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Characterization of naturally derived calcium compounds used in food industry

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In this work, naturally derived calcium containing compounds from dairy, corals, seashells, and bovine bones are analyzed and characterized using thermal analysis (TG/DSC), infrared spectroscopy (FTIR), X-ray diffraction analysis (XRD) and scanning electron microscopy (SEM). These methods are indispensable tools for identifying some special features of calcium compounds, their composition and structure as well as describing particle size and surface morphology. From the obtained results, functional properties, usage in food industry and theoretical bioavailability of these compounds are evaluated.

Key words: calcium, food industry, characterization, XRD, SEM, FTIR

INTRODUCTION

Health benefits of calcium compounds are widely acknowledged [1–6]. An adequate intake of both calcium and vitamin D is important for bone health and is recognized as an important component of any osteoporosis prescription-drug regimen. The risk of one or another health condition arises when people do not consume the Recommended Daily Intake (RDI) of calcium through their daily diet. Recent studies revealed that calcium intake is inadequate in both children and elderly people [7–9]. Calcium supplements and calcium-fortified foods are additional sources of calcium for people unable to consume sufficient dietary calcium [10, 11]. Calcium supplements are available in a variety of different calcium salts.

Apparently, both scientists and the general public are becoming increasingly aware of the importance of dietary calcium due to the vital role of this element in human body. Calcium, the most abundant mineral in the body, is found in some foods, added to others, available as a dietary supplement, and present in some medicines (such as antacids). Calcium is required for vascular contraction and vasodilatation, muscle function, nerve transmission, intracellular signalling and hormonal secretion, though less than 1% of total body calcium is needed to support these critical metabolic functions [12, 13]. Serum calcium is maintained within a narrow concentration range and does not fluctuate with changes in dietary intakes; the body uses bone tissue as a reservoir for, and source of calcium, to maintain constant concentrations of calcium in blood, muscle, and intercellular fluids [12–14].

The remaining 99% of the body's calcium supply is stored in bones and teeth [12]. Calcium is a major structural element in bones and teeth. The mineral component of the bone consists mainly of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] crystals, which contain large amounts of calcium and phosphate [13]. The bone is a dynamic system consisting of living cells embedded in a mineralized matrix, with constant desorption and deposition of calcium into new bones [15]. Balances between bones desorption and deposition change with age. Bone formation exceeds desorption in periods of growth in children and adolescents, whereas in early and middle adulthood both processes are relatively equal. In aging adults, particularly among postmenopausal women, bone breakdown exceeds formation, resulting in bone loss that increases the risk of osteoporosis over time [12–14]. Depending on age, gender and

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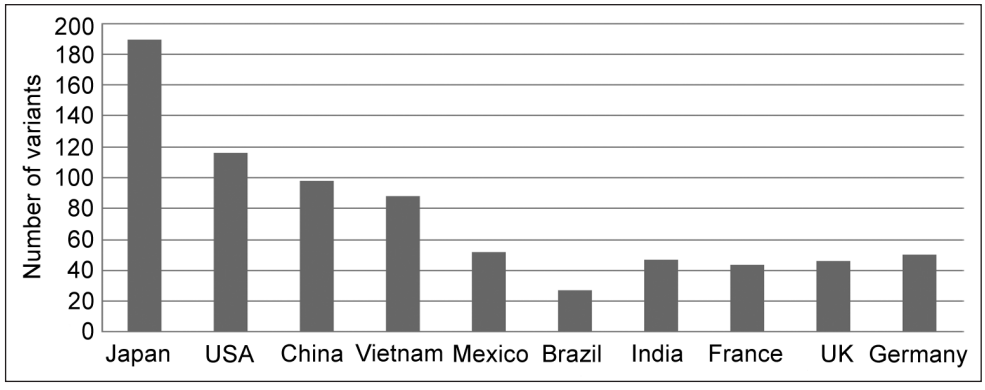

Fig. 1. Calcium fortified food production in 2010 by country (Mintel GNPD data)

medical conditions, the RDI of calcium slightly varies (i. e., from 200–260 mg for 1 year, 1 300 mg for 18 years, 1 000 mg for 50 years to 1 300 mg for more than 71 years) [12].

About 70% of dietary calcium in a human diet is available from milk and dairy products, mainly cheese in adults [16]. Non-dairy sources include vegetables, such as Chinese cabbage, kale, and broccoli (16% of intake) [12, 16]. Drinking water, including mineral water, provides 6% to 7% of dietary calcium [16]. Even though there are good dietary sources of calcium, studies demonstrate that several of the population segments mentioned above have low calcium intake [16, 17]. Dairy consumption and calcium intake remain low in most countries with large populations examined as compared with the recommended amounts of dairy products and calcium. Promotion of consumption of dairy products does not necessarily increase total calcium intake [17]. Thus, calcium supplementation and fortification have become increasingly important and calcium is being incorporated into food products around the world (Fig. 1).

Obviously, to maintain the proper composition of mineral in the bone, there must be adequate absorption and delivery of all bone minerals to bone sites in the human body. The scientific literature is lacking substantial information concerning maintenance of appropriate dietary mineral balances, particularly in relation to bone health. Some people have health issues that impair the absorption of calcium and may lead to poor calcium status [16]. Such factors include hypochlorhydria, a condition characterized by insufficient secretion of stomach acid. This condition affects many people and is especially common in the elderly. Due to the reasons (insufficient bioavailability of calcium from food, medical conditions, poor diet, etc.) demand of calcium fortified foods or calcium supplements arises in order to prevent calcium deficiency related diseases.

It is well known that bioavailability of different compounds depends very much on their concentration, chemical and phase composition, microstructure and morphology, crystal structure and other physical properties [18–23]. Therefore, in this article the chemical and phase composition, as well as the morphology of naturally derived calcium compounds (from dairy, corals, sea-shells, and bovine bones) used in food industry are analyzed and characterized.

EXPERIMENTAL

Calcium compounds for this work were selected from the ones that are already used in food and food supplements industry. All samples are derived from natural sources and their brand names as well as manufacturers are known for the authors. Samples were divided into three categories: coral / seashell calcium, dairy calcium and bovine bone calcium. In this part of the article, technological aspects of the production / extraction procedures of calcium compounds would be presented along with the rest of information and specifications received from suppliers.

Coral calcium. Coral calcium powder

This ingredient is produced from pristine white fossilized coral heads which are protected beneath a layer of soil. After corals are harvested using eco-friendly sustainable methods, they are taken to a grinding facility where they undergo a three stage grinding process before purification and water filtration. It is claimed that above sea coral does not contain pollutants such as high amounts of heavy metals, chemicals, fertilizers, etc. found in the contaminated oceans. The sample is available in 325 μm and 18 μm mesh powder.

Coral water

This sample is mashed coral grains with calcium and magnesium as major minerals present. Grains of coral water have porous structure and due to this feature they are able to absorb harmful impurities of regular tap water. When added to water, coral water sachets raise pH, buffer chlorine, provide antioxidants, add ionic minerals.

White oyster shell powder

Oyster Shell Powder for the preparation of this sample is harvested from oyster shell beds located in the Caribbean islands, hence its white colour. The sample is available in 325 μm mesh powder. According to the supplier's data, it contains 39% of calcium as well as at least 3% of magnesium and alkali salts.

Dairy derived calcium. Sample 1

It is a complex of natural milk minerals rich in calcium (Ca), phosphorus (P), and magnesium (Mg). It is made from milk

by means of a unique isolation process and jet-micronisation. According to the manufacturer, their product contains high levels of minerals from milk, mainly in the form of calcium phosphate and citrate complexes. This sample is distinguished by a fine particle size that is proved to prevent sedimentation.

Sample 2

It is a milk mineral concentrate that is obtained from selected cheese whey with a calcium content of 30% dry matter. It is available in standard (particle size <150 μm) and micronized (<7 μm) references. When micronized, this sample is told to avoid sandy sensation in mouth and minimizes settling in liquid products. This dairy calcium compound is extracted through physical process. It is known that besides calcium it contains Mg and P. Ca : P ratio is claimed to be optimal for a greater retention of calcium.

Sample 3

It is manufactured using a proprietary extraction technology combined with further purification techniques. This ingredient is the mineral fraction of milk that has been concentrated and spray dried. It contains milk minerals including calcium (24%), phosphorus (12.5%), magnesium (1.5%) as well as traces of other minerals such as potassium (0.8%), zinc (0.008%) and copper (0.0004%). There were conducted both *in vivo* studies and human clinical trial involving this ingredient. It was concluded that Sample 3 was more effective at increasing bone tensile strength and bone density in young growing rats than calcium carbonate. Human clinical study demonstrated that this ingredient was more effective than calcium carbonate at decreasing bone loss and building strong bones during six-week study.

This sample is also available in two references: with particle size of <7 μm (for optimizing suspension stability in neutral environments) as well as size <90 μm (greater solubility in acidic environment).

Bovine bone calcium

In this category one sample was selected, which is beef bone derived. It is manufactured from New Zealand sourced bone of export grade, free range, pasture-fed beef. This ingredient is manufactured using proprietary low temperature processes to ensure retention of bioactive components. It is told to contain minimum 24% calcium and 10% phosphorus in a microcrystalline structure, minimum 22% bone protein, including type I collagen and bioactive growth factors as well as a broad range of trace minerals and glycosaminoglycans. The particle size of the ingredient varies as follows: <850 μm, <250 μm and <150 μm.

The thermal decomposition of selected samples was analyzed through thermogravimetric and differential scanning calorimetric analysis (TG-DSC) using a Perkin Elmer STA 6000 Simultaneous Thermal Analyzer. Dried samples of about 5–15 mg were heated from 30 to 900 °C at a heating rate of 10 °C/min in a dry air flow (20 ml/min). All samples were characterized by infrared spectroscopy (FTIR), X-ray powder diffraction (XRD) analysis and scanning electron microscopy (SEM). The IR spectra were recorded as KBr pellets on a Perkin-Elmer FTIR Spectrum BX II spectrometer. The XRD studies were performed on a Rigaku miniFlex II diffractometer operating with Cu $K_{\alpha 1}$ radiation (step size: 0.02, time per step: 0.5 s). In order to study the morphology and microstructure of the samples a scanning electron microscope EVO 50 EP (Carl Zeiss SMT AG) was used.

RESULTS AND DISCUSSION

The XRD patterns of coral and seashell derived sample powders are shown in Fig. 2. The obtained *d* spacings were com-

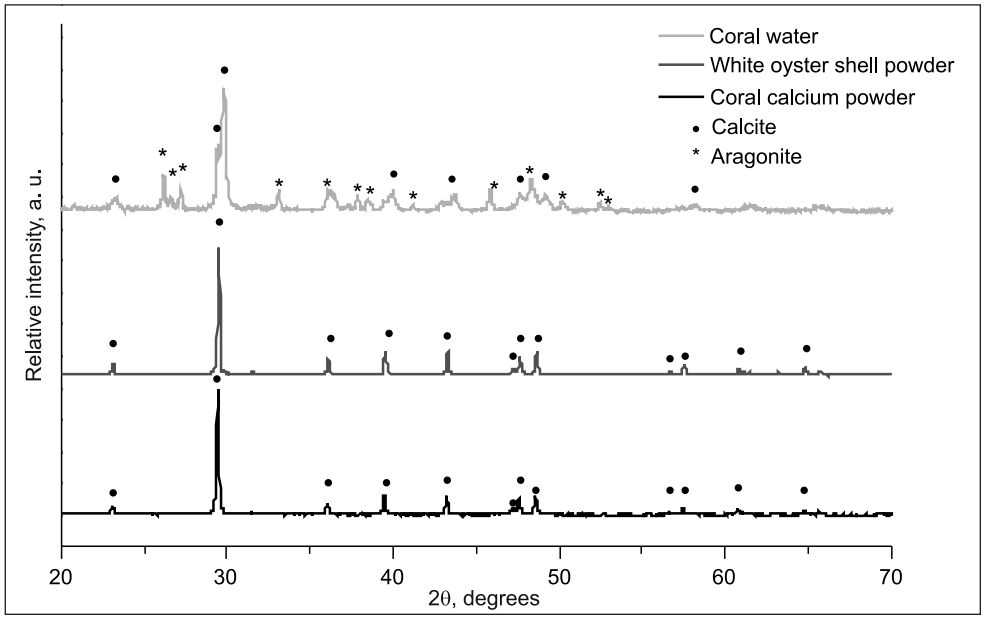

Fig. 2. XRD patterns of coral and sea shell derived samples

pared with the standard data (ICSD). It can be seen that only one component of the coral calcium powder sample is calcium carbonate in the form of calcite with a rhombohedral crystal system. The XRD spectrum of calcium water implies that the major compound present in this sample is calcite and, however, aragonite phase was also determined. Magnesium calcite, (Ca, Mg)CO_3, is a variety of calcite consisting of randomly substituted magnesium carbonate in disordered calcite lattice. XRD pattern of white oyster shell powders is also given in Fig. 2, indicating that the dominating crystal phase in this sample is calcium carbonate in the form of calcite having a rhombohedral crystal system. Data from the XRD analysis of all investigated coral and seashell derived samples are summarized in Table 1. According to XRD results, the predominant compound in the coral and seashell derived sample is calcite, however, in the sample of coral water the aragonite phase is also present.

The FTIR spectra of the corresponding coral and seashell derived compounds are presented in Fig. 3. As it can be seen, all three spectra are almost identical, confirming that the predominant compound contains ionic carbonate. The corresponding characteristic bands of stretching vibrations of CO_3^{2-} are at 1 083 cm^{-1} (ν_1), 875 cm^{-1} (ν_2), 1 433 cm^{-1} (ν_3) and 712 cm^{-1} (ν_4) [24–26]. The observed non-split peaks ν_2 and ν_4 in the FTIR spectra indicate the presence of calcite, and if splitting occurs, the aragonite phase is present. The band assigned to the adsorbed water is also present at 3 432 cm^{-1}. The absorptions observed between 3 000 and 2 850 cm^{-1} are due to vibrations of aliphatic C-H. As it was mentioned, the aragonite phase could be detected with the characteristic bands at 1 082 cm^{-1} and 700 cm^{-1}. The splitting of the 713 cm^{-1} band is characteristic only of the aragonite phase.

SEM micrographs of the corresponding coral and seashell derived compounds are displayed in Fig. 4. As seen from Fig. 4 (top, left), coral calcium powders consist of irregular shaped particles with a size of 5–10 μm forming porous agglomerates. The powders essentially show a mixture of

Table 1. **XRD data of coral and seashell derived samples**

Sample name	Mineral name	Space group	Crystal system	Cell parameters, Å	Cell volume, Å^3
Coral calcium powder	Calcite (ICSD [00-005-0586])	R-3c (167)	Rhombohedral	a = 4.989 c = 17.062	367.78
White oyster shell powder	Calcite (ICSD [00-083-1762])	R-3c (167)	Rhombohedral	a = 4.990 c = 17.061	367.85
Coral water	Aragonite (ICSD [00-076-0606])	Pmcn (62)	Orthorhombic	a = 4.960 b = 7.964 c = 5.738	226.65
	Calcite, magnesian (ICSD [00-071-1663])	R-3c (167)	Rhombohedral	a = 4.941 c = 16.864	356.55

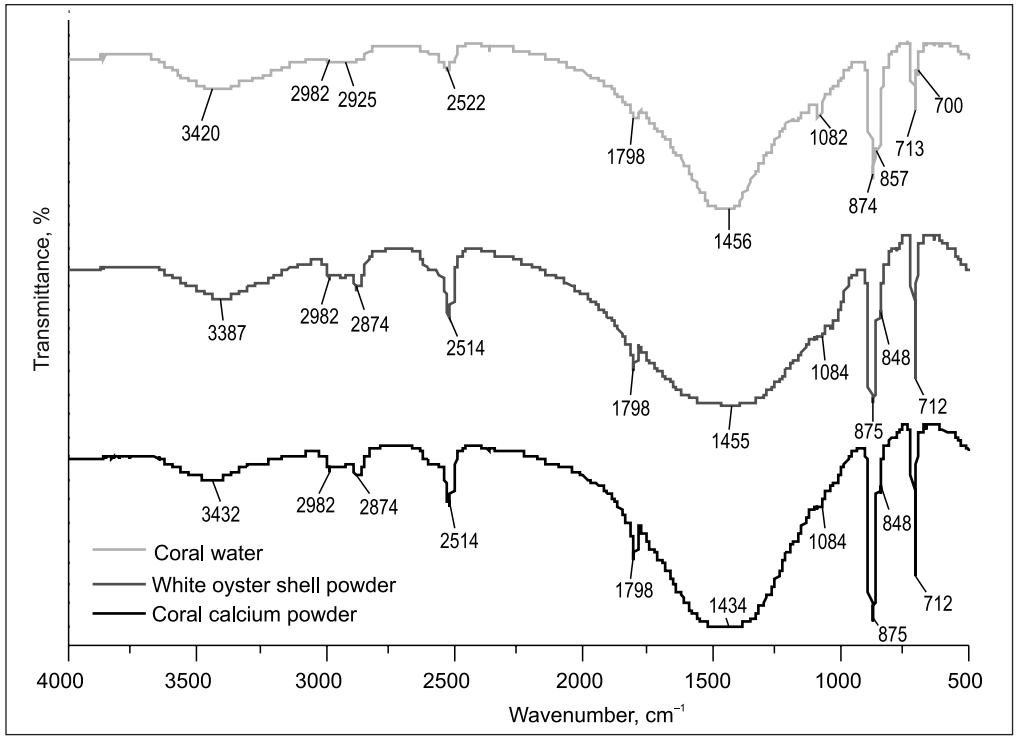

Fig. 3. IR spectra of the corresponding coral and seashell derived compounds

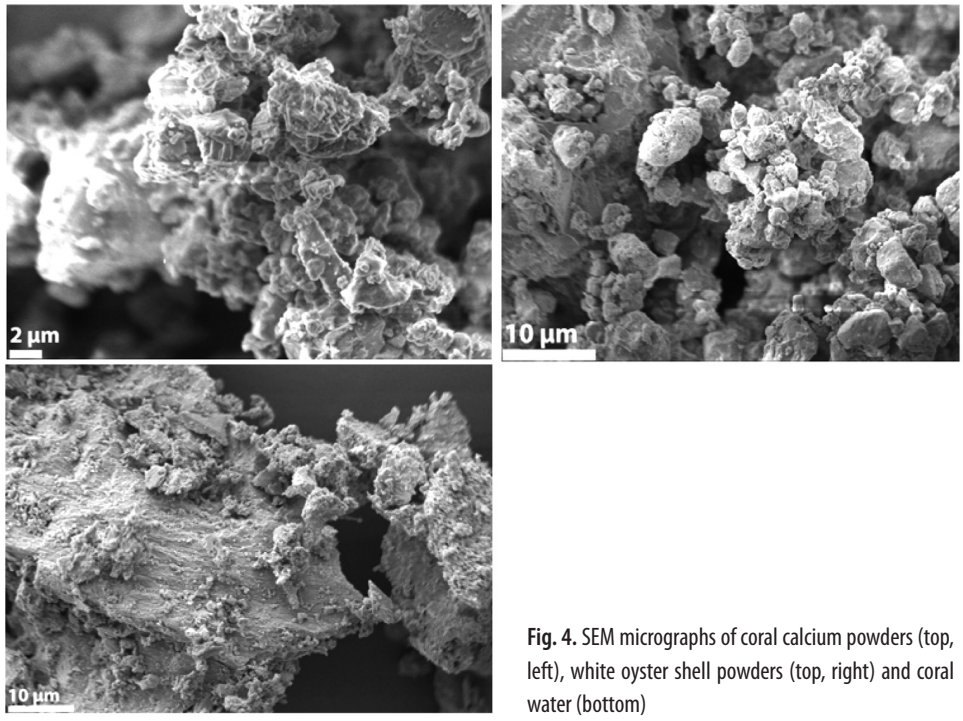

Fig. 4. SEM micrographs of coral calcium powders (top, left), white oyster shell powders (top, right) and coral water (bottom)

different size particles, whereas each particle is an assembly of numerous distinguishable angular grains with the size between approximately 5 and 10 µm.

In Fig. 4 (top, right), the surface morphology of white oyster shell powders is shown. It is obvious that grain shaped particles with a size of 2–10 µm tend to form porous agglomerates. The SEM image (Fig. 4, bottom) of coral water indicates that this sample consists of plate shape particles with a size of approximately 1–10 µm. It can be also observed that the particles form rigid agglomerates. In principle, a very similar surface morphology was determined for all three investigated samples.

TG/DTG and DSC curves of as-received samples of coral calcium and white oyster shell powders are shown in Fig. 5. As expected, analysis data for both samples are very similar. From 600 °C to 810 °C, a continuous weight loss was observed, which can be associated with decarbonation of the samples [27]. This conclusion is supported by the DSC analysis, which showed a small exothermic peak at about 800 °C [28]. There was no weight loss between 810 °C and 995 °C indicating that all carbonates were completely removed at about 800 °C. Total weight loss associated with heat treatment of coral calcium and white oyster shell powders was calculated to be approximately 44% in both cases. Thus, the thermal analysis results confirmed once again that the

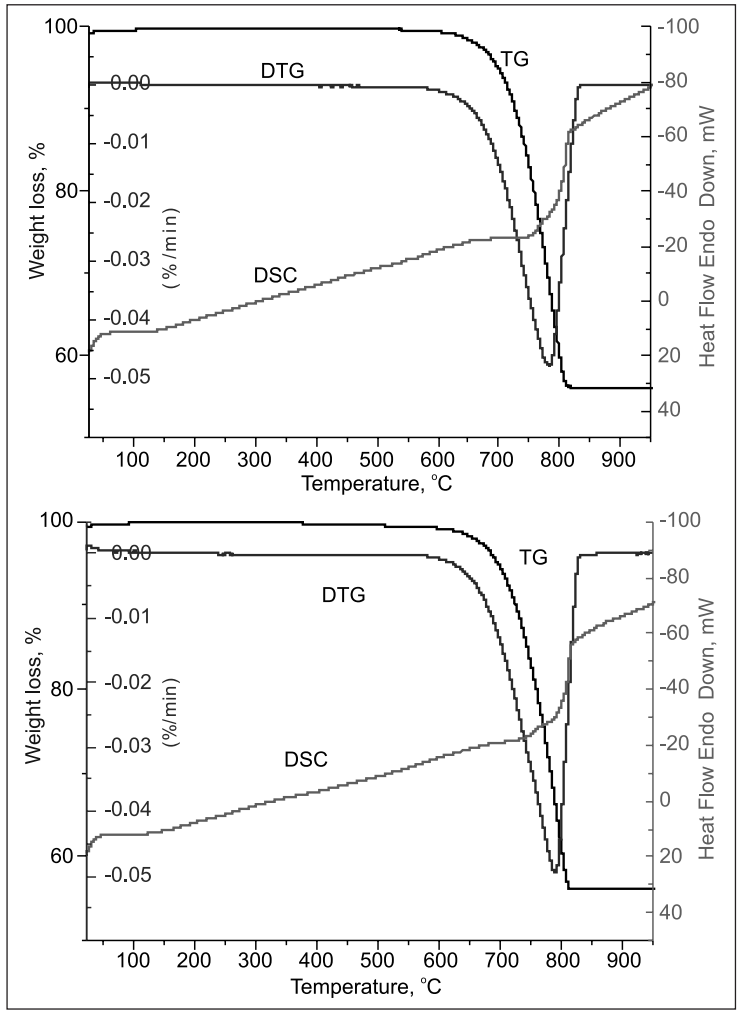

Fig. 5. TG/DTG-DSC curves of coral calcium (at top) and white oyster shell (at bottom) powders

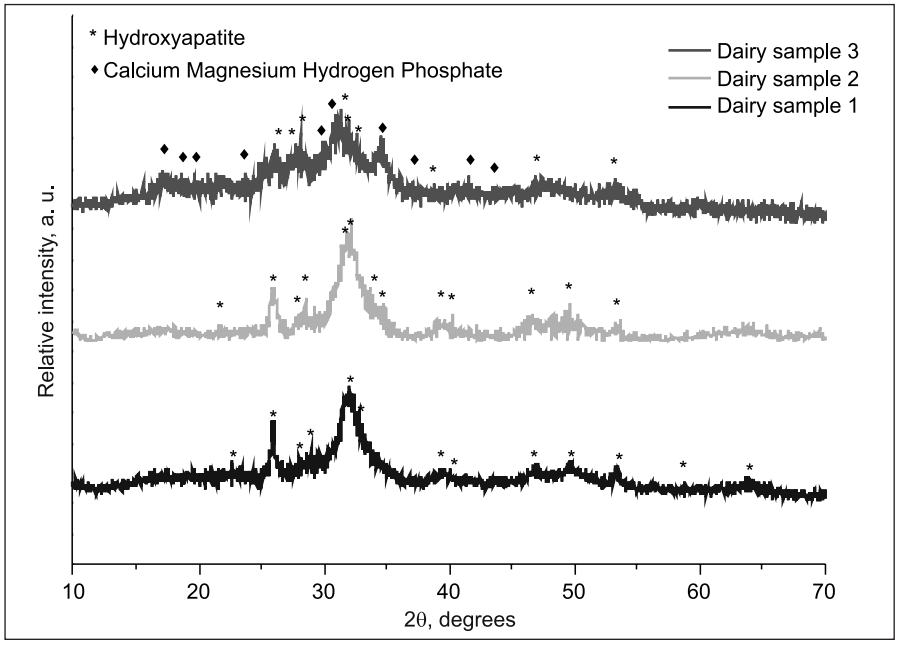

Fig. 6. XRD patterns of dairy derived samples

major compound presented in the investigated samples is $CaCO_3$. Calculations were based on TG data and the following reaction: $CaCO_3 \rightarrow CaO + CO_2$.

So we can conclude that limited usage of coral and seashell derived sample powders in food fortification is due to its low solubility. Therefore these calcium compounds could be mainly used in food supplements.

It is well known that the chemical composition of dairy derived calcium compounds highly depends on the isolation (extraction, filtration, precipitation, etc.) method used by the company. The XRD patterns of dairy derived powder samples are shown in Fig. 6. It is evident that the main crystalline component of Sample 1 is calcium hydroxyapatite (CHAp) with a hexagonal crystal system. However, the obtained XRD pattern has well-pronounced background. This observation let us to conclude that this sample consists of the amorphous phase along with crystalline. Apparently, the phase composition of Sample 2 is very close to the previous one. There were identified two crystalline phases, namely hydroxyapatite with a hexagonal crystal system and calcium magnesium hydrogen phosphate having a rhombohedral crystal system for Sample 3. XRD data from all dairy samples investigated during this study are summarized in Table 2.

FTIR spectra of the corresponding dairy derived compounds are shown in Fig. 7. All free FTIR spectra are almost identical. The absorption bands of significant intensity located at 1 037 cm^{-1} and 1 095 cm^{-1} could be attributable to the factor group splitting of the ν_3 fundamental vibration mode of PO_4^{3-} tetrahedral. The bands observed at 956–960 cm^{-1} and the doublet at 563–603 cm^{-1} correspond to ν_1 and ν_4 symmetric P-O stretching vibration of the PO_4^{3-} ion, respectively [29–33]. FTIR analysis also indicated that the apatites present in dairy Samples 1, 2 and 3 have CO_3^{2-} substitutions and hence the powders were carbonated hydroxyapatites. The characteristic band from the inorganic carbonate ion is present at 1 418 cm^{-1}. The ν_3 carbonate bands at 1 418 cm^{-1} and the ν_2 mode at 876 cm^{-1} (Fig. 6) correspond to carbonated calcium hydroxyapatite [24, 33], i. e. PO_4^{3-} substituted by CO_3^{2-}. A broad band located at 3 600–3 350 cm^{-1} as well as the band centered at ~1 600 cm^{-1} correspond to H_2O adsorbed on the surface [30].

SEM micrographs and EDX spectra of dairy samples are shown in Fig. 8. It could be observed that Sample 1 consists of

Table 2. **XRD data of dairy derived samples**

Sample name	Mineral name	Space group	Crystal system	Cell parameters, Å	Cell volume, Å³
Sample 1	Hydroxyapatite (ICSD [00-073-0294])	P63/m (176)	Hexagonal	*a* = 9.352 *c* = 6.882	521.26
Sample 2	Hydroxyapatite (ICSD [00-073-0294])	P63/m (176)	Hexagonal	*a* = 9.432 *c* = 6.881	530.14
Sample 3	Hydroxyapatite (ICSD [00-084-1998])	P63/m (176)	Hexagonal	*a* = 9.417 *c* = 6.875	527.91
	Calcium magnesium hydrogen phosphate; $Ca_{19.68}Mg_{.12}H_{1.8}(PO_4)_{13.80}$ (ICSD [00-079-2186])	R3c (161)	Rhombohedral	*a* = 10.360 *c* = 37.176	3 455.51

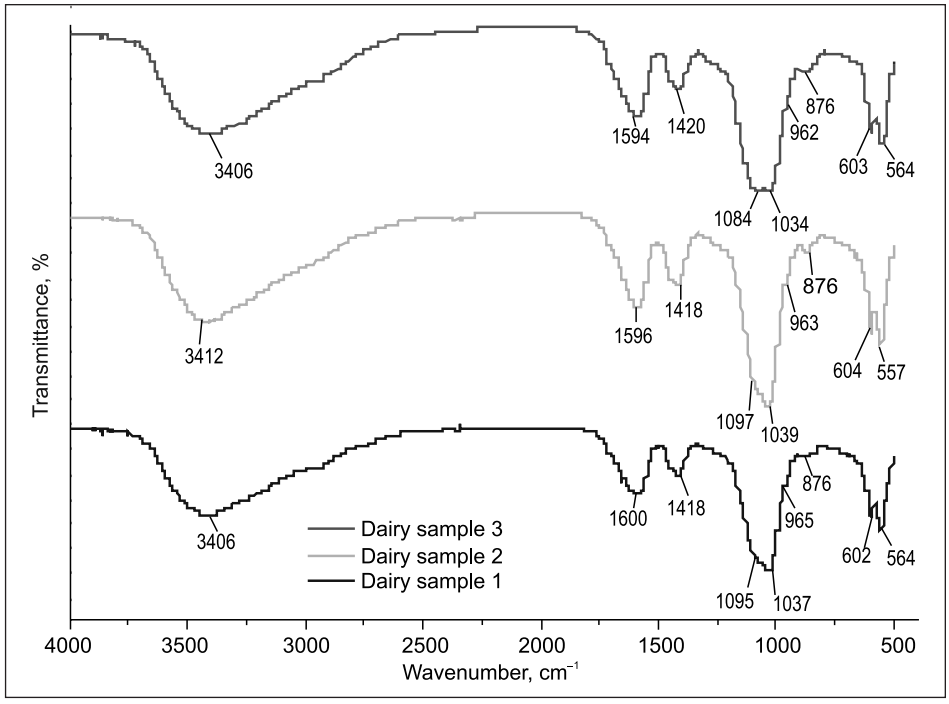

Fig. 7. IR spectra of the corresponding dairy derived compounds

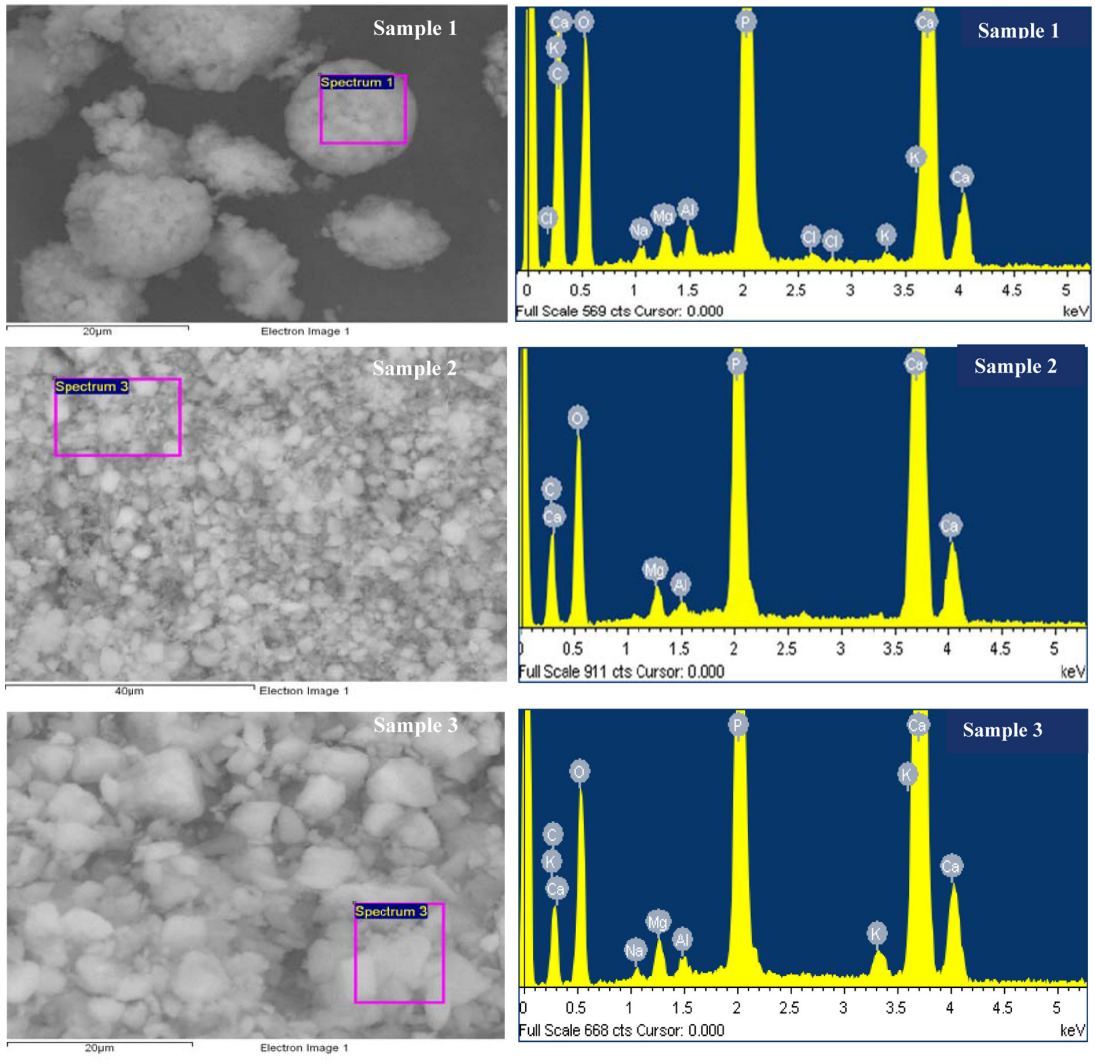

Fig. 8. SEM micrographs (at left) and EDX spectra (at right) of dairy samples 20 µm, 40 µm

porous spherical particles with a size of 10–15 μm. From EDX measurements, the Ca/P ratio was calculated to be 1.92. It also reveals that there are other minerals present in the sample, e. g. magnesium (0.45%). Grain shape angular particles with the size of approximately 2–5 μm are observed in the SEM image of Samples 2 and 3. Again, from EDX measurements, the Ca/P ratio was calculated to be 1.7 (Samples 2) and 1.51 (Samples 3). These samples also contain a minor amount of magnesium (0.98% for Samples 2 and 1.2% for Samples 3). Aluminium is observed in all three dairy samples due to the fact that samples were measured on the aluminium substrate.

Thus, dairy derived calcium compounds have higher solubility and could be widely used for the enrichment of food (dairy products, beverages, biscuits, ice cream, etc.) and food supplements.

Fig. 9 presents the XRD pattern of the bovine bone derived sample. It shows the presence of nanocrystalline calcium hydroxyapatite (ICSD [00-086-0740]) in the bone matrix. The FTIR spectrum of the beef bone sample is shown in Fig. 10. It indicates the presence of ionic phosphate (PO_4^{3-}), hydroxyl (OH^-) and carbonate (CO_3^{2-}) groups in the sample. Moreover, the N-H stretching band around 2 928 cm^{-1} and

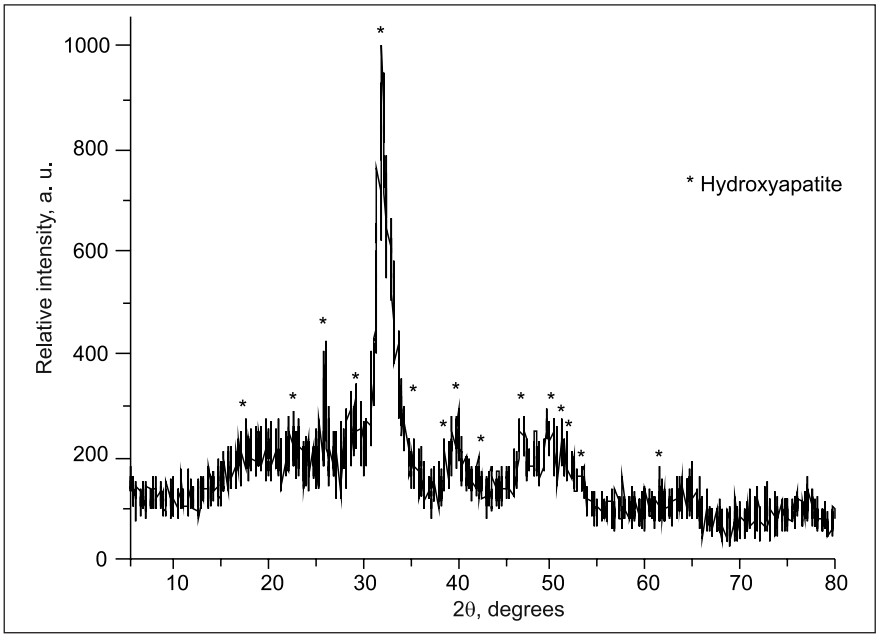

Fig. 9. XRD pattern of the beef bone derived sample

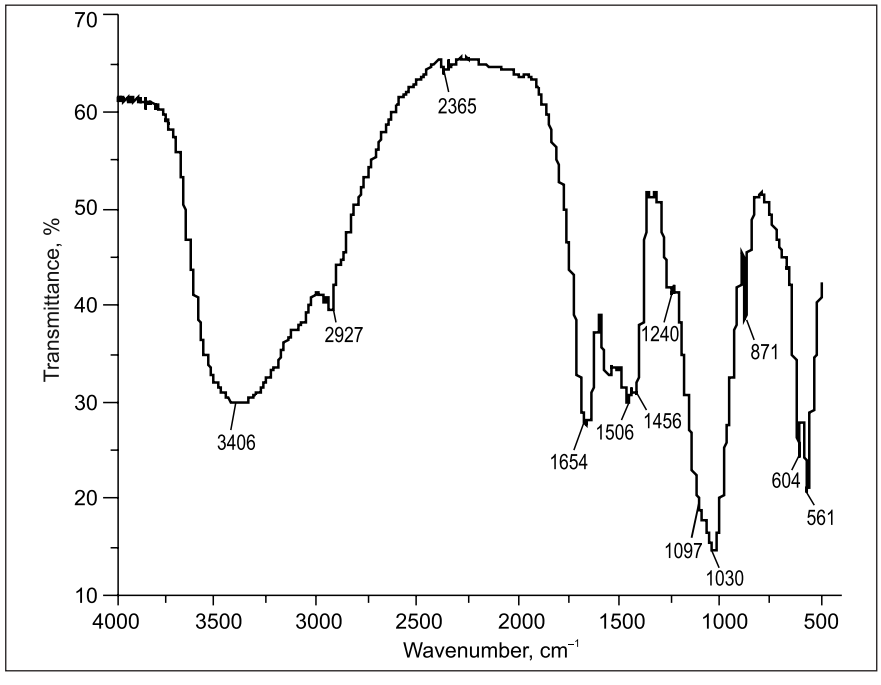

Fig. 10. IR spectrum of the beef bone derived sample

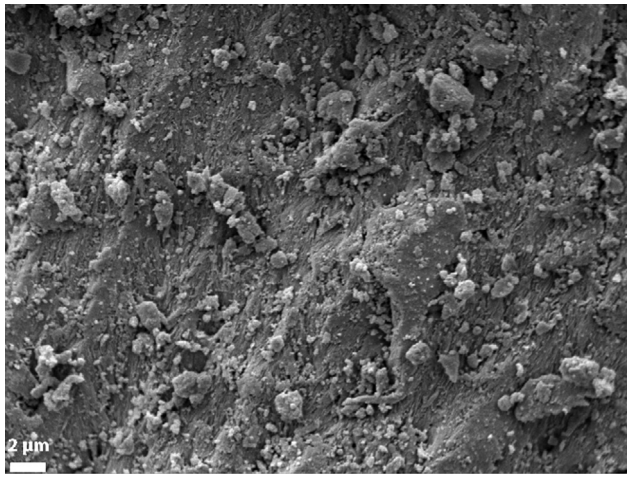

Fig. 11. SEM micrograph of as-received bovine bone

amide bands at 1 506 cm^{-1} were also observed [34–36]. These two bands are characteristic of protein macromolecules in the bovine bone matrix, collagen in particular. Fig. 11 shows the representative SEM picture of as-received bovine bone. The microstructure of this sample appears to be dense due to the presence of organic substances in the beef bone matrix.

Since beef bone derived calcium material is predominantly composed of calcium hydroxyapatite with a microcrystalline structure, it could be suggested for usage in food supplements.

CONCLUSIONS

Naturally derived calcium containing compounds from dairy, corals, sea-shells, and bovine bones vary significantly in their size, composition, chemical environment as well as the amount of calcium present depending on their natural origin. Coral and seashell derived calcium compounds are predominantly composed of calcite. However, in the sample of coral water the aragonite phase was also detected. SEM micrographs of coral and seashell derived calcium compounds indicated that irregular shaped particles are 1–10 µm in size forming agglomerates. One of the benefits of coral and seashell calcium compounds is sufficient porosity. Due to these natural pores, coral calcium has more surface area allowing stomach acid to come in contact more efficiently for easier absorption by the body. The dairy derived calcium compounds investigated in this study were composed mainly of calcium hydroxyapatite or a mixture of CHAp and calcium magnesium hydrogen phosphate. Additionally, amorphous phases along with crystalline ones co-exist in the samples. SEM and EDX analyses showed that samples of the same origin vary highly in their size and shape (from 5 µm to 20 µm and from spherical particles to agglomerates). One of the advantages of dairy derived calcium compounds is that they offer an optimal Ca/P ratio that contributes to efficient calcium absorption. Beef bone derived calcium material is predominantly composed of calcium hydroxyapatite. The microcrystalline structure of bovine bone ensures its absorption and bioavailability. The bone also contains protein, which enhances absorption and promotes bone formation. It is mainly suggested for usage in food supplements.

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KALCIO JUNGINIŲ, RANDAMŲ GYVOJOJE GAMTOJE IR NAUDOJAMŲ MAISTO PRAMONĖJE, APIBŪDINIMAS

S a n t r a u k a

Gyvojoje gamtoje (jūros kriauklėse, koraluose, pieno produktuose ir galvijų kauluose) randami kalcio junginiai buvo tirti terminės analizės (TG/DSC), infraraudonosios spektroskopijos (FTIR), rentgeno spindulių difrakcinės analizės (XRD) ir skenuojančios elektroninės mikroskopijos (SEM) metodais. Parodyta, kad šie tyrimo metodai yra nepakeičiamos priemonės norint nustatyti gyvojoje gamtoje randamų kalcio junginių savitus bruožus, cheminę bei fazinę sudėtį, sandarą, paviršiaus morfologiją ir dalelių dydį. Gauti kalcio junginių apibūdinimo rezultatai leido pateikti rekomendacijas galimam jų panaudojimui maisto pramonėje.