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QUALITY CONTROL OF ALOE VERA BEVERAGES

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KEYWORDS

Aloe vera juice, Aloe barbadensis MILLER, solid-phase microextraction, gas chromatography, mass spectrometry, thin-layer chromatography.

ABSTRACT

Aloe vera beverages have to be produced exclusively using material of the plant species A*loe barbadensis* MILLER. Commercial material was reported to be frequently adulterated by artificial preservatives or to lack significant amounts of Aloe ingredients. HPTLC and HS-SPME/GC/MS methods to assess the authenticity of Aloe vera beverages were developed in this study, allowing to differentiate between authentic and adulterated products. In one case a commercially available Aloe vera juice could be proven to be exceedingly watered down. Parallel to the authenticity control, the HS-SPME method employed in this work allowed to detect the preservatives benzoic acid, sorbic acid and pHB-esters. In 17 of 24 (71%) currently available Aloe-food products an illegal addition of preservatives of up to 1000 mg/l could be ascertained. The presented analyses of Aloe vera beverages lead to the conclusion, that this product line does not give any cause for hygienic but rather legal concerns: controls have to be intensified to ensure sufficient product quality with regard to preservatives

INTRODUCTION

By law Aloe vera beverages have to be produced using material of the plant species A*loe barbadensis* MILLER, which is a tropical or subtropical plant characterized by lance-shaped leaves with jagged edges and sharp points [1-3]. In the production process of Aloe vera juices and gels the manufactures often employ artificial preservatives (e.g. benzoic or sorbic acid) to stabilize the plant extract and conserve the valued ingredients. Apart from this illegal addition of preservatives to Aloe-beverages, adulterated products containing no significant amount of Aloe ingredients are also marketed. Aloe juices and gels are generally high-priced, for 1l Aloe vera juice for example the consumer is charged up to 30 EUR. It cannot be excluded that manufacturers try to enlarge their profit margins by watering down the original Aloe juice. Studies from the USA showed that a considerable part of commercial material labelled as Aloe vera material was not consistent with the label claim [4,5].

Aloe vera products are only suitable for human consumption if they are free from aloin, a native Aloe vera constituent that acts as a laxative and is supposed to be a DNA-damaging and carcinogenic agent. Aloin can be prevented from entering the production process if the outer layers of the Aloe plant, containing the highest quantities of aloin, are discarded before extracting the juice. Aloe vera products

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may be considered aloin-free if the maximum limit of 0.1 mg/l is not exceeded [6]. Therefore, for a thorough quality control of Aloe beverages, the following analyses have to be performed:

- Investigation of authenticity (identity, adulteration, dilution)
- Test for inadmissible preservatives
- Determination of aloin content

For the detection of preservatives as well as aloin, standard methods are available [7,8]. This paper, however, describes for the first time HPTLC and HS-SPME methods to assess the authenticity of Aloe vera beverages.

Solid-phase microextraction (SPME), discovered and developed by Pawliszyn and co-workers [9], has recently emerged as a versatile solvent-free alternative to conventional liquid-liquid extraction procedures. Headspace solid-phase microextraction (HS-SPME) is based on the distribution of analytes between the sample, the headspace above the sample and a coated fused-silica fibre. Analytes are absorbed by the coating of the fibre where they adhere until equilibrium concentrations between the phases are reached. Subsequently, the fibre can be introduced directly into a GC injection port for thermal desorption. In HS-SPME no matrix interferences can result in a diminished chromatographic background, solvent consumption is markedly reduced and its overall technical performance is fast and simple. The use of SPME in food analysis was recently reviewed by Kataoka [10].

MATERIALS AND METHODS

Reagents and materials

Cyclodecanone, which was used as internal standard, was purchased as a solid from Fluka (Buchs, Switzerland). It was stored at 8 °C, and used after dilution to the required concentrations. Further chemicals were purchased from Merck (Darmstadt, Germany). An SPME device for the autosampler with a replaceable 100 µm polydimethylsiloxane (PDMS) fibre was obtained from Supelco (Deisenhofen, Germany). The fibre was conditioned at 250°C for 1 h in the injection port of the GC according to the supplier's instructions.

Aloe vera beverages (n=17) were sampled in the context of the official food control in the German Federal State of Baden-Württemberg. Further samples (n=7) were obtained from chemists and pharmacies.

Thin-layer chromatography (HPTLC)

Classic thin-layer chromatographic methods are suitable to investigate the authenticity of Aloe beverages. Separation was performed on pre-coated 10x10cm HPTLC glass plates (sorbent: silica gel; pore size: 60Å; fluorescence indicator: F254; Merck, Darmstadt, Germany). Sample volumes of 20 µl were applied to the plates as bands with a width of 10 mm using a TLC applicator (Automatic TLC Sampler III, Camag, Berlin, Germany). The plates were developed using a freshly prepared mobile phase of n-Butanol : n-Propanol : glacial acetic acid : water (30 : 10 : 10 : 10, v/v/v/v).

After drying at room temperature, the spots were stained with a solution of anisaldehyde reagent R (0.5 ml of anisaldehyde, 10 ml acetic acid, 85 ml methanol, and 5 ml sulphuric acid) by dipping, followed by heating for 5 min at 105-110°C.

Following chromatographic separation bands can be compared to Aloe-typical bands of an authentic Aloe vera-standard and authenticity of the sample can be evaluated.

Gas chromatography – mass spectrometry (GC/MS) method

For further confirmation, a detection method combining solid-phase microextraction (SPME) and GC/MS was developed. The applicability of this novel method could be assured by the successful identification of volatile analytes, which have already been recommended for the characterization of Aloe, e.g. limonene, eucalyptol and β-pinene [11,12].

The analyses were performed on a model 6890 Series Plus gas chromatograph, in combination with an Agilent 5973 N MSD mass spectrometer (Chromtech, Idstein, Germany). Substances were separated on a fused silica capillary column (HP-5MS, 30 m x 0.25 mm I.D., film thickness 0.25 µm). The temperature program was applied as follows: start temperature 35°C, 10 °C/min increase up to 300 °C. The temperatures for the injection port, ion source, quadrupole and interface were set at 260 °C, 230 °C, 150 °C and 280 °C, respectively. Injection was carried out in splitless injection mode, and helium at a flow rate of 1.0 ml/min was used as carrier gas. Electron impact (EI) mass spectra for the analytes were recorded in Full Scan mode.

Headspace-SPME method

500 µl aliquots of each sample were deposited into two 10 ml headspace vials with 50 µl cyclodecanone (50 ng/ml) each. No further sample preparation was necessary. The vials were sealed using silicone septums and magnetic caps. One vial was shaken for 5 min at 40° C, the other one at 80° C in the agitator of the autosampler (650 rpm, agitator on time: 0:05 min, agitator off time: 0:02 min). For absorption the needle of the SPME device containing the extraction fibre was exposed to the vapours in the headspace of the vials for 11 min. The absorbed compounds were desorbed from the fibre by incubating it in the injection port for 5 min.

In order to develop optimal conditions in the sample preparation step, the influence of extraction, incubation temperature, incubation time and desorption on recovery was determined. Samples (500 µl plus 50 µl ISTD) were either diluted with buffer solutions (500 µl phosphate buffer pH 4-11) to solubilize the analytes or hydrolysed using acid or base (500 µl 0.1 M HCl and 0.1 M NaOH). The following preparation and analysis was as described above. Furthermore, samples were incubated at different temperatures (30-100° C). The incubation time was evaluated between 5 and 15 min. Optimal conditions were defined as those yielding the highest recoveries, i.e. the highest peak areas, of the internal standard cyclodecanone, which was chosen to represent all volatile compounds.

Multivariate data analysis

The quantitative data of HS-SPME/GC/MS analyses were exported to the software Unscrambler v9.0 (CAMO Process AS, Oslo, Norway). The data set was pre-processed by standardization to give all variables the same variance. Then Principal Component Analysis (PCA) was used to transform the original measurement variables into new variables called principal components (PC). The technique of cross-validation was applied to determine the number of principal components (PCs) needed. During cross-validation, one sample at a time (out of n samples) is left out, and the prediction ability is tested on the omitted sample. This procedure is repeated n times resulting in n models and giving an estimate on the average prediction ability for the n models. This result is used to select the number of PCs needed. By plotting the data in a coordinate system defined by the two largest principal components, it is possible to identify key relationships in the data as well as to find similarities and differences.

RESULTS AND DISCUSSION

HS-SPME method optimization

The SPME-method parameters extraction temperature, extraction time and desorption conditions were successively optimized. The desorption velocity of analytes from the fibre can be regarded as uncritical at and above 260° C. A desorption time of 5 min was proven to eliminate all residual analytes from the PDMS-phase. The fully automated extraction of the samples either in buffer solution (phosphate buffer pH 4-11) or after acidic or alkaline hydrolysis did not significantly affect recovery as compared to untreated samples. The influence of different pH-values was shown to be negligible (Fig. 1). Extraction time and extraction temperature were most critical for the optimization of the method. The optimal extraction temperature of cyclodecanone was found to be 60° C (Fig. 2). However, it was shown that peak areas of the volatile substances were greater at lower temperatures (30-50° C), while peak areas of fatty acids where significantly greater in the upper temperature ranges (80-100°C). For this reason samples were analysed in duplicate (40° C and 80°C), to cover the whole spectrum of analytes. For HS-SPME it is necessary, that a 3-phase equilibrium between the liquid sample, the gas phase and the solid fibre is formed. The optimal extraction time for sample analysis was 11 min and it can be presumed that at this time equilibrium prevails. Longer extraction times lead to a decrease in recovery (Fig. 3). A typical HS-SPME/GC/MS chromatogram of an Aloe vera juice is shown in Fig. 4.

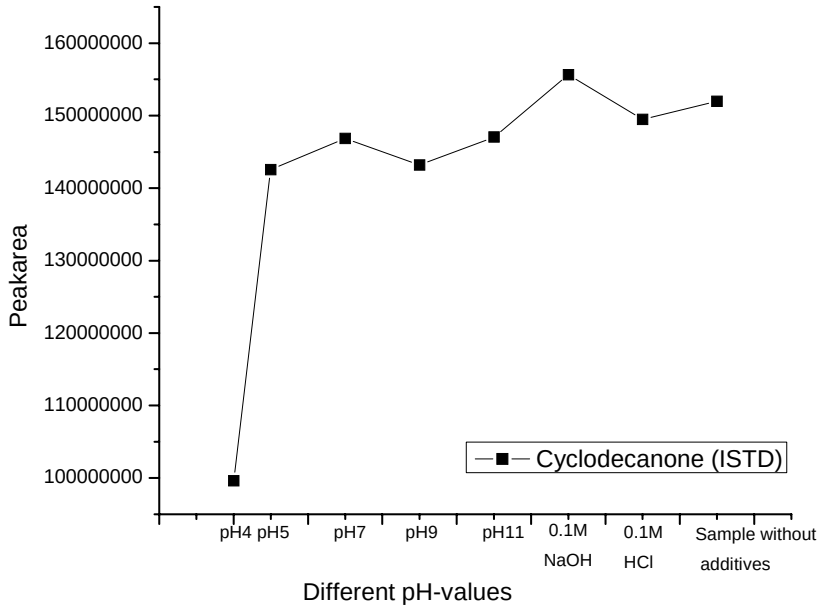

Fig. 1. Influence of different pH-values on the recovery of cyclodecanone.

The new HS-SPME procedure proved to be suitable for the determination of volatile compounds of Aloe vera in food products in an automated and therefore convenient procedure. All steps (e.g. heating and shaking of the sample, absorption, pre-concentration and desorption into the injector of the GC) are programmable and automatically executed, thereby reducing the possible sources of error and distinctly improving reproducibility.

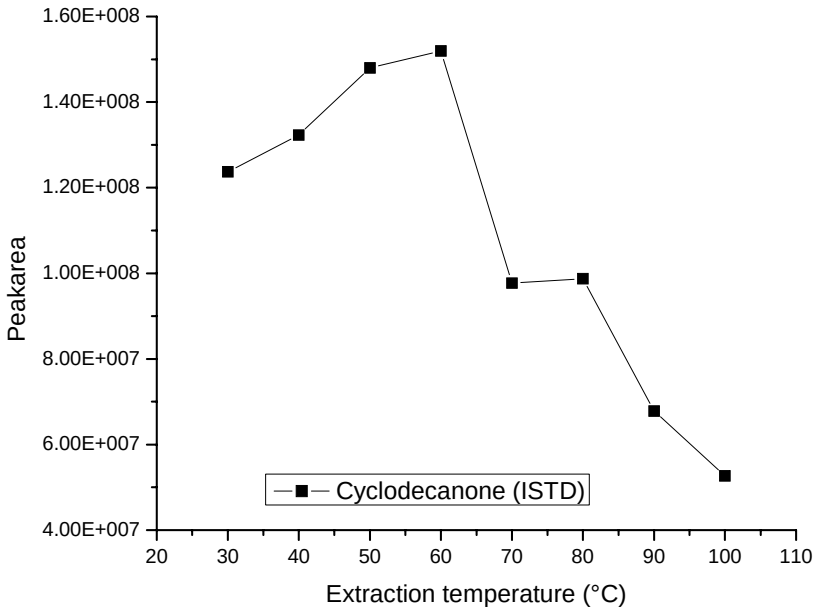

Fig. 2. Optimization of SPME extraction temperature.

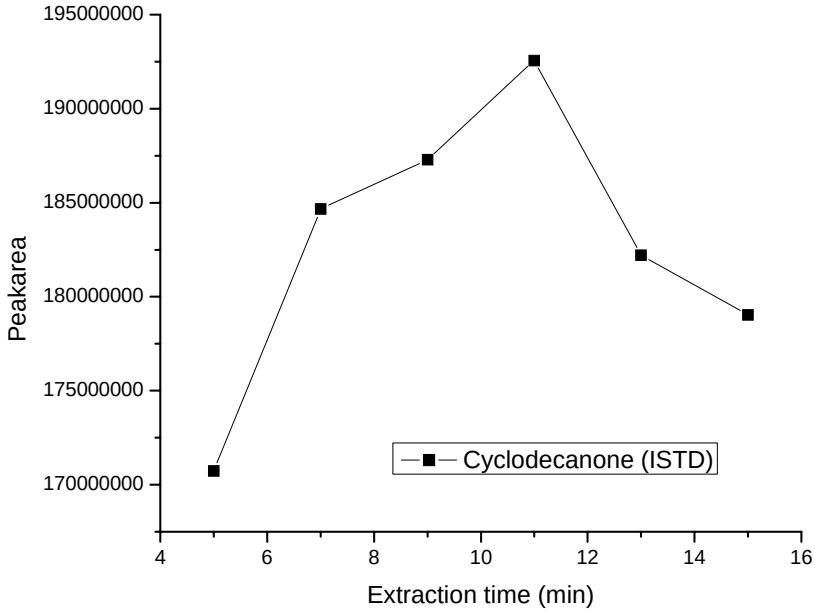

Fig. 3. Optimization of SPME extraction time.

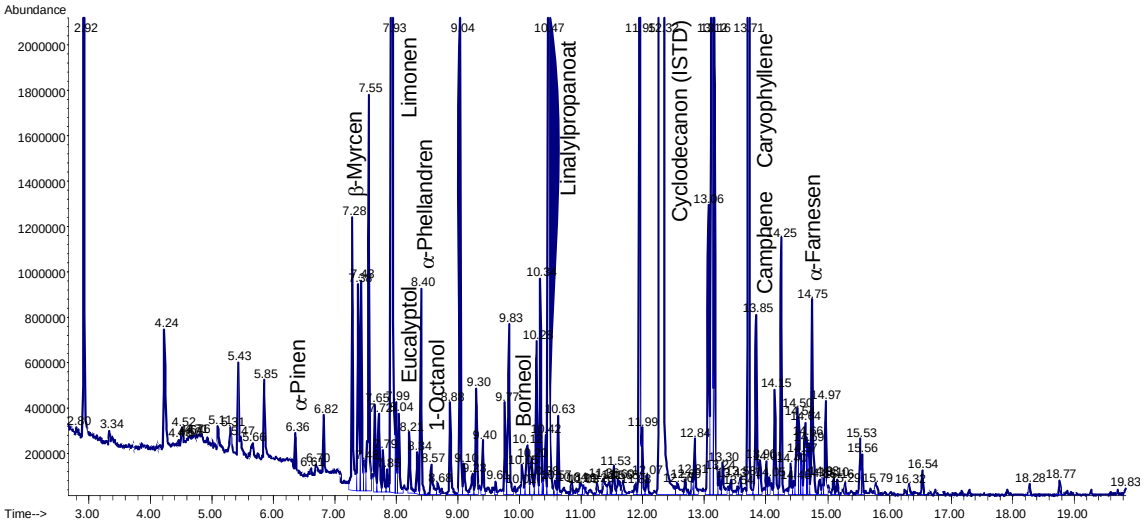

Fig. 4. HS-SPME/GC/MS chromatogram of an Aloe vera-juice (100%).

Authenticity control using HPTLC and HS-SPME/GC/MS

Thin-layer-chromatography is a simple and rapid method to detect adulterations of Aloe vera products (Fig. 5). In one case a commercially available Aloe vera juice could be proven to be exceedingly watered down. In comparison to HPTLC, HS-SPME/GC/MS-chromatograms contain considerably more data, which can be made accessible using multivariate data analysis (Fig. 6).

Table 1. Analysis results of 24 Aloe vera products (+ positive, - negative)

No.	Sample	HPTLC identity	SPME identity	Sorbic acid	Benzoic acid	pHB-Esters
1	Bio Juice	+	+	-	+	-
2	Juice from concentrate	+	+	-	+	-
3	Juice	+	+	-	-	-
4	Juice	+	+	+	+	-
5	Juice	+	+	+	+	-
6	Juice	-	-	-	+	-
7	Juice	+	+	+	+	-
8	Juice	+	+	+	+	-
9	Juice	+	+	-	+	-
10	Gel	+	+	-	+	+
11	Juice	+	+	+	+	-
12	Juice	+	+	+	+	-
13	Juice	+	+	+	+	-
14	Premium juice	+	+	+	+	+
15	Juice	+	+	+	+	-
16	Juice	+	+	-	+	-
17	Juice	+	+	-	-	-
18	Juice	+	+	-	-	-
19	Juice	+	+	-	-	-
20	Gel	+	+	-	-	-
21	Fresh juice	+	+	-	-	-
22	Gel	+	+	-	-	-
23	Tea	-	-	-	+	-
24	Gel	+	+	+	+	-

This technique was used successfully to differentiate between products containing just a minimal amount of Aloe ingredients and material made exclusively from Aloe (Table 1). However, substantial differences in the HS-SPME/GC/MS-fingerprints of different products were detected, thus it was impossible to identify a single compound as a characteristic marker for Aloe in drinks. Parallel to the authenticity control, the HS-SPME method employed in this work allowed to detect the preservatives benzoic acid, sorbic acid and pHB-ester, which was confirmed using standard HPLC.

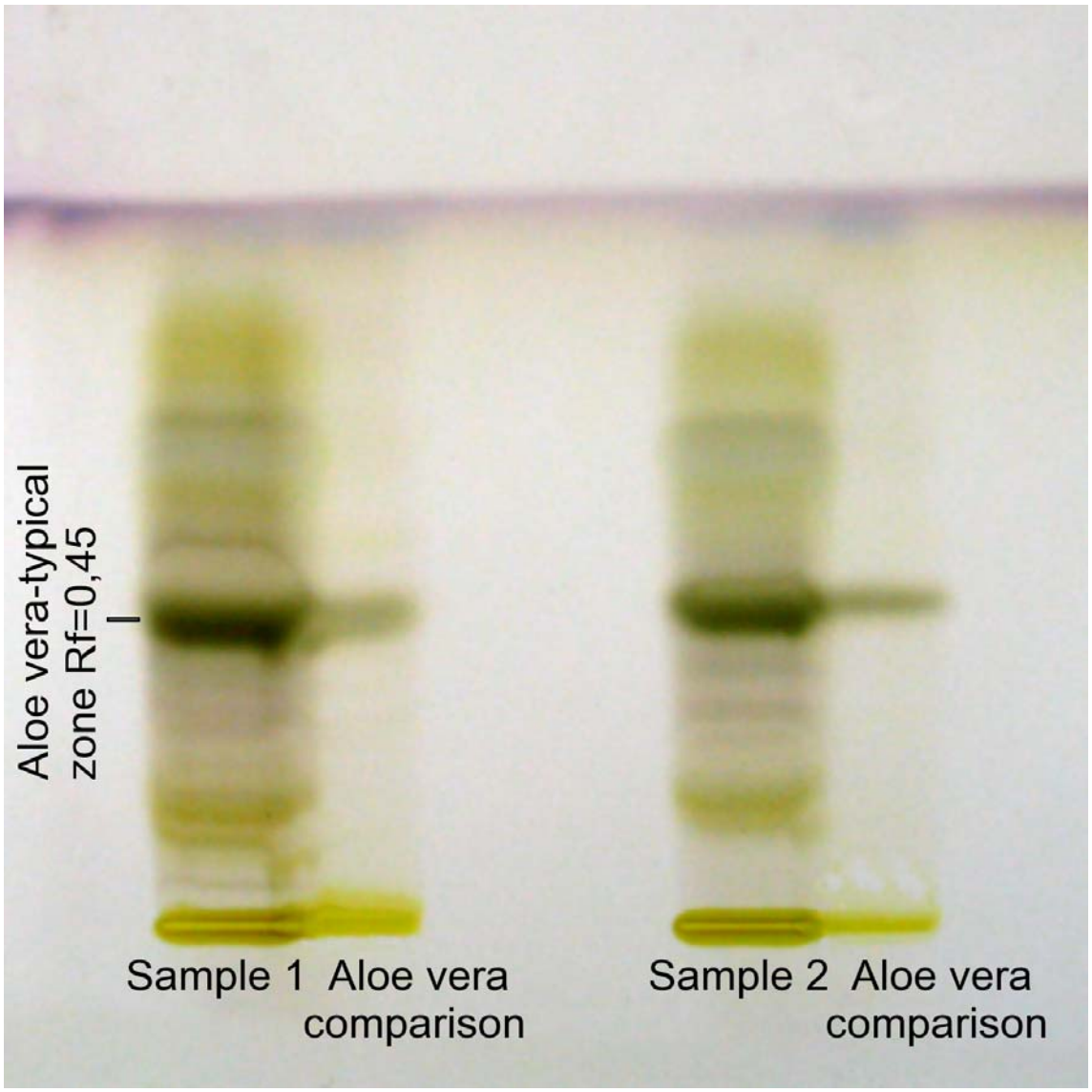

Fig. 5. HPTLC chromatogram of Aloe vera products and authentic samples for comparison (Aloe vera gel in pharmaceutical quality.

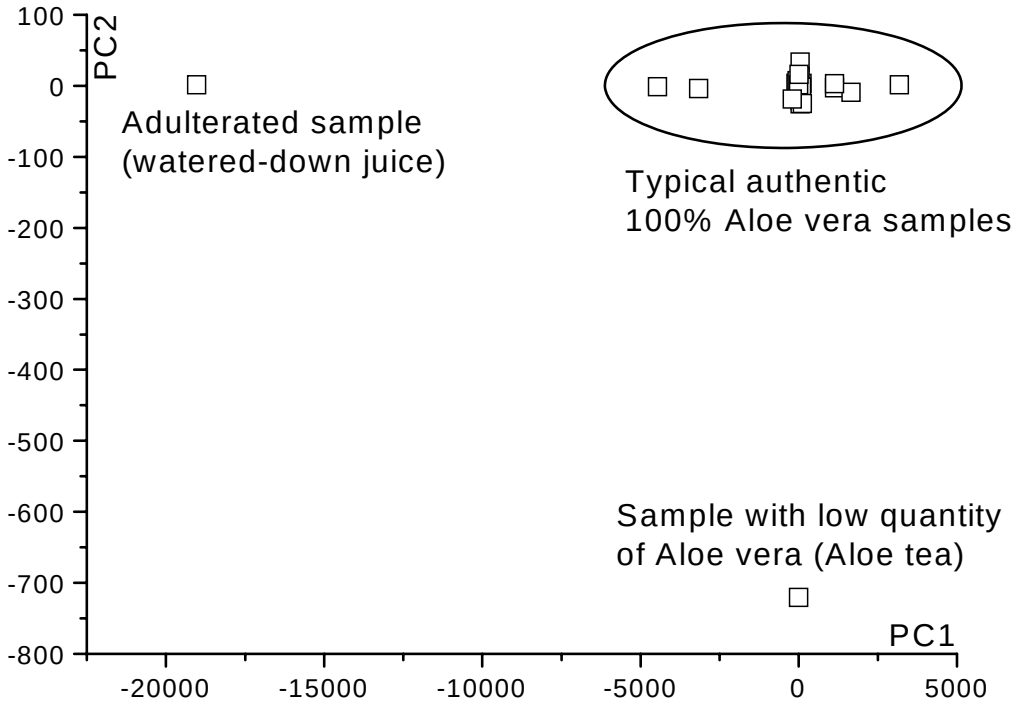

Fig. 6. PCA scores plot of 24 Aloe vera products

Problem of artificial preservatives

In 17 of 24 (71%) currently available Aloe-food products an illegal addition of preservatives was ascertained (Table 1). Many Aloe vera juices are preserved by addition of sorbic and/or benzoic acid in concentrations of up to 1000 mg/l. As preservation of Aloe juices is prohibited in the European Union (EU) [13], several manufacturers add ascorbic acid to their products and declare their Aloe-juices to be "dietary supplements". This is done to legally bypass the above mentioned EU-directive, as dietary supplements may contain preservatives. It has to be considered though that Aloe juices or gels do not contain concentrated nutrients or other ingredients of dietary or physiological value, and even with the addition of ascorbic acid Aloe juices do not qualify as dietary supplements. Otherwise bottling plants could vitaminize all their fruit- or vegetable-juices, declare them to be "liquid dietary supplements" and add preservatives up to a concentration of 2000 mg/l, thereby circumnavigating the current ban on food preservatives. The classification of vitamin C-enriched Aloe vera juice/gel as a dietary supplement is therefore not admissible.

CONCLUSIONS

The presented analyses of Aloe vera beverages lead to the conclusion, that this product line does not give any cause for hygienic but rather legal concerns.

Using standard HPLC only one of the tested products had to be reprehended due to an Aloin concentration above the legal limit. In earlier quality control experiments such an excess of Aloin was more frequent, leading to the interpretation that manufacturers have improved their production methods and/or the quality assurance. Aloe vera beverages are extensively controlled by the official food control and nowadays most products abide by the legal aloin limit. The considerable percentage of illegally

preserved products we found during this study makes us postulate that controls have to be intensified to ensure sufficient product quality with regard to preservatives.

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