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Effect of household processing on reduction of pesticide residues in Cauliflower (*Brassica oleraceae var. botrytis*)

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ABSTRACT

Cauliflower is widely cultivated in almost all the parts of the world. In India, it is cultivated in about 90 thousand hectares area. There has been a substantial increase in the area of cauliflower (about 20%) during the last two decades. The important cauliflower growing states are Uttar Pradesh, Karnataka, Maharashtra, Bihar, West Bengal, Punjab and Haryana. 387.41, 356.75, and 321.15 tones cabbage produced by Gujarat state during 2010-11, 2009-10 and 2008-09 respectively (Source: National Horticulture Board; NHB). Cauliflower or phul gobhi is used as a cooked vegetable in curries, soups, etc. It is frequently employed as an ingredient of mixed pickles. Traditionally cauliflower is eaten in the form of raw, boiled and cooked as a subji and soups and therefore raw, washed, boiled and cooked samples were selected for the pesticide residual analysis. The effects of household processing on pesticide residues were also studied. Analysis of cauliflower for pesticidal contamination was carried out on Gas Chromatograph-Electron Capture and TID Detector with capillary columns. Cauliflower was found contaminated with diclorvos, monocrotophos, phorate, parathion, pendamethalin, endosulphan-II, captafol, permethrin and cypermethrin. The study revealed that cauliflower was found contaminated maximum with dichlorvos and minimum with phorate in the range of 85.1-95.12 and 0.076-0.096 $\mu g g^{-1}$ respectively Findings show that washing; boiling and cooking process minimized the pesticide residues of nine pesticides in the range of 3.32-70.0, 21.08-70.67 and 31.63-85.30 percent respectively. The percentage reductions in the present study are supported by both early and most recent publications. These reductions are extremely important in evaluating the risk associated with ingestion of pesticide residues, especially in vegetables, which are eaten by almost all income groups' people. The present study showed that cooking was found more effective than washing and boiling.

Key words: Cauliflower, household processing, minimizing pesticide residue, op &oc

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INTRODUCTION

India has made significant achievements in the production of food grains, yet malnutrition is still very real impediment to the development of the country. Fruits and vegetables, being rich sources of nutrients, vitamins and minerals, have an indisputable role to overcome this problem. Cauliflower is one of the most important winter vegetables of India with a variety of uses. Cauliflower (*Brassica oleraceae var. botrytis*) is one of the *cruciferous* vegetables namely, cabbage, Brussel's sprouts and broccoli. It was introduced in lndia in 1822. The initial introductions were "Cornish" types from England followed by the European types. The Indian cauliflower or the

tropical types are a result of intercrossing among these types. Cauliflower is a delicate crop and gets damaged near harvest by freezing weather. The name cauliflower consists of two Latin words namely 'caulis' which means cabbage and 'floris' which means flower. In cauliflower, the edible portion, known as curd, is made up of abortive flowers, the stalks of which are short, fleshy and closely crowded. Cauliflower is probably the native of Western Europe and the northern shore of Mediterranean region. All cole crops (cabbage, cauliflower, knol-knol, Brussel's sprouts, etc) have originated from a single ancestor, *Brassica oleraceae var. sylvestris*. The important pests attacking cauliflower are mustard saw -fly (*Athalia proxima*), mustard aphid (*Lipahis erysilnl*), painted bug (*Bagrada Cruciferum*), diamond back moth *(Plutella maculipensis*), cabbage butterfly (*Pieris sp*.) and root -knot nematodes [1,2]. In order to combat the insect pest problem, lot of pesticides is used by the vegetable growers for better yield and quality. Insecticides are repeatedly applied during the entire period of growth and sometimes even at the fruiting stage. It accounts for 13-14% of total pesticide consumption as against 2.6% of cropped area [3]. Pesticide exposure has been associated with human health risk of arthritis, skin disease, bone disorder, cancer and nerve disorder [4,5]. Contamination of vegetables with pesticide residues has been reported by many researchers [6-8]

Cauliflower is widely cultivated in almost all the parts of the world. In India, it is cultivated in about 90 thousand hectares area. There has been a substantial increase in the area of cauliflower (about 20%) during the last two decades. The important cauliflower growing states are Uttar Pradesh, Karnataka, Maharashtra, Bihar, West Bengal, Punjab and Haryana. 387.41, 356.75, and 321.15 tones cabbage produced by Gujarat state during 2010-11, 2009-10 and 2008-09 respectively (Source: National Horticulture Board; NHB). Cauliflower or phul gobhi is used as a cooked vegetable in curries, soups, etc. It is frequently employed as an ingredient of mixed pickles. Traditionally cauliflower is eaten in the form of raw, boiled and cooked as a subji and soups and therefore raw, washed, boiled and cooked samples were selected for the pesticide residual analysis. The effects of household processing on pesticide residues were also studied.

MATERIALS AND METHODS

Chemicals

a. Reagents: Standard pesticides which were >98% pure were procured from RFCL, Delhi, India. HPLC grade hexane, acetone and ethyl acetate, and AR grade anhydrous sodium sulphate, sodium chloride, Florisil, Activated charcoal, Silica gel for column chromatography were procured from RFCL, Delhi, India.
b. Standard materials: Standard pesticides which were >98% pure were procured from RFCL, Delhi, India. The standard stock solutions (100 ppm) were prepared in ethyl acetate and stored at -4^{0}C. Working standard mixtures of 8 OC and 15 OP pesticides in ethyl acetate, containing 1.0 µg/ml of each pesticide, were used for spiking the samples and preparing calibration standards.

Instruments

a. Blender-Boss Appliances, Daman, India
b. Centrifuge-Kumar Industries, Bombay, India
c. Mechanical shaker -Modern Industrial corporation, Bombay, India
d. Rotary evaporator -Jain Scientific, India
e. GC- Thermofisher 1000 GC equipped with capillary columns using ^{63}Ni electron capture detector (ECD) and TID.
f. Capillary column- 1. SPB-5 of 5% diphenyl/ 95% dimethyl fused silica capillary column (30 m×0.32 mm ID, 0.25 μm film thickness) 2. HP-1 of methyl silicone (10 m×0.53 mm ID, 2.65 μm film thickness).

Instrument conditions

For OC: Temperatures (^{0}C):150 (5 min) → 8 ^{o}c min^{-1} → 190 (2 min) → 15 ^{o}c min^{-1} 280°c (10 min); injection port: 280 ^{o}c; detector: 300 ^{o}c; carrier gas: (N_2), flow rate 60 ml min^{-1}, 2 ml through column and split ratio 1:10. Carrier gas, N_2, flow rate 60 ml min^{-1}, 2 ml through column.

For op: Temperatures(^{0}C): Oven: 100 (1 min) → 10 ^{o}c min^{-1} → 200 ^{o}c (0 min) → 20 ^{o}c min^{-1} → 260 ^{o}c (3 min); injector port, 250 ^{o}c , detector, 275 ^{o}c , carrier gas N_2 18 ml min^{-1}, H_2, 1.5 ml min^{-1} and zero air 130 ml min^{-1}.

Sampling
A total of 45 samples of cauliflower were commercially purchased from the local market of Rajkot city, Gujarat, India, and served as the blank or spiked sample. All the samples were extracted fresh. The unit was generally more than 250 g [9]. For the analysis, only the edible portions were included, whereas bruised or rotten parts were removed.

Processing vegetables
Cauliflower samples, after washing, were hand peeled into slices with a stainless steel peeling knife and cooked. Cauliflower samples (raw) were dry, cleaned to remove soil contamination with a disposable paper towel and blended to mace a homogeneous sample for pesticide analysis.

Washing
Cauliflower was washed by placing in a plastic colander and rinsed under normal tap water (25-30^{0}c) for 30 second [10].with gentle rotation by hands and blotted dry with a paper towel. These samples were divided into two portions, of which one was analyzed as such after homogenizing in blender and other was further boiled and cooked.

Boling
Sliced cauliflower was boiled by placing 75 ml of water in saucepan. Vegetable (50g) was added immediately to boil for 5-10 min / boiled still softness was subjected to pesticide analysis.

Cooking
Sliced cauliflower was cooked [11] by placing 15 ml of water in saucepan. Vegetable (50g) was added immediately to cook for 10-12 min was subjected to pesticide analysis. Washed, boiled and cooked samples were processed in a similar manner as of unprocessed samples (Raw).

Extraction
Commercially purchased cauliflower served as the blank or spiked sample. All the samples were extracted fresh. Each vegetable was chopped into small pieces and after quartering, a representative sample (50g) was macerated with 5-10g anhydrous sodium sulphate in blender to make a fine paste. The macerated sample was extracted with 100ml acetone on mechanical shaker for 1 h by using the method of [12]. Extract was filtered, concentrated up to 40ml and subjected to liquid-liquid partitioning with ethyl acetate (50, 30, 20 ml) after diluting 4-5 times with 100 ml 10% aqueous NaCl solution. Concentrated the organic phase up to 10ml on rotary evaporator and divide it into two equal parts. One part was kept for OC and second for OP.

Clean-up
For OC, clean-up was carried out by using column chromatography. Column (60cm × 22mm) was packed with, Florisil and activated charcoal (5:1 w/w) in between the two layers of anhydrous sodium sulphate. Extract was eluted with 125ml mixture of ethyl acetate: hexane (3:7 v/v). Eluate was concentrated to 2ml for residue analysis.

Residues of OP were also cleaned by adopting column chromatographic technique. Column was packed with silica gel and activated charcoal (5:1 w/w) in between the layers of anhydrous sodium sulphate. Extract was eluted with 125ml mixture of acetone: hexane (3:7 v/v). After concentrating the eluate on rotary evaporator, final volume was made to 2ml for analysis by gas liquid chromatography (GC).

Quantization
An external method was employed in the determination of the quantities of residues in the sample extracts. A standard mixture of known concentration of pesticide was run and the response of the detector for each compound ascertained. The area of the corresponding peak in the sample was compared with that of the standard. All analyses were carried out in triplicates and the mean concentrations computed accordingly.

Recovery rate and limit of detection
Cauliflower samples were fortifies at 0.01, 0.02 and 0.1 mg/kg by adding 5.0 mL of a mixed standard solution. Recovery and precision (expressed as relative standard deviation) were calculated for three replicate samples. Percent recoveries in spiked samples ranged 87.3% -104.0 % [13]. Accordingly, the sample analysis data were corrected for these recoveries. Detection limit(s) of the method were also assessed based on the lowest concentrations of the residues in each of the matrices that could be reproducibly measured at the operating

conditions of the GC; which were 0.001 mg/kg. Blank analyses were also carried in order to check any interfering species in the reagents.

Estimation

The cleaned extracts were analyzed on Thermofisher 1000 GC equipped with capillary columns using ^{63}Ni electron capture detector (ECD) and TID. Operating conditions were as per details: **For OC**: Detector : ECD (^{63}Ni), column: SPB-5 of 5% diphenyl/ 95% dimethyl fused silica capillary column (30 m×0.32 mm ID, 0.25 μm film thickness) with split system. Temperatures (^{0}C):150 (5 min) → 8 ^{o}c min^{-1} → 190 (2 min) → 15 ^{o}c min^{-1} 280°c (10 min); injection port: 280 ^{o}c; detector: 300 ^{o}c; carrier gas: (N_2), flow rate 60 ml min^{-1}, 2 ml through column and split ratio 1:10. Carrier gas, N_2, flow rate 60 ml min^{-1}, 2 ml through column.

For OP: Detector: TID, megabore column: HP-1 of methyl silicone (10 m×0.53 mm ID, 2.65 μm film thickness). Temperatures(^{0}C): Oven: 100 (1 min) → 10 ^{o}c min^{-1} → 200 ^{o}c (0 min) → 20 ^{o}c min^{-1} → 260 ^{o}c (3 min); injector port, 250 ^{o}c , detector, 275 ^{o}c , carrier gas N_2 18 ml min^{-1}, H_2, 1.5 ml min^{-1} and zero air 130 ml min^{-1}.

RESULTS AND DISCUSSION

In the analyzed samples, the detected pesticides comprised of diclorvos, monocrotophos, phorate, parathion, pendamethalin, endosulphan-II, captafol, permethrin and cypermethrin. The study revealed that cauliflower was found contaminated maximum with dichlorvos and minimum with phorate in the range of 85.1-95.12 and 0.076-0.096 µgg^{-1} respectively. Although all the samples were found contaminated with OC insecticides but none of the samples contained residues of any of these insecticides above maximum residue limits (MRL) fixed by Prevention of Food Adulteration Act (PFA) 1954 and FAO/WHO (1996). As many organohalogen pesticides like BHC and DDT have been banned with effect from April 1993, In India, but they have remained in the environment where they continue to be incorporated into plant biomass. In India, practically, DDT has not been phased out completely because it is still used to control the mosquito in public health programmes from where it could enter the agricultural soils and water systems and possibly find its way into crops. Presence of endosulfan in the present study is due to use of endosulfan in almost every crop in Gujarat, India among the OC pesticides after banning of use of DDT and HCH in 1993. Residues of monocrotophos (2.01-3.24 µgg^{-1}), parathion (5.99-7.99 µgg^{-1}), pendamethalin (0.29-0.41 µgg^{-1}), endosulphan-II (0.70-1.64 µgg^{-1}), captafol (0.31-0.51 µgg^{-1}), permethrin (0.25-0.35 µgg^{-1}) and cypermethrin (0.40-0.60 µgg^{-1}) were detected in cauliflower. The results obtained from the present study are consistent with an earlier study that show residues of these pesticides are present in different vegetables [14, 15, 16, 17, 18].

Effects of household processing

Among household processes washing process reduced the pesticide residues by 3.32-70 percent. Maximum reduction of residue was observed in case of monocrotophos and parathion where the residues decreased to the extent of 70 and 48.26 percent by washing process respectively. In the present study washing was found effective in the decontamination of pesticide residues as it depends on a number of factors like, location and age of residues, water solubility, temperature and type of washing solution. In earlier studies also, effect of these factors were observed in different vegetables by various researchers' vegetables [14, 15, 16, 17, 18, and 19]. Washing found comparatively less effective in reducing the residues of phorate (16.27), permethrin (6.80) and dichlorvos (3.32).

Boiling was observed to be effective in reducing the residues. By this process, reduction of residues of nine pesticides was observed in the range of 21.08-70.67 percent. Maximum reduction was observed in the case of monocrotophos, parathion and pendamethalin where the residues decreased to the extent of 70.67, 70.26 and 59.82 percent respectively.

Table-1: Pesticide residues* (µg g^{-1}) in cauliflower

Sr.No	Pesticide	Raw					Washing					Boiling					Cooking				
1	**Dichlorvos**	95.11	85.1	90.12	94.1	93.1	94.1	74.1	88.93	93.1	92.1	40.5	30.5	35.02	30.21	48.5	31.22	21.2	26.78	39.5	30.2
2	**Monocrotophos**	2.53	2.01	3.24	2.43	2.51	0667	0.567	1.26	0.657	0.665	0.516	0.416	1.10	0.98	0.91	0.19	0.10	0.89	0.506	0.185
3	**Phorate**	0.086	0.076	0.096	0.081	0.085	0.072	0.062	0.082	0.067	0.071	0.048	0.038	0.058	0.045	0.047	0.02	0.01	0.04	0.043	0.019
4	**Acephate**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5	**Dimethoate**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	**Fenitrothion**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	**Diazinon**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8	**Malathion**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
9	**Parathion**	6.99	5.99	7.99	6.94	6.89	3.77	2.77	4.77	3.72	3.67	2.04	2.14	2.04	2.17	1.94	1.22	1.20	1.23	1.98	0.99
10	**Chlorpyriphos**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	**Quinalphos**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
12	**Profenophos**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
13	**pendamethalin**	0.31	0.34	0.41	0.29	0.33	0.22	0.24	0.32	0.20	0.24	0.114	0.124	0.214	0.108	0.116	0.11	0.18	0.21	0.112	0.12
14	**Ethion**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
15	**Carbaryl**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
16	**Aldrin**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
17	**Captan**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
18	**Pp DDT**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
19	**Endosulphan-II**	1.54	1.59	1.64	1.52	0.70	0.892	0.98	.992	0.890	0.91	0.71	0.88	0.81	0.58	0.82	0.59	0.58	0.69	0.70	0.71
20	**P p DDD**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
21	**Captafol**	0.31	0.41	0.51	0.33	0.36	0.22	0.32	0.42	0.24	0.27	0.15	0.25	0.35	0.098	0.20	0.098	0.01	0.11	0.17	0.015
22	**Permethrin**	0.25	0.30	0.35	0.30	0.27	0.23	0.28	0.33	0.28	0.25	0.21	0.26	0.31	0.152	0.23	0.148	0.198	0.248	0.26	0.15
23	**Cypermethrin**	0.40	0.50	0.60	0.42	0.45	0.284	0.384	0.484	0.304	0.334	0.212	0.312	0.412	0.148	0.217	0.128	0.228	0.318	0.222	0.133

Table-2: Effect of processing on pesticide residues (μg g^{-1}) in cauliflower

Sr. no	Name of Pesticide	Raw	Washing	Boiling	Cooking
1	Dichlorvos	85.1-95.12 (91.51)	74.1-94.1 (88.46) [3.32]	30.21-48.5 (36.95) [51.62]	21.2-39.5 (29.78) [67.46]
2	Monocrotophos	2.01-3.24 (2.544)	0.567-1.26 (0.763) [70.00]	0.410-0.98 (0.746) [70.67]	0.10-0.890 (0.374) [85.30]
3	Phorate	0.076-0.096 (0.0848)	0.062-0.082 (0.071) [16.27]	0.038-0.058 (0.0472) [44.34]	0.01-0.043 (0.0264) [68.87]
4	Parathion	5.99-7.99 (6.96)	2.77-4.77 (3.74) [48.26]	1.94-2.17 (2.07) [70.26]	0.99-1.23 (1.324)[80.98]
5	Pendamethalin	0.29-0.41 (0.336)	0.20-0.32 (0.244) [27.38]	0.108-0.214 (0.135) [59.82]	0.11-0.18 (0.129) [61.61]
6	Endosulphan-II	0.70-1.64 (1.398)	0.890-0.992 (0.933) [33.26]	0.58-0.88 (0.76) [45.64]	0.58-0.70 (0.654) [53.22]
7	Captafol	0.31-0.51 (0.384)	0.22-0.42 (0.294) [23.44]	0.15-0.35 (0.210) [45.31]	0.01-0.170 (0.081) [78.91]
8	Permethrin	0.25-0.35 (0.294)	0.23-0.33 (0.274) [6.80]	0.152-0.310 (0.232) [21.08]	0.148-0.26 (0.201) [31.63]
9	Cypermethrin	0.40-0.60 (0.474)	0.284-0.484 (0.358) [24.47]	0.148-0.412 (0.217) [54.22]	0.133-0.318 (0.206) [56.54]

Cooking was observed to be more effective in reducing the residues. By this process, reduction of residues of nine pesticides was observed in the range of 31.63-85.30 percent. The great variation in reduction of residues by boiling/cooking was observed which may be attributed to the rates of degradation and volatilization of residues as the concentration of residues increases by heat involved in boiling/cooking. Maximum reduction was observed in the case of monocrotophos, parathion and captafol where the residues decreased to the extent of 85.30, 80.98 and 78.91 percent respectively. Holland *et al.*, [20] reported appreciably reduction in pesticide residues in different commodities by using different processing methods. Hence, the present results are in consistent with the earlier results.

CONCLUSION

It can be concluded that residues of dichlorvos exceeded their respective maximum residue limits and it is 67.46 % minimized by cooking process. It was found that washing; boiling and cooking process minimized the pesticide residues of nine pesticides in the range of 3.32-70.0, 21.08-70.67 and 31.63-85.30 percent respectively. The percentage reductions in the present study are supported by both early and most recent publications. These reductions are extremely important in evaluating the risk associated with ingestion of pesticide residues, especially in vegetables, which are eaten by almost all income groups' people. The present study showed that cooking was found more effective than washing and boiling.

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