Fresh

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HUMAN HEALTH | ENVIRONMENTAL HEALTH

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Editorial

Dear Readers of Fresh,

In this day and age of better health awareness, we are proud to bring to you two articles on edible oils.

The first article is a technical note from PerkinElmer that discusses the harmful role of antioxidants in edible oils.

The second article brings to light a very important aspect on the level of trans-fatty acids found in edible oil today.
We at PerkinElmer believe that Health comes first. So please exercise caution the next time you're ordering that deep-fried snack.

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Rapid UHPLC Determination of Common Antioxidants in Edible Oils

Introduction

Phenolic antioxidants and ascorbyl palmitate (Figure 1 – Page 2) are commonly used in food to prevent the oxidation of oils. Oxidized oils will cause foul odor and rancidity in food products. This application note will present a UHPLC analysis of edible oils to determine the type and amount of ten different antioxidants.

The method was developed with a 1.9 µm column to achieve very high throughput at a low flow rate, reducing solvent consumption. The throughput of an HPLC method with a 5 µm particle-size column will be compared with that of a UHPLC method and 1.9 µm particle-size column. In addition to throughput comparisons, method conditions and performance data, including precision, linearity, and recovery from spiked samples, will be presented.

Experimental

The separation was characterized and system calibrated with a mixture of antioxidants diluted from neat material. One stock solution contained 0.5 mg/mL of propyl gallate (PG), octyl gallate (OG), dodecyl gallate (DG), nordihydroguaiaretic acid (NDGA), 2 (or 3)-tert-butyl-4-hydroxyanisole (BHA), butylated hydroxytoluene (BHT), 2,6-di-ter-butyl-4- hydroxymethyl phenol (Ionox 100) in methanol; a second stock contained about 0.5 mg/mL of 2,4,5-trihydroxybutyro phenone (THBP) in methanol; a third stock contained about 0.5 mg/mL of t-butylhydroquinone (THBQ) in methanol; and a fourth stock contained about 0.5 mg/mL of ascorbyl palmitate (AP) in methanol with 1 mg/mL of citric acid and 1 mg/mL of isoascorbic acid. The isoascorbic acid and the citric acid as an oxygen quencher and chelating agent respectively were added to the methanol to prevent the degradation of ascorbyl palmitate. Working standards with 10 µg/mL of each antioxidant were prepared from the stock standards.

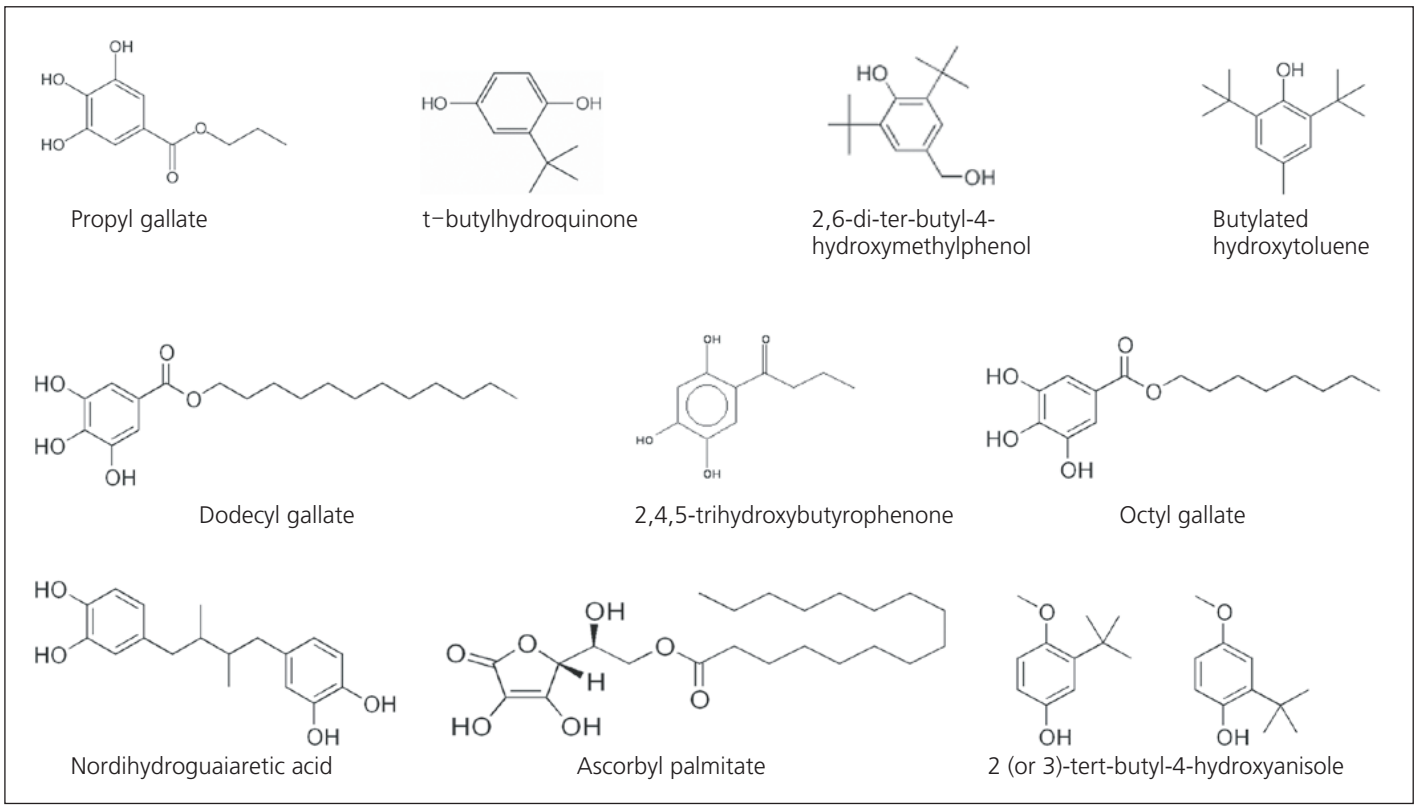


Figure 1. Names and codes of antioxidants.

Repeatability was studied with six injections of the working standard. Linearity was determined across the range of 0.2 – 10 µg/mL, with injections at: 0.2, 0.5, 1, and 10 µg/mL. Recovery from sample analysis was tested with oils samples spiked with 50 mg/kg of each antioxidant. A canola-oil and a corn-oil sample were tested. The samples were diluted with methanol containing 1 mg/mL of citric acid and 1 mg/mL of isoascorbic acid, vortexed for 5 minutes and centrifuged at 5000 rpm for 10 minutes. The supernatant were filtered with a 0.2 µm nylon syringe filter prior to dispensing into UHPLC vials.

A PerkinElmer® Flexar™ FX-15 with a Flexar UV/Vis detector provided the UHPLC platform for this application. The separation was completed on a PerkinElmer Brownlee™ Analytical C18, 1.9 µm 50 mm x 2.1 mm column. The run time was approximately 2 min with a back pressure of 8000 psi (552 bar).

Results and Discussion

Initially, the method was developed with phosphoric acid as the modifier in mobile phase A and samples were run using a C18 100 x 4.6 mm, 5 µm particle-size column. The optimal flow rate of this method was determined to be 1.8 mL/min at ambient temperature. All the antioxidants eluted in 7 min. By using a UHPLC shorter column with smaller particle size (C18 50 x 2.1 mm, 1.9 **µ**m particle size), the run time was dramatically reduced from 7 min to about 2 min. The resolution of analyte peaks and sensitivity of the determination were improved by changing the phosphoric acid modifier to formic acid. The optimal flow rate with formic acid was 0.7 mL/min at a temperature of 44 ˚C. An improved separation with sharper peaks and better signal-to-noise characteristics was obtained.

The final analysis was completed in 2.2 minutes with a total solvent usage of 1.5 mL for each injection, an impressive improvement from 7 min run time and 12.6 mL solvent usage when the conventional HPLC column was used. Representative chromatograms of standard solution analysis under conventional HPLC and UHPLC conditions are presented in Figures 2 and 3 (Page 4), representative chromatograms of spiked canola oil and corn oil under UHPLC conditions are presented in Figures 4 and 5 (Page 5).

Table 1. Detailed UHPLC system and chromatographic conditions.

Autosampler:	Flexar FX UHPLC, Part No. N2930664	
	Setting: 50 µL loop and 15 µL needle volume, partial loop injection	
	Injection: 10 µL C18 Conventional HPLC column 2 µL C18 HPLC column	
Detector:	Flexar UV/Vis Detector, Part No. N2920013	
	280 nm for phenolics antioxidants and 255 nm for ascorbyl almitate	
Pump:	Flexar FX-15, Part No. N2910531	
Columns:	PerkinElmer Brownlee Analytical C18, 1.9 µm, 50 x 2.1 mm, Part No. N9303853	
	PerkinElmer C18, 5 µm, 100 x 4.6 mm	
Column temperature:	Ambient, 44 ˚C	
Mobile phase:	**B:** 70/30 (v/v) acetonitrile/methanol,	
	A: 1% phosphoric acid in water	
	B: 70/30 (v/v) acetonitrile/methanol,	
	A: 0.02% formic acid in water	
	HPLC and ACS® reagent-grade solvents	
Flow rate:	1.8 mL/min C18 Conventional HPLC column	
	0.7 mL/min C18 UHPLC column	
Gradient:	A with phosphoric acid modifier	A with formic acid Modifier
	(C18 Conventional HPLC column)	(C18 UHPLC column)
	0.5 min 35% B	0.3 min 38% B
	2 min 35% - 45% B	0.5 min 38% - 70% B
	2 min 45% - 100%	B 0.7 min 70% - 100% B
	2.5 min 100% B	0.7 min 100% B
Software:	Chromera® Version 2.1.0.1631	
Sampling rate:	50 pts/s	

The method performance was outstanding. The linearity of the analysis achieved an average r2 value of 0.998. The average precision was less than 1% relative standard deviation (n=6). The sample preparation resulted in recovery results between 97% and 114% for both corn and canola oils, with an average recovery of approximately 103%. Details of the method performance are presented in Table 2.

Conclusion

The application of UHPLC to the analysis of common antioxidants in edible oils has resulted in a 4.8 min or about 70% reduction in run time as well as a reduction of solvent usage of 11.1 mL or about 90%. The PerkinElmer Flexar FX-15 UHPLC system and Brownlee Analytical C18, 1.9 µm 50 x 2.1 mm column resolved all antioxidants in about 2 minutes. The method was shown to be linear, the antioxidant peaks were well resolved and the recovery was good.

Reference

1. Perrin C., and Meyer L., J. Am. Oil Chem, vol 80, no.2 (2003) 115-118.

Table 2. Precision, linearity and recovery.

	PG	THBP	TBHQ	NDGA	BHA	Ionox-100	OG	BHT	DG	AP
Precision (% RSD)	0.9	0.7	0.9	0.8	1.1	1.0	0.8	1.3	1.0	1.3
Linearity ®²)	0.9996	0.9999	0.9991	0.9985	0.9990	0.9931	0.9999	0.9944	0.9959	0.9999
Corn oil recovery (%)	107	102	103	102	102	101	101	100	100	105
Canola oil recovery (%)	97	105	105	105	105	104	106	108	106	114

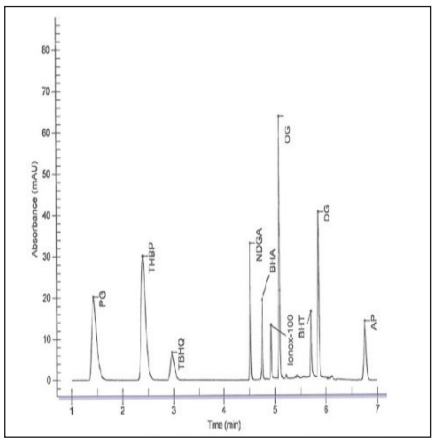


Figure 2. Chromatogram from the analysis of a standard solution with 10µg/mL of 10 antioxidants using a conventional HPLC C18 100 x 4.6 mm, 5 µm particle-size column.[1]

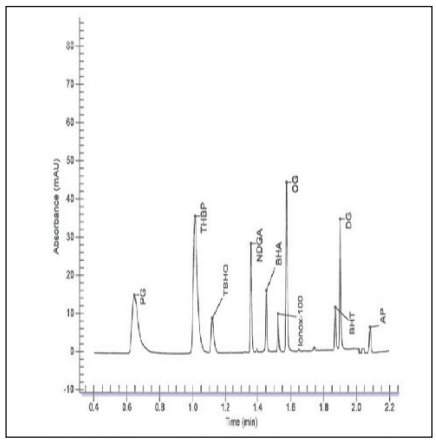


Figure 4. Chromatogram from the analysis of canola oil spiked with common antioxidants.

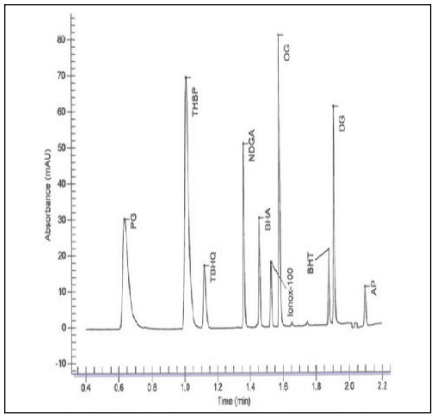


Figure 3. Chromatogram from the analysis of a standard solution with 10 µg/mL of 10 antioxidants using a UHPLC C18 50 x 2.1 mm, 1.9 µm particle-size column.

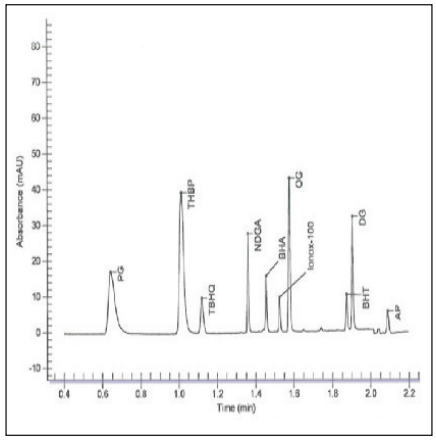


Figure 5. Chromatogram from the analysis of corn oil spiked with common antioxidants.

Trans Fatty Acid (TFA) content in Edible Oil

Higher yield as well as increasing margins are key economical factors to be addressed in today's growing edible oil market. Safety and product quality, as well as new regulations requires more information during the processing of oils. Traditionally, analytical methods are labour intensive and time consuming and are being replaced by new, faster and easy to

use systems as well as on-line methods. Edible Oil using FTIR tool changes traditional laboratory wet chemistry activities into a single method, one touch operation Analyzer.
Peanuts, popcorn and potato chips, though we may enjoy these simple snacks, determining which oil to use to make or bake these foods, and ensuring

its quality requires a great deal of preparation. Govt. of India has already announced the legislation mentioning that total consumption per service of TFA shall not exceed 0.2g. Indian edible oil industry needs to develop and adopt alternative technologies to produce zero

TIMES BUSINESS

Norms to cap bad fats in vanaspati

Trans Fatty Acid Content In Vegetable Oils Will Be Brought Down To 10%

Rupali Mukherjee | TNN

Mumbai: The government has decided to fix norms for trans fatty acids content in vanaspati and in partially hydrogenated vegetable oils (PHVOs), limiting it at 10%, and later proposes to bring it down to 5% within three years.

Further, to help consumers make informed choices, the government plans to introduce a regulation for mandatory labelling of trans fatty acids and saturated fat content on vanaspati packs, edible oils or other products containing TFA from vanaspati sources.

At present, there are no limits under the PFA on TFA content in hydrogenated edible fats or products made from these oils like namkeens, chips and other fried eatables and sweets.

A committee set up under the Food Safety and Standards Authority has in consultation with National Institute of Nutrition decided the limits of TFAs in edible oils, which will be notified within the next couple of months.

Earlier, the Prime Minister's Office had directed that norms for TFAs be laid down by December 2009.

Official sources said that manufacturers of edible oils and vanaspati will be given some time to conform to the proposed changes in their products, once the notification comes into effect. A detailed review of the impact will be carried out after three years to decide whether further reduction may be attempted.

CHECK ON CALORIES

Industrially produced trans fats are formed during partial hydrogenation, a process used by the vanaspati industry to harden and stabilise liquid vegetable oils. This process maintains the taste and smell characteristics of oils while enabling a longer shelf life for final food products. Majority of trans fats in food are industrially produced and are typically found in foods made with partially hydrogenated oil, baked and fried foods. Vanaspati, margarine, desi ghee, butter, etc are sources of TFA. Also, commercially fried, processed ready to eat bakery foods are potential sources.

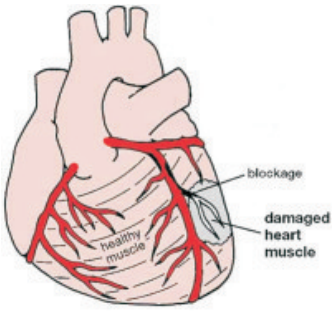
There are growing concerns about potential health effects of TFAs particularly those derived from vanaspati.

There is a significant and growing body of scientific evidence linking trans fats to coronary heart disease indicating that trans fats may do even more harm than saturated fats. Several countries such as Denmark, the United States and Canada have introduced limits on trans fatty acids in edible oils.

The World Health Organisation recently completed an exhaustive review of a vast research on links of TFA to cardiovascular diseases and diabetes, recommending all countries should take urgent regulatory steps to limit trans fats in their diet so that clear danger to heart health in vulnerable groups is avoided.

Sources said that implications of replacing high TFA partially hydrogenated oil by substitutes were also considered by FSSAI. While the availability of alternative sources of fats is not likely to be a problem, test results of fatty acids profile in the market indicate that technological solutions are available for reducing trans fats to acceptable limits.

TFA. Consumer education about negative health effects of TFA and providing food based guidelines to reduce TFA consumption in the entire population need to be actively pursued. Edible oils contain fatty acids which are essential to maintain a healthy diet, so they are vital to the food processing industry. Consumers and manufacturers have a variety of oils to choose in different foodstuffs including soybean, palm, sunflower, and cottonseed and also fish oil to name just a few. A wide range of specialty products can be produced through the hydrogenation of natural oils. Hydrogenation increases the hardness of fats by reducing the level of unsaturation. Iodine Value (IV) is the traditional method used to measure the degree of unsaturation of an oil or fat and is a key parameter used in the industry today.



TFA Causing heart blockages

Hydrogenation can lead to increased levels of trans fatty acids (TFA). This has become undesirable as consumption and the resulting high levels of TFA in human diets have been linked to an increased risk of cancer and heart disease. It is critical to carefully monitor the hydrogenation process so that the right functional form of oil is obtained with the lowest level of TFA. It can be measured by Gas Chromatography (GC), which is time consuming, or infrared spectroscopy which is faster tool.

Fourier Transform infrared spectroscopy to measure simultaneously a range of key quality parameters, as detailed above, which are important at various stages of the edible oil production process.

The Government of India has released the document through the Ministry of health and family welfare to regulate the TFA in edible oils an other fat containing products to be marketed for the human consumption. The abstract of it is given along side.

Making your Food & Drugs Safer

Ministry of Health and Family Welfare has Introduced mandatory labelling provisions for prepackaged goods vide Notification No. OSR 664 (E) in the interest of consumers. The provisions will help consumers in making an informed choice for healthy and nutritive food. The salient features arc:

- Nutritional information on the following would be declared on the label in quantitative terras:
  i) Energy
  ii) Protein
  iii) Carbohydrate
  iv) Fat
- List of ingredients would be declared in descending order of ingoing weight/volume.
- The amount of nutrient for which a health claim is made has to be declared.
- Where any hydrogenated vegetable fat is used in preparation of any food, the declaration that it contains trans fatty acid shall be given.
- In case any claim that the product is free from trans fatty acids is made, then the amount of trans fatty add shall not be more than 0,2g per serving.
- These provisions will come in to effect from 19.3.2009.

In the Interest of consumers health and patient safety, the penal provisions under the Drugs ane Cosmetics Act. 1940 have been made more stringent:

- Enhancement of punishment for manufacturing, supplying or selling of substandard, spurious, adulterated and misbranded drugs.
- Punishment goes upto life imprisonment in case of death of a patent.
- Fine upto Rs. 10 lakhs or three times the value of confiscated goods, whichever is higher, in case of death and grave injuries.
- The fine Imposed on the offenders will be paid ay way of compensation to person who has suffered grievous injury on account of adulterated or spurious drugs.
- In case of death of the person due to consumption of spurious or adulterated drugs, the compensation shall be payable to the relative of victim.

The Ministry of Health and Family Welfare has further undertaken a country wide survey to assess the extent of spurious drugs in the country.

Ministry of Health and Family Welfare