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CONDENSATION OF FRUCTOSE IN ACID WATER-SOLUTIONS

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Condensation of fructose in water solutions of 0.1-1.2 g/cm^3, pH 1.3 or 3.5, and temperatures of 60° and 98°C, was investigated. Three products of condensation were identified with the use of the paper and the preparative chromatographies. It was ascertained that fructose undergoes condensation mainly at moderate temperatures (60°C) and the pH values (1.3-3.5). When the temperature is high (98°C) and pH is 1.3, the condensation of fructose is accompanied by dimerization and decomposition.

Fructose has attracted considerable attention as a sweetener superior to sacharose. Introduction of new production techniques results in higher yields thus making fructose more readily available for a broad range of applications in standard and dietetic foods. This leads to a number of problems that have to be taken care of, such as the highly pragmatic question concerning the behaviour of this monosaccharide in water solutions of varying concentration and acidity.

In an acidic water solution monosaccharides are condensed to reducing oligosaccharides [12, 22, 26]. Condensation of monosaccharides is a process taking place simultaneously with hydrolysis of oligo- and polysaccharides [12, 14, 23]. Consequently, products of condensation are sometimes taken for products of reversion [24, 25, 29].

The most extensively investigated products of condensation (reversion) so far are those of aldoses, mainly of glucose [12, 16, 23—25, 30].

Glucose primarily yields disaccharides with a 1.6 glycoside bond: isomaltose and gentiobiose; the 1.4 bond disaccharides: maltose, and the 1.3 bond: nigerose. Trisaccharides and tetrasaccharides also develop. Silberman [22] proved that monosaccharides undergo condensation more readily than disaccharides.

The literature on fructose condensation provides contradictory data. According to some authors [22, 25] ketoses — fructose and sorbose — when heated in a sulphuric or hydrochloric acid solution with its pH < 1 at 70° to 100°C, do not condense but yield products of dimerization and polycondensation. According to Schneider [20], however, prolonged heating of a neutral solution of fructose results in three disaccharides of the reducing type in which inulobiose β-D-fructofuranozyl-0-1-D fructofuranose) was identified. This disaccharide was also found among the products of inulin hydrolysis [14]. The second difructoside was regarded by Schneider as having alternating structure of dipyranoses or pyrano--furanosides with 2.1 and 2.4 bonding, respectively. The third disaccharide was described by him as a fermenting fructoside. These discrepant opinions quoted here as regards condensation of fructose follow from investigations on the behaviour of fructose in extreme conditions. Particularly intensive study was made on the effect of acids on fructose in drastic conditions, which lead to profound transformations of that sugar and formation of products of dehydration, dimerisation and polycondensation. The products of dehydration are 5-hydroxymethylofurfural and its derivatives [8, 21, 28], levulinic acid, and humus substances, which simultaneously are the products of polycondensation [8]. The products of dimerisation, fructose dianhydrides, are the final products of the treatment of acids, both concentrated at room temperature or lower as well as diluted at nearly 100°C [14, 17, 19]. The structure and properties of seven fructose dianhydrides are well known and described [6, 7, 26, 27]. Three of the fructose dianhydrides are composed exclusively of fructofuranose residua, and the other four dianhydrides, the so-called heterolevulosanes, contain simultaneously residua of fructofuranose and fructopyranose, or residua of fructopyranose only. Recently there have been discoveries and identification of monoanhydrides of fructose [11, 13].

It is worth mentioning that certain products of fructose condensation are identical with the products of synthesis of oligosasccharides, which involves enzymes from the group of transglycosilases [4]. Bell and Edelman [1, 4] and Andersen [1] proved that inulobiose and levanobiose — β-D-fructofuranosilo-0-6-D-fructofuranose — are the only products of transfructosylase accompanied by β-fructofuranosidase. The reaction products show a greater presence of inulobiose.

The aim of the present study was to investigate the composition of the products of fructose condensation in water solutions of different concentration and pH 3.5, at which fructose is most durable; and in water solutions of different concentration and pH 1.3, at which complete hydrolysis of saccharose is performed under technological conditions.

MATERIALS AND METHODS

Recrystallized fructose (specific rotation — 92.5°) was used. 0.1 to 1.2 g fructose per 1 ml solutions were adjust with hydrochloric acid to $pH_{20} = 1.30$, or 3.50 (ELPO 512 pehameter). The ready solutions were put into closed vessels (100 ml portions) and placed in a thermostat, where the temperatures were kept at 60° or 98°C for 120 min. After cooling-off the solutions were subjected to chemical and chromatographic analyses. The specific rotation of solutions was determined as well as the contents of reducing substances (by Luff-Schoorl method), the contents of 5-hydroxymethylofurfural (spectrophotometrically [21], and fermentation tests. The latter were performed according to the methodology given by Zerban [19] and Bachman [3].

The quality and quantity compositions were analysed with paper chromatography. Here, Whatman paper was used, and the ratio of solvents was this — propanol: ethyl acetate: water $(7 : 1 : 2)$. Chromatograms were developed with an aniline reagent as well as resorcinol and triphenyltetrasolic reagents [15]. The quantitative determinations in eluates were made with resorcinol reagent, after Roe [18]. The preparation chromatography was following Bidwell's method [5].

RESULTS AND DISCUSSION

The study of condensation products in solutions of different concentration was made with pH kept identical all the time since it is a fact that durability of sugars and their vulnerability to hydrolysis primarily depend on the concentration of hydrogen ions. The heating time for the solutions was selected after preliminary tests. Prolongation of the heating of the acidified fructose solutions leads to interesting changes, which call for a separate discussion.

Results of the chemical and chromatographic analyses are in Table 1 and Table 2. Figures 1 and 2 provide give specimens of chromatograms of the fructose condensation products. Fig. 1 presents the chromatogram of fructose solutions heated at 60°C for 120 min. (concentrations: 0.1; 0.3; 0.6; 0.9 and 1.2 g/cm³; pH = 1.3). It was developed with a resorcinol reagent. Solutions concentrated at 0.6 g/cm³ provided three spots with R_F in relation to fructose: $R_{FF} = 0.63$; 0.49; 0.31. The 0.9 and 1.2 g/cm³ solutions provided an additional spot with $R_{FF} = 0.14$. As concerns the solution of 0.1 g/cm³ and pH 1.3, a distinct spot is visible near the starting line corresponding to a polyfructosane.

The results of tests with invertase affecting fructose, the fermentation tests, the preparation chromatography, and the developing of chromatograms with different reagents (Table 1), made it possible to report that

T a b l e 1. Characteristics of fructose condensation products separated by preparation chroma-
tography

| Fractions | Reducibility | Fermenta-bility | Reaction to re-agent | | Specific rotation | |
			TTC	resorcinol	presently obtained	data in literature
Levanobiose	1/2 F	+	+	+	−14.0	−20.8[2]
Inulobiose	1/2 F	+	+	+	−62.0	−66.0[10]
Fructopyranosi-lofructose	1/2 F	−(+)	+	+	−47.0	no data
Mixture of crystalline di-fructose dia-nhydrides	—	—	—	+	−39.0	−39[27]

TTC — trifenyltetrasoliose reagent specific for reducing sugars
+ reducing properties present
— non- reducing component
resorcinol — re-cagent specific for ketoses (fructose):
+ fructose present
fermentability of disaccharides by *Saccharomyces cerevisiae*:
+ complete decomposition of a disaccharide
— complete immunity to yeast activity
— (+) insignificant decomposition of a disaccharide by yeast
(possible presence of a β—fructofuranoside bonded disaccharide in this fraction

the R_{FF} 0.63; 0.49 and 0.32 spots correspond, respectively, to levanobiose
(β-D-fructofuranosyl-0-6-D-fructofuranose), inulobiose (β-D-fructofurano-
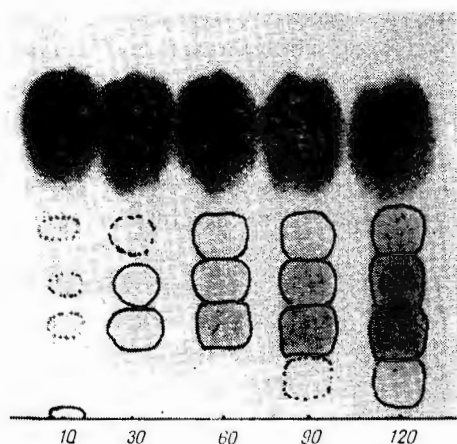syl-0-1-D-fructofuranose), and fructopyranosilofructose, whose precise

Fig. 1. Chromatogram of condensation
products of fructose obtained by 120
min heating at 60°C of solutions con-
taining 10, 30, 60, 90, 120 g of fructose in
100 ml (pH = 1.3)

structure was not determined. It was also observed that levanobiose was
accompanied by a nonreducing component with $R_{FF} = 0.66$.

Supplementary tests showed it to be a difructofuranose dianhydride.
Complementary experiments were run with a fructose solution heated at

T a b l e 2. Results of chemical analyses of fructose solutions in different concentrations and different pH heated at 60°C (samples A) and 98°C (samples B) for two hours

| Type of analysis | Fructose solution concentration; pH=1.3 | | | | | | | | | | Fructose solution pH=3.5 0.9 g/cm³ | |
| | 0.1 g/cm³ | | 0.3 g/cm³ | | 0.6 g/cm³ 0.9 g/cm³ 1.2 g/cm³ | | | | | | | |
	A	B	A	B	A	B	A	B	A	B	A	B
Specific rotation	-89		-90.4	-83.4	-89.8	-78.0	-89.9	-72.7	-85.5		-91.4	-87.5
Reducibility; Luff-Schoorl method; %/dry substance	96.9		97.0	89.6	96.0	85.8	93.0	76.8	89.0		98.5	94.0
Total content of fructobioses; %/dry substance	0.6		2.2	2.8	5.3	6.0	11.5	9.2	15.4		2.5	5.8
Content of non-reducing substances; %/dry substance	2.8		1.9	9.0	1.4	10.9	1.3	13.4	3.3		0.4	3.1
5-HMF content; %/dry substance	0.021		0.025	1.1	0.026	0.83	0.025	0.84	0.029		0.00	0.01
Total content of products of condensation; dimerisation and decomposition; %/dry substance	3.4		4.1	12.9	6.7	17.7	12.8	23.6	18.7		2.9	8.9
Proportion of condensation products in relation to processed fructose; %/dry substance	17.7		53.5	21.6	79	34.0	83	39.0	82.5		86	65

80°C for several hours. It was fermented and desalinated and then sub-
jected to crystallization in a solution of ethyl acetate and petroleum
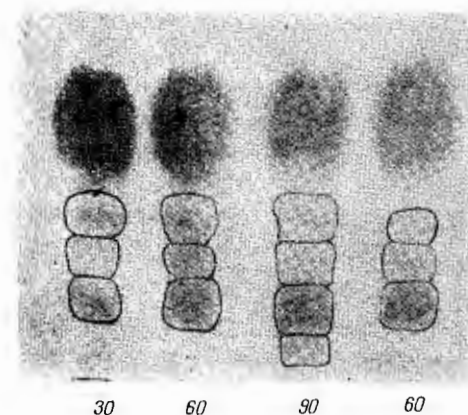benzin. In effect a mixture pf anhydrides (specific rotation: 39°) was
obtained.

Fig. 2. Chromatogram of condensation
products of fructose obtained by 120
min heating at 98°C of solutions con-
taining 30, 60, 90 g of fructose in 100
ml (pH = 1.3). Additionely a sample
of solution 60 g of fructose in 100 ml
heated at 60°C have been analysed (60′)

After a chromatographic analysis it turned out that the mixed group
consisted of 30⁰/o of difructofuranose dianhydride R_{FF} = 0.66 and 70⁰/o
heterolevulosane II, i.e. dianhydride of fructopyranosylfructofuranose
(R_{FF} = 0.39).

The latter (heterolevulosane II) appears in a mixture of products of
condensation. During the developing of a chromatogram with a resorcinol
reagent it begins to show as the last one, also giving a partial darkening
of the background. For this reason the coloring caused by that compound
as given in the present chromatogram is rather insignificant and shows
between the spots of inulobiose and levanobiose. The spots with R_{FF} =
= 0.14 probably correspond to a reducing fructotriose.

Comparison of the intensity of colour of the chromatographic spots
reveals a distinct increase of concentration of all the products of fructose
condensation as the concentration of the initial solution grows. The do-
minating product of fructose condensation is fructopyranosylfructose. The
concentration of inulobiose is two times lower, and that of levanobiose —
four times lower than the dominant component. The above observations
are confirmed by the results of the chemical analyses which are given in
Table 2.

Specific rotation and reducibility of the pH 1.3 solutions heated at
60°C decrease while the concentration of fructose in the initial solution
increases. This is due to the presence of reducing disaccharides which
have lower specific rotation and their reducing ability cut down to 50⁰/o
(Table 1). The contents of nonreducing dianhydrides of fructose, calcu-
lated from the difference in reducibility of the initial and final solutions,
taking into account the concentration of two main fructobioses, is rather

small (2 to 3%). The concentration of anhydrides is practically indepen-
dent of the initial concentration of fructose solution.

The total contents of condensation products in the pH 1.3 solutions —
0.6 to 15.4%, in terms of dry substance of solution, clearly depends on the
initial concentration of fructose solution. In view of these facts, the con-
densation of fructose can be classified with bimolecular reactions.

The proportion of the condensation products in ratio of the processed
fructose in diluted solutions (0.1-0.3g/ml) is 17 to 53.5%. In solutions
concentrated above 0.6 g/ml this proportion is almost constant — 79 to
83%.

The pH 3.5 solutions heated at 60°C, compared to the pH 1.3 solutions
treated same way, show four-times lower concentration of products of
condensation and dimerisation. The level of condensation products in
ratio of processed fructose is 86%. In fructose solutions, heated at 60°C
for 2 hours, the 5-HMF concentration is virtually independent of the con-
centration — 0.020 to 0.025%.

The chromatogram in Fig. 2 illustrates changes in the fructose solution
compositions at concentration levels of 0.3; 0.6 and 0.9 g/ml; pH = 1.3,
heated at 98° for two hours. For comparison a sample of the pH 1.3;
0.6 g/ml solution heated at 60°C was used here as well. The 0.3 g/ml
solution provided three spots of nearly the same size: R_{FF} = 0.63; 0.49,
and 0.31, and one more spot near the starting line. For the 0.6 and 0.9
solutions an additional spot with R_{FF} = 0.14 was obtained. Colouring in-
tensity and size of the spots are somewhat different here than for the
solutions heated at 60°C, particularly the spots with R_{FF} = 0.63 have
larger areas. This is induced by the dianhydride of difructofuranose pre-
sent in the solution. Concentrations of fructopiranosylfructose and inulo-
biose, the main products of condensation, are similar in both cases. It is
evident in the chromatograms and in the results given in Table 2.

The results thus confirm a supposition that condensation of fructose
can be classified with bimolecular reactions. The data given in the table
ascertain that the heating of the pH 1.3 solution at 98°C leads to a con-
siderable decrease in specific rotation and reducibility. It occurs on ac-
count of development of large quantities (9 to 13%) of nonreducing sub-
stances. The ratio of fructopyranosylfructose to inulobiose in the pH 1.3
solutions heated at 60° and 98°C is similar, respectively, 70:30 and 65:35.
It was observed that at 40°C the ratio is 75:25 while for the pH 3.5 so-
lutions in both cases it is approximating 50:50. The above ratios and
different concentrations of these sugars in solutions are related to pH
and temperature thus reflecting specific conditions of equilibrium betwe-
en products of fructose condensation and concentration of diverse forms
of fructose in a solution. It is reported [9] that at 20° and pH = 5.4 the
proportion of the pyranoside form is 67%, and of the furanoside form —
33%.

In the mixture isomers β dominate while isomer α presence is small. With growing temperature and concentration and decreasing pH the presence of β-fructofuranose rises. Therefore, products of condensation include disaccharides with the β-fructofuranoside structure (inulobiose and levanobiose) appear; it is also quite likely that the chromatographically identified fructopyranosilofructose has the structure of β-D-fructopyranosilofructofuranose or β-fructopyranosylfructopyranose. With growing temperature an increasing quantity of oligosaccharides with the ß-fructofuranoside bond is to be expected. The obtained results indicate that the quantity of the main products of condensation at 60° and 98°C for solutions with similar pH and concentrations is similar. At the higher temperature (98°), however, there appears a larger amount of fructose anhydrides (Table 2), including the difructofuranose dianhydride — the basic product of dimerisation of β-fructofuranose. It also follows from Table 2 that under similar conditions of fructose concentration and pH 1.3 the products of condensation account for $80^0/o$ processed fructose at 60°C, and only 34 to $39^0/o$ processed fructose at 98°C. For the pH 3.5 fructose solutions the proportion of the products of condensation is, respectively, $86^0/o$ and $65^0/o$. In view of this it can be said that fructose heated in mild conditions (optimum pH, moderate temperature), particularly in concentrated solutions, mainly undergoes condensation to three reducing fructobioses, of which one does not ferment. Fructose condensation is probably a bimolecular reaction. The heating of fructose solutions at temperatures approximating 100°C in acidified environment with the pH being either optimum or lower (3.5 to 1.3) enhances dimerisation and decomposition, which accompany condensation. The process of dimerisation and decomposition does not depend on fructose concentration.

CONCLUSIONS

Fructose, when heated for two hours in solutions with pH = 3.5 or pH = 1.3 at either 60° or 98°C undergoes condensation to fructopyranosylfructose, inulobiose and levanobiose. The ratios of the three components depend on pH and temperature.

The summary concentration of condensation products — 0.6 to $15.4^0/o$ per dry substance solution increases with the growth of the initial concentration of fructose.

Under moderate temperature (60°C) and pH (3.5-1.3) fructose in solutions concentrated above 0.6 g/ml is mainly subject to condensation, the products of which stand for ca $80^0/o$ of the processed fructose.

At temperatures near 100°C fructose in solutions with pH = 1.3 or 3.5 and concentrated below 0.3 g/ml undergoes not only condensation but

also dimerisation, decomposition, and polycondensation. The yield of the products of condensation amount then to no more than about 20% of the processed fructose.

LITERATURE

1. Andersen B.: Acta Chim. Scand., 1967, **21**, 828.
2. Aspinell G. O., Tefler G. J.: J. Chem. Soc., **1955**, 1106.
3. Bachman B.: Zeszyty Naukowe Politechniki Łódzkiej, Chemia Spożywcza, 1957, **16**, (2), 43.
4. Bell D. J., Edelman L.: J. Chem. Soc., **1954**, 4652.
5. Bidwell R. G. S., Krotkew G., Reed G. B.: Arch. Biochem. Biophys., 1954, **48**, 72.
6. Binkley R., Binkley W.: Carb. Res., 1974, **36**, 196.
7. Binkley W.: Int. Sugar J., 1971, **73**, 259.
8. Bretschneider R.: Listy cukrov. 1971, **87**, 126.
9. Curtius H., Muller M., Vollmin J.: J Chromatog., 1968, **37**, 216.
10. Dikerson A. G., Moor J.: Carb. Res., 1975, **39**, 162.
11. Goldschmidt M. R., Perlin A. S.: Can. J. Chem., 1960, **38**, 2178.
12. Hollo J., Szejtli J.: Nahrung, 1961, 5, 506.
13. Leibnitz J.: J. Org. Chem., 1959, **24**, 1897.
14. MacDonald E. J.: Adv. Carb. Chem., 1946, **2**, 265.
15. Opieńska-Blauth J.: Chromatografia, Warszawa 1957, str. 583.
16. Pazur J. H.: J. Am. Chem. Soc., 1956, **78**, 1885.
17. Picet A., Chavan J.: J. Helv. Chim. Acta, 1926, 9, 809.
18. Roe J. H., Epstein J. H.: J. Bid. Chem., 1949, **178**, 839.
19. Sattler L., Zerban F. W.: Ind. Eng. Chem., 1945, **37**, 1135.
20. Schneider F., CITS X, 1957, 32.
21. Shaw P., Tatum J., Berry E.: Carb. Res. 1967, **5**, 266.
22. Silberman J.; J. Org. Chem., 1967, **26**, 1961.
23. Sroczyński A., Boruch M.: Die Stärke, 1964, **16**, 21.
24. Teufel K., Muller K.: Z. Lebensmittel Unt., 1955, **100**, 437.
25. Teufel K., Muller K.: Z. Lebensmittel. Unt., 1952, **94**, 401.
26. Verstraeten L. M. J.: Adv. Carb. Chem. 1967, **21**, 258.
27. Wickberg A. B.: Acta Chim. Scand., 1954, **8**, 436.
28. Wiggins L. F.: Adv. Carb. Chem., 1949, **4**, 293.
29. Wohl A.: Ber., 1890, **23**, 2084.
30. Wolfrom M. L.: J. Am. Chem. Soc., 1953, **75**, 3003.

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KONDENSACJA FRUKTOZY W KWASOWYCH ROZTWORACH WODNYCH

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Streszczenie

Badano skład jakościowy i ilościowy produktów kondensacji fruktozy, uzyskanych przez ogrzewanie w temperaturze 60°C lub 98°C w czasie 120 min roztworów czystej fruktozy o stężeniu 0,1-1,2 g/cm³ oraz o pH 3,5 lub 1,3. Analizę produktów

kondensacji prowadzono głównie za pomocą chromatografii bibułowej i preparatywnej.

Stwierdzono występowanie trzech produktów kondensacji: fruktopiranozylofruktozy $[\alpha]_D^{20} = -47°$, inulobiozy $[\alpha]_D^{20} = -62°$ oraz lewanobiozy $[\alpha]_D^{20} = -14,0°$. Stosunek stężeń trzech wymienionych dwucukrów zależy od warunków kondensacji. Sumaryczne stężenie produktów kondensacji wynoszące od 0,6 do 15,4% licząc na s.s. roztworu wzrasta z podwyższeniem początkowego stężenia roztworu fruktozy. W umiarkowanych warunkach temperatury (60°C) i pH 3,5; 1,3 w roztworach o stężeniu powyżej 0,6 g/cm³ 80% przereagowanej fruktozy stanowią produkty kondensacji, natomiast w temperaturach 98°C przy zachowaniu pozostałych parametrów produkty kondensacji zawierają jedynie 20% przereagowanej fruktozy. Większość bowiem przereagowanej fruktozy stanowią produkty dimeryzacji i rozkładu.