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

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Abstract

A novel method for the determination of the fruit content of strawberry fruit preparations based on the quantification of hemicellulose is presented. For this purpose, the hemicellulose fraction was isolated from the alcohol-insoluble residue (AIR) of strawberry fruits (*Fragaria* × *ananassa* cv. 'Senga Sengana' and 'Camarosa') to calculate the amount of fresh fruit per gram hemicellulose. Fruit preparations with fruit contents ranging from 30% to 60% were produced using starch, pectin, xanthan and guar gum as hydrocolloids. For the determination of the fruit content, added hydrocolloids were removed by enzymatic digestion and alkaline degradation, respectively. The hemicellulose fraction resulting from AIR fractionation was quantified gravimetrically. Due to the characteristic composition of neutral sugars obtained after hydrolysis, the hemicellulose fractions may be used for authenticity control. Excellent agreement between specified and determined contents (30% vs. 31.5%; 45% vs. 44.7%; 60% vs. 64%; 40% vs. 37.6–42.2%) was obtained irrespective of the composition of the fruit preparation. This method is considerably more reliable than those based on the determination of low-molecular compounds which can easily be added to feign a higher fruit content. Furthermore, fruit juice concentrates added to fruit preparations as a food colorant do not affect the quantification of the fruit content.

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

1. Introduction

Fruit preparations are important intermediates for the production of bakery products, ice cream and especially of dairy products. According to the guideline of the German Federation of Food Law and Food Science (BLL), a fruit content of 35% is demanded for fruits in general, while for certain fruits such as raspberry, black and red currant, banana and pineapple fruit content is lowered to 25–30%. The BLL guideline also governs the fresh fruit content of fruit yogurts which shall be 1.5–6%, depending on the fruit.

Adulteration of fruit-based products such as fruit preparations, jams and spreads is a serious economic problem and may encompass both the admixture of cheaper fruits and non-compliance with the specified fruit content. For consumer protection, as well as reception inspection and quality control, the availability of suitable analytical methods for the determination of the fruit content of fruit-based products would therefore be highly desirable. The complexity of the matrix of such products has so far been the main reason for the lack of analytical methods. Fruit preparations contain both genuine fruit constituents and a wide range of ingredients such as sugars, essences, flavours, coloring foodstuff, organic acids and hydrocolloids. Methods reported so far for the determination of the fruit content are mostly based on the quantification of low-molecular

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compounds, e.g. amino acids, organic acids, sugars, and minerals (Nehring, Prehn, & Skott, 1978; Pilando & Wrolstad, 1992; Wallrauch, 1995). However, a prerequisite for the validity of these methods is that all compounds originate from the fruits and not from additives. Furthermore, depending on cultivar, fruit maturing and cultivation conditions, most fruit constituents are subject to considerable variations in their contents and can easily be manipulated. Due to these tremendous variations, methods of linear approximation had to be applied, resulting in rather vague predictions (Klinger & Nehring, 1966; Nehring et al., 1978; Prehn, Bosch, & Nehring, 1977a, Prehn, Bosch, & Nehring, 1977b; Prehn & Nehring, 1977a, 1977b; Prehn, Thaler, & Nehring, 1977). Rheological indices in combination with chemical parameters represent an alternative approach to the estimation of the fruit content (Carbonell, Costell, & Duran, 1991a, 1991b, 1991c; Costell, Carbonell, & Duran, 1987, 1993), however, due to the effect of added hydrocolloids, the predicted fruit contents of jams were not consistently satisfying. Chemometric methods such as FTIR spectroscopy require a great number of well-defined, authentic samples for calibration and extensive statistical treatment of the data (Contal, León, & Downey, 2002; Defernez, Kemsley, & Wilson, 1995; Holland, Kemsley, & Wilson, 1998; Wilson, Slack, Appleton, Sun, & Belton, 1993).

In a comprehensive review very recently published by Waldron, Parker, and Smith (2003) the role of plant cell walls in relation to food quality has been assessed. Attention to the response of cell wall constituents to enzymatic and thermal processing of canned cherries and strawberries has been paid by Carle, Borzych, Dubb, Siliha, and Maier (2001). In continuation of these studies we have demonstrated that the neutral sugar profile of the hemicellulose fraction may be used for the differentiation of strawberry, cherry and apple fruits (Fügel, Carle, & Schieber, 2004). Even more important, independent of processing the content of the hemicellulose fraction proved to be constant in the alcohol-insoluble residue (AIR). It has therefore been hypothesised that quantification of hemicellulose would also allow the determination of the fruit content of fruit-based products. The present paper marks the first report on the determination of the fruit content of strawberry fruit preparations by gravimetric quantification of the hemicellulose fraction.

2. Materials and methods

2.1. Materials

2.1.1. Strawberries

Individually quick frozen strawberries (*Fragaria × ananassa* Duch. cv. 'Senga Sengana', 'Camarosa') harvested in 2001 were obtained from Schwartau (Bad Schwartau, Germany) and 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 from Meyhall Chemical (Kreuzlingen, Switzerland), and Starch NATIONAL 67-0029 from National Starch (Bridgewater, NJ, USA). Pectin, xanthan and guar gum were suspended with distilled water before admixture.

2.2. Production of the fruit preparations

Fig. 1 shows a flow diagram of the experimental procedure used for the treatment of the fruit preparations. The basic formulations of the fruit preparations were adopted from industrial recipes. Typical compositions with a fruit content ranging from 30% to 60% are listed in Table 1. The thawed whole strawberries, 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.

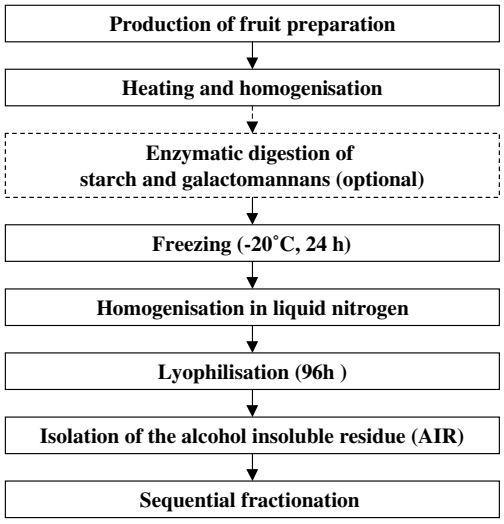

Fig. 1. Sample treatment of strawberry fruit preparations.

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

Sample code	Strawberries		Fruit preparation				Enzymatical digestion			
	Cultivar	Origin	Fruit (%)	Water (%)	Sucrose (%)	Hydrocolloid	Enzyme	Temperature	Dosage[a] (mL)	Time (h)
S-P30	Senga Sengana	Poland	30	49.4	20	Pectin (0.6%)				
S-P45	Senga Sengana	Poland	45	34.4	20	Pectin (0.6%)				
S-P60	Senga Sengana	Poland	60	19.4	20	Pectin (0.6%)				
S-S40	Senga Sengana	Poland	40	31.0	25	Starch (4.0%)	α-Amylase (Fructamyl©)	55 °C	1	1
							Amyloglucosidase/ α-Amylase (Hazyme©)	55 °C	1	5
S-PX40	Senga Sengana	Poland	40	39.4	20	Pectin (0.5%) Xanthan (0.1%)				
S-PG40	Senga Sengana	Poland	40	39.0	20	Pectin (0.5%) Guar gum (0.5%)	Galactomannanase	50 °C	1	5
C-P40	Camarosa	Spain	40	39.4	20	Pectin (0.6%)				

[a] Per 2.5 kg fruit preparation.

2.3. Pretreatment of the fruit preparations

After heating the mixture was cooled to the digestion temperature and enzymes were added as shown in Table 1. The digest was cooled to room temperature, filled on metal trays and frozen at −20 °C for 24 h. After immersion of the frozen sample in liquid nitrogen a very firm and brittle mass was obtained. Subsequently, the mass was minced, homogenised in a pre-cooled cutter and finally lyophilised for 96 h.

2.4. Isolation of the alcohol-insoluble residue

2.4.1. Strawberries

Freeze-dried strawberries were roughly ground. The lyophilisate (30 g) was homogenised in 300 mL of boiling ethanol (80%, v/v) using an Ultra-Turrax blender. After boiling for 1 h, the insoluble solids were collected on a Büchner funnel. This procedure was repeated five times until a clear extract was obtained. The residue was stirred overnight in pure acetone, passed through a G3 glass sinter filter and air-dried at 40 °C for 24 h. Isolation of AIR was performed in triplicate ($n = 3$). The AIR preparations were weighed (output weight of the AIR) and subsequently pooled for sequential fractionation.

2.4.2. Fruit preparations

Amounts of 30 g of freeze-dried fruit preparation were roughly ground and homogenised in boiling ethanol (300 mL, 80% v/v) using an Ultra-Turrax blender. After boiling for 1 h, the slurry was centrifuged at 15,000 *g* (40 °C) for 10 min. Insoluble solids were collected on a Büchner funnel and again submerged in boiling ethanol. Rinsing was carried out until a clear extract was obtained. The residue was thereafter stirred in pure acetone (12 h), passed through a G3 glass sinter filter and air-dried for 24 h. Isolation of AIR was also performed in triplicate ($n = 3$). The AIR preparations were weighed (output weight of the AIR) and subsequently pooled for sequential fractionation.

2.5. Sequential extraction of the AIR

The procedure of AIR fractionation is shown in Fig. 2. A shortened sequential fractionation based on the procedure described by Voragen et al. (1983) was implemented. AIR (0.8 g) was suspended in 50 mL of alkaline EDTA solution (0.05 M NaOH; 0.5 mM EDTA) and

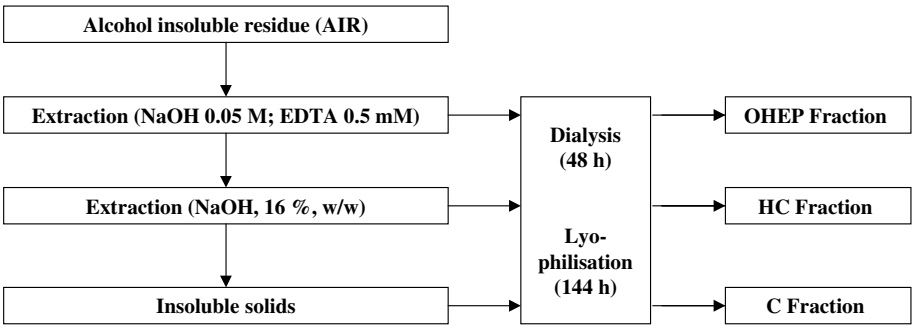

Fig. 2. Shortened sequential fractionation of the AIR.

Table 2
Gravimetrical data of strawberries and calculation of the conversion factor

Cultivar	Dry matter (%)	AIR isolation[a]		Sequential fractionation[b]		
		Initial weight lyophilisate (g)	Output weight AIR (g)	Initial weight AIR (g)	Output weight HC (mg)	Conversion factor[c]
Senga Sengana	10.9	14.600	3.022	0.75978	137.13	232.3 ± 7.0
				0.71857	141.54	
		15.048	2.938	0.72926	136.50	
				0.71587	142.26	
		14.419	3.237	0.70112	130.55	
Camarosa	9.1	24.244	4.637	1.49264	305.02	297.5 ± 5.6
				1.57060	279.92	
		24.441	4.669	1.46527	280.04	
				0.76121	148.18	
		25.250	4.972	0.70453	127.87	

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

stirred at 30 °C for 1 h. After centrifugation at 15,000 *g* for 20 min, the residue was resuspended in alkaline EDTA solution (50 mL), extracted at 30 °C for 1 h under stirrring and centrifuged again. The pellet from EDTA extraction was washed twice with 50 mL of distilled water. After pooling and adjusting to pH 6.5 using HCl, the supernatants were dialysed against distilled water for two days using dialytic membranes (type 36/32, pore size 25–50 Å, Roth, Karlsruhe, Germany). Subsequently, the NaOH–EDTA-soluble pectin (OHEP) extract was freeze-dried. The pellet from EDTA extraction was suspended and stirred in 50 mL of aqueous sodium hydroxide solution (16%, w/w) at 30 °C for 5 h. 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 described for the previous fraction in order to yield the hemicellulose (HC) fraction. After lyophilisation, the HC fraction was weighed (output weight of the HC fraction). The remaining pellet consisted of insoluble solids such as lignin and cellulose (C fraction). The C fraction was finally suspended in 100 mL of distilled water, dialysed and lyophilised. The sequential fractionation was performed repeatedly ($n = 5$ for strawberries, $n = 3$ for fruit preparations).

2.6. 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

In our previous study, only minor variations of the hemicellulose content were found within a given fruit species, as shown for strawberry, cherry and apple (Fügel et al., 2004). Furthermore, since concomitant investigations revealed exceptional stability of the hemicellulose fraction during processing of fruit preparations, the basic idea was to use this fraction as a parameter for the determination of the fruit content. For this purpose, the amount of the hemicellulose fraction needed to be correlated with the fresh weight of strawberry fruits. This correlation will hereinafter be referred to as the conversion factor.

Dry matter, AIR and hemicellulose content of the two cultivars 'Senga Sengana' and 'Camarosa' which are usually employed for the production of strawberry fruit preparations and jams were determined (Table 2). AIR isolation was performed in triplicate, while sequential fractionation of the pooled AIR was replicated five times. The conversion factor was calculated according to the equation

$$F = \frac{I_S * I_{AIR} * 100\%}{O_{AIR} * O_{HC} * DM_S},$$

where I_S is the initial weight of the lyophilised strawberries (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_S the dry matter of the strawberries (%). In the equation the term I_S*100%/DM_S indicates the quantity of the fresh fruit, while the quotient I_{AIR}/O_{AIR} is an aliquot factor which marks the part of the pooled AIR used for sequential fractionation. Gravimetrical data obtained from AIR isolation were not averaged, but each pair of parameters (I_S, O_{AIR}) was combined with the data obtained from sequential fractionation (I_{AIR}, O_{HC}) instead. The conversion factor given in Table 2, therefore, represents the mean value ± relative standard deviation of 15 combinations and expresses the amount of strawberry fruit per gram hemicellulose.

Six types of fruit preparations with varying fruit contents ranging from 30% to 60% using four different hydrocolloid systems were made from strawberries of the cultivar 'Senga Sengana', while one sample was produced from cultivar 'Camarosa'. Gravimetrical data were obtained as described above for the strawberry fruits. The fruit contents of the fruit preparations were calculated according to the equation

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

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), with the product of the conversion factor F and output weight of the hemicellulose fraction (O_{HC}) representing the fruit weight of the strawberries. The parameters DM_{FP} and I_{FP} are required for the calculation of the fresh weight of the fruit preparation. Since the set of parameters obtained from gravimetrical determination of the AIR ($n = 3$) was combined with each set obtained from AIS fractionation ($n = 3$), the fruit contents given in Table 3 are the mean ± relative standard deviation of nine values.

From Table 3 it becomes evident that excellent agreement of specified and determined fruit content was obtained in most cases. Only the fruit content of sample S-P60 was overestimated (64.0 ± 7.5% vs. 60%). Except for sample S-P45, relative standard deviations did not exceed 10%, demonstrating a good reproducibility of the method, especially in consideration of the experimental expenditure.

While the pectins were exhaustively extracted from the preparations containing up to 45% fruit using diluted alkali, it is assumed that minor amounts of pectin remained in the residue after alkali extraction, thus interfering with the gravimetric determination of the HC fraction and leading to a 4% overestimation of the fruit content. Another possibility for this comparatively large deviation is that exhaustive extraction of the HC fraction was not achieved, due to the increased amount of cell wall compounds present. However, it is of particular importance that the type and amount of thickening agents used for the production of the fruit preparations did not affect the determination since these hydrocolloids could efficiently be removed by enzymatic

Table 3
Gravimetrical data of strawberry fruit preparations and calculation of their fruit content

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)		
S-P30	24.5	14.549	0.653	0.6779	86.95	31.5 ± 9.4	+1.5
		14.602	0.663	0.66123	75.83		
		15.240	0.733	0.7166	83.26		
S-P45	26.2	15.006	0.818	0.70958	106.00	44.7 ± 11.9	−0.3
		15.357	0.858	0.75441	102.33		
		16.264	0.757	0.63781	87.06		
S-P60	27.8	15.618	1.016	0.69351	107.65	64.0 ± 7.5	+4.0
		15.289	0.971	0.7685	114.14		
		17.651	1.157	0.67861	105.58		
S-S40	34.6	16.175	0.573	0.61458	87.21	40.6 ± 10.0	+0.6
		14.958	0.564	0.60011	83.87		
		14.458	0.499	0.26231	32.54		
S-PX40	26.6	14.027	0.677	0.64653	76.26	37.6 ± 8.3	−2.4
		15.570	0.771	0.62498	77.19		
		13.983	0.691	0.71061	92.49		
S-PG40	26.8	14.484	0.815	0.67206	66.83	39.3 ± 10.1	−0.7
		15.588	0.907	0.64396	74.56		
		15.665	0.948	0.64685	71.09		
C-P40	27.16	16.165	0.696	0.63135	76.56	42.4 ± 5.6	+2.4
		17.119	0.746	0.64813	79.37		
		15.768	0.675	0.67383	81.72		

[a] $n = 3$.
[b] $n = 3$.
[c] ± Standard deviation (rel., %), mean of $n = 9$.

digestion (samples S-S40 and S-PG40) and by alkaline degradation, respectively.

The employed enzymes α-amylase, amyloglucosidase and galactomannanase were devoid of hemicellulolytic and cellulolytic side activities. Therefore, the essential cell wall fractions were not affected by enzymatic starch and guar gum degradation. In contrast, commercial pectinases usually display hemicellulolytic and cellulolytic side activities. For this reason, water-, oxalate- and acid-soluble pectins were degraded with dilute alkali. Since no commercial β-glucanase was available, xanthan was also removed by alkaline extraction. Although the hydrocolloids used in the present study do not cover all possible thickening agents, starch, pectins, xanthan and guar gum are the most important hydrocolloids used in fruit preparations (Unterholzner & Unterholzner, 1998) and were therefore chosen to demonstrate the general applicability of the method. Since fruit juice concentrates used as colouring foodstuffs are devoid of high-molecular cell wall constituents due to mash enzymation, quantification of the fruit content of fruit preparations based on purees or fruit pulp is not affected by the method applied.

The natural heterogeneity of the strawberries becomes apparent in the differences in AIR content and dry matter of the investigated varieties. Since both parameters are crucial for the calculation of the conversion factor, the variation limit of the fruit content inevitably increases by averaging conversion factors of different strawberry varieties. While the influence of the AIR content is of minor importance, results are affected to a greater extent by dry matter variation. Since in most cases the latter parameter is unknown for the processed strawberries, the establishment of a database containing dry matters of economically important strawberry cultivars and origins is a prerequisite to improve the reliability of the newly developed method.

Far from being a particularly rapid method, the procedure presented in this paper represents a completely new approach to the determination of the fruit content of fruit-based products, as demonstrated for strawberry fruit preparations. In contrast to methods reported so far, satisfactory results were obtained without the use of sophisticated analytical methods and extensive statistical treatment. Instead, virtually all parameters relevant for quantification are determined by gravimetry. Therefore, the procedures can be adopted by many laboratories of the food industry and the Food Inspection Board, which is a prerequisite for widespread application and standardisation. To minimise systematical as well as incidental errors, keeping to the conventions as described is highly recommended. Since lyophilisation has proved to be the most tedious and time-consuming step of sample preparation, alternative drying methods, e.g. microwave vacuum drying, might be advantageous to provide a higher sample throughput.

4. Conclusion

The results obtained in the present study demonstrate that the isolation and gravimetrical quantification of the hemicellulose fraction is a promising approach to the determination of the fruit content of strawberry fruit preparations. Moreover, this method also appears to be suitable for the determination of the fruit content of jams and marmelades. Since all ingredients usually added during manufacture can efficiently be removed by extraction or enzymatical digestion, detailed knowledge of the composition of the fruit preparations is not required. However, when fruit juice concentrates are used instead of fruit pulp and puree, determination of fruit content should still be based on low-molecular fruit constituents. Admixture of hemicellulose-containing fibers to feign a higher fruit content may be detected by determination of the neutral sugar profile, as described previously (Fügel et al., 2004). Only in the case of identical neutral sugar pattern of ingredients fraudulently added, the method may have its limitations. On the other hand, it must be taken into consideration that the availability of the novel method present here dramatically increases the expense necessary for adulterating fruit preparations and related products. Comprehensive databases of the dry matter of economically important strawberry, cherry, peach and apricot cultivars are currently being established to further improve the reliability of the results. Furthermore, methods for the determination of the fruit content of dairy products containing strawberry fruit preparations (yogurts and curd cheese) are being developed.

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