Aspects of the methodology validation for light filth in fruit pulp

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Abstract

Methodology validation is an important tool to support the activities of metrology in the quantification analysis. Extraneous materials may be present in the pulps due to the conditions or the practices of production, storage and distribution. Since there is no specific methodology for fruit pulp light filth quantification a research has been carried out to test two methods for isolation of light filth in mango, strawberry, tomato and guava pulps. For guava, mango and tomato pulps the AOAC Official Method 964.23 was used, and for strawberry pulp AOAC Official Method 950.89, with adaptations. The micro-analytic standard for insect fragment and rodent hair was prepared in the laboratory. The study has been conducted on 63 samples of 100 g of pulp analyzed in duplicate after contamination with 5, 15, 30 insect fragments (IF) per 100 g and 5, 10, 15 rodent hair (RH) per 100 g, with blank samples as a control. The validation parameters used were precision and accuracy. Recovery of insect fragment was considered satisfactory in mango, tomato, guava pulps, and the average recovery ranged from 87 to 96%. For strawberry pulp, the recovery rate ranged from 68 to 80%. For rodent hairs, the best recovery rate was observed in mango pulp ranging from 73 to 81%, followed by guava pulp ranging from 65 to 76%, tomato pulp ranging from 50 to 67%, and strawberry pulp ranging from 33 to 35%. It was concluded that the methodologies used to detect light filth in guava, mango, tomato and strawberry pulps can be adopted in the monitoring routine of fruit pulp extraneous material contamination even though they were found not very efficient for rodent hairs.

Keywords: Insect fragment, Rodent hair, AOAC determination method, Recovery rate, Fruit pulp.

1. Introduction

The validation of the analytical method is an important requirement to support the activities of metrology in the measurement and analysis to show that the method is suitable for its intended use. A validation process has to be well defined and documented. In microscopic analysis the micro-analytical methods used for light filth isolation can be found among the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC, 2005).

Food contaminated with light filth looks normal, not allowing the consumer to identify it by naked eye. Examples of light filth are insect fragments, animal hairs, bird barbules, mites, among others. Analytical methods require a large number of steps to prepare the sample for the final extraction. Light filth has a specific affinity for oil and can be trapped off in an oil layer, filtered and examined microscopically.

The fruit pulp is the product obtained by crushing the edible parts of fleshy fruits through appropriate processing. To meet the regulation governing hygienic and sanitary condition of fruit pulp, the product should be prepared with healthy fruit, clean, without parasites and other animal or plant waste and without inedible parties (Brazil, 2003).

A growing number of products derived from fruit that has been developed and launched in the domestic market and for exportation demand that require the compliance with satisfactory quality and safety standards. Extraneous materials may be present in the pulp due to poor conditions or practices of manufacturing, storage and distribution which include decayed organic materials, miscellaneous materials such as soil, sand, glass and others.

Since there is no specific methodology for fruit pulp in the AOAC Official Methods considering that they are Brazilian products, a study to evaluate the performance of two methodologies (pasta and fruit jelly) for isolation of light filth in mango, strawberry, tomato and guava pulps has been carried out.

2. Materials and Methods

2.1. Material

Specifically for this study the fruits that were used for fruit pulp processing that are guava, mango, tomato and strawberry have been grown in the city of Matão-SP, Brazil.

2.2. Methods

The experiment took place from May to December 2009, in the Microscope Food Section at Adolfo Lutz Institute. To detect light filth in guava, mango and tomato pulps we used the AOAC Official Method 964.23 (16.10.05), Chapter 16, pp. 29, 2005, with the following adaptations:

- The sample was weighed in the 2 L Wildman trap flask.
- The agitation was limited to 15 min per sample.
- Heptanes was used as flotation oil.
- Before the first trapping sequence, the waiting time was 20 min before trap off.
- Then, before the second trap off the waiting time was fixed at 10 min.
- During extractions, the rod and neck of the bottle trap were washed with hot water.

To search for light filth in strawberry pulp, AOAC Official Methods 950.89 (16.10.06, Chapter 16, pp. 30, 2005) was used with the following adaptations:

- The sample was weighed in 1L capacity Wildman trap flask.

During the extraction process:

- The magnetic stirring was fixed at 5 min.
- After filling the flask with water, stirring was done every 5 min on a period of 20 min.
- The waiting time was fixed at 10 min.
- The rod and the neck of the bottle trap were washed with hot water.

2.3. Sample Contamination

Micro-analytical standards for insect fragments (Order: Dyctioptera; Family: Blatellidae) and rodent hair (Mus musculus L.) were prepared in the laboratory using the technique described by Brickey et al. (1968). Contamination was made using the following spike levels: 5, 15, 30 for insect fragments (IF) per 100 g and 5, 10, 15 for rodent hairs per 100 g.

2.4. Collaborative Study

The study has been performed by 9 analysts during 4 months. Sixty-three samples of 100 g of pulp were analyzed in duplicate totaling 126 determinations contaminated with 5, 15, 30 insect fragments (IF) per 100 g and 5, 10, 15 rodent hairs (RH) per 100 g, with blind samples as a control.

3. Results and discussion

The presence of insects and rodent hairs count method was used to evaluate the sanitary conditions of the fruit pulps during their production, storage and distribution. Insect fragments and rodent hairs were chosen based on the Brazilian legislation (Brazil, 2003), which enforces a strict regulation on the presence of extraneous materials harmful for human consumption. The validation parameters used were precision and accuracy.

Recovery of insect fragment was considered satisfactory in mango, tomato, guava pulps, for which the average recovery rate remained in the range of 87 to 96%, and for strawberry pulp for which the range was 68 to 80%, as shown in Table 1. For recovery of rodent hairs, the best recovery rate was observed with mango pulp ranging from 73 to 81%, followed by guava pulp ranging from 65 to 76%, tomato pulp ranging from 50 to 67%, and strawberry pulp ranging from 33 to 35%, as mentioned in Table 2.

Table 1 Average recovery rate of insect fragments (IF) in mango, guava, tomato and strawberry pulps contaminated by different numbers of IF (5, 15 and 30 IF/100 g).

	Recovery Rate (%)		
	Insect Fragments Added (#/100 g)		
Plant	**5**	**15**	**30**
Mango**	93.94	95.94	92.76
Guava**	93.33	89.26	90.37
Tomato**	87.41	90.58	87.12
Strawberry*	79.68	67.96	71.18

*AOAC Method No. 950.89 (16.10.06); ** AOAC Method No. 964.23 (16.10.05)

Table 2 Average recovery rate of rodent hair (RH) in mango, guava, tomato and strawberry pulps at different contamination levels (5, 10 and 15 RH / 100 g).

	Recovery Rate (%)		
	Rodent Hair (RH)		
Plant	**5**	**10**	**15**
Mango**	73,45	72,54	80,85
Guava**	75,56	65,00	70,74
Tomato**	57,22	66,82	50,41
Strawberry *	33,43	33,58	35,22

*AOAC Method No. 950.89 (16.10.06); ** AOAC Method No. 964.23 (16.10.05)

Strawberry pulp was tested using the same methodology for other pulps, but the results were not acceptable due to large amount of residue in the extracted material. Then, another methodology was tried (AOAC Official Method 950.89). Strawberry pulp has its own characteristics, like great quantity of fruit (akene) and hairs. The large amount of residues in strawberry pulp might have influenced the recovering of insect fragments and rodent hairs. It was believed that the rodent hair was stuck to strawberry hair, style, stigma that remained at the bottom of the Wildman trap flask. Although its recovery rate was lower than at other pulps, this data could not be ignored and should be improved in future studies. According to Dimov et al., (2004), recovering of rodent hair could be a problem linked with the stirring conducted by the analyst. Brickey (1968) also reported that the stirring method plays an important role in recovering this kind of contaminant and depends on the formation of a vortex that barely touches the stir bar and no audible or visible splashing occurs. The recovery of rodent hair when compared to insect fragment was poor in all pulps, showing that the techniques used were more effective for insect fragments.

Figures 1 and 2 showed the data obtained by analysts for both contaminants in tomato pulp. It was observed more precision for insect fragments which average recovery rate remained in the range of 80 to 100% in the 3 contamination levels. For the rodent hair, average recovery rate remained in the range of 20 to 100%. The variability among analysts and contamination level was high, showing more spread results than for insect fragments recovery.

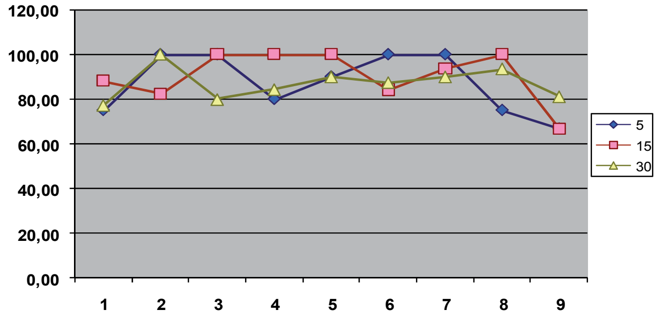

Figure 1 Variability of the recovery rate of insect fragment (IF) in mango, guava, tomato and strawberry pulps by different laboratory assistants (IF contamination levels: 5, 15 and 30 IF/100 g).

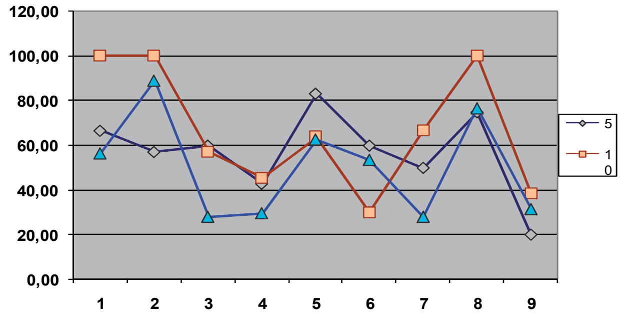

Figure 2 Variability in the recovery rate of rodent hair (RH) in mango, guava, tomato and strawberry pulps by different laboratory assistants (contamination levels 5, 10 and 15 RH/100 g).

4. Conclusion

Based on the results obtained, it was concluded that the methodologies applied in this validation study can be used for the isolation of light filth in guava, mango, tomato and strawberry pulp, and might be adopted in routine monitoring due to the lack of appropriate techniques until now. The methodology used for the recovery of rodent hairs may underestimate the actual amount of this contaminant. More studies are needed to improve the techniques for routine use development.

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