JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2003,** *51,* 1749−1752 **1749**

Source of Arsenic in Licorice Confectionery Products

ÁNGEL ANTONIO CARBONELL-BARRACHINA,* PEDRO ARACIL, ELENA GARCÍA, FRANCISCO BURLÓ, AND FRANCISCO MARTÍNEZ-SÁNCHEZ

División Tecnología de Alimentos, Universidad Miguel Hernández, Carretera de Beniel, km 3.2, 03312 Orihuela, Alicante, Spain

Spanish legislation sets a maximum level for total arsenic (As) in confectionery products at 0.1 μg g^{-1}. The U.S. Food and Drug Administration limitations for glycyrrhizic acid in hard and soft candies are 160 and 31 mg g^{-1}, respectively. Arsenic and glycyrrhizic acid were determined in 22 different confectionery products: 9 throat pearls, 4 hard candies, and 9 soft candies. Arsenic and glycyrrhizic acid were quantified by atomic absorption spectrometry with hydride generation and high-performance liquid chromatography, respectively. Levels of glycyrrhizic acid were always below the maximum limits established by the U.S. FDA; however, the As concentration in seven of nine throat pearls (0.55 $\pm$ 0.15 μg g^{-1}) were above the Spanish maximum limit. A clear empirical relationship between the arsenic and glycyrrhizic acid concentrations was observed ($R^2 = 0.9357$), implying that to avoid high levels of potentially toxic arsenic in licorice confections high-quality licorice extract should be used.

KEYWORDS: ***Glycyrrhiza glabra*; glycyrrhizic acid; hard candy; soft candy; throat pearls**

INTRODUCTION

The consumption of confectionery products has increased tremendously in the past two decades, especially by children and teenagers. Candy currently is being retargeted as functional food (*1*); however, in addition to beneficial properties (inclusion of functional ingredients: vitamins, minerals, herbal remedies, etc.), when consumed in excessive amounts, these products may cause potential health hazards, for example, dental cavities (*2*) and high intake of metals (*3*, *4*).

If the origin of the toxic element is not known, it is impossible to recommend measures for reducing the content of potentially toxic elements that do not satisfy national and/or international legislations.

Carbonell-Barrachina et al. (*5*) studied the effects of raw materials, ingredients, and production lines on arsenic and copper concentrations in four confectionery products: chewing gum, two licorice items, and soft candy (all of them elaborated by the same company) and concluded that the licorice extract and molasses seemed to be the common source for both As and Cu pollution.

Thus, the main objective of this study was to confirm that a relationship exists between the licorice extract and the arsenic concentration in a wide range of confectionery products, different flavors, manufacturing companies, and even countries where these items were elaborated. The information provided by this study will assist confectionery manufacturers in reducing arsenic pollution of their final licorice products through a better quality control of raw materials, ensuring their products meet the requirements of international legislation and consumer demands for quality and innocuousness.

* Author to whom correspondence should be addressed [telephone (34) 966 749656; fax (34) 966 749677; e-mail angel.carbonell@umh.es].

MATERIALS AND METHODS

Experimental Design. A total of 22 different confectionery products were analyzed; 16 of these candies contained licorice extract in their formulation (**Table 1**). All products were purchased from pharmacies and candy shops. At least five replicates of each confectionery product were analyzed.

Reagents. Deionized water, 18.2 MΩ cm^{-1}, was used for the preparation of reagents and standards. Water quality was obtained by filtering distilled water through a Milli-Q purifier (Millipore, Gif-sur-Yvette, France). All chemicals were of pro analysi quality or better and were obtained from Sigma-Aldrich (Steinheim, Germany). Standard solution of pentavalent arsenic (1000 $\pm$ 2 mg L^{-1}) was prepared by dilution of Titrisol standards (Merck, Darmstadt, Germany).

All glassware was treated with 10% v/v HNO_3 for 24 h and then rinsed three times with deionized water before being used.

Sample Digestion. A multiplace digestion block, Selecta Block Digest 20 (Barcelona, Spain), was used for sample mineralization. Candy samples were frozen with liquid nitrogen and then homogenized using a meat grinder. A 0.250-g portion of homogenized sample was treated with 5 mL of 65% (w/v) HNO_3 in Pyrex tubes, placed in the digestion block, and heated at 60 °C for 60 min and at 130 °C for 120 min (*6*). A maximum temperature below 140 °C was utilized to prevent arsenic volatilization (*7*). Solutions were cooled at room temperature, transferred to a volumetric flask, and diluted to a final volume of 25 mL with ultrahigh-purity deionized water.

Total Arsenic Analysis. Determination of "total" arsenic in previously acid-mineralized samples was performed with a Unicam model Solaar 969 atomic absorption spectrometer equipped with a continuous hydride generator Unicam Solaar VP90.

10.1021/jf026057h CCC: $25.00
Published on Web 02/05/2003

Table 1. Total Concentrations of Arsenic in Different Commercially Available Confectionery Products

product			arsenic (μg g^{-1} of fresh matter)	licorice extract (%)
type		flavor		
throat pearls	1	licorice, menthol	0.33 ± 0.08[a]	np[b]
	2	licorice, menthol	0.50 ± 0.01	86.27
	3	licorice, menthol	1.13 ± 0.06	np
	4	licorice	1.06 ± 0.08	np
	5	licorice, menthol	0.18 ± 0.03	np
	6	licorice, menthol, eucalyptol	0.30 ± 0.09	np
	7	licorice, menthol, eucalyptol, terpineol	0.34 ± 0.02	96.97
	8	licorice, anethole	0.08 ± 0.01	np
	9	licorice, aromatic herbs	0.06 ± 0.02	np
hard candy	1	licorice, menthol, eucalyptol, terpineol	0.02 ± 0.01	np
	2	licorice, menthol, eucalyptol	0.01 ± 0.01	np
	3	licorice, menthol	0.03 ± 0.01	0
	4	licorice, lemon, honey	0.03 ± 0.01	0
soft candy	1	menthol	0.05 ± 0.01	np
	2	licorice	0.07 ± 0.01	1.0
	3	licorice	0.05 ± 0.01	1.0
	4	licorice	0.04 ± 0.01	1.0
	5	blackberry	0.02 ± 0.01	0
	6	strawberry	0.02 ± 0.01	0
	7	blackberry	0.04 ± 0.01	0
	8	strawberry	0.02 ± 0.01	0
	9	strawberry, menthol	0.01 ± 0.01	0

[a] Data in this table are mean values of five replicates for each product; the ± values indicate the standard error of the mean. [b] np indicates that no data on the amount of licorice extract were provided on the label of the product.

A mixture containing 5% m/v KI and 5% m/v ascorbic acid was employed as a prereducing solution for all of the samples before the quantification of total arsenic. The $NaBH_4$ was prepared daily and filtered through Whatman no. 42 paper.

The instrumental conditions used for arsenic determination by HG-AAS were as follows: reducing agent, 1.0% (m/v) $NaBH_4$ in 0.1% NaOH, 5 mL min^{-1}; HCl solution, 10% (v/v), 10 mL min^{-1}; carrier gas, argon, 250 mL min^{-1} flow rate. Conditions for atomic absorption spectrometry were as follows: wavelength, 193.7 nm; spectral band-pass, 0.5 nm; hollow cathode lamp current setting, 8 mA; air/acetylene flame with a fuel flow rate of 0.8 L min^{-1}.

The detection limit of this method was 0.25 μg L^{-1}. The detection limit was translated into the limit of quantification (LOQ: smallest arsenic concentration in the original specimen that could be measured) based on the amount of sample used for digestion and other procedural processes, such as dilutions; the LOQ value for arsenic was 8 μg kg^{-1}.

Analytical Quality Control. All instruments were calibrated using matrix-matched standards. In each analytical batch, at least two reagent blanks, one internationally certified reference material (CRM) and one spike, were included to assess precision and accuracy for chemical analysis. The certified materials selected for the current experiment were GBW07603 (bush, branches, and leaves) and SRM1548a (typical diet: proximates and trace elements), which have certified values for arsenic of 1.25 and 0.20 μg g^{-1}, respectively. The certified reference material was provided by LGC Deselaers S.L. (Barcelona, Spain) and produced by the Institute of Geophysical and Geochemical Exploration of China (GBW07603) and the U.S. National Institute of Standards and Technology (SRM1548a).

Glycyrrhizic Acid. Glycyrrhizic acid in fresh samples was determined using AOAC Official Method 982.19 (*8*). Fifty milligrams of fresh confectionery product was dissolved in 50 mL of ultrapure H_2O and vortex-shaken until the sample was completely dissolved. The solution was then filtered through a 0.45-μm filter, using an aqueous sample clarification kit. Glycyrrhizic acid was analyzed by HPLC (Hewlett-Packard series 1100). The elution system consisted of $H_2O/CH_3COOH/CH_3CN$ (61:1:38) solvent, running isocratically with a flow rate of 2.0 mL min^{-1}. The glycyrrhizic acid was eluted through a reversed-phase column (LiChroCart 250-4.5 μm) and detected by absorbance at 254 nm. Standards of monoammonium glycyrrhizinate (available from Sigma-Aldrich) were used in the calibration procedure.

RESULTS AND DISCUSSION

Analytical Quality Control for Arsenic Analyses. The mean recoveries for arsenic in the reference materials GBW07603 and SRM1548a were 93.2 ± 1.6% (n = 10) and 91.1 ± 2.1% (n = 10), respectively. The spike recoveries were 96.5 ± 1.3% (n = 10; 5 for GBW07603 and 5 for SRM1548a) and 97.0 ± 1.3% (n = 10; 5 for GBW07603 and 5 for SRM1548a) for arsenite and arsenate, respectively. These spike recoveries were carried out using both reference materials due to the different nature of the samples analyzed. Some of the candy samples were also analyzed by a different analytical technique, dry ashing in a muffle furnace with addition of ashing aid suspension 20% (w/v) $Mg(NO_3)_2$ and 2% (w/v) MgO (*9*), and the results were consistent with those previously reported; the mean recovery for the reference materials was 95.2 ± 0.9% (n = 6; 3 for GBW07603 and 3 for SRM1548a).

Arsenic. Spanish legislation (*10*) sets the maximum level for total As in confectionery products at 0.1 μg g^{-1}. However, no such limits exist for raw materials or essential/optional ingredients.

Arsenic concentrations in the confectionery products studied ranged from trace levels (0.01−0.02 μg g^{-1}) in some soft candies not containing licorice extract according to their labeling to 1.13 ± 0.06 μg g^{-1} in licorice throat pearls with menthol flavor (**Table 1**). The mean and median values for the As concentrations were 0.20 and 0.05 μg g^{-1}, respectively. These statistical results denoted that the experimental arsenic concentrations in most of the studied candies (15 of 22) were below the maximum concentrations for total As established by the Spanish legislation. On the other hand, five products had concentrations ranging from 0.1 to 1.0 μg g^{-1}. Two products had an As concentration >1.0 μg g^{-1}.

The relatively high levels of arsenic found especially in the throat pearls clearly illustrated the effect of chemicals used in agriculture (arsenic fungicides, herbicides, etc.) on food pollution. Following As application to soils and/or plants and uptake by the plants, the arsenic accumulates mainly in the root system (*11*). Therefore, the high As content in licorice-flavored products could be expected because the licorice extract used in their formulation is isolated from the dried root of *Glycyrrhiza glabra*.

Further research is needed to obtain information regarding the speciation of arsenic throughout the manufacture of the licorice confectionery items due to the different toxicities of As species. Arsenic differs from many of the common trace elements in that the majority of the organoarsenicals are less toxic than inorganic compounds; arsenite is more phytotoxic than arsenate, and both are much more phytotoxic than methylarsonic and dimethylarsinic acids (*12*).

Glycyrrhizic Acid. Glycyrrhizic acid is a natural constituent of licorice isolated from the dried root of *G. glabra*. Salts of glycyrrhizic acid are widely used as sweeteners and aromatizers in sweets, drugs, beverages, chewing gums, chewing tobacco, and toothpastes (*13*, *14*).

Glycyrrhizic acid concentrations have been determined in confectionery products on the market in Germany, Belgium, the United Kingdom, and the United States (*14*, *15*). A similar range of glycyrrhizic acid concentrations (0.29−7.9 mg g^{-1}) was found in these studies, with only 2 of 18 products containing >3.5 mg of glycyrrhizic acid g^{-1} in the United Kingdom and

Table 2. Total Concentrations of Glycyrrhizic Acid in Different Commercially Available Confectionery Products

product			glycyrrhizic acid
type		flavor	(mg g^{-1} of fresh matter)
throat pearls	1	licorice, menthol	32.4 ± 2.4[a]
	2	licorice, menthol	22.6 ± 0.9
	3	licorice, menthol	65.5 ± 1.3
	4	licorice	53.4 ± 0.3
	5	licorice, menthol	12.2 ± 0.4
	6	licorice, menthol, eucalyptol	28.0 ± 0.7
	7	licorice, menthol, eucalyptol, terpineol	14.6 ± 0.2
	8	licorice, anethole	nd[b]
	9	licorice, aromatic herbs	0.7 ± 0.1
hard candy	1	licorice, menthol, eucalyptol, terpineol	nd
	2	licorice, menthol, eucalyptol	7.4 ± 0.8
	3	licorice, menthol	1.9 ± 0.1
	4	licorice, lemon, honey	0.7 ± 0.1
soft candy	1	menthol	nd
	2	licorice	1.4 ± 0.1
	3	licorice	nd
	4	licorice	0.8 ± 0.1
	5	blackberry	nd
	6	strawberry	nd
	7	blackberry	nd
	8	strawberry	nd
	9	strawberry, menthol	nd

[a] Data in this table are mean values of five replicates for each product; the ± values indicate the standard error of the mean. [b] nd indicates that no glycyrrhizic acid was detected.

3 products containing >4.2 mg of glycyrrhizic acid g^{-1} in Germany (*14*).

The concentrations of glycyrrhizic acid found in the current study ranged from trace levels (<0.1 mg g^{-1}), especially in soft candies with no licorice extract or powder addition, to 65.5 ± 1.3 mg g^{-1}, in the licorice throat pearls that presented the highest arsenic concentration (**Table 2**). Our experimental data appeared to be significantly higher than those previously reported in Germany, Belgium, the United Kingdom, and the United States (*14*, *15*). This difference is probably due to the fact that 9 of the 22 confectionery products analyzed in this study were throat pearls. Certain "health products", such as licorice-flavored diet gum, licorice tea, and throat pearls, may contain much higher levels of glycyrrhizic acid than regular food (*15*).

It is possible to find much higher glycyrrhizic acid concentrations in the literature; however, it is probable that the methods employed in the analysis of these samples have overestimated the true figures (*16*). An indication of the probable overestimates of glycyrrhizin contents resulting from the application of nonspecific methodology is seen in the study of Bell (*17*), who compared gravimetric and HPLC methods, the latter yielding figures ∼30% of the former (*16*).

The FDA has taken the view that "adverse effects of glycyrrhizin are generally associated with the consumption of foods that are characterized by a distinctive licorice flavor, such as licorice-flavored candies". Therefore, the FDA has set maximum levels of glycyrrhizin in food: (1) chewing gum, 11 mg g^{-1}; (2) hard candy, 160 mg g^{-1}; (3) soft candy, 31 mg g^{-1} (*16*). In the current study, only hard and soft candies were analyzed. In 100% of the cases, the experimental glycyrrhizic acid concentrations were significantly below these maximum levels (hard candies, including throat pearls, from <0.1 to 65.5 mg g^{-1}; soft candies, from <0.1 to 1.4 mg g^{-1}).

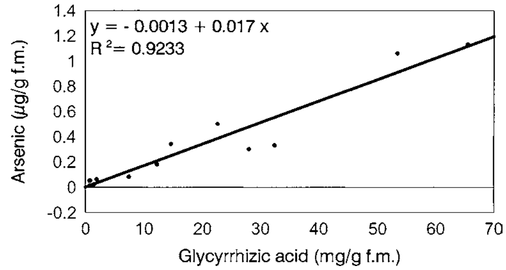

Figure 1. Total arsenic concentration (μg g^{-1}) versus glycyrrhizic acid concentration (mg g^{-1}) in different commercial confectionery products. Only those products having measurable levels of both arsenic and glycyrrhizic acid have been included.

Relationship between Arsenic and Glycyrrhizic Acid. After quantifying the contribution of raw materials and ingredients to the arsenic content in confectionery products, Carbonell-Barrachina et al. (*5*) recommended strict control of the quality of licorice extract (the raw material with the highest As concentration) and molasses (the raw material with the highest percentage in the final product) because these two raw materials seemed to be the primary sources for the arsenic.

With this precedent, the experimental arsenic concentration (micrograms per gram) was plotted versus the glycyrrhizic acid concentration (milligrams per gram) in all confections with measurable amounts of both arsenic and glycyrrhizic acid (**Figure 1**). A correlation coefficient (R^2) of 0.9233 was obtained ($y = 0.017x - 0.0013$), showing that a positive empirical relationship exists between these two chemicals, arsenic and glycyrrhizic acid. A similar pattern was obtained when all 22 samples were considered ($R^2 = 0.9357$). Perhaps this close empirical relationship would indicate that arsenic and glycyrrhizic acid could be chemically associated in licorice plants. Although the mechanisms of this chemical interaction are beyond the aims of the current experiment, they deserve further research.

Therefore, it is reasonable to state that products such as pellets, cachous, and small rhombic lozenges generally having a high content of licorice extract (heavily flavored) also contain high levels of arsenic. The main technological conclusion to be extracted from this experiment is that confectionery manufacturers should use high-quality licorice extract to reduce the arsenic content in these widely consumed confectionery items.

ACKNOWLEDGMENT

We thank H. Mínguez for cooperation in the laboratory work.

LITERATURE CITED

(1) Rogers, P. Helping the medicine go down. *Candy Ind.* **2001**, *166*, 42−61.
(2) Kandelman, D. Sugar, alternative sweeteners and meal frequency in relation to caries prevention: New perspectives. *Br. J. Nutr.* **1997**, *77*, 121−128.
(3) Dorfman, J. S.; Quattrone, A.; Jacobs, R. M.; Batarseh, N.; Bird, C. W.; Carlson, W. T.; James, M. R.; Reinke, E. V.; Rowney, R.; Butler, K. D.; Park, J. D.; Johnson, D. Lead poisoning associated with imported candy and powdered food coloring (California and Michigan). *Morbidity and Mortality Weekly Report* **1998**, *47*, 1041−1043.
(4) Wu, C. N.; Yang, G. Y.; Shen, C. Y.; Liou, S. H. Lead contamination of candy: An example of crisis management in public health. *Lancet* **1995**, *346*, 1437−1438.
(5) Carbonell-Barrachina, A. A.; García, E.; Sánchez Soriano, J.; Aracil, P.; Burló, F. Effects of raw materials, ingredients, and production lines on arsenic and copper concentrations in confectionery products. *J. Agric. Food Chem.* **2002**, *50*, 3738−3742.

(6) García, E.; Cabrera, C.; Lorenzo, M. L.; López, M. C. Chromium levels in spices and aromatic herbs. *Sci. Total Environ.* **2000**, *247*, 51−56.

(7) Marin, A. R.; Masscheleyn, P. H.; Patrick, W. H., Jr. The influence of chemical form and concentration of arsenic on rice growth and tissue arsenic concentration. *Plant Soil* **1992**, *139*, 175−183.

(8) Horwitz, W. *Official Methods of Analysis of AOAC International*; AOAC International: Gaithersburg, MD, 2000.

(9) Lario, Y.; Burló, F.; Aracil, P.; Martínez-Romero, D.; Castillo, S.; Valero, D.; Carbonell-Barrachina, A. A. Organic arsenicals toxicity and total arsenic accumulation in bush beans. *Food Addit. Contam.* **2002**, *19*, 417−426.

(10) BOE (Boletin Oficial del Estado). Real Decreto 1810/1991: *Aprobación de la Reglamentación Técnico-Sanitaria para la Elaboración, Circulación y Comercialización de Caramelos, Chicles, Confites y Golosinas*; Madrid, Spain, 1991.

(11) Carbonell-Barrachina, A. A.; Burló, F.; Valero, D.; López, E.; Martínez-Romero, D.; Martínez-Sánchez, F. Arsenic toxicity and accumulation in turnip as affected by arsenic chemical speciation. *J. Agric. Food Chem.* **1999**, *47*, 2288−2294.

(12) Carbonell-Barrachina, A. A.; Burló, A.; Valero, D.; Martínez-Romero, D.; Martínez-Sánchez, F. Environmental factors affecting arsenic incorporation in fruits and horticultural products. *Recent Res. Dev. Agric. Food Chem.* **2000**, *4*, 95−121.

(13) Gordon, M. H.; An, J. Antioxidant activity of flavonoids isolated from licorice. *J. Agric. Food Chem.* **1995**, *43*, 1784−1788.

(14) Størmer, F. C.; Reistad, R.; Alexander, J. Glycyrrhizic acid in liquorice. Evaluation of health hazard. *Food Chem. Toxicol.* **1993**, *31*, 303−312.

(15) Spinks, E. A.; Fenwick, G. R. The determination of glycyrrhizin in selected UK liquorice products. *Food Addit. Contam.* **1990**, *7*, 769−778.

(16) Fenwick, G. R.; Lutomski, J.; Nieman, C. Liquorice, *Glycyrrhiza glabra* L. composition, uses and analysis. *Food Chem.* **1990**, *38*, 119−143.

(17) Bell, J. H. Determination of glycyrrhizic acid in liquorice extracts and chewing tobaccos. *Tobacco Sci.* **1980**, *24*, 126−129.

Received for review October 23, 2002. Revised manuscript received January 2, 2003. Accepted January 6, 2003.

JF026057H