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ORIGINAL ARTICLE

Evaluation of the sweetener content in diet/light/zero foods and drinks by HPLC-DAD

Daniela de Queiroz Pane[1] · **Cíntia Botelho Dias**[2] · **Adriana Dillenburg Meinhart**[1] · **Cristiano Augusto Ballus**[1] · **Helena Teixeira Godoy**[1]

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Abstract Artificial sweeteners are widely used in foods and beverages to replace sugars. It is essential to monitor the content of these compounds, as amounts over the legal limit may create harmful effects to the health of consumers. Therefore, the aim of this paper was to determine the amount of acesulfame-k, saccharin, cyclamate, aspartame, and neotame in eleven food and beverage products classified as diet, light and zero, using high performance liquid chromatography (HPLC). We also aimed to verify whether their amount in the products was in agreement with the effective legislation and the values indicated on the product label. Soft drinks, nectars, instant juices, puddings and cappuccinos, drinking chocolate powder, jams, jellies, barbecue and tomato sauce and tabletop sweeteners were among the products evaluated. The amount of artificial sweetener in the analysed samples ranged from 0.3 to 25.8 mg.100 mL^{-1} for acesulfame-k; 1.3 to 15.8 mg.100 mL^{-1} for saccharin; 36.7 to 79.4 mg.100 mL^{-1} for cyclamate; 2.7 to 55.9 mg.100 mL^{-1} for aspartame. All tomato and barbecue sauce samples contained concentrations above the legal limit. Furthermore, several samples contained concentrations above the concentrations stated on the labels, which indicates the need for more efficient control and inspection in the food industry.

Keywords Artificial sweeteners · Diet · Light · HPLC · Legislation

Introduction

In the past few years, there has been an increasing demand for products that impact sweetness while improving health and appearance, and as a result the availability of artificial sweeteners has increased. Additionally, the worldwide increase of conditions such as diabetes *mellitus* and obesity, which require calorie or sucrose restriction, has led doctors to advise the use of diet and light products, containing artificial sweeteners (Torloni et al. 2007). However the use of artificial sweeteners is still controversial with suggested risk to health (Kroger et al. 2006; Renwick 2006; Leth et al. 2008) and they must be subject to a rigorous assessment before use in food products and beverages (Moraes 2008). Furthermore, the use of artificial sweeteners is limited to specific products, under specific conditions and restricted to the lowest levels needed to reach the desired effect (Brazil 1997), and their use is controlled in each country by regulatory agencies such as the National Agency for Sanitary Vigilance (ANVISA) in Brazil and the Food and Drugs administration in the United States. The Resolution – RDC n° 18/2008 has established the limits for artificial sweetener use in foods and beverages in Brazil (Brazil 2008).

A common practice in the food and beverage industries is to combine artificial sweeteners in a formulation, as they work synergistically to obtain the desired sweetness and texture, allowing their use in smaller amounts. Therefore, analytical

✉ Helena Teixeira Godoy
helena@fea.unicamp.br

[1] Department of Food Science, Faculty of Food Engineering, University of Campinas (UNICAMP), Rua Monteiro Lobato 80, 13083-862 Campinas, SP, Brazil

[2] Nutraceuticals Research Group, School of Biomedical Science and Pharmacy, University of Newcastle, Callaghan, Australia

methods able to determine different artificial sweeteners simultaneously are requires for the identification and quantification of artificial sweeteners (Huang et al. 2006; Zygler et al. 2009). Additionally, the need to analyse those compounds on a wide variety of matrices has resulted in the development of different analytical methods (Pietra et al. 1990). Within the chromatographic separation techniques, methodologies were developed for gas chromatography (Nakaie et al. 1999; Farhadi et al. 2003), high performance liquid chromatography – HPLC (Wasik et al. 2007; Yang and Chen 2009), liquid chromatography with mass spectrometry (Huang et al. 2006; Loos et al. 2009), and ion-exchange chromatography (Chen and Wang 2001; Zhu et al. 2005). Among them, high performance liquid chromatography with diode array detector (DAD) is the most commonly employed in the simultaneous determination of artificial sweeteners, due to its more affordable operating costs and equipment availability in laboratories (Kritsunankula and Jakmuneeb 2011; Wang et al. 2011).

Considering the wide use of artificial sweeteners in diet and light food products and their toxicity to human beings when used in concentrations above the legal limits, the aim of this study was to evaluate the amount of five artificial sweeteners in a wide variety of foods and beverages classified as diet, light and zero using HPLC-DAD, in order to determine whether the values obtained were in accordance with the current Brazilian legislation and the values declared on the product label.

Materials and methods

Reagents

Standard of acesulfame-k was acquired from Fluka (USA); aspartame, hydrated sodium saccharin and sodium cyclamate, from Sigma-Aldrich (USA); and neotame was donated by the company Sweetmix (Brazil). Chromatographic degree acetonitrile was acquired from the company J. T. Baker (USA); sodium phosphate monobasic monohydrate and orthophosphoric acid, from Merck (Germany); and sodium hydroxide, from Carlo Erba (Italy). The water used to prepare the reagents and the mobile phase was purified using the Milli-Q system (Millipore, USA). All reagents and the mobile phase were filtrated in HAWP membrane 0.45 μm pore diameter (Millipore, USA).

Samples

Foods and beverages classified as diet, light and zero were acquired in supermarkets in Campinas and Sao Paulo (SP, Brazil). Forty five food products and beverages were analysed in total, among them soft drinks (cola, guarana and lemon flavour), nectars (grape, peach, guava, passion fruit and orange), instant juices (apple and orange flavour), puddings (chocolate and vanilla flavour) and cappuccinos (traditional and with cinnamon), drinking chocolate powder, strawberry jam, jelly (grape and strawberry flavour), barbecue sauce, tomato sauce and tabletop sweeteners. Products from at least two different manufacturers were analysed, except for guava, passion fruit and orange nectar, strawberry jelly, barbecue sauce and tomato sauce, for which only one brand was available in the supermarkets. For tabletop sweeteners six different brands were analysed. For each of the 45 products, 3 batches were acquired in triplicate (individual packages), totalling 9 replicates for each food product or beverage. Each sample (individual package) was then homogenized and subject to analyses.

Sample preparation

The sample preparation methods were based on the study by Wasik et al. (2007). Soft drinks were subjected to ultrasound for 5 min, in order to remove all the carbon dioxide, before the dilution and filtration stages. Liquid tabletop sweeteners were diluted in water. Nectars were diluted, centrifuged for 5 min at 2415 $g.s^{-1}$ and then filtered.

One gram powdered foods (instant juice, pudding and cappuccino, drinking chocolate, jelly and tabletop sweeteners), and 2.5 g barbecue sauce, tomato sauce and jam, were weighed directly into 25 mL volumetric flasks and the volume was made up with water. These solutions were then subjected to ultrasound for 15 min, for complete dissolution of the artificial sweeteners. Except for the tabletop sweeteners, the samples were centrifuged for 5 min at 2415 $g.s^{-1}$ after the ultrasound stage. For jellies, 350 μL of trichloroacetic acid was added, for the precipitation of proteins, before the volumetric flask had its volume made up to 25 mL with water.

All samples were further diluted 10 times in water and filtered in a HV membrane 0.45 μm pore diameter (Millipore, USA). From the filtered solution, 10 μL was immediately injected.

The concentration of the artificial sweeteners was evaluated according to the "Technical Regulation to establish the identity and quality of special purpose foods" (Brazil 1997) and the "Technical Regulation that authorizes the use of sweetening additives in foods, within their respective maximum limits" (Brazil 2008). Analysis of variance and Tukey's test were used for the statistical processing, and *p*-values lower than 0.05 were considered statistically significant. The software Statistica 7.0 (Statsoft, USA) was used to perform the statistical analysis.

Instrumentation

A liquid chromatograph Agilent (USA) series 1100, with an automatic injector, degasser, and quaternary pump, equipped

with an ultra violet visible (UV–vis) diode array detector was used. The system was controlled using the software HP-Chemstation, which also managed the data acquisition system. For the chromatographic process, a C18 Pinnacle II column, with 5 μm particle diameter, 150 mm in length and 4.6 mm of internal diameter (Restek, USA) was used.

Chromatographic conditions

The artificial sweeteners were separated using a reversed-phase system with a linear gradient, based on the study by Dias et al. (2014), and the mobile phase was constituted by A (5 mmol.L^{-1} monobasic sodium phosphate buffer at pH 7.0, adjusted with sodium hydroxide) and B (acetonitrile), at 40 °C temperature and 1 mL.min^{-1} flow rate. The gradient started with 94 % A for 8 min; from minutes 8 to 9, A was linearly reduced up to 85 %, and the concentration was maintained until minute 16. From minutes 16 to 17, A was reduced to 70 % and the concentration was maintained until minute 26. The initial conditions were then resumed and the column was re-equilibrated for 5 min, before the next injection.

Detection was conducted using DAD, and the monitored wavelengths were: 192 nm (for sodium cyclamate, aspartame and neotame), 201 nm (for sodium saccharin) and 227 nm (for acesulfame-K). The sweeteners were identified by comparison with retention times obtained for the standards analysed in isolation by co-chromatography, and comparison of the spectrums obtained by DAD.

Validation

The method was validated according to the Harmonized Guidelines for Single Laboratory Validation of Methods of Analysis (Thompson et al. 2002). The evaluated parameters were precision (repeatability and intermediate precision), accuracy (through recovery tests), limit of detection (LOD), limit of quantification (LOQ), linearity range and selectivity.

Precision was studied through ten consecutive determinations on the same day (repeatability) and across three days (intermediate precision). Precision was investigated on an artificial sweetener free jam sample with analytical standards added (matrix-standard mix). Jam was used as matrix for validation due to its complexity compared to the other samples analysed. Strawberry jam (2.5 g) was weighed, transferred to a 25 mL volumetric flask and solubilized in a small volume of water; the artificial sweeteners standards were then added up to the concentrations of 9 μg.mL^{-1} acesulfame-k, 10 μg.mL^{-1} sodium saccharin, 45 μg.mL^{-1} sodium cyclamate, 25 μg.mL^{-1} aspartame and 30 μg.mL^{-1} neotame. After that, the flask volume was made up with water and homogenized. The solution was centrifuged for 5 min at 2415 g.s^{-1}. It was then filtered on a Millex HV membrane 0.45 μm pore diameter (Millipore, USA). From the filtered solution, 10 μL was immediately injected.

In order to evaluate the accuracy of the method, recovery tests were conducted using the sweetener-free jam matrix added with standards in two different concentration levels. One of the levels represented 85 % of the likely amount of artificial sweetener in the samples, and the other level represented 110 % of the likely amount. The likely amount of artificial sweeteners in a sample was determined using the overall mean of the reported amounts in the labels of the products.

The limit of detection was estimated using serial dilutions of the matrix-standard solution mix. The limit of detection was considered as the concentration that produced a sign three times greater than the noise signal ($S/N \geq 3$; when S = signal and N = noise). The limit of quantification was considered as two times the limit of detection.

The linearity range was investigated on at least 7 different levels, studied randomly in triplicate. The analytical curves were built from the addition of the standards to an artificial sweetener-free jam sample, and the linearity was evaluated using the mathematical model for linear regression, the distribution of the residues, and the test of lack of fit, using the software Statistica 7.0 (Statsoft, USA).

Selectivity was analysed by calculating the retention factor (k), the separation factor (α), and the resolutions (Re) for each of the sweetener.

Results and discussion

Validation of the HPLC-DAD method

Table 1 presents the figures of merit for the separation method.

To prevent peak overlapping, the separation factor must be greater than 1 and the minimal desirable resolution, equal to 1.5 (Snyder et al. 1997). Therefore, the k, α, and Re values observed (Table 1) demonstrate an effective separation between peaks.

Since the $F_{calculated}$ values were greater than the F_{critic} values, the mathematical models suggested for the linear regressions were fitted. The residues showed a random distribution and the linear regression was significant, indicating an adequate linearity of the method.

The variation coefficients were below 0.68 % for repeatability and below 1.67 % for intermediate precision.

Application in samples

Figures 1, 2 and 3 present the chromatographic profiles for the standards of the five sweeteners separated and some of the samples analysed.

Table 1 Figures of merit for the separation methods

Parameter		Sweeteners				
		Acesulfame-k	Saccharin	Cyclamate	Aspartame	Neotame
LOD ($\mu g.mL^{-1}$)		0.2	0.1	18	0.142	0.45
LOQ ($\mu g.mL^{-1}$)		0.4	0.2	36	0.284	0.9
Retention factor (k)		1.04	1.56	2.64	8.901	16.37
Separation factor (α) [a]		1.5	1.68	3.36	1.83	–
Resolution (Re) [a]		2.95	8.56	34.02	94.29	–
Linearity ($\mu g.mL^{-1}$)		0.4–250	1.0–50	36–100	1.0–80	2.5–50
Equation		y=843.92× – 886.87	y=3118.4× – 1870.4	y=1571.5× - 1194	y=788.68×+8664.8	y=1068.4×+8368.2
$F_{calculated}$ [b]		167.06	92.3	88.1	101.45	55.9
F_{critic} [b]		6.39	9.28	19.25	9.28	9.28
Repeatability n=10 [c]	Day 1	0.68	0.31	0.19	0.37	0,2
	Day 2	0.67	0.29	0.2	0.12	0,24
	Day 3	0.57	0.42	0.2	0.24	0,33
Intermediate precision (n=3) [c]		1.67	1.03	0.2	0.45	0.30

[a] Between the peak in question and the next peak

[b] F for the fit test of the models, considering that F_{critic} should be lower than $F_{calculated}$ in order for the model to fit

[c] Values shown on a relative standard deviation

Among the products analysed, only the samples of soft drink, nectar and instant juice presented reference to the amount of artificial sweeteners in their labels. Table 2 contains the values determined for all samples analysed.

Soft drinks

Among the soft drinks analysed, we observed that aspartame was the most frequently used sweetener, followed by acesulfame-k.

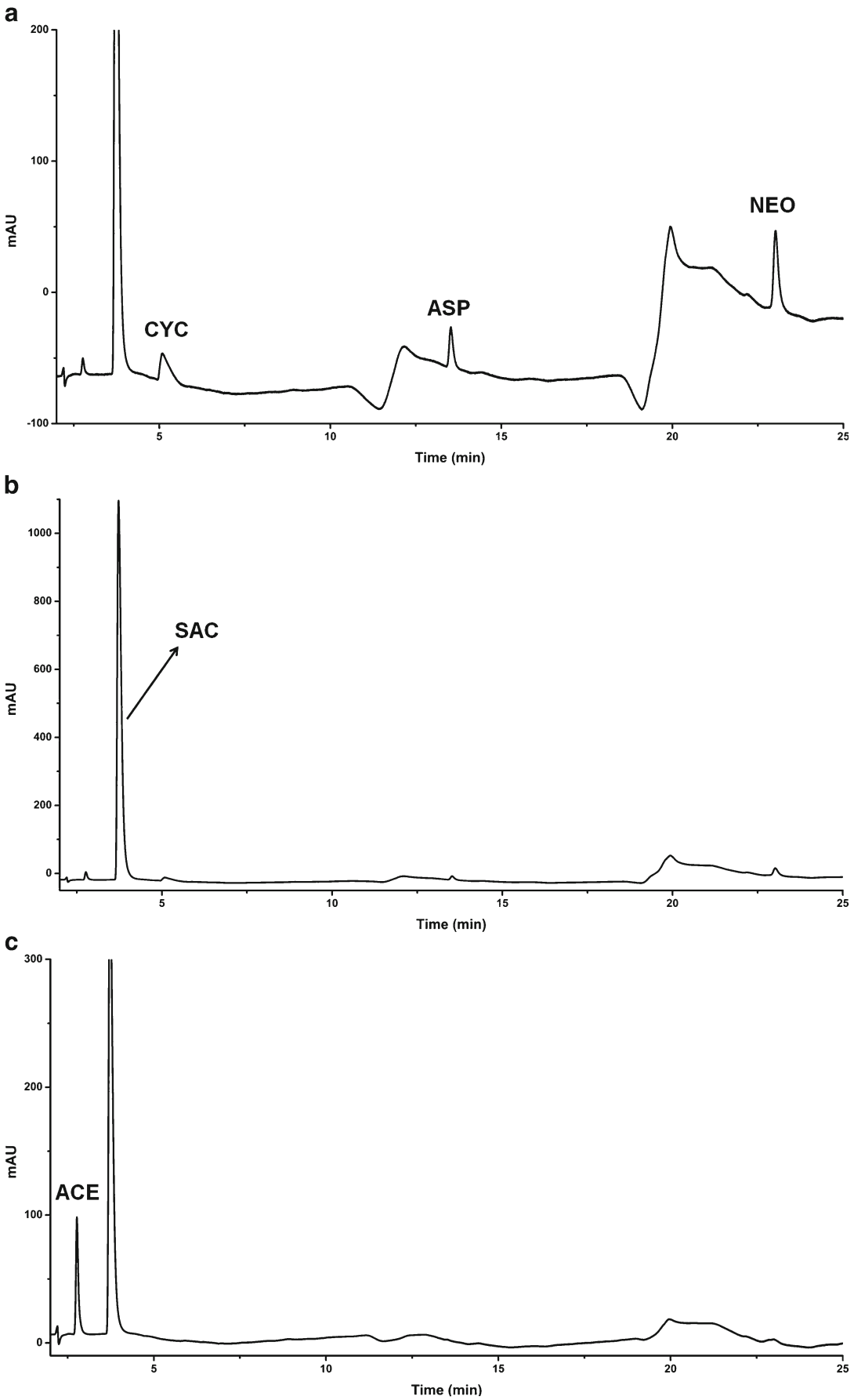
For the lemon-flavoured low calorie drink from Manufacturers 1 and 2, only acesulfame-k and aspartame were found. The amount of acesulfame-k in the drink from Manufacturer 1 was significantly different across batches; and in addition its amount was over four times above the amount stated on the product label. For the same manufacturer (1), the concentration of aspartame was 29 % below that stated on the label. Furthermore, it is noticeable that both manufacturers used different concentrations for acesulfame-k and the same concentration for aspartame.

The guarana-flavoured drink from Manufacturers 2 and 3 contained saccharin and cyclamate. The drink from Manufacturer 2 also contained aspartame. The amount of aspartame on Manufacturer 2's drink was significantly different across batches, while Manufacturer 3's drink contained significant difference across batches for saccharin and cyclamate, indicating a possible homogenization flaw in their process. Additionally, the concentration of cyclamate was 19 % higher than that stated on the label for Manufacturer 3. The fact that aspartame was not used by this manufacturer made necessary the use of higher amounts of saccharin and cyclamate (three and two times more, respectively), when compared to Manufacturer 2. This fact evidenced the synergism among sweeteners when used in combination, which allows the achievement of the same sweetness using smaller amounts of different sweeteners (Zygler et al. 2009).

For the cola drink from Manufacturers 1 and 3, only acesulfame-k and aspartame were identified. For Manufacturer 1, the concentrations were 49 % higher than the stated on the label for acesulfame-k and 17 % for aspartame. The amount of acesulfame-k from Manufacturer 2 and aspartame from Manufacturer 1 were significantly different across batches.

The artificial sweeteners' maximum legal limit for soft drinks classified as zero are 35.0 mg.100 mL^{-1} for acesulfame-k, 15.0 mg.100 mL^{-1} for saccharin, 40.0 mg.100 mL^{-1} for cyclamate, and 75.0 mg.100 mL^{-1} for aspartame. Regarding light soft drinks, the maximum limits are 26.0 mg.100 mL^{-1} for acesulfame-k, 10.0 mg.100 mL^{-1} for saccharin, 30.0 mg.100 mL^{-1} for cyclamate, and 56.0 mg.100 mL^{-1} for aspartame. None of the samples presented amounts of artificial sweeteners above the legal limits.

Fig. 1 Chromatographic profile obtained for the determination of artificial sweeteners (ACE: acesulfame - 227 nm; SAC: saccharin - 201 nm; CYC: cyclamate - 192 nm; ASP: aspartame - 192 nm; NEO: neotame - 192 nm), related to standards (**a**, **b**, **c**). Chromatographic conditions: Pinnacle II column, C18, 5 µm, 150×4.6 mm d.i. (Restek); mobile phase composed by monobasic sodium phosphate buffer (5 mM, pH 7.0) and acetonitrile; diode array detection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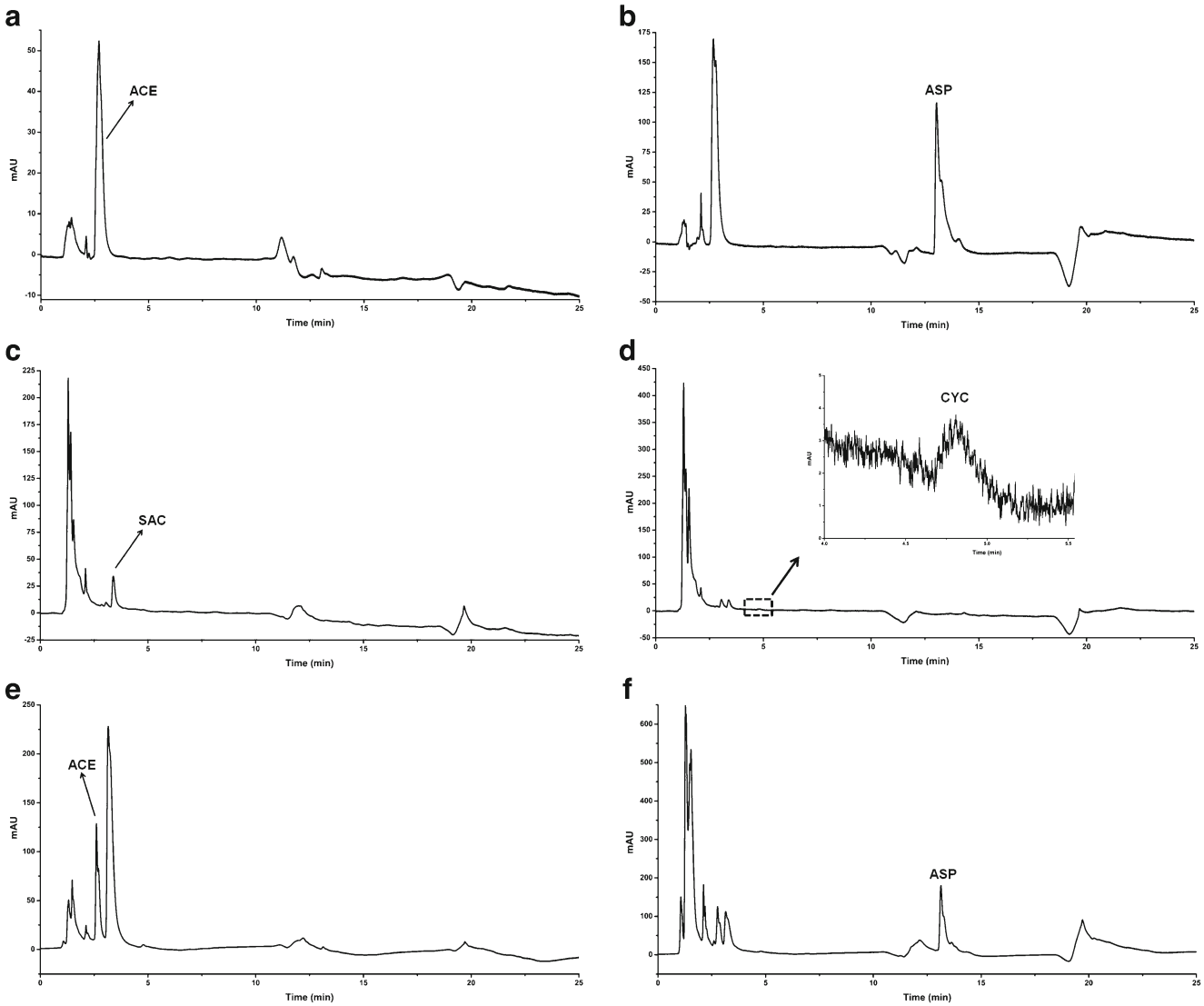

Fig. 2 Chromatographic profile obtained for the determination of artificial sweeteners (ACE: acesulfame −227 nm; SAC: saccharin −201 nm; CYC: cyclamate −192 nm; ASP: aspartame - 192 nm; NEO: neotame - 192 nm), on the soft drink cola flavour samples Brand 1 (**a**, **b**), grape nectar Brand 4 (**c**, **d**), jam Brand 11 (**e**, **f**). Chromatographic conditions described in Fig. 1

Nectars

In the composition of the analysed nectars, acesulfame-k dominated, except for the light grape nectar from Manufacturer 4. This sweetener has been the most used possibly due to its prolonged stability in low pH conditions in aqueous solution, and its resistance to heat treatment (Lipinski and Hangler 2001; Kemp 2006). Manufacturer 4 used different sweeteners for different nectar flavours.

There was a significant difference across batches for cyclamate on the light grape nectar from Manufacturer 4, for acesulfame-k on the light peach nectar from Manufacturer 4, and for the light passion fruit nectar from Manufacturer 2.

For light nectars, the maximum legal limits are 26.0 mg.100 mL^{-1} for acesulfame-k, 10.0 mg.100 mL^{-1} for saccharin, 30.0 mg.100 mL^{-1} for cyclamate, and 56.0 mg.100 mL^{-1} for aspartame. None of the samples presented amounts of artificial sweeteners above the legal limits.

Powder products

The use of acesulfame-k and aspartame dominated in powder products.

For the apple-flavoured light instant juice, the concentrations of both sweeteners were above the values stated on the labels in some batches, on average 14 % for acesulfame-k and 5 % for aspartame. The concentrations of aspartame for the orange-flavoured light instant juice and for the orange-flavoured zero instant juice were also above the values stated on their labels (9 and 31 % above, respectively). Although the

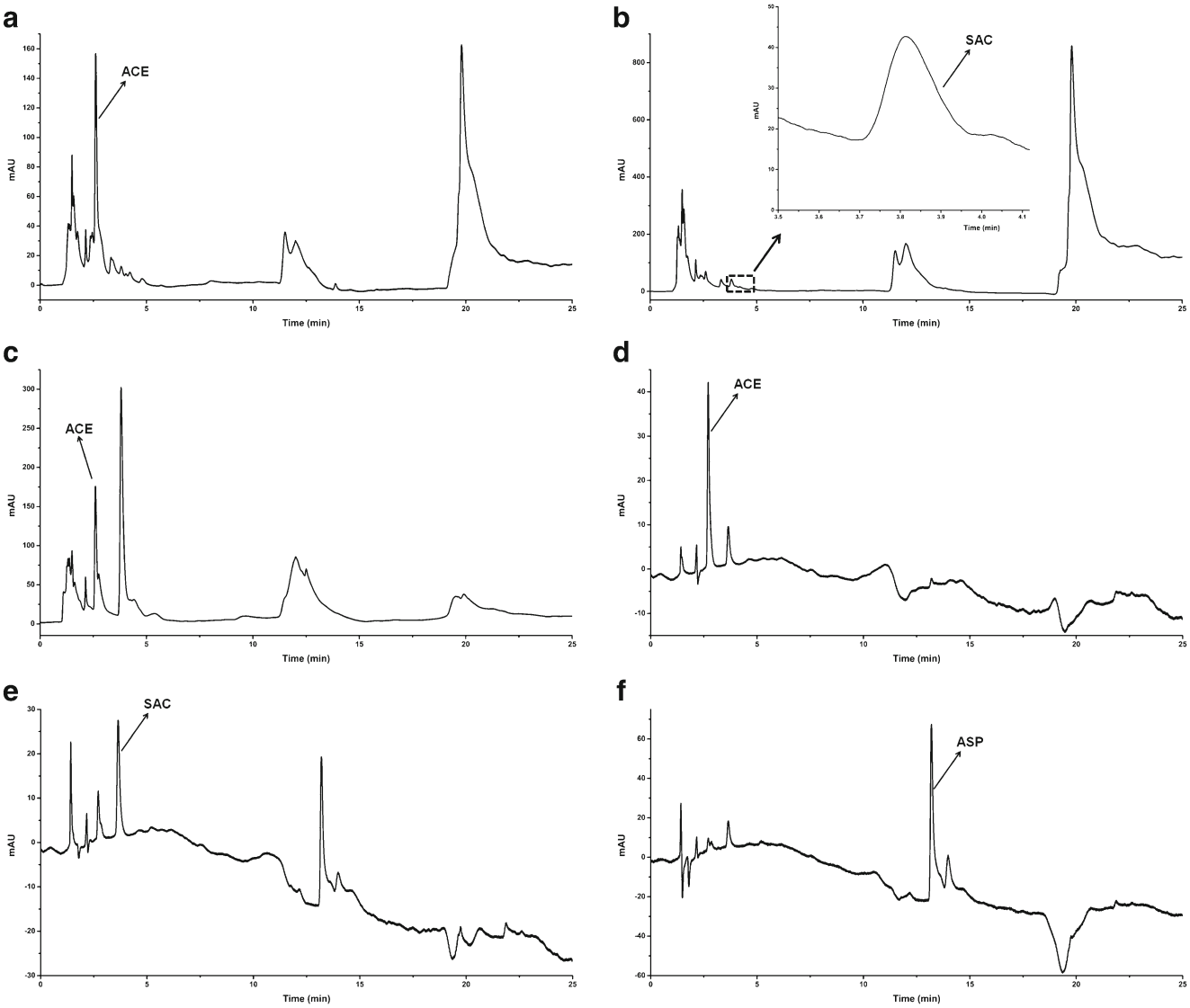

Fig. 3 Chromatographic profile obtained for the determination of artificial sweeteners (ACE: acesulfame −227 nm; SAC: saccharin - 201 nm; CYC: cyclamate −192 nm; ASP: aspartame - 192 nm; NEO: neotame - 192 nm), on cappuccino samples Brand 8 (**a**, **b**), drinking chocolate powder Brand 6 (**c**) and vanilla instant pudding Brand 10 (**d**, **e**, **f**). Chromatographic conditions described in Fig. 1

three batches analysed for each food product were not statistically different, there was a pronounced difference across samples within batches, evidenced by high standard deviations, which masked the differences across batches and demonstrated an inefficient quality control.

For light instant juices, the maximum legal limits are 26.0 mg.100 mL^{-1} for acesulfame-k, 10.0 mg.100 mL^{-1} for saccharin, 30.0 mg.100 mL^{-1} for cyclamate, and 56.0 mg.100 mL^{-1} for aspartame. For zero instant juices, the limits are 35.0 mg.100 mL^{-1} for acesulfame-k, 15.0 mg.100 mL^{-1} for saccharin, 40.0 mg.100 mL^{-1} for cyclamate, and 75.0 mg.100 mL^{-1} for aspartame. None of the samples presented amounts of artificial sweeteners above the legal limits.

For drinking chocolate powder, only acesulfame-k was identified, and its concentration differed significantly across batches for Manufacturers 1 and 6.

For cappuccinos, acesulfame-k was identified in products from Manufacturers 7 and 8, in addition to aspartame for Manufacturer 7, and saccharin for Manufacturer 8. There was a significant difference across batches for the concentration of aspartame on the product from Manufacturer 7 and saccharin for Manufacturer 8's product.

The maximum legal limits for light drinking chocolate powder are 26.0 mg.100 mL^{-1} for acesulfame-k, 10.0 mg.100 mL^{-1} for saccharin, 30.0 mg.100 mL^{-1} for cyclamate, and 56.0 mg.100 mL^{-1} for aspartame. The maximum legal limits for light instant cappuccinos are 26.0 mg.100 mL^{-1} for acesulfame-k, 10.0 mg.100 mL^{-1} for

Table 2 Amount of artificial sweetener (mg.100 mL^{-1}) in the samples analysed [a]

Sample	Batch	Acesulfame-k	Saccharin	Cyclamate	Aspartame
Lemon flavoured low calorie soft drink Manufacturer 1	1	23.6±0.5a			15.4±0.7a
	2	25.3±0.4b			15.9±0.2a
	3	24.5±0.1b			14.9±0.1a
	Mean	24.5±0.8			15.4±0.6
Stated on the label	5	–	–	21	
Lemon flavoured low calorie soft drink Manufacturer 2	1	5.0±0.6a			22.3±0.6a
	2	5.2±0.1a			21.9±1.1a
	3	5.3±0.1a			21.8±0.6a
	Mean	5.2±0.3			22.0±0.7
Stated on the label	5	–	–	21	
Zero Guarana Manufacturer 2	1		6.2±0.1a	36.69±1.81a	7.61±0.22a
	2		6.4±0.1a	40.17±1.91a	8.52±0.28b
	3		6.3±0.1a	37.13±0.53a	8.39±0.27b
	Mean		6.3±0.1	37.99±2.08	8.17±0.48
Stated on the label	–	5	31	12	
Zero Guarana Manufacturer 3	1		14.3±0.5a	49.8±0.7a	
	2		14.0±0.4a	66.4±0.2b	
	3		13.2±0.1b	79.4±0.1c	
	Mean		13.8±0.6	65.2±12.9	
Stated on the label	–	16	69.7	–	
Light cola soft drink Manufacturer 1	1	13.5±0.4a			38.6±1.3a
	2	13.6±0.2a			42.3±0.5b
	3	13.2±0.1a			41.5±0.5b
	Mean	13.5±0.3			40.8±1.8
Stated on the label	8,99	–	–	34.69	
Light cola soft drink Manufacturer 2	1	12.6±0.2a			25.5±0.5a
	2	11.6±0.5b			25.6±0.9a
	3	11.5±0.3b			23.4±1.2a
	Mean	11.9±0.6			24.8±1.3
Stated on the label	13	–	–	24	
Light grape nectar Manufacturer 4	1		3.4±0.3a	38.3±1.4a	
	2		3.6±0.1a	36.7±3.3a	
	3		3.8±0.2a	37.0±3.8a	
	Mean		3.7±0.4	37.3±2.8	
Stated on the label	–	4	40	–	
Light grape nectar Manufacturer 2	1	3.7±0.1a			
	2	3.4±0.1a			
	3	3.7±0.3a			
	Mean	3.6±0.2			
Stated on the label	3	–	–	–	
Light peach nectar Manufacturer 4	1	2.5±0.4a			
	2	3.2±0.1b			
	3	2.6±0.1a			
	Mean	2.7±0.4			
Stated on the label	3	–	–	–	
Light peach nectar Manufacturer 2	1	2.7±0.1a			
	2	2.7±0.1a			
	3	2.6±0.0a			
	Mean	2.7±0.1			

Table 2 (continued)

Sample	Batch	Acesulfame-k	Saccharin	Cyclamate	Aspartame
Stated on the label	3	–	–	–	
Light guava nectar Manufacturer 2	1	1.5±0.2a			
	2	1.4±0.1a			
	3	1.5±0.1a			
	Mean	1.5±0.1			
Stated on the label	3	–	–	–	
Light passion fruit nectar Manufacturer 2	1	11.8±0.8a			14.0±1.1a
	2	10.2±0.5b			13.6±1.7a
	3	9.9±0.4b			14.6±0.9a
	Mean	10.6±1.0			14.1±1.2
Stated on the label	10	–	–	–	
Light Orange nectar Manufacturer 2	1	2.0±0.1a			
	2	1.9±0.1a			
	3	1.9±0.1a			
	Mean	1.9±0.1			
Stated on the label	3	–	–	–	
Apple-flavoured light instant juice Manufacturer 5	1	2.4±1.2a			33.6±8.1a
	2	8.6±3.2a			28.3±4.2a
	3	4.2±3.0a			29.5±5.1a
	Mean	5.2±3.7			30.4±5.9
Stated on the label	4.5	–	–	–	
Apple-flavoured zero instant juice Manufacturer 5	1	5.1±2.8a			27.1±4.0a
	2	3.7±1.0a			26.9±1.2a
	3	3.6±3.4a			26.2±11.6a
	Mean	4.1±2.4			26.7±6.2
Stated on the label	4.6	–	–	–	
Orange-flavoured light instant juice Manufacturer 5	1	9.2±0.1a			16.8±4.8a
	2	8.7±0.7a			30.7±8.5a
	3	8.1±0.5a			24.4±2.4a
	Mean	8.7±0.7			24.0±7.9
Stated on the label	9.4	–	–	–	
Orange-flavoured zero instant juice Manufacturer 5	1	1.5±0.2a			45.4±3.3a
	2	3.2±2.4a			47.8±2.6a
	3	3.3±0.9a			42.6±2.4a
	Mean	2.7±1.6			45.8±2.6
Stated on the label	3.9	–	–	–	
Light drinking chocolate powder Manufacturer 6	1	16.4±3.3a		**	
	2	15.7±2.4a		**	
	3	12.2±0.7b		**	
	Mean	14.8±2.8			
Stated on the label	-	–	–	–	
Light drinking chocolate Manufacturer 1	1	4.1±0.1a		**	
	2	2.5±0.5b		**	
	3	5.3±0.2c		**	
	Mean	4.0±1.3			
Stated on the label	-	–	–	–	
Light instant cappuccino Manufacturer 7	1	25.8±6.9a			51.0±4.9a
	2	18.3±5.8a			24.1±3.3b
	3	24.5±6.4a			28.9±5.3b

Table 2 (continued)

Sample	Batch	Acesulfame-k	Saccharin	Cyclamate	Aspartame
	Mean	22.9±6.5			34.7±13.0
Stated on the label	-	–	–	–	
Light instant cappuccino with cinnamon Manufacturer 8	1	12.8±4.7a	2.8±0.9a		
	2	13.3±5.8a	1.7±0.2b		
	3	12.0±6.1a	1.3±0.2b		
	Mean	12.8±4.8	1.9±0.8		
Stated on the label	-	–	–	–	
Vanilla-flavoured diet instant pudding Manufacturer 9	1		6.7±0.2a		6.5±0.2a
	2		6.7±0.3a		6.0±0.3a
	3		7.8±0.1b		6.4±0.2a
	Mean		7.1±0.6		6.3±0.3
Stated on the label	–	–	–	–	
Vanilla-flavoured zero instant pudding Manufacturer 10	1	1.0±0.7a	3.0±0.7a		13.7±0.4a
	2	0.6±0.3a	3.4±0.7a		14.7±2.5a
	3	0.3±0.2a	3.3±0.6a		13.9±0.5a
	Mean	0.6±0.5	3.2±0.6		14.1±1.4
Stated on the label	–	–	–	–	
Chocolate-flavoured diet instant pudding Manufacturer 9	1		11.8±0.6a	**	2.7±0.6a
	2		8.4±0.2b	**	3.1±0.3a
	3		8.3±0.3b	**	3.1±0.2a
	Mean		11.1±7.8		3.0±0.4
Stated on the label	–	–	–	–	
Chocolate-flavoured zero instant pudding Manufacturer 10	1		15.8±0.5a	**	11.6±0.1a
	2		13.9±1.5a	**	12.1±0.6a
	3	0.5±0.2	15.8±1.1a	**	12.9±0.9b
	Mean		15.1±1.3		12.1±0.8
Stated on the label	–	–	–	–	
Grape-flavoured zero jelly preparation powder Manufacturer 10	1				13.9±2.7a
	2				11.6±1.1ab
	3				10.8±0.3b
	Mean				12.1±2.2
Stated on the label	–	–	–	–	
Grape-flavoured zero jelly preparation powder Manufacturer 8	1				45.2±5.3a
	2				55.9±13.5a
	3				48.8±7.8a
	Mean				50.0±9.5
Stated on the label	–	–	–	–	
Strawberry-flavoured zero jelly preparation powder Manufacturer 10	1				14.7±1.0a
	2				17.9±0.6ab
	3				13.1±5.2b
	Mean				15.0±3.4
Stated on the label	–	–	–	–	
Diet strawberry jam Manufacturer 8					
	1	1.6±0.1a			
	2	1.4±0.2ab			
	3	1.2±0.1b			
	Mean	1.4±0.2			

Table 2 (continued)

Sample	Batch	Acesulfame-k	Saccharin	Cyclamate	Aspartame
Stated on the label	–	–	–	–	
Diet strawberry jam Manufacturer 2	1	14.3±2.2a			22.5±3.1a
	2	14.3±2.0a			47.3±5.6b
	3	16.0±3.4a			43.4±3.4b
	Mean	14.9±2.4			37.8±15.4
Stated on the label	–	–	–	–	
Light tomato sauce Manufacturer 8	1	110.7±9.2a			
	2	118.2±10.7a			
	3	118.4±8.1a			
	Mean	115.8±9.0			
Stated on the label	–	–	–	–	
Light barbecue sauce Manufacturer 8	1	215.1±21.3a			
	2	164.2±15.9b			
	3	217.5±19.6a			
	Mean	198.9±30.8			
Stated on the label	–	–	–	–	
Powdered tabletop sweetener Manufacturer 1	1		1882.2±0.4a	1122.3±1.6a	
	2		1882.3±1.4a	1125.3±2.2a	
	3		1881.9±1.4a	1125.6±1.9a	
	Mean				
Powdered tabletop sweetener Manufacturer 2	1	34.5±2.04a			169.0±2.1a
	2	36.3±3.02a			167.6±1.9a
	3	35.7±2.20a			170.7±2.9a
	Mean	36.15±2.50			169.1±2.6
Powdered tabletop sweetener Manufacturer 3	1	37.2±1.9a			170.3±1.9a
	2	36.1±3.2a			167.7±1.4a
	3	35.7±1.4a			169.3±4.7a
	Mean	36.3±1.9			169.1±2.9
Powdered tabletop sweetener Manufacturer 4	1				483.9±1.8a
	2				483.4±2.0a
	3				486.0±3.2a
	Mean				484.4±2.4
Powdered tabletop sweetener Manufacturer 5	1				632.6±0.9a
	2				631.8±0.6a
	3				632.9±2.1a
	Mean				632.4±1.3
Liquid tabletop sweetener Manufacturer 3	1		929.4±2.2a	637.2±4.4a	
	2		926.3±2.6a	639.2±1.8a	
	3		929.7±2.7a	637.3±1.5a	
	Mean		928.5±2.7	637.9±2.8	
Liquid tabletop sweetener Manufacturer 1	1		727.5±3.7a	603.45±2.1a	
	2		725.6±3.3a	603.72±2.4a	
	3		724.7±2.8a	602.54±0.6a	
	Mean		725.9±3.1	603.24±1.7	
Liquid, crystal, tabletop sweetener Manufacturer 5	1		947.3±1.1a	640.6±1.3a	
	2		947.2±1.0a	641.9±1.6a	

Table 2 (continued)

Sample	Batch	Acesulfame-k	Saccharin	Cyclamate	Aspartame
	3		947.1±1.4a	641.2±1.5a	
	Mean		947.2±1.0	641.3±1.4	
Liquid tabletop sweetener Manufacturer 6	1	29.7±1.1a	360.6±1.5a	531.8±2.0a	
	2	30.6±2.0a	360.6±2.8a	533.5±2.5a	
	3	30.5±3.0a	359.5±1.5a	534.9±1.5a	
	Mean	30.2±2.2	360.2±2.0	533.4±2.0	
Liquid aspartame tabletop sweetener Manufacturer 5	1				815.9±2.0a
	2				817.6±1.8a
	3				816.4±2.1a
	Mean				816.7±1.7

[a] Data presented as mean±standard deviation for the triplicate determination (n=3). Values on the same column indicated with the same lowercase letter do not differ according to Tukey's test $p<0.05$)

** The label of these samples indicated the presence of cyclamate, however, it was not possible to identify it due to the presence of an unidentified interferent not found in any of the other samples

saccharin, 30.0 mg.100 mL^{-1} for cyclamate, and 56.0 mg.100 mL^{-1} for aspartame. In both products, none of the samples presented amounts of artificial sweeteners above the legal limits, except for the amount of aspartame on Manufacturer 7's product.

For diet instant pudding, the maximum legal limits are 35.0 mg.100 mL^{-1} for acesulfame-k, 15.0 mg.100 mL^{-1} for saccharin, 40.0 mg.100 mL^{-1} for cyclamate, and 75.0 mg.100 mL^{-1} for aspartame. For zero instant pudding, the limits are 35.0 mg.100 mL^{-1} for acesulfame-k, 15.0 mg.100 mL^{-1} for saccharin, 40.0 mg.100 mL^{-1} for cyclamate, and 75.0 mg.100 mL^{-1} for aspartame. None of the samples presented amounts of artificial sweeteners above the legal limits.

Saccharin and aspartame were identified in samples of instant pudding from Manufacturers 9 and 10. Samples from Manufacturer 10 also contained acesulfame-k. Saccharin concentrations were statistically different across batches of vanilla and chocolate flavour puddings from Manufacturer 9; acesulfame-k and aspartame concentrations were also significantly different for the chocolate flavour. The amount of saccharin in the chocolate-flavoured instant pudding from Manufacturer 10 was slightly above the legal limit for its product category.

For zero jelly preparation powders, the legal limits are 35.0 mg.100 mL^{-1} for acesulfame-k, 15.0 mg.100 mL^{-1} for saccharin, 40.0 mg.100 mL^{-1} for cyclamate, and 75.0 mg.100 mL^{-1} for aspartame. None of the samples presented amounts of artificial sweeteners above the legal limits.

For grape and strawberry jelly preparation powder samples, only aspartame was identified. There was a significant difference across batches for jellies from both flavours for Manufacturer 10. For this manufacturer, the product labels informed the presence of acesulfame-k, saccharin, cyclamate and aspartame, although the first three sweeteners were not found in the samples.

The labels of the drinking chocolate powder and instant cappuccino from Manufacturer 8 and the chocolate instant pudding samples indicated the presence of cyclamate. However, it was not possible to identify the cyclamate in these samples due to the presence of an unidentified interferent not found in any of the other samples. Nine samples of powdered products presented variation on the concentrations of the replicates, in which the batches differed among themselves, indicating a low quality control by the industry in its production or flaws related to the processing of powdered foods.

The processing of powdered food is still governed by cost control. Most of the pieces of equipment used are old, having been designed several decades ago, and the food industry is still trying to understand and control the performance of powdered foods. Powdered mixes usually contain several ingredients, which present different properties each (particularly the size of the particle, its density and porosity), hence segregation may occur after mixing different powder-like substances (Ahrné and Fitzpatrick 2005). Furthermore, there is no standard to be followed regarding powder processing (Cuq et al. 2010). Indeed, in the document from the European project "Food Powders" (2003), researches highlighted the difficulty to obtain an ideal mixing method.

Jams

The most prevailing sweetener used in the jams and sauces analysed was acesulfame-k.

For diet jams, the maximum legal limits are 35.0 mg.100 mL^{-1} for acesulfame-k, 15.0 mg.100 mL^{-1} for

saccharin, 40.0 mg.100 mL^{-1} for cyclamate, and 75.0 mg.100 mL^{-1} for aspartame. None of the samples presented amounts of artificial sweeteners above the legal limits.

For the strawberry diet jam samples, acesulfame-k was found in products from Manufacturers 8 and 11, in addition to aspartame in Manufacturer 11's jam. A significant difference across batches was observed for acesulfame-k in the jam from Manufacturer 8 and for aspartame in the jam from Manufacturer 11. Manufacturer 8 declared in its label the presence of sucralose, which justifies the low concentration of acesulfame-k found in this sample.

Barbecue and tomato sauces

The maximum legal limits for barbecue and tomato sauces are 26.0 mg.100 mL^{-1} for acesulfame-k, 10.0 mg.100 mL^{-1} for saccharin, 30.0 mg.100 mL^{-1} for cyclamate, and 56.0 mg.100 mL^{-1} for aspartame.

In both sauces, only acesulfame-k was found, and there was a significant difference across batches for the barbecue sauce. Both products presented higher concentrations of acesulfame-k than the amount allowed by the legislation, 4 times higher for the tomato sauce, and 7 times for the barbecue sauce.

Tabletop sweeteners

Among the tabletop sweeteners analysed, different combinations and proportions of artificial sweeteners were observed. Saccharin and cyclamate combinations were the most frequently observed. All samples were homogeneous, which is possibly explained by the fact that these are almost pure solutions. The powdered tabletop sweetener from Manufacturer 3 presented acesulfame-k and aspartame, while its label only indicated the presence of aspartame.

Conclusions

The artificial sweeteners were used in combination in most of the samples, which contained up to three sweeteners in their composition. Among soft drinks, aspartame was the most used sweetener, followed by acesulfame-k. In nectars and powdered products, acesulfame-k prevailed, followed by aspartame.

Tomato and barbecue sauce samples presented acesulfame-k concentrations above the legal limit, while one sample of the chocolate instant pudding presented saccharin above the limit. Soft drinks and instant juices presented concentrations of sweeteners higher than the amounts stated on the labels. Additionally, one of the tabletop sweeteners contained a sweetener which was not declared on the label. The results obtained in this study indicate the need for a more rigorous quality control by the manufacturers of products containing artificial sweeteners, as well as a more effective inspection by the Brazilian agencies responsible for inspecting foods and beverages, in order to avoid consumers' exposure to excessive amounts of artificial sweeteners.

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References

Ahrné L, Fitzpatrick JJ (2005) Food powder handling and processing: industry problems, knowledge barriers and research opportunities. Chem Eng Process 44(2):209–214

Brazil. Agência Nacional de Vigilância Sanitária/ Ministério da Saúde (2008) Resolução RDC n° 18, de 24 de março de 2008. Regulamento Técnico que autoriza o uso de aditivos edulcorantes em alimentos, com seus respectivos limites máximos. Diário Oficial da União, Brasília

Brazil. Agência Nacional de Vigilância Sanitária/Ministério da Saúde (1997) Portaria n° 540, de 27 de outubro de 1997. Regulamento Técnico: Aditivos Alimentares. Diário Oficial da União, Brasília

Chen Q, Wang J (2001) Simultaneous determination of artificial sweeteners, preservatives, caffeine, theobromine and theophylline in food and pharmaceutical preparations by ion chromatography. J Chromatogr A 937(1/2):57–64

Cuq B, Rondet E, Abecassis J (2010) Food powders engineering, between knowhow and science: constraints, stakes and opportunities. Powder Technol 208(2):244–251

Dias CB, Meinhart AD, Pane DQ, Ballus CA, Godoy HT (2014) Multivariate optimisation and validation of a method for the separation of five artificial sweeteners by UPLC-DAD in nine food matrices. Food Anal Methods. doi:10.1007/s12161-014-0056-8, Accepted Manuscript

Farhadi A, Keshavarzian A, Holmes BEW, Fields J, Zhang L, Banan A (2003) Gas chromatographic method for detection of urinary sucralose: application to the assessment of intestinal permeability. J Chromatogr B 784(1):145–154

Food Powders, Documento Estratégico para Pesquisa em Pós Alimentícios (2003) Available at: http://www.foodpowders.net/strategic.htm, accessed on October 2011

Huang Z, Ma J, Chen B, Zhang Y, Yao S (2006) Determination of cyclamate in foods by high performance liquid chromatography–electrospray ionization mass spectrometry. Anal Chim Acta 555(2):233–237

Kemp SE (2006) Low-calorie sweeteners. In: Spillane WJ (ed) Optimizing sweet taste in food. Woodhead Publishing Limited, England, pp 175–250

Kritsunankula O, Jakmuneeb J (2011) Simultaneous determination of some food additives in soft drinks and other liquid foods by flow injection on-line dialysis coupled to high performance liquid chromatography. Talanta 84(5):1342–1349

Kroger M, Meister K, Kava R (2006) Low-calorie sweeteners and other sugar substitutes: a review of the safety issues. Compr Rev Food Sci F 5(2):35–47

Leth T, Jensen U, Fagta S, Andersen R (2008) Estimated intake of intense sweeteners from non-alcoholic beverages in Denmark, 2005. Food Addit Contam Part A 25(6):662–668

Lipinski G R, Hangler L Y (2001) Acesulfame K. In: NABORS, L.O. Alternative Sweeteners, 3ed, revised and expanded, Switzerland, Marcel Dekker:13–30

Loos R, Gawlik BM, Boettcher K, Locoro G, Contini S, Bidoglio G (2009) Sucralose screening in European surface waters using a solid-phase extraction-liquid chromatography–triple quadrupole mass spectrometry method. J Chromatogr A 1216(7):1126–1131

Moraes P C B T (2008) O impacto do uso de edulcorantes em bebidas de café solúvel e café torrado/moído como substitutos da sacarose. 107p, Tese de doutorado em Alimentos e Nutrição, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, Campinas.

Nakaie Y, Yogi T, Kakehi K, Inoue D, Hirose H, Hashimoto S, Tonogai Y (1999) Simultaneous and simple determination of saccharin and acesulfame K in foods by GC–NPD. J Food Hyg Soc Jpn 40(3): 223–229

Pietra AM, Cavrini V, Bonazzi D, Benfenati L (1990) Analysis of aspartame and saccharin in pharmaceutical and dietary formulations. Chromatographia 30(3/4):215–219

Renwick AG (2006) The intake of intense sweeteners – an update review. Food Addit Contam 23(4):327–338

Snyder LR, Kirkland JJ, Glajch JL (1997) Pratical HPLC method development. 2 ed., Wiley-Interscience, Hoboken, NJ, USA p. 765

Thompson M, Ellison SLR, Wood R (2002) Harmonized guidelines for single laboratory validation of methods of analysis. IUPAC 74:835–855

Torloni MR, Nakamura MU, Megale A, Sanchez VHS, Mano C, Fusaro AS, Mattar R (2007) O uso de adoçantes na gravidez: uma análise dos produtos disponíveis no Brasil. Rio de Janeiro, Rev Bras Ginecol Obstet 29(5):267–275

Wang Y, Xu HT, Xie YY, Tian YX, Shen YD, Young G, Wang H, Lei HT, Sun YM (2011) Development of polyclonal antibody-based indirect competitive enzyme-linked immunosorbent assay for sodium saccharin residue in food samples. Food Chem 126(1):815–820

Wasik A, McCourt J, Buchgraber M (2007) Simultaneous determination of nine intense sweeteners in foodstuffs by high performance liquid chromatography and evaporative light scattering detection - Development and single-laboratory validation. J Chromatogr A 1157(1/2):187–196

Yang DJ, Chen B (2009) Simultaneous determination of nonnutritive sweeteners in foods by HPLC/ESI-MS. J Agric Food Chem 57(8): 3022–3027

Zhu Y, Guo Y, Ye M, James FS (2005) Separation and simultaneous determination of four artificial sweeteners in food and beverages by ion chromatography. J Chromatogr A 1085(1):143–146

Zygler A, Wasik A, Namiesnik J (2009) Analytical methodologies for determination of artificial sweeteners in foodstuffs. Trend Anal Chem 28(9):1082–1102