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Microbiological Quality and Organochlorine Pesticide Residue in Commercially Available Ready-To-Eat Raisins

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Abstract Microbiological quality and organochlorine pesticide residual contamination in raisins in a restricted area of Lucknow city, India was assessed in 20 samples. Total bacterial count was found in both open and packed raisin samples within the acceptable range (10^5–$<10^6$). The presence of food pathogens like *Salmonella* spp. and *Enterobacteriaceae* was detected more in open samples whereas *Staphylococcus* spp. and *Pseudomonas* spp. were absent. *Lactobacilli* spp. was found in all open samples and two packed samples. Presence of OCP residue was also found below the MRL although low levels of α-HCH and γ-HCH were detected in samples. The study shows presence of spoilage and pathogenic microorganisms as well as OCP residue within permissible limits which was more in open samples than in packed ones.

Keywords Raisins · Organochlorine pesticides · Total bacterial count · *Enterobacteriaceae* count

The use of food safety management systems such as hazard analysis critical control point (HACCP) has resulted in an increased awareness and understanding of risk associated with microbiological contamination within the food industry. However, in developing countries, post harvest losses are often severe due to the lack of adequate handling and refrigerated controlled atmosphere storage facilities. Toxic or pathogenic fungi could pose a health risk for the consumer; as they are responsible for the change of appearance, taste and flavor of the products, reduction of nutritional value, allergies and production of mycotoxins (Tournasa and Katsoudas 2005). Regulation of microbial contamination is difficult because of the dynamic nature of microbial populations at normal ambient conditions. Reports of *Shigella* spp. on tomatoes and cantaloupes (Warren et al. 2007), *E. coli* 0157:H7 on lettuce and in apple juice (Li and Mustapha 2004) and hepatitis A on strawberries (Butot et al. 2007) have shaken consumer confidence in the safety of fruits and vegetables. Pesticide is routinely used in the cultivation of fruits to control insects, weeds, spoilage due to bacteria and fungi. Organochlorine pesticides are persistent in the environment, and accumulate in plants and soil. These organochlorines have a wide range of both acute and chronic health effects and suspected as endocrine disruptors (Cabras and Angioni 2000). HCH, an organochlorine insecticide, widely used in agriculture and public health is considered to be number one environmental contaminant in many parts of the world particularly India and China (Srivastava and Shivanandappa 2005). Intermediate moisture foods (IMF's) such as raisin, prunes, nectarines, apricot etc are acquiring major attention of consumers now-a-days regarding contaminant levels. Raisins refer to dried-grape, manufactured by exposure to sunlight or oven-drying. They have high amount of sugar but resist spoilage due to low moisture and low pH. Raisins are composed of carbohydrates i.e. fructose and glucose (60% w/w), fruit acids (folic acid and pantothenic acid) and mineral salts (Robson 1976). They have also been found to contain several chemical compounds such as oleanolic acid, oleanolic aldehyde etc. that may assist in fighting oral bacteria and slow the growth of *Streptococcus mutans*, the main bacteria behind tooth

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decay (Kozai et al. 1999). Thus, contributing as a good therapeutic/medicinal food product. This study investigates the microbiological quality and persistent organochlorine pesticide residues in various types of raisins sold in local market in order to assess their quality and effect of storage conditions.

Materials and Methods

The sample collection area was crowded residential areas of the city. Onsite observation of the location included food display, hand-food contact and handling of the food among the sites visited. Total of twenty samples of ready-to-use raisins (50 g) were procured from different local markets of the Lucknow city area in open and packed conditions from Aug–Sept 2007. The average environmental temperature at the time of sample collection ranged from 40 to 45°C. All the samples were purchased and aseptically transferred into sterilized aluminum foil stand up pouches and kept at 2–6°C till analysis. Microbial determination, organochlorine pesticide content, moisture content and pH values were evaluated in all the raisin samples.

Ten grams of each food sample was homogenized with 95 mL of 0.1% sterile peptone water (Himedia laboratories). Serial dilutions were performed as required and plating was done in triplicate into selective agar medium (Anon 1992). The media used and experimental conditions were nutrient agar media for total aerobic counts at 37°C for 24 h, De Man Rogosa and Sharpe Agar (MRS) for lactic acid bacteria (*Lactobacilli*) count at 30°C for 72 h; violet red bile agar (VRBA) for *Enterobacteriaeceae* counts at 37°C for 24 h; Sabouraud dextrose agar (SDA) for yeast and moulds counts at 25°C for 3–4 days; Baired-Parker agar (BPA) for *Staphylococcus* spp. counts at 37°C for 48 h; cetrimide base agar (CBA) for *Pseudomonas* spp. counts at 37°C for 48 h and brilliant green agar (BGA) for *Salmonella* spp. counts at 37°C for 48 h. Typical colonies were counted on each plate using Colony Counter Meter (Sigma, USA) and transferred to nutrient agar slants. These observed colonies were confirmed by biochemical characterization using HiStaph Identification kit KB004, HiSalmonella Identification kit KB001. *Pseudomonas* spp., *Lactobacilli* spp. and Enteropathogens were confirmed by using Hicarbohydrate kit as per instructions. All the bacterial culture medium and biochemical identification kit used in the study were purchased from Himedia laboratories, India. Permissible limits according to categories of ready-to-eat foods were: Aerobic colony count $\geq 10^7$ CFU, *Enterobacteriacea* $\geq 10^4$ CFU, *Staphylococcus aureus* 100 $>10^4$ CFU and *Salmonella* spp. were not detected in each 25 g/sample for a single food sample. The moisture content in raisins was determined by oven drying method (The Ayurvedic Pharmacopoeia of India 2001). Five grams of raisin samples were dried at 80–85°C in oven for 3 h to remove most of the moisture and finally for 1 h. The process was repeated till concordant readings were obtained or difference between them was not more than 0.25%. The moisture content was calculated as per formula: Moisture content (%) = Final wt. of sample − Initial wt. of sample/Initial wt. of sample. pH was also determined for open and closed raisin sample using digital cyberscan 510 pH meter, according to the AOAC (1990) method. Statistical analysis of the data was performed for S.D. and Student's *t*-test for the analysis of variance (ANOVA) and significance was determined at the $p \leq 0.05$ level.

The solvents used for pesticide analysis such as n-hexane, acetonitrile, benzene were of HPLC grade (Merck, India). Deionized water was used throughout the study. Glassware used for the study was Borosil 'A' grade, including volumetric flask, conical flask, measuring cylinders, test tubes and pipettes. Samples were washed in fresh running water to eliminate dust, dirt and possible parasites, and then washed again with deionized water (Zurera et al. 1987). The samples were air dried prior to analysis. Estimation of organochlorine pesticides was carried out by taking 2 g materials and extracting it with 150 mL of n-hexane using a soxhlet apparatus. The extract was passed through an anhydrous sodium sulfate filter column, which was prepared by putting about 10 g of anhydrous sodium sulfate in a glass wool column to remove traces of water. Hexane extract was then evaporated at 60°C (temperature of water bath) under reduced pressure in a rota vapor (Buchi R-114). The concentrated extract (1–2 mL) was transferred to a clean-up column with small washing of n-hexane. The sample was further extracted with acetonitrile/n-hexane saturated solvent system for oil removal followed by clean-up done with the n-hexane + benzene mixture elution method. Four grams of deactivated adsorbent Florisil was packed in a borosil glass column (150 × 5 mm^2) topped with anhydrous sodium sulphate and tapped firmly. Concentrated extract (in n-hexane) was transferred to the top of the column and eluted with 5 mL hexane. Elute was collected carefully and 5 mL benzene: n-hexane mixture (20:80) was added to the column and collected. The elute was again concentrated to 1–5 mL, as required, and analyzed on GLC (NUCON 5765) according to the standardized method in the laboratory (Naithani and Kakkar 2006). The temperature of the injection port (250°C), oven (190°C) and detector (250°C) was adjusted as recommended for optimum efficiency and thermal stabilization. Carrier (N^2) gas flow rate was adjusted to 60 mL/min. Five-microliter mixed standard solution (HCH and its isomers, α-endosulphan, DDT and its metabolite) followed by 5 μL of the samples were injected into the GLC, and chromatograms were recorded. Detection limit of equipment was 1 μg/kg, and recovery obtained under the experimental conditions was found to be 90–93%.

Results and Discussion

The world raisin production in 1995 was estimated to be 1,072,000 kg. Unites States and Turkey are the major producers and the exporters of raisin (Borriss et al. 2006). In the opinion of the Advisory Committee for Food and Dairy Products (Gilbert et al. 2000) the ready-to-eat foods should be free from *Salmonella*, *Camplylobacter* and *E. coli O157:H1* and other verocytotoxin producing *E. coli* (VTEC). The infectious doses vary from less than 10 to more than 10^6 microorganisms and their presence even in small numbers, results in such foods being of unacceptable quality/potentially hazardous food items. Microbiological contamination in open and packed raisins is shown in Tables 1 and 2, respectively. The total bacterial count (TBC) of the open samples ranged between 10^3–10^6 CFU/g as against 10^2–10^6 CFU/g in the packed samples. Only two packed raisin samples showed acceptable range for TBC (10^5–10^6) as per recommended permissible limits for food safety monitoring. Rest was found to have satisfactory microbiological quality. In open raisins six raisin samples were found in acceptable range whereas four were found in satisfactory range. In open samples, moisture content was found to be 4.07–19.60% (Table 1) i.e. much higher than the limits (>10%) prescribed for raisins by Codex Alimentarius Committee (1987). In packed samples it was 1.44–9.78% i.e. well within the prescribed limits. pH of all the raisins varied from 3.63 to 4.24 indicating acidic nature.

Yeast and mould counts (YMC) were lower (10–5 $\times$ 10^5 CFU/g) in packed raisins and ranged from 10^2–10^6 CFU/g in open samples (Table 3) and no sample was found in unsatisfactory range. Fungal profiles of various grapes and its product have been studied by Tournasa and Katsoudas (2005). Moulds commonly isolated from grapes and its products (juice, wine, vinegar and dried grapes) can be contaminated with Orchratoxin A. (OTA), a mycotoxin with nephrotoxic, carcinogenic, genotoxic and immunotoxic properties (Gomez et al. 2006). Statistical correlation between Total bacterial count and *Enterobacteriaceae* was found to be 0.96 in packed raisins, similarly correlation between *Lactobacilli* and moisture content in open raisins was found to be 0.708. There was no statistical difference in the mean values of (ANOVA) of the microbial counts among raisin groups.

Incidence of specific pathogens like *Salmonella* spp. was observed in both the forms of raisin. Presence was more frequent in open sample (4/10) than in packed ones (2/10). Indicator microorganisms in packed samples i.e. Enteropathogens were found to be absent, whereas two open samples were found to have <100 CFU/g, i.e. showing satisfactory microbiological quality (Table 3). *Staphylococcus* spp. was found to be absent in all the samples of raisins. *Pseudomonas* spp. was not detected in any of the raisins tested. *Lactobacilli*/Lactic acid bacteria were also found in all the open raisins and in only two packed samples. The findings indicate the presence of spoilage and pathogenic organisms in raisins but within limits, providing an evidence base for possible microbiological risk to consumer health.

Pesticides are widely used in the cultivation of fruits and vegetables (Kumari et al. 2006). Their chemical composition can pose a risk to consumer health and for this reason; most countries have legislation that governs the permissible concentration of pesticide in foods. The level of nine organochlorine pesticides including HCH (α-HCH, β-HCH,

Table 1 pH, moisture content and microbiological examination of different commercially available open raisin samples (CFU/g) (average of triplicates)

Sample	pH	Moisture content (%)	NAM	SDA	BGA	VRBA	MRS	BPA	CAB
O1	3.68 ± 0.07	7.50	3.0×10^3	5.0×10^5	5.0×10^2	Absent	5.0×10^2	Absent	Absent
O2	3.88 ± 0.02	8.86	1.0×10^6	1.0×10^6	Absent	0.1×10^2	1.0×10^2	Absent	Absent
O3	3.63 ± 0.02	8.53	4.0×10^4	5.0×10^5	2.5×10^2	Absent	4.0×10^2	Absent	Absent
O4	4.05 ± 0.05	9.27	1.0×10^4	3.0×10^6	0.1×10^2	0.2×10^2	4.5×10^2	Absent	Absent
O5	3.92 ± 0.01	9.82	1.0×10^6	3.0×10^6	Absent	Absent	0.5×10^2	Absent	Absent
O6	3.75 ± 0.05	19.60	2.0×10^6	4.0×10^6	Absent	Absent	1.0×10^3	Absent	Absent
O7	3.89 ± 0.03	15.76	15×10^5	1.0×10^6	1.0×10^2	Absent	1.5×10^2	Absent	Absent
O8	4.04 ± 0.03	9.03	1.0×10^5	1.0×10^2	Absent	Absent	1.0×10^2	Absent	Absent
O9	3.74 ± 0.03	4.07	3.0×10^4	2.0×10^3	Absent	Absent	1.0×10^2	Absent	Absent
O10	3.71 ± 0.01	11.36	2.0×10^6	1.0×10^6	Absent	Absent	4.0×10^2	Absent	Absent

O, open raisin sample; NAM, nutrient agar medium (total aerobic count); SDA, Sabouraud's agar medium (yeast and mould count); BGA, brilliant green agar (*Salmonella* spp.); VRBA, violet red bile agar (*Enterobacteriaceae*); MRS, De Man Rogosa and Sharpe Agar (*Lactobacilli* spp.); BPA, Baired pair agar (*Staphylococcus* spp.); CAB, cetrimide agar base (*Pseudomonas* spp.)

Table 2 pH, moisture content and microbiological examination of different commercially available packed raisin samples (CFU/g) (average of triplicates)

Sample	pH	Moisture content (%)	NAM	SDA	BGA	VRBA	MRS	BPA	CAB
P1	3.66 ± 0.03	6.81	2.6×10^4	Absent	Absent	Absent	Absent	Absent	Absent
P2	4.20 ± 0.01	9.78	0.5×10^2	0.1×10^2	Absent	Absent	Absent	Absent	Absent
P3	3.96 ± 0.17	7.22	1.0×10^4	5.0×10^3	Absent	Absent	3.0×10^2	Absent	Absent
P4	4.01 ± 0.17	9.33	Absent	Absent	Absent	Absent	1×10^2	Absent	Absent
P5	3.64 ± 0.01	6.98	6.0×10^5	5.0×10^4	1.0×10^2	Absent	Absent	Absent	Absent
P6	4.07 ± 0.04	1.44	0.5×10^2	Absent	Absent	Absent	Absent	Absent	Absent
P7	4.24 ± 0.05	9.62	0.5×10^2	0.2×10^2	5.0×10^4	Absent	Absent	Absent	Absent
P8	4.09 ± 0.00	2.30	1.0×10^2	1.0×10^6	Absent	Absent	Absent	Absent	Absent
P9	4.12 ± 0.00	8.54	1.0×10^6	1.0×10^2	Absent	Absent	Absent	Absent	Absent
P10	3.61 ± 0.01	2.60	1.0×10^3	Absent	Absent	Absent	Absent	Absent	Absent

P, packed raisin sample; NAM, nutrient agar medium (total aerobic count); SDA, Sabouraud's agar medium (yeast and mould count); BGA, brilliant green agar (*Salmonella* spp.); VRBA, violet red bile agar (*Enterobacteriaceae*); MRS, De Man Rogosa and Sharpe agar (*Lactobacilli* spp.); BPA, Baired pair agar (*Staphylococcus* spp.); CAB, cetrimide agar base (*Pseudomonas* spp.)

Table 3 Microbiological quality of commercially available open and packed raisin sample

Criterion (n = 10)	Microbiological quality (CFU/g)			
	Range (CFU/g)	Satisfactory	Acceptable	Unsatisfactory
TBC[o]	$(3.0 \times 10^3$–$2.0 \times 10^6)$	$6(<10^5)$	$4(10^5$–$<10^{6)}$	$(\geq 10^6)$
TBC[P]	0.3×10^2–1.0×10^6	8	2	–
YMC[O]	1.0×10^2–4.0×10^6	N/A	N/A	N/A
YMC[P]	0.1×10^2–1.0×10^6			
Salmonella spp.[a O]	0.1×10^2–5.0×10^2	4	N/A	N/A
Salmonella spp.[a P]	1.0×10^2–5.0×10^6	2 (not detected in 25 g)		
Enterobacteriaceae[b O]	0.1×10^2–0.2×10^2	$2(<100)$	$(100$–$10^4)$	$(\geq 10^4)$
Enterobacteriaceae[b P]	0.1×10^2–6.0×10^2	–	–	–
Staphylococcus spp.[O]	0	(<20)	$(20$–$<100)$	$(\geq 10^4)$
Staphylococcus spp.[P]	0	–	–	–
Lactobacillus[c O]	0.5×10^2–1.0×10^3	N/A	N/A	N/A
Lactobacillus[c P]	0			
Pseudomonas spp.[O]	0	N/A	N/A	N/A
Pseudomonas spp.[P]	0			

O = open, P = packed

NA = not available, values in parenthesis are the permissible limits

[a] Pathogens

[b] Indicator organism

[c] Spoilage bacteria

δ-HCH and γ-HCH), Endosulfan, DDE and its metabolites (pp′DDE, op′DDT, pp′DDT, pp′DDD) selected for monitoring of their residue in raisins are given in Table 4. Results indicate that α-HCH and γ-HCH (except two) were detected in all the samples. The Organochlorine pesticides (γ-HCH) content in the open tested raisins ranged from 0.011 to 0.9620 mg/kg whereas as in packed raisins it was 0.032 to 0.766 mg/kg but below the maximum residue level (MRL) i.e. 3.0 mg/kg in fruits and vegetables. It was also found that the level of γ-HCH was higher than α-HCH in almost all the raisins. α-HCH was the dominant contaminant followed by γ-HCH in all the tested raisins. DDT and its metabolites (op′DDT, pp′DDD, pp′DDT) concentration level in all the samples was found to be below detection level. Residues of Endosulfan were not detected in any of the raisin samples. Gaun et al (2001) were the first researchers to significantly

Table 4 Level of organochlorine pesticide residue (mg/kg) in open and packed market raisin samples

Organochlorine pesticide residue (mg/kg)									
Open sample	α-HCH	β-HCH	δ-HCH	γ-HCH	Packed sample	α- HCH	β- HCH	δ-HCH	γ-HCH
O1	0.328	BDL	BDL	BDL	P1	0.039	BDL	BDL	BDL
O2	0.035	BDL	BDL	0.283	P2	0.092	BDL	BDL	0.766
O3	0.065	BDL	BDL	0.566	P3	0.048	BDL	BDL	0.355
O4	0.061	BDL	BDL	0.524	P4	0.017	BDL	BDL	0.155
O5	0.052	BDL	BDL	0.011	P5	0.056	BDL	BDL	0.470
O6	0.067	BDL	BDL	0.577	P6	0.079	BDL	BDL	0.066
O7	0.518	BDL	BDL	0.962	P7	0.019	BDL	BDL	0.205
O8	0.065	BDL	BDL	0.517	P8	0.038	BDL	BDL	0.359
O9	0.040	BDL	BDL	0.307	P9	0.054	BDL	BDL	0.438
O10	0.031	BDL	BDL	0.419	P10	0.004	BDL	BDL	0.032

BDL, below detection limit; O, open sample; P, packed sample

question the microbiological risks associated with pesticide use in food production. They realized that pesticide preparation are normally reconstituted and diluted in non-potable farm or agricultural waters, and that bacterial contaminants, including pathogens from this source might then grow in the preparations prior to application to the produce.

In our study a more detailed examination of the microbiological contamination in ready-to-eat food/food products and the estimation of organochlorine pesticides were carried out. Although present study cannot pinpoint the source of contamination or conditions prevailing during distribution or sale particularly when products are sold in open containers, it does advocate quality control in ready-to-eat foods. Although implementation of HACCP is the best way to assure the lowest possible risk by food borne pathogens in ready-to-eat foods like raisins but good post processing storage practices are also equally important and need to be enforced for quality control.

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