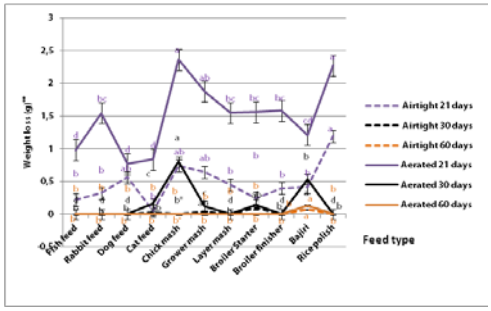
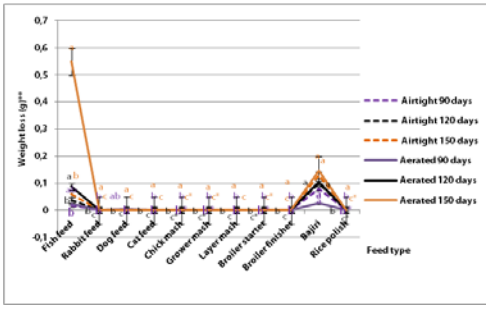
3. Results



*Fungal contamination
**For a combination of a 'given aeration (aerated or air tight) and duration', weight loss followed by the same letter are not significantly different at P=0.05 according to Tukey's test following ANOVA.
Fig. 1. Weight loss in animal feed at 21, 30 and 60 days following infestation under aerated/air tight condition.



* Fungal contamination
**For a combination of a 'given aeration (aerated or air tight) and duration', weight loss followed by the same letter are not significantly different at P=0.05 according to Tukey's test following ANOVA.
Fig. 2. Weight loss of animal feed at 90, 120 and 150 days following infestation under aerated/air tight condition.

4. Discussion

Aerated samples of a given animal feed demonstrated higher weight loss than air-tight samples. The maximum weight loss occurred in chick mass and Bajiri. The minimum weight loss was recorded in dog feed and cat feed. Discard of certain animal feed samples due to fungal contamination seemingly interrupted the smooth increase of weight loss when the duration increased.

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Quality and Safety Conditions of Flocked Oats (*Avena Sativa* L.) Stored in Bags

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Abstract

Oats (*Avena sativa* L.) have reached the healthy food market worldwide due to its special nutrients composition and fiber high quality. Therefore, quality & safety control is a must, both during the storage and commercialization stages. The current study evaluated the physicochemical characteristics (flakes size/variation %, pH, moisture content-mc, water activity-aw), living organisms (insects & mites / mycoflora - fungi load& genera identification), mycotoxins(ochratoxin A – OTA / zearalenone – ZON / aflatoxins – AFLs / esterigmatocistin – EST)andthe storage conditions of flocked oats stored inbags.Regarding the oats physicochemical characteristics, flakes particle size varied, however most of the samples present size uniformityand only one sample had high percentage of residue. That indicates high insects and other living organisms activity (consumption / proliferation) of oats starch and other nutrients. The analysis through

stereomicroscope showed intense presence of insects and mites. Samples were seen also sheltering those living organisms (27%), which are not allowed by regulation (no soils, parasites and larvae presence). As expected, mc (10.8-13.2%) and/or aw (0.61-0.90) varied, however they kept on the safer levels (< 13% / 0.90) insects/mites and fungi growth wise. With respect to pH, it varied from4.1to 5.85, indicating some rancidity/fermentation reactions taking place, thus changes in organoleptic parameters. The total fungi load ranged from $3x10^2$ to $1.29x10^5$ CFU/g, with*Aspergillus* and *Rhizopus*the genera more identified. Only one sample was toxin contaminated (OTA - 80 µg/kg). Insects are known vectors of fungal spores and can spread their hyphae on their dead/live skeleton, apart from mites that can trigger allergies in humans and animals. Therefore, current data demonstrate that despite the storage conditions control application, living organisms can occur in flocked oats (stored in bags) and it is necessary to apply decontamination methods to control/prevent their proliferation.

Key words: oats, storage, bags, insects, fungi, toxins.

Introduction

As part of the demand for a healthy diet, oats (*Avena sativa* L.) have gained more and more popularitydue to its functional claims (mainly due to its composition). Oats have large amounts of beta-glucans and soluble fibers, which are able toreduceglucose absorption and increase intestinal transit. Several studies have shown its effectiveness in preventing diabetes and cardiovascular diseases, and in reducingglucose levels and blood pressure. It is important to emphasize that processing steps do not alter the concentration of oats nutrients (De Sá et al, 1998).

During grain storage, moisture and temperature reduction and control is required. Despite that, grains need to be harvested in the most efficient way so that there is no mechanical damageallowing insect infestation and fungiproliferation. Storage locations vary fromwarehouses, bags or silos, and also in more modern ways, such as hermetic silos (Marini et al., 2007).

Oats, like others cereals, are susceptible to a number of fungi, including those of field and storage, such as *Fusarium*, *Aspergillus*, and *Penicillium*. Fungi, under favorable conditions, can cause deterioration in grains and produce mycotoxins. These toxins canaffect animal and human health, being more severe in some animals, such as swines and equines.We are exposed to fungal spores at all times as they are easily transported through the air. These fungalspores can be toxigenic - those that are able toproduce toxins harmful to health.As these spores are not easily perceived due to their microdimensions, we only identify their presence in foods when they are well developed, spoiling their tissues and producing mycotoxins. More than 300 mycotoxins have been isolated in food, however there are five main ones, among them aflatoxins (AFLs), ochratoxin A (OTA), T-2 toxin, deoxynivalenol, and fumonisins (Scussel, 2002; Agais, 2005).

Some foods may contain mycotoxins and are apparently healthy, which leads us to consume these foods without the full certainty of safety. It is necessary to monitor the quality of the grains stored and marketed, so that the population is aware of what they are consuming.

Therefore, this work evaluated the quality and safety conditions of flocked oats stored in bags.

Materials and Methods

Material

Samples: flocked oats stored in bags.

*Culture media and reagents:*potato dextrose agar (PDA) and peptone bacteriology media were purchased from Himedia (Curitiba, Parana, Brazil) and chloramphenicol were from Vetec (Duque de Caxias, RJ, Brazil), phenolphthalein and sodium hydroxide from Merck (Darmstadt, Germany).

*Equipment:*autoclave, Phoenix (Araraquara, SP, Brazil); microwave oven, Philco (Sao Paulo, SP, Brazil); tweezers, Prolab (São Paulo, SP, Brazil);caliper, Digimatic (Mitutoyo, Tokyo, Japan); drying oven, Olidef-cz (Ribeirao Preto, SP, Brazil); a_w meter, Aqua- Lab4TE, Decagon (Sao Jose dos Campos, SP, Brazil); Peagameter, Model Schott-gerate CG 818 (Schott,, Mainz, Germany); laminar flow cabinet, Veco (Campinas, SP, Brazil); fume cabinet, Quimis (Diadema, SP, Brazil); rotary shaker, Marconi (Piracicaba, SP, Brazil); microbiological incubator, Quimis (Diadema, SP, Brazil); colonies counter,

Phoenix (Araraquara, SP, Brazil); sieve system, mesh (2-1mm) Beffer (Caieiras,SP, Brazil).Microscopes - light (LM), CH-Bl45-2, Olympus (Shinjuku, Tokyo, Japan); stereo microscope (SM), Opzt coupled to a color image-capture camera, model OPT14 MP, Opticam Microscopy Technology (Doral, Fl., USA).

Methods

Sample collection and preparation: samples (300 g) were collected from stored bags, then sealed, labeled, and transported to the Laboratory of Mycotoxicology and Food Contaminants for analysis; *(b) preparation* - each oat sample was homogenized and then divided into two main portions: (b.1) integral i.e., its original flakes characteristics (analysis: pH, mycology, and aw) and (b.2) ground, for mc and mycotoxins.

Granulometry of oat flakes: sample portions (100 g) were subjected to separation by a Screen System (sieves) with different apertures (Mesh: 9; 16; 200, corresponding to 2.0; 1.0 and 0.75 mm) (Lorini et al., 2015) then %/mesh calculated.

Physicochemical analysis: pH, acidity, and moisture content (mc) were determined by the international official AOAC methods (Peisino et al., 2015; AOAC 2005). The water activity (a_w)was determinedusingthe Aqualab apparatus at 25ºC (n=3) (Decagon, 2001).

Total fungi load and genera identification: the enumeration technique and genera identification of Da Silva et al. (2007) and Pitt (1979) were used.

Storage conditions: the environmentalconditions of the storage- ventilation / refrigeration, application of pest control system, cleaning of the premises - were evaluated (Souza et al., 2013).

Multi-toxin analysis: the method of Soares&Rodrigues-Amaya (1989) was applied for the determination of multi-toxins [AFLs (AFB1, AFB2, AFG1, AFG2), ZON, EST and OTA].

Results

From the data obtained, it was possible to observe that part of the flocked oats stored in bags showed that the flocculation process applied and the bags storage condition in which they were submitted were efficient. Despite that, some oat flakes presented different physicochemical conditions ideal for the development of insects, mites, andfungi.Table 1 shows the total fungal load, genera, and humidity of flocked oats samples (*Avena sativa* L.).

Insects and mites: they were detected in all oats samples (at different percentages), enphasizing the concern on the storage conditions and safety. Part of the samples (32%) presented insects and mites when analyzing under stereoscopic microscope. Thereboth living and dead insects present. Figure 1shows by sterescopy (a, b) *insects*and (b) *mites* isolated from oat samples.

Humidity: the mc of the samples analyzed varied from 10.8 to 13.2%, indicating a small variation of the products stored and process. With respect to a_w, the samples varied from 0.4782 to 0.5906 and the pH ranged from 4.1 to 5.85 indicating some rancidity and fermentation process, thus flavor alterations.

*Fungi:*as expected, the total load was high ranging from $3x10^2$ to $1.29x10^5$ CFU / g.The genera isolated were *Aspergillus* and*Rhizopus*, the first with possible toxin formation and the second only deterioration. Figure 2presents the light microscopy of fungi (a) *Aspergillus* and (b) *Rhyzopus*isolated from oat samples.

Mycotoxins: a single sample showed contamination by OTA (80 µg / kg) well above the regulations of several countries (OTA daily intake: 3; maximum tolerant level: 50 µg / kg – FAO/WHO, 2017).

Table.1 Physico-chemical characteristicsand fungi of flocked oats (*Avena sativa* L.) stored in bags

Flakes oats		Physico-chemical			Fungi		Mycotoxins* (ug/kg)			
Number	Code	Humidity		pH	CFU/g	Genera	AFLs	EST	ZON	OTA
		mc (%)	a_w							
1	A	11.48	0.5429	5.75	ND	ND	ND	ND	ND	ND
2	B	11.97	0.5712	5.535	ND	ND	ND	ND	ND	ND
3	C	11.92	0.5547	5.853	$1.05x10^4$	*Aspergillus/ Rhizopus*	ND	ND	ND	ND
4	D	12.94	0.5514	5.6	$3x10^2$	*Rhizopus*	ND	ND	ND	ND
5	E	12.42	0.5159	5.39	$7.0x10^4$	ND	ND	ND	ND	ND
6	F	12.88	0.5904	4.095	$6x10^{-2}$	*Aspergillus/Rhizopus*	ND	ND	ND	80
7	G	12.11	0.5239	5.53	$1.47x10^4$	*Aspergillus*	ND	ND	ND	ND
8	H	10.81	0.4781	5.25	ND	ND	ND	ND	ND	ND
9	I	13.08	0.5728	4.61	$1.29x10^5$	*Aspergillus*	ND	ND	ND	ND
10	J	12.83	0.5416	4.595	$8.85x10^4$	*Aspergillus*	ND	ND	ND	ND
11	K	13.24	0.5061	5.665	ND	ND	ND	ND	ND	ND

Mc: moisture content a_w: water activity CFU/g: colony-forming units AFLs: aflatoxins OTA: ochratoxin A EST: sterigmatocistin ZON: zearalenoneND: not detected *LOQ: 2 ug/kg each

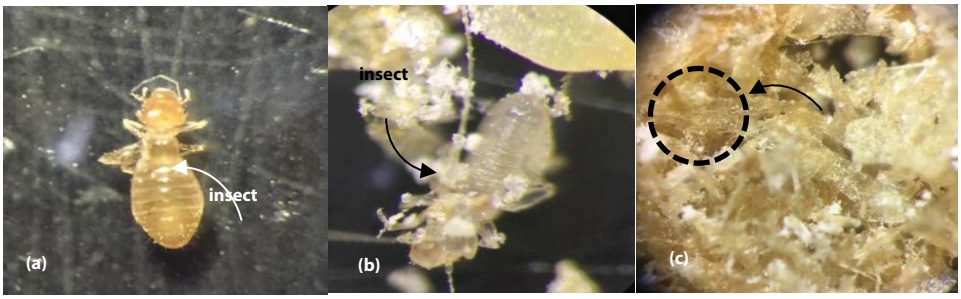

Fig.1 Insects and mites detected in flocked oats (*Avena sativa* L.) samples stored in bags understereomicroscopy [x60].

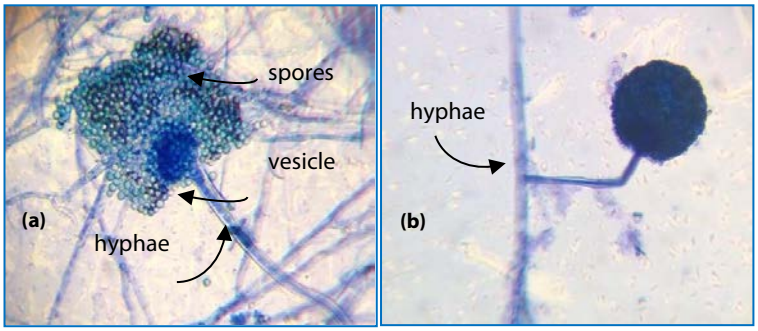

Fig.2 Reproductive structures of fungiisolated from flocked oats (*Avena sativa* L.)by light microscopy (a)*Aspergillus* and (b) *Rhyzopus*genera [x400].

Discussion

Insects and mites are vectors of fungal contamination, as spores and hyphae may develop and be carried / transported in their exoskeleton.In addition, mites can trigger allergies in humans. Franzolin (1998) has identified that when spores of *Aspergillus flavus* adhere to the body of mites, they do so as a means of spreading, causing viable spores to proliferate in grains stored incorrectly.

Soares et al. (2018) isolated fungi of the genus *Aspergillus* and *Penicillium* adhered to the exoskeleton of beetles *Alphitobiusdiaperinus*, considered a secondary pest in storage units.

The mc values detected in the samples were higher than those found by Sandrin (2013) in oat flakes where authors got 10.04%. Gutkoski &El Dash(1999) suggest that after hydrothermal processing followed by flocking, the oats flakes should reach 10% mc.When the mc is higher than 13% over time, the acidity index increases very fast, indicating grain deterioration (Gutkoski and Pedó, 2007; Rupollo et al, 2004). All samples analyzed showed high pH, which leads us to conclude that the deterioration and rancidity process started, generating a characteristic odor, however, only in one sample studied. The oil extracted from oats presents a great amount of unsaturated fatty acids, with linoleic being the main one. Unsaturated fatty acids have double bonds in their structure, making themmore unstable to the rancidity process. Hydrolytic and oxidative rancidity are factors that adversely affect quality (Gutkoski and El-dash, 1999). They can be caused by enzymes (active at acidic pH) present naturally in the grain or by contaminating microorganisms.The acidity of sample F (pH: 4.1), followed by samples J (pH: 4.60) and I (pH: 4.61), can be explained by that reason (enzyme catalyte activities). Samples I and J both presented microorganisms contamination, one of the causes of rancidity byoxidative enzymes.

Only 2.8% the samples were out of the standards required for total fungal load. Unsatisfactory conditions at the storage time, such as high temperature and humidity favor the development of fungi (Scussel, 2002).With the data obtained from the total fungal load it is concluded that there was a high contamination in storage due to the presence of *Aspergillus* (storage) and *Rhyzopus* (deterioration) genera. The presence of fungi in the product can cause spoilage, alter organoleptic properties, and present health risks (DallaVecchia&Castilhos-Fortes, 2007).

Influenced by extrinsic factors such as a_w, fungi can develop, causing serious problems for the grain. According to Iamanaka et al. (2013) the values that favor fungal development and toxin formation vary between 0.60 and 0.90. However, these values obtained from samples C (aw: 0.5547), F (aw: 0.5904), and I (aw: 0.5728) were favorable to the development of some genus of filamentous fungi. The mycotoxin production isdirectly related to the quality of storage. According to Gerez et al. (2014), the optimal conditions for *Aspergillus niger* to produce OTA was a_w of 0.995. Despite that, the toxin can be produced from a_w as low as 0.60, depending on other factors such as temperature and substrate composition.Other authors such as Esteban et al. (2006) also reported lower values of $a_w$for OTA production.

In a single sample (F) the highest $a_w$detected was 0.590.Consequently it was contaminated by OTA. Kuzdralinski et al. (2013), when analyzing oat grains, reported that 42 of58 samples were contaminated with OTA. In another study by Sacchi et al. (2009) authors did not find AFLs and ZON in their samples, corroborating what was found in the present study.

Conclusion

The samples showed uniformity in the flakes size. However, the presence of insects and mites, which exposes the grain to other types of contaminants such as fungi, was registered along with high colony forming units of *Aspergillus* and *Rhyzopus*. The high pH detected in the samples leads us to conclude that deterioration and rancidity were in the process of initiation. Other characteristics such as the levels of mc and a_w also favor the development of fungi and their metabolites.

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The impact of two drying methods on the quality of high-moisture rice

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Abstract

In this experiment, freshly harvested rice was dried by natural and mechanical methods. For natural drying, paddy rice was spread on a cement floor under a shelter at a thickness of 4cm, and it was turned twice a day. At a temperature of 19.3°C and a relative humidity of 58.8%, a total of 28 days was needed to reduce the water content from 23.11 to 14.38%. For mechanical drying, the Guwang 5HXG-15B circulating dryer was used, drying temperature was set to 42°C, and it took a total of 5 hours to reduce the water content from 23.1 to 11.8%. The changes in spore count, fatty acid value, germination rate, waist burst rate, whole polished rice rate, and taste value of rice mold after drying were studied. The results showed that compared with mechanical drying, the