

Research Paper

Factors Affecting the Levels of Heavy Metals in Juices Processed with Filter Aids

ZHENGFANG WANG,[†] LAUREN S. JACKSON,^{*} AND JOSEPH E. JABLONSKI*U.S. Food and Drug Administration, Division of Food Processing Science and Technology, Office of Food Safety, Center for Food Safety and Applied Nutrition, 6502 South Archer Road, Bedford Park, Illinois 60501, USA*

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ABSTRACT

This study investigated factors that may contribute to the presence of arsenic and other heavy metals in apple and grape juices processed with filter aids. Different types and grades of filter aids were analyzed for arsenic, lead, and cadmium with inductively coupled plasma–tandem mass spectrometry. Potential factors affecting the transfer of heavy metals to juices during filtration treatments were evaluated. Effects of washing treatments on removal of heavy metals from filter aids were also determined. Results showed that diatomaceous earth (DE) generally contained a higher level of arsenic than perlite, whereas perlite had a higher lead content than DE. Cellulose contained the lowest level of arsenic among the surveyed filter aids. All samples of food-grade filter aids contained arsenic and lead levels that were below the U.S. Pharmacopeia and National Formulary limits of 10 ppm of total leachable arsenic and lead for food-grade DE filter aids. Two samples of arsenic-rich (>3 ppm) food-grade filter aids raised the level of arsenic in apple and grape juices during laboratory-scale filtration treatments, whereas three samples of low-arsenic (<1 ppm) food-grade filter aids did not affect arsenic levels in filtered juices. Filtration tests with simulated juices (pH 2.9 to 4.1, Brix [°Bx] 8.2 to 18.1, total suspended solids [TSS] 0.1 to 0.5%) showed that pH or sugar content had no effect on arsenic levels of filtered juices, whereas arsenic content of filtered juice was elevated when higher amounts of filter aid were used for filtration. Authentic unfiltered apple juice (pH 3.6, °Bx 12.9, TSS 0.4%) and grape juice (pH 3.3, °Bx 16.2, TSS 0.05%) were used to verify results obtained with simulated juices. However, body feed ratio did not affect the arsenic content of filtered authentic juices. Washing treatments were effective at reducing arsenic, but not cadmium or lead, concentrations in a DE filter aid. This study identified ways to reduce the amount of arsenic transferred to juices during filtration.

Key words: Arsenic; Cadmium; Diatomaceous earth; Filtration; Fruit juices; Lead

Arsenic has been classified by the International Agency for Research on Cancer of the World Health Organization as “carcinogenic to humans,” based on evidence from human studies (15, 42). Chronic exposure to the inorganic forms of arsenic, which are generally more toxic than the organic forms, has been associated with the development of cancers in humans (15, 23, 42). Since January 2006, the maximum level of total arsenic allowed in public drinking water, which is regulated by the U.S. Environmental Protection Agency (EPA), is 10 µg/L (ppb) (27). Although acute arsenic intoxication is rare, chronic exposure to levels higher than the current recommended limit of 10 ppb may be a threat to public health (15, 27, 42).

The U.S. Food and Drug Administration (FDA) has been monitoring total arsenic concentrations in foods through its Total Diet Study program since 1964 (20). In July 2013, the FDA proposed a draft action level of 10 ppb

for inorganic arsenic in single-strength apple juice (33). This is the same level set by the FDA for total arsenic in bottled water (29). In addition, regulatory limits of 15 ppb for lead and 5 ppb for cadmium in public drinking water have been established by the EPA (27). The FDA “Guidance for Industry: Juice HACCP Hazards and Controls Guidance” indicates that lead levels in juice above 50 ppb may constitute a health hazard (28).

The FDA continues to take actions to minimize the presence of arsenic in the U.S. food supply (24, 37). However, arsenic contamination of food is still not unusual. For example, an Import Alert for surveillance of arsenic in fruit juices and fruit juice concentrates was issued by FDA in 2015 (34). It is suspected that some of the arsenic present in juices may be due to contamination of the soil where fruits are grown (40, 41). Filter aids used during the production of beverages have also been identified as a possible source of arsenic contamination. The discovery of elevated levels of arsenic in 140 German beer products led to speculation that the arsenic was released into the beer from a filter aid known as kieselguhr, or diatomaceous earth (DE), during filtration treatments (2). However, information is currently lacking on the factors affecting transfer of arsenic and other heavy

* Author for correspondence. Tel: 708-924-0616; Fax: 708-924-0690; E-mail: Lauren.Jackson@fda.hhs.gov.

[†] Present address: International Food Safety Training Laboratory, Joint Institute for Food Safety and Applied Nutrition, University of Maryland, 5145 Campus Drive, Patapsco Building, Suite 2134, College Park, MD 20742, USA.

metals from filtering aids to beverages such as juices during filtration processing.

Filtration is a critical step in the production of some juice products. Filter aids are used during filtration treatments to remove solids or sediment present in freshly pressed or expressed juices. DE is the most common type of filter aid in use today due to its versatility and low cost (3, 19, 22, 26). There are different grades of commercial DE-based filter aids used for different applications (3). Other common filter aids are perlite, rice hulls, and rice hull ash. Cellulose is commonly mixed with DE or perlite to provide optimal clarification results (16). Rice hulls and rice hull ash are relatively new types of filter aids (17).

In a typical filter aid filtration process, a thin layer of filter aid is first deposited on the surface of a filter cloth (or mesh) by recirculating a small amount of filter aid slurry within the filtration device (also known as precoating). After precoating, additional filter aid is added to the liquid to be filtered (also known as body feed), and juice filtration is then initiated. During the filtration process, the filter aid and suspended solids in the body feed are retained by the precoating layer. As more juice with filtering aid is applied to the filtration device, a new filtering surface is continuously formed. The micron-size filter aid particles provide microscopic channels that entrap suspended impurities but allow liquid to pass. As filtration progresses, a filter cake composed of retained filter aid and juice pulp is formed, which progressively becomes thicker, increasing the filtration resistance (4). The filtration process is complete when the optimum operating pressure is reached and the desired juice clarity is obtained.

The U.S. Pharmacopeia and National Formulary (USP-NF) specifications require that the total leachable arsenic and lead concentrations in food-grade DE filter aids are less than 10 mg/kg (ppm) for each metal (1). A systematic study is needed to investigate the transfer of heavy metals present in filter aids to juices during filtration treatments. Because filter aids are widely used in the juice industry (19, 22), it is prudent to identify factors affecting arsenic content of juices processed with filter aids in order to minimize arsenic contamination (31). There are a number of factors that can influence heavy metal transfer to juice during filtration. This study focused on the composition and grade of filter aid, types of juice to be filtered (with different pH values, sugar contents, and amounts of total suspended solids [TSS] to be removed), and filtration parameters (body feed ratio and filtration flow rate), and investigated their contributions to the levels of arsenic and other heavy metals in processed juices. The study also evaluated the effects of washing treatments on their ability to remove heavy metals from a DE-based filter aid.

MATERIALS AND METHODS

Materials. Samples of seven food-grade DE filter aids, six food-grade perlite filter aids, two food-grade cellulose products, and one rice hull product were purchased from BSG CraftBrewing (Shakopee, MN), Brewers Supply Group (Kasota, MN), and Riceland Foods (Stuttgart, AR). Other samples of 12 DE filter aids and four perlite filter aids, which were not specified as being food grade, were purchased from EP Minerals (Reno, NV), Imerys

Filtration Minerals (San Jose, CA), Sigma-Aldrich (St. Louis, MO), Silbrico Corporation (Hodgkins, IL), Fisher Science Education (Rochester, NY), Schundler Company (Edison, NJ), Gilson Company (Lewis Center, OH), MP Biomedicals (Solon, OH), Alfa Aesar (Ward Hill, MA), Gusmer Enterprises (Fresno, CA), and Acros Organics (Bridgewater, NJ). All product names were blinded in this study. The median pore size of food-grade DE filter aids ranged from 3.5 to 7 μm . The median pore size of food-grade perlite filter aids ranged from 13 to 20 μm . Three subsamples were randomly drawn from each lot of filter aid and were analyzed for arsenic, lead, and cadmium.

Unfiltered, commercial apple and grape juices were purchased from a local grocery store. TSS content of the juices was measured. Pulp, or water-insoluble solids in unfiltered juice, was retained on Whatman grade 4 filter paper (Sigma-Aldrich) in a Büchner funnel, and the retained solids were dried thoroughly in a fume hood for over a week. The weight percentage of TSS of unfiltered apple and grape juices, defined as weight of dried retained solids/initial weight of juice to be filtered, was determined to be 0.40 and 0.05%, respectively. The average pH values of the unfiltered apple and grape juices, as measured with a calibrated pH meter (S20 SevenEasy pH meter and the InLab Routine Pro pH electrode, Mettler Toledo, Columbus, OH) were 3.6 and 3.3, respectively. The average Brix ($^{\circ}\text{Bx}$) values of unfiltered apple and grape juices, as measured with a refractometer (Quick-Brix 90, Mettler Toledo, Schwerzenbach, Switzerland), were 12.9 and 16.2, respectively.

Chemicals and reagents. Trace CERT arsenic (As), lead (Pb), and cadmium (Cd) standards for inductively coupled plasma-mass spectrometry (ICP-MS) were purchased from Sigma-Aldrich. Dimethylarsinic acid (DMA) and disodium methyl arsonate hexahydrate (MMA) analytical standards were purchased from Chem Service Inc. (West Chester, PA). Standards of arsenic species, As(III) and As(V), were purchased from SPEX CertiPrep (Metuchen, NJ). Optima nitric acid (67 to 70 wt% as HNO_3) and hydrogen peroxide (30 to 32 wt% as H_2O_2) were purchased from Fisher Scientific (Fairlawn, NJ). EDTA (99.995% trace metals basis), citric acid (ACS reagent, $\geq 99.5\%$), sucrose (BioXtra, $\geq 99.5\%$), and DL-malic acid (ReagentPlus, $\geq 99\%$) were purchased from Sigma-Aldrich. Deionized water (DIW) was collected with a DI EXPRESS water purification system (Crossbow Water, Glenwood, IL).

Washing treatments for DE filter aid SSC. A 10% (w/w) slurry of filter aid was made by mixing 5 g of a DE filter aid, SSC, with 45 g of DIW. Citric acid, malic acid, or EDTA was used to adjust the SSC slurry to a pH range of 2.6 to 3.0. The pH values were measured with a calibrated pH meter. After 25 min of stirring with a magnetic stir bar, the slurry was subjected to vacuum filtration in a Büchner funnel lined with Whatman grade 4 qualitative filter paper. Retained filter aid solids were further rinsed with 500 mL of DIW and then were dried in a fume hood.

A separate experiment compared the effectiveness of DIW or a sucrose solution with an acid washing treatment for arsenic removal from the SSC filter aid. A slurry (10%, w/w) of filter aid was made by mixing 5 g of the SSC filter aid solids with 45 g of DIW. No pH adjustment was performed. Typically, the pH value of DIW and the sucrose solution used in this study was 6.0 ± 0.2 , and the pH value of the 10% SSC slurry was 8.5 ± 0.2 . The mixture of solids and liquid was poured through Whatman grade 4 qualitative filter paper in a Büchner funnel. Retained filter aid solids were rinsed with 500 mL of DIW or a sucrose solution ($^{\circ}\text{Bx}$ 17.0 ± 0.5) and then were dried in a fume hood.

Preparation of simulated juices. Simulated juices with specific pH and °Bx values were used as experimental test materials to systematically evaluate the effects of these factors on transfer of heavy metals from filtering aids to juices. A series of simulated juices (pH 2.9 ± 0.1 , °Bx 8.2 ± 0.1 ; pH 3.4 ± 0.1 , °Bx 8.2 ± 0.1 ; pH 4.1 ± 0.1 , °Bx 8.2 ± 0.1 ; pH 2.9 ± 0.1 , °Bx 18.1 ± 0.1 ; pH 3.4 ± 0.1 , °Bx 18.1 ± 0.1 ; pH 4.1 ± 0.1 , °Bx 18.1 ± 0.1) were prepared with malic acid (a major acid in apple and grape), sucrose (a major sugar in apple and grape), and DIW. These pH and °Bx values used for the model system were within the range of pH and °Bx values of authentic apple and grape juices (8, 12, 30, 36). All chemicals were analyzed and were determined to be arsenic free before preparing simulated juices. The trace levels of heavy metals introduced by water were deducted from ICP–tandem mass spectrometry (MS/MS) analytical signals as background.

Laboratory-scale filtration of juices. A laboratory test filtration system was assembled in-house with the Posiseal filter housing (Gusmer Enterprises), a 0 to 30 lb/in² pressure gauge (Cole-Parmer Instrument Company, Vernon Hills, IL), a Masterflex L/S digital drive with two Masterflex standard pump heads (Cole-Parmer Instrument Company), a stainless steel 128 by 36 PZ80 Reverse Plain Dutch Weave wire mesh (GKD-USA, Cambridge, MD), and Masterflex Tygon E-LFL L/S 16 pump tubing (Cole-Parmer Instrument Company). The custom laboratory-scale filtration apparatus as shown in Supplementary Figure 1 is a pressure vessel complete with filter leaf, control unit, and pump. It was operated to simulate production conditions in a plant and was used to evaluate filtration conditions of liquids to be filtered.

A laboratory-scale filtration test run consisted of three steps. For the first step, the filtration device was filled with DIW, and air bubbles in the Posiseal filter housing were expelled by inverting the housing several times. In the second step (precoating phase), 0.98 ± 0.01 g of filter aid solids was mixed with 300 mL of DIW in a 400-mL Nalgene polypropylene beaker (Thermo Fisher Scientific, Waltham, MA). Both the inlet and the outlet tubing were placed in the beaker, and the pump was set to approximately 160 mL/min. The slurry of precoating filter aid was recirculated through the filtration device until there were no visible particles in the Posiseal filter housing, indicating that the precoat was established. In the third step (body feeding phase), the desired amount of filter aid solids (body feed) was added to $1,000.0 \pm 0.1$ g of the liquid to be filtered in a 2-L Nalgene polypropylene beaker. The pump was stopped, and the inlet tubing was placed in the beaker containing the unfiltered juice to prevent introducing air into the filtration device. The pumping speed was set to the operational speed (50, 63, or 100 mL/min, depending on the experimental design). The outlet tubing was placed in another 2-L Nalgene polypropylene beaker. Clarified juice was continuously collected until all juice was filtered. Samples (at least three) of clarified juice were obtained in 15-mL *DigitUBE* disposable tubes (SCP Science, Champlain, NY) after all juice was filtered. Juice samples were stored in a freezer (-20°C) until ICP-MS/MS analysis.

The two major filtration variables, flow rate and body feed ratio, were controlled in each experiment. Filtration flow rate, or pumping speed, was controlled by monitoring the display on a graphical interface in the unit of milliliters per minute. For experiments with simulated juices, the filtration flow rate remained constant throughout the experiment. However, during the filtration of commercial juices, the flow rate was decreased gradually as solids accumulated on the precoat and formed an increasingly thicker filter cake.

The body feed ratio was defined as follows: required weight of a filter aid/amount of TSS to be removed from the unfiltered juice. In industrial filtration, the body feed ratio is usually determined by the nature of the solids being removed and the capacity of the filtration device. In this study, body feed ratios of 1:1 and 2:1 were used for filtering apple and grape juices, based on industry recommendations (8, 13). The amount of a filter aid required for the filtration treatment was defined as the initial weight of an unfiltered juice \times weight percent of TSS \times body feed ratio. Samples of juices were collected and were stored at -20°C prior to heavy metal analysis.

To estimate the transfer of arsenic from filter aid to juice during clarification, batches of commercial apple and grape juices were filtered with a variety of filter aids (three food-grade DE-based filter aids and two food-grade perlite-based filter aids). For each filter aid, filtration experiments were performed in triplicate. Filtration flow rate, starting at 50 mL/min, was gradually decreased to ensure that the operating pressure was lower than 30 lb/in², the maximum allowable working pressure of the laboratory-scale filtration apparatus. Samples of the clarified juices were obtained and were stored as described previously.

To investigate factors associated with filtration parameters, filtration tests were performed with a simulated juice (pH 3.4 ± 0.1 , °Bx 8.2 ± 0.1) using a food-grade DE-based filter aid, SSC, which contained a relatively high level of arsenic (7.13 ppm), and using different filtration conditions with respect to TSS, body feed ratio, and filtration rate. Because the simulated juice did not contain suspended solids, the TSS values were all assumed. To investigate factors associated with juice pH and sugar content, filtration tests were performed with a series of simulated juices and the SSC filter aid under the same filtration conditions (TSS 0.1%, body feed ratio 2:1, filtration flow rate 63 mL/min).

Experimental findings obtained with simulated juices were compared with those acquired for authentic commercially available juices. For this experiment, three batches of commercial apple and grape juices were subjected to a series of filtration experiments with the SSC filter aid before and after an acid-washing treatment, and using a body feed ratio of 1:1 or 2:1.

Microwave-assisted acid extraction of filter aids. The microwave-assisted acid extraction method was adapted from the FDA *Elemental Analysis Manual for Food and Related Products* (EAM) section 4.7 (35). Acid extraction of filter aids was performed with a closed-vessel Discover SP-D Microwave Digestion System (CEM Corporation, Charlotte, NC). In each Teflon digestion vessel liner, 9 mL of HNO₃ and 1 mL of H₂O₂ were added to 0.25 ± 0.01 g of filter aid solids. Temperature was ramped to 200°C in 10 min and was held for 20 min with medium stirring. After extraction was complete, vessels were depressurized and cooled to room temperature. Approximately 35 mL of DIW was added to the liner, and the entire solution was transferred to a 50-mL *DigitUBE* disposable tube. The dilution factor was the final weight of the diluted extract divided by the initial weight of the filter aid solids.

Acid digestion of juices. Microwave-assisted acid digestion of juices was performed by adding 2 mL of HNO₃ and 1 mL of H₂O₂ to 2.00 ± 0.01 g of juice in each quartz digestion vessel. Temperature was ramped to 200°C in 5 min and was held for 30 min with medium stirring. On completion of the digestion, vessels were depressurized and cooled to room temperature. Approximately 35 mL of DIW was added to each quartz tube, and the entire solution was transferred to a 50-mL *DigitUBE* disposable

tube. The dilution factor was the final weight of the diluted digest divided by the initial weight of the juice.

ICP-MS/MS quantification of total heavy metals. Total leachable arsenic, cadmium, and lead content was measured with an Agilent 8800 ICP-MS/MS (Agilent Technologies, Santa Clara, CA) in MS/MS mode with helium as the collision gas (0.50 to 0.60 mL/min) and argon as the carrier gas. The internal standard solution was 1 ppm of germanium in 10% (w/w) of HNO₃, which was prepared from the ICP-MS Internal Standard Mix (Agilent Technologies). Prior to the ICP-MS/MS analysis, each sample was further filtered with a 0.20- μ m-pore-size polytetrafluoroethylene syringe filter (EMD Millipore Corporation, Billerica, MA), and the first 1 mL of filtrate was discarded to prevent cross-contamination from the syringe filter. Data processing was conducted with MassHunter Workstation software version 4.2 (Agilent Technologies) and was exported as Excel worksheets (Microsoft Office 2013, Microsoft Corporation, Redmond, WA).

Arsenic speciation analysis with high-pressure liquid chromatography (HPLC)–ICP-MS. Arsenic speciation was performed in eight DE-based filter aids and three perlite-based filter aid products. The protocol for arsenic speciation analysis was adapted from the FDA EAM section 4.11 (32). Arsenic species were extracted from 0.20 \pm 0.01 g of each filter aid sample with 10 mL of 0.28 M HNO₃ at 95°C for 90 min in a *Digi*PREP heating block digestion system (SCP Science). After cooling to room temperature, the extract was diluted with approximately 6.7 g of DIW and was centrifuged at 1,100 \times *g* for 10 min. Dilution factor 1 was the final weight of the diluted extract divided by the initial weight of the filter aid solids. The supernatant was filtered with a 0.45- μ m-pore-size nylon syringe filter (Agilent Technologies). The first 1 mL through the filter was discarded. The rest of the filtrate was collected.

The chromatographic mobile phase, 10 mM ammonium phosphate dibasic at a pH of 8.25 (\pm 0.05), was prepared fresh daily with (NH₄)₂HPO₄ and 28% NH₃ in H₂O for adjusting pH value. A pH adjustment solution (pH of 9.85 \pm 0.05) was prepared fresh daily with approximately 0.6 g of 28% NH₃ (aq) in H₂O and 100 g of mobile phase.

Prior to analysis, 1 g of filter aid extract was further diluted with 2 g of the pH adjustment solution. The pH of the diluted solution was between 6.0 and 8.5. Dilution factor 2 was the final weight of the diluted extract divided by the initial weight of the filter aid extract. A portion of this solution (typically 1.5 mL) was transferred to a plastic auto-sampler vial for HPLC-ICP-MS analysis by Agilent 1200 HPLC coupled with Agilent 7700 ICP-MS (Agilent Technologies). The total dilution factor was dilution factor 1 multiplied by dilution factor 2.

Analytical and guard HPLC columns used for separation of arsenic species were a Hamilton PRP-X100 anion exchange column (4.1 by 250 mm, particle size 10 μ m) and respective guard column (Hamilton Company, Reno, NV). Instrument standard operating procedure for startup, initialization, and tuning was performed prior to sample analysis. ICP-MS carrier gas was argon at 1.1 L/min. The HPLC injection volume was 100 μ L, and the mobile phase flow rate was 1 mL/min. Multianalyte calibration standards (0.25, 1, 5, and 20 ng/g) were prepared fresh daily with As(III), As(V), DMA, and MMA hexahydrate analytical standards. The postcolumn internal standard for monitoring and correcting signal drift was 2 ng/g As(V). Data processing was conducted with MassHunter Workstation software (Agilent Technologies) and was exported as Excel worksheets.

Data analysis. All experimental treatments were performed in triplicate, and at least three samples were obtained for each treatment. Two-way analysis of variance (ANOVA) was performed with Excel (Microsoft Office 2013) to (i) determine whether the arsenic content of simulated juices was affected by juice properties (pH and sugar content) and (ii) determine whether filtration conditions (flow rate and amount of filter aid) affected arsenic content of simulated juices. One-way ANOVA was used to determine whether body feed ratio significantly affected arsenic content of apple or grape juices. One-way ANOVA followed by Tukey's multiple comparison test was used to determine which of the filter aids significantly ($P < 0.05$) affected arsenic, cadmium, and lead concentrations in filtered apple and grape juices. Similarly, significant differences ($P < 0.05$) among washing treatments on removal of heavy metals were verified by one-way ANOVA followed by Tukey's multiple comparison test.

RESULTS AND DISCUSSION

Heavy metal content of filter aids. Figure 1 illustrates the total leachable arsenic, cadmium, and lead content of 32 filter aids available in the U.S. market for food and nonfood applications. Because DE is the most popular type of filter aid used for beverage filtration, this study included 22 samples of DE products. In contrast, perlite, cellulose, and rice hulls are less often used and, thus, had smaller sample sizes (10 for perlite, two for cellulose, and one for rice hulls). Figure 1 shows that levels of arsenic, lead, and cadmium in various filter aids varied considerably with respect to type, grade, and manufacturer, but generally, DE tended to have higher levels of arsenic than perlite. The levels of total leachable arsenic in all but one sample of DE-based and perlite-based filter aids (food-grade and non-food-grade) were lower than the USP-NF limit for total leachable arsenic (10 ppm). The only product that exceeded the 10-ppm limit was a DE-based filter aid (DH5). Recommended applications for DH5 were not specified on the package or the safety data sheet, but personal communications with industrial experts revealed that DH5 is usually used in the production of paint. Although it is less likely that this filter aid would be used in juice clarification, it was included in this survey to understand differences in arsenic levels between food-grade filter aids and those not for use for food processing. A food-grade DE filter aid (SSC) contained the second highest level of arsenic (7.13 ppm) among 32 samples of filter aids. The levels of total leachable lead in all samples of filter aids were lower than the USP-NF limit for total leachable lead (10 ppm). A non-food-grade perlite filter aid (17S) and a non-food-grade DE filter aid (C0) contained the highest levels of lead (6.74 ppm) and cadmium (0.36 ppm), respectively. Currently there is no USP-NF limit for cadmium in food-grade DE-based and perlite-based filter aids.

As shown in Figure 1, arsenic levels of DE-based filter aids used for food and beverage applications ranged from 0.56 to 7.13 ppm, whereas arsenic concentrations in food-grade perlite-based filter aids ranged from 0.16 to 0.89 ppm. The range of lead concentrations in DE-based filter aids used for beverage filtration was from 0.29 to 4.17 ppm, whereas the concentrations in perlite-based filter aids ranged from 0.80 to 3.02 ppm. Cadmium levels in DE-based filter aids

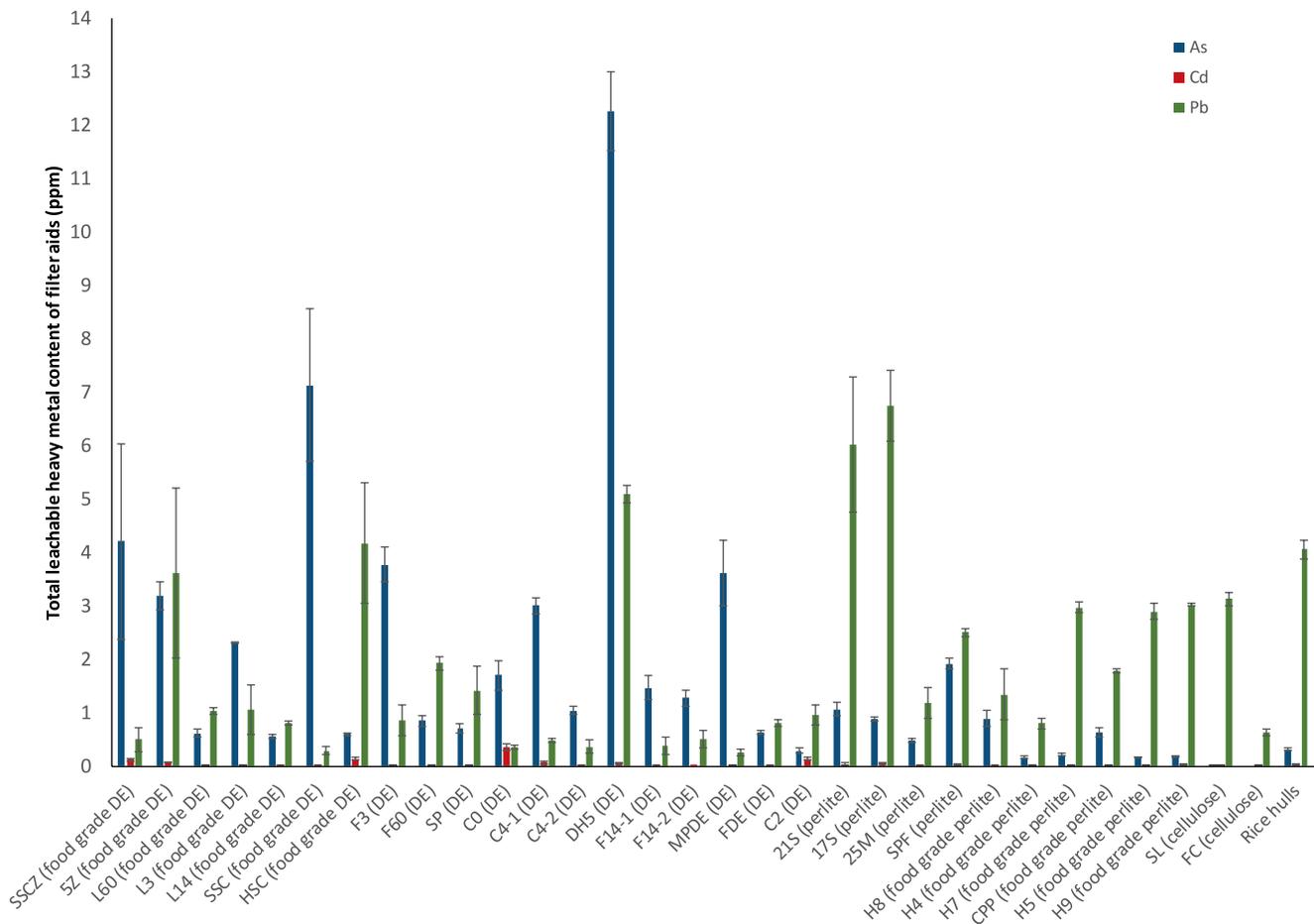


FIGURE 1. Survey of total leachable heavy metal content of 32 representative filter aids available in the United States. Three subsamples of each product were analyzed individually for total leachable arsenic, cadmium, and lead. Each bar represents the average arsenic, cadmium, or lead content of the three subsamples, whereas the error bar represents the standard deviation. All product names were blinded. The composition and grade of each filter aid product are indicated in parentheses.

used in the beverage industry ranged from 0.01 to 0.12 ppm, whereas in perlite-based filter aids, they ranged from 0.01 to 0.03 ppm. All test results of heavy metal content shown in Figure 1 were the averages of three subsamples from each lot of filter aids. Because the analytical variability (the variability associated with the heavy metal analyses) was typically low (% coefficient of variation <5%), the high degree of variability in the concentrations of arsenic in some of the filter aids suggests that heavy metals were heterogeneously distributed throughout lots of filter aids. The variability in concentrations of heavy metals within a lot of filter aids is not unexpected because DE and perlite filter aids are mined materials (5, 7).

Figure 2 shows the average heavy metal content of three batches of apple juice independently processed with each of five food-grade filter aids, including three that were DE-based (5Z, SSC, and L14) and two that were perlite-based (CPP and H7). A laboratory-scale filtration device was used for filtration studies, because filtration conditions and other parameters could be easily controlled. In addition, similar laboratory-scale filtration devices are often used by the filtration industry to optimize conditions prior to commercial production of filtered juices and liquid pharmaceutical products (9, 14). The commercial apple juice before

filtration treatments contained 4.71 ppb of total arsenic, 1.61 ppb of cadmium, and 17.19 ppb of lead (average of three batches). The concentrations of arsenic and lead found in the commercial apple juice are similar to those reported in other studies (6, 25, 39, 41), but cadmium levels were higher than those previously reported (6). After filtration, the levels of arsenic in 5Z-filtered and SSC-filtered apple juice significantly increased ($P < 0.05$) to 16.55 and 27.05 ppb, respectively. As filter aids 5Z and SSC themselves contained elevated levels of arsenic (3.19 and 7.13 ppm, respectively), apple juice processed with these arsenic-rich filter aids contained relatively high arsenic levels. In contrast, the arsenic content of apple juice did not significantly change ($P > 0.05$) when filtered with L14, CPP, and H7 filter aids, because these products contained comparatively low levels of arsenic (0.56, 0.63, and 0.22 ppm, respectively). Cadmium concentrations in apple juice decreased slightly, but significantly ($P < 0.05$), for all treatments with filter aids, except L14. No changes were observed in the lead concentrations in apple juice due to the filtration treatments (Fig. 2).

Figure 3 shows the average arsenic, lead, and cadmium concentrations in three batches of grape juice processed with the 5Z, SSC, L14, CPP, and H7 food-grade filter aids. The

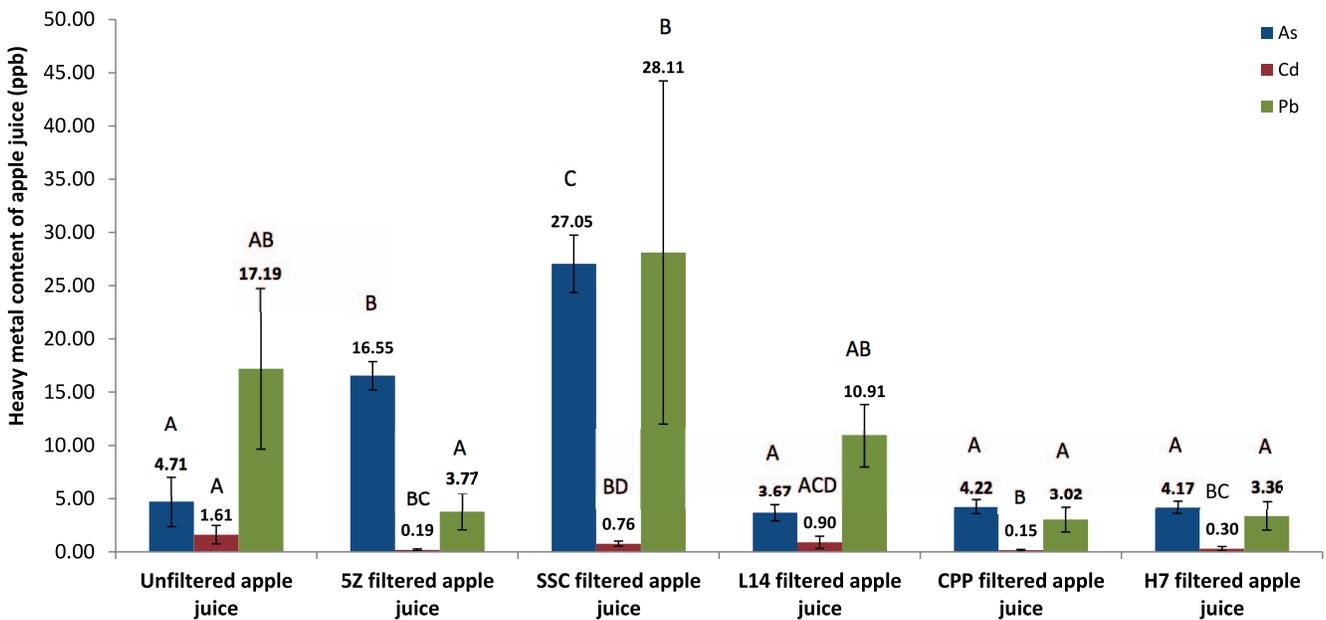


FIGURE 2. Total heavy metal content of apple juice processed with different types of filter aids and a body feed ratio of 1:1. Three batches of apple juice were individually processed with each type of filter aid. For each batch, three samples of filtrate were collected. Each sample was analyzed for arsenic, cadmium, and lead content. Each bar represents the average arsenic, cadmium, or lead content of the three batches, whereas the error bar represents the standard deviation. 5Z, SSC, and L14 were the blinded names of three DE filter aids. CPP and H7 were the blinded names of two perlite filter aids. Bars (for each heavy metal) with different letters (A, B, C, D) are significantly different from each other (Tukey's multiple comparison test; $P < 0.05$).

arsenic, cadmium, and lead levels of the commercial grape juice prior to filtration studies were 6.13, 2.11, and 26.27 ppb, respectively. The levels of the arsenic found in this grape juice product were similar to those previously reported (39), whereas lead concentrations were slightly higher than

those published for grape juice products (41). Published information is lacking on the cadmium content of commercial grape juices.

After filtration with the three DE-based filter aids, the levels of arsenic in 5Z-filtered, SSC-filtered, and L14-filtered

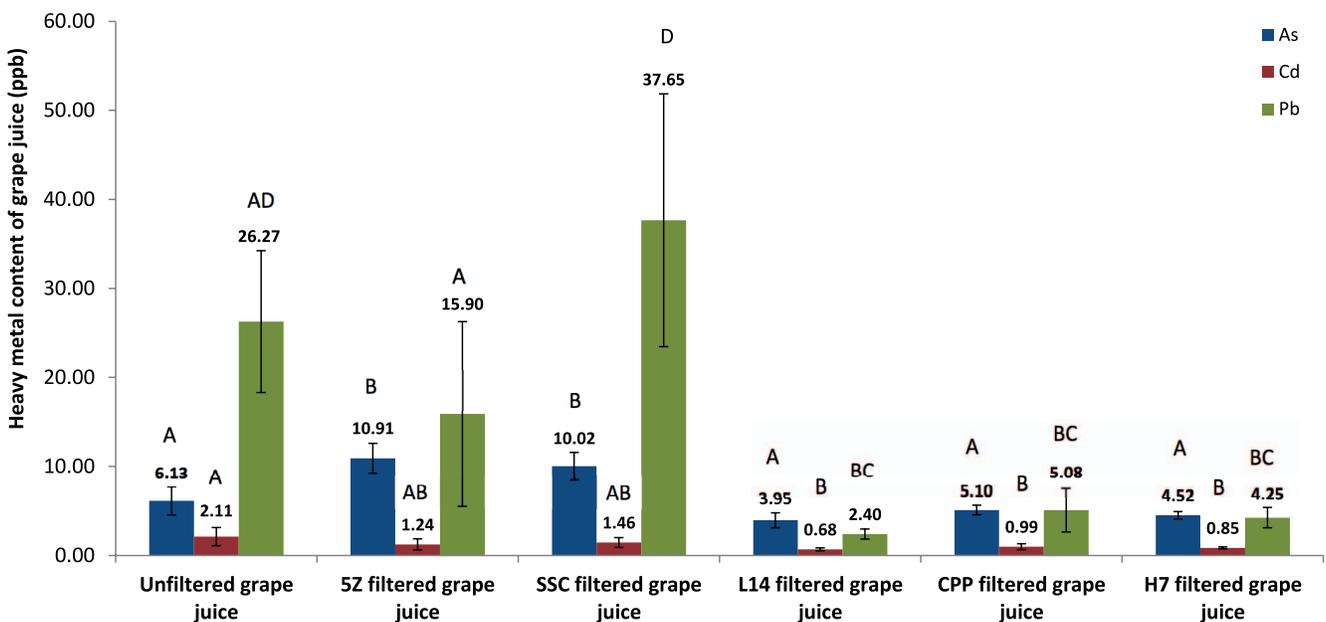


FIGURE 3. Total heavy metal content of grape juice processed with different types of filter aids and a body feed ratio of 1:1. Three batches of apple juice were individually processed with each type of filter aid. For each batch, three samples of filtrate were collected. Each sample was analyzed for arsenic, cadmium, and lead content. Each bar represents the average arsenic, cadmium, or lead content of the three batches, whereas the error bar represents the standard deviation. 5Z, SSC, and L14 were the blinded names of three DE filter aids. CPP and H7 were the blinded names of two perlite filter aids. Bars (for each heavy metal) with different letters (A, B, C, D) are significantly different from each other (Tukey's multiple comparison test; $P < 0.05$).

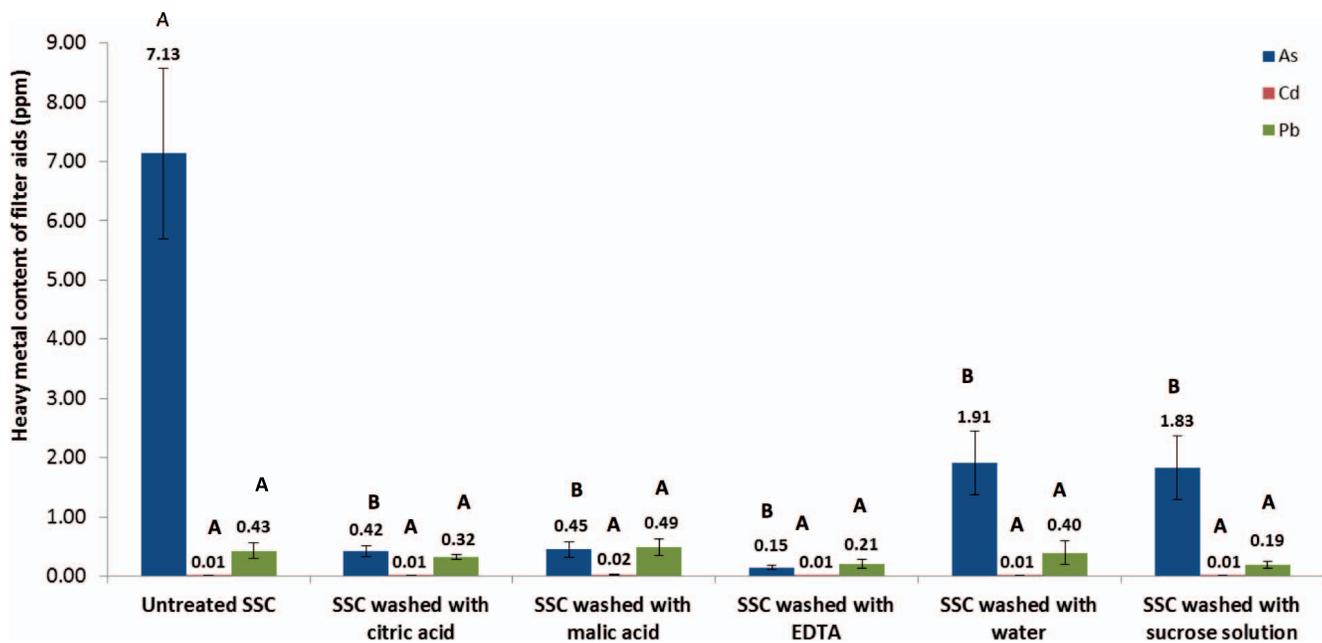


FIGURE 4. Total leachable heavy metal content of untreated and washed SSC filter aid. Samples of SSC were treated with citric acid, malic acid, EDTA, water, and sucrose solution washing treatments. All washing treatments were done in triplicate. Each bar represents the average arsenic, cadmium, or lead content of the SSC filter aid for the three trials, whereas the error bar represents the standard deviation. Bars (for each heavy metal) with different letters (A, B) are significantly different from each other (Tukey's multiple comparison test; $P < 0.05$).

grape juice were 10.91, 10.02, and 3.95 ppb, respectively. Filtration treatments with CPP and H7, perlite-based filter aids, resulted in arsenic concentrations in grape juice of 5.10 and 4.52 ppb, respectively. Similar to the results obtained for apple juice, the only significant increases ($P < 0.05$) in arsenic content were found for grape juice filtered with 5Z and SSC filtering aids. Because the commercial grape juice contained lower amounts of TSS than commercial apple juice, less filter aid was used for the filtration of grape juice. This explains the lower arsenic concentrations for the grape juice filtered with 5Z and SSC filter aids than those found for apple juice. The concentrations of cadmium and lead in the grape juice decreased when filtered with L14, CPP, and H7 filter aids (Fig. 3).

Speciation analysis of eight DE-based filter aids (SSC, SSCZ, 5Z, L14, L3, F3, F60, and F14-1) and three samples of perlite-based filter aids (H8, CPP, and 17S) showed that arsenic in the filter aids was predominantly (>95% for perlite and >99% for DE) in inorganic forms [As(III) and As(V)] of the element. Although speciation analysis was not performed for arsenic present in the juices, it is likely that the predominant forms of arsenic transferred from filter aids to clarified juices were inorganic arsenic. Based on surveys of fruit juices produced in the United States and elsewhere, other researchers (6, 39) found that the majority of arsenic present in fruit juices is inorganic arsenic.

Because the USP-NF limit for total leachable arsenic in food-grade filter aids (10 ppm) is 1,000 times higher than the FDA draft action level of 10 ppb for inorganic arsenic in apple juice and the EPA limit of 10 ppb for total arsenic in drinking water, using an arsenic-rich filter aid is likely a major cause of arsenic contamination in clarified juices. To

reduce the amount of transfer of arsenic, the amount of filter aid used for filtration, particularly for arsenic-rich (>3 ppm) filtering aids, should be minimized. Alternatively, use of filter aids containing a low level of arsenic (such as <1 ppm) would be a prudent measure for minimizing arsenic contamination during industrial clarification of juices.

Effects of acid washing treatments on heavy metal content of a DE-based filtering aid. An experiment investigated the use of simple washing treatments for reducing the arsenic concentrations in a DE-based filter aid. Washing treatments involved the use of citric acid and malic acids, weak organic acids that have metal-chelating properties (11). Citric acid treatments have been useful in removing heavy metals from contaminated soils (21, 43) and fish products (10). Another acid washing treatment used EDTA, a chelating agent that exhibits strong metal-complexing ability, and has proven effective for remediating soils contaminated with arsenic, lead, and other heavy metals (21, 38, 43).

As shown in Figure 4, adjusting the pH of 10% (w/w) DE slurry with citric acid to approximately 3 was effective in significantly reducing ($P < 0.05$) arsenic concentrations in SSC, a DE-based filter aid, from 7.13 ppm of arsenic to 0.42 ppm. Malic acid and EDTA, also used to treat the SSC filter aid, caused significant reductions ($P < 0.05$) in the arsenic content of the filter aid (Fig. 4). The arsenic levels of malic acid-washed and EDTA-washed SSC were 0.45 and 0.15 ppm, respectively. Figure 4 also shows results from water washing treatments that were used as experimental controls for the acid washing treatments. The arsenic levels of water-washed and sucrose solution-washed SSC were

TABLE 1. Arsenic content of a simulated juice processed with the SSC filter aid under different filtration conditions^a

Amt of SSC used for filtration (as body feed) ^b	Arsenic content (ppb) (mean \pm SD)	
	Flow rate 63 mL/min	Flow rate 100 mL/min
(A) 1 g	16.25 \pm 2.70	16.54 \pm 1.97
(B) 2 g	28.05 \pm 4.25	25.07 \pm 3.91
(C) 5 g	61.99 \pm 8.13	62.84 \pm 10.23
(D) 10 g	119.68 \pm 20.77	113.24 \pm 17.74

^a A simulated juice (pH 3.4 \pm 0.1, °Bx 8.2 \pm 0.1) was subjected to eight different filtration treatments. With each filtration treatment, three batches of the simulated juice were processed individually. For each batch, three samples of filtrate were collected and analyzed for arsenic concentration. SSC, blinded product name; TSS, total suspended solids.

^b Amount of the SSC filter aid used as body feed = weight of simulated juice to be filtered (1,000.0 \pm 0.1 g) \times TSS% \times body feed ratio. (A) TSS = 0.1%, body feed ratio = 1:1; (B) TSS = 0.1%, body feed ratio = 2:1; (C) TSS = 0.5%, body feed ratio = 1:1; (D) TSS = 0.5%, body feed ratio = 2:1.

1.91 and 1.83 ppm, respectively, which were significantly ($P < 0.05$) lower than the concentrations present in the unwashed filter aid. It was predicted that the acid washing treatments would remove more arsenic from the filter aid than the water or sucrose treatments (with an average pH of 6) because of the chelating abilities of citric acid, malic acid, and EDTA. However, the unexpected results may have been due, in part, to the fact that arsenic tends to be more soluble at alkaline than at acidic pH values (18). The concentrations of cadmium and lead in the SSC filter aid did not significantly change ($P > 0.05$) with any of the washing treatments (Fig. 4). These findings were not expected because EDTA, citric acid, and other organic acids have been shown to chelate, solubilize, and remove lead from soils and foods (10, 11, 21). Based on the findings of this experiment, the washing treatments were identified as a simple and practical method for removing arsenic from DE filter aids.

Factors affecting transfer of heavy metals from a DE-based filter aid to juices. A series of experiments evaluated the effect of juice composition and filtration parameters on transfer of heavy metals from the SSC filter aid to simulated and authentic juices. Table 1 shows the arsenic content of a simulated juice (pH 3.4 \pm 0.1, °Bx 8.2 \pm 0.1) processed with the SSC filter aid under different filtration conditions with respect to the amount of filter aid used and the filtration flow rate. Under each condition, three filtration tests were independently conducted, and three samples of filtrate were collected in each test. Results of two-way ANOVA indicate that the amount of filter aid had a significant effect ($P < 0.05$) on the arsenic content of clarified juices, whereas filtration flow rate had no effect ($P > 0.05$). There was no interaction between the two factors.

Table 2 shows the arsenic content of simulated juices with different pH values (2.9 \pm 0.1, 3.4 \pm 0.1, and 4.1 \pm

TABLE 2. Arsenic content of different simulated juices processed with the SSC filter aid under the same filtration condition^a

	Arsenic content (ppb) (mean \pm SD)	
	°Bx 8.2 \pm 0.1	°Bx 18.1 \pm 0.1
pH 2.9 \pm 0.1	33.55 \pm 9.39	56.18 \pm 27.17
pH 3.4 \pm 0.1	33.18 \pm 9.04	42.39 \pm 15.86
pH 4.1 \pm 0.1	23.82 \pm 6.26	37.67 \pm 12.94

^a Six types of simulated juice were subjected to the same filtration treatment (TSS 0.1%, body feed ratio 2:1, filtration flow rate 63 mL/min). For each type of simulated juice, three batches were filtered individually. For each batch, three samples of filtrates were collected and analyzed for arsenic concentration. SSC, blinded product name; TSS, total suspended solids.

0.1) and °Bx values (8.2 \pm 0.1 and 18.1 \pm 0.1) processed with the SSC filter aid under identical filtration condition. For each type of simulated juice, three batches were individually filtered with SSC. Results of two-way ANOVA demonstrated that neither pH nor sugar content (°Bx value) had a significant effect ($P > 0.05$) on the arsenic content of the simulated juice processed with SSC. The lack of an effect of juice pH on arsenic transfer may be due to the narrow pH range used in this study and to the fact that the pH values evaluated were all in the acidic pH range. Because arsenic tends to be more soluble at alkaline pH values (18), use of simulated juices that were close to neutral pH might have revealed significantly higher levels of arsenic transfer than the acidic juices used in this study.

Table 3 shows the heavy metal content of authentic apple and grape juices clarified with SSC. Because the body feed ratio was different for each set of experiments, the amount of filter aid used for the filtration treatment varied. Three batches of each type of juice were independently subjected to each filtration treatment. Results of one-way ANOVA indicate that the amount of filter aid used for filtration had no significant effect ($P > 0.05$) on the level of arsenic in filtered apple and grape juices. Similarly, the amount of filter aid had no significant effect ($P > 0.05$) on transfer of lead and cadmium from the filter aid to the authentic apple and grape juices. The effect of filtration flow rate on the levels of heavy metals in filtered juice was not investigated with authentic apple and grape juices, because the flow rate decreased and pressure increased as solids were accumulated on the filter. Interestingly, body feed ratio had a significant effect on arsenic transfer in the simulated juice with pH of 3.4 and °Bx of 8.2, whereas no effects were observed with authentic juices. Although an attempt was made to carefully control the filtration conditions used for the authentic juices, slight differences in the conditions may have resulted in the variable results obtained and the subsequent lack of statistical significance found for the effect of body feed ratio.

In a final experiment, citric acid-washed SSC was used to process commercial apple and grape juices with different body feed ratios. Heavy metal concentrations of filtered apple and grape juices are shown in Table 4. Compared with apple and grape juices processed with the untreated SSC

TABLE 3. Heavy metal content of apple and grape juices processed with the SSC filter aid and different body feed ratios^a

	Heavy metal content (ppb) (mean ± SD)		
	Arsenic	Cadmium	Lead
Filtered apple juice			
Body feed ratio 1:1	19.57 ± 5.91	1.18 ± 0.88	19.75 ± 9.61
Body feed ratio 2:1	24.30 ± 3.85	0.60 ± 0.57	20.13 ± 15.56
Filtered grape juice			
Body feed ratio 1:1	8.91 ± 3.00	2.00 ± 1.21	18.89 ± 21.64
Body feed ratio 2:1	10.33 ± 3.11	1.76 ± 0.95	10.20 ± 10.78

^a Unfiltered apple juice contained 4.71 ppb of arsenic, 1.61 ppb of cadmium, and 17.19 ppb of lead, as shown in Figure 2. Unfiltered grape juice contained 6.13 ppb of arsenic, 2.11 ppb of cadmium, and 26.27 ppb of lead, as shown in Figure 3. Three batches of each juice were subjected to each of two filtration treatments (body feed ratio of 1:1 or 2:1). Three samples were obtained for each replicate treatment. SSC, blinded product name.

(Table 3), the levels of arsenic in filtered juices were reduced by using citric acid–washed SSC. With a body feed ratio of 1:1, the arsenic level of filtered apple juice was reduced from 19.57 to 1.56 ppb, and the arsenic level of filtered grape juice was reduced from 8.91 to 4.92 ppb. With a body feed ratio of 2:1, the arsenic level of filtered apple juice was reduced from 24.30 to 1.85 ppb, and the arsenic level of filtered grape juice was reduced from 10.33 to 3.81 ppb. Even when higher amounts of citric acid–washed SSC were used (a body feed ratio of 2:1) during the filtration process, the arsenic levels in the filtered apple and grape juices were less than the FDA draft action level of 10 ppb for inorganic arsenic in apple juice. The lead and cadmium concentrations in filtered apple and grape juices were not reduced by using the citric acid–washed SSC rather than untreated filter aid. These findings are in agreement with the results obtained from previous experiments, in which acid washing treatments did not affect lead and cadmium levels in the SSC filter aid.

In conclusion, types and grades of filtering aids vary considerably with regard to arsenic, lead, and cadmium content. This study showed that filtering aids can transfer significant amounts of arsenic to juices, particularly if the filter aids are arsenic rich. To reduce concentrations of

arsenic in juices, it is advisable that filter aids used for juice clarification contain as low a content of this toxic metal as possible (i.e., <1 ppm). Washing proved to be an effective way for removing arsenic from DE-based filter aids. Further work is needed to evaluate the effects of washing treatments on other types of filtering aids, to optimize washing treatments for removing arsenic and other toxic metals from filtering aids, and to study the transfer of heavy metals from filtering aids to other types of beverages.

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SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at: <https://doi.org/10.4315/0362-028X.JFP-16-464.s1>.

TABLE 4. Heavy metal content of apple and grape juices processed with the citric acid–washed SSC filter aid and different body feed ratios^a

	Heavy metal content (ppb) (mean ± SD)		
	Arsenic	Cadmium	Lead
Filtered apple juice			
Body feed ratio 1:1	1.56 ± 0.66	1.36 ± 0.71	8.14 ± 9.31
Body feed ratio 2:1	1.85 ± 0.62	0.73 ± 0.70	11.28 ± 14.08
Filtered grape juice			
Body feed ratio 1:1	4.92 ± 2.84	2.85 ± 1.31	12.63 ± 8.77
Body feed ratio 2:1	3.81 ± 1.97	1.55 ± 0.66	13.00 ± 11.92

^a Apple juice, before filtering treatments, contained 4.71 ppb of arsenic, 1.61 ppb of cadmium, and 17.19 ppb of lead, as shown in Figure 2. Grape juice, before filtering treatments, contained 6.13 ppb of arsenic, 2.11 ppb of cadmium, and 26.27 ppb of lead, as shown in Figure 3. Three batches of each juice were subjected to each of two filtration treatments (body feed ratio of 1:1 or 2:1). Three samples were obtained for each replicate treatment. SSC, blinded product name.

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