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Information Bulletin

Number 43/2006



Determination of the pear content in apple juice

By means of an SPE-cartridge module for the Syncore® Analyst the process flow for determining the quality of apple juices is optimized.

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Determination of the pear content in apple juice

A method for determining of the pear content in fruit juices is introduced. By means of the SPE-cartidge module Syncore® Analyst parallel evaporator the number of samples analyzed per day can be increased by three times.

The method was assessed successfully with 17 samples, the analytical results of which are detailed and evaluated.

Introduction

As cider pears have a lower market value than cider apples, it is frequently suspected that apple juice is diluted with the cheaper pear juice. According to Swiss law, apple juice can contain a maximum 10% of pear juice and pear juice a maximum 10% of apple juice [1]. For any other ratios a different designation, e.g. sweet must or fruit juice has to be used, so that the composition is clear for the consumer.

The question posed is how to check that such a regulation has been followed. To ensure the purity of a fruit juice, the SLMB (Swiss Handbook for Food) suggests the analysis of proline [2]. According to these guidelines, a pure apple juice should contain <15 mg/l of proline, whereas pear juice has a proline content of 30 – 250 mg/l. Additionally, according to the SLMB, pear juice contains higher amounts of sorbitol (averaged 15g/l vs. 4g/l) and higher amounts of citric acid (0.1 – 4 vs. 0.05 – 0.2 g/l) compared to apple juice.

Our own analyses have shown increased concentrations of proline and sorbitol in apple juice, indicating the presence of excess pear juice, but the parameters are unreliable for a quantitative assessment of the actual contents.¹

The reason for this is that although these compounds are present in both apples and, in higher concentrations, in pears, the natural deviations are relatively high. Thus, these parameters only allow for a

more or less qualitative conclusion concerning the actual pear content.

Another group of compounds used for the determination of fruit juice concentrations are the phenolic glycosides. Schieber *et. al.* describe a method to detect any addition of pear juice by determining the presence of isorhamnetin-3-glucoside [3]. Furthermore Spanos and Wrolstad show that the phenolic profiles of apple and pear juices differ most obviously when comparing the content of 4-hydroxyphenyl-β-D-glucopyranoside, trivially named arbutin [4, 5]. During subsequent years, other research groups drew the same conclusion [6, 7]. As to the analytic proof, the advantage of arbutin compared to other phenolic glycosides is the fact that the molecule is acid-resistant [4].

Method

The established method for the determination of arbutin by means of HPLC and UV-detection is based on the work of Picinelli [8] and Andrade [9]. As for the sample preparation and subsequent HPLC-separation, these methods had to be considerably improved.

The arbutin contained in the apple juice is enriched by SPE (Solid Phase Extraction), (HR-P by Macherey-Nagel), eluted with methanol, and evaporated (Büchi Rotavapor® EL-131). The residue is then dissolved in a HPLC-eluate. 20 µl of this solution are injected into the HPLC-system (Agilent 1100), where arbutin is separated from residual substances on a ODS3-column (Chrompack) with an aqueous phosphoric acid acetonitrile gra-

Sample designation	Declared pear content	Arbutin [mg/l]	Calculated pear content
Sweet must	with pears	11.3	10.4%
Sweet must	with pears	25.5	23.5%
Sweet must	30%	21.2	19.5%
Sweet must	< 10%	3.5	3.2%
Sweet must	with pears	45.0	41.5%
Sweet must	with few pears	0.0	0.0%
Fruit juice	with pears	10.3	9.5%
Apple juice	0%	0.0	0.0%
Apple juice	0%	10.7	9.9%
Apple juice	0%	0.0	0.0%
Apple juice 100%	0%	0.0	0.0%
Apple juice 100%	0%	0.0	0.0%
Apple juice	10%	2.2	2.0%
Apple juice	0%	0.0	0.0%
Apple juice	0%	0.0	0.0%
Apple juice	0%	0.0	0.0%
Apple juice	0%	0.0	0.0%

Table 1: Fruit juices, their arbutin content and the pear content resulting from it

¹ This is also valid for citric acid, which, according to SLMB can also be used as an indicator for elevated pear content.

dient and detected at 282 nm by means of a diode array detector (DAD). The UV absorption spectrum (maxima at 220 nm and at 282 nm) serves as verification. The detection limit of this method is 1 mg/l, the limit of determination 3 mg/l and the recovery rate is in the range of 92% to 101%. The uncertainty of measurement calculated according to the SLMB amounts to 7%.

Results and discussion

The detected arbutin content allows the amount of pear juice in a fruit or apple juice to be calculated. For this purpose, the amount of arbutin in a pear is needed. According to our own analyses, the average arbutin content of the most common Swiss pear, the so-called "Gelbmöstler" [10] amounts to approximately 100 mg/kg. In addition to this, the upgrade coefficient caused by musting has to be considered as the production of 1 liter of fruit juice requires 1.4 kg of fruit.

Our own analyses have shown that a quantity of arbutin remains in the pomace, so the upgrade coefficient used in the calculation is 14%. Whether or not the "Gelbmöstler" was used can be confirmed by further analysis of the

amino acid proline and the sugar alcohol sorbitol.

Table 1 shows the results of 17 analyzed fruit juices. According to the food law, a must declared as apple juice is allowed to contain a maximum pear content of 10%, furthermore the used fruit sorts have to be declared. If these conditions are not fulfilled, the correct designation would be must, sweet must or fruit juice. Only one apple juice analyzed showed a presence of arbutin without an addition of pear being declared.

The fruit juices without quantitative declaration of the pear content are freshly pressed musts, which are not industrially bottled and which simply have a qualitative declaration.

Optimizing the sample upgrade with the Syncore® Analyst

Conventional method

Solid phase extraction vacuum manifolds with 12 cartridge ports are typically used. This setup requires the vacuum manifold to be ventilated after each step, such as conditioning, enrichment, washing, and to exchange the receiving vessels below

the cartridges, all of which introduces the risk of cross-contamination. A weak vacuum is applied for the following elution, after which the collected eluates are evaporated individually with a rotary evaporator and the residue dissolved into a defined volume. This solution is subsequently used for HPLC-analysis.

Optimization with the Analyst

To reduce the manual sample handling during sample preparation, an SPE-module for the Syncore® Analyst was developed in collaboration with Büchi Labor-technik AG. The system requirements are as follows:

- The operational steps for the SPE-concentration (conditioning, sample enrichment, washing, elution) shall be carried out without interruption and sample handling.
- Avoidance of cross-contamination.
- The outlet of each SPE-cartridge must be individually adjustable to the positions "stop", "waste", and "elute" (see **Figure 1**).

Consistent with the Analyst rack 12 an SPE-module with 12 cartridge ports (see **Figure 2**) was developed. The re-

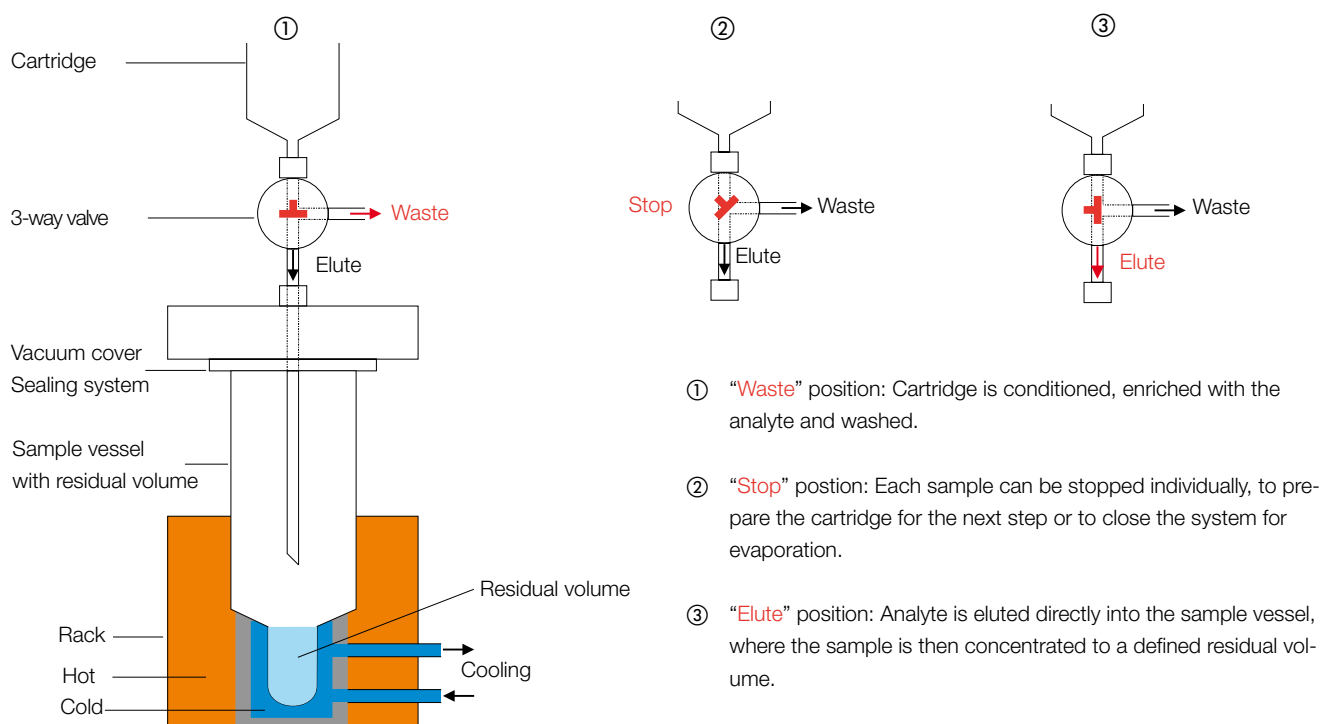


Figure 1: Schematic representation of the different positions for conditioning, enrichment, washing and elution with subsequent concentration to a residual volume of 1 ml.

sults presented in **Table 1** were obtained using this SPE-module.

The SPE-module for the Analyst enables the enrichment of 12 apple juice samples at the same time, it allows the elution of arbutin and subsequent concentration to 1 ml, which leads to a considerable increase of the sample throughput.

With the help of the three-way stopcock, connecting the SPE-cartridge to the Analyst vessel and a waste container allows the easy selection between "waste", "elute" and "stop" positions (see **Figure 1**).

This setup enables the user to carry out all steps – from the solid phase extraction to the complete evaporation of the eluate – without changing the instrument setup. Thus, a great time saving is achieved as samples are processed at the same time, which is especially relevant when evaporating the solvent.

In reality a trained laboratory assistant could carry out 12 concentrations three times within one working day with this setup.

Conclusion

With the aid of the analyzed pear samples, the content of pear juice in various fruit juices due to arbutin content can be qualitatively analyzed in a reliable way.

Furthermore, a method is available enabling to check whether the corresponding legal regulations have been fulfilled.

By using the SPE-module for the Syncore® Analyst the sample preparation throughput in routine analyses could be tripled.

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Figure 2: Syncore® Analyst with rack 12 and vacuum cover with SPE-cartridges

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