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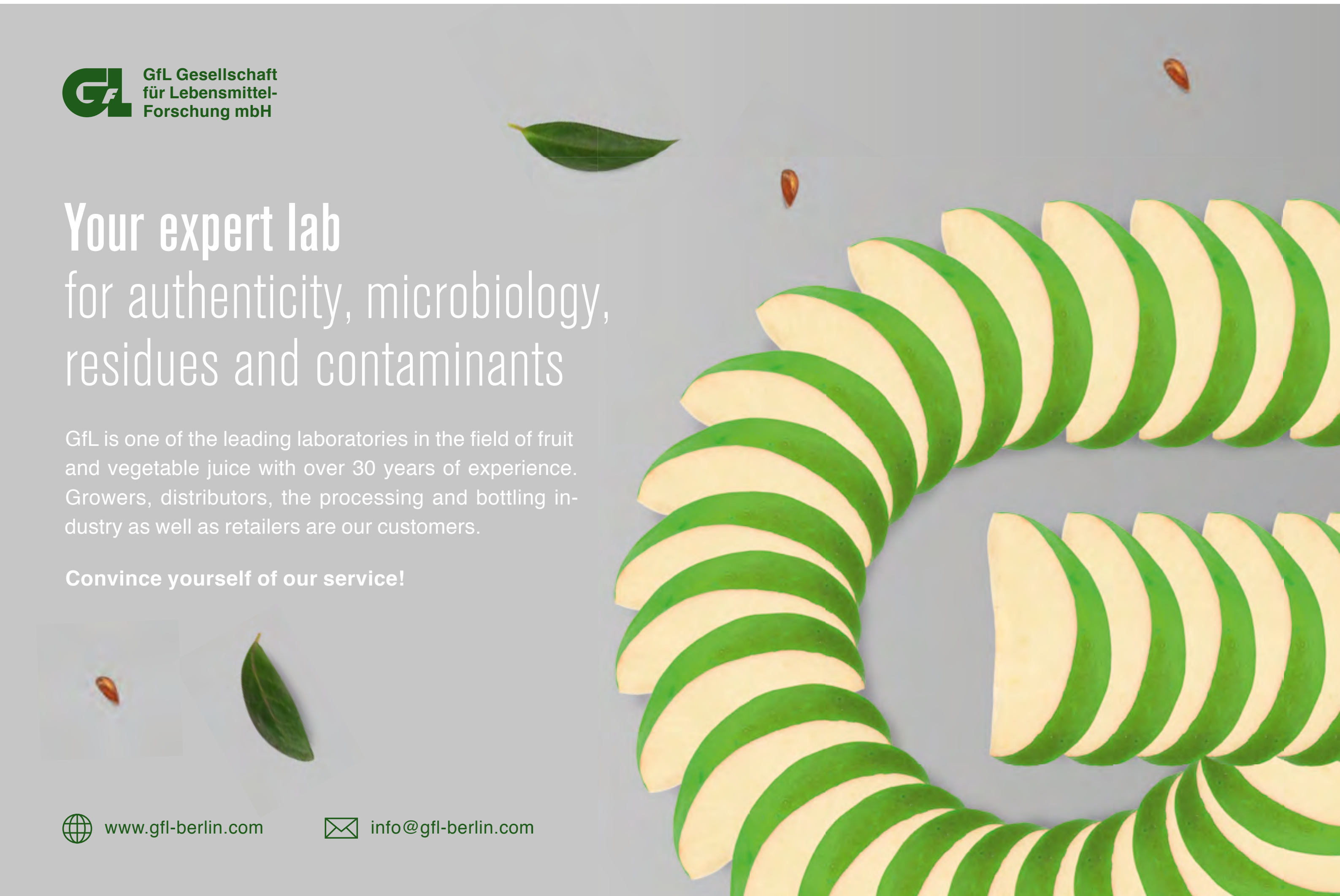
Instability of fruit-based beverages – clouds, hazes, and sediments

by
Prof Dr Frank Will, Jeannette Zacharias, Prof Dr Helmut Dietrich



Instability of fruit-based beverages – clouds, hazes, and sediments*

| Clouds | Fruit-Based Beverages | FTIR-spectroscopy | Hazes | Instability | Sediments |

Introduction

During the production of clear beverages a lot of processes are carried out to reach and maintain stability within the recommended shelf life. Technological processes to avoid haze formation in clear fruit based beverages start normally with an enzyme treatment to degrade pectin and in case of apples and pears also starch with pectinases and amylases. In the juice stage, mechanical treatment steps like flotation, racking, decanting, and centrifugation are used to clarify the product. Additionally, colloidal stabilisation is done with classical fining (gelatin/plant proteins, silica sol, bentonite) or polyphenol removing with active carbon, adsorber resins, or PVPP. Finally, different filtration techniques (diatomaceous earth, sheet, cross-flow, dead end filtration) are used to achieve clear products. In spite of this high clarification effort, clouds, hazes, precipitations, and sediments are occurring in an estimated low, but unknown percentage of readily produced fruit beverages.

The most frequent reasons for undesired haze formation are:

- insufficient enzymatisation, clarification, stabilisation, and filtration
- solubility product of constituents exceeded (often during concentration)
- temperature changes (improper storage or shipping conditions)
- cross-reaction of different ingredients (pectin and starch fragments, polyphenols, proteins)
- chemical oxidation and condensation of polyphenols

The fruit juice industry is affected twice: first with semi-finished products like semi- or full concentrates in the global trade, where various, often non-standardised tests are used to predict stability (Will and Dietrich, 1992; Will 1993). Secondly the problems occur with finished products in the food retailing sector. Customers expect crystal-clear products and do not accept precipitates and reject the products. In a worst-case scenario retailers require recall actions, products have to be returned on own costs, and products, manufacturers or bottlers may be delisted combined with inevitable financial and image losses.

Based on our long-time experience with the characterisation of sediments from fruit based beverages we would divide them into 5 categories:

1. organic non-crystalline (residual pectins, polyphenols, starch, complexes thereof)
2. organic crystalline (organic acids, amino acids, sugars, polyols)
3. inorganic, crystalline or amorphous particles (metal-based, silicate)
4. microbiological (microorganisms or their metabolites)

Isolation and analytics

The "Geisenheim approach" (Dietrich *et al.*, 1995) for characterisation of beverage haze starts with the microscopic examination of an incoming beverage sample to exclude or determine microorganisms or other easily detectable causes. If nothing can be detected, we make a sedimentation at least overnight and in difficult cases up to some days. The supernatant is decanted under vacuum, the residue is centrifuged to achieve a compact sediment. The sediment is cleaned up by 1-3 successive washings with water or low-concentrated ethanol and dried at 40-60 °C. Finally the dried sediment is weight for yield determination and examined under the light microscope using different techniques (bright field, dark field, phase contrast, polarisation). The microscopic assessment is only significant, if characteristic microorganisms or typical crystals are present. In most cases there are uncharacteristic, amorphous findings. *Figure 1* shows the isolation steps of sediments from a pear juice resulting in about 100 mg final sediment from 6 bottles.

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* based on a presentation given at the IFU Technical Workshop in Athens, 6th March 2019

Fig. 1: Isolation of sediments from a pear juice © all Geisenheim University

The first analytical step is always FTIR-spectroscopy (Fourier-Transform-Infrared-Spectroscopy), because there we get substance-specific adsorption bands in the fingerprint range between the wavenumbers 800 and 1800 cm^{-1}. The dried and finely ground sediment is loaded on the ATR-probe (attenuated total reflection) of the Bruker Tensor 27 FTIR-machine, and the resulting sample spectrum will be compared with the spectra of our electronic library. In many cases, there is a spectral match and the analysis is already complete unless customers request a confirmation with a second method. If the FTIR-spectrum is unclear e. g. with starch, pectins, and composed precipitates, additional analytical methods have to be used to characterise the sediments. Target analytes and required analytical techniques are summarised in *table 1*.

Case studies

Haze formation happens in all kind of alcoholic and non-alcoholic beverages. We will focus here on selected and typical examples found in fruit juices and concentrates and investigated in the last 25 years in our lab. Microorganisms are not considered, because they are only rarely occurring as clouds in practice. If so, mainly yeasts, lactic/acetic acid bacteria, and mould are found which all are easy to determine under the microscope.

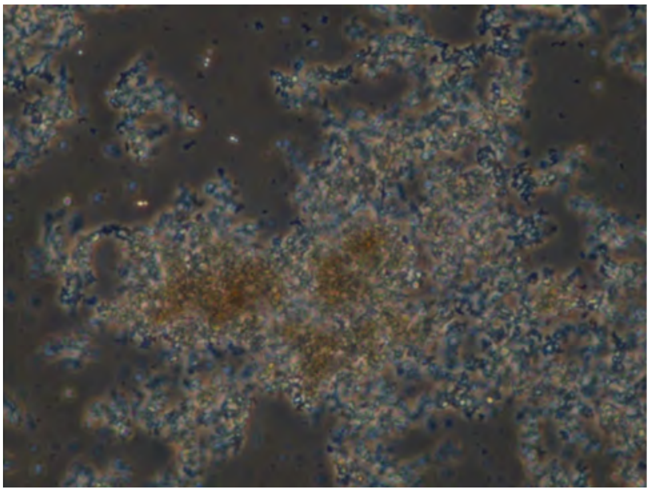

Fig. 2: Protein from grape juice (500x, phase contrast)

Tab. 1: Target analytes and required analytical techniques

	HPAEC /PAD	AA-analysator	RP-HPLC UV/VIS	TXRF-spectr.	LCMS	AAS, ICP-MS/ OES
amino acids, proteins		x				
anthocyanins			x		x	
carbohydrates	x					
element analysis				x		
metals				x		x
organic acids			x			
pectins	x					
polyphenols			x		x	
starch	x					
sugar alcohols	x					
sugars	x					
unknowns			x		x	

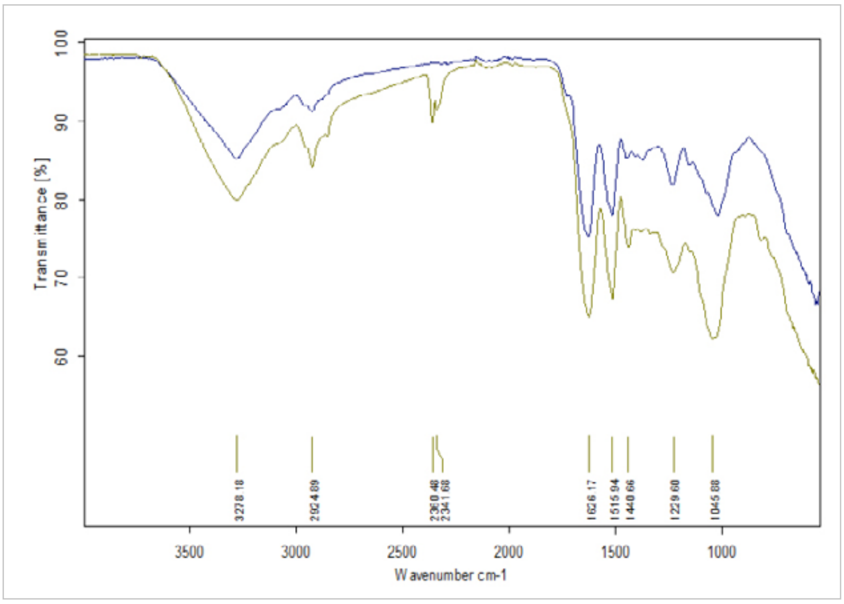

Fig. 3: FTIR-spectrum of grape protein. Blue: isolated and purified standard protein from the spectra library reference; green: precipitate from commercial white grape juice.

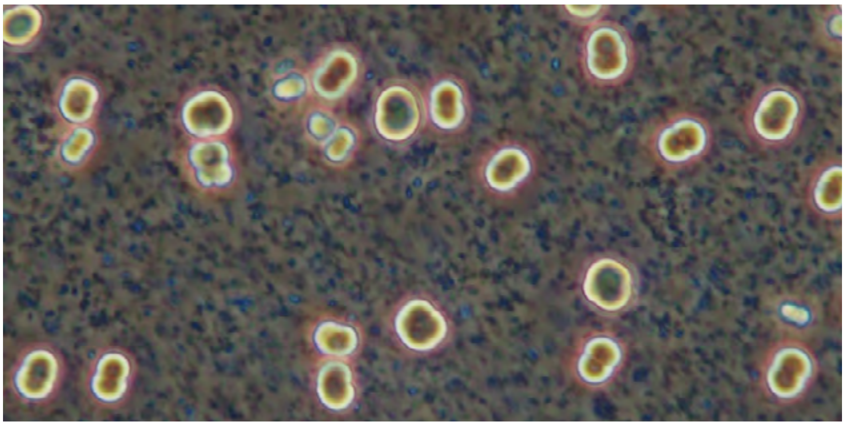

Fig. 4: Arabinan from AJC (500x, phase contrast)

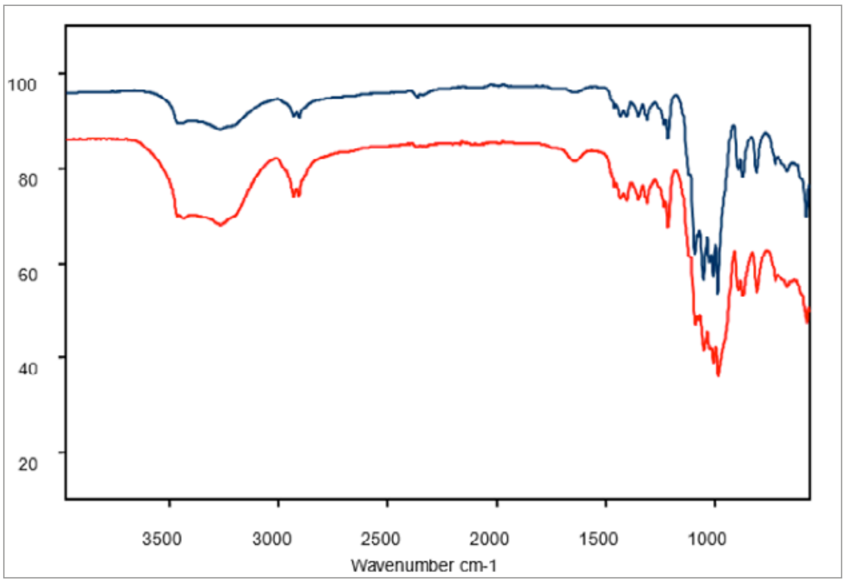

Fig. 5: FTIR-spectrum of arabinan. Blue: isolated and purified arabinan reference standard; red: precipitate from commercial AJC

Figure 2 shows a typical picture of an uncharacteristic, amorphous precipitation originating from a grape juice sediment. Contrary to most fruits used in the fruit juice industry, grapes and grape juices contain considerable amounts of protein. Protein is the most frequent haze in grape juices and also in the corresponding wines. There is nothing recognisable under the microscope than amorphous crumbs. FTIR-spectroscopy revealed clearly the typical protein bands at 1515 and 1630 cm^{-1} (*fig. 3*). This result was confirmed after hydrolysis of the material with 6M hydrochloric acid and separation of the resulting amino acids with an amino acid analyzer. Here the typical amino acid composition of grape protein was found with 17 amino acids and the dominating component proline (not shown).

Figure 4 shows the microscopic picture of an arabinan isolated from a 70 °bx apple juice concentrate (AJC). Arabinan is a typical pectin side chain composed of α-1,5-linked arabinose. It was a frequently occurring precipitate in apple and pear juice concentrates of the 1980s, where there was a lack of arabinase activity in enzyme preparations for the treatment of mash and juice. The problem disappeared in the 1990s and occurred again more frequently in the last 3-5 years. FTIR-spectroscopy (*fig. 5*) showed a good matching between a standard arabinan isolated and purified from an apple juice (Will and Dietrich, 1992; Will *et al.*, 1994) and the precipitated sample. Under the microscope it is very easy to mix up with the shape of diplococci, so these findings should always be confirmed with sugar component analysis. After hydrolysis with 2M sulphuric acid and separation of resulting sugars with HPAEC/PAD (high performance anion exchange chromatography/pulsed amperometric detection) arabinose was found with more than 60 mas% which stands for the positive proof.

Figure 6 shows the microscopic picture of a crystalline sediment from a raspberry concentrate. Typical for crystals, it is polarising. Because of the strong sour taste of the precipitate we subjected the material to RP-HPLC/UV for organic acids and found exclusively citric acid. Beside small amounts of malic acid citric acid is the main organic acid of raspberries ranging from 10 to 20 g/kg depending on the cultivar. Citric acid is a highly soluble substance and the reason for its precipitation in the concentrate stage could not be determined. The only explainable possibility is that the solubility product was exceeded during evaporation.

A similar case was observed with a precipitate from black carrot concentrate. Again there was a strong sour taste, and therefore the substance was not washed before the analysis. By means of RP-HPLC/UV we found 496 g/kg citric acid in the material. As reported from the manufacturer, citric acid was added in the juice stage to lower the pH for microbiological reasons, which is a usual practice. During juice evaporation the added citric acid precipitated. The material was intensely red coloured, and with a second RP-HPLC/VIS-method we found 4.7 g/kg anthocyanins. *Figure 7* shows the chromatogram of the typical anthocyanin composition of black carrot: cyanidin-3-xyl-glc-gal, cyanidin-3-sambubioside-5-glc, cyanidin-3-snapinoyl-xyl-glc-gal, cyanidin-3-feruloyl-xyl-glc-gal, and cyanidin-3-coumaroyl-xyl-glc-gal. Like citric acid, anthocyanins are highly soluble in aqueous media like juice. But both substances precipitated during evaporation and the only remaining explanation was the breach of the solubility products. Although the precipitate dissolved after reconstitution to juice strength, customers refused the product.

As mentioned above, the dried precipitate was not washed. During washing, citric acid and anthocyanins were removed completely. After purifying, another insoluble residue remained. Under the microscope typical octahedron shape crystals were found (*fig. 8*). FTIR and RP-HPLC/UV identified the crystals clearly as Ca-oxalate. Oxalic acid is the main organic acid of rhubarb juice (10-18 g/l, Will and Dietrich, 2012, 2016); in most other fruit and vegetable juices it occurs only in minor amounts like here. Even natural trace amounts of oxalic acid are able to react with endogeneous calcium to hardly soluble Ca-oxalate, which precipitates easily. We found Ca-oxalate precipitations in a number of different fruit and vegetable juices. Confirmation of Ca-oxalate findings is possible with FTIR and RP-HPLC/UV.

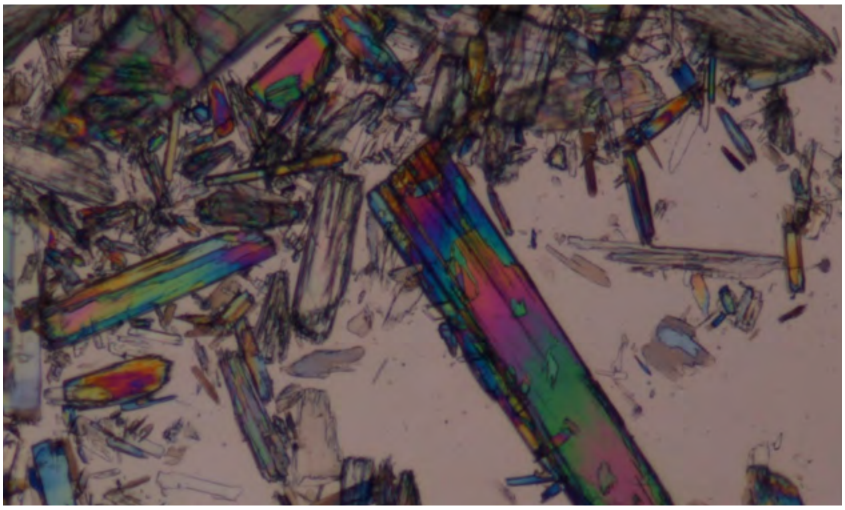

Fig. 6: Citric acid precipitate from a raspberry concentrate (125x, polarisation filter)

In a large number of sent in commercial samples we found more or less pure anthocyanins as precipitates. The corresponding sources were mostly concentrates from anthocyanin-rich berries like aronia, blackcurrant, blueberry, and cranberry. They occur often in form of platelets, which are documented in *figure 9*. In all cases we could determine the fruit-characteristic anthocyanin compositions by means of RP-HPLC/VIS in combination with mass spectrometry detection (LCMS).

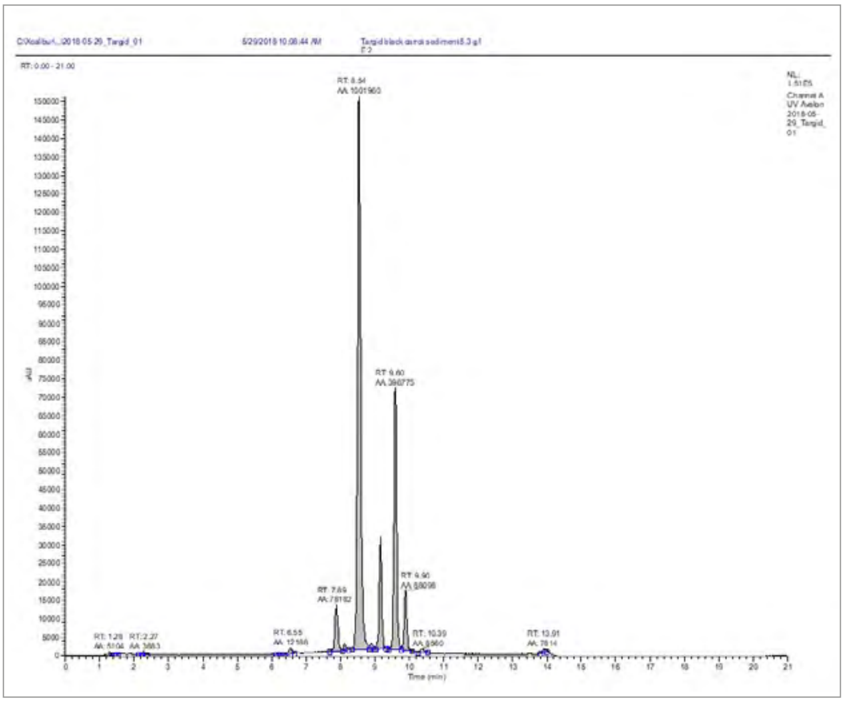

Fig. 7: RP-HPLC/VIS chromatogram of the typical black carrot anthocyanin composition from the isolated precipitate

Like anthocyanins, also colourless polyphenols are causing pure and composed precipitates in juices and concentrates. They are often difficult to analyse, in case of e. g. condensed or polymerised proanthocyanidins they are hardly analytically accessible with FTIR and the routine methods presented in *table 1*. We found only three single polyphenols being responsible for relatively pure precipitates in concentrates: ellagic acid from raspberry, quercetin from blueberry, and hesperidin from orange (*figure 10*).

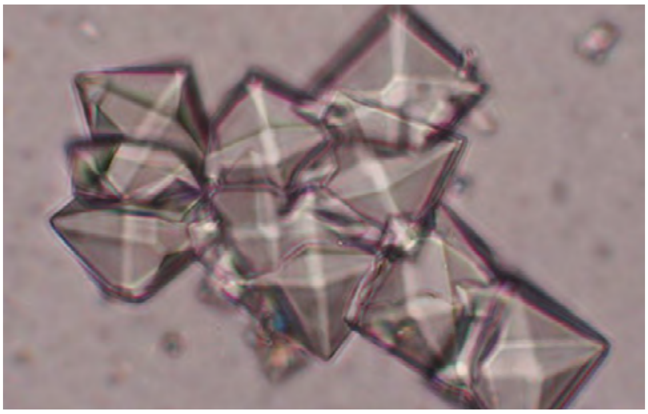

Fig. 8: Oxalate-crystals isolated from a black carrot precipitate (500x, polarisation filter)

All were determined by means of FTIR and RP-HPLC/UV/MS. Ellagic acid is a typical and not very well soluble polyphenol in many processed fruits (raspberry, strawberry, pomegranate, some grape varieties). The same is true for quercetins, which mainly occur as glycosides. In acidic juice or concentrate media they may hydrolyse into the aglycone and the sugar supported by heat (pasteurisation, wrong storage or shipping conditions). The solubility of the aglycone is lower, so that quercetin can precipitate (Will *et al.*, 2005). Hesperidin is the main flavonoid from oranges, during precipitation in OJCs it forms voluminous aggregates with crystal needles. Condensed tannin haze formations are present as uncharacteristic, amorphous aggregates under the microscope and they do not form needle-like structures like the polyphenols mentioned above. More frequently than pure tannins are complexes of condensed polyphenols with starch fragments and protein, which often were found as sediments in apple and pear concentrates or juices or nectars reconstituted from these concentrates. This underlines the importance of a complete starch degradation with amylases during processing.

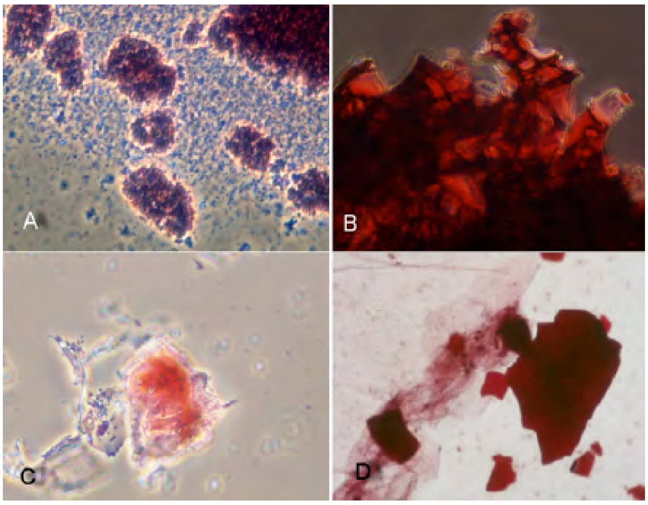

Fig. 9: Precipitated anthocyanin platelets from anthocyanin-rich concentrates: A Blueberry; B and D Aronia, C Cranberry

The prevention of polyphenol precipitates is only possible by previous removal. The problem is the non-selective reaction of fining measures, so other valuable substances like anthocyanins will be removed in parallel. On the other hand it is evidenced by meanwhile innumerable literature references that polyphenols are healthy or at least health-promoting. This leads to a situation known as "polyphenol dilemma". They have undoubted health properties, but they are chemically reactive substances which have to be removed to avoid stability problems in processed fruits and vegetables.

Like all so far mentioned haze-relevant substances, amino acids are natural ingredients of fruit juices too. During our investigations we found two examples, where they were responsible for precipitations. Tyrosine was isolated from an elderberry concentrate, where it formed small needles. It was first described as a typical elderberry precipitate in 1984 (Otto and Wittenschläger) occurring during juice evaporation. The other one was asparagine found in a peach concentrate. Under the microscope large crystal packets were visible (*figure 11*). Amino acids are very small molecules thus being an advantage for FTIR analysis. Both substances were very reliable analytically identified by their sharp FTIR absorption bands (spectra not shown).

Last examples for precipitates which were only found in concentrates are shown in *figure 12*. We isolated sorbitol (12A) from apple and pear concentrates where it occurs naturally in a range of 9-40 g/kg juice. It is also a natural substance in other fruits like e. g. plums and cherries. Sorbitol is highly soluble in aqueous media, shows a typical FTIR-spectrum and a typical HPAEC/PAD-chromatogram and the precipitate tasted very sweet. Calcium-malate (12B) was isolated from AJC. Malate is the major organic acid of many fruits, especially in apple. Like

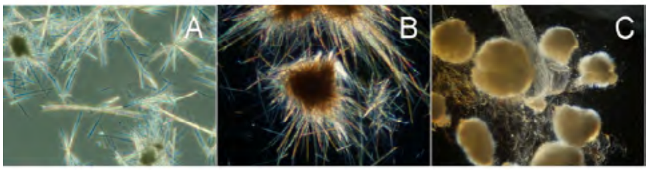

Fig. 10: Polyphenol precipitates in juice concentrates. A ellagic acid from raspberry, B quercetin from blueberry, C hesperidin from orange

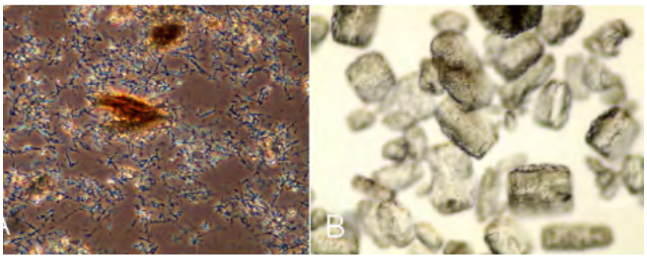

Fig. 11: Microscopic pictures of amino acid precipitates. A tyrosine from elderberry concentrate; B asparagine from peach concentrate (both 125x, bright field)

sorbitol, it showed a typical FTIR-spectrum and a typical RP-HPLC/UV-chromatogram. The glucose precipitate (12C) originated from persimmon concentrate and was also analytically confirmed with FTIR and HPLC.

Conclusions

As demonstrated, numerous natural fruit ingredients can be responsible for hazes and sediments in concentrates or juices. It is not an exhaustive presentation, and among the examples are many slightly soluble substances, where the formation of precipitates should not be expected. Isolation and characterisation requires manual skills and a broad analytical spectrum. Nevertheless, there is also the possibility to fail in spite of a full analytical equipment. This is often the case with sediments which are composed of different substance classes. Considering the large effort of personnel, time and instruments, laboratories may reach their limits during routine work. A clear strategy to avoid haze formation cannot be recommended. The production of clear beverages includes the risk of instability. Sometimes all possible stabilisation measures have been done and it still happens, because the products are simply natural and there are too many reactive and interactive substances coming together. Stability tests (temperature change tests) give no absolute results, because the mechanisms of haze formation are still poorly understood. Systematic research approaches to investigate critical moments of haze generation are hardly present, because the risk of failure is very high. It remains unsafe, whether possible results will come out at all, and if so, whether they could be usefully transferred into practice.

In relation to hazes and sediments in readily produced fruit beverages we cannot expect more tolerance from retail customers. A normal customer will think more of strange contaminations or other residues than of e. g. precipitated natural fruit ingredients. From the view of food safety it is correct for the consumer to reject the product. As shown above, many precipitations are occurring only in the concentrate stage and they often re-dissolve completely during reconstitution. Hence a little bit more technical insight and tolerance could be expected in the global trade of concentrates to prevent expensive and time-consuming recall actions, price dumping or legal disputes.

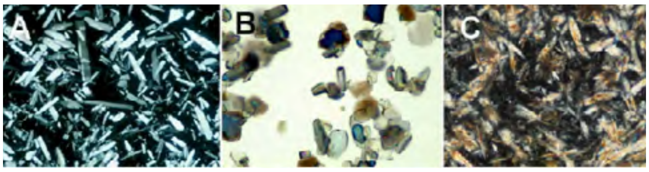

Fig. 12: Microscopic pictures of precipitates found in juice concentrates: A sorbitol from AJC (125x, polarisation); B Ca-malate from AJC (500x, polarisation); C glucose from persimmon concentrate (50x, polarisation)

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Authors:

Prof Dr Frank Will
Hochschule Geisenheim University –
Institute of Beverage Research
frank.will@hs-gm.de

Jeannette Zacharias
Hochschule Geisenheim University –
Institute of Beverage Research

Prof Dr Helmut Dietrich
Hochschule Geisenheim University –
Institute of Beverage Research