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EXAMINATION OF PESTICIDES IN WINE, BEER AND THEIR CONSTITUENT PRODUCTS USING HIGH-THROUGHPUT TECHNIQUES TO MAXIMIZE EXTRACTION AND EFFICIENCY

ABSTRACT

There are hundreds of commercial pesticides in use in industrial and private agriculture. Concern over the health effects of residual pesticides on fruits and vegetables has led to increased testing of these products to determine the levels of pesticides on produce when it goes to market.

In this study, commercial red wine and beer samples were examined for their pesticide concentrations. In addition to the examination of the finished alcoholic beverage, the constituent agricultural products of wine and beer production (grains, malts, hops, and wine grapes) were also examined to determine the levels of pesticides found in those products. The sample preparation and extraction process efficiency and recovery were examined by processing samples using manual versus high-throughput techniques. The QuEChERS method was used to process a greater number of samples in a shorter period of time than other extraction methods.

1. MATERIALS AND METHODS

Samples

The wine and wine component samples studied included two varietals of wine grapes (Malbec and Syrah) obtained from a commercial urban vintner, and six red wines of the same varietals manufactured at vineyards in South America, US, Europe, and Australia.

The beer component samples included two Briess grain samples (organic and non-organic), a dark malt sample, and four hop samples (Cascade, Magnum, Centennial, and CitraHops). Six US craft beer samples representing the same hop varieties were also examined.

Solid Sample Preparation

Solid samples such as the grain samples and hop samples were ground 2.5 g at a time using a Spex SamplePrep FreezerMill and the following program: precool for twenty minutes followed by 5 cycles of grinding (16 impacts/second) at two minutes per cycle. Each cycle had an additional two minutes of cooling before the subsequent grinding cycle.

Sample Extraction

The AOAC 2007.1 methods for the extraction and clean-up of agricultural products using QuEChERS were followed. QuEChERS Kits were used for the initial extraction step in which ten to fifteen grams of sample were placed in a 50 mL centrifuge tube of 10 mL to 15 mL of 1% acetic acid in acetonitrile (HPLC grade). Samples were shaken mechanically for one minute in the Spex SamplePrep ShaQer 1500 using ceramic mixing media to ensure mixing and homogenization of the samples. An identical set of samples was hand-shaken for one minute to compare the pesticide recovery when using mechanical versus hand-shaken methods. The contents of 75 µL I.S. solution and a spiking standard solution were added to the samples, and the tubes were then shaken for an additional one minute using both the mechanical and hand-shaken methods. The samples were then centrifuged at 1,500 U/min for one minute.

Eight mL of the acetonitrile layer of the samples were transferred to a 15 mL tube. The mechanically shaken samples were again shaken for one minute in the Spex SamplePrep ShaQer 1500 before being centrifuged at 1,500 U/min for one minute while the hand-shaken samples were agitated for one minute by hand before centrifugation. The supernatant was transferred to a clean tube and evaporated to near dryness in a Reacti-Therm at low temperature with nitrogen gas. The solvent was exchanged to 1 mL dichloromethane (pesticide grade). The resulting sample was transferred to a GC vial and spiked with a GC/MS internal instrument standard (CLPS-I90) prior to being analyzed by GC/MS.

Analytical Conditions

- Agilent 5975 GC/MS in scan mode with EIC (35-450 m/z)
  - DB-5 (eq): 30 m x 0.25 mm, 0.25 um
- Run program:
  - 55 °C x 1 minute; 20 °C/min to 200 °C, hold for 1 minute; 30 °C/min to 310 °C, hold for 3 minutes
  - Detector: 280 °C and injector 150 °C All samples were spiked with an internal standard (CLPS-I90) as well as compared to an external standard mix prepared at multiple levels to obtain a calibration curve.

2. RESULTS AND DISCUSSION

Wine and Wine Grapes

Only one pesticide, imidacloprid, was found at levels above the spiked level with a concentration of 0.9 ppm. The pesticide residue was only detectable in the mechanically shaken samples.

The wine samples contained increased pesticide residues for several compounds: carbaryl (3.7 ppm), imidacloprid (2.8 ppm) and pesticide residues under 1.5 ppm for ethion, bifenthrin, chlorpyrifos methyl, endosulfan sulfate, and lambacyhalothrin. The pesticide residues were increased by 5%-15% using mechanical shaking techniques over manual shaking. Comparison of the wine grapes and the finished wine products showed that imidacloprid was found in both materials.

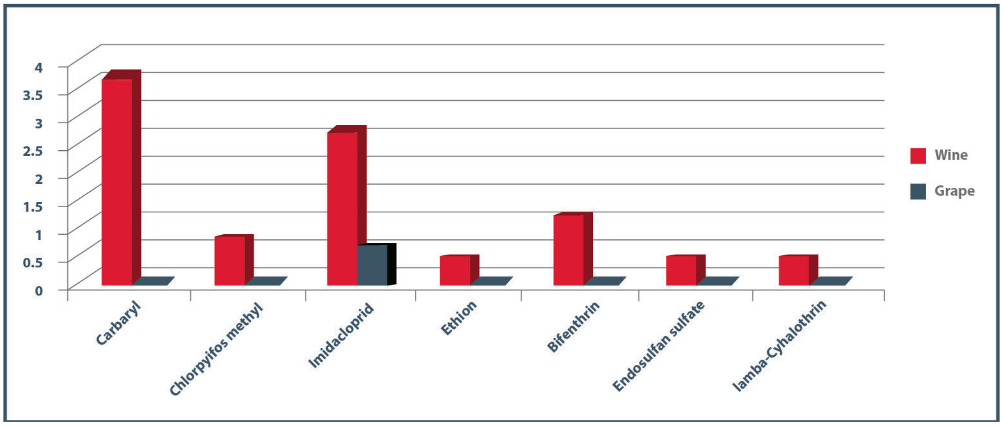

Figure 1. Pesticide residue levels (ppm) found in wine and grapes.

Beer, Grain, Malt, And Hops

Two samples of the Briess grain were examined for pesticide residues. One sample was from organic Briess and the other from a traditionally grown Briess sample. The overall concentration of pesticide residues was lower in the organic Briess sample than in the traditionally grown Briess sample. The highest pesticide residues for both products was for bifenthrin at 0.5 ppm in each sample type and ethion which had a concentration of 3.6 ppm in the traditional grain samples and 1.4 ppm in the organic grain samples. The use of mechanical shaking increased the pesticide residue recovery by 10%-25% over manual shaking methods.

The dark malt sample, which is a toasted ground grain product, contained three detectable pesticide residues above the spiked range: imidacloprid (0.75 ppm), bifenthrin and endosulfan sulfate (< 0.5 ppm).

The hop samples showed the greatest concentrations of pesticides of all of the products tested. These samples also contained numerous organic compounds such as: beta-pinene, caryophyllene, alpha-humulene, lupulon, Vitamin E, and phytosterol which potentially interfered with the detection and quantitation of some pesticides which were seen in other beer components. Additional sources of sample clean-up would need to be employed to remove more of these interfering organic compounds.

One compound was consistently mis-identified by the GC/MS library software as the pesticide folicur, and was seen up to 18 ppm in some hop samples. This compound was finally identified as a diketopiperazine compound, which is a bittering agent. This compound was only found in the beer samples and the hops samples.

The hop varieties show high levels of ethion (1.6-9.0 ppm), imidacloprid (8.5 ppm) and carbaryl (up to 6.5 ppm).

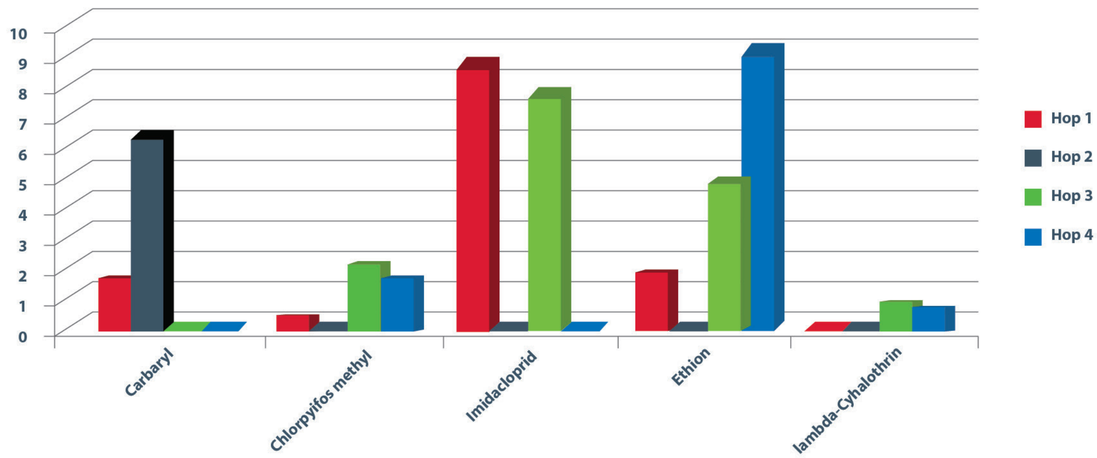

Figure 2. Pesticide levels (ppm) detected in four varieties of beer hops.

The beer samples contained detectable pesticide residue levels for carbaryl (3 ppm), ethion (2.5 ppm) and imidacloprid (5.25 ppm). The bittering agent observed in the hop samples was also present in the beer samples. The use of the mechanical shaking techniques increased the pesticide recoveries up to 35% over manual shaking.

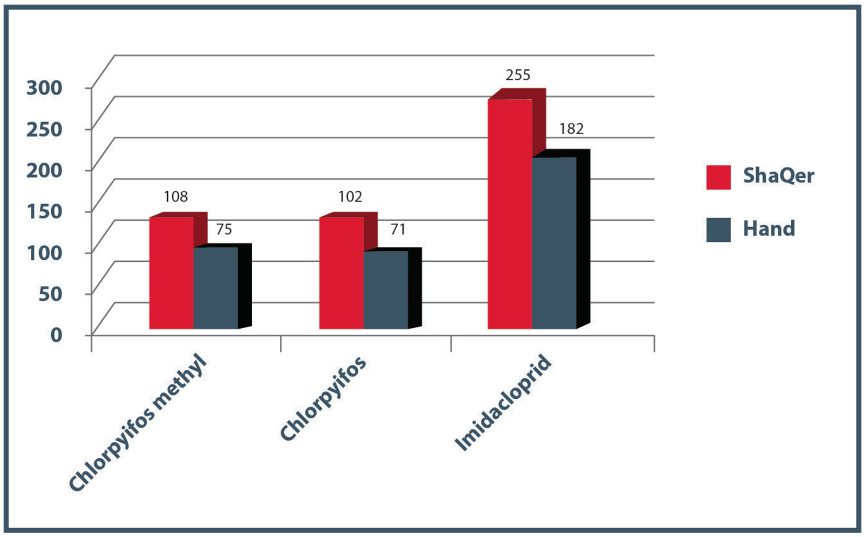

Figure 3. Recovery levels (%) of pesticides spiked into beer using hand-shaking versus mechanical shaking methods.

The most commonly detected pesticides in the beer and beer components were carbaryl, ethion, bifenthrin, chlorpyrifos methyl, chlorpyrifos, Imidacloprid, lambda-cyhalorthrin, and endosulfan sulfate. Imidacloprid was found at the highest levels in the hops and the finished beer products with a smaller concentration found in the malt. Ethion was found in all the beer and beer components with the highest levels in the hops, grains and finished beer. Bifenthrin was found highest in the finished beer with residue also found in the malt and both grains.

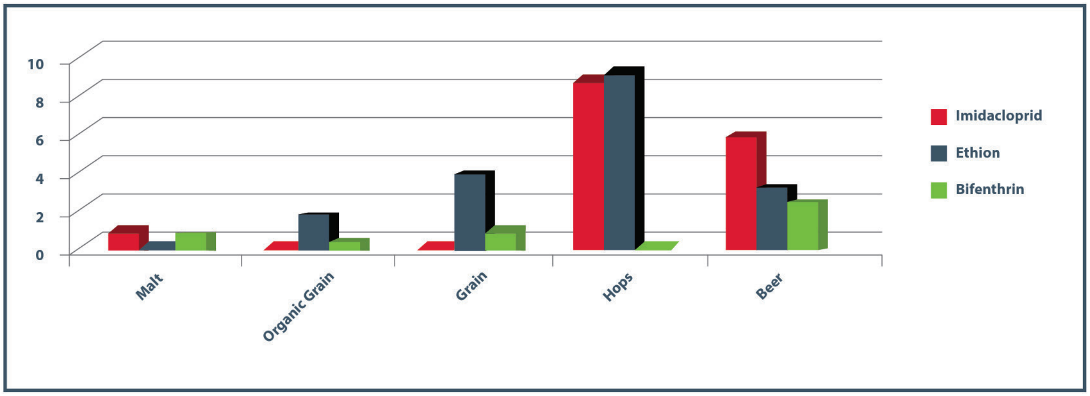

Figure 4. Comparison of pesticide levels (ppm) found in various beers and beer components.

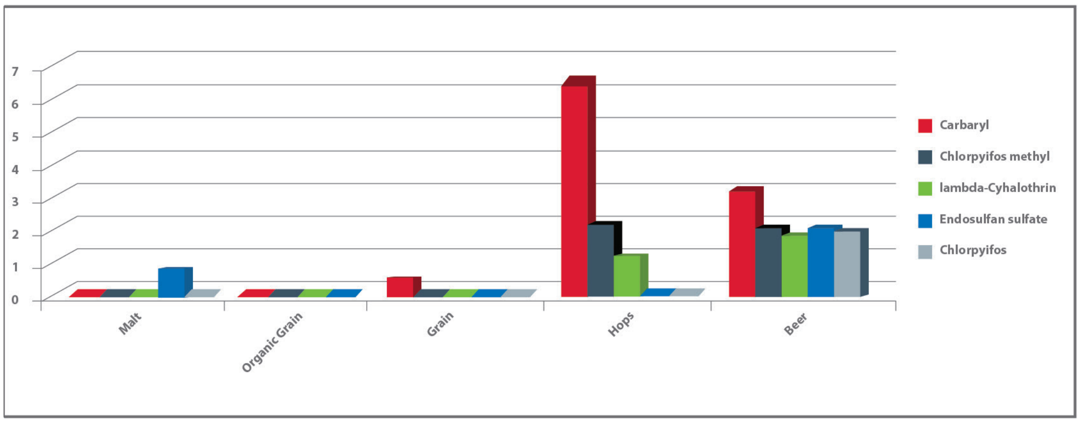

Figure 5. Comparison of pesticide levels (ppm) found in various beers and beer components.

The highest pesticide levels detected in all of the products tested were found in the beer hops with imidacloprid detected at 8.5 ppm. The pesticides found in the beer components were also found in the finished beer products at up to 5 ppm (imidacloprid) with most pesticides in the finished beer products being detected below 1-2 ppm. The use of mechanized shaking increased the recovery of the pesticides in all of the sample types from 5%-35%.

The wine and wine grapes showed one consistent pesticide residue (imidacloprid) up to 3 ppm in the finished wine. The wine grapes only showed a detectable pesticide residue level in samples that were mechanically shaken versus handshaken.