Analysis of Phospholipids in Eggs

Introduction

Some portion of an egg, be it the yolk, white or whole egg, is found in countless finished food products. As with many precursor ingredients, the properties of the eggs must be verified prior to being added into the final product. Historically, the fat content of eggs has been analyzed by acid hydrolysis. The acid hydrolysis gravimetric method requires the use of expensive solvents along with dangerous acids. With the introduction of the ORACLE™, CEM has endeavored to replace these gravimetric methods.

The ORACLE has performed very well with a multitude of products and has proven to be as accurate, and in some cases, more precise than gravimetric techniques for triglyceride-based fat analysis. However, certain industries characterize fat differently than others. To better encapsulate what is globally defined as fat, CEM is expanding the use of the ORACLE to include the analysis of phospholipids. To better study phospholipids, we chose to study eggs, due to their relatively high phospholipid content.

Current Issues

When analyzing egg yolks, whole eggs, and egg yolk powders, the current ORACLE methodology results in a fat result that is slightly lower than the acid hydrolysis gravimetric method. It has been reported that dry egg yolk is approximately made of 62.5% lipids, 33.0% proteins, 1.2% carbohydrates and 3.5% minerals.[1] Lipids in yolk are associated with lipoprotein assemblies. These lipoproteins are made up of 62% triglycerides, 33% phospholipids and less than 5% cholesterol. The ORACLE has had no difficulties in the analysis of triglycerides, so this led to the hypothesis that the discrepancy was due to phospholipid content. Phospholipids are amphiphilic, containing a hydrophilic phosphoric acid head group and a hydrophobic tail of two fatty acids. In egg yolk, phosphatidylcholine makes up 76% of the present phospholipids. Phosphatidylethanolamine represents 22% of the phospholipids while the other 2% is made up of other various phospholipids. Looking at the percentages of the components in egg yolk, phosphatidylcholine corresponds to approximately 23% of the total fat in the yolk.

Triglycerides are a neutral ester and have a fairly standard hydrocarbon NMR relaxation rate. The ORACLE utilizes time-domain NMR, which excites the protons in the sample with a specific sequence. As the protons in the sample relax, the different proton environments will relax at different speeds. The ORACLE recognizes that the protons on the fatty acids of a triglyceride will relax within a certain time frame and record that signal, which is used in calculating the fat. A phospholipid is a diglyceride and is amphiphilic. The differences in the electronic properties between a triglyceride and phospholipid will cause the relaxation time of the protons on the fatty acids to be different. Comparatively, the relaxation times of triglycerides and phosphatidylcholine can be separated by several orders of magnitude. Phosphatidylcholines relax between 400 - 800 µs while triglycerides relax anywhere between 2500 – 100k µs.[2, 3] The electronic difference is what causes the discrepancy, especially with phosphatidylcholine and phosphatidylethanolamine. The addition of the choline and amine head groups to the phospholipid makes phosphatidylcholine and phosphatidylethanolamine zwitterions.

The charges in these molecules have a significant effect on the relaxation times of the protons on the fatty acids.

Method and Results

To accurately analyze phospholipids, adjustments needed to be made to the original ORACLE pulse sequence to accommodate the rapid relaxation time of the phospholipids. The original ORACLE pulse sequence already accurately measures the triglycerides in a sample so the new pulse sequence needs to work in conjunction with the original signal to give a new total fat. The new phospholipid tag first measures the original ORACLE signal, then measures the separate signal for phospholipids, then combines the results for total fat. The total time taken for the two signals is approximately 90 seconds. Because the phospholipid relaxation time is much faster, the resolution of the signal is lower than the normal triglyceride signal.

To accommodate this difference in resolution, more scans are performed. This tag has resulted in a significant decrease in the difference between the ORACLE and the reference chemistry, with an average difference of 0.04% total fat, as shown in **Table 1**.

Conclusion

Egg yokes contain a very unique mixture of lipids that are not common in other food products. By incorporating the ability to include phospholipids in total fat analysis, the ORACLE provides more flexibility in the fat analysis of egg products. Using the phospholipid tag, the ORACLE is able to measure total fat in a way that closely matches gravimetric reference chemistry, with good reproducibility.

References

[1] Huopalathi, R. Bioactive Egg Compounds; Springer-Verlag Berlin Heidelberg: New York, 2007.

[2] Chan, S. I.; Feigenson, G. W.; Seiter, C. H. Nature 1971, 231, 110–112.

[3] Hickey, H.; Macmillan, B.; Newling, B.; Ramesh, M.; Eijck, P. V.; Balcom, B. Food Research International 2006, 39(5), 612–618.

Table 1. Percent Fat of Egg Products Compared Across Various Methods

	Reference	ORACLE			Phospholipid Tag		
Sample	Fat %	Fat %	STDEV	Difference	Fat %	STDEV	Difference
White Egg Yolk	30.30	22.37	0.07	-7.93	30.32	0.09	0.02
Cage-Free Egg Yolk	29.10	21.61	0.05	-7.49	29.27	0.11	0.17
Brown Egg Yolk	29.23	21.65	0.04	-7.58	29.48	0.10	0.25
Duck Egg Yolk	28.92	22.7	0.13	-6.22	28.75	0.11	-0.17
Quail Whole Egg	12.60	9.43	0.03	-3.17	12.72	0.09	0.12
White Whole Egg	9.85	7.25	0.04	-2.6	9.99	0.09	0.14
Brown Whole Egg	10.53	7.82	0.01	-2.71	10.59	0.07	0.06
Cage-Free Whole Egg	8.88	6.44	0.02	-2.44	8.76	0.09	-0.12
Egg Yolk Powder	57.21	41.47	0.05	-15.74	57.1	0.13	-0.11

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