Assessment of hygienic conditions of ground pepper (*Piper nigrum L.*) on the market in São Paulo city, by means of two methodologies for detecting the light filth

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Abstract

Pepper should to be collected, processed, and packed under optimum conditions to avoid the presence of foreign matter. The hygienic conditions of ground pepper marketted in São Paulo city were assessed in determining the presence of foreign matter by means of two extraction methodologies. This study was carried out during a six-month time period from May to September 2006. The occurrence of light impurities was determined either by the flotation technique following the methodology recommended by AOAC or by enzyme – linked immunosorbent assay (ELISA). It was observed that 100% of the examined samples contained insect fragments, and many samples were housing more than one type of foreign matter. Twenty-two percent of samples were unqualified for consumption owing to the occurrence of rodent hairs. For the calibration of ELISA test for quantification of insect contamination level in pepper samples, a range of standard-infested samples was prepared in adding 1, 2, 4, 8 and 10 insects in a control sample to estimate the number of insects in the analyzed samples by measuring optical densities (OD) values with a spectrophotometer. Among the 22 samples, 36.4% of samples presented OD values close to that corresponding to the standard infested with eight insects, 40.9% of samples were comparable to OD of the standard infested with four insects, 18.2% comparable to standard with 10 insects, and 4.5% to the standard with two insects. According to the results observed in the present study, the technique described in AOAC official methods manual was found more suitable for detecting not only the insects but also the additional impurities in analyzed samples, while ELISA is specific to detect myosin from the insect muscle, which undergoes serious degradation with time.

Keywords: Pepper, ELISA test , Light filth, AOAC official method.

1. Introduction

Brazil is a remarkable black pepper producer and its large use as a condiment may lead to a health hazard, since it can be directly added to food without any procedure to neutralize eventual contaminants. Black pepper of good quality should be harvested, processed, packed, delivered and stored under conditions that avoid the development and/or addition of physical, chemical or biological substances potentially harmful for the consumer’s health (Brasil, 2005). In tropical and subtropical regions, weather changes and the natural drying processes increase the chance of spices to be contaminated by microorganisms and insects. These, in turn, facilitate the growth of fungi due to their metabolic activity that increases the humidity and environment temperature (Correia et al., 2000). The study of extraneous materials in food (black pepper) has been of fundamental importance in order to maintain the physical, sanitary and nutritional qualities of the product. The Association of Official Analytical Chemists (AOAC) defines as extraneous materials any different materials which do not take part of the existing food, resulting from inadequate practices during production, storage or distribution. Light filth such as whole insect and insect fragments, mites, animal hair and bird feathers are some of the extraneous materials among many others (AOAC, 2005). Detection and quantification of these contaminants in dry foodstuffs represent a constant challenge for industry, and require periodical control analyses of the filth contamination of stored products either in the raw or processed condition.

The Consumer Rights Code, effective since 1991 in Brazil, has induced significant changes on the relationship between consumers and manufacturers in the Brazilian food-processing industries. The current legislations that regulate microscopic extraneous matter in food are Resolution RDC no. 175/MS and Regulation no. 326/MS which describe the general steps of the procedure for assessing the presence of extraneous material that may be harmful to human health in packed food. These regulations establish the general hygienic requirements and the good manufacturing practices that are convenient as well as for raw agricultural product and for finished food ready for consumption, respectively (Brasil, 1990, 1997, 2003)..Flotation methodology for the light filth extraction from black pepper has been regulated by the AOAC and it has long been used by official laboratories. Today, an immunoenzymatic assay (enzyme-linked immunosorbent assay– ELISA) has been developed for the detection of insect fragments in food samples via the reactivity of antibodies to insect myosin – the protein found in insect muscles. In comparing this technique with Flotation Method, ELISA may be considered as a rapid method, with a relative low cost and allowing the analysis of a large number of samples in a test run of a few hours. Myosin is found in large quantities in both larvae and adult insects and can be easily extracted. Some technical variations of the original extraction method were performed according to the species of insects in study (Kitto, 1991, Bair and Kitto, 1992). Thus, a comparative study evaluated the hygienic-sanitary conditions of black pepper in powder available in various sale points in the city of São Paulo, by using the flotation technique (AOAC) and enzyme-immunoassay – ELISA.

2. Materials and methods

From May to September 2006, 22 samples of ground black pepper were collected and analyzed for their light filth content. These samples from different trademarks, batches numbers and consumption deadline time delay were bought in local markets of São Paulo city. The study was carried out in two laboratories of Instituto Adolfo Lutz-Central Laboratory, being Food Microscopy Laboratory and Laboratory of HIVAids that are, Laboratório de Microscopia Alimentar e HIV/Aids do Instituto Adolpho Lutz – Laboratório Central (HIV/AIDS Laboratory of Adolpho Lutz Institute) and in the laboratory of the Grain Marketing and Production Research Center of the United States Department of Agriculture, KS, USA. For extraction of light impurities in ground black pepper the Flotation Method no. 972-40A described in the Association of Official Analytical Chemists (AOAC) International manual (AOAC, 2005) was used as a reference method. In comparison, the immunoenzyme assay – ELISA commercial kit (Biotec - Austin, TX, USA) was used for insect myosin detection using polyclonal antibodies, and following the procedures recommended by the manufacturer (AOAC, 2005). Samples were processed in duplicate, and results were expressed in arithmetic average.

3. Results and discussion

Tables 1 and 2 show the results found in ground back pepper samples analyzed by means of flotation methodology. In Tables 1 and 2, respectively, 100% (22/22) of black pepper samples were housing insect fragments, and several kinds of extraneous material were found in more than one sample. The origin of the contamination cannot be determined and may be from either. The pepper tree plantation or from the storage and processing operations. Although these kinds of insects do not cause any harm to consumer's health, industries are reluctant to process dirty, dusty or spoiled raw products, and are compelled to follow suitable procedures and to observe good manufacturing, storage, and distribution practices (Brasil, 1997, 2003).

Table 1 Number and percentage of samples with light filth in 22 samples of Ground Black Pepper purchased in the city of São Paulo in 2008.

Black Pepper	**Presence** No.	**%**	**Absence** No.	**%**
Insect	4	18.2	18	81.8
Insect Fragment	22	100	0	0
Larvae	3	13.6	19	86.4
Larvae Fragment	4	18.2	18	81.8
Mites	9	41	13	59
Rodent Har	5	22.7	17	77
Fragment of bird feather	1	4.5	21	95.5
Unidentified animal Hair	1	4.5	21	95.5

No. = Number of samples

Table 2 Various types of extraneous material found in ground black pepper samples purchased on São Paulo city markets, 2008.

Sample Number	Fragment of Insect	Larvae	Fragment of bird feather	Insect	Fragment of larvae	Mites	Rodent Hair	Unidentified Animal Hair
1	3.5	1	0	0	0	0	0	0
2	34	0	0	0	0	0	0	0
3	22.5	0	0	0	0	0	0	0
4	7	0	0	1	0	0	0	0
5	11	1	0	1	5	1	0	0
6	6.5	0	0	0	0	0	0	0
7	17	0	0	0	0	0	0	0
8	49.5	0	0	0	0	1	0	0
9	293	1	0	0	1	1	0	1
10	67.5	0	0	0	0	1	0	0
11	40.5	0	0	3	1	2	1	0
13	352	0	0	0	0	0	2	0
14	65	0	0	0	0	0	0	0
15	10.5	0	0	1	0	1	0	0
16	19	0	0	0	0	0	1	0
17	56.5	0	0	0	0	0	2	0
18	12.5	0	0	0	0	1	0	0
19	6	0	0	0	0	1	0	0
20	466	0	1	0	1	9	1	0
21	23	0	0	0	0	0	0	0
22	14	0	0	0	0	0	0	0
23	55	0	0	0	0	0	0	0

Insect fragments are composed of chitin. When eaten by man, chitin is not metabolized due to the lack of the chitinase enzyme in the human digestive system. Nevertheless, even if there is no nutritional hazard associated to the ingestion of insect fragments, it may cause harm to the intestinal mucous (Gorham, 1981). The raw agricultural product may be attacked and harmed by insects which grow in the crop and harvest areas. The most common insects in pepper plantations belong to the following Orders: Lepidoptera, Coleoptera, Hymenoptera, Homoptera and Hemiptera. During pepper storage, the most common insects to attack the product are of the Coleoptera and Lepidoptera. Many of the insects found in the ground black pepper samples are homopteran, which are phytophagous: many species appear as plagues in cultivated plants (FDA, 1982; Wirtz, 1991). In the present study, 41% (9/22) of samples were contaminated with mites which may carry bacteria, yeast and fungi (Franzolin et al., 1999). The small part of excrement from mites contains allergenic substances that can cause reactions in human or animals, and may induce acute enteritis and also macrotis lesions in intestinal mucous; as in case of a massive mould mite pollution on humid food material (Gorham, 1981). Mites found in this study belonged to Mesostigmata and Cryptostigmata, mostly from Oribatidae family which are ground mites (Baggio and Franzolin, 1991; Fletchman, 1986).

Gecan et al. (1986) reported a study on various types of condiments sold in the retailer market, carried out to determine their sanitary conditions. As far as black pepper is concerned, authors verify that of 1523 analyzed samples, 98.4% held insect fragments, 46% were contamined with mites and 20% contained rodent hairs. These results are similar to those data found in the present study. Graciano et al. (2006) examined ground black pepper and found 98.5% of samples contaminated with fragments of insects; 24.6% with mites and 23.2% with rodent hairs. Samples housing rodent hairs are improper for human consumption, because: 1 of the presence of extraneous materials (e.g., pathogenic bacteria) regarded as harmful for human health, and 2 considering that rodents are potential carriers of several diseases, such as leptospirosis, salmonellosis, plague (bubonic plague, pneumonia, septicemia) (Gecan et al., 1986; Carvalho Neto, 1987).

Finally, in comparison with data found in a similar study carried out in year 1999-2000, it was found that the quality control of manufacturers and black pepper packing industries in the state of São Paulo was not improving during this period. The totality of analyzed samples (22/22) do not comply with the current lawful requirements for insects and fragments, mites, unidentified animals hair, food products contamination. These findings indicate the non-adoption and/or lack of maintenance of Good Manufacturing Practices (Brasil, 1997). According to Domestic Regulation RDC 175/2003 which regulates the extraneous macro- and microscopic materials, harmful to health, only the samples contaminated with rodent hairs (22.7%) can be considered as improper to consumption and should be withdraw from market places (Brasil, 2003; FDA, 1982).

The series of results obtained through enzyme-immunoassay – ELISA are shown in Fig. 1. In this figure, the extracts from samples showing a similar OD than the standards spiked with one, two, four, eight insects before grinding were associated in order to estimate the “quantity” of insects present on raw material in the 22 samples by optical density (OD) values. According to the above mentioned pairing, 36.4% (8/22) of samples presented OD values close to “pattern four insects”; 18.2% (4/22) close to “pattern 10 insects”; and 4.5% (1/22) similar to “pattern two insects”. The number of additional insects that may be estimated by the ELISA test was found at 0.5 insect per 50 g sample. Samples with contamination higher than 10 insects per 50 g sample were at the value of the highest contamination rate of 10 insects per 50 g sample. Regarding to the accuracy and limit of detection of the AOAC recommended method, the ELISA myosin antibody test was unable to quantify the level of pollution of pepper by insect fragments, even when insects occur in large number in the raw material.

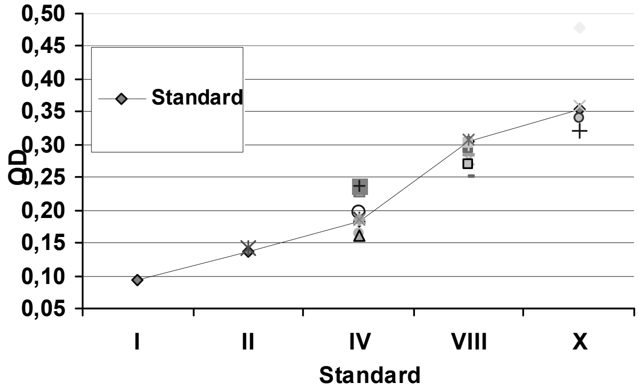

Figure 1 Results (OD) of samples and Standards using enzyme – linked immunosorbent assay (ELISA) to quantify insect fragment contamination level in ground pepper samples

Following the literature, the polyclonal antibody used in ELISA reacts with the myosin from various species that may infest ground material. The Immuno-sorbent assays based on a colorimetric reaction give consistent quantitative answer only in the case of raw product infestation with a large quantity of insects (Chen and Kitto, 1993). This study used ELISA kits containing polyclonal antibodies and the patterns are specific for *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). For samples infested with other insect species, the myosin antibody reaction values may be lower than those found in our study. Most insects found in these black pepper samples were not identified and those identified as homopteran (field pests). Thus, the quantity of insect myosin may be underestimated considering that the test was developed for coleopteran insects (beetles) belonging to the (Atui, 2002).

In the study reported by Kitto (1991), ELISA tests showed an excellent correlation between the obtained coloured reaction and the number of *Sitophilus granarius* added to the samples of clean wheat. The intensity of developed color is proportional to the quantity (in percentage) of myosin present and to be evaluated, which indicates a very good correlation with muscle mass of insect present in wheat or flour sample, and that may be considered as an extrapolation from the numbers of insects in the sample (Kitto, 1991).

In the study performed by Atui (2002) it was reported that myosin present in flour (after grinding grain) was degraded after 2 wk. The same situation may occur with the concentration of myosin in black pepper purchased from different market sale points in São Paulo city, because the date of pepper batches grinding date and delay before purchase of ground material was unknown.

The standard samples, infested by known numbers of insects, have high co-relation with optical density on ELISA, as reported in several studies. In this investigation, there was no correlation between data for the flotation method or those observed with ELISA tests, since there was no previous knowledge on contamination of black pepper samples (Atui, 2002; Kitto et al., 1992; Kitto et al., 1996). The immuno-enzymatic assay – ELISA is specific to detect insect myosin. Hence, it can detect insect fragments only. The flotation method can determine which species is infesting a sample by identification of insect recovered fragments. So, the immune-enzymatic ELISA assay is not able to determine the species of insect at the origin of the contamination.

Myosin concentration may serve to estimate the number of insects and fragments. ELISA is a rapid, sensitive, cheap and easy technique. In can be used for assessing insect presence in freshly processed products susceptible to infestation, but it is unsuitable for analyzing samples sold on the market.

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