METHOD FOR DETERMINING FAT IN FREEZE-DRIED COW'S MILK AS PART OF AN AUTOMATED PROCEDURE FOR HALOGENATED ORGANIC POLLUTANTS

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Introduction

Persistent organic pollutants (POPs) such as polychlorinated dibenzo-p-dioxins/furans (PCDD/Fs), polychlorinated biphenyls (PCBs), and polybrominated biphenyls (PBDEs) are associated with lipid compartment in foods. FDA plans to monitor milk POPs levels on a lipid weight basis and needs to determine the lipid content of all milk samples as part on the PCDD/F analysis. Traditional liquid-liquid extraction (LLE) methods such as AOAC 970.52L, AOAC methods, require large amount solvent and tedious manual manipulations[1]. Pressurized liquid extraction (PLE) methods, on the other hand, use less solvent and are automated. Recent publications regarding the accelerated PLE for lipid extraction and fat removal methods for PCDD/Fs and/or PCBs analysis have demonstrated various parameters for the extraction cell-packing systems[2-5]. In this work the optimum condition for the automated system for milk lipid extraction and fat removal was investigated. The fat extracting and the fat determining method for cow's milk will precede a purification method for POPs analysis[6].

Materials and Methods

1. Milk Lipid Extraction

Milk lipids were extracted using the two methods, a manual LLE and an automated PLE. Milk samples, labeled as grade A whole milk, were obtained at a local grocery store

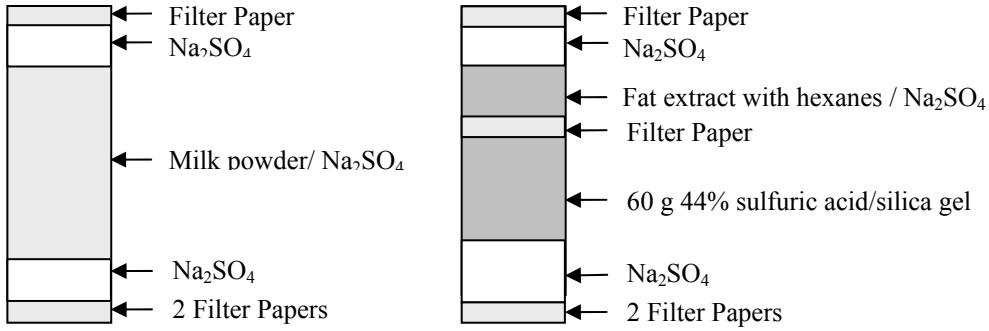
Liquid-Liquid Fat Extraction (LLE)

The LLE of Cow's Milk was performed by a modified AOAC fat extracting method[1]. The ratio of solvents: ethanol/ diethyl ether/ n-hexane, was 1:1:1 (v/v/v). The collected organic layer was filtered through dried anhydrous sodium sulfate Na_2SO_4 placed on the filter paper into a 2 L round-bottomed flask. The extract was evaporated by a rotary evaporator and placed in an aluminum boat to determine the lipid content gravimetrically.

Pressurized Liquid Extraction (PLE) using Accelerated Solvent Extractor (ASE)

The milk sample aliquots were frozen at -39°C and freeze-dried for 30 hours at -40°C and at 300×10^{-3} m Bar. A 6 g of the freeze-dried milk sample, equivalent to 100 g milk, was ground finely with Na_2SO_4 and was filled into a 100 ml-cell for ASE 300 (Dionex, Sunnyvale, CA) as illustrated in Figure 1(a). The ASE parameters used for the PLE method is listed in Table 1. During evaporating the extracts, a small amount of dichloromethane (DCM) was added to in order to evaporate methanol completely. The lipid extract was dried in a tared aluminum boat to determine the lipid content gravimetrically.

Figure 1. Cell Packing System for 100-ml cell of ASE 300



(a) PLE fat extraction cell packing (b) Fat removal cell packing

2. Automated butter fat removal

Fat removal using a fat retainer was performed using butter samples by ASE 300. The butter sample used was commercially available at a local grocery store. The method performed was a modification method developed by Björklund et al[2]. Instead of 40 %, 44 % (w/w) sulfuric acid/ silica gel fat retainer was used. The fat removal procedure is carried as the first step of the automated purification for POPs analysis[6].

A 4 g butter sample was liquefied at approximately 50 °C and was transferred with small amount of hexane onto the dried Na_2SO_4 layer above 60g 44% sulfuric acid/silica layer as illustrated in Figure 1 (b). The ASE parameters set up for the fat removal are shown in Table 1. The two different solvents, 100% cyclohexane (Cyc-hex) and 100% petroleum ether (Pet ether), were used for comparison. The extract was evaporated until the extracts became concentrated to approximately 1ml. The residue obtained after lipid removal was dried onto an aluminum boat and measured gravimetrically.

Table 1. ASE conditions

Parameters	PLE Method	Fat removal with sulfuric acid/silica gel fat retainer	
Solvent	1-11[a]	100% Cyc-Hex	100% Pet-ether
Temperature (°C)	80	100	100
Pressure (psi)	1500	1500	1500
Heat time (min.)	5	5	5
Static time (min.)	5	10	10
Purge time (min.)	100	100	100
Flush volume (%)	60	5	5
Number of Cycle	2	3	3

[a]PLE methods: 1.Acetone/Cyc-Hex (2/1), 2. Acetone/DCM/Cyc-Hex (4/3/3), 3. EtOH/toluene (7/3), 4. MeOH/DCM (1/4), 5. MeOH/DCM (1/2), 6. DCM/Hex (1/1), 7. EtOH/DCM/Hex (1/2/2), 8. MeOH/DCM/pentane (1/2/2), 9.MeOH/DCM/Hex (1/2/5) 10.MeOH/DCM/Hex (1/4.5/4.5), 11.MeOH/DCM/Hex (1/2/2)

Results and Discussion

1. Lipid Extraction Experiment

The mean % lipid from the LLE was 3.1 ± 0.2 % as described in Table 2. The experimental value was not significantly different from the % milk lipid 3.3%, labeled on the milk cartons, by t-test (α= 0.05, P>0.05).

Eleven different solvent systems were tested under the same ASE extraction conditions listed in Table 1. The % lipids extracted from the systems were compared to the % lipids extracted by LLE as shown in Table 3. The PLE method with solvent system 9 was developed by She et al[7]. A modification of this method with solvent system 11 was the most efficient system. A few more replicates were done to compare their efficiency. The mean % lipid from the LLE was 3.1 ± 0.2 % as described in Table 2. The experimental value was not significantly different from the % milk lipid 3.3%, labeled on the milk cartons, by t-test (α= 0.05, P>0.05).

The optimal solvent system using the PLE was determined to be MeOH/ DCM/ Hex (1/2/2). The % lipid for milk samples using PLE was estimated to be 3.22 ± 0.03% as shown in Table 2. The % lipid extracted with MeOH/DCM/ Hex (1/2/5) was 2.5 ± 0.5 %. The difference in these values was significantly different by t-test (α= 0.05, P< 0.05). The mean % lipid extracted by PLE with 1/2/2 solvent mixture was not significantly different from the labeled % lipid, 3.3 %, by t-test (α= 0.05, P< 0.05).

Table 2. The % lipids extracted from cow's milk by LLE method and PLE method

Extraction Methods	LLE	PLE	
Samples	EtOH/ DEE/ n-hexane (1/1/1)	MeOH/$MeCl_2$/ hexane (1/2/5)	MeOH/$MeCl_2$/ hexane (1/2/2)
1	3.23	2.93	3.24
2	3.43	2.00	3.19
3	2.88	2.68	3.22
4	3.04	2.80	3.22
5	3.10	2.21	
Mean ± CI (95%)	3.1 ± 0.2	2.5 ± 0.5	3.22 ± 0.03

Table 3. PLE solvent systems comparisons

Table 2. Solvent system comparisons

PLE method	% of LLE	N
1	71	1
2	66	1
3	81	1
4	72	1
5	90	1
6	80	1
7	71	2
8	97	1
9	81	5
10	100	1
11	104	4

2. Automated % Fat Removal of butter sample

The % fat removal of butter sample using 100% cyclohexane and 100% pet-ether was 86.2 ± 0.2% and 99.7 ± 0.7% respectively. The 100% Pet-ether was found to be the optimal solvent to remove fat using ASE system. These two values were significantly different by t-test (α= 0.05, P< 0.05).

Table 4. Fat removal efficiency by solvent

Milk samples	100% Cyc-Hex	100% Pet-ether
1	84.57	99.79
2	89.97	99.39
3	84.17	99.91
Mean ± CI (95%)	86.2 ± 2.0	99.7 ± 0.7

References

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