COMPARATIVE METHODS FOR FAT SEPARATION IN CHEESE PRODUCTS TYPE "PRESSED CHEESE"

Chiş Adriana, Purcărea Cornelia

University of Oradea, Environmental Protection Faculty, 26 Gen. Magheru St., 410048 Oradea, Romania; e-mail: andichis@yacoo.com

Abstract

The determination in fat foods of certain liposoluble compounds, such as the case of the organochlorine pesticides and polychlorinatedbiphenils has as a first step the separation of fat from the respective matrix.. This is why it is useful to compare the results obtained through the application of various methods presented by the specialized literature that refer to the fat separation in cheese products. The validity of the methods has been tested through the comparison of the determined values with the ones determined through the reference method indicated for the determination of fat from cheese products.The application of the results of various methods alternative to the reference method shows that there are differences between them; however, some of them get closer to the reference methods. This thing is good to know in order to be able to apply in safety conditions, the fastest method, the less expensive or, simply, the most accessible in the laboratory that performs the determination.

Key words: fat, alternative methods, cheese products

INTRODUCTION

Despite the fact that OCP usage has been limited due to the toxic effects and bioaccumulation, research on this kind of food contaminant is necessary because they occur under sanitary - veterinary surveillance (Savu and Georgescu, 2004, Hura, 2005). Organochlorine pesticides are liposoluble substances, which leads to their accumulation in animal and vegetal fat (Bara et al, 1998). For this reason, in the qualitative and quantitative determination of this kind of toxic the first phase is the separation of fat from the matrix elements. (Hura, 2006) However, for this kind of food product the organochlorine pesticides-kind residues content, is expresses in mg/kg fat reported to the dry substance (Bradley, 2001, 2003). So in order to be sure of an accurate extraction of the toxic for the purpose of its quantitative determination is useful to know the efficiency of fat separation methods. This is the preliminary phase of quantitative determination of pesticides because the contaminant are separated together with fats.

MATERIALS AND METHODS

Materials: We worked with samples of "Dalia" pressed cheese coming from three different producers from the Bihor county and who collect the milk as raw material from different geographical areas: the Oradea area, the East-Oradea area, confining with the Hungarian border and the hills area, in the western side of Oradea. There were three determinations (1,2 3) for each (named as C1, C2 and C3) product through each applied method.

Methods: Determination of fat content

Reference method

The fat content of tests was determined using as reference method the gravimetric method (SR EN ISO 1735, 2005) that is based on the Schmid-Bondynski-Ratzlaff and uses Mojonnier-type fat extraction flasks. The representative sample was prepared by grinding the cheeses.

We worked with tests of about 3 g weighted with 4 exact decimals so that/because the testing of the products through the Gerber method indicated a fat content under 30%. The acid digestion was performed with hydrochloric acid in the lower bulb of the fat-extraction flask where was delivered the sample. We used for extraction ethanol, and the acid-ethanol solution was extracted with diethyl ether and light petroleum. The separation of fat was performed by maintaining it in a vertical position in a support of adequate dimensions. The separation time was at least 30 minutes while in the purpose of the clear separation of fat we have cooled the vials under running water. The tests have been worked in parallel with a blank test, using the same procedure and reagents, but omitting the test portion.For the calculation of the mass fraction of fat of the sample the following equation has been used:

$$\text{Fat \%} = [(m_1 - m_2) - (m_3 - m_4)]/ m_0 \bullet 100, \qquad \text{where:}$$

m_0 – the mass of the test portion, gr
m_1 – the mass of the fat-collecting vassel and extracted matter in it, gr
m_2 – the mass of the prepared fat-collecting vassel, gr
m_3 – the mass of the fat-collecting vessel used in the blank test and any extracted matter in it, gr
m_4 - the mass of the fat-collecting vessel used in the blank test, gr

Application of alternative methods of cheese fat separation

For the extraction of fat from cheese we used the following four methods (SR EN 1528-2, October 2004).

<u>Soxhlet extraction</u>

We used 500ml, round bottom Soxhlet extraction balloons. The balloons are brought to constant weight through drying in the drying oven at 105 ^{0}C. The cheese consists of about 10g of products, exactly weighted after grind. The homogenization of the test was executed through grinding with a 1:1 (m/m) sea sand: anhydrous sodium sulfate. We used sea sand purified through boiling with hidrochlorhydric acid until we obtained a dry, friable mass. The homogenized mixture was transferred in quantity on filter paper with the help of a cotton pad soaked in light petroleum. The cotton will also be put on the filter paper that we introduced in the extractor of the device. We added 250 ml light petroleum in the balloon and we execute the extraction for 6 hours under reflux. The solvent will be removed through evaporation with the spinning evaporator at appreciatively 50 ^{0}C under low pressure. (Beck and Mathar , 1985)

Extraction through the AOAC method (Association of Analytical Communities) modified

The method has been modified (Sprecht, W. in 1987 and Cuniff, P. in 1997) in the proportional diminution (1:5) of all reactive quantities used, due to the volume of the test tubes for centrifugation at our disposal. So, in a 100 ml centrifugation test tube, put 20 ml ethanol, 0,4 g potassium oxalate and 10 g of cheese. The cheese has been firstly grinded and homogenized very well in a lab homogenizer. Mix it very well with all the reagents. Add 10 ml ethylic ether and agitate it thoroughly for 1 minute, then add 10 ml light petroleum and agitate it thoroughly for 1 minute. Afterwards, centrifuge it for 5 minutes at 1500 rot/min; afterwards transfer the solvent layer in a 250 ml separation funnel with 100 ml water and 6 ml solution of saturated sodium chlorine. Extract the residue twice agitating thoroughly with 10 ml ethylic ether: light petroleum 1 : 1 (v/v) mix solvent. Centrifuge it and transfer the solvent layer in the separation funnel after each extraction. Carefully mix the combined extracts with water. Drain and remove water layer. Wash the solvent layer, twice, with two portions of water, of 20 ml each, remove water each time. For dehydration, pass the solvent solution through a column of anhydrous sodium sulphate of 50 mm length and exterior diameter of 25 mm (chromatographic column with PTFE taper and stopcock plug); collect the elute in a Berzelius glass. Wash the column with small portions of ether (three times, 10 ml each time). Gather all etheric extracts in a weighted vase and evaporate the solvent from the combined extracts at steam bath temperature under a current of air, to obtain the fat.

Reflux extraction

Mix 10g g of test cheese with 25 g of sodium sulphate. Transfer the mixture in an Erlenmeyer vase of 250 ml and extract subsequently with four portions of 100 ml mixture 2:1 (V/V) methylene chlorine: acetone through

heating under reflux for 15 minutes. Evaporate the combined extracts. The remaining residue is dissolved, after the evaporation, in 20 ml petroleum ether, the solution is decanted carefully through a cotton tap in a 50 ml round bottom balloon and is evaporated at appreciatively 50° C under low pressure. (Stijve,1987)

Column extraction

We used a 3g test, grinded and weighted with four exact decimals. We homogenized the test in a mortar with pestle with see sand and anhydrous sodium sulphate (1 + 1 mix) to obtain a dry friable product. (We used appreciatively 30 and 40 g of mixture for the homogenization). Transfer the mix in the extraction glass-tube (interior diameter 12 mm and 300 mm length-chromatographic column with PTFE taper and stopcock plug). Before this, introduce in the tube a tap of "glass cotton" and a layer of 2 cm of sodium sulphate, for dehydration. Elute the column with a mix 2:1 (v/v) n-hexane and acetone (250 ml) Collect the elute and evaporate it in a spinning evaporator at approximately 50 ºC under reduced pressure, using balloons of constant weight to determine fat (Beck and Mathar 1985)

RESULTS AND DISCUSSIONS

For each alternative separation method, the final phase is the one when the solvent is removed through evaporation and the fat is determined gravimetrically. In each method the collecting recipient of the final fat extract has been brought to constant weight through drying in the drying oven at 105^0C, before each determination and we have executed blank tests, similar to the reference method**.**

The results obtained are shown in the graphics 1, 2 and 3 for the three types of studied tests. In each case, the values obtained through the three methods, for each sample and the average value are shown in distinct graphics. The values have been calculated with two exact decimals and they are written to the right of the represented point while average value is written with three exact decimals, the numbers being places to the left of the point.

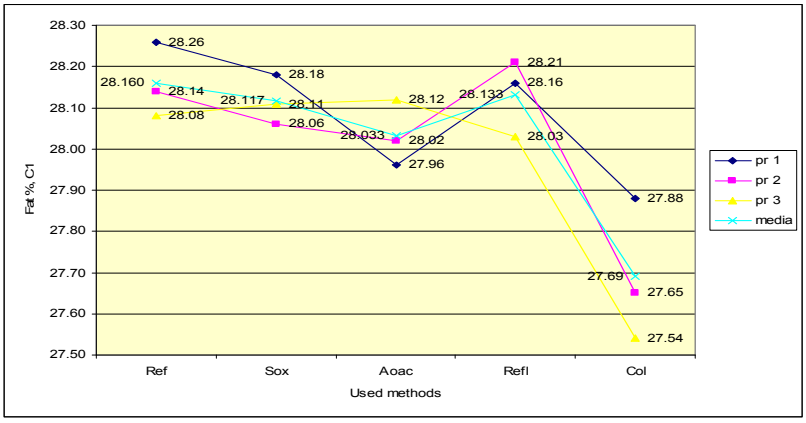

Figure 1

Variation %Fat for C1

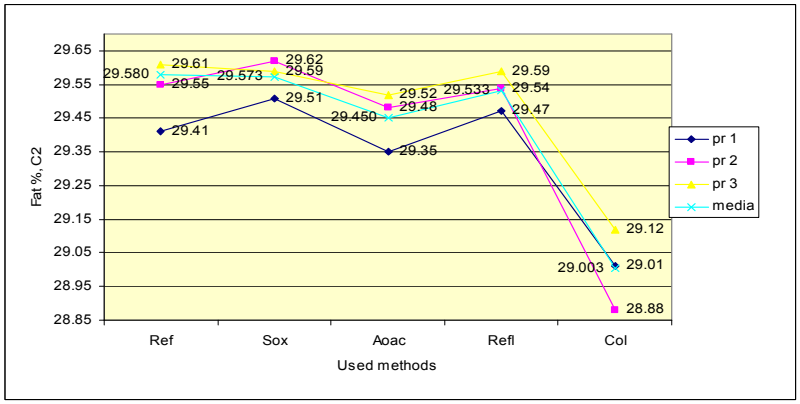

Figure 2

Variation %Fat for C2

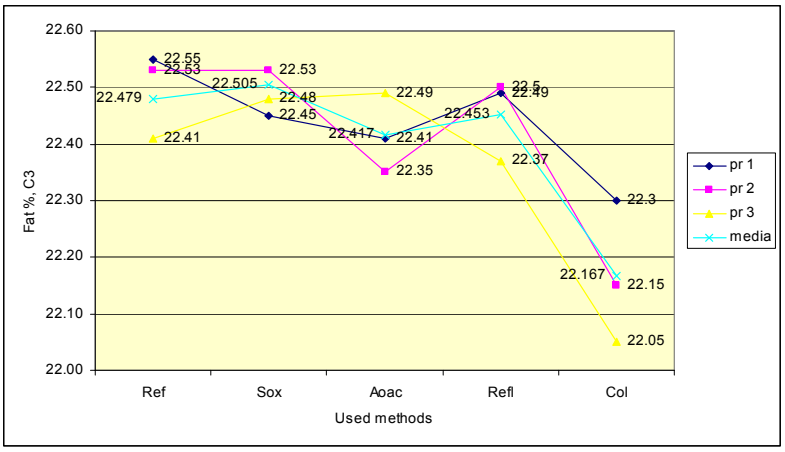

Figure 3

Variation %Fat for C3

The values written in the graphics at figures 1, 2 and 3 show at first sight that the Soxhlet method and the reflux method show the most appropriate results to the reference method. The values obtained through the AOAC method are farther and more oscillating among the three types of pressed cheese, while the ones obtained through the column separation method are much different.

A rigorous analysis has been made by a statistic analysis performed through the t – student test (Ardelean 2005) using an computer program run under Windows. http://www.graphpad.com. The results obtained are shown for the three series of determinations in tables 1, 2 and 3.

Table 1

Statistic analisis of the determinations at test C1 t – test

Media	28.160	28.117	28.033	28.133	27.690
Abatere medie	0.092	0.060	0.081	0.093	0.173
t		0.678	1.7407	0.3575	4.1546

Table 2

Statistic analysis of determinations at sample C2 t – test

Media	29.580	29.573	29.450	29.533	29.003
Abatere medie	0.042	0.057	0.089	0.060	0.120
t		0.171	2.9920	1.1257	5.6138

Tabel 3

Statistic analysis of determinations at sample C3 t – test

Media	22.497	22.505	22.417	22.453	22.167
Abatere medie	0.076	0.035	0.070	0.072	0.126
Valoarea t		-1656	1.3411	728	3.8844

CONCLUSIONS

The statistic analysis was performed through the t – student test (Ardelean M. 2005) and it shows the fact that the differences are not significant between the reference method and the Soxhlet and reflux methods.

However the AOAC method values are farther from the reference one and the "t" value for 4 degrees of freedom are very close to the value that makes the distinction from un-significant values to significant values in two cases and significantly different in one case 2,9920 value). But, since we worked on this case on a reduced scale (1:5), as shown in the description of the work methods, it is possible that the deviation is influenced by this aspect and it is recommended that the method should be redone on a full-amount of product or, at limit, 1:2, as in the case of milk (Chiş, 2007).

In what regards the column separation method, the results are significantly different in all the cases. It may seem that the difference of fat content and the different consistency noticed when analysing the sample might not permit such an efficient separation through this method, related to the other ones applied. The method will be verified on other types of cheese too, in order to draw more complex conclusions.

However, the Soxhlet method and the reflux method have satisfying results compared to the reference method, the closest being in all the cases the Soxhlet method. So we can consider these alternative methods as viable alternatives to the reference method in the fat separation from "Dalia" pressed cheese, for the purpose of calculation of pesticide residues in cheese tests that are supposed to be contaminated.

Later, the separated fraction is submitted to purification and qualitative/quantitative determination of organochlorine pesticides through instrumental methods. The most used method is the gas-chromatography.

REFERENCES

1. Ardelean M., 2005, Principles of agronomic and medical-veterinary research, Academicpress Publishing House, Cluj-Napoca
2. Bara V., C. Laslo., Bara Camelia, 1998, Practical ecotoxicology, Publishing House of the Oradea University.
3. Beck H. and W. Mathar, 1985, Bundesgessundheitsdl. 28, pag 1 -12
4. Bradley, R.L. Jr, 2003, Moisture and total solid analysis, Chap. 6, in Part II, Food Analysis, Third Edition, Springer Science (Suzanne Nielsen Ed.), USA Kluwer Academic New York, 81-103
5. Bradley, R.L. Jr., 2001, Determination of moisture in cheese and cheese products, Journal of AOAC International, 84, 570-592
6. Chiş Adriana, 2007, Determinări practice de laborator în ecotoxicologia alimentelor, Publishing House of the Oradea University.
7. Chiş Adriana and Bara V, 2007, Comparative tests for separation methods of organochlorine pesticides in milk, Joint International Conference an Long-Term Experiments, Agricultural Research and Natural Resources, Debrecen, Nyirlugos, 31 May- 1st June, 2007, p. 453
8. Cunniff, P. (Ed): Official Methods of Analysis of the AOAC INTERNATIONAL, 16IH edition, Arlington VA USA 1995, Vol. 1, Chapter 10 pp 1-10, Method No 970.52
9. Hura Carmen, 2005, Chemical contamination of food in Romania, Cermi Publishing House, Iaşi
10. Hura Carmen, 2006, Laboratory guide – Analysis methods for food products, Cermi Publishing House, Iaşi
11. Savu C-tin and Georgescu Narcisa, 2004, Food safety, "Semne" Publishing House, Bucharest
12. Specht, W,.1987,: Organochlorine and organophosphorus pesticides in: Deutsche Forschungsgemeinschaft, Manual of Pesticide Residue Analysis, VCH Verlagsgesellschaft Weinheim, Voi. 1, pp 309-319, Method S 10.
13. Stijve, T,.1987, Organochlorine and organophosphorus pesticides [Pesticide organoclorurate şi organofosforice] In: Deutsche Forschungsgemeinschaft, Manual of Pesticide Residue Analysis, VCH Verlagsgesellschaft Weinheim, Vol1,5, pp 297-308, Method S 9
14. *** SR EN 1528-2, October 2004, Fat food products. Determination of pestidices and polychlorbiphenyls (PCB), Part 2: Extraction of fat and PCB and determination of fat content
15. *** SR EN ISO 1735, September 2005, Cheese and processed products. Determination of fat content.Gravimetric method (Reference method).