ORACLE vs. Conventional Fat Methods

Challenges and Limitations of Gravimetric and Gas Chromatography Methodology

Introduction

Fat determination in foodstuffs is typically carried out by either solvent extraction-based gravimetric methods such as Soxhlet and Mojonnier, or by gas chromatography (fatty acid methyl esters or FAMEs). While these methods, particularly the gas chromatography method, are considered to be the "gold standards," they have inherent limitations.

Though ubiquitous for foodstuff production and process control applications, gravimetric methods (regarded as "crude" fat) are often not optimized for a given matrix and the results are heavily dependent on a host of experimental conditions, such as extraction time, temperature, solvent composition, and hydrolysis conditions (if applicable). Moreover, gravimetric methods can tend to overestimate fat, due to extraction of nonfat components such as low molecular weight carbohydrates, amino acids, organic acids, and glycerol.[1] Low-moisture samples with high protein-to-fat ratios (e.g. whey protein concentrates) have been shown to be particularly susceptible to overestimation, with nonfat components comprising nearly 50% of the extraction value.[2]

The gas chromatography method, often used for food labeling and research applications, is able to provide very detailed information about the fat composition, including the amounts of individual fatty acids. While it is viewed as being more accurate than gravimetric methods (since it is less prone to overestimation), results are heavily dependent on experimental factors such as derivatization conditions and efficiency, and column and hydrolysis conditions. Accurate quantitation can also be difficult, due both to imprecision in chromatographic peak integration as well as volatility of short-chain fatty acids.[3]

Illustrating the challenges in the reproducibility of conventional methods, the USDA recently published an extensive study whereby commonly available foodstuff CRM samples (sourced from NIST and LGC) were submitted to several contract laboratories for analysis. As summarized in **Table 1** (page 2), it was found that nearly 30% of the gravimetric method results were outside the 95% confidence interval (CI) of the certified values. In addition, approximately 17–56% of the gas chromatography results (depending on fatty acid composition) were outside the 95% confidence intervals.[4] Significantly more variation was observed for mono and polyunsaturated fatty acids as compared to saturated fatty acids, which suggests results for samples with significant amounts of vegetable oils (e.g. food dressings) may be particularly unreliable.

Materials and Methods

CRM Samples

In light of these challenges, the ORACLE™ rapid NMR fat analyzer has been shown in many cases to be more accurate and repeatable than typical conventional method results from contract laboratories. As shown in **Table 2** (page 2) and **Table 3** (page 3), ORACLE and contract laboratory results (single lab) were compared to certified values for several common foodstuff CRM samples. Outliers (outside 99% confidence interval) were observed for the conventional methods in every case, except for Peanut Butter (NIST 2387); outliers comprised 50% of all results for both (fatty acid methyl esters, or FAMEs) and gravimetric analyses.

In a few cases (baking chocolate and frozen diet composite; **Table 2**), the outliers were repeatable, which would have made them difficult to detect if a certified value was not available. No outliers were observed for the ORACLE analyses, though in the case of baking chocolate (NIST 2384), the ORACLE compared closer with the gravimetric result.

Non-CRM Samples

Despite the usefulness of CRMs for validating ORACLE results, relevant CRMs are not available for many matrices. This necessitates the use of non-CRM samples, which must be characterized by outside laboratories. However, obtaining reliable conventional method results can be difficult, particularly when it isn't feasible to submit samples to multiple laboratories. A typical scenario is summarized in **Table 4** (page 3), where a store-bought mayonnaise sample was analyzed both by the ORACLE as well as by two well-known contract laboratories (sample submitted in blind duplicate). While the gas chromatography results for Lab A were quite repeatable, they were nearly 2% different than the results for Lab B. This highlights the importance of submitting samples to multiple laboratories in order to better estimate the true result. Likely due to overestimation by the gravimetric methods, the ORACLE values had much better agreement (within the 95% CI) with the gas chromatography results.

Conclusion

While the conventional methods are widely used in foodstuff testing, they suffer from several limitations (e.g. overestimation, and poor precision) which can make comparisons to the ORACLE difficult. In validating ORACLE results, it is ideally suggested that CRM samples should be obtained. In cases where relevant CRM samples are unavailable, samples of interest should be outsourced for replicate analyses by preferably multiple laboratories.

References

[1] Aued-Pimentel, et al. Quim. Nova 2010, 33, 76-84.

[2] Vaghela, M.N. and Kilara, A. JAOCS 1995, 72, 1117–1121.

[3] Kostic, Vesna. IOSR Journal of Pharmacy 2015, 5, 11–19.

[4] Phillips, Katherine et al. Anal Bioanal. Chem. 2007, 389, 219–229.

Table 1. Analysis of CRMs by Contract Laboratories[4]

Method	Determination	CRMs	Total Labs	Total Values	% Outside 95% CI*	% Outside 99% CI*
Gravimetric	Total Fat	11	5	107	29.5	17.8
Gas Chromatography	Monounsaturated Fatty Acids	3	4	36	55.6	27.8
	Polyunsaturated Fatty Acids	3	4	69	49.3	26.1
	Saturated Fatty Acids	4	4	107	16.8	7.5

**Confidence intervals roughly approximated from Z-scores, as presented by Phillips and coworkers.*

Table 2. Single Contract Lab FAMEs vs. ORACLE

Sample	Certified Value (FAMEs)	Method	Lab/ORACLE Value			# Outside 95% CI	# Outside 99% CI
			1	2	3		
Meat Homogenate (NIST 1546a)	18.96 ± 0.40	FAMEs	18.80	19.70	20.10	2	2
		ORACLE	18.87	19.29	19.03	0	0
Baking Chocolate (NIST 2384)	50.3 ± 1.1	FAMEs	53.06	53.33	52.59	3	3
		ORACLE	51.50	51.67	51.25	2	0
Peanut Butter (NIST 2387)	49.8 ± 1.9	FAMEs	49.25	51.46	49.95	0	0
		ORACLE	50.35	50.62	50.78	0	0
Frozen Diet Composite (NIST 1544)	3.7 ± 0.6	FAMEs	3.65	4.60	4.63	2	1
		ORACLE	3.99	3.94	3.86	0	0
				Total FAMEs		7 (58.3%)	6 (50%)
				Total ORACLE		2 (16.7%)	0 (0%)

Table 3. Single Contract Lab Gravimetric vs. ORACLE

Sample	Certified Value (FAMEs)	Method	Lab/ORACLE Value			# Outside 95% CI	# Outside 99% CI
			1	2	3		
Baking Chocolate (NIST 2384)	51.4 ± 1.1	Gravimetric	44.8	47.2	48.7	3	3
		ORACLE	51.5	51.67	51.25	0	0
Peanut Butter (NIST 2387)	51.6 ± 1.4	Gravimetric	49.7	49.7	50.5	2	0
		ORACLE	50.35	50.62	50.78	0	0
				Total FAMEs		5 (83.3%)	3 (50%)
				Total ORACLE		0 (0%)	0 (0%)

Table 4. Analysis of Non-CRM Foodstuff Sample (Mayonnaise)

Replicate	ORACLE	Contract Lab	Gas Chromatography	Gravimetric
Jar A – 1	77.07	A	78.62	79.6
Jar A – 2	76.99		78.59	79.8
Jar B –1	76.68	B	76.08	79.29
Jar B –2	76.66		77.26	76.96
Average	76.85	---	77.64	79.45
95% CI	0.34		1.94	2.10

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