

Lipid Extraction for Determination of trans-Fatty Acid in Food

SpeedExtractor E-916, Rotavapor R-300:

Pressurized Solvent Extraction of Food for the Determination of trans-Fatty Acid in Cookies and Brown Rice

Trans-fatty acids have been associated with coronary heart diseases. In this Short Note, the determination of trans-fatty acids using the SpeedExtractor E-916 for the extraction of the lipid of foodstuff is shown. The results obtained with SpeedExtractor E-916 were compared to the ones obtained with conventional methods (BUCHI Extraction Unit E-816 Hot Extration [1], Acid Hydrolysis [2], and Folch [3]). The results showed good correlation and it was demonstrated that neither oxidative degeneration nor new trans isomerization of unsaturated fatty acid occurred during the pressurized solvent extraction.

1. Introduction

Trans-fatty acids in foods derive from hydrogenation of vegetable oils and the refinement of crude oils to obtain edible oils. The determination of trans-fatty acids is a routine procedure for quality assurance and labeling. The determination is usually done by extraction using Folch method (cold extraction using methanol:chloroform mixture), followed by a methylation step and the determination with GC-MS. A less laborious method that reduces the exposition of the operator to toxic solvent is desired.

2. Experimental

Equipment: SpeedExtractor E-916, Rotavapor R-300

Samples: Cookies (A + B), brown rice

Determination: The milled samples were extracted using the SpeedExtractor E-916 using the parameters shown in Table 1.

Table 1: Parameters for SpeedExtractor E-916

Parameter	Value
Temperature	100 °C
Pressure	100 bar
Solvent	Hexane (100%)
Cell size	10 mL
Vial size	60 mL
No. of Cycles	3
Heat up-time	1 min
Hold time	10 min
Discharge	2 min
Flush with solvent	1 min
Flush with gas	3 min
Total Extraction time	60 min

The extracts were evaporated to dryness under nitrogen flow using a Rotavapor R-300. The amount of total lipids was then determined gravimetrically. For the determination of the *trans*-fatty acids, an aliquote of 20 mg was methylated and quantified according to JOCS Standard Method 17-2007 [4] using heptadodecanoic acid (C 17:0) as internal standard. The quantification was done gaschromatographically on a Shimadzu GC-2010, equipped with a CP-2506 column (Supelco).

3. Results

The results of the fat determination and the quantification of the *trans*-fatty acids using SpeedExtractor E-916 were comparable to those determined with the reference methods, see Table 2 and 3.

Table 2: Determined fat contents [%, +/- SD] of cookies A and B and brown rice

Method	Cookie A	Cookie B	Brown rice
SpeedExtractor E-916	25.0 +/- 0.2	17.4 +/- 0.3	2.6 +/- 0.1
Folch method	25.8 +/- 0.4	17.8 +/- 0.4	2.5 +/- 0.1
Acid hydrolysis	26.6 +/- 0.7	17.5 +/- 0.1	-
E-816 HE	24.5 +/- 0.1	17.2 +/- 0.1	-

Table 3: Determined contents of unsaturated fatty acids, c:cis; t:trans [g/100 g lipids, +/- SD] of cookie A

Method	C18:1t	C18:1c	C18:2t	C18:2c	C18:3t	C18:3c
SpeedExtractor	1.97	27.93	0.39	6.51	0.09	0.49
E-916	+/-0.10	+/- 0.75	+/- 0.04	+/- 0.18	+/- 0.01	+/- 0.03
Folch method	2.01 +/- 0.05	27.37 +/- 0.22	0.37 +/- 0.02	6.43 +/- 0.10	0.09 +/- 0.01	0.50 +/-0.02
Acid hydrolysis	1.95 +/- 0.08	27.07 +/- 0.46	0.39 +/- 0.02	6.43 +/- 0.20	0.08 +/- 0.01	0.49 +/- 0.03
E-816 HE	1.99 +/- 0.08	27.77 +/- 0.50	0.36 +/- 0.02	6.20 +/- 0.15	0.07 +/- 0.01	0.47 +/- 0.03

4. Conclusion

The fat contents determined by pressurized solvent extraction using BUCHI's SpeedExtractor E-916 correspond to those obtained with reference methods. Although the samples are extracted at high temperature (100°C) there is no formation of *trans*-fatty acids or degradation of fatty acids. This is due to the fact, that the sample is hardly exposed to oxygen because of the nitrogen flushing of the instrument.

Pressurized solvent extraction is a very well suited extraction method for the determination of *trans*-fatty acids in foodstuff. The detailed procedure and the results have been published in peer review journals [5-6].

5. Acknowledgements

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6. References

- [1] JOCS Standard Method 3.1.1.-1996
- [2] JOCS Standard Method 3.1.3-1996
- [3] Folch, J. et al. J. Biol. Chem. 1957, 226, 497
- [4] JOCS Standard Method 17-2007.
- [5] Tsuzuki et al. Chem. Lett. 2013, 1026-1028
- [6] Tsuzuki et al. Food Sci and Technol. 2014, 58, 222-229