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A study on aflatoxin content in rolled oats available in domestic market of India

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ABSTRACT

Oat is an important cereal crop in developing worlds and the one of most commonly cultivated species is *Avena sativa* L. Oats possess beneficial effects against gastrointestinal problems, has anticancerous effects. **Rolled oats** are a type of lightly processed whole-grain food. Traditionally, they are made from oat groats that have been dehusked and steamed, before being *rolled* into flat flakes under heavy rollers and then stabilized by being lightly toasted. Rolled oats that are sold for porridge usually have had the tough outer bran removed. They have often, but not always, been lightly baked, pressure-cooked, or otherwise processed in some fashion. **Thick-rolled oats** are large whole flakes, and **thin-rolled oats** are smaller, fragmented flakes. Rolled whole oats, without further processing, can be cooked into a porridge and eaten as **old-fashioned oats**, but more highly fragmented and processed rolled oats absorb water much more easily and therefore cook faster into a porridge, so they are sometimes called "**quick**" or "**instant**" oats. Oats have high nutritional value. Oat based food products like breads, biscuits, cookies, probiotic drinks, rolled oats (instant oatmeal) are gaining increasing consideration. Rolled oat, which is more susceptible to absorb water, is often contaminated with mycotoxins such as aflatoxins. 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. To control the Contamination of Aflatoxin in Rolled oats, the oats to be free from Aflatoxin contamination or contain the permissible limit of same. The objectives of this study was to determine the concentrations of Aflatoxin B1 in Rolled Oats collected from different parts of India and also to assess whether the Rolled oats were safe for human consumption.

The aflaoxin in Rolled oat has been analysed using HPTLC. Out of 59 samples of rolled oats analysed for Aflatoxin, all the samples were found to be free from Aflatoxin i.e. Below detection limit. As per FSSAI, the maximum permissible limit for Aflatoxin is 30 ppb. The study showed that the Rolled oats samples collected from different parts of India were safe for human consumption.

Keywords: Rolled oats; India; Aflatoxin; HPTLC

INTRODUCTION

Oats are uniquely nutritious food as they contain an excellent lipid profile and high amounts of soluble fibre. However, an oat kernel is non-digestible and thus must be utilized in milled form to reap its nutritional benefits. Milling is made up of numerous steps such as dehulling to expose the digestible groat, heat processing to inactivate enzymes that cause rancidity, and cutting, rolling or grinding to convert the groat into a product that can be used directly in oatmeal or can be used as a food ingredient in products such as bread, ready to eat breakfast cereals and snack bars [1]. Oats have a hull that consists mainly of cellulose, hemicelluloses and lignin. Within the hull is the groat, which comprises 68 to 72 % of the kernel [2]. The outer layer of the groat is an important source of protein, neutral lipids, β -glucan while germ contains mainly lipids and proteins. To make oats more digestible and easier to eat, the starch in the groat must become gelatinized. Starch consists of granules made up of two different glucose containing polysaccharides i.e. amylose and amylopectin. Converting oats into a more desirable food requires heating the starch in the presence of water so the water can move into a granule, allowing it to swell and become soft. Quick cooking oatmeal contains oatmeal so their starch can absorb water more quickly and thus decrease the cooking time [3, 4]. The creamy texture of oatmeal comes from the water binding properties of its soluble fibres. An added advantage of oats as a food ingredient is that they do not contain gluten, they have avenins as their storage proteins. Thus they can be used in gluten free foods targeted at people with celiac disease because avenins are less likely to cause allergies even among celiac sufferers [5, 6]. However, oats cannot be used in bread making due to lack of gluten which prevent oat flour from being used as the sole flour in raised bread. Hence most oat breads still contain wheat flour. Rolled oats are a type of lightly processed whole-grain food. Traditionally, they are made from oat groats that have been dehulled and steamed, before being *rolled* into flat flakes under heavy rollers and then stabilized by being lightly toasted. Rolled oats that are sold for porridge usually have had the tough outer bran removed. They have often, but not

always, been lightly baked, pressure-cooked, or otherwise processed in some fashion. Thick-rolled oats are large whole flakes, and thin-rolled oats are smaller, fragmented flakes. Rolled whole oats, without further processing, can be cooked into a porridge and eaten as old-fashioned oats, but more highly fragmented and processed rolled oats absorb water much more easily and therefore cook faster into a porridge, so they are sometimes called "quick" or "instant" oats.

Proper storage and handling of oats is important to decrease nutrition loss and to minimize the formation of off-flavors resulting from lipid oxidation. Storage conditions are an important food preservation factor as improper moisture control can result in the growth of micro-organisms that can cause spoilage and impose food safety risks such as formation of aflatoxins from mould growth. Excess temperatures can also decrease quality by increasing enzyme reactions, nutrient degradation and microbial growth, so it has been recommended that oats are stored at <0.65 water activity (approximately 13% moisture in the kernels) between 5 and 20°C [7, 8].

Aflatoxins are toxic and carcinogenic metabolic products of *Aspergillus* (*A. flavus*, *A. parasiticus* and *A. nomius*) [9-11]. 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 [12-14]. Aflatoxin producing fungi may contaminate Cereal, 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 [15-17]. Prolonged storage and/or contamination during

storage or transport have also been associated with higher Aflatoxin levels.

Investigation of Aflatoxin levels in Oat products 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 Rolled Oats 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 Safety and Standard 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. 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[18].

The Rolled Oats (Oat meal), which is being consumed by human being should be free from Aflatoxin contamination or contain the permissible limit of same. There are no reports available regarding aflatoxin content in rolled oats available in India. In the present study, the concentration of Aflatoxin B₁ content have been determined in Rolled Oats (Oat meal) obtained from different parts of India and also to assess whether the Rolled oats were safe for human consumption.

Materials and Methods

Total 59 Rolled Oats were investigated for Aflatoxin B₁ levels. The samples were collected from different parts of country.

Extraction of aflatoxins from Dates

For detection and estimation of aflatoxins from Rolled oats, 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

Whatman 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 171005, CAMAG Visualizer 171113 and operated with winCATs software.

Method of Spotting and Development of TLC plate

Pre-coated TLC sheets silica gel Merck 60 F₂₅₄ 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 B₁ (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 B₁ in µg/kg has been calculated as follows:

$$\mu\text{g/kg} = \frac{B \times Y \times S \times V}{\text{-----}}$$

$$Z \times X \times W$$

Where, B = average Aea/Height of Aflatoxin B₁ peaks in test aliquots.

- Y = concentration of Aflatoxin B₁ standards, µg/ml
- S = µl of Aflatoxin B₁ 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 content in Rolled Oat samples in region wise are indicated in Table 1

Table-1 Aflatoxin content in Rolled Oats

S. No.	Region	Result (in ppb)	S. No.	Region	Result (in ppb)	S. No.	Region	Result (in ppb)
1	Amritsar	ND	21	Nagpur	ND	41	Nagpur	ND
2	Amritsar	ND	22	Nagpur	ND	42	Nagpur	ND
3	Chennai	ND	23	Nagpur	ND	43	Nagpur	ND
4	Bhopal	ND	24	Nagpur	ND	44	Nagpur	ND
5	Bhopal	ND	25	Nagpur	ND	45	Nagpur	ND
6	Bhopal	ND	26	Nagpur	ND	46	Nagpur	ND
7	Bhopal	ND	27	Nagpur	ND	47	Nagpur	ND
8	Bhopal	ND	28	Nagpur	ND	48	Nagpur	ND
9	Bhopal	ND	29	Nagpur	ND	49	Nagpur	ND
10	Nagpur	ND	30	Nagpur	ND	50	Nagpur	ND
11	Nagpur	ND	31	Nagpur	ND	51	Nagpur	ND
12	Nagpur	ND	32	Nagpur	ND	52	Nagpur	ND
13	Nagpur	ND	33	Nagpur	ND	53	Nagpur	ND
14	Nagpur	ND	34	Nagpur	ND	54	Nagpur	ND
15	Nagpur	ND	35	Nagpur	ND	55	Nagpur	ND
16	Nagpur	ND	36	Nagpur	ND	56	Nagpur	ND
17	Nagpur	ND	37	Nagpur	ND	57	Nagpur	ND
18	Nagpur	ND	38	Nagpur	ND	58	Nagpur	ND
19	Nagpur	ND	39	Nagpur	ND	59	Nagpur	ND
20	Nagpur	ND	40	Nagpur	ND			

ND- Not detected and may be taken as "0".

59 samples of Rolled Oats were collected from Amritsar, Chennai, Bhopal and Nagpur. These samples were analysed for Aflatoxin content. The results of analysis are summarized in Table-1. It has been observed that all the samples are free from Aflatoxin B₁ contamination.

The Food Safety and Standard 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 Safety and Standards Act, 2006* and *Food Safety and Standards*

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. In India, a tolerance limit of 30 µg/kg has been prescribed for Aflatoxin under the Food Safety and Standards (Contaminants, Toxins and Residues) Regulation 2011, for all foods meant for human consumption (FSSAI, 2011). All the samples of Rolled Oats are free from Aflatoxin contamination.

A survey of cereal grains (wheat, sorghum and oats) and Soyabean for the presence of Aflatoxin was carried out in USDA [19], it has been reported that out of 304 oat samples analysed for presence of aflatoxins, only 3 samples were found to be positive with aflatoxin content of 6 ppb. Surveillance programme for Mycotoxin in foods was conducted by Food standards Agency, UK in November, 2010. As per Food Survey Information Sheet 04/10, out of 35 oats based cereals and cereal products samples tested, none of the samples were found to contain Mycotoxin above the regulatory limits. Aflatoxin B₁ were found be not detected in the samples in the present study which is similar to results obtained in study conducted by Food standards Agency, UK in November, 2010.

CONCLUSION

In present study, Rolled Oats are collected from different parts of India 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 present study showed that all the samples of Rolled Oats are having aflatoxin below detection limit. i.e free from Aflatoxin B₁ and safe for human consumption.

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