



# Sequential Microwave-Assisted Extraction of Oil from Layer Poultry Feeds and GC-MS Quantification of the Fatty Acids

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## Abstract

The present study reports the effect of sequential microwave-assisted extraction (SeMAE) on fatty acids composition (FAC) of layer poultry feed oil as compared to conventional Soxhlet extraction (SE) method. The FAC of extracted oil was determined by gas chromatography–mass spectrometry (GC-MS). There was no significant difference found in the amount of total extracted oil and FAC by SeMAE and SE. However, slightly greater content of *trans* fat in the samples revealed that SE lead to the formation of a little higher level of *trans* fat as compare to SeMAE. Therefore, the SeMAE could be used as a remarkable substitute to conventional SE for extraction of oil from the poultry feeds due to its faster speed, lesser solvent consumption, more environmental friendly.

**Keywords:** Layer poultry feed oil; Sequential microwave assisted extraction; Soxhlet extraction; Fatty acid composition; *trans* Fat.

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## Introduction

One of the most important aspects of lipid chemistry is the extraction of oil and fat from animal and plant sources. Traditionally, it is carried out by several ways using different types of solvents depending upon the sample characteristics [1]. The most commonly used methods for the extraction of lipids in the laboratories from solid samples are the Soxhlet, Goldfish, and Folch methods. Quantitatively lipids can be accurately determined by these methods, but all of these methods require 8 to 24 hour long extraction time. The Soxhlet extraction technique was developed by Franz von Soxhlet in 1879 for the determination of fat in milk [2] and it is widely used for extraction of oil in agricultural chemistry. However, high temperature and the large volume of organic solvents are the main deficiencies of Soxhlet extraction, and also employ some changes in the extract quality. These changes can influence on the level of *trans* fatty acids [1], and free fatty

acids [3]. Because of the exhaustive long extraction time, in early 1970s Randall [4] developed an efficient and less time consuming extraction technique based on the Soxhlet method, known as Soxtec system which was based on three-step procedure involving boiling, rinsing and solvent removal.

Also few years ago, other new techniques were explored including supercritical fluid extraction (SFE) [5], accelerated solvent extraction (ASE) [6], microwave-assisted extraction (MAE) [7] and focused microwave-assisted Soxhlet extraction (FMASE) [8]. An other technique developed alternative to Soxhlet extraction (SE) is an ultrasound-assisted extraction (UAE), which has been successfully applied for the seed oil extraction [9-10] and known as proficient extraction technique that significantly decrease extraction time, increasing yield and enhancing the

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quality of the extract. Recently, a new improved microwave assisted SEx process has been developed by the French group called microwave integrated soxhlet extraction (MIS) [11] for the total fats and oils from food products.

In our previous study [3] we used domestic microwave oven for the extraction of oil from broiler poultry feeds and reported that the small amount of hazardous solvent was used for the oil extraction by SeMAE, and the extracted oil contained lower quantity of free fatty acid (FFA) than conventional SE. Present study deals with the effect of SeMAE extraction on fatty acid composition of layer poultry feeds with special reference to *trans* fat which is very harmful to the human health.

## Experimental

### *Reagents, standards and samples*

All chemicals and reagents used were of analytical grade. Hexane was obtained from Fisher Scientific Ltd UK. Methanol, potassium hydroxide and anhydrous sodium sulfate were purchased from Merck (Darmstadt, Germany). Cis and *trans* fatty acid methyl esters (FAMES) standards (GLC 481-B and 607) were purchased from Nu-Check-Prep, Inc (Elysian, MN, USA). Poultry feed samples were specified for layer finisher and collected from a local industry suppliers commercially available in Sindh, Pakistan.

### *Sample preparation and extraction*

Feed samples were ground using a Mammonlex Super blender Mill Grater 3 (No: 4AO- 0018, Type JW-1001, Taiwan), sifted through a stainless steel screen having a mesh size of 1.0 mm to obtain a uniform particle size and kept in air tight plastic bags until required for extraction.

### *Sequential microwave assisted extraction*

Sequential microwave assisted extraction was carried out as described by Mahesar et al. [3]. A domestic microwave oven (Pell-PM 023, Japan) with power settings range from 100-900W was used for oil extraction. 5 g of ground feed was taken in a 30 ml vial containing 12 ml hexane and subjected to full power (900 W) of microwave

irradiation. After 20 sec microwave heating the vial was taken out and shaken vigorously to cool. The vial was again placed into the oven for further 20 sec. The same practice was repeated after each 20 sec so that to obtain the 2 minutes of microwave oven exposure. After extraction, the miscella was collected and replaced with fresh solvent. The process was repeated four more times to attain a 10 min of microwave exposure. After oil extraction, the solvent was recovered by simple distillation using a rotary evaporator (R-210, Büchi, Zurich, Switzerland) and the residual oil was oven-dried at 75 °C for one hour. The oil was then transferred to a desiccator and allowed to cool before being weighed. The drying, cooling and weighing was repeated until a constant dry weight within 0.01 g was obtained. Extracted oil from feed was analyzed for fatty acids composition using gas chromatography coupled with mass spectrometry (GC-MS).

### *Soxhlet extraction*

A 250 ml capacity soxhlet extractor was used for the extraction of oil from the poultry feed samples for comparison as a standard method. 5 g of the ground samples were placed in a Whatman thimble and inserted into the Soxhlet extractor and 100 ml hexane (analytical grade) was used as the extracting solvent on a water bath at 80 °C [12]. The period of continuous extraction was 5 hour. At the end of this period, the solvent was recovered by simple distillation and the residual oil was oven-dried at 75 °C for one hour. The extract was analyzed for fatty acids composition using GC-MS.

### *Determination of fatty acid composition and trans Isomers by GC-MS*

For the determination of fatty acids composition of the poultry feed oil, fatty acid methyl esters (FAMES) were prepared using standard IUPAC method 2.301 [13]. An Agilent GC-MS was used with ChemStation 6890 Scale Mode software. Separation and quantification of the FAMES were carried out using a gas chromatograph 6890 N (Agilent Technologies Network GC system ) equipped with an Agilent MS-5975 inert XL Mass selective detector, automatic sample injector 7683-B (Agilent

Technologies, Little Fall, NY, USA). Highly polar Rt-2560 biscyanopropylsiloxane capillary column (100m x 0.25mm i.d x 0.2µm film thickness) was used for the separation of individual *cis trans* isomers. Helium was used as carrier gas at a flow rate of 1.2 mL/min, samples were injected using a split mode with the ratio (50:1) and 1µL of sample solution was injected and the temperature of injector was kept at 250 °C. The oven temperature was held at 150 °C with 2 min hold time, ramp at 4 °C/min, final temperature 230 °C. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV; ion source temperature 230 °C; quadrupole temperature 150 °C; translating line temperature 270 °C; the mass scan ranged from 50 - 550 *m/z*; Em voltage, 1035 V. The comparison and identification of fatty acids methyl esters of poultry feed oil was performed using two libraries (NIST & Wiley).

### Calculations and statistical analyses

Peak identification of the fatty acids in the analyzed poultry feed oil samples were carried out by the comparison with retention times and mass spectra of known standards. Two samples of each brand were collected and each sample was analyzed three times. The data obtained were put into Origin 7 program and reported as mean ± SD (n = 2 x 3).

### Results and Discussion

The total crude lipid extracts of poultry feeds obtained by the sequential microwave assisted extraction (SeMAE) and conventional soxhlet extraction (SE) methods are shown in Table 1. Quantitative results of the both techniques are comparable. However slightly higher oil content was observed by the SeMAE as compared to the conventional SE method. The mean fat content derived from SeMAE and SE in the layer poultry feeds was 3.15 and 3.11 %, respectively.

Table 2 shows the composition of the fatty acids present in the poultry feed lipid extract. The dominant saturated fatty acids (SFAs) found in the poultry feed oils were palmitic acid (C16:0) and stearic acid (C18:0), and represented (12-18%) of the total fatty acids composition. Whereas the remaining SFAs were myristic acid (C14:0),

arachidic acid (C20:0), behenic acid (C22:0) and lignoceric acid (C24:0) each fatty acid was present less than 1%. Among unsaturated fatty acids the major fatty acids were oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) and palmitoleic acid (C16:1), these four fatty acids represent (76-82%) of the total fatty acids while gadoleic acid (C20:1) and erucic acid (C22:1) were present less than 1% of the total fatty acids. Elaidic acid (C18:1 *n-9 t*) ranged from 0.9 to 3.04% in the lipid extracted from layer poultry feeds. The results of fatty acid profile obtained by the SeMAE and SE were comparable as shown in Table 2.

**Table 1. Lipid content of layer poultry feeds by sequential microwave assisted and Soxhlet extraction.**

Sample	Lipid contents (%)	
	SeMAE	SE
LPF-1	3.64±0.05	3.62±0.07
LPF-2	3.45±0.06	3.40±0.06
LPF-3	2.90±0.04	2.86±0.05
LPF-4	3.24±0.05	3.20±0.06
LPF-5	2.52±0.03	2.50±0.04
Average	3.15	3.11

LPF: Layer poultry feed  
SeMAE: Sequential microwave assisted extraction  
SE: Soxhlet extraction

Caponio et al. [14] has also reported that heat treatments caused an increase in the *trans*-isomers of unsaturated fatty acids and this was more evident after microwave treatment. Recently a study carried out by Perez-Serradilla et al. [15] using focused microwave-assisted soxhlet extraction to extract the oil from acorn with comparison to the other extraction reference methods, and no *trans* fatty acids (TFAs) were detected in the extracts obtained by the FMASE method. While little amount of TFAs was determined in soxhlet and ISO reference extraction methods. The present study indicated that both lipid extracts were found to contain some amounts of TFAs, but in the oil extracted by SeMAE comparatively contained less amount of TFA than SE. The possible reason for the lower TFAs in SeMAE extract is the controlled temperature (~ 45 °C) and much shorter time 40 min extraction as compared to the exhaustive 6 hours of Soxhlet extraction.

Table 2. Effects of SeMAE and Conventional Soxhlet Extraction on the Fatty Acids Composition (%) of Layer Poultry Feed oil.

FA	LPF-1		LPF-2		LPF-3		LPF-4		LPF-5	
	SeMAE	SE	SeMAE	SE	SeMAE	SE	SeMAE	SE	SeMAE	SE
<b>14:0</b>	nd	nd	0.17 ± 0.02	0.21 ± 0.01	0.16 ± 0.07	0.27 ± 0.04	0.17 ± 0.07	0.09 ± 0.08	nd	nd
<b>16:0</b>	12.25 ± 0.67	12.39 ± 0.70	14.42 ± 0.45	14.60 ± 0.45	10.71 ± 0.33	11.09 ± 0.34	9.14 ± 0.65	10.51 ± 0.72	13.64 ± 0.26	14.02 ± 0.41
<b>16:1 n-9 c</b>	nd	nd	1.39 ± 0.014	1.48 ± 0.011	0.30 ± 0.07	0.25 ± 0.033	0.55 ± 0.05	0.40 ± 0.06	0.92 ± 0.01	0.70 ± 0.03
<b>18:0</b>	2.24 ± 0.07	2.33 ± 0.09	3.31 ± 0.16	3.21 ± 0.12	2.40 ± 0.11	2.31 ± 0.09	2.59 ± 0.04	2.47 ± 0.06	3.28 ± 0.01	3.07 ± 0.02
<b>18:1 n-9 c</b>	30.72 ± 0.49	30.78 ± 0.51	35.81 ± 0.21	35.96 ± 0.28	35.06 ± 0.11	35.28 ± 0.14	40.56 ± 0.71	40.71 ± 0.81	35.99 ± 0.43	37.08 ± 0.48
<b>18:1 n-9 t</b>	0.96 ± 0.07	1.01 ± 0.05	1.96 ± 0.02	2.05 ± 0.08	3.04 ± 0.06	3.21 ± 0.04	2.92 ± 0.03	3.02 ± 0.04	1.97 ± 0.02	2.44 ± 0.03
<b>18:2 n-9, 12 cc</b>	51.49 ± 0.81	51.32 ± 0.90	40.96 ± 0.12	40.84 ± 0.19	45.08 ± 0.25	44.88 ± 0.30	39.35 ± 0.46	38.97 ± 0.14	40.45 ± 0.22	39.54 ± 0.37
<b>18:3 n-9, 12, 15 ccc</b>	1.29 ± 0.08	1.06 ± 0.06	1.18 ± 0.02	1.12 ± 0.02	2.06 ± 0.05	1.88 ± 0.07	3.45 ± 0.06	2.85 ± 0.02	1.62 ± 0.03	1.47 ± 0.01
<b>20:0</b>	0.44 ± 0.02	0.49 ± 0.03	0.36 ± 0.01	0.39 ± 0.04	0.48 ± 0.04	0.41 ± 0.06	0.52 ± 0.05	0.35 ± 0.03	0.52 ± 0.02	0.44 ± 0.04
<b>20:1 n-11 c</b>	0.35 ± 0.05	0.27 ± 0.06	nd	nd	0.48 ± 0.02	0.31 ± 0.04	0.75 ± 0.08	0.63 ± 0.06	0.60 ± 0.04	0.44 ± 0.03
<b>22:0</b>	0.12 ± 0.03	0.14 ± 0.05	nd	nd	0.23 ± 0.01	0.11 ± 0.04	nd	nd	nd	nd
<b>22:1 n-13 c</b>	nd	nd	0.44 ± 0.021	0.14 ± 0.012	nd	nd	nd	nd	1.01 ± 0.014	0.80 ± 0.020
<b>24:0</b>	0.14 ± 0.004	0.21 ± 0.002	nd	nd	nd	nd	nd	nd	nd	nd

The presence of TFAs in the poultry feed oil indicated that during manufacturing of the poultry feed, the additional dietary lipid may be provided from animal-vegetable blend using tallow from rendering sources and restaurant grease or hydrogenated oil from the food industry. Lipids from these sources are rich in *trans* fat [16]. The amount of TFAs in the meat of nonruminant animals is generally low [17] and is dependent on the presence of TFAs in the feeds. On the basis of the quality of poultry feeds, these TFAs have shown various harmful effects on the poultry i.e. adversely affect EFA metabolism, poor growth and feed conversion rate, and lower metabolizable energy [18]. While the presence of TFAs in the

poultry meat could be harmful for the health of consumers [19].

Table 3 shows the fatty acid classes and their ratios present in the extracted poultry feeds oil. The mean value of saturated and unsaturated fatty acids in poultry feed samples were 15.46-15.82 and 84.23-84.55 for SeMAE and SE, respectively. The ratio of unsaturated/saturated FA shows the relation between the two major FA groups of the fat composition; its value varies from 4.48 to 7.05 and 4.43 to 6.45 for SeMAE and SE, correspondingly. This ratio is one of the predominant factors that contribute to the ability of the chick to digest and utilize fatty acids [20].

Table 3. Fatty acid classes in total fat and their ratio of layer poultry feed by sequential microwave assisted and Soxhlet extraction.

Classes	LPF-1		LPF-2		LPF-3		LPF-4		LPF-5	
	SeMAE	SE	SeMAE	SE	SeMAE	SE	SeMAE	SE	SeMAE	SE
$\Sigma$ SFA	15.19	15.56	18.26	18.41	13.98	14.19	12.42	13.42	17.44	17.53
$\Sigma$ MUFA	32.03	32.06	39.60	39.63	38.88	39.05	44.78	44.76	40.49	41.46
$\Sigma$ PUFA	52.78	52.38	42.14	41.96	47.14	46.76	42.8	41.82	42.07	41.01
$\Sigma$ MUFA+ $\Sigma$ PUFA	84.81	84.44	81.74	81.59	86.02	85.81	87.58	86.85	82.56	82.47
PUFA/SFA	3.47	3.37	2.31	2.28	3.37	3.30	3.45	3.12	2.41	2.34
$\Sigma$ MUFA+ $\Sigma$ PUFA/ $\Sigma$ SFA	5.58	5.43	4.48	4.43	6.15	6.05	7.05	6.45	4.73	4.70
n-6	51.49	51.32	40.96	40.84	45.08	44.88	39.35	38.97	40.45	39.45
n-3	1.29	1.06	1.18	1.12	2.06	1.88	3.45	2.85	1.62	1.47
n-6/ n-3	39.91	48.42	34.71	36.46	21.88	23.87	11.41	13.67	24.97	26.84

According to the common nutritional instructions of the Department of Health UK [21], the recommended ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (P:S) should be or above 0.45. Since some meats naturally have a P:S ratio of around 0.1, meat has been implicated in causing the imbalanced fatty acid intake of today's consumers. Therefore, ratio should be maintained near to the recommended value in the feed formulation because it has direct impact on the meat of poultry. The values for the PUFA/SFA ratio in the analyzed poultry feed oil varied from 2.28 to 3.47, which is very high.

Recommendations for n-6 and n-3 classes of PUFA are also important because scientists recognize differences in metabolism and physiological function between these fatty acid families [22]. The n-6/ n-3 fatty acids ratio is an important index to evaluate the nutritional value of a fat. The recommended ratio of n-6/ n-3 fatty acids for healthy diet should be less than 4 [23], and in present study extracted poultry feed oil contains much higher ratio than recommended value by the both extraction methods, SeMAE and SE (26.57- 29.85). The decrease in amount of n-3 fatty acids in poultry feed has lead to an imbalance diet for poultry, because industrially processed feeds were poor in n-3 fatty acids. Increase in the ratio of n-6/n-3 leading to production of meat

which may be higher in n-6 and lower in n-3 fatty acids.

### Conclusions

In the present study, SeMAE based on domestic microwave oven was carried out and compared to conventional Soxhlet extraction in terms of crude lipid extract and fatty acid profile. The results indicated that the oil extracted by SeMAE were quantitatively and qualitatively comparable to those obtained by conventional Soxhlet extraction. Therefore, SeMAE using domestic microwave energy can be regarded as alternative for the extraction of poultry feed lipids. Due to the selection of appropriate column and temperature programming during our investigation for screening of fatty acids including *trans* fatty acids, GC-MS found to be very capable and efficient tools for the separation and identification of individual fatty acids without using any costly standards.

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