Società Chimica Italiana
Divisione di Spettrometria di Massa

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Florence (Italy) - August 26-31, 2018

ABSTRACT BOOK

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WP-236 / UHPLC-HRMS ANALYSIS OF THEOBROMINE IN COSTA RICAN THEOBROMA CACAO

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Keywords: High Resolution Mass Spectrometry, Costa Rican Cacao, UHPLC-HRMS, Theobromine

Introduction
Costa Rican Cacao has been a staple within Costa Rican culture for many centuries. Within the past 10 years, Costa Rican cacao has made a return for chocolate enthusiasts, helping to provide antioxidants to humans and satisfy sweet cravings. One of the major components of chocolate is Theobromine. Theobromine is a bitter alkaloid/flavonoid beneficial in the treatment of hypertension, arteriosclerosis, and angina pectoris.

Methods
The samples were centrifuged and vortexed numerous times to give a purified product. A three-minute gradient method with a flow rate of 300 µL/min was developed on the UHPLC-HRMS using HPLC-grade water and acetonitrile. Quantitative analysis on Theobromine was completed on the Thermo Scientific UHPLC and LTQ Orbitrap Discovery equipped with an ESI ion source. 2-Hydroxyethyl theophylline was used as the internal standard.

Results
In a previous study from our laboratory, Headspace GC-MS analysis was used to identify flavonoids from Cocoa Beans. Theobromine was the major component found. To give the precision of the theobromine extraction process, the recovery analysis was 86%. Raw, unroasted, roasted, and 100% cacao was analyzed in concentrations of ng/mL. Raw cocoa had a concentration of 586 ng/mL and unroasted cocoa had a concentration of 747 ng/mL. Roasted cocoa had a concentration of 521 ng/mL and 100% cacao had a concentration of 1018 ng/mL. Roasted cocoa had the lowest concentration of theobromine.
Unroasted cocoa had a higher concentration of theobromine prior to roasting cacao. Theobromine could have been lost based on the roasting process or choice of roasting. The 100% cacao chocolate bar had the highest amount of theobromine. Additional theobromine existed and contributed to a more bitter and rich cocoa flavor. Theobromine levels in popular commercially available chocolates such as Lindt, Hershey, Mars, etc will be discussed.

Conclusions
Theobromine levels in popular commercially available chocolates such as Lindt, Hershey, Mars, etc. were determined by UHPLC-HRMS and compared between raw, unroasted, and roasted chocolates.

Novel Aspect
A sensitive and selective UHPLC-HRMS method to quantify Theobromine levels in chocolates.

WP-237 / LC-MS/MS METHOD TO EVALUATE THE PRESENCE OF EXOGENOUS GLYCEROL IN WINES

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Keywords: LC/MS/MS, glycerol, wine, cyclic diglycerols, 3-methoxypropane-1,2-diol

Introduction
Glycerol is a natural constituent of wine being produced by yeast during fermentation and its concentration ranges between 5 and 11 g/L depending on yeast strain. It plays an important role on the sweet taste of

wine [1]. The addition of synthetic glycerol to wines in order to increase this parameter is not allowed by the European oenological legislation [2]. Actually, the strategy to investigate the presence of exogenous glycerol in wine is based on the detection of impurities produced by the industrial processes on its production [3], which are absent in wines. Synthetic glycerol contains the follows impurities: 3-methoxypropane-1,2-diol (3-MPD) and cyclic diglycerols (CycDs) formed during the purification of the rough industrial product conducted through a distillation process. Currently, the determination of these impurities are carried out by an GC/MS method after an extraction step in ethyl ether. As an alternative to this "old" method has been proposed an innovative approach based on liquid chromatography coupled to the mass spectrometry (LC-MS/MS).

Methods

The chemical analysis of 3-methoxypropane-1,2-diol (3-MPD) and cyclic diglycerols (CycDs) has been performed using an Acquity Liquid Chromatographer (UPLC Waters Corporation, Milford, MA, USA) coupled to an Xevo TQ Mass Spectrometer (Waters Corporation, Milford; MA; USA). Chromatographic separation has been performed with an C18 HSS T3 column (2.1 mm x 100 mm, 1.8 µm); flow rate 0.45 ml/min; Eluent A, Water + NH_4Ac (5mM); Eluent B, Methanol. Mass spectrometer was equipped with an electrospray ion source operating in positive ion mode; capillary voltage 1.5 kV; nitrogen gas flow, 1000 L/h; source temperature, 150°C. Acquisition were carried out in MRM (multiple reaction monitoring).

Results

Gas chromatography mass spectrometry (GC-MS) is the analytical method adopted by the OIV to detect the fraudolent addition of glycerol by measure these two contaminants [4]. To the best of our knowledge, this is the only validated method to detect and quantify 3-MPD and CycDs in wine. However, the application of the GC-MS method shows serious difficulties: sample preparation and time analysis (runtime about 42 min); dirty injections with early consumption of the column (particularly in the case of sweet wines); mass spectrometer ion source must be cleaned very often to improve the required sensitivity. In order to overcome these limitations a simple LC-MS/MS method has been developed.

Conclusions

The proposed method could be a useful tool to rapidly screening wine suspected of fraudulent glycerol addition. Keeping GC-MS analysis to further confirm positive results.

Novel Aspect

High throughput LC-MS/MS method respect the official method (using GC/MS) proposed by OIV.

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WP-238 / APPROACHES TO MINIMIZE AND CONTROL EXTERNAL CONTAMINATION DURING SAMPLE HANDLING AND ANALYSIS OF UBIQUITOUS ENVIRONMENTAL PHENOLIC COMPOUNDS IN FOOD SAMPLES

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