The Reducing Property of Tobacco Smoke

1. Modified Flue-Cured Tobacco and Other Smoking Products*

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INTRODUCTION

On account of our interest in the chemical reactivity of tobacco smoke as an indicator of its biological activity, we have examined [I] the redox property of smoke (1, 2), [II] photosensitized oxidation of aqueous extracts of smoke (3), [III] inhibition by smoke of radical-initiated polymerisation of vinyl acetate (4, 5), [IV] inhibition by smoke of the terminal respiratory enzyme, cytochrome oxidase (6), and [V] the antioxidant activity of smoke utilising the coupled oxidation of β -carotene and linoleic acid (7). The redox property of tobacco smoke has been the subject of research in several laboratories (8, 9, 10, 11), and our interest in this property stemmed from an observation made during enzyme inhibition studies in which it was found that cigarette smoke itself could bring about reduction of cytochrome c (1). We considered such a property of possible importance in the biochemical effects of tobacco smoke and hence worthy of further investigation.

Reported in this paper is an extension of the work on reducing activity (1) with several objectives. Firstly, to try to identify the major groups of compounds in tobacco as well as in non-tobacco material which, on combustion, give rise to reducing activity. The problem has been approached by modification of tobacco by fermentation, solvent extraction or addition to tobacco of materials which seemed likely to change reducing activity. Secondly, we wished to explore the effect of physical changes in cigarette design on the oxidation state of condensates. In this area, changes in cigarette diameter, form of tobacco and porosity of paper were studied, plus the effect of burn retardants as well as other additives. Finally, in parallel to the exploratory lines, a range of different cigarette types and smoking products were examined for reducing activity. The results of these tests are reported.

MATERIALS AND METHODS

Smoking Procedures

Methods used in smoking and collection of condensate have already been described (1). When different types of tobaccos, natural as well as extracted, were compared,

they were smoked in granulated form in cigarette tubes (12).

Reduction of Dichlorophenolindophenol (DCIP)

The procedure followed as well as the arbitrary "unit" employed have been described in an earlier publication (1). Most of the values reported are the averages of three separate determinations each made using 3 different dilutions. Only specific activities are recorded. Total activities may be obtained by multiplying specific activity by the dry TPM* weight in mg.

Fermentation Procedures

A strain of *Saccharomyces cerevisiae* (baker's yeast) and an unidentified yeast isolated from a tobacco product were used to "ferment" the flue-cured tobacco. Yeast cells were harvested from a commercial malt extract medium (Difco), or a tobacco extract medium (10 percent tobacco extract containing 0.1% Difco yeast extract) prepared in the Laboratory.

Extraction Procedures

The flue-cured tobacco or wood shavings were ground in a Wiley mill and steeped in the solvent in question at 4°C or ambient temperature, and the solvent was removed by straining through muslin cloth or by filtration on a Buchner funnel and drying. The solvent systems used are detailed in the text. For elucidating the role of polymeric materials such as lignin and cellulose, in generating reducing compounds on burning, the extraction procedures used were adapted from the method recommended for extracting lignin from wood (13). This involved 4 to 5 successive extractions each with 10 to 20 volumes of benzene-ethanol (1:2), water and 5% NaOH.

Application of Additives

When flue-cured tobacco was impregnated with known compounds to find out if they were precursors, the compound under study was dissolved in a suitable quantity of water and added uniformly to the tobacco. An appropriate control was also run by adding the same amount of water to another lot of tobacco. The tobaccos were then air-dried.

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* Total particulate matter.

RESULTS AND DISCUSSION

Precursors of Reducing Compounds in Smoke

Since the DCIP-reducing activity of smoke from flue-cured tobacco had been found to be higher than that from air-cured types (cigar and Burley), it was felt that the higher level of sugars in the flue-cured tobacco could explain the larger amount of reducing substances in its smoke. To test this hypothesis, most of the sugars in the sample of flue-cured tobacco were removed by yeast fermentation using baker's yeast harvested from a malt extract medium or using an unidentified yeast harvested from a tobacco extract medium. Both the untreated and yeast-treated flue-cured tobaccos were dried, granulated and smoked in specially made cigarette tubes. The results obtained using baker's yeast are presented in Table 1. From the results it will be seen that although there were distinct drops in specific reducing activities, they did not exactly parallel the removal of sugar. Also, total activities did not change, since TPM values from the yeast-treated tobacco are distinctly higher. A similar reduction of specific reducing activity was recorded using the unidentified yeast harvested from the tobacco extract medium. Apparently, yeast treatment results in an as yet unknown modification of tobacco that is reflected in the specific reducing activity of smoke.

That the sugars played no role in the generation of reducing activity was confirmed when application of [I] 20% sucrose and [II] 20% glucose to flue-cured tobacco did not result in any increase in the reducing activity of the smoke. A similar application of [I] a protein preparation (obtained from flue-cured tobacco by water extraction and $(\text{NH}_4)_2\text{SO}_4$ precipitation, followed by dialysis), [II] 10% albumin, [III] 10% starch, and [IV] 2% pectin, too, did not result in any increase in reducing activity of the smoke.

The negative results reported above induced us to look for alternative ways of approaching the problem of the origin of reducing activity in smoke. Solvent extraction appeared to be a good alternative. Consequently, flue-cured tobacco extracted in [I] water and [II] 70% ethanol in water was dried, powdered and subsequently smoked in specially made cigarette tubes. The reducing activities of the total particulate matter obtained from the smoking of these extracted tobaccos have been compared with unextracted flue-cured tobacco condensate under identical smoking conditions. From the results presented in Table 2 it will be seen that the specific reducing activity of the TPM obtained from the water-extracted tobacco is much lower than the specific activities of both the ethanol-extracted and control flue-cured tobaccos. An analysis of variance followed by the *Tukey's* test (14) has shown that the lower value obtained in the case of the water extracted tobacco is indeed significant. It will also be seen that, as in the case of yeast-fermented tobacco, the total activities of the extracted and unextracted tobaccos are similar.

It is possible that the reduction in specific activity by yeast fermentation and water extraction is a result of a significant effect on the physical nature of the tobacco and should be of value in obtaining smoke of lower reducing activity, since other ways of removing total activity, such as filtration, are available. In any case, it appears that both the above treatments bring about significant modification of tobacco and that specific reducing activity is a useful way of monitoring tobacco modification.

Other extraction studies involved [I] sequential extraction with toluene, acetone and methanol, and [II] chloroform-methanol extraction. The former treatment gave increases in specific activities, indicating, once again, significant modification of the tobacco, while the latter had no effect.

The persistence of reducing activity in smoke in spite of all the above treatments, suggested that polymeric materials such as cellulose, hemicellulose, and lignin may be the source of the reducing activity in tobacco smoke. Thus, dried beech leaves and cut rolled tobacco stems were smoked, and these gave only slightly higher specific reducing activities than did flue-cured tobacco, while dried green tobacco leaves gave values close to those obtained for flue-cured tobacco (see Table 8). However, three types of wood (pine, red cedar and

Table 1. DCIP*-reducing activity of yeast-treated flue-cured tobacco.

Tobacco	Weight of dry TPM from 5 cigarettes (mg)	Sugar content (%)		DCIP-reducing activity Specific activity — "units" per mg TPM
		Reducing	Total	
Yeast-treated (24 hours)	196.7	4.9	5.0	5.34
Yeast-treated (96 hours)	179.1	3.4	3.9	4.64
Flue-cured control	101.4	23.2	23.8	7.17

* Dichlorophenolindophenol.

Table 2. DCIP*-reducing activities of water and 70% ethanol-extracted tobaccos.

Cigarettes from	Weight of dry TPM from 5 cigarettes (mg)	No. of puffs per cigarette	DCIP-reducing activity Specific activity — "units" per mg TPM
Water-extracted flue-cured tobacco	129.5	20	5.81
70% ethanol-extracted flue-cured tobacco	103.7	15	7.29
Flue-cured control	88.4	12	7.64

* Dichlorophenolindophenol.

birch), smoked in a similar way, gave the very high specific reducing activities of 10, 11 and 16, respectively. When birch wood was extracted successively using benzene-ethanol, water and 5% NaOH to remove all constituents except cellulose and lignin, its smoke still retained its high specific reducing activity. Further extraction of this birch wood with dioxane did seem to lower the reducing activity, but the results were vitiated by the fact that cigarettes made with dioxane-extracted birch had to be lighted for every puff. Tobacco when extracted in the same manner as birch wood also gave similar results in that there was no significant lowering of reducing activity up to the dioxane extraction. On dioxane extraction the same problem of keeping the cigarettes lighted was faced. However, a lowering of activity was recorded. When finely powdered wood cellulose was smoked, only a trace of tar and no reducing activity was obtained. Thus, in spite of all the above studies, only indirect evidence has been obtained that lignin and possibly the phenolics derived therefrom contribute mainly to the reducing activity of smoke.

The Effect of Some Cigarette Parameters on Smoke Reducing Properties

1. **Strand Width:** The physical form of the tobacco in the combustion zone could be expected to exert some influence on smoke composition. An easily controlled variable in cigarette manufacture is strand width. Consequently, we investigated how this parameter influenced reducing activity. Condensates from cigarettes made using tobaccos cut at 20, 45, 54 and 105 cuts per inch were examined for reducing activity, and from the results presented in Table 3, it will be seen that no significant differences between the strand widths were noted. Similarly, in earlier studies no significant differences were found in going from cut tobacco to ground tobacco (unpublished observations).
2. **"Expanded" Tobacco:** A low-density tobacco was produced by a process involving "expansion" of the tobacco. We have examined the effect of this physical alteration of the tobacco on the oxidation state of the condensates. Cigarettes containing 50% and 100% expanded tobacco were smoked, along with control flue-cured cigarettes, and from the results presented in Table 4, it will be seen that there was no difference in the specific reducing activities of the condensates.

Table 3. Effect of tobacco strand width on DCIP*-reducing activity of cigarette smoke condensate.

Strand width of tobacco (cuts per inch)	Weight of dry TPM from 5 cigarettes (mg)	DCIP-reducing activity
		Specific activity — "units" per mg TPM
105	84.5	7.86
54	109.0	7.05
45	100.5	7.04
20	107.0	6.32

* Dichlorophenolindophenol.

3. **Paper Porosity and Burn Control Additives:** The intention of this experiment was to measure the effects of paper porosity as well as the addition of $MgCl_2$ and KNO_3 , which are known to, respectively, retard or accelerate combustion. The experimental design was arranged to examine the effects of both these simultaneously. From the results of comparisons made with the DCIP reduction test and summarised in Table 5, it will be seen that neither paper porosity nor salt application influenced the reducing activities of the condensates. The small differences seen were not statistically significant.

4. **The Role of Polyol Additives on Smoke Reducing Properties:** In attempting to explain the low reducing activity of pipe smoke we found that the smoking vehicle plays an important role (15). Both flue- and air-cured tobaccos when smoked in a pipe gave very low reducing activities. However, we also observed that pipe tobaccos when smoked in cigarette tubes gave reducing values which were markedly lower than that obtained for flue-cured tobacco. This suggested to us that processing of pipe tobacco was implicated in the low reducing activities recorded.

It is well known that many pipe tobaccos contain significant amounts of non-tobacco additives such as

Table 4. DCIP*-reducing activity of cigarettes made from expanded tobacco.

Type of tobacco in cigarette	No. of puffs per cigarette	Weight of tobacco smoked in 5 cigarettes (g)	Weight of dry TPM from 5 cigarettes (mg)	DCIP-reducing activity
				Specific activity — "units" per mg TPM
50% expanded	6	1.91	93.75	7.72
100% expanded	4	1.17	65.57	7.25
Flue-cured control	9	2.96	135.35	7.09

Table 5. Effect of paper porosity and salt application on DCIP* reduction by cigarette smoke condensate.

Salt application	Specific DCIP-reducing activity — "units" per mg TPM		
	Paper porosity — Greiner seconds		
	61	45	15
5% $MgCl_2$	6.53	7.34	7.54
Slow burning			
1% $MgCl_2$	6.60	6.80	6.25
Medium burning			
5% KNO_3	6.73	7.16	7.11
Fast burning			
Flue-cured control	7.77	7.39	6.89

* Dichlorophenolindophenol.

Table 6. Effect of polyol additives on DCIP*-reducing activity of cigarette smoke.

Additive	Weight of tobacco smoked in 5 cigarettes (g)	Weight of dry TPM from 5 cigarettes (mg)	DCIP-reducing activity
			Specific activity – "units" per mg TPM
Experiment 1 – flue-cured tobacco			
Water control	5.34	165	7.3
10 % Ethylene glycol**	4.67	114	6.9
10 % Propylene glycol	5.08	124	6.1
10 % Polypropylene glycol	5.47	217	4.4
10 % Carbowax	5.34	202	5.0
10 % Glycerol	5.19	163	4.3
Experiment 2 – flue-cured blend			
Water control	3.05	113	7.3
3.4 % Glycerol	3.05	121	5.4
1.7 % Glycerol	3.03	115	5.9
1.7 % Sorbitol			

* Dichlorophenolindophenol.

** Ethylene glycol is not used in commercial tobacco products.

licorice, glycerol and other polyols (16), and it appeared to us that these additives could influence the production of reducing activity in smoke. To study the effect of some of these, aqueous solutions of glycerol, ethylene glycol*, propylene glycol, polypropylene glycol and carbowax were applied to samples of flue-cured tobacco to give levels of 10%. A sample of tobacco treated with water was used as a control. The tobaccos were dried, ground, and smoked under standard conditions. From the results presented in Table 6 it will be seen that addition of all these polyols resulted in a lowering of both total and specific reducing activities.

Since the amounts of polyols applied to tobacco in the above experiment were perhaps unrealistically high, a second experiment was conducted in which cigarettes containing a flue-cured tobacco blend, cut at 45 cuts per inch, to which glycerol and/or sorbitol were added, were smoked. As will be seen from Table 6 the cigarettes containing 3.4 % glycerol gave a lower reducing activity, both total and specific, than cigarettes containing 1.7 %

glycerol and 1.7 % sorbitol, and both were distinctly lower than flue-cured control cigarettes.

Redox Properties of Condensates from a Range of Tobacco Products and Tobacco Types

We have already reported that granulated air-cured tobaccos when smoked in the form of cigarettes gave lower reducing activities than granulated flue-cured tobacco when smoked under identical conditions (1). As part of our continuing effort at identifying tobacco smokes of low reducing activity, we decided to examine other types of tobacco as well as products by the DCIP test.

1. *Indian "bidis"*: Since it was difficult to keep the bidis alight on a standard cigarette smoking machine set up for taking a 2-second puff every minute, the bidis were smoked on a pipe-smoking machine devised in this laboratory. The smoking involved taking a 35 ml puff of 2 seconds duration every 10 seconds. Five bidis and regular flue-cured cigarettes were smoked to butt lengths of approximately 25 mm and

* Ethylene glycol is not used in commercial tobacco products.

Table 7. DCIP*-reducing activity of Indian "bidis".

Brand No.	No. of puffs per bidi/cigarette	Weight of bidi/cigarette (g)	Weight of tobacco in bidi/cigarette (g)	Weight of dry TPM from 5 bidis/cigarettes (mg)	DCIP-reducing activity	
					TPM Specific activity — "units" per mg TPM	Vapour phase "Units" per 5 bidis/cigarettes
1	18	0.3297	0.1669	157	7.07	36
2	19	0.3298	0.1590	153	7.02	31
3	13	0.3969	0.1723	169	7.24	39
Canadian flue-cured control	18	1.0381	0.9852	190	7.22	46

* Dichlorophenolindophenol.

30 mm respectively, and the total particulate matter was collected on weighed Cambridge pads. The vapour phase (VP) was collected in spiral condensers cooled to -70°C .

From the results presented in Table 7, it will be seen that all bidi brands and standard cigarettes have similar specific reducing activities. The high TPM value recorded for regular flue-cured cigarettes in this experiment is due to the different puffing regime. However, in the case of the bidis, which weigh only 30–40% the weight of standard cigarettes and contain an amount of tobacco equal to half their weight, the high TPM values obtained reflect not only the large number of puffs but also a significant effect of the wrapper leaf. It should be mentioned here that a large amount of moisture (about 40% of TPM weight) accumulates on the Cambridge pad in bidi smoking. Vapour phase fractions from both bidis and cigarettes are low in reducing activity and contain only about a thirtieth of the activity obtained in the TPM.

2. *Indian Cigarettes*: Cigarettes in India vary considerably in price, the most expensive being those made exclusively from flue-cured Virginia tobacco. Cheaper brands are made from Natu, a country, sun-cured tobacco grown in Andhra Pradesh. Blended brands presumably contain different amounts of flue-cured tobacco blended with air-cured White Burley or the sun-cured Natu.

We have examined several brands of Indian cigarettes by the DCIP-test and found that a regular flue-cured brand (No. 3), gave reducing activity equal to that obtained for Canadian flue-cured brands (see Table 8). No. 5, a brand made exclusively from Natu tobacco, gave the lowest values. The others (Nos. 1, 2 and 4), gave intermediate values indicating that these cigarettes are made from tobacco blends.

3. *French Cigarettes*: French cigarettes may be considered a particular kind, in as much as they are made from dark fermented tobacco. Consequently, we considered these interesting from the point of view of determining reducing activities of their smoke condensates, which, as will be seen from Table 8, are much lower than those obtained for air- and flue-cured tobacco cigarettes.

4. *Other Smoking Materials*:

a) *Lettuce and green leaf cigarettes*: Lettuce cigarettes are made from leaves that have been fermented by a patented process (17). An examination of condensates from these cigarettes gave results presented in Table 8. The table also records reducing values for rapidly dried, freshly harvested tobacco (green) as well as beech leaves which have been dried, both of which after granulation were smoked in cigarette tubes. While the green tobacco and beech leaf cigarettes gave specific reducing values close to those obtained for regular flue-cured cigarettes, the lettuce cigarettes gave distinctly lower values.

Table 8. DCIP*-reducing activity of miscellaneous smoking materials.

Smoke from tobacco/cigarette brand No.	Weight of dry TPM from 5 cigarettes (mg)	No. of puffs per cigarette	DCIP-reducing activity Specific activity — "units" per mg TPM
Indian cigarettes			
1. Filter, regular	117	9	5.33
2. Filter, king-size	148	13	4.80
3. Filter, king-size	132	10	7.72
4. Non-filter, regular	118	9	4.88
5. Non-filter, regular	127	10	3.89
French cigarettes			
6. Filter, king-size	78	10	2.08
7. Non-filter, regular	78	7	3.11
8. Filter, regular	54	7	2.84
9. Non-filter, regular	83	6	2.95
10. Filter, regular	55	7	2.91
11. Non-filter, regular	104	7	2.81
Other smoking materials			
Lettuce	56	6	4.26
Lettuce-menthol	51	6	5.06
Green tobacco	52	9	7.35
Beech leaves	35	17	8.02
Turkish	130	16	3.92
Cuban-Cigar Type 1	57	6	3.47
Cuban-Cigar Type 2	94	9	2.52
Latakia	208	17	2.37
Blend 1	129	12	2.96
Perique-granulated	203	32	0.73
Canadian flue-cured control	113	9	7.17

* Dichlorophenolindophenol.

b) *Miscellaneous tobaccos*: The tobaccos examined in this study included Turkish, Cuban and Latakia tobaccos, and a blend of Burley and dark air-cured tobaccos (referred to as Blend 1 in Table 8). While Latakia, a fire-cured Oriental tobacco, acquires its flavour and aroma from the curing that is employed, the Turkish and Cuban tobaccos develop their characteristic aroma through a fermentation step. Also examined was Perique, which is unique in that after air-curing it is made to undergo a fermentation which gives it a rich fragrant aroma (16). A yeast, *Hansenula anomala*, dominates the late stage of fermentation and is believed to be important in producing the characteristic fragrance of Perique tobacco (18). All of these different tobaccos gave reducing activities which were distinctly lower than that obtained for flue-cured tobacco (see Table 8). Indeed, the reducing activity of Perique is the lowest we have ever recorded for any tobacco smoked in cigarette form. This tobacco has also given lower values in another test designed to measure smoke reactivity (4).

In conclusion, it may be stated that by employing a chemical property of smoke we have been able to study not only number of cigarette parameters, but also differentiate tobacco products from different parts of the world. A study of pipe smoking has also been undertaken to find out why this mode of smoking gives smoke of extremely low reducing activity, and this is subject of the accompanying paper (15). Finally, it must be emphasized here that mention of low values for certain smokes in tests such as DCIP reduction, which are merely designed to obtain a measure of groups of reactive compounds, does not in any way suggest that these also relate to low biological activity as measured by skin painting tests.

SUMMARY

In order to determine which tobacco constituents give rise to reducing compounds in smoke as well as to find out what factors affected the reducing property of the latter, modifications of flue-cured tobacco by solvent extraction and chemical impregnations were carried out. While no unequivocal evidence has been obtained as to the main source of reducing activity, all the evidence suggests that lignin and phenolic compounds derived therefrom, are the main contributors to reducing activity. A number of cigarette parameters, strand width, expansion of tobacco, paper porosity and burn control additives were studied and found to have no significant effect, within the limits tested, on the reducing power of the smoke. On the other hand, additives such as glycerol, ethylene glycol, polyethylene glycol, polypropylene glycol and carbowax gave distinctly lower reducing activities.

Examination of tobacco products showed lower reducing activities in smoke from Cuban, Latakia and Turkish tobaccos, as well as in smoke from cigarettes made from Indian sun-cured and French dark, fermented tobaccos. The lowest reducing activity was recorded for the highly fermented Perique tobacco. Indian "bidis" have specific reducing activities similar to those of standard flue-cured cigarettes. Freshly harvested, rapidly dried green tobacco as well as dried beech leaves, gave specific reducing activities close to those obtained for regular cigarettes. On the other hand, commercially available lettuce cigarettes, which undergo a fermentation step in their manufacture, gave distinctly lower specific activity.

ZUSAMMENFASSUNG

Zur Bestimmung der Tabakinhaltsstoffe, die im Rauch die reduzierenden Verbindungen bilden, und zur Ermittlung der Faktoren, die auf diese Eigenschaft einwirken, wurde „flue-cured“-Tabak mittels Lösungsmittelextraktion und chemischer Imprägnierung modifiziert. Während der hauptsächliche Ursprung der reduzierenden Eigenschaft nicht eindeutig aufgeklärt werden

konnte, deuten alle Befunde darauf hin, daß Lignin und dessen phenolische Abkömmlinge den Hauptbeitrag zur Bildung der reduzierenden Aktivität liefern. Die Untersuchung einer Reihe von Cigarettenparametern, wie Schnittbreite, Tabakausdehnung, Papierporosität und Zusätze zur Regulierung der Verbrennung, ergab innerhalb der Grenzen der Versuche keinen signifikanten Einfluß auf die reduzierende Wirksamkeit des Rauches. Andererseits führten Zusätze wie Glycerin, Äthylenglykol, Polyäthylenglykol, Polypropylenglykol und Carbowax zu deutlich niedrigerer reduzierender Aktivität.

Die Untersuchung von Tabakprodukten ergab eine geringere reduzierende Aktivität sowohl im Rauch von Kuba-, Latakia- und türkischen Tabaken als auch im Rauch von Cigaretten aus indischen sonnengetrockneten und französischen dunklen fermentierten Tabaken. Die niedrigste reduzierende Aktivität fand sich in dem stark fermentierten Perique-Tabak. Indische „bidis“ haben eine spezifische reduzierende Aktivität, die der von „flue-cured“-Standardcigaretten ähnlich ist. Sowohl frisch geernteter und schnell getrockneter grüner Tabak als auch getrocknete Buchenblätter hatten eine spezifische reduzierende Aktivität, welche der normaler Cigaretten vergleichbar war. Andererseits hatten handelsübliche Cigaretten aus Salatblättern, die bei der Verarbeitung fermentiert worden waren, eine deutlich niedrigere spezifische Aktivität.

RESUME

On a modifié des tabacs «flue-cured» par extraction au solvant et par imprégnation chimique, dans le double but de déterminer les constituants du tabac engendrant des composés réducteurs dans la fumée, ainsi que de trouver les facteurs affectant les propriétés réductrices de la fumée. Quoique l'on n'ait pas pu isoler avec certitude la cause principale de l'activité réductrice, toutes les données suggèrent la lignine et ses dérivés phénoliques comme apportant une contribution majeure à l'activité réductrice. Un certain nombre de paramètres de la cigarette, tels que la largeur de coupe, l'expansion du tabac, la porosité du papier, et l'action d'additifs contrôlant la combustion, ont été étudiés et n'ont pas eu d'influence significative, dans les limites de l'expérience. Par contre des additifs tels que le glycérol, l'éthylène-glycol, le polyéthylène-glycol, le polypropylène-glycol et le carbowax ont provoqué une activité réductrice nettement moindre.

En examinant différents tabacs, on a trouvé une activité réductrice plus faible dans la fumée des tabacs de Cuba, de Turquie et du tabac Latakia, ainsi que dans la fumée de cigarettes confectionnées avec des tabacs indiens séchés au soleil et des tabacs noirs français fermentés. La plus faible activité réductrice a été obtenue à partir du tabac Périque très fermenté. Les «bidis» indiens ont une activité réductrice spécifique similaire à celle des cigarettes «flue-cured» normales. Le tabac fraîchement récolté et séché rapidement ainsi que les feuilles de hêtre séchées ont donné une activité réductrice

spécifique proche de celle des cigarettes ordinaires. Par contre les cigarettes de laitue, qui passent par un stade de fermentation durant leur fabrication, ont produit une activité spécifique nettement moindre.

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