Analysis of pesticide residue concentration in exported quality ceylon black tea by GC-MS.

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Abstract

Contamination of food commodities with pesticide residues have become a major concern throughout the world as it causes adverse health effects to the humans. Different regulatory bodies have been established MRLs for pesticide residues, in order to control the health risks. This study distinguishes the selected Ten pesticide's residual contamination of the exported quality Ceylon black tea. The analysis was carried out by the modified QuEChERS extraction method using GC-MS. And the results were compared with the Codex, EU and Japanese MRLs. Tea samples were collected from the major tea exporters in the country based on five tea grades namely OP, BOP, FBOP, BOPF and PEKOE. Method validation was performed by spiking the blank tea samples with two concentrations levels 200 ppb, 500 ppb respectively. [Recovery percentage=[Detected concentration/Spiked concentration] × 100%]. Calculated recovery percentage was 80% -120%. For accurate quantification, representative matrix-spiked calibration curves were applied to compensate matrix effects. The limit of detection for all the targeted pesticides in this study was 0.005 ppm. The results pointed out that exported quality Ceylon black tea are safe from the established limits of Codex, EU and Japanese MRL's for the selected pesticides. The obtained data concludes that among the analyzed 15 samples, some were found with detectable amounts of pesticide residues. But none of the sample exceeded the Maximum Residue Levels established by Codex, EU and Japan. Endosulfan and bifenthrin was the main focus on the study and only one tea grade had residues more than 0.005 ppm. Other than that Chlorpyrifos, Methyl parathion, O,P-DDT were detected and at a much lower frequency. None of the analyzed teas contained residues that may be considered as being detrimental to health.

Keywords: Ceylon black tea, Pesticide residue, QuEChERS method, Maximum residue limits, GC-MS, Extraction.

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Introduction

Tea *Camellia sinensis* is one of the most preferred beverages worldwide especially in Asian countries. According to the recent studies and researches it has been found that tea has been a treasured elixir for thousands of years. Recent research studies are flaking light on why this beverage has earned its reputation for abundant wellness benefits. The prevailing tea lifestyle is evolving as people look to take a moment of respite and explore tea to address a myriad health issues, ranging from weight loss, to stress reduction, osteoporosis and even helps for cancer preventing. The role antioxidants play in the prevention of disease has positioned loose leaf tea as the ideal health beverage [1].

As like other agricultural commodities, tea is also prone to pest's attacks. Each tea growing areas has its own idiosyncratic pests and diseases, though several of them might have been recorded from more than one region [2]. Number of pests and diseases associated with tea plants in an area depends on the duration of time for which it is cultivated in that area. More than one thousand species of arthropod pests and nearly 400 pathogens are known to attack tea all over the world. Crop loss due to pest and diseases varies between 15 and 20%. Magnitude of the losses is bound to be higher today in view of the increased production and productivity besides the variations in climatic conditions. Therefore, pesticides [insecticides, fungicides] used to reduce the severity of infestation [3]. Pest management in tea largely depends on the use of broad-spectrum, synthetic chemical pesticides, including organophosphates, carbamates, synthetic pyrethroids, neonicotinoids and zimidazoles [4].

Manufacturing process of tea has a greater effect on pesticides. Black tea manufacturing process involves leaf harvesting, withering, rolling, fermentation and drying. No significant reduction in residue level resulted from the rolling and fermentation steps in black tea. Residue levels are reduced during final drying [5]. In addition, pesticide levels decrease due to the natural factors, including rainfall, dew, volatilization, airflow, photolysis, biodegradation, moisture, pH and growth dilution [6] and the pre-harvest interval between the last application and harvesting [7]. Recent study shows that pesticide residues were likely transferred from tealeaves to brewed tea during the brewing process, and may therefore pose a risk to consumers.

As pesticides cause adverse health effects to the human beings, regulatory authorities set limits for pesticides in agricultural products to minimize the impact. Primarily they aim to maintain consumer safety through defined sets of limits and regulations, but these regulations could have a trade deterring effect when exporters cannot comply with the standards (Table 1).

Table 1. *Maximum residue levels of analyzed pesticides.*

Compound	**MRL (mg/Kg)**		
	CODEX	**EU**	**JAPAN**
Bifenthrin	30	30	25
Chlorpyrifos	2	0.15*	10
Cypermethrin	20 (15*)	0.5	20
Endosulfan sulfate	10	30	30
Fenitrothion	NA	0.05	0.2
Malathion	NA	0.05	NA
Methidathion	0.5	0.1*	1
Methyl parathion	NA	0.05	0.2
Profenofos	0.5	0.5	0.2
O, P-DDT	NA	NA	0.2
*Updated MRLs			

Table 2. *Optimized conditions for the injection and GC analysis of the pesticides.*

GC-MS method parameters			
Column	DB1701capillary column (30 m × 0.25 m × 0.25 µm)		
Injection source	GC ALS		
Inlet pressure	13.064 psi		
Inlet temperature	Initial 250°C		
Injection mode	Split less		
Injection volume/ type	3 µl/Standard		
Septum purge flow	3 ml/min		
Purge flow to spilt vent	50 ml/min at 1.5min		
Gas saver	20 ml/min after 5 min		
Oven temperature program	Rate	Value	Hold Time
Initial	0°C/min	50°C	1 min
Ramp 1	25°C/min	125°C	0 min
Ramp 2	10°C/min	300°C	10 min
Run Time	31.5 min	--	--

Due to these legal regulations, analysis of pesticide has become mandatory. Different analytical techniques have been developed to fill the gap. QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) is one of the efficient pesticide analysis technique [8].

QuEChERS method covers a very wide scope of analytes, including polar, semi-polar and non-polar pesticide residues in various food matrices. The procedure involves initial single-phase extraction of the sample with acetonitrile, followed by liquid–liquid partitioning by the addition of anhydrous magnesium sulfate $MgSO_4$ and Sodium Chloride. Removal of water and clean up was perform simultaneously on an aliquot of the acetonitrile: toluene extract with dispersive solid phase extraction [9].

Objectives

Pesticides always pose a threat to human health and nowadays people pay more attention on what they consumed. The purpose of this study was to determine the pesticide residual concentration in exported quality Ceylon black tea and ensure the quality of the product by comparing the results with codex, Eu and Japanese maximum residue limits. Selected grades of exported quality Ceylon black tea used for this analysis. These results would provide information to customers that the Ceylon black tea is a safe product to consume.

Methods

Sample collection for the analysis

Five pure black tea grades namely, OP, BOP, FBOP, BOPF and PEKOE were selected based on the demand of the export market. Samples were collected from five top leading tea exporters in Sri Lanka as per Export Development Board report 2016.

Chemicals and solutions

Pesticide analytical standards, all 95% or higher purity, were obtained Chemservice (West Chester, PA) Each pesticide standard contained 100 ppm. Each standard was transferred to amber color 10 ml volumetric flask and diluted up to the mark. Concentration of the prepared pesticide solution was 10ppm. Similarly 10 individual pesticides stock standards were prepared and stored in 0-4°C. Matrix spiked calibration curves at five levels of 50 ppb, 100 ppb, 200 ppb, 500 ppb and 1000 ppb were prepared by addition of 25 µl, 50 µl, 100 µl, 250 µl, and 500 µl of 10-ppm stock solution respectively, to 5.0000 g of blank tea samples. Analyzed pesticides were divided into two mixes. Appropriate aliquots of the individual stock solution of each were dilute with MeCN to prepare a mixed stock solution for GC-MS experiments. MeCN and toluene were high purity grade solvents for pesticide residue analysis from Sigma-Aldrich (St. Louis, MO, USA). Anhydrous $MgSO_4$ and NaOAc from Sigma-Aldrich and were analytical grade. The $MgSO_4$ and NaOAc was dried at 500°C for >5 h to remove phthalates and residual water. Dispersive-SPE cartridges-Cleanert TPT from AGELA Technologies. In addition, internal standard was from Chemservice (West Chester, PA).

Sample preparation

QuECHERS method with cleanup modifications from AOAC 2014 was used for sample preparation. Weighed 5 g homogenized dry tea powder (accurately to 0.01 g) into a 50 ml Fluorinated ethylene propylene (FEP) centrifuge tube. 10 ml of water was added to hydrate the tea powder. 10 mL acetonitrile [containing 1% acetic acid],also added and vortex for 3 min. 1.5 g anhydrous Sodium acetate [CH_3COONa] and 4 g, anhydrous Magnesium Sulphate ($MgSO_4$) was added and vortex mixer was applied for mixing along 1 min. The tube was cooled in an ice-water bath immediately, for 5 min. The mixture was centrifuged for 5 min at 5000 r min-1. Placed a pear-shaped flask and mount the Cleanert TPT cartridge above the pear-shaped flask. Anhydrous Sodium Sulphate, was added approximately 2 cm onto the cleanert TPT packing material. 10 ml Acetonitrile: Toluene [3+1, v/v] was added to activate the cartridge and Eluted the solvents. Transferred 1 mL from the extracted solution to the column. Eluted the column with 20 mL Acetonitrile/toluene [3:1]. Added 40 µl heptachlor epoxide [internal standard; ISTD] Evaporated to dryness under a stream of nitrogen in a 35°C water bath. Dissolved the dried residue in 1.5 ml hexane, ultra-sonicated the sample to mix, and filter through a 0.2 µm membrane filter. The sample was subjected to GC/MS analysis [10].

For recovery fortitude, spiked blank tea samples at concentration levels of 50 ng/g, 1000 ng/g were prepared in triplicates and kept for one hour at ambient temperature prior to use and prepared the sample as described. The recovery percentages were calculated using the calibration curves obtained from the spiked samples.

Instrumentation

The study was performed using a gas chromatograph Agillent 7890A GC (Agilent Technologies) coupled to a mass spectrometer 5975C (Agilent Technologies). The GC-MS was applied with high purity helium as carrier gas at a flow rate of 1.5429 ml/min. Optimized condition for the analysis are summarized in Table 2. ChemStation quantitative analysis software [Agilent Technologies] was used for data processing. The concentration of the analyzed pesticides were determined by interpolation of the relative peak areas for each pesticide to internal standard peak area in the sample on the spiked calibration curve.

Analysis was performed in the SIM mode based on the use of one target ion and two or three qualifier ions. Analyzed pesticides were recognized according to their retention times and target and qualifier ions. Table 3 shows the retention time and target and qualifier ions of the studied pesticides (Figure 1).

Results and Discussion

Method validation

Linearity: All pesticide shows linearity in SIM mode. Linear spiked calibration curves for all the tested pesticides were obtained with >0.995.

Limit of detection and limit of quantification: Limit of detection of the method and Limit of Quantification were measured in spiked samples and calculated by considering a value 3 and 10 times that of background noise. For all the tested pesticides, LOD value was 0.005 mg/l and LOQ was 0.015 mg/l.

Recovery: The accuracy of the method was determined by recovery experiments. Recovery of an analyte was obtained from the known concentration added to the tea sample blank and recovered from the sample blank. To evaluate the recovery concentration, spiked the tea sample with two different concentration levels in triplicates. Mean recoveries for initial validation in the range of 70-120% [11]. Recovery percentages for the analyzed pesticides were on between 70% -120%. Table 4 presents the obtained recoveries and linearity's for tested pesticides.

Pesticide residues in real samples

Tea is cultivated in about 36 countries all over the world, but production is heavily concentrated in just a handful. In 2000, five countries (India, China, Sri Lanka, Kenya and Indonesia) together produced almost 80% of the world's tea [12]. Tea provides numerous health benefits. Nowadays, people not only require better quality of tea, but also pay attention to the harmful substances in it [13]. Since tea is susceptible to suffer from pests and diseases, farmers usually spray pesticides to reduce the adverse effects, which results in pesticide residues [14,15]. The level of pesticide residues in tea brew is directly related to the intake amount of human consumed.

Due to the fact, responsible regulatory bodies have established MRL's for pesticide to control the pesticide usage. There are some methods and strategies for control and monitoring related pesticides, which used in tea. Some studies focused on evaluation of a variety of solvents for extraction, such as acetonitrile, methanol, isopropanol, and multiple d-SPE cleanup methods, including C18, PSA, GCB, $MgSO_4$, and their combinations for improvement of pesticide analysis method in tea [11]. The major source of inaccuracy in pesticide residue analysis by GC-MS, especially in food, is related to the presence of interfering components in the sample and it is called as "matrix effect" [16]. In this current study, spiked calibration curves used to overcome the matrix effect. Calibration curves are prepared by the addition of standard solution to the blank tea samples and subjected to the same sample preparation and extraction procedure, which is envisioned to be used for the unknown samples.

Randomly selected five samples from each grade mixed and made a composite sample. The composite sample was analyzed for seven replicates to determine pesticide residues for selected ten pesticides. The collected data were analyzed using Minitab (16 version) statistical software packages. One-way ANOVA used to analyses the significant differences of mean values of each sample, followed to descriptively analyze the samples that had significant difference from control sample. All test procedures were made at 5% significant level. Mean values obtained from the data analysis has compared with the Codex, EU, and Japanese MRL's to prove that the residue levels are lower than the established levels. Table 5 shows, that all the tested samples contained pesticide residues below MRLs. Major concern of these study was concentrations of bifenthrin and endosulfan in selected tea grades. Obtained results shows that none of the tea grade contained residues, which can cause adverse health effects. According to the analysis results, maximum allowed residue limit of Bifenthrin is 30 ppm in both Codex and EU regulation. In all the tested tea grades 0.044 ± 0.05 ppm of Bifenthrin is the maximum detected pesticide residual content and this value is below the maximum residual levels as per the above three standards. 0.074 ± 0.05 ppm of Chlorpyrifos

Table 3. *Target ion, qualifier ions and the retention times of the studied pesticides.*

Compound	Diagnostic ions (m/z)			Quantification ion (m/z)	Retention time, min
Heptachlor epoxide	353	272	237	353	22.150
Bifenthrin	181	165	166	181	18.010
Cypermethrin	207	77	181	207	20.511
Endosulfan sulfate	272	389	274	272	17.123
Chlorpyrifos	199	97	197	97	13.836
Fenitrothion	125	109	277	109	13.341
Malathion	125	93	173	121	13.621
Methidathion	145	85	125	145	14.648
Methyl parathion	109	263	125	263	12.950
Profenofos	208	97	139	208	12.475
O, P-DDT	235	165	236	235	16.320

Table 4. *Recovery percentage for tested pesticides.*

Compound	% Recovery at 50 ppb	% Recovery at 1000 ppb	RSD/CF
Bifenthrin	79.23	83.26	1.0000
Chlorpyrifos	80.11	108.89	0.9991
Cypermethrin	79.55	94.22	1.0000
Endosulfan Sulphate	82.32	103.58	1.0000
Fenitrothion	79.48	97.88	0.9990
Malathion	81.36	103.25	1.0000
Methidathion	81.88	103.48	1.0000
Methyl parathion	80.33	96.37	1.0000
Profenofos	79.39	92.88	1.0000
O,P –DDT	80.14	93.22	1.0000

Table 5. *Descriptive analysis in tested tea samples.*

Type of Pesticide	Tea Grade	Average pesticide residue concentration(mg/l)	Type of Pesticide	Tea Grade	Average pesticide residue concentration(mg/l)
Residue			Residue		
Bifenthrin	OP	0.041 ± 0.05	Malathion	OP	0.019 ± 0.03
	PEKOE	0.044 ± 0.05		PEKOE	0
	BOP	0.010 ± 0.03		BOP	0
	BOPF	0.022 ± 0.04		BOPF	0
	FBOP	0.018 ± 0.05		FBOP	0
Chlorpyrifos	OP	0.042 ± 0.04	Methidathion	OP	0.049 ± 0.05
	PEKOE	0.067 ± 0.05		PEKOE	0
	BOP	0.039 ± 0.04		BOP	0.069 ± 0.05
	BOPF	0		BOPF	0
	FBOP	0.074 ± 0.05		FBOP	0
Cypermethrin	OP	0	Methyl Parathion	OP	0.038 ± 0.05
	PEKOE	0		PEKOE	0.051 ± 0.05
	BOP	0		BOP	0.027 ± 0.05
	BOPF	0.005 ± 0.02		BOPF	0.067 ± 0.05
	FBOP	0		FBOP	0
Endosulfan Sulfate	OP	0.014 ± 0.02	Profenofos	OP	0.002 ± 0.003
	PEKOE	0.014 ± 0.02		PEKOE	0
	BOP	0.034 ± 0.03		BOP	0
	BOPF	0.033 ± 0.03		BOPF	0
	FBOP	0		FBOP	0
Fenitrothion	OP	0.018 ± 0.02	O, P-DDT	OP	0.002 ± 0.04
	PEKOE	0		PEKOE	0.036 ± 0.05
	BOP	0		BOP	0
	BOPF	0		BOPF	0.007 ± 0.03
	FBOP	0		FBOP	0.036 ± 0.05

is the recorded highest residue concentration in tested tea grades and this is below than the EU commission Maximum Residue Limit of 0.15 ppm as this is the lowest allowed limit out of three standards. Cypermethrin 0.5 ppm is the lowest allowable limit of pesticide residue as per three standard and recorded pesticide residue content of tested tea samples is 0.005 ± 0.02 ppm, and below all the international standards. As per Codex standard 10 ppm of Endosulfan sulfate is the minimum level of pesticide residue out of three international standards for Maximum Residue Limits and recorded maximum pesticide level of tested tea samples is 0.034 ± 0.03 ppm. Fenitrothion 0.018 ± 0.02 ppm is the maximum detected limit in tested tea grades and this limit is below 0.05 ppm of maximum residue limit as per EU commission regulation.

For Malathion, the maximum residue limit of 0.05 ppm has

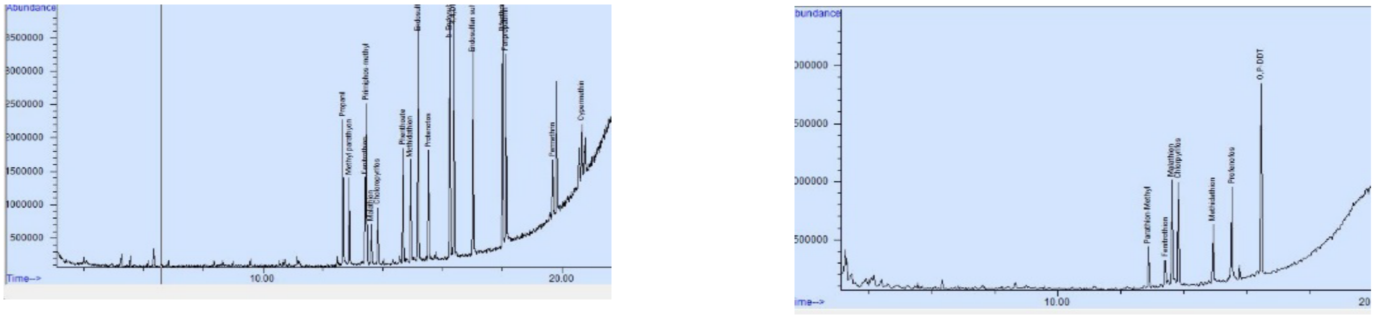

Figure 1. A representative chromatogram obtained for the analyzed pesticide mix 01 and Mix 02.

given only by EU commission regulation and the detected limit of pesticide residue limits is 0.019 ± 0.03 ppm, which is below the given MRL.

When considering Methidathion, Methyl parathion, Profenofos and o,p-DDT maximum residue limits out of three considered international standards are 0.1 ppm, 0.05 ppm, 0.2 ppm and 0.2 ppm and maximum detected pesticide residuals in tested tea grades are 0.069 ± 0.05 ppm, 0.067 ± 0.05 ppm, 0.002 ± 0.003 ppm and 0.036 ± 0.05 ppm respectively. This confirms that the detected pesticide residual levels are below the maximum residue limits as per above three international standards. According to the study for the determination of endosulfan and bifenthrin in Ceylon tea, they have concluded that detectable amounts of α-endosulfan, β-endosulfan and bifenthrine residues were not observed [17]. The study Houab et al. [18] conducted to monitoring of some pesticide's residue in consumed tea in Tehran market, concluded that, consumed tea in Iran are free from pesticide residues or lower than established MRLs. Analysis of 53 samples in 2011 showed the presence of endosulfan sulfate in 18.9% of the samples with residues ranged from 5-20 µg/kg and the presence of bifenthrin in 9.4% of the samples with residues ranged from 5-35 µg/kg, these contaminated samples were lower than MRL established by regulation authorities from India and England. This study includes samples from Sri Lankan origin too. A large-scale survey of teas produced in the south Indian tea factories had been examined for the pesticide residues such as dicofol, ethion, quinalphos, hexaconazole, fenpropathrin, fenvalerate and propargite. The results obtained from the survey proved that only less than 0.5% of tea samples contained the above pesticides but the residue levels were lower than the maximum residue limits set by the European Union, Codex Alimentarius Commission of FAO/WHO and Prevention of Food Adulteration Act of Govt. of India [19]. According to the study, none of the detected pesticides does not exceed the maximum residue levels set by EU, Codex and Japan.

Conclusion

According to the study, it is inevitable that the pesticide residual contamination in Ceylon black tea is below than the Maximum Residue Levels set by the Eu, Codex and Japan.

Bifenthrin and Endosulfan were the targeted two pesticides among the selected ten. But in each tea grade both the bifenthrin and endosulafan concentrations did not exceed 0.04 ppm. And their MRL's are much higher in Eu, Codex and Japan.

Results have shown that the residual concentrations of profenofos, fenitrothion, malathion and methidathion are zero in almost all samples. Cypermethrin and chlorpyrifos were detected at very low concentrations. However, all the detected pesticides were below the Maximum Residue Levels. Results obtained from this study make sense that, Ceylon black tea is a safe product for consumption.

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