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Biorefinery concept in the meat industry: From slaughterhouse biowastes to superaborbent materials

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ABSTRACT

The expansion of food production has a large environmental impact in many ways. More specifically, 30–40% of total food production is lost as wastes and/or by-products before it reaches the market. In this sense, blood is an inevitable by-product in the meat industry that typically consists of 3–5% of the total weight of the animal. The dry organic matter present in blood is mostly protein, which can be employed more efficiently as raw material in the development of biodegradable materials. In the present manuscript, the blood collected after slaughtering of Iberian pigs was centrifuged and the upper (i.e., plasma) and bottom (i.e., red cells) layers were separated. Three freeze-dried fractions were characterized and evaluated on terms of their potential in the field of bioplastics: whole blood, plasma and bottom layer. Albumin was detected clearly in the plasma fraction, while globulins in red cells. After their characterization samples were mixed thoroughly with glycerol and injection molded at 120 °C. Special applications may be proposed for every fraction (i.e., whole blood, plasma or red cells), as the materials displayed different properties depending on the raw material employed. Thus, plasma resulted in materials with a greater deformability and swelling capacity during immersion, resulting in superabsorbent materials when processed at milder conditions (80 °C).

1. Introduction

Up to 30% of incoming raw materials used in food processing becomes waste, which can pollute significantly land, air, and water because of its high Chemical Oxygen Demand (COD) and sheer volume [1,2]. Considering the increasingly restrictive regulations that have prohibited some of the previously used disposal practices, an adequate waste management is an urgent matter. Animal feeding, composting, anaerobic digestion or recycling are some of the most pursued options when dealing with wastes from the food industry. Another viable alternative method is the reuse of waste for utilization in other industries, as can be the production of biodegradable materials which could be employed in several fields, such as packaging, biomedicine, or agriculture [3,4]. This biorefinery approach to waste management supports the circular bioeconomy concept, which is in accordance with the Sustainability Development Goals of the United Nations.

More specifically, the enormous amounts of wastes and by-products generated globally in the meat industry (i.e., low-value protein meals, bones) is expected to increase due to globalization and free trade policies. An effective valorization of these wastes and under-utilized by-products (biorefinery) would promote circular economy concepts (zero waste generation), minimizing negative environmental impacts, and enhancing food security and sustainability[5].

The European Union (EU) is the second biggest producer of pork in the world after China and the biggest exporter of pork and pork products. Germany, Spain and France are the leaders in the sector, as they represent half of the total production in the EU [6]. Pork meat is one of the most consumed meat in the world and the most consumed in Europe, where Spain is the first consumer (14.1 kg per capita), followed by Austria and Germany [7,8]. A high production is required to answer the demand of increasing population. Thus, 109.84 million tons of meat were produced in 2020 [9], which generate an enormous amount of blood as a by-product per year [10], considering that each pig has an amount of blood of around 3.2 % of its weight (3.5 L approximately) [11]. The direct disposal of blood to the environment is not permitted, as it is highly pollutant due to its high organic content that causes an

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important COD [12]. Thus, blood is typically submitted to combustion or anaerobic digestion [13], even if these waste management practices cause high economic and energetic costs.

Blood composes of 75–82% moisture and 15–17% protein [11], which is albumin (around 50 %), α-, β- and γ-globulin (around 40%) and fibroin (around 5 %), although their levels vary depending on age and animal species [11,14]. Its protein content can be used in several applications where its high nutritional value and functional quality can be profited [15]. Blood is, next to the liver, the major ingredient of offal-derived food products for humans [16,17] or animals [18]. As a result, blood is widely used in the food industry to be consumed after being conveniently processed to avoid the presence of Hepatitis E virus (HEV) which is likely infectious [17,18]. Also, it is used as a binder, emulsifier, colorant, or color enhancer [16,19]. As its dark color may limit its use in the industry, blood may be separated through centrifugation into two well-differentiated fractions: plasma as supernatant and denser red platelets at the bottom. In the food industry, plasma is used as a hot-set binder due to its facility to form a gel when heated [20,21]. Previously, several works have been focused on the development of green materials from plasma with application as superabsorbent materials of interest for several industries (e.g., personal care, horticulture) [22–27]. Different strategies were pursued for that purpose, such as processing at mild conditions (i.e., low temperature) [24] or lowering the pH [25]. As only commercial plasma was used in all those studies, the present work aims to assess the feasibility of using an actual biowaste of a local slaughterhouse, comparing the properties of the materials developed from extracted plasma to those obtained from blood and other extracted fractions (i.e., red cells). Moreover, the characterization of the different fractions employed was made, as well as the sustainability of the extraction process through a Life Cycle Analysis. The proposed biorefinery represents a strategic mechanism for achieving an effective circular bioeconomy that addresses multiple and complex challenges (economic, environmental, social) of the industrial-sector [28].

Besides the food industry, blood-derived products are widely used in the pharmaceutical industry (e.g., nutraceuticals), agriculture (e.g., fertilizers), the paper industry (e.g., glue), or biotechnology (e.g., reagents) [29]. However, most of the blood coming from slaughterhouses is discarded, as only 30% is employed as by-product. The remaining blood could be better used as raw material after centrifugation and atomization [24,30] in the development of biodegradable materials, as different potential uses such as packaging [31], pots [32] or hydrophilic materials [24,33] have been reported. Injection molding acquires special importance as a processing technique thanks to its parameters can be conveniently defined to produce materials covering a wide range of properties for different suitable applications where they can replace traditional plastics from non-renewable sources, hindering the environmental impact caused by them [34–36].

The present work focuses on the extraction of two different fractions from blood discarded from a local slaughterhouse of Iberian pigs in the south of Spain. Those fractions (supernatant plasma (PP) and denser red cell concentrate (RC)) are obtained after centrifugation and freeze-drying. Environmental analysis is made for this extraction procedure to provide a comprehensive and quantitative analysis of the environmental impact of the process. The potential of those two fractions together with the freeze-dried whole blood (WB) as raw materials in the production of biodegradable materials is assessed. WB, PP and RC are characterized through elemental analysis, z-potential, electrophoresis, calorimetric analysis, among others. Additionally, each of them is blended with a plasticizer (i.e., glycerol) and then injection molded. Both blends and plastic materials obtained are also studied through rheological, thermomechanical and water absorption tests. Finally, PP is considered for the development of superabsorbent materials which may be used for personal care or horticultural applications, adding value to this biowaste.

2. Materials and methods

2.1. Materials

2.1.1. Extraction from blood fractions

Porcine blood was directly collected from a local slaughterhouse, Mataderos del Sur S.A., in Seville, Spain. In total, three batches of 4L of blood were collected from the slaughterhouse at different times of the year. A sodium citrate solution (0.3–0.4% wt/vol) was directly added to avoid the coagulation of blood during the transport. WB was obtained after freezing at −40 °C and subsequent freeze-drying of the whole blood in a LyoQest freeze-dryer (Telstar Technologies, Spain) at −80 °C and 1 Pa. PP and RC were obtained after centrifugation (4136x g, 4 °C, 10 min), separation and similar freeze-drying of the whole blood.

2.1.2. Blends and plastic processing

The three powders obtained after freeze-drying (WB, PP and RC) were thoroughly mixed with glycerol in a ratio of 1/1 to ease their processability. A two-blade counter-rotating batch mixer Haake Polylab QC (ThermoHaake, Karlsruhe, Germany) was used at 50 rpm at room temperature, obtaining homogeneous protein-based blends.

Those blends were then injection molded into rectangular probes (10x1x60 mm^3) using a lab scale Minijet Piston Injection Molding System (ThermoHaake, Karlsruhe, Germany). Temperatures used during the processing of the materials were 40 and 120 °C in the pre-injection chamber and the mold, respectively; pressure was set at 50 MPa for both the injection and holding stages, with an overall duration of the process of 150 s. An attempt was made to keep the cylinder temperature as low as possible, as it has been shown that there is better water absorption in milder conditions [24]. Only the PP sample allows the temperature in the mold to be lowered to 80 °C, the other two samples are injectable under these conditions.

2.2. Methodology

2.2.1. Environmental assessment

ISO 14,040 guidelines and recommendations [37] were used to carry out the environmental analysis of the extraction procedure, using as functional unit 1 g of porcine plasma powder. The environmental analysis was carried out using SimaPro 9.4 software (PRé Consultants, The Netherlands) and considering all the materials used and the energy consumed throughout the pretreatments and the preparation steps. The transportation of porcine blood from the slaughterhouse (Salteras, Sevilla) to the lab where the extraction takes place was also considered. Inventory data were obtained from Ecoinvent database, and environmental impacts were assessed according to the Hierarchist version of ReCiPe 2016, midpoint. The impact categories analyzed were global warming, stratospheric ozone depletion, ionizing radiation, ozone formation (human health), fine particulate matter formation, ozone formation (terrestrial ecosystems), terrestrial acidification, freshwater eutrophication, marine eutrophication, terrestrial ecotoxicity, freshwater ecotoxicity, marine ecotoxicity, human carcinogenic toxicity, human non-carcinogenic toxicity, land use, mineral resource scarcity, fossil resource scarcity, and water consumption.

2.2.2. Characterization of the protein powders

2.2.2.1. Elemental analysis. The protein content of WB, PP and RC was determined using a Kejdhal factor of 6.25 to multiply the N percentage estimated through a LECO CHNS-932 Nitrogen analyzer (Leco Corporation, St. Joseph, MI, USA). Ashes content was determined according to the Official Methods of Analysis Chemists International (AOAC international) [38]. It consists of calcining a known amount of sample at 550 °C for 5 h in an HD-230 muffle furnace (Hobersal, Spain).

2.2.2.2. Electrophoresis. The protein subunits present in the three protein powders studied (WB, PP and RC) were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) [39]. Continuous and stacking gels of 10 and 3.5% of acrylamide, respectively, were prepared. The separating gel included a buffer of 2 M Tris-base, containing 0.15% SDS pH 8.8. A running buffer consisted of 0.027 M Tris-base, 0.38 M glycine pH 8.3 with the addition of 0.15% SDS, and Coomassie Brilliant Blue was used as colorant agent.

Precision Plus Protein standards (Bio-Rad-Calibration kit, Richmond, CA, USA) containing ten protein bands were used: 10 kDa, 15 kDa, 20 kDa, 25 kDa, 37 kDa, 50 kDa, 75 kDa, 100 kDa, 150 kDa, ad 250 kDa.

2.2.2.3. Zeta potential. Aqueous dispersions of WB, PP, and RC (1 mg protein/1 mL) were prepared in different buffers with the same ionic strength (0.5 M NaCl) to adjust pH values, and then dispersions obtained were homogenized and centrifuged for 15 min at 15,000g and 10 °C. Z-potentials of the resulting dispersions were finally measured using a Zetasizer Nano ZS (Malvern Instruments, UK), which uses the Smoluchowski equation [40] to evaluate the electrophoretic mobility of the protein chains.

2.2.2.4. Amino acid composition. A study of the amino acid profile was carried out using the Biochrom + 30 (Biochrom) automatic analyzer. This device uses a quantitative method based on ion exchange chromatography with post-column derivatization with ninhydrin. The concentrate sample underwent an acid digestion for 24 h with HCl (50 mg of sample in 1 mL HCl), producing total hydrolysis. Subsequently, it was neutralized to pH 2.5 and passed through 0.25- and 0.02-μm filters. Finally, before derivatization, the sample was reconstituted with phosphate buffer at pH 2.5.

2.2.2.5. Fourier transform infrarred (FTIR) spectroscopy. FTIR measurements were carried out using an Attenuated Total Reflection (ATR) device to obtain the FTIR spectra of the treated samples in a BRUKER spectrometer (USA) using a model Invenio X. The tests were performed with a resolution of 4 cm^{-1}, in a range of wave numbers from 400 to 4000 cm^{-1}.

2.2.2.6. Thermogravimetric analysis (TGA). The thermogravimetric analysis was conducted in a SDT Q600 V20.9 Build 20 thermogravimetric analyser (TA Instruments (USA)) from 0 °C to 600 °C under N_2 atmosphere.

2.2.2.7. Differential scanning calorimetry (DSC). 12–14 mg of sample were placed into aluminum pans which were then hermetically sealed. This sealed pan containing the sample was placed next to an empty pan which was used as reference in an 822 calorimeter (Mettler Toledo, Worthington, OH, USA). The tests were run at a rate of 10 °C/min from − 25 to 300 °C. For the study of the data Mettler Toledo Star System software was used.

2.2.3. Characterization of the protein-based blends and materials

2.2.3.1. Rheological tests. Small amplitude oscillatory tests within the Linear Viscoelastic Range (LVR) were carried out in a DMA580 rheometer (TA Instruments, USA) to determine the viscoelastic properties of blends and bioplastics prepared from WB, PP and RC. A 12 mm diameter cylindrical geometry in compression mode was used for blends and a rectangular geometry in tensile mode was used for bioplastics. First, the mechanical spectra of blends and bioplastics were obtained from frequency sweep tests from 0.1 to 10 Hz at 20 °C. Then, temperature ramp tests were carried out at constant frequency (1 Hz) from 0 to 120 °C, and from −30 to 180 °C for blends and bioplastics, respectively.

2.2.3.2. Tensile tests. An Insight 10 kN Electromechanical Testing System (MTS, USA) was used to perform uniaxial tensile tests at 1 $mm{\cdot}min^{-1}$ until the rupture of the samples at room temperature, following ISO-527–2 standard [41]. Dumbbell bioplastic probes were used and at least 5 replicates were made. Mechanical properties, such as Young's modulus (E), tensile strength (σ_{max}) and maximum strain (ε_{max}) were estimated from stress-strain curves.

2.2.3.3. Water uptake capacity (WUC) and soluble mater loss (SML). The ability to absorb water of bioplastics prepared from WB, PP and RC was analyzed by weight differences before and after water immersion. First, bioplastics were stored in an oven at 50 °C for 24 h to remove the moisture of the samples (w_1). Subsequently, dried samples were introduced into distilled water for 24 h (w_2), and finally the probes were freeze-dried (w_3). WUC and SML were calculated using the following equations:

$$WUC\,(\%) = \frac{w_2 - w_3}{w_3} x\,100 \quad (1)$$

$$SML\,(\%) = \frac{w_1 - w_2}{w_1} x\,100 \quad (2)$$

2.2.4. Statistical analysis

In the current work, all measurements were performed at least in triplicate. Statistical studies were determined using Statgraphics software (ANOVA comparisons). Uncertainty was expressed as mean values ± standard deviations whose values were plotted in all the parameters calculated.

3. Results and discussion

3.1. Environmental assessment

Blood is an important by-product of the meat industry, where it can be obtained from different animals, like pig, cattle, sheep, deer or horse, being commonly obtained from bovine or porcine sources at commercial scale [42]. In this work, blood was obtained from a local Iberian pigs slaughterhouse with a production capacity of 8,360 tons per year. Most of the blood produced is disposed, as commonly happens in the meat industry. However, this opposes the desirable zero waste approach, which promotes the rethink/redesign of industrial processes over the traditional procedures (i.e., incineration, waste-to-energy). Through that approach, wastes are integrated again in the consumption chain as fully recycled products [43]. Moreover, blood has been named "liquid protein" due to its high protein content (generally, around 18%) [18], which could be better used in several applications, either as whole blood or separated in any of the fractions resulting after centrifugation. Plasma is the most widely used in the food industry due to the loss of dark color related to red blood cells that mostly remain in the bottom sediment [18]. Its potential in the field of superabsorbents has also been explored recently [24,25]. As described in the methodology section, blood is collected the same day of the killings, where anticoagulant is added conveniently, and then transported to the lab where it is centrifuged and separated into the top layer (i.e., rich in plasma) and the bottom sediment (i.e., rich in red cells). The three samples are freezed at −40 °C overnight and freeze-dried at −80 °C until a powder is obtained from each fraction. In the analysis, the distilled water production procedure is also considered. The global environmental results of the production of 1 g of plasma powder are reported in Table 1 for all the impact categories evaluated.

Results showed that terrestrial ecotoxicity, global warming, human carcinogenic toxicity, and ionizing radiation cause low environmental damage in the plasma powder production process, whereas the contribution of the other categories was minimal to the overall environmental burden. Moreover, in order to evaluate the contribution of each stage over the final product, environmental results are disaggregated in percentage ratios (Fig. 1). Thus, contributions from the different stages of

Table 1
Impact category values related to plasma powder.

Impact category	Unit	Total
Global warming	kg CO_2 eq	0.23078685
Stratospheric ozone depletion	kg CFC11 eq	1.17E-07
Ionizing radiation	kBq Co-60 eq	0.14842805
Ozone formation. Human health	kg NOx eq	0.000754515
Fine particulate matter formation	kg PM2.5 eq	0.000526696
Ozone formation. Terrestrial ecosystems	kg NOx eq	0.000759447
Terrestrial acidification	kg SO_2 eq	0.0013728
Freshwater eutrophication	kg P eq	9.33E-05
Marine eutrophication	kg N eq	8.47E-06
Terrestrial ecotoxicity	kg 1.4-DCB	0.37537218
Freshwater ecotoxicity	kg 1.4-DCB	0.008139047
Marine ecotoxicity	kg 1.4-DCB	0.010633085
Human carcinogenic toxicity	kg 1.4-DCB	0.012914321
Human non-carcinogenic toxicity	kg 1.4-DCB	0.20236182
Land use	m^2a crop eq	0.006225915
Mineral resource scarcity	kg Cu eq	0.000502991
Fossil resource scarcity	kg oil eq	0.062664583
Water consumption	m^3	0.002311646

the whole process were determined in relation to global results. As can be seen, freezing and freeze-drying steps were the main contributors to the impact category values, representing around 98% of the total impact. The environmental assessment identified these two processes as the most relevant indicators with higher potential of improvement for decision-making in future works. However, it is worth mentioning that the freeze-drying step used at laboratory scale in this work is replaced by spray drying at industrial scale, which is a cheaper, faster and more energy efficient alternative to freeze drying. Therefore, scaling up processes would certainly lead to achieve the goal of the reduction in the environmental impacts abovementioned.

3.2. Characterization of different blood fractions extracted

Whole blood (WB) and the different fractions obtained after centrifugation (PP, RC) were studied through different techniques.

3.2.1. Elemental analysis

All samples were free of moisture, as they were freeze dried in the last stage of their production. As can be observed in Table 2, both WB and RC were protein isolates (% protein > 90%), while PP was a protein concentrate. The lower protein content in PP samples should be associated to its higher ash content. The excess of ashes in the composition of all fractions, more importantly in plasma, should be related to the presence of the anticoagulant (i.e., sodium citrate). Previously, some authors reported that the pyrolysis of citrates takes place at higher temperatures (800 °C) than those employed for the ashes determination [44]. The protein and ash contents were higher than those reported by Sorapukdee & Narunatsopanon (91 and 7.8%, respectively) [20].

Table 2
Protein and ash contents of the powders obtained from the different blood fractions (WB, PP and RC) extracted directly from blood.

Sample	Protein (%)	Ashes (%)
WB	94.5 ± 0.7	14.3 ± 0.8
PP	74.8 ± 0.2	26.5 ± 0.5
RC	97.0 ± 0.5	8.7 ± 1.9

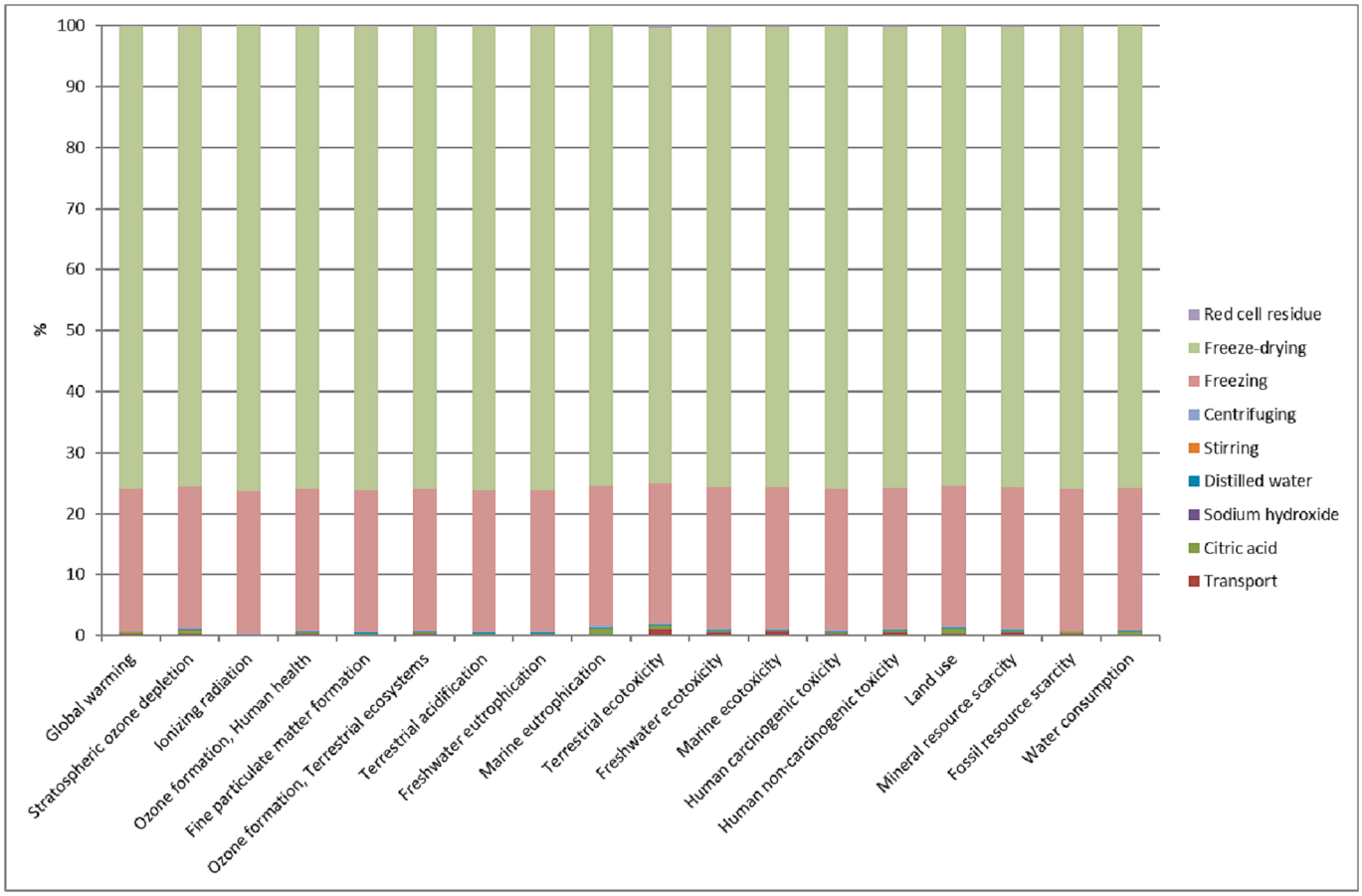

Fig. 1. Relative contributions in each impact category for the most relevant processes (stirring, centrifuging, freezing, freeze-drying and transport) and chemicals (sodium hydroxide, citric acid, distilled water, and red cell residue) involved in the extraction of plasma powder. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Variations may be related to different sampling conditions, the health of the animals, or the season of the year. Porcine plasma protein and ash contents were previously reported to be around 70 and 20% [45], respectively.

3.2.2. Electrophoresis

Fig. 2 shows the electrophoretic bands obtained for the three samples studied (WB, PP, RC) together with those of the standard used. Blood proteins are mainly globulin, albumin and fibrinogen [15,46]. For every sample, an intense band could be observed just at the top of the lane, which corresponds to proteins with a molecular weight higher than that admitted by the pore size distribution of the gel employed and ends up entrapped at the top of the gel [15].

When observing the bands for PP (lane C), the most intense band can be detected around 50 kDa, which has been associated before with albumin, the most abundant protein in plasma [47,48]. This albumin band has been typically found in plasma samples from different species, such as deer, sheep or cattle [42]. The rest of the bands corresponds to globulins, such as γ-globulins (172 kDa), although those bands were not easily detectable in this study.

In the case of the RC (lane D), two different bands can be distinguished at 27 kDa and around 10 kDa, which have been previously associated with haemoglobin. A prominent band at 30 kDa has been linked to gamma globulin. In lane B, which corresponds to the whole blood (WB sample), all the bands above commented can be detected with different intensities, which could be expected as WB contains all protein subunits in PP and RC, although with different percentages.

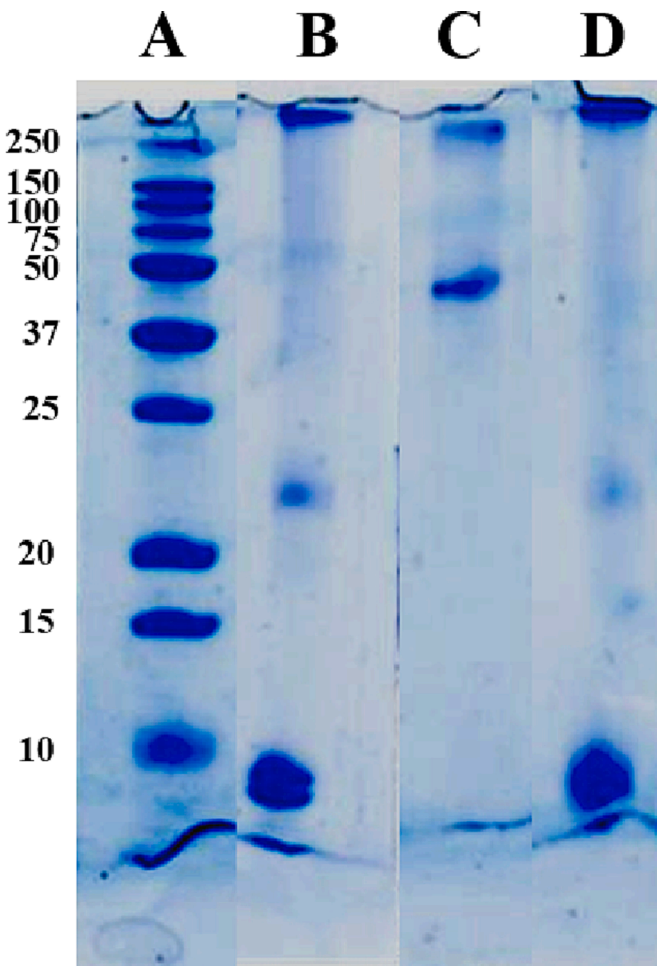

Fig. 2. Electrophoresis image obtained from the powders obtained from the different blood fractions (WB, PP and RC) extracted directly from blood.

3.2.3. Zeta potential

Zeta potential is the effective charge energy of protein molecules in solution and can be used to determine the isoelectric point (IEP) as the charge of proteins change with pH from positive below the IEP to negative above. Thus, the IEP is the pH at which the net charge of the protein system is zero [49]. The three blood samples studied (WB, PP, RC) showed similar zeta potential distribution (Fig. 3A), with an IEP around 4.5 [25,50] for the PP sample and higher than 5 for WB and RC. This difference is due to the different nature of the protein samples, as plasma is rich in albumin with a reported IEP around 4.8. On the other hand, globulins are a heterogeneous group of globular proteins with a IEP between 5 and 7 [48]. As expected, the solubility of blood proteins was generally high [51] (data not shown) but showed a minimum around the isoelectric point that should be related to the relatively neutral charge hindering interactions with water, as observed previously [25].

3.2.4. Amino acid composition

Even if the composition of amino acids in the blood can vary depending on the age or specie of the animal [20], Fig. 3B compares the amino acid composition of whole blood protein (WB) and the two fractions obtained after its centrifugation (PP, RC). A relatively low amount of essential amino acids, such as methionine and isoleucine, can be confirmed [52]. PP sample is richer in Histidine, Leucine, Alanine, Valine and Aspartic acid (His, Leu, Ala, Val and Asp, respectively) than WB or RC. These two materials are richer in Threonine and Glutamic Acid (Thr and Glu, respectively). In general terms, the big amount of the polar amino acids Glu and Asp could explain their good solubility in water due to promoted interactions. It should be highlighted that a high content of polar amino acids, like Glu and Asp, favours to protein–water interactions, which are pursued in the development of superabsorbent materials. The promotion of the interactions with water is due to the presence of hydrophilic groups (e.g., –COOH, –COO–,–$CONH_2$)[34].

3.2.5. FTIr

FTIR is an useful tool for studying the structure of proteins and their changes [53]. As may be observed in the FTIR spectra of whole blood (WB) and its different fractions (PP, RC) (Fig. 4), they display the typical peaks shown by protein sources such as a broad peak around 3300 cm^{-1}, related to amide A type, and a smaller peak at 3100 cm^{-1}, representing the presence of amide B type. Furthermore, two intense peaks are found at around 1653 and 1567 cm^{-1} representing amide I and amide II, respectively; another small peak may be observed around 1360 cm^{-1} referred to amide III. Some authors reported that a deep study of the changes depicted in amide I peak can be of high importance as it gives information about different conformational structure of the protein [54], for example, under pH alterations [55]. Noticeable differences can be observed in the amide I peak between PP and PP or RC which indicate that there are some conformational differences between the protein subunits in the samples studied.

In order to observe clearly the differences between the samples, the amide I band has been deconvoluted into different bands corresponding to different secondary structures of the protein (Fig. 5). The wavenumbers of those conformations studied are: a) 1674–1695 cm^{-1}: β-sheet (antiparallel), b) 1662–1686: β-turns, c) 1648–1657: α-helix, d) 1642–1657: non-organized, e) 1623–1641 cm^{-1}: β-sheet (parallel) [54]. Results obtained for PP sample agree those reported by Saguer et al. where the broader peak observed at 1651 cm^{-1} is associated with the serum fraction and concretely with the essential α-helical conformation of serum albumin in which plasma is rich [55]. Blood has an important amount in serum albumin, as denoted by the high peak at 1651 cm^{-1}. Most of the serum fraction goes to PP, so that RC displays a much smaller peak at that wavenumber, as expected. The opposite happened with non-organized and β-sheet (parallel) conformation, which bands are more important for the RC sample.

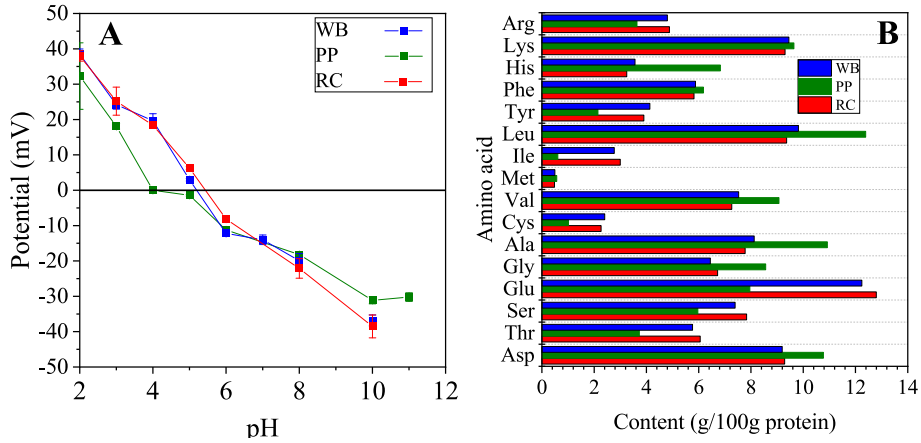

Fig. 3. Z potential distribution (A) and amino acids composition (B) for the powders obtained from the different blood fractions (WB, PP and RC) extracted directly from blood.

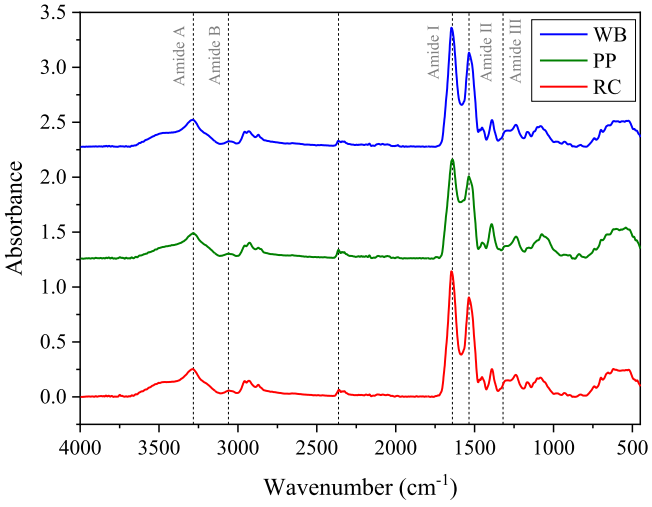

Fig. 4. FTIR spectra of the powders obtained from the different blood fractions (WB, PP and RC) extracted directly from blood.

3.2.6. TGa

The thermal stability of WB, PP and RC was analyzed through thermogravimetric essays, observing some differences between the samples. The removal of the water, either adsorbed on the surface or included within the powder, is the first step in the TGA curve (Fig. 6A). Therefore, all samples showed a plateau around 100 to 200 °C after an initial matter loss, which can be related to the moisture loss [56]. From this temperature on, a more dramatic diminution in the weight of the samples took place between 200 and 600 °C due to the burning of the organic matter (i.e., protein degradation) in the samples. The highest ratio degradation for proteins was observed at around 300–320 °C. Considering the temperatures at which peaks are located for the first derivative (Fig. 6B) and the amount of residual weight at the end of the test, it can be concluded that PP showed the lowest thermal stability of the studied samples, followed by RC and WB. The three samples displayed a double peak around 300 °C, although displaced onto higher temperatures in the PP-WB-RC sequence, which may be related to a higher thermal stability of globulins when compared to albumins [57].

3.2.7. DSc

Thermal transitions of biopolymers can be deeply studied through DSC thermograms. Fig. 7 shows two different endothermic peaks for the three samples studied. The first one is a double peak at lower temperatures (150–175 °C), which overlaps for WB and PP, while the second peak is sharper and located at higher temperatures (200–230 °C) which might be related to a decomposition process of the proteins. PP shows a lower thermal stability, as all the thermal transitions take place at lower temperatures, which matches the above-commented TGA results (Fig. 6). Results obtained for PP are similar to those reported in previous works for commercial plasma [27]. Comparable peaks and temperatures were also reported by Nuthong [56] for plasma based samples. Those peaks are associated with the disruption of the ordered phase of the protein molecules during the heating. Analogous thermal transitions appear in the thermograms of WB and RC samples but placed at higher

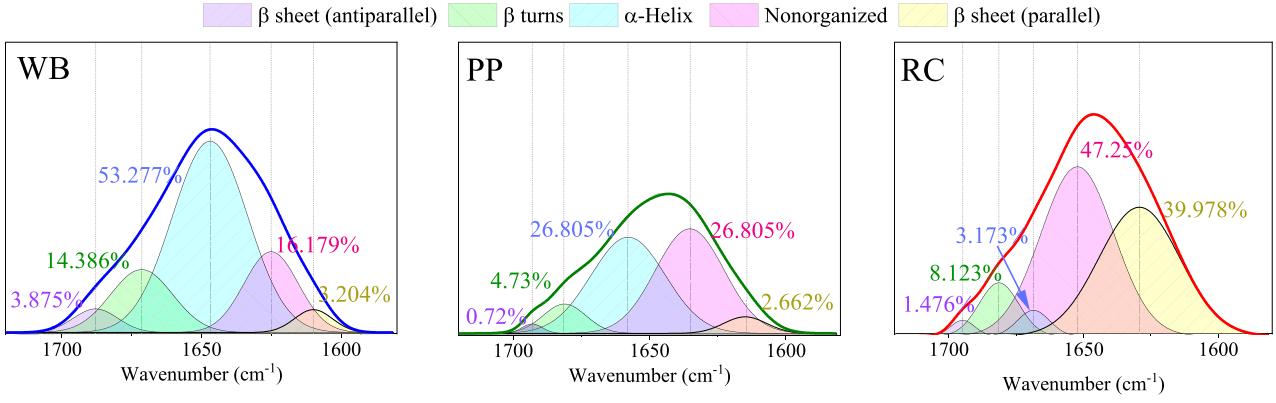

Fig. 5. Deconvolution of the amide I band from FTIR spectra for the powders obtained from the different blood fractions (WB, PP and RC) extracted directly from blood.

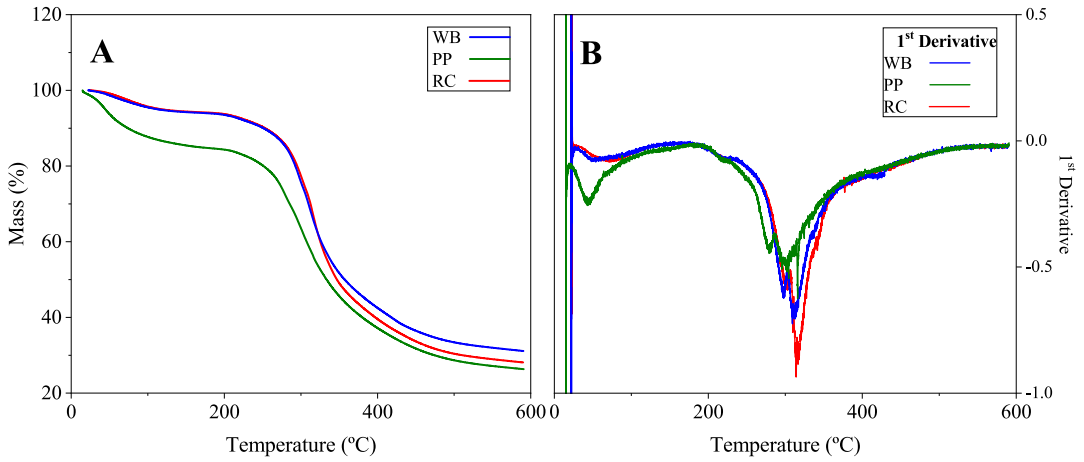

Fig. 6. Thermogravimetric analysis for the powders obtained from the different blood fractions (WB, PP and RC) extracted directly from blood.

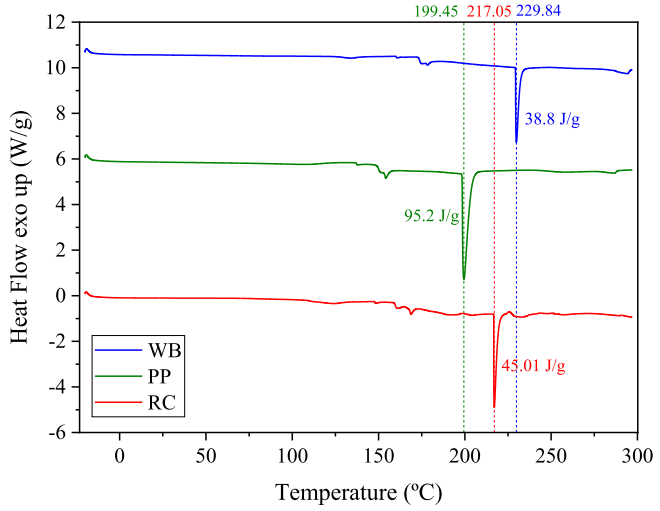

Fig. 7. DSC thermograms for the powders obtained from the different blood fractions (WB, PP and RC) extracted directly from blood.

temperatures. Thus, the peak found for all cases takes place at around a temperature range between 200 and 230 °C.

The absence of a denaturation peak within the 40–70 °C range should be due to the phenomenon known as cold denaturation, which occur at temperatures below the freezing point of water [58]. Freezing and drying stresses resulting from the freeze-drying stage employed in the extraction procedure have resulted presumably in the denaturation of the different protein fractions.

3.3. Evaluation of the blood fractions extracted as raw materials for the production of bioplastic

3.3.1. Blends

3.3.1.1. Mixing. The different samples from porcine blood (WB, PP and RC) were mixed with the plasticizer (i.e., glycerol) in a 50/50 ratio for 5 min at room temperature. Fig. 8 shows the evolution of torque and temperature with mixing time for every sample. A maximum torque value is observed at the beginning of the mixing stage due to the initial compression to which samples are submitted when closing the mixing chamber. As mixing evolved, an asymptotic decrease took place until a plateau value. As torque mirrors the resistance of samples to the rotating blades during the mixing, the achievement of a steady value may be associated to the development of a homogeneous blend. Similarly, temperature generally increased at the beginning of the mixing process until plateau region was reached. The lowest values in both torque and temperature were achieved by PP, the sample with the lowest protein content. Higher torque values could be associated with a higher friction between chains. However, no important increase in torque or temperature difference is observed in any of the systems, with eventual torque values around 0.5–1 N·m and no relevant temperature increases (i.e., equal or lower to 1 °C).Thus, the absence of a torque or temperature increase implies that any of the sample underwent through excessive crosslinking during the mixing time.

3.3.1.2. Viscoelastic properties. Fig. 9A shows the dependence of viscoelastic moduli (E', E'') on frequency from 0.1 Hz to 10 Hz for the blends based on WB, PP and RC obtained after mixing the extracted raw materials and glycerol. A cross point between E' and E'' could be detected for WB and PP blends at a characteristic frequency (f_{cross}), while a tendency onto a similar cross point was observed for the RC blend at frequencies lower than 0.1 Hz. At frequencies below that cross point, a predominantly viscous behavior was observed. At higher frequencies, blends approached a solid elastic behavior implying an important dependence of the rheological behavior of the blends with time. The value of f_{cross} followed the sequence RC < PP < WB, implying that WB blend was specially viscous until 1 Hz. PP sample showed higher viscoelastic moduli than RC and WB, so that the values of E' at 1 Hz (E'_1) were around 1.86, 0.82 and 0.39 MPa for the PP, RC and WB blends respectively.

Fig. 9B shows the effect of heating WB, PP and RC blends from 30 to 150 °C on viscoelastic moduli. At 25 °C, viscoelastic data are in agreement with the frequency sweep tests (from Fig. 9A), where higher values for E' and E'' were presented by PP, followed by RC and WB. When increasing temperature, there is a general decrease in both viscoelastic moduli due to the increased mobility of polymeric chains because of the temperature effect [24]. Finally, a minimum was reached in E' and E'' around 60–80 °C for all systems, before an increase occurred either in one or two steps depending on the sample. This increase might be associated to the aggregation of one or two proteins fractions with different nature that occurs at different range of temperatures [59]. In case of blood, the most common types of proteins are albumin and globulins. On the one hand, the increase observed for PP sample takes place in a single step, which might be related to the aggregation of mostly one protein fraction (i.e., albumin). Albumin is the main fraction of protein present in plasma and aggregates in a temperature range between 60 and 82 °C [60]. On the other hand, a two-step reinforcement was distinguished for RC and WB samples due to their similar amino acid composition (Fig. 3B). The first reinforcement might be associated with some small amount of albumin still present in the sample. The second

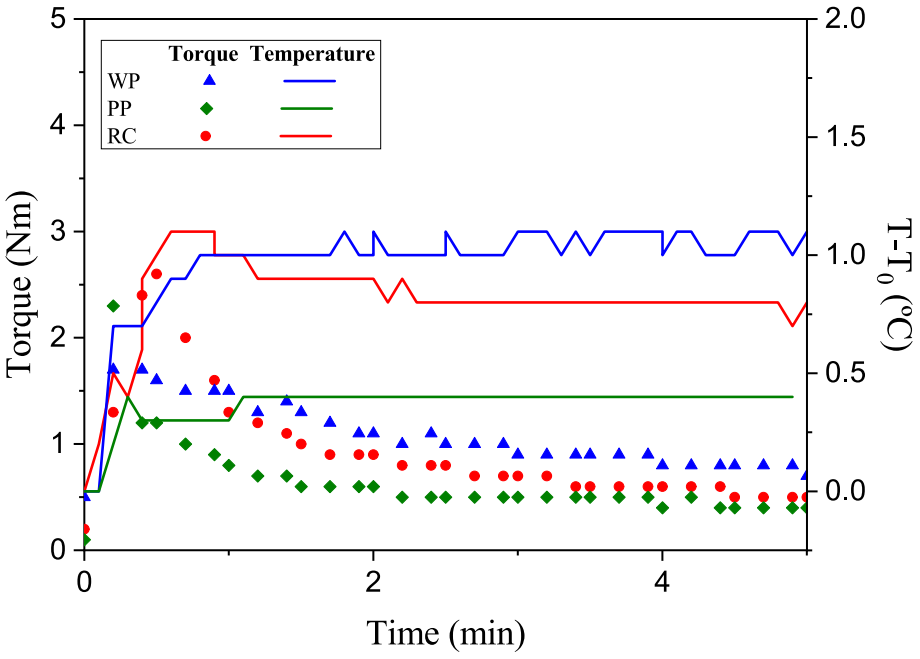

Fig. 8. Torque and ΔT (where T is temperature over mixing and T_0 the initial temperature of each system) values of the blends obtained from the different blood fractions (WB, PP and RC) extracted directly from blood during the mixing stage.

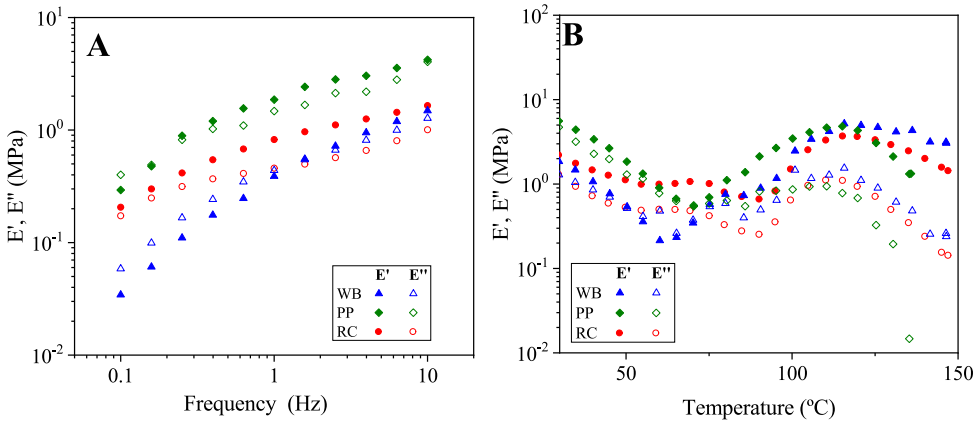

Fig. 9. Evolution of elastic (E') and viscous (E'') moduli with frequency (A) and with temperature (B) for the blends obtained from the different blood fractions (WB, PP and RC) extracted directly from blood, before injection moulding.

increase located at higher temperatures (i.e., around 100 °C) might be explained by the aggregation of globulins that, as commented, possess a higher thermostability, with a denaturation temperature of around 100 °C [57]. The observed reinforcement of the blends at higher temperatures was attributed to thermally induced protein aggregation, as previously reported for egg albumen [61] or industrial plasma protein [24] blends with glycerol.

Even if the blend based on whole blood (WB) showed a more viscous character at room temperature compared to PP or RC, it displayed the highest thermal reinforcement after heating, which might be due to the higher presence of both proteins (i.e., albumin and globulin).

3.3.2. *Bioplastics*

3.3.2.1. Viscoelastic properties. After injection molding, all systems reinforced and showed a solid-like behavior at all frequencies studied (Fig. 10A), with the elastic modulus always above the viscous one. Moreover, all samples displayed a lower dependence on frequency and, presumably, on time, as they all showed similar slopes, much smaller than the ones observed for blends. This implies a reinforcement of the samples due to temperature and pressure effects on blends during the injection molding process. E' and E'' values obtained for injection molded bioplastics based on PP molded at 120 °C were much lower than for WB or RC molded at the same temperature, which should be due to its lower protein content.

Fig. 10B shows the evolution of E' and tan δ for bioplastics based on WB, PP and RC injection molded when heated from −25 to 150 °C. The mold temperature (120 or 80 °C) selected was above than both thermal transitions observed in Fig. 9B, which corresponded to the denaturation of albumin and globulins (~60 °C and ~100 °C, respectively). A general softening is observed in Fig. 10B for all bioplastics, with a decrease in E' values when heating. As commented before, this decrease is caused by a higher mobility of the polymeric chains leading to a relaxation of the protein chains interactions. During the softening, a maximum is observed in tan δ at ~80, ~70 and 95 °C for WB, PP and RC, respectively. The peak in tan δ for PP matched the gelation temperature for albumin (60–82 °C), as is the most abundant protein in plasma when extracted from blood in animals [62]. As the plasma content of the

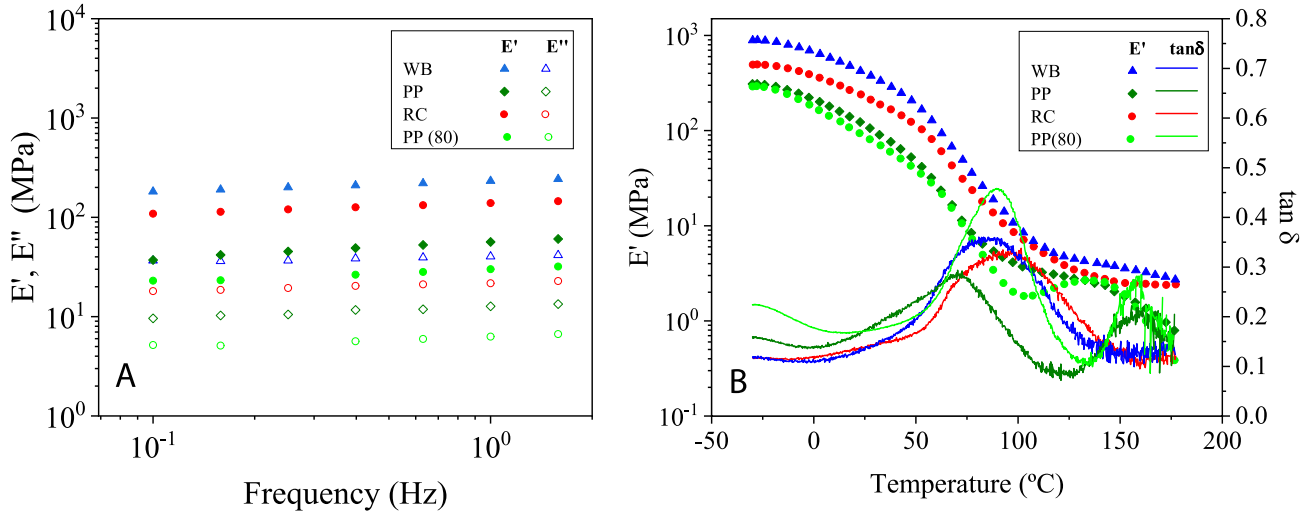

Fig. 10. Evolution of elastic (E') and viscous (E'') moduli with frequency (A) and of elastic (E') and loss tangent (tan δ) with temperature (B) for the materials obtained from the different blood fractions extracted from blood, injection molded at 120 °C (WB, PP and RC) and 80 °C (PP(80)).

systems decreases (PP > WB > RC), the position of the peak of tan δ is displaced onto a higher temperatures until it coincides with the denaturation temperature of other secondary proteins present in animal blood such as globulin or fibrinogen [46]. The peaks in tan δ have been related to a glass transition temperature of different bioplastic materials made of gluten or egg white using glycerol as plasticizer [61,63].

Mold temperature is a key parameter to control when pursuing a high water uptake, as high temperature promotes thermal crosslinking which typically hinders swellability and, hence, hydrophilicity [24]. Thus, as a preliminary test to assess the feasibility of using blood or their fractions as superabsorbent materials, milder temperatures are tested (i.e., 80 °C). However, only PP blends result in adequate bioplastics, as WB and RC cannot be processed at 80 °C. As expected, when PP is processed at lower temperatures (PP(80)), relatively lower viscoelastic values are obtained. Moreover, when that sample is heated at higher temperatures, a thermal reinforcement can be inferred when a minimum in E' takes place at around 100 °C.

3.3.2.2. Tensile properties. Fig. 11A shows the stress–strain curves obtained for the studied bioplastics obtained from tensile tests performed at 1 mm·min^{-1}. All curves initially displayed an elastic region, with a constant slope corresponding to the Young's modulus. Once a certain stress was overpassed (i.e., tensile strength), a decrease in the stress–strain slope was observed and plastic deformation took place. In the samples studied, the tensile strength corresponded to the maximum stress σ_{max} in the curves. When WB bioplastics reached σ_{max}, it fractured abruptly, not showing any plastic deformation. In contrast, PP and RC bioplastics showed plastic deformation, reaching a plateau region where the materials deformed not requiring an increase in the stress. Bioplastic based on PP deformed in a greater extent than RC, finally reaching a significantly higher ε_{max} value.

Fig. 11B shows the mechanical parameters determined from the stress–strain curves for the three samples. Regarding the Young's modulus (E), the WB bioplastic has a higher value of E and σ_{max} than the rest, corroborating what was observed in the frequency tests (Fig. 10A), where that sample displayed greater viscoelastic moduli. The value of σ_{max} for the whole blood is similar to that reported for injection molded bioplastics made from a commercial blood meal [32]. This greater values of E for WB and RC compared to PP may be due to the greater crosslinking that occurred in these systems during injection molding which can be expected considering their higher protein content. Moreover, WB blend was the one which showed a greater thermal reinforcement in Fig. 10B. The higher ε_{max} value of PP plastic can be associated to its lower rigidity which allows a greater deformation of the sample leading to an elastomeric behavior [64].

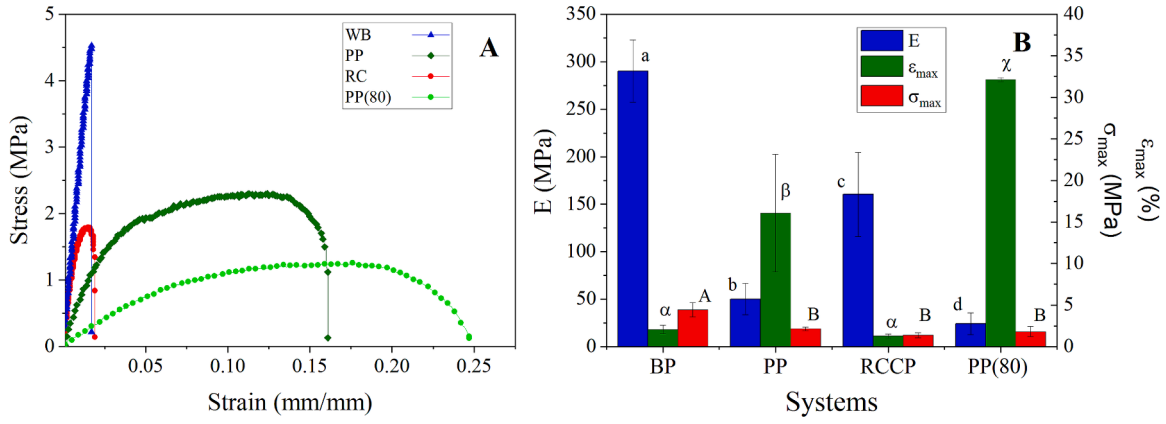

Fig. 11. Stress–strain curves (A) and mechanical parameters (E, ε_{max} and σ_{max}) (B) for the materials obtained from the different blood fractions extracted from blood, injection molded at 120 °C (WB, PP and RC) and 80 °C (PP(80)). Different letters within the same column parameter (E, σ_{max}, ε_{max}) indicate significant differences ($p < 0.05$).

The PP sample processed at 80 °C instead of 120 °C was quite more deformable than the rest, which should be associated to the lower amount of crosslinking that keeps the structure tighter. Conversely, this is the sample also showing less rigidity and strength.

One of the main constraints for certain applications of the materials developed is the relatively poor mechanical properties found, which could be fixed through different approaches (e.g., addition of cross-linkers) [26].

3.3.2.3. Water absorption. The higher WUC value (Table 3) was achieved by PP bioplastics, which is at least twice the value obtained for WB and RC. These two samples did not show significant differences between them. The higher ability to absorb water displayed by PP bioplastic agrees with that observed in Fig. 11 as WB and RC have a less deformable structure, preventing water from entering their structure but, in contrast, PP sample possesses more ability to swell as the water can penetrate in a more deformable framework. Similar results of WUC for commercial PP processed at a T_{mold} of 120 °C were obtained in a previous work [24]. It is remarkable that a superabsorbent material can be developed from PP when processed at temperatures low enough. Thus, PP molded at 80 °C absorbs around 7 times more water than the same blend processed at 120 °C.

When SML values are analyzed, it can be observed that the mass lost during immersion is approximately the amount of plasticizer used for samples processed at 120 °C, especially for PP and RC samples. This is explained by the remarkable hydrophilic behavior of glycerol [65]. As the SML value is much lower than the percentage of glycerol in WB bioplastics, it is possible that not all glycerol was lost during immersion due to the greater reinforcement displayed by that sample after processing, which can lessen the swelling ability of the sample and impart a less porous structure. Also, some exudation may have taken place during processing considering the high molding temperatures used [66]. PP (80) possesses the greater soluble matter loss, which probably includes not only glycerol lost during immersion, but some protein that was less integrated in the whole structure as it has been previously reported for other bioplastics processed by injection molding [67].

4. Concluding remarks

The present study characterized the freeze-dried porcine blood protein obtained as biowaste of a local slaughterhouse and two fractions obtained after its centrifugation and decanting, plasma and red blood cells. The whole blood and red cells fraction obtained were protein isolates, rich in globulins and some albumin, while plasma was a concentrate, containing mainly albumin. Both whole blood and the red cell fraction possessed a similar amino acid composition, but with some differences when compared to that of plasma.

After the three samples were characterized, their potential in the field of bioplastics was assessed. First, the heating of blends revealed that rheological parameters, like elastic modulus, underwent a significant increase that was dependent on the type of protein more abundant in the corresponding fraction. Thus, elastic modulus increased at temperatures higher than 60 °C in a single step, related to the presence of exclusively albumin in plasma, in the case of plasma; but in two steps for whole blood and red cell concentrate caused by both albumin and globulin, and even fibrinogen in a lesser extent. When blends were injection molded, the greatest reinforcement was achieved in the bioplastic based on the whole blood due to a greater crosslinking during processing. This resulted in a greater rigidity and lower deformability, especially when compared to the samples prepared from the plasma fraction. The greater deformability of the plasma sample could be promoted by lowering the mold temperature, as it permits a greater swelling of the sample during immersion, enhancing its water uptake even to the point of surpassing the threshold required for superabsorbent materials (1000%).

Table 3
Water uptake capacity (WUC) and soluble matter loss (SML) for the materials obtained from the different blood fractions extracted from blood, injection molded at 120 °C (WB, PP and RC) and 80 °C (PP(80)). Different letter within a column indicates significant differences ($p < 0.05$).

System	WUC (%)	SML (%)
WB	111.0 ± 7.1 a	39.8 ± 1.6 a
PP	221.3 ± 18.2b	46.4 ± 1.2b
PP(80)	1422.2 ± 115.2c	64.2 ± 1.7c
RC	109.4 ± 3.2 a	51.8 ± 0.9 d

It was clear that different properties were achieved selecting either the whole blood or one of the two fractions obtained after centrifugation, which should be directly linked to their different composition. The final application pursued for the waste can determine the best option among the three systems considered, either greater mechanical resistance or better hydrophilicity and deformability. Porcine plasma is confirmed as a solid candidate for superabsorbent applications, like personal care (mainly for corporal fluids) or agricultural (for retaining soil moisture in dry regions). The main benefits would result from the replacement of non-degradable polymers by a green alternative, as well as by the enhancement of the sustainability of the industrial process.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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