R.J. Reynolds Tobacco Company

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Title: Burley Study (III): The Use of DOF Analyses to Monitor the Chemical Changes that take place during Heat-treatment when Tobaccos are cased with Various Sugars

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Objective: To determine whether DOF analyses correlate with the chemical changes that occurred when Burley tobacco is cased with various sugars and heat-treated in a Sargeant Dryer

Summary: Routine analyses do not provide enough information on the chemical changes that take place when tobacco is heat-treated. Analyses such as pH, the level of reducing sugar and headspace analyses can only give us a direction trend on the heat-treatment ,and not very conclusive and condition sensitive. We applied the DOF results to confirm our prediction and to correlate the condition change of heating process with chemical change. We have established the correlation from analyzing the DOF formation from the tobaccos cased with different sugars and undergo high, medium and low temperature. Higher temperature or longer duration of heating produce higher DOF, and vice versa. Fructose flavor the formation of 2,5-DOF and glucose favor the 2,6-DOF formation. Ammonia added would definitely enhance DOF formation. DOFs are the flavor precursors of lower alkylpyrazines, and hydroxylalkylpyrazines. The amounts of DOF formed in cased tobaccos (fructose=HFCS > glucose >>sucrose) are similar to the results obtained when sugar and DAP are heated together. It is postulated that both reactions followed the same type of reaction kinetics and mechanism. Sucrose did not show much effect due to the fact that the heating was not very severe, even when the tobacco was treated with ammonia. It was of interest to note that when HFCS is treated with ammonia, 2,5-DOF is found in preference to 2,6-DOF. This finding is the reverse of what happens when ammonia is absent.

Key Words: Burley, DOF, Casing sugars, fructose, glucose, HFCS, sucrose,heat-treatment, chemical change, Sargeant dryer, tobacco, HPLC, Maillard, monitor, ammonia, isomers

Status: This is the third paper in a series of studies related to the casing and heat treatment

of Burley tobacco.

Results and Discussion:

In our second paper on the heat-treatment study of cased burley[1], we described that none of existing routine analytical methods (i.e. nicotine, ammonia, reducing sugars, pH, etc.) (Tables 1-4), except a weak trend correlation between the decrease in pH and reducing sugars with the increase in temperature of heat-treatment was found.

Specific analyses (such as amino acid, glucosamine,etc.) were examined; however, they also did not clearly demonstrate the quantitative chemical changes that take place during heating the different sugar-cased tobaccos[2-7]. Although the sophisticated headspace analyses gave a trend on the formation of certain specific volatile components, alone it was not very conclusive as to what reactions took place and to what extent did they proceed[8]. The HPLC method of deoxyfructosazine (DOF) measurement has been demonstrated to be an indicator of the chemical changes that take place with of sugar after ammoniation (quantitatively shown a certain degree of Maillard reaction of the processed tobacco)[9]. We have recently applied the DOF method[10] to study a number of heat-treated cased burley tobacco samples (those used in a previous report)[1].

The DOF results of all samples from burley tobacco cased with different sugars and at different heating conditions using the Sargeant Dryer are collectively summarized in four separate Tables (6-9). Under constant heating and time, tobaccos were separately heated at high (330°F), medium (300°F) and low temperature (230-260°F). The highlights of these DOF studies are described as follows:

A) Fructose Cased Tobacco

1)Temperature factor--- $DOF_{330} > DOF_{300} >> DOF_{230}$

2)Time factor----- $DOF_{3.5min} > DOF_{2.5min}$

3)Ammonia content factor---- $DOF_{300,\ 1\%\ NH3} > DOF_{300}$

4)Isomer predominate factor--- 2,5-DOF > 2,6-DOF

B) Glucose Cased Tobacco

1)Temperature factor--- $DOF_{330} = DOF_{300} >> DOF_{248}$

2)Ammonia content factor---- $DOF_{300,\ 1\%\ NH3} > DOF_{300}$

3)Isomer predominate factor-- 2,6-DOF > 2,5-DOF

C) HFCS Cased Tobacco

1)Temperature factor--- $DOF_{330} = DOF_{300} >> DOF_{248}$

2)Ammonia content factor---- $DOF_{300,\ 1\%\ NH3} >> DOF_{300}$

3)Isomer predominate factor-- 2,6-DOF > 2,5-DOF

but after added 1% NH_3 2,5-DOF >> 2,6-DOF

C) Sucrose Cased Tobacco

Sucrose was very slightly affected by

1)Temperature 2)Duration of heating and 3)The addition of ammonia.

D) DOF Content in tobaccos cased with various sugars

Fructose =HFCS > glucose >>sucrose

E) Different Intermediate Mechanism for 2,5-DOF and 2,6-DOF:

In order to explain the preference of certain isomer of DOF formation, the following Scheme 1 and 2 would demonstrate their direct relation with the kind of sugars cased on tobaccos, which confirmed by the above-mentioned results after heat-treatments. Their differences in the kinetics of formation are similar to the kinetics results when sugars and DAP reacted[11] .

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Distribution List:

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T-1 **Temperature Effect on the Tobacco pH of Fructose Cased Burley**

Sample #	Cased	Heated	Reordered	Cased	Heated	Reordered
DL0630, 10# tobacco, AN16211				DL0708, 10#, AN16319		
T=280-285 up for 4min., 280-285 down for 3min				T=308-298, up, 2.5 min; T=258-304, for2 min.		
Tob pH	5.8	5.23	5.2	5.69	5.34	5.23
reducing sugar	9.3	5.3	5.0	8.8	4.1	5.2
Moisture %	31	0.4	11.8	30.07	1.8	9.53
Nicotine	2.29	2.24	2.26	2.45	2.40	2.31
Fructose	6.85	2.5	2.63	7.36	2.71	3.28
DL0709 a, 10# tobacco, AN16318				DL0701 a, 10# tobacco, AN16225		
T=308-296 up for 2.5min., 286-320 down for 2min				T=310-306 up for 3.5min., 290-327 down for 1.6m		
Tob pH	5.73	5.21	5.25	5.7	5.3	5.24
reducing sugar	10	4.2	4.7	8.8	3.0	3.0
Moisture %	29.09	0.95	9.08	30	9.4	15.6
Nicotine	2.47	2.47	2.4	2.1	2.03	2.14
Fructose	6.5	1.95	2.72	7.43	1.73	1.63
DL0710, 10# tobacco, AN16357				DL0709 b, 10# tobacco, AN16323		
T=332-306 up for 2.5min., 294-326 down for 2 min				T=229-224 up for 2.5min., 184-204 down for 2min		
Tob pH	5.9	5.34	5.31	5.84	5.81	5.76
reducing sugar	8.9	3.5	3.9	8.2	8.0	8.7
Moisture %	30.77	1.08	11.7	31.38	5.64	12.32
Nicotine	2.25	2.14	2.36	2.43	2.62	2.37
Fructose	6.88	1.93	2.08	6.03	5.65	6.13
DL0615, 20# tobacco, AN15970				DL0618, 20# tobacco, AN16024		
T=310 up for 4min., 270-307 down for 1.6min, cooling 210 for 2min, 1st reorder 3.4 min, 2nd reorder 2 min				T=290-308 up for 4min., 270-314 down for 1.6min Cooling 200 for 2.4min, reorder in drum externally		
Tob pH	5.72	5.32	5.4	5.84	5.54	5.43
reducing sugar	9.5	6.6	6.6	9.3	8.0	8.0
Moisture %	30.6	2.42	4.5	30.3	8.16	12.67
Nicotine	2.41	2.48	2.6	2.45	2.62	2.55
Fructose	8.36	4.94	4.4	6.92	5.23	5.53
DL0624, 20# tobacco, AN16127				DL0701 b, 20# tobacco, AN16237		
T=290-300 up for 5min., 240-314 down for 5min				T=301-292 up for 4min., 275-302 down for 2.5min		
Tob pH	-	5.30	5.25	5.82	5.37	5.32
reducing sugar	-	4.2	4.2	9.2	5.0	5.2
Moisture %	-	1.27	12.0	30.23	2.59	10.1
Nicotine	-	2.30	2.31	2.01	2.18	2.1
Fructose	-	2.23	2.34	6.67	2.91	3.16
DL0702, 20# tobacco, AN16244						
T=280-292 up for 5min., 280-312 down for 5min						
Tob pH	5.76	5.33	5.23			
reducing sugar	10	4.2	4.7			
Moisture %	29.09	0.95	9.08			
Nicotine	2.47	2.47	2.4			
Fructose	8.59	2.35	2.48			

T-2

Temperature Effect on the Tobacco pH of HFCS Cased Burley

Sample #	Cased	Heated	Reordered	Cased	Heated	Reordered
DL0721 A, 10# tobacco, AN16515 T=303-295 up for 2.5min., 265-285 down for 2min				DL0721 B, 10#, AN16525 T=304-296, up, 2.5 min; T=290-305, for2 min.		
Tob pH	5.75	5.42	5.39	5.98	5.47	5.44
reducing sugar	6.75	4.05	4.8	7.3	4.3	5.1
Moisture %	32.4	1.53	11.87	32.44	1.04	13.64
Nicotine	2.48	2.49	2.46	2.38	2.57	2.39
Fructose	3.28,3.56	1.32,1.53	1.55,1.91	3.16	1.55	1.89
Glucose	2.17,2.3	0.3,0.43	0.02,0.6	2.27	0.4	0.6
DL0722, 10# tobacco, AN16538 T=238-234 up for 2.5min., 192-248 down for 2min				DL0723 A, 10# tobacco, AN16565 T=334-338 up for 2.5min., 284-339 down for 2min		
Tob pH	5.98	5.76	5.72	5.72	5.41	5.31
reducing sugar	7.5	6.2	7.1	7.3	1.9	1.8
Moisture %	32.9	3.97	13.9	33.58	1.31	10.82
Nicotine	2.62	2.56	2.46	2.41	2.46	2.43
Fructose	3.50	2.76	3.1	3.31	0.59	0.52
Glucose	2.52	1.61	1.7	2.07	0.12	0.11
DL0723 B, 10# tobacco, AN16567 T=297-310 up for 2.5min., 246-312 down for 2min add 1% NH3						
Tob pH	6.61	5.62	5.6			
reducing sugar	8.4	4.5,3.5	3.3			
Moisture %	32.53	4.56,0.76	11.24			
Nicotine	2.59	2.58,2.46	2.48			
Fructose	3.52	1.55,125	1.11			
Glucose	2.43	0.56,0.28	0.26			
Ammonia	0.81	0.11,0.45	0.35			

T-3 **Temperature Effect on the Tobacco pH of Sucrose Cased Burley**

Sample #	Cased	Heated	Reordered	Cased	Heated	Reordered
DL0724, 10# tobacco, AN16584 T=308-293 up for 2.5min., 254-307 down for 2min				DL0727, 10#, AN16603 T=301-294, up, 2.5 min; T=243-3 Added 2% NH3		
Tob pH	5.67	5.49	5.54	6.41	6.11	6.09
reducing sugar	5.0	1.4	1.2	1.2	0.7	0.7
Moisture %	31.99	1.21	11.84	37.54	2.19	13.59
Nicotine	2.68	2.13	2.24	2.01	2.09	2.03
Fructose	0.60	0.55	0.55	0.94,0.83	0.75,0.86	0.71,0.82
Glucose	0.73	0.63	0.60	0.96,0.85	0.76,0.87	0.73,0.82
Sucrose	6.54	5.94	5.71	7.22,7.48	7.44,7.83	7.0,7.50
Ammonia	–	–	–	0.8,0.63	0.47,0.59	0.73,0.33
DL0728 A, 10# tobacco, AN16635 T=338-334 up for 2.5min., 291-330 down for 2min						
Tob pH	5.84	5.46	5.41			
reducing sugar	0.9	0.8	0.7			
Moisture %	32.4	11.91	10.59			
Nicotine	2.21	2.15	2.06			
Fructose	0.73	0.92	0.92			
Glucose	0.72	0.71	0.72			
Sucrose	7.11	6.75	6.51			

T–4

Temperature Effect on the Tobacco pH of Glucose Cased Burley

Sample #	Cased	Heated	Reordered	Cased	Heated	Reordered
DL0729 A, 10# tobacco, AN16656 T=307–302 up for 2.5min., 245–308 down for 2min				DL0729 B, 10#, AN16658 T=304–296, up, 2.5 min; T=290–305, for2 min. Added 1% NH3		
Tob pH	5.74	5.33	5.30	6.41	5.50	5.48
reducing sugar	8.4	4.0	5.8	8.75	3.65	4.8
Moisture %	30.68	0.72	10.92	33.32	0.62	12.83
Nicotine	2.23	2.21	2.18	2.18	2.28	2.11
Fructose	0	0	0	0.1,0.1	0.15,,0.15	0.16,0.17
Glucose	6.60	1.19	2.07	6.32,6.84	1.05,0.62	1.4,1.49
Ammonia	–	–	–	0.56,0.67	0.38,0.48	0.59,0.47
DL0728 B, 10# tobacco, AN16642 T=341–338 up for 2.5min., 273–318 down for 2min				DL0730, 10# tobacco, AN16694 T=226–248 up for 2.5min., 221–228 down for 2		
Tob pH	5.70	5.26	5.88	5.92	5.80	5.67
reducing sugar	8.5	2.8	3.4	8.7	8.5	8.5
Moisture %	32.83	11.02	11.05	33.82	4.17	12.63
Nicotine	2.12	2.26	2.23	2.2	2.28	2.24
Fructose	0.20	0.17	0.2	0	0	0
Glucose	7.41	0.69	1.03	6.42	5.09	4.75

Correlation of Deoxyfructosazine levels and Fructose Caseed/heating processed Burleys

Sample #	Control	Cased	Dried	Final
DL0710, 10# tobacco, AN16357				
T=332–306 up for 2.5mh., 294–326 down for 2 mh				
		AL	BL	CL
Tob pH		5.9	5.34	5.31
reducing sugar		8.9	3.5	4.1
Fructose		6.88	1.93	2.19
2,5–DOF(mg)		0.02	1.89	1.59
2,6–DOF		0.03	0.98	0.78

Control	Cased	Dried	Final
DL0709 b, 10# tobacco, AN16323			
T=229–224 up for 2.5mh., 184–204 down for 2mh			
AP	BP	CP	DP
5.67	5.84	5.81	5.76
0.8	8.2	8.0	8.7
0.58	6.03	5.65	6.13
0.05	0.30	0.57	0.66
0.04	0.17	0.12	0.12

	AP	BP	CP,CL	DP,DL
DL0709 a, 10#, AN16318				
T=308–296, up, 2.5 min; T=285–320, down for 2 min.				
Tob pH		5.79	5.21	5.23
reducing sugar		8.4	3.4	5.2
Fructose		5.58	1.95	3.28
2,5–DOF(mg)	0.2	0.57	1.73,1.91	2.18,1.83
2,6–DOF	0.12	0.315	0.89,0.95	1.13,1.10

AL			
DL0708, 10#, AN16319			
T=308–298, up, 2.5 min; T=258–304, for2 min.			
5.74	5.69	5.34	5.23
0.6	8.8	4.1	5.2
0.21	7.36	2.71	3.28
0.05	xx	xx	xx
0.07	2.45	2.40	2.31

	AP	BP	DP	GP
DL0630, 10# tobacco, AN16211				
T=280–285 up for 4mh., 260–265 down for 3mh				
Tob pH	5.66	5.78	5.22	5.16
reducing sugar	0.032	9.3	5.5	4.4
Fructose	0.21	7.11	2.63	2.17
2,5–DOF(mg)	0.06	0.99	2.69	1.79
2,6–DOF	0.05	0.27	1.36	0.67

	BP	CP	EP
DL0701 a, 10# tobacco, AN16225			
T=310–306 up for 3.5mh., 290–327 down for 1.5mh			
	5.69	5.32	5.25
	8.5	2.7	3.0
	7.09	1.47	1.70
	0.62	2.28	2.51
	0.23	1.2	1.29

	AP	BP	CP	
DL0729 B, 10#, AN18152				
T=304–296, up, 2.5 mh; T=290–305, for2 mh. Added 1% NH3				
Tob pH		5.74	5.33	5.30
reducing sugar	xxx	8.4	4.0	5.8
Fructose		0	0	0
Glucose		6.60	1.19	2.07
2,5–DOF(mg)		0.06	0.21	0.18
2,6–DOF		0.17	1.40	1.49
Ammonia		–	–	–

DL103092 a, 10# tobacco, AN18151			
T=250–271 up for 2.5mh., 217–265 down for 2mh			
AP	BP	CP	
xxx	5.86	5.79	5.41
	0.6	8.6	6.0
	0.1	7.2	3.97
0.12	0.10	1.23	1.17
0.09	0.08	0.62	0.62

	AP		BP	
DL0624, 20# tobacco, AN16127				
T=290–300 up for 5min, 240–314 down for 5min				
Tob pH	5.75	–	5.30	5.25
reducing sugar	0.7	–	4.2	4.2
Fructose	0.17	–	2.23	2.34
2,5–DOF(mg)	0.09		2.25	2.04
2,6–DOF	0.06		1.17	1.10

DL0702, 20# tobacco, AN16244			
T=280–292 up for 5min, 280–312 down for 5min			
AP	BP	CP	
5.76	5.33	5.23	
10	4.2	4.7	
8.59	2.35	2.48	
0.27	2.43	2.25	
0.16	1.38	1.16	

	AP	BP	EP,CP
DL0615, 20# tobacco, AN15970			
T=310 up for 4min., 270–307 down for 1.5min, cooling 210 for 2min, 1st reorder 3.4 min, 2nd reorder 2 mh			
Tob pH	5.72	5.32	5.4
reducing sugar	9.5	6.5	6.5
Fructose	8.36	4.94	4.4
2,5–DOF(mg)	0.75	1.53	1.89,1.79
2,6–DOF	0.30	0.6	0.72,0.4

DP	AP	BP	CP
DL0618, 20# tobacco, AN16024			
T=290–308 up for 4mh., 270–314 down for 1.5mh, Cooling 200 for 2.4mh, reorder in drum externally			
5.70	5.84	5.54	5.43
0.9	9.3	8.0	8.0
0.12	6.92	5.23	5.53
0.16	1.33	1.34	1.61
0.07	0.34	0.51	0.62

	AP,BP	CP	DP,EP
DL0701 b, 20# tobacco, AN16237			
T=301–292 up for 4 mh., 275–302 down for 2.5mh			
Tob pH	5.76,5.88	5.37	5.32
reducing sugar	9.7, 8.6	5.0	5.2
Fructose	6.81,6.54	2.91	3.16
2,5–DOF(mg)	0.32,1.65	2.25	2.13,1.91
2,6–DOF	0.13,0.33	0.92	0.78,0.72

AP	BP	CP	DP
DL103092 a, 10# tobacco, AN18151			
T=250–271 up for 2.5mh., 217–265 down for 2mh			
5.86	5.79	5.41	5.44
0.6	8.6	6.0	6.1
0.1	7.2	3.97	4.06
0.12	0.10	1.23	1.17
0.09	0.08	0.62	0.62

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Correlation of Deoxyfructosazine levels and HFCS Cased/heating processed Burleys

DL0723 A, 10# tobacco, AN16565
T=334-338 up for 2.5min., 284-339 down for 2min

Sample #	Control	Cased	Dried	Final
	AP	BP	CP	DP
Tob pH	5.77	5.72	5.41	5.31
reducing sugar	0.5	7.3	1.9	1.8
Fructose	0.18	3.31	0.59	0.52
Glucose	0.09	2.07	0.12	0.11
2,5-DOF(mg)	0.05	0.53	0.87	0.99
2,6-DOF	0.09	0.6	1.17	1.25

DL0722, 10# tobacco, AN16538
T=238-234 up for 2.5min., 192-248 down for 2min

Control	Cased	Dried	Final
AP	BP	CP	DP
5.80	5.98	5.76	5.72
0.7	7.5	6.2	7.1
0.0	3.50	2.76	3.1
0.2	2.52	1.61	1.7
0.06	0.12	0.32	0.39
0.06	0.14	0.40	0.60

DL0721 A, 10# tobacco, AN16515
T=303-295 up for 2.5min., 265-285 down for 2min

	BP	CP	EP
Tob pH	5.72	5.39	5.41
reducing sugar	6.9	3.7	4.4
Fructose	3.56	1.32	1.55
Glucose	2.3	0.3	0.02
2,5-DOF(mg)	0.20	1.00	0.78
2,6-DOF	0.21	1.25	0.98

DL0721 B, 10#, AN16525
T=304-296, up, 2.5 min; T=290-305, for2 min.

AP	BP	CP
5.98	5.47	5.44
7.3	4.3	5.1
3.16	1.55	1.89
2.27	0.4	0.6
0.20	0.93	0.9
0.27	1.25	1.19

DL0723 B, 10# tobacco, AN16567
T=297-310 up for 2.5min., 246-312 down for 2min
add 1% NH3

	AP	BP	DP
Tob pH	6.61	5.62	5.6
reducing sugar	8.4	4.5	3.3
Fructose	3.52	1.55	1.11
Glucose	2.43	0.56	0.26
2,5-DOF(mg)	0.27	1.41	1.71
2,6-DOF	0.29	1.76	2.16
Ammonia	0.81	0.28	0.35

Correlation of Deoxyfructosazine levels and Sucrose Cased/heating processe

Sample #	Cased	Heated	Reordered
DL0728 A, 10# tobacco, AN16635			
T=338–334 up for 2.5min., 291–330 down for 2min			
	AP	BP	CP
Tob pH	5.84	5.46	5.41
reducing sugar	0.9	0.8	0.7
Fructose	0.73	0.92	0.92
Glucose	0.72	0.71	0.72
Sucrose	7.11	6.75	6.51
2,5–DOF(mg)	0.08	0.09	0.06
2,6–DOF	0.08	0.15	0.14
Ammonia	–	–	–
DL0724, 10# tobacco, AN16584			
T=308–293 up for 2.5min., 254–307 down for 2min			
	AP	BP	CP
Tob pH	5.67	5.49	5.54
reducing sugar	5.0	1.4	1.2
Fructose	0.60	0.55	0.55
Glucose	0.73	0.63	0.60
Sucrose	6.54	5.94	5.71
2,5–DOF(mg)	0.045	0.06	0.06
2,6–DOF	0.03	0.05	0.05

Cased	Heated	Reordered	Control
BP	CP	EP	GP
DL0727, 10#, AN16603			
T=301–294, up, 2.5 min; T=243–308, for 2 mir			
Added 2% NH3			
BP	CP		
5.93	5.92	6.18	5.67
1.4	0.7	0.7	0.6
0.83	0.75	0.71	0.12
0.85	0.76	0.73	0.0
7.4	7.44	7.0	0.13
0.08	0.08	0.08	0.05
0.08	0.06	0.08	0.03
0.63	0.47	0.73	0.49

T-9

Correlation of Deoxyfructosazines with Glucose Cased/Heat processed Burley Tobacco

DL0728 B, 10# tobacco, AN16642
T=341-338 up for 2.5min., 273-318 down for 2min

Sample #	Cased	Heated	Reordered
	AP	BP	CP
Tob pH	5.70	5.26	5.88
reducing sugar	8.5	2.8	3.4
Fructose	0.20	0.17	0.2
Glucose	7.41	0.69	1.03
2,5-DOF(mg)	0.09	0.23	0.17
2,6-DOF	0.78	1.44	1.41

DL0729 A, 10# tobacco, AN16656
T=307-302 up for 2.5min., 245-308 down for 2min

	AP	BP	CP
Tob pH	5.74	5.33	5.30
reducing sugar	8.4	4.0	5.8
Fructose	0	0	0
Glucose	6.60	1.19	2.07
2,5-DOF(mg)	0.08	0.21	0.18
2,6-DOF	0.17	1.40	1.49
Ammonia	-	-	-

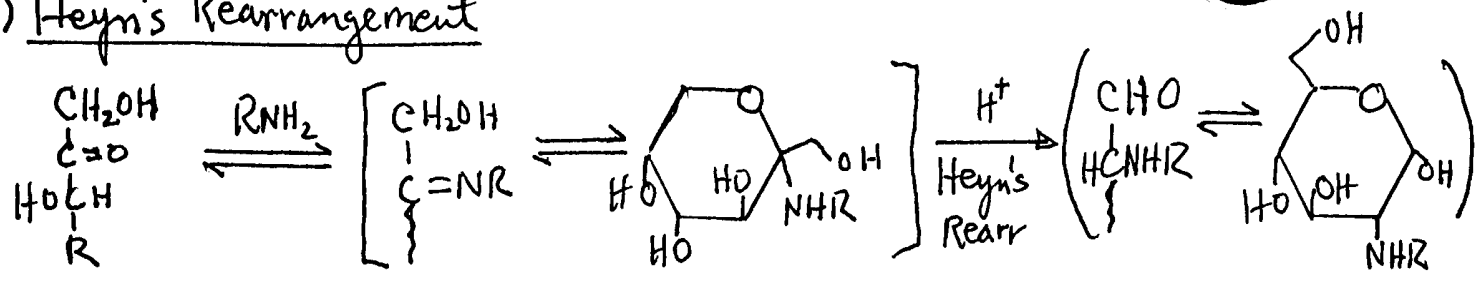
Cased	Heated	Reordered
DL0730, 10# tobacco, AN16694		
T=226-248 up for 2.5min., 221-228 down for 2min		
AP	BP	CP
5.92	5.80	5.67
8.7	8.5	8.5
0	0	0
6.42	5.09	4.75
0.05	0.11	0.03
0.29	0.33	0.50

DL0729 B, 10#, AN16658
T=304-296, up, 2.5 min; T=290-305, for2 min.
Added 1% NH3

	DL	EL	GL
6.41	5.50	5.48	
8.75	3.65	4.8	
0.1,0.1	0.15,0.15	0.16,0.17	
6.32,6.84	1.05,0.62	1.4,1.49	
	0.08		
	0.65		

Scheme I Aminosugar Formation

(1) Heyn's Rearrangement



Fructose (keto form)

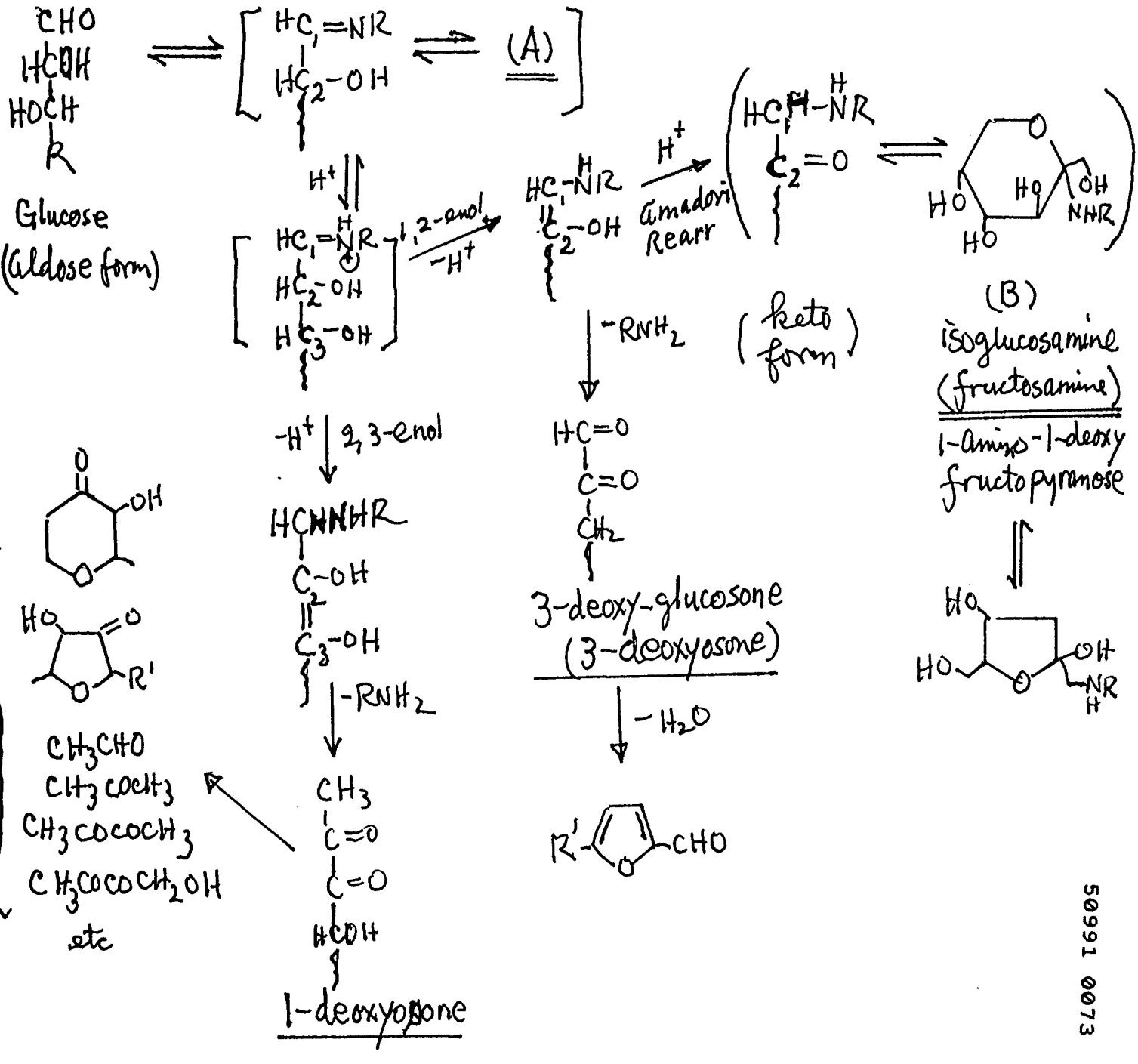
α-D-Fructopyranosamine

(Aldose form)

2-amino-2-deoxy α-D-glucopyranose (if R=H)

Glucosamine (A)

(2) Amadori Rearrangement and Strecker Degradation



Scheme II DOF Formation