The Effects of Pretreatment with Dipping Solution on Variation of Boscalid Residue in Grape Samples During Drying Process with Natural Sunlight

Mustafa Cittan*, Ali Çelik, Kenan Dost

Manisa Celal Bayar University, Faculty of Science and Letters, 45140, Manisa, Turkey, Phone: +90 236 201 31 92,
mustafa.cittan@cbu.edu.tr
ali.celik@cbu.edu.tr
kenan.dost@cbu.edu.tr
*Corresponding author

Received: 13 December 2016
Accepted: 19 June 2017
DOI: 10.18466/cbayarfbe.339312

Abstract

Field experiments were carried out to investigate the variation of boscalid level during the drying period in grape samples that were pretreated and untreated with a dipping solution named POTAS prior to natural sunlight drying. Grape samples were collected at harvest and on the 3rd, 7th and 10th days after harvest (during the drying process of the pretreated and untreated grapes). Initially, 360 different pesticides were scanned qualitatively by GC-MS and LC-MS/MS after QuEChERS (Quick, easy, cheap, effective, rugged and safe) extraction method and only boscalid was observed in grape samples. Consequently, study was concentrated on boscalid extraction and analysis. Boscalid residues were extracted and cleaned up using QuEChERS method. The analysis method shows a good linearity over the concentration range of 0.010-0.125 mg kg⁻¹ with a correlation coefficient value of 0.998. Recoveries of the extraction and the analysis methods ranged from 74.8 to 100.8% within 0.7-1.7 % RSD at three different concentration levels. Detection and quantification limits were 0.77 and 2.55 µg kg⁻¹, respectively. The uncertainty of the method was calculated as 0.100±0.040 mg kg⁻¹. The results showed that grapes pretreated with the dipping solution prior to drying process showed shorter drying times and higher boscalid degradation rate than those untreated.

Keywords — Boscalid, degradation, GC-MS, QuEChERS, raisin

1. Introduction

Raisins are produced commercially by drying of harvested grapes. In order to dry grapes, water inside the grape must be removed completely from the interior of the cell onto the surface of the grape where the water droplets can evaporate. However, this diffusion process is very difficult because the grape skin contains wax in its cuticle, which prevents the water from passing through. In addition to this, the physical and chemical mechanisms located on the outer layers of the grape are adapted to prevent water loss [1]. Raisin, for fresh consumption as well as for using in baking and confectionery, is an important fruit because of its high nutritional value. Raisins are not only a good source for necessary vitamins and minerals, including potassium, iron, calcium, and vitamin B, but also fatless, cholesterol-free, and contain 70% fructose that is easily digestible [2]. The health benefits of raisins include relief from constipation, acidosis, anemia, fever, and sexual dysfunction. Raisins have also been known to help in attempts to gain weight in a healthy way, as well as its positive impact on eye health, dental care, and bone quality.

Raisins have a great economic importance for Turkey. The majority of seedless grapes are cultivated in the Aegean region in Turkey, especially in Manisa district. Therefore, drying of grapes is an important activity in the grape industry. Sun drying is the commonly employed method for this activity in Turkey. The harvested seedless grapes are traditionally pretreated with a dipping solution and then dried with natural sunlight on canvas sheets or concrete surfaces [3]. Pretreatment is a necessary step in raisin production in order to ensure the increased rate of water removal during the drying process. A faster water removal rate decreases
the rate of browning and helps to produce more desirable raisins. The historical method of completing this process was developed in the Mediterranean and Asia Minor areas by using a dry emulsion cold dip made of potassium carbonate and olive oil. Conventionally used dipping solution is named POTAS and prepared by dissolving 0.5 kg K$_2$CO$_3$ in 10 L water and adding 0.05 kg olive oil. These methods can encourage water transfer to the outer surface of grapes which helps to increase the efficiency of the drying process. Although, the exact role of the constituents of the dipping solution is not fully understood, present knowledge indicates that the fatty acids mainly modify the outer wax layer while the POTAS neutralizes the free acids and their electrical charges in the cuticular membrane [4].

Due to the great economic importance of raisins for Turkey, the main aim here is to focus on increasing the efficiency of grape production in vineyards. Increasing agricultural productivity may require application of pesticides which are generally a chemical or biological agent that control the pests and diseases. Although pesticides have benefits, some also have drawbacks, such as potential toxicity to humans and other desired species. Their use must be controlled and employers should be aware of possible adverse effects on human health and the natural environment. Improper use of pesticides reduce agricultural sustainability by causing environmental problems such as underground and surface water pollution, destruction of beneficial organisms and acquisition of resistance by pests, and at the same time can have harmful effects on the health of both farmers and consumers [5].

Grapes contain high levels of sugars and other nutrients, low pH and ideal water activity for microbial growth that makes them particularly susceptible to fungal spoilage [6]. Therefore, the application of fungicides, as boscalid, is a common practice in the field. Boscalid [2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide; C$_{18}$H$_{12}$Cl$_2$N$_2$O] (see Figure 1) is one of the most important fungicides used to destroy highly destructive plant pathogens, such as Botrytis cinerea in fruits and vegetables. It inhibits mitochondrial respiration, thereby inhibiting spore germination, germ tube elongation, mycelial growth and sporulation of pathogenic fungi on the leaf surface [7].

A few numbers of analytical methods for the determination of residues of boscalid have been developed previously. Boscalid has been determined by using gas chromatography (GC) in various matrices by mass spectrometric (MS) detection [8,9], and in blueberry samples by µ-electron capture (µECD) detection [10]. Determination of boscalid was also carried out by using liquid chromatography-tandem mass spectrometry (LC-MS/MS) [11,12].

In recent years simplification and increasing automation of sample preparation steps are one of the modern trends in analytical chemistry [13]. The traditional sample preparation methods used for extraction of pesticide residues in food matrices are usually multi-stage procedures and require one or more cleanup steps. Therefore, they are time-consuming, labour-intensive, complicated, expensive and produce considerable amounts of wastes. Quick, easy, cheap, effective, rugged and safe (QuEChERS) method is particularly popular for extraction of pesticide residues in food matrices [14–19] because of its simplicity, inexpensiveness, amenability to high throughput, and relatively high efficiency results with a minimal number of steps. The QuEChERS approach is very flexible and it serves as a template for modification depending on the analyte properties, matrix composition, equipment and analytical technique available in the lab [17]. This technique involves microscale extraction using acetonitrile and purifying the extract using dispersive solid-phase extraction (d-SPE) [19].

Boscalid is a certified pesticide in Turkey and is widely used by farmers in vineyards for protection against Botrytis cinerea. The aim of the study was to investigate the degradation rate of boscalid in respect to the time of sun drying process in pretreated and untreated grapes with the dipping solution.

![Figure 1. Chemical structure of boscalid](image-url)

2. Material and Methods

2.1 Chemicals and reagents

Reference standard of Boscald was purchased from Dr Ehrenstorfer (Augsburg, Germany). QuEChERS extraction kits including anhydrous magnesium sulfate, sodium acetate and primary secondary amine (PSA) were obtained from Agilent Technologies (Wilmington, USA). HPLC grade acetonitrile and glacial acetic acid were supplied by Merck KGaA (Darmstadt, Germany). Acetone was of HPLC grade and obtained from Lab Scan (Sowinski, Poland). Distilled water was deionized by using a Milli-Q system from Millipore (Bedford, MA, USA).
2.2 Preparation of standard solutions
The stock solution of boscalid was prepared by accurately weighing 5 mg (±0.01 mg) of boscalid in volumetric flasks (certified “A” class) and dissolving in 10 mL acetone. This solution was stored in dark at 4 °C. The standard solutions of boscalid used to make the calibration graphs and validation of method were prepared before use by dilution of the calculated volume of stock solution to appropriate volume.

2.3 Instrumentation
GC analyses were performed with a Shimadzu GC-2010 Plus (Shimadzu, Japan) gas chromatography system hyphenated to a MS-QP2010 Ultra (Shimadzu, Japan) mass spectrometer. A Rtx-CLPesticides2 column (20 m x 0.10 mm I.D. and film thickness 0.14 µm) was used. The PTV injector was operated at 250 °C. The ion source and the transfer line temperatures were set at 220 and 250 °C, respectively. The sample (1 µL) was injected in splitless mode. A Rtx-CLPesticides2 column (20 m x 0.10 mm I.D. and film thickness 0.14 µm) was used. The PTV injector was operated at 250 °C. The ion source and the transfer line temperatures were set at 220 and 250 °C, respectively. The sample (1 µL) was injected in splitless mode.

3. Results and Discussion
3.1 Determination, recovery and uncertainty
The linearity of the analysis method was quite good over concentration range of 0.010-0.125 mg kg⁻¹ given a correlation coefficient (R²) of 0.998. The recovery of the method was evaluated by spiking control samples with boscalid at three concentration levels (at low, middle and high concentrations of the calibration graph). The results of the recovery studies are presented in Table 1. Average boscalid recovery results in GC-MS for the QuEChERS spiked at 0.010 mg kg⁻¹, 0.050 mg kg⁻¹ and 0.100 mg kg⁻¹ were 86.1, 74.8 and 100.8% with 0.7, 1.2 and 1.7% RSD respectively (n=3). Similar recoveries have been reported by Lagunas et al. [20].

Table 1. Recovery of boscalid in grape samples
<table>
<thead>
<tr>
<th>Conc. (mg kg⁻¹)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
<th>Rep.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.010</td>
<td>86.1</td>
<td>0.7</td>
<td>3</td>
</tr>
<tr>
<td>0.050</td>
<td>74.8</td>
<td>1.2</td>
<td>3</td>
</tr>
<tr>
<td>0.100</td>
<td>100.8</td>
<td>1.7</td>
<td>3</td>
</tr>
</tbody>
</table>

Conc., concentration; RSD, relative standard deviation and Rep., replicates.
The uncertainty of the method was calculated as 0.100 ± 0.040 mg kg⁻¹. This value was calculated using the bottom-up approach by taking into account all the uncertainty of the both extraction and analysis methods, such as, uncertainty in weighting and pipetting, volume of extraction solvent, repeatability, recovery and calibration graph [21].

The overall combined uncertainty of the used method could be calculated using the following equation:

\[
\frac{U(W_R)}{W_R} = \sqrt{\frac{U(V_{End})^2}{V_{End}}} + \left[ \frac{U(M_{sample})}{M_{sample}} \right]^2 + \left[ \frac{U(C_A)}{C_A} \right]^2 + \left[ U(\text{Rec})/\text{Rec} \right]^2 + U(\text{Repeat})^2
\]

(2.1)

where, \(W_R\) is the amount of boscalid residues in grape samples in mg kg⁻¹; \(C_A\) is the concentration of the boscalid in the sample solution in mg L⁻¹; \(V_{End}\) is the volume of the sample solution in mL; \(M_{sample}\) is the sample mass in g; \(\text{Rec}\) is the recovery [13].

The limit of detection (LOD) and quantification (LOQ) of the method including QuEChERS and GC-MS were calculated as three and ten times the signal-to-noise ratio, respectively. The LOD was 0.77 and LOQ was 2.55 µg kg⁻¹. These values satisfy the MRLs established by the European Commission (EC) and means that the method is sufficiently sensitive. The LOD and LOQ of our method were lower than those obtained by Čuš et al. [22] and Munitz et al. [10]. In addition, Pizzutti et al. [23] developed a more sensitive methodology based on liquid extraction and HPLC-MS/MS detection for soil grains, where the LOD was lower than 0.25 µg kg⁻¹.

### 3.2 Variation of boscalid residue levels

Results of boscalid variation in grape samples are presented in Table 2 and Figure 2. Values reported are means of three parallel samples that were analyzed in triplicate. The initial deposits of boscalid were 0.032±0.013 and 0.016±0.006 mg kg⁻¹ for grapes that were pretreated and untreated with the dipping solution, respectively. Boscalid residue level in grapes which were pretreated with the dipping solution after harvest were higher than those untreated samples. This can be attributed to the accumulation of boscalid in the dipping solution, because of repeated use, and the adsorption of the boscalid on grape surfaces during the dipping pretreatment.

Increases in the level of boscalid were observed due to loss of water until the 7th day of harvest in both grape samples pretreated and untreated with the dipping solution respectively. Boscalid residue level in grapes which were pretreated with the dipping solution after harvest were higher than those untreated samples. This can be attributed to the accumulation of boscalid in the dipping solution, because of repeated use, and the adsorption of the boscalid on grape surfaces during the dipping pretreatment.

Table 2. Variation of boscalid residue levels on grape samples depending on time and dipping solution, (n=3)

<table>
<thead>
<tr>
<th>Days</th>
<th>Boscalid concentration (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grapes pretreated with dipping solution</td>
</tr>
<tr>
<td>At harvest</td>
<td>0.032±0.013</td>
</tr>
<tr>
<td>3rd of harvest</td>
<td>0.037±0.015</td>
</tr>
<tr>
<td>7th of harvest</td>
<td>0.079±0.032</td>
</tr>
<tr>
<td>10th of harvest</td>
<td>0.029±0.012</td>
</tr>
<tr>
<td>180th of harvest (6 months)</td>
<td>&lt;LOD</td>
</tr>
</tbody>
</table>

4. Conclusions

It was found that, boscalid level in grapes began to increase due to loss of water while drying process until the 7th day for both pretreated and untreated grapes with the dipping solution. Boscalid level decreased for a value of 63 and 13% between the 7th and the 10th days of harvest (during the drying period) in grape samples that were pretreated and untreated with the dipping solution, respectively. Higher degradation rate in pretreated grape samples is attributed to the effect of chemical composition of the pretreatment solution on boscalid structure.

It has been discussed in the literature that boscalid undergoes photolytic and metabolic degradation in various me-
of pesticides and their reflection on practices: The case of Kemalpasa, grapes and citrus fruits, Turkey.

After 6 months from harvest, boscalid level in grape samples pretreated with the dipping solution decreases below the limit of detection of the method due to the degradation of boscalid in time. Similar degradation of boscalid was also observed for the grapes which were untreated with the dipping solution, but degradation rate was lower than those pretreated. This result proves that the pretreatment with the dipping solution causes not only accelerate the drying process, but also increases the degradation of boscalid in grapes with the support of sunlight. Merchants who dry grapes should take this into consideration.

Acknowledgments
M. Cittan thanks TÜBİTAK for scholarship (BİDEB 2211-A).

References


