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Enzymatic determination of inulin in food and dietary supplements

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Abstract An enzymatic method for the determination of the oligofructoside inulin based on the enzymatic hydrolysis of inulin by inulinase was developed. Fructose formed in this reaction was phosphorylated by adenosine-5′-triphosphate and hexokinase, and isomerized to glucose-6-phosphate by phosphoglucose isomerase. Glucose-6-phosphate was finally oxidized to gluconate-6-phosphate by glucose-6-phosphate dehydrogenase. Reduced nicotinamide adenine dinucleotide phosphate formed in this sequence of reactions is determined spectrophotometrically by its absorbance at 340 nm. The absorbance is directly proportional to the initial amount of inulin. Precision, accuracy, repeatability and recovery rates of inulin following standard addition to sample solutions were evaluated. All steps of the enzymatic analysis were carried out on an automatic centrifugal analyzer. This, together with rapid and simple sample preparation, makes the method suitable for the routine determination of inulin in food surveilance and for quality control purposes.

Key words Inulin · Inulinase · Dietetic food · Dietary supplements · Artichokes · Viper's grass · Topinambur

Introduction

Inulin is an oligofructofuranoside containing up to 35 fructose units linked via β-1,2-glycosidic bonds. The fructose chain is usually terminated by a 2,1-bonded α-glucose. Inulin is found as a storage carbohydrate in many plant roots and tubers, for example dahlia tubers or chicory root. Some of these plants, such as artichokes (*Cynara scolymnus* L.), viper's grass (*Scorzonera hispanica* L.) and topinambur (*Helianthus tuberosus* L.), are regularly used as vegetables in human nutrition.

Inulin is used for the production of dietetic foods, such as dietetic chocolates or dietetic bread, for diabetes mellitus patients. More recent interest in inulin stems from the fact that it is used as a so-called praebiotic. Praebiotics are substances added to food that are not reabsorbed in the digestive system and therefore pass undegraded into the colon, where they stimulate the propagation and metabolic activity of certain microorganisms (mainly bifidobacteria) [1, 2].

Finally, inulin is used by the food industry to produce fructose syrups of various types after either enzymatic or acid hydrolysis.

In food inulin has previously mainly been determined by high-performance liquid chromatography (HPLC) methods [3–8]. Although enzymatic methods figure prominently in the analysis of carbohydrates such as glucose, fructose, saccharose, oligosaccharides and starch in food [9], there are no reports dealing with the enzymatic determination of inulin in food. This is in marked contrast to the situation in clinical chemistry, where enzymatic analyses of inulin in urine and plasma are routinely carried out to determine the so-called inulin clearance, an important parameter in assessing renal function [10–15].

In the present work we investigated the application of an enzymatic method to the determination of inulin in vegetables, dietetic chocolates and dietary supplements. All stages of the determination were carried out on an automatic centrifugal analyzer.

Materials and methods

Method

Inulin is enzymatically hydrolyzed to fructose by the enzyme inulinase. Fructose is then phosphorylated to fructose-6-phosphate by adenosine-5′-triphosphate (ATP) in the presence of hexokinase (HK). Fructose-6-phosphate is isomerized to glucose-6-phosphate by phosphoglucose isomerase (PGI). Glucose-6-phosphate

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is then oxidized to gluconate-6-phosphate by nicotinamide adenine dinucleotide phosphate (NADP) in the presence of glucose-6-phosphate dehydrogenase (G6P-DH). The amount of the reduced form of NADP that is formed in this reaction (NADPH) is determined by its absorbance at 340 nm and is equivalent to the amount of inulin initially present in the sample solution. These reactions are summarized as follows:

$$\text{inulin} + (n-1)\,H_2O \xrightarrow{\text{inulinase}} n \text{ fructose}$$

$$\text{fructose} + \text{ATP} \xrightarrow{\text{HK}} \text{fructose-6-phosphate} + \text{ADP}$$

$$\text{fructose-6-phosphate} \xrightarrow{\text{PGI}} \text{glucose-6-phosphate}$$

$$\text{glucose-6-phosphate} + \text{NADP}^+ \xrightarrow{\text{G6P-DH}} \text{gluconate-6-phosphate} + \text{NADPH} + \text{H}^+$$

To quantify inulin by this sequence of reactions it is necessary to determine the amounts of glucose and fructose present in the sample prior to the analysis of inulin because they contribute to the measured total absorbance in the assay of inulin. To avoid having to do this for glucose, and because very high amounts of glucose in the sample tend to make the quantification of inulin less precise, glucose can be removed from the reaction system by oxidation with oxygen in the presence of glucose oxidase (GOD) before the determination of inulin, according to the following reaction:

$$\text{glucose} + H_2O + O_2 \xrightarrow{\text{GOD}} \text{gluconate} + H_2O_2$$

The amount of fructose in the sample still has to be determined prior to the analysis of inulin.

Two different assay protocols (A and B) have been developed, one without (A) and one with (B) the oxidation of glucose in the reaction system. These protocols are described in further detail below.

Reagents and standards

Citrate buffer pH 4.6 (0.127 M) was prepared by dissolving 6.9 g citric acid monohydrate and 9.1 g trisodium citrate dihydrate in approximately 200 ml deionized water and adjusting the pH to 4.6 with NaOH. The volume was made up to 500 ml with deionized water. Carrez I was prepared by making 150 g potassium hexacyanoferrateII up to 1000 ml with deionized water. Carrez II was prepared by making 300 g zinc sulphate heptahydrate up to 1000 ml with deionized water. All chemicals were from Merck, Darmstadt, Germany.

The test combination (TC) D-Glucose for biochemical analysis and food analysis was obtained from Boehringer, Mannheim, Germany. Hexokinase/glucose-6-phosphate dehydrogenase (EC 2.7.1.1/1.1.1.49) suspension (340 and 170 U/ml), phosphoglucose isomerase (EC 5.3.1.9) suspension (175 U/ml) and glucose oxidase (EC 1.1.3.4) lyophilisate (grade I, 250 U/mg) (were all obtained from Boehringer, Mannheim, Germany) and *inulinase* (EC 3.2.1.7) lyophilisate (17 U/mg) was obtained from Fluka, Buchs, Switzerland.

Glucose, fructose, saccharose and maltose monohydrate (for biochemical purposes were all obtained from Merck) and maltodextrine (glucose equivalent 4–7) was obtained from Aldrich. *Inulin* from dahlia tubers was obtained from Fluka, inulin from chicory root was obtained from Sigma (Wien, Austria) and inulin for biochemical purposes was obtained from Merck.

Start reagent 1a (SR1a) (protocol A) was prepared by dissolving 5 mg inulinase in 2 ml citrate buffer. Start reagent 1b (SR1b) (protocol B) was prepared by dissolving 5 mg inulinase and 1 mg glucose oxidase in 2 ml citrate buffer. Start reagent 2 (SR2) (protocols A and B) was prepared by mixing PGI suspension and HK/G6P-DH suspension (1:1, v/v). Reagent solution (protocols A and B) was prepared by dissolving the contents of bottle 1 of TC D-glucose (7.2 g powder containing triethanolamine buffer pH 7.6, 110 mg NADP, 260 mg ATP, magnesiumsulphate and stabilizers) in 45 ml deionized water. For the determinations 10 ml of this solution was mixed with 19 ml deionized water.

Apparatus

The main items of apparatus used were an automatic centrifugal analyzer (COBAS FARA II, Hoffmann LaRoche, Basel, Switzerland), an ultrasonic bath (heating range: room temperature to 80 °C) (Bandelin, Berlin, Germany) and an Ultra-Turrax homogenizer (IKA-Werk, Staufen, Germany). Paper filters (Schleicher & Schüll, Dassel, Germany, 595½, $\phi = 125$ mm) and various volumetric flasks, pipettes, etc. were also used.

Sample preparation

Viper's grass and topinambur were peeled and cut into small pieces with a household knife. The outer parts of the artichokes were removed and the inner parts cut into pieces with a household knife. Samples (from 2 to 15 g) of these foods were accurately weighed into a beaker and approximately 50 ml deionized water added. This mixture was homogenized for 5 min with an Ultra-Turrax homogenizer. The resulting suspension was quantitatively transferred into a 100 ml volumetric flask and sonicated at 60 ± 5 °C for 30 min. After cooling to room temperature the contents of the flask was treated with 1 ml Carrez I and II and the resulting mixture was brought to volume with deionized water and filtered through a paper filter. The clear filtrate was diluted such that the concentration of inulin was less than 700 mg/l.

Samples (2–5 g) of dietetic chocolates and dietary supplements (effervescent tablets, chewing tablets) were weighed accurately, suspended or dissolved in approximately 50 ml of hot deionized water, quantitatively transferred into a 100 ml volumetric flask and sonicated at 60 ± 5 °C for 30 min. After cooling to room temperature the contents of the flask was treated with 1 ml Carrez I and II, and the resulting mixture was brought to volume with deionized water and filtered through a paper filter. The clear filtrate was diluted such that the concentration of inulin was less than 700 mg/l.

As was verified in experiments with standard inulin solutions of different concentrations, the treatment with Carrez reagents did not influence the amount of inulin determined.

Centrifugal analyzer program

In all determinations the following conditions were used: temperature 37 °C, l = 340 nm, end-point mode. absorption was measured against a reagent blank.

Reaction without GOD (protocol A). 10 µl sample solution was treated with 20 µl SR1a and incubated for 900 s. To this sample was added 250 µl reagent solution and the absorbance was read after 10 s (E_1). Next 20 µl SR2 was added and after a reaction period of 730 s the absorbance was read again (E_2) and the difference in the absorbances was calculated ($\Delta A = (E_2 - E_1)_{\text{sample}} - (E_2 - E_1)_{\text{blank}}$).

Reaction with GOD (protocol B). All parameters were as above except that instead of SR1a, start reagent SR1b was used and after addition of SR1b the incubation time was increased from 900 to 2700 s.

Calculation

The amount of inulin in the sample solution was determined by comparison with a standard calibration plot.

Results and discussion

As already mentioned above, because of the nature of the determination, glucose and fructose contribute fully

to the measured absorbance difference in the inulin assay, thus giving ΔA_{tot}. The differences in absorbance due to glucose (ΔA_{glu}) and fructose (ΔA_{fru}) must therefore be determined in separated runs by the usual enzymatic procedures [9]. Further investigation also showed that saccharose and maltose were totally hydrolyzed by the inulinase used. Higher oligoglucosides were degraded incompletely (maltodextrin, glucose equivalent 4–7, was only broken down to approximately 80%). The differences in absorbance caused by saccharose (ΔA_{sacc}) and oligoglucosides (ΔA_{oligo}) (where maltose is already included) therefore also have to be determined in separate runs. Lactose is not hydrolyzed by inulinase. It was found that inulin does not interfere with the determination of glucose, fructose, saccharose, oligoglucosides and starch under the usual reaction conditions [9].

When oligoglucosides are absent from the sample, or only present in small amounts compared to the other carbohydrates, the determination of inulin in the sample can be carried out following reaction protocol A: the differences in absorbance due to glucose (ΔA_{glu}), fructose (ΔA_{fru}), saccharose (ΔA_{sacc}) and oligogluocosides (ΔA_{oligo}) are determined in separate runs. Afterwards the determination of inulin is carried out, yielding a total difference in absorbance, ΔA_{tot}. The difference in absorbance due to inulin (ΔA_{inulin}) is then calculated as follows:

$$\Delta A_{inulin} = \Delta A_{tot} - \Delta A_{glu} - \Delta A_{fru} - 2 \times \Delta A_{sacc} - \Delta A_{oligo} \quad (1)$$

Figure 1 shows the absorbance-time plots for a reagent blank, two inulin standard solutions and an artichoke extract. The time-scale of the plot starts at the time of addition of SR2 to the reaction system. After that time the absorbance increases until it reaches a constant value after about 5 min. The reaction is therefore well suited for end-point measurement.

A calibration plot of the concentration of inulin standard solutions against the difference in absorbance, ΔA_{inulin}, was rectilinear for inulin concentrations up to 700 mg/l [correlation coefficient 0.9999, c (mg/l inulin) $= (725.16 \times \Delta A_{inulin}) + 1.46$].

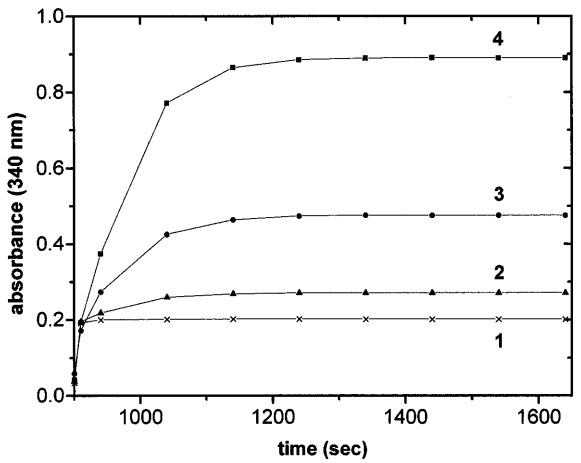

Fig. 1 Absorbance-time plots for a reagent blank solution (*1*), a 50 mg/l inulin standard solution (*2*), an artichoke extract (*3*) and a 500 mg/l inulin standard solution (*4*)

The repeatablity of the method was tested with a standard solution, which nominally contained 377 mg/l glucose and 220 mg/l inulin. The inulin concentration found was 212.6 ± 2.2 mg/l (95% confidence interval of the mean, $n = 7$), resulting in a recovery rate of 96.6 ± 1% and $r_{lab} = 6.7$ mg/l. The repeatability, r_{lab}, is defined as the largest absolute difference expected between two individual measurements based on a 95% probability basis, if the analysis is carried out by one operator using one piece of equipment over a relatively short time span.

The influence of other carbohydrates on the determination of inulin was studied using two test mixtures, T1 and T2, with the composition and nominal concentrations of carbohydrates shown below. The following results were obtained (the measured difference in absorbance, the resulting concentration and the recovery rate are given in parentheses):

T1: glucose 117 mg/l ($\Delta A_{glu} = 0.1594$, 117.0 mg/l, 100.0%), fructose 129 mg/l ($\Delta A_{fru} = 0.1788$, 129.6 mg/l, 100.5%), maltose 95 mg/l ($\Delta A_{oligo} = 0.1461$, 97.3 mg/l, 102.4%), inulin 244 mg/l ($\Delta A_{tot} = 0.8088$, 236.8 mg/l, 97.0%)

T2: saccharose 213 mg/l ($\Delta A_{sacc} = 0.1549$, 217.5 mg/l, 102.1%), inulin 99 mg/l ($\Delta A_{tot} = 0.4415$, 97.0 mg/l, 98.0%)

It can be seen from these results that inulin can be determined without difficultly in the presence of moderately high amounts of other carbohydrates.

Table 1 presents the results of the inulin assay for various dietary supplements, obtained with protocol A. In one sample (chewing tablets) the amount of inulin was declared to be 200 mg/tablet. The found value of 221 mg/tablet is in reasonably good agreement with this value. In two other samples inulin was declared on the label and amounts of 7.17 g/100 g and 2.15 g/100 g were detected. In two other samples 2.62 g/100 g and 0.54 g/100 g inulin were found but inulin was not declared on the label.

Table 1 Inulin content of selected dietary supplements; + indicates that inulin declared on the label; – indicates that no declaration of inulin was made on the label

	Inulin (found)	Inulin (declared)
Multivitamin effervescent tablets	2.62 g/100 g	–
Iron-vitamin C effervescent tablets	0.54 g/100 g	–
Calcium-magnesium effervescent tablets	7.17 g/100 g	+
Children's effervescent tablets	2.15 g/100 g	+
Chewing tablets	221 mg/tablet	200 mg/tablet

Table 2 Inulin content of selected dietetic chocolates together with the amount of inulin formulation added, as provided by the manufacturer, and the corresponding recovery rates

	Inulin (found)	Inulin formulation (added)[a]	Recovery
Dietetic chocolate 1	13.3 g/100 g	15.0 g/100 g	101.4%
Dietetic chocolate 2	11.7 g/100 g	13.4 g/100 g	99.9%
Dietetic chocolate 3	10.8 g/100 g	11.9 g/100 g	103.8%
Dietetic chocolate 4	10.7 g/100 g	12.2 g/100 g	100.4%
Dietetic chocolate 5	12.3 g/100 g	13.4 g/100 g	105.0%

[a] The inulin content of the inulin formulation was ~90 g/100 g in dry matter according to the manufacturer. The recovery rates are corrected for this

For the iron-vitamin C effervescent tablets recovery studies were carried out (100 mg/l and 200 mg/l inulin were added to sample solutions). The recoveries of inulin were 98.4% and 99.2%, respectively.

Table 2 shows the results of inulin determination in different dietetic chocolates. Added amounts of an inulin formulation, known to contain about 90% inulin (in dry matter), were provided by the manufacturer. The recoveries given in the table are based on this value. The mean recovery for all five chocolates studied was $102.1 \pm 2.7\%$ (95% confidence interval of the mean), which is very near to the expected value, indicating good accuracy of the method.

Repeatability measurements were carried out with dietetic chocolate 1. A mean value of 13.34 ± 0.34 g/100 g inulin (95% confidence interval of the mean, $n = 6$), resulting in $r_{lab} = 0.91$ g/100 g, was obtained.

When the ratio of mono- and disaccharides to inulin is too high, the inulin assay according to protocol A tends to be less precise because of the subtractions of large absorbance differences, as shown in Eq. 1. In the presence of oligoglucosides in amounts $\geq 1\%$ the determination of inulin using protocol A becomes impossible because of the incomplete hydrolysis of the oligoglucosides by inulinase. Delanghe et al. [13] have described a very elegant solution of this problem, which was tested by us for its applicability in food analysis. Glucose was oxidized enzymatically in the presence of O_2 by GOD. This was not done in a separate run but by directly adding GOD to SR1 before the first incubation step. The advantage of doing this in comparison with the procedure described elsewhere in the literature [16] lies in the fact that glucose produced during the enzymatic cleavage of saccharose, maltose and oligoglucosides in the incubation period is also immediately oxidized and thereby removed from the reaction system. As our results below show, it is not even necessary to remove the H_2O_2 formed during this reaction by catalase, as is usually done. After the incubation with GOD and inulinase the determination of fructose was carried out as in protocol A. Since fructose initially present in the sample is not removed by GOD it is still necessary to determine the differences in absorbance due to fructose (ΔA_{fru}) and saccharose (ΔA_{sacc}) in separate runs before the total absorption difference (ΔA_{tot}) is measured. The absorbance difference due to inulin (ΔA_{inulin}) is then calculated as follows:

$$\Delta A_{inulin} = \Delta A_{tot} - \Delta A_{fru} - \Delta A_{sacc} \quad (2)$$

This method of determination, protocol B, was used to determine the inulin concentration of two test samples, T3 and T4, containing glucose and inulin in the nominal concentrations given below (the measured difference in absorbance, the resulting concentration and the recovery rate are given in parentheses):

T3: glucose 360 mg/l, inulin 281 mg/l ($\Delta A_{tot} = \Delta A_{inulin} = 0.3885$, 283.2 mg/l, 100.8%)
T4: glucose 992 mg/l, inulin 292 mg/l ($\Delta A_{inulin} = 0.4085$, 295.7 mg/l, 101.3%)

Using protocol B it was possible to accurately determine inulin in the presence of up to 1000 mg/l glucose (5.5 mM).

A test mixture T5 containing maltodextrin and inulin was also studied with the following results:

T5: maltodextrin (glucose equivalent 4–7) 237 mg/l, inulin 123 mg/l ($\Delta A_{inulin} = 0.1691$, 124.1 mg/l, 100.9%).

Test mixtures T1 and T2 were re-examined using assay protocol B and gave the following results (ΔA_{fru} and ΔA_{sacc} are already subtracted):

T1: inulin 244 mg/l ($\Delta A_{inulin} = 0.3245$, 236.8 mg/l, 97.0%)

T2: inulin 99 mg/l ($\Delta A_{inulin} = 0.1329$, 97.8 mg/l, 98.8%)

Inulin in test mixtures is therefore determined with very good accuracy using protocol B.

Inulin was determined using protocol B in artichokes, viper's grass and topinambur and the results are shown in Table 3. The amounts of glucose, fructose, saccharose and oligoglucosides determined enzymatically in these samples are also given. Table 3 includes a comparison of our results with inulin values found in literature. This comparison is not always easy because of the dietary fibre (praebiotic) nature of inulin. In the case of topinambur and viper's grass only the sum of total carbohydrates and total dietary fibre was given in the literature. The values of this sum parameter are 18.6 g/100 g for viper's grass [18] and 16.5 g/100 g for topinambur [17], and these values compare quite well with our found values for the sum of carbohydrates and inulin of 20.1 g/100 g and 16.8 g/100 g, respectively. In the case of artichokes our measured value of 2.6 g/100 g inulin (14.9% on a dry matter basis using a value of 17.5 g/100 g for dry matter content [18]) is higher than the tabulated amount of 9.4% (in dry matter) [18]. The reason for this difference may be that we only used the inner parts of the artichokes, whereas the tabulated data relate to a 100 g edible portion.

Table 3 Amounts of glucose, fructose, saccharose, oligoglucosides and inulin in various vegetables (g/100 g of fresh product)

	Glucose	Fructose	Saccharose	Oligoglucosides	Inulin	Literature
Viper's grass	0.4	1.3	3.2	<0.1	15.2	18.6[a] [18]
Topinambur	<0.1	0.1	7.0	<0.1	9.7	16.5[a] [17]
Artichoke	0.9	0.1	0.6	<0.1	2.6	9.4%[b] [18]

[a] Sum of total carbohydrates and total dietary fibre
[b] In dry matter

For all three vegetable samples recovery studies were carried out following standard addition to sample solutions. The recovery rates of inulin were (100 mg/l inulin added to sample solutions): 94.7 and 103.3% (artichokes), 97.2% (viper's grass) and 98.6% (topinambur).

The enzymatic determination of inulin, as implemented by protocols A and B, does not differentiate between oligofructosides of variable degrees of polymerization, but gives instead the total amount of oligofructosides present in the sample. The distribution of chain lengths of fructose oligomers in inulin obtained from different natural sources can be conveniently studied by HPLC methods [4–8], but quantification of the total oligofructoside content is not as straightforward as with our present method.

Conclusion

We have developed a simple and reliable enzymatic method for the determination of inulin in various foodstuffs and dietary supplements. It can easily be implemented on an automatic centrifugal analyzer and needs only a minimum effort for sample preparation, and therefore is ideally suited for quality control purposes.

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References

1. De Vrese M (1997) Ernährungs-Umschau 44:398–402
2. Kneifel W (1997) Milchwirtschaftl Ber 128/129:93–99
3. Dall'Amico R, Montini G, Pisanello L, Piovesan G, Bottaro S, Cracco AT, Zacchello G, Zacchello F (1995) J Chromatogr B Biomed Appl 672:155–159
4. Simms PJ, Haines RM, Hicks KB (1993) J Chromatogr 648:131–137
5. Feste AS, Khan I (1993) J Chromatogr 630:129–139
6. Ruo TI, Wang Z, Dordal MS, Atkinson AJ (1991) Clin Chim Acta 204:217–222
7. Praznik W, Beck RHF, Nitsch E (1984) J Chromatogr 303:417–421
8. Wight AW, Van Niekerk PJ (1983) J Agric Food Chem 31:282–285
9. Gombocz E, Hellwig E, Vojir F, Petuely F (1981) Dtsch Lebensm Rdsch 77:1–26
10. Wong ECC, Wilhite TR, Miller R, Smith CH, Landt M (1994) Clin Chem 40:1788- 1789
11. Summerfield AL, Hortin GL, Smith CH, Wilhite TR, Landt M (1993) Clin Chem 39:2333–2337
12. Hellerstein S, Berenbom M, Alon U, Warady BA (1993) Clin Chem 39:2211–2212
13. Delanghe J, Bellon J, De Buyzere M, Van Daele G, Leroux G, Leroux-Roels G (1991) Clin Chem 37:2017–2018
14. Apple FS, Benson P, Abraham PA, Rosano TG, Halstenson CE (1989) Clin Chem 35:312–314
15. Degenaar CP, Frenken LAM, van Hooff JP (1987) Clin Chem 33:1070–1071
16. Boehringer Mannheim (ed) (1994) Methoden der enzymatischen Bioanalytik und Lebensmittelanalytik mit Test-Combinationen. (Application notes) Boehringer, Mannheim, pp 52–61
17. Deutsche Forschungsanstalt für Lebensmittel-Chemie, Garching b. München (ed) (1986) Souci-Fachmann-Kraut: Die Zusammensetzung der Lebensmittel, Nährwerttabellen 1986/87. Wissenschaftliche Verlagsgesellschaft, Stuttgart
18. Eisenbrand G, Schreier P (eds) (1995) Römpp Lexikon Lebensmittelchemie. Thieme, Stuttgart