Kinetic Study of the Acid Hydrolysis of Various Oligofructose Samples

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The kinetic of acid hydrolysis of five commercially available oligofructose samples used as food ingredients has been investigated as a function of the dry matter concentration, reaction pH, and temperature. The initial fructose release rate is found to be roughly proportional to the inverse of the average polymerization degree in number. A pseudo first order kinetic is found with respect to the fructosyl chain end concentration and to the proton concentration. Arrhenius plot is found to reasonably fit the data in a relatively wide temperature range (7−130 °C). The results allow the estimation of the fructose release rate in many foodstuff processing conditions.

KEYWORDS: inulin; oligofructose; acid hydrolysis; fructose

INTRODUCTION

During the past decade, inulin and its derivatives have received considerable interest as food ingredients under the generic name of oligofructose. Oligofructose is used as a nondigestible dietary fiber and for its bifidogenic and texturing properties in many foodstuff preparations such as milk products, low fat products, bread, and drinks (*1*, *2*).

A lot of studies are focused on the inulin properties with respect to its average molecular weight and polydispersity. Few or none take into account the fact that the molecular weight distribution may be affected during food processing.

Actually, it has been stated that the chemical breakdown of inulin (hydrolysis) is easy and may occur under relatively mild pH and temperature conditions (*3*). Conditions during which partial acid hydrolysis of the inulin occurs are frequently met in foodstuff processing or during longterm storage. The main effects are a shortening of the inulin chains and the production of free sugars (fructose, glucose, and sucrose) in nonnegligible amounts. Thus, it seems important to determine whether or not hydrolysis occurs during an industrial process. Moreover, the estimation of the amount of released sugars, the resulting increase of the calorific content, and loss in fiber content are of great importance.

Many research studies deal with the total hydrolysis of inulin into its 2 constitutive monomers, glucose and fructose, for the preparation of high content fructose syrups, with special care to avoid the formation of byproducts. Enzymatic or acidic catalysis can be used to reach this goal. Both are well documented (*4−11*). Other techniques such as hydrolysis in ion-exchange resins or other catalysts have also been tested (*5*, *12*, *13*). Kinetic of degradation of inulin at high temperature in the presence of citric acid has also been studied (*14*). On the other hand, only a few studies concern partial acid hydrolysis of inulin.

The present work is devoted to the study of the partial acid hydrolysis of 5 different oligofructose samples derived from chicory (*Cichorium intybus*) inulin or enzymatically synthesized. Only the first stages of the hydrolysis are investigated. As fructose is the major end product of acid hydrolysis, its concentration increase is the main point investigated here. The initial reaction kinetic has been studied for various dry matter concentrations, pH levels, and temperatures. The final aim is to provide a convenient method for estimating the fructose release rate during acid hydrolysis in given temperature and pH conditions.

MATERIALS AND METHODS

Materials. The five oligofructose samples used in this study are commercially available products. Actilight 950P is a product of Béghin-Meiji industries (Neuilly-sur-Seine, France). Raftilose P95 is a product of Raffinerie Tirlemontoise (Tienen, Belgium), and Fibrulose 97, Fibruline Instant, and Fibruline Long Chain are products of Cosucra (Fontenoy, Belgium). All are white powders with a moisture content around 5%. Some of their characteristics are given in **Table 1**.

Semiquantitative estimation of the molecular weight distribution of these samples was done by high-performance anion exchange chromatography coupled with pulse amperometric detection (HPAEC−PAD) on a Dionex DX500 chromatographic system operating at 1 mL/

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Table 1. Main Characteristics of the Various Oligofructose Samples

	fru[a] (%)	glu[a] (%)	sac[a] (%)	free sugars[a] (%)	$\overline{Dp_n}$[b]	GF$_n$[c] (%)	F$_n$[c] (%)	FCE[d]
Actilight 950P	0.5	0.2	2.6	3.3	3	100	0	0.19
Raftilose P95	1.5	0.5	3.0	5.0	4	50	50	0.23
Fibrulose 97	0.5	0.2	2.5	3.2	5.5	80	20	0.11
Fibruline Instant	3.0	1.0	3.0	7.0	9	90	10	0.071
Fibruline Long Chain	0.5	0.1	0.3	0.9	22	95	5	0.036

[a] Mass percentage estimated by HPLC. [b] Average polymerization degree in number estimated by HAPEC–PAD. [c] Mass percentage estimated by HAPEC–PAD. [d] Number of fructosyl chain ends estimated by HAPEC–PAD (in arbitrary units).

min. Aliquots of 25 μL of a 0.8 g/L solution were injected. Separation of the various chain lengths was achieved on a PA100 column. Sodium hydroxide (150mM) was used as eluent. Sodium acetate gradient was applied and amperometric detection was used (*15*). Relative response factors of the various species were taken from references (*16*, *17*). The relative fructosyl chain end (FCE) concentration was estimated by multiplying the relative concentration of a molecular species by its corresponding fructosyl chain ends content, 1 for fructooligosaccharides (GF$_n$) and 2 for inulooligosaccharides (F$_n$), and this for the total Dp range except the monomers.

Hydrolysis. Limpid solutions of these samples were prepared by dissolving the required amount of powder in boiling water at neutral pH. The solutions were then cooled and sodium acetate (or sodium citrate) (Sigma, 99.0%) was added as buffer to reach a final concentration of 0.05 M. The pH was then adjusted to the desired value. Hydrochloric acid, sulfuric acid, and acetic acid (UCB, Leuven, Belgium, analytical-reagent grade) were used. However, as the hydrolysis rate was found to be independent of the type of acid used, hydrochloric acid was used in most of the experiments.

For long time experiments sodium benzoate was added as a preservative.

For the experiments above 40 °C, 15-mL glass tubes were filled with 2 mL of solution, tightly closed, and immersed in a water bath controlled to 0.05 °C. In most cases less than 40 s was needed to reach the desired temperature ±1 °C. Tubes were periodically removed from the bath and cooled, and one drop of sodium hydroxide 3 M was then added to stop the reaction. The maximum pH variation during hydrolysis was found to be 0.15. Experiments above 100 °C were done in stainless steel tubes in an oil bath. For long time experiments (below 40 °C), the tubes were stored at 7 ± 2 °C or 20 ± 1 °C or 37 ± 1 °C. Hydrolysis was generally conducted with 10% (w/w) solutions. Some experiments were also realized with 2, 5, 20, 30, and 40% dry matter solutions.

Analysis. Assessment of the fructose concentration was done by HPLC after dilution to 2% of dry matter. The HPLC line included a Waters 515 pump, a Waters 717 plus Autosampler, and a 2410 refractive index detector. Millenium software was used for data acquisition and processing.

The Waters Sugar Pak 1 chromatography column was used for the determination of fructose. The flow rate was 0.5 mL/min and a volume of 10 μL of the sample was injected.

Hydrolysis generally leads to an increase of the total dry matter of the solution as a water molecule is added to each broken bond. However, as most of the analysis reported here concerns only the beginning of the hydrolysis, no correction was made to account for this minor effect.

The initial rates of the hydrolysis reaction were graphically determined as the slope at zero time of the curve giving the fructose concentration as a function of time.

RESULTS AND DISCUSSION

The five commercially available oligofructose samples used in this study are described in **Table 1**. Actilight 950P, which is obtained by enzymatic synthesis from sucrose, has the shortest

average chain length. Raftilose P95 results from hydrolysis of chicory inulin with endoinulinase; Fibruline Instant has roughly the same composition as chicory inulin. Fibrulose 97 and Fibruline LC are both obtained by fractionation of chicory inulin. The former is mainly composed of short inulin chains up to a polymerization degree (Dp) of 20 (Dp20), and the latter is composed of the longer inulin chains with very little amount of chains shorter than Dp10.

Though the general term oligofructose is often used to name inulin and inulin derivatives, a distinction has to be made between molecules containing only fructosyl residues, designated as inulooligosaccharides (IOS or F$_n$), and molecules containing fructosyl residues ended by a glycosyl residue, which are designated fructooligosaccharides (FOS or GF$_n$). Both are produced in the course of the hydrolysis reaction. The five starting samples differ principally by their average chain length and also by their relative F$_n$ and GF$_n$ concentrations as can be seen in **Table 1**.

The main problem treated here is the fructose production rate and its dependence on oligofructose type, oligofructose concentration, reaction pH, and temperature.

Fructose (*F*) and glucose (*G*) are the final products of several consecutive and competitive reactions catalyzed by acid. The exact description of the whole mechanism would involve a large number of reaction constants, most of which are unknown. In a first step, the release rate of fructose, which is the major product, is analyzed as a function of the oligofructose type and concentration, the reaction pH, and temperature. Results concerning the other small sugars (glucose and sucrose released during hydrolysis of oligofructose) will be published elsewhere. The reactions yielding fructose may be summarized as follows:

$$R - F \xrightarrow{H^+} R' - F + F \qquad (1)$$
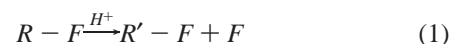
where $R - F$ is a reaction intermediate which can be either an inulooligosaccharide or a fructooligosaccharide. Its concentration will vary during hydrolysis in a complex fashion. From a kinetic point of view, it seems obvious that the chemical nature of the radical R will influence the end scission reaction rate. For example, it has been stated that the breakdown rate was much greater for inulobiose (F$_2$) than for sucrose (GF). However, it was also found that the kinetic constant is only slightly dependent on the chain length from Dp2 to Dp7 (*18*). For a given oligofructose sample, the initial rate method is used here to compare reaction kinetic in different pH and temperature conditions. This method is also used to compare the hydrolysis rate of different samples in the same reaction conditions.

Influence of the Starting Fructooligosaccharide. As already mentioned, the various starting materials investigated here exhibit very different average chain lengths. It seems obvious that the rate of fructose release during hydrolysis is dependent on the fructosyl chain ends concentration and thus on average chain length distribution.

The fructose release is expected to be much slower for long molecules for which the probability of inside chain scission is much higher. HPAEC−PAD has been used to roughly estimate the amount of fructosyl chain ends (FCE) of the various starting samples (**Table 1**).

It can be seen in **Figure 1a** that for identical pH and temperature conditions, the shorter oligomers actually transform into fructose faster than the longer ones. The largest fructose release rate is observed for Raftilose P95 which presents the highest FCE concentration. This is due to the fact that this compound has a relatively small average chain length and also the highest content of Fn molecules, which possess 2 ending
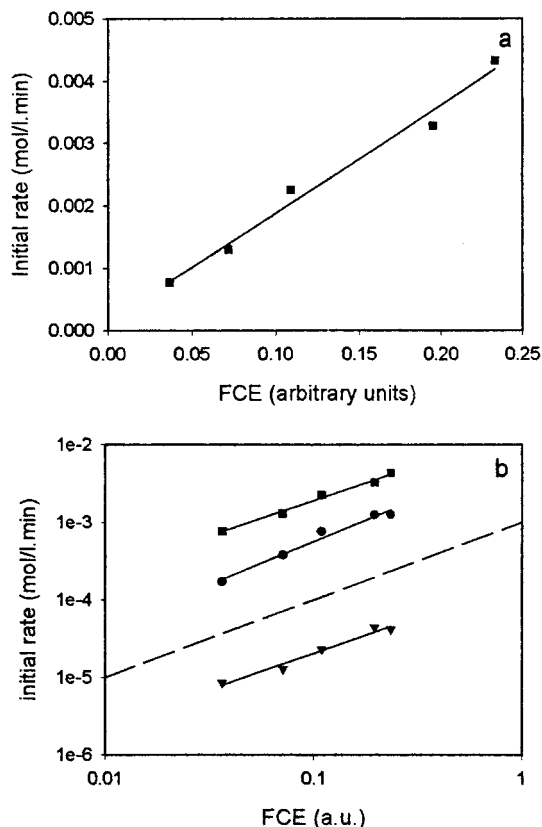
Figure 1. (a) Initial fructose release rate as a function of the fructosyl chain ends concentration for the hydrolysis of the various oligofructose samples at 70 °C, pH = 2.75 (■). (b) Double logarithmic plot of the initial rate as a function of the number of fructosyl chain ends for the experiment at pH = 4 and T = 90 °C (●), pH = 3 and T = 40 °C (▼), and pH = 2.75 and T = 70 °C (■). The dashed line has a slope of 1.

fructosyl residues instead of only one for the GFn series. The initial reaction rate is found to linearly increase with the FCE concentration. A double logarithmic plot (**Figure 1b**) indicates that the reaction order with respect to fructosyl chain ends concentration is independent of the hydrolysis conditions (pH and temperature).

The chemical composition of the starting material has, thus, a marked influence on the initial reaction rate, but also on the shape of the curve giving the fructose concentration as a function of time as shown in **Figure 2** for hydrolysis at 70 °C at pH = 3. The major feature is the change of concavity observed when going from the smaller to the longer average chain length. This behavior may be explained by considering the evolution of the FCE concentration during hydrolysis. For the long chain inulin, this concentration is initially very low, so the scissions mainly occur inside the chain, and yield two smaller chains. The probability for end-of-chain scissions is low so that the concentration of free fructose only slightly increases while the number of ends of chain increases rapidly. When the chains are sufficiently shortened, end-of-chain scissions take place, and lead to an acceleration of the fructose production. This explains the upward concavity observed for Fibruline long chain and for Fibruline Instant. In contrast, the smaller oligomers have a relatively high initial concentration of chain ends, with the resulting free fructose production rate being large. During the hydrolysis only some new ends of chain are created from scissions of the longer oligomers, which are few. The total concentration of fructose precursors thus continuously decreases in the course of the reaction and so does the fructose release
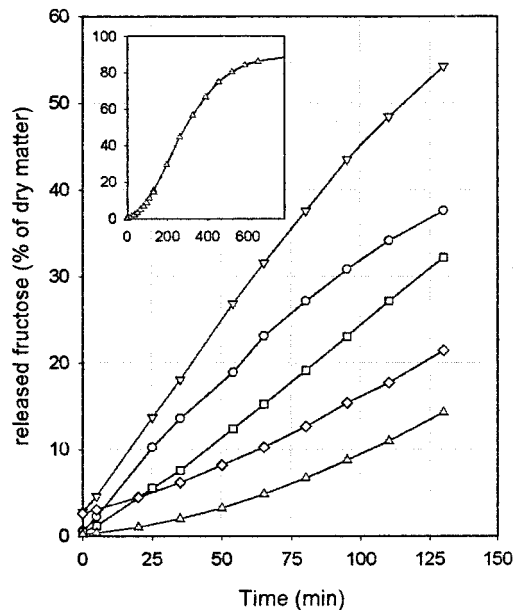

Figure 2. Evolution of the fructose concentration versus time at pH = 3, T = 70 °C for 5 different oligosaccharides: Actilight 950P (○), Raftilose P95 (▽), Fibrulose 97 (□), Fibruline Instant (◇), and Fibruline Long Chain (△). The inset illustrates the change of concavity observed during long time hydrolysis of Long chain inulin.

rate. This leads to the downward concavity for Actilight 950P and Raftilose P95 as one would have expected for a real first order kinetic. A somewhat intermediary case is that of Fibrulose 97, for which the disappearance of chain ends seems to be compensated by the creation of new ones by scissions of longer molecules. This explains the straight line observed for Fibrulose 97, i.e., pseudo constant reaction rate. Note that this is valid only at the early stages of the reaction. If total hydrolysis of Fibruline long chain is considered, the curve giving the fructose concentration versus time contains an inflection point (see inset of **Figure 2**). The second part of the curve is then comparable to what is obtained at the beginning of the hydrolysis of smaller oligomers.

Influence of Oligofructose Concentration. Typical inulin concentration in foodstuff ranges from 1−2% to 20%. Expressed on a water basis these values can be somewhat higher. Most of the data presented in this study were obtained for 10% (weight by weight) dry matter solutions. However, to determine a potential influence of the concentration on the hydrolysis rate some experiments were carried out with 2, 5, 10, 20, 30, and 40% solutions. Because of its relatively low solubility, LC was tested only up to 20%. For a given starting compound, the initial reaction rate expressed in mol/L·min is found to be proportional to the concentration of the oligofructose (see some examples in **Figure 3a**) except for a high concentration of Fibruline Instant. In this case, the initial rate is found to be lower than what would have been expected from extrapolation of low concentrations data. This decrease of the reaction rate at high concentration was already documented for total hydrolysis of inulin (*6, 7*). This may be due to a reduced solubility at high concentration, as well as to an increase of the viscosity of the reaction medium. On the other hand, for the smaller oligofructose samples which show a high solubility, the reaction rate is found to be directly proportional to the dry matter concentration up to 40% (w/w). A double logarithmic plot (**Figure 3b**) yields a slope of ∼1 indicating a first order kinetic with respect to polysaccharide concentration. This result was foreseeable, as it
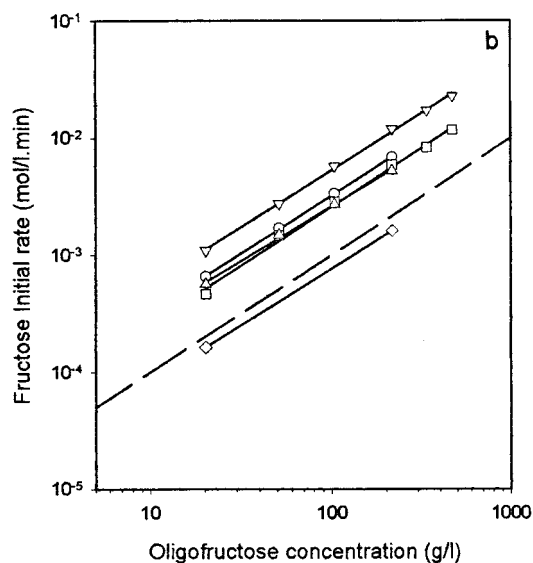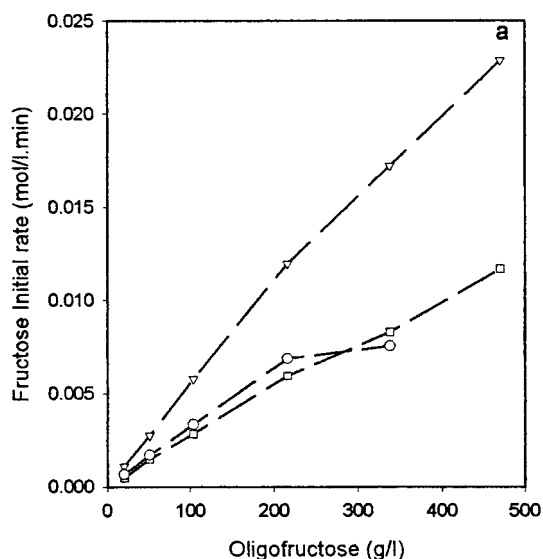
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Figure 3. (a) Initial fructose release rate as a function of the oligofructose concentration for hydrolysis of Fibruline Instant at pH = 2.75 and T = 80 °C (O), Raftilose P95 at pH = 3 and T = 80 °C (▽), and Fibrulose 97 at pH = 3 and T = 80 °C (□). (b) Double logarithmic plot of the initial fructose release rate as a function of the oligofructose concentration for hydrolysis of Fibruline Instant at pH = 2.75 and T = 80 °C (O), Raftilose P95 at pH = 3 and T = 80 °C (▽), Fibrulose 97 at pH = 3and T = 80 °C (□), Fibrulose 97 at pH = 3 and T = 70 °C (◇), and Fibruline Long Chain at pH = 3 and T = 80 °C (△).The dashed line has a slope of 1.

was found in the previous section that the reaction rate was directly proportional to the concentration of fructosyl ends of chain which is also directly related to dry matter concentration for a given oligofructose sample.

The fructose concentration is commonly expressed in percent of the total oligofructose content. In such a case, the reaction rate expressed in percent fructose per time unit is thus independent of the initial oligofructose concentration.

Influence of the Reaction pH. As far as acid catalysis is concerned, it was stated that the rate of hydrolysis of inulin was dependent on the proton concentration [H⁺] but did not depend on the type of acid used (*4*), so that a single pH measurement allows determination of whether hydrolysis occurs or not. It is reported that production of fructose syrups is possible
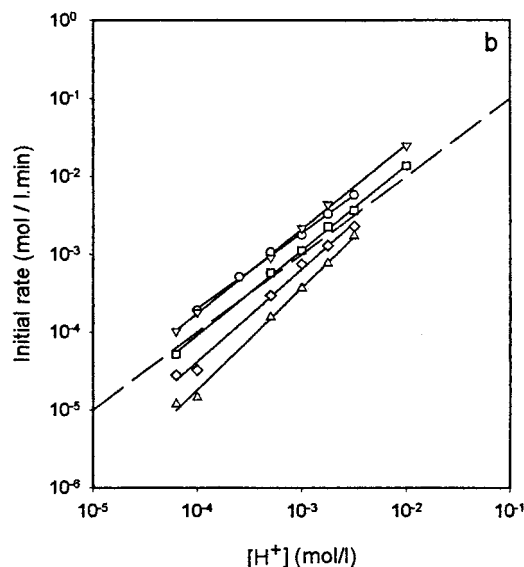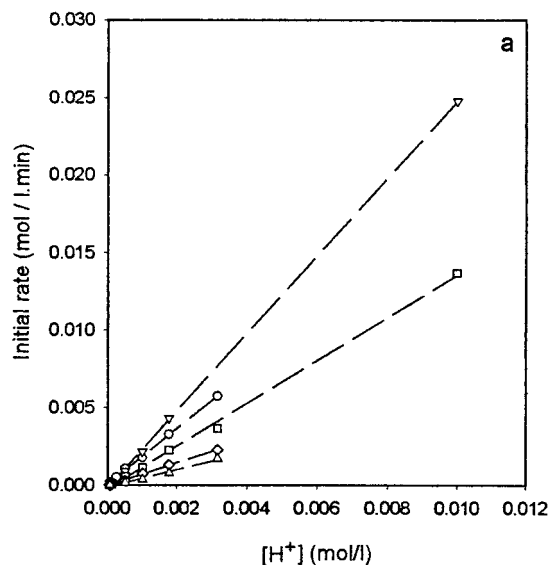
Figure 4. (a) Initial fructose release rate as a function of the H⁺ concentration during hydrolysis at 70 °C for the 5 oligofructose samples: Actilight 950P (O), Raftilose P95 (▽), Fibrulose 97 (□), Fibruline Instant (◇), and Fibruline Long Chain (△). (b) Double logarithmic plot of **Figure 4a**. Same symbols. The dashed line has a slope of 1.

in relatively mild acidic conditions (at high temperature) as well as at low pH (at moderate temperature) (*7*).

The results presented here are restricted to a relatively small pH range (2.0−4.2) which corresponds to the conditions usually met in foodstuffs (preparation and storage) and for which hydrolysis is expected to occur to a significant level.

Figure 4a shows the evolution of the fructose release rate as a function of proton concentration for hydrolysis experiments conducted at 70 °C. The initial rate is found to be directly proportional to [H⁺]. Similar results are obtained for other temperatures. A log−log plot (see **Figure 4b**) yields a slope of 1, indicating a first order with respect to [H⁺] at 70 °C over the pH range investigated here. In other words, decreasing the solution pH by 1 unit will increase the hydrolysis rate by a factor of 10. **Table 2** presents the values obtained for the various oligofructose samples as a function of temperature, and it can be seen that an partial order 1 is observed over the whole temperature range. Let us note that side reaction(s) may occur during hydrolysis of inulin. It was namely found to happen

Table 2. Partial Reaction Order with Respect to the Proton Concentration for the 5 Oligofructose Samples at Several Temperatures

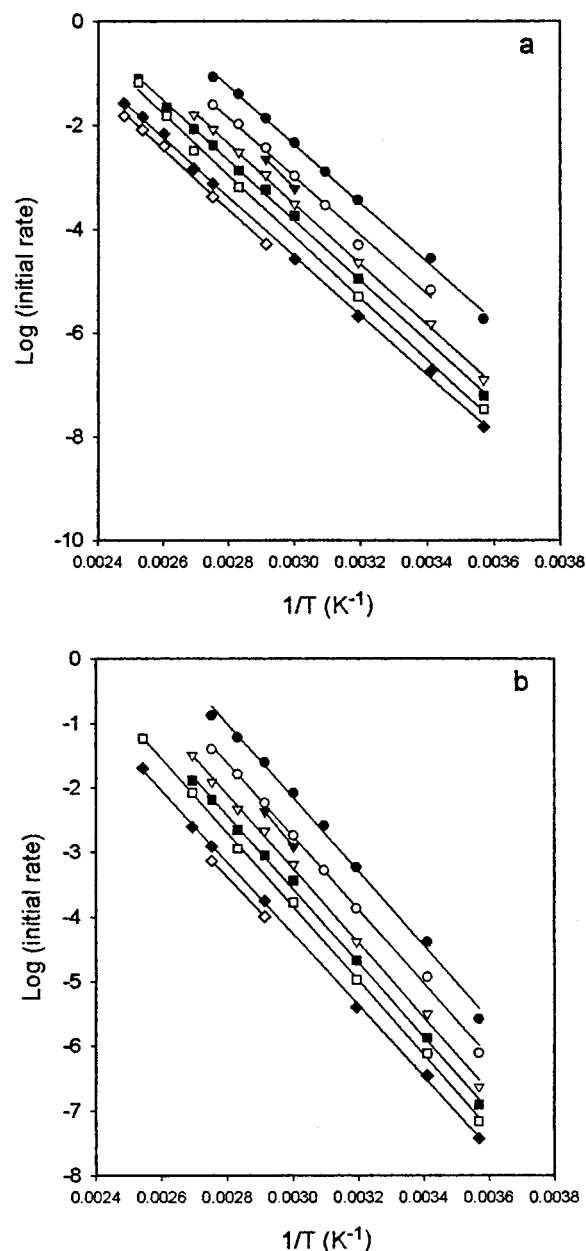| T (°C)	Actilight 950P	Raftilose P95	Fibrulose 97	Fibruline Instant	Fibruline Long Chain
7	0.9	0.9	0.8	0.9	1.0
20	1.0	1.1	1.1	1.0	1.0
37	1.0	1.1	1.1	1.0	1.0
60	1.1	0.9	1.1	1.1	
70	1.0	1.1	1.1	1.2	1.3
80	1.0	1.1	1.1	0.9	1.0
90	1.1	1.0	1.0	1.1	1.3
98	1.0	1.1	1.1	0.9	0.9
120		1.2			
122			1.2		
130			1.2		
av. order	1.0	1.0	1.1	1.0	1.1
st. dev.	0.1	0.1	0.1	0.1	0.2

during total hydrolysis for the preparation of fructose syrups in relatively drastic conditions. In this case byproduct (difructose-dianhydrides) concentration remains limited to a few percent. The experimental conditions used in the present study are relatively mild, one may thus expect the side reactions to be much more limited. Moreover, no byproducts were found during analyses.

Thermal Evolution of the Rate Constant. In most of the works dealing with total hydrolysis of inulin toward the production of high concentration fructose syrups the reaction temperature was investigated. Low and high-temperature experiments were conducted in order to minimize color and caramel formation. However, only a few studies have been published concerning the thermal evolution of the reaction rate at the beginning of the reaction. Foodstuff preparation generally involves high-temperature steps such as sterilization and also long-term storage at low temperature. The hydrolysis experiments of the present study were conducted at various pH levels for temperatures ranging from 7 to 100 °C. Some experiments were also conducted under pressure up to 130 °C. As the corresponding hydrolysis rates vary by several orders of magnitude, the time scales of these experiments vary from a few minutes to several months. The data were analyzed assuming an Arrhenius type thermal dependence of the initial reaction rate. The results obtained for Fibrulose 97 and Raftilose P95 are presented in **Figure 5a** and **5b**, respectively. Similar results are observed for the other oligofructoses. Linear behaviors are found over the whole temperature range for the various pHs investigated. Activation energy E* may be extracted from the slope of the Arrhenius plots. Values of E* are found to be independent of the reaction pH of the oligofructose type. The average value of E* is (109 ± 10) kJ/mol which is surprisingly very close to that of acid hydrolysis of sucrose (106 kJ/mol).

The major interest, for those who are involved in processing of food containing oligofructose, is to avoid the degradation of this relatively expensive ingredient and thus to find the acceptable conditions for which the hydrolysis is minimized. A fitting procedure was applied to our results and the following relations are found to reasonably fit the data:

$$\text{Log (initial rate)} = y_0 - \frac{a}{(273.1 + T)} - \text{pH}$$

where T is the temperature in °C, and y_0 and a are fit parameters that depend on oligofructose type (see **Table 3**).

Figure 5. Arrhenius plot for hydrolysis of Fibrulose 97 (a) and Raftilose P95 (b) at pH = 2 (●), pH = 2.5 (○), pH = 2.75 (▼), pH = 3 (▽), pH = 3.3 (■), pH = 3.6 (□), pH = 4 (◆), and pH = 4.2 (◇).

Table 3. Fit Parameters for the Various Oligofructose Samples

	y_0	a
Actilight 950P	19.045	5680
Raftilose P95	19.048	5672
Fibrulose 97	18.647	5620
Fibruline Instant	18.011	5497
Fibruline Long Chain	17.873	5520

As the initial rate is given in % of fructose created per min on total oligofructose concentration, these equations are independent of the oligofructose concentration. Note that these easy to handle semiempirical equations involving only two parameters allow a rough estimate (±20%) of the initial fructose release rate over a very wide range of values (5 orders of magnitude). However, this is quite enough to determine the potential risk of degradation.

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Concluding Remarks. Acid hydrolysis of 5 different oligofructose samples was investigated over wide ranges of temperature and pH. The fructose production rate was found to be much more important for the smaller oligomers, which have a relatively high content of fructosyl ends of chain. Pseudo first order kinetics were found with respect to the oligofructose concentration (or fructosyl chain ends concentration) and to the proton concentration. For each oligofructose sample, the thermal dependence of the initial reaction rate was found to follow an Arrhenius type law over the whole temperature, and pH ranges usually met in foodstuff preparation and storage.

The model used in this work shows that hydrolysis may occur during food processing, so that the resulting composition might be considerably different from that of the initially added oligofructose. The results obtained here for binary systems allow estimation of the potential risks of degradation of the oligofructose for any commonly used pH and temperature conditions.

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