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Assessment of the Levels of Degradation in Fat Co- and Byproducts for Feed Uses and Their Relationships with Some Lipid Composition Parameters

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This paper discusses the levels of degradation of some co- and byproducts of the food chain intended for feed uses. As the first part of a research project, "Feeding Fats Safety", financed by the sixth Framework Programme—EC, a total of 123 samples were collected from 10 European countries, corresponding to fat co- and byproducts such as animal fats, fish oils, acid oils from refining, recycled cooking oils, and other. Several composition and degradation parameters (moisture, acid value, diacylglycerols and monoacylglycerols, peroxides, secondary oxidation products, polymers of triacylglycerols, fatty acid composition, tocopherols, and tocotrienols) were evaluated. These findings led to the conclusion that some fat by- and coproducts, such as fish oils, lecithins, and acid oils, show poor, nonstandardized quality and that production processes need to be greatly improved. Conclusions are also put forward about the applicability and utility of each analytical parameter for characterization and quality control.

KEYWORDS: Fat co- and byproducts; levels of degradation; characterization; feed uses

INTRODUCTION

The use of fats as ingredients in feed formulation has generated increasing interest among feed producers, because fats provide the highest energy value. Thus, the percentage of fat included in animal feeds has been increasing in recent years to reach higher productive results (*1*). In addition, fats provide several essential nutrients, such as polyunsaturated fatty acids (*2*), and behave as a solvent for several important nutrients, such as liposoluble vitamins. However, the inclusion of fats in feed may lead to a number of side effects. First, they supply minor components, such as persistent pollutants, that may accumulate in meat and fat tissues and may have undesirable effects on animals and consumers. For this reason, maximum levels of contaminants in feeds and feedstuffs are regulated by the European Union (EU). Moreover, several lipid oxidation products may deposit or be formed in meat and other animal tissues, which may decrease their quality (*3*) and pose a health risk to the consumer (*4*). Finally, the lipid composition, stability, and nutritional quality of meat depend directly on the fat composition and quality of fat included in the feed (*5*). However, there is a lack of information about several aspects of these fats: strict composition characterization, nutritional quality for different animal production systems, ability to modify lipid composition of meat, effect on the level of oxidation in meat and in other tissues, and toxicological or physiological effects of some fat degradation compounds and of some undesirable contaminants in these fats. The quantification of these characteristics and effects is mandatory, in order to assess the safety of the use of these fats in animal production and to protect both consumer and animal. The production processes of some of these fat materials (animal fats) have recently become more strictly regulated by the EU. However, there is a lack of standardization for the rest, which means little attention is paid to the assessment of the presence of lipid alteration components in these fat materials. The repercussions of this presence are relevant, because feeds include usually between 2 and 10% of added fat as ingredient, depending on the animal species, reaching in the case of some fish species 35−40%. Only some of these fat by- and coproducts are forbidden or not intended now for feed use (recycled cooking oils and oils from exhausted bleaching earths). The rest are not clearly regulated, and particular attention should be paid to the control of quick oxidation in highly polyunsaturated fats and to persistent contaminant levels by health reasons. There is confusion in the market about the classification of these fats as feedstocks, allowing people to use all kinds of feedstock of unknown quality, low cost, and occasionally contaminated recycled fat materials. This lack of knowledge hinders the

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Table 1. References of Procedures Followed for the Analysis of Each Parameter

	method reference
sampling	EN ISO 5555: 2001, animal and vegetable fats and oils, sampling (*6*)
moisture	EN ISO 662: 1998, animal and vegetable oils and fats, determination of moisture and volatile matter content (*19*)
acid value	EN ISO 660: 2005, animal and vegetable oils and fats, determination of acid value and acidity (*20*)
mono- and diacylglycerols	UNI 22053:1996, biodiesel, determination of bonded glycerol: methyl esters, mono-, di-, and triglyceride contents, adapted for the different matrices (*21*)
fatty acid composition	ISO 5509: 2000, animal and vegetable oils and fats, preparation of methyl esters of fatty acids (*22*) ISO 5508: 1990, animal and vegetable oils and fats., analysis by gas chromatography of methyl esters of fatty acids (*23*)
tocopherols and tocotrienols	adapted from Hewavitharana et al., 2004 (*24*)
peroxide value	Official EEC method, regulation 2568/91, determination of peroxide value (*25*)
peroxide content	Navas et al., 2004 (*26*)
p-anisidine value	AOCS Official Method Cd 18-90, *p*-anisidine value (*27*)
TBA value (only in fish oils)	adapted from Grau et al., 2000 (*28*)
polymer content (>3%)	International Union of Pure and Applied Chemistry (IUPAC), 1992, standard method 2508 (*29*)
polymer content (<3%)	Munster, DGF standard methods [C-III 3d(00)] (*30*)

development and diffusion of special fatty materials and the correct use of several natural oil refining by- and coproducts and is also the main barrier to the development of legislation on this subject.

Our paper discusses the results of the first part of a project, "Feeding Fats Safety" (FFS), financed by the sixth Framework Programme-EC, dealing with the characterization of the quality and safety of the use as feedstuffs of some co- and byproducts of the food chain, such as animal fats and fish oils (recovered from animal tissues by hot or cold rendering); lecithins (insoluble oil recovered after the treatment of oils by hot water or steam); acid oils from chemical refining (lipid fraction recovered as byproduct of the chemical neutralization of oils and fats); acid oils from physical refining (last byproduct of the physical refining of oils and fats by steam/vacuum distillation); hydrogenated byproducts (obtained by hydrogenation of fatty acid fractions coming from oil refining processes); fatty acid calcium soaps (obtained by neutralization of fatty acid fraction or saponification of fatty acid or triacylglycerol fractions from palm oils); recycled cooking oils (byproduct obtained from oils discarded by industries and catering kitchens and collected by authorized companies); and oils from exhausted bleaching earths (lipid fraction recovered by solvent extraction from exhausted bleaching earths used in refining oils and fats). The authors try to establish the usual levels of degradation of several fat by- and coproducts of the food chain that may be useful feedstuffs, because the origin and quality of these fats are quite variable. These fat co- and byproducts are obtained through the application of a wide variety of technological unit operations. The control of their degradation characteristics needs to be directly related to the system of production. For instance, acid value (AV) is a characteristic value in acid oils, whereas in other fats it is a degradation marker. By analyzing the values of all these parameters in the different types of fat co- and byproducts, any sample can be properly classified. Moreover, the range and variability of the values found for each type of fat could lead to the detection of problems caused by specific technologies, which could then be modified to improve the levels of standardization and, thus, the fat's quality and safety for feed uses. Because these fats are not at present well standardized, acceptable levels of degradation are not easy to establish. The first aim of this paper, within the framework of the results of the entire FFS project, is to assess the usual levels of degradation in European feed fat samples, which are several fat co- and byproducts from the food chain, in order to reach conclusions about the quality and safety aspects of their use. No studies on this subject exist in Europe yet. A second aim of this paper is to define the most suitable and reliable analytical parameters to assess the degradation in these samples.

MATERIALS AND METHODS

Samples. The project was designed to study the main fat co- and byproducts of the food chain that are or could be used as feeding stuffs. We classified these fats in nine categories, such as animal fats (ANFA), fish oils (FISH), acid oils from chemical refining (AOCHE), acid oils from physical refining (AOPHY), lecithins (LECI), recycled cooking oils (RECY), hydrogenated byproduct (HYBY), oils from exhausted bleaching earths (EBE), and a miscellaneous group. Two of these categories, RECY and EBE, are now forbidden or not intended for feed uses. A total of 123 samples were collected from 10 European countries. The distribution between families ranged from 36 samples for ANFA to 2 for EBE. A sampling procedure extracted from ISO 5555 (*6*) and adapted for the scope of the FFS project was prepared and distributed to fat suppliers, together with a sampling form, especially designed to get the maximum of information about the source, the starting material, and the production technology. Moreover, to have the same contamination background, if contamination is present, we sent previously to each supplier the corresponding polyethylene high-density food containers with a capacity of 10 L. On receipt, samples were subdivided into 500 mL new dark glass containers, properly sealed and labeled, for distribution to the laboratories in charge of the analysis.

Analytical Methods. We chose to evaluate several degradation parameters, mainly those relating to lipid oxidation, which are shown in **Table 1**. Moreover, we also determined some complementary parameters relating to fat composition (moisture, mono- and diacylglycerols, fatty acid (FA) composition, tocopherols, and tocotrienols), because in some samples their values may be relevant to explaining the oxidation results. The analytical procedures followed to measure each parameter are also given in **Table 1**. For the three methods we adapted from certain literature references, we give detailed procedures as Supporting Information with this paper.

Statistics. All calculations were carried out using the SPSS software package (version 14.0). The correlations between the analytical parameters were assessed according to the number of samples. For categories with a number of samples $N > 30$, the Pearson correlation coefficient was calculated. When the categories had a number of samples between 10 and 30, normality was checked using the Kolmogorov−Smirnov test. For $5 < N < 10$, the Spearman correlation coefficient was determined. As a result, the Pearson correlation coefficient was used for the following categories: AOCHE, AOPHY, ANFA, and all types of combined fat; and the Spearman correlation was used for FISH and RECY categories. P values of <0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Oxidation Values. The oxidation parameters evaluated, such as peroxide value (PV), peroxide content, *p*-anisidine value (*p*-AnV), TBA value, and triacylglycerol polymer content, showed relevant differences and in general varied within a wide range of values (**Table 2**) according to the type of fat. The TBA value was only performed in FISH samples because it was the only

Table 2. Values Found for Parameters Related to Lipid Oxidation

	peroxide value (mequiv O_2/kg)	peroxide content (mmol CHP/kg)[c]	*p*-anisidine value	TBA value (μg MDA/kg)	polymers (%)
		Primary Production: Co- and Byproducts			
ANFA ($n = 36$)	0−6.8 (1.8; 2.2)[a]	0.2−4.1 (1.0; 1.2)	0.1−13.4 (3.2; 3.8)	NA[b]	0.1−1.1 (0.1; 0.2)
AOCHE ($n = 25$)	0−2.8 (0.3; 0.5)	0−2.1 (0.3; 0.5)	2.0−57.7 (14.7; 16.7)	NA	0−7.6 (0.4; 0.7)
AOPHY ($n = 16$)	0−2.4 (1.2; 1.2)	0−0.9 (0.2; 0.3)	13.4−62.6 (36.3; 35.0)	NA	0−0.3 (0.0; 0.0)
FISH ($n = 9$)	0−5.1 (1.2; 1.5)	0.3−2.4 (1.4; 1.4)	16−200.5 (57.8; 73.5)	528−6266 (757; 1851)	0.4−5.1 (2.0; 2.1)
LECI ($n = 8$)	0−0	NA	19.3−57.3 (44.6; 42.8)	NA	0−0.1 (0; 0)
		Secondary Production: Technical Lipids			
FACS ($n = 3$)	1.4−2.4 (2.3; 2.0)	NA	NA	NA	NA
HYBY ($n = 6$)	0.3−1.6 (0.8; 0.9)	0.1−0.4 (0.3; 0.2)	0.6−6.2 (3.4; 3.0)	NA	0−0.8 (0; 0.1)
		Waste Materials			
EBE ($n = 2$)	0−1.4 (0.7; 0.7)	0.3−0.4 (0.3; 0.3)	9.8−31.8 (20.8; 20.8)	NA	8.8−10.4 (9.6; 9.6)
RECY ($n = 8$)	0−12.2 (8.5; 7.6)	0.2−7.9 (2.5; 3.6)	36.9−69.0 (56.0; 55.0)	NA	5.0−11.3 (10.1; 9.2)

[a] Results expressed as min−max (median; average). [b] NA, not analyzed due to solubility problems. [c] CHP, cumene hydroperoxide equiv.

Table 3. Values Found for Tocol and Fatty Acid Composition

	total tocopherols (mg/kg)	total tocotrienols (mg/kg)	SFA (%)[a]	MUFA (%)[a]	n-3 PUFA (%)[a]	n-6 PUFA (%)[a]
			Primary Production: Co- and Byproducts			
ANFA ($n = 36$)	0.1−32.2 (3.4; 6.1)[b]	0−50.5 (0.8; 7.6)	27.1−59.7 (44.1; 41.8)	38.0−55.6 (45.7; 45.6)	0.3−3.0 (0.7; 1.1)	1.8−28.7 (8.9; 11.5)
AOCHE ($n = 25$)	0.6−1725.8 (271.4; 415.2)	0−411.8 (2.9; 32.1)	10.8−87.3 (19.0; 26.9)	10.2−72.3 (40.6; 42.8)	0−8.6 (1.1; 2.7)	2.5−63.0 (21.2; 27.6)
AOPHY ($n = 16$)	2.8−18735.7 (214; 2595.5)	0−4290.5 (74.6; 507)	11.7−94.1 (52.4; 43.8)	4.7−72.5 (36.8; 40.3)	0.0−6.7 (0.3; 0.8)	1.2−61.6 (9.1; 15.2)
FISH ($n = 9$)	15.6−88.2 (51.5; 50.6)	0−1.6 (0; 0.4)	24.6−34.6 (32.5; 30.6)	26.8−44.1 (33.4; 34.0)	22.2−33.3 (29.2; 28.7)	2.1 −6.5 (3.8; 3.9)
LECI ($n = 8$)	195.1−460.9 (376.2; 350.5)	0.6−20.5 (2.9; 6.0)	10.2−22.0 (20.8; 18.7)	13.8−55.3 (19.7; 23.7)	0.1−8.6 (5.4; 5.1)	26.5−66.0 (53.7; 52.5)
			Secondary Production: Technical Lipids			
FACS ($n = 3$)	15.2−237.5 (49.5; 100.7)	28.0−84.8 (84.2; 65.6)	55.0−63.9 (55.8; 58.2)	25.9−36.3 (35.4; 32.6)	0.2−0.3 (0.3; 0.3)	8.4−9.9 (8.5; 9.0)
HYBY ($n = 6$)	1.0−1299.1 (97.4; 295.3)	0−499.0 (140; 165.8)	60.2−96.5 (88.8; 85.1)	3.5−38.5 (11.2; 14.7)	0−0 (0; 0)	0−1.3 (0; 0.2)
			Waste Materials			
EBE ($n = 2$)	103.1−326.9 (215.0; 215.0)	2.4−9.2 (5.8; 5.8)	26.2−31.4 (28.8; 28.8)	41.5−42.2 (41.8; 41.8)	1.1−1.5 (1.3; 1.3)	25.4−30.8 (28.1; 28.1)
RECY ($n = 8$)	80.7−314.2 (160.3; 175.4)	3.7−16.7 (9.5; 10.2)	12.1−27.8 (19.6; 19.5)	34.0−50.7 (41.5; 40.9)	0.1−3.5 (0.8; 1.0)	20.7−53.8 (37.9; 38.7)

[a] Compensated area normalization. [b] Results expressed as min−max (median; average).

type of fat giving a significant formation of malondialdehyde, whereas the rest of the fats did not give enough response. These oxidation results indicate that good discrimination can be achieved, within each fat category, between good- and bad-quality fats. PV and peroxide content show, in general, low values, with a maximum for RECY (PV 12.2), clearly higher than the rest of the fat materials. In contrast, secondary oxidation values (*p*-anisidine and percent polymers) showed high variability among the different fats (**Table 2**). Thus, ANFA showed the lowest *p*-AnV values (maximum 13.4), whereas AOPHY (maximum 62.6), RECY (maximum 69), LECI (maximum 57.3), and particularly FISH (maximum 200.5) showed much higher levels. Percent polymers showed high values only for RECY and EBE (maximum values over 10%), whereas for the rest only AOCHE and FISH showed some samples reaching 5−7%, although the variability of content is very high. In contrast, the rest of the fats always had very low percent polymers. This is due to the higher temperatures involved in the processes for obtaining EBE and RECY and, in the case of FISH, also to the polymerization occurring during storage and transportation. This corroborates other studies on oils' and fats' oxidation levels reporting that secondary oxidation values increase according to several factors that we have discussed above. Fatty acid composition is clearly the most relevant factor. Thus, the most saturated fats (animal, hydrogenated) show the lowest oxidation values, whereas the most unsaturated (fish oils) show the highest ones. However, some types of coproducts of similar origins and with similar fatty acid composition (i.e., AOPHY vs AOCHE) show differences in oxidation. This suggests that temperature and other conditions in the respective ways they were obtained must be taken into account to explain the differences observed. In some fats from animal or mixed origin, the TBA value is a more reliable parameter than *p*-anisidine in assessment of moderate levels of oxidation. In our case, we tried to apply this methodology to several fats, but found enough sensitivity only in fish oil evaluation (**Table 2**). The polymer percentage could be a parameter to complement the assessment of secondary oxidation. Values found for polymers (**Table 2**) were maximum for EBE and RECY and intermediate for FISH, with their contents for the remaining samples always $\leq 1\%$.

Tocol and Fatty Acid Composition. Tocopherol and tocotrienol contents showed high relevance as control parameters because they possess antioxidant properties. As a consequence, they prevent oxidation in fats and are also transferred to the animal, increasing the oxidative stability of meat and fatty tissues (*7*, *8*). Our results (**Table 3**) show a very interesting distribution of tocopherols among the analyzed fat co- and byproducts. Lecithins (mean 350.5 mg/kg of fat), AOCHE (mean 415.2), and particularly AOPHY (mean 2595.5) showed the highest tocopherol contents, whereas ANFA (mean 6.1) and FISH (mean 50.6) had very low values. The highest values of tocotrienols were obviously found in AOPHY (mean 507 mg/kg of fat) and HYBY (mean 165.8), because palm oil was the predominant raw material of these byproducts. These results indicate that, despite aggressive temperature conditions, certain fat by- and coproducts recover a higher concentration of tocols, or at least maintain concentrations similar to those of raw fat materials. This gives added value to their use in feeds. For all these reasons, as well as their relevance to lipid oxidation prevention, tocopherol and tocotrienol are very significant as composition parameters and can be used for the analytical characterization of these fats. FA composition (**Table 3**) is one of the most significant parameters for the identification and characterization of a fat material. In these fat byproducts, it

Table 4. Values Found for Moisture and Triacylglycerol Hydrolytic Compounds

	moisture (%)	acid value (mg of KOH/g)	monoglycerides (%)	diglycerides (%)
		Primary Production: Co- and Byproducts		
ANFA ($n = 36$)	0−0.70 (0.08; 0.14)[a]	0−47.5 (6.6; 8.7)	0−1.4 (0; 0.12)	0−13.5 (2.1; 2.7)
AOCHE ($n = 25$)	0.34−4.53 (1.30; 1.56)	80.6−191.0 (111.9; 120.7)	0.4−4.8 (1.6; 1.7)	0−13.1 (5.9; 5.9)
AOPHY ($n = 16$)	0.17−6.10 (0.36; 0.93)	103.1−201.2 (160.1; 155.2)	0−9.0 (1.0; 1.4)	0.5−17.2 (2.9; 4.0)
FISH ($n = 9$)	0−3.00 (0.14; 0.50)	0.5−50.7 (8.6; 13.0)	0−0.2 (0.0; 0.0)	0−3.6 (1.0; 1.1)
LECI ($n = 8$)	0.23−28.90 (1.48; 5.22)	16.2−145.3 (35.25; 52.8)	NA[b]	NA
		Secondary Production: Technical Lipids		
FACS ($n = 3$)	1.80−3.84 (3.10; 2.91)	NA	NA	NA
HYBY ($n = 6$)	0.09−0.40 (0.29; 0.25)	3.0−206.5 (171.1; 149.4)	0−1.3 (0.5; 0.5)	0−3.2 (1.6; 1.6)
		Waste Materials		
EBE ($n = 2$)	0.56−0.57 (0.57; 0.57)	39.2−65.3 (52.3; 52.3)	0.4−1.2 (0.8; 0.8)	8.2 −9.4 (8.8; 8.8)
RECI ($n = 8$)	0.10−1.17 (0.31; 0.39)	1.3−19.3 (5.5; 9.3)	0−0.7 (0; 0.1)	1.3−7.8 (4.1; 4.3)

[a] Results expressed as min—max (median; average). [b] NA, samples not analyzed.

demonstrated some limitations because of the variety of origins that are found in each type of fat and particularly because of the possibility of finding fat blends (in the case of animal fats, recycled cooking oils, etc). However, FA composition determination also gives nutritional information that is relevant for the animal and for humans as final meat consumers. Our results show that this composition is quite variable within all fat categories, even in those that can be quite homogeneous such as FISH. Specific statistical analysis of FA composition results and spectroscopic techniques are used more and more for fat identification and classification (*9*). Thus, on its own or combined with other composition and classification parameters, this is a good routine control parameter. However, poor standardization and the presence of mixed fat samples in the market can sometimes make it difficult to interpret FA composition results in these fat by- and coproducts.

Moisture, Acid Value, and Mono- (MG) and Diacylglycerols (DG). We detected a low level of standardization for moisture content in several fat categories analyzed. For this reason (and for overall quality, of course), this must be a mandatory control parameter in all cases. Percent moisture in these fats (**Table 4**) showed high values only for some categories, such as AOCHE, LECI, and FACS. These are fats showing a high proportion of free fatty acids (**Table 4**) or soaps, in which the conditions of processing and particularly the high proportion of water or steam used in obtaining the fats can lead to higher recovery of moisture in the final product. For the rest, mean moisture values were always ≤1%. AV, % MG, and % DG are also valuable parameters to control hydrolytic processes in fats due to their relationship with production technology and, in some cases, with their origin (palm oil products). In pure fats, AV is considered more as a quality or degradation parameter, but in some fat byproducts (i.e., acid oils) free fatty acids are major constituents. Thus, acid oils are the recovered fraction from neutralized oils and mainly consist of free fatty acids. AOPHY showed the highest AV levels (mean 155.2) because they are separated by distillation, whereas AOCHE maintained higher proportions of triacylglycerols and other components of the initial oil and showed lower AV (mean 120.7). HYBY showed the highest acidity values because they were obtained by hydrogenation of palm fatty acid distillates. For the remaining categories, AV should be much lower because it indicates a lack of quality or a low level of technological attention during processing. However, LECI showed quite high acid values (maximum 145.3 and mean 52.8), whereas ANFA and FISH showed the lowest. One of the most interesting general conclusions is that there is a lack of AV homogeneity in each type of fat, suggesting that low levels of standardization exist in the technology process and in quality control of these fat co- and byproducts. For the same reason, MG and DG contents can also be regarded as complementary parameters. Of the other two hydrolytic parameters, MG values were slightly lower than DG values, and we found that their values are not strictly related to AV in certain cases. For instance, AOCHE and AOPHY showed high mean values of DG, but EBE and RECY showed the highest values, although their corresponding AV are quite a bit lower than acid oils'. This is because EBE and RECY are fats from waste materials, of very mixed origin and, particularly in RECY's case, suffer very complex processes. As a consequence, further studies are needed to confirm the relevance of AV/MG/DG as characterization parameters. These studies must always take into account the technological processes applied to each fat source.

Correlations between Parameters. First we discuss the correlations found between the values of all the analytical parameters applied to the general pool of fat samples, taking all categories together. **Table 5** shows a summary of all the significant correlations found ($p \leq 0.05$). There is a strong, direct correlation between all of the oxidation parameters, peroxide value (PV), peroxide content (FOX), percent polymers, and *p*-anisidine value (*p*-AnV). The TBA value (when it was performed) also correlates well with the *p*-AnV value and percent polymers. Only percent polymers and *p*-AnV correlate directly with n-3 PUFA and inversely with SFA, indicating that the highest values of secondary oxidation compounds were found in the most polyunsaturated fats (i.e., fish oils). Other authors had reported similar findings. Thus, Tompkins and Perkins (*10*), working on frying with different soybean and hydrogenated soybean oils, with linolenic and linoleic acid increasing percentages, found that percent linolenic and percent linoleic acids correlated clearly with *p*-AnV and percent polymers (*r* values around 0.73). However, we have to take into account that *p*-AnV varies highly according to the heat treatment of the fat and other conditions such as oxygen contact, catalysts, and antioxidants present in the fat matrix (*11*). For this reason, it is very relevant that we found these strong correlations of *p*-AnV and percent polymers with fatty acid composition parameters, because it indicates that *p*-AnV is a good control parameter in various fat co- and byproducts with different origins and ways of being obtained. Oxidation parameters analyzed are quite simple and rapid, with good applicability, except the TBA value, and a high level of discrimination. In primary oxidation, the levels of peroxides were always low in all types of fats evaluated, which seems to reveal the low significance of PV as a quality control parameter. We assayed in parallel the determination of peroxide content by the colorimetric FOX method, which gave more reliable peroxide value results. As can be seen in the correlations we

Table 5. Correlation between Analytical Parameters Considering All Types of Fat[a]

parameters correlated		r	P	N	parameters correlated		r	P	N
moisture	MG	0.252	0.011	100	*p*-AnV	% polymers	0.416	<0.001	111
AV	MG	0.417	<0.001	112	*p*-AnV	SFA	−0.253	0.007	111
AV	PV	−0.340	<0.001	120	*p*-AnV	n-3 PUFA	0.563	<0.001	111
AV	FOX	−0.395	<0.001	109	TBA	*p*-AnV	0.733	0.025	9
AV	% polymers	−0.253	0.005	120	TBA	% polymers	0.817	0.007	9
MG	DG	0.699	<0.001	112	% polymers	SFA	−0.242	0.008	120
MG	PV	−0.246	0.009	112	% polymers	n-6 PUFA	0.191	0.036	120
MG	FOX	−0.292	0.002	109	total T	n-6 PUFA	0.195	0.03	123
DG	FOX	−0.213	0.026	109	total T3	SFA	0.192	0.033	123
DG	% polymers	0.201	0.034	112	SFA	MUFA	−0.463	<0.001	123
PV	FOX	0.747	<0.001	109	SFA	n-3 PUFA	−0.223	0.013	123
PV	% polymers	0.548	<0.001	120	SFA	n-6 PUFA	−0.638	<0.001	123
FOX	*p*-AnV	0.205	0.037	103	MUFA	n-6 PUFA	−0.283	0.002	123
FOX	% polymers	0.426	<0.001	109					

[a] AV, acid value; MG, % monoglycerides; DG, % diglycerides; total T, total tocopherol content; total T3, total tocotrienol content; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; n-3 PUFA, n-3 polyunsaturated fatty acids; n-6 PUFA, n-6 polyunsaturated fatty acids; PV, peroxide value; FOX, peroxide content measured by the ferrous oxidation−xylenol orange method; p-AnV, *p*-anisidine value; TBA, thiobarbituric acid method.

gave above, FOX results correlated better with the rest of the oxidation parameters than PV. As mentioned above, most fat co- and byproducts have been submitted to certain processes inducing higher levels of oxidation, and we can find low levels of PV in fat materials that have undergone strong oxidative processes. For this reason, any control program should complement PV with the determination of some secondary oxidation products index. Of all the oxidation parameters evaluated in fat co- and byproducts, *p*-AnV can be recommended for secondary oxidation control, taking into account all of the composition and technological factors affecting their values (*11, 12*). The TBA value showed only a good application for fish oils, not being sensitive enough for the rest, including animal fats. Moreover, *p*-AnV is a very simple and rapid colorimetric determination. However, some fat co- and byproducts (i.e., RECY, AOCHE, FISH) are obtained by using high-energy conditions that lead to high levels of polymerization. In these cases, the percent polymer should be recommended as a complementary control parameter, because its measurement is more robust than other secondary oxidation products and gives additional information, and because it correlates well with *p*-AnV and TBA and also supplies more information about all degradation characteristics. Moreover, polymers are relevant because they are poorly digested by animal and could have undesirable biological effects according to their level. Several combinations of these parameters could be recommended for routine control analysis, according to the type of fats and the type of control program (control of feedstocks, control of processes, etc).

For the composition parameters, certain direct and inverse correlations are found between all of the fatty acid classes (SFA, MUFA, n-6 PUFA, and n-3 PUFA). Thus, SFA correlates inversely with the rest of the FA classes, whereas MUFA correlates inversely with SFA and n-6 PUFA. n-3 PUFA correlates inversely only with SFA. The correlations found between FA and oxidation parameters indicate that the oxidative stability of fats is clearly dependent on their PUFA composition. FA composition is always a recommended determination because, in one analytical operation, we may have the values of major FA classes (% SFA, % MUFA, % n-6 PUFA, and % n-3 PUFA), but also quantify certain individual fatty acids that characterize some types of fat or give a clue to the origin of the fat materials from which the co- or byproduct derived (lauric and miristic acids, C18:1 trans, EPA, and DHA, etc). Total tocopherols correlates positively with n-6 PUFA, which can be explained by unsaturated fats having a seed oil origin and being high in these minor components. In contrast, total tocotrienols correlate directly with SFA, which is because palm byproducts are particularly rich in tocotrienols, and they are also more saturated than other plant oils. For the correlations found for moisture, acidity, and MG and DG values, there are some interesting comments. Moisture correlates directly with % MG. As mentioned above, this suggests a certain relationship between remaining water in fats and the hydrolytic reactions occurring while fats are being obtained. Percent MG also correlates directly with the other hydrolytic parameters and inversely with oxidation parameters. First, we observed direct correlations between MG and AV and between MG and DG, which seems obvious because they are products of the same reaction. However, AV does not correlate with DG, which can be explained by the fact that a certain number of samples analyzed corresponded, totally or partially, to palm oil co- and byproducts. Palm oil is characterized by an unusually high percentage of DG in fresh oil and, for this reason, in their co- and byproducts the % DG found did not correspond only to hydrolytic reactions occurring while they were being obtained, handled, and stored. Similar results were reported by other authors (*13, 14*), who found lack of correlation between the AV and % DG in fresh palm oil and fractions and in recycled frying oils containing palm oils. Moreover, our results show a particular pattern of correlation between acidity and oxidation values. Thus, AV and MG both correlate inversely with peroxide value (PV) and peroxide content (FOX), and DG also correlates inversely with FOX, although in all cases the correlation values are low (*r* values between 0.4 and 0.2). However, these inverse correlations can be explained by the fact that values of peroxides found in almost all samples analyzed were low, due to the high temperatures involved in the processes of obtaining fat co- and byproducts, which in parallel induce triacylglycerol hydrolysis. In contrast, the pattern for secondary oxidation was unclear, because AV correlates inversely with percent polymers, whereas DG correlates directly with the level of polymerization. However, although they are significant, the level of these correlations is quite low (*r* value around 0.2) and they disappear when we analyze each type of fat in separate pools. We should take into account that secondary oxidation parameters showed quite variable values among the different fat coproducts. Several authors have reported similar patterns of behavior for hydrolysis and oxidation processes, working on commercial discontinuous frying and heating (*15, 16*) or commercial continuous frying (*14*), with oils having different levels of unsaturation. A common observation in these studies is that in all types of heating

processes and under various conditions, hydrolysis does not correlate well with the oxidation process in highly polyunsaturated oils. Moreover, a clear tendency exists to find lower % DG values in frying oils when % PUFA increases. This correlation pattern observed in the whole pool of samples leads to conclusions about a general approach to the analytical control of fat co- and byproducts. Obviously, because there are very different kinds of fat, if we analyze the values and correlations between parameters within each fat group, the general pattern will change according to the particular composition and degradation characteristics of each type of fat. This is why we discuss below the main correlations found in the individual fat groups, avoiding comments about those groups when the sample number was too small for statistical significance to be reached.

ANFA Samples. Animal fat samples ($n = 36$) followed in most cases the same pattern of correlations between parameters as the general samples. However, there are some interesting differences. For instance, two new correlations appear between moisture and hydrolytic parameters: moisture/AV ($r = 0.611$, $p < 0.001$) and moisture/DG ($r = 0.502$, $p = 0.003$). Moisture also correlates directly with n-6 PUFA ($r = 0.504$, $p = 0.003$) and n-3 PUFA ($r = 0.461$, $p = 0.007$) and inversely with SFA ($r = -0.437$, $p = 0.011$), correlations which suggest that the most unsaturated animal fats (i.e., poultry) tend to include more left-over water. Strong direct correlations were found between all hydrolytic parameters (AV, MG, and DG), always with a significance $p < 0.001$, AV/MG $r = 0.699$, AV/DG $r = 0.740$, and MG/DG $r = 0.848$. Unlike the negative correlations found between hydrolytic and peroxide values in the general samples, for ANFA we found a strong negative correlation only between DG and PV ($r = -0.480$, $p = 0.003$) and between DG and peroxide content ($r = -0.484$, $p = 0.004$). Regarding oxidation values, there are fewer parameters correlated in ANFA samples than in the general sample. Thus, only PV to peroxide content ($r = 0.832$, $p < 0.001$) and *p*-AnV to percent polymers ($r = 0.428$, $p = 0.026$) correlate directly. Moreover, PV correlates inversely with tocopherol content ($r = -0.472$, $p = 0.004$) and tocotrienol content ($r = -0.435$, $p = 0.008$), and FOX also correlates inversely with tocopherol content ($r = -0.340$, $p = 0.049$). For composition parameters, we found direct correlation of tocopherol content with n-6 ($r = 0.641$, $p < 0.001$) and n-3 PUFA ($r = 0.529$, $p = 0.001$) and tocotrienol with n-6 ($r = 0.524$, $p = 0.001$) and n-3 PUFA ($r = 0.450$, $p = 0.005$). Inverse correlations of tocopherol with SFA ($r = -0.620$, $p < 0.001$) and tocotrienol with SFA ($r = -0.509$, $p = 0.001$) were also observed. These correlations between tocols and several FA classes are explained by the fact that ruminant fats (more saturated) are poorer in tocopherol than other animal fats such as chicken fat, which is at the same time more polyunsaturated.

FISH Samples. Fish oil samples ($n = 9$), unlike the whole sample ensemble and animal fats, showed no correlation for moisture, AV, MG, or DG. The only relevant correlation is that between MG and TBA values ($r = 0.730$, $p = 0.025$). A similar correlation is only observed in another type of fat (AOCHE), in which MG and DG correlate well with secondary oxidation values. The explanation may be that the two types of fat are the ones with the highest values of oxidation. For oxidation values, PV correlates directly with FOX ($r = 0.823$, $p = 0.006$). It should be noted that n-6 PUFA correlates inversely with both primary oxidation parameters, PV ($r = -0.681$, $p = 0.044$) and FOX ($r = -0.837$, $p = 0.005$), correlation that is not seen in the general fat sample. This may be because n-6 PUFA are in a minority in fish oils and the high level of oxidation reduces them significantly. All secondary oxidation parameters correlate well with each other. Thus, *p*-AnV correlates directly with TBA value ($r = 0.733$, $p = 0.025$) and with percent polymers ($r = 0.833$, $p = 0.005$), and TBA value also correlates directly with percent polymers ($r = 0.817$, $p = 0.007$). Correlations between FA classes are very interesting; we observed a direct and strong correlation between SFA and n-3 PUFA ($r = 0.733$, $p = 0.025$), which is explained by the fact that fish oils are the only natural oils particularly rich in both long-chain SFA and n-3 PUFA, suggesting higher elongase activity shared for both FA categories. Moreover, MUFA percentage correlates inversely with both SFA ($r = -0.950$, $p < 0.001$) and n-3 PUFA ($r = -0.850$, $p = 0.004$).

AOPHY Samples. Correlations found in AOPHY samples ($n = 16$) must be analyzed by taking into account the particular composition of this fat coproduct, which consists of a high proportion of free fatty acids and unsaponifiable components and which is obtained by means of a distillation process. For this reason, AV, MG, and DG do not correlate with any other parameter. Obviously, MG correlates well and directly with DG ($r = 0.884$, $p < 0.001$), because both are present in high amounts. AOPHY samples, because of their particular nature and the way they are obtained, have a particular oxidation profile. They show high *p*-anisidine values (see **Table 2**), but low levels of polymers, in comparison with other types of fats also high in secondary oxidation products (FISH and RECY). This lack of correlation in AOPHY between polymers and *p*-AnV, which we found only in FISH and RECY samples, can be explained by the fact that oxidation processes are effectively catalyzed between 20 and 140 °C and polymerization between 120 and 200 °C (*17*). Moreover, the correlation found in the case of FISH could be also due to the fact that polymerization occurs during storage and, the higher secondary oxidation, the higher polymer formation. Thus, we only found a certain level of polymers in fat co- and byproducts that undergo the highest processing temperatures, but treatments using temperatures around 100 °C induce secondary oxidation but much less polymer formation. However, some correlations were found between some oxidation parameters and some FA classes. Thus, FOX correlates directly with n-6 PUFA ($r = 0.615$, $p = 0.011$) and *p*-AnV with MUFA ($r = 0.590$, $p = 0.021$). The correlation between peroxide (FOX) and n-6 PUFA contents seems obvious, as it reflects the higher susceptibility to oxidation of most polyunsaturated fats, but the correlation between MUFA and *p*-AnV is difficult to understand, because the formation of secondary oxidation products involved in the *p*-anisidine reaction is higher for polyunsaturated fats than for monounsaturated ones (*11*).

Similar to the global fat pool, an inverse correlation was found between SFA and MUFA ($r = -0.752$, $p = 0.001$) and between SFA and n-6 PUFA ($r = -0.583$, $p = 0.018$). Direct correlations were also observed between tocopherols and n-3 PUFA ($r = 0.813$, $p < 0.001$) and between tocopherols and n-6 PUFA ($r = 0.550$, $p = 0.027$), as we observed for the general ensemble of fats. Among AOPHY, samples of seed oil origin showed together higher values of PUFA and tocopherols, whereas those from olive or palm oils had lower PUFA and tocopherol values.

AOCHE Samples. AOCHE samples ($n = 25$) had fewer parameters that correlated than AOPHY ones did. Like AOPHY, AOCHE showed no correlations between AV, MG, and DG, because free fatty acid content is very high in the two types of fat. Only DG, strongly and very significantly, correlates with MG ($r = 0.748$, $p < 0.001$). Another similarity between

AOCHE and AOPHY is the absence of correlations between the oxidation parameters. Direct correlation was found only between p-AnV and MG ($r = 0.544$, $p = 0.007$). As for AOPHY samples, direct correlation was observed in AOCHE between total tocopherol content and n-6 PUFA ($r = 0.498$, $p = 0.011$). Finally, MUFA correlates inversely with n-6 PUFA ($r = -0.542$, $p = 0.005$).

RECY Samples. In recycled cooking oils ($n = 8$), direct correlation was found between AV and % MG ($r = 0.768$, $p = 0.026$). Inverse correlations were also found between peroxide value and AV ($r = -0.790$, $p = 0.020$) and between peroxide value and % MG ($r = -0.764$, $p = 0.027$). There was direct correlation between *p*-AnV and percent polymer ($r = 0.833$, $p = 0.010$). This correlation is quite strong, and we had already found the same direct, strong correlation in FISH samples. Both types of fats are those showing the lowest AVs, almost the highest *p*-AnV, and the highest percent polymers. Other authors reported similar correlations between *p*-AnV and percent polymers (*18*) or between *p*-AnV and polar compounds (*15*). These authors worked in heated oils and recycled frying oils that were obtained under different conditions and that had different acid values. They concluded that percent polymers (or total polar compounds) increase in parallel to *p*-AnV, except when low acid values are found in the used oil. Moreover, because conditions for obtaining RECY involve high-temperature processes, a critical factor for polymer formation (*17*), other co- and byproducts analyzed showed high *p*-AnV but low percent polymer. Finally, a direct correlation exists in RECY between tocopherol content and n-6 PUFA ($r = 0.786$, $p = 0.021$). There was also an inverse and very significant correlation between tocopherol and the rest of the FA classes: with SFA ($r = -0.881$, $p = 0.004$), with MUFA ($r = -0.857$; $p = 0.007$), and with n-3 PUFA ($r = -0.755$, $p = 0.031$). These correlations between tocopherol contents and the different classes can be explained by the origin and type of the mixture of fats in the recycled oil. We have already commented that seed oils are particularly rich in n-6 PUFA and have the highest contents of tocopherols. Olive oils, rich in MUFA, have lower tocopherol content, and tropical oils and animal fats are more saturated and poorer in tocopherols than seed and olive oils. The strong negative correlation between n-3 PUFA and tocopherol content could be explained by the very low content of tocopherol found in fish oils, which are the main contributors to n-3 PUFA content in recycled cooking oils.

Global Comment. Our results taken together led us to conclude that some fat by- and coproducts, such as fish oils, lecithins, and acid oils, show bad and nonstandardized quality. Therefore, production, storage, and handling conditions need to be improved and better controlled to prevent degradation. EBE and recycled cooking oils showed the lowest quality, but they are at present not allowed or intended for feed uses. Acid oils, particularly those from chemical refining, seem to need optimization in the technological conditions of their production, but they seem to be quite valuable fats for feeding uses because of their wide variety of fatty acid composition and their high tocopherol content. Animal fats seem to be the best quality products at this moment.

ABBREVIATIONS USED

ANFA, animal fats; AOCHE, acid oils from chemical refining; AOPHY, acid oils from physical refining; FISH, fish oils; LECI, lecithins; RECY, recycled cooking oils; HYBY, hydrogenated byproduct; EBE, oils from exhausted bleaching earths; FACS, fatty acid calcium soaps; FFS, "Feeding Fats Safety" project; AV, acid value; PV, peroxide value; TBA, thiobarbituric acid value; MG, monoacylglycerols; DG, diacylglycerols; FA, fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FOX, ferrous oxidation−xylenol orange method.

NOTE ADDED AFTER ASAP PUBLICATION

After this paper was published ASAP February 9, 2009, changes were made to the paragraph titled "RECY Samples"; the corrected version was reposted February 13, 2009.

Supporting Information Available: Detailed description of the analytical procedures adapted from several methods cited in the literature; these methods are mono- and diacylglycerols, tocopherols and tocotrienols, and TBA value determination. This material is available free of charge via the Internet at http://pubs.acs.org.

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