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FORMATION OF OLIGOSACCHARIDES DURING LACTOSE HYDROLYSIS WITH β-GALACTOSIDASE IN CHEESE-WHEY PERMEATES

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Key words: oligosaccharides formation, lactose hydrolysis, β-galactosidase, cheese-whey permeate.

Hydrolysis of lactose in the cheese-whey permeates was conducted using β-glactosidase of yeast and mold origin. Both enzymatic preparations showed similar transgalactosidase activity. The amount of oligosaccharides was 1-7% of total sugars and depended on the reaction temperature and lactose concentration in the cheese-whey.

The hydrolysis of lactose in cheese-whey and cheese-whey permeates in order to obtain sweet galactose-glucose sirups has been reported in the number of papers [6, 7, 11, 12, 15]. It was stated that β-galactosidase shows catalytic effect not only on the hydrolysis of lactose but also on transgalactosidation of lactose [10]. The enzym transfers galactose to various acceptors with hydroxyl groups. When water is an acceptor — a hydrolysis of lactose takes place, when sugar molecules — oligosaccharides are formed. The data concerning their chemical nature and factors affecting their formation are not uniform [3, 13, 16]. In earlier papers of the authors the possibility of utilisation of concentrated cheese-whey permeates for galactose-glucose sirups production was demonstrated by using commercial enzymatic preparations: Maxilact R (β-galactosidase from yeast) [5], and Lactase N β-galactosidase from molds [15].

The purpose of this study was to investigate the influence of the conditions of lactose hydrolysis in the cheese-whey permeates on the qualitative and quantitative composition of the reaction products, especially oligosaccharides.

MATERIALS AND METHODS

Cheese-whey ultrafiltration: dehydrated cheese-whey (New York Research and Development Corp.) was re-hydrated at 45°C to the concentration of 25% of lactose and than ultrafiltrated using Abcor Model 225 aparatus. After ultrafiltration the permeates were demineralized by electrodialysis in the Ionics Stackpack Elektrodialysis Unit to the resistance of 220Ω/cm.

Enzymatic hydrolysis of lactose in permeates: two enzymatic preparation have been used:

1) β-galactosidase from yeast *Kluyveromyces lactis* Mixilact R 40 000 (Enzyme Development Corp.),

2) β-galactosidase from *Aspergillus niger*-Lactase N (G.B. Fermentation Industries INC).

The hydrolysis was conducted at two temperatures:
Maxilact R — at 4°C and 30°C
Lactase N — at 30°C and 55°C
— and by three concentrations of lactose: 5, 15 and 25%. Permeates containing 15% and 25% of lactose were hydrolysed after demineralization. The temperature, lactose concentration and time of hydrolysis is given in Table 1.

For yeast β-galactosidase activity pH was corrected to the value of 6.8-7.0 with potassium citrate. In case of Lactose N application pH was corrected to the value 4.5 with HCl. Concentrations of lactose for experiments were obtained by dilution of 25% lactose whey permeates. En-

Table 1. The time of hydrolysis of lactose in the cheese-whey permeates according to concentration of lactose and temperature

Concentration of lactose % w/V	Temperature			
	4°C	30°C	30°C	55°C
5	12	5	12	5
15	24	12	24	12
26	48	24	48	24
The kind of enzyme	Maxilact		Lactase N	

zymatic preparations were added directly to the hydrolysed samples in amount of 0.5% of lactose concentration. Reaction was stopped by heating samples in boiling water for 5 min.

GLC analysis of sugars: sugars were transformed into volatile silyl derivatives according to Sweeley et al. method [14]. GLC analysis of derivatives was carried out using gas chromatograph Finnigan with flame-ionisation detector. Capillary SCOT, type column 30 m long (diameter

0.36 mm) with SE-30 on Silonox 101 was used. Carrier gas hel; flow speed — 10 ml/min. Temperature programmed in the range of 110°C-260°C with the rate of 4°C/min. Detector and evaporator temperature — 260°C. Quantitative estimation was done by integrating sugar peaks areas using Minigrator (Spectra Physics Comp.) against the area of internal standard peak (mezoinositol). To estimate correction factors GC-standards of sugar (Supelco. Inc., USA) were applied. Sugars were identified by comparing their retention times with those of standars.

RESULTS

Results of lactose hydrolysis of different concentration in the permeates with both enzymes are given in Table 2 and 3. Maxilact caused almost complete hydrolysis at temperature of 4°C as well at 30°C. Although in lactose concentration 25% some amount of unhydrolysed sugar was observed (4-5%). Activity of Lactase N was much lower. This caused the remaining of 30% of unhydrolysed lactose at temperature of 90°C.
In the samples hydrolysed by Lactase N remarcable amount of saccharose was identified. Saccharose was introduced with preparation where it serves probably as a carrier agent.

The amount of galactose obtained in hydrolysis experiments was lower when compared to the amount of glucose. It is the result of oligosaccharides formation. The amount of estimated oligosaccharides was given as a sum of di- and trisaccharides in Tables 2 and 3. Total amount of oligosaccharides formed in this study is rather limited and varies in the range of 2-5% of total sugars. It depends on the concentration of lactose in permeates and the reaction temperature. No oligosaccharides were formed in concentration of lactose similar to the normal concentration in cheese-whey. Higher concentrations of lactose caused the increase of oligosaccharides quantity for both enzymatic preparation.

Especially high content of oligosaccharides was found in 25% of lactose and not deionized permeates.

The activity of transgalactosidation increase when the reaction temperature rised up to the optimum. It concerned especially to the β-galactosidase from the yeast. However, the relative higher portion of trisaccharides comparing to disaccharides was observed when enzyme from molds was used. The partial chromatograms showing separation of di- and trisaccharides after hydrolysis of 25% lactose permeates was given in Fig. 1 and 2. Generally the composition of oligosaccharides was similar as concerned of their main constitients. Hower, molds β-galactosidase action caused the formation of higher number of different oligosaccharides especially trisaccharides. The oligosaccharides profiles consisted of several peaks and depended on hydrolysis conditions. For example, in the not de-

Table 2. Relative amounts of sugars formed by the hydrolysis of lactose in whey permeates with Maxilact[R]

Temperature of incubation	Lactose concentration (% w/v)		Sugars concentration (% of total sugars)				
			glucose	galactose	lactose	disaccharides[x3)]	trisaccharides
4°C	5	I[x2)]	48.53	50.12	—	—	—
		D[x1)]	49.55	44.60	1.36	1.85	1.36
	15	I	50.73	41.73	2.45	3.27	1.80
		D	47.54	46.22	0.60	3.84	2.40
	25	I	46.75	40.18	3.70	5.40	3.86
30°C	5	I	49.37	48.21	—	—	—
		D	50.32	46.41	0.70	2.21	2.73
	15	I	47.53	42.35	2.05	3.56	3.32
		D	45.91	43.24	1.08	4.97	4.31
	25	I	48.90	37.84	4.36	8.15	7.86

x2/ — not deionized cheese-whey permeates
x1/D — deionized cheese-whey permeates
x3/ — disaccharides without lactose

Table 3. Relative amounts of sugars formed by the hydrolysis of lactose in whey permeates with lactase N

Temperature of incubation	Lactose concentration (% w/v)		Sugars concentration (% of total sugars)				
			glucose	galactose	lactose	disaccharides x/3	trisaccharides
30°C	5	I[x1]	37.80	35.54	24.23	0.5	1.61
	15	D[x2]	33.36	29.18	32.99	2.55	1.90
		I	33.93	26.71	35.19	3.24	2.70
	25	D	32.24	30.14	30.39	3.67	3.59
		I	32.15	25.60	31.35	4.82	5.62
55°C	5	I	45.11	44.18	3.25	—	—
	15	D	43.28	39.56	10.98	2.85	3.26
		I	43.65	41.33	12.08	2.81	1.21
	25	D	43.32	38.64	9.36	3.51	4.23
		I	40.73	35.14	15.17	3.99	4.87

x1/ I — not deionized cheese-whey permeates
x2/D — deionized cheese-whey permeates
x3/ — disaccharides without lactose and sucrose

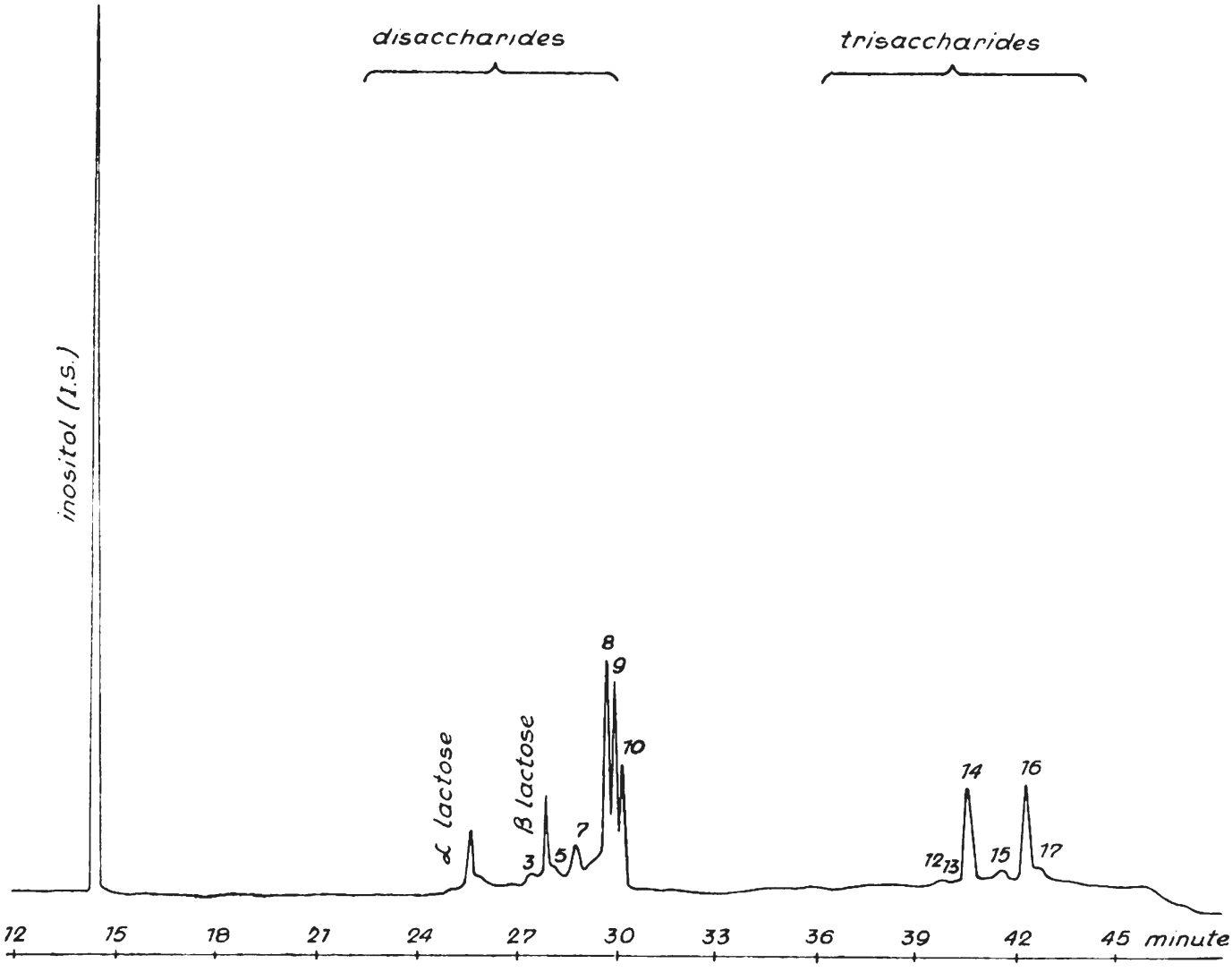

Fig. 1. Partial gas chromatogram of the TMS ethers of carbohydrates obtained after hydrolysis of lactose with Maxilact R in not deionized cheese-whey permeates (concentration of lactose 25%, temperature of hydrolysis 30°C)

ionized permeates peak No 8 is always higher than peak No 9, whereas in deionized permeates reverse relation was observed. In this study the authors did not identify GC-separated oligosaccharides because of their low quantities and lack of suitable standards.

DISCUSSION

Formation of di-and trisaccharides during hydrolysis of lactose has been shown in experimental part of this study. When lactose concentration is low (5%) quantitative estimation of oligosaccharides is hadrly possible. However by the concentration of lactose 15% and 25% the amounts of oligosaccharides were quantitatively measurable. The relationship between concentration of lactose and formation of oligosaccharides has been observed what confirms similar findings of Kosikowski et al. [15, 16] — mold β-galactosidase and of Burvall et al. [3] yeast β-galactosidase. The later authors stated twice as high concentration of oligosaccharides comparing with the concentration observed in this study. General relations concerning the activity of β-galactosidase are in agreement.

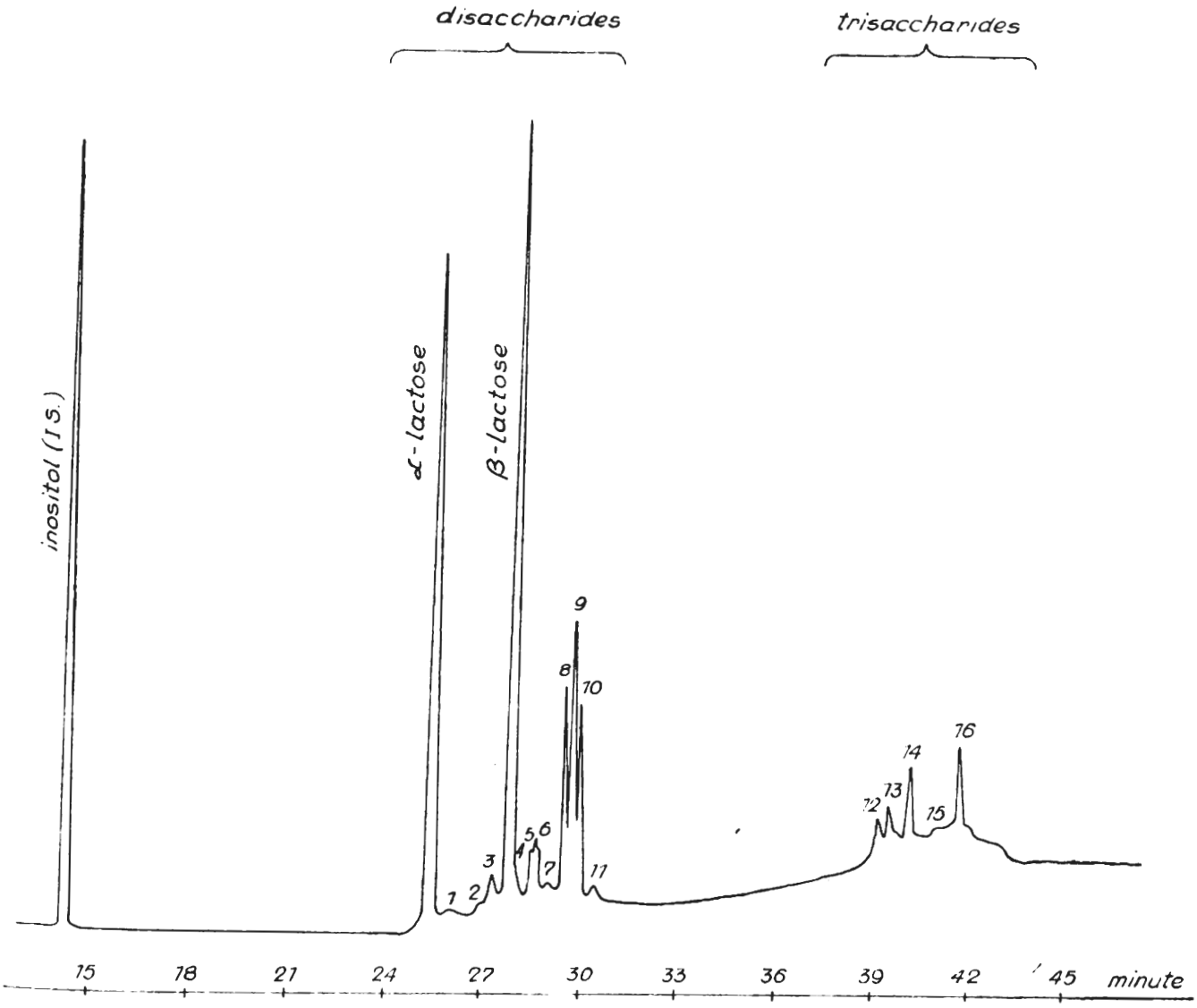

Fig. 2. Partial gas chromatogram of the TMS ethers of carbohydrates obtained after hydrolysis of lactose with Lactase N in deionized cheese-whey permeates (concentration of lactose 25%, temperature of hydrolysis 30°C)

Detailed investigations of β-galactosidase from *Escherichia coli* K-12 conducted by Wallenfels et al. [8, 9] showed that it was the multi-directional reactions of trans-galactosidation and hydrolysis effect of treating lactose by this enzyme. The first product of above reactions was allo-lactose formed as a result of replacement of galactose in the lactose from fourth to sixth position of glucose. Also lactose can be consecutively hydrolysed by the enzyme and can act as acceptor of the further galactose molecules in transgalactosidation reactions. High initial concentrations of lactose inhibit the hydrolysis of already — formed oligosaccharides what allows their identification after the hydrolysis is ended (terminated).

The similarity of the way of acting of β-galactosidase from yeast and from mold seems to explain the phenomena observed in this study as well as in the other studies on the topic. Above mentioned discrepancies with the results obtained by Burvall et al. [3] may be explained by much lower concentration of the enzyme and higher concentration of lactose applied by these authors.

Hydrolysis of oligosaccharides formed, going paralelly with the hydro-

lysis of lactose was probably partially eliminated by unsufficient (comparing with lactose) concentration of the enzyme. That resulted in relatively high amount of oligosaccharides in the final product.

The fact that application by Burvall et al. [3] of additional portions of the enzyme during the hydrolysis resulted total destruction of disaccharides, confirms also above hypothesis.

Results obtained by Robersts and Pettiniati [13] are more difficult to be explained. These authors observed extremely high amounts of oligosaccharides (up to 40% of lactose).

Deionisation of cheese-whey permeates effect not only hydrolysis of lactose — as it has been pointed out earlier [5], but also the formation of oligosaccharides. It is probably related to the specific action of some ions which influence the balance between hydrolytical and transgalactosidase activity of the enzyme. The last activity is remarkably inhibited in the low temperature — what is an argument to conduct the production of galactose-glucose sirups using β-galactosidase from the yeast at 4°C [5].

There is still a lack of agreement in the literature concerning qualitative identification of di- and trisaccharides — and also tetrasaccharides which have been found by Huber et al. [9]. Most of the authors identified only allolactose a first sugar formed as a result of enzym activity. It was identified against self-synthetized standards [2, 9]. Qualitative identification of all oligosaccharides formed as a result of β-galactosidase activity remains open.

CONCLUSIONS

1. Investigated β-galactosidase preparations: yeast origin — Maxilact R and mold origin — Lactase N showed transgalactosidase activity. The final effect of their activity is formation of di- and trisaccharides in the cheese-whey permeates.

2. In the experimental conditions the amount of oligosaccharides was in the range of 1-7% of total sugars. It depends on the several factors, mainly on the temperature and lactose concentration.

3. Qualitative composition of oligosaccharides formed by both enzymatic preparations are generally very close as far as concerns main components. Quantitative ratios among single peaks very depending on the conditions of hydrolysis.

Hydrolysis of lactose in the cheese-whey permeates was conducted using β-galactosidase of yeast and mold origin. Both enzymatic preparations showed similar transgalactosidase activity. The amount of oligosaccharides was 1-7% of total sugars and depended on the reaction temperature and lactose concentration in the cheese-whey.

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TWORZENIE OLIGOSACHARYDÓW PODCZAS HYDROLIZY LAKTOZY PRZY UŻYCIU GALAKTOZYDAZY W ODCIEKACH POULTRAFILTRACYJNYCH SERWATKI

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Streszczenie

Odcieki poultrafiltracyjne serwatki o stężeniach laktozy 5%, 15% i 25% poddano działaniu preparatów handlowych β-galaktozydazy. Zastosowano enzym pochodzenia drożdżowego o optimum działania w pH = 6,8-7,0 — Maxilact R oraz pochodzenia pleśniowego — Lactase N, którego optimum aktywności przypada na pH = 4,5. Badania preparatu β-galaktozydazy, oprócz daleko posuniętej hydrolizy laktozy, wywołały powstanie dwu- i trójcukrów w wyniku reakcji transgalaktozydacji.

Ilość wytworzonych oligosacharydów jest niewielka (1-7% ogólnej ilości cukrów) i zależy w głównej mierze od stężenia laktozy oraz temperatury działania enzymu. Czynniki te, obok pH działania i obecności soli w odciekach determinują również niewielkie zmiany w zakresie profilu jakościowego tworzonych oligosacharydów, który dla obydwu badanych enzymów jest zbliżony pod względem głównych jego składników.

Stosując metodę chromatografii gazowej estrów sililowych stwierdzono powstawanie kilkunastu oligosacharydów.