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Effects of pre-treatments and organic matter on oxygen and carbon isotope analyses of skeletal and inorganic calcium carbonate

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Abstract

The oxygen and carbon isotope composition of skeletal and inorganic calcites and aragonites was measured with or without pre-treatments (vacuum roasting, NaOCl and H_2O_2 treatments) used for removing organic matter. In addition, mixtures of pure calcium carbonate and organic matter (simple organic compounds, coal and kerogen) were analyzed with or without the thermal and oxidizing treatments. The studied organic substances have no effect on isotope analyses of untreated samples carried out by using the phosphoric acid method. The experimental results indicate that the roasting treatment may produce carbon isotope exchange between calcium carbonate and organic matter. Changes in original $\delta^{18}O$ and $\delta^{13}C$ values of calcites and $\delta^{13}C$ values of aragonites were observed after the NaOCl treatment of the skeletal and inorganic samples. The H_2O_2 treatment causes minor deviations in original δ values, although may cause partial dissolution of calcium carbonate. Unnecessary usage of pre-treatment methods may be a source of errors in stable isotope analyses and palaeotemperature determinations based on the oxygen isotope composition of skeletal carbonates.

Keywords: Calcium carbonate; Stable isotope; Roasting; Oxidizing treatment

1. Introduction

Organic impurities are often thought to affect measured $\delta^{18}O$ and $\delta^{13}C$ values of biogenic carbonates. The volatile organic impurities introduced into a mass spectrometer along with carbon dioxide may form molecules or radicals of the masses 44–46 in the ionization chamber. Due to a facility for amplification of the weak 45 and 46 ion beams the contaminants (e.g., C_2H_5OH, CS, BCl, NO_2, N_2O) may increase measured $\delta^{18}O$ and $\delta^{13}C$ values of carbonates [1–4]. Furthermore, Epstein et al. [5] believed that the reaction of organic matter with orthophosphoric acid could liberate carbon dioxide different in the isotopic composition from that produced from calcium carbonate.

Diverse methods of removal of organic matter were employed in palaeoenvironmental studies based on stable isotope composition of biogenic carbonates. These procedures usually involve roasting or oxidizing pre-treatments. Epstein et al. [1,5] roasted aragonites and calcites in a slow helium stream and a vacuum roasting was adopted in later studies [6–8]. Roasting temperatures varied between 200 °C and 500 °C at duration time from 20 min to 1 h [cf. 5,6,9–13]. Typical oxidizing techniques include: 1–10% sodium hypochlorite bleaching [cf. 4,14–18], 30–35% hydrogen peroxide treatment [cf. 19–21] or low-temperature oxygen plasma ashing [cf. 22–24].

As a rule, treatments cause δ values of skeletal carbonate to decrease—usually less than 1‰ (see Table 1). Roasting often results in a depletion of ^{18}O in biogenic carbonates at only slight modifications of their $\delta^{13}C$ values. The isotope composition of inorganic carbonates does not alter during pre-treatments [1,4,7,8,25]. Appreciable depletions of ^{18}O and ^{13}C isotopes found in skeletal carbonates after roasting, oxygen plasma ashing or NaOCl treatment were sometimes inferred to be caused by isotope exchange processes [16,21,23,26–28]. However, experiments on isotope exchange between gaseous CO_2 and skeletal calcium carbonates by Epstein et al. [5] provided no clear evidence for a source of observed changes in carbonate $\delta^{18}O$ values. Besides, experiments involving analyses of pure calcium carbonate mixed with organic matter showed no modification of original isotope values of the carbonate [20,28,29].

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Table 1
Changes in δ values of calcium carbonate observed after various methods of pre-treatments—literature data

Material	T (°C)/C (%)	$\Delta\delta^{18}O$ (‰)	$\Delta\delta^{13}C$ (‰)	Reference
Vacuum roasting				
Marble	475 °C	−0.10 to 0.00	No data	[7]
Inorganic $CaCO_3$, unknown mineralogy	470 °C	−0.17 to +0.11	−0.14 to +0.15	[8]
Skeletal calcite (fossil belemnites and foraminifers)	475 °C	−0.40 to 0.0	No data	[7]
Skeletal calcite (modern foraminifers)	400 °C	−0.47 to +0.01	−0.19 to −0.01	[23]
Skeletal calcite (modern bivalves and gastropods)	390 °C	−0.21 to −0.11	−0.03	[19]
Skeletal aragonite (modern bivalves)	475 °C	**−0.50** to −0.20	No data	[7]
Skeletal aragonite (fossil gastropods)	400 °C	−0.22 to +0.05	−0.04 to +0.41	[52]
Skeletal aragonite (modern gastropods)	470 °C	**−0.70** to **−0.54**	−0.07 to +0.02	[64]
Skeletal aragonite (modern gastropods)	390 °C	−0.21 to −0.18	−0.27 to −0.10	[19]
Skeletal aragonite (modern corals)	470 °C	**−0.87**	−0.19	[16]
Skeletal aragonite (modern corals)	350 °C	**−0.65** to −0.49	−0.30 to −0.10	[21]
Skeletal aragonite (modern corals)	250–300 °C	+0.08	+0.05	[20]
Skeletal $CaCO_3$, unknown mineralogy (modern bivalves)	470 °C	**−0.73** to −0.35	−0.27 to −0.19	[8]
Skeletal $CaCO_3$, unknown mineralogy (modern bivalves)	350 °C	**−0.70** to 0.00	−0.20 to 0.00	[65]
Roasting in a helium flow				
Marble	475 °C	−0.20 to 0.00	No data	[7]
Inorganic $CaCO_3$, unknown mineralogy	470 °C	−0.03 to +0.16	−0.08 to +0.12	[8]
Skeletal calcite (fossil belemnites and foraminifers)	475 °C	**−0.50** to 0.00	No data	[7]
Skeletal aragonite (modern bivalves)	475 °C	**−0.80** to −0.30	No data	[7]
Skeletal aragonite (modern gastropods)	470 °C	**−1.10** to −0.15	No data	[5]
Skeletal $CaCO_3$, unknown mineralogy (modern bivalves)	470 °C	**−0.74** to −0.39	−0.30 to −0.13	[8]
Low-temperature plasma oxidation				
Skeletal calcite (modern foraminifers)	100 °C	**−0.95** to −0.04	**−0.68** to −0.21	[23]
NaOCl treatment				
Organic matter-free calcite	5%	−0.16	−0.02	[4]
Skeletal calcite (modern foraminifers)	10%	−0.29	−0.22 to −0.17	[66]
Skeletal calcite (modern ostracods)	5%	−0.05 to −0.04	−0.27 to −0.01	[15]
Skeletal calcite (modern bivalves and gastropods)	5%	**−0.77** to −0.38	**−1.19** to −0.33	[19]
Skeletal aragonite (modern gastropods)	5%	+0.02 to +0.20	−0.23 to +0.27	[64]
Skeletal aragonite (modern gastropods)	5%	**−0.50** to +0.01	−0.25 to −0.24	[19]
Skeletal aragonite (modern corals)	5% (?)	−0.29	−0.20	[16]
H_2O_2 treatment				
Skeletal calcite (modern bivalves and gastropods)	35%	−0.05 to −0.04	−0.15 to −0.08	[19]
Skeletal aragonite (modern gastropod)	35%	+0.25	−0.11	[19]
Skeletal aragonite (modern corals)	30%	−0.26 to −0.10	−0.45 to −0.25	[21]
Skeletal aragonite (modern corals)	No data	+0.08	+0.02	[20]

Deviations (Δ) in δ values of treated samples are reported with respect to untreated samples. Deviations higher than 0.5‰ are in bold.

Many isotope geologists have abandoned pre-treatment techniques due to debatable results, preparation difficulties or slight isotope effects [cf. 29–31]. Yet, there is no agreement on results of pre-treatments (cf. http://list.uvm.edu/archives/isogeochem.html). A decrease in the δ values of skeletal carbonates, found after pre-treatments, may be linked to removal of organic contaminants, alteration of mineral phases or isotopic exchange with organics or other substances occurring during treatments. A role of organic matter on isotopic analyses of carbonates has not been eventually verified. Due to different pre-treatment methods or analyses of untreated samples, isotope values of skeletal carbonates reported in different studies appear not to be fully comparable.

The aim of this study is to determine the reliability of pre-treatment methods and the effects of organic matter on isotope analyses of calcium carbonate. The measured isotopic values of untreated inorganic carbonates mixed with organic matter are compared with the δ values of pure samples. The effects of NaOCl, H_2O_2 and heat treatment on the isotope values of calcium carbonate are evaluated by the comparison between the isotope compositions of treated and untreated samples of biogenic and inorganic origin.

2. Material and methods

The effects of roasting, NaOCl and H_2O_2 treatments on isotope analyses of skeletal samples were studied on powdered and homogenized modern calcitic oyster shells from Cuba and Jurassic belemnite rostra from Poland and Scotland. Modern aragonitic shells (*Cardium edule*) from Baltic Sea were also investigated (Table 2). Three powdered and homogenized inorganic carbonates: a Carrara marble (MAB 27/11/96 internal standard), a synthetic calcite (>99% Merck) and a synthetic aragonite were studied (Table 2). The synthetic aragonite, which contained less than 1% of calcite, was precipitated by slow addition of Na_2CO_3 (0.1 M) solution into $CaCl_2$ (0.12 M) solution at

Table 2
Description and isotope composition of studied, homogenized samples (untreated)

Sample	Material	Age	$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)
Hibolithes sp. 1	Calcite	Late Jurassic	−0.14	+2.64
Hibolithes sp. 2	Calcite	Late Jurassic	−0.12	+2.65
Hibolithes sp. 3	Calcite	Late Jurassic	+0.51	+0.90
Pachyteuthis sp.	Calcite	Late Jurassic	−0.91	+1.41
Ostrea sp. 1	Calcite	Modern	−0.98	−2.75
Ostrea sp. 2	Calcite	Modern	−1.30	−4.73
Carrara marble (MAB)	Calcite	Early Jurassic	−2.44	+2.41
Synthetic calcite (Merck)	Calcite	Modern	−19.43	−8.40
Cardium edule 1	Aragonite	Modern	−6.16	+0.18
C. edule 2	Aragonite	Modern	−6.14	+0.31
C. edule 3	Aragonite	Modern	−6.03	+0.23
Synthetic aragonite	Aragonite	Modern	+1.34	−4.91

75 °C in the presence of $MgCl_2$ (0.06 M). All reference samples were investigated by means of X-ray diffraction.

During the preliminary studies powdered and homogenized, inorganic carbonates were mixed (without grinding) with powdered organic reagents: glycine, L+ lysine monochloride, oleic acid, vanillin as well as with powdered Carboniferous coal from Silesia (Poland) and liquid oleic acid. Further studies involved investigations of skeletal samples and inorganic calcites that were ground in agate mortar for 30 min with glycine, lysine monochloride, vanillin, coal and kerogen type III characterized by low vitrinite reflectance and low nitrogen values from Spitsbergen [cf. 32]. The studied organic matter was selected because it is found in modern or fossil carbonate skeletons. Amino acids (glycine and lysine) occur in recent and fossil organic matrices extracted from calcareous shells [33–35]. Kerogen and coal are present in ancient sediments. Oleic acid and vanillin were studied to check effects of fatty acids and aromatic organic compounds on the stable isotope analysis.

Effects of prolonged grinding on the isotope composition of inorganic calcites were studied. The particle size distribution in the initial and the manually ground calcites mixed with glycine was measured by means of laser sizer (Malvern Instruments, Mastersizer 2000). Although partial conversion of calcite into aragonite by prolonged grinding in mechanical mills is reported [36,37] no such transformation was observed for the investigated specimens after manual grinding. The grinding resulted in a decrease in particle size. The initial modes of two major populations of synthetic calcite particles were 0.7–1 μm and 6–20 μm, while two modes of Carrara marble grains averaged 0.7–1 μm and 50–100 μm. Both finely ground specimens consisted of two major populations of the size 0.7–1 μm and 6–20 μm. Manual milling caused the specific surface area of the samples to increase moderately as it was calculated with granulometric analyses. The specific surface area increased from 0.341 m^2/g to 2.23–2.33 m^2/g and from 1.24 m^2/g to 2.36–2.59 m^2/g in the Carrara marble and the synthetic calcite, respectively.

Samples of ca. 30–40 mg of pure, powdered carbonates and the carbonates mixed or ground with organic matter were roasted under vacuum at 200 °C, 340–350 °C and 450 °C for 45 min. A liquid-nitrogen cold trap, which was placed adjacent to the oven, was employed to freeze volatiles and prevent contamination of a vacuum line. Effects of thermal treatments on phase composition of carbonates were investigated using X-ray analysis. The amount of calcite and aragonite was calculated after Davies and Hooper [38]. Inorganic calcites ground with vanillin, coal and kerogen, skeletal carbonates as well as pure inorganic calcites and aragonite were additionally treated at room temperature with 5% NaOCl solution and with 30% H_2O_2 solution [cf. 17,21] for 18 h and 12 h, respectively. The samples were filtered through a cellulose acetate membrane with 0.45 μm pore diameter, rinsed two times with twice distilled water, each time filtered and eventually dried at 40 °C.

The isotopic values of treated and untreated samples were measured. About 10 mg of powdered samples was analyzed for stable isotopes using the standard method of McCrea [39]. The samples were reacted overnight in sealed vessels at 25 °C with 100% orthophosphoric acid prepared without addition of CrO_3. The liberated gas passed through a cold trap at the temperature of dry ice (−78.5 °C) before carbon dioxide was frozen in a collection vessel immersed in liquid nitrogen. Analyses of five untreated inorganic calcites that contained 15 wt% of glycine, lysine monochloride, oleic acid, vanillin and coal were additionally carried out without using the trap. The isotope composition of the extracted CO_2 was analyzed by means of Finnigan Mat Delta and Delta Plus mass spectrometers. The acid fractionation factors of 1.01025 (calcite) and 1.01034 (aragonite) were applied in this study [40,41]. The oxygen and carbon isotope results are reported in δ notation in per mil relative to the VPDB international standard. Precision of results, which was measured on repeated analyses of a laboratory reference sample calibrated versus NBS-19, was close to ±0.10‰ (2σ, $n = 47$) and ±0.05‰ (2σ, $n = 47$) for $\delta^{18}O$ and $\delta^{13}C$ values, respectively. The isotope analyses of inorganic carbonate samples that indicated changes in original δ values higher than the analytical reproducibility were made in duplicate. The average value was reported. The reproducibility of duplicated analyses of inorganic carbonates was within the range of analytical precision measured on laboratory reference. Preliminary studies were carried out at the Department of Earth Sciences, Parma University in Italy. A major part of the experiments was undertaken at the Institute of Geological Sciences, Polish Academy of Sciences in Warsaw, Poland.

3. Results

Results of isotope analyses of treated samples as well as untreated samples mixed and ground with organic matter are presented in terms of deviations in measured δ values from the values of the pure, untreated original samples (Tables 3–5 ; Figs. 1–6). Because of the analytical precision (±0.10‰ and ±0.05‰) the difference between two repeated analyses of a single specimen may, in a worst case, amount to 0.20‰ and 0.10‰ for the $\delta^{18}O$ and the $\delta^{13}C$ values, respectively. The samples whose deviations in the δ values exceeded reproducibility limits were regarded as altered during treatments.

Table 3
Changes in δ values of pre-treated skeletal samples relative to the untreated samples

Material	Method	$\Delta\delta^{18}O$ (‰)	$\Delta\delta^{13}C$ (‰)
C. edule 1 (aragonite)	Vacuum roasting (200 °C)	−0.15	+0.02
C. edule 2 (aragonite)	Vacuum roasting (200 °C)	**−0.23**	0.00
C. edule 3 (aragonite)	Vacuum roasting (200 °C)	**−0.23**	−0.01
C. edule 1 (aragonite)	Vacuum roasting (340 °C)	−0.18	−0.08
C. edule 2 (aragonite)	Vacuum roasting (340 °C)	−0.15	−0.07
C. edule 3 (aragonite)	Vacuum roasting (340 °C)	**−0.22**	−0.08
Ostrea sp. 1 (calcite)	Vacuum roasting (340 °C)	−0.15	+0.01
Ostrea sp. 2 (calcite)	Vacuum roasting (340 °C)	−0.16	−0.03
Hibolithes sp. 1 (calcite)	Vacuum roasting (350 °C)	−0.09	−0.01
Hibolithes sp. 2 (calcite)	Vacuum roasting (350 °C)	−0.16	+0.01
Hibolithes sp. 3 (calcite)	Vacuum roasting (350 °C)	**−0.21**	−0.02
C. edule 1 (aragonite)	Vacuum roasting (450 °C)	−0.03[a]	**−0.14**
C. edule 2 (aragonite)	Vacuum roasting (450 °C)	−0.11[a]	**−0.19**
C. edule 3 (aragonite)	Vacuum roasting (450 °C)	−0.07[a]	**−0.21**
Ostrea sp. 1 (calcite)	Vacuum roasting (450 °C)	−0.04	+0.02
Ostrea sp. 2 (calcite)	Vacuum roasting (450 °C)	+0.03	**−0.11**
Hibolithes sp. 1 (calcite)	Vacuum roasting (450 °C)	−0.01	−0.06
Pachyteuthis sp. (calcite)	Vacuum roasting (450 °C)	−0.11	−0.03
C. edule 1 (aragonite)	NaOCl treatment	+0.04	**−0.17**
C. edule 2 (aragonite)	NaOCl treatment	+0.01	**−0.14**
C. edule 3 (aragonite)	NaOCl treatment	−0.04	**−0.19**
Ostrea sp. 1 (calcite)	NaOCl treatment	**−0.38**	**−0.23**
Ostrea sp. 2 (calcite)	NaOCl treatment	**−0.34**	**−0.30**
Hibolithes sp. 1 (calcite)	NaOCl treatment	**−0.28**	**−0.43**
Hibolithes sp. 2 (calcite)	NaOCl treatment	**−0.32**	**−0.51**
Hibolithes sp. 3 (calcite)	NaOCl treatment	**−0.26**	**−0.40**
C. edule 1 (aragonite)	H_2O_2 treatment	+0.14	+0.03
C. edule 2 (aragonite)	H_2O_2 treatment	+0.11	+0.03
C. edule 3 (aragonite)	H_2O_2 treatment	+0.15	+0.05
Ostrea sp. 1 (calcite)	H_2O_2 treatment	+0.01	+0.08
Ostrea sp. 2 (calcite)	H_2O_2 treatment	0.00	+0.06
Hibolithes sp. 1 (calcite)	H_2O_2 treatment	+0.11	+0.06
Hibolithes sp. 2 (calcite)	H_2O_2 treatment	+0.10	+0.03
Hibolithes sp. 3 (calcite)	H_2O_2 treatment	+0.15	+0.02
Pachyteuthis sp. (calcite)	H_2O_2 treatment	+0.17	+0.03

Deviations higher than the reproducibility range are in bold.
[a] Deviations in $\delta^{18}O$ values calculated by using calcite–carbon dioxide acid fractionation factor because of aragonite to calcite conversion at 450 °C.

3.1. Skeletal samples

Some aragonitic *Cardium* samples indicated detectable ^{18}O depletions (0.22–0.23‰) after roasting at 200 °C and 340 °C (Table 3; Fig. 1). Besides, three *Cardium* samples showed $\delta^{13}C$ decrease of as much as 0.21‰ after roasting at 450 °C. The effect of roasting on modern oyster samples and fossil belemnite rostra was normally undetectable lying within reproducibility of measurements except two samples that indicated slight depletions of 0.21‰ and 0.11‰ in ^{18}O and ^{13}C, respectively.

The NaOCl treatment led to significant decreases in oyster and belemnite $\delta^{18}O$ (0.26–0.38‰) and $\delta^{13}C$ values (0.23–0.51‰; Table 3; Fig. 1). The $\delta^{18}O$ values of *Cardium* samples were not noticeable affected by bleaching. Nevertheless, the $\delta^{13}C$ values of *Cardium* samples decreased of as much as 0.19‰ after the NaOCl treatment.

The effect of H_2O_2 treatment on the isotope composition of all skeletal samples was below the reproducibility error (Fig. 1; Table 3).

3.2. Inorganic samples untreated

The effect of prolonged grinding on the isotope composition of inorganic calcites lay within the reproducibility range of measurements (Table 4; Figs. 3 and 4). Similarly, the addition of organic matter (glycine, lysine monochloride, vanillin, coal and oleic acid) does not noticeable affect the original $\delta^{13}C$ and $\delta^{18}O$ values of inorganic calcites and aragonite (Table 4; Fig. 2). The same applies to results of measurements carried out without using the cold trap at −78.5 °C. The only exception was a Carrara marble sample containing coal, which indicated ^{18}O depletion of 0.24‰ when non-ground, and 0.32‰ when long ground in agate mortar (Table 4; Fig. 2).

3.3. Inorganic samples thermally treated

The effect of thermal treatment on the isotope composition of pure inorganic calcites lay within the reproducibility of measurements except a long-ground Carrara marble, whose $\delta^{13}C$

Table 4
Changes in δ values of untreated inorganic carbonates, including samples mixed and ground with organic matter, relative to the pure original carbonates

Sample	Method	$\Delta\delta^{18}O$ (‰)	$\Delta\delta^{13}C$ (‰)
Synthetic aragonite + 5 wt% glycine	Untreated	+0.03	+0.02
Synthetic aragonite + 10 wt% glycine	Untreated	+0.03	−0.02
Synthetic calcite (ground)	Untreated	+0.05	0.00
Synthetic calcite + 5 wt% glycine	Untreated	−0.02	−0.03
Synthetic calcite + 15 wt% glycine	Untreated	+0.02	+0.02
Synthetic calcite + 5 wt% lysine chlor.	Untreated	−0.04	+0.02
Synthetic calcite + 15 wt% lysine chlor.	Untreated	+0.02	+0.03
Synthetic calcite + 5 wt% vanillin	Untreated	+0.02	−0.02
Synthetic calcite + 15 wt% vanillin	Untreated	+0.12	+0.04
Synthetic calcite + 5 wt% coal	Untreated	+0.02	+0.04
Synthetic calcite + 15 wt% coal	Untreated	+0.07	+0.06
Synthetic calcite + 1drop oleic acid	Untreated	−0.03	−0.03
Synthetic calcite + 5 wt% glycine (ground)	Untreated	+0.01	+0.03
Synthetic calcite + 10 wt% glycine (ground)	Untreated	+0.03	+0.05
Synthetic calcite + 15 wt% glycine (ground)	Untreated	−0.14	−0.03
Synthetic calcite + 5 wt% lysine chlor. (ground)	Untreated	−0.06	−0.04
Synthetic calcite + 5 wt% vanillin (ground)	Untreated	−0.02	−0.01
Synthetic calcite + 5 wt% coal (ground)	Untreated	+0.06	−0.01
Synthetic calcite + 5 wt% kerogen (ground)	Untreated	−0.02	−0.01
Carrara marble (ground)	Untreated	−0.04	−0.01
Carrara marble + 5 wt% glycine	Untreated	+0.06	+0.05
Carrara marble + 15 wt% glycine	Untreated	−0.02	−0.01
Carrara marble + 5 wt% lysine chlor.	Untreated	+0.05	+0.05
Carrara marble + 15 wt% lysine chlor.	Untreated	+0.03	+0.02
Carrara marble + 5 wt% vanillin	Untreated	+0.09	+0.06
Carrara marble + 15 wt% vanillin	Untreated	+0.11	+0.06
Carrara marble + 5 wt% coal	Untreated	+0.01	−0.03
Carrara marble + 15 wt% coal	Untreated	−0.18	−0.01
Carrara marble + 1 drop oleic acid	Untreated	+0.05	0.00
Carrara marble + 5 wt% glycine (ground)	Untreated	−0.04	+0.02
Carrara marble + 10 wt% glycine (ground)	Untreated	−0.08	−0.01
Carrara marble + 15 wt% glycine (ground)	Untreated	−0.07	−0.02
Carrara marble + 5 wt% lysine chlor. (ground)	Untreated	0.00	−0.02
Carrara marble + 5 wt% vanillin (ground)	Untreated	−0.01	+0.02
Carrara marble + 5 wt% coal (ground)	Untreated	**−0.32**	−0.05
Carrara marble + 5 wt% kerogen (ground)	Untreated	−0.03	0.00
Samples collected without using the trap at the temperature of dry ice (−78.5 °C)			
Carrara marble + 15 wt% glycine	Untreated	+0.02	−0.01
Carrara marble + 15 wt% lysine chlor.	Untreated	+0.05	+0.01
Carrara marble + 15 wt% vanillin	Untreated	+0.05	−0.01
Carrara marble + 15 wt% coal	Untreated	**−0.24**	−0.04
Carrara marble + 5 wt% coal	Untreated	+0.01	−0.03
Carrara marble + 1 drop oleic acid	Untreated	+0.10	0.00

Deviations higher than the reproducibility range are in bold.

value was depleted by 0.12‰ after roasting at 450 °C (Table 5; Figs. 3 and 4). Powdered and homogenized inorganic carbonates mixed without grinding with vanillin, coal and oleic acid, were characterized by no detectable bias in δ values after roasting. However, little deviations in $\delta^{13}C$ values (−0.11‰ to −0.18‰) were stated for a few samples mixed without grinding with glycine and lysine monochloride after roasting at 340 °C and 450 °C (Table 5).

It is of importance that roasting at 340 °C and 450 °C produced significant deviations in $\delta^{13}C$ values in calcites ground for 30 min with glycine and lysine monochloride (Table 5; Figs. 5 and 6). The $\delta^{13}C$ shifts from 0.12‰ to 0.81‰ were noted for all samples containing 5–15 wt% of glycine and three samples containing 5 wt% of lysine monochloride. The $\delta^{18}O$ values of calcites ground with glycine and a sample ground with natural coal decreased or increased by 0.23‰ and 0.25‰, respectively (Table 5; see also Fig. 2). The isotope composition of samples ground with other organic substances (vanillin, coal, kerogen) was not noticeable modified by roasting (Table 5; Figs. 5 and 6).

3.4. Inorganic samples treated with NaOCl

The NaOCl treatment produced a 0.23‰ negative offset of the $\delta^{18}O$ value of a powdered and homogenized pure synthetic calcite and a 0.22‰ negative offset in the $\delta^{13}C$ value of the long-ground synthetic calcite sample (Table 5; Figs. 3 and 4).

Table 5
Changes in δ values of treated inorganic carbonates, including samples mixed and ground with organic matter, relative to the untreated pure carbonates

Sample	Method	$\Delta\delta^{18}O$ (‰)	$\Delta\delta^{13}C$ (‰)
Synthetic aragonite	Vacuum roasting (340 °C)	−0.04	+0.04
Synthetic aragonite + 5 wt% glycine	Vacuum roasting (340 °C)	−0.11	−0.03
Synthetic aragonite + 10 wt% glycine	Vacuum roasting (340 °C)	−0.13	−0.04
Synthetic calcite	Vacuum roasting (340 °C)	+0.05	−0.03
Synthetic calcite (ground)	Vacuum roasting (340 °C)	+0.18	−0.09
Synthetic calcite + 5 wt% glycine	Vacuum roasting (340 °C)	+0.03	+0.02
Synthetic calcite + 15 wt% glycine	Vacuum roasting (340 °C)	+0.04	−0.01
Synthetic calcite + 5 wt% lysine chlor.	Vacuum roasting (340 °C)	+0.04	−0.02
Synthetic calcite + 15 wt% lysine chlor.	Vacuum roasting (340 °C)	+0.04	**−0.11**
Synthetic calcite + 5 wt% vanillin	Vacuum roasting (340 °C)	+0.08	+0.08
Synthetic calcite + 15 wt% vanillin	Vacuum roasting (340 °C)	+0.03	+0.01
Synthetic calcite + 5 wt% coal	Vacuum roasting (340 °C)	−0.04	−0.04
Synthetic calcite + 15 wt% coal	Vacuum roasting (340 °C)	−0.04	−0.03
Synthetic calcite + 1 drop oleic acid	Vacuum roasting (340 °C)	+0.14	+0.01
Synthetic calcite + 5 wt% glycine (ground)	Vacuum roasting (340 °C)	−0.05	**−0.14**
Synthetic calcite + 10 wt% glycine (ground)	Vacuum roasting (340 °C)	−0.07	**−0.27**
Synthetic calcite + 15 wt% glycine (ground)	Vacuum roasting (340 °C)	−0.05	**−0.20**
Synthetic calcite + 5 wt% lysine chlor. (ground)	Vacuum roasting (340 °C)	+0.01	**−0.12**
Synthetic calcite + 5 wt% vanillin (ground)	Vacuum roasting (340 °C)	−0.11	−0.03
Synthetic calcite + 5 wt% coal (ground)	Vacuum roasting (340 °C)	+0.04	−0.05
Synthetic calcite + 5 wt% kerogen (ground)	Vacuum roasting (340 °C)	−0.02	−0.01
Carrara marble	Vacuum roasting (340 °C)	+0.03	−0.02
Carrara marble (ground)	Vacuum roasting (340 °C)	−0.05	−0.10
Carrara marble + 5 wt% glycine	Vacuum roasting (340 °C)	+0.06	+0.06
Carrara marble + 15 wt% glycine	Vacuum roasting (340 °C)	+0.05	+0.05
Carrara marble + 5 wt% lysine chlor.	Vacuum roasting (340 °C)	0.00	−0.02
Carrara marble + 15 wt% lysine chlor.	Vacuum roasting (340 °C)	−0.03	−0.08
Carrara marble + 5 wt% vanillin	Vacuum roasting (340 °C)	+0.09	+0.04
Carrara marble + 15 wt% vanillin	Vacuum roasting (340 °C)	−0.06	−0.02
Carrara marble + 5 wt% coal	Vacuum roasting (340 °C)	−0.01	+0.01
Carrara marble + 15 wt% coal	Vacuum roasting (340 °C)	−0.03	+0.05
Carrara marble + 1 drop oleic acid	Vacuum roasting (340 °C)	+0.05	−0.08
Carrara marble + 5 wt% glycine (ground)	Vacuum roasting (340 °C)	−0.13	**−0.25**
Carrara marble + 10 wt% glycine (ground)	Vacuum roasting (340 °C)	**−0.22**	**−0.39**
Carrara marble + 15 wt% glycine (ground)	Vacuum roasting (340 °C)	**−0.23**	**−0.35**
Carrara marble. + 5 wt% lysine chlor. (ground)	Vacuum roasting (340 °C)	−0.13	**−0.20**
Carrara marble + 5 wt% vanillin (ground)	Vacuum roasting (340 °C)	−0.05	−0.03
Carrara marble + 5 wt% coal (ground)	Vacuum roasting (340 °C)	−0.19	−0.04
Carrara marble + 5 wt% kerogen (ground)	Vacuum roasting (340 °C)	0.00	+0.01
Synthetic aragonite	Vacuum roasting (450 °C)	+0.10[a]	−0.06
Synthetic aragonite + 5 wt% glycine	Vacuum roasting (450 °C)	+0.04[a]	**−0.14**
Synthetic aragonite + 10 wt% glycine	Vacuum roasting (450 °C)	+0.01[a]	**−0.18**
Synthetic calcite (ground)	Vacuum roasting (450 °C)	+0.18	−0.05
Synthetic calcite + 15 wt% glycine	Vacuum roasting (450 °C)	−0.13	−0.08
Synthetic calcite + 1 drop oleic acid	Vacuum roasting (450 °C)	+0.12	−0.02
Synthetic calcite + 5 wt% glycine (ground)	Vacuum roasting (450 °C)	**+0.23**	**−0.28**
Synthetic calcite + 10 wt% glycine (ground)	Vacuum roasting (450 °C)	**+0.25**	**−0.56**
Synthetic calcite + 15 wt% glycine (ground)	Vacuum roasting (450 °C)	+0.03	**−0.34**
Synthetic calcite + 5 wt% lysine chlor. (ground)	Vacuum roasting (450 °C)	+0.14	−0.06
Synthetic calcite + 5 wt% vanillin (ground)	Vacuum roasting (450 °C)	−0.06	−0.02
Synthetic calcite + 5 wt% coal (ground)	Vacuum roasting (450 °C)	+0.11	−0.03
Synthetic calcite + 5 wt% kerogen (ground)	Vacuum roasting (450 °C)	−0.05	−0.01
Carrara marble (ground)	Vacuum roasting (450 °C)	−0.01	**−0.12**
Carrara marble + 15 wt% glycine	Vacuum roasting (450 °C)	−0.08	**−0.12**
Carrara marble + 5 wt% glycine (ground)	Vacuum roasting (450 °C)	−0.05	**−0.49**
Carrara marble + 10 wt% glycine (ground)	Vacuum roasting (450 °C)	−0.13	**−0.80**
Carrara marble + 15 wt% glycine (ground)	Vacuum roasting (450 °C)	**−0.21**	**−0.81**
Carrara marble + 5 wt% lysine chlor. (ground)	Vacuum roasting (450 °C)	−0.07	**−0.21**
Carrara marble + 5 wt% vanillin (ground)	Vacuum roasting (450 °C)	−0.18	−0.04
Carrara marble + 5 wt% coal (ground)	Vacuum roasting (450 °C)	**−0.21**	−0.08
Carrara marble + 5 wt% kerogen (ground)	Vacuum roasting (450 °C)	−0.02	0.00

Table 5 (*Continued*)

Sample	Method	$\Delta\delta^{18}O$ (‰)	$\Delta\delta^{13}C$ (‰)
Synthetic aragonite	NaOCl treatment	−0.07	0.00
Synthetic calcite	NaOCl treatment	**−0.23**	−0.03
Synthetic calcite (ground)	NaOCl treatment	+0.16	**−0.22**
Synthetic calcite + 5 wt% coal (ground)	NaOCl treatment	−0.03	−0.02
Synthetic calcite + 5 wt% kerogen (ground)	NaOCl treatment	−0.01	−0.02
Synthetic calcite + 5 wt% vanillin (ground)	NaOCl treatment	−0.10	−0.05
Carrara marble	NaOCl treatment	−0.17	−0.07
Carrara marble (ground)	NaOCl treatment	**−0.49**	**−0.69**
Carrara marble + 5 wt% coal (ground)	NaOCl treatment	**−0.30**	**−0.16**
Carrara marble + 5 wt% kerogen (ground)	NaOCl treatment	**−0.32**	**−0.27**
Carrara marble + 5 wt% vanillin (ground)	NaOCl treatment	**−0.21**	**−0.14**
Synthetic aragonite	H_2O_2 treatment	−0.14	−0.02
Synthetic calcite	H_2O_2 treatment	**−0.40**	−0.04
Synthetic calcite (ground)	H_2O_2 treatment	−0.08	−0.01
Synthetic calcite + 5 wt% coal (ground)	H_2O_2 treatment	−0.02	−0.02
Synthetic calcite + 5 wt% kerogen (ground)	H_2O_2 treatment	−0.12	−0.01
Synthetic calcite + 5 wt% vanillin (ground)	H_2O_2 treatment	**−0.30**	−0.09
Carrara marble	H_2O_2 treatment	−0.14	−0.04
Carrara marble (ground)	H_2O_2 treatment	−0.12	−0.03
Carrara marble + 5 wt% coal (ground)	H_2O_2 treatment	**−0.46**	−0.04
Carrara marble + 5 wt% kerogen (ground)	H_2O_2 treatment	−0.10	−0.03
Carrara marble + 5 wt% vanillin (ground)	H_2O_2 treatment	−0.17	−0.01

Deviations higher than the reproducibility range are in bold.

[a] Deviations in $\delta^{18}O$ values calculated by using calcite–carbon dioxide acid fractionation factor because of aragonite to calcite conversion at 450 °C.

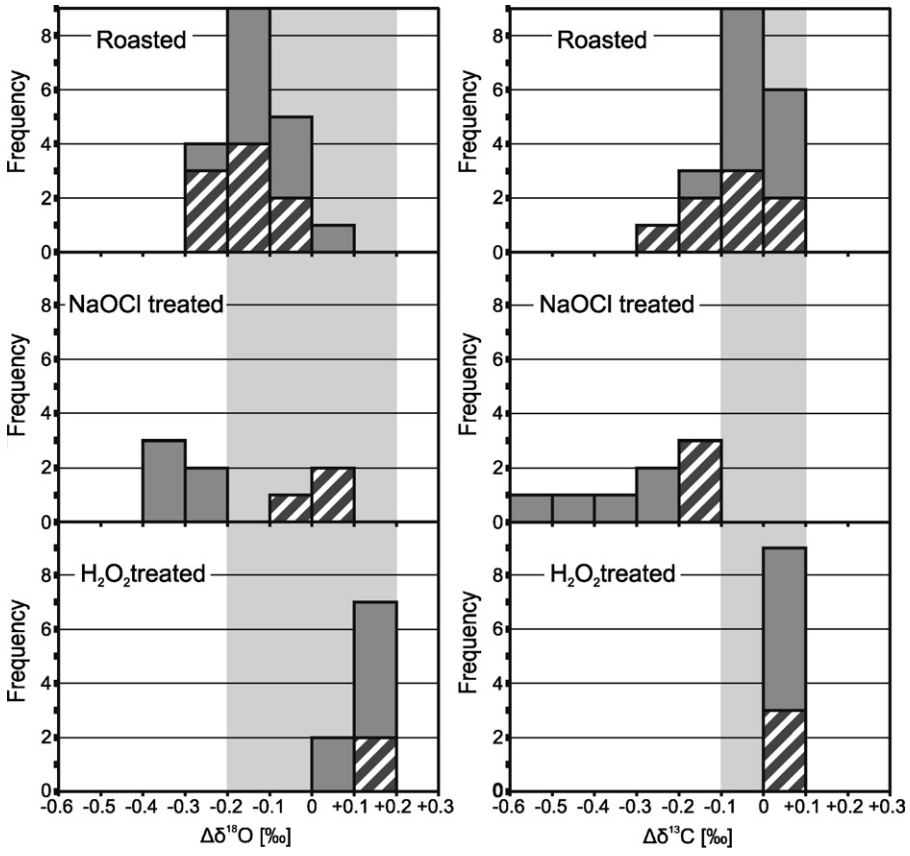

Fig. 1. Histograms showing the frequency distribution of deviations in $\delta^{18}O$ and $\delta^{13}C$ values of pre-treated skeletal calcites (grey columns) and aragonites (striped columns) with respect to untreated samples. Error areas are shaded.

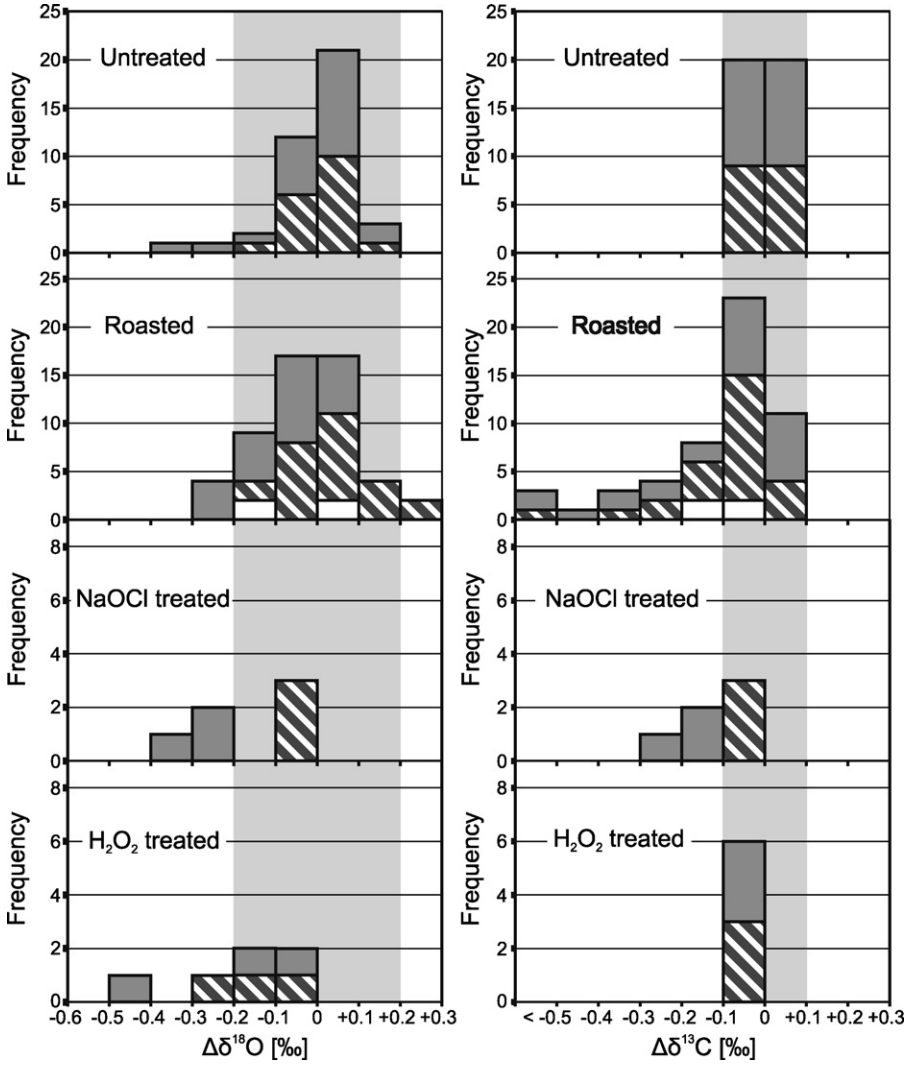

Fig. 2. Histograms showing the frequency distribution of deviations in $\delta^{18}O$ and $\delta^{13}C$ values of untreated and pre-treated Carrara marble samples (grey columns), synthetic calcite samples (striped columns) and synthetic aragonite samples (white columns) mixed and ground with organic matter, with respect to untreated, pure carbonates. Error areas are shaded.

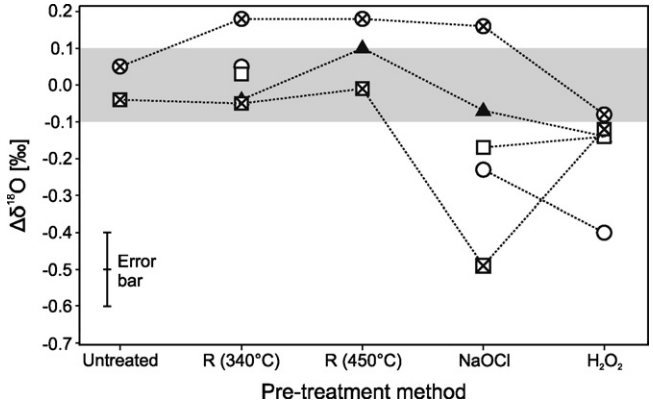

Fig. 3. Plot of deviations in $\delta^{18}O$ values of long-ground and pre-treated inorganic carbonates with respect to untreated samples of the homogenized carbonates. Synthetic aragonite (triangles); synthetic calcite (open circles); long-ground synthetic calcite (crossed circles); Carrara marble (open squares) and long-ground Carrara marble (crossed squares). Reproducibility area for untreated homogenized carbonates is shaded.

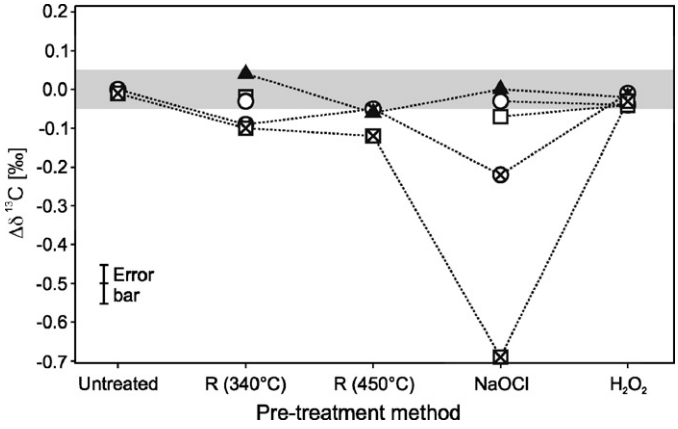

Fig. 4. Plot of deviations in $\delta^{13}C$ values of long-ground and pre-treated inorganic carbonates with respect to untreated samples of the homogenized carbonates. Symbols as in Fig. 3.

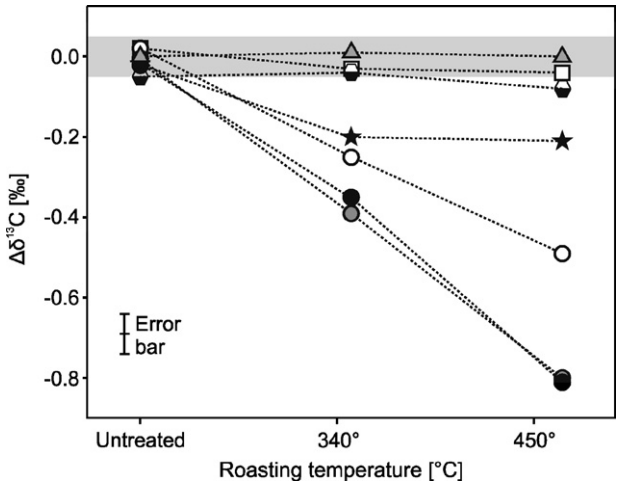

Fig. 5. Plot of deviations in $\delta^{13}C$ values of untreated and thermally treated Carrara marble samples ground with organic matter with respect to the untreated sample of the pure homogenized marble. Samples ground with 5 wt% of kerogen (triangles); samples ground with 5 wt% of coal (black-white hexagons); samples ground with 5 wt% of vanillin (squares); samples ground with 5 wt% of lysine monochloride (stars); samples ground with 5 wt% of glycine (open circles); samples ground with 10 wt% of glycine (grey circles) and samples ground with 15 wt% of glycine (black circles). Reproducibility area for the untreated sample of the pure homogenized marble is shaded.

Synthetic calcites ground with organic matter indicated no detectable deviations in the δ values after NaOCl treatment (Table 5; Fig. 2).

No deviations above the reproducibility limits was measured in δ values of the powdered and homogenized pure Carrara marble and the pure synthetic aragonite after NaOCl treatment (Table 5; Figs. 3 and 4).

The NaOCl treatment led to 0.49‰ and 0.69‰ depletions of ^{18}O and ^{13}C isotopes, respectively, in the long-ground pure Carrara marble (Table 5; Figs. 3 and 4). Modifications of $\delta^{18}O$ and $\delta^{13}C$ values of −0.32‰ and −0.27‰, respectively, were also revealed in Carrara marble ground manually with three organic substances and treated with NaOCl solution (Table 5; Fig. 2).

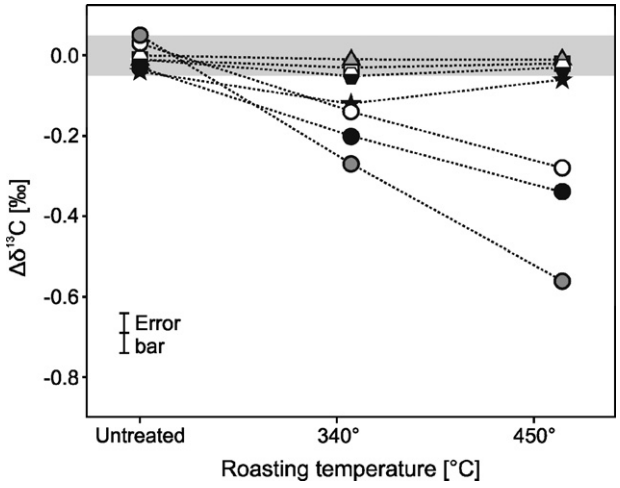

Fig. 6. Plot of deviations in $\delta^{13}C$ values of untreated and thermally treated synthetic calcite samples ground with organic matter with respect to the untreated sample of the pure homogenized calcite. Symbols as in Fig. 5.

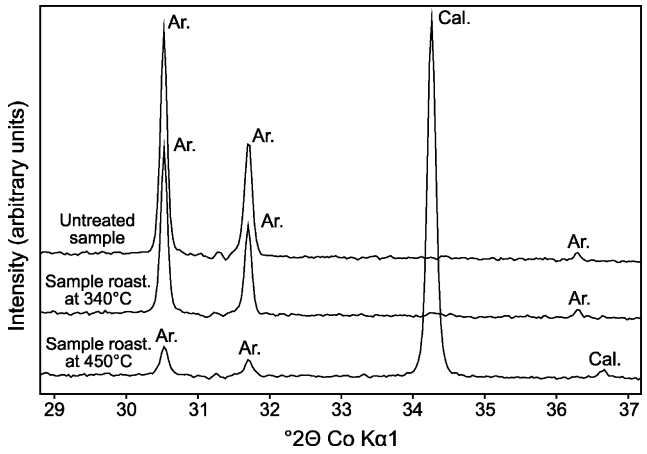

Fig. 7. X-ray diffraction patterns of synthetic aragonite untreated and roasted in vacuum at 340 °C and 450 °C. About 80% of original aragonite transformed to calcite in the sample roasted at 450 °C.

3.5. Inorganic samples treated with H_2O_2

The effect of H_2O_2 treatment on $\delta^{13}C$ values of all studied samples was within the reproducibility range of measurements (Table 5; Figs. 2 and 4). $\delta^{18}O$ negative offsets (0.30–0.46‰) were only stated in a powdered and homogenized pure synthetic calcite, the calcite ground with vanillin as well as a Carrara marble ground with coal (Table 5; Figs. 2 and 3).

3.6. Mineralogical alteration produced by thermal treatment

No transformation of mineral phase occurred after roasting of calcitic samples. Roasting of aragonite samples resulted in partial or complete conversion of aragonite to calcite. The calcite content in the inorganic postaragonitic sample reached 1–2% and 81% after roasting at 340 °C and 450 °C, respectively (Fig. 7). *Cardium* samples, which were originally composed of pure aragonite, did not alter at 200 °C. However, the calcite concentration in these samples increased from 8% to more than 99% after roasting at 340 °C and 450 °C, respectively (Fig. 8).

4. Discussion

4.1. Skeletal calcium carbonate

Modern carbonate skeletons contain less than 5 wt% of organic matter except for some algae, bryozoans, annelids and ostracods. For example, the nacre of molluskan shell contains between 1% and 5% of organics and the concentration of total organic carbon in corals is below 0.5 wt% [35,42,43]. Organic matter in carbonate skeletons occurs between and within calcium carbonate crystals forming so-called "intercrystalline" and "intracrystalline" phases. The organic matter usually consists of acidic glycoproteins or polysaccharides, which play important role in biomineralization processes. In addition, chitin–protein complexes constitute a framework of carbonate shells [44]. Organic molecules are present in calcareous fossils. Diagenetic

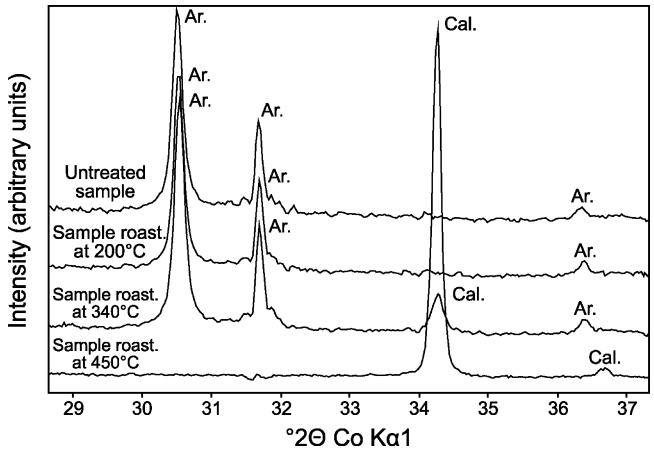

Fig. 8. X-ray diffraction patterns of *Cardium* samples untreated and roasted in vacuum at 200 °C, 340 °C and 450 °C. About 8% of original aragonite transformed to calcite in the sample roasted at 340 °C. The sample roasted at 450 °C is composed of pure calcite.

alteration of organic matter produces small breakdown products (e.g., amino acids, fatty acids and alkanes) and complex macromolecules [33,45]. Carbonate skeletons also contain water and hydroxyl ions. The amount of water in modern calcareous shells is estimated at up to 3 wt% [46,47]. Carbonate shells contain several water species: liquid water in fluid inclusions, water associated with an organic matrix, water bound to carbonate minerals and bound OH^- ions [47].

Because of a shielding effect of carbonate minerals the "intracrystalline" organic matter is considerably resistant to oxidizing treatments [48–50]. A significant contact area between both "intracrystalline" and "intercrystalline" organic matter (or water) and the shell carbonate may enable isotope exchange during roasting.

Processes leading to the creation of biogenic calcium carbonate, which is intimately bound with organic matter and water, cannot be mimic without precipitation of the mineral. On the other hand, the precipitation from solution containing organics may result in the isotope offset of the precipitated carbonate in comparison with the isotope composition of the pure carbonate, which grew under the same physical conditions. Therefore, only the prolonged grinding of inorganic carbonates (of known isotope composition) with organic substances was undertaken to obtain better mixing and coating of small crystal grains by organics.

4.2. *Effect of organic matter on isotope analyses of untreated samples*

A comparative study of untreated and pre-treated biogenic carbonates does not allow assessment of the effect of organic matter on the isotope analysis because one cannot differentiate between the isotope shifts caused by the isotope exchange with organic matter during treatments and the shifts in measured δ values resulting from removal of organic molecules from evolved CO_2. Both processes may theoretically result in negative bias in measured δ values [cf. 1–4,51].

A lack of any perceptible changes in δ values of untreated samples consisting of inorganic calcites or aragonite mixed and ground with glycine, lysine monochloride, oleic acid, vanillin and kerogen, collected with or without the indirect cold trap, indicates that these substances have no effect on isotope analyses of CO_2 derived from the decomposition of carbonates with orthophosphoric acid (Fig. 2; Table 4).

Measured $\delta^{18}O$ deviations in two samples consisting of Carrara marble and natural coal (−0.32‰ to −0.24‰) might be linked to organic matter or sulphide impurities (Table 4) [cf. 3,4]. However, the effect of coal on the $\delta^{18}O$ values of other untreated samples is undetectable (Table 4; Fig. 2). Significant depletions of ^{18}O (0.21‰ and 0.46‰) were additionally noted for two Carrrara marble samples ground with 5 wt% of coal that were roasted at 450 °C and soaked in the H_2O_2 solution, respectively (Table 5). It is apparent that H_2O_2 treatment caused no decrease in the δ values of Carrara marble samples. If the samples mixed with coal had contained organic impurities, which affected isotope analyses, the H_2O_2 treatment would have diminished their concentrations and as a consequence would have reduced the $\delta^{18}O$ bias. This is not observed. Therefore, the $\delta^{18}O$ isotope offset stated for two samples of the Carrara marble blended with coal may be linked to contamination of coal by natural carbonate.

Cellulose, glucose, mixture of short chain fatty acids and organic matter extracted from marine plankton using HCl are also reported to have no impact on isotope analyses of carbonates [20,28,29]. In addition, contamination of evolved gasses by nitrogen dioxide (NO_2), which was described for some samples of low carbonate content, was not an effect of the presence of organic matter but ammonium-bearing clay minerals [51]. Further studies are needed to determine effects of complex organic molecules on the isotope analysis including sulphur-bearing organic species, which were reported to affect measured isotope ratios of specific carbonates from sulphide ore deposits [4]. It is also necessary to carefully examine calcium carbonate skeletons of high organic matter content including those whose organic compounds were claimed to affect stable isotope analyses (e.g., coral *Montastrea* sp.) (see [3]). Nevertheless, it appears that organic matter does not affect isotope analyses of skeletal carbonates characterized by its relatively low contents.

It should be emphasized that the present observations are valid for the digestion of calcium carbonate with pure orthophosphoric acid at 25 °C in sealed vessels. Other methods of carbonate decomposition like remarkable excess of CrO_3 in acid, higher reaction temperatures, or abandonment of using cold trap may lead to obtaining impure gas or addition of CO_2 derived from the decomposition of organic matter.

4.3. *Effect of thermal treatment on isotope analyses*

Vacuum roasting is considered an equivalent of the roasting in a helium flow but it is regarded to be more reliable as its conditions are independent from the rate of gas stream [6–8]. Thus, current results should be relevant to assessment of both roasting techniques.

Isotope analyses of roasted samples indicate that the thermal treatment cannot significantly affect the isotope composition of

pure inorganic calcites (Table 5). This is consistent with observations of various authors [cf. 1,4,7,8,25]. The transformation of pure synthetic aragonite into calcite at 450 °C is associated with no alteration of the isotope composition of calcium carbonate (Table 5; Figs. 3 and 4).

Appreciable depletions of ^{18}O (>0.5‰) were found after helium flow roasting in skeletal calcites and aragonites or after vacuum roasting in aragonitic samples (Table 1). Current data (Table 3; Fig. 1) and the data presented by D'Eugenio and Leone [19], McConnaughey [20], Erez and Honjo [23] and Leone et al. [52] do not confirm the occurrence of remarkable deviations in oxygen isotope values of skeletal carbonates due to the thermal treatment (Fig. 1; Tables 1 and 3). Alteration of the measured isotope composition of roasted natural samples may be linked to removal of organic matter, isotope exchange with organics, water and evolved gases as well as to some decarbonation processes taking place above 400 °C [cf. 53]. The aragonite to calcite transformation may decrease activation energy necessary for the isotope exchange [5]. Therefore, higher ranges of isotope shifts that occur after roasting of skeletal aragonites or in calcite samples after poorly controlled helium flow roasting may be an indicator of the isotope exchange [cf. 16,21,26].

A lack of any detectable changes in $\delta^{18}O$ values and slight changes in (<0.18‰) $\delta^{13}C$ values of roasted inorganic calcites and aragonite mixed without grinding with organics indicates that the thermal decomposition of organic matter which is poorly mixed and has little contact area with calcite cannot affect significantly the isotope composition (Table 5). The effect of better-mixed organic matter on the isotope values of roasted inorganic calcites is different. Coal and kerogen cannot easily exchange oxygen and carbon isotopes with calcite likely because of their thermal stability and hardness, which disables strict joining with calcite particles (Table 5; Figs. 5 and 6). Oleic acid, vanillin and partly lysine monochloride, all of which are susceptible to thermal treatment, decompose or evaporate rapidly in the temperature that does not allow isotope exchange with calcium carbonate. Nonetheless, roasted reference calcites ground with glycine indicated significant depletion of ^{13}C (up to 0.81‰), and minor $\delta^{18}O$ deviations (−0.23‰ and +0.25‰ for the Carrara marble and the synthetic calcite, respectively; Figs. 5 and 6; Table 5). The $\delta^{13}C$ changes clearly resulted from the isotope exchange. A decrease (or increase) in the $\delta^{18}O$ values of the roasted inorganic calcites appears to be a consequence of the same process, although the direction of isotope shifts was different probably due to major dissimilarity in the oxygen isotope composition of the synthetic calcite and the Carrara marble (Table 5).

Glycine may partially survive thermal treatment at 400–500 °C. Its recovery is reported to be around 10% of the starting amount after pyrolysis at 400 °C [54]. Glycine as other neutral amino acids is easily sorbed on the calcium carbonate surface layer. The amount of the sorbed amino acids increases with a decrease in grain size and an increase in the specific surface area of carbonate particles [55,56]. Thus, the thermal stability and strict binding of glycine with calcite are likely responsible for the isotope exchange occurring during roasting of ground samples. This partly reflects situation found in skeletal carbonates, which consist of intimately bound carbonate minerals and the thermally persistent organic framework [44,57]. Organics may also facilitate isotope exchange between evolved gases and the solid carbonate [cf. 5].

The carbon isotope exchange between calcites and glycine is significant likely due to glycine carbon percentage, which is much higher than the oxygen content. Similar or lower oxygen to carbon ratio is distinctive of other amino acids and proteins. Therefore, these components do not appear to be responsible for observed decreases in the $\delta^{18}O$ values of roasted skeletal carbonates (Table 1). The reported $\delta^{18}O$ decreases (up to 1‰) could hardly be linked to removal of volatile organic matter impurities that interfere with 45 and 46 ion masses in the mass spectrometer since:

1. Many calcites or aragonites indicate smaller $\delta^{18}O$ shifts after roasting than a few vacuum roasted aragonites or calcite–aragonitic samples roasted in a helium flow (see Tables 1 and 3; Fig. 1) and there is no information that the latter samples are characterized by higher organic matter content.
2. NaOCl and H_2O_2 treatments do not cause a significant decrease in the $\delta^{18}O$ values of the same aragonites, which were roasted [cf. 16,17,21,26].
3. Organic sulphide impurities, whose impact on isotope analyses of $CaCO_3$ is well-demonstrated, affect measured $\delta^{13}C$ values to a much higher degree [4].
4. Epstein et al. [1] did not find detectable changes in measured isotope values of CO_2, derived from acidification of skeletal carbonates, after treatments used to remove impurities (treatment with Van Slyke oxidizing solution, mixing with traces of ammonia and water, mixing with bromine gas and irradiating, passing over hot CuO). Therefore, Epstein et al. [5] excluded the possibility of the interference of organic compounds with CO_2 molecules. Instead they claimed that the reaction of organic matter with phosphoric acid might liberate CO_2 different in the isotopic composition from that produced from calcium carbonate. However, this possibility seems to be minor or unlikely because of the reasons mentioned above (points 1 and 2) as well as due to little modifications of the $\delta^{13}C$ signatures of biogenic carbonates.

On the other hand, skeletal carbonates contain water and OH^- ions, which may be responsible for the oxygen isotope exchange [47]. Let us consider the water content of 2% as typical of skeletal carbonates [46,47,58]. The oxygen isotope composition of the water should be in equilibrium with $CaCO_3$ at the temperature of its growth and storing (about 20 °C). Thus, one can assume the calcium carbonate $\delta^{18}O$ value of −1‰ VPDB and the $\delta^{18}O$ value of its internal water of −0.07‰ VSMOW as calculated from the calcite–water fractionation factor given by Friedman and O'Neil [41] for 20 °C. The water may re-equilibrate its oxygen isotope composition with carbonate at elevated temperatures during roasting. The most significant loss of water during roasting of coral skeletons occurs at 270–335 °C [57]. Therefore, the temperature of ca. 200 °C, which is far below the limits of major water expulsion in corals, may approxi-

mate the conditions of the isotope exchange in roasted biogenic carbonates.

A maximal change in the $\delta^{18}O$ value of the skeletal calcium carbonate due to equilibration with internal water at elevated temperature may be calculated from the mass balance and equilibrium partitioning equations for a close fluid-rock system after Banner and Hanson [59]:

$$\delta^{18}O_{total} = \frac{(\delta^{18}O_w)(CO_w)F + (\delta^{18}O_c)(CO_c)(1-F)}{F(CO_w) + (1-F)(CO_c)} \quad (1)$$

where $\delta^{18}O_{total}$ is the oxygen isotope composition of the entire carbonate–water system, $\delta^{18}O_w$ and $\delta^{18}O_c$ the initial values of the water and calcium carbonate, CO_w and CO_c the weight concentration of oxygen in water and in calcium carbonate, and F is the weight fraction of the water in the system.

The $\delta^{18}O$ value of the calcium carbonate after the isotopic equilibration with water ($\delta^{18}O_{c,eql}$) is calculated from the equation after Banner and Hanson [59]:

$$\delta^{18}O_{c,eql} = \frac{(\delta^{18}O_{total})[F(CO_w) + (1-F)(CO_c)](\alpha_{calcite\text{-}water}) - 1000(CO_c)(1-F)}{(CO_c)(1-F)(\alpha_{calcite\text{-}water}) + (CO_w)F} \quad (2)$$

Substitution of the given values and the appropriate calcite–water fractionation factor for 200 °C [41] gives a −0.71‰ change in the $\delta^{18}O$ value of calcite due to potential isotope exchange with internal water. The sum of the calculated change in the $\delta^{18}O$ value of calcite caused by isotope equilibration with internal water and the measured shift in the $\delta^{18}O$ value of the roasted Carrara marble mixed with glycine (see Table 5) amounts to −0.94‰. This may approximate a maximal range of a change in the $\delta^{18}O$ values of skeletal carbonates due to roasting (see Table 1), although does not take into consideration possible decarbonation processes of natural samples [cf. 53]. It is worth noting that a 1‰ decrease in $\delta^{18}O$ may result in 4 °C error in palaeotemperature determinations based on oxygen isotope composition of skeletal calcium carbonate. The isotope bias introduced by roasting may also be a source of errors in commonly used paleotemperature equations that were established on roasted skeletal carbonates [cf. 5,60,61].

4.4. Effect of NaOCl treatment on isotope analyses

Significant decreases in the $\delta^{13}C$ and the $\delta^{18}O$ values of long-ground Carrara marbles along with a lack of the isotope alteration of the normally powdered and homogenized marble indicate that the increase in surface area of calcite particles (from 0.341 m^2/g to 2.23–2.33 m^2/g) enabled isotope changes after NaOCl treatment (Figs. 3 and 4; Table 5). 5% NaOCl is assumed to produce no appreciable dissolution of calcium carbonate after prolonged treatment [62,63]. However, observed changes in the $\delta^{18}O$ values of the bleached samples may result from the isotope exchange with NaOCl solution or the replacement of calcium carbonate by calcium hydroxide at exposed grain surfaces [cf. 62]. The latter process may also affect the $\delta^{13}C$ values.

A lack of any detectable changes in the oxygen isotope ratios of a majority of long-ground synthetic calcites may have been due to their low $\delta^{18}O$ value (−19.43‰ VPDB), which might have been closer to equilibrium with the solution than did the oxygen isotope composition of the Carrara marble (Fig. 3; Table 5).

Significant modifications of the oxygen and carbon isotope composition (>0.2‰) of some biogenic carbonates after NaOCl treatment were shown in both the present study and the geological literature (cf. Fig. 1; Tables 1 and 3) and [28]. However, the data presented suggest that negligible changes in the $\delta^{18}O$ values of skeletal and synthetic aragonites occur after the NaOCl treatment (Tables 3 and 5). This is consistent with results of Grossman and Ku [17] and Grottoli et al. [31] who reported that bleaching may cause minor shifts (<0.2‰) in the oxygen isotopic composition of skeletal aragonites.

Due to possible modification of the isotope composition the NaOCl treatment should be avoided in carbon isotope studies of aragonites and calcites as well as oxygen isotope studies of the latter. The NaOCl treatment does not remove all organic matter from natural samples [4]. Removal of organic matter from coarsely ground specimens by bleaching may be dubious due to a possibility of the occurrence of "shielding effects" of carbonate minerals.

4.5. Effect of H_2O_2 treatment on isotope analyses

Hydrogen peroxide dissolves calcium carbonate faster than deionized water and is more corrosive to calcite than aragonite [62,63]. However, Boiseau and Juillet-Leclerc [21] claimed that that there is no isotope exchange between $CaCO_3$ and the 30% H_2O_2 solution. Furthermore, observed changes in δ values of aragonitic corals after H_2O_2 treatment (on average −0.04‰ and −0.07‰ for the $\delta^{18}O$ and the $\delta^{13}C$ values, respectively) are only statistically detectable and there is no single correction factor for different coral species [31].

A lack of any detectable changes in $\delta^{18}O$ values of the majority of studied skeletal and inorganic samples after H_2O_2 treatment may confirm that the isotope exchange between the 30% H_2O_2 solution and calcium carbonates is of secondary importance for isotope analyses (Figs. 1–4; Tables 3 and 5).

Remarkable decreases in $\delta^{18}O$ values of powdered and homogenized synthetic calcite and synthetic calcite long ground with vanillin (Table 5; see also Fig. 3) indicate that partial dissolution may be responsible for the isotope alteration during H_2O_2 treatment. Coarser grinding of the former sample and the presence of vanillin, which might have hampered small-grinding of the latter sample, along with internal variations in the oxygen isotope composition of synthetic calcite crystals may have induced $\delta^{18}O$ offset after partial dissolution. It is of importance that long-ground synthetic calcite samples normally did not indicate $\delta^{18}O$ deviations after H_2O_2 treatment.

The 30% H_2O_2 treatment is assumed to cause minor modifications of the isotope signatures of calcium carbonate. However, attention should be paid to the possibility of partial dissolu-

tion of carbonate if studied samples consist of various carbonate phases or crystals that are characterized by internal differentiation of the isotope composition (fine-grinding could minimize isotope changes in the latter case). Besides, the 30% H_2O_2 treatment cannot remove all organic matter from natural carbonate samples and it is less efficient in removing organics than 2.5% NaOCl solution treatment [63].

5. Conclusions

1. Studied organic matter (simple amino acids, oleic acid, vanillin, coal and kerogen) is considered to have no effect on oxygen and carbon isotope analyses carried out by the calcium carbonate decomposition with pure orthophosphoric acid at 25 °C in sealed vessels.
2. Roasting treatment of skeletal calcium carbonate may lead to isotope exchange with organic matter—particularly carbon isotope exchange, which has been for the first time shown experimentally. However, negative deviations in $\delta^{18}O$ values of thermally treated skeletal carbonates may result from isotope exchange with internal water and such process cannot be experimentally evaluated.
3. NaOCl treatment often causes a decrease in $\delta^{18}O$ and $\delta^{13}C$ values of calcium carbonate, which has presently been demonstrated. However, negligible changes in $\delta^{18}O$ values occur after the NaOCl treatment of aragonitic samples. The NaOCl treatment is not recommended for calcitic samples or studies of the carbon isotope composition of aragonites.
4. H_2O_2 treatment induces minor isotope changes. However, it may result in partial dissolution of calcium carbonates, which is important for samples containing different calcium carbonate phases. The H_2O_2 treatment is less efficient in removing organic matter than the other methods [cf. 63].

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