INCREASE OF SORBITOL IN PEAR AND APPLE JUICE BY WATER STRESS, A CONSEQUENCE OF CLIMATIC CHANGE?

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INTRODUCTION

Plants use several sugar derivatives, like sucrose, sorbitol or oligosaccharides, for the translocation of assimilates into the plant organs. Within the botanical family Rosaceae, sorbitol is an important transport form of the assimilates (Heldt, 1996). For example, growing apple fruit is supplied with the C-assimilates sorbitol and sucrose produced by leaf photosynthesis. These C-sources enter the fruit metabolism and accumulate mainly as fructose, sucrose, malic acid and starch (Berüter et al. 1997).

In many fruits and fruit juices this sugar alcohol contributes significantly to the sugar free extract. Its concentration is typical for the fruit species and shows a large range, as can be seen in Tab. 1. Sorbitol is therefore often used as an indicator for the authenticity of fruit juices. For example, apple juice contains sorbitol in the range of 2.5-7 g/l whereas pear juice has higher amounts up to 10-25 g/l. An addition of pear juice would increase the sorbitol content of apple juice.

TAB. 1: SORBITOL CONCENTRATIONS IN DIFFERENT FRUIT JUICES

Fruit juice from	Concentration (g/l)
Apples	2.5 - 7
Pears	10 – 25
Apricots	1.5 – 10
Peaches	1.5 – 5
Aronia	65-100
Sour cherry	10 - 35
Black currant	Max. 0.15
Raspberry	Traces
Strawberry	Max. 0.25

Sorbitol plays an important role in osmotic adjustment of mature apple leaves under water stress, and can therefore be influenced by growing conditions. Wang et al. (1996) conducted a study to determine whether water stress increases the conversion of glucose to sorbitol in mature apple leaves as a function of applied water stress. Water stress inhibited the conversion of both glucose and sorbitol to starch and led to an accumulation of this sugar alcohol; this was explained by the preferential conversion of glucose to sorbitol rather than to sucrose and starch.

Stone fruits also contain high amounts of sorbitol. In peach, sorbitol and sucrose are the two main forms of photosynthetic and translocated carbon and may have different functions depending on the organ of utilization and its developmental stage. The role and interaction of sorbitol and sucrose metabolism was studied in mature leaves (source) and shoot tips (sinks) of peach under drought stress (Bianco et al. 2000). The authors concluded from their results that a loss of sorbitol dehydrogenase activity in sinks leads to osmotic adjustment via sorbitol accumulation in the fruit. Thus, sorbitol metabolism plays an adaptive role in peach under drought stress.

Within the last years we found sorbitol in some authentic pear and apple juices in rather high concentrations which exceed the maximum values given in Tab. 1. This was pronounced in 2003 and 2006 when the weather was hot and dry in Germany (Tab. 2). The aim of this study was to investigate the influence of water stress on pear and apple juices.

MATERIALS AND METHODS

PROCESSING OF CLOUDY PEAR JUICES:

Cloudy pear juices of the harvest year 2005 were produced from three different varieties: 'Alexander Lukas' is

TAB. 2: TEMPERATURE, RAINFALL AND EVAPORATION DURING THE VEGETATION PERIOD IN 2001 TO 2006 AT GEISENHEIM IN COMPARISON TO THE LONG-TIME AVERAGE

	Longtime average (1970-2000)	2001	2002	2003	2004	2005	2006
	Temperature °C (monthly average)						
May	14.7	15.4	15.9	15.5	13.8	14.9	14.6
June	17.0	18.4	19.2	21.7	17.4	19.0	18.8
July	19.1	16.3	18.8	20.8	18.6	20.2	24.6
August	18.8	19.4	19.7	23.3	19.9	17.4	16.6
September	14.7	15.4	14.4	15.3	15.9	16.9	18.1
Ø	16.9	17.4	17.6	19.3	17.1	17.7	18.5
	Rainfall in mm (monthly average)						
May	48.1	18.6	53.0	80.1	40.0	51.8	56.7
June	52.8	48.4	39.2	20.1	23.4	33.5	16.1
July	59.3	80.2	81.5	42.8	64.5	43.5	15.4
August	43.3	41.5	45.0	26.4	61.0	41.7	99.3
September	42.7	76.6	12.3	41.2	25.9	38.3	58.1
Sum	246.2	265.3	231.0	210.6	214.8	208.8	245.6
	Evaporation in mm (monthly average)						
May		133.6	93.4	109.7	96.2	103.8	108.9
June		110.0	125.7	161.6	116.0	142.5	145.7
July		136.0	103.5	155.1	114.6	133.2	215.0
August		124.5	102.8	216.9	124.0	96.7	77.2
September		44.0	76.2	100.4	90.1	99.2	87.7
Sum		548.1	501.6	743.7	540.9	575.4	634.5

Source: German weather service Geisenheim

an old french variety traditionally grown in Germany, 'Harrow Sweet' is a new variety from Canada, and 'Concorde' is also a new variety bred in England.

In order to estimate the effect of drought stress, 7 year-old pear tress of cv. 'Alexander Lukas' on rootstock 'Pyrodwarf' were subjected to water stress in 2006. After a long period of a pronounced negative climatic water balance (1 June until 18 July: 23.8 mm rainfall and 263.1 mm evaporation) rainfall was excluded from the root zone of 16 trees by covering the herbicide bar with small plastic roofs on the 18 July). 16 control trees were exposed to normal rainfall (130.1 mm between 18 July and harvest; Fig. 1). In addition, they were irrigated 7 times with a total water amount of 80 l/m2 to maintain soil moisture at -350 hPa in 25 cm soil depth. Pears from irrigated and non-irrigated trees were harvested in healthy condition on the 19 September 2006.

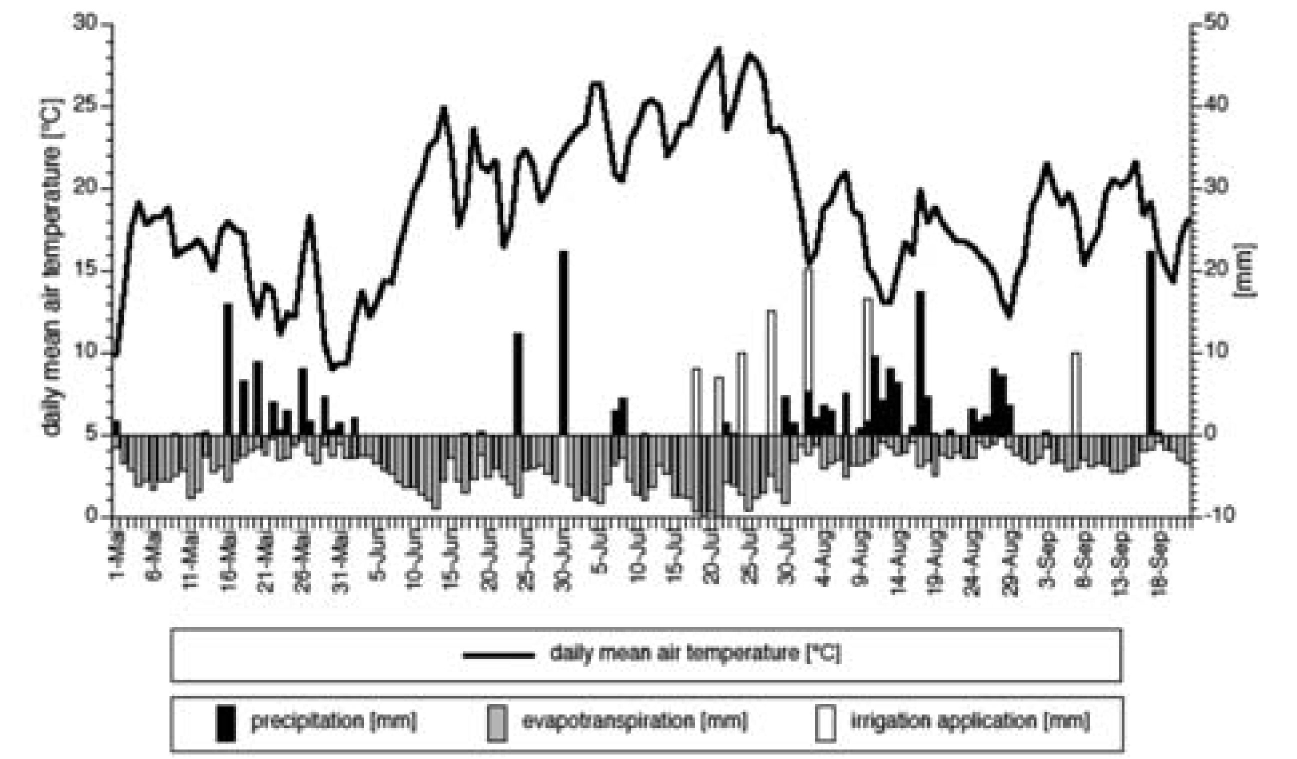
In both years, the processing of the cloudy pear juice was done as follows: Lots of 50-80 kg fruits were used: Grinding with a Rätz mill, addition of 200 mg/kg ascorbic acid into the mash, pressed with a rack-and-cloth-press (Wahler, 40 x 40 cm), heated to 82°C in a flow pasteurizer (fruit juice dispenser "Mabo", Eppingen, Germany) and hot-filled into 0.7 l glass bottles. The cloudy pear juices were stored until analysis at 13-15 °C.

For the sake of comparison, data of cloudy apple juices from dessert and cider apples from the seasons 2001 to 2003 will be presented as recently described by (Thielen et al. 2006).

ANALYTICAL METHODS:

The IFU methods were used in general, if not indicated otherwise. Relative density was measured with an oscillation-type density meter (Anton Paar DMA 48) and soluble solids (°Brix) with a digital refractometer (Dr. Kernchen ABBEMAT). The total extract and the sugar-free extract were calculated. Titratable acidity (as citric acid, pH 8.1) and pH were determined potentiometrically. L-malic acid, citric acid, lactic acid, glucose, fructose, and sucrose were analysed enzymatically.

Sorbitol was determined by enzymatic analysis and HPLC. The enzymatic analysis was performed with a se-



Source: German weather service Geisenheim

Fig. 1: Temperature, rainfall and evaporation at Geisenheim in 2006

quential analyser (Thermo, Arena) . The method is based on the procedure of Boehringer Mannheim with the enzyme sorbitol dehydrogenase (SDH) as follow:

$$D - \text{Sorbitol} + NAD^{+} \xrightleftharpoons{SDH} D - \text{Fructose} + NADH + H^{+}$$

The reaction was carried out at 37 °C, reaction time was 1500 seconds and the NADH formed was measured at 340 nm. The results were verified with the standard addition method.

Sorbitol was additionally analysed by means of anion-exchange chromatography on a Dionex BioLC system (HPAEC/PAD, Dionex, Idstein, Germany). Chromatographic separation was achieved on a 250*4 mm Dionex CarboPac MA-1 column protected with a 50*4 mm guard column of the same material. Juices were injected after centrifugation and 0.45 µm membrane filtration. The injection volume was 10 µl, and isocratic elution was carried out with 612 mmol/l NaOH at a flow rate of 0.4 ml/min at 25 °C. For detection we used a pulsed amperometric detector. Quantification was carried out using peak areas from external calibration with the reference substance purchased at Sigma (Taufkirchen, Germany). HPLC analysis was done in duplicate.

Selected samples were analysed in a second laboratory to confirm the results (enzymatic method according to IFU No. 62).

Sodium, potassium, calcium, magnesium, copper, iron, and zinc were analysed with AAS (Perkin Elmer AS 4100). Total polyphenols were estimated photometrically with Folin-Ciocalteu reagent (Merck, Darmstadt) according to Singleton and Rossi (1965), and calculated as (+)-catechin. For the determination of antioxidant capacity, the method of Re et al. (1999) was applied.

Polyphenols were determined according to Schieber et al. (2001), using a Dionex HPLC system (P 680 HPLC Pump, ASI-100 Automated Sample Injector, STH 585 Column Oven, PDA-100 Photodiode Array Detector) and the Aqua-column (5µ C18 125A, 250x4,60 mm, Phenomenex) with a C18–precolumn (ODS, Octadecyl, 4 mm l x 3,0 mm id, Phenomenex). The gradient was: A = Water/-acetic acid conc. (98:2), B = Water/acetonitrile/acetic acid conc.(500:500:5); temperature 40 °C, Flow rate 1 ml/min. Flavanols were detected at 280 nm, phenolic acids at 320 nm and quercetins at 360 nm.

RESULTS AND DISCUSSION

PEAR JUICES

The chemical composition of cloudy pear juices from three varieties of the year 2005 is given in Tab. 3. Concentrations between 39.7-58.3 g/l were found for sorbitol. Thus, for all three varieties, these values signifi-

TAB. 3: CHEMICAL COMPOSITION OF CLOUDY PEAR JUICES FROM THREE VARIETIES IN 2005

2005		Concorde	Harrow Sweet	Alexander Lucas
Conductivity	µS/cm	2440	2050	2350
relative density	20/20	1,06577	1,06401	1,06869
Brix	° Brix	16,07	15,61	16,78
Total extract	g/L	171,2	166,4	178,7
sugar-free extract	g/L	57,2	47,7	71,5
total sugars, before inversion	g/L	110,7	114,5	98,5
total sugars, after inversion	g/L	114,0	118,7	107,2
Glucose	g/L	23,0	23,5	18
Fructose	g/L	87,7	91	80,5
Sucrose	g/L	3,3	4,2	8,7
Glucose/Fructose - Ratio		0,26	0,26	0,22
pH- value		4,11	4,26	4,12
Titratable acidity at pH 7,0 as tartaric acid	g/L	2,61	2,07	3,1
Titratable acidity at pH 8.1 as citric acid	g/L	2,31	1,94	2,74
Titratable acidity at pH 8.1 as malic acid	g/L	2,42	2,03	2,87
Volatile acid	g/L	n.n.	n.n.	n.n.
citric acid	g/L	0,08	0,19	0,06
L-malic acid	g/L	2,8	3,3	4,9
L-lactic acid	g/L	0,5	n.n.	n.n.
Ascorbic acid	mg/L	108	166	141
Ethanol	g/L	n.n.	n.n.	n.n.
Sorbitol. Enz.	g/L	58,3	39,7	56,1
Sorbitol. HPLC GfL	g/L	not determined	37,2	55,9
Total polyphenols (Folin)	mg/L	469	527	481
Calcium	mg/L	51	61	47
Potassium	mg/L	1447	1105	1379
Magnesium	mg/L	53	53	57
Copper	mg/L	0,3	0,5	0,2
Iron	mg/L	0,1	0,3	0,4
Zinc	mg/L	0,5	0,5	0,7
sodium	mg/L	9,4	5,2	4,9

cantly exceed the normal range (10-25 g/l) of pear juices. The same holds for relative density and uncorrected Brix (normal range for pear juices 1.044-1.055 and 11-13.5°, resp.). Glucose, fructose and the glucose : fructose ratios were in the normal range whereas the sugar-free extracts (47.7-71.5 g/l) exceeded the normal values for pear juices (24-40 g/l). This is predominantly due to sorbitol. The acidity is essentially determined by malic and citric acid. The titratable acidity (1.94 – 2.74 g/l, as citric acid) was low, malic acid (2.8 – 4.9 g/l) was in the normal range for pear juices, whereas citric acid was only found in traces (0.08-0.19 g/l). It is known that citric acid is highly dependent on the degree of maturity and the pear variety. The minerals and trace elements were in the normal range for pear juices as the comparison with CoP revealed.

Based on the findings for pear juices of the year 2005, pear trees cv. Alexander Lukas were exposed to drought stress in 2006 as described above. Pears juice from these and the irrigated trees were analysed. As can be seen from Tab. 4, the sorbitol content increases drastically from 47.2 g/l to 60.3 g/l under drought stress. There is also a pronounced effect of drought stress on total extract, uncorrected Brix and sugar free extract. Due to sorbitol, drought stress leads to an increase of sugar-free extract (67 g/L compared to 55.8 g/l in the control). On the other hand, the formation of glucose and fructose is reduced under water stress. The atypically high value for sorbitol of the irrigated sample (47.2 g/l) may be also an indication of the special climatic conditions in 2006 which was even warmer than 2003. Especially, September, and therefore the weeks before harvest, were 3.4 °C warmer in comparison to the long-time average and by 2.8 °C higher than September 2003 (Tab. 2). The influence of climatic conditions or drought stress on titratable acidity and the single acids is rather small. The same is valid for the minerals.

Surprisingly, there is not much effect on total polyphenols or antioxidant capacity as is shown in Tab. 4. Normally, it is said

TAB. 4: INFLUENCE OF WATER STRESS ON THE COMPOSITION OF IRRIGATED AND NON-IRRIGATED CLOUDY PEAR JUICE CV. ALEXANDER LUKAS IN 2006

2006		Pear juice non-irrigated	Pear juice irrigated
Relative density	20/20	1,0677	1,05948
Brix	°	16,54	14,57
Conductivity	µS/cm	2510	2480
Total extract	g/L	176,1	154,9
sugar-free extract	g/L	67,0	55,8
Glucose	g/L	24,8	20
Fructose	g/L	82	74,7
Sucrose	g/L	2,3	4,4
Sorbitol enz.	g/L	60,3	47,2
titratable acidity at pH 8.1, as tartaric acid	g/L	3,70	3,90
titratable acidity at pH 8.1 as citric acid	g/L	3,33	3,14
titratable acidity at pH 8.1 as malic acid	g/L	3,49	3,32
pH - value		3,81	3,77
Ethanol	g/L	0,02	0,02
volatile acids as acetic acid	g/L	0,04	0,04
L-lactic acid	g/L	n.n.	n.n.
D-lactic acid	g/L	n.n.	n.n.
L-malic acid	g/L	5,8	5,5
Citric acid	g/L	0,1	0,1
Total polyphenols (Folin)	mg/L	499	479
Ascorbic acid	mg/L	75	135
Calcium	mg/L	48	52
Potassium	mg/L	1322	1244
Magnesium	mg/L	66	60
Antioxidative capacity (TEAC)	mmol/L Trolox	3,9	3,6

TAB. 5: IMPACT OF WATER STRESS ON THE POLYPHENOL COMPOSITION (HPLC, mg/L) OF THE PEAR JUICES CV. ALEXANDER LUKAS IN 2006

2006	Pear juice non-irrigated	Pear juice irrigated
280 nm:		
Arbutin	13,9	12,9
Procyanidin B1	0,6	3,7
Catechin	0,4	0,4
Procyanidin B2	3,2	2,5
Epicatechin	2,9	2,8
Procyanidin C1	0,0	0,0
320 nm:		
Neochlorogenic acid	0,0	0,0
Chlorogenic acid	92,5	88,0
Caffeic acid	0,1	0,2
3-Cumaroyl quinic acid	1,1	0,9
4-Cumaroyl quinic acid	1,4	1,1
Cumaric acid	0,9	0,7
360 nm:		
Quercetin-3-rutinoside	0,2	0,2
Quercetin-3-galactoside	1,2	1,3
Quercetin-3-glucoside	0,7	0,8
Isorhamnetin-3-rhamnogalactosid	2,0	2,1
Isorhamnetin-3-rhamnoglucoside	3,9	4,3
Isorhamnetin-3-galactoside	0,6	0,7
Isorhamnetin-3-glucoside	0,1	0,1
Total	125,8	122,6

that polyphenols generally increase due to drought stress but this is not always the case. This is shown in Tab. 5 with essentially the same concentrations for both juices from irrigated and non-irrigated pear trees.

Figure 2 gives a summary of all results for sorbitol in pear juices from 2005 and 2006. The maximum limit for pear juices according to the AIJN Code of Practise is exceeded significantly. Climatic conditions and corresponding drought stress lead to higher concentrations of this sugar alcohol.

Besides the effects of climate and drought a certain influence of the pear varieties cannot be excluded. Harrow Sweet and Concorde are rather new varieties, and they were probably not included in the RSK values or the AIJN CoP. This question cannot be answered by the data presented here and will be considered in future studies. Nevertheless, the upper limit for sorbitol in the RSK values and CoP should be scrutinized.

APPLE JUICES

In 2002 and especially in 2003 we found rather high values of sorbitol in some apple juices produced from certain cider apple varieties grown in Germany. These traditional varieties are often chemically different from new varieties and dessert apples. They are characterized by high acidity and high polyphenol concentrations (Keller und Streker et al. 2001,Schmitz-Eiberger et al. 2003,Thielen et al. 2006).

In most cases, the analyses of cloudy apple juices from cider apples revealed normal sorbitol concentrations in the range 1.5-7 g/l. An extreme value was found for Bittenfelder apple juice of 2003 (25.8 g/L sorbitol, relative density 1.0798, 19 °Brix, sugar-free extract 63.4 g/l, 8.8 g/l titratable acidity) whereas 8.8 g/l sorbitol was measured in 2002 for the same variety. For Boskoop apple juice we found 10.8 g/L sorbitol and 37.7 g/l sugar-free extract in 2003 (Thielen et al. 2006). As the fruit were processed in our own laboratory, the addition of pear juice to the apple juices, which would increase sorbitol, is excluded; this was further verified by HPLC analysis of the polyphenols (absence of arbutin and isorhamnetin derivatives). The extreme value of sorbitol for Bittenfelder juice is definitely an exception, but values about 10 g/l can sometimes be found for classical cider apples.

Additionally we analysed 12 apple juice concentrates of a fruit juice company in South West Germany. As usual, the apple juice concentrates were from mixed apple varieties. According to the producer these concentrates from 2003 contained a significant but variable part of Bittenfelder apples. The analyses were done enzymatically and with HPAEC. Five out of twelve samples exceeded the value of 7 g/l (up to 11.6 g/l at 11.2 °Brix). The data are shown in Tab. 6. The polyphenol content sup-

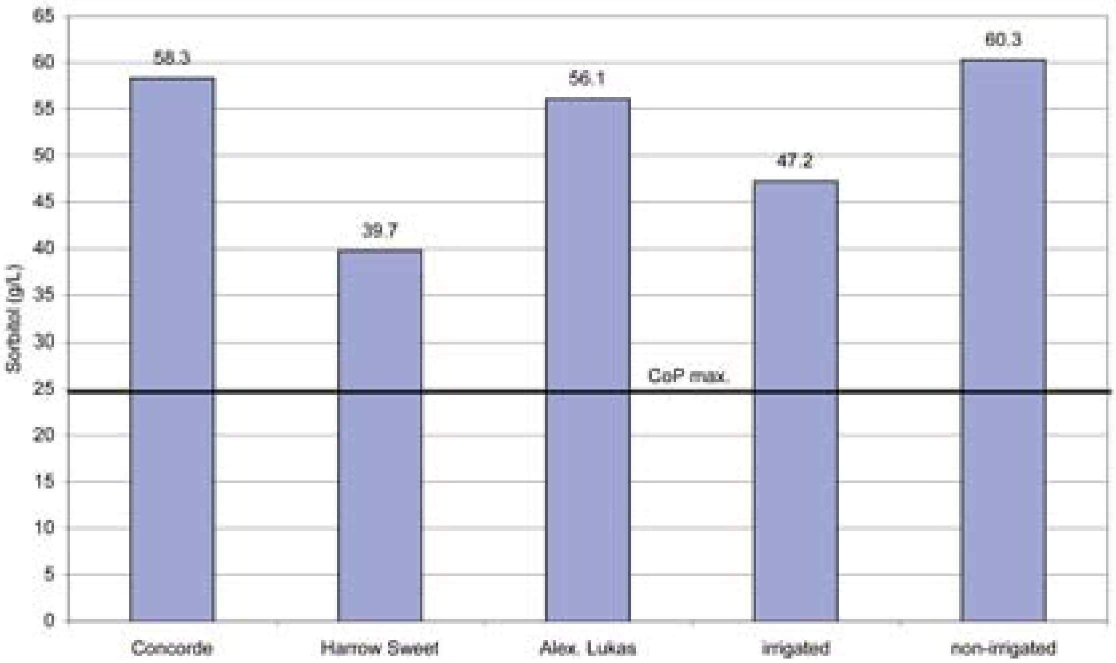


Fig. 2: Sorbitol in pear juices and comparison with the maximum concentration according to CoP. Concorde, Harrow Sweet and Alex. Lukas are from 2005, irrigated and non-irrigated samples (Alex. Lukas) from 2006

TAB. 6: APPLE JUICE CONCENTRATES 2003 MEASURED AFTER REDILUTION TO SINGLE STRENGTH JUICE

No.	Rel. density	Brix	Sorbitol enz.	Sorbitol HPLC	Total polyphenols
		°	g/L	g/L	mg/L
1	1,0590	14,14	9,9	9,9	743
2	1,0579	14,00	6,4	5,4	225
3	1,0580	13,95	7,1	6,2	399
4	1,0583	13,88	8,4	7,9	348
5	1,0590	14,16	10,8	10,8	372
6	1,0602	14,36	8,8	9,1	418
7	1,0582	14,00	11,2	10,0	304
8	1,0585	14,07	11,9	10,5	340
9	1,0592	14,10	8,3	8,7	379
10	1,0595	14,16	8,3	7,7	351
11	1,0600	14,33	16,1	15,5	734
12	1,0620	14,88	8,2	5,5	371

ports the presence of polyphenol-rich varieties, like Bittenfelder. In total, high sorbitol contents can also be found in industrial apple juices. This has to be regarded in authenticity studies.

Figure 3 summarizes the results on apple juices from 2002-2003 based on the data of Thielen et al. (2006) and Tab. 6. In several samples of 2003 sorbitol is found in rather high concentrations. There is also a tendency that sorbitol was higher in 2003 compared to climatically "normal" years.

The results presented in this study are in accordance to the literature which supports the idea of sorbitol as an osmoregulator (Wang et al. 1996, Bianco et al. 2000). A tendency to higher sorbitol values was also found by Mills und Behboudian et al. 1994. They studied five-year-old 'Braeburn' apple trees on MM106 rootstock to determine plant and fruit quality responses to reduced plant water status. Trees were irrigated or not irrigated. Irrigated trees developed reduced xylem water potential and stomatal conductance. However, they showed no reduction in photosynthetic rates. Fruits had higher soluble solids concentrations, enhanced red skin pigmentation, and a tendency for higher sorbitol concentrations. In addition, (Mpelasoka et al. 2001) found after deficit irrigation an improved fruit quality at harvest in terms of increased firmness, total soluble solids, total sugar concentration and dry matter. Sorbitol in pear and apple juice can be rather high, exceeding the typical values, as was shown by this study.

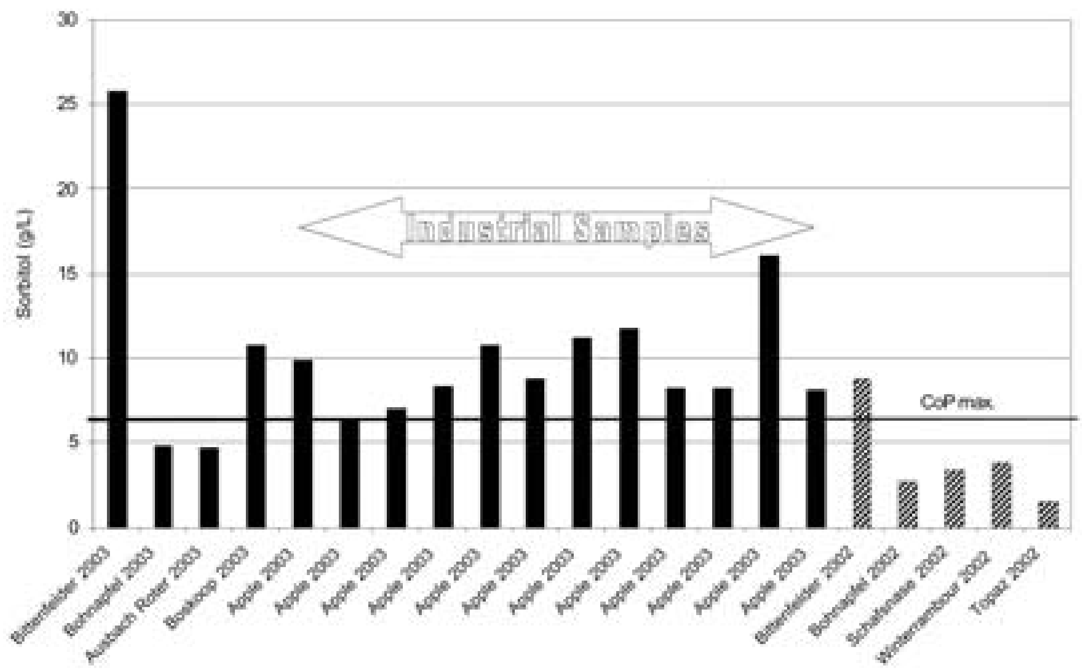


Fig. 3: Sorbitol in apple juices from 2002-2003 and comparison with the maximum concentration according to CoP

This must be taken into consideration when sorbitol is used as a marker of authenticity, for example the question of illegal addition of pear juice to apple juice without declaration. The concentration seems to be dependent on climatic conditions including water stress and temperature and therefore dependent on the harvest year. Another aspect is the variety. Some varieties show a different behaviour in terms of chemical composition than most of the dessert apple varieties which are used more and more in the fruit juice world.

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