Korean Society for Biotechnology and Bioengineering Journal 31(1): 1-7 (2016)
http://dx.doi.org/10.7841/ksbbj.2016.31.1.1 ISSN 1225-7117 / eISSN 2288-8268

총설

돌산갓 김치와 돌산갓 피클의 Glucosinolates의 LC-PDA/MS/MS분석

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LC-PDA/MS/MS Analysis of Glucosinolates in Dolsan Leaf Mustard Kimchi and Dolsan Leaf Mustard Pickles

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Received: 4 November 2015 / Revised: 9 January 2016 / Accepted: 9 February 2016

Abstract: Changes in the concentrations of glucosinolates and related compounds in different extracts of Dolsan leaf mustard kimchi (DLMK) and Dolsan leaf mustard pickles (DLMP) were during storage investigated. Samples were kept at 0ºC for 35 days and collected at 7 day intervals. The leaves and stems of DLMK and DLMP were refluxed for 24 h with 50% acetonitrile, and the extracts were analyzed by LC-PDA/MS/MS. The main glucosinolates detected in DLMK were sinigrin, gluconapoleiferin, glucobrassicanapin, and gluconapin, whereas those in DLMP were sinigrin, gluconapoleiferin, glucobrassicanapin, glucobrassicin, and glucoerucin. Sinigrin concentrations were quantified by UV absorption at 228 nm. Sinigrin concentrations in the leaves and stems of DLMK on the day of preparation were 2.14 mg/g and 2.25 mg/g, respectively, and those on day 35 after preparation were 1.25 mg/g and 1.00 mg/g, respectively. DLMP showed a similar trend: the concentrations in the leaves and stems on the day of preparation were 2.04 mg/g and 0.29 mg/g, respectively, whereas those on day 35 after preparation were 0.59 mg/g and 0.41 mg/g, respectively. Thus, sinigrin concentrations decreased during storage.

Keywords: Leaf mustard kimchi, Leaf mustard pickle, Glucosinolates, LC-MS/MS

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1. INTRODUCTION

Kimchi is a representative traditional fermented food in Korea, with a long history and many variations. Yeosu, Korea is a famous city were kimchi is produced from Dolsan leaf mustard (*Brassica juncea*). Brown mustard, leaf mustard and belongs to the cruciferous vegetables. Myrosinase acts on sinigrin, which is a kind type glucosinolates, and it is generated. Leaf mustard contains large amounts of thiosulfates and organosulfur compounds, which are known to hinder the development of chemically-derived tumors [1]. Crude extracts of Dolsan leaf mustard kimchi (DLMK) have been reported to have physiological functionalities, such as anti-bacterial activity, anti-oxidative activity, angiotensin-converting enzyme (ACE)-inhibiting effects and cytotoxicity in many types of cancer cells [2-7]. Nevertheless, research on the transformation of sinigrin or its derivatives and functionality of the fermentation process is currently insufficient. The supply of Western food has rapidly expanded, by the using of various vegetables; the pickle is similar to the Korea traditional food jangajji and is prepared using the same manufacturing method as that for jangajji, cucumber has been used

to be predominantly the main ingredient of the pickle, but other vegetables, such as turnip, cauliflower, ginseng, or deodeok are now actively used. DLM can also be used to prepare pickles (DLMP), producing a food that is different from DLMK. The purpose of this study was to prepare DLMK and DLMP and to evaluate changes in the concentration of sinigrin, and the composition of glucosinolates during the storage of DLMK and DLMP.

2. MATERIALS AND METHODS

2.1. Materials and Reagents

DLM was cultivated at Dolsan, Yeosu, Jeonnam, Korea, and harvested in October 2014. Ingredients to prepare kimchi and pickles were purchased at a traditional market in Yeosu. Sinigrin was purchased from Sigma Chemical Co. (St. Louis, MO, USA), and acetonitrile was purchased from J. T. Baker of Avantor (Center Valley, PA, USA).

2.2. Preparation of DLMK and DLMP

After washing DLM, excess water was removed, and DLM was pickled in 10% (w/v) salted fresh water for 3 h. After washing the salted DLM, excess water was removed. The other DLM was blanched at 94°C for 13 s and washed with cold water three times. Excess water was removed by washing for 10 min. Samples were mixed with the ingredients listed in Table 1. After preparation of DLMK and DLMP, samples were weighed and packaged (500 g each) in food-grade polyethylene bags. Samples were stored at 0°C for 35 days and evaluated at 7 day intervals.

2.3. Sinigrin extraction

The leaves and stems of DLMK and DLMP (10 g each) were collected on a thimble filter. Samples were added to a Soxhlet extractor with 200 mL of 50% (v/v) acetonitrile in a round flask and extracted at 92°C for 24 h. After cooling at room temperature, the extracts were filtered using Whatman No. 4 filter (Whatman International Ltd., Maidstone, England) [8].

2.4. HPLC analysis for quantification of sinigrin concentrations

Sinigrin, a typical glucosinolates extracted from DLMK and DLMP, was analyzed by HPLC based on retention time. HPLC was performed using a Shimadzu JP/LC-20AD series system, fitted with PDA-10A detector set at 228 nm. Inertsil Ph-3 column (4.6×250 mm) was used with a flow rate of 1 mL/min and the injection volume of 20 μL. The mobile comprised water (A) and acetonitrile (B) for a total running time of 35 min, using gradient program, as follows: 2 min 0% B; 14 min linear gradient to 31% B, held for 3 min; 2 min linear gradient down to 0% B, held for 14 min.

2.5. Sinigrin standard curve

The sinigrin standard was analyzed in triplicate at concentrations of 0.125 mg/g, 0.25 mg/g, 0.50 mg/g, and 1.00 mg/g. Mean values were used to create a standard curve. The coefficient of correlation between the peak area and sinigrin concentration (mg/g) was $R^2 = 1.000$.

2.6. LC-PDA/MS/MS analysis for identification of glucosinolates

To identify glucosinolates in DLMK and DLMP, LC-PDA/MS/MS was performed with a Shimadzu UFLC Prominence system connected with a Thermo Orbitrap XL under HPLC conditions similar to those used for sinigrin quantification. Solvent A (water) gradient elution program was started with 0% solvent B (acetonitrile), and followed a linear gradient to 35% B over 16 min period, after which solvent B was increased to 100% within 2 min and held for 2 min. Solvent B was then reduced to 0% over 18.01 min and held for 17 min. The running time

Table 1. The ratios of ingredients used for the production of DLMK and DLMP

DLMK		DLMP	
Ingredient	Weight (%)	Ingredient	Weight (%)
Salted DLM	75	Blanched DLM	65.3
Powdered red pepper	12	Soy sauce	15
Fermented sand lance juice	4	Vinegar	7
Sugar	1	Sugar	3
Garlic	1	Garlic	1
Salt	1	Red pepper	0.5
Ginger	1	Ginger	0.3
Seasoning powder	1	Seasoning powder	0.3
Green onion	1	Shiitake (mushroom)	0.3
Glutinous rice paste	1	Leek	0.3

was 18 min and the flow rate was 1.0 mL/min. Glucosinolates were identified by the *m/z* of the parental ion and fragment ion peaks by tanderm mass spectrometry (MS/MS) with electrospray ionization (ESI) in positive ion mode at the *m/z* range from 50 to 1500. MS conditions were as follows: source voltage, 5.40 kV; capillary voltage, 48.00 V; capillary temperature, 275°C; sheath gas flow: 50.00 L/min; aux gas flow: 10.00 L/min; source current, 1000 μA; and tube lens, 100 V.

2.7. Statistical analysis

All experiments were performed at least in triplicate and are presented with standard deviation calculated in Microsoft Office Excel 2013.

3. RESULTS AND DISCUSSION

3.1 Changes in sinigrin concentrations in DLMK and DLMP during storage

On the day of the preparation (0 days after preparation [DAP]) of DLMK, the concentrations of sinigrin in the leaves and stems were 2.14 mg/g and 2.25 mg/g, respectively, whereas those at 21 DAP were 0.74 mg/g and 0.78 mg/g, respectively (Fig. 1). According to Lim [9], leaf mustard kimchi fermented at 20°C has the highest sinigrin content on the fourth day, which is the optimal maturity period, rather than in the early stage of fermentation and sinigrin content of leaf mustard kimchi fermented at 0°C peaks on 0 days and unexpectedly increases for 28 days after 21 days of fermentation. This observation is attributed to an improper activation of myrosinase owing to the extremely low fermentation temperature in the experiment, because Park et al. [10] found ≥ 50% loss of myrosinase activation after days of storage and slight activation after 10 days of storage, although tissues of cruciferous vegetables are supposed to be destroyed and secreted by myrosinase to generate the breakdown product. For DLMP, the concentrations of sinigrin in the leaves decreased from 2.04 mg/g at 0 DAP to 0.54 mg/g at 7 DAP (Fig. 1). However, the in the stems were 0.29 mg/g at 0 DAP, which was lower than the concentration in the stems at 0 DAP, and increased slightly at 7 DAP and 14 DAP. During storage of DLMP, the sinigrin concentrations in the leaves and stems reached similar concentrations of approximately 0.50 mg/g.

3.2. Glucosinolates identified from DLMK and DLMP

Sinigrin, which has a molecular mass [M+H] of *m/z* 359.03 according to the mass spectrum of the standard material, eluted at 8.74 min but showed *m/z* 279.1570, which was identical to that of desulfo-sinigrin $[M\text{-}SO_3+H]^+$, calculated to be 279.08,

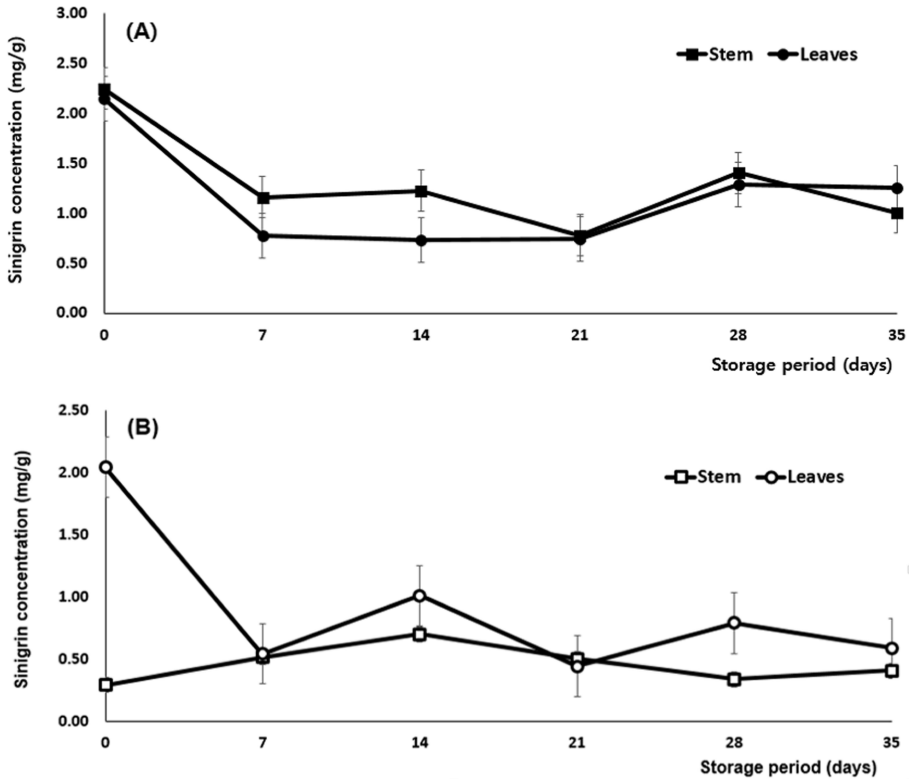

Fig. 1. Changes in sinigrin concentrations in DLMK (A) and DLMP (B) during storage.

with three typical fragment ions at *m/z* 102.13, 83.06, and 74.09, respectively (Fig. 2). Gluconapoleiferin ($[M+H]^+$, *m/z* 402.05) was detected as a sodium adduct of deglucosyl-desulfo-gluconapoleiferin ($[M-C_6H_{12}O_6-SO_3+Na]^+$ calculated *m/z* 166.0297), at 0 DAP, 7 DAP, and 21 DAP, respectively. However, well-defined MS/MS fragments of the deglucosyl component (detected at *m/z* 166.06) were not obtained (Fig. 3). Gluconapin ($[M+H]^+$, *m/z* 371.0351) was also detected as a potassium adduct of deglucosyl-desulfo-gluconapin ($[M-C_6H_{12}O_6-SO_3+K]^+$,calculated *m/z* 135.0113). Glucoerucin, as a potassium adduct of deglucosyl-desulfo-glucoerucin ($[M-C_6H_{12}O_6-SO_3+K]^+$, calculated *m/z* 200.1311) was detected from DLMK at 0 DAP. From DLMP, gluconapoleiferin as ($[M-C_6H_{12}O_6-SO_3+Na]^+$, calculated *m/z* 166.0297) was detected at 0 DAP, 7 DAP, 14 DAP, and 21 DAP respectively. Glucoerucin was detected at 21 DAP and 35 DAP, as a potassium adduct of deglucosyl-desulfo-glucoerucin ($[M-C_6H_{12}O_6-SO_3+K]^+$, calculated *m/z* 200.1311) (Fig. 4). Desulfo-sinigrin (MW, 279) and desulfo-glucoiberin (MW, 343) were found among the glucosinolates of green and red leaf mustards, the two major ingredients of DLMK [11]. Cruciferous vegetables, including cabbage, Brussels sprouts, and broccoli, were shown to have glucosinolates, such as progoitrin, glucobrassicanapin, sinigrin, and gluconapin as well as desulfo-glucosinolates, similar to the results of this experiment [12-18].

4. CONCLUSION

We used LC-PDA/MS/MS to identify glucosinolate components in the DLMK and DLMP. Standard sinigrin, which should be identified as $[M+H]^+$ at *m/z* 359.03341, was detected as desulfo-sinigrin ($[M-SO_3+H]^+$, calculated *m/z* 279.0716) with *m/z* 279.1570. The other glucosinolates were also detected as sodium or potassium adducts of deglucosylated or desulfonated forms. It is possible that the ionization method (ESI in

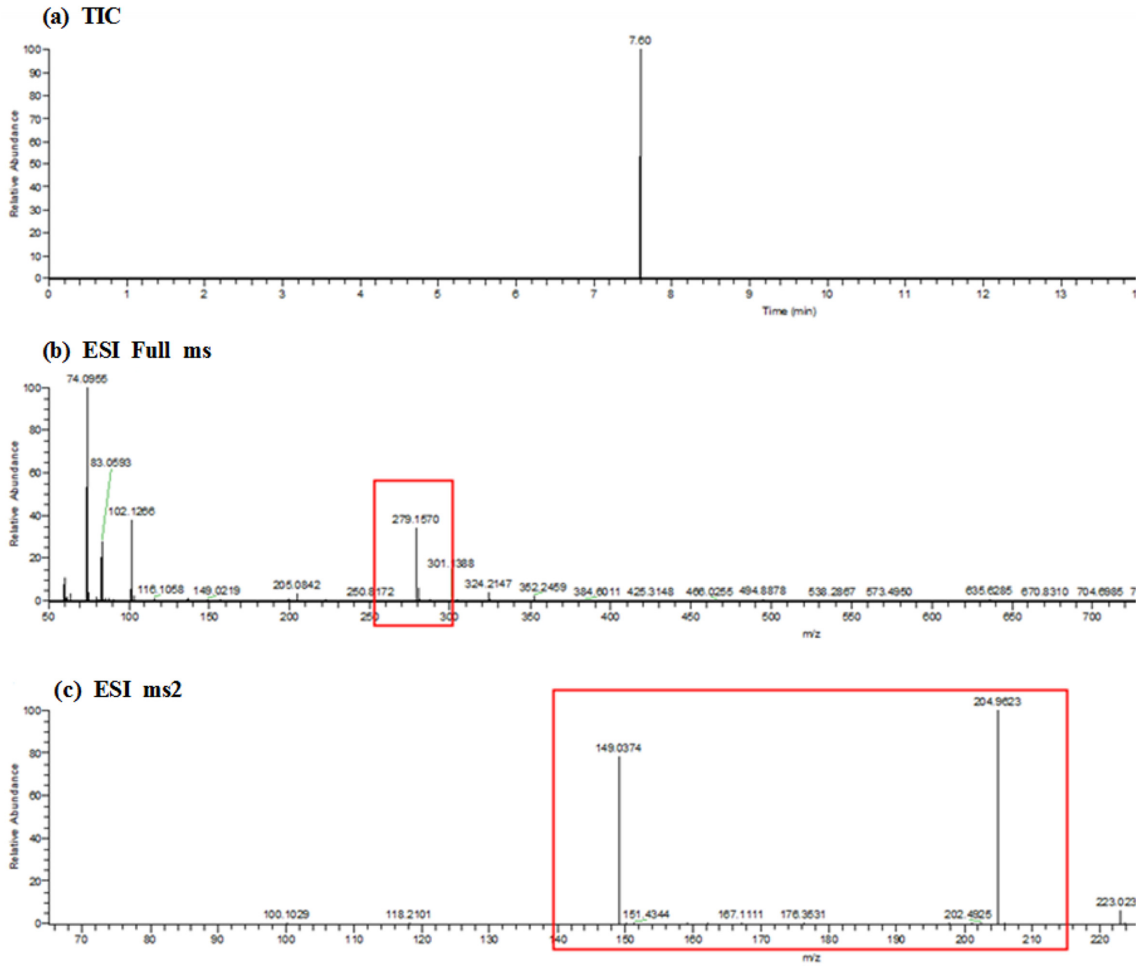

Fig. 2. Selected ion chromatography spectra of standard sinigrin in the desulfo-sinigrin form ($[M-SO_3+H]^+$, *m/z* 279.08), and detected at 7.60 min (a). The mass spectrum the peak component is also shown (b, c).

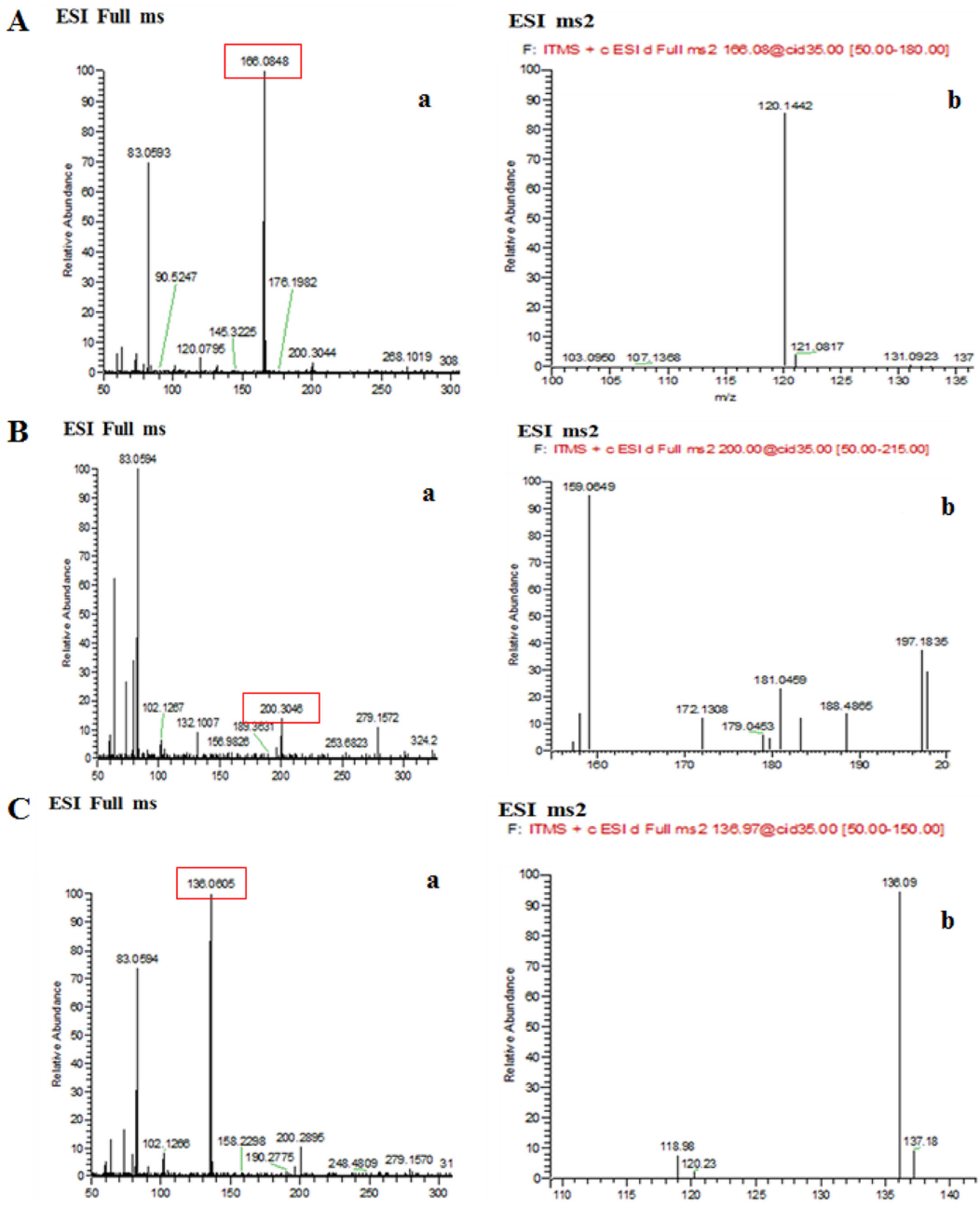

Fig. 3. Mass spectra showing retention times and MS/MS fragments at *m/z* 166.08, 200.13, and 136.03, representing deglucosyl-desulfo-gluconapoleiferin (A), deglucosyl- desulfo-glucoerucin (B), and deglucosyl-desulfo-gluconapin (C), respectively, in DLMK after storage. It represents ESI full scan mode (a) and ESI ms2 (b).

positive-ion mode) used in this experiment was not suitable to detect glucosinolates. ESI in negative-ion mode or atmospheric pressure chemical ionization (APCI) method should be applied.

During storage, total sinigrin concentrations decreased, and sinigrin derivatives were formed. Fermentation conditions, composition and content of ingredients, storage period, and other factors are thought to affect the composition and content of glucosinolates in DLMK and DLMP. These factors would also be expected to affect the health functionalities of these foods. Further studies are required.

Acknowledgements

This work was supported by the BK21 program.

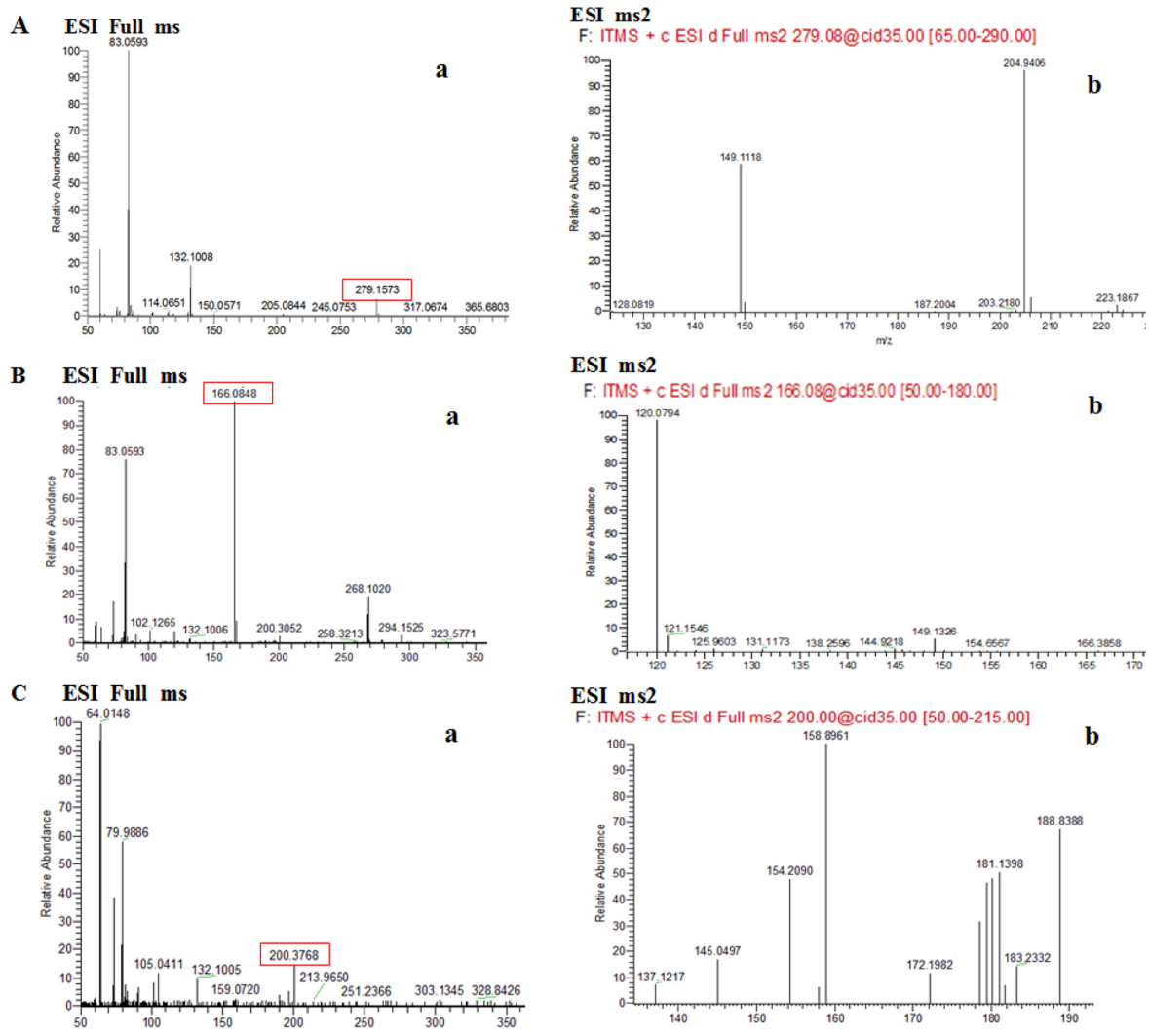

Fig. 4. Mass spectra showing retention times and MS/MS fragments at *m/z* 279.08, 166.08, and 200.13, representing desulfo-sinigrin (A), deglucosyl-desulfo-gluconapoleiferin (B), and deglucosyl-desulfo-glucoerucin (C), respectively, in DLMP after storage. It represents ESI full scan mode (a) and ESI ms2 (b).

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