



HAL
open science

Estimated intake of sweeteners acesulfame-K and aspartame from soft drinks, soft drinks based on mineral waters and nectars in a scholar group of Portuguese teenagers

Celeste de Matos Lino, Isabel M. Costa, Angelina Lopes Simões Pena, Rui Ferreira, Salvador M. Cardoso

► **To cite this version:**

Celeste de Matos Lino, Isabel M. Costa, Angelina Lopes Simões Pena, Rui Ferreira, Salvador M. Cardoso. Estimated intake of sweeteners acesulfame-K and aspartame from soft drinks, soft drinks based on mineral waters and nectars in a scholar group of Portuguese teenagers. *Food Additives and Contaminants*, 2008, 25 (11), pp.1291-1296. <10.1080/02652030802195309>. <hal-00577402>

HAL Id: hal-00577402

<https://hal.science/hal-00577402v1>

Submitted on 17 Mar 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization



Estimated intake of sweeteners acesulfame-K and aspartame from soft drinks, soft drinks based on mineral waters and nectars in a scholar group of Portuguese teenagers

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2008-051.R1
Manuscript Type:	Review
Date Submitted by the Author:	30-Apr-2008
Complete List of Authors:	Lino, Celeste; University of Coimbra, Laboratory of Bromatology, Faculty of Pharmacy; University of Coimbra, Laboratory of Bromatology, Faculty of Pharmacy Costa, Isabel; Group of Bromatology, CEF, Faculty of Pharmacy, University of Coimbra, 3000-295, Coimbra, Portugal, University of Coimbra Pena, Angelina; Faculty of Pharmacy- University of Coimbra, Laboratory of Bromatology Ferreira, Rui; Faculty of Humanities, University of Coimbra, 3000, Centre of Geographic Studies Cardoso, Salvador; Faculty of Medicine, University of Coimbra, 3000, Hygiene and Public Health Laboratory
Methods/Techniques:	Exposure
Additives/Contaminants:	Sweeteners
Food Types:	Beverages

SCHOLARONE™
Manuscripts

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review Only

1
2
3 **Estimated intake of sweeteners acesulfame-K and aspartame from soft**
4 **drinks, soft drinks based on mineral waters and nectars for a group of**
5
6 **Portuguese teenage students**
7
8
9

10
11
12
13
14 **C. M. LINO*¹, I. M. COSTA¹, A. PENA¹, R. FERREIRA³ & S. M. CARDOSO²**
15

16 *¹Group of Bromatology, CEF, Faculty of Pharmacy, University of Coimbra, P-3000-*
17 *295, Coimbra, Portugal,*

18 *²Hygiene and Public Health Laboratory, Faculty of Medicine, University of Coimbra,*
19 *P-3000, Coimbra, Portugal*

20 *³Faculty of Humanities, Centre of Geographic Studies, University of Coimbra, P-3000,*
21 *Coimbra, Portugal*
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42

43 *Celeste M. Lino

44 Group of Bromatology

45 CEF

46 Faculty of Pharmacy

47 University of Coimbra

48 Rua do Norte s/n

49 3000-295 COIMBRA - PORTUGAL

50 Tel. 351-239859994

51 Fax. 351-239827126

52 E-mail: cmlino@ci.uc.pt
53
54
55
56
57
58
59
60

Abstract

A survey of levels of acesulfame-K and aspartame in soft drinks and in light nectars was undertaken, from which the intake of these intense sweeteners was estimated for a group of teenage students. Acesulfame-K was detected in 72% of the soft drinks, with a mean concentration of 72 mg/L and aspartame was found in 92% of the samples with a mean concentration of 89 mg/L. When data on content of these sweeteners in soft drinks was analyzed according to flavour, cola drinks had the highest mean levels for both the sweeteners containing 98 and 103 mg/L for acesulfame-K and aspartame, respectively. For soft drinks based on mineral water, aspartame was found in 62% of the samples, with a mean concentration of 82 mg/L and acesulfame-K was found in 77%, with a mean level of 48 mg/L. All samples of nectars contained acesulfame-K, with a mean concentration of 128 mg/L and aspartame was detected in 80% of the samples with a mean concentration of 73 mg/L. A frequency questionnaire, designed to identify adolescents having high consumption of these drinks, was completed by a randomly selected sample of teenagers (n=65) living in the city of Coimbra, in the year 2007. The estimated daily intakes (EDI) of acesulfame-K and aspartame for the average consumer were below the acceptable daily intakes (ADIs). For acesulfame-K the EDI was 0.7 mg/kg b.w./day for soft drinks, 0.2 mg/kg b.w./day for soft drinks based on mineral waters, and 0.5 mg/kg b.w./day for nectars, representing 8.0%, 2.2%, and 5.8% of the ADI, respectively. A similar situation was observed for aspartame. In this way, the EDI for soft drinks was 1.1 mg/kg b.w./day, representing only 2.9% of the ADI. In respect to nectars, the EDI was 0.2 mg/kg b.w./day, representing 0.5% of the ADI. Soft drinks based on mineral waters showed the lowest EDI values of 0.3 mg/kg b.w./day, accounting for 0.7 % of the ADI.

Keywords: *Estimated intake, sweeteners, acesulfame-K, aspartame, soft drinks, soft drinks based on mineral waters, nectars, Portuguese teenagers*

Introduction

Food additives play a vital role in the modern food industry, and are generally used for maintaining food quality characteristics as well as ensuring food safety. Among them, artificial sweeteners are widely used throughout the world. For many year artificial sweeteners have been commonly used in the food, beverage, confectionary and pharmaceutical industries because of their sweet taste with fewer calories (Zhu et al. 2005). The use of sweeteners is of significance due to increase in obesity problems in developed countries, the general growing awareness of health implications connected to a high calorie diet, to the worldwide increase in the number of people suffering from diabetes, and also to reduce tooth decay (Leth et al., 2007).

Acesulfame-K and aspartame are used in about 100 countries (Zhu et al. 2005). In Portugal, the most common artificial sweeteners used by the food industry are aspartame, acesulfame-K, and sodium cyclamate. Nowadays, in this country, sucralose begins to be introduced in some food items, and the use of saccharin is decreasing. Scrutiny of the labels from soft drinks, soft drinks based on mineral water, and nectars shows that aspartame and acesulfame-K are those preferentially incorporated.

The acceptable daily intake (ADI) values established are 9 and 40 mg/kg b.w./day for acesulfame-K and aspartame, respectively (SCF, 1985; SCF, 2002). Recently, in an update review of the intake estimations, Renwick (2006) showed that few data are available for the population in general, and for teenagers, only two studies from Italy are reported.

1
2
3 As far as we know, this is the first study of sweeteners intake for Portuguese
4 populations, namely adolescents, which is a group with high consumption. The main
5 objective of this study was to check for the presence and concentration of acesulfame-k
6 and aspartame, and verify if below maximum permitted levels in light soft drinks, soft
7 drinks based on mineral waters, and nectars. The data were subsequently used to
8 estimate intake of the sweeteners for a group of Portuguese teenage students aged
9 between 13 and 15.
10
11
12
13
14
15
16
17
18
19
20
21

22 **Materials and methods**

23 *Studied population*

24
25
26 One public high school was selected in the city of Coimbra (central zone of
27 Portugal). In the school year 2006–2007, students were randomly selected (“Random
28 sample”) (n=65). All students, aged between 13 and 15 years old, 50.8% males and
29 49.2% females, were asked to fill in a questionnaire related to the duration and
30 frequency of intake of sugar-free soft drinks and nectars. Students were also asked to
31 record their own personal data, such as age, sex, rural/urban residence area and
32 education level of parents. Permission was obtained from those responsible for their
33 education.
34
35
36
37
38
39
40
41
42
43
44
45
46
47

48 The mean body weight was 56.5 kg ranging from 36.5 to 106.1 kg. The Body Mass
49 Index was calculated, and it was found that 62% of individuals had normal weight,
50 23% had low weight, and 15% had excess weight or obesity.
51
52
53
54
55
56

57 The consumption data for the Portuguese population in general were obtained through
58 the annual intake estimated per Portuguese inhabitant in 2006, according the ANIRSF,
59
60

1
2
3 the National Association of Soft Drink and Fruit Juice Industry. The estimated mean
4 body weight was 60 kg.
5
6
7

8 9 *The drink survey*

10
11
12 A total of 48 samples were chosen in accordance with products available for sale at the
13 canteen of the school and the consumption of the teenagers. Of these samples, 38 were
14 soft drinks comprising 25 light soft drinks and 13 soft drinks based on mineral waters (a
15 new group of cooled drinks recently introduced in the Portuguese market) and 10 were
16 light nectars. Samples were collected in the school canteen, and in supermarkets
17 between December 2006 and January 2007.
18
19
20
21
22
23
24
25
26
27
28

29 *Chemical analysis*

30
31 Soft drinks were filtered through 0.45 µm membrane filter and ultrasonicated, before
32 HPLC analysis. Nectars were submitted to centrifugation at 3400g for 15 min, filtration
33 through membrane filter and ultrasonication. The two sweeteners were separated using
34 reverse phase liquid chromatography (Pump model 305 from Gilson, France, an
35 injection valve with one 20 µL loop model 7125 Rheodyne, Cotati, California, USA)
36 with an Hichrom C18 column (5 µm, 250 x 4.6 mm) and a buffered mobile phase
37 (KH₂PO₄ 0.02M / ACN (90:10) / phosphoric acid), at 1 mL/min, which pH was
38 rigorously controlled at 4.2-4.3 for an adequate resolution between aspartame and
39 benzoic acid, since this preservative is present in almost analysed samples. Detection
40 was performed with an UV detector, model 116 from Gilson, France, at 220 nm.
41
42 External standard method was used for quantification. Integration was performed with
43 an integrator; model SP4290 from Spectra-Physics.
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Recovery studies

Recoveries were determined by spiking one soft drink free for the two sweeteners, in triplicate, with known amounts of acesulfame-K and aspartame at final concentrations between 35 and 350 mg/L for the first, and 35 and 600 mg/L for the second sweetener.

Results

The methodology was shown to have adequate linearity, sensitivity, accuracy and precision. Correlation coefficients (r^2) were 0.9906 for acesulfame-K and 0.9973 for aspartame, both between 0.025 µg and 0.4 µg injected. Limits of quantification of acesulfame-k and aspartame were 1.7 mg/L and 3.5 mg/L, respectively. Recovery values were between 91% for acesulfame-K at fortification levels of 350 mg/L and 102% for aspartame at spiked levels of 35 mg/L. Intra-day repeatabilities (% RSD) ranged between 1.0% for aspartame at 35 mg/L and 7.9% for acesulfame-K at 35 mg/L. The inter-day repeatabilities ranged between 7.3 and 11.5% for aspartame at 600 mg/L and acesulfame-K at 100 mg/L, respectively (Table I).

Figure I shows the chromatograms of a standard of acesulfame-K and aspartame containing 0.3 µg of each compound; of a blank sample without sweeteners; and the same sample fortified at 35 mg/L (0.175 µg) with each sweetener.

Table II shows the number of the samples, the range, and the mean of the sweeteners in the different types of analysed beverages. Acesulfame-K was detected in 18 (72%) of the 25 soft drinks analysed, with levels ranging between 7.8 mg/L e 166 mg/L, and a mean concentration of 72 mg/L. Twenty three samples (92%) contained aspartame at

1
2
3 levels between 8.8 mg/L and 339 mg/L. The mean concentration was 89 mg/L (Table
4
5 II).
6
7
8
9

10 When the data on sweeteners soft drinks were analyzed according the flavour, the cola
11 drinks (n=5) had the highest mean levels for both sweeteners, 98 and 103 mg/L for
12 acesulfame-K and aspartame, respectively (Table III). In respect of soft drinks based on
13 mineral waters, aspartame was found in 8 samples (62%), between 19 and 154 mg/L.
14 The mean concentration value was 82 mg/L. Acesulfame-k was detected in ten samples
15 (77%), at levels ranging between 3.7 and 182 mg/L, with a mean level of 48 mg/L
16 (Table II). In five of these ten samples, faulty declarations were found as this sweetener
17 was not declared on the ingredient labels. Those samples represent four Portuguese
18 brands. One of them did not specify the presence of any sweetener, but indicated
19 “without addition of sugar”, and four samples only referred to the presence of
20 aspartame.
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38

39 All samples of nectars showed the presence of acesulfame-K, with levels between 35
40 and 356 mg/L, being the mean concentration 128 mg/L. Aspartame was detected in 8
41 (80%) samples, at levels between 39 mg/L and 129 mg/L. The mean concentration was
42
43
44
45
46 73 mg/L.
47
48
49
50

51 According Portuguese legislation (Decreto-Lei nº 394/98 de 10 de Dezembro de 1998),
52 the maximum permitted level for acesulfame-K in soft drinks and in nectars is 350
53 mg/L. This level was exceeded in one sample of nectar, with a concentration of 356
54 mg/L. For aspartame, the maximum permitted level for the same kind of products, 600
55 mg/L. For aspartame, the maximum permitted level for the same kind of products, 600
56 mg/L. For aspartame, the maximum permitted level for the same kind of products, 600
57 mg/L. For aspartame, the maximum permitted level for the same kind of products, 600
58 mg/L. For aspartame, the maximum permitted level for the same kind of products, 600
59 mg/L. For aspartame, the maximum permitted level for the same kind of products, 600
60 mg/L, was not exceeded.

1
2
3 The estimated daily intake (EDI) for each sweetener by the teenager population was
4 calculated by the following equation $EDI = (\sum c) (CN^{-1} D^{-1} K^{-1})$ (Table IV), where $\sum c$ is
5 the sum of sweetener concentration in the analyzed samples related to the questionnaire
6 (mg/L), C is the mean annual intake estimated per teenager, N is the total number of
7 analysed samples, D is the number of days in a year, and K is the mean body weight. The
8 estimated intakes of acesulfame-K and aspartame for the average intake, by the
9 consumers were below the acceptable daily intakes (ADIs). For acesulfame-K the EDI
10 was 0.7 mg/kg b.w./day for soft drinks, 0.2 mg/kg b.w./day for soft drinks based on
11 mineral waters, and 0.5 mg/kg b.w./day for nectars, representing 8.0%, 2.2%, and 5.8%
12 of the ADI, respectively (Table IV).
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

29 Similar situation was observed for aspartame. The EDI for soft drinks was 1.1 mg/kg
30 b.w./day, representing only 2.9% of the ADI. For soft drinks based on mineral waters
31 0.3mg/kg b.w./day representing 0.7% of the ADI. In respect to nectars, the EDI was 0.2
32 mg/kg b.w./day, what represents 0.5 % of the ADI (Table IV).
33
34
35
36
37
38
39
40

41 Three of the well-known international trade mark soft drinks, being two cola type
42 drinks, were responsible for the maximum daily intake of the sweeteners by the studied
43 population, assuming the maximum consumption of the soft drinks. One of them was
44 responsible for the maximum intake of aspartame, with 4.5 mg/kg body weight, and
45 occupying the second place for the acesulfame intake of 1.3 mg/kg body weight. The
46 international trade mark beverage contributed the maximum intake for acesulfame of
47 1.6 mg/kg body weight. Another soft drink gave an aspartame intake of 2.8 mg/kg body
48 weight. The maximum daily intake for both sweeteners given by nectars was lower than
49 that for soft drinks.
50
51
52
53
54
55
56
57
58
59
60

1
2
3 The EDI of the teenager population was higher than the Portuguese population in
4 general. For the latter, the EDIs of acesulfame-K were 0.18 mg/kg b.w./day and 0.057
5 mg/kg b.w./day when soft drinks and nectars were consumed, respectively, representing
6 2.0 and 0.6% of the ADI. For aspartame, the EDI values are 0.3 mg/kg b.w./day and
7 0.026 mg/kg b.w./day for soft drinks and nectars respectively, which means 0.7% and
8 0.07 % from the ADIs (Table V).
9
10
11
12
13
14
15
16
17

18 Discussion

19
20 The comparison of data on the levels in this study with other countries is difficult,
21 as result of scarcity of data. In what concerns the presence of aspartame in soft drinks,
22 Prodoliet and Bruelhart (1993) found levels of this sweetener of 153 mg/L and 286
23 mg/L in 2 diet cola drinks in Switzerland. In the same kind of drinks, in Barcelona, the
24 levels of aspartame were 130 mg/L and 676 mg/L (Suárez et al. 1997). A Danish study
25 reported 159 mg/L and 56 mg/L for soft drinks with (n=20) and without (n=43) carbon
26 dioxide, respectively (Leth et al. 2007). The same sweetener was found by Tyler (1984),
27 in USA, in two *diet* cola drinks with 97 mg/L and 92 mg/mL. In USA, Walker et al.
28 (1997) detected aspartame in two soft drinks, one of *diet* coca with 206 mg, and another
29 of diet lemon carbonated soft drink with 185 mg, by scan of 355 mL, which corresponds
30 to 580 mg/L and 521 mg/L, respectively. In China, Zhu et al. (2005) found levels of
31 7234.6 and 2826.3 mg/L in 2 samples of cola soft drinks. The values in different
32 countries are higher than those found in the samples analysed in the present study,
33 except for soft drinks without carbon dioxide from Denmark.
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57

58 As far as we know, only one study of nectar samples has been previously reported. In
59 this study, aspartame was found in one exotic fruit nectar at concentrations of 2416
60

1
2
3 mg/L, in Switzerland (Prodoliet and Bruelhart 1993). One drink of fruit juice, in China,
4
5 had levels of aspartame and acesulfame-K of 317.2 and 4.5 mg/L, respectively (Zhu et
6
7 al. 2005). The aspartame level is excessively high when compared with the
8
9 concentrations found in identical samples involved in the present study, ranging in this
10
11 case between 39 mg/L and 129 mg/L.
12
13
14

15
16
17 Concerning acesulfame-K levels, Suárez et al. (1997) reported its presence in two cola
18
19 drinks at 59 mg/L and 146 mg/L. Recently, in Denmark, a study of *light* soft drinks
20
21 with (n=21) and without (n=60) carbon dioxide showed mean levels of acesulfame-K of
22
23 129 mg/L and 62 mg/L, respectively (Leth et al. 2007). Also in China, Zhu et al. (2005)
24
25 found levels of acesulfame-K of 1105.4 and 1475.2 mg/L, in 2 cola drinks. The levels
26
27 found in Portuguese study, 56 mg/L e 137 mg/L, are between those reported by Suárez
28
29 et al. (1997), are higher than those found by Leth et al. (2007), and lower than those
30
31 referred by Zhu et al. (2005).
32
33
34
35
36
37
38

39 The results of the present study are consistent with the percentage of ADI for
40
41 acesulfame and aspartame reported for other countries. The lowest percentage of ADI
42
43 for both sweeteners of 0.1%, was found in Italy for a population aged between 13 and
44
45 19 years old (Leclercq et al. 1999). Studies carried out in another teenager population,
46
47 from Italy, had ADI percentages of 0.2 and 0.3% for aspartame and acesulfame-k,
48
49 respectively (Arcella et al. 2004). The highest values for acesulfame-K, 9%, were found
50
51 in individuals aged between 3 and 74, in United Kingdom (Wilson et al. 1999). For the
52
53 aspartame, the highest values were found in one Canadian population, aged between 2
54
55 and 6 years, with 10% of the ADI (Devitt et al. 2004).
56
57
58
59
60

1
2
3 It should be stressed that youngest population group aged between 1.5 - 4.5 years
4 across different areas of the United Kingdom (Food Standards Agency UK. 2003), and
5
6 2-6 years in Toronto, Canada (Devitt et al. 2004), had some of the highest percentages
7
8 of the ADI for aspartame and acesulfame-K. In the UK those values are 6% and 8% for
9
10 acesulfame and aspartame, respectively, and in Canada represented 4% and 10% for the
11
12 same sweeteners. The first European conference to review the safety of aspartame, held
13
14 in Paris in 2006, showed that the intakes are about 10% of the acceptable daily intake,
15
16 even by high consumers of aspartame (Renwick and Nordmann 2007).
17
18
19
20
21
22
23
24
25

26 **Conclusions**

27 The analytical methodology is well-suited for quality control, providing adequate
28
29 linearity, sensitivity, accuracy and precision for the determination of both sweeteners in
30
31 soft drinks and nectars. Moreover, the speed of analysis and simple sample preparation
32
33 allows for its use in a quality control laboratory. In this study it was demonstrated that
34
35 nectars showed 100% frequency of detection and the highest mean concentration of 128
36
37 mg/L for acesulfame-K, and soft drinks contained aspartame in 92% of the samples
38
39 with mean levels of 89 mg/L. The highest estimated daily intakes of both sweeteners
40
41 for the teenage population was for soft drinks, giving 0.7 and 1.1 mg/kg b.w./day, being
42
43 far below their respective ADI-values. It was also shown that EDIs of both sweeteners
44
45 by the general Portuguese population is lower than the teenager population. It can be
46
47 concluded that, with the current observed consumption patterns and occurrence levels,
48
49 the risk of an excessive intake of intense sweeteners by Portuguese teenagers is low.
50
51
52
53
54
55
56
57
58
59
60

References

- Arcella D, Le Donne C, Piccinelli R, Leclercq C. 2004. Dietary estimated intake of intense sweeteners by Italian teenagers. Present levels and projections derived from the INRAN-RM-2001 food survey. *Food and Chemical Toxicology* 42:677–685.
- ANIRSF (Associação Nacional dos Industriais de Refrigerantes e Sumos de Frutos), “Nutrição e estilos de vida”. <http://estilosdevida.anirsf.pt/tema>. Accessed in: 28/04/2007.
- Decreto-Lei nº 394/98 de 10 de Dezembro de 1998 in Diário da República – I Série-A, Nº 284 de 10/12/1998, p. 6731 – 6738.
- Devitt L, Daneman D, Buccino J. 2004. Assessment of intakes of artificial sweeteners with type I diabetes mellitus. *Canadian Journal of Diabetes* 28:142-146.
- Food Standards Agency UK. 2003. Dietary survey on the intake of intense sweeteners by young children from soft drinks. No 36/03 Month 2003.
- Leclercq C, Berardi D, Sorbillo MR, Lambe J. 1999. Intake of saccharin, aspartame, acesulfame-k and cyclamate in Italian teenagers. Present levels and projections. *Food Additives and Contaminants* 16:99-109.
- Leth T, Fabricius N, Fagt S. 2007. Estimated intake of intense sweeteners from non-alcoholic beverages in Denmark. *Food Additives and Contaminants* 24:227-235.
- Prodoliet J, Bruelhart M. 1993. Determination of aspartame and its major decomposition products in food. *Journal of AOAC International* 76:275-282.
- Renwick AG. 2006. The intake of intense sweeteners – an update review. *Food Additives and Contaminants* 23:327-338.
- Renwick AG, Nordmann H. 2007. First European Conference on aspartame: Food and Chemical Toxicology 42: Putting safety and benefits into perspective. Synopsis of presentations and conclusions. *Food and Chemical Toxicology* 45:1308-1313.

1
2
3 SCF Scientific Committee on Food "Sweeteners", Reports of the Scientific Committee
4
5 for Food, N° 16, Commission of the European Communities (CEC), Luxembourg,
6
7 1985.
8
9

10 Scientific Committee on Food. Opinion of the Scientific Committee on Food: Update on
11
12 the Safety of Aspartame. SCF/CS/ADD/EDUL/222 Final 10 December 2002.
13
14

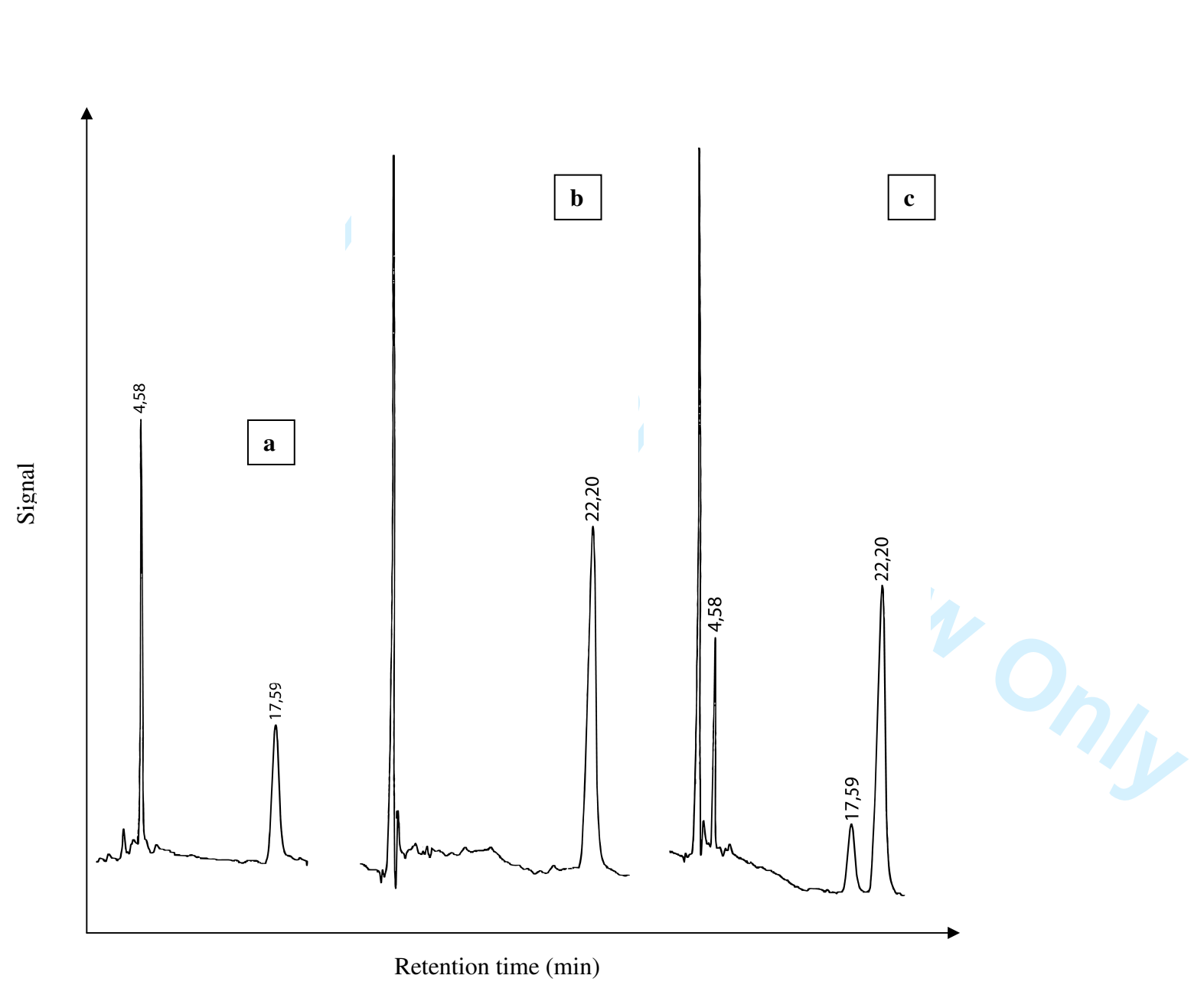
15 Suárez MA, Masferrer D, Vázquez L, Centrich F. 1997. Analisis de aditivos en bebidas
16
17 refrescantes. *Alimentaria* 97:43-48.
18
19

20 Tyler TA. 1984. Liquid chromatographic determination of sodium saccharin, caffeine,
21
22 aspartame, and sodium benzoate in cola beverages. *Journal of Assoc.Off. Anal.*
23
24 *Chem.* 67:745-747.
25
26

27 Walker JC, Zaugg SE, Walker EB. 1997. Analysis of beverages by capillary
28
29 electrophoresis. *Journal of Chromatogr. A* 781:481-485.
30
31

32 Wilson LA, Wilkinson K, Crews HM, Davies AM, Dick CS, Dumsday VL. 1999.
33
34 Urinary monitoring of saccharin, and acesulfame-k as biomarkers of exposure to
35
36 these additives. *Food Additives and Contaminants* 16:227-238.
37
38

39 Zhu Y, Guo Y, Ye M, James FS. 2005. Separation and simultaneous determination of
40
41 four artificial sweeteners in food and beverages by ion chromatography. *Journal of*
42
43 *Chromatogr. A* 1085:143-146.
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



1
2
3 Figure I. Chromatograms of: **a)** standard of acesulfame-K (rt= 4.58) and aspartame
4 (rt=17.59) containing 0.3 μg of each compound; **b)** blank (sample without sweeteners);
5
6
7
8 **c)** sample fortified at 35 mg/L (0.175 μg) with each sweetener. The rt=22.20 represents
9
10 benzoic acid.
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review Only

Table I. Recovery, intra and inter-day repeatability (% RSD) for soft drinks spiked at different levels of acesulfame-K and aspartame

Sweeteners	Fortification level (mg/L)	Exactitude*	Intra-day repeatability	Inter-day repeatability
Acesulfame-K	35	92	7.9	8.2
	100	94	1.1	11.5
	350	91	5.3	9.7
Aspartame	35	102	1.0	8.5
	100	85	1.7	9.9
	600	89	2.8	7.3

*n=3

Table II. Frequency, range, and mean concentration¹ of the sweeteners in the different types of analysed beverages.

Type of sample	Analysed samples	Acesulfame-K			Aspartame		
		Positive samples	Range	Mean	Positive samples	Range	Mean
Soft drinks	25	18	7.8 - 166	72 ¹	23	8.8 - 339	89 ¹
Soft drinks based on mineral waters	13	10	3.7 - 182	48 ¹	8	19 - 154	82 ¹
Nectars	10	10	35 - 356	128	8	39 - 129	73 ¹

¹ Mean concentration for samples with the sweetener

Table III. Mean levels (mg/L) of acesulfame-K and aspartame in *light* soft drinks according to the flavor.

Samples	Acesulfame-K	Aspartame
Orange (n=3)	-	70
Cola (n=5)	98	103
Lemon (n=2)	70	87
Pineapple (n=3)	51	52
Soy (n=2)	57	49

Table IV. Estimated daily intake (EDI)^a of acesulfame-K and aspartame by scholar teenagers through soft drinks, soft drinks based on mineral water, and nectars and respective comparison with acceptable daily intake (ADI)

Sweeteners	Soft drinks		Soft drinks based on mineral water			Nectars	
	ADI ^b (mg/kg b.w./day)	EDI (mg/kg b.w./day)	ADI (%)	EDI (mg/kg b.w./day)	ADI (%)	EDI (mg/kg b.w./day)	ADI (%)
Acesulfame-K	9	0.7	8.0	0.2	2.2	0.5	5.8
Aspartame	40	1.1	2.9	0.3	0.7	0.2	0.5

^aEDI was calculated using the equation $EDI = (\sum c) (CN^{-1} D^{-1} K^{-1})$, where $\sum c$ is the sum of sweetener concentration in the analyzed samples related to the questionnaire (mg/L), C is the mean annual intake estimated per teenager (288 L for soft drinks, 111.3 L for soft drinks based on mineral water, and 84 L for nectars), N is the total number of analysed samples, D is the number of days in a year, and K is the mean body weight, which was considered 56.5 Kg (mean of body weight of the studied population).

^bADIs proposed by SCF (1985).

Table V. Estimated daily intake (EDI)^a of acesulfame-K and aspartame by Portuguese population in general through soft drinks, , and nectars and respective comparison with acceptable daily intake (ADI)

Sweeteners	Soft drinks			Nectars	
	ADI ^b (mg/kg b.w./day)	EDI (mg/kg b.w./day)	ADI (%)	EDI (mg/kg b.w./day)	ADI (%)
Acesulfame-K	9	18×10^{-2}	2.0	57×10^{-3}	0.6
Aspartame	40	0.3	0.7	26×10^{-3}	7×10^{-2}

^aEDI was calculated using the equation $EDI = (\sum c) (CN^{-1} D^{-1} K^{-1})$, where $\sum c$ is the sum of sweetener concentration in the analyzed samples related to the questionnaire (mg/L), C is the annual intake estimated per Portuguese inhabitant in 2006 (77.6 L for soft drinks, and 9.8 L for nectars according the ANIRSF, 2007), N is the total number of analyzed samples, D is the number of days in a year, and K is the mean body weight, which was considered 60 Kg (mean of body weight of the Portuguese population).

^bADIs proposed by SCF (1985).