Determination of Bis(2-ethylhexyl) Adipate in Food Products

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A new method of extraction of DEHA, bis(2-ethylhexyl) adipate, plasticizer from a matrix containing a high amount of fat matter, such as cheese, has been developed. By using an ultrasonic bath and hexane as the extracting solvent, the cleanup step to separate the fat matter before the analysis of DEHA is unnecessary. The method is successfully applied in the study of migration of DEHA to several cheeses.

INTRODUCTION

The use of poly(vinyl chloride) (PVC) cling film to wrap food has greatly increased due to many advantages, such as flexibility, transparency, and hygienic factors, which enable the consumer to observe the external aspect and the quality of the product, and at the same time it is convenient.

These films contain additives such as plasticizers, which are organic compounds (esters of high molecular weight). The plasticizers are used to give flexibility to the film, and when the film is directly in contact with the food, the plasticizer can migrate from the film into the food. The European Community is interested in this field as was shown by the publication of a list containing the substances and monomers which may be used in the manufacture of plastic material (EEC, 1990).

There have been migration studies of cling films containing plasticizers such as citrates, sebacates, and phthalates (Castle et al., 1988a,b and 1989), with epoxided soya bean oil (Gilbert et al., 1988; Castle et al., 1988a,b) and bis(2-ethylhexyl) adipate (DEHA) (Startin et al., 1987a,b; Castle et al., 1987) being the most commonly used and also the most frequently studied.

There are many different types of cling films on the Spanish market now, but none of them specifies the recommended or not recommended use for wrapping fatty food. Consequently, everybody can buy the cling film and use it to wrap cheese, ham, or other food. Similarly supermarkets use cling films to wrap small pieces of food sold on retail. The DEHA content in these common cling films was analyzed, and a high content of DEHA (22% w/w) was found. The major problem in the analysis of DEHA in foods is the fat which is extracted together with DEHA. In order to avoid this problem the fat has to be separated before injecting the sample into the gas chromatography. There are several methods which have been reported for the determination of the plasticizers, such as the use described by Shepherd et al. (1981) for the determination of DEHA, which involve acetone/hexane extraction and fat separation by size-exclusion chromatography (SEC) and analyzing the plasticizer by gas chromatography (GC). The most recent described by Startin et al. (1987a,b) involves gas chromatography–mass spectrometry detection. Most of the time the separation procedure used is size-exclusion chromatography.

This paper describes a new method, which has been used to analyze cheese samples. The fat interference has been minimized because the sample has not been completely dissolved but only extracted using hexane as extraction agent solvent. The extraction process is carried out using an ultrasonic bath in which the plasticizer is quantitatively extracted without dissolving the sample.

EXPERIMENTAL PROCEDURES

Materials. The most common films were selected and bought in national supermarkets. Some Spanish cheeses with a fat content between 40% and 50% were chosen for the study, taking care that they had not previously been in contact with cling film or other plastics.

The DEHA standard used for calibration and tetracosane used as internal standard were from Fluka. A capillary gas chromatograph (HP 5890) was used with an integrator. An ultrasonic bath was used to extract and prepare the samples.

Sample Preparation. To prepare the spike samples, some cheese slices (5 g) of 4-cm × 4-cm size were cut into small pieces of about 1-cm × 1-cm size, and they were placed in a round-bottom flask (A), and an organic solution (1–5 μL) of DEHA (1 mg/mL) in hexane was injected into the cheese sample. The flask was shaken for some minutes until the solvent evaporated. This spiked cheese sample was transferred to another round-bottom flask, and then the recommended procedure was followed.

The first round-bottom flask (A) was rinsed with hexane, and the solution obtained was analyzed to determine the content of DEHA which could be deposited on the glass walls. The difference between the amount of DEHA added to the cheese and the amount of DEHA found on the round flask (less than 10% of the amount was found in the first flask in all cases) was the DEHA contained in the cheese samples.

Analysis of Cheese. The cheese sample was cut in several pieces of about 6 g with maximum thickness from 0.6 cm to 1.0 cm. All the pieces were transferred to a round flask of 100 mL, and hexane was added until they were covered. The flask was placed in an ultrasonic bath at 30 °C for 15 min. After this period of time the organic extract was decanted and the extraction was repeated again twice under the same conditions. The pieces of cheese were washed with 25 mL of hexane. After extraction this hexane was added to the combined extracts, and the mixture was filtered through 50 g of anhydrous Na_2SO_4, and 1 mL of a tetracosane solution (0.1 mg/mL) used as internal standard was added. The mixture was concentrated in a rotary evaporator to 5 or 25 mL depending on the content of DEHA expected in the cheese. A blank and a spike from another piece of cheese were prepared simultaneously with the sample. The final extract was analyzed by capillary gas chromatography.

Analysis of Films. A portion of film (1 dm^2) was placed in a round flask, and 20 mL of hexane-containing tetracosane (0.1 mg/mL) used as internal standard was added. The flask was placed in an ultrasonic bath at 30 °C for 1 h. A second extraction was carried out using 10 mL of hexane and keeping the solution in the ultrasonic bath at 30 °C for 15 min. Both extracts were combined, concentrated to 10 mL, and analyzed by GC. This film extraction method was validated with another extraction procedure using $CHCl_3$ as extracting agent (Castle et al., 1987).

Chromatographic Conditions. A nonpolar capillary column (SPB-1) of 30-m length and 0.25-mm i.d. was used under the

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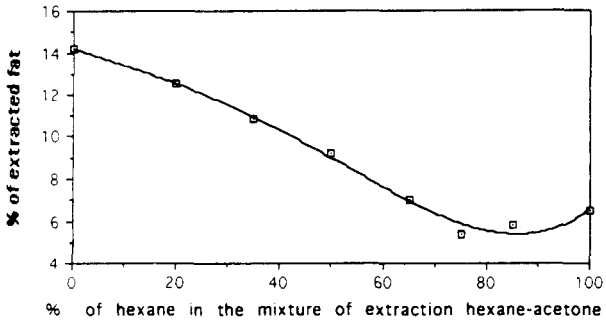

Figure 1. Solvent influence in the fat extraction for an extraction mixture acetone–hexane.

following conditions: oven temperature, isothermal at 225 °C; injector temperature, 250 °C; detector temperature (FID), 300 °C; carrier gas, N_2 flow, 1 mL/min; split ratio, 1/10; volume injected, 1 μL.

Retention times obtained were DEHA, 12.0 min, and tetracosane, 12.9 min.

RESULTS AND DISCUSSION

The method described in this paper has been developed by taking into account the different variables which can affect the extraction process, such as solvent used as extraction agent, the sample size, and the fat content. These variables are described as follows.

Influence of the Solvent. The first choice of solvent was the one used previously in the literature in which a mixture of acetone/hexane (1/1) was recommended (Startin et al., 1987a,b).

The results obtained for the extraction of DEHA were very good, but together with the plasticizer a high amount of fat was coextracted or dissolved, too. This organic extract was directly injected into the capillary column of the gas chromatograph, and no interference from the fat matter was obtained. However after several injections new peaks appeared due to the interference of the fat matter. For this reason, other mixtures of different polarity were examined as extracting agent. Figure 1 shows the results obtained. When the percentage of acetone in the mixture diminished, the fat extracted decreased and the interference in the GC column was lower. Consequently in the following work only hexane was used.

Influence of the Sample Size. To study the influence of the sample size, two series of experiments were carried out, one of them changing the sample weight and the other varying the thickness of the sample. When the sample was ground, most of the fat was dissolved in the solvent. In this case the organic extract had a very high fat content together with the DEHA, and the direct gas chromatographic analysis was impossible because of the interference from the fat after the first injection. Consequently, a cleanup step was necessary to eliminate the fat.

On the other hand, when the sample was cut into small pieces but not ground, the solvent was able to extract the DEHA, but under these conditions, not all the fat was dissolved. DEHA, being of a smaller molecular size than the fat, can migrate through the sample into the solvent.

However, when the size of the cheese pieces was too large, the extraction recovery of DEHA diminished. The results of different extractions with several sample sizes showed that approximately 8 mm was enough to permit the contact between DEHA in the cheese and the extraction solvent but was not enough to dissolve all the fat matter. In such conditions, the extraction recovery after the first extraction was over 83% (Table I) and the extract could be directly injected into the gas chromatograph without interferences. More than 50 injections can be injected in the same column of the gas chromatograph without problems.

Table I. Extraction Recovery of DEHA from Some Spiked Cheese Samples

no. of extractions	sample 1	sample 2	sample 3
1	82.9%	85.5%	88.7%
2	5.7%	6.8%	4.6%
3	2.8%	2.0%	1.3%

Table II. Analysis of DEHA after Three Extractions with Hexane in the Same Spiked Cheese Sample

DEHA added, mg/kg	DEHA found, mg/kg	error, %
704.4	697.6	1.0
430.2	421.8	2.0
1036.6	1018.2	1.8
618.0	599.2	3.1
531.4	528.8	0.5
773.2	780.2	1.0

Influence of the Composition of the Cheese. The study was carried out by adding a known amount of DEHA to several cheese samples, each containing different amounts of fat. The results obtained for the recoveries showed that the fat content influenced the extraction. However in real samples, it is not only the fat but the other compounds present in the sample which might affect the extraction. Thus, a new series of extractions were carried out on other cheese samples in which all had the same fat content but different composition. These were of cheese from cow's milk, lamb's milk, sheep's milk, or mixtures. Also in these cases some significant differences in the total recovery of DEHA appeared. Table I shows the results obtained with hexane in the ultrasonic bath. It can be observed that the values vary between 83 and 89% for total recovery in the first step. The extraction recovery for the second and third extraction steps were lower when the recovery of the first step was higher. The whole process gave a range of recoveries between 90 and 95%, which is appropriate for this type of analysis. The three sequential extractions are recommended to obtain an optimum recovery of DEHA from cheese.

ANALYTICAL PARAMETERS

To check the accuracy of the proposed method a series of nine cheese samples were wrapped with cling film containing 21.9% DEHA; this film can be considered as representative of the films used in many supermarkets in Spain. After 24 h, the samples were extracted and the above procedure was followed. The results obtained show a relative standard deviation of 2.8%.

The precision and the accuracy of the method were checked with six independent samples of the same cheese containing a known amount of plasticizer. A representative example of the results obtained are given in Table II. The result for the lower concentration was checked, and similar results were obtained.

To establish if the results of migration obtained by this procedure were similar to those obtained using the other published methods (Startin et al., 1987a,b), a series of samples were prepared and analyzed by the two procedures. The results are shown in Figure 2. Both are similar in the range of 200–1500 μg/g of DEHA in cheese, which means that the proposed procedure is acceptable for this type of samples.

Migration of DEHA to Several Cheeses. The migration of DEHA to several cheeses was studied. The samples and the cling film were bought in normal supermarkets. All the samples were wrapped in the film for 24 h at 20 °C. It is worth noting that these time/

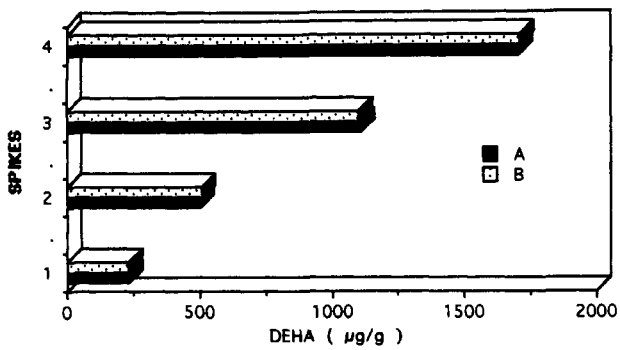

Figure 2. Bar diagram comparing the two analytical procedures: (A) Startin method and (B) new method.

Table III. Migration of DEHA for Different Cheeses and Different Cling Films[a]

cling film (% DEHA w/w)	food	DEHA migration[b] mg/kg	mg/dm^2
1	cheese 1	71.2	1.7
(1.1% DEHA)	2	28.3	0.6
	3	82.0	1.9
2	cheese 1	1663	24.8
(21.9% DEHA)	2	628	
	3	1747	22.1
3	cheese 1	2003	25.3
(23.2% DEHA)	2	637	
	3	2100	25.9

[a] Cheese 1 and 3 have a 50% fat matter. Cheese 2 has between 30 and 35% fat matter and having the highest moisture of all samples. [b] Average of three or more independent determinations with a RSD between 3.6 and 8.7%.

temperature conditions are not appropriate for using these migration data to assess potential consumer exposure to DEHA (MAFF, 1989). The results obtained following the procedure described above are shown in Table III.

Cheese 2 was fresh with a high moisture content. For this reason its behavior was very different from that observed in the other samples 1 and 3 which were old cheese and obviously of a high fat content. This fat allows the plasticizers to be dissolved and thus the levels of contamination by plasticizers which can be reached are quite high.

CONCLUSIONS

The proposed method has several advantages over the methods existing in publication. The most important aspects to be emphasized are as follows:

(a) It is faster than other methods because of the use of an ultrasonic bath and the elimination of the cleanup step.

(b) It is cheaper due to the lower amount of solvent.

(c) The cleanup step can be eliminated and consequently the method is simpler than others.

(d) It has fewer sources of error because there are fewer steps in the analysis and less sample handling.

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