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Estimation of heavy metal contamination and antioxidant potential of Pakistani condiments and spices

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Key words: Condiments, Heavy Metals, Antioxidant Activity, Atomic Absorption Spectroscopy, UV/Vis Spectroscopy.

Abstract

This study was conducted to detect and estimate some toxic metals present in branded (BS) and non branded (NS) condiments and spices widely used in Punjab, Pakistan. Flame atomic absorption spectroscopy was applied to determine the concentration of Cadmium (Cd), Copper (Cu), Nickel (Ni), Lead (Pb) and Zinc (Zn) in the studied samples where as the UV/Vis spectroscopy was used for evaluating the antioxidant potential of samples via 1, 1- diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. On comparing the standards set by FAO/WHO, concentration of Cd and Ni was found higher in many of the samples. Minimum risk level (MRL) was also calculated by assessing the daily intake of samples and t test was applied using SPSS 13.0. Higher metal concentration in studied samples correlates with the metal pollution in soil, air and water, while higher antioxidant potential of NS substantiates the medicinal importance of condiments and spices.

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Introduction

Pakistan is an agricultural country with eastern values. The Pakistani food is famous for its spices and condiments which add flavor, color, preservation and aroma to it. Spices and condiments can be divided into branded and non branded form on the basis of packaging and processing. These are usually extracted or separated from the leaves, flowers, berries, seeds, barks, buds or rhizomes of the plants (Nkanash *et al.*, 2010). Metals in adequate concentration are essential for healthy functions of body and also for proper growth and development of plants (Nakansah *et al.*, 2010) but at higher concentration are likely to behave as toxins (Hina *et al.*, 2012).

As these spices and condiments have shown biological activities like antioxidant, anti inflammatory, anti tumor, immuno modulatory and anti radical so it is essential to maintain the level of heavy metals in plants (Kochhar *et al.*, 2008). Generally *Zingiber officinale* is advised for medicinal purpose due to its anti inflammatory, cholesterol lowering and anti blood clotting properties (Mishra *et al.*, 2007) similarly Star Anise possess the anti carcinogenic and anti oxidant properties (Belewa *et al.*, 2009; *Shu et al.*, 2010).

There are various routes of exposure of plants, spices and condiments to the heavy metals including from air, water and soil. Likewise Lead (Pb) is transported and distributed from fixed, mobile and natural sources through air. Airborne Pb can contaminate the food, water, and can affect the lungs directly through inhalation. Similar is the case with copper (Cu), cadmium (Cd) , Nickel (Ni) and Zinc (Zn).

As discussed previously, that the spices have antioxidant properties too. Antioxidants actively participate in delaying or prevention of oxidation by donating electrons to the free radicals and removing the reactive oxygen species and reactive nitrogen species initiators (Borek, 1991). Condiments are widely used in Eastern cuisines particularly in South Asian region. The main objective of this research was to check the toxic metal content in BS and NS of spices and condiments available in local markets. Heavy metal concentration present in spices and condiments has been compared with international standards WHO/FDA and antioxidant characteristics were estimated.

Experimental

Heavy Metal Analysis

The description of seasoning, culinary condiments and dry spices purchased from local market of Rawalpindi/Islamabad as non branded samples (NS) and brands (Shan foods, Chand and Chef's choice) were selected as branded samples (BS) is given in table 1. All the collected samples were carefully opened, dried, grinded and sieved through 60 µm mesh size (Nkanash *et al.*, 2010). Wet digestion procedure was followed by taking few gram of each sample and was digested with 2:1 HNO_3 /H_2O_2. Digestion was confirmed with the turning of color of emitting fumes from reddish brown to white. The remaining mixture was filtered, diluted with deionized water and was analyzed using FAAS (Varian Inc. Model: Spectra AA 220) for Pb, Cd, Cu, Zn and Ni content (Soylak *et al.*, 2004). All the reported results are average of triplicates.

Antioxidant Activity

Radical scavenging activity of condiments was studied by using 1,1 diphenyl -2-picrylhydrazyl DPPH radical scavenging assay according to procedure mentioned in (William *et al.*, 1995; Khalaf *et al.*, 2008).

After this spectra were recorded on UV spectrophotometer at wavelength of 517 nm. The results were calculated by the following formula:

% DPPH scavenging activity= [Absorption of control –Absorption of sample] x 100

The daily intake (mg/kg/day) was calculated based on these suppositions i.e. 1) The human weight is 50 kg 2) The human intake from spices per day is 20 g. For calculating daily intake mg/kg/day for each metal following equation was used in (Mubeen *et al.*, 2009).

The daily intake (mg/kg/day) = Metal concentration in spice × 20/1000 /50 Eq.....1.

Results and discussion

Concentration of Heavy Metals in Condiments

Studied samples of spices and condiments showed different concentrations of heavy metals which vary according to plant and its origin and growing conditions. The concentration of each heavy metal in condiments given in the following tables, and has been compared with the permissible values proposed by WHO.

Table 1. Sample coding for branded and non branded samples.

Serial No	Sample Code		Sample Description
1	BS 01	NS 01	Red Chilli
2	BS 02	NS 02	Black Pepper
3	BS 03	S 03	Fenugreek
4	BS 04	NS 04	Turmeric
5	BS 05	NS 05	Cumin
6	BS 06	NS 06	Cinnamon
7	BS 07	NS 07	Garlic
8	BS 08	NS 08	Ginger
9	BS 09	NS 09	Coriander
10	BS 10	NS 10	Black cumin/Onion Seed
11	BS 11	NS 11	Ajwain
12	BS 12	NS 12	Ispaghol
13	BS 13	NS 13	Khash khash
14	-	NS 14	Badiyan k Phool
15	BS 15	NS 15	Meat Tenderizer

Cadmium (Cd)

Cd is considered to be a highly toxic metal as it can cause many severe diseases. The results calculated for Cd in condiments were parallel to those found in research reported by Mubeen *et al.*, 2009. The maximum concentration of Cd was recorded in NS 8 shown in table 2. As compared to WHO permissible limit for condiments (0.3 µg/g) (Ziarati, 2012).

Table 2. Heavy metal content in studied samples (BS & NS).

S.No	Sample Code	Cd µg/g	Pb µg/g	Cu µg/g	Zn µg/g	Ni µg/g
1	NS 01	0.514 ±0.231	BDL	1.280±0.104	6.580±0.294	1.020±0.010
	BS 01	1.582±0.728	1.295±0.053	1.747±0.337	0.151±0.029	4.939±0.182
2	NS 02	1.010±0.338	1.25±0.126	1.240±0.020	5.520±0.375	2.460±0.406
	BS 02	0.2253±0.006	0.928±0.043	1.198±0.351	0.438±0.203	1.519±0.131
3	NS 03	0.983±0.418	4.49±0.524	1.610±0.006	12.170±0.208	6.970±0.107
	BS 03	1.021±0.040	1.735±0.527	1.196±0.287	0.340±0.028	3.080±0.155
4	NS 04	0.760±0.227	BDL	0.110±0.031	8.860±0.811	1.430±0.741
	BS 04	1.117±0.103	BDL	0.2391±0.084	0.217±0.033	2.089±0.637
5	NS 05	0.820±0.289	5.290±0.218	1.570±0.021	9.680±0.406	5.440±0.517
	BS 05	0.071±0.399	1.273±0.405	1.299±0.431	3.186±4.050	1.449±1.092
6	NS 06	0.925±0.275	4.270±0.625	0.190±0.049	7.570±0.918	1.240±1.555
	BS 06	1.036±0.070	0.094±0.007	BDL	0.083±0.016	2.265±0.883
7	NS 07	0.776±0.217	4.030±0.404	BDL	8.240±1.562	BDL
	BS 07	BDL	0.750±.586	0.460±0.053	0.410±.325	2.093±0.037
8	NS 08	1.740±1.500	4.970±0.975	BDL	8.550±.227	1.440±0.721
	BS 08	BDL	5.155±0.425	0.234±0.003	0.243±.055	3.133±0.188
9	NS 09	0.613±2.340	2.340±0.350	0.620±0.036	12.050±.570	2.380±0.294
	BS 09	1.124±0.025	1.724±0.749	1.148±0.051	0.467±.011	3.970±0.176
10	NS10	0.506±0.160	2.650±1.429	0.480±0.002	19.80±.873	2.200±0.267
	BS 10	0.313±0.083	1.292±0.146	1.863±0.437	1.348±.666	5.610±0.197
11	NS11	0.614±0.132	5.260±1.790	0.566±0.008	82.830±.604	5.270±0.836
	BS 11	0.343±0.113	3.513±0.288	0.794±0.504	0.726±.301	5.820±0.127
12	NS12	0.421±0.292	2.920±1.297	**BDL	2.550±.082	0.840±0.428
	BS 12	0.181±0.067	0.182±0.043	BDL	0.292±.156	1.940±0.881
13	NS13	0.457±0.072	7.970±0.181	7.860±0.015	97.080±.585	16.10±3.825
	BS 13	0.444±0.066	2.169±0.222	2.698±0.325	1.300±.819	5.550±0.160
14	NS 14	0.361±0.050	3.880±0.082	1.070±0.034	6.740±.228	8.910±0.234
15	NS 15	0.359±0.119	7.830±2.297	1.520±0.007	10.610±0.664	3.550±0.183
	BS 15	0.256±0.082	1.336±0.516	1.356±0.022	0.462±0.350	4.360±0.325

It is evident from the results that Cd content is quite high in nearly all the NS. This high level of cadmium might be due to the use of cadmium containing phosphate fertilizers, or from the practice of growing these plants on soil amended with sewage sludge, or both (Kumar *et al.*, 2006). The daily intake values were much lower than MRL values (0.002mg/kg/day). So there was no harm due to cadmium intake of these spices under study.

Table 3. Single t Test for Branded and Non branded samples.

Serial No	Metals	Sample Code	Test Value	Mean n= 45	Std. Dev	Std error Mean	t (df=44)	Sig. (2 tailed)
1	Lead	Branded	10	1.4252	1.39330	0.20770	-41.284	0.000
		Non-branded	10	3.8144	2.46439	0.36737	-16.838	0.000
2	Cadmium	Branded	0.3	0.5288	0.52688	0.07854	2.913	0.006
		Non-branded	0.3	0.7247	0.50962	0.07597	5.591	0.000
3	Nickel	Branded	0.5	3.1902	1.78098	0.26549	10.133	0.000
		Non-branded	0.5	4.0156	4.17954	0.62305	5.643	0.000
4	Copper	Branded	20	0.9512	0.81950	0.12216	-237.78	0.000
		Non-branded	20	1.2117	1.89341	0.28225	-101.995	0.000
5	Zinc	Branded	50	0.6417	1.19589	0.17827	-276.87	0.000
		Non-branded	50	19.9259	28.16084	4.19797	-7.164	0.000

Copper (Cu)

Although copper is an essential element in trace amount but can be toxic at higher level. As revealed by analytical results (see Table 2) copper content of spices samples ranged between BDL to7.860 µg/g. The highest mean level of copper was found in NS 13 samples, and was not detected in BS & NS 06, 07, 08 , 12 and Badiyan k Phool sample, comparable to previous findings (Loutfy *et al.*, 2012; Kahalaf *et al.*, 2008; Hina *et al.*, 2012). The permissible limit of Cu was reported to be 50 µg/g (Nkansah & Amoako, 2010). The Proposed value for Cu MRL is 0.4mg/kg/day and all of the studied samples are markedly within permissible limit. So there is no harm on health due to intake of Cu from studied spices and condiments. The waste water from electronic industry is a significant source of Cu in soil and water.

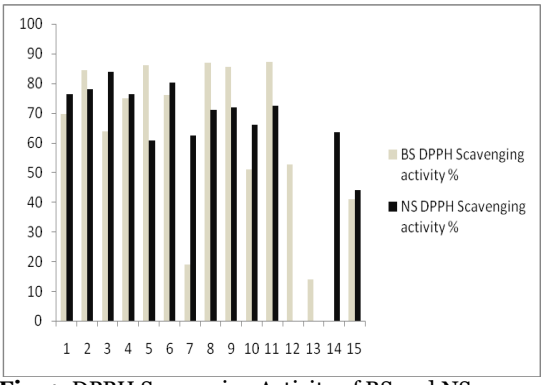

Fig. 1. DPPH Scavenging Activity of BS and NS.

Nickel (Ni)

The results calculated for Ni in BS and NS presented in table 2.The results showed variation in concentration from16.1µg/g to BDL. The permissible limit proposed by FDA 1999 was 0.5µg/g. All of the samples had values above permissible limit except NS -7 and on comparison the higher amount of Ni was present in NS. The results reported for Ni in Nigella sativa and in ginger by Ziarati (2012) were found similar with present study results. The primary reason for heavy metal contamination in edible portion of vegetables is possibly due to the use of treated and untreated waste water for irrigation (Ozcan, 2004).

Zinc (Zn)

As shown in table 2 the highest concentration of Zn was calculated in NS 13. The permissible limit of Zn was 50µg/g (WHO 1989) and all samples were well below the Permissible limit except NS, BS & NS 11.Findings for Zn in ajwain, black pepper and cinnamon were similar to those reported by (Khattak & Khattak, 2011). While for NS-7, BS-4, BS-8, NS-4 and NS-8 results were similar to those findings calculated by Krejpcio *et al.*, 2007. Just as inordinately high amounts of zinc could be more deleterious than nutritious, the WHO limit is not to be exceeded. The proposed value for Zn MRL is 0.3mg/kg/day and all of the studied samples are in

safe limit. There is no harm on health by taking Zn from using the studied spices and condiments.

Lead (Pb)

In BS and NS, concentration of Pb ranged between BDL to7.970 µg/g, i.e. greater than permissible standard limit of Pb (0.3mgkg^{-1}) for herbs . However, the values fall within the range of the WHO 2007 permissible limit of Pb (10 µg/g). All of the samples showed pretty good trend and all were within safe limits. Values for Pb in BS-6, NS-6 and BS-7 were similar to findings made by Krejpcio *et al.*, 2007. Similar trend as mentioned above in table 2 were calculated by Abou-Arab & Abou Donia, 2000 for BS-2 and BS-5. The MRL value for Pb is 0.0002 mg/kg/day (ATSDR, 2001). In most of the studied samples calculated MRL value is above the permissible limit. Electronic industry, plumbing and paint industry is a source of Pb in air, water and soil.

Heavy metals contents in spices varied depending on the country of origin, environmental pollution levels, plant part and technological processes. So the difference in metal concentration in BS and NS condiments heavily depends upon the technological processes involve

T Test

T test was applied in order to reject the null hypothesis (H) and the decision rule was : if the one tailed critical t value is less than the observed t and the means are in the right order, then Ho is rejected that is 1.068 in this case. It was evident that the mean value of Cd and Ni metals at both samples was significantly higher than the test value of 0.3µg/g and 0.5µg/g respectively. As showed by t values which were 2.931 and 5.591> 1.068 for Cd and 10.63 and 5.643 > 1.068 for Ni and null hypothesis was rejected for Cd and Ni in branded and non branded samples respectively. So this affirmed the presence of Cd and Ni in condiments.

Antioxidant Assay

DPPH Scavenging Activity of Branded Samples

The methanolic extract of studied samples (1mg/10ml) of spices and condiments was monitored for DPPH scavenging activity with method as described above. The results of this screening were compared with ascorbic acid a known antioxidant. The DPPH scavenging activity recorded for ascorbic acid was 89.6% and it was compared with scavenging activity of BS and NS shown in fig.1. The highest scavenging in BS was recorded for Ajwain 87.126%, Ginger 86.85%, Cumin 86.07%, Coriander 85.55%, and black pepper 84.52%.

DPPH Scavenging Activity of Non Branded Samples

The highest scavenging activity in NS (fig 1) was recorded in turmeric 83.74 %, cinnamon 80.36 % and black pepper 78.02% while samples of Ispaghol and khush khash (Poppy seeds) showed < 1% scavenging activity. The significant DPPH scavenging activity of both types of studied samples showed that these samples behaved as free radical scavenger and can convert free radicals to stable products.

Conclusion

This study was conducted to detect and estimate some toxic metals present in branded and non branded condiments and spices widely used in Punjab, Pakistan. The concentration of metals in non branded samples was found higher than the branded samples which support the unhygienic conditions of processing and storage involved for non branded samples. The antioxidant potential of non branded samples is higher than branded condiments. This antioxidant potential of condiments substantiates the medicinal importance of condiments.

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