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Food-O4

Removal of Some Pesticide Residues From Capia Peppers by Different Washing Treatments

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OVERVIEW

What is RAC and PAC (OECD,2008)

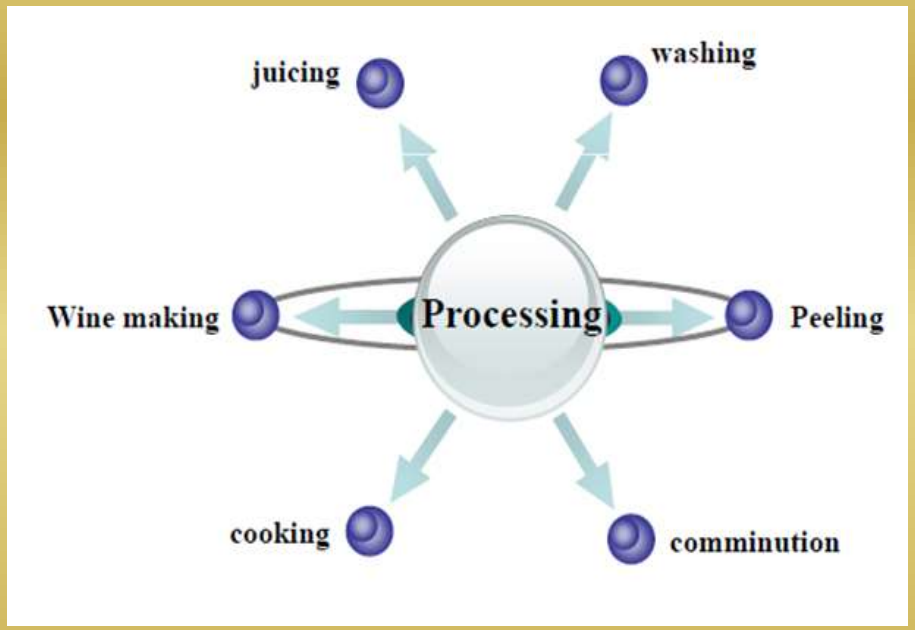
- ✓ **Raw Agricultural Commodity (RAC):** Any agricultural product in natural state, including all fruits that are unpeeled natural form prior to marketing.

- ✓ **Processed Agricultural Commodity (PAC):** Any agricultural commodity that has been subjected to processing, like tomato paste and orange juice.

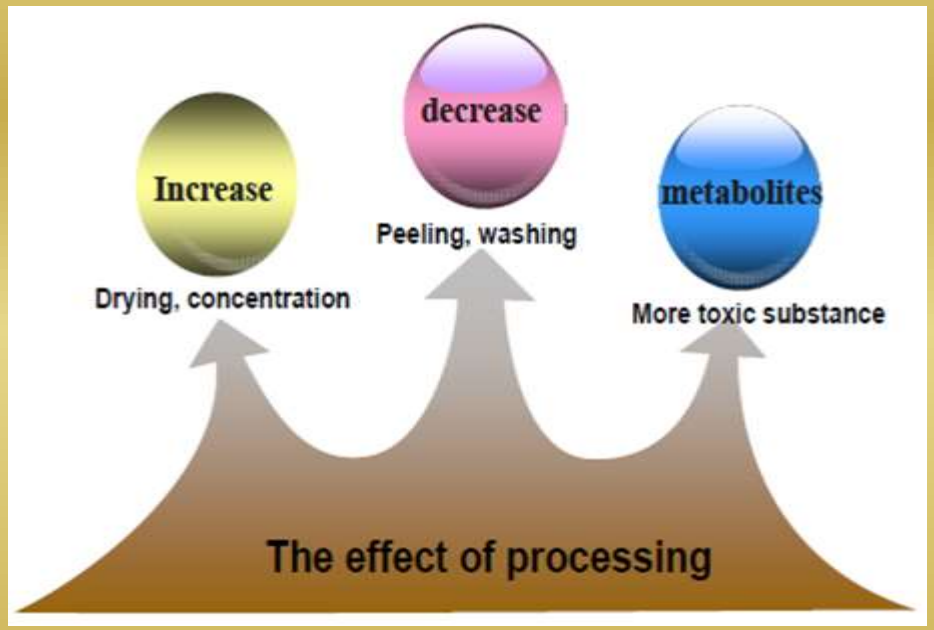
Why do we consume PAC?

- ✓ Perishable **RAC** can be transformed to value-added product by processing
- ✓ Residues are important for PAC. MRL regulations should be set for PAC

Some Processes Affecting Pesticide Residues



Three effects of food processing (Dong, 2012)



Decreasing or increasing pesticide residues in PAC is depend on;

- ✓ processing type,
- ✓ pesticide type **(i.e.,mechanism of action; systemic/contact)**,
- ✓ commodity type.

What is processing factor ?

(OECD, 2008; Bonnechère et al. 2012)

Processing Factor (PF) ; It is the ratio between a residue concentration in the processed commodity and the raw commodity,

PFs is calculated for any process by using this equation:

$$PF = \frac{\text{Residue level in processed commodity (mg/kg)}}{\text{Residue level in the raw commodity (mg/kg)}}$$

IF *(Kong et al, 2012);*

PF<1 reduction factor	indicates the reduction of a pesticide in processed product
PF>1 concentration factor	indicates a concentration in processed product.
PF= 1	No change in the processed food

MRL for Processed products

Pf ≤1 ;

- ✓ According to EU and Codex regulations , no MRL is set for processed product if the Pf ≤1.
- ✓ In this case the MRL for raw commodity is applicable for processed product as well (Personal Comuncation with Dr. Arpad Ambrus; Center for Plant Protection and Soil Conservation, Budao¨rsi, Hungary)

Pf> 1 ;

According to Turksih Food Codex (TGK 2016), ;

- ✓ If Pf> 1 , the amount of residue in the processed product is divided by the processing factor and the value obtained is compared with the LOD value.
- ✓ If no MRL or LOD is available, 0.01 mg/kg of MRL is used for processed food.

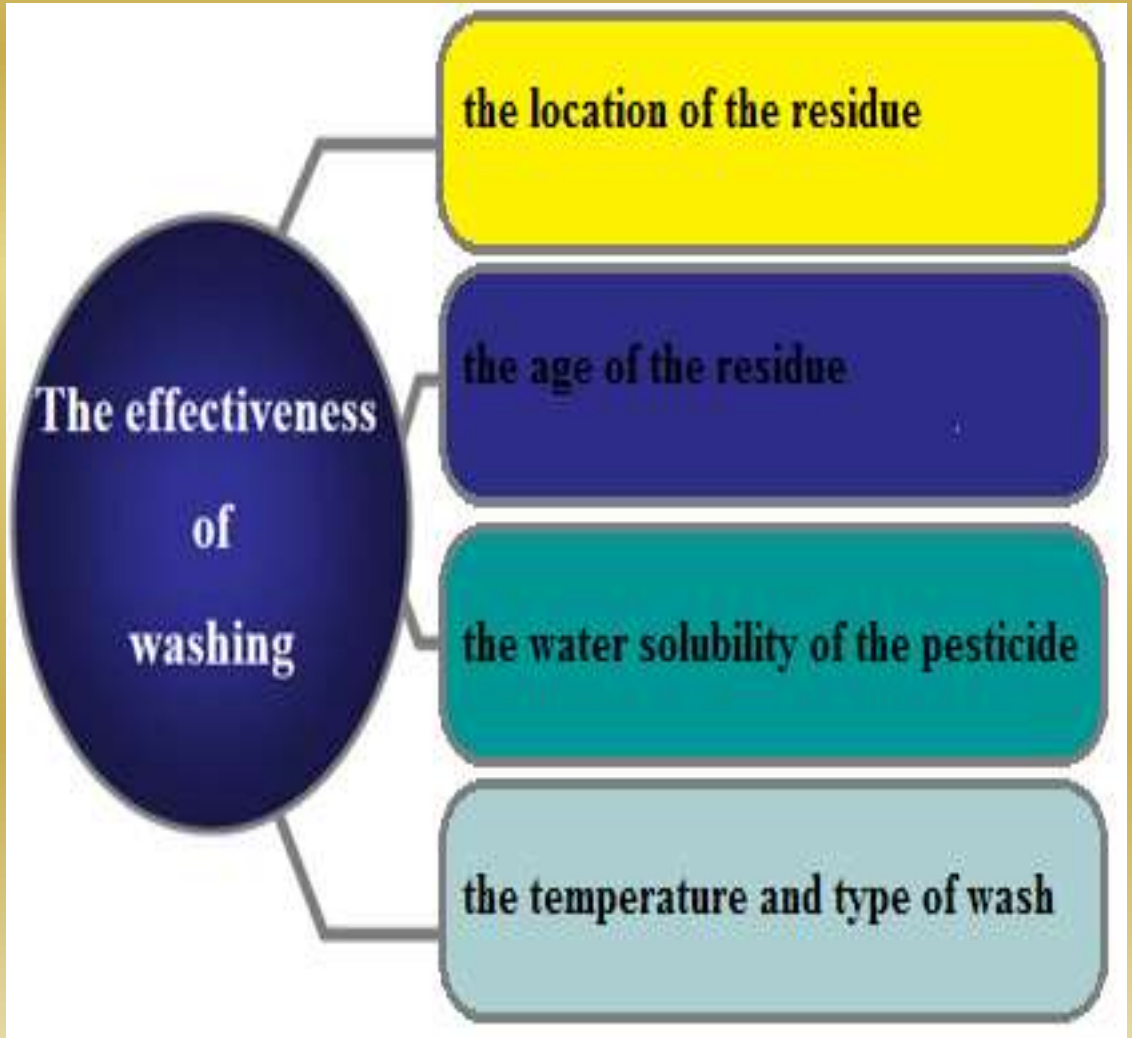
MRL for Processed products-2

- ✓ There is a need to optimize newer protocols for fixing MRLs in Processed Foods,
- ✓ It should include pesticide residue levels at various stages of processing.
- ✓ To arrive at fixing the MRLs for Processed Foods;
  - ✓ final residue levels and
  - ✓ the food consumption data

are essential to bring out meaningful information.

Washing Process

Washing is the first step in household and commercial food preparation. The proportion of residues may reduce 9- 99% **but it depends on:**



Easy to remove **surface residues**, but systemic residues can not remove

The proportion of residue that can **be** removed by washing declines with time

Polar, water soluble pesticides such as carbaryl **are** more readily removed than low polarity materials

Hot washing is more effective than cold washing

Washing;

- ✓ can be done with easily available plain water
- ✓ several washing solutions and chemicals have also been proven successful in removal of pesticide residues (Krol et al 2000);

* tap water
* acetic acid
* peroxyacetic acid solutions
* salt
* distilled water
* citric acid
* ultrasonic cleaning
* ozonated water
* baking soda
* $KMnO_4$

Reduction rates of treatments on pesticide residues in tomato (Baltacı-Yiğit,2015)

Pesticides	Reduction rate, %	
	Ozonated Water	Tap Water
Imidacloropid	40.9	32.6
Fenazaquin	57.8	57.9
Lambda-cyhalothrin	20.4	8.3

Effect of washing treatment on pesticide residues in strawberry (Lozowicka et al,2016)

Pesticide	Treatment	Time 1 min			Time 2 min			Time 5 min		
		Mean concentration (mg/kg)	PF	Reduction %	Mean concentration (mg/kg)	PF	Reduction %	Mean concentration (mg/kg)	PF	Reduction %
Fenhexamid MRL=5.0	Raw	0.444								
	tap water	0.323	0.73	27	0.298)	0.67	33	0.190	0.43	57
	ozonated water	0.366	0.83	17	0.363)	0.82	18	0.190	0.5	5
	ultrasonic cleaning	0.371	0.84	16	0.360	0.81	19	0.244	0.55	45
Pyraclostrobin MRL=1.5	Raw	0.905								
	tap water	0.720	0.80	20	0.670)	0.74	26	0.620	0.69	31
	ozonated water	0.500	0.55	45	0.480	0.53	47	0.390	0.43	57
	ultrasonic cleaning	0.169	0.19	**81**	0.153	0.17	**83**	0.096	0.11	**89**
Chlorpyrifos MRL=0.2	Raw	0.100								
	tap water	0.054	0.54	46	0.052	0.52	48	0.032	0.32	**68**
	ozonated water	0.045	0.45	55	0.034	0.34	66	0.025	0.25	**75**
	ultrasonic cleaning	0.058	0.58	42	0.050	0.50	50	0.021	0.21	**79**

PF<1 reduction factor ; PF>1 concentration factor

Pesticide residues remained and reduction (%) in pepper after processing (Ghani et al., 2010)

Treatment	Pesticide								
	Myclobutanil (2.09/0.5)*			Fenhexamid (2.29/2)*			Boscalid (0.64/2)*		
	Conc. found mg/kg	PF	Reduction %	Conc. found mg/kg	PF	Reduction %	Conc. found mg/kg	PF	Reduction %
Tap water	1.34	0.64	35.75	1.06	0.46	53.76	0.22	0.35	65.47
Sodium carbonate	1.58	0.76	24.13	1.34	0.59	41.37	0.23	0.36	64.00
Acetic acid	1.47	0.70	29.74	1.62	0.71	29.42	0.3	0.47	53.06
Sodium hypochloride	1.52	0.73	27.43	1.49	0.65	34.89	0.31	0.48	52.33
Glycerol	1.73	0.83	17.3	1.83	0.80	19.87	0.35	0.55	45.44
*Initial residue/ MRL (mg/kg)									

Imidacloprid residue level and its reduction of cucumber and bell pepper through washing with organic acid solutions(Randhawa et al., 2014)

Treatments	Cucumber (mg / kg)	Reduction (%)	Bell pepper (mg/kg)	Reduction (%)
Control (tap water)	1.92 ± 0.04a	—	1.89 ± 0.061a	—
Citric acid (1.5%)	0.48 ± 0.02e	75.00	1.02 ± 0.031e	46.03
Citric acid (3%)	0.31 ± 0.01g	83.85	0.87 ± 0.040g	53.96
Citric acid (6%)	0.24 ± 0.09h	87.50	0.78 ± 0.028g	58.73
Citric acid (9%)	0.12 ± 0.04j	93.75	0.52 ± 0.019i	72.48
Acetic acid (1.5%)	0.71 ± 0.013c	63.02	1.60 ± 0.058b	15.34
Acetic acid (3%)	0.48 ± 0.026e	75.00	0.95 ± 0.034ef	49.73
Acetic acid (6%)	0.37 ± 0.018f	80.72	0.68 ± 0.024h	64.02
Acetic acid (9%)	0.34 ± 0.018fg	82.29	0.59 ± 0.021i	68.78
Citric acid (0.75%) + Acetic acid (0.75%)	0.99 ± 0.036b	48.43	1.67 ± 0.067a	11.64
Citric acid (1.5%) + Acetic acid (1.5%)	0.53 ± 0.019d	72.39	1.49 ± 0.053c	21.16
Citric acid (3.0%) + Acetic acid (3.0%)	0.32 ± 0.011g	83.33	1.12 ± 0.040d	40.74
Citric acid (4.5%) + Acetic acid (4.5%)	0.17 ± 0.06i	91.14	0.82 ± 0.029g	56.61

Values are triplicates and expressed as mean ± SD. The same letters in a column showed non-significant differences among treatments.

METHODOLOGY

Experimental

- ✓ Capia peppers (*Capsicum annuum* L. var. *capia*) were cultivated in Çanakkale in 1 da plot.

Detailed information on pesticide application and harvesting

Active ingredient (a.i.)	Formulation – registrated dose	Pest	Number of Appl.	Necessary time between application and harvest, day	Harvesting (sampling) time*
Acetamiprid %20	MOSPILAN 20 SP- 25 g/100 l water	Whitefly Aphid	3	3	Zero time**
Chlorpyrifos 480 g/l	DURSBAN 4- 180 ml/da	Cotton bollworm		7	2nd day ***
Formetanate hydrochloride 500 g/kg	DICARZOL 50 100 g/100 l water	Thrips		7	3rd day**** of last application

* About 10 kg

** July 30, 2018; Zero time samples were taken after 4 h of pesticide application

*** August 1,2018

**** August 2,2018

Pesticide applied on;
July 16,2018 , July 23,2018 and July 30,2018

Washing Treatments

- ✓ Peppers were subjected to (for 2 and 5 min) washing with;
  - ✓ tap water,
  - ✓ acetic acit solution
  - ✓ citric acid solution
  - ✓ ultrasonic cleaning process.
- ✓ Then samples were stored in deep-freezer until the analyses.

Detailed information of treatments

Harvest (PHI, day)	TREATMENT									∑AP
	Non-treated (Tr_0)	Tap water (Tr_1)		Acetic acid solution, 9% (Tr_2)		Citric acid solution, 9% (Tr_3)		Ultrasonic cleaning (Tr_4)		
	0 min	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min	
0	3 AP/1 kg	3 AP*/1 kg	3 AP/1 kg	3 AP/1 kg	3 AP/1 kg	3 AP/1 kg	3 AP/1 kg	3 AP/1 kg	3 AP/1 kg	27
2	3 AP/1 kg	3 AP/1 kg	3 AP/1 kg	3 AP/1 kg	3 AP/1 kg	3 AP/1 kg	3 AP/1 kg	3 AP/1 kg	3 AP/1 kg	27
3	3 AP/1 kg	3 AP/1 kg	3 AP/1 kg	3 AP/1 kg	3 AP/1 kg	3 AP/1 kg	3 AP/1 kg	3 AP/1 kg	3 AP/1 kg	27
∑AP	9	9	9	9	9	9	9	9	9	81

*Each analytical portion will be analysed in triplicate (3 GC vial) with LC-MS/MS.

Treatments

Treatments-1 (TR_1)

TR_4)

TR_2 and TR_3

Treatments


0 day / U1 A
0 day / U2 B
0 day / U4 B
0 day / U1 B
0 day / U3 A
0 day / U4 A
0 day / U2 A
0 day / U3 B

Fortification

- ✓ For the method validation study, blank homogenized peppers were spiked.
- ✓ 15 g analytical portion (AP) was supplemented with 100 µl MeCN mixed 3 pesticide solution corresponded 0.1, 1.0, and 10.0xMRL spiking levels, with 4 replications (9).
- ✓ Analiytical procedure illustrated in Fig 1 followed.

Fortification pattern of Capia pepper with pesticides

Fortification level	Code	Acetamiprid, µg/kg	Chlorpyrifos, µg/kg	Formetanate hydrochloride, µg/kg
0.1 X MRL	F1/1-4	30	1	1
1.0 X MRL(*)	F2/1-4	300	10	10
10.0 X MRL	F3/1-4	3000	100	100
Control	F0/1-4	-	-	-

(*) EU MRL, µg/kg ; Regulation (EC) No 396/2005

- ✓ For the analysis of spiked and processed samples, **QuEChERS-*AOAC* Method 2007.01** (Lehotay 2007) was/will be employed.
- ✓ 1 kg pepper (EC, 2002) was/will be chopped and homogenized with blender.
- ✓ 3 analytical portions of 15 g was/will be taken for the analysis (Fig 1).

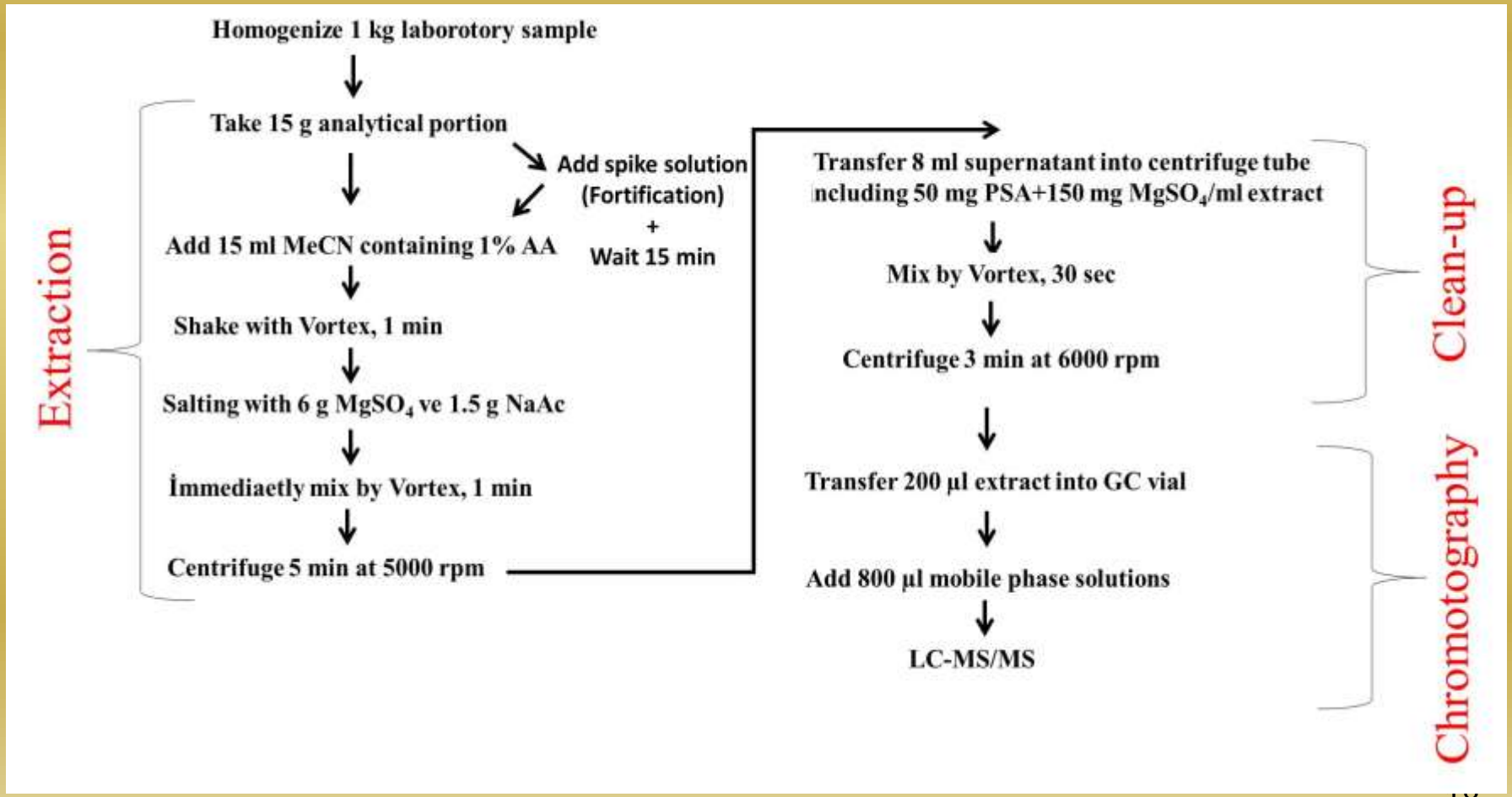


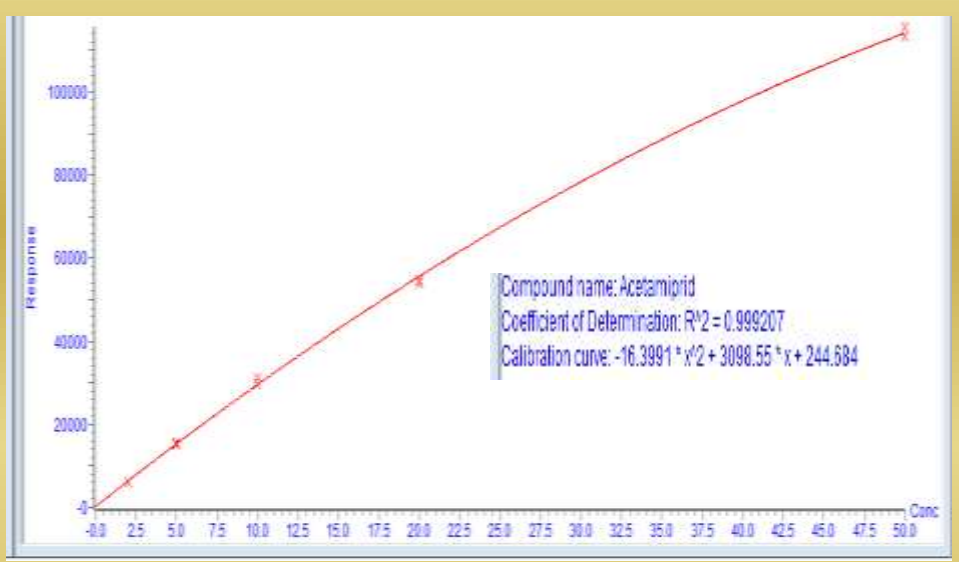
Fig 1.Schematic diagram of QuEChERS-*AOAC* official method 2007.01

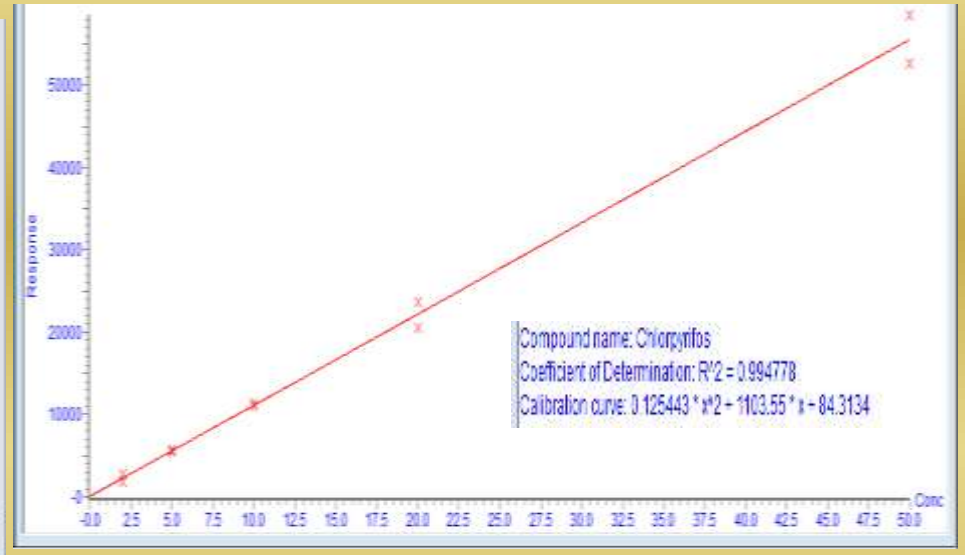
Chromatohgraphy

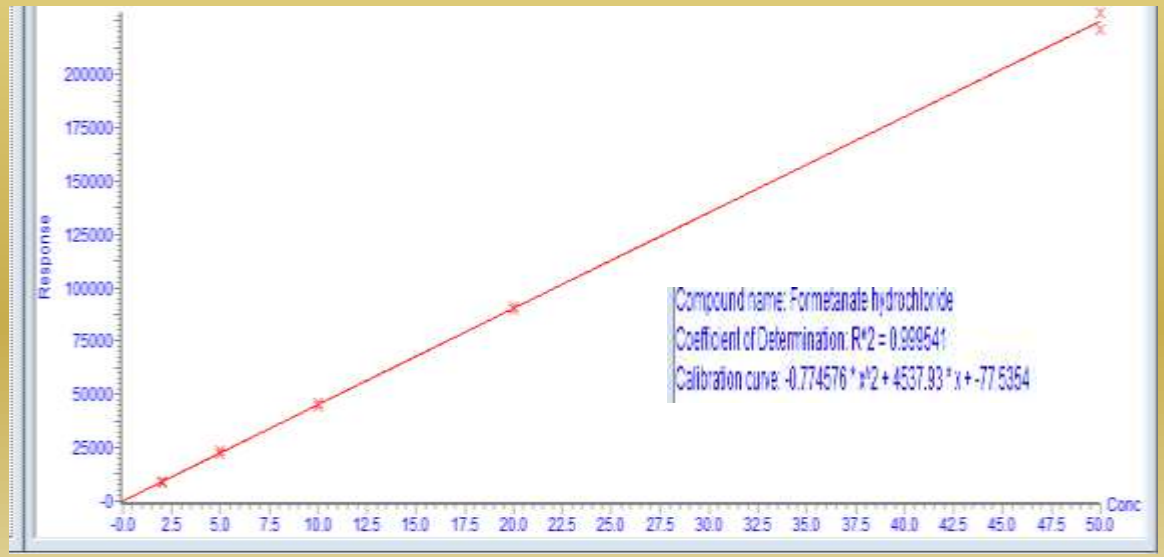
- ✓ Samples were/will be analyzed with Waters Acquity UPLC+Acquity TQD for identification and quantification.
- ✓ For LC-MS/MS, injection volume will be 20 μl onto an ACQUITY UPLC® BEH C_{18} 1.7 μm 2.1 mm x100 mm.
- ✓ Flow rate will be 0.3 mL/min and a gradient program will be used consisting of 5 mM Ammonium acetate+5% MeOH in water (A1) and 5 mM Ammonium acetate 95% MeOH (B1).
- ✓ Flow rate will be 0.3 mL/min and total run time will be 15 min.

RESULTS

Matrix-matched calibration curves (analytical function) of pesticides*







*As indicated in "**CAC/GL 40-1993**" and "**SANTE/11813/2017 Guidelines** ", representative sample matrix (apple) calibration was used instead of peper. Because both samples are in Class II (high water and low or no chlorophyll content).

**The curve of 3 compounds was linear over the range 1-50 pg/µL, with R^2 (≥ 0.99).

***Some samples (i.e., 10.0xMRL spiking level samples) were **diluted to fit the calibration range.**

Detection limits of analytes, µg/kg

Acetamaprid	**2**
Chlorpyrifos	10
Formatenate hydrochloride	5

Recoveries for pesticides

- The method validation was assessed with the recovery, RSD and precision.
- The recovery, precision and accuracy were identified with 4 parallels of analytical portions at 3 fortification levels of acetamaprid, chlorpyrifos and formatenate hydrochloride.
- The recovery was expressed as the ratio of determined quantity to fortification level of the analyte.

Method validation parameters obtained recovery studies with the 3 fortification levels by QuEChERS method

Analyte	Fortification, µg/kg	Found, µg/kg	Recovery, %	SD	RSD, %	Accuracy, %
Acetamaprid	30	34.29	114.31	3.77	3.29	
	300	346.66	115.55	2.76	2.39	
	3000	2342.09	78.07	1.68	2.16	
	Mean, n=36		**102.64**	**18.04**	**17.58**	**102.64**
Chlorpyrifos	1	1.05	99.5	7.84	7.88	
	10	10.34	103.37	7.47	7.21	
	100	94.91	98.96	6.83	7.20	
	Mean, n=36		**94.91**	**7.6**	**7.68**	**94.91**
Formatenate hydrochloride	1	1.01	101.00	10.01	9.91	
	10	11.26	112.59	5.21	4,63	
	100	115.55	115.55	3.52	3.09	
	Mean, n=36		**109.71**	**9.33**	**8.51**	**109.71**

Overall recovery of the method (accuracy of the method):104.52 (n=108, RSD=13.68)

- Pesticide recovery rations varied between 78.07- 115.55%.
- Recovered acetamaprid from pepper matrix was 102.64%. Considering the precision of the method (closeness of repeated analyses), RSD value was identified as 17.58 % (n= 36).
- Overall recovery of the method (Q), RSD values (n=108) were respectively calculated as 104.52%, 13.68%.

- **All individual recoveries, accuracy as overall recovery and repeatability as RSD were within the specified acceptance criteria (60% ≤ Q ≤ 140% and RSD ≤ 20%) for 3 compounds in pepper (SANTE, 2017).**

Remaining work of the project:

- ✓ Residues for field sample, subjected to washing treatments, will be determined.
- ✓ PF will be calculated for each washing treatments (OECD 2008).
- ✓ Pesticide reduction rate of each treatments will be determined.
- ✓ The differences reduction or concentration rate between treatments will be assessed with ANOVA (SAS V8 programme). Statistical programme

I wish you all a pesticide-free and a healty life

Thank you for your attention