

# Comparison of the methods for determination of pesticide residue in fruits and vegetables in the locations of India and Costa Rica

## Comparación de los métodos para la determinación de plaguicida residual en frutas y vegetales en India y Costa Rica

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## Keywords

Determination of pesticide residue; Quechers; National Institute of Occupational Health; Servicio Fitosanitario del Estado; Instituto Tecnológico de Costa Rica; India; Costa Rica.

## Abstract

This study is a comparison of the methods followed for the determination of pesticide residue in fruits and vegetables in India and Costa Rica. The procedures followed are in both cases the QuEChERS method for which the National Institute of Occupational Health, Ahmedabad executes it and in the case of Costa Rica the Servicio Fitosanitario del Estado laboratory is the one in charge to carry it out.

## Palabras clave

Determinación de plaguicida residual; Quechers; National Institute of Occupational Health; Servicio Fitosanitario del Estado; Instituto Tecnológico de Costa Rica; India; Costa Rica.

## Resumen

Este artículo corresponde a la comparación de los métodos seguidos para la determinación de plaguicida residual en frutas y vegetales tanto en India como en Costa Rica. Tanto en el National Institute of Occupational Health, Ahmedabad en India y el encargado de realizar dicho procedimiento en Costa Rica, el Laboratorio del Servicio Fitosanitario del Estado, utilizan el procedimiento llamado QuEChERS.

## Introduction

The Instituto Tecnológico de Costa Rica (known as ITCR or TEC) is one of the four public universities in Costa Rica, located in Cartago Province. One of the budgets handled by this university finances the program Movilidad Estudiantil which has as a main goal the internationalization of personnel and students. For this goal, they sponsor every year students of different majors to go abroad and participate of academic experiences as per the requirements of the program [1].

For the year of 2016, the school of Ingeniería en Seguridad Laboral e Higiene Ambiental, approved the visit to the National Institute of Occupational Health (NIOH), Ahmedabad, India. The student executed an internship based in the participation of the on-going activities of the NIOH.

The National Institute of Occupational Health (NIOH), Ahmedabad is the premier institute under the aegis of the Indian Council of Medical Research (ICMR) of the Ministry of Health and Family Welfare of India's Government. It was during 1945 where the Industrial Health Advisory Committee (IHAC) was set up due to the need of research in the field of Occupational Health. Then, in 1947 the Industrial Health Research Unit was created at the Institute of Hygiene and Public Health in Calcutta. In 1966 that the ICMR, New Delhi, established the Institute at the Medical College in Ahmedabad as the Occupational Health Research Institute (OHRI) and in the 1970 was given the name of National Institute of Occupational Health. Also, two Regional Occupational Health Centers (ROHC) were established at Bangalore (1977) and Calcutta (1980). The National Institute of Occupational Health, Ahmedabad, has the objective of promoting research for evaluation of environmental stress/factors at the workplace; promote

highest quality of occupational health through fundamental and applied research and the development of control technologies and programs in the field of health through research and to generate human resources in the field [2].

According to the Department of Agriculture Cooperation and Farmers Welfare [3] they are regularly monitoring the presence and levels of pesticide residues in food commodities and environmental samples under the central sector scheme, "Monitoring of Pesticide Residues at National Level". The scheme was initiated during 2005-06 and is being implemented through Indian Agricultural Research Institute, New Delhi and has 23 participating laboratories from the Ministry of Agriculture, Indian Council of Agriculture Research, Ministry of Health and Family Welfare, Ministry of Environment and Forest, Council of Scientific and Industrial Research, Ministry of Chemical and Fertilizers, Ministry of Commerce and State Agricultural Universities. The Ministry of Agriculture and Farmers Welfare, India, under the scheme "Monitoring of Pesticide Residues at National Level" regularly monitors the presence and level of pesticide residues, including food commodities. It was initiated in 2005-06 and implemented through a wide list of laboratories, which includes the Pesticide Toxicology Division of the National Institute of Occupational Health, Ahmedabad.

Moreover, down in Costa Rica, the Phytosanitary Service of the State (*Servicio Fitosanitario del Estado*) of Costa Rica, a dependency of the Ministry of Agriculture and Livestock of Costa Rica, is in charge of monitoring pesticide residue in vegetables and fruits and in other commodities as well. According to *Ley de Protección Fitosanitaria No. 7664* (Phytosanitary Protection Law) is part of their responsibilities the protection and regulation of the vegetables and fruits as it is said in chapter 2, section e, chapter 5 section o and chapter 36 [4]. This is the reason why this dependency is in charge of monitoring the pesticide residue in vegetables and fruits.

## Methos

The following paper presents one of different activities carried on the National Institute of Occupational Health, Ahmedabad, India, as part of an internship by one student of Instituto Tecnológico de Costa Rica. This visit was sponsored in great part by the Instituto Tecnológico de Costa Rica. As well, the Indian Council of Medical Research, in India, approved the internship to be executed in this institute located in Ahmedabad, India.

One of the activities carried out in the internship was the visit in the Pesticide Unit and the study of the QuEChERS procedure for the determination of pesticide residue in fruits and vegetables. The whole procedure was taught by qualified personnel of the laboratory, all were conducted visits to the laboratories and practical experiences were also executed.

Moreover, once back in Costa Rica, it was coordinated a visit to the Servicio Fitosanitario del Estado (Phytosanitary Service of the State) laboratory. According to Costa Rican Law, this is the laboratory certified to coordinate the determination of pesticide residue in fruits and vegetables and approve other laboratories to execute this activity as well. The observational visits coordinated to this laboratory were all conducted by qualified personnel of the laboratory.

## Results

### Determination of Pesticide Residues in Fruits and Vegetables at the National Institute of Occupational Health, Ahmedabad

During the visit to NIOH, Ahmedabad, it was shown one of the on-going projects in the field of pesticide exposure. For this project it is followed different Standard Operating Procedure (SOP). The method followed in the procedures for the determination of pesticide residues in fruits and vegetables is QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe).

The QuEChERS method uses in one single step buffered Acetonitrile extraction and salting out liquid-liquid partitioning from water in sample using  $MgSO_4$ . Dispersive solid phase extraction (Dispersive SPE) cleanup is performed using Primary Secondary Amine (PSA) and  $MgSO_4$ , which allows the removal of organic acids, water (in excess) and other compounds. Later, after chromatographic analytical separation is performed, the extracts are analyzed by mass spectrophotometry (MS) techniques.

Table 1 brings a summary of every step carried out following the QuEChERS procedure, requirements and other details.

#### Sampling procedure

Different vegetables and fruits are collected. The vegetables collected are: Brinjal, Cabbage, Cauliflower, Capsicum, Green Chilli, Tomato, Okra (Ladies Fingers), Cucumber, Bittergard, Green Peas. The fruits collected include: Apple, Banana, Grapes, Pomegranate, Sweet Orange, Guava, Pineapple, Mango and Chiko.

The time, weight and temperature ( $^{\circ}C$ ) of environment is recorded. For collection, one pair of clean gloves is worn. The commodities are kept in separate zip bags, for avoiding contamination.

#### Sampling points

Every month, one kilogram of every sample is collected from different markets from locations as per the project plan (locations may vary). Every location has a data sheet and sample labels. The samples collected are identified with location and sample ID. The information of each location recorded includes: Date, Time, Personnel (person going to the field), Weather conditions, Sample identification number used, Deviation if sample is not collected and reason, Description of any deviation to the collection point Vegetable Sampling Plan and reason of Deviation, Samples taken from vendors with visible staining or other indications of non-homogenous conditions

#### Transport and storage

The transport and storage of the commodities must be done at  $4^{\circ}C$ .

#### Sample Analysis Procedure: QuEChERS

The use of mask, head cover, lab coat and gloves is mandatory as part of the Personal Protective Equipment (PPE) worn during performance of the whole procedure.

Table 1. Details of the QuEChERS procedure used at NIOH, Ahmedabad

Step	QuEChERS step	Requirements		Reagents purpose	Details
		Equipment	Solvents and Absorbance		
1	Homogenize the commodities to get a uniform sample representative of the product.	Knife Chopping tab Container Blender (household type)			<b>Commodities chopping:</b> is performed using the knife and table tab to chop all the commodities, after they are kept into the containers. Later, they are mixed and blended using the blender.
2	Weight 15 grams of homogenized product into a clean 50mL tube and in deep freeze overnight.	Weighing analytical balance 50 mL tubes Fridge			<b>Weighing analytical balance:</b> Is used for weighing the 15 grams of product in the tubes. <b>Fridge:</b> is used to deep in freeze the weighted sample over night.
3	Add 15 mL of 1% Acetic Acid in Acetonitrile (v/v).	Pipette	15 mL of 1% Acetic Acid in Acetonitrile (v/v)	Use of Acetic Acid: the homogenization is also performed by adding the Acetic Acid in Acetonitrile. The Acetic Acid is used to adjust pH, as well.	<b>Pipette: Eppendorf</b> pipette is used to make up the 15mL volume required
4	Further add 6.0 grams MgSO <sub>4</sub> and 1.5 grams Sodium Acetate Anhy. Shake vigorously for 1.0 min by hand.	Weighing analytical balance	6.0 grams Magnesium Sulfate (MgSO <sub>4</sub> ) 1.5 grams Sodium Acetate Anhy	Use of Magnesium Sulfate (MgSO <sub>4</sub> ): facilitates solvent portioning and improves recovery of polar analytes. <b>Use of Sodium Acetate Anhy:</b> The addition is done for the extraction of pesticide residue.	<b>Weighing analytical balance:</b> Is used for weighing solid reagents.

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Step	QuEChERS step	Requirements		Reagents purpose	Details
		Equipment	Solvents and Absorbance		
5	Centrifuge for 1.0 min at > 1500 RCF to separate the solid material. Clean up.	Centrifuge			<b>Centrifuge:</b> is carried on using a Centrifuge at 1500 RCF <sup>1</sup> for separating purposes.
6	<b>Clean up:</b> Steps 6 and 7 are performed for clean up purpose. Is important to clean up the sample so interferences are reduced and analytical instrumentation is not damaged. Transfer 6.0 mL supernatant to the tube containing 300mg PSA <sup>2</sup> + 900mg Magnesium Sulfate (MgSO <sub>4</sub> ). Shake vigorously for 30 seconds.	Pipette 50mL tubes	300mg PSA <sup>2</sup> 900 mg Magnesium Sulfate (MgSO <sub>4</sub> )	<b>Use of PSA<sup>2</sup>:</b> removes sugar, fatty acids, organic acids, lipids and some pigments.  <b>Use of Magnesium Sulfate (MgSO<sub>4</sub>):</b> Removes water from organic phase.	<b>Pipette: Eppendorf</b> pipette is used to make up the 6.0mL volume of supernatant required.
7	Centrifuge for 1.0 min at > 1500 RCF <sup>1</sup> to separate the solid material.	Centrifuge			<b>Centrifuge:</b> is carried on using a Centrifuge at 1500 RCF <sup>1</sup> for separating purposes.
8	Withdraw the 2.0 mL supernatant and evaporate to dryness and makeup to 2.0 mL with Hexane: Acetone (3:1) for FTD/NPD).	Pipette N <sub>2</sub> evaporator Vials	Hexane: Acetone (3:1)	<b>Use of Hex: Acetone (3:1):</b> it is used as a solvent and also for injecting purposes.	<b>Pipette: Eppendorf</b> pipette is used to make up the 2.0mL volume of sample and poured into the vials for the analysis.
9	For ECD/FPD/MS - Inject directly without solvent transfer.				

1 RCF: Relative Centrifugal Force

2 Primary Secondary Amine

**Table 2.** QuEChERS procedure used at Phytosanitary Service State Laboratory, Costa Rica

No. of the step	Details of the steps
1	The samples are carried inside a safety bag with a mark and an unambiguous identification number.
2	The sample is reviewed by laboratory personnel so that it meets the entry specifications.
3	It is entered into the system (developed specifically for the laboratory); it is identified with a unique number for each sample and labeled.
4	The sample is delivered, filling the respective register in order to continue the homogenization step.
5	Check mark is removed that ensures integrity.
6	Quartets the sample to reduce its size, if necessary. Then sample sections are taken. The sample must be completely homogenized with the help of mills.
7	The respective record is filled
8	A security tag is placed on counter sample Type B. The type B counter sample is stored in refrigeration at -20°C for one month, in case the user requests that his analysis be repeated.
9	The type A sample and registration are delivered to the personnel in charge of the extraction process by QuEChERS, where the pesticides are separated from the matrix.
10	Extraction process begins: The sample is weighed and immediately stored in refrigeration at 4°C, which is discarded once the results report is issued
11	The sample is extracted with 1% acidified acetonitrile and shaken manually for 10 minutes and then automatically shaken for 10 minutes more.
12	A package of salts of the QuEChERS kit (magnesium sulphate + sodium acetate) is added.
13	The sample is shaken manually for 1 minute and then centrifuged at 4500 min <sup>-1</sup> for 5 minutes
14	In the already centrifuged sample the phase separation (analyte-matrix-water and impurities) is clearly observed.
15	A portion of the supernatant is taken to another tube containing the PSA salts and magnesium sulfate, stirred 1 minute again.
16	Centrifuge at 4500-1 for 5 minutes.
17	From the extract portion is taken and filtered through a membrane of maximum porosity of 0.45µm, collecting the filtrate in a properly labeled 2mL vial.
18	A portion is taken for liquid chromatography. Another portion is taken for chromatography in which 30µL of the internal standard ParathionD10 and 30µL of the mixture of protective analytes (Glucolactone and Sorbitol) are added to the vial.
19	The gas chromatography vial is stirred for about 1 minute on the automatic stirrer.
20	The respective record is filled.
21	The vials are delivered to the personnel in charge of the sample injection in each equipment (LC-MS/MS and GC-MS/MS).
22	Samples are injected and data analysis is performed, then results are reported.
23	The personnel in charge enters the data in the system to generate the laboratory report.
24	System where official reports are made by the laboratory.
25	The completed analysis report is submitted to the head office, who reviews and approved it.
26	The final official analysis report is submitted to the Secretariat of the Residues Control Unit.

### **Possibility of application of the NIOH on-going project in Costa Rica**

The “*Monitoring of Pesticides Residues at National Level*” project is a great example of monitoring the exposure to pesticides for workers and for general population.

The development of a project of this much extension, can be a great contribution to data collection, and after this will be helpful for professionals, institutions and authorities to support their decisions and research.

### **Determination of Pesticide Residues in Fruits and Vegetables in Costa Rica**

Table 2 shows the procedure followed by the Phytosanitary Service of the State Laboratory.

## **Conclusions**

### **General differences and similarities of the QuEChERS procedure used in the National Institute of Occupational Health in India and the Phytosanitary Service of the State Laboratory in Costa Rica**

First of all, both of the monitoring programs are conducted by Governmental Institutions, in which laboratories of the dependencies of Ministries are the responsible of this kind of study in fruits and vegetables. In the case of India, the Pesticide Toxicology Unit of National Institute of Occupational Health is a collaborating laboratory which belongs to the Ministry of Health and Welfare of India. In the case of Costa Rica, the Phytosanitary Service of the State belongs to the Ministry of Agriculture and Livestock of Costa Rica.

These two institutions have this activity in a form of a program, which is conducted every certain period of time. In the case of India, every month the samples are collected. In the case of Costa Rica, the samples are collected every day.

Both of the laboratories carry out the pesticide residue monitoring using the QuEChERS procedure, which is worldwide well known and somehow is a very standardized methodology.

One of the main differences in the sampling is the collection of fruits and vegetables. In India, the bags used are common plastic bags which are given in the sampling points. In Costa Rica, the bags are made out with special material which in case the sample is not carried in this bag, they will not be accepted. Also the bags must be completely sealed and labeled, if not the laboratory will not proceed with the monitoring as well.

The NIOH laboratory is focused only into fruits and vegetables specified in the sampling procedure section of this document. In the case of Costa Rica, the fruits and vegetables include a wide and extended list, also other food products like grains and seeds.

In the case of the laboratories equipment and amenities, they all are very similar. Variations may be in the kind of equipment, models or way of using. Nevertheless, the purposes for all of them are the same, for example: to homogenize, to centrifuge, to shake, others.

In general terms, methods, amenities and the QuEChERS procedure is performed in a very similar way.

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