Preliminary studies on the valorization of animal flour ash for the obtainment of active glasses

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Abstract

Animal flour ash, rich in phosphorous, calcium and alkaline oxides, has been used to formulate (i) controlled-release fertilizers, since they manage to release the nutrient elements (P, K) at a low rate, and (ii) bioactive glasses.

(i) Four formulations were tailored using different amounts of animal flour ash (35–48 wt%), potassium carbonate (10–25 wt%) and a fixed amount of glassy sand (40 wt%) in order to get glasses and glass-ceramics. The materials were characterised from a chemical (XRF), crystallographic (XRD) and microstructural (SEM/EDS) point of view. Moreover, in order to check the ability to release the macro- and micro-nutrients, tests were conducted to determine the kinetics of glass dissolution in different media (2% citric acid solution, 1% hydrochloric acid solution and ammonium citrate solution). The results obtained allowed to confirm all samples show a very low solubility in water (less than 1%) and high values (>40%) of P, Ca, K and Na in the other media.

(ii) The rich content of phosphorous and calcium oxides makes the animal waste-derived ash a potential low-cost raw material to produce bioactive glasses. The analysis was focused on a bioactive glass, named BG_Ca, whose composition comes from the standard Bioglass® 45S5, got by increasing as much as possible the CaO content to combine a controlled behaviour during processing and a good apatite-forming ability in a simulated body fluid (SBF).

This preliminary investigation shows that animal flour ash is a versatile material, which may be successfully used for several applications as various as the production of fertilisers and the preparation of bioactive glasses.

1. Introduction

The slaughter bovine industry generates a significant amount of residues and by-products of animal origin that could be enhanced through recovery process and/or further transformation instead of simply being disposed as waste.

On the other hand, the National and European legislation in force since 2002 (derived mainly from the need to prevent and contain the spread of BSE – Bovine Spongiform Encephalopathy – and other transmissible encephalopathies) has allowed very limited technological options for the treatment of waste from animal origin.

Normally, animal by-products are generated on a regular basis throughout the year and they are only transformed into flour. The animal flour is in fact made from the carcasses of slaughter, dried and compressed to remove fat. The resulting powder, which is characterised by a specific smell and a yellow-brown colour range, can be used as an alternative fuel because of its high heating value.

Among the different methods of energy recovery from animal waste, the most obvious one is the direct combustion (incineration) with energy recovery (the so-called "energy plant"). It should be specified that the direct incineration of by-products like this is hardly feasible because of their high moisture content. Therefore they are usually subjected to energy-intensive treatments appropriately designed to reduce the moisture content. The ashes used in the present study were produced by an experimental facility simulating a fixed-bed gasifier. In this way, the animal waste is converted into ash, which is enriched in phosphorous (P_2O_5=30 wt%), calcium (CaO=40 wt%) and alkaline oxides such as Na_2O and K_2O (~6 wt%). On account of the chemical composition of the ash, it was possible to hypothesise its use to prepare active glasses where the presence of P compounds imparts specific features.

In particular, in this work, preliminary studies on the valorisation of this animal flour ash have been performed with the aim to verify its suitability to obtain active glasses such as: (i) controlled-release fertilizers rich in nutrient elements (P, K) and (ii) bioactive glasses. Indeed, in spite of their dramatically different applications, both controlled-release fertilizers and bioactive glasses must contain an appropriate amount of phosphorous and alkaline oxides, and therefore

animal flour ash may represent an interesting option to produce them in place of conventional raw materials.

The ecological technologies leading up to the decrease of chemical treatments, that are aimed to the reduction of the pollutant impact of agricultural activities on the environment and oriented towards the obtainment of agricultural products with superior biological quality are supported and privileged by the European Community, with respect to intensive agriculture that utilises synthetic fertilizers and pesticides in order to satisfy the requirements of nutrients and to control bad diseases, and damages [1]. Conventional fertilisation, for example, can lead to some environmental risks, such as soil nutrient leaching. Therefore, it is important to use fertilisers that can reduce the nutrient input by optimising the mineral uptake thus limiting the risk of nutrient loss in the environment. An 'ideal' fertiliser has to release nutrients slowly, according to plant demand, in relation to its phenological stage. Controlled-release fertilisers can provide nutrients according to plants' needs and growth phases, so they can be added in a relatively low amount. In the last few years, the use of traditional fertilizers with a sparingly soluble coating has been proposed. The main advantages of new fertilisers, over classical ones, are various: they offer an increased grade of assimilation by plants; they do not release insoluble compounds in soil; they remain in soil during the entire period of plant development, and they do not pollute the water phreatic layers [2], [3], [4] and [5].

In this context, glass matrix fertilizers are a new kind of fertilizers, made of a vitreous matrix with a low and controlled solubility in water (composed by macro elements useful for plants, such as K, P, Mg) and there is the chance to incorporate many useful microelements in the vitreous matrix, such as Mo, B, Mn, V, Fe, Zn, etc., which are necessary for plants growth and development. The quantity of microelements as oxides is 1–5%.

Some researchers prepared fertilizer glasses using pure grade reagents as starting materials [6], [7], [8] and [9] and several studies have been patented [10] and [11], but only few contributions reported the production of glass matrix fertilisers using low-cost raw materials or residues as components [12].

As far as bioactive glasses (BG or bioglasses) are concerned, they are special glasses, which are able to directly bond to bone tissue after implantation. The implant-tissue integration is mediated by the development of a hydroxyapatite surface layer at the interface with bone tissue [13], since hydroxyapatite (HA, $Ca_5(PO4)_3(OH)$) is the main mineral component of bone.

Several bioactive glasses for orthopaedic applications are based on the $Na_2O–CaO–P_2O_5–SiO_2$ system, which was previously investigated by Hench and co-workers [14]. The easiest way

to produce such bioactive glasses consists in melting them from commercially available raw powders, often including Na_2CO_3, $CaCO_3$, $Ca_3(PO_4)_2$ and SiO_2, which provide the required elements for the final glass composition. Raw materials on the market usually ensure a very controlled purity grade, but they are quite expensive. For this reason, in the present contribution a bioactive glass of known composition was formulated by using the animal flour ash in substitution of commercial tricalcium phosphate ($Ca_3(PO_4)_2$) with a consequent economical and environmental saving. The analysis was focused on a glass, named BG_Ca, whose composition was derived from the standard Bioglass® 45S5 [15] by increasing as much as possible the CaO content [16], in order to control its thermal behaviour during sintering and manufacturing processes [17]. Great attention was paid to the reaction of the glass in a simulated body fluid (SBF), i.e. a solution mimicking the blood plasma of mammalians [18], in order to fix the consequence of the use of the animal flour ash, instead of a conventional commercial raw powder, on the ability of the glass to develop a hydroxyapatite (HA) surface layer when it is exposed to a physiological environment.

2. Experimental

This work is focused on development of active glasses starting from waste materials, in particular animal flour ash, according to two different guidelines: the first one is planning and realizing fertilizers glasses, the second one is aimed to the formulation of bioactive glasses.

2.1. Fertilizer glasses

Fertilizer glasses have been prepared starting from: (1) animal flour ash produced by a pilot plant simulating a counter-current fixed bed gasifier; (2) glassy sand supplied by Sasil S.p.A (Biella, Italy) derived from a secondary processing of the scraps from treating packaging glass waste and (3) potassium carbonate of industrial grade.

On the basis of the chemical analysis carried out on the starting materials (Table 1), in order to obtain the series of glasses and glass-ceramics, four formulations were tailored using different amounts of animal flour ash (35–48 wt%), which supplies mainly P_2O_5 and CaO, ceramic grade potassium carbonate (12–25 wt%) and a fixed amount of glassy sand (40 wt%), which acts as SiO_2 carrier. The formulations are reported in Table 2. The particle size distribution of the glass was measured by a laser particle-size analyser (Mastersizer 2000 Ver. 5.22 Malvern Instruments Ltd.).

Table 1. Chemical analysis (wt% oxides) of the materials used.

Main oxides (wt%)	Ash	Glassy sand	K$_2$CO$_3$
CaO	41.12	10.00	0.05
P$_2$O$_5$	29.20	0.00	0.00
Na$_2$O	3.89	12.68	0.46
SiO$_2$	1.72	71.30	0.00
MgO	1.37	2.23	0.00
K$_2$O	2.72	0.92	74.17
Fe$_2$O$_3$	0.36	0.37	0.02
SO$_3$	0.13	0.00	0.00
Al$_2$O$_3$	0.95	2.00	0.00
Cl	1.21	0.00	0.00
L.O.I	(1150 °C) 17.04	(450 °C) 0.17	(450 °C) 25.30

Table 2. Composition of the different batches.

Mix	Ash (%)	Glassy sand (%)	K$_2$CO$_3$ (%)
35–40–25	35	40	25
40–40–20	40	40	20
45–40–15	45	40	15
48–40–12	48	40	12

After a pre-treatment at 900 °C with a heating rate of 10 °C/min, all mixes were melted in refractory crucibles following a thermal cycle with Tmax: 1450 °C, heating rate of 10 °C/min and 60 min of soaking time. The final material was quenched in cold water and subsequently dried for 24 h in an oven at 105 °C.

The above materials were characterised with different analyses. The chemical composition of the glasses was determined by scattering wavelengths X-ray fluorescence (XRF, Bruker S8 TIGER endowed with the QUANT EXPRESS SPECTRA plus software package). For the XRF analysis, samples were prepared in fused bead form using a XRF S Phoenix Scientific bead maker furnace and Pt/Au crucible and mould. The beads were obtained by melting at ~1150 °C a homogeneous mixture of 1 g of glass powder (<63 µm), 10 g of Li-borate mix (34% LiBO$_2$+66% Li$_2$B$_4$O$_7$) and 2 drops of LiBr.

Phase identification was carried out by qualitative X-ray diffraction (XRD, BRUKER D8 Advance diffractometer) using Ni-filtered CuKα radiation and operating at 40 kV and 30 mA. For XRD, powder samples (sieved<63 µm) were scanned in the 5°–60° (2θ) interval at a scanning rate of 0.5°/min.

The microstructure of the glasses was examined by field emission scanning electron microscopy (FESEM, JEOL model JSM 6500F) using an acceleration voltage of 20 kV. The SEM specimens were polished using 6, 3 and 1 µm diamond pastes after grinding with silicon carbide paper and water. The polished surfaces were etched for 10 s in a 5 vol% HF solution, washed ultrasonically with distilled water and ethylic alcohol, dried and subsequently Au–Pd coated in a Balzers SCD 050 sputter. Secondary electron images (SEI) were used for microstructural examination. Semi-quantitative analyses of different phases were obtained by energy dispersive X-ray spectroscopy (EDS) by using a Link eXL detector equipped with a beryllium (Be) window. The distribution of ions among the different phases was determined by means of digital X-ray mapping, which is an imaging technique used to examine the two-dimensional distribution of elements in a specimen. Each two-dimensional map is associated with a single element and the colour variations on the map represent differences in weight percentage of the elements from point to point.

Besides, in order to check the releasing ability of macro- and micro-nutrients present in the formulations, kinetics of glass dissolution were conducted in different media and conditions.

The eluates were analysed by Inductively Coupled Plasma (ICP Varian Liberty 200) detecting the main components of the glass, namely Si, Ca, P, K, Na, Al, Fe. The detection limits for the instrument used in the present research are as follows: Si: 18 ng/mL; Ca: 6 ng/mL; P: 33 ng/mL; K: 20 ng/mL; Na: 1.5 ng/mL; Al: 18 ng/mL; and Fe: 2.5 ng/mL.

For each test the glassy materials were ground and sieved following the requirements of the specific rules. Indeed the European norm (CE 2003/2003) prescribes grain size <500 μm, treatment time 30 min, 35–40 rpm, whereas the Italian legislation (D. Lgs. 75/2010) indicates grain size <100 μm, treatment time 30 min and 300 rpm for soluble P determination; they both consider water as leaching medium.

Furthermore other different release media, following CE 2003/2003, were used:

–2% citric acid solution (30 min, <100 μm, Troom), which is a common root exudate and thus suitable to simulate plant action. In order to investigate the long-term release of nutrients more carefully, a period of 21 days was investigated too [19];

–ammonium citrate solution (<500 μm, 60 min, pH=7, T=65 °C).

Finally, 1% hydrochloric acid solution (<100 μm, 30 min, Troom) was also tested to study the effect in strong acid condition, according to the Italian Legislation.

2.2. Bioactive glass

As previously mentioned, the tailored formulation of the BG_Ca glass derives from the standard Bioglass® 45S5, but it is characterised by a higher content in CaO and a lower content in Na2O (Na$_2$O: 4.7 wt%; CaO: 42.3 wt%; P$_2$O$_5$: 6 wt%; SiO$_2$: 47 wt%) [16] and [17].

Taking into account the chemical composition of the animal flour ash reported in Table 1, according to the oxide contents, the raw materials in powder form (animal flour ash, and reagent-grade Na$_2$CO$_3$, CaCO$_3$ and SiO$_2$ by Carlo Erba Reagenti, Italy) were properly weighed as reported in Table 3. As a term of comparison, Table 3 lists also the raw materials which would be required to produce the same BG_Ca glass, using standard commercial products (reagent-grade tri-calcium phosphate instead of animal flour ash).

Table 3. Raw materials required to obtain the BG_Ca glass (using flour ash and using commercial raw powders only).

Raw material	Weight (g/100 g batch)	
	With ash	*With Ca$_3$(PO$_4$)$_2$*
Animal flour ash	15.6	//
Ca$_3$(PO$_4$)$_2$	//	10.2
Na$_2$CO$_3$	4.9	6.2
CaCO$_3$	44.8	47.8
SiO$_2$	34.7	35.8

The powders were weighted, mixed in a rotating sealed vessel for 2 h and then melted in a platinum crucible at 1450 °C for 1 h (heating rate from room temperature to 500 °C:5 °C/min; heating rate from 500 °C to 1100 °C:10 °C/min: isothermal step at 1100 °C:90 min; heating rate from 1100 °C to the maximum temperature: 10 °C/min). The molten glass was poured in a pre-heated graphite mould, introduced in a kiln previously pre-heated at 650 °C and then annealed at this temperature for two hours. Afterward, the glass was left to cool down slowly inside the kiln.

Thus, the obtained glass bulk was cut into 1 cm×1 cm×0.5 cm regular pieces, which were polished with a 2000 grid SiC grinding paper, rinsed in distilled water and used to perform in-vitro tests according to the procedure proposed by Kokubo [18]. The glass samples were immersed in a simulated body fluid (SBF) for increasing times of 1, 3, 7 and 14 days. The plastic containers were held at 37 °C in a controlled environmental chamber (MPM Instruments S.r.l., Bernareggio-MI, Italy). Even if the plastic containers were sealed, the humidity in the chamber was set to 90%, so as to limit the SBF evaporation. During the test, the soaking fluid was replaced every two days to create dynamic test conditions. After the extraction from the SBF, each sample was gently rinsed in bi-distilled water and left to dry in a closed and safe place. The samples were observed with an environmental scanning electron microscope (ESEM Quanta 200-FEI Company, Eindhoven, The Netherlands) equipped with an X-ray energy dispersion spectroscopy (X-EDS) system (Inca, Oxford Instruments, UK). The microscope was operated in low-vacuum mode (pressure: 60 Pa) not to metalize the sample surface; back-scattered electron (BSE) images were acquired to investigate the surface morphology. The analysis by X-EDS aimed at detecting the nature of the elements on the surface and to monitor the Ca-to-P ratio, which may be useful to notice the possible precipitation of calcium phosphates, such as HA. The surface of the samples soaked in SBF was also characterised by means of micro-Raman Spectroscopy (Jobin-Yvon Raman Microscope spectrometer; diode laser emitting at 632.8 nm, output power of 20 mW at the sample). For each sample, a spectrum collection setup of 10 acquisitions, each of them taking 60 s, was used.

In addition, in order to evaluate the nature and concentration of the elements released by the glass in SBF, the in vitro tests were repeated in static conditions. In particular, the glass pieces were soaked for 1, 3, 7 and 14 days, but the soaking fluid was not replaced meanwhile. After each extraction, the corresponding soaking fluid was chemically analysed by ICP. The analysis was focused on Na, Ca, Si, K, Mg, Fe, Al. In fact, the nominal composition of the glass includes Na, Ca, and Si, whereas the presence of K, Mg, Fe, Al in the glass and the possible release of such elements in SBF may result from the use of the animal flour ash as a raw material (see

following section). The detection limits are the same as before; for Fe the detection limit is as 2.5 ng/mL. To complete the analysis, the concentration of chlorides was evaluated with the Mohr titration method (method sensibility+0.6%).

3. Results and discussion

3.1. Fertilizer glasses

From the data reported in Table 1 it is possible to observe a high content of CaO and P_2O_5 in the ash due to its bone origin. Other oxides such as Na_2O, SiO_2, K_2O and MgO permit to confirm that this kind of ash has mainly inorganic composition and it is not hazardous. The as-received ash contains an unburned fraction of organic carbon corresponding to 4.64% (elementary analysis not shown here) and a percentage of inorganic carbon of 1.02% (this value was calculated from carbonates content). The ash used in this study is constituted by hydroxyapatite ($Ca_5(PO_4)_3(OH)$) (JDCS-00-073-0293), as main phase, calcite ($CaCO_3$) (JDCS-01-072-1937) and $Ca_9MgK(PO_4)_7$ (JDCS-01-088-0798). The glassy sand (Table 1) is a commercial product obtained from the secondary treatment of glass cullet of packaging. The particle size distribution of the glassy sand supplied by the producer indicates that the 90% has a diameter lower than 710 µm and the 50% of the particles present a diameter lower than 283 µm and only a small fraction of particles is below 73 µm.

From the chemical point of view, it is a sodium–calcium silicate glass. The particle size distribution after the treatment process is within 100–800 μm. To conclude, the potassium carbonate of ceramic grade used to produce the fertilizer glasses has a 74% K_2O content.

The chemical analyses (XRF) of the glassy frits are reported in Table 4. It may be noted that, as the ratio between ash and K_2CO_3 decreases, the content of Ca and P reduces and the amount of K greatly increases. Instead no significant changes (variation <0.5 wt%) can be observed in the percentages of Mg, Fe and Al oxides and the other elements present in small quantities.

According to the Statement of the European Union on low release fertilizers [1], a fertilizer is classified as "PK" type if the content of each element (P and K) is higher than 5 wt% (individually) and the total content (P plus K) is higher than 18 wt% (together). All of the glass compositions are within this range.

Table 4. Chemical analysis (XRF) of the glasses obtained.

Oxide (%wt)	35–40–25	40–40–20	45–40–15	48–40–12
SiO_2	33.21	34.00	33.09	31.90
CaO	22.92	24.21	26.24	26.37
P_2O_5	13.45	14.79	16.74	16.87
K_2O	17.33	13.08	10.28	8.17
Na_2O	8.50	8.40	8.41	8.07
Al_2O_3	2.06	2.91	2.71	5.53
MgO	1.68	1.70	1.59	1.68
Fe_2O_3	0.44	0.47	0.48	0.49
Cl	0.33	0.22	0.39	0.50
Other oxides	0.40	0.38	0.40	0.56

The glasses were characterised by X-ray diffraction in order to identify the crystalline phases eventually developed during thermal treatment or during cooling. Fig. 1 shows that, as the amount of animal flour ash increases, the obtained materials have a partially glassy nature, thus confirming their glass-ceramic nature. The mix 48–40–12 with high amount of ash is characterised by a high crystallisation degree. The peaks, well defined and visible, correspond to a new crystalline phase rich in sodium and calcium phosphate (specifically: $Na_3Ca_6(PO_4)$; JDCS-00-011-0236). Other weak peaks are imputable to crystalline phases of flour ash.
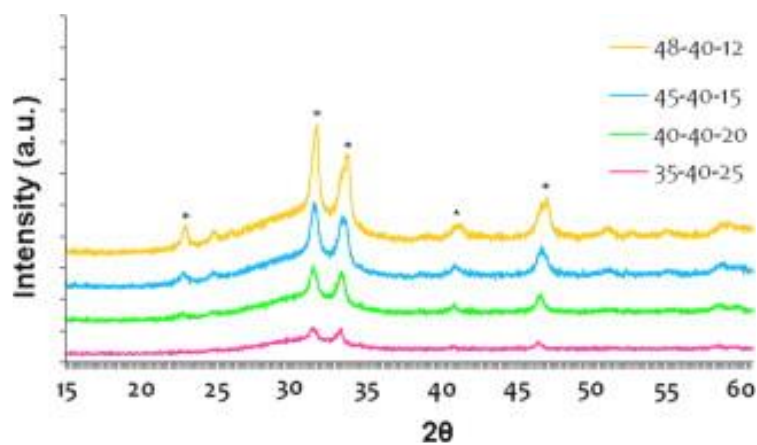

Fig. 1. X-ray diffraction spectra of the obtained glassy materials (*)=$Na_3Ca_6(PO_4)$ (JDCS-00-011-0236). From bottom to up flour ash amount increases from 35 to 48 wt%.

The behaviour and degree of crystallinity of the mixes 45–40–15 and 40–40–20 are very similar. They have peaks of crystallisation smaller and slightly shifted with respect to the mix 48–40–12, however, such variation does not indicate the formation of a different crystalline phase but it is due to the rapid cooling and to the fact that the starting materials are not perfectly homogeneous. The mix 35–40–25, which is characterised by the lowest ash content, turns out to be nearly amorphous. Indeed, even if its spectrum is similar to those of the other mixes, the intensity of the peaks is extremely low. No other crystalline phases are present (i.e. K_2CO_3 because of its easy solubility in a sodium–calcium silicate melt).

In Table 5 the chemical analysis of mix 48–40–12 is reported. In particular columns 1–3 correspond to analysis of single crystals, identified as points 1–3 in Fig. 2(a); these crystals are rich in CaO, P_2O_5 and Na_2O, thus confirming the presence of traces of $Na_3Ca_6(PO_4)$ phase, identified by XRD analysis. On the contrary, the average composition of the material, column 4, is characterised by a greater percentage of SiO_2 due to the predominant presence of glassy phase.

Table 5. EDS chemical analysis of different areas for 48–40–12 composition.

	Crystal 1	Crystal 2	Crystal 3	Sample average composition
CaO	47.07	61.14	43.57	30.71
P_2O_5	36.00	26.28	36.20	17.57
Na_2O	8.02	5.73	10.18	6.30
K_2O	3.47	4.68	3.29	9.18
Al_2O_3	3.26	1.24	3.29	6.09
SiO_2	2.12	0.92	3.28	28.29
MgO	0.07	0.00	0.18	1.23
FeO	0.02	0.01	0.01	0.64

Fig. 2 shows microscopic images of glass mix 48–40–12. The image 2(b) confirms the presence of glass spherical phase separation, inclusions dispersed within the amorphous matrix can be observed. Other authors have shown that silicate–phosphate glasses of the $NaCaPO_4$–SiO_2 system are also characterised by the occurrence of the phenomenon of glass phase separation [20].
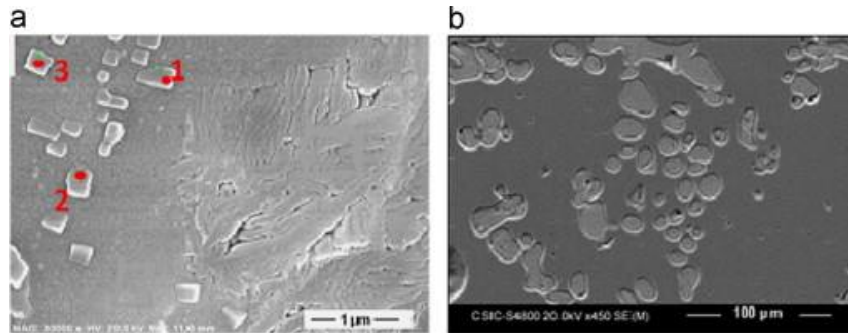
Fig. 2. SEM micrograph of mix 48–40–12 (a) single point analysis of the sample and (b) phase separation.

Mapping analysis (Fig. 3) shows that the inland areas are rich in P and Ca (Fig. 3b and e) confirming EDS analysis of crystals while Si (Fig. 3c) remains localised in the glassy matrix. For K and Na (Fig. 3f and d) the distribution is less separated between matrix and crystals. In particular Na is more abundant in crystals, confirming XRD results ($Na_3Ca_6(PO_4)$) than in the matrix; on the contrary K is more concentrated in the matrix (see column 4 in Table 5) with respect to crystals. It can therefore be assumed that the residual glass is mainly constituted by Si, Al and K according to SEM/EDS micro-analysis.
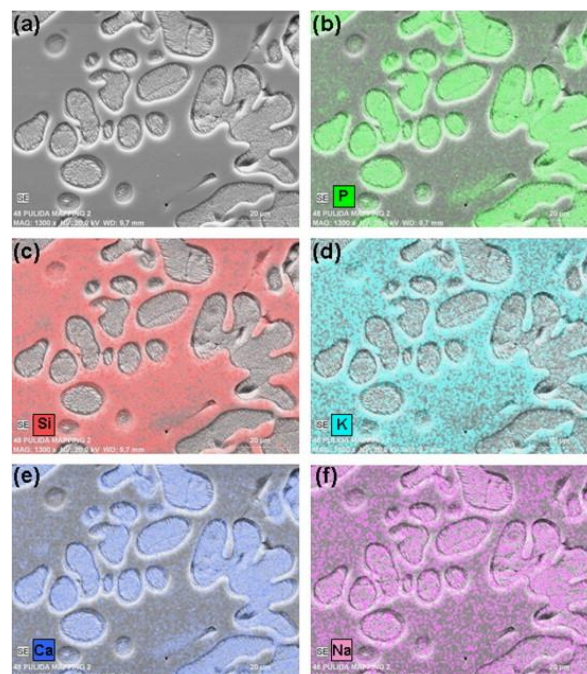

Fig. 3. (a) EDS analysis of mix 48–40–20 and mapping analysis of (b) P, (c) Si, (d) K, (e) Ca and (f) Na.

In order to check the ability to release macro- and micro-nutrients present in the formulations, the kinetics of glass dissolution were conducted in different media, namely water (30 min.), 2% citric acid solution (30 min and 21 days), 1% hydrochloric acid solution (30 min), and ammonium citrate solution (pH=7, 60 min). It was possible to detect a very low solubility in water for all compositions, independently of the test conditions (Table 6). Vice versa the percentage of nutrient elements (P and K) released in 2% citric acid solution, 1% hydrochloric acid and in neutral ammonium citrate are very high, mainly around 70–80% as observed by Waklawska and Szumera [8] for glasses containing a comparable amount of phosphorous (15 wt%). In fact, Fig. 4 clearly shows the higher leaching capacity of both the elements by strong acid, followed by weak acid and salt.

The comparison between the tests performed in 2% citric acid ($C_6H_8O_7$) solution for 30 min and 21 days highlights the effect of time on the kinetic of dissolution of the macro-nutrients (Table 7).

Table 6. Comparison of percentage dissolved in water according to Italian law ($*$) and European Standard ($**$).

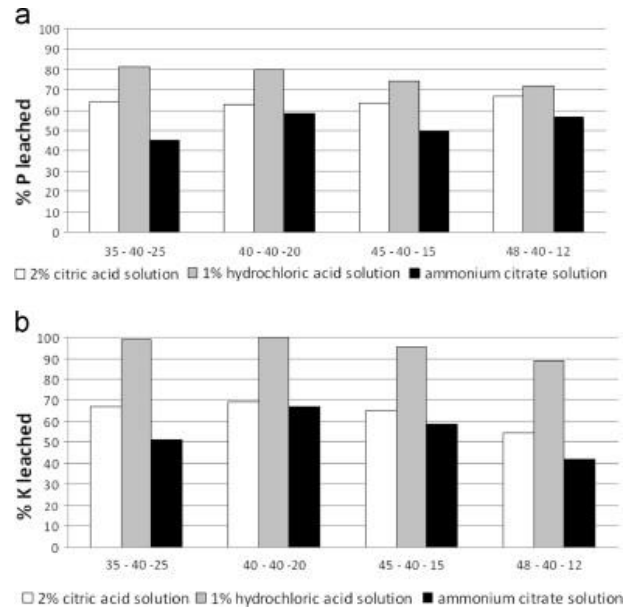| Element	35–40–25		40–40–20		45–40–15		48–40–12	
	($*$)	($**$)	($*$)	($**$)	($*$)	($**$)	($*$)	($**$)
Si	0.30	0.25	0.23	0.07	0.14	0.03	0.03	0.03
Ca	0.09	0.35	0.33	0.24	0.30	0.21	0.35	0.27
P	0.37	0.42	0.44	0.27	0.39	0.25	0.46	0.35
K	1.07	0.53	0.95	0.33	0.70	0.28	0.92	0.35
Na	0.48	0.21	0.36	0.14	0.28	0.11	0.30	0.15
Al	0.11	0.04	0.01	0.01	0.01	0.01	0.01	0.01

Fig. 4. Phosphorus (a) and potassium (b) leached percentage for all compositions in different media.

Table 7. Percentage released in citric acid after 30 min and 21 days.

Element	35–40–-25		40–40–20		45–40–15		48–40–12	
	30 min	21 days	30 min	21 days	30 min	21 days	30 min	21 days
Si	5.50	4.05	5.98	5.10	5.03	5.34	6.96	12.61
Ca	57.50	68.40	58.98	78.42	57.73	77.83	55.15	77.78
P	64.18	78.07	62.64	84.46	63.73	100.00	66.79	100.00
K	66.94	80.75	69.05	95.49	65.14	91.73	54.32	100.00
Na	65.82	73.41	67.43	84.03	67.55	86.24	11.17	100.00
Al	51.55	88.50	50.87	100.00	40.39	100.00	36.40	100.00
Fe	53.90	71.43	53.99	79.56	47.74	76.39	36.48	98.25

Looking at the compositions, for long periods the 48–40–12 system shows complete release percentage for the highest number of elements (P, K, Na and Al), while for short times, it evidences lower release for the elements contained in the glassy phase (K, Na and Al). From obtained data, it is possible to hypothesise that the crystallisation degree improves the release of

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nutrient elements as a function of time. Si release, on the other hand, shows a different behaviour in composition 48–40–12, in fact, for long time all the samples present constant Si release except 48–40–12 one. This composition shows a double leaching of Si for 21 days. This can be imputable to a weakening of the glassy structure due to the higher content of flour ash and traces of crystalline phases as observed by Waklawska and Szumera [8] for glasses with high content of phosphorous (15 wt%).

It can therefore be assumed that some of the formulations prepared have a high release in the short term, but it is maintained below certain levels and does not reach complete dissolution, which is instead the case of the mixes 48–40–12 and 45–40–15.

3.2. Bioactive glass

The BG_Ca glass produced using animal flour ash was perfectly transparent and homogeneous, without any appreciable trace of phase separation and/or devetrification. This suggests that the animal flour ash may be effectively used as a raw material to obtain glasses. Moreover, as proposed in Table 3, the animal flour ash provides the glass not only with phosphorous, but also with other useful elements such as sodium, calcium and silicon, thus reducing the required amounts of all the commercial raw materials. However, as revealed by the XRF analysis (Table 1), the animal flour ash also contains additional elements, such as potassium, iron and aluminium, which may alter the bioactivity of the original glass [21]. For this reason, great attention was paid to the glass behaviour in SBF. Even if the SBF represents a simplified model of a physiological environment, since it is both protein- and cellular-free [22] and [23], it is very useful for a preliminary evaluation of the bioactivity. In fact, since the development of a HA layer at the interface between bone tissue and implanted glass is required for the glass integration in vivo, the in vitro formation of such a layer is usually considered as a fundamental prior condition [18].

As shown in Fig. 5(a), the surface of the as-cast and polished glass (not immersed in SBF) is perfectly flat. After soaking in SBF, the surface is progressively covered by dome-like aggregates (Fig. 5(b)–(e)), "cauliflower-like" structures are frequently observed in the literature on the surface of bioactive materials soaked in SBF and they are generally identified as hydroxyapatite precipitates [24]. The globular precipitates are clearly detectable already after one day in SBF and they gradually cover the entire surface for increasing immersion times. The chemical analysis by EDS is consistent with the identification of the precipitates as HA formations, since calcium and phosphorous are predominantly present, with a Ca/P ratio rising

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from 1.42 to 1.85 (Fig. 6). These values are comparable to the Ca/P ratio of stoichiometric HA, which is 1.67 [25], and they are similar to the data usually reported in the literature for the HA precipitated in SBF on bioactive glasses [26].
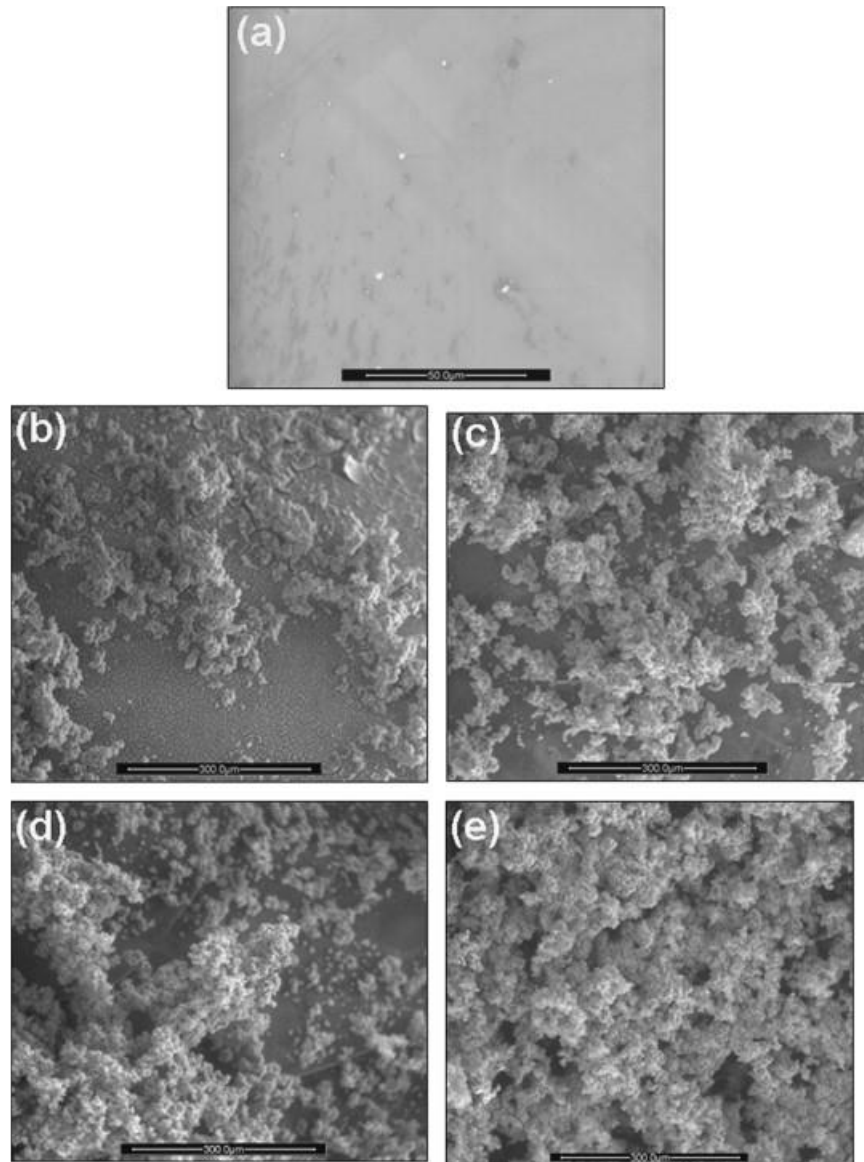

Fig. 5. Surface of glass samples as-cast (a) and after soaking in SBF for 1 day (b), 3 days (c), 7 days (d) and 14 days (e).
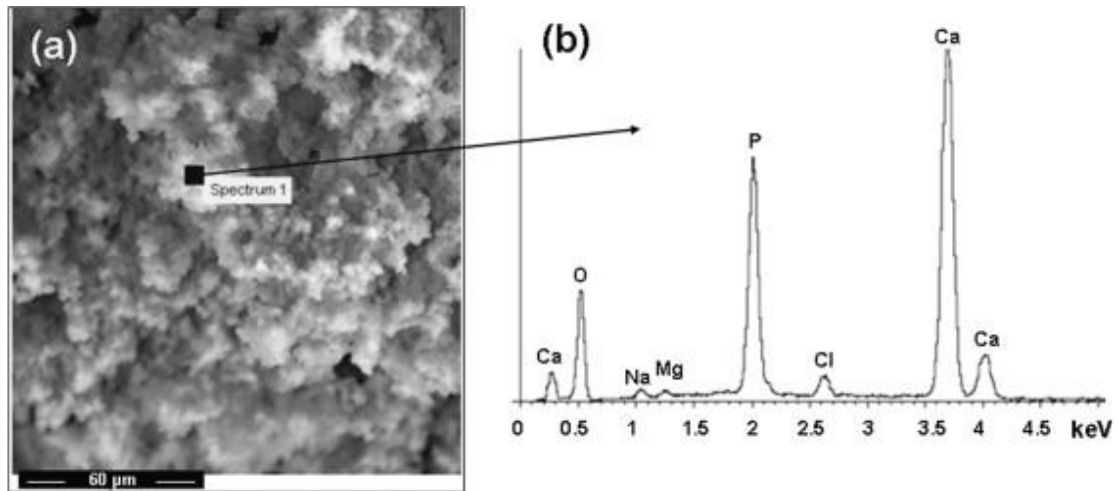
Fig. 6. EDS chemical analysis of the dome-like precipitates on the sample soaked for 14 days.

Further information about the composition of the dome-like aggregates comes from the micro-Raman analysis. The patterns in Fig. 7 (which, as a term of comparison, also includes the graph from not-immersed glass) display the peculiar peaks of HA. In fact, the strong peak at 950 cm^{-1} is due to the vibration (symmetrical stretching) of the $(PO_4)^{3-}$ groups; as often happens for precipitated HA, such peak gets stronger and shifts to higher wave numbers as the immersion time in SBF increases. Also the peaks at 430 cm^{-1} and 590 cm^{-1} are associated to characteristic modes of the $(PO_4)^{3-}$ groups of precipitated HA: the former corresponds to the ν_2 mode (bend, asymmetric stretch), the latter to the ν_4 mode (bend, asymmetric stretch) [27], [28] and [29]. Moreover a peak at about 1070 cm^{-1} appears after the immersion in SBF. This peak is related to a carbonate group [30], thus suggesting that the precipitates consist of carbonated HA. As stated in the literature [31], the OH vibration mode usually results in a shoulder of the Raman spectrum at about 631 cm^{-1} for HA or in a weak peak in close proximity to this wavenumber for carbonated HA. Unfortunately it was not possible to identify this peak in the spectra acquired after soaking in SBF, probably due to its weakness, which is masked by the very strong peaks associated to the $(PO_4)^{3-}$ and carbonate groups. In conclusion, the results of the ESEM observation, X-EDS analysis and Raman spectroscopy converge to support the identification of the precipitates as newly formed (carbonated) HA.
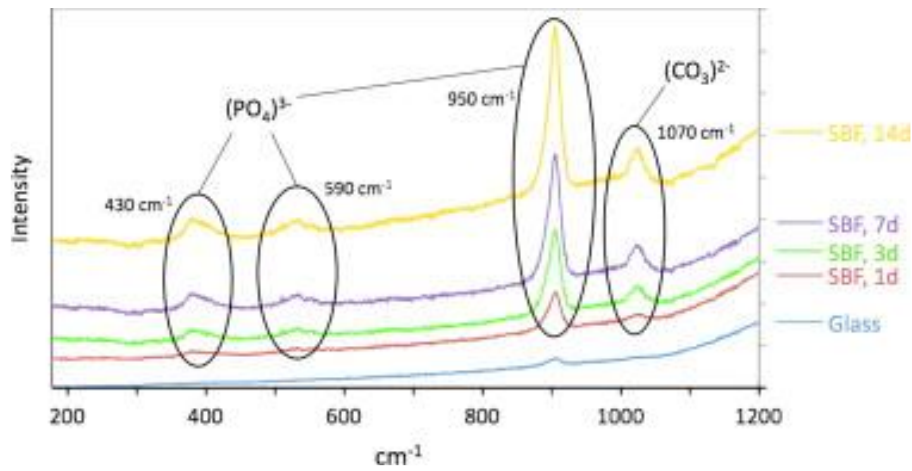
Fig. 7. Raman spectra of the BG_Ca glass produced with animal flour ash, before and after immersion in SBF for increasing times.

The elements concentrations in SBF after 1, 3, 7 and 14 days of static immersion are detailed in Table 8, where the values of the original SBF have been included as a term of comparison. The calcium concentration in SBF increased during the first days of immersion, since the glass starts its dissolution by releasing alkali and alkaline-earth cations, according to the original model proposed by Hench [32]; at the same time, the Si–O–Si bonds of the glass network break up, soluble silica is released in the solution (a rapid increase in Si concentration is reported in Table 8 already after one day in SBF) and silanol groups form at the interface between SBF and glass, giving rise to a silica gel layer. After 7 and 14 days the calcium concentration in SBF decreases, since calcium and phosphate groups migrate to the glass surface from the SBF and promote the development of a calcium–phosphate rich film which finally crystallises to hydroxycarbonate apatite.

Table 8. Elements released (mg/L) by the glass after 1, 3, 7 and 14 days of immersion in SBF.

Sample	Na	Ca	P	Si	K	Mg	Cl	Fe	Al
SBF	2917	9.8	43.4	0.36	340.1	28.55	4460	0.005	0.022
1d SBF	2864	146.0	11.3	19.93	343.4	31.49	5815	0.003	0
3d SBF	2789	160.3	5.1	20.57	349.7	30.73	5319	0.030	0.098
7d SBF	2822	124.4	82.7	21.03	330.3	34.12	5460	0.025	0
14d SBF	2730	117.6	5.1	19.33	341.1	31.22	5600	0.028	0

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4. Conclusions

The animal flour ash could be successfully used to formulate special active glasses, where the presence of P-based compounds imparts a feature with the aim to apply them as (i) controlled-release fertilizers, since they are able to release nutrient elements (P, K) at a low rate, and (ii) bioactive glasses. In particular, for fertilizer glasses the results obtained allow to confirm that all samples showed a very low solubility in water, while high values in acid and salt environment were detected.

For the CaO-rich bioactive glass, apatite-forming ability in a simulated body fluid (SBF) was demonstrated, in spite of the presence of secondary elements such as potassium, iron and aluminium. The animal flour ash is therefore a potential alternative to expensive commercial raw materials.

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