The odour activity value of aroma-active esters – An appropriate means to assess the aroma quality of apple juices

JOHANNA KREISSL, Katharina Lehmann and Peter Schieberle
Leibniz-Institute for Food System-Biology at the Technical University of Munich, Lise-Meitner-Str. 34, 85354 Freising, Germany*
**formerly Deutsche Forschungsanstalt fuer Lebensmittelchemie (German Research Centre for Food Chemistry)*

Abstract

Aroma quality of apple juice from concentrate depends on an appropriate rearomatisation. Nevertheless, until now, available concepts to analytically evaluate the aroma quality of apple juices are non-satisfying. Most concepts focused only on the concentration of esters to rate the aroma quality. However, without consideration of the different odour thresholds of the esters no successful results were obtained. To address this challenge, odour-active compounds were characterized in apple juices by means of the Sensomics concept including gas chromatography-olfactometry and GC×GC-MS(TOF) and a set of 16 esters was selected for quantitation. The concentrations and OAVs of these esters were determined in 23 defined apple juices using a fast, multicomponent stable isotope dilution assay (SIDA), based on headspace-solid phase micro extraction (HS-SPME) in combination with GC×GC-MS(TOF). Thus, for each ester limits in terms of OAV ranges were determined representing a correct re-aromatisation and a good aroma quality of apple juice. The new method enabled a high and fast throughput of samples due to the absence of any sample work-up.

Introduction

Beside orange, multivitamin, and grape juices, apple juice is one of the most favoured juices in Germany [1]. About 700 million litres of apple juice are consumed per year in Germany. However, its consumption dropped from more than 12 litres in 2005 to 8 litres per person and year in 2010. Beside juice not from concentrate (NFC), mainly juice from concentrate (FC) is produced. For its manufacturing, the fresh juice is concentrated, while recovering the valuable apple aroma. After storage and/or transportation, the reconstitution of the juice concentrate, the apple aroma and water takes place, followed by a pasteurisation step. However, thermal stress and an inappropriate re-aromatisation may affect the aroma quality. This has also been criticised by consumer protection organisations [2]. However, until now, there is no appropriate concept available to evaluate the aroma quality respectively an adequate re-aromatisation of apple juices by means of analytical parameters.

Esters are known as important odorants in apple juice. Former proposed concepts (sum of esters, aroma index) included the concentration of esters to rate the aroma quality of apple juices, but these concepts did not consider the (big) difference in odour thresholds of individual odorants. To evaluate the aroma of apple juice not only the amount of an aroma compound is important, the potential (odour threshold) of an odorant has to be regarded, as well. For example, while hexyl acetate has an odour threshold of 15 µg/L (water), ethyl 2-methylbutanoate has a threshold of 0.013 µg/L. This is the reason why, when considering the sensory potential, of the 72 esters already found in apple juices,

B. Siegmund & E. Leitner (Eds): Flavour Sci., 2018, Verlag der Technischen Universität Graz
DOI: 10.3217/978-3-85125-593-5-103, CC BY-NC-ND 4.0

only a few (~7) are sensory relevant [3-6], because not all esters reach or exceed their individual odour threshold.

The aim of the study was to develop a reliable and fast, multicomponent quantitation method, taking the sensory potential of single esters into consideration for the evaluation of the aroma quality of apple juices.

Experimental

Materials

23 apple juices (freshly pressed, NFC, and FC) and in cased of FC, correctly re-aromatized, were obtained from defined processes directly provided by the manufacturers. The apples were from different varieties (not known) from South Tyrol, Germany, and Poland and harvested in the years 2010 to 2013. In addition, 17 commercially available apple juices of different origin and appearance were purchased form several supermarkets: 10 from Germany, 2 from United Kingdom, and 1 each from Poland, Hungary, France, Belgium, and Netherlands. Among them 4 cloudy NFC juices, 1 clear NFC juice, 2 cloudy FC juices, and 10 clear FC juices.

Hedonic, ranking order

Hedonic evaluation was performed using an incomplete balanced block plan with 18 tests and 4 juices per test. 40 panellists were asked to rank the juices on a scale from 1 (most wanted) to 4 (most unwanted).

Screening for esters

For the screening of esters volatiles were extracted with dichloromethane, distilled in high vacuum (SAFE) at 40 °C, concentrated, and analysed by GC-olfactometry (GC/O) on a GC 8000 (Fisons Instruments, Mainz, Germany). After the separation on an FFAP column (30 m×0.25 mm, 0.25 µm film thickness, J&W, Köln) using the following temperatures: 40 °C (2 min), 6 °C/min, 190 °C (0 min), 12 °C/min, 230 °C (5 min) the effluent was split 1:1 via an Y-splitter and transferred to an FID and a sniffing port.

Quantitation of esters

For the quantitation of the esters, stable isotope dilution assays (SIDA) were applied. Measurements were done by HS-SPME/GC×GC-MS(TOF) analysis on a Pegasus 4D instrument (Leco, St. Joseph, MI) consisting of a 7890A GC (Agilent), a dual-stage quad-jet thermal modulator and a secondary oven coupled to the mass spectrometer. Apple juice samples (0.5 mL) and stable isotope internal standards were mixed in a SPME vial (20 mL), equilibrated for 30 min and afterwards, the enrichment of the esters was done by exposing a Stable Flex fibre (65 µm, PDMS/DVB, Supelco, Sigma-Aldrich, Taufkirchen) for 30 min at 40 °C in the headspace of the sample. Desorption was performed in the GC Multimode inlet system at 250 °C. In the first dimension an FFAP column (30 m×0.25 mm, 0.25 µm film thickness, J&W, Köln) was installed running the following temperatures: 40 °C (3 min), 6 °C/min, 230 °C (7 min). In the second dimension a VF-5 column (1.5 m×0.15 mm, 0.30 µm film thickness, Agilent, Böblingen) was installed running the following temperature program: 80 °C (3 min), 6 °C/min, 250 °C (10 min).

Results and discussion

Screening for esters and additive effects

First, aroma-active esters in the solvent extract of an NFC apple juice were screened by GC/O to detect esters in trace amounts but with a high aroma potential. In addition, less odour-active esters were identified by GC×GC/MS(TOF). In total 29 esters were found whereof 17, with low odour thresholds and/or present in huge amounts, were selected for quantitation. These compounds were quantitated in the NFC apple juice by HS-SPME/GC×GC/MS(TOF) using stable isotope dilution assays. Finally, odour activity values (OAV, ratio of the concentration of an odorant to its odour threshold) were calculated (Table 1).

Table 1: Odour activity values of 17 esters in NFC apple juice

No.	*Compound/variety*	*Odour activity value (OAV)**
1	ethyl 2-methylbutanoate	1700
2	ethyl butanoate	100
3	2-ethylbutyl acetate	64
4	methyl 2-methylbutanoate	27
5	ethyl 2-methylpropanoate	20
6	hexyl acetate	11
7	butyl acetate	9.6
8	propyl 2-methylbutanoate	4.4
9	ethyl hexanoate	3.7
10	ethyl propanoate	1.9
11	pentyl acetate	< 1 (0.77)
12	2-methylpropyl acetate	< 1 (0.31)
13	butyl 2-methylbutanoate	< 1 (0.17)
14	butyl butanoate	< 1 (0.09)
15	hexyl 2-methylbutanoate	< 1 (0.07)
16	hexyl butanoate	< 1 (0.03)
17	butyl propanoate	< 1 (0.03)

** Ratio of the concentration of an odorant to its odour threshold.*

The calculation of OAVs served as basis for the investigation of additive effects of esters in sensory tests. For this purpose, a mixture of all esters (no. 1-17) was compared in a triangle test to a mixture of esters containing only esters with an OAV ≥ 1 (no. 1-10). The sensory evaluation clearly showed that the esters with OAVs < 1 did not contribute to the overall aroma. Due to this fact, further investigations were done with ethyl 2-methylbutanoate, ethyl butanoate, 2-methylbutyl acetate, ethyl 2-methylpropanoate, methyl 2-methylbutanoate, butyl acetate, propyl 2-methylbutanoate, hexyl acetate, ethyl propanoate, and ethyl hexanoate (ester no. 1 to 10).

Determination of OAV ranges and application to commercial juices

Esters 1 to 10 were quantitated in all 23 defined juice samples (freshly pressed, NFC, and FC) and OAV were calculated. Thus, OAV ranges for a well-balanced apple juice aroma were established (Table 2).

These OAV ranges were applied to 17 commercially available apples juices. A sensory trained panel ranked these juices concerning their hedonic preference. In addition, esters 1 to 10 were quantitated and OAVs were calculated. The OAVs of the (significantly) most appreciated (best) and the most unpopular (worst) juice were applied to the established OAV ranges and highlighted a clear trend: while all OAVs were within these OAV ranges for the best, only the OAVs of 2-methylbutyl acetate and hexyl acetate were within its range for the most unpopular juice (Table 2).

Table 2: OAV Ranges of 10 Selected Esters and OAVs of Juice 6 and 13

Compound/variety	*OAV range* *	*OAV best juice*	*OAV worst juice*
ethyl 2-methylbutanoate	900 – 12000	**12000**	280
ethyl butanoate	55 – 390	**270**	17
2-methylbutyl acetate	11 – 110	**31**	**11**
ethyl 2-methylpropanoate	8 – 110	**95**	3
methyl 2-methylbutanoate	12 – 80	**75**	3
butyl acetate	5 – 45	**10**	4
propyl 2-methylbutanoate	4 – 37	**23**	< 1
hexyl acetate	1 – 26	**4**	**2**
ethyl propanoate	1 – 12	**11**	< 1
ethyl hexanoate	1 – 11	**4**	< 1

** Values resulted from the investigation of 23 sensory proper juices (freshly pressed, NFC, FC) from defined processes directly provided by different apple juice manufacturers.*

The results of these investigations showed that OAV ranges of only 10 aroma-active esters may serve as markers for the fruity aroma quality of apple juices. Using HS-SPME/GC×GC/MS a suitable method was established for the simultaneous quantitation of all esters of interest. By considering the aroma perception of a sensory panel (consumer) and the analysis of the aroma quality of apple juices, expressed in OAV ranges, the production and also a correct re-aromatisation of apple juices can be lifted to a reliable basis.

References

1. Unknown (2014) VdF (Verband der deutschen Fruchtsaft-Industrie e.V.), Bonn, brochure.
2. Unknown (2009) Stiftung Warentest, Test 9:20-26. (www.test.de/Apfelsaft-Drei-sind-durchgefallen-1801978-0/, last access 01.2018)
3. Flath R.A., Black D.R., Guadagni D.G., McFadden D.G., Schultz T.H. (1967) J Argic Food Chem 1:29-35.
4. Poll (1988) LWT Food Sci Technol 21:87-91.
5. Young H., Gilbert J.M., Murray S.H., Ball R.D. (1996) J Agric Food Chem 71:329-336.
6. Bogen J. (2010) Ph.D. Thesis, Verlag Deutsche Forschungsanstalt für Lebensmittelchemie Freising