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STUDIES ON STORED CEREAL DEGRADATION BY *ALTERNARIA TENUISSIMA*

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ABSTRACT

The ability of a strain of *Alternaria tenuissima* Kunze (IMI 301005) Wiltshire to degrade and alter the nutrient profile of three common Nigerian cereals was studied. The grains involved were *Pennisetum glaucum*, *Sorghum vulgare* and *Oryza sativa*. Fat, fibre and protein decreased in the inoculated substrates, and this was accompanied by an increase in ash content. The highest levels of cereal degradation by the mould were attained at pH 6.8 and at temperature range of 30-35 °C. Within 48 hours of incubation, extracellular proteases were detected in all the culture media.

Key words: *Alternaria tenuissima*, cereal, nutrient's degradation.

RESUMEN

Se estudió la habilidad de una cepa de *Alternaria tenuissima* Kunze (IMI 301005) Wiltshire de degradar y alterar el perfil de nutrientes de tres cereales nigerianos comunes. Los granos seleccionados fueron *Pennisetum glaucum*, *Sorghum vulgare* y *Oryza sativa*. Las grasas, fibras y proteínas disminuyeron en los sustratos inoculados, hecho acompañado de un incremento en el contenido de ceniza. Los niveles más altos de degradación de cereal por el hongo (moho) se obtuvieron en cultivos con pH de 6.8 en un intervalo de temperatura de 30-35 °C. A partir de las 48 horas de incubación se detectaron proteasas extracelulares en todos los medios de cultivo.

Palabras clave: *Alternaria tenuissima*, cereal, degradación de nutrientes.

INTRODUCTION

Cereals are important staple foods in Nigerian homes, and their biological value on the basis of nutrient components has been documented (Oyenuga, 1968). The characteristic relatively high environmental temperatures and humidities of the tropic further worsen the integrity of stored grains, under fungal attack whether whole or in blended state (Christensen and Kaufmann, 1969). The interrelationship between *Alternaria* species and some substrates had also been recorded (Sulaiman and Hussain, 1984; Sanchis et al., 1993; Samson et al., 1995; Pettybridge et al., 2001; Grishkan et al., 2003; Osono, 2003) and the dangers this can pose to man, as a consumer, highlighted (Fapohunda and Ogundero, 1990).

The dangers include toxin consumption and malnutrition arising from consuming grains having a depleted nutrient profile. *Alternaria* contamination specifically leads to sinusitis, hay fever, skin infection and asthma, these body reactions, being the combined results of accumulation of spores and the production of "alternariol", a unique mycotoxin in the body (Shresthra et al., 1996). The general health implications of the attendant mycotoxins on the affected cereals and on the likely consumer are well documented (Fink-Grennels, 1999; Steyn and Stander, 1999). In 1998, Nielsen et al. had discovered that various forms of mycotoxins on artificially inoculated building materials resulted in cancerous expressions among persons directly exposed to them. The same year, Ren et al. demonstrated the destructive effects on the organs of those who were in contact with ceiling tiles infected by *Alternaria alternata*. Also, *Alternaria* species have been reported as mycodeteriogens in some cereals in the field (Ilhan and Asan, 2001). Health implications include cancer of the liver, digestive complications and respiratory difficulties (Zureik et al., 2002).

Millet (*Pennisetum glaucum* (L.) Leeke*)*, guinea corn (*Sorghum vulgare* (L.) Moench) and rice (*Oryza sativa* L.) constitute a group of grains eaten daily by a large number of Nigerians from peasants through the elites. The aim of this study is to highlight the unique role of a strain of *Alternaria tenuissima* in the bio-degradation of cereals under the stated conditions.

MATERIALS AND METHODS

A strain of *Alternaria tenuissima* Kunze Wiltshire IMI 301005, was maintained by growing in a sporulation medium described earlier (Fapohunda, 1992). All sterilizations before inoculation were done at 121 ^{o}C for 15 minutes.

Fifty grains each of the cereals: millet, guinea corn and rice, purchased from markets in South Western Nigeria, were selected for experimentation after conducting viability tests on them by earlier methods (Huff, 1980; Okafor and Aniche, 1980). Also, the grain samples were held at 0 ^{o}C for 92 hours to kill any mites present, as mites are primary source of cross contamination in a mycology laboratory. Surface sterilization was carried out by dipping inside 2% NaOCl for 1 minute and rinsing with distilled water.

Twenty grams of blended grains were placed in flask and 50 ml distilled water added to make a suspension. The fungus was cultivated on nutrient broth for seven days and filtered with sterile cotton wool. Dilution of the filtrate was carried out to give 3.5 x 10^{6} conidia/ml. These asexual spores serve as inoculum for the blended grains and incubation was at 28 ^{o}C for 14 days. The biochemical analyses expressed as % ash, crude fibre, protein and fat and moisture contents were determined (Anonymous, 1975) using dry samples. The initial moisture content, which was critical to mould invasion, was also determined. Controls were set up with no inoculum.

The pH of a 2:1 (w/v) suspension of blended grains in 250 ml conical flasks, containing 100 ml/flask, were adjusted with 0.1 N-NaOH or 0.1 N-HCl, as appropriate, to pH values between 3.8-7.6. pH stability was controlled by citric acid phosphate buffers (Ogundero, 1981). Also, the effect of temperature (15-35 ^{o}C) on the bio-degrading ability of the *Alternaria tenuissima* was tested by incubating cultures at 5 ^{o}C intervals. For each treatment, three replicates were prepared and incubation was done as earlier described. The % ash, crude fibre, protein and fat contents were equally determined as described above after seven days of incubation. Uninoculated flasks similarly treated served as controls.

Suspensions of the cereals were prepared (15 g/litre) in distilled water. Four grams of casein and 30 ml portion of the suspension were dispensed in 250 ml conical flasks, and inoculation carried out as described earlier. Three replicates were made and incubation was at 35^{o} C and over a 10-day period. At 2-day intervals, protease activity of the culture filtrate was determined, while using filtrates from the control flasks as assay blanks. The protein contents of the filtrates were also determined using the Folin phenol reagent method of Lowry et al. (1951). The Folin phenol reaction was designed to detect proteolysis as carried out by enzyme protease and the residues include tyrosine, which was measured in this research.

RESULTS AND DISCUSSION

Fat, fibre and protein decreased in the inoculated substrate with a significant increase in ash (Table 1). The ash content increased as other materials were utilized.

Table 1. Biochemical analysis of cereal substrates inoculated with conidia of *Alternaria tenuissima* and incubated for 14 days at 28 °C.

Composition (%)*	Millet		Sorghum		Rice	
	Not inoculated	Inoculated	Not inoculated	Inoculated	Not inoculated	Inoculated
Ash	13.00±0.24	16.80±0.18	6.05±0.34	15.68±0.01	5.98±0.11	11.11±0.08
Crude fibre	3.61±0.01	3.01±0.22	2.65±0.02	1.90±0.25	1.26±0.30	1.05±0.05
Protein	3.68±0.20	2.08±0.01	7.01±0.09	4.12±0.05	6.08±0.16	3.86±0.06
Fat	2.11±0.63	1.18±0.45	3.22±0.70	2.67±0.12	3.01±0.17	2.51±0.02
Moisture	7.25±0.10	9.89±0.11	10.56±0.08	15.60±0.02	8.89±0.04	13.68±0.04

*Results and means of three replicates

High fibre content had been noted in most cereals (Oyenuga, 1968), which, together with the available protein and fat formed ready substrates on which fungal enzymes acted. This accounted for the reduction in the relative quantities for fibre, protein and fat (Filtenborg et al., 1996). *Alternaria* spp. had earlier been implicated in the degradation of crops like melon in store by decreasing the germination percentage and increasing the risk of mycotoxicosis (Bankole et al., 1999). The highest percent reduction in protein was recorded when rice was the substrate, followed by sorghum, whereas millet gave the highest percent reduction in fat contents (Table 1). Appreciable increase in water content, which was recorded in all the inoculated cereals, was in normal response to fungal respiration in an environment of oxygen.

Optimization of pH and temperature regimes

When the effect of pH on mould degradation was investigated on the cereals, pH 6.8 proved optimal for all nutrients particularly protein (Table 2). Table 3 shows

Table 2. Effect of pH on the degradation of blended cereals at 28 ºC for seven days by *Alternaria tenuissima*.

	Proximate analysis	pH of substrate						
		Control value	3.8	4.8	5.6	6.2	6.8	7.6
Millet	Ash	0.98±0.11	1.00±0.01	1.16±0.01	4.02±0.03	6.08±0.01	7.02±0.02	6.98±0.00
	Fibre	3.56±0.08	3.11±0.12	3.01±0.20	2.08±0.05	2.01±0.07	1.89±0.10	1.98±0.10
	Protein	29.00±0.22	26.40±0.80	23.80±0.08	24.00±0.01	12.8±0.11	11.60±0.03	11.6±0.10
	Fat	4.60±0.10	3.50±0.01	3.30±0.02	2.05±0.11	1.96±0.10	1.44±0.01	1.06±0.00
Sorghum	Ash	1.45±0.02	1.98±0.01	3.00±0.01	4.08±0.03	6.74±0.02	7.18±0.03	7.08±0.10
	Fibre	3.61±0.08	3.61±0.05	2.68±0.01	2.58±0.08	2.01±0.16	1.80±0.02	1.11±0.10
	Protein	31.90±0.18	27.90±0.11	24.80±0.05	21.60±0.02	14.70±0.13	11.80±0.11	10.10±0.10
	Fat	3.89±0.11	3.01±0.04	2.91±0.09	1.48±0.08	1.46±0.27	1.60±0.04	1.08±0.00
Rice	Ash	1.80±0.01	2.08±0.17	3.89±0.16	3.96±0.18	4.08±0.21	4.11±0.11	4.60±0.10
	Fibre	2.89±0.16	2.64±0.02	2.48±0.04	1.89±0.01	1.89±0.03	1.76±0.11	1.66±0.10
	Protein	28.60±0.08	25.80±0.18	24.90±0.02	18.90±0.01	17.80±0.11	17.60±0.01	1.66±0.10
	Fat	5.00±0.20	4.56±0.02	4.00±0.01	3.98±0.07	3.98±0.07	3.96±0.06	3.95±0.10

Figures are % means of three readings with standard deviations

Table 3. Effect of temperature on the degradation of blended cereals by *Alternaria tenuissima* after seven days of incubation.

	Proximate analysis	Temperature of substrate (° C)					
		Control value	15	20	25	30	35
Millet	Ash	0.98±0.03	1.56±0.01	2.18±0.04	3.65±0.02	3.56±0.03	3.41±0.01
	Fibre	3.56±0.10	3.18±0.20	2.99±0.26	2.60±0.02	1.85±0.02	2.46±0.06
	Protein	29.00±0.22	28.70±0.11	24.80±0.07	13.90±0.21	14.00 ±0.71	16.90±0.12
	Fat	4.58±0.10	4.00±0.40	1.89 ±0.05	1.80±0.11	1.05 ±0.11	2.45±0.21
Sorghum	Ash	1.45±0.02	1.87 ±0.01	2.56 ±0.14	3.75±0.01	3.90 ±0.01	3.08±0.05
	Fibre	36.00±0.07	2.96±0.04	2.97±0.05	2.11±0.01	145.00 ±0.02	180.00±0.11
	Protein	32.00±0.18	28.80±0.11	16.10±0.12	15.40±0.01	17.8±0.24	19.5±0.17
	Fat	3.89±0.10	3.40±0.14	2.56±0.71	1.56±0.05	1.80±0.07	1.99±0.42
Rice	Ash	1.80±0.01	1.84±0.02	1.84±0.11	3.66±0.06	3.68±0.11	2.48±0.27
	Fibre	2.89±0.61	2.48±0.07	2.08±0.05	1.89±0.04	0.98±0.01	0.18±0.20
	Protein	28.6±0.07	25.50±0.16	23.40±0.17	15.80±0.13	11.60±0.14	19.60±0.17
	Fat	5.00±0.21	4.50±0.21	3.08±0.40	2.48±0.16	2.00±0.08	2.05±0.04

Figures are % means of three readings with standard deviations

that the temperature range for maximum mould degradation was 25-30 °C. At 15 °C, the ability to breakdown the nutrients decreased. However, with rice as substrate the rate of degradation of fibre was highest at 35 °C. The results showed that fibre content in rice was decreased with an increase in temperature.

Extracellular protease production

The secretion of protease by the fungus in the presence of the substrates (Fig. 1) explains the high rate of protein degradation under the various pH and temperature conditions, a significant result. Within 48 hours of inoculation, soluble peptides were excreted in the growth medium (Fig. 2). For rice, protein production fell at day 5. This was not so in the other two cereals where increases were observed until day 5.

Adequate precautions like immersing of the moist grains in 0.5% propionic acid and potassium sorbate (Liewen and Marth, 1984; Fapohunda, 1992) for a two minutes could be taken to guarantee significant safety and economic advantage to prospective consumers of the grains, while some human pathogenic fungi have been

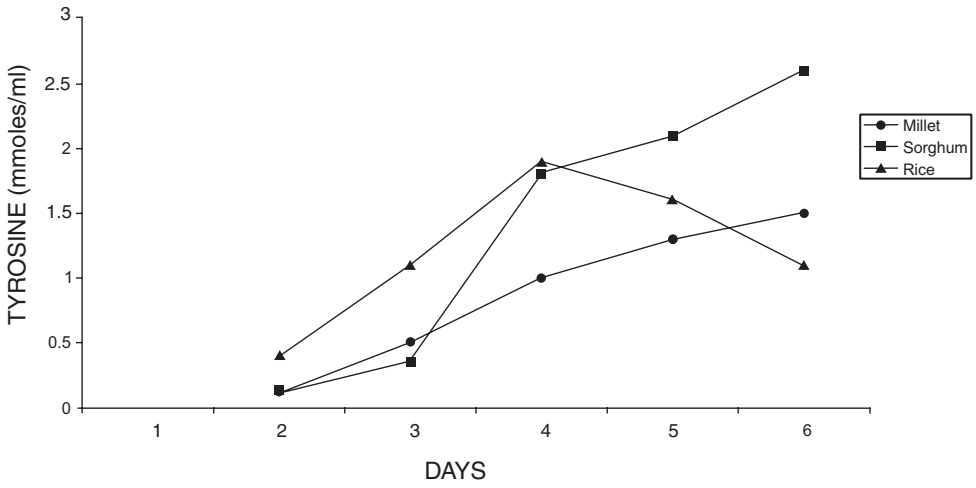

Fig. 1. Protease activity of culture filtrates of *Alternaria tenuissima* grown on millet, sorghum and rice.

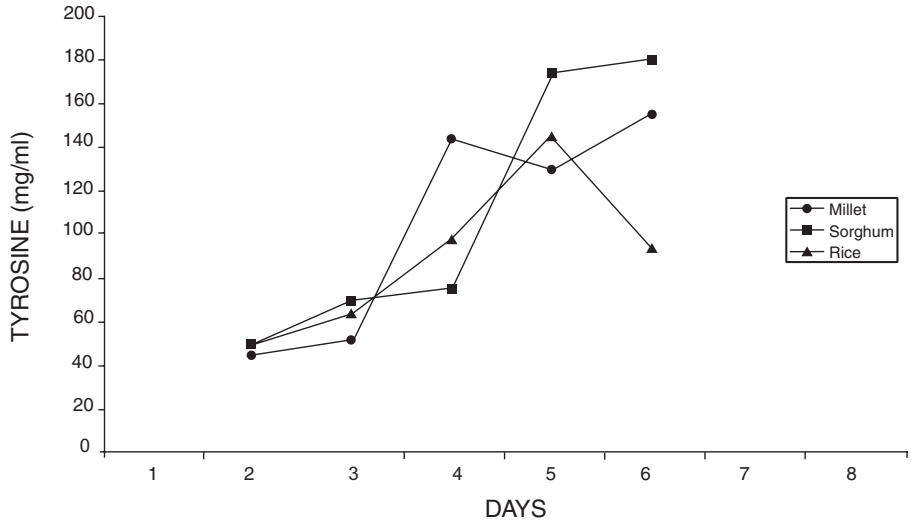

Fig. 2. Protein content of culture filtrates of *Alternaria tenuissima* grown on millet, sorghum and rice.

known to be inhibited by ethnobotanically selected plant extracts (Brandwagt et al., 2001; Ficker et al., 2003).

We are carrying out further studies on the prevention of fungal contamination, isolation and characterization of specific toxic metabolites using the High Performance Liquid Chromatography (HPLC) and the development of more antifungal agents with respect to this species. Also, the exploitation of inexpensive antifungal plant extracts is attracting further investigation, as some fungi express resistance to synthetic drugs (like propionic acid) and encourage new opportunists in the course of infection (Perfect and Schell, 1996). From this study it is observed that common cereals like sorghum, rice and millet are degraded, among others, by the proteolytic action of the fungus *Alternaria tenuissima*. The fungus, which incites various body disorders through its spores and toxic metabolites, can be controlled by a brief immersion in 0.5% propionic acid and sorbate. Since similar microbes had been inhibited by ethnobotanically selected plant extracts from, e.g., ginger and butternut, the next stage of our work is to formulate inexpensive antifungal agents from plant extracts in order that cereals in storage can be fairly safe from its contamination.

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