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Fast quality monitoring of oil from prefried and fried foods by focused microwave-assisted Soxhlet extraction

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Abstract

A new method for fast quality monitoring of fat from prefried and fried meat and fish is proposed. Prefried and fried samples were extracted with a focused microwave-assisted Soxhlet extractor. The main factors contributing to the extraction efficiency, namely microwave irradiation power, number of cycles and microwave irradiation time were optimized by means of a central composite design based on two level-three factors factorial design. This method has allowed us to carry out the extraction of lipids from prefried and fried samples with qualitative and quantitative results similar to those provided by the usual methods (both manual and conventional Soxhlet extraction). A drastic reduction of the procedure time (55 min versus 8 h) is achieved with similar reproducibility to that provided by the conventional method. In addition, the proposed method is cleaner than conventional Soxhlet as 75–80% of the extractant is recycled.

Keywords: Focused microwaves; Lipid extraction; Prefried and fried foods; Response surface methodology

1. Introduction

During deep-fat frying, a complex series of reactions takes place resulting in hydrolysis, oxidation and polymerisation of the oil. As quality of fried foods is affected by that of the oil, regulations or guidelines have been established in many countries to guarantee high quality precooked and cooked foods. Determination of polar compounds by adsorption chromatography has become the most accepted method for quality evaluation of used frying fats as demonstrated by the present official European regulations in which a level around 25% has been adopted as the maximum permitted for human consumption (Firestone, 1996).

Over the last 40 years the consumption of frying fats and oils has undergone a great increase, following the development of a wide range of new products among which frozen prefried food and fast food stand out. The growing volume of frying fats and oils applied and the loss of oil quality during the frying process has stressed even more the need for quality control as some compounds are suspicious of impairing the nutritional value of the fats (Márquez-Ruiz & Dobarganes, 1996).

Most information on the quality of used frying fats has been obtained by analysing the oil in the fryer on the assumption that acceptability of fried foods depends on the quality of the used frying oil or fat. Nevertheless, as pointed out by Pokorny, quality of food lipids, which are those ingested in the fried foods, were much less studied (Pokorny, 1998), mainly due to the requirement of a previous extraction step.

The use of microwave energy as a heat source in wet ashing procedures was first demonstrated in 1975 (Abu-Samra, Morris, & Koirtyohann, 1975). Since then, microwaves have shown their suitability for accelerating extraction processes. A focused microwave assisted Soxhlet extractor is a patented device (PCT Application, WO97/44109, 1998), which has been checked in the extraction of different compounds in solid samples such as pollutants (García-Ayuso, Luque-García, & Luque de Castro, 2000) and also in food analysis as an alternative to traditional methods for lipid extraction from different samples (García-Ayuso, & Luque de Castro, 1999; García-Ayuso, Velasco, Dobarganes, & Luque de Castro, 1999). The key aspect of focused microwave

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assisted Soxhlet extraction (FMASE) is that it maintains the advantages of conventional Soxhlet extraction, (namely: sample-fresh solvent contact during the whole extraction step, no filtration required after extraction, easy manipulation, well-known procedures and a large experience in the extraction field for more than a century) and circumvents the shortcomings of conventional Soxhlet by accelerating the process and minimizing environmental pollution due to the small amount of solvent released into the atmosphere.

In this paper, two commercialized prefried foods accepted worldwide, i.e. chicken nuggets and hake fingers, were extracted before and after a final frying operation by FMASE. The efficacy of the extraction as well as the analytical results for fatty acid composition, polar compounds and the main groups of polar compound constituents were compared with those obtained by conventional extraction methods.

2. Experimental

2.1. Sampling

2.1.1. Types of samples

Two types of food samples (meat and fish) were used. In the case of meat, chicken nuggets prefried and fried under commercial conditions were used. For fish, prefried hake fingers under commercial conditions were used, and the same hake was fried under domestic conditions. This selection was aimed at comparing the effect of frying under controlled or uncontrolled quality oils conditions.

2.1.2. Sample preparation

Each kind of sample was separated into two fractions by removing the external layer or batter. The fractions (batter and unbattered food, either meat and fish) were ground independently in a mechanical mill. They were placed in a plastic flask and stored at 4 °C until use. Meat from fried chicken nuggets was used in the microwave factors optimization study, and the optimal values found were used in the fat extraction from other samples.

2.2. Determination of the moisture and volatile matter

Ten grams of the foodstuff under study was placed on a desiccated tared capsule that was transferred into an electrically heated oven with ±2 temperature control (Selecta, Barcelona, Spain) at 80 °C for 2 h. After this, the capsule was removed from the oven and cooled to room temperature in a dessicator. After weighing (Explorer analytical balance, Ohaus, USA), the procedure was repeated until the difference between two consecutive weightings was smaller than 2 mg.

2.3. Manual extraction

An amount of 10 g of the corresponding sample was placed in a flask containing 200 ml of n-hexane. The mixture was stirred in an electrical agitator. After stirring for 15 min, anhydrous sodium sulphate was added to remove water and the suspension was filtered. The solvent was released by a rotary-evaporator (Rikakikai Co., Ltd., Japan) and the extract was dried under nitrogen stream until the difference between two consecutive weighings was smaller than 2 mg.

2.4. Conventional Soxhlet extraction

Five grams of the sample under study was placed in an oven at 80 °C until their moisture were less than 6% m/m. The dried sample was placed in a cellulose thimble (25×88 mm, Albet, Barcelona, Spain). The overall Soxhlet glassware was fitted to a distillation flask containing 70 ml of n-hexane and 2–3 boiling glass regulators. After extraction for 8 h, the solvent was released to a rotary-evaporator and the extract was dried under a nitrogen stream until the difference between two consecutive weightings was smaller than 2 mg.

2.5. FMASE

A conventional Soxhlet extractor was modified in order to facilitate accommodation of the sample cartridge compartment in the irradiation zone of a Microdigest 301 device of 200-W maximum power (Prolabo, France). The latter was also modified: an orifice at the bottom of the irradiation zone enabled connection of the cartridge zone to the distillation flask through a glass siphon.

Fig. 1 illustrates the operation of the overall device (PCT Application, WO97/44109, 1998). A Megal 500 thermometer (Prolabo, France) was used to monitor the extraction temperature. Two microprocessor programmers were used to control the microwave unit and thermometer.

One hundred milliliters of n-hexane and some pieces of pumice stone were poured into a tared distillation flask. Five grams of the sample was mixed with 1–2 g of pre-washed sea sand as a dispersion agent. The mixture was put into a cellulose extraction thimble (25×88 mm, Albet, Barcelona, Spain), which was covered with cotton wool and inserted into the quartz extraction vessel placed in the microwave-irradiation zone. The distillation flask was positioned on an electrical isomantle (Prolabo, France) and connected to the sample vessel by a siphon and a distillation tube. After the extraction step, (9 cycles with 85 s of microwave irradiation at 60% of maximum power each cycle) the extractant was collected in a reservoir by actuating a valve, which switched

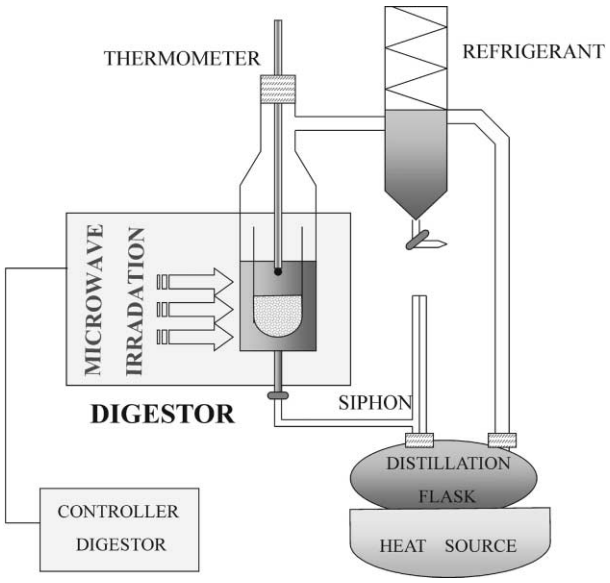

Fig. 1. Scheme of the prototype operation.

between the usual way of distilling in the Soxhlet extractor and the reservoir. Seventy-five to eighty milliliters of the solvent was thus recovered. Removal of solvent traces from the extracted fat and gravimetric determination was performed as in the conventional Soxhlet procedure.

2.6. Chromatographic determination

2.6.1. Fatty acid composition

Fatty acids were analyzed by gas chromatography after derivatisation to fatty acid methyl esters (FAME) with 2 N KOH in methanol, according to the IUPAC standard method (IUPAC, 1992). A HP 6890 chromatograph on an HP Innowax capillary column (polyethylene glycol, 30 m×0.25 mm i.d., film thickness 0.25 μm; Hewlett Packard, USA), was used under the following temperature programme: 180 °C (4 min), 4 °C min^{-1} to 230 °C (15 min). Samples were introduced into the column via a split injector (1:40 split ratio) at 250 °C and the flow rate of hydrogen, used as carrier gas, was 1 ml min^{-1}. The temperature of both split injector and flame ionization detector was 250 °C.

2.6.2. Quantitation of polymers

Triacylglycerol polymers were quantified by high-performance size exclusion chromatography (HPSEC) following the IUPAC standard method (Wolf, Mordret, & Dieffenbacher, 1992).

2.6.3. Quantitation of total content and distribution of polar compounds

The evaluation of the samples was approached by applying a methodology developed for quantitative analysis of different groups of altered compounds in small samples (Márquez-Ruiz, Jorge, Martín-Polvillo, & Dobarganes, 1996). Briefly, 2 ml of a sample solution containing 50 mg of extracted oil and 1 mg monostearin, used as internal standard, was separated by solid-phase extraction (silica cartridge) into a first fraction (essentially unaltered triglycerides) eluted with 15 ml 90:10 hexane:diethyl ether and a second fraction, containing polar compounds and the internal standard, eluted with 15 ml of diethyl ether. Subsequently, fractions of polar compounds were analyzed by HPSEC using a Rheodyne 7725y injector with a 10 μm sample loop, a Waters 510 HPLC pump (Waters Associates, Milford, MA, USA), two 100 and 500 Å Ultrastyragel columns 25×0.77 cm inner diameter, packed with a porous, highly cross-linked styrenedivinylbenzene copolymer (<10 μm) connected in series, and a refractive index detector (Hewlett Packard, CA, USA). HPLC-grade tetrahydrofuran was the mobile phase with a flow of 1 ml min^{-1}. The peaks resolved corresponded to triglyceride polymers, oxidized triglyceride monomers, diglycerides, monostearin, and a final peak constituted by free fatty acids and polar unsaponifiables.

3. Results and discussion

Fried chicken nuggets after removal of the batter were used in the optimization study.

3.1. Optimization of the focused microwave-assisted Soxhlet extraction

The variables susceptible to be optimized in FMASE are always the irradiation power, the irradiation time and number of cycles needed for total extraction of the analytes. The multivariate optimization procedure used was based on the response surface methodology.

The selection of a central composite design (CCD) based on a two-level full factorial design was supported on the low number of factors to be optimized. Statistical software (Statgraphics for windows, 1996) was used to analyze the data from the experiments.

The overall optimization procedure was developed as follows: a full factorial design was built for a screening study of the behavior of the main factors and interactions present within the domain imposed by the limits. The experimental matrix and results obtained are shown in Table 1.

Analysis of variance (ANOVA) was performed on the design to assess the significance of the model with the initial summary of the model statistic given in Table 2a. The *F*-ratio in this table is the ratio mean-squared error to pure error obtained from the replicates at the design center. The significance of the *F* value depends on the

Table 1
Experimental design and results of the surface response methodology

Coded power (%)	Time (s)	Cycles	Decoded power (%)	Time (s)	Cycles	Fat recovery (%)
Full factorial design						
+1	+1	−1	80	60	8	24.60
+1	+1	−1	80	90	8	26.14
0	0	0	50	75	10	26.00
0	0	0	50	75	10	25.74
0	0	0	50	75	10	25.62
-1	+1	+1	20	90	12	25.31
-1	+1	−1	20	90	8	24.37
+1	−1	+1	80	60	12	26.07
-1	−1	+1	20	60	12	23.56
-1	−1	−1	20	60	8	22.51
+1	+1	+1	80	90	12	25.72
Star points						
0	0	−α	50	75	8	25.73
0	0	0	50	75	10	25.83
0	0	+α	50	75	12	26.28
+α	0	0	80	75	10	25.57
−α	0	0	20	75	10	24.28
0	0	0	50	75	10	26.15
0	+α	0	50	90	10	25.56
0	0	0	50	75	10	25.86
0	−α	0	50	60	10	25.15

number of degrees of freedom (d.f.) in the model, as shown in the *P*-value column (95% confidence level). Thus, the effects, <0.05 in this column, namely: irradiation power, irradiation time, number of cycles and the squared terms of the irradiation power and time, are significant. The ANOVA (Table 2a) indicates a significant value for the lack of fit's test which could be due to the existence of a normal behavior deviation produced when a first order polynomial is used to represent the response surface. Thus, it was mandatory to convert the first order polynomial into a second order one. This conversion was performed by the construction of a CCD, which involved the full factorial design and the juxtaposition of a face centered star design in which the two centers of the design coincided. The axial distance of the star design is ± 1 due to the impossibility of using an orthogonal or rotatable star because of the technical features of the microwave device (only 5% changes of the irradiation power were allowed). The results of the complete CCD are shown in Table 1 and the ANOVA in Table 2b.

Table 2
Analysis of the variance (ANOVA)

Effect	Sum of squares	DF	Mean square	*F*-ratio	*P*-value
a) Full factorial design[a]					
A: Power	5.746	1	5.746	152.28	0.0065
B: Time	2.880	1	2.880	76.33	0.0128
C: Cycles	1.155	1	1.155	30.61	0.0311
AB	0.732	1	0.732	19.40	0.0479
AC	0.110	1	0.110	2.93	0.2292
BC	0.500	1	0.500	13.25	0.0679
Lack of fit	2.585	2	1.292	34.26	0.0284
Pure error	0.075	2	0.038		
Total (corr.)	13.784	10			
b) Central composite design[b]					
A: Power	6.512	1	6.512	184.66	0.0000
B: Time	2.714	1	2.714	76.97	0.0003
C: Cycles	1.289	1	1.289	36.54	0.0018
AA	2.019	1	2.019	57.25	0.0006
AB	0.732	1	0.732	20.76	0.0061
AC	0.110	1	0.110	3.13	0.1370
BB	0.501	1	0.501	14.21	0.0130
BC	0.500	1	0.500	14.18	0.0131
CC	0.137	1	0.137	3.88	0.1058
Lack of fit	0.753	5	0.150	4.27	0.0686
Pure error	0.176	5	0.035		
Total (corr.)	18.093	15			

[a] $R^2 = 0.081$; R^2 (adj. for d.f.) $= 0.052$.
[b] $R^2 = 0.949$; R^2 (adj. for d.f.) $= 0.903$.

The second order polynomial equation obtained was as follows:

$$Y = 25.83 + 0.81 \times \text{Power} + 0.52 \times \text{Time} + 0.36 \times \text{Cycles} - 0.86 \times \text{Power}^2 - 0.30 \times \text{Power} \times \text{Time} - 0.12 \times \text{Power} \times \text{Cycles} - 0.43 \times \text{Time}^2 - 0.25 \times \text{Time} \times \text{Cycles} + 0.22 \times \text{Cycles}^2$$

The optimal values, 61.82% for power irradiation, 83 s for irradiation time and 9.25 cycles, were obtained by equalizing to zero the first derivatives of the polynomial,

solving the resulting equations system and de-codifying the results. These values were approximated to 60%, 85 s and 9 cycles and applied to other samples.

3.2. Reproducibility of the results obtained by both procedures

The optimal conditions were used in a reproducibility study involving seven replicates of the same sample (i.e. fried chicken nuggets unbattered fraction) in different days, and the results compared with those obtained using a conventional Soxhlet (8 h) procedure. The percent relative standard deviation using FMASE (1.69%) is similar to that provided by the conventional method (1.53%). This precision study was good enough to compare both extraction methods but it must be augmented just in case of a complete validation for routine purposes.

3.3. FMASE versus conventional methods

After the optimization study and using the optimum values found in it, the comparison study between FMASE and conventional methods was performed using the samples described under "Sampling" in Section 2.

3.3.1. Quantitative aspects

The fat content from the eight different samples under study, namely: prefried batter nuggets (PBN), prefried unbattered nuggets (PUN), fried batter nuggets (FBN), fried unbattered nuggets (FUN), prefried batter hake (PBH), prefried unbattered hake (PUH), fried batter hake (FBH) and fried unbattered hake (FUH), were extracted using both FMASE and Soxhlet extraction.

A two-tailed *t*-test was used to compare the means of related (paired) samples in order to evaluate if both methods yield similar results at the 95% confidence level. Table 3 shows the statistical parameters.

The null hypothesis was that both methods yield the same results or, in other words, that the observed differences between FMASE and conventional Soxhlet were not significant. H_0 is formulated as a two-tailed test required:

$$H_0 : \bar{d} = 0 \; H_1 : \bar{d} \neq 0$$

The result obtained was:

$$\bar{d} = \frac{\sum d_i}{n} = 0.512$$

$$s_d = \sqrt{\frac{\sum d_i^2 - \left[\left(\sum d_i\right)^2 / n\right]}{n-1}} = 0.892$$

$$t = \frac{\bar{d}}{S_d/(n)^{1/2}} = 1.623$$

The calculated *t*-value was compared with the theoretical value at $\alpha = 0.05$ and seven degrees of freedom, i.e. 2.262. As the calculated value (i.e. 1.623) is smaller than the theoretical value, H_0 is accepted. This means that at the chosen significance level, no differences between two methods were found.

3.3.2. Qualitative aspects

Chromatographic determination of the extracts was carried out in order to know the influence of the different extraction procedures on the composition of the lipids extract. Table 4 shows the results obtained for the polymers amount, total polar compounds and their distribution, and the fatty acid composition in lipid samples. Only the batter samples were analyzed by manual extraction methods due to the low fat content of the unbattered samples. As can be seen, the results obtained were similar in all cases. Thus, it is clear that the microwave irradiation does not affect the fat composition.

3.4. Comparison of samples and frying processes

Chicken nuggets samples were prefried and fried under commercial conditions. The main difference between prefried and fried samples was the palmitic acid content, which is the typical fatty acid present in

Table 3
Sattistical parameters for the two-tailed *t*-test

Sample	X_{FMASE}[a]	$X_{conventional\ Soxhlet}$[a]	$d_i = X_{FMASE} - X_{conventional\ Soxhlet}$
PBN	26.64	25.63	+1.01
PUN	8.85	8.04	+0.81
FBN	24.85	24.64	+0.21
FUN	26.91	26.04	+0.87
PBH	35.25	35.87	−0.62
PUH	8.69	9.53	−0.84
FBH	47.54	46.74	+0.8
FUH	12.38	10.52	+1.86

[a] Corresponding to the median of three values.

Table 4
Chromatographic results obtained from lipid extracts

(a) Polymer amount (%) and total polar compounds (%)

Sample	PC (%)	PTAG (%)	OxMTAG (%)	DAG (%)	FA (%)
Manual Extraction					
PBN	11.0	4.5	3.6	1.8	1.1
FBN	13.9	3.8	4.9	3.5	1.7
PBH	28.0	17.2	6.7	2.3	1.8
FBH	8.6	2.1	2.4	2.4	1.7
Conventional Soxhlet					
PBN	10.4	4.2	3.8	1.4	1.0
PUN	8.4	2.3	1.7	1.9	2.5
FBN	14.1	3.8	5.0	3.6	1.7
FUN	6.3	0.6	2.1	1.2	2.4
PBH	28.6	17.5	7.2	2.4	1.5
PUH	31.9	15.4	6.2	3.9	6.4
FBH	10.2	3.2	2.5	2.6	1.9
FUH	10.5	1.8	1.9	2.7	4.1
FMASE					
PBN	10.9	4.4	3.6	1.7	1.2
PUN	11.1	2.2	2.7	2.0	4.2
FBN	13.7	3.4	5.2	3.3	1.8
FUN	5.1	0.7	1.1	1.1	2.2
PBH	28.9	17.6	6.9	2.5	1.9
PUH	30.8	13.0	6.2	3.5	8.1
FBH	10.7	3.3	2.4	2.6	2.4
FUH	11.6	2.1	2.2	2.8	4.5

PC, Polar compounds; PTAG, Polymer triacylglycerols; oxMTAG, oxidised monomeric triacylglycerols; DAG, Diacylglycerols and FA, fatty acids.

(b) Major fatty acid composition (%)

Sample	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	Others
Manual extraction							
PBN	8.2		4.0	26.7	58.4	1.2	1.5
FBN	7.5	0.5	7.0	49.4	33.8		1.7
PBH	9.0	0.6	5.0	28.1	54.5		2.8
FBH	10.6	0.7	3.0	69.7	13.7	0.7	1.6
Conventional Soxhlet							
PBN	8.2		4.5	27.0	57.3	1.5	1.5
PUN	41.4	1.1	5.2	31.6	43.9	1.1	2.7
FBN	7.7	0.5	6.9	49.3	32.7	0.9	2.0
FUN	22.9	6.2	6.9	42.2	18.9	0.8	2.1
PBH	9.8	0.5	5.3	29.6	51.8		3.0
PUH	10.8	1.1	4.8	27.7	44.1		11.5
FBH	11.8	1.1	3.0	67.0	13.9	0.7	2.5
FUH	15.0	2.5	3.1	53.0	10.6	2.2	13.6
FMASE							
PBN	8.7		4.0	26.8	47.4	1.5	1.5
PUN	14.7	1.4	5.1	31.2	44.1	1.1	2.7
FBN	7.6	0.5	7.0	49.7	32.4	1.0	2.0
FUN	22.3	6.0	6.7	49.1	13.5		2.1
PBH	9.4	0.5	5.3	29.8	52.1		3.0
PUH	10.7	1.3	4.5	26.6	42.2		11.5
FBH	10.9	1.0	3.1	68.6	14.0	0.7	2.5
FUH	14.8	2.5	3.1	53.8	11.4	0.7	13.6

chicken meat. The normal amount is around 25%. The difference shown in samples, 14.7% for prefried and 22.3% for fried sample, are not attributable to the frying processes because palmitic acid is absent in the composition of frying oils. Thus, it must conclude that a product with the same composition seemingly, may have a different number of calories. Likewise, the differences in the quantity of polymers and polar compounds confirm this assertion.

Hake fingers samples were prefried under commercial conditions and the same hake was fried under domestic conditions using high quality olive oil. The results, on a dry basis expressed, show a high fat absorption in the batter during the final cooking (from prefried to fried). Since the results are expressed on a dry basis, so the lipid:dry matter ratio is significantly higher in the latter. In the case of the unbattered samples, the increase on the ratio is not significant.

One of the most important conclusions of this comparison is the exchange of the prefried samples fat during the frying process. The prefried hake was fried using sunflower oil which has an important content in linoleic acid, while the final frying process was carried out using high quality olive oil. As can be seen (Table 4b) the content of linoleic acid in prefried samples is higher than in fried samples, so the fat has been exchanged. The results obtained for polymers and polar compounds confirm this fact. In this way, the prefrying oil has a high content in polymers and polar compounds (higher than 25%, which is the limit accepted in most of the European Community countries). However, these quantities decrease drastically after the final frying process.

4. Conclusions

The use of a prototype consisting of a conventional Soxhlet extractor assisted by focused microwaves on the extraction chamber has been used in this study for leaching the oil content from different prefried and fried food samples. The results obtained (quantitative and qualitative) are in agreement with those of conventional methods.

The use of FMASE provides the following advantages:

1. Substantial shortening of the extraction time (from 8 h to less than 1 h).
2. Saving of extractant in such a way that only 20–25 ml are consumed per extraction.
3. Use of samples as received without the moisture adjustment usually required in conventional Soxhlet method.
4. Extraction efficiencies and reproducibility comparable with those provided by conventional Soxhlet extraction.

It is worth emphasizing that the most remarkable characteristic of FMASE is the use of a new technology for circumventing the drawbacks associated with a well-tested and useful old technique as conventional Soxhlet. Thus, FMASE has shown its potential to become one of the best alternatives for displacing conventional Soxhlet in routine uses.

Other conclusions from this research are related with:

1. The similar fat composition obtained by FMASE and the conventional methods based on both manual and conventional Soxhlet extraction.
2. The improvement of food quality when a food sample prefried under industrial and low quality oil conditions is fried with an appropriate high quality oil because of the fat exchange during the frying process.

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