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A study on aflatoxin content in dates available in domestic market in India

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ABSTRACT

Phoenix dactylifera, commonly known as **date** or **date palm**, is a flowering plant species in the palm family, Arecaceae, cultivated for its edible sweet fruit. Although its place of origin is unknown because of long cultivation, it probably originated from lands around Iraq. Dates have been a staple food of the Middle East and the Indus Valley for thousands of years. The Ancient Egyptians used the fruits to make date wine, and ate them at harvest. **Dates** make a sweet, nutritional snack and can be eaten as they come, or glazed with syrup. The date fruit consists of 70 % carbohydrates (mostly sugars), making it one of the most nourishing natural foods available to man. In most varieties, the sugar content of a date fruit is almost entirely of the inverted form (namely glucose and fructose), important for persons who cannot tolerate sucrose. The invert sugar in dates is immediately absorbed by the human body without being subjected to the digestion that ordinary sugar undergoes.

Dates are of two types Unpitted and pitted. Pitted dates are fruits that had their stones removed. **Pitted dates** are without the pit are loaded with natural sweetness and can be used for noshing or baking. Unpitted dates is containing the pits or stones. P**itted dates** are firm, easy to chop up and can be used in variety of recipes. The dates are directly consumed without any processing. The aflatoxins are a group of chemically similar toxic fungal metabolites (mycotoxins) produced by certain moulds of the genus *Aspergillus* growing on a number of raw food commodities. Aflatoxins are highly toxic compounds and can cause both acute and chronic toxicity in humans and many other animals. The aflatoxins consist of about 20 similar compounds belonging to a group called the difuranocoumarins, but only four are naturally found in foods. These are aflatoxins B1, B2, G1 and G2. Aflatoxin B1 is the most commonly found in food and also the most toxic and classified by the International Agency for Research on Cancer (IARC) as 1st class carcinogen. The Dates, which is directly being consumed by human being, to be free from Aflatoxin contamination or contain the permissible limit of same. In the present study Aflatoxin B1 contamination have been carried out in two varieties of dates(pitted and unpitted) collected from different parts of India and also to assess whether the fruits were safe for human consumption.

The aflaoxin in dates have been analysed using HPTLC. 50 Samples of unpitted dates analyzed for the estimation of Aflatoxin. 49 samples are having Aflatoxin content below detection level. Only one sample contains 4.771 ppb of

Aflatoxin. In FSSAI Max limit for Aflatoxin is 30 ppb. 51 Samples of pitted dates analyzed for the estimation of Aflatoxin. All the 51 samples are having Aflatoxin content below detection level.

Keywords: Dates; Aflatoxin; HPTLC.

INTRODUCTION

Phoenix dactylifera, commonly known as **date** or **date palm** [1], is a flowering plant species in the palm family, Arecaceae, cultivated for its edible sweet fruit. Although its place of origin is unknown because of long cultivation, it probably originated from lands around Iraq [2]. The species is widely cultivated and is naturalized in many tropical and subtropical regions worldwide [3]. Dates have been a staple food of the Middle East and the Indus Valley for thousands of years. The Ancient Egyptians used the fruits to make date wine, and ate them at harvest. **Dates** make a sweet, nutritional snack and can be eaten as they come, or glazed with syrup. The date fruit consists of 70 % carbohydrates (mostly sugars), making it one of the most nourishing natural foods available to man. In most varieties, the sugar content of a date fruit is almost entirely of the inverted form (namely glucose and fructose), important for persons who cannot tolerate sucrose. The invert sugar in dates is immediately absorbed by the human body without being subjected to the digestion that ordinary sugar undergoes. The flesh of dates contains 60 to 65 % sugar, about 2.5 % fibre, 2 % protein and less than 2 % each of fat, minerals, and pectin substances. Date fruits are also a good source of iron, potassium and calcium, with a very low sodium and fat content. In addition, moderate quantities of chlorine, phosphorous, copper, magnesium, silicon and sulphur are also found in the date fruit.

To grow as they should, date palms require the right balance of environmental factors including a sufficient direct sunlight and just the right amount of water as allowing the soil to dry out can be disastrous and stop the date palm from growing completely. They also require fertilizer and just the right temperature (above 20°C). Because of these requirements, the areas in which they can be grown are restricted. Typically, they are grown tropical or sub-tropical countries which can offer the right climate. In India, it is cultivated in Kachchh district of Gujarat with a production of 85351tones per annum of fruits.

Dates are of two types Unpitted and pitted. Pitted dates are fruits that had their stones removed. **Pitted dates** are without the pit are loaded with natural sweetness and can be used for noshing or baking. P**itted dates** are firm, easy to chop up and can be used in variety of recipes. They can be stuffed with various fillings such as nuts (walnuts, almonds and pecans), In fact, dates are so versatile that different countries have found their own novel uses for date fruit. They actually make an effective natural binding agent in baked treats. Date pectin, dietary fibre and syrup are some of the date substances which can find a plethora of applications as a thickener or gelling agent in processed foods, i.e., confectionery products, jams, table jellies, soft cheeses, yoghurts, etc.

Aflatoxins are toxic and carcinogenic metabolic products of *Aspergillus (A. flavus*, *A. parasiticus* and *A. nomius)* [4, 5, 6]. Aflatoxins are highly toxic compounds and can cause both acute and chronic toxicity in humans and many other animals. The aflatoxins consist of about 20 similar compounds belonging to a group called the difuranocoumarins, but only four are naturally found in foods. These are aflatoxins B1, B2, G1 and G2. Aflatoxin B1 is the most commonly found in food and also the most toxic and classified by the International Agency for Research on Cancer (IARC) as 1st class carcinogen [7, 8, 9]. Aflatoxin producing fungi may contaminate fruit, nuts or corn if grown, stored and/or processed under conditions which favour fungal growth. Hot, humid climates and any pest pressures resulting in bruising or cuts on the commodity will favour the growth of the Aflatoxin producing fungi, either in the field or in storage. Growth of these fungi on certain foods and feeds may result in Aflatoxin production which results in illness or death in humans and animals and thus is an important public health concern [10, 11, 12]. Prolonged storage and/or contamination during storage or transport have also been associated with higher Aflatoxin levels. Another factor affecting

the levels of Aflatoxin in dried fruits specifically is the type of drying method used. There are numerous variations and/or combinations of drying processes used by the food industry depending on the desired characteristics of the finished product, cost, and equipment availability. The typical temperatures in conventional drying processes do not exceed 120 °C and so are too low to cause appreciable Aflatoxin degradation. The most commonly used drying method for fruit is sun-drying.

Investigation of Aflatoxin levels in nut products and dried fruits was undertaken because of the human health effects of Aflatoxin exposure and the widespread consumption of these products in the Indian market. Frequent monitoring was thus carried out to assess the levels of contaminants in dry fruits in commercial markets of different parts of India. The Food Standard and Safety Authority of India (FSSAI) is responsible for enforcing safety laws and regulations on the production, sale, composition and content of foods and food products as outlined in the *Food and Drugs Act & Regulations* 2011. It also establishes health-based limits for contaminant residues in food. Tolerances are established as a risk management tool and are generally set only for foods that significantly contribute to the total dietary exposure. The tolerance level for Aflatoxin range from 0 to 50 µg/kg under food safety act. In India, a tolerance limit of 30 µg/kg has been prescribed under the Food Safety and Standards(Contaminants, Toxins and Residues) Regulation 2011, for all foods meant for human consumption [13].

The Dates, which is directly being consumed by human being, to be free from Aflatoxin contamination or contain the permissible limit of same. In the present study, Aflatoxin B1 determination have been carried out in two varieties of dates(pitted and unpitted) collected from different parts of India and also to assess whether the fruits were safe for human consumption.

MATERIALS AND METHODS

Total 101 Dates samples, consisting of 50 Unpitted Dates and 51 Pitted Datess were investigated for Aflatoxin B_1 levels. The Dates were collected from different parts of country.

Extraction of aflatoxins from Dates

For detection and estimation of aflatoxins from Dates, samples collected from different parts of India, the analytical procedure of solvent extraction and subsequent analysis by HPTLC was employed. About 20 g. dried finely crushed sample accurately weighed in 500 ml. Conical flask containing mixture of 1 gm NaCl, 50 ml Hexane and 125 ml Methanol: Water (55:45) and allowed to stand for 30 minutes with intermittently shaken Thereafter, the mixture was filtered through Whattman filter paper and solution has been taken in separating funnel. Discard Hexane layer. Wash again with Hexane ,if require. Collected Methanol: Water layer. 25 ml of this layer taken in separating funnel, and added 25 ml of Chloroform and shake. After layer being separated, discarded the aqueous layer, and Chloroform layer collected. The chloroform layer evaporated to dryness on water bath. The residue was dissolved with 2.5 ml of chloroform and stored in darkness for quantitative analysis.

Quantitative estimation of aflatoxins

Quantitative estimation of aflatoxin was done by High performance thin layer chromatography (HPTLC). The analytical equipment for HPTLC (CAMAG Linomat 5) with CAMAG TLC Scanner 3/081123 and operated with win CATs software.

Method of Spotting and Development of TLC plate

Pre-coated TLC sheets silica gel Merck 60 F_{254} 10x10 cm was taken.

Sample application

Apply band with CAMAG Linomat, distance from lower edge of sheet 12 mm, and distance from left edge 12 mm. Spotted 10 µl volume samples extract with band length 5 mm.

Standards application

Apply side by side, 3.0, 6.0 and 10.0 µl standard Aflatoxin B_1 (Concentration 0.5µl/ml).

Chromatography

The development chamber should be filled up with chloroform-acetone (9:1) upto a depth of about 8 mm and insert the sheet, The solvent migrates up to 70 mm. Then plate is air dried.

Scanning of TLC

Mounted air dried plate on Scanner Tray and fixed with the magnets. Scanned plate in TLC scanner, under UV light at 366 nm.

Calculation

The concentration of Aflatoxin B_1 in µg/kg has been calculated as follows:

$$\mu g/kg = \frac{B \times Y \times S \times V}{Z \times X \times W}$$

Where, B = average Area/Height of Aflatoxin B_1 peaks in test aliquots.

Y = concentration of Aflatoxin B_1 standards, µg/ml

S = µl of Aflatoxin B_1 standards spotted

V = final volume of test solution, µl

Z = average Area/Height of Aflatoxin peaks in standards aliquots.

X = µl test solution spotted.

W = gm test portion represented by test solution.

The final results have been obtained by taking average of concentration of Aflatoxin after calculation with respect to Height and Area.

RESULTS & DISCUSSION

The results of Aflatoxin in region wise have been mentioned in Tabe-1 for Unpitted dates and Pitted dates.

Table-1

Aflatoxin in Unpitted Dates			Aflatoxin in Pitted Dates		
Sl. No.	**Region**	**Result(in ppb)**	**Sl. No.**	**Region**	**Result(in ppb)**
1	Amritsar	ND	1	Nagpur	ND
2	Amritsar	ND	2	Nagpur	ND
3	Amritsar	ND	3	Nagpur	ND
4	Amritsar	ND	4	Nagpur	ND
5	Amritsar	ND	5	Nagpur	ND
6	Bhopal	ND	6	Nagpur	ND
7	Bhopal	ND	7	Nagpur	ND
8	Bhopal	ND	8	Amritsar	ND
9	Guntur	ND	9	Amritsar	ND
10	Guntur	ND	10	Bhopal	ND
11	Guntur	ND	11	Bhopal	ND
12	Guntur	ND	12	Bhopal	ND
13	Guntur	ND	13	Bhopal	ND
14	Guntur	ND	14	Guntur	ND
15	Jaipur	ND	15	Guntur	ND
16	Jaipur	ND	16	Guntur	ND
17	Jaipur	ND	17	Guntur	ND
18	Kanpur	ND	18	Guntur	ND
19	Kanpur	ND	19	Guntur	ND
20	Kanpur	ND	20	Jaipur	ND
21	Kanpur	ND	21	Jaipur	ND
22	Kanpur	ND	22	Jaipur	ND
23	Kanpur	ND	23	Kanpur	ND
24	Kanpur	ND	24	Kanpur	ND
25	Kochi	ND	25	Kanpur	ND
26	Kolkata	ND	26	Kanpur	ND
27	Kolkata	ND	27	Kanpur	ND
28	Mumbai	ND	28	Kanpur	ND

29	Mumbai	ND	29	Kanpur	ND
30	Mumbai	ND	30	Kanpur	ND
31	Mumbai	ND	31	Kochi	ND
32	Mumbai	ND	32	Mumbai	ND
33	Mumbai	ND	33	Mumbai	ND
34	Mumbai	ND	34	Mumbai	ND
35	Mumbai	ND	35	Mumbai	ND
36	Rajkot	ND	36	Mumbai	ND
37	Rajkot	ND	37	Mumbai	ND
38	Rajkot	ND	38	Mumbai	ND
39	Rajkot	ND	39	Mumbai	ND
40	Rajkot	ND	40	Rajkot	ND
41	Rajkot	ND	41	Rajkot	ND
42	Rajkot	ND	42	Rajkot	ND
43	Rajkot	ND	43	Rajkot	ND
44	Rajkot	ND	44	Rajkot	ND
45	Nagpur	ND	45	Rajkot	ND
46	Nagpur	ND	46	Rajkot	ND
47	Nagpur	ND	47	Rajkot	ND
48	Nagpur	ND	48	Rajkot	ND
49	Nagpur	ND	49	Rajkot	ND
50	Nagpur	4.771	50	Rajkot	ND
			51	Rajkot	ND

ND- Not detected and may be taken as “0”.

50 samples of Unpitted dates and 51 samples of Pitted Dates were collected from the different parts of India. These samples were analysed for Aflatoxin content. The results of analysis are summarized in Table-1. Out of the 50 Unpitted Dates samples analysed, only one tested positive for Aflatoxin B1 having value 4.771 ppb. In case of Pitted dates, none gives positive results out of 51 samples analysed. The Aflatoxin contamination is due to ubiquitous prevalence of toxigenic *Aspergillus flavus* as a natural contaminant in the samples. Although only one sample of unpitted dates is contaminated, but the level is within the limits of 30 µg/kg set by FSSAI. The results show that all 51 samples of Pitted dates are Aflatoxins free. In previous study carried out by Abdel-Sater and Saber (1999) [14] reported that Aflatoxin B1 was found in 2 samples out of 20 tested of dry dates. Limited reports have been published about the occurrence of aflatoxins in date palm. In this concern, the date fruit may be are less suspected contaminated with Aflatoxins because the chemical composition of the date fruit.

CONCLUSION

In present study, the two varieties of dates i.e. pitted and unpitted are collected from different parts of countries mentioned in Table-1, and the content of Aflatoxin has been determined using HPTLC. In India, a tolerance limit of 30 µg/kg has been prescribed under the Food Safety and Standards (Contaminants, Toxins and Residues) Regulation 2011, for all foods meant for human consumption. The dates are being directly consumed by human being, should be free from Afltatoxin or should be within permissible limit as prescribed by FSSAI. While going through the results obtained for Aflatoxin in Pitted and Unpitted dates, it has been found that none of the samples of pitted dates give positive results, and only one sample of unpitted dates gives positive results, which has been collected from “Nagpur” region, but the value is within the permissible limit fixed by FSSAI. It has been concluded that the dates collected from different parts of India are safe for human consumption in respect of Aflatoxin contamination.

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