Fat Content of Oilseeds Used as Foods Is Dependent on the Method of Determination

Sir:

In earlier work from the Grain Research Laboratory (GRL), methods commonly used for determination of total fat in foods, including the Bligh and Dyer extraction method (1) and the AOAC acid hydrolysis–GC method (2), did not give accurate results when applied to whole oilseeds, in particular to flaxseed and mustard seed (3–5). In general, the cited methods gave lower results for these oilseeds when compared with an AOCS method that requires exhaustive extraction of neutral lipids with hexane accompanied by regrinding (6). Recently, at the request of the USDA, we compared the AOCS method with the method used by some commercial laboratories providing data for the USDA National Nutrient Database for Standard Reference (7) and found similar results (Table 1). In this case, the AOAC method used by the commercial laboratory to determine oil content was the gravimetric method AOAC Official Method 933.05 Fat in Cheese (8) in which samples are hydrolyzed first with ammonia and then with hydrochloric acid before being triple-extracted in a Mojonnier-style flask with ethyl ether and ethyl ether/alcohol.

The samples used in this study were provided by the USDA Nutrient Data Laboratory (Beltsville, MD) as part of the samples being studied for upgrading the USDA National Nutrient Database for Standard Reference (7). Fat content, moisture content, nitrogen content, and FA composition were determined both by commercial laboratories and the GRL. Commercial laboratories used the AOAC or AOCS (for FA composition of flax) method, as shown in Table 1. Several of these methods were not developed for use on oilseeds, and the fat and nitrogen methods were developed particularly for use on dairy products only. The GRL used the AOCS or ISO methods specifically developed for oilseeds, as shown in Table 1.

As expected from previous studies (9,10), the nitrogen contents as determined by the Dumas combustion method were slightly higher than the nitrogen contents as determined by the Kjeldahl digestion method. For three of the four samples, results from the forced-air oven moisture determination (11) were lower than results from the AOAC vacuum oven method (12). Since the amount of sample available was limited, the moisture contents by the GRL were conducted on the ground samples used for oil content determination. It is possible that, especially in the case of sesame seeds, moisture was lost in the grinding process. It is also notable that the AOAC method used for moisture content is actually specific for the determination of solids in canned vegetables. It does, however, provide a mechanism for removing moisture from samples with minimal possible impact of heat.

The greatest differences in the results were for fat contents: In three of the four samples, the exhaustive regrind extraction method employed by the GRL found significantly more fat content than the AOAC hydrolysis Mojonnier extraction method. It is probable that the main reason for the difference was the lack of clear grinding instructions for the AOAC method (which was, after all, designed for cheese). Our previous work (3–5) has shown that it is extremely important to reduce the particle size of oilseeds to less than 100 μm to ensure complete extraction of the neutral lipids. This cannot be achieved in a single step using grinders such as a simple coffee mill, and the AOCS method calls for grinding in a wet ball mill. It is likely that the omission of a regrinding step is the main reason for the differences noted for the flax and sesame samples. The ground flax sample did not have particle sizes sufficiently reduced to allow complete extraction. The ground mustard sample, on the other hand, had a very fine particle size and it is likely that both the methods resulted in a complete extraction of the neutral lipids. It is possible that the mustard results from the AOAC method were slightly higher because the method used would result in the release of the mustard oil from the glucosinolates in the mustard. This would add slightly to the total oil found. Also, the difference between the results from the two methods was not more than might be expected based on reproducibility data from the methods used (6,8).

The lower results for fat content translate to lower results for FA contents as well. In addition, it appears that the commercial laboratory misidentified some of the long-chain FA in the mustard sample. The misidentification of erucic acid, an important and defining FA in mustard. is most serious, but most analysts should also be aware that C20:4 is not found in lipids from many plant species. Perhaps providing known samples to the technicians carrying out the analyses might minimize this problem.

Use of inappropriate methods to determine factors such as fat content may have negative consequences if the results are used in defining diets for nutritional studies. For example, if one is designing a dietary study on n-3 FA in flax and underestimates the fat by 5%, the calculated caloric content of the diet will be in error as will the true linolenic acid content. Results from such a study would be of questionable value. In addition, there may be commercial consequences if contracts are based on the content of lipids or specific FA, as may be the case for flax. In any case, this example should show that it is important that analysts (i) use methods that are suitable and have been proven for the matrix on which they are used and (ii) develop a

Paper no. J11113 in *JAOCS 82*, 767–769 (October 2005).

TABLE 1
Results for Nitrogen, Fat, Moisture, and FA Content of Different Oilseeds by Different Analytical Methods

Samples (as is)	Nitrogen (%)[a]	Fat (%)[b]	Moisture (%)[c]	FA (g/100 g)[d]																	
				C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C20:3	C20:4	C22:0	C22:1	C22:2	C24:0	C24:1	Others	Total FA
Ground flaxseed																					
GRL	3.6	41.1	7.8	ND	1.9	Tr.	1.1	7.3	5.3	23.1	Tr.	0.1	ND	ND	ND	Tr.	ND	ND	Tr.	0.1	0.3	39.1
Commercial laboratory	3.5	35.7	8.8	ND	1.5	ND	0.9	5.5	4.1	17.1	Tr.	0.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	29.1
Whole flaxseed																					
GRL	4.1	36.8	8.7	ND	1.7	Tr.	1.3	7.8	5.2	18.7	Tr.	0.1	ND	ND	ND	Tr.	ND	ND	Tr.	0.1	0.2	35.0
Commercial laboratory	4.0	28.9	9.1	ND	1.3	ND	1.0	5.7	3.8	13.3	Tr.	0.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	25.1
Sesame seeds, hulled																					
GRL	3.9	61.2	3.8	ND	5.3	0.1	3.3	23.6	25.2	0.3	0.4	0.1	ND	ND	ND	0.1	0.1	ND	0.1	Tr.	0.1	58.5
Commercial laboratory	3.8	53.4	6.3	Tr.	5.2	0.1	2.6	19.6	23.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	50.7
Ground mustard seed																					
GRL	5.0	38.5	5.1	ND	1.0	0.1	0.5	9.0	6.3	4.0	0.3	4.3	0.3	ND	ND	0.2	10.0	0.1	0.1	0.7	0.5	36.8
Commercial laboratory	4.9	40.3	4.6	ND	1.4	0.1	0.5	9.8	7.1	4.5	0.3	4.4	0.3	9.1[e]	0.1[e]	0.2	ND[e]	0.1	0.7[e]	ND[e]	ND	38.5

Samples (dry matter basis)	Nitrogen (%)[a]	Fat (%)[b]	Moisture (%)	FA (g/100 g)[d]																	
				C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C20:3	C20:4	C22:0	C22:1	C22:2	C24:0	C24:1	Others	Total FA
Ground flaxseed																					
GRL	3.9	44.6	0.0	ND	2.1	Tr.	1.2	7.9	5.8	25.1	0.1	0.1	ND	ND	ND	Tr.	ND	ND	Tr.	0.1	0.3	42.4
Commercial laboratory	3.8	39.2	0.0	ND	1.6	ND	1.0	6.1	4.5	18.9	Tr.	0.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	31.9
Whole flaxseed																					
GRL	4.4	40.3	0.0	ND	1.8	Tr.	1.4	8.5	5.7	20.5	0.1	0.1	ND	ND	ND	0.1	ND	ND	0.Tr.	0.1	0.3	38.3
Commercial laboratory	4.4	31.7	0.0	ND	1.4	ND	1.1	6.3	4.1	14.6	Tr.	0.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	27.6
Sesame seeds, hulled																					
GRL	4.0	63.6	0.0	ND	5.5	0.1	3.4	24.5	26.2	0.3	0.4	0.1	ND	ND	ND	0.1	0.1	ND	0.1	Tr.	0.1	60.8
Commercial laboratory	4.1	57.0	0.0	Tr.	5.6	0.1	2.8	20.9	24.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	54.1
Ground mustard seed																					
GRL	5.2	40.6	0.0	ND	1.1	0.1	0.5	9.5	6.6	4.3	0.3	4.5	0.3	ND	ND	0.2	10.5	0.1	0.1	0.7	0.5	38.8
Commercial laboratory	5.1	42.2	0.0	ND	1.4	0.1	0.6	10.2	7.4	4.8	0.3	4.6	0.3	9.5[e]	0.1[e]	0.2	ND[e]	0.1	0.7[e]	ND[e]	ND	40.4

[a]The Grain Research Laboratory (GRL) used the AOCS Dumas Combustion Method Ba 4e-93 (13) [reproducibility (R) = 0.2%]; the commercial laboratories used AOAC Kjeldahl Method 991.20 (14) [relative standard deviation of reproducibility (RSDR) = 0.5%].
[b]The GRL used AOCS Method Am 2-93 (6) (R = 1.5%); the commercial laboratories used AOAC method 933.05 (7) (R = 0.7%).
[c]The GRL used ISO forced air oven method 665 (11) (R = 0.4%); the commercial laboratories used AOAC vacuum oven method 964.22 (12).
[d]The GRL used AOCS Method Ce 1-62 (15) ; the commercial laboratories used AOCS Ce 1-62 (15) (for flax) and AOAC 996.06 (2) (for sesame and mustard seed). Tr., <0.05%; ND, not detected.
[e]These results may be misidentified. Probable correct assignments are C22:1 for C20:3, C24:0 for C20:4, and C24:1 for C24:0.

knowledge of an acceptable range of results for the matrix they are studying based on data published in the scientific literature and ensure that their results are comparable to those previously published.

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[Received April 18, 2005; accepted August 4, 2005]

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