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## 용존유기탄소의 $\delta^{13}\text{C}$ 분석시 고형화 전처리 방법 비교: 알칼린 과황산칼륨산화-탄산침전과 동결건조

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### Comparison of Solidification Pre-treatment Methods for the Determination of $\delta^{13}\text{C}$ of Dissolved Organic Carbon: Alkaline Persulfate Oxidation-Carbonate Precipitation vs. Freeze Drying

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#### Abstract

**BACKGROUND:** The carbon (C) isotope ratio ( $\delta^{13}\text{C}$ ) of dissolved organic C (DOC) is an indicator of water pollution source. In this study, the potential use of two pre-treatments for the  $\delta^{13}\text{C}$  analysis, alkaline persulfate oxidation coupled with carbonate precipitation (precipitation) and freeze drying (drying), were compared to suggest a more feasible pre-treatment method.

**METHODS AND RESULTS:** Two reference materials with different  $\delta^{13}\text{C}$  values were used for the experiments; chemical grade glucose ( $-12.0 \pm 0.02\%$ ) and pig manure

compost extract ( $-23.3 \pm 0.04\%$ ). In the precipitation method, the measured  $\delta^{13}\text{C}$  values were consistently lower than the theoretically calculated values as dissolved  $\text{CO}_2$  could not be removed due to the alkaline property of the reagents and the dissolution of air  $\text{CO}_2$  into the alkaline solution. The drying method also resulted in more negative  $\delta^{13}\text{C}$  than the calculated  $\delta^{13}\text{C}$ ; however, the difference was systematic ( $3.9 \pm 0.3\%$ ) and there was a strong correlation ( $\delta^{13}\text{C}_{\text{calculated}} = 0.87 \times \delta^{13}\text{C}_{\text{measured}} - 0.624$ ,  $r^2 = 0.98$ ) between the calculated and measured  $\delta^{13}\text{C}$ . Calibration of  $\delta^{13}\text{C}$  using the relationship between the calculated and the measured  $\delta^{13}\text{C}$  values produced reliable and accurate  $\delta^{13}\text{C}$  values.

**CONCLUSION:** Our results suggest that the drying method is more accurate pre-treatment method to minimize the influence of air  $\text{CO}_2$  compared to the precipitation method for the determination of  $\delta^{13}\text{C}$  of DOC.

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**Key words:** Carbon isotope ratio, Dissolved organic carbon, Freeze drying, Stable isotope ratio mass spectrometer, Strontium carbonate precipitation

**서론**

(DOC, Dissolved Organic Carbon) 가 (Tu *et al.*, 2011). DOC (Yoo *et al.*, 2012). DOC 가 가 (Zhou *et al.*, 2006). 가 (Ongley *et al.*, 2010; Sun *et al.*, 2012).

(Choi *et al.*, 2017). ( $^{13}\text{C}/^{12}\text{C}$ ,  $\delta^{13}\text{C}$  %) 가 가 가 C4  $\delta^{13}\text{C}$  (Yanagi *et al.*, 2012). C4 ( -11.2‰, -13.0‰)  $\delta^{13}\text{C}$  C3 ( -29.1‰, -25.3‰) (Yanagi *et al.*, 2012), C4  $\delta^{13}\text{C}$  (-12.6‰) C3  $\delta^{13}\text{C}$  (-26.8‰) (Dungait *et al.*, 2010).

가 DOC  $\delta^{13}\text{C}$  DOC  $\delta^{13}\text{C}$  (TOC analyzer) (Stable Isotope Ratio Mass Spectrometer, SIRMS) (Kirkels *et al.*, 2014), DOC  $\delta^{13}\text{C}$

DOC CO<sub>2</sub> Gas Chromatography (GC)-SIRMS (van Geldern *et al.*, 2013; Yu *et al.*, 2015), 가 (Elemental Analyzer) EA-SIRMS 가 (50~60°C) EA-SIRMS (Parker *et al.*, 2010; Tu *et al.*,

2011). , 가 DOC (Amiotte-Suchet *et al.*, 2007; Lambert *et al.*, 2011).

$\delta^{13}\text{C}$  가 . DOC CO<sub>2</sub> SrCl<sub>2</sub> SrCO<sub>3</sub> (Harris *et al.*, 1997). SrCO<sub>3</sub> (DIC, Dissolved Inorganic Carbon) (Parker *et al.*, 2010; van Geldern *et al.*, 2013), DOC  $\delta^{13}\text{C}$  DOC  $\delta^{13}\text{C}$  2가 -SrCO<sub>3</sub> EA-SIRMS DOC  $\delta^{13}\text{C}$

**재료 및 방법**

**표준 물질**

(Glucose) -SrCO<sub>3</sub> DOC  $\delta^{13}\text{C}$  1:10 500 mL ( $^{13}\text{C}/^{12}\text{C}$ ) EA-SIRMS (IsoPrime-EA, Micromass, Manchester, UK) 10  $\delta^{13}\text{C}$

$$\delta^{13}\text{C} (\text{‰}) = [(R_S - R_R) / R_R] \times 1000 \quad (1)$$

R<sub>S</sub> R<sub>R</sub> Vienna-Pee Dee Belemnite (CaCO<sub>3</sub>) 0.0112372 <sup>13</sup>C atom % (Choi and Lee, 2012).  $\delta^{13}\text{C}$  -12.0±0.02‰ -23.3±0.04‰

**과황산칼륨 산화-SrCO<sub>3</sub> 침전 후  $\delta^{13}\text{C}$  분석 방법**

-SrCO<sub>3</sub>  $\delta^{13}\text{C}$  1)  $\delta^{13}\text{C}$  (12.0±0.02‰) (10 mg C/L) (0, 5, 10, 20, 30 mL; : G<sub>0</sub>, G<sub>5</sub>, G<sub>10</sub>, G<sub>20</sub>, G<sub>30</sub>) DOC (0, 0.05, 0.1, 0.2, 0.3 mg C) -SrCO<sub>3</sub>  $\delta^{13}\text{C}$  2)  $\delta^{13}\text{C}$  (10 mg C/L) 6가 DOC

**Table 1. Experimental settings for the pre-treatment of glucose samples using alkaline persulfate oxidation method and freeze-drying method**

Sample code	Glucose C added (mg)	Compost extract C added (mg)	Total C added (mg)	$\delta^{13}\text{C}$ of total C calculated ( $\text{‰}$ ) <sup>a)</sup>
G <sub>10</sub> C <sub>0</sub>	0.10	0.00	0.10	-12.0
G <sub>8</sub> C <sub>2</sub>	0.08	0.02	0.10	-14.3
G <sub>6</sub> C <sub>4</sub>	0.06	0.04	0.10	-16.5
G <sub>4</sub> C <sub>6</sub>	0.04	0.06	0.10	-18.8
G <sub>2</sub> C <sub>8</sub>	0.02	0.08	0.10	-21.0
G <sub>0</sub> C <sub>10</sub>	0.00	0.10	0.10	-23.3

a) The  $\delta^{13}\text{C}$  was calculated using isotope mass balance equation.

$\delta^{13}\text{C}$  가 (Table 1).  
 0.185 M K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (Cabrera and Beare, 1993), DOC  
 0.42 M NaOH and Beare, 1993), DOC  
 2 N SrCl<sub>2</sub> (Harris *et al.*, 1997).  
 50 mL septa  
 10 mL (Cabrera and Beare, 1993), 2 N SrCl<sub>2</sub> 2 mL  
 121 °C 30  
 (SrCO<sub>3</sub>) 65 °C  
 V<sub>2</sub>O<sub>5</sub> 5 mg  
 가  $\delta^{13}\text{C}$  3  
 $\delta^{13}\text{C}$   
 (Isotope mass balance)  
 (Nitschelm *et al.*, 1997).

$$\delta^{13}\text{C}_{\text{glucose+compost}} = [(\delta^{13}\text{C}_{\text{glucose}} \times C_{\text{glucose}} + (\delta^{13}\text{C}_{\text{compost}} \times C_{\text{compost}})] / C_{\text{glucose+compost}} \quad (2)$$

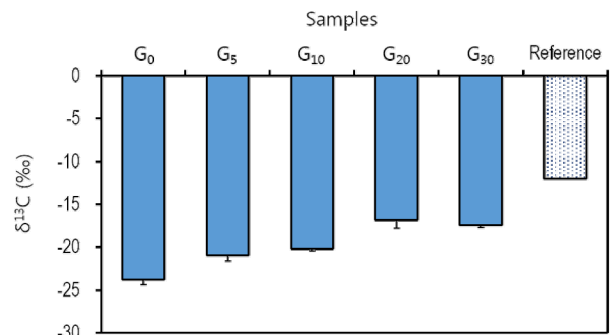
$\delta^{13}\text{C}_{\text{glucose+compost}}$ ,  $\delta^{13}\text{C}_{\text{glucose}}$ ,  $\delta^{13}\text{C}_{\text{compost}}$  는 각각 글루코스 와 퇴비침출액 분말 혼합시료, 글루코스, 퇴비침출액 분말의  $\delta^{13}\text{C}$  이고,  $C_{\text{glucose}}$ ,  $C_{\text{compost}}$ ,  $C_{\text{glucose+compost}}$  는 각각 이들의 탄소 함량이다.

**동결건조 후  $\delta^{13}\text{C}$  분석 방법**

-SrCO<sub>3</sub> (Table 1)  
 pH 2  
 DIC  
 K<sub>2</sub>SO<sub>4</sub> 100 mg  
 $\delta^{13}\text{C}$  3

**통계 분석**

(Homogeneity of variance)



**Fig. 1.** The <sup>13</sup>C of glucose samples processed with alkaline persulfate digestion coupled with SrCO<sub>3</sub> precipitation. 0, 5, 10, 20, and 30 mL of glucose solution (10 mg C/L) were used for sample codes, G<sub>0</sub>, G<sub>5</sub>, G<sub>10</sub>, G<sub>20</sub>, and G<sub>30</sub>, respectively. Reference is glucose of which <sup>13</sup>C was directly measured with EA-SIRMS. Vertical bars are standard errors of the means (n=3). All measured <sup>13</sup>C for G<sub>0</sub>~G<sub>30</sub> differ from the <sup>13</sup>C of reference ( $P < 0.001$ ).

(Normality of distribution) Shapiro-Wilk's test, Levene's test 가

$\delta^{13}\text{C}$  가 ANOVA 가

-SrCO<sub>3</sub>  
 $\delta^{13}\text{C}$  F-test  
 SPSS 18.0 (SPSS Inc. Chicago, USA)  
 95% ( $\alpha=0.05$ )

**결과 및 고찰**

과황산칼륨 산화-SrCO<sub>3</sub> 침전 후  $\delta^{13}\text{C}$  분석 결과  
 -SrCO<sub>3</sub>  
 $\delta^{13}\text{C}$  -23.8 ~ -17.4 ‰ (-19.9 ‰)  
 (-12.0 ‰) (Fig. 1).

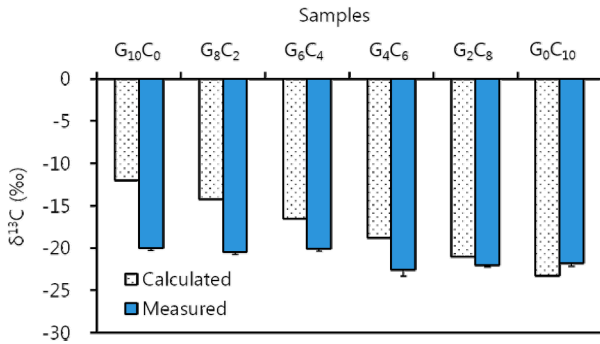


Fig. 2. The <sup>13</sup>C of glucose+compost-extract mixed samples calculated with isotope mass balance equation (calculated) and processed with alkaline persulfate digestion coupled with SrCO<sub>3</sub> precipitation (measured). Sample codes are provided in Table 1. Vertical bars are standard errors of the means (n=3). All measured <sup>13</sup>C differ from the calculated <sup>13</sup>C (*P*<0.001).

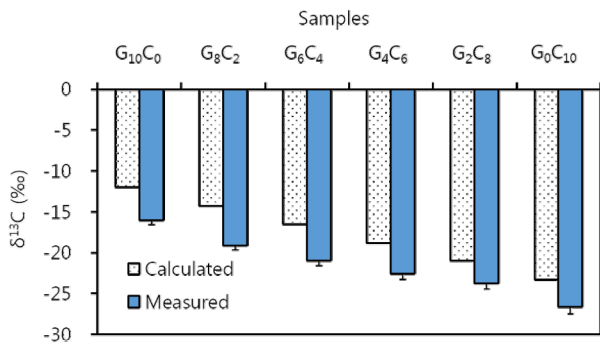


Fig. 3. The <sup>13</sup>C of glucose+compost-extract mixed samples calculated with isotope mass balance equation (calculated) and processed with freeze drying (measured). Sample codes are provided in Table 1. Vertical bars are standard errors of the means (n=3). All measured <sup>13</sup>C differ from the calculated <sup>13</sup>C (*P*<0.001).

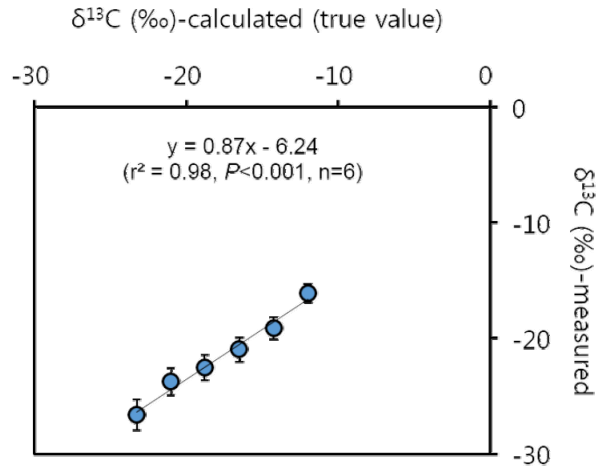


Fig. 4. Regression analysis between the <sup>13</sup>C calculated (true value) and measured for freeze drying pre-treatment using data shown in Fig. 3. Vertical bars are standard errors of the means (n=3).

-23.2~-12.0‰ (Fig. 2), -22.6~-20.0‰ (Fig. 2) C<sub>0</sub>C<sub>10</sub> δ<sup>13</sup>C  
 가 , -SrCO<sub>3</sub> δ<sup>13</sup>C  
 DOC δ<sup>13</sup>C  
 pH 2.0 DIC CO<sub>2</sub>  
 (Yu *et al.*, 2015). ,  
 -SrCO<sub>3</sub> DOC CO<sub>2</sub>  
 DIC 가 가 ,  
 CO<sub>2</sub>가  
 (Harris *et al.*, 1997).  
 CO<sub>2</sub> δ<sup>13</sup>C

-10‰ , CO<sub>2</sub> δ<sup>13</sup>C -20‰  
 (Choi and Lee, 2012).  
 가 δ<sup>13</sup>C -23.8‰  
 (Fig. 1),  
 Harris *et al.* (1997) CO<sub>3</sub><sup>2-</sup> SrCl<sub>2</sub>  
 SrCO<sub>3</sub> EA-SIRMS δ<sup>13</sup>C  
 SrCO<sub>3</sub> CO<sub>2</sub>  
 δ<sup>13</sup>C -13.6‰  
 가 SrCO<sub>3</sub> CO<sub>2</sub> δ<sup>13</sup>C  
 CO<sub>2</sub> SrCl<sub>2</sub> SrCO<sub>3</sub>  
 δ<sup>13</sup>C CO<sub>2</sub> δ<sup>13</sup>C  
 -SrCO<sub>3</sub>  
 δ<sup>13</sup>C , DOC  
 동결건조 후 δ<sup>13</sup>C 분석 결과  
 δ<sup>13</sup>C -26.7~-16.1‰  
 -SrCO<sub>3</sub> δ<sup>13</sup>C (-23.8~-17.4‰)  
 가 , -SrCO<sub>3</sub>  
 (-23.3~-12.0‰) (Fig. 3). ,  
 (Fig. 4), (r<sup>2</sup>)가 0.98  
 가 3.9±0.3‰  
 가  
 (Systematic error)  
 (Fig. 4)  
 (3.9±0.3‰)  
 δ<sup>13</sup>C (Fig. 5).  
 DOC δ<sup>13</sup>C  
 (Amiotte-Suchet *et al.*, 2007; Lambert *et*

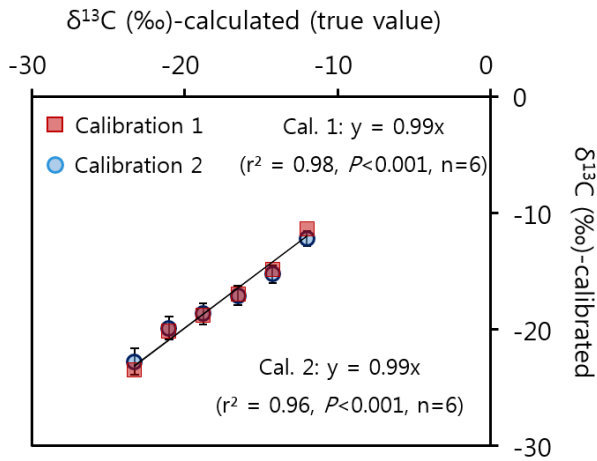


Fig. 5. Regression analysis between the <sup>13</sup>C calculated (true value) and calibrated using the regression equation in Fig. 4 (Calibration 1) and using the difference (3.9‰) between the calculated and measured <sup>13</sup>C for freeze drying pre-treatment. Vertical bars are standard errors of the means (n=3).

al., 2011), <sup>13</sup>C  
 가  
 DOC <sup>13</sup>C  
 , <sup>13</sup>C  
 가  
 .  
 요약  
 DOC <sup>13</sup>C  
 (EA-SIRMS) DOC <sup>13</sup>C  
 가 ( -SrCO<sub>3</sub>  
 ) 가  
 ( <sup>13</sup>C; -12.0±0.02‰  
 -23.3±0.04‰ ) ( <sup>13</sup>C;  
 -SrCO<sub>3</sub>  
 DIC 가  
 CO<sub>2</sub> 가  
 가 가 DIC  
 가 가  
 , EA-SIRMS  
 DOC <sup>13</sup>C

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