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Application News

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Liquid Chromatography Mass Spectrometry

Application of Direct Analysis in Real Time (Part 1) Rapid Analysis of Fatty Acids and Amino Acids in Food Using LCMS-2020

DART (Direct Analysis in Real Time) is a method which permits direct ionization of the sample, and when used in combination with a mass spectrometer, analysis of the target compound can be conducted quickly without the need for pretreatment. Furthermore, analysis is possible regardless of the sample form, be it gas, liquid or solid, providing that the sample can be ionized via exposure to the gas emitted from the DART ion source.
Typically, a cumbersome pretreatment process, such as extraction, is required for analysis of a specific component in a solid food. However, due to the considerable time and effort associated with this process, there is demand for a method which permits convenient screening analysis. We believe that DART is suitable for such a purpose.
Here, we introduce an example of direct analysis of free fatty acids and amino acids in solid dried bonito samples without conducting sample pretreatment. Normally, such an analysis requires considerable time and effort for sample pretreatment.
Katsuobushi (dried bonito), which was included on the Representative List of the Intangible Cultural Heritage in 2013 as an essential Japanese food item, is manufactured using processes including thorough boiling, roasting, drying and preservation using mold. The mold, in this case, is intentionally used to impart a mellow flavor. Bonito is referred to as "arabushi" when in the state prior to molding, and "hongarebushi" after molding. Here, we conducted characterization analysis of the product in these two states.

Analytical Conditions Used for Katsuobushi

The two kinds of katsuobushi (Fig. 1), arabushi and hongarebushi, were sliced to divide them into internal and surface parts. The sliced pieces of dried bonito were then held up to DART with pincers, and measured by DART.
The DART SVP ion source (IonSense, Inc., MA, USA), was used in combination with the LCMS-2020 single quadrupole mass spectrometer (Fig. 2). The LCMS-2020, with its maximum 15,000 u/sec high-speed scanning and 15 msec ultra-high-speed polarity switching, permits one-second multiple scanning over the range of *m/z* 50 to 1500 using dual, positive – negative polarity. These features made it possible to simultaneously detect a spectrum of amino acids (positive ion detection) and fatty acids (negative ion detection). And, since analysis could be carried out by simply exposing the sample to the gas, measurement time was kept to about ten seconds per sample, thereby achieving high-throughput analysis.

Table 1 Analytical Conditions

DART Heater Temperature	: 100, 200, 300, 400, 500 ˚C
Scan type	: *m/z* 50 - 1500 (Positive / Negative)
Neburizing Gas Flow	: 1.5 L/min.
Drying Gas Flow	: 5.0 L/min.
DL Temperature	: 250 ˚C
Block Heater Temperature	: 400 ˚C

Fig. 1 Katsuobushi Samples (A: Arabushi, B: Hongarebushi)

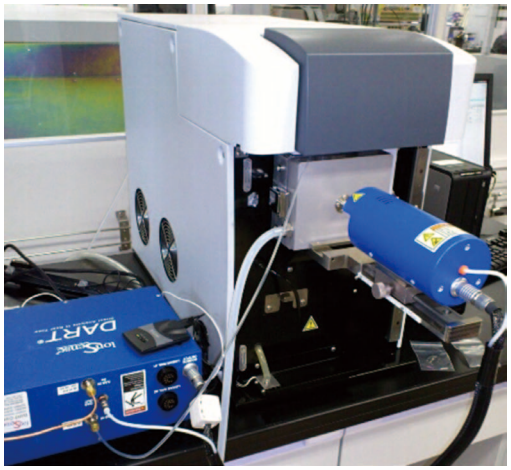
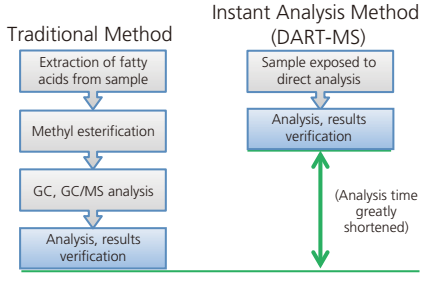


Fig. 2 DART-SVP Ion Source Coupled with LCMS-2020 Single Quadrupole Mass Spectrometer

Analysis of Fatty Acids in Katsuobushi

Typically, fatty acids in food are analyzed by GC or GC/MS following preparation which includes extraction from the sample and methyl esterification. This sample preparation process is quite time-consuming, but the analytical method presented here requires only that the sample be exposed to the gas, without any pretreatment, thereby dramatically shortening the analysis time.
The free fatty acids, including palmitic acid, oleic acid, and stearic acid, were detected in both the arabushi and hongarebushi types of katsuobushi (Fig. 3).



In addition, the distinctive free fatty acids of fish, namely docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), were also easily detected.

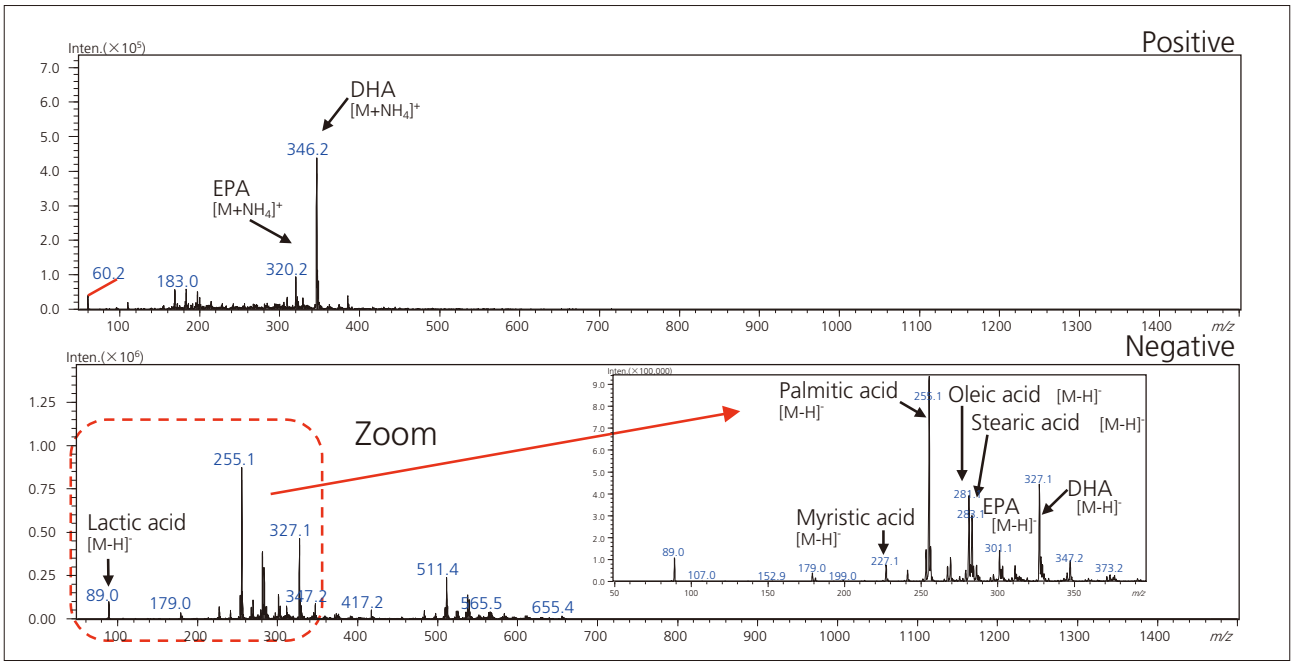


Fig. 3 Mass Spectra Obtained from Surface of Hongarebushi; DART Heater Temperature: 200 °C

Analysis of Amino Acids in Katsuobushi

Although GC-GC/MS or LC-LC/MS can be used for analysis of amino acids in foods following pretreatment which includes extraction and derivatization, DART-MS was similarly applied to this analysis using direct detection without pretreatment.

The peak of the amino acid histidine, characteristic of pelagic and migratory fish, and those of the dipeptides carnosine and anserine, commonly contained in highly migratory species, were confirmed from the positive mass spectrum.

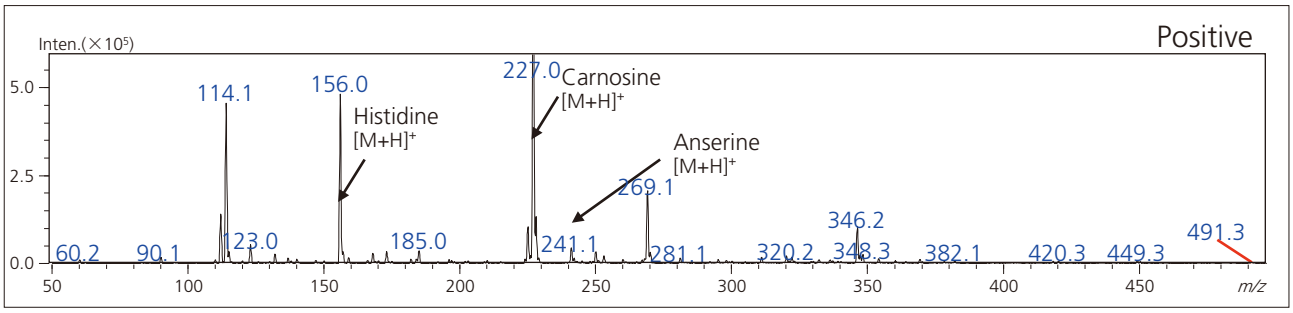


Fig. 4 Mass Spectrum from Internal Surface of Hongarebushi; Heater Temp: 400 °C

Katsuobushi Differences According to Type and Sample Site

To verify differences in fatty acid detection between arabushi and hongarebushi, or between their surfaces and internally, the signal intensities of four typical fatty acids were compared. The results are shown in Table 2. Regardless of the fatty acid, the quantity was greatest on the surface of the hongarebushi type. Since the hongarebushi is the bonito in which the molding process was applied, it is believed that the mold may be associated with the increase in free fatty acid content.

Table 2 Fatty Acids Detected from Each Katsuobushi

Fatty Acid \ Katsuobushi Site/Type	Hongarebushi Surface	Hongarebushi Internal	Arabushi Surface	Arabushi Internal
Palmitic acid	+++	++	++	+
Oleic acid	+++	++	++	+
EPA	+++	+	+	−
DHA	+++	++	++	−

(+: Abundant, −: Scarce)

[Reference Literature]
Shun Wada et al., High throughput characterization of Katsuobushi using DART-MS with high-speed polarity switching (Poster No.ThP633), ASMS 2014 in Baltimore, June 15-19, 2014.

[Acknowledgment]
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*DART is a product of IonSense Inc. (http://www.ionsense.com/).

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