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MESOPHILIC AND THERMOPHILIC FILAMENTOUS FUNGI ISOLATED FROM PROCESSED OATS (*Avena sativa* L.) IN BRASIL

*(Hongos filamentosos mesofílicos y termofílicos aislados de avena procesada (**Avena sativa** L.) en Brasil)*

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Palabras clave: Avena, hongos, mesófilos, termófilos.
Key words: Oat, fungi, mesophilic, thermophilic.

RESUMEN

Con la finalidad de verificar la presencia de hongos filamentosos mesófilos y termófilos, se analizaron muestras de avena procesada envasada en: contenedores metálicos, caja de cartón y bolsas plásticas, ya sea en forma de escamas finas o gruesas, así como en harinas. Las muestras fueron procesadas mediante tres diluciones (1:10, 1:100, 1:100) y sembradas en placas de Petri en Agar Sabouraud + CAF, incubandose a 3 temperaturas: ambiente 28± 2°C, 45°C y 50°C. Se aislaron un total de 48 taxa de hongos filamentosos, representados en 14 géneros.

***Aspergillus** y **Penicillium** fueron los géneros más frecuentes, seguidos por **Cladosporium, Rhizopus, Syncephalastrum, Curvularia, Acremonium, Nigrospora, Paecilomyces, Tritirachium, Sporothrix, Oidiodendron** y un representante de los **Aphyllophorales**.*

*A las temperatura de 45°C y 50°C, se aislaron **Aspergillus fumigatus, A. duricaulis, Rhizopus microsporus, R. oryzae,** siendo **A. fumigatus** el que presentó un mayor número de aislamientos*

SUMMARY

*In order to determine the occurrence of mesophilic and thermophilic filamentous fungi on processed oats, samples of powered, thin and thick oat flakes, kept in cans, cardboard boxes, and plastic bags were analysed. For each sample, 3 dilutions were prepared: 1:10, 1:100, 1:1000 and they were placed on Petri dishes with Sabouraud agar plus antibiotic and incubated at 3 temperatures, 28± 2° C, 45° C, and 50° C. Forty eight taxa of filamentous fungi representing 14 genera were isolated, the most common being **Aspergillus** and **Penicillium**. Other genera recorded in order of their frequency were: **Cladosporium, Rhizopus, Syncephalastrum, Alternaria, Curvularia, Acremonium, Nigrospora, Rhinocladiella, Paecilomyces, Tritirachium, Sporotrix, Oidiodendron** and an unidentified representative of **Aphyllophorales**.*

***Aspergillus fumigatus, A. duricaulis, Rhizopus microsporus,** and **R. oryzae** were isolated at 45° C and 50° C temperatures. Among these, **A. fumigatus** was the most commonly found.*

INTRODUCTION

Oat (*Avena sativa* L.), has been worldwide produced for human and animal consumption, due to its great nutritive value and other digestive qualities (14).

The microbiota responsible for causing the wheat deterioration in food, specially in oats, corn, barley, wheat, and rye, is greatly influenced by internal factors connected directly with the food, such as nutritive value, pH, humidity, oxygen reduction potential, inhibitory compound, as well as by external factors like temperature, time of storage, relative humidity contained in the grain, insects, mechanical damage, and the presence and concentration of gases (12, 17). Among the contaminating microorganisms of food , fungi are responsible for the deterioration (2, 4). The aflatoxin, secondary metabolite

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produced by fungi , is also encountered in many food, such as soybean, corn, wheat, rye, oats, rice, among others (15). On the other hand, the occurrence of thermophilic and thermotolerant fungi in food has been the objective of taxonomic studies because of the great damage caused to processed foods (8).

In Brazil studies focused on the occurrence of fungi in grains and processed oats were poor. Consequently to support this, it was suggested to carry out research work on corn, barley, wheat and rice in grain according by Stenwing & Liven (32) and Sauer et al (30) methodology.

The objectives of the present work were: the isolation and identification of filamentous fungi on processed oats, to determine the presence of mesophilic and thermophilic fungi, and the quality - quantity analysis of these fungi detected, considering the methods of processing and packing of the oat.

MATERIAL AND METHODS

The following processing and packaging of oats were utilized: thin and thick flaked oats in cardboard boxes and cans; powered oat in cardboard boxes; unrefined oat bran in cans and in plastic bags.

Samples were obtained in many supermarkets and selected those unopened and within the valid date for consumption. The thin and thick flaked oats in cardboard boxes and cans were obtained from two industries, the powered oat in cardboard boxes was obtained from three industries, and the unrefined oat bran in cans and plastic bags were obtained only from one industry. Thirteen samples were tested. The following culture media were used for isolation and identification of the fungi: Sabouraud agar plus yeast extract and CAF (0,100 g/l), Potato dextrose agar, Czapek agar, Oat agar, and Malt extract agar (20). The Warcup method (33), modified isolation technique based on diluition method was used to isolate fungi in one gram of each oat. Sample was aseptically weighted and diluted in 10 ml of distilled sterilized suspension. From this diluted solution with concentration of 1:100 and 1:1000 were prepared. A 0,2 ml sample of each dilution was plated in triplicate on Petri dishes in Sabouraud-yeast extract agar + CAF, and homogenize equally. Cultures were grown at three temperatures: in a 28 °C (± 2 °C) incubator room, or in a electric incubator at 45 °C, and 50 °C, for an up to seven day period. After 72 hours processed fungal colonies were counted and different isolates were transferred to specific culture media used for the corresponding groups of fungi.

Identifications were made with macroscopic and microscopic observations of the isolates. Microscopic observation was based in the morphological analysis of the structures using, when necessary, the Riddell (28) slide culture technique. Identifications were made with the use of a number of keys and manuals (11, 13, 25, 26, 27).

Each fungi isolates at 45°C and 50°C were inoculated on potato dextrose agar and incubated at 45°C and 50°C for seven days.

The species isolated were storaged in the Micotheca URM, of the Mycology Department of the Biological Center of Science (CCB), at the Federal University of the Pernambuco (UFPE).

RESULTS AND DISCUSSION

In the analysis of 13 samples,a total of 282 colonies representative of 48 taxa of filamentous fungi distributed in 14 genera were isolated. The most frequent genera were ***Aspergillus*** 45,40%, and ***Penicillium*** 24,11%, ***Cladosporium*** 11,34%, ***Rhizopus*** 5,68% (Tabla 1). Other interesting taxa of smaller occurrence such as ***Nigrospora, Rinocladiella, Paecilomyces, Tritirachium, Sporotrix, Oidiodendron,*** and one representative of ***Aphyllophorales*** were also isolated (Table 1.) Among the most frequent species it can be mentioned: ***Aspergillus fumigatus, A. parasiticus, A. niveus, A. sydowi,*** and ***Penicillium implicatum*** (Table 1).

A total of 30 colonies of termophilic and thermotolerant fungi in three genera and five species, represented the 10,64% of total fungi isolated, where ***A. fumigatus*** was the most frecuent (Table 2).

Christensen & Kaufmann (4), classified the fungi that contaminated grains of agricultural products, based upon their moisture requirements and their predominance in the fields and in storage. The storaged fungi occur predominantly in grains that have 13 to 18% of moisture and belong mainly to the genera ***Aspergillus*** and ***Penicillium***. From the analysed samples, the predominant genera were ***Aspergillus***, specially in powered oat packed in cardboard and ***Penicillium*** from thin oat flakes and flour packed in cans and cardboards. From those, the most common species were ***A.fumigatus*** and ***P. implicatum. Cladosporium spp.*** and ***Alternaria alternata***, although isolated with less frecuency, were also obseved by Christensen & Kaufmann (4), who referred to them as "storage fungi". In relation to the occurrence of the ***A. fumigatus*** in the sample from processed oats, this species is referred to as pathogenic for men and animals by Lacaz (20), Rippon (29); Conant (6), Know-Chung & Bennett (19).

Within the isolated species, there are some of them bearing potential abilities to produce micotoxins in food, mainly ***A. flavus*** and ***A.parasiticus.*** The former was isolated just once while the latter (6% of the isolates), a

Table 1. Filamentous fungi isolated from processed oats packed under different conditions

Genera/species	Ff cx	Ff lt	Fg cx	Fg lt	Far sp	Far lt	Fh cx	Total
Acremonium fusidioides (Nicot) W. Gams	3							3
A. griseoviride (Onions & Barron) W. Gams		1						1
Alternaria alternata (Fr.) Keissi							6	6
Aspergillus aureolatus Munt			1	1				2
A. brevipes Smith						3		3
A. caesiellus Saito					7			7
A. duricaulis Rapper & Fennell				1			4	5
A. flavus Link						1		1
A. fumigatus Fres.		4	4	16		1	14	**39**
A. janus Rapper & Thom					4			4
A. janus var. ***brevis*** Rapper & Thom					4		5	9
A. niveus Blochwitz				16				**16**
A. parasiticus Speare	3	3				4	8	**18**
A. sydowii Bain. & Sarf.	4				4		6	**14**
A. terreus Thom		3		3				6
A. variabilis Gasperine	1							1
Basidiomycete (Aphyllophorales)	1							1
Cladosporium cladosporioides (Fres.) de Vries	10						1	11
C. oxysporum Berk. & Curt.	14		8	2		1		**25**
C. sphaerospermum Pens.	1				1			2
C. tenuissimum Cooke					1			1
Curvularia pallescens Boedjin	5			1				6
Eupenicillium sp. Ludwig						2		2
Eurotium chevalieri Mangin						1		1
Nigrospora sphaerica (Sacc.) Mason		1						1
Oidiodendron griseum Robak					1			1
Paecilomyces lilacinus (Thom) Samson	2							2
Penicillium aurantiogriseum Diercky					3		3	6
P. bilaii Chalabuda							5	5
P. citrinum Thom		2	1			1		4
P. corylophilum Durckx	1		1					2
P. decumbens Thom	1			2				3
P. expansum Link		1	1					2
P. funiculosum Thom	2	3						5
P. implicatum Biourge	2	12						**14**
P. islandicum Sopp	1		2	1				4
P. janthinellum Biourge			5					5
P. melinii Thom		1						1
P. oxalicum Currie & Thom							1	1
P. pinophilum Hedgcock							4	4
P. sclerotiorum Van Beyma					1	2		3
P. waksmanii Zaleski						1		1
Rhinocladiella atrovirens Nannf.					1			1
Rhizopus microsporus V. Tieghem			3			1		4
R. oryzae Went & Prinsen Geerligs.		3				1	1	5
Syncephalastrum racemosum Cohn ex. Schrot			2	2		1		5
Sporothrix cyanescens (de Hoog) de Vries							2	2
Tritirachium oryzae (Vicens)de Hoog	17							**17**
Total	**68**	**34**	**28**	**45**	**27**	**20**	**60**	**282**

Ff cx = (Thin flaked oats in cardboard boxes). **Ff lt** = (Thin flaked oats in cans). **Fg cx** = (Thick flaked oats in cardboard boxes). **Fg lt** = (Thick flaked oats in cans). **Far lt** = (Oat bran in cans). **Far sp** = (Oat bran in plastic bags). **Fh cx** = (Oats bran in cardboard boxes).

TABLE 2 - Thermophilic fungi isolated from processed oats at 45° C and 50° C. temperatures

Genera/species	Ff cx		Ff lt		Fg cx		Fg lt		Far sp		Far lt		Fh cx		Total
	45°	50°	45°	50°	45°	50°	45°	50°	45°	50°	45°	50°	45°	50°	
Aspergillus duricaulis													1	1	2
A. fumigatus					2						1		3	18	24
Rhizopus microsporus	1										1				2
R. oryzae					2										2
Total	**1**				**4**						**2**		**4**	**19**	**30**

Ff cx = (Thin flaked oats in cardboard boxes). **Ff lt** = (Thin flaked oats in cans). **Fg cx** = (Thick flaked oats in cardboard boxes). **Fg lt** = (Thick flaked oats in cans). **Far lt** = (Oat bran in cans). **Far sp** = (Oat bran in plastic bags). **Fh cx** = (Oats bran in cardboard boxes).

closely related species, is also a greather producer of micotoxis, and the most strains producing B an G aflatoxin, two compounds which are toxic for animals. Aflatoxin B1 is certainly acutely toxic for humans, and responsible for liver necrosis following chronic exposure, and mainly involved in human liver cancer, perhaps synergistically with hepatitis B virus (9, 10, 21).

With regard to processed oats, the first step involves the improvement of the phase of sanitation, that consists in the removal of foreign materials, followed by a slow heating designed to reduce the moisture content of the grain until it remains at about 6% and to avoid fungal development.

The large number of fungi found in the various forms of packing, can be explained by the procedures connected to the last stage of the process of production. Johnson &Peterson (18), state that oats do not receive an efficient sterilization, since they are exposed only to fast boiling and heating, and the flaked material is later ground into flour and then packed. This form of processing is easily susceptible to fungal contamination.

In relation to the type of packaging, it was observed that there was more contamination in cardboard boxes than in cans, which indicates that the material utilized for packaging may enable an increase of moisture and temperature enough to allow later germination of propagules existing in a latent stage. Since ***Aspergillus*** and ***Penicillium***, classified as "storage fungi" occurred frequently in the samples of processed oats, it is possible to suggest that these were acquired during the storage period of the grain . This fact was observed by Christensen (5) who concluded that fungal propagules on the seed coat of cereals can remain dormant inside the grain and develop when proper conditions arise.

Most of the taxa isolated in this work were also observed by other researchers, in grains of oats, wheat, corn, and barley (3, 24, 30, 32). Some of the fungi isolated from these natural substrates are thermotolerant and thermophilic. Physiological and biochemical factors influence their thermophilic capacity allowing them to grow and reproduce effectively at high temperatures (7, 8). Thermophilic species detected in this work are commonly isolated from soil (3, 22), but were also found in wheat, barley, hay and bean leaves (1, 23, 31).

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