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Assessment of non-essential heavy metals in ready- to-eat chicken meat products of Chennai city

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Abstract
A study was undertaken to analyse the non-essential heavy metals viz. aluminium, arsenic, cadmium, chromium, mercury and lead in commercially available Ready-To-Eat chicken meat products (chicken 65, chilly chicken, grilled chicken and tandoori chicken) collected from street food outlets at different regions of Chennai city. Totally 288 samples (72 samples for each product) were collected and analysed using inductively coupled plasma-optical emission spectrometry (ICP-OES). Results revealed that, among the three regions southern region had higher concentration of cadmium (2.21±0.65 ppm), chromium (2.82±1.58 ppm) and lead (4.00±2.07 ppm) whereas aluminium (12.30±2.99) and arsenic (0.38±0.18) were noticed in central region. Higher mercury (0.12±0.10) was noticed in northern region. Among the products grilled chicken samples had higher concentrations of aluminium, arsenic, and lead. Based on the results it was concluded that, heavy metals like arsenic and mercury in ready-to-eat chicken meat products were within the limits of prescribed standard whereas aluminium, cadmium, chromium, and lead were slightly higher than the permissible limits set by different regulatory agencies for meat and meat products. The reason for these higher values may be due to the ingredients, cooking methods and cooking utensils used for the preparation of chicken meat products and also the extraneous contaminants.

Keywords: Street food outlets, chicken meat products, extraneous contaminants, heavy metals

Introduction

Environmental pollution is considered as one of the most serious problem in the world over the decades. The constituents like air, water, soil, and food are vital to the human lives which directly influence the quality of human life and risk of contamination with various pollutants in this industrialized area. Contaminant is defined as any substance not intentionally added to food, which is present in such food as a result of the production, manufacture, processing, preparation, treatment, packaging, transport, or holding of such food or as a result of environmental contamination (Codex Alimentarius, 1995) [8]. Therefore, all food products are at risk of contamination from several resources, and there are no exemptions for chicken meat and products.

The development of a primary production and processing standard for poultry meat uses an approach that investigate the sources of potential risks, which may be introduced at different points through the primary production and processing chain. Poultry meat and products supply chain is divided into four distinct steps: primary production, processing, retail, and consumer. At each of these steps, poultry meat and products may be directly or indirectly exposed to contamination (Reyes-Herrera, 2012) [20]. Direct exposure results when a compound is present in raw meat, whereas in indirect exposure, contaminants cross into meat during processing, storage, packaging, or preparation. Indirect contaminants also include substances that become toxic and harmful to people due to food-processing practices. Indirect pollution is most frequently the result of unawareness, lack of education of food handlers or inappropriate handling applications (Botsoglou, 2001) [7].

Among the environmental contaminants, pollution by heavy metals is posing as the most serious threat to the people in the world. The majority of metals are natural components of the earth's crust. Metals and other elements can be naturally present in food or can enter food as a result of human activities such as industrial and agricultural processing. The sources of toxic metals in the environment are the fossil fuels, mining industries, waste disposal and municipal sewage through air, water and soil. Farming and forestry also contribute to the metal content in the environment due to the use of fertilizer, pesticide and herbicides.

Consequent to environmental pollution, the contaminants may also enter into the food chain. The major route of entry of most of the metals into the body is through food.

Presently, the society is sparing little attention and time towards food and health due to the technology advancement and busy chores at home as well as at work place. There is a rapid development and attraction towards the street foods, fast foods and foods from restaurants due to their spicy and tasty preparation in a short time. Most of the urban people living a mechanical life and they don't find time to prepare food regularly due to their work nature. Mainly for time saving and to reduce the work load they prefer to eat outside. These types of consumers consider only the taste of products and hygiene of surroundings or location of the outlets which are comfortable to eat and buy. But these heavy metals gain entry into the food chain through air, water and the raw ingredients which are used for the product preparation.

Street food outlets are defined as those ready-to-eat foods and beverages prepared and/or sold by vendors and hawkers in the streets and other public places for immediate consumption or consumption at a later time without further processing or preparation (WHO, 1996) [21]. Especially the street food outlets, which are nearer to road, factory, industries and sewage have more possibilities to be contaminated with more heavy metals than the usual preparations. Hence, this study will be helpful to understand the conditions of the street foods sold in Chennai city.

Materials and methods

The study was conducted to analyse the non-essential heavy metals in Ready-To-Eat chicken meat products samples viz. chicken 65 (72), chilly chicken (72), grilled chicken (72) and tandoori chicken (72). A total of 288 Ready-To-Eat chicken meat products were collected from street food outlets of northern, central and southern regions of Chennai as mentioned by Greater Chennai Corporation. The samples each weighing approximately 200g were carefully collected and homogenized thoroughly using blender. The homogenized samples were then packed in polyethylene zip-lock bags and stored at the temperature of -18ºC in the laboratory until further use as per the procedure of Hoha *et al.* (2014) [13].

Preparation of Glassware and Plasticware

Glassware such as screw cap test tubes, pipettes, funnels, volumetric flasks, measuring cylinders, conical flasks and round bottom flasks were initially soaked in detergent solution and thoroughly cleaned using tap water. Subsequently, they were rinsed and soaked in 5 per cent nitric acid solution overnight. Then, the glassware soaked in the nitric acid were rinsed with single glass distilled water and soaked again overnight. The next day it was rinsed with Millipore water and allowed to remain soaked overnight. Finally, they were drained and dried in hot air oven at 70 ˚C for 8-10 hours.

Sterile 50 ml disposable polypropylene conical bottom centrifuge tubes with screw caps were used at the final stage of sample preparation to collect the final extract and to make up the final volume to 50 ml.

Preparation of chemicals for sample digestion

For sample digestion Nitric acid 69% (HNO3), Hydrogen Peroxide 30% (H2O2) and Millipore water were used.

Wet Digestion Method

The homogenized frozen samples were thawed overnight in a refrigerator at 4±1 ˚C before starting the digestion process. The samples were digested by wet digestion method for the analysis of various heavy metals by following the procedure of Jankeaw *et al.* (2015) [15]. One gram of sample was taken in a 25ml glass screw cap test tube. Five ml of 69% concentrated nitric acid (Sigma aldrich) was added to the sample and kept in water bath at 80 ˚C for 15 minutes subsequently the test tubes were cooled to room temperature and dried in hot air oven at 135 ˚C for 3 minutes. The samples were cooled to room temperature and 1 ml of 30% hydrogen peroxide solution was added to the contents and filtered through Whatman filter paper No.1 and followed by No.42 filter paper. The extracted solution was quantitatively transferred into a 50 ml volumetric flask and the volume was made upto the mark with millipore water and immediately used for heavy metal analysis.

Analysis of heavy metals

Samples were analysed for the estimation of heavy metals using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (The Agilent Technologies 720 series).

Table 1: Standard operating conditions

Instrument set up				
Parameters	**Purge**	**Delay**	**Ignite**	**Run**
Plasma (L/min)	22.5	22.5	1.5	15
Auxillary (L/min)	2.25	2.25	1.5	1.5
Mass Flow Control (L/min)	0.900	0.000	0.000	0.900
Power (kw)	0.00	0.00	2.00	1.20
Pump (RPM)	0	0	50	7
Time (s)	15	10	5	3
Nebulizer flow (L/min)	0	0	0	0.75

Instrument stabilization delay (s)	:	15
Replicates	:	3
Replicate Read time (s)	:	1
Sample introduction setting		
Sample uptake delay (s)	:	30
Pump rate (RPM)	:	15
Rinse time (s)	:	10

Estimation of heavy metals

Calibration was done with multi-element calibration standard for the heavy metals viz. aluminium, cadmium, chromium, and lead in aqueous medium linear range of 0 ppb to 1 ppm. Calibrations of Arsenic and Mercury elements were done using the VGA (Vapour Generation Accessory) unit of ICP-OES in aqueous medium linear range of 0 ppb to 100 ppb.

Table 2: Wavelength used for quantification.

S. No.	Elements	Wavelength (nm)
1.	Aluminium (Al)	396.152
2.	Arsenic (As)	228.812
3.	Cadmium (Cd)	228.802
4.	Chromium (Cr)	205.560
5.	Mercury (Hg)	253.652
6.	Lead (Pb)	220.353

Quantitative determination of heavy metals

Multi-element including aluminium, arsenic, cadmium, chromium, mercury, and lead were recorded directly from the analysis scale of ICP and were calculated by the following equation according to ASTM, (2002) [4].

Element in mg/kg = R x D/W

R= Reading of elemental concentration (mg/kg) from the digital scale of ICP system

D = Dilution of prepared sample
W = Weight of the sample
The results obtained in this study (ppb (μg/kg)) were converted into ppm (mg/kg) for the calculation.

Statistical analysis

The raw data were analysed by using one way ANOVA for statistical analysis with statistical package SPSS 14.0 version (SPCC Inc., Chicago, USA 2005).

Results and discussion

The results of non-essential heavy metals in the Ready-To-Eat chicken meat products of street food outlets at different regions of Chennai city, Tamil Nadu were as follows:
The results of non-essential heavy metals viz. Aluminium, Arsenic, Cadmium, Chromium, Mercury and Lead are presented in Table No.1 and Figure 1-6.

Table 1: Mean ± SE values of non-essential heavy metals (ppm) in chicken products collected from the street food outlets of different Regions of Chennai

Elements (ppm)	Products	Northern Region	Central Region	Southern Region	F Value
Aluminium (Al)	Chicken 65	6.14±1.99	7.38±2.25	5.31±1.97	0.97^{NS}
	Chilly chicken	2.41±0.64	6.23±1.68	3.81±1.40	2.16^{NS}
	Grilled chicken	7.72±2.25	10.24±3.37	8.88±2.90	0.66^{NS}
	Tandoori chicken	4.64±1.54^{a}	10.30±2.99^{b}	5.56±1.75^{a}	3.65*
Arsenic (As)	Chicken 65	0.10±0.10	0.30±0.15	0.00±0.00	2.26^{NS}
	Chilly chicken	0.09±0.09	0.31±0.17	0.03±0.03	1.78^{NS}
	Grilled chicken	0.22±0.15	0.38±0.18	0.00±0.00	1.96^{NS}
	Tandoori chicken	0.26±0.15	0.23±0.13	0.07±0.05	0.79^{NS}
Cadmium (Cd)	Chicken 65	0.99±0.09^{a}	0.78±0.12^{a}	2.21±0.65^{b}	3.94*
	Chilly chicken	1.04±0.10^{ab}	0.75±0.14^{a}	1.96±0.54^{b}	3.72*
	Grilled chicken	0.97±0.11^{a}	0.80±0.12^{a}	1.95±0.52^{b}	3.87*
	Tandoori chicken	0.89±0.10	0.76±0.14	1.81±0.65	2.13^{NS}
Chromium (Cr)	Chicken 65	0.71±0.22	0.87±0.28	2.82±1.58	1.57^{NS}
	Chilly chicken	0.71±0.26^{a}	1.23±0.35^{ab}	1.95±0.50^{a}	2.65*
	Grilled chicken	1.13±0.32	1.16±0.29	1.38±0.36	0.17^{NS}
	Tandoori chicken	1.37±0.33	1.24±0.30	1.17±0.36	0.10^{NS}
Mercury (Hg)	Chicken 65	0.03±0.01	0.06±0.01	0.06±0.01	2.65^{NS}
	Chilly chicken	0.03±0.01^{ab}	0.06±0.01^{a}	0.06±0.01^{b}	3.33*
	Grilled chicken	0.12±0.10	0.04±0.01	0.07±0.01	0.51^{NS}
	Tandoori chicken	0.06±0.03	0.05±0.01	0.06±0.01	0.11^{NS}
Lead (Pb)	Chicken 65	0.28±0.28	0.60±0.44	2.04±1.56	0.98^{NS}
	Chilly chicken	0.69±0.33	1.29±0.65	3.30±1.68	1.67^{NS}
	Grilled chicken	0.65±0.37	0.96±0.59	4.00±2.07	2.16^{NS}
	Tandoori chicken	0.35±0.25	1.41±0.71	3.69±1.55	2.93^{NS}

Aluminium: Grilled chicken collected from central region had the highest aluminium content (10.30±2.99) whereas the lowest content (2.41±0.64) was observed in chilly chicken collected from northern region. There was no significant difference ($P>0.05$) between regions in all the products whereas a significant difference ($P<0.05$) was observed between the regions in tandoori chicken (Fig.1).

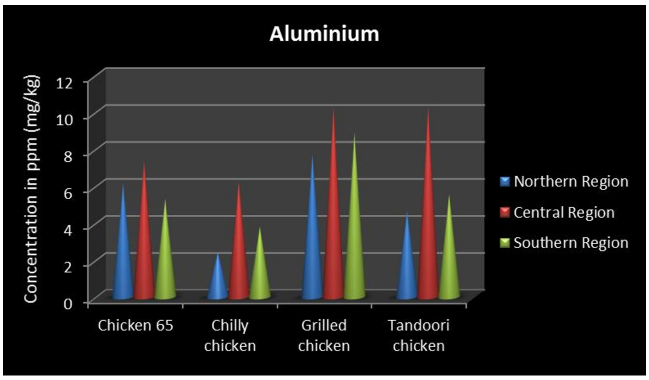

Fig 1: Aluminium concentration (ppm) of Ready-To-Eat chicken meat products (Street food outlets) in different regions of Chennai city

Results of this study were higher than the results reported by Filippini *et al.* (2019) who found 1.227 ppm of Aluminium in processed meat products. The values of this study were within the limits of FAO/WHO (1989) Experts Commission for food additives. The committee concluded that the daily intake of aluminium in children is 2-6 mg/kg, adults 6-14 mg/kg and the PTWI parameter for aluminium is 7 mg/kg body weight. In Meat products pollution with aluminium may be due to the use of aluminium utensils, acidic solutions for the preparation of chilly chicken, Tandoori chicken like products, butter, oil and spices, as well as the packaging materials like aluminium foil. Aluminum is also used to make beverage cans, pots and pans, airplanes, siding and roofing, and foil. Powdered aluminum metal is often used in explosives and fireworks.
Aluminum compounds are used in many diverse and important industrial applications such as alums (aluminum sulfate) in water-treatment and alumina in abrasives and furnace linings. Aluminum is found in consumer products including antacids, astringents, buffered aspirin, food additives, antiperspirants and cosmetics. There are chances of these products entering into the food chain and contaminate the meat products (ATSDR, 2008) [5].
Arsenic: Grilled chicken collected from Central Region had the highest Arsenic content (0.38) whereas no arsenic content in chicken 65 (BDL) and grilled chicken (BDL) collected from southern region. There was no significant differences ($P>0.05$) between the regions in all the four products (Fig.2). The arsenic contents were below the observation made by Mariam *et al.* (2004) [17] who found higher arsenic concentrations in lean meats of beef (46.46 ± 3.41 ppm), mutton (42.40 ± 4.95 ppm) and poultry (44.09 ± 3.62 ppm) and concluded that the arsenic pollution in the environment,

which may be due to copper smelting, coal combustion, burning of firewood and cow dung. The present study was within the permissible limits (0.5-2 PPM) of ANZFA, 2001 [3].

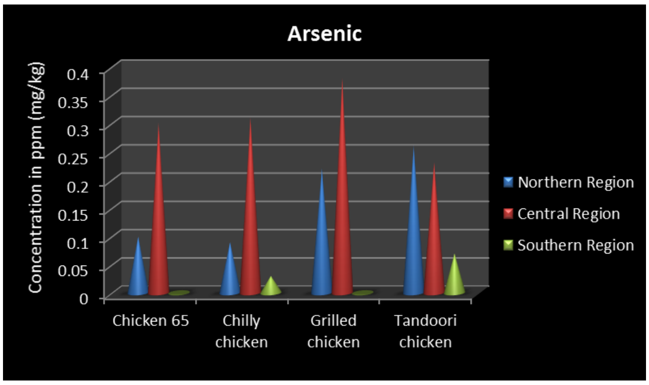

Fig 2: Arsenic concentration (ppm) of Ready-To-Eat chicken meat products (Street food outlets) in different regions of Chennai city

Cadmium: Chilly chicken collected from northern region had the highest Cadmium content (1.96±0.54) whereas the lowest content (0.75±0.14) was observed in Chilly chicken collected from Central region. There was a significant difference ($P<0.05$) observed between the regions in chicken 65, chilly chicken and grilled chicken whereas no significant difference ($P>0.05$) was observed between regions in Tandoori chicken (Fig.3).

Similar study made by Abraham *et al*. (1999) [1] in different meats and found 0.42±0.04 ppm of cadmium in goats muscle. The cadmium content in chilly chicken were slightly higher than the results obtained by Mariam *et al.* (2004) [17] in poultry meat (0.31 ppm) and higher than the maximum permissible limit of 0.5 ppm prescribed by FAO/WHO, (2000) [11] and FSSAI (2011) [12] prescribed standard of 1.5ppm for cadmium in food.

However, Contamination of meat products with cadmium would occur due to phosphate fertilizers or the lands contaminated with industrial cadmium effluent (Koh and Judson, 1986) [16]. Secondary cadmium contamination of food occurs in food processing (Zurera-Cosano, 1993) [22]. Muller *et al.* (1996) [19] reported that sausages had higher cadmium content than the raw meat. The addition of spices during production of sausages might be the main reason since spices could contain cadmium concentrations up to 200 ng g-' (Muller *et al.* 1992) [18].

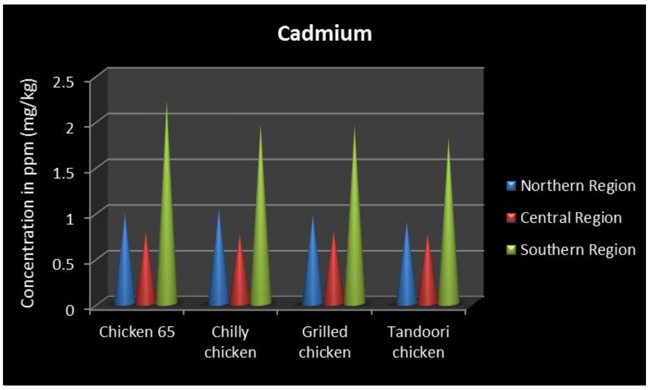

Fig 3: Cadmium concentration (ppm) of Ready-To-Eat chicken meat products (Street food outlets) in different regions of Chennai city

Chromium: Chicken 65 collected from southern region had the highest content of Chromium (2.82±1.58) whereas the lowest content (0.71±0.22) was observed in Chicken 65 collected from northern region. There was a significant difference ($P<0.05$) observed between the regions in Chilly chicken and no significant difference ($P>0.05$) was observed between regions in all other products (Fig.4).

In general, the total concentrations of chromium in meat and fish ranged from 0.01 to 1.3 mg/kg (Anonymous, 1996) [2]. However, in the current study the values exceeded the permissible limits of Codex, 1994 (0.05 ppm). In India, there is no specific standard limit for chromium content in fresh meat and processed meat (FSSAI, 2011) [12]. Similar study made by Iwegbue *et al.* (2008) [14] who also found 0.01 and 4.83 mg/kg of chromium in chicken meat. The reasons for higher chromium in meat products may be due to the extraneous contamination in food chain by wood treated with copper dichromate, leather tanned with chromic sulfate, and stainless steel cookware (ATSDR, 2012) [6].

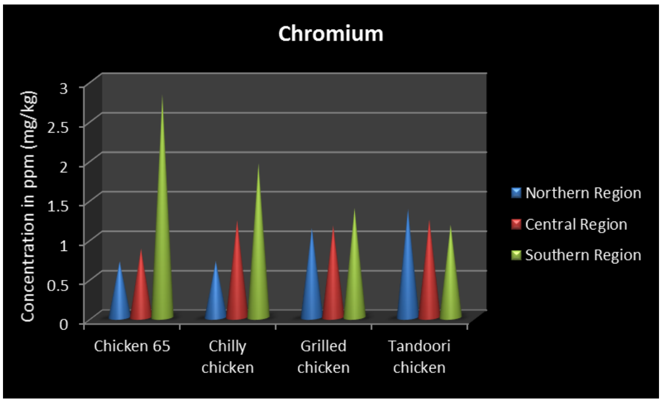

Fig 4: Chromium concentration (ppm) of Ready-To-Eat chicken meat products (Street food outlets) in different regions of Chennai city

Mercury: Grilled chicken collected from Northern region had the highest Mercury content (0.12±0.10) whereas the lowest content (0.03±0.01) was observed in Chicken 65 and Chilly chicken collected from Northern region. There was no significant difference ($P>0.05$) was observed between regions in all the products (Fig.5). Results of this study were incongruence to the study of Mariam *et al.* (2004) [17] who found more Mercury content in poultry meat (76.10 + 27.44 ppm).The values of the present study were within the limits of FSSAI, 2011 [12] (1.0 ppm) and ANZFA, 2001 [3] (0.03 ppm).

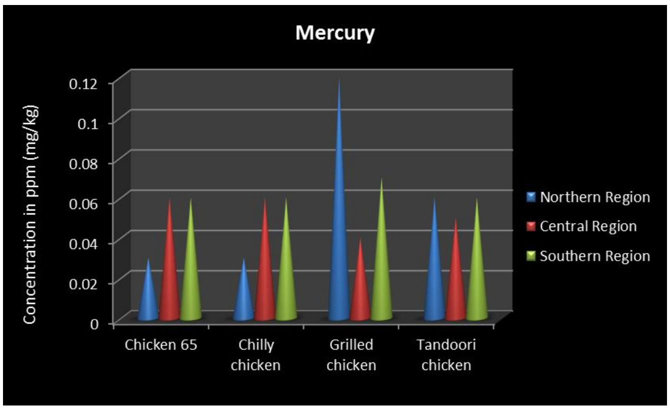

Fig 5: Mercury concentration (ppm) of Ready-To-Eat chicken meat products (Street food outlets) in different regions of Chennai city

Lead: Grilled chicken collected from southern region had the highest Lead content (4.00±2.07) whereas the lowest content

(0.28±0.28) was observed in chicken 65 collected from northern region. There was no significant difference ($P>0.05$) observed between regions in all the products (Fig.6). The standards prescribed by FSSAI for foods (2011) [12] 2.5 ppm, Codex Alimentarius, (1999) 0.5 ppm for meat and Ireland standard 5 ppm for meat products. The results of this study were exceeded the permissible limit of FSSAI.

Iwebgue *et al*. (2008) [14] who also found high range of lead concentrations in their study between 0.01−4.60 mg.kg-1 for chicken meat and mentioned that the excessive amount of Pb in chicken meat could not be attributed to industrialization alone. High contents of metals in poultry products emanate mainly from contamination of feeds and water sources. Mariam *et a*l. (2004) [17] also found higher Lead values in poultry meat (3.1 + 0.58) which was more than the permissible limits of Codex Alimentarius (1999) 0.5 ppm. Similar study made by Abraham (1994) and found 20.22±3.39 ppm of lead content in cattle muscle and below the detection level in goats and pigs muscle.

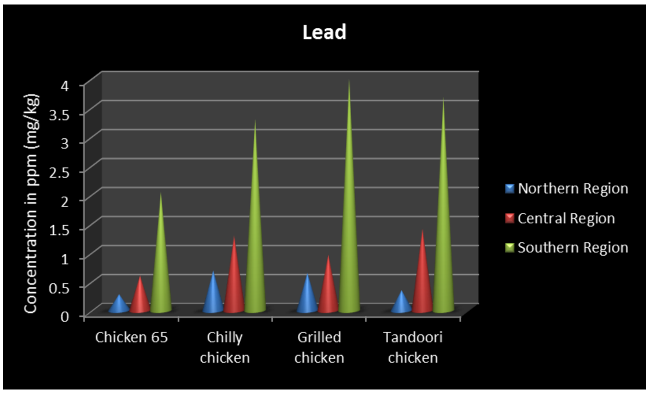

Fig 6: Lead concentration (ppm) of Ready-To-Eat chicken meat products (Street food outlets) in different regions of Chennai city

The higher lead concentration is may be due to lead pollution in the environment. The major source of lead contaminant is automobile exhaust gases, which arise from anti-knocking agents added in gasoline, paints, water pipes and untreated waste effluents of industry, which find their way to irrigation channels and hence pollute the fodder through soil.

Conclusion

A study made to find out the non-essential heavy metals viz. aluminium, arsenic, cadmium, chromium, mercury and lead in Ready-To-Eat chicken meat products revealed that the arsenic and mercury levels were within the limits in all the products and three regions. All the three regions had higher aluminium content than the permissible limit of 1 ppm in all the products. Southern region had higher cadmium, chromium and lead content in all the products. There is scanty literature to discuss the results and no standard limits are available for many heavy metals in meat products. Based on the results it can be concluded that the presence of higher levels of some heavy metals in Ready-To-Eat chicken meat products collected from street food outlets of Chennai city mostly due to the extraneous contaminants only not from the chicken meat. Because the chickens are slaughtered around 37 days old that is not possible to accumulate the heavy metals in the meat especially the cumulative poisoning like arsenic, cadmium, mercury and lead.

The reason for higher values may be due to the raw ingredients, cooking methods and cooking utensils used for the preparation of the chicken meat products and also environmental pollution. The levels exceeded in this study were not of health risk, because the products were not consumed daily as a whole diet to accumulate in the body. Hence, it is concluded that the Ready-To-Eat chicken meat products sold in the street food outlets of Chennai city can be consumed as a part of the diet in regular interval and also the contamination of heavy metals in meat products were mainly through some extraneous contaminants.

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