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Varietal change in Nutritional composition of *Aspergillus flavus* infested with Wheat grain

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ABSTRACT
Newly harvested five commonly grown wheat varieties (WH-542, PBW-343, UP-2003, Kundan & WH-502) of wheat grains samples are collected from different places of Bihar and mixed well for a year (2010-2011). Surface sterilized wheat grains were inoculated with 0.5 ml of A .flavus spore suspension ($2x10^{-2}$ spores/ml) of the respective fungal species. Inoculated wheat grains were kept in conical flask at different temperatures i.e. 10^0,20^0,30^0,40^0 and 50^0C for 7, 14, 21 and 28 days respectively. Wheat grains deterioration was studied based on biochemical qualities –Total Carbohydrate (Starch, Maltose), Protein (crude protein, gluten), Fiber (crude fiber) and Fats after 28 days at 30^0C. Maximum deterioration of all the nutritive contents along with minerals was recorded as 23% after 28 days at 30^0C. Maximum deterioration was noticed at 30^0C after 28 days in Kundan and UP 2003 varieties followed by WH- 542, PBW-343 and WH-502.
Key word: *Wheat grains, Nutritional quality, Aspergillus flavus, Grain deterioration.*

INTRODUCTION

Bihar is potentially an important wheat growing state that contributes 5.7% towards. National production from 8% of wheat growing area of the country with a low productivity of 1.9 tonnes/ha. Improper drying and storage under unhygienic condition make the grain vulnerable to microbial attack. The variable Indian climatic condition accompanied with heavy rains and increased level of relative humidity also stimulates the microbial activities on deterioration of quality of food value. During the journey of grain from field to storage and further effect of moulds under storage though depends on several factors but the genomic profile of the host also play important role moreover there is degree of variation of infection on different varieties, vis a vis loss in food value which might be due to variation in genomic sequence of wheat varieties. This leads to the association of moulds with them. During storage, moulds and microbes establish over seeds by utilizing their contents and as such deteriorate their quality. In addition some of these moulds (especially *Aspergillus* group) also elaborate toxic metabolites. Colonization of storage fungi led to decrease in Carbohydrates, Protein, Fats & Fiber content in most cases [1]. Decrease in enzyme activity indicates loss of viability of grains. The scuteller amylase activity increase in un-aged grains while it decrease in aged seeds [2]. In present study, an attempt has been made to evaluate the extent of damage caused to the nutritional quality of different varieties of wheat grains viz., Total Carbohydrates (starch, maltose) Protein (Crude protein, gluten) Fiber (Crude fiber) & Fat activity of storage wheat grains due to the invasion by *Aspergillus flavus.* Contamination of various microorganisms in cereal grain is a common phenomenon [3]. Amongst these, some of the fungal contaminants are known to cause deterioration of grains and may also poison them [4]. Researches on biodeterioration of cereals, pulses and other agricultural commodities by mould and natural contamination of mycotoxins during storage have been advanced by several earlier investigators [5-10]. Several earlier workers including Ushamalini *et al,* [11] have reported the infestation of wheat grains by fungi during storage. The storage fungi/Moulds thrive on wheat grains by deriving nutrition there by cause significant reduction in the weight and quality of grain. However, such biological event is not informally true for all varieties of wheat. Some of them vulnerable to fungal association where as some varieties behave differentially in terms keeping in view.

MATERIALS AND METHODS

Newly harvested five different varieties (WH-542, PBW-343, UP-2003, Kundan & WH-502) of wheat grains samples were collected from different collection centers (Farmer's houses, Ware houses & F.C.I Storage) of Bihar, India. Biochemical and nutritional changes in the grains were studied infested by

Aspergillus flavus for Total Carbohydrate (Starch, Maltose), Protein (crude protein, gluten), Fiber (crude fiber) and Fats after 28 days at 30^0C.

Estimation of Starch. Starch content of wheat grain was determined by the procedure of Hedge *et al*., [12]. 100 mg of the sample was homogenized in hot 80% ethanol to obtain sugars. The solution was centrifuged and the supernatant was collected in test tube for starch estimation. The supernatant was then dried well over a water bath. In the dried sample added 5.0 ml of water and 6.5 ml of 52% perchloric acid and left for 20 min at 0°C. It was centrifuged and the supernatant was saved. The extraction was repeated by using fresh perchloric acid, Centrifuged and pooled the supernatants to make 100 ml. 0.1 ml of the supernatant was pipetted out and made up the volume to 1 ml with water. 4 ml of anthrone reagent to each tube was added and heated for eight minutes on a boiling water bath. It was cooled rapidly and the reading was taken at 630 nm in UV-Vis spectrophotometer and the glucose content was determined with the standard graph. Multiply the value by a factor 0.9 to arrive at the starch content.

Estimation of Maltose. Estimation of Maltose was determined by the procedure of Somogyi [13]. 100 mg of the samples was weighed and extracted the sugars with hot 80% ethanol twice. The supernatant was collected and evaporated it on a water bath at 80°C. 10 ml water was added and dissolves the sugars. 0.1 ml of the supernatant was pipetted out and made up the volume to 2 ml with water. 2ml distilled water was pipeted out in a separated tubes to set a blank. 1 ml of alkaline copper tartrate reagents was added to each tube. The tubes were placed in boiling water for 10 minutes. The tubes were cooled and 1 ml of arsenomolybolic acid reagent was added to all the tubes. The volume in each tube was made up to 10 ml with water. The absorbance was taken after 10 min at 620 nm in UV-Vis spectrophotometer. The amount of reducing sugars (maltose) present in the sample was calculated with following formula.

Absorbance corresponds to 0.1 ml of test = *x* mg of glucose

$$10 \text{ ml contains } = \frac{x}{0.1} X10 \text{ mg of glucose} = \% \text{ of Maltose}$$

Estimation of crude protein: Estimation of crude protein was made by Microkjeldahl method [14]. Each seed sample was powdered and 300mg of each sample was placed in 50-ml. Microkjeldahl flask and added with 60 mg catalyst and 7.5ml. H_2SO_4 the flasks were digested for 6-8h, after cooling digest was diluted to 50ml in a volumetric flask and 5ml of liquid was introduced in Markhma's distillation unit through the side of funnel to which glass stopper was fitted. NH_3 librated was collected in 50ml conical flask containing 2% boric acid with an indicator and the distillate was titrated against 0.035 NH_3Cl_2 till end point was achieved. The crude protein was calculated as percent NX6.25 = crude protein.

Estimation of Gluten. Gluten content was determined by employing the procedure of Paul [15] with slight modification. 10 gm sample of wheat flour is mixed with water to form dought and placed into the glutomatic washing chamber on top of the polyester screen. The sample was then hydrolysis with a 2% salt solution for 5 minutes then washed in running tap water in the washing chamber. The gluten content is then dried in hot air oven for 200^oc at 30 minutes and determined dry weight of the sample with the help of following formula.

$$Dry\ weight\ = \frac{100\ x\ dry\ weight\ of\ the\ gluten}{\text{Weight of the sample}} X \frac{100}{100 - \text{ Moisture content}}$$

Estimation of Crude Fibre: Crude Fibre content was determined by employing the procedure of Maynard *et al.* [16]. 2 g of ground material was extracted with ether or petroleum ether to remove fat (Initial boiling temperature 35–38°C and final temperature 52°C). If fat content is below 1%, extraction may be omitted. After extraction with ether 2 gm of dried material was boiled with 200 ml of sulphuric acid for 30 min with bumping chips, Filtered through muslin and washed with boiling water until washings were no longer acidic and boiled with 200 ml of sodium hydroxide solution for 30 min. It was filtered through muslin cloth again and washed with 25 ml of boiling 1.25% H_2SO_4, three 50 ml portions of water and 25 ml alcohol. The residue was removed and transferred to ashing dish (preweighed dish W1). The residue was dried for 2 h at 130 ± 2°C, the dish was cooled in a desiccators and weighed (W2), left for 30 min at 600 ± 15°C. Cooled in desiccators and reweighed (W3).

$$\% \text{ crude fibre in ground sample} = \frac{\text{Loss in weight on ignition (W2-W1) (W3-W1) X 100}}{\text{Weight of the sample}}$$

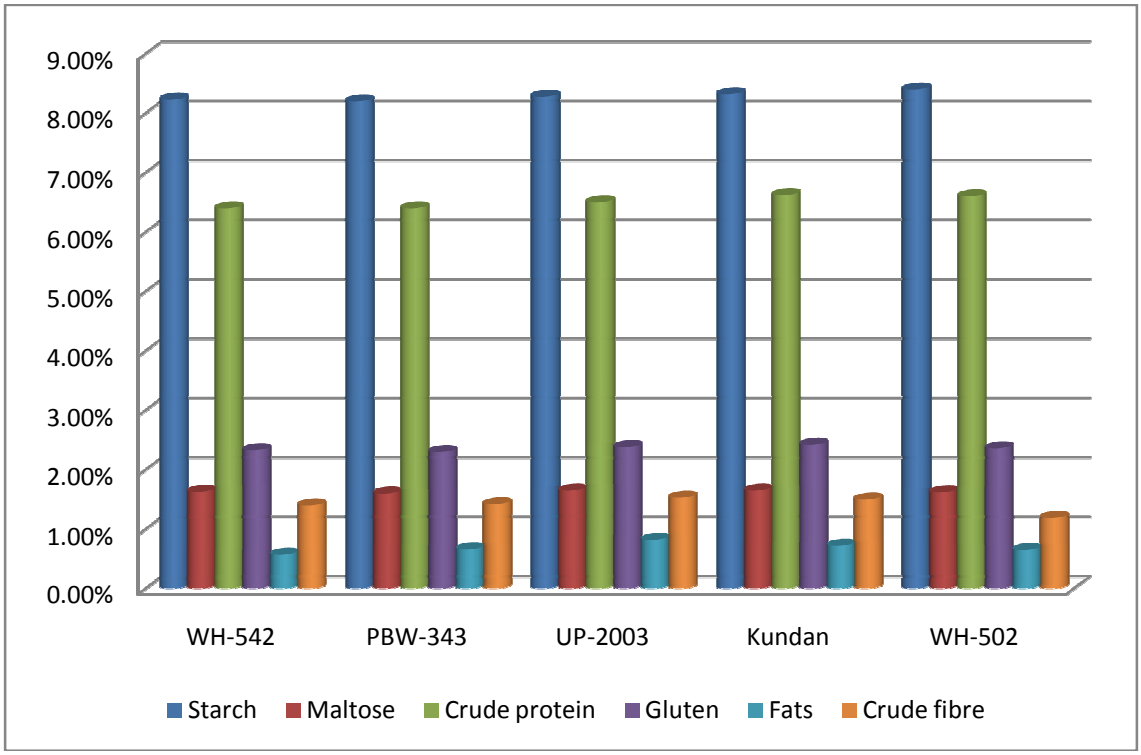
Estimation of Fat: - Estimination of fat content A.O.A.C Leslic (Association of official Agricultural chemists) method was used is mentioned by Leslei Hart and Fisher (1971). An amount of 1gm of oven dried and powdered sample material were transferred to whatman no.1 filter paper with a porosity permitting rapid flow of paper with a porosity permitting rapid flow of petroleum ether 60-80˚C depending on the type of petroleum ether. The weight of the sample together with the dried whatman no.1 filter paper and the weight of the dry soxhlet flask were recorded the fat content of the samples were extracted using soxhlets extract for 12hrs. Using petroleum ether as solvent for fats. The ether was then removed from the mixture by cautions evaporation of the soxhlets flask in an oven at 100˚C for 30min., the extracted fat were left behind in the flask. The flask then cooled and weight again. The final weight of the sample and filter paper were also recorded again.
Fat content= (Initial weight of the sample and the filter paper)-(Final weight of the sample and filter paper).

RESULT AND DISCUSSION

Changes in Nutritional composition: - Maximum deterioration of all the nutritive contents along with minerals was recorded as 23% after 28 days at 30^0C. Maximum deterioration was noticed at 30^0C after 28 days in Kundan and UP 2003 varieties followed by WH- 542, PBW-343 and 502.
Starch: Maximum deterioration was noticed at i.e. 8.4% in WH-502 varieties after 28 days at 30^0C followed by WH- 542, PBW-343, UP-2003 and Kundan
Maltose: Maximum deterioration was noticed at i.e. 1.65% in UP-2003 varieties after 28 days at 30^0C followed by WH- 542, PBW-343, Kundan and WH-502.
Crude protein: Maximum deterioration was noticed at i.e. 6.62% in Kundan varieties after 28 days at 30^0C followed by WH- 542, PBW-343, UP-2003 and WH-502.
Gluten: Maximum deterioration was noticed at i.e. 2.45% in Kundan varieties after 28 days at 30^0C followed by WH- 542, PBW-343, UP-2003 and WH-502.
Crude fiber: Maximum deterioration was noticed at i.e. 1.53% in UP-2003 varieties after 28 days at 30^0C followed by WH- 542, PBW-343, Kundan and WH-502.
Fats: Maximum deterioration was noticed at i.e. 0.82%. in UP-2003 varieties after 28 days at 30^0C followed by WH- 542, PBW-343, Kundan and WH-502.

Table:-Showing changes in Nutritional composition of *Aspergillus flavus* infested with Wheat grain

Percentage decrease over control due to *Aspergillus flavus*						
Incubation period after 28-days						
Varieties / **Nutritive profile**		**WH-542**	**PBW-343**	**UP-2003**	**Kundan**	**WH-502**
Starch	**Control**	65.90%	64.94%	66.85%	65.18%	63.95%
	(± % change)	57.05 (-8.85%)	56.22 (-8.72%)	57.05 (-9.80%)	53.60 (-11.58%)	54.45 (-9.50%)
Maltose	**Control**	2.3%	2.1%	2.5%	2.4%	2.2%
	(±% change)	0.68 (-1.62%)	0.50 (-1.60%)	0.85 (-1.65%)	0.76 (-1.64%)	0.60 (-1.60%)
Crude protein	**Control**	16.8%	16.60%	17.15%	16.75%	16.18%
	(± % change)	10.40 (-6.40%)	10.20 (-6.40%)	10.65 (-6.50%)	10.13 (-6.62%)	9.58 (-6.60%)
Gluten	**Control**	7.59%	7.48%	8.02%	7.95%	7.38%
	(±% change)	5.26 (-2.33%)	5.18 (-2.30%)	5.64 (-2.38%)	5.50 (-2.45%)	5.02 (-2.36%)
Fats	**Control**	1.25%	1.5%	1.6%	1.4%	1.15%
	(± % change)	0.68 (-0.57%)	0.84 (-0.66%)	0.78 (-0.82%)	0.68 (-0.72%)	0.5 (-0.65%)
Crude Fiber	**Control**	2.28%	2.32%	2.38%	2.3%	2.15%
	(± % change)	0.88 (-1.4%)	0.90 (-1.42%)	0.85 (-1.53%)	0.8 (-1.50%)	0.96 (-1.19%)



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