	3.7	4.3	5.6	5.2	6.6
Propionaldehyde	47	49	47	48	46	46	47	48	45	50	50	52 ^a	51
sd (<i>n</i> = 3)	1.6	1.8	3.4	2.2	0.7	2.8	3.2	1.9	1.0	4.6	2.7	2.1	2.7
<i>n</i> -Butyraldehyde	38	38	39	39	38	39	41	42 ^a	38	40	42	43 ^a	44 ^a
sd (<i>n</i> = 3)	2.1	1.4	3.8	1.1	0.7	2.0	2.7	1.2	1.3	1.8	3.6	1.2	2.4
Crotonaldehyde	25	27	22	26	18 ^a	19 ^a	21 ^a	22 ^a	19 ^a	24	24	23	19 ^a
sd (<i>n</i> = 3)	1.2	0.8	3.0	3.3	0.2	1.0	2.1	1.3	1.9	1.5	1.7	1.1	1.9
Methyl ethyl ketone	79	81	79	82	78	76	79	80	73	78	80	80	86
sd (<i>n</i> = 3)	5.1	3.8	5.4	2.6	2.5	5.7	3.4	1.8	0.9	3.9	5.7	1.9	4.0

sd: standard deviation for *n* replicates.

^a Significantly different to D1 (control) value at 95% confidence level—this data set.

^b Also significantly different to D1 (control) value at 95% confidence level when long-term analytical variability is included.

Table 4
Experimental cigarettes with added sugars (2004): mainstream smoke yields, ISO smoking machine regime

Cigarette	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15
Sugar	–	Brown sugar	Brown sugar	Brown sugar	Brown sugar	Fructose	Glucose	Fructose + glucose	Invert sugar (UK)	Corn syrup	Honey	Molasses	Invert sugar (USA)	Maple syrup	Sugar cane syrup
Level added (%)	–	2.1	4.2	6.2	8.2	6.2	6.2	4.1 + 4.1	8.2	6.2	3.1	2.0	6.2	7.0	2.5
Puff count	7.6	7.6	7.6	7.2 ^{a,b}	7.4	7.2 ^{a,b}	7.2 ^{a,b}	7.1 ^{a,b}	6.8 ^{a,b}	7.2 ^{a,b}	7.0 ^{a,b}	7.1 ^{a,b}	6.9 ^{a,b}	7.2 ^{a,b}	7.2 ^{a,b}
sd (<i>n</i> = 5)	0.3	0.2	0.3	0.1	0.3	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.1	0.2	0.2
TPM (mg/cig)	18.2	18.4	18.0	14.6 ^{a,b}	16.1	16.9	15.0 ^{a,b}	16.8	15.8 ^{a,b}	17.2	15.9	16.8	15.7 ^{a,b}	16.4	16.6
sd (<i>n</i> = 5)	1.96	1.51	2.15	1.47	1.68	0.81	0.34	0.79	0.71	0.79	1.20	1.09	0.91	0.59	0.66
NFDPM (mg/cig)	14.1	14.2	13.6	11.9 ^{a,b}	13.4	13.4	12.4 ^{a,b}	13.8	12.6 ^{a,b}	13.8	12.9	13.7	13.1	13.6	13.4
sd (<i>n</i> = 5)	0.93	0.75	0.90	1.41	1.60	0.37	0.22	0.46	0.42	0.3	0.73	0.53	0.63	0.41	0.44
Nicotine (mg/cig)	1.19	1.16	1.10	1.04 ^{a,b}	1.07 ^{a,b}	1.11	1.05 ^{a,b}	1.09 ^a	0.99 ^{a,b}	1.13	1.10	1.18	1.06 ^{a,b}	1.12	1.12
sd (<i>n</i> = 5)	0.08	0.06	0.08	0.03	0.05	0.04	0.01	0.03	0.03	0.04	0.05	0.05	0.03	0.04	0.06
CO (mg/cig)	12.2	12.3	11.8	11.4	11.6	11.7	10.8 ^{a,b}	11.7	11.4	11.9	11.3	11.8	11.5	11.9	11.4
sd (<i>n</i> = 5)	0.62	0.66	0.71	0.49	0.34	0.27	0.29	0.58	0.56	0.30	0.58	0.52	0.73	0.32	0.39
<i>Carbonyls (µg/cig)</i>															
Formaldehyde	41	44	48 ^a	52 ^a	56 ^{a,b}	54 ^{a,b}	55 ^{a,b}	66 ^{a,b}	64 ^{a,b}	58 ^{a,b}	42	43	50	51 ^a	48
sd (<i>n</i> = 3)	3.0	3.2	1.8	3.1	0.9	2.3	3.5	5.8	1.9	3.2	3.0	3.8	5.0	4.7	4.7
Acetaldehyde	637	640	594	613	570 ^a	649	582 ^a	603	608	640	571	585 ^a	586	632	606
sd (<i>n</i> = 3)	25.9	29.8	47.1	34.3	24.7	31.2	3.8	45.1	27.0	5.0	45.7	8.2	41.8	25.6	37.1
Acetone	359	355	339	355	336	370	323 ^a	342	348	361	316 ^a	326 ^a	329	357	339
sd (<i>n</i> = 3)	4.9	22.3	25.8	19.6	14.2	22.0	6.7	25.6	18.0	6.2	24.6	2.7	24.3	9.5	18.8
Acrolein	70	72	72	73	72	77	71	75	78	81 ^a	67	68	68	75	71
sd (<i>n</i> = 3)	5.6	2.9	3.0	4.4	2.6	4.4	2.0	6.7	2.1	1.6	3.8	2.3	4.2	6.3	6.5
Propionaldehyde	55	60	57	59	56	64 ^a	54	57	56	60	53	54	54	58	60
sd (<i>n</i> = 3)	4.9	3.2	1.9	3.6	2.0	2.3	0.3	5.7	2.0	1.8	1.4	1.4	4.0	2.4	2.6
<i>n</i> -Butyraldehyde	48	48	48	50	46	47	45	46	46	51	43	45	45	47	46
sd (<i>n</i> = 3)	3.9	2.3	2.8	3.5	1.9	4.2	1.8	3.9	3.2	0.8	1.5	0.8	3.2	1.7	1.4
Crotonaldehyde	26	33 ^a	25	25	24	29 ^a	29	30	29	33 ^a	33 ^a	30 ^a	31 ^a	32 ^a	31 ^a
sd (<i>n</i> = 3)	1.6	2.9	2.7	2.1	0.8	1.8	0.5	2.1	3.1	0.9	1.5	0.4	2.3	2.8	1.6
Methyl ethyl ketone	93	89	91	96	91	93	88	92	91	98	82	85	87	92	88
sd (<i>n</i> = 3)	6.4	5.8	7.8	5.8	3.6	7.5	1.7	5.9	5.6	2.2	7.3	2.3	7.3	2.8	4.4

sd: standard deviation for *n* replicates.

^a Significantly different to E1 (control) value at 95% confidence level—this data set.

^b Also significantly different to E1 (control) value at 95% confidence level when long-term analytical variability is included.

Table 5
Experimental cigarettes with added sugars (2005): mainstream smoke yields, ISO smoking machine regime

Cigarette	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Sugar (7.0 % added)	–	Honey	Fructose	Glucose	White sugar	Brown sugar	Corn syrup	Molasses	Sugar cane syrup	Maple syrup	Invert sugar
Puff count	7.6	7.8	7.7	7.3	7.4	7.5	7.4	7.7	7.4	7.3	7.5
sd (<i>n</i> = 5)	0.3	0.1	0.1	0.2	0.1	0.2	0.1	0.2	0.3	0.2	0.2
TPM (mg/cig)	18.1	18.0	18.9	17.5	16.6 ^{a,b}	16.6	16.4 ^{a,b}	16.8	16.9	16.3 ^{a,b}	17.0
sd (<i>n</i> = 5)	1.21	0.89	0.69	0.95	0.67	0.83	0.41	0.93	0.71	0.79	0.68
NFDPM (mg/cig)	14.0	13.8	15.0 ^a	14.3	13.3	13.4	13.2	13.6	13.6	13.3	13.7
sd (<i>n</i> = 5)	0.73	0.53	0.49	0.72	0.37	0.54	0.34	0.47	0.51	0.59	0.38
Nicotine (mg/cig)	1.33	1.24 ^a	1.29	1.23	1.14 ^{a,b}	1.14 ^{a,b}	1.16 ^{a,b}	1.15 ^{a,b}	1.12 ^{a,b}	1.12 ^{a,b}	1.16 ^{a,b}
sd (<i>n</i> = 5)	0.08	0.02	0.03	0.06	0.03	0.04	0.04	0.04	0.05	0.03	0.02
CO (mg/cig)	13.2	13.4	13.8	12.7	11.8 ^{a,b}	11.9 ^{a,b}	11.4 ^{a,b}	12.0 ^{a,b}	12.4	12.1 ^a	12.3
sd (<i>n</i> = 5)	0.75	0.56	0.20	0.92	0.40	0.61	0.14	0.39	0.65	0.50	0.72
Ammonia (µg/cig)	21.5	22.8	23.6	24.9 ^a	21.9	22.1	23.6	21.8	23.1	22.2	21.9
sd (<i>n</i> = 3)	1.37	0.35	0.63	1.29	1.22	0.22	0.88	0.68	0.66	1.68	0.68
<i>Carbonyls (µg/cig)</i>											
Formaldehyde	29.9	35.6 ^a	40.0 ^{a,b}	36.6 ^a	38.7 ^{a,b}	35.1 ^a	34.9 ^a	35.5 ^a	37.0 ^a	32.3	36.0 ^a
sd (<i>n</i> = 3)	0.89	2.22	3.73	2.87	2.50	2.14	2.64	2.35	2.43	1.91	2.04
Acetaldehyde	555	559	580 ^a	516	525	541	541	547	543	523	543
sd (<i>n</i> = 3)	11.2	19.0	10.4	34.4	6.26	32.1	36.2	23.6	13.5	21.5	26.7
Acetone	311	335 ^a	348 ^a	301	320	327	313	321	324	312	319
sd (<i>n</i> = 3)	8.42	10.5	6.39	18.6	9.68	21.7	25.8	18.5	8.86	17.0	15.1
Acrolein	65.6	70.0	74.2 ^a	66.7	68.6	68.6	67.9	67.1	67.7	62.9	67.9
sd (<i>n</i> = 3)	0.97	5.32	4.70	4.33	2.17	4.46	4.22	0.25	1.91	2.18	2.84
Propionaldehyde	52.8	55.2	57.8 ^a	51.1	52.1	53.1	52.3	53.6	53.5	51.0	53.7
sd (<i>n</i> = 3)	0.87	1.93	1.65	3.12	0.92	2.84	3.74	2.17	1.71	2.34	2.37
<i>n</i> -Butyraldehyde	46.0	48.7	50.9 ^a	45.2	46.2	47.4	47.1	48.1	48.3	46.5	47.6
sd (<i>n</i> = 3)	0.71	1.59	2.21	2.50	1.88	2.39	4.48	2.25	1.03	2.80	1.49
Crotonaldehyde	22.5	21.8	23.5	21.2	21.1	21.9	22.1	22.4	23.2	21.9	23.1
sd (<i>n</i> = 3)	0.21	1.30	1.16	1.56	0.81	1.55	2.02	0.64	0.44	0.61	1.52
Methyl ethyl ketone	75.7	78.4	82.7	73.0	74.4	76.9	75.7	77.0	77.4	72.6	77.6
sd (<i>n</i> = 3)	4.15	2.06	1.79	4.60	1.80	6.24	7.10	5.15	3.64	3.59	1.93

sd: standard deviation for *n* replicates.

^a Significantly different to F1 (control) value at 95% confidence level-this data set.

^b Also significantly different to F1 (control) value at 95% confidence level when long-term analytical variability is included.

Yoshida et al., 2003). It is well known that ammonia as well as amino acids can react with sugars to form adducts (Maillard reactions) that then re-arrange to form non-volatile sugar–amino compounds called Amadori compounds. These reactions would thus be in competition with the reactions of sugars that generate formaldehyde. A lower increase in formaldehyde would then be expected from sugars that contain small amounts of amino compounds relative to sugars that do not, as observed. Furthermore, the variability in the effect with different samples of honey and maple syrup is explicable since different samples of these natural substances will contain different levels of protein and amino acids. This subject is examined further in Section 5.3.

The generalities discussed above for formaldehyde are consistent over the three series of cigarettes made in 2003, 2004 and 2005, namely that all sugars and cellulose added to tobacco increase the yield of formaldehyde in mainstream cigarette smoke under ISO standard smoking machine conditions. The increases are mostly statistically significant. The magnitude of the increases are variable, probably because of various levels of sugars being present

in the syrups added to the tobacco, as discussed in Section 3.1 above, the presence of minor amino impurities in some of the sugars, and the general variability of the analytical methodology. The results with formaldehyde are also consistent with all previously published studies on the subject (Rustemeier et al., 2002; Paschke et al., 2002; Baker et al., 2004a,b,c, 2005).

As stated in Section 1, the objectives of the present study were aimed specifically at the generation of formaldehyde in a burning cigarette. However, seven other carbonyl compounds were analysed in smoke at the same time as formaldehyde and these are all on the Hoffmann list of toxic components of smoke. For completeness, it is therefore of interest to assess the effects of the experimental parameters on the levels of these other Hoffmann carbonyl smoke constituents and the results are also listed in Tables 3–5. The addition of the various saccharides in the three series of cigarettes also produced some statistically significant effects in the mainstream yields of the other carbonyl smoke constituents (Tables 3–5). These were generally small, less than 16% in most cases and were not consistent amongst the various cigarette series. Most of these effects

Table 6

Increases in cigarette mainstream smoke formaldehyde yields when saccharides are added to the tobacco in three series of cigarettes, ISO smoking machine regime

Ingredient (maximum cigarette level ^a , %)	Increase in formaldehyde								
	Series D ^b			Series E ^c			Series F ^d		
	Ingredient level (%)	µg	%	Ingredient level (%)	µg	%	Ingredient level (%)	µg	%
D-Fructose (0.37)				6.2	13 ^{e,f}	32	7.0	10 ^{e,f}	34
Glucose (0.15)				6.2	14 ^{e,f}	34	7.0	6.7 ^c	22
Fructose + glucose				4.1 + 4.1	25 ^{e,f}	61			
Invert sugar (7.0)	7.0	19 ^{e,f}	63	8.2	23 ^{e,f}	56	7.0	6.1 ^e	20
White sugar (5.0)	2.5	6.0	20				7.0	8.8 ^{e,f}	29
	5.0	9.0 ^c	30						
	7.5	14 ^{e,f}	47						
	10.5	12 ^{e,f}	40						
White sugar + 0.9% acetic acid	5.0	11 ^{e,f}	37						
	10.5	27 ^{e,f}	90						
Brown sugar (5.7)	6.2	16 ^{e,f}	53	2.1	3.0	7.3	7.0	5.2 ^c	17
				4.2	7.0 ^c	17			
				6.2	11 ^c	27			
				8.2	15 ^{e,f}	37			
Corn syrup (1.8)				6.2	17 ^{e,f}	41	7.0	5.0 ^c	17
Molasses (5.35)	5.3	8 ^c	27	2.0	2.0	4.9	7.0	5.6 ^c	19
Sugar cane syrup (1.5)				2.5	7.0	17	7.0	7.1 ^c	24
Maple syrup (6.5)				7.0	10 ^c	24	7.0	2.4	8.0
Honey (4.1)				3.1	1.0	2.4	7.0	5.7 ^c	19
Cellulose (2.2)	0.8	6	20						
	1.6	9 ^{e,f}	30						
	2.4	8 ^c	27						

^a Typical maximum level used on British American Tobacco cigarettes.

^b Control (no ingredient) cigarette for Series D had a formaldehyde yield of 30 µg/cig (Table 3) under standard ISO machine smoking conditions.

^c Control (no ingredient) cigarette for Series E had a formaldehyde yield of 41 µg/cig (Table 4) under standard ISO machine smoking conditions.

^d Control (no ingredient) cigarette for Series F had a formaldehyde yield of 29.9 µg/cig (Table 5) under standard ISO machine smoking conditions.

^e Significantly different to control (no ingredient) cigarette for this series of cigarettes at the 95% confidence level—this data set.

^f Also significantly different to control (no ingredient) cigarette for this series of cigarettes at the 95% confidence level when long-term analytical method variability is included.

are likely to be due to analytical variability, as discussed in detail previously (Baker et al., 2004a). Indeed, the changes were found to be not significant when the long-term analytical variability was taken into account, as described previously (Baker et al., 2004a). However, the analyses in the present study were undertaken with three replicates for each analysis. Statistical power calculations indicate that in most, but not all, cases this number of replicates was sufficient to detect significant differences above 10% between the test and control cigarettes at the 95% confidence level (when the statistical power of the test was generally 0.99). However, this small sample size will not allow the statistical significance to be given with confidence for small increases below about 10%, particularly when the coefficient of variation was above about 7% (in such cases the statistical power of the test can be below 0.5). For such differences to be observed with significance at the 95% confidence level, ten or more replicates would be needed to get a statistical power of the test of 0.95. For the record, the significant effects with the other carbonyls that were observed are briefly summarised, as follows.

Mainstream smoke acetaldehyde was increased by 11–12% when 1.6–2.4% cellulose was added to the tobacco

(Table 3). Previous studies have reported both increases and decreases in smoke acetaldehyde by the addition of cellulose (Paschke et al., 2002). Mainstream smoke acetaldehyde was also decreased by up to 11% after addition of brown sugar, glucose and molasses to the tobacco (cigarette series E, Table 4) but was unaffected by the same ingredients in cigarette series D and F (Tables 3 and 5). Acetaldehyde was increased by 4.5% after addition of fructose in cigarette series F (Table 5) but unaffected by fructose in series E (Table 4). Clearly, the effect of sugars on acetaldehyde smoke yield is variable. This is consistent with the conclusions from a recent review on acetaldehyde formation in smoke: acetaldehyde is formed in about the same amount from sugars added to the tobacco blend than are produced from the tobacco itself on a weight-for-weight basis (Seeman et al., 2002).

The levels of acrolein were increased by 1–16% in 30 of the 36 samples across all three years in which the samples were made, Tables 3–5. However, the increases were only significant in seven of the samples: after the addition of white sugar, invert sugar and cellulose in cigarette series D (Table 3), by the addition of corn syrup in cigarette series E (Table 4), and by the addition of fructose in cigarette

series F (Table 5). These significant increases were not consistent across the three series of cigarettes. Thus:

- The addition of 2.5% and 7.5% white sugar in series D (Table 3) produced significant increases of 11–13% in smoke acrolein yield relative to the control (no additive) cigarette, but the addition of 5% and 10.5% white sugar produced no significant changes to smoke acrolein, in fact a non-significant decrease in one case. The addition of 7% white sugar in series F (Table 5) produced a 4.5% non-significant increase in smoke acrolein yield.
- The addition of 7% invert sugar in produced a significant increase of 16% in smoke acrolein in series D (Table 3) and no significant changes in series E (Table 4).
- The addition of 7% fructose in series F (Table 5) produced a significant increase in smoke acrolein but the increase in series E (Table 4) was not significant.
- The addition of 6% corn syrup in series E (Table 4) produced a significant increase of 16% in smoke acrolein, while 7% corn syrup produced a non-significant 3.5% increase in smoke acrolein in series F (Table 5).

The coefficient of variation for the analytical method for acrolein analysis was 11% for over the time that these analyses were being undertaken, (Baker et al., 2004a). This is of similar magnitude to that found in other studies, for example Rickert and Wright (2002) have reported a coefficient of variation of 15% for acrolein measured over an eleven month period using the same analytical methodology in the same laboratory with the University of Kentucky 1R4F reference cigarette. When this analytical variability was taken into account, as described previously (Baker et al., 2004a), none of the changes in smoke acrolein yields were found to be statistically significant.

Acetone was decreased by up to 12% after the addition of glucose, honey and molasses in cigarette series E (Table 4), and increased by the same amount by the addition of honey and fructose in cigarette series F (Table 5). Propionaldehyde was increased by up to 16% by the addition of cellulose in cigarette series D (Table 3), and by the addition of fructose in cigarette series E (Table 4) and in cigarette series F (Table 5). *n*-Butyraldehyde was increased by up to 16% by the addition of invert sugar and cellulose in cigarette series D (Table 3), and by the addition of fructose in cigarette series F (Table 5). Finally, crotonaldehyde was decreased by up to 28% by the addition of white sugar, invert sugar, molasses and cellulose in cigarette series D (Table 3), and was increased by 12–27% by the addition of brown sugar, fructose, corn syrup, honey, molasses, maple syrup, sugar cane syrup and one of the two samples of invert sugar in cigarette series E (Table 4). None of these ingredients had any significant effect on crotonaldehyde yield in cigarette series F (Table 5). None of the ingredients had any effect on the smoke yields of methyl ethyl ketone in any of the three series of cigarettes.

5.3. Effect of sugars in the presence of amino compounds on mainstream smoke yields

Some of the results discussed in Section 5.2 above imply that the presence of small amounts of amino acids and protein in some of the sugars could inhibit the generation of formaldehyde in the burning cigarette. This is consistent with work published in the scientific literature and discussed in Section 3. In order to investigate this effect further, a fourth series of experimental cigarettes has been made, cigarette series G, in which two levels of invert sugar have been added to the tobacco in the presence of diammonium phosphate or L-proline, the most abundant amino acid in honey (White, 1978). The results on the yields of some smoke constituents in the cigarette mainstream smoke under ISO standard machine smoking conditions are shown in Table 7 and, for the formaldehyde yield, in Fig. 2.

The results on formaldehyde yield in Fig. 2 show, firstly, that increasing the level of invert sugar added to the tobacco produced a systematic and linear increase in smoke formaldehyde. Secondly, when diammonium phosphate was also added to the tobacco, the levels of smoke formaldehyde generated were decreased. This inhibiting effect on formaldehyde generation increased as the level of diammonium phosphate increased. Thirdly, the addition of 1.0% L-proline also inhibited the generation of formaldehyde, although 0.2% L-proline did not, presumably because that level of L-proline was too low to have any observable effect. These results are in strong agreement with the concept of an inhibiting effect of amino compounds on the generation of formaldehyde in a burning cigarette, as depicted in Scheme 2, shown and discussed in Section 3.2.

Some of the experimental cigarettes in series G also produced other interesting effects (Table 7). Virtually all the experimental cigarettes had an increased puff count relative to the control (no ingredient) cigarette. The cigarette (G5) with 1% diammonium phosphate and no added sugar had its mainstream ammonia yield increased by 25%, and its NFDPM and nicotine yields increased by about 5%; none of these changes were significant when the long-term analytical variability was taken into account. Armitage et al. (2004) have also reported similar increases in mainstream ammonia, nicotine and NFDPM yields when diammonium phosphate was added to the tobacco. None of the other experimental cigarettes with added diammonium phosphate or L-proline had mainstream ammonia yields that were significantly different to the control cigarette. Furthermore, some of the other experimental cigarettes with added diammonium phosphate or L-proline had mainstream yields of nicotine that were up to 10% lower than the control, and carbon monoxide yields that were up to 13% lower than the control. Again, none of these changes were significant when the long-term analytical variability was taken into account.

The addition of diammonium phosphate and no sugar increased the mainstream smoke acetaldehyde yield by up

Table 7

Experimental cigarettes with added invert sugar, diammonium phosphate and L-proline (2005): mainstream smoke yields, ISO smoking machine regime

Cigarette	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11
Invert sugar (%)	–	4.0	8.0	–	–	4.0	4.0	8.0	8.0	4.0	4.0
DAP (%)	–	–	–	0.5	1.0	0.5	1.0	0.5	1.0	–	–
L-Proline (%)	–	–	–	–	–	–	–	–	–	0.2	1.0
Puff count	7.2	7.6 ^{a,b}	7.4	7.9 ^{a,b}	7.7 ^{a,b}	7.7 ^{a,b}	7.7 ^{a,b}	7.7 ^{a,b}	8.0 ^{a,b}	7.7 ^{a,b}	7.9 ^{a,b}
sd (<i>n</i> = 5)	0.2	0.2	0.2	0.2	0.4	0.3	0.1	0.2	0.2	0.3	0.1
TPM (mg/cig)	18.1	19.4	18.3	18.3	18.7	17.6	17.8	17.5	17.8	18.4	17.8
sd (<i>n</i> = 5)	0.41	1.43	1.62	1.31	0.62	0.87	0.59	0.28	0.76	0.76	0.78
NFDPM (mg/cig)	14.0	15.0 ^a	14.2	14.3	14.7 ^a	13.9	14.2	14.0	14.3	14.6 ^a	13.9
sd (<i>n</i> = 5)	0.24	0.61	0.75	0.66	0.29	0.45	0.64	0.23	0.70	0.44	0.18
Nicotine (mg/cig)	1.24	1.28	1.19	1.29	1.31 ^a	1.22	1.16 ^a	1.11 ^{a,b}	1.17 ^a	1.23	1.22
sd (<i>n</i> = 5)	0.06	0.07	0.04	0.05	0.03	0.03	0.02	0.02	0.01	0.03	0.02
CO (mg/cig)	13.3	14.0	13.4	12.5	12.9	12.2 ^a	11.8 ^{a,b}	11.9 ^{a,b}	12.2 ^a	13.2	13.2
sd (<i>n</i> = 5)	0.30	1.07	0.86	0.56	0.57	0.35	0.08	0.46	0.92	0.59	0.34
Ammonia (µg/cig)	24.7	22.9	22.6	27.0	30.9 ^a	22.8	25.4	21.0	22.2	21.6	21.5
sd (<i>n</i> = 3)	1.53	1.46	1.85	1.61	0.84	1.18	1.31	0.90	0.45	0.51	2.04
<i>Carbonyls (µg/cig)</i>											
Formaldehyde	28.8	34.2 ^a	38.9 ^{a,b}	27.7	26.3	29.9	28.9	35.1 ^a	30.4	35.1 ^a	29.9
sd (<i>n</i> = 3)	1.21	0.79	3.64	1.34	1.79	3.01	1.86	2.01	2.82	3.60	1.60
Acetaldehyde	451	493	469	532 ^a	517 ^a	565 ^{a,b}	475	475	528 ^a	514 ^a	491
sd (<i>n</i> = 3)	31.4	33.2	33.5	18.9	4.63	36.6	34.0	1.11	17.6	19.0	4.78
Acetone	301	266 ^a	296	310	329	332	273	274 ^a	316	292	287 ^a
sd (<i>n</i> = 3)	4.50	20.5	21.2	8.61	25.4	22.4	21.9	3.11	14.4	12.0	0.41
Acrolein	55.8	60.2	61.3	62.9 ^a	64.4 ^a	66.6	63.3 ^a	65.5 ^a	67.1 ^a	64.7 ^a	62.8
sd (<i>n</i> = 3)	1.91	4.67	4.53	3.09	2.09	6.78	3.69	5.45	2.90	3.29	5.97
Propionaldehyde	47.9	47.9	50.2	52.0	51.1	52.8	46.1	49.0	50.6	49.4	50.8
sd (<i>n</i> = 3)	3.30	3.06	3.50	2.70	0.61	4.26	3.21	4.36	1.57	2.32	5.07
<i>n</i> -Butyraldehyde	40.6	35.7	42.5	46.0	45.7	47.0	39.4	38.8	44.5	42.4	46.4
sd (<i>n</i> = 3)	3.97	2.51	4.64	1.95	1.97	4.41	3.22	0.21	1.78	2.06	4.98
Crotonaldehyde	20.8	23.9	21.6	23.7	25.4 ^a	25.1	20.3	21.4	22.9	21.1	21.4
sd (<i>n</i> = 3)	2.06	1.29	1.10	1.03	1.54	2.77	1.57	2.06	1.11	1.02	2.38
Methyl ethyl ketone	71.0	68.9	73.1	73.4	77.3	75.7	65.9	69.6	73.1	69.4	68.0
sd (<i>n</i> = 3)	6.57	4.38	4.74	3.13	5.39	7.38	4.83	6.46	2.46	3.10	2.44

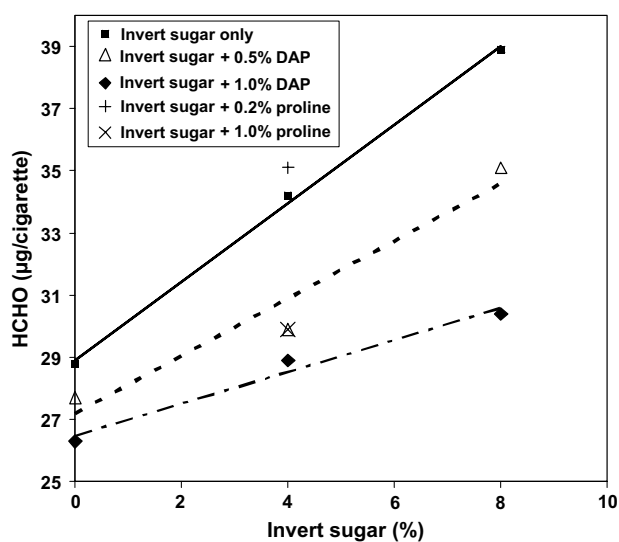
sd: standard deviation for *n* replicates.^a Significantly different to G1 (control) value at 95% confidence level—this data set.^b Also significantly different to G1 (control) value at 95% confidence level when long-term analytical variability is included.

Fig. 2. Yield of formaldehyde in cigarette mainstream smoke as a function of added invert sugar and diammonium hydrogen phosphate (DAP) and L-proline, ISO standard smoking machine conditions.

to 18%, the acrolein yield by up to 15%, and the crotonaldehyde yield by up to 22%. Again, none of these changes were significant when the long-term analytical variability was taken into account.

5.4. Effect of sugars on puff-by-puff yields

As reviewed in Section 3.2 above, the puff-by-puff profile of formaldehyde is somewhat unusual in that its yield in the first puff is abnormally high, being up to six times higher than the yield in the second puff, under standard ISO machine-smoking conditions (Parrish and Harward, 2000; Plunkett et al., 2001; Li et al., 2002a; Wagner et al., 2005). Experiments have been undertaken to determine how the addition of sugars affects the puff-by-puff profile for formaldehyde and other carbonyl smoke constituents under standard ISO machine-smoking conditions. The puff-by-puff analyses of mainstream carbonyl constituents in smoke were determined by Arista Laboratories in Richmond, Virginia, USA using methodology that they have recently published (Wagner et al., 2005). The analyses were

done on cigarette codes D1 (control), D8 (7.0% invert sugar) and D10 (6.2% brown sugar).

The puff-by-puff results for all eight of the carbonyl smoke constituents determined in this study are shown in Fig. 3. Clearly, the formaldehyde yield in the first puff is

substantially higher than that in the subsequent puffs for all three cigarettes, as reported in other studies (Parrish and Harward, 2000; Plunkett et al., 2001; Li et al., 2002a; Wagner et al., 2005). The formaldehyde yield in the first puff is three to four times higher than in the second puff,

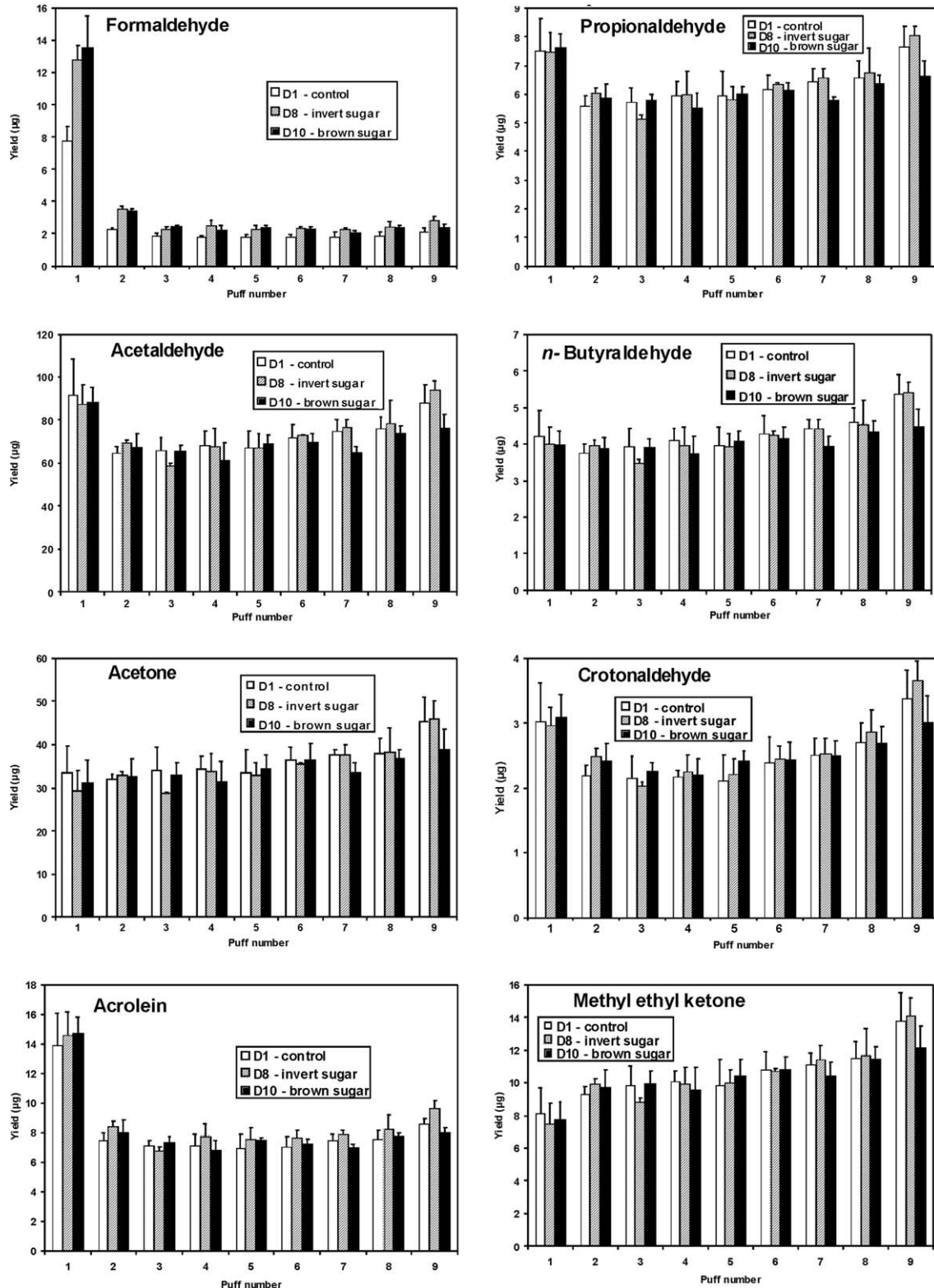


Fig. 3. Puff-by-puff yields of mainstream smoke carbonyls, ISO standard smoking machine conditions.

and the first puff formaldehyde yield contributes 32–41% to the total yield (Table 8). These ratios are similar to those reported in the other studies. Furthermore, with the cigarettes with added sugar (D8 and D10), the formaldehyde is increased more in the first puff relative to the control cigarette (D1) than in the total formaldehyde yield (Table 9).

The puff-by-puff profiles for acetaldehyde, acrolein, propionaldehyde and crotonaldehyde also show a slightly elevated yield in the first puff (Fig. 2, Table 8), varying from about 1.3 times the second puff yield for crotonaldehyde,

to about 1.8 times the second puff yield for acrolein. The first puff contributes 13–20% to the total yield for these carbonyl smoke constituents.

The puff-by-puff profiles for acetone, *n*-butyraldehyde and methyl ethyl ketone are very similar to the profiles seen for the majority of smoke constituents, namely a systematic increase in yield as the tobacco rod is consumed during smoking (Fig. 2, Table 8). The increasing yield is caused by a decrease in filtration by the tobacco rod for products in the particulate phase of smoke, or a decrease in air dilution and outward gaseous diffusion for products in the gaseous phase (Baker and Crellin, 1977; Baker and Robinson, 1990). Recent studies using single ionisation/time-of-flight mass spectrometry have also shown that the puff-by-puff profiles for acetone and acetaldehyde in the mainstream smoke of various cigarettes increase systematically with each puff (Adam et al., in press; Mitschke et al., 2005).

Even allowing for the small variations in puff-by-puff profiles for the other carbonyl smoke constituents, the puff-by-puff profiles for formaldehyde are noticeably unusual, with their abnormally high first puff yield, as discussed in Section 3.2. This first puff effect persists, and is even enhanced, in the presence of added sugars. With all of the other carbonyl smoke constituents, the presence of sugar has no significant effect on yield in a given puff.

5.5. Effect of smoking machine parameters on mainstream formaldehyde yields

All of the results presented so far in this paper have been obtained using the standard ISO smoking machine regime, which takes a 35 ml puff of 2 s duration every 60 s, abbreviated to 35/2/60 (ISO Standard 3402, 1999). Other, more intense machine smoking regimes are also used by regulatory authorities in Canada and the USA. The effects on mainstream carbonyl yields of increasing the puff volume to 70 ml (a regime of 70/2/60), and increasing the puff volume to 55 ml taken twice per minute (a regime of 55/2/30), have been determined. The results are given in Table 10. Clearly, the formaldehyde yields from the cigarettes containing sugars (D8 and D10) were also significantly higher than that from the control cigarette (D1) at the two intense smoking regimes. The relative increases were, however, lower for cigarette D10 (23–31%), which contained brown sugar at the two intense regimes, than for cigarette D8 (52–56%), which contained invert sugar. Other factors must also be occurring that affect the generation of formaldehyde from the sugars. These include the effects of puff volume on the temperatures generated in the cigarette burning zone, and the residence time of the generated products in the burning zone.

The use of the two intense smoking regimes did not produce any major effects in the other carbonyl smoke constituents that had not been observed using the ISO smoking regime. A few small, spurious changes were observed, for example the 13% decrease in acetone yield for cigarette

Table 8

Yields of carbonyl smoke constituents in first puff relative to second puff and total yield, ISO standard smoking machine conditions

Carbonyl; cigarette	Carbonyl yield ratio	
	Puff 1/puff 2	Puff 1/total yield (%)
<i>Formaldehyde</i>		
D1—control	3.2	32
D2—invert sugar	3.6	38
D3—brown sugar	4.0	41
<i>Acetaldehyde</i>		
D1—control	1.4	14
D2—invert sugar	1.3	13
D3—brown sugar	1.3	14
<i>Acetone</i>		
D1—control	1.1	10
D2—invert sugar	0.90	9.3
D3—brown sugar	0.95	10
<i>Acrolein</i>		
D1—control	1.9	19
D2—invert sugar	1.7	19
D3—brown sugar	1.8	20
<i>Propionaldehyde</i>		
D1—control	1.4	13
D2—invert sugar	1.2	13
D3—brown sugar	1.3	14
<i>n-Butyraldehyde</i>		
D1—control	1.1	11
D2—invert sugar	1.0	11
D3—brown sugar	1.0	11
<i>Crotonaldehyde</i>		
D1—control	1.4	13
D2—invert sugar	1.2	13
D3—brown sugar	1.3	13
<i>Methyl ethyl ketone</i>		
D1—control	0.88	8.6
D2—invert sugar	0.76	8.0
D3—brown sugar	0.80	8.4

Table 9

Comparison of formaldehyde ratios in first puff and total yield

Cigarette	Formaldehyde yield ratios		
	Puff 1/(puff 1 _{D1})	Total/(total _{D1}) ^a	Total/(total _{D1}) ^b
D1—control	1.0	1.0	1.0
D8—invert sugar	1.7	1.5	1.6
D10—brown sugar	1.8	1.4	1.5

^a Total yields from summation of the puff-by-puff yields (data in Fig. 3).

^b Total yields determined directly (data in Table 3).

Table 10
Effect of smoking machine regime on yields

Smoking regime	ISO (35/2/60)			70/2/60			55/2/30		
	D1	D8	D10	D1	D8	D10	D1	D8	D10
Cigarette									
Sugar	–	Invert sugar	Brown sugar	–	Invert sugar	Brown sugar	–	Invert sugar	Brown sugar
Level (%)	–	7.0	6.2	–	7.0	6.2	–	7.0	6.2
Puff count	7.5	7.3	7.2 ^a	6.4	6.3	6.7	10.1	10.9	10.6
sd (<i>n</i> = 5)	0.2	0.3	0.2	0.3	0.3	0.08	0.5	0.6	0.7
TPM (mg/cig)	17.1	15.7	16.2	33.4	31.3 ^a	33.7	43.9	43.0	41.6
sd (<i>n</i> = 5)	1.14	1.47	0.72	1.09	1.05	1.89	1.86	2.72	1.26
NFDPM (mg/cig)	13.3	12.4	12.6	21.2	19.6 ^{a,b}	21.4	28.9	28.4	27.4
sd (<i>n</i> = 5)	0.67	0.84	0.48	0.75	0.49	0.29	0.42	1.27	1.12
Nicotine (mg/cig)	1.17	1.07 ^a	1.13	1.70	1.54 ^a	1.62	2.40	2.23	2.25
sd (<i>n</i> = 5)	0.04	0.07	0.04	0.10	0.044	0.066	0.094	0.16	0.067
CO (mg/cig)	12.4	11.1 ^{a,b}	11.3 ^{a,b}	18.1	17.6	18.7	22.4	21.8	21.6 ^a
sd (<i>n</i> = 5)	0.48	0.64	0.45	0.73	0.29	0.30	0.25	0.64	0.67
<i>Carbonyls (µg/cig)</i>									
Formaldehyde	30	49 ^{a,b}	46 ^{a,b}	52	81 ^a	64 ^a	54	82 ^a	71 ^a
sd (<i>n</i> = 3)	3.2	2.1	5.4	6.5	4.4	2.7	3.0	4.4	8.5
Acetaldehyde	535	574	562	799	708	748	726	746	763
sd (<i>n</i> = 3)	19.5	18.0	27.3	55.5	16.7	23.8	29.3	43.0	23.2
Acetone	315	334	326	455	394 ^a	436	425	434	458 ^a
sd (<i>n</i> = 3)	12.9	6.6	16.2	32.8	12.5	14.1	13.3	26.7	9.0
Acrolein	67	78 ^a	74	100	98	98	92	104	92
sd (<i>n</i> = 3)	0.8	4.1	4.3	6.2	3.6	4.6	3.9	6.7	11
Propionaldehyde	47	48	50	81	73	77	63	66	62
sd (<i>n</i> = 3)	1.6	1.9	4.6	5.9	2.2	2.8	2.7	3.8	4.0
<i>n</i> -Butyraldehyde	38	42 ^a	40	65	59	63	57	60	55
sd (<i>n</i> = 3)	2.1	1.2	1.8	4.1	2.4	1.8	3.2	4.0	2.1
Crotonaldehyde	25	22 ^a	24	41	38	40	33	36	34
sd (<i>n</i> = 3)	1.2	1.3	1.5	2.7	1.8	1.0	1.7	2.4	1.3
Methyl ethyl ketone	79	80	78	114	98 ^a	110	111	117	117
sd (<i>n</i> = 3)	5.1	1.8	3.9	8.4	3.9	3.7	4.7	8.4	2.1

sd: standard deviation for *n* replicates.

^a For that smoking machine regime, significantly different to D1 (control) value at 95% confidence level—this data set.

^b For that smoking machine regime, also significantly different to D1 (control) value at 95% confidence level when long-term analytical variability is included.

D8 relative to the control (D1) at the 70/2/60 smoking regime, and the 8% increase in acetone yield for cigarette D10 at the 55/2/30 smoking regime. These changes were small compared to the systematic changes that occurred with the formaldehyde yields.

5.6. Effect of sugars on sidestream yields

The effect of added sugars on the sidestream yields are given in Table 11, when the cigarette was smoked in a fish-tail chimney, taking a puff of 35 ml volume and 2 s duration once per minute (Proctor et al., 1988). Rather surprisingly, at the 95% confidence level the presence of added sugars has no significant effect on the sidestream yields. This is dramatically different to their effect on the mainstream formaldehyde yields. There are two possible explanations for this lack of a sidestream effect.

(1) Sufficient temperatures are not produced in the cigarette burning zone during the inter puff smoulder period to pyrolyse the sugars to formaldehyde. The solid phase components inside the smouldering cigarette and immediately behind the paper burn line of the

cigarette, where the sidestream products are generated, are below 500 °C (Baker, 1999). Referring to Scheme 1, as discussed in Section 3.2 above, below 500 °C the precursors of formaldehyde (sugars) are likely to decompose into products other than formaldehyde inside the cigarette burning zone, based on the work of Li et al. (2002a). Thus, past studies would support the proposition that sufficient temperatures are not generated during smoulder to generate formaldehyde directly from sugars. With this mechanism, the sidestream formaldehyde that is observed in the test and control cigarettes must be generated from other components of the tobacco.

(2) Any excess formaldehyde generated during the smoulder period would react with other smoke constituents in the sidestream plume. In fact, it has been shown that ammonia and formaldehyde react almost instantaneously in the sidestream plume of a smouldering cigarette to produce hexamethylenetetramine, which resides almost exclusively in the particulate phase of sidestream smoke (Borgerding et al., 1984; González and Sarabia, 1992). This mechanism, therefore, is a likely possibility.

Table 11
Sidestream yields of carbonyl smoke constituents, at a machine smoking regime of 35/2/60

Cigarette	D1	D4	D8	D10	E1	E8
Sugar	–	White sugar	Invert sugar	Brown sugar	–	Fructose + glucose
Level (%)	–	7.5	7.0	6.2	–	4.1 + 4.1
Puff count	8.3	7.7	8.0	8.7	8.0	8.0
sd (<i>n</i> = 3)	0.6	0.6	0.0	0.6	0.0	0.0
<i>Carbonyls (µg/cig)</i>						
Formaldehyde	375	412	375	408	401	426
sd (<i>n</i> = 3)	32	17	44	49	42	37
Acetaldehyde	1530	1510	1450	1510	1610	1600
sd (<i>n</i> = 3)	43	59	44	101	82	8
Acetone	881	888	860	893	917	936
sd (<i>n</i> = 3)	33	35	18	64	56	2
Acrolein	371	408	356	377	402	395
sd (<i>n</i> = 3)	20	16	16	24	26	7
Propionaldehyde	154	150	148	151	160	160
sd (<i>n</i> = 3)	3	8	7	10	8	0
<i>n</i> -Butyraldehyde	115	107	109	112	115	112
sd (<i>n</i> = 3)	5	6	8	8	6	1
Crotonaldehyde	75.5	84.3 ^a	79.7	83.5	76.2	86.7 ^b
sd (<i>n</i> = 3)	3.4	3.1	1.9	6.4	0.8	2.2
Methyl ethyl ketone	224	217	218	224	225	233
sd (<i>n</i> = 3)	9	14	10	16	13	3

sd: standard deviation for *n* replicates.

^a Significantly different to D1 (control) value at 95% confidence level—this data set.

^b Significantly different to E1 (control) value at 95% confidence level—this data set.

In general, the presence of added sugars also has no significant effect on the sidestream yields of the other carbonyl smoke constituents (Table 11). A possible exception to this is the 12–14% increased yield of sidestream crotonaldehyde with added white sugar and the fructose/glucose mixture (Table 11).

6. Overall summary and conclusions

- Using three series of cigarettes made on different occasions, all sugars added to tobacco, and cellulose, increase the yield of formaldehyde in mainstream cigarette smoke under ISO standard smoking machine conditions. Increases up to 60% are observed at maximum sugar levels used on cigarettes. The increases are mostly statistically significant. The magnitude of the increases are variable, probably because of various levels of sugars being present in the syrups added to the tobacco, the presence of minor amino impurities in some of the sugars, and the general variability of the analytical methodology. The results with formaldehyde are consistent with all previously published studies on the subject.
- The increases in mainstream formaldehyde yields, in the presence of added sugars, are also observed under two more intense smoking regimes.
- Different sugars increase formaldehyde to different extents, which may be due at least partially to the presence of varying levels of amino compounds in the sugars.
- Using a fourth series of cigarettes, it has been shown that the presence of ammonium compounds and amino acids inhibit the generation of formaldehyde from sugars.
- In general, the first puff is abnormally high in formaldehyde, three to four times higher than in the second puff and the first puff contributes 30–40% to the total mainstream formaldehyde yield. This effect persists in the presence of added sugars.
- In contrast to the situation with mainstream smoke, the sidestream yields of formaldehyde are not affected by the addition of sugars to the tobacco.
- These results on the smoke analysis of cigarettes containing added saccharides are consistent with a large body of pyrolysis and other mechanistic studies that have been conducted over the last 30 years.
- The addition of the various saccharides also produced some statistically significant effects in the mainstream yields of six of the seven other Hoffmann carbonyl smoke constituents analysed at the same time as formaldehyde. These effects were generally small, less than 16%, were not consistent amongst the various cigarette series and lost their significance when the long-term analytical variability was taken into account. The effects, when they did occur, were always increases for acrolein, propionaldehyde and *n*-butyraldehyde, and were both increases and decreases for acetaldehyde, acetone, and crotonaldehyde. In order to further investigate the significance, if any, of these changes it would be necessary to increase the number of replicate analyses and improve the long-term variability of the analytical methodology.

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