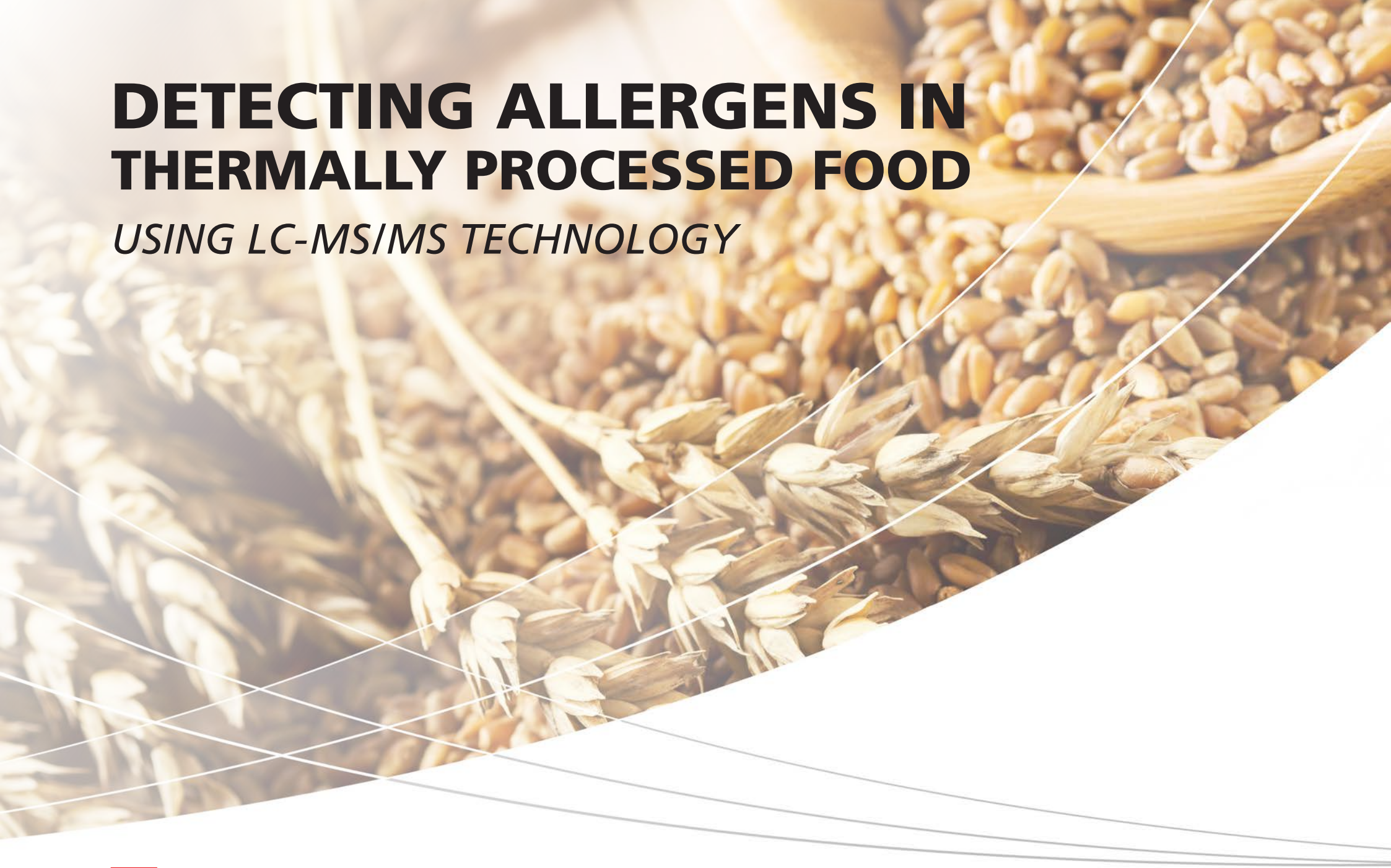
DETECTING ALLERGENS IN THERMALLY PROCESSED FOOD

USING LC-MS/MS TECHNOLOGY



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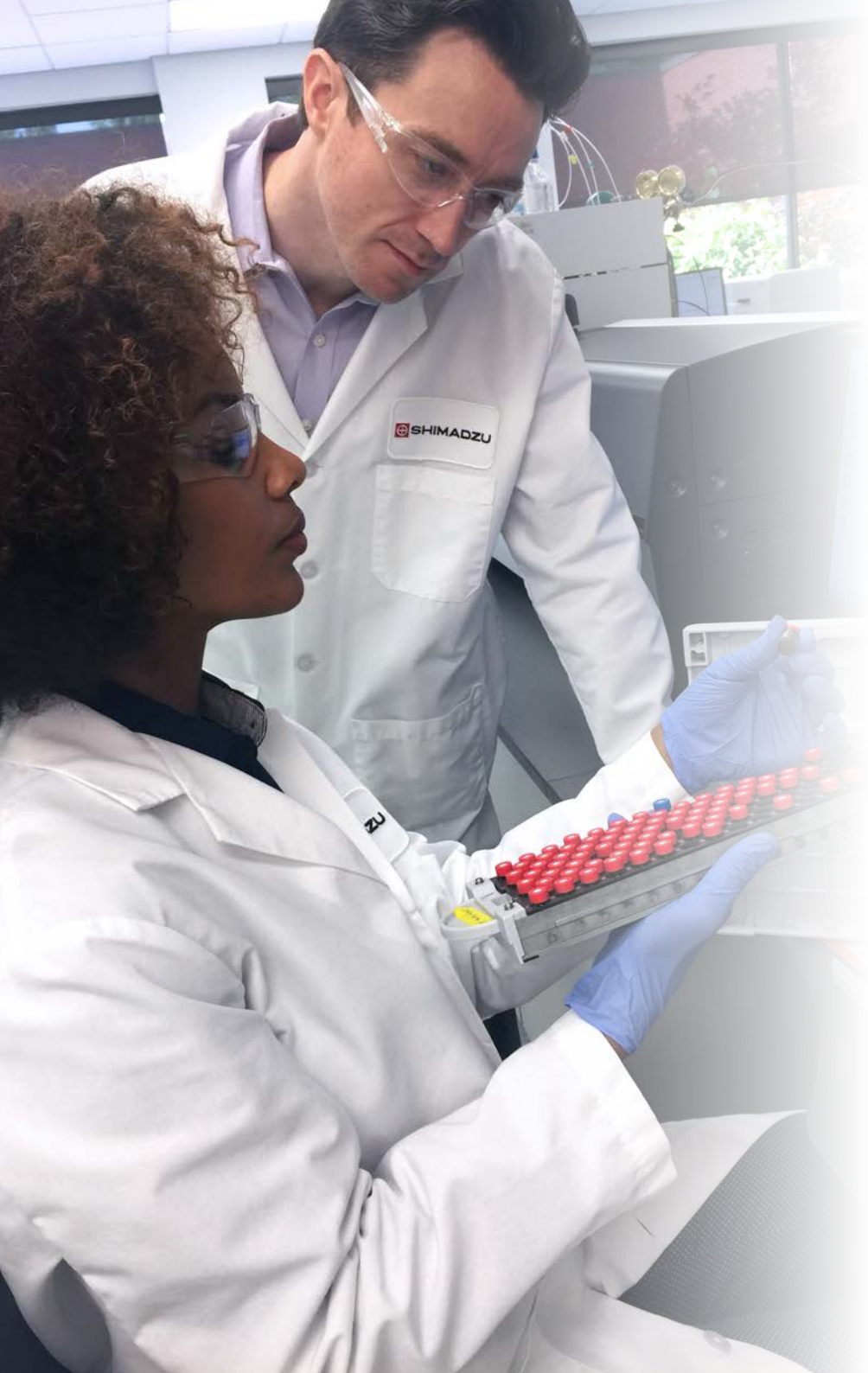
Millions of people ***every year experience allergic reactions to food.***

Symptoms range from mild rashes and hives to severe, life-threatening anaphylaxis. Because there is no cure for food allergies, avoiding food allergens is an important measure to prevent health consequences.

The FDA's Food Allergen Labeling and Consumer Protection Act (FALCPA) requires labeling of all food products regulated by the agency to clearly identify the presence of ingredients designated as major food allergens. These eight major allergens are milk, eggs, fish, crustacean shellfish, tree nuts, wheat, soybeans and peanuts.

To ensure compliance with FALCPA and to help reduce or avoid allergic reactions, food manufacturers and testing facilities must be able to identify food allergens quickly, easily and accurately. Adding to the challenge of identifying major allergens is detecting contamination from hidden allergens in processed food that contains many ingredients. ***If left undeclared, these ingredients can lead to mislabeling and can cause serious health problems for consumers.***

Shimadzu developed a method that successfully ***detects specific food ingredients in thermally processed foods.***



To overcome the challenges of identifying food allergens, Shimadzu offers advanced analytical instruments and methods, including triple quadrupole mass spectrometers and ultra-high-performance liquid chromatography systems.

Processed food contains a large number of ingredients, which makes liquid chromatography tandem mass spectrometry (LC-MS/MS) an ideal alternative to the enzyme-linked immunosorbent assay (ELISA) analytical technique. That's because LC-MS/MS provides high selectivity and sensitivity, as well as the capability to analyze multiple allergens simultaneously. While ELISA is frequently used to detect allergenic proteins, it often produces false detection of allergens in processed foods.

With this in mind, we developed a method for monitoring significant peptides derived from 13 food allergens and then evaluated this method in thermally processed foods.

Detecting Multiple Allergens in Thermally Processed Food Using LC-MS/MS

Using a Nexera UHPLC with our LCMS triple quadrupole mass spectrometer, we developed an LC-MS/MS method for simultaneous monitoring of 13 allergenic food ingredients. This method can be used to analyze both raw material and thermally processed food, including the eight foods that are subject to FALCPA's labeling requirements. ***We set a total of 245 transitions to monitor 50 peptides selected from 21 proteins for simultaneous monitoring.***

To evaluate the performance of this method, we analyzed a mixture of peptides prepared from raw food materials of milk, eggs, cod, shrimp, lobster, almonds, brazil nuts, cashew nuts, hazelnuts, walnuts, peanuts, wheat and soybeans. As the figure below shows, we confirmed that this method can detect all of the signals from the mixture of peptides prepared from raw materials.

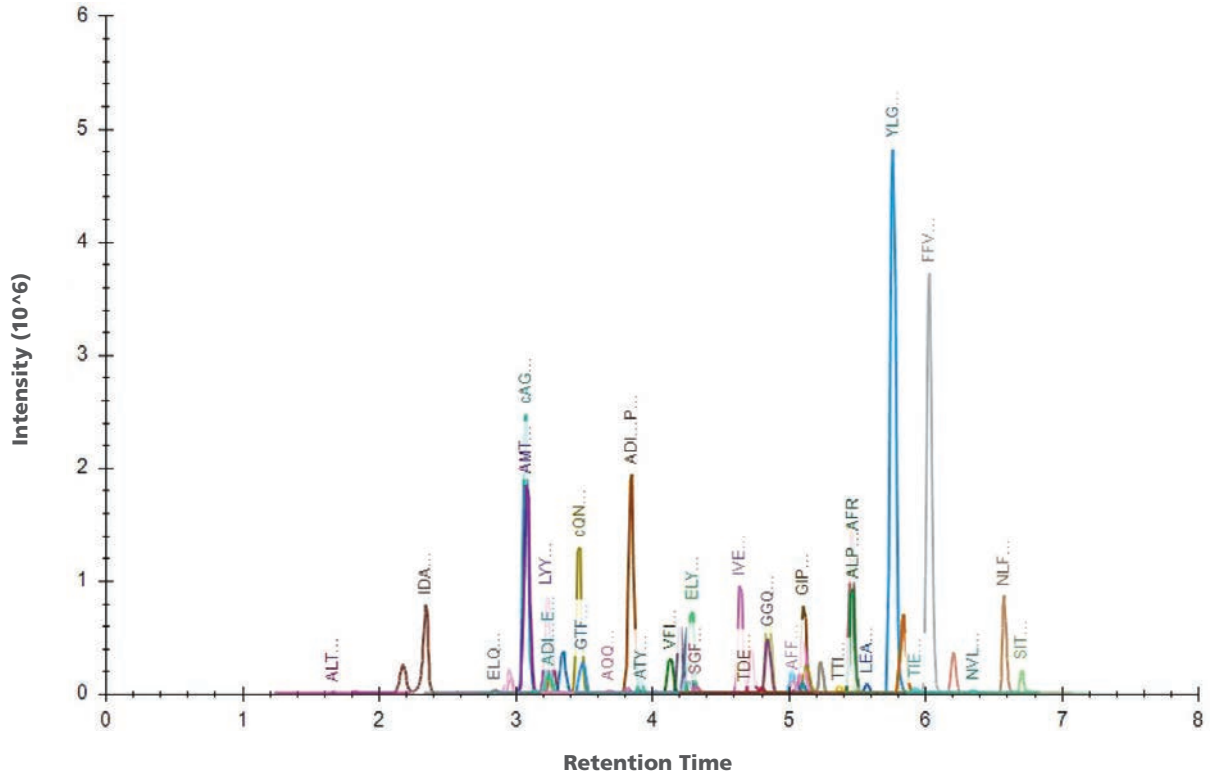


Figure 1. Chromatogram of a mixture of milk, eggs, cod, shrimp, lobster, almonds, brazil nuts, cashew nuts, hazelnuts, walnuts, peanuts, wheat, and soybeans.

We also analyzed thermally processed foods such as bread, cookies, fried fish and frozen pasta.

These samples have labels declaring that they contain or can reasonably be expected to contain wheat, milk, eggs, peanuts, soybeans, cod or shrimp as allergenic food ingredients. ***The results enabled us to determine the presence of ingredients both listed and not listed on the labels.***

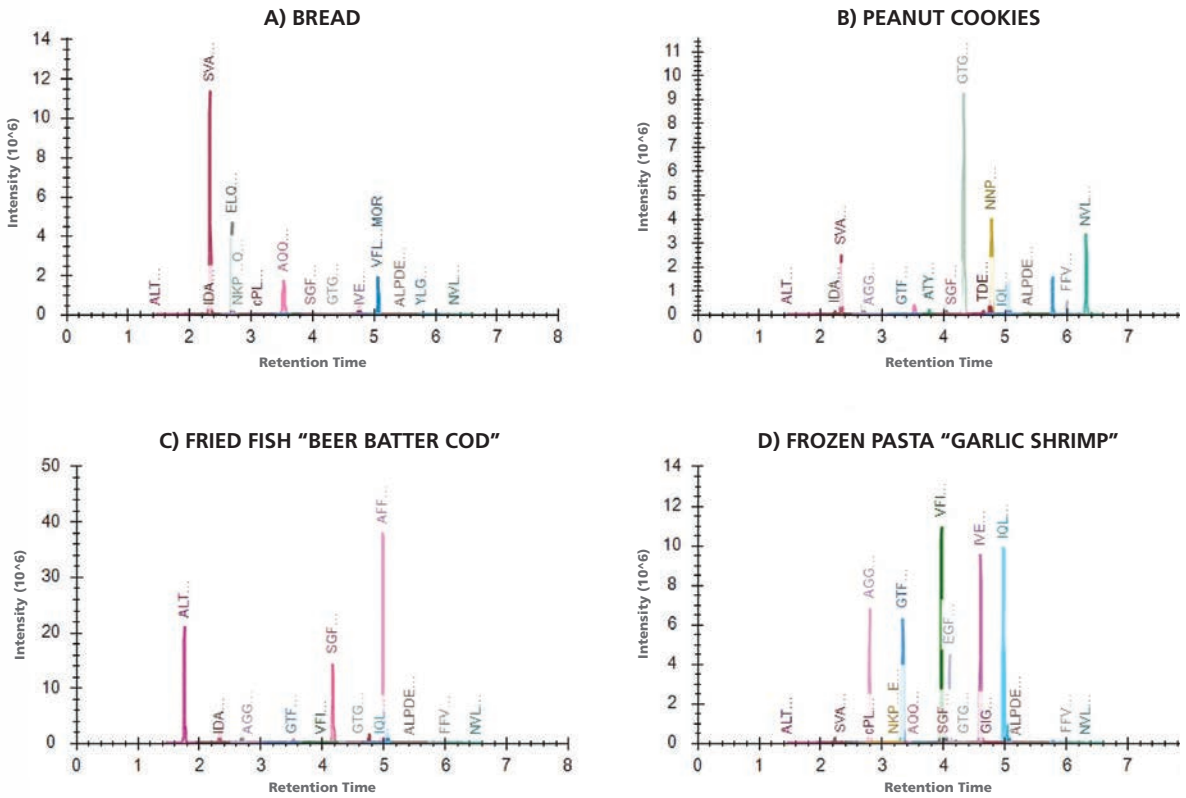


Figure 2. Chromatograms of four thermally processed foods: (a) bread, (b) cookies, (c) fried fish, and (d) frozen pasta.

The table below shows a summary of the food labeling and analytical results of the thermally processed food samples.

Food	Allergens		Bread		Peanut cookies[a]		Fried fish "Beer batter cod"		Frozen pasta "garlic shrimp"	
			LABEL	DETECT	LABEL	DETECT	LABEL	DETECT	LABEL	DETECT
Wheat	High-molecular-weight glutenin	Tri a 26	●	●	–	●	●	●	●	●
	High-molecular-weight glutenin	Tri a 36		●		●		●		●
Milk	Caseins	Bos d 8			–	●	●	●	●	●
	Beta-lactoglobulin	Bos d 5				●		●		●
Eggs	Ovalbumin	Gal d 2	●	●	–	●				
	Ovotransferrin	Gal of 3		●		●				
Peanuts	Cupin, vicillin-type, 75 globulin	Ara h 1			–	●				
Soybeans	Trypsin inhibitor	Gly m TI			–				●	
Atlantic cod	Beta-parvalbumin	Gad m 1			–		●	●		
Whiteleg shrimp	Tropomyosin	Lit v 1			–					●
	Myosin, light chain 2	Lit v 3							●[b]	●
	Sarcoplasmic CBP	Lit v 4								●

[a]No allergen information was provided.
[b]It was declared as shrimp.

We also demonstrated the method's ability to quantitate wheat peptides.

As the figures show, the ratio of wheat-related peptides in frozen pasta suggests the existence of both common wheat and durum wheat.
A majority was durum wheat, which is commonly used to produce dry pasta.

The manufacturer listed soybean oil as an ingredient on the product label of the frozen pasta. However, no soybean peptides were detected in the frozen pasta. This suggests that the food samples did not contain soybean proteins.

False positives are common with the popular ELISA method. Our LC-MS/MS method eliminates such errors.

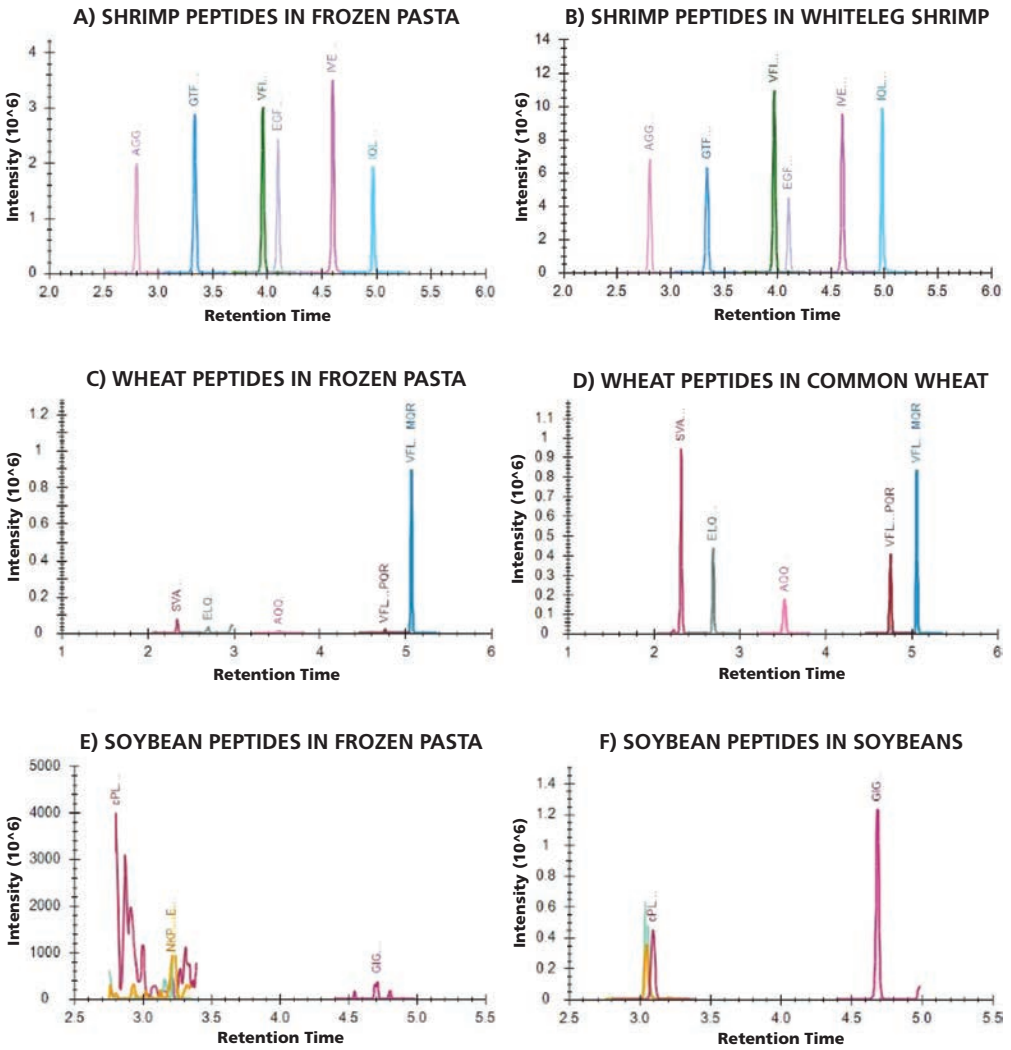


Figure 3. Comparison of chromatograms of each ingredients in pasta and raw materials: (a) shrimp peptides in frozen pasta, (b) shrimp peptides in whiteleg shrimp, (c) wheat peptides in frozen pasta, (d) wheat peptides in common wheat, (e) soybean peptides in frozen pasta, (f) soybean peptides in soybeans.

These results confirm that this method can be used for monitoring the 13 allergenic food ingredients from both raw material and thermally processed food.

Accurately detecting food allergens in raw and processed food

is critical to quality control measures of food manufacturers. More importantly, this information is vital for the safety of consumers. ***Shimadzu's advanced LC-MS/MS instruments and technology provide the high sensitivity and specificity food analysts need to generate high-quality data for multiple complex samples.***

For more information on performing high-specificity and -sensitivity analysis of food allergens with LC-MS/MS, visit

www.FeedYourLab.com

To learn more about how Shimadzu can help you optimize your food analysis, visit

www.FeedYourLab.com

7102 Riverwood Drive, Columbia, MD 21046, USA
Phone: 800.477.1227 / 410.381.1227
www.ssi.shimadzu.com