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

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Summary

The applicability of a recently developed method for the determination of the fruit content of fruit preparations to strawberry yogurts was investigated. For this purpose, the protein matrix of the yogurts was enzymatically digested using commercial protease preparations. The alcohol-insoluble residue (AIR) was isolated and subsequently differentiated in the pectin, hemicellulose, and cellulose fractions. The calculation of the fruit content of the strawberry yogurts was based on the amount of hemicellulose, which was quantified gravimetrically. A good agreement of initial and calculated fruit content was obtained for yogurt containing 30 % strawberries (31.5 %), whereas larger deviations were observed with decreasing strawberry contents.

Introduction

Dairy products, in particular fruit yogurts, have experienced a considerable increase in popularity during the past decades. According to the recently established 'Quantitative Ingredient Declaration' (QUID) regulation (article 7 of EU directive 79/112/EWG), the ingredients of compositional foods have to be specified on a quantitative base. Currently, there is an evident lack of methods for the determination of the fruit content of fruit yogurts and related products. However, such methods for the detection of adulterations are urgently needed for quality assurance and consumer protection. Our recent studies had revealed constant amounts of hemicellulose (HC) and cellulose in cell walls of strawberry, cherry and apple (FÜGEL et al., 2004). Furthermore, a sufficient process stability of the HC fraction was observed. Since further investigations demonstrated a good correlation of the fruit content and the amount of the hemicellulose fraction, the fruit content of strawberry and cherry fruit preparations could be calculated by gravimetrical quantification of hemicellulose (FÜGEL et al., 2005; SCHIEBER et al., 2005). The objective of this work was to extend this method to dairy products by the implementation of an enzymatic digestion of the protein matrix considering strawberry yogurts as an example.

Materials and methods

Individually quick frozen strawberries (cv. Senga sengana and Camarosa) harvested in 2001 were obtained from Wild (Heidelberg, Germany) and Schwartau (Bad Schwartau, Germany). After thawing, the strawberries were homogenised using a blender and mixed with a commercial yogurt (3.8 % fat, Milchwerke Schwaben, Ulm, Germany) in proportions of 30, 20, 10 and 6 % (cv. Senga sengana) and 6 % (cv. Camarosa), respectively. Subsequently, the proteolytic enzyme preparations Beerzym P7 and Beerzym Chill (Erbslöh, Geisenheim, Germany) were added in dosages of 0.5 mL kg^{-1} yogurt. The digestion was performed at 40°C and pH 7.5 under stirring for 1 min in intervals of 30 min. The slurry was then lyophilised for 96 h. From the resulting powder the alcohol-insoluble residue (AIR) comprising the high-molecular compounds, in particular polysaccharides, was prepared by precipitation in ethanol (80 %, v/v) to remove interfering alcohol-soluble low molecular compounds. An aliquot of the lyophilisate (15 g) was homogenised in boiling ethanol (150 mL, 80 %, v/v) using an Ultra-Turrax blender. After stirring at 60°C for 1 h, the suspension was centrifuged at 15,000 *g* (20 min) and the insoluble residue was collected on a Büchner funnel. The extraction was performed repeatedly until a clear supernatant was obtained. The insoluble solids were immersed in pure acetone, stirred overnight, passed through a G1 glass sinter filter, and air-dried at 40°C for 24 h. Finally, the AIR was weighed (output weight of the AIR) and subsequently pooled.

AIR (0.8 g) was suspended in 50 mL of alkaline EDTA/urea solution (0.05 M NaOH; 0.5 mM EDTA; 8 M urea) and 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 stirring 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 2 days using dialytic membranes (type 36/32, pore size 25-50 Å, Roth, Karlsruhe, Germany). Subsequently, the NaOH-EDTA-soluble pectin 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 output weight of the hemicellulose (HC) fraction. The remaining insoluble solids mainly consist of lignin and cellulose (C fraction). The C fraction was finally suspended in 100 mL of distilled water, dialysed and lyophilised. The complete procedure of sample preparation is shown in Fig. 1.

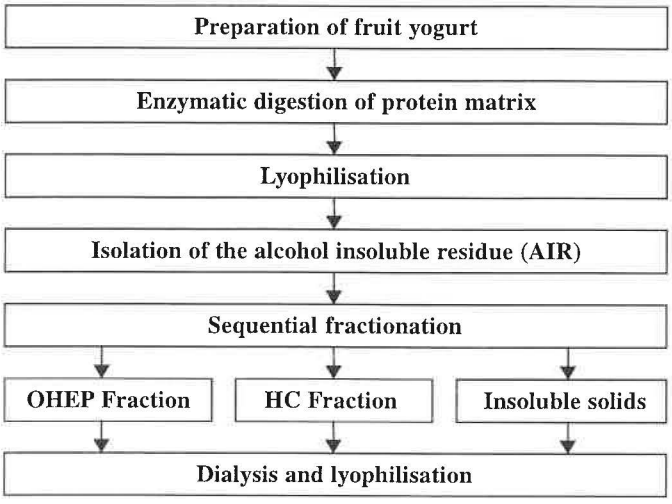

Fig. 1: Sample preparation for the determination of the fruit content of fruit yogurts

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Results and discussion

In preliminary studies, several combinations of technical commercial enzyme preparations (proteases, peptidases) were applied to yogurts, revealing that only very low quantities of high-molecular compounds remained after AIR isolation and fractionation. Enzymatic digestion was also performed for both strawberry cultivars without the addition of yogurt to exclude potential hemicellulolytic side activities of the technical proteases, which would affect quantification of the hemicellulose content of the fruits. Subsequently, AIR isolation and sequential fractionation of the fruits were carried out to relate the amount of hemicellulose and the strawberry fresh weight. This correlation was expressed as the conversion factor **F** which was calculated according to the equation

$$(1) \qquad F = \frac{I_S * I_{AIR} * 100\ \%}{O_{AIR} * O_{HC} * D_{MS}},$$

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 [%]. The quotient I_{AIR}/O_{AIR} defines the part of the pooled AIR used for the sequential fractionation, while the term $(I_S/DM_S)*100$ % is utilized for the conversion from dry matter into fresh weight of the strawberries. Finally, the factor F specifies the amount of fresh strawberries per gram hemicellulose (Tab. 1).

AIR, hemicellulose and dry matter of the strawberry yogurts were quantified gravimetrically and the fruit content was calculated according to the equation

$$(2) \qquad \text{Fruit content [\%]} = \frac{F * O_{HC} * O_{AIR} * DM_Y}{I_{AIR} * I_Y},$$

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_Y the dry matter of the fruit yogurt [%], I_{AIR} the initial weight of the AIR [g], and I_Y the initial weight of the lyophilised fruit yogurt [g]. The aliquot of the fractionated AIR is again specified in the quotient O_{AIR}/I_{AIR}, while the term DM_Y/I_Y refers to the fresh weight of the fruit yogurts.

The product of conversion factor F and output weight of the hemicellulose indicates the fresh weight of the strawberries contained in the yogurts (Tab. 2).

From Tab. 2 it can be seen that satisfactory results for the determination of the fruit contents of the strawberry yogurts were obtained in most cases. While for sample FYS-30 an excellent agreement with the initial fruit content was observed (31.5 *vs.* 30 %), marked overestimations were found for the yogurts with lower fruit contents (26.0 *vs.* 20 %, 13.5 *vs.* 10 %, 9.2 *vs.* 6 %, respectively). Furthermore, relative deviations of these samples increased from 5 % to 53.3 % (FYS-6), depending on the strawberry content. Compared to the samples of the strawberry cultivar 'Senga sengana', a relatively small deviation from the specified content was determined for the yogurt made from strawberries of the cultivar 'Camarosa' (7.8 *vs.* 6.0 %). The loss of precision might have been caused by incomplete enzymatic digestion of the proteins, resulting in increased output weights of the AIR since these matrix compounds also precipitate after addition of ethanol. The protein fraction in the AIR increases with the amount of yogurt, confirming the comparatively large overestimations found for the samples with low fruit and high yogurt content. Therefore, further optimization of the enzymatic digestion is required to obtain an AIR devoid of proteins.

Additionally, the low amounts of hemicellulose obtained for the fruit yogurts, in particular for sample FYS-10, are unfavourable to the precision of the method, because gravimetrical determination of quantities lower than 50 mg leads to inaccuracies. Initial weights of the AIR used for fractionation were therefore doubled, but the hemicellulose quantities obtained for samples FYS-6 and FYC-6 were still low. On the other hand, a further increase of the AIR used for fractionation would require the AIR to be isolated in a larger amount, which in turn is associated with additional experimental expenditure during the complete procedure of sample preparation.

From our studies it becomes evident that the method recently developed for the determination of the fruit content of fruit preparations is also promising for fruit containing yogurts. Considering the fact that so far an appropriate methodical concept for the quantification of the fruit content in dairy products has not been developed, the present work marks an innovative approach to

Tab. 1: Gravimetrical data of the lyophilised strawberries and calculation of the conversion factor

Strawberries		AIR isolation[a]		Sequential fractionation[b]		
Cultivar	**Dry matter [%]**	**Initial weight lyophilisate [g]**	**Output weight AIR [g]**	**Initial weight AIR [g]**	**Output weight HC [mg]**	**Conversion factor[c]**
Senga sengana	13.1	14.734	2.963	0.74401	93.29	291.9 ± 4.1[c]
		14.414	2.905	0.78163	98.52	
		15.046	3.274	0.74426	96.38	
Camarosa	10.5	15.422	3.747	0.77898	93.77	332.9 ± 6.5[c]
		15.369	3.715	0.79966	92.11	
		15.332	3.722	0.81054	96.05	

[a] $n = 3$

[b] $n = 3$

[c] ± standard deviation [rel. %]; mean of $n = 9$

Tab. 2: Gravimetrical data of the lyophilised fruit yogurts and calculation of their fruit contents

Fruit yogurts		AIR isolation[a]		Sequential fractionation[b]			
Sample Code	Dry matter [%]	Initial weight lyophilisate [g]	Output weight AIR [g]	Initial weight AIR [g]	Output weight HC [mg]	Calculated fruit content[c]	Deviation from initial fruit content [rel. %]
FYS-30	15.5	14.761	1.547	0.75550	49.73	31.5 ± 9.4	+ 5.0
		14.827	1.597	0.76285	47.19		
		15.021	1.599	0.76423	43.00		
FYS-20	15.5	20.248	1.855	0.80950	49.78	26.0 ± 6.0	+ 30.0
		20.881	1.914	0.82294	49.80		
		20.560	1.883	0.83548	55.51		
FYS-10	16.1	15.610	1.073	0.83664	33.17	13.5 ± 7.1	+ 35.0
		15.595	1.032	0.82856	37.28		
		15.531	1.046	0.84089	36.48		
FYS-6	18.4	21.812	1.704	1.59217	35.78	9.2 ± 14.5	+ 53.3
		20.952	1.597	1.66238	41.38		
		21.469	1.728	1.61131	29.16		
FYC-6	18.6	31.040	2.336	1.63857	29.41	7.8 ± 6.6	+ 30.0
		30.104	2.27	1.64867	27.22		
		30.748	2.342	1.65282	26.53		

[a] $n = 3$
[b] $n = 3$
[c] ± standard deviation [rel. %]; mean of $n = 9$
FYS: Fruit yogurt from strawberry cultivar 'Senga sengana'; FYC: Fruit yogurt from strawberry cultivar 'Camarosa'

overcome this analytical problem. Although far from being a rapid procedure, the availability of such a method considerably increases the expense necessary for adulterations of fruit-derived products and may therefore contribute to avoid unfair competition. In principal, the applicability of the new method could be shown for yogurts with high fruit contents, while for lower contents unsatisfying results were found, suggesting that sample preparation needs to be optimised. In particular, a scale-up of AIR isolation and fractionation is required in order to obtain sufficient HC quantities, resulting both in a higher precision and a lower detection limit. Because the drying process is the most tedious step, the feasibility of the method may further be improved using an alternative drying method. Moreover, most commercial fruit yogurts contain hydrocolloids which are either part of the recipe or originate from the starter cultures or fruit preparations used. Since these high-molecular compounds also precipitate in boiling ethanol, their interference with the AIR isolation and the subsequent fractionation is to be expected. Therefore, the methods established for hydrocolloid separation from fruit preparations (FÜGEL et al., 2006; SCHIEBER et al., 2005) also need to be adapted to fruit yogurts.

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