



Variation of Sugar Levels in Tobaccos Upon Heating

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Background

- During tobacco processing, preparation of expanded tobacco, as well as when tobacco is used in “heat not burn” type cigarettes, the tobacco is exposed to different degrees of heat.
- Sugars, and in particular glucose, fructose and sucrose are present at high level in flue-cured and Oriental tobaccos (up to 15-16% reducing sugars and about 7% sucrose in some flue-cured tobaccos).
- Several tobacco components, including sugars start decomposing at temperatures above 150 °C.
- Sugars decomposition has significant impact on the sensory properties of tobaccos.
- Some undesirable compounds such as acetaldehyde are generated during sugar thermal decomposition.
- The present study evaluated changes in the overall chemical composition of six common tobaccos when the tobacco was subject to heating up to 250 °C, with particular focus on the variation of the levels of fructose, glucose and sucrose.
- The tobacco types evaluated in the study were flue-cured, burley, and Oriental, as typically used in cigarette production.

Heating of the tobaccos

- The tobacco leaf was ground in a coffee grinder to a fine powder with particles of about 0.2 mm diameter.
- The heating of the tobacco samples was performed in sealed glass tubes.
- Two types of Pyrex glass tube were used for containing the tobacco, one type had 8 mm i.d. and a length of 15 cm and the other had 6 mm i.d. and a length of 30 cm. Both tubes had 1 mm wall thickness.
- About 600 mg tobacco were weighed in each tube which was sealed at one end and was subject to mild vacuum before being sealed at the second end. The purpose of applying vacuum was to prevent the tubes from breaking when exposed to heat due to the expansion of the gases from the tube.
- The heating was performed in a Thermoline Furnace 62700 (Thermo Fisher, Waltham, MA, USA).
- Four different temperatures were used: 100 °C, 150 °C, 200 °C, and 250 °C. Two times intervals were selected for heating: 2 min and 5 min.
- Only the time and the temperature of heating of the glass tube containing the tobacco were known, and not the actual temperature of the tobacco. Delays in the temperature reached by the tobacco occurred during heating but also during cooling of the tubes.
- The sugar levels in the initial tobacco and in the tobacco removed from the glass tubes after heating were measured.

Tubes containing burley tobacco before heating



Screening of tobacco composition

- The tobacco composition was evaluated using a screening analytical technique that consisted of direct silylation of tobacco followed by GC/MS analysis [1].
- This technique allows the identification of up to 58 compounds, including acids such as lactic, maleic, succinic, butendioic, malic, trihydroxybutanoic, gluconic, citric, caffeic, linoleic, stearic, chlorogenic, etc., sugars and sugar alcohols such as mannose, altrose, glucose, fructose, sucrose, maltotriose, xylitol, ribitol, etc., and also compounds such as inositol, propylene glycol, glycerin, phosphate, nicotine, and deoxyfructosazines.
- Polymeric tobacco components such as cellulose, hemicellulose, pectin, proteins, starch, as well as most of amino acids, and inorganic components (except for the phosphate) are not detected by this screening procedure.
- The results for individual compounds were expressed as area counts of the chromatographic peak. This type of presentation of results does not provide the quantitative level of different compounds in the tobacco sample, but still allows a quantitative comparison indicating which compound is at a higher or a lower level in the compared samples.

[Alford, E.D. and J.H. Lauterbach: 41st Tobacco Chemist's Research Conference, Program, Vol. 41, No. 56, 1987, p. 41.]

Details on the GC/MS screening technique

- 50 mg of each sample of tobacco was weighed (with 0.1 mg precision) in 2 mL GC vials and directly silylated without using a preliminary extraction.
- For silylation, 400 μ L of DMF that contained an internal standard was added first to each vial containing the sample, followed by 800 μ L BSTFA with 1% TMCS.
- The vials were kept at 78 $^{\circ}$ C (in a heating block) for 30 min, and subsequently allowed to cool at room temperature for another 30 min.
- After cooling the solution from each vial was filtered through 0.45 μ m PVDF filters and analyzed by GC/MS.
- The GC/MS separation was performed on a DB-5 MS column, 30 m x 0.25 mm i.d. with 0.25 μ m film with He as a carrier gas.
- The temperature gradient of the GC oven was varied between 50 $^{\circ}$ C to 300 $^{\circ}$ C in an interval of 85.5 min.
- The mass spectrometer was acquiring in the mass range 33 to 550 amu.

Tobacco samples evaluated in the study with the temperatures and the exposure time

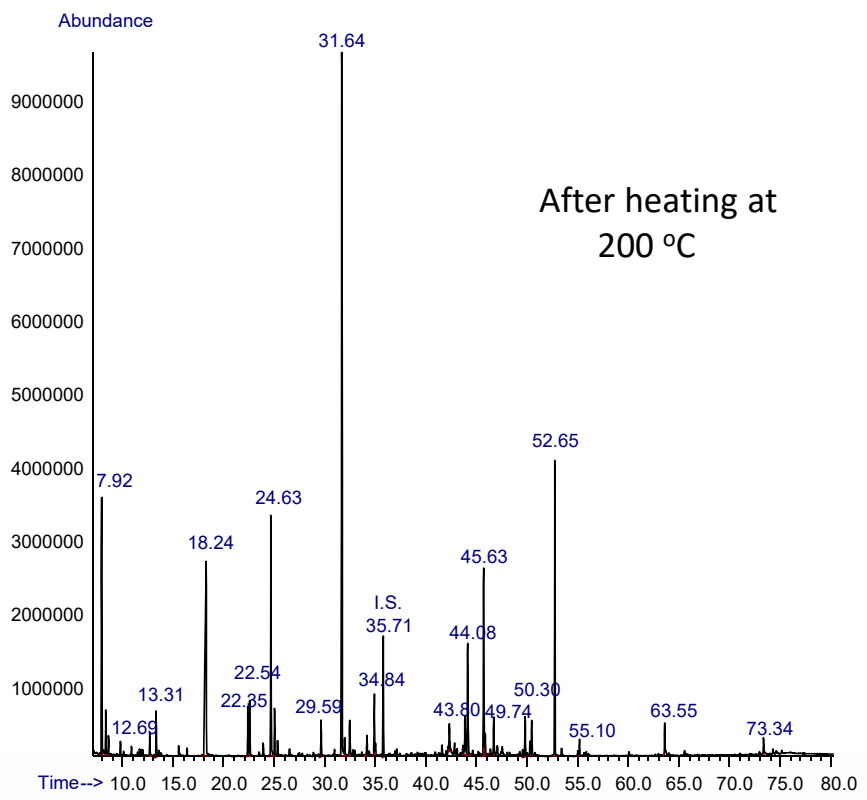
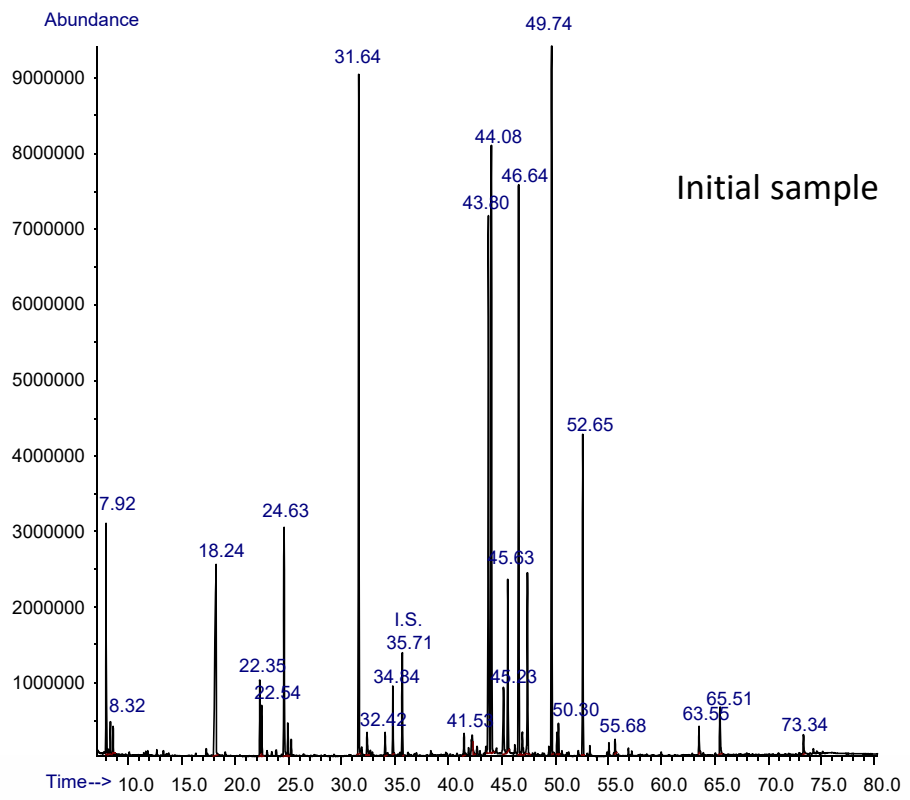
Name	Tobacco description	Temperatures °C	Time of exposure
FC-Lo	Lower stalk flue-cured	100, 150, 200, 250	2 min, 5 min
FC-Up	Upper stalk flue-cured	“	“
Bu-Lo	Lower stalk burley	“	“
Bu-Up	Upper stalk burley	“	“
Or (1)	Oriental	“	“
Or (2)	Oriental	“	“

The initial levels in % (dry weight basis) of reducing and total sugars and total alkaloids in the starting tobaccos

Name	Reducing sugars %	Total sugars %	Alkaloids %
FC-Lo	10.3	11.0	2.07
FC-Up	8.86	9.29	2.85
Bu-Lo	BQL*	BQL	2.51
Bu-Up	BQL	BQL	3.46
Or (1)	6.54	6.99	1.13
Or (2)	10.3	11.3	1.02

* BQL indicates below quantitation limit.

Changes in the chromatographic profile of a flue-cured tobacco sample when heated at 200 °C for 5 min



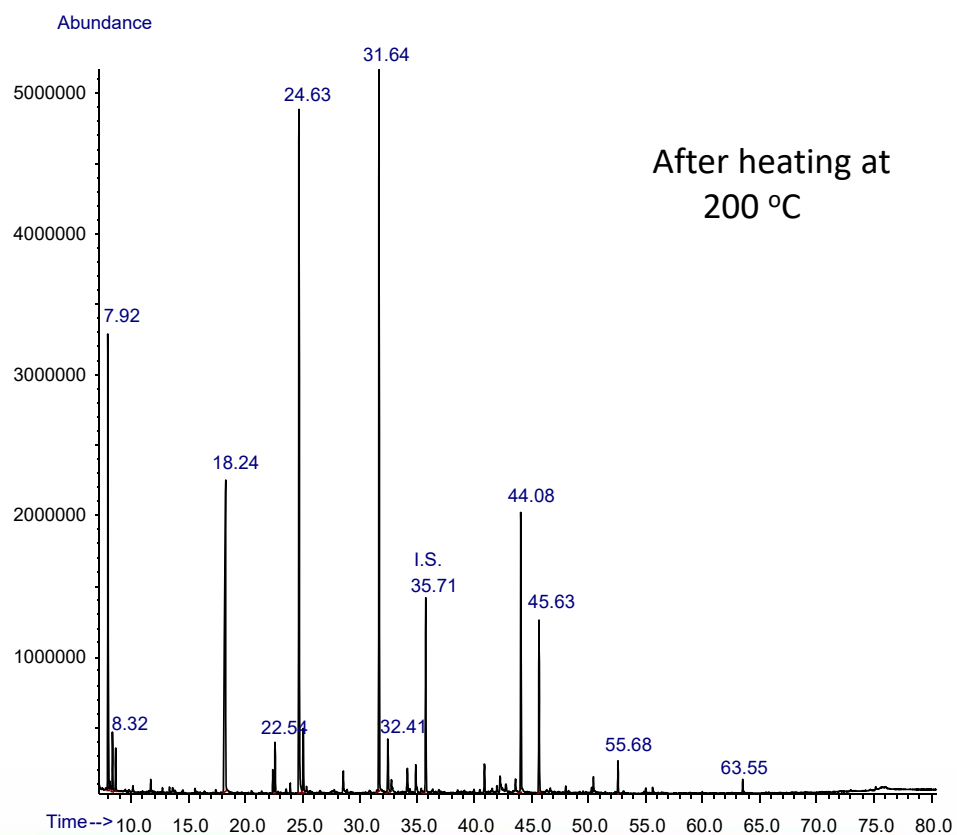
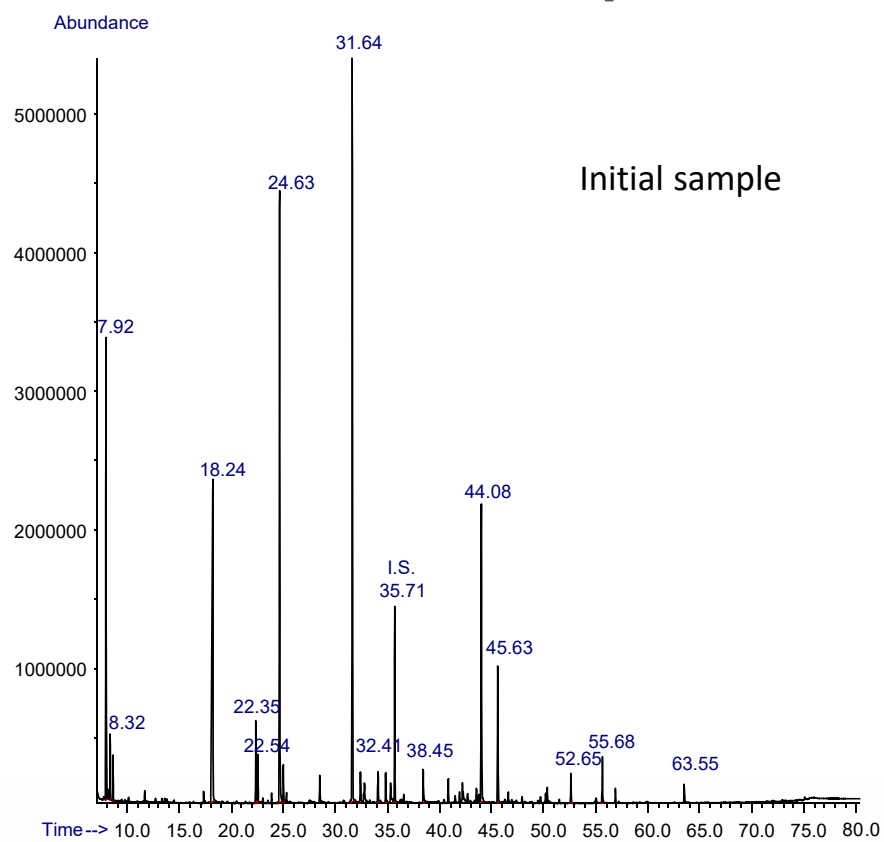
Changes in the chemical composition of a flue-cured tobacco sample when heated at 200 °C for 5 min

	Compound	Ret. time min	Ratio 200/initial	Type of change
1	Propylene glycol	10.12	1.01	No change
2	Lactic acid	12.69	3.89	Up
3	Glycolic acid	13.31	8.90	Up
4	Pyridinol	15.25	-	New
5	Propanoic acid	16.34	0.98	No change
6	Phosphate	22.36	0.99	No change
7	Glycerin	22.54	1.10	No change
8	(E)-2-Butenedioic	23.49	0.94	No change
9	Nicotine	24.63	0.99	No change
10	Glyceric acid	24.99	1.48	Up
11	(Z)-2-Butenedioic	25.30	0.97	No change
12	2-Hexenedioic acid	29.56	-	New
13	Malic acid	31.65	1.04	No change
14	Pyroglutamic acid	32.42	1.59	Up
15	Trihydroxybutanoic acid (1)	34.12	0.90	Small change
16	Trihydroxybutanoic acid (2)	34.85	0.90	Small change
17	Levogluconan	41.52	0.45	Down
18	Arabinose	42.30	0.69	Down
19	Xylose	43.34	0.97	No change
20	Fructose (1)	43.79	0.06	Down
21	Fructose (2)	44.08	0.07	Down
22	Citric acid	44.04	1.40	Up
23	Neophytadiene	43.57	1.06	No change

Changes in the chemical composition of a flue-cured tobacco sample when heated at 200 °C for 5 min (cont.)

	Compound	Ret. time min	Ratio 200/initial	Type of change
24	Quinic acid	45.63	1.17	Small change
25	Sorbose	46.36	0.60	Down
26	Glucose (1)	46.63	0.05	Down
27	Sugar acid	47.47	0.05	Down
28	Glucosamine	46.63	0.62	Down
29	Mannitol	49.04	0.58	Down
30	Glucose (2)	49.73	0.05	Down
31	Gluconic acid	50.23	0.68	Down
32	Hexadecanoic acid	50.38	1.08	No change
33	Myoinositol	52.65	0.94	No change
34	Caffeic acid	53.33	0.98	No change
35	Linoleic acid	54.94	1.07	No change
36	Linolenic acid	55.11	1.04	No change
37	Galactopyranose	57.24	0.06	Down
38	Glucuronic acid	63.55	1.15	Small change
39	Disaccharide	65.51	0.10	Down
40	Sucrose	65.52	0.10	Down
41	2,5-Deoxyfructosaz	65.62	1.02	No change
42	2,6-Deoxyfructosa	65.80	0.95	No change
43	Chlorogenic acid (1)	73.35	0.97	No change
44	Tocoferol	72.95	1.02	No change
45	Chlorogenic acid (2)	74.59	1.00	No change
46	Stigmasterol	75.15	0.99	No change

Changes in the chromatographic profile of a burley tobacco sample when heated at 200 °C for 5 min



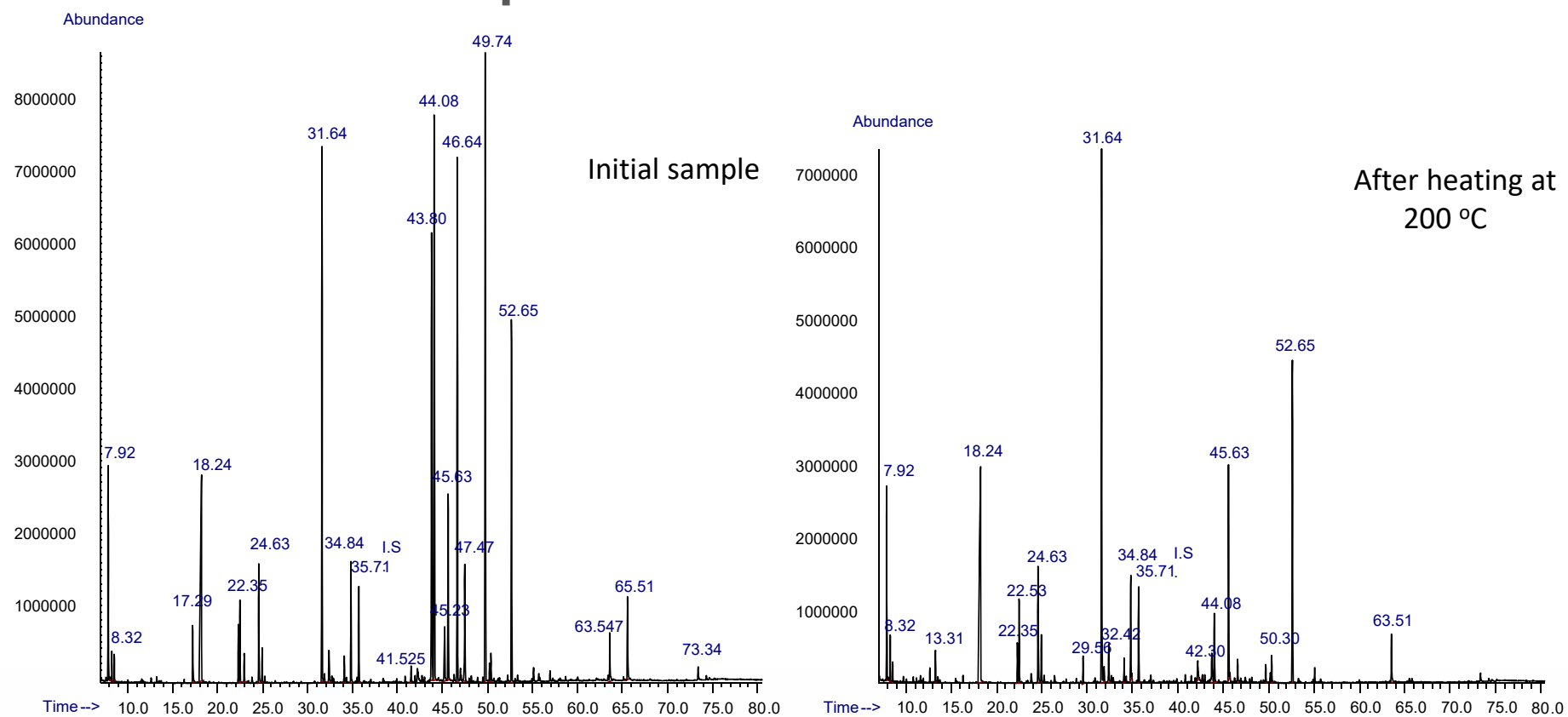
Changes in the chemical composition of a burley tobacco sample when heated at 200 °C for 5 min

	Compound	Ret. time min	Ratio 200/initial	Type of change
1	Propylene glycol	10.12	0.99	No change
2	Lactic acid	12.69	1.49	Up
3	Glycolic acid	13.31	1.18	Small change
4	Alanine	13.59	1.04	No change
5	Phosphate	22.35	0.99	No change
6	Glycerin	22.54	1.01	No change
7	(E)-2-Butenedioic	23.49	1.08	No change
8	Nicotine	24.63	1.04	No change
9	Glyceric acid	0.00	1.61	Up
10	(Z)-2-Butenedioic	25.30	0.61	Down
11	Malic acid	31.63	0.93	No change
12	Pyroglutamic acid	32.41	1.74	Up
13	Trihydroxybutanoic acid (1)	34.12	0.72	Down
14	Trihydroxybutanoic acid (2)	34.85	0.92	No change
15	Asparagine	38.45	7.26	Up
16	Levogluconan	41.52	0.59	Down
17	Arabinose	42.30	0.54	Down
18	Fructose (1)	43.79	0.07	Down

Changes in the chemical composition of a burley tobacco sample when heated at 200 °C for 5 min (cont.)

	Compound	Ret. time min	Ratio 200/initial	Type of change
19	Citric acid	44.04	0.92	No change
20	Neophytadiene	43.57	0.97	No change
21	Quinic acid	45.63	1.24	Up
22	Glucose (1)	46.63	0.18	Down
23	Sugar acid	47.47	0.01	Down
24	Glucosamine	46.61	0.04	Down
25	Galacturonic acid	48.88	0.05	Down
26	Glucose (2)	49.73	0.15	Down
27	Gluconic acid	50.23	0.58	Down
28	Hexadecanoic acid	50.38	0.99	No change
29	Myoinositol	52.65	1.08	No change
30	Caffeic acid	0.00	0.98	No change
31	Linoleic acid	54.93	1.02	No change
32	Sugar acid	55.68	9.54	Up
33	Galactopyranose	57.24	0.04	Down
34	Glucuronic acid	63.55	0.71	Down
35	Chlorogenic acid (1)	73.34	1.08	No change
36	Tocopherol	72.94	1.05	No change
37	Stigmasterol	75.15	1.00	No change

Changes in the chromatographic profile of a Oriental tobacco sample when heated at 200 °C for 5 min



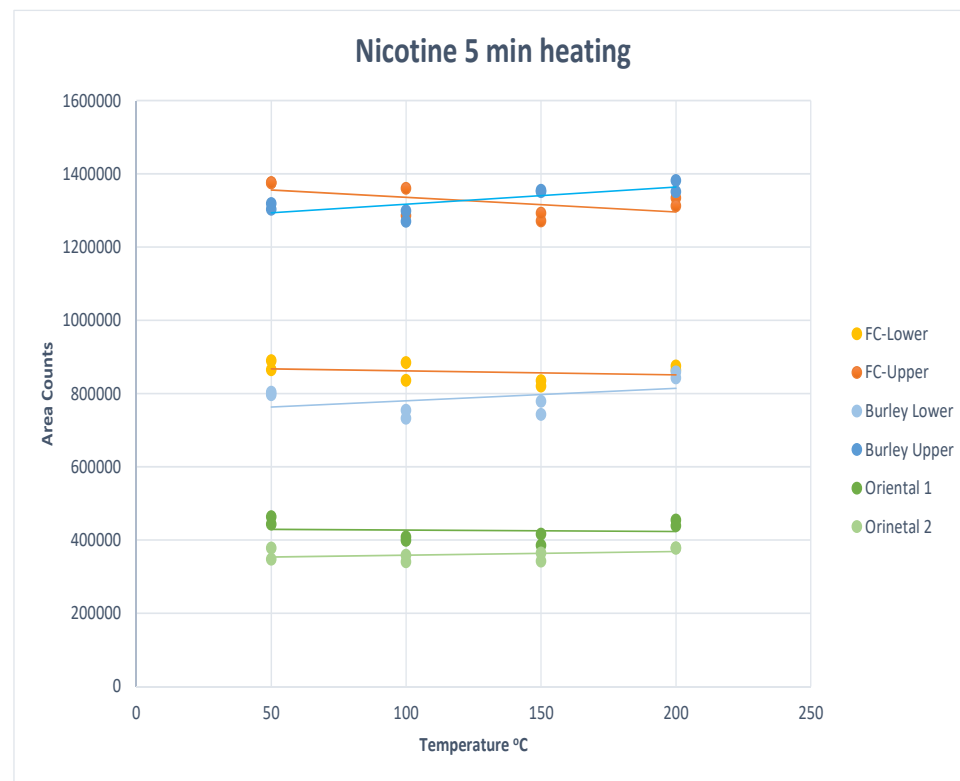
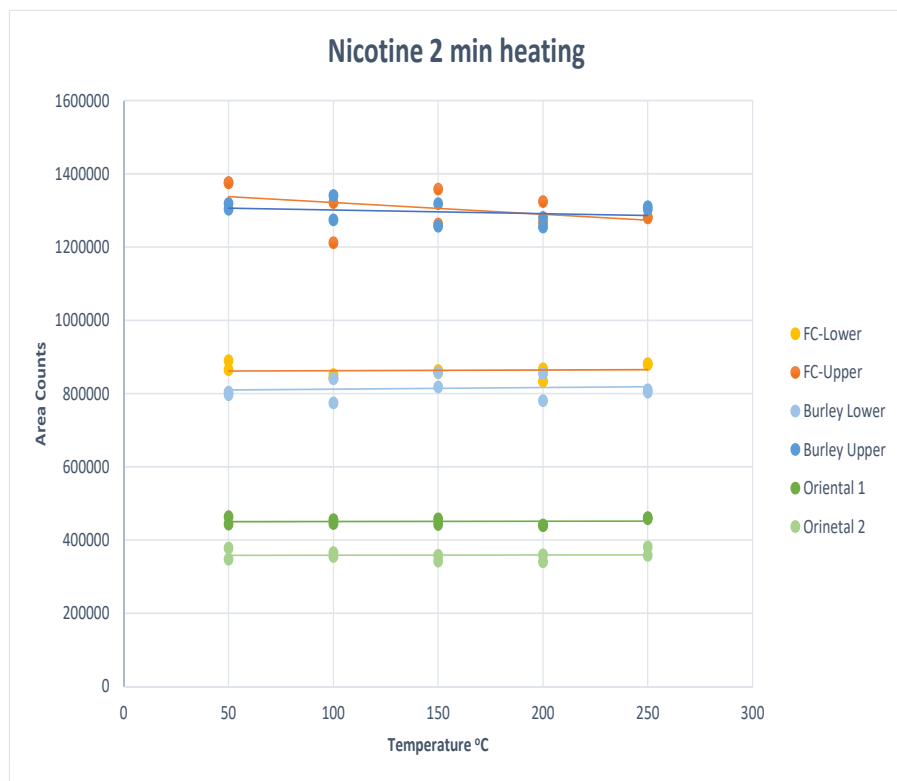
Changes in the chemical composition of a Oriental tobacco sample when heated at 200 °C for 5 min

	Compound	Ret. time min	Ratio 200/initial	Type of change
1	Propylene glycol	10.11	1.04	No change
2	Lactic acid	12.69	2.92	Up
3	Glycolic acid	13.31	5.10	Up
4	Alanine	13.58	3.14	Up
5	Proline	17.29	0.06	Down
6	Phosphate	22.35	1.01	No change
7	Glycerin	22.54	1.01	No change
8	(E)-2-Butenedioic	23.49	1.23	Up
9	Nicotine	24.63	0.99	No change
10	Glyceric acid	24.99	1.37	Up
11	(Z)-2-Butenedioic	25.30	1.16	Up
12	2-Hexenedioic acid	29.56	-	New
13	Malic acid	31.63	0.99	No change
14	Pyroglutamic acid	32.41	1.19	Up
15	Trihydroxybutanoic acid (1)	34.12	0.92	No change
16	Trihydroxybutanoic acid (2)	34.85	0.88	Small change
17	Levogluconan	41.53	0.43	Down
18	Arabinose	42.30	0.89	Down
19	Xylose	43.36	1.07	No change
20	Fructose (1)	43.80	0.05	Down
21	Fructose (2)	44.10	0.06	Down
22	Citric acid	44.04	0.83	Down
23	Neophytadiene	43.57	1.00	No change
24	Galactofuranose	45.23	0.00	Down
25	Quinic acid	45.63	1.15	Small change

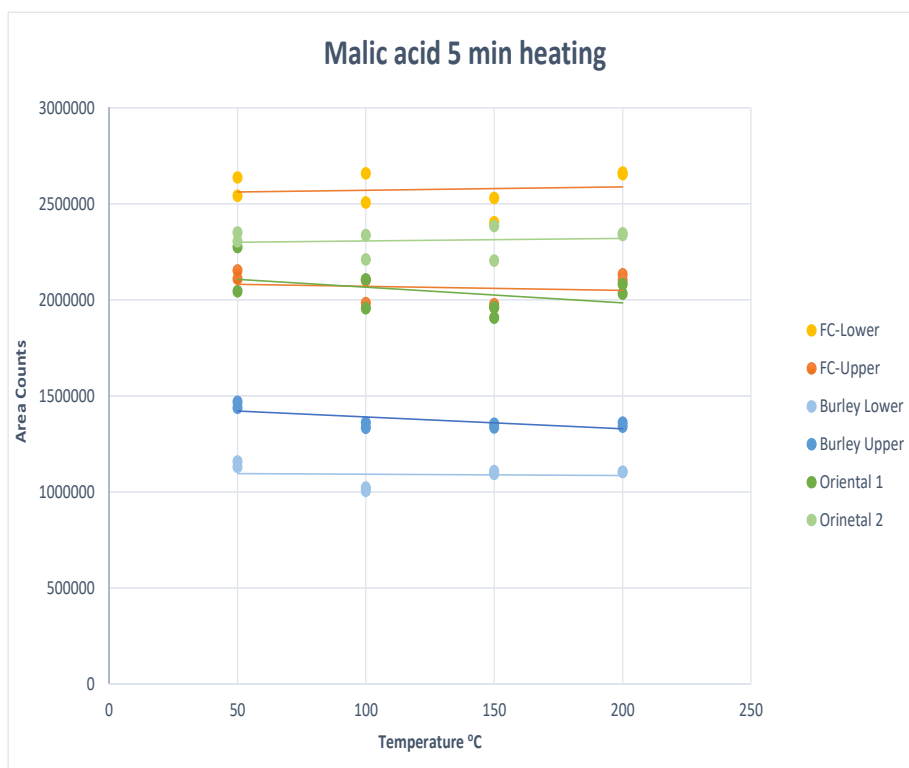
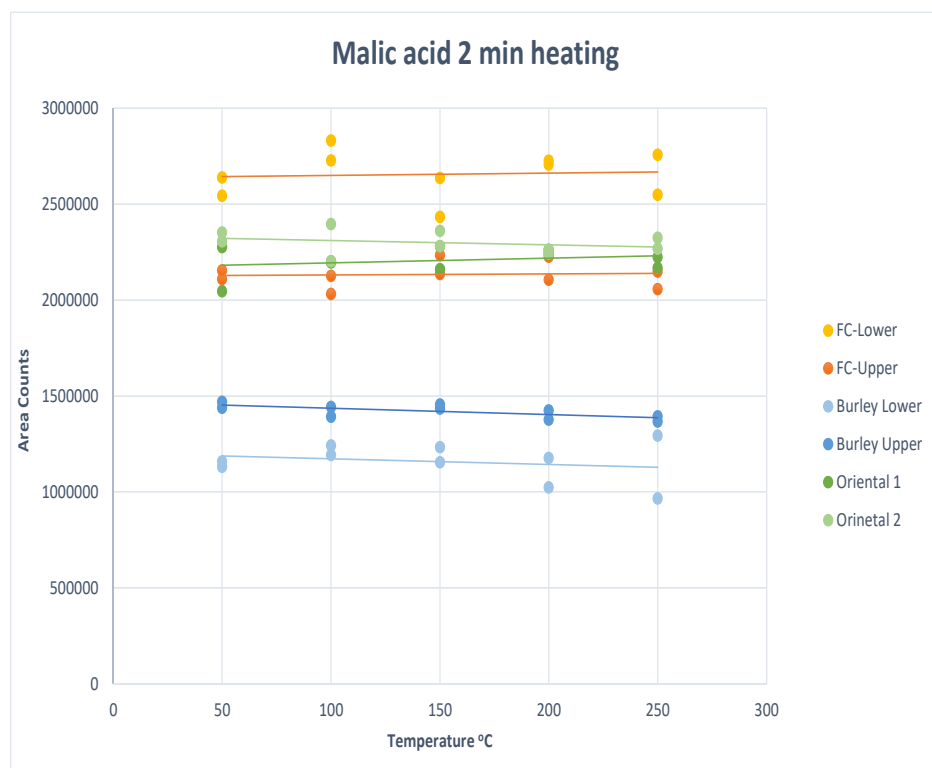
Changes in the chemical composition of a Oriental tobacco sample when heated at 200 °C for 5 min (cont.)

	Compound	Ret. time min	Ratio 200/initial	Type of change
26	Sorbose	46.36	0.76	Down
27	Glucose (1)	46.64	0.03	Down
28	Sugar acid	47.47	0.04	Down
29	Glucosamine	46.60	0.20	Down
30	Mannitol	49.04	0.65	Down
31	Sorbitol	47.47	0.00	Down
32	Galacturonic acid	48.89	0.58	Down
33	Glucose (2)	49.74	0.02	Down
34	Gluconic acid	50.23	0.56	Down
35	Hexadecanoic acid	50.38	0.96	No change
36	Myoinositol	52.65	0.96	No change
37	Caffeic acid	53.32	0.95	No change
38	Linoleic acid	54.93	1.04	No change
39	Linolenic acid	55.11	1.08	No change
40	Galactopyranose	57.24	0.00	Down
41	Glucuronic acid	63.55	0.98	No change
42	Disaccharide	65.51	0.04	Down
43	Phytosterol (1)	65.08	0.05	Down
44	Sucrose	65.51	0.04	Down
45	2,5-Deoxyfructosaz	65.51	0.93	No change
46	2,6-Deoxyfructosa	65.80	1.06	No change
47	Maltose	66.90	0.00	Down
48	Chlorogenic acid (1)	73.34	0.58	Down
49	Tocoferol	72.94	1.06	No change
50	Chlorogenic acid (2)	74.59	0.80	Down

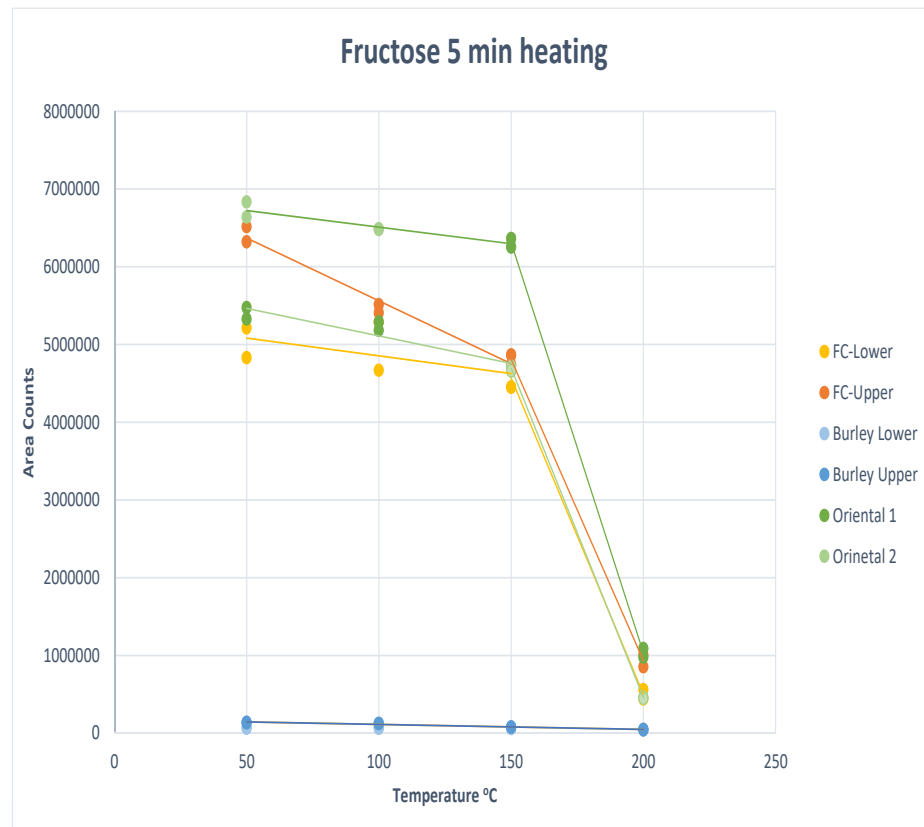
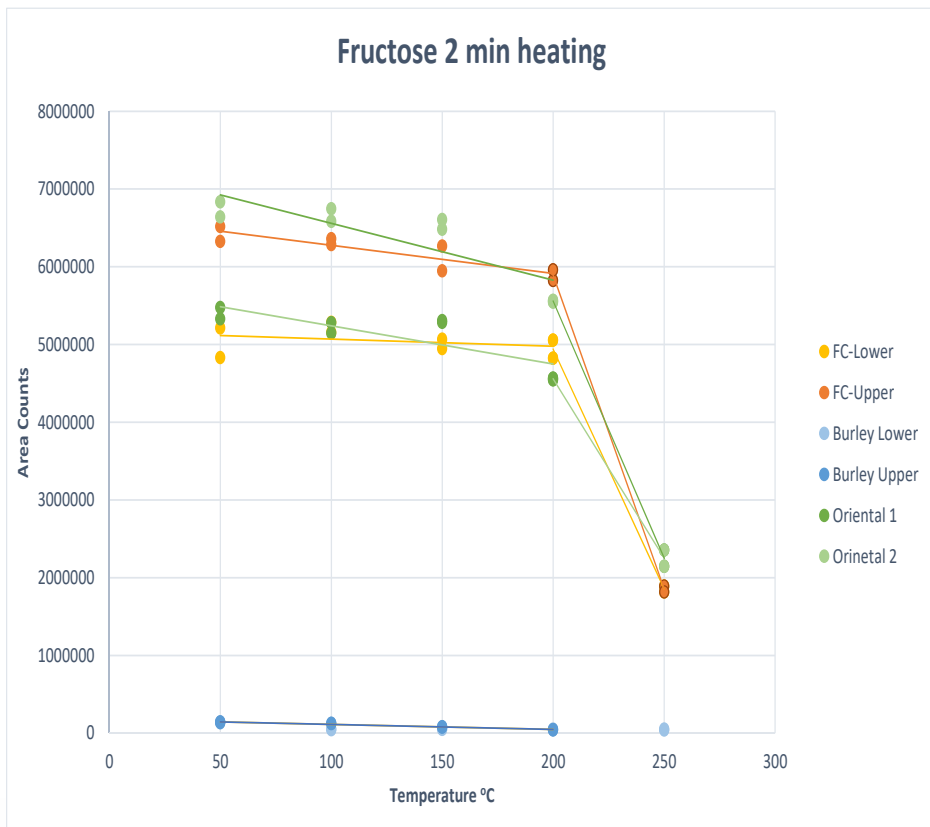
Changes in nicotine level in heated tobaccos



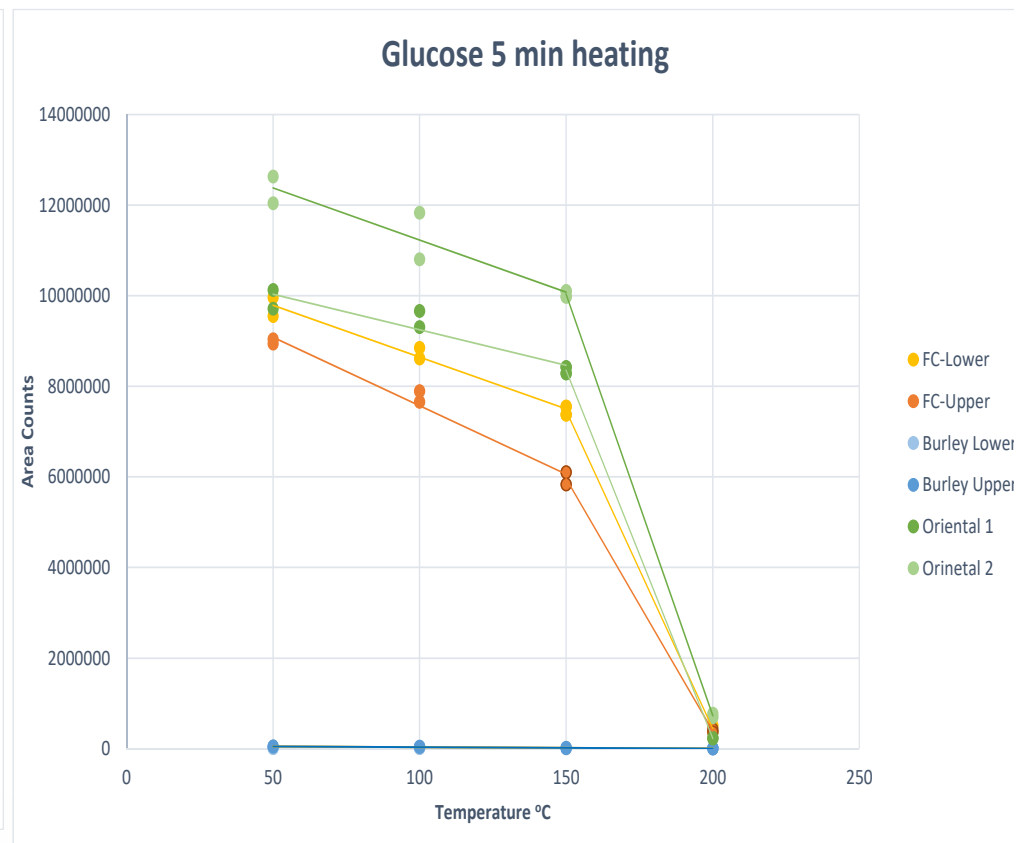
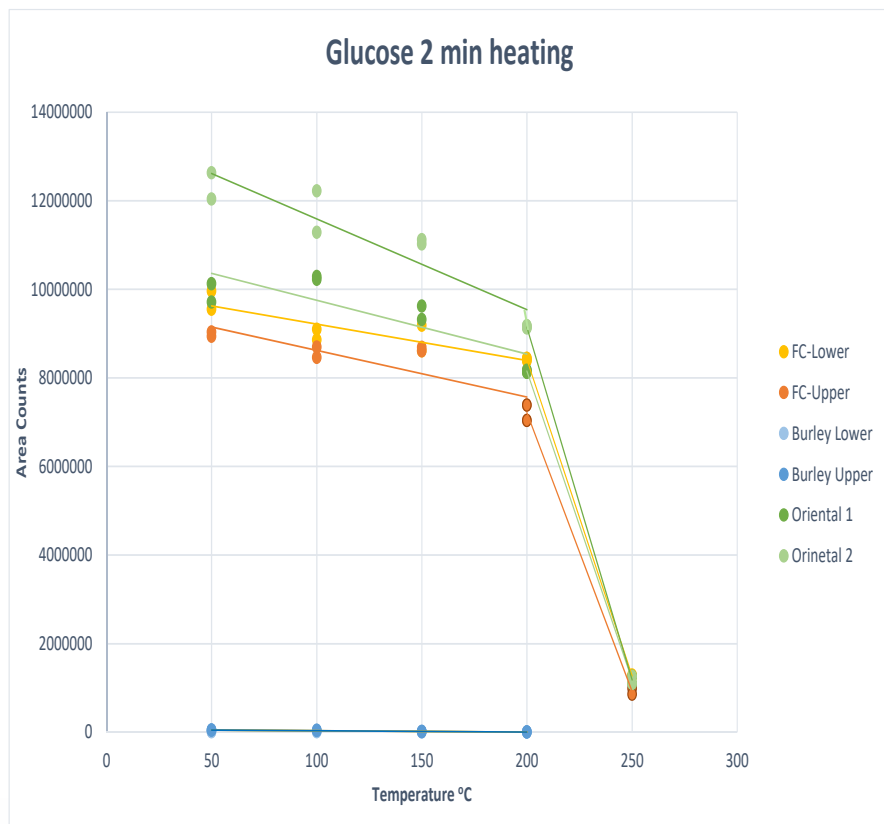
Changes in malic acid level in heated tobaccos



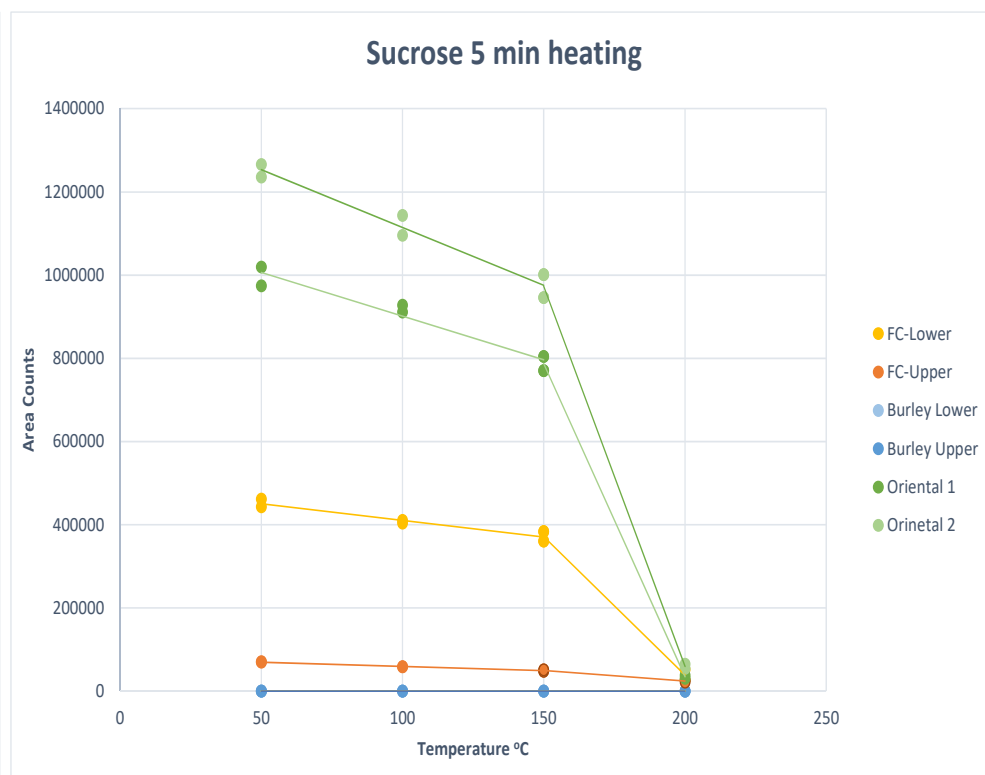
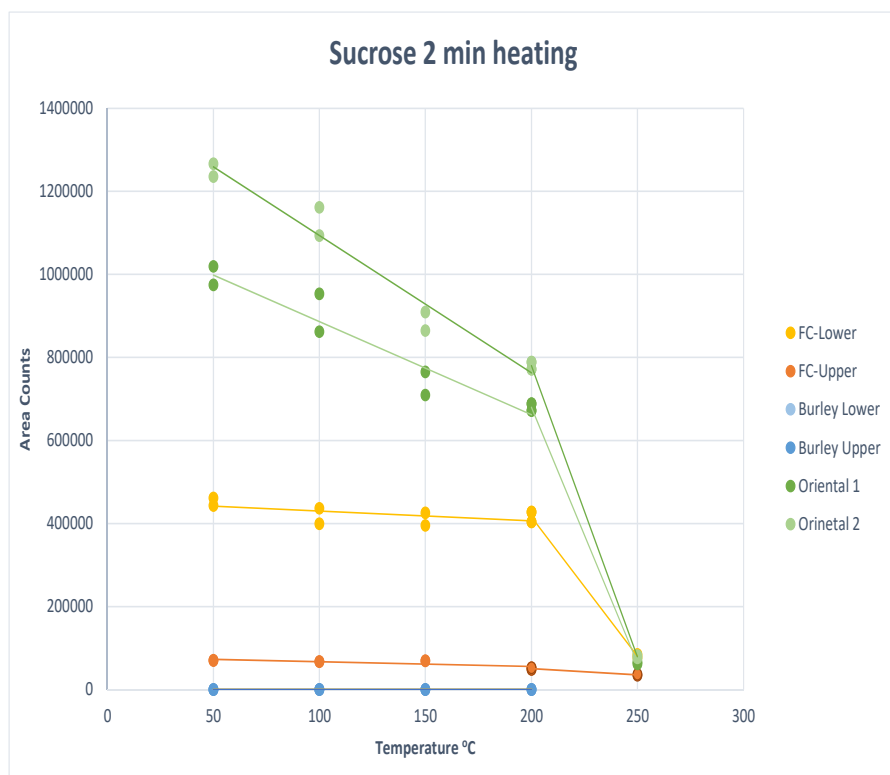
Changes in fructose level in heated tobaccos



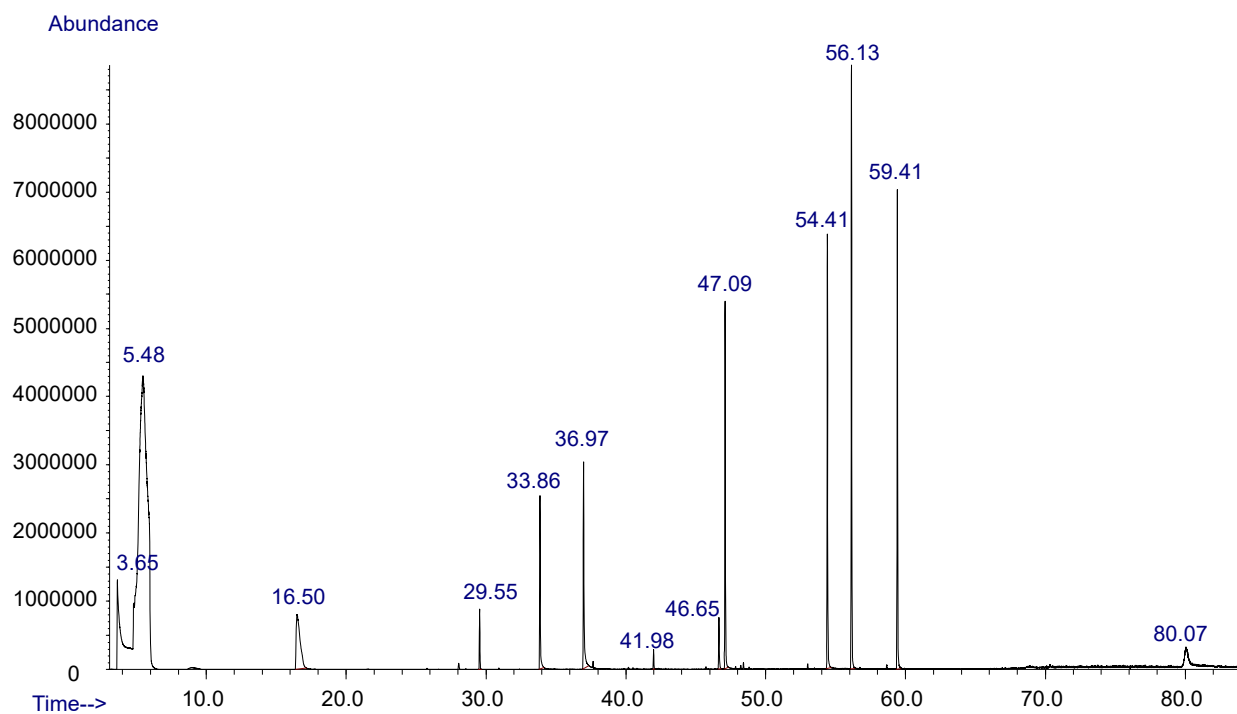
Changes in glucose level in heated tobaccos



Changes in sucrose level in heated tobaccos



Pyrogram of pure glucose, at 300 °C for 40s



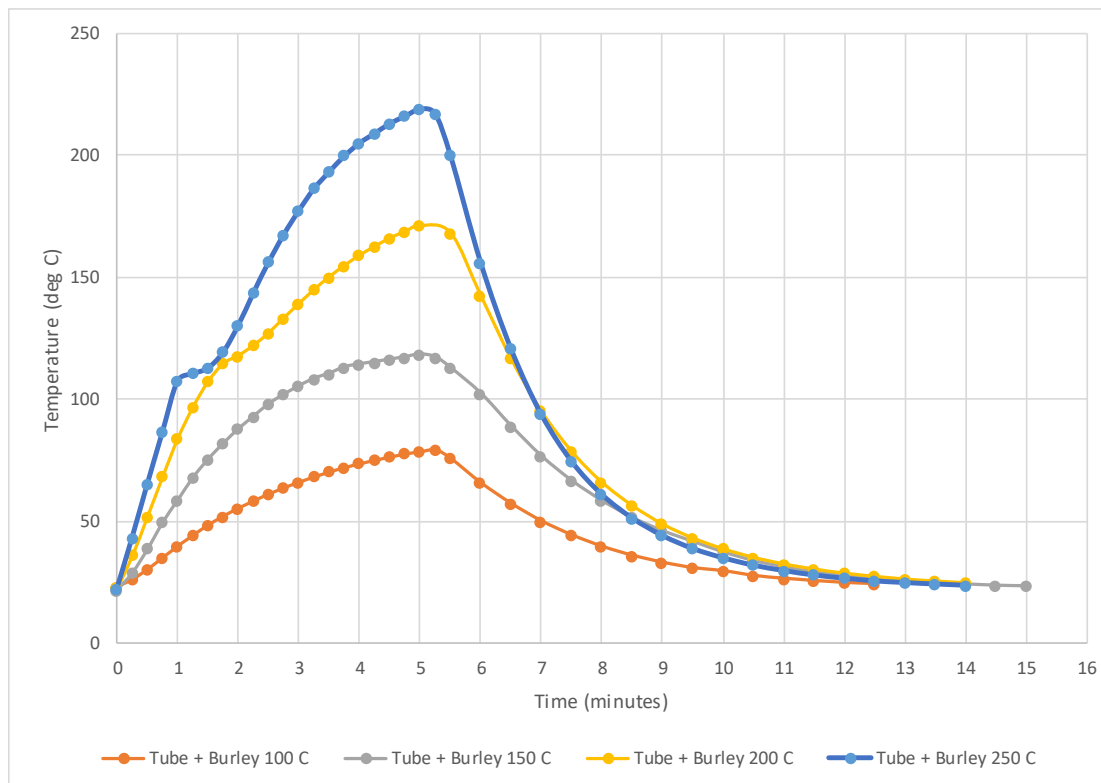
Peak identification for the pyrogram of glucose

	Compound	Ret. time	Area %
1	Air	3.65	4.47
2	Water	5.49	54.54
3	Hydroxyacetone	16.50	5.01
4	Furfural	29.55	0.86
5	Glyceraldehyde	33.86	2.74
6	Dihydroxyacetone	36.97	3.75
7	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	41.98	0.25
8	Glycerol	46.65	0.80
9	5-Hydroxymethylfurfural	47.10	4.87
10	2-Ethyl-2-(hydroxymethyl)-1,3-propanediol	54.41	5.98
11	1,6-Anhydro-.beta.-D-glucopyranose	56.13	8.49
12	1,6-Anhydro-.beta.-D-glucofuranose	59.41	6.86
13	Hydrocarbon (from the pyrolyzer)	80.07	1.39

Conclusions

- Six common tobaccos including two flue-cured, two burleys and two Orientals were heated in sealed glass containers at temperatures of 100, 150, 200 and 250 °C for two min or for five min.
- Some compounds such as nicotine or malic acid are stable when tobacco is heated at these moderate temperatures.
- All sugars start decomposing at temperatures as low as 150 °C and depending on the heating time their level is significantly reduces when heating at 250 °C for two minutes or at 200 °C for five minutes.
- The decomposition products of sugars include water, several anhydrosugars, short chain organic acids such as lactic acid and glycolic acid, furfural, hydroxymethylfurfural, hydroxyacetone, dihydroxyacetone.

Variation of tobacco temperature in time



Results from a study by F. K. St. Charles and S. C. Moldoveanu