Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 95 (2006) 163–168
Food Chemistry
www.elsevier.com/locate/foodchem

Determination of the fruit content of cherry fruit preparations by gravimetric quantification of hemicellulose

Ralf Fügel, Andreas Schieber *, Reinhold Carle

Hohenheim University, Institute of Food Technology, Section Plant Foodstuff Technology, August-von-Hartmann-Strasse 3, D-70599 Stuttgart, Germany

Received 29 September 2004; received in revised form 15 February 2005; accepted 15 February 2005

Abstract

A method recently developed for the determination of the fruit content of strawberry fruit preparations by gravimetric quantification of hemicellulose was extended to cherry fruit preparations. Isolation of the alcohol-insoluble residue (AIR) and sequential fractionation of the cell wall compounds from cherries (*Prunus cerasus* L. cv. 'Oblacinska') was performed yielding the amount of fresh cherries per gram hemicellulose. Cherry fruit preparations with varying fruit contents (30–40%) were produced using different hydrocolloid systems (pectin, starch, guar gum, xanthan gum, carrageenan, and combinations thereof). After separation of the hydrocolloids by enzymatical digestion and successive extraction, the fruit preparations were subjected to AIR isolation. The AIR was fractionated to yield the hemicellulose fraction, which was quantified gravimetrically for the calculation of the fruit content. Compared to strawberries, modifications including additional extraction steps for the sequential fractionation were required to separate the pectin of the cherries exhaustively. Calculated and initial fruit contents were in good agreement for the single hydrocolloid components pectin and starch as well as for the combinations pectin/xanthan gum and pectin/carrageenan (26.8% vs. 30%, 38.6% vs. 40%, 42.5% vs. 40%, 37.6% vs. 40%, and 41.2% vs. 40%), whereas the preparations produced with more complex hydrocolloid systems (pectin/xanthan gum/guar gum and starch/xanthan/guar gum) showed larger deviations in their contents (46.2% vs. 40%, 49.6% vs. 40%). It is concluded that the novel method is generally suitable for the determination of the fruit content of fruit preparations, but steps of sample preparation need to be individually adapted to the different fruit matrices.

Keywords: Alcohol-insoluble residue; Cherry; Fractionation; Fruit content; Fruit preparations; Hemicellulose; Hydrocolloids

1. Introduction

Fruit preparations as intermediate products are used in a multitude of dairy products, as well as in bakery products and confectionery. Although no regulation is available in Germany with respect to their composition, manufacturers are obliged to meet the requirements of the German Association for Food Law and Food Science (BLL) definition. According to this guideline, fruit preparations are products meant for the production of dairy products and, as a rule, are produced from fruits or fruit constituents and various sugars, and also essences, flavours, colouring foodstuffs, thickening agents and consumable acids, and which are preserved by appropriate methods. The production of fruit preparations in Germany amounted to 325,338 tons in 2002, with strawberry and cherry fruit preparations accounting for the major part. The fruit content of the fruit preparations is regulated and generally amounts to 35%. In the case of raspberry, raspberry–blackberry, red currant, gooseberry, plum and pineapple, the minimum fruit content is 30%, and for banana and black currant 25%. Colouring foodstuffs such as juices from grape or red beet are not considered part of the fruit

* Corresponding author. Tel.: +49 7114 593125; fax: +49 7114 594110.
E-mail address: schieber@uni-hohenheim.de (A. Schieber).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.02.011

content. The BLL guideline also governs the fresh fruit content of fruit yogurts which shall be 1.5–6%, depending on the fruit.

Diverse approaches have so far been made for determining the fruit content of fruit derived products in order to maintain product quality and consumer protection. These methods were mostly based on the quantification of low-molecular weight compounds which vary in a wide range and can easily be added for fraudulent purposes (Nehring, Prehn, & Skott, 1978; Pilando & Wrolstad, 1992; Wallrauch, 1995). To circumvent these problems, recent studies have focussed on high-molecular weight fruit compounds which represent more promising parameters. Specific cell wall fractions were shown to be constant in the AIR of different apple, strawberry and cherry cultivars, and also exhibited characteristic neutral sugar profiles suitable for authenticity control (Fügel, Carle, & Schieber, 2004). Further investigations of strawberry fruit preparations demonstrated that the gravimetric quantification of the hemicellulose is a suitable tool for the determination of the fruit content (Schieber, Fügel, Henke, & Carle, 2005). In continuation of these studies, the method was extended to cherry fruit preparations produced by addition of complex hydrocolloid systems. Particular attention was paid to the comparison of these results to those obtained from strawberry fruit preparations in order to draw conclusions as to the general applicability of the method.

2. Materials and methods

2.1. Materials

2.1.1. Cherries

Individually quick frozen cherries (*Prunus cerasus* L. cv. 'Oblacinska') harvested in Serbia in 2001 were obtained from Wild (Berlin, Germany).

2.1.2. Enzymes

Galactomannanase solution (5 U/ml) from *Aspergillus niger* was obtained from Fluka (Buchs, Switzerland). The preparation Fructamyl© (α-amylase) was kindly provided by Erbslöh (Geisenheim, Germany). Hazyme©, a mixture of amyloglucosidase and α-amylase, was a gift from DSM Food Specialties (Seclin, France).

2.1.3. Hydrocolloids

Hydrocolloids used for the production of fruit preparations included Guar Gum VIDOGUM GH 175 from Unipektin (Eschenz, Switzerland), Pectin Amid AF 010-A from Herbstreith & Fox (Neuenbürg, Germany), xanthan gum from Meyhall Chemical (Kreuzlingen, Switzerland), Starch NATIONAL 67-0029 from National Starch (Bridgewater, NJ, USA), and carrageenan from FMC BioPolymer (Brussels, Belgium). Pectin, xanthan gum, carrageenan, and guar gum were dispersed with distilled water before admixture to the fruits.

2.2. Production and pretreatment of the fruit preparations

Fruit preparations with a fruit content of 30–40% were produced in quantities of 2.5 kg according to typical compositions adopted from industrial recipes (Table 1). The pitted thawed whole cherries, water, sucrose and the hydrocolloid suspensions were blended. Subsequently, the mixture was homogenised for 2 min using an Ultra-Turrax blender and heated at 96 °C for 6 min. The resulting fruit preparation was cooled to the digestion temperature and enzymes were added as shown in Table 1. After cooling to room temperature, the digest was filled on metal trays and frozen at −20 °C for 24 h. The frozen sample was immersed with liquid nitrogen to yield a firm and brittle mass which was immediately minced, homogenised in a pre-cooled cutter, and finally lyophilised for 96 h.

Table 1
Specification of cherry fruit preparations and conditions of enzymatic digestion of the added hydrocolloids

Sample code	Fruit preparation				Enzymatical digestion			
	Fruit (%)	Water (%)	Sucrose (%)	Hydrocolloid	Enzyme	Temperature	Dosage[a]	Time
O-P30	30	49.4	20	Pectin (0.6%)				
O-P40	40	39.4	20	Pectin (0.6%)				
O-PX40	40	39.4	20	Pectin (0.5%) Xanthan (0.1%)				
O-PC40	40	39.4	20	Pectin (0.5%) Carrageenan (0.1%)				
O-S40	40	31.0	25	Starch (4.0%)	α-Amylase (Fructamyl©) Amyloglucosidase (Hazyme©)	55 °C 55 °C	1 mL 1 mL	1 h 5 h
O-PXG40	40	39.3	20	Pectin (0.5%) Xanthan (0.1%) Guar gum (0.1%)	Galactomannanase	50 °C	1 mL	5 h
O-SXG40	40	35.8	20	Starch (4.0%) Xanthan (0.1%) Guar Gum (0.1%)	α-Amylase (Fructamyl©) Amyloglucosidase (Hazyme©) Galactomannanase	55 °C 55 °C 55 °C	1.5 mL 1.5 mL 1.5 mL	1 h 5 h 5 h

[a] Per 2.5 kg fruit preparation.

2.3. Isolation of the alcohol-insoluble residue (AIR)

Isolation and gravimetric determination of the AIR output weight were performed as described by Schieber et al. (2005) including triplicates for the cherries and quadruplicates for the fruit preparations.

2.4. Sequential extraction of the AIR

Different from the simplified fractionation procedure used for the AIR isolation from strawberries and strawberry fruit preparations (Schieber et al., 2005), a more extensive fractionation method had to be applied for cherries (Fig. 1). AIR (0.8 g) was suspended in 50 mL of distilled water and stirred at 40 °C for 30 min. After centrifugation at 15,000*g* for 25 min (20 °C) the cake was resuspended in distilled water (50 mL), extracted at 40 °C for 1 h under stirring and centrifuged again. The combined supernatants were exhaustively dialysed against distilled water for 2 days using dialytic membranes (type 36/32, MWCO 14,000, Roth, Karlsruhe, Germany). The water-soluble pectin (WSP) extract was then freeze-dried. The pellet from water extraction was suspended and stirred in 50 mL of ammonium oxalate solution (0.5%, w/v) at 40 °C for 90 min. The suspension was centrifuged for 25 min at 15,000*g* (20 °C) and the pellet washed twice with 100 mL of distilled water. The supernatants were pooled, dialysed for 2 days against distilled water, and freeze-dried to yield the oxalate-soluble pectin (OXP) fraction. Dilute hydrochloric acid (0.05 M) was used for further extraction of the residue at 60 °C for 90 min. The homogenates were centrifuged at 15,000*g* for 25 min (20 °C). The remaining pellet was washed twice with 50 mL of distilled water. The supernatants were pooled and referred to as HCl-soluble pectin (HSP) fraction. The HSP extract was finally treated as described for the WSP and OXP fractions. The residue was then extracted with 50 mL of aqueous sodium hydroxide (0.05 M) at 30 °C for 90 min. After centrifugation at 15,000*g* for 20 min, the pellet was rinsed twice. The supernatants were pooled and the pH adjusted to 6.5 with HCl, followed by the treatment according to the previous fractions in order to obtain the NaOH-soluble pectin (OHP) fraction. The final extraction was carried out using 50 mL of aqueous sodium hydroxide (16%, w/w) at 30 °C for 5 h. After centrifugation at 15,000*g* for 25 min (20 °C) and rinsing, the supernatants were combined and the pH adjusted to 6.5. The extract was then treated as described above, yielding the output weight of the hemicellulose (HC) fraction. The remaining pellet consisted of insoluble solids such as lignin and cellulose (CL fraction). The CL fraction was finally suspended in 50 mL of distilled water, dialysed and lyophilised. The sequential fractionation was performed repeatedly ($n = 5$ for cherries, $n = 3$ for fruit preparations).

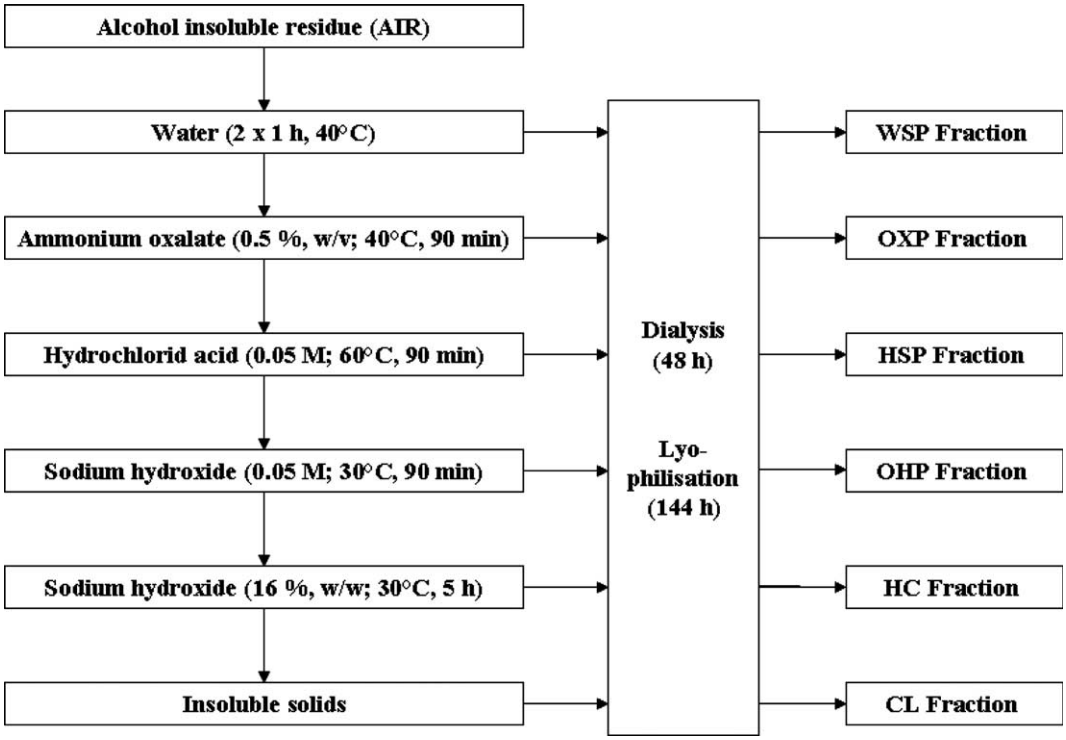

Fig. 1. Sequential fractionation of the cherry AIR.

2.5. Determination of the dry matter

Dry matter was determined after lyophilisation of the fruits and fruit preparations, respectively. The samples were weighed before and after freeze-drying for 96 h on a metal tray.

3. Results and discussion

As reported in our previous study on strawberry fruit preparations, the hemicellulose fraction was used as a parameter for the determination of the fruit content since only minor variations in its content within the AIR were found (Schieber et al., 2005). Based on these findings, a correlation between the amount of hemicellulose and the fresh weight of the cherries was established, hereinafter referred to as the conversion factor F, which is essential for the calculation of the fruit content.

The dried AIR, the lyophilised hemicellulose fraction of the cherries and the dry matter of the cherries were gravimetrically quantified (Table 2) and utilised for the calculation of the conversion factor according to the equation

$$F = \frac{I_C * I_{AIR} * 100\%}{O_{AIR} * O_{HC} * DM_C},$$

where I_C is the initial weight of the lyophilised cherries (g), I_{AIR} the initial weight of the AIR (g), O_{AIR} the output weight of the AIR (g), O_{HC} the output weight of the HC fraction (g), and DM_C the dry matter of the cherries (%). In this equation, the term $I_C * 100\%/DM_C$ refers to the fresh weight of the cherries, and the quotient I_{AIR}/O_{AIR} indicates the fractionated part of the pooled AIR. Since the AIR was pooled, each of the respective data pairs (I_C, O_{AIR}) was combined with the data obtained from sequential fractionation (I_{AIR}, O_{HC}). The mean conversion factor resulting from the fifteen calculations specifies the amount of fresh cherries per g hemicellulose.

Gravimetric parameters (dry matter, weight of AIR and HC) were determined for cherry fruit preparations in order to quantify the respective fruit contents (Table 3). For this purpose, the equation

$$\text{Fruit content } (\%) = \frac{F * O_{HC} * O_{AIR} * DM_{FP}}{I_{AIR} * I_{FP}}$$

was used for the calculation, where F specifies the conversion factor, O_{HC} the output weight of the HC fraction (g), O_{AIR} the output weight of the AIR (g), DM_S the dry matter of the fruit preparation (%), I_{AIR} the initial weight of the AIR (g), and I_{FP} the initial weight of the lyophilised fruit preparation (g). The product of conversion factor F and output weight of the hemicellulose fraction (O_{HC}) specifies the fresh weight of the cherries, while the remaining parameters again refer to the aliquot of the pooled AIR and to the fresh weight of the fruit preparations. The resulting fruit contents represent the mean value of 15 calculations, since the quantification of AIR and hemicellulose was carried out five and three times, respectively.

For the fruit preparations O-P40, O-PX40, O-PC40, and O-S40 only marginal deviations (<3.0%) between initial and determined fruit content were found (Table 3). In contrast, overestimations of 6.2% and 9.6% were obtained for the preparations O-PXG40 and O-SXG40, respectively, while the fruit content of sample O-P30 was underestimated by 3.2%. Most strikingly, relative standard deviations consistently exceeded 10%, ranging from 13.8% to 16.7%. In our previous study on the determination of the fruit content of strawberry fruit preparations a better reproducibility with standard deviations between 5.6% and 11.9% was observed (Schieber et al., 2005). It is assumed that modification of the procedure for the sequential extraction, implementing three additional steps in comparison to strawberry fruit preparations, was the major reason for these findings, thus compromising precision and reproducibility. However, since preliminary investigations had revealed large overestimations applying the shortened fractionation method to cherry fruit preparations, this modification was indispensable to isolate the pectins exhaustively, which considerably differ from strawberry pectins with respect to their qualitative composition (Fügel et al., 2004; Voragen, Timmers, Linssen, Schols,

Table 2
Gravimetric data of cherries (cv. 'Oblacinska', 25.5% dry matter) and calculation of the conversion factor

AIR isolation[a]		Sequential fractionation[b]		Conversion factor[c]
Initial weight lyophilisate (g)	Output weight AIR (g)	Initial weight AIR (g)	Output weight HC (mg)	
25.152	2.195	0.74974	156.48	243.0 ± 13.7
		0.75397	121.40	
25.017	2.285	0.69020	131.85	
		0.67945	116.91	
24.809	2.233	0.81001	142.53	

[a] $n = 3$.
[b] $n = 5$.
[c] ±Standard deviation (rel., %), mean of $n = 15$.

Table 3
Gravimetric data of cherry fruit preparations and calculation of their fruit contents

Sample code	Dry matter (%)	AIR isolation[a]		Sequential fractionation[b]		Fruit content[c]	Deviation from initial fruit content (abs., %)
		Initial weight lyophilisate (g)	Output weight AIR (g)	Initial weight AIR (g)	Output weight HC (mg)		
O-P30	31.3	15.342	0.523	0.74377	85.86	26.8 ± 16.7	−3.2
		15.337	0.509	0.62241	62.77		
		15.422	0.513	0.45321	42.73		
		15.000	0.543				
O-P40	33.9	15.020	0.569	0.68294	80.75	38.6 ± 14.0	−1.4
		15.020	0.593	0.73086	91.68		
		15.019	0.575	0.75512	91.69		
		15.003	0.575				
O-PX40	33.4	17.449	0.625	0.68004	98.63	42.5 ± 15.0	+ 2.5
		16.217	0.578	0.82651	130.85		
		16.588	0.588	0.69701	95.99		
		17.482	0.625				
O-PC40	33.0	15.000	0.541	0.70748	93.77	37.6 ± 14.1	−2.4
		15.003	0.542	0.67962	84.28		
		15.000	0.543	0.72803	96.49		
		15.005	0.542				
O-S40	42.3	15.461	0.272	0.73047	154.14	41.2 ± 16.7	+ 1.2
		16.279	0.370	0.68935	128.65		
		18.669	0.371	0.62045	126.27		
		15.494	0.299				
O-PXG40	32.9	18.750	0.770	0.77080	107.66	46.2 ± 13.8	+ 6.2
		18.152	0.736	0.71911	103.40		
		16.129	0.650	0.85903	123.95		
		15.945	0.642				
O-SXG40	47.5	17.308	0.503	0.62885	100.75	49.6 ± 16.5	+ 9.6
		16.662	0.470	0.56112	75.28		
		18.380	0.556	0.33801	52.98		
		17.287	0.459				

[a] $n = 4$.
[b] $n = 3$.
[c] ±Standard deviation (rel., %), mean of $n = 12$. (%)

& Pilnik, 1983). Furthermore, large relative standard deviations of 13.7% were also observed for the conversion factor, which represents an essential parameter for the calculation of the fruit content. In the case of strawberries, standard deviations of 7.0% and 5.6% of the conversion factor had been found for the cultivars 'Senga Sengana' and 'Camarosa', respectively (Schieber et al., 2005). Most probably these findings must be ascribed to the more pronounced variation of the total soluble solids (dry matter) of cherries. While AIR and hemicellulose contents were found to be constant in ripe cherries, which are usually employed in the production of the fruit preparations, the dry matter of the cherries represents a critical parameter subject to intrinsic variations, largely influencing the calculation of the conversion factor. A conversion factor universally applicable to fruit preparations of unknown cherry cultivars is difficult to establish in view of the observed differences in the dry matter. In order to obtain a more comprehensive range of data of cherry cultivars, particularly with respect to their dry matter, acquisition of gravimetric data of various cherry cultivars of different proveniences and harvest seasons is currently underway for compilation in a database. Although a conversion factor based on this data would undoubtedly reduce the accuracy of the calculated fruit content because of the inherent variations in the parameters, it is expected that at least a more reliable range for the fruit content may be provided. Nevertheless, it must be conceded that the method is most efficient when the actually used cherries are available for analysis.

The applicability of the novel method to cherry fruit preparations produced by the addition of complex hydrocolloid systems was a major interest of the present study. While the thickening agents pectin, xanthan gum, and carrageenan could efficiently be removed by sequential extraction of the cell wall fractions, enzymatical digestion was required for the degradation and separation of guar gum and starch using amylase, amyloglucosidase, and galactomannanase, respectively. The

latter enzyme was shown to be devoid of interfering hemicellulolytic side activities. In addition, relevant cell wall fractions were not affected by degradation when technical enzyme preparations containing α-amylase and amyloglucosidase were used (Schieber et al., 2005). As can be seen from Table 3, satisfactory results for the determined fruit contents were obtained by the successive extraction of one and two hydrocolloid components (O-P30, O-P40, O-PC40, O-PX40) using a broad spectrum of extraction agents, as well as by the enzymatical degradation of starch (O-S40). In contrast, the presence of more complex hydrocolloid systems such as pectin, xanthan gum, and guar gum (O-PXG40), and starch together with xanthan gum and guar gum (O-SXG40), respectively, resulted in overestimations, indicating that high-molecular alcohol insoluble degradation products accumulated in the HC fraction. It is assumed that, as a result of incomplete enzymatical degradation, the high-molecular fragments of thickening agents could not exhaustively be extracted with water, oxalate, dilute acid, and alkali, and therefore remained in the HC fraction. Since the dry matter largely affects the calculation of the fruit content, overestimation of the fruit content of the sample O-SXG40 could be ascribed to this parameter, which was determined as 47.5% compared to the real value of 34.4%. It is concluded that this hydrocolloid system, together with sucrose, forms a stable gel displaying increased water-binding capacity and stability against enzymatical degradation. This hypothesis is confirmed by previous studies reporting synergistic effects with respect to gelation properties and viscosity particularly for the combination of xanthan and guar gum (Sanderson, 1982; Tako & Nakamura, 1985). Xanthan gum as a cellulose derivative is degradable using suitable cellulases, however, since these enzymes usually show also hemicellulosic side activity, their application may result in a decrease in hemicellulose content, thus affecting the calculation of the fruit content.

4. Conclusion

As reported by Schieber et al. (2005), the gravimetric quantification of hemicellulose proved to be a suitable tool for the determination of the fruit content of strawberry fruit preparations. Our present investigations revealed that the applicability to cherry fruit preparations could not be accomplished without modifications of the sample preparation, leading to larger relative standard deviations in comparison to strawberry fruit preparations. Furthermore, it needs to be elucidated whether the difficulties, which became evident for the preparations containing complex hydrocolloid systems, are typical of cherries only, or whether the same limitations also apply to other fruit species. However, promising results were obtained for the cherry fruit preparations containing pectin as a single hydrocolloid. It is therefore assumed that the method also allows the determination of the fruit content in jams and marmelades with pectin as the exclusive stabilising agent. These investigations, as well as the extension of the method to fruit containing dairy products are currently underway.

Acknowledgements

The present work was supported by the Research Association of the German Food Industry (FEI), the German Federation of Industrial Research Associations (AiF) and the Federal Ministry of Economics and Labour (BMWA). Project No.: AiF-FV 12971N. The authors thank Mr. M. Henke for his excellent technical assistance.

References

Fügel, R., Carle, R., & Schieber, A. (2004). A novel approach to quality and authenticity control of fruit products using fractionation and characterisation of cell wall polysaccharides. *Food Chemistry, 87*, 141–150.

Nehring, P., Prehn, H. J., & Skott, W. P. (1978). Fruchtgehaltsbestimmung in Konfitüren. *Industrielle Obst- und Gemüseverwertung, 63*, 559–566.

Pilando, L. S., & Wrolstad, R. E. (1992). Compositional profiles of fruit juice concentrates and sweeteners. *Food Chemistry, 44*, 19–27.

Sanderson, G. R. (1982). The interaction of xanthan gum in food systems. *Progress in Food and Nutrition Science, 6*, 77–87.

Schieber, A., Fügel, R., Henke, M., & Carle, R. (2005). Determination of the fruit content of strawberry fruit preparations by gravimetric quantification of hemicellulose. *Food Chemistry, 91*, 365–371.

Tako, M., & Nakamura, S. (1985). Synergistic interaction between xanthan and guar gum. *Carbohydrate Research, 138*, 207–213.

Voragen, A. G. J., Timmers, J. P. J., Linssen, J. P. H., Schols, H. A., & Pilnik, W. (1983). Methods of analysis for cell wall polysaccharides of fruit and vegetables. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 177*, 251–256.

Wallrauch, S. (1995). Berechnungsformel zur Abschätzung des Fruchtgehaltes in Erdbeerfruchtzubereitungen. *Lebensmittelchemie, 49*, 40–45.