PROCESSING PERACETIC ACID TREATED BLOODMEAL INTO BIOPLASTIC

Aaron Low, Casparus Johannes Reinhard Verbeek, Mark Christopher Lay

The University of Waikato
Faculty of Science and Engineering, Private Bag 3105, Hamilton 3240, New Zealand
awkl1@waikato.ac.nz

ABSTRACT

Renewable and biodegradable bioplastics can be produced from biopolymers such as proteins. Animal blood is a by-product from meat processing and is rich in protein. It is dried into low value bloodmeal and is used as animal feed or fertiliser. Previous work has shown that bloodmeal can be converted into a thermoplastic using water, urea, sodium dodecyl sulphate (SDS), sodium sulphite and triethylene glycol (TEG). To increase its range of applications and acceptance from consumers, the colour and odour was removed from bloodmeal using peracetic acid (PAA). The aim of this study was to investigate the bioplastic processing of 3-5% (w/w) PAA treated bloodmeal.

3-5% PAA treated bloodmeal powder was compression moulded using different combinations of water, TEG, glycerol, SDS, sodium sulphite, urea, borax, salt and sodium silicate at concentrations up to 60 parts per hundred bloodmeal (pph_{BM}). Partially consolidated extrudates and fully consolidated compression moulded sheets were obtained using a combination of water, TEG and SDS. 4% PAA treated bloodmeal produced the best compression moulded sheets and extrudates and was chosen for investigating the effects of water, TEG and SDS concentration on consolidation, specific mechanical energy input (SME) and product colour during extrusion.

Analysis of variance (ANOVA) showed SDS was the most important factor influencing its ability to be extruded because it detangled protein chains and allowed them to form new stabilising interactions required for consolidation. The best extruded sample, which was 98% consolidated and 49% white, contained 40 pph_{BM} water, 10 pph_{BM} TEG and 6 pph_{BM} SDS.

INTRODUCTION

Plastics are a part of everyday life used in many applications including packaging, automobiles and computers. The main problem associated with plastic use is that it takes a long time to degrade once discarded. As a result attention has turned to biodegradable bioplastics produced from natural polymers such as starch, cellulose and proteins. Agricultural by-products such as bloodmeal have limited applications so are attractive feedstocks for bioplastic production. Bloodmeal is low value dried animal blood which is used as a fertiliser or animal feed in some countries.

Previous work has shown that bloodmeal can be converted into a thermoplastic using water, urea, SDS, sodium sulphite and TEG (Verbeek and van den Berg, 2011a, Verbeek and van den Berg, 2011b). This material is currently being commercialised as Novatein Thermoplastic Protein (NTP). To increase its range of applications and acceptance from consumers, its colour and odour must be removed without compromising its ability to be processed into a bioplastic.

Consolidation of a biopolymer into a bioplastic is the detangling and flow of the original biopolymer allowing the reformation of new stabilising interactions between the biopolymer chains and the subsequent formation of a new homogenous bioplastic

material. The complex structure and large number of interactions possible between protein chains make processing them into bioplastics a difficult task and consolidation is usually only possible in a small window of processing conditions and additives.

Oxidation of blood proteins has previously been shown to remove the colour by degrading the haem group (Low et al., 2012). However treating proteins with oxidising agents can also lead to excessive protein degradation and reduce molecular mass which are undesirable when used in bioplastic applications.

PAA has been used to decolour and deodorise bloodmeal without reducing its molecular mass. However it has been shown that treating bloodmeal with PAA caused reduced disulphide cross-linking, crystallinity, glass transition temperature and stabilising interactions such as hydrogen bonding and increased solubility (Low, 2012). Therefore processing PAA treated bloodmeal into a bioplastic may require different additives and processing conditions to untreated bloodmeal. This paper investigated the use of 3-5% PAA decoloured bloodmeal powders for bioplastic production. The aim of this paper was to identify the additives and processing conditions required to produce a consolidated bioplastic from PAA decoloured bloodmeal powders and then investigate the contribution of each additive to extrusion properties such as consolidation, specific mechanical energy and extrudate colour.

MATERIALS

Bloodmeal (98% solids) was obtained from a local rendering company. 5% (w/w) industrial grade PAA equilibrium mixture (5% PAA, 7.5% acetic acid, 25% hydrogen peroxide) was obtained from Solvay and diluted to the required concentration using distilled water. The following additives were used during thermo-processing trials.

Table 1: Additives used to process PAA treated bloodmeal.

Material	**Supplier**	**Grade**	**Purpose**	**Amount (pph_{BM})**
Sodium Dodecyl Sulphate (SDS)	Biolab	Technical	Surfactant	0-6
Sodium Sulphite	BDH Lab Supplies	Analytical	Remove cross-links	0-6
Urea	Agrinutrients-Balance	Agricultural	Denaturant	0-10
Triethylene Glycol (TEG)	BDH Lab Supplies	Analytical	Plasticiser	0-40
Glycerol	BDH Lab Supplies	Analytical	Plasticiser	0-45
Sodium Chloride	Ajax Finechem	Analytical	Increase Aggregation	0-10
Sodium Silicate	Ajax Finechem	Analytical	Increase Electrostatic Interactions	0-15
Borax	Ajax Finechem	Analytical	Increase Hydrogen Bonding	0-15

METHODS

Bloodmeal Decolourisation

Bloodmeal was decoloured by treating 100 g bloodmeal with 300 g of 3, 4 or 5% PAA solution. After 5 minutes of continuous mixing, 300 g distilled water was added to create a slurry which was then filtered. Acetic acid was neutralised by submerging the filtered and treated bloodmeal in 300 g distilled water and adjusting to pH 7 by adding 1 mol/L sodium hydroxide solution. The treated bloodmeal was filtered and washed again with 200 g distilled water. It was frozen and freeze dried overnight using a Labconco Freezone 2.5 freeze dryer to 5-8% moisture. The powders were then ground with a bench top grinder and sieved using a 700 μm sieve.

Compression Moulding

3 pphBM SDS was dissolved in 25 pphBM distilled water. The solution was heated and stirred to 60°C. The hot solution was added to 3-5% PAA decoloured bloodmeal and mixed in a high speed mixer for 5 minutes. 20 pphBM TEG was added and mixed for further 5 minutes. 50 g of the mixture was compression moulded using a hydraulic press with top and bottom heated plates. The mixture was placed in the preheated mould (size 150 x 220 x 2 mm) and compression moulded at 110°C (top and bottom plate) under 2.2 MPa of pressure for 5 minutes. Heating was turned off after 5 minutes and the mould was left under pressure for a further 5 minutes. The pressure was released, the sheet removed and left to cool. Sheets were examined visually for consolidation.

Extrusion

The decoloured bloodmeal powders were processed into different pre-extrusion mixtures using the additives shown in Table 1. Additives were dissolved in distilled water heated to 60˚C while stirring. The hot mixture was added to the decoloured bloodmeal powder and mixed in a high speed mixer for 5 minutes. Plasticiser (TEG or glycerol) was added and the pre-extrusion mixture mixed for a further 5 minutes. The mixture was transferred to an air tight bag and left in the fridge at 2°C overnight to equilibrate.

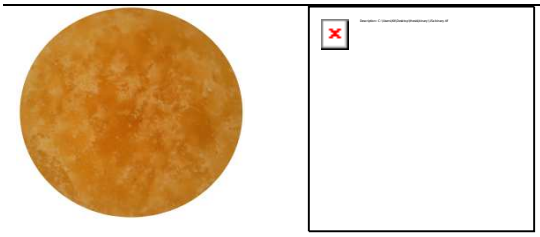
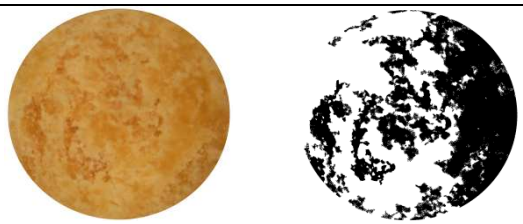
Extrusion was carried out using a ThermoPrism TSE-16-TC twin screw extruder fed with a rotating auger. The feed rate was adjusted to approximately 35 g/min. The extruder temperature profile was 80, 100, 100, 110, 125°C from the feed zone to the die. The screws were operated at 150 rpm and the die was 10 mm in diameter. Pressure, torque and mass flow rates were recorded during extrusion and used to calculate specific mechanical energy input using the equation:

$$SME\left(\frac{kJ}{kg}\right) = \frac{[Torque\ (N.m) \times Screw\ Speed\ (rpm)]}{Mass\ Flow\ Rate\left(\frac{kg}{\min}\right)}$$

Measuring Consolidation

Extrudate cross sections were examined using a Heerbrugg Wild 38 microscope and photographed with a Nikon DS 5MC digital sight camera attached to the microscope. The images were converted into black and white using ImageJ image processing software. Consolidated regions appeared black and unconsolidated regions appeared white. Percent consolidation was calculated by dividing the black area by the total area. Example conversions are shown in Table 2.

Table 2: Example black and white cross section conversions showing complete, high, average and poor extrudate consolidation.

Cross Sections Converted into Black and White Images	
Complete Consolidation (NTP 100%)	*High Consolidation (Above 95%)*
	
Average Consolidation (80-95%)	*Poor Consolidation (Less than 80%)*
	

RESULTS AND DISCUSSION

Initial attempts to extrude 3-5% PAA treated bloodmeal using the existing formulation for producing NTP were unsuccessful. Extrusions resulted in the extrudate exiting the extruder as either a powder or small sections of compressed powder.

Extrusion trials with PAA treated bloodmeal resulted in the formation of four general types of extrudate as shown in Table 3.

Table 3: General types of extrudates formed when processing PAA treated bloodmeal with different additives.

Type 1. Powder/Compressed Powder
 
There was no melt formation and extrudate exited the extruder as powder or small sections of compressed powder. The sections of compressed powder had high amounts of surface defects and broke apart easily if bent or compressed.

Type 2. Dark Poorly Consolidated/Compressed Powder Extrudate


No melt formation and extrudate flowed poorly out of the extruder causing high pressures and torque. Extrudate was not flexible or rubbery and broke when bent. Surface defects such as voids, cracks and shark skinning were also present.

Type 3. Semi-Consolidated with Rough Surface and Powdery Sections


Extrudate flowed well out of the extruder with moderate pressures and torque. Extrudate was flexible and rubbery but had large sections with defects such as voids, cracks, compressed powder sections and shark skinning. They also broke easily when bent or compressed.

Type 4. Semi-Consolidated with Semi Smooth Surface


Extrudate flowed well out of extruder with moderate pressures and torque. Extrudate was flexible and rubbery and its surface was reasonably smooth. Some surface defects such as small cracks, powder and shark skinning were present.

Extrusions with Additives to Increase Interactions

It appeared that PAA treated bloodmeal required more interactions to stabilise the extrudate and help aid consolidation. This could be achieved by removing denaturants or adding additives which increased interactions. Extrusion with just water and no denaturants resulted in compressed powder extrudates (type one). Sodium chloride, sodium silicate and borax have been used to increase aggregation, cross-linking and hydrogen bonds in proteins (Lin and Gunasekaran, 2010, van der Zalm et al., 2010, Kaewmanee et al., 2011, Coviello et al., 2010, Coradin et al., 2004, Coradin et al., 2003). These were added to try and improve consolidation.

When sodium chloride was added to extrusion mixtures it produced type one extrudates which showed increased aggregation when exiting the extruder but no melt formation. Sodium silicate and borax have been used to increase electrostatic interactions and hydrogen bonding between biopolymers. When these were used in extrusion, type two

and three extrudates were produced. Interactions were increased as shown by increased extrudate length, aggregation and semi-consolidation, but melt formation was poor and extrudate surfaces were rough. These extrusions often had high pressures and torque due to the increased interactions. They were also often dark which could be due to the high pressures and torques which have been shown to cause protein degradation (Areas, 1992, Pommet et al., 2005). It was decided that denaturants were still required to reduce interactions but not at the levels or combinations that were required for untreated bloodmeal.

Extrusions with SDS, Urea, Sodium Sulphite, TEG and Water

Using various combinations of water, urea, sodium sulphite, SDS and TEG resulted in type three extrudates. The presence of sodium sulphite and urea resulted in non-continuous extrudates which showed some consolidation but had a lot of defects and broke apart easily. When sodium sulphite was excluded it was still possible to process the PAA decoloured powder and extrudate quality was reasonable (type four). Processing without urea was also possible and extrudate quality was reasonable (type four). Processing without SDS resulted in poor extrudates (type three). Insufficient water and TEG (below 40 pph_{BM} total) produced poor flow, high pressures and torques, as well as brittle extrudates with various surface defects (type three and type four). This suggested that successful processing may be possible with just water, SDS and TEG if their concentrations and processing conditions could be optimised.

Extrusion had limited success and this was caused by the wide range of variables such as the die size, screw speed, feed rate and five temperature zones making it difficult to understand the processing requirements of the decoloured bloodmeal powders. A simpler method such as compression moulding with fewer variables could help to identify what conditions and additives were required for consolidation of PAA treated bloodmeal powders. These conditions and additives could then be applied to extrusion. Compression moulding was used with the aim of investigating what processing conditions and additives were required for consolidation of PAA decoloured bloodmeal powders. After these were found, they could then be applied to extrusion.

Compression Moulding

Compression moulding is a simpler technique with fewer variables and has been used in previous studies to produce bioplastic sheets (Jerez et al., 2007). 3-5% PAA treated bloodmeal was compression moulded using only water (25 pph_{BM}), SDS (3 pph_{BM}) and TEG (20 pph_{BM}). These additives were used for compression moulding as they gave the most promising results from extrusions.

Initial trials involved varying temperature and pressure until a temperature of 110˚C (top and bottom plate) and 2.2 MPa pressure was selected. It was found that at temperatures above this the sheets would be brittle due to the evaporation of water and below this the material would not form a melt. Excessive pressures (above 3 MPa) would cause the material to flow out the sides of the mould. This was not good for compression moulding but was a good sign that melting and consolidation was possible during extrusion.

Compression moulding showed that processing PAA decoloured bloodmeal into a bioplastic was possible using water (25 pph_{BM}), SDS (3 pph_{BM}) and TEG (20 pph_{BM}). Observations from compression moulding showed that at 110°C and high pressures the decoloured powders formed a melt and squirted out the edges of the mould as consolidated ribbons. This showed that processing temperatures around 110-120˚C were sufficient. In addition it was observed that 3% PAA treated bloodmeal darkened slightly

when compression moulded and 5% PAA treated bloodmeal produced sheets which were very flexible and easy to break.

From these results it was decided to return to extrusion using 4% PAA treated bloodmeal and investigate the effects of water, TEG and SDS. 4% PAA treated bloodmeal was chosen to be investigated because it produced the best compression moulded samples.

Effect of SDS, Water and TEG on Extrusion Properties of 4% PAA Treated Bloodmeal

During extrusion several general observations were made. Samples with SDS showed better consolidation than samples without SDS. At high water and TEG content consolidation was reduced. In addition these samples also had difficulty feeding and water was also seen in the feed zone. The powders would clump and block in the feed zone leading to build up in the feed hopper. Water in the feed zone was probably due to water being evaporated in the extruder barrel.

These observations suggested that SDS was the most important component for consolidation during extrusion. The primary role of SDS is to detangle the bloodmeal protein allowing it to interact and form new stabilising interaction with other protein chains during extrusion. The majority of extruded samples were a transparent yellow/orange colour which means their colour could be modified with the use of pigments if desired.

The best consolidation results were achieved using 6 pph_{BM} combined with 40 pph_{BM} water and 10 pph_{BM} TEG or 30 pph_{BM} water and 20 pph_{BM} TEG. These samples were 98.19 and 98.47% consolidated respectively. High consolidation (97.37 and 97.13%) was also achieved using 3 pph_{BM} SDS with 40 pph_{BM} water and 10 pph_{BM} TEG or 30 pph_{BM} water and 20 pph_{BM} TEG. The highest consolidation at 0 pph_{BM} SDS (85.65%) was also achieved using this combination of water and TEG. This suggested that 50 pph_{BM} total plasticiser is the optimum level for consolidation during extrusion. These extrusion trials were summarised in Table 4.

Table 4: Percentage consolidation, SME and percentage whiteness when 4% PAA treated bloodmeal was extruded with different combinations of SDS, water and TEG.

Formulation	SDS (pph_{BM})	Water (pph_{BM})	TEG (pph_{BM})	SME (kJ/kg)	Standard Error	Extrusion Consolidation (%)	Standard Error	Whiteness (%)
1	6	30	10	30.4	0.9	96	0.5	43
2	6	40	10	23.7	0.1	98	0.6	49
3	6	50	10	23.4	0.1	96	0.2	48
4	6	30	20	21.0	0.2	98	0.4	44
5	6	40	20	18.3	0.0	96	1.4	49
6	6	50	20	17.6	0.3	96	0.6	52
7	6	30	30	17.0	0.2	93	0.3	51
8	6	40	30	16.2	0.3	93	0.4	52
9	6	50	30	16.9	0.6	92	0.0	53
10	3	30	10	29.3	0.3	95	0.2	43
11	3	40	10	26.1	0.7	97	1.1	38
12	3	50	10	20.5	0.2	94	1.7	37
13	3	30	20	19.2	0.0	97	0.3	37
14	3	40	20	18.4	0.4	95	1.2	42
15	3	50	20	18.8	0.9	87	2.3	50
16	3	30	30	18.3	0.6	95	1.2	42
17	3	40	30	22.3	1.5	90	1.1	53
18	3	50	30	23.3	2.1	83	1.5	58
19	0	30	10	11.8	0.2	83	5.6	52
20	0	40	10	10.1	0.1	86	2.1	49
21	0	50	10	10.3	0.1	62	6.7	63
22	0	30	20	8.7	0.1	74	6.9	58
23	0	40	20	10.6	1.7	68	3.5	63
24	0	50	20	9.4	0.2	63	5.8	65
25	0	30	30	11.1	0.9	62	9.3	62
26	0	40	30	10.2	2.3	38	8.3	67
27	0	50	30	8.4	0.5	54	11.0	64

Observations suggested SDS had the greatest impact on consolidation, extrudate colour and SME. This was confirmed using analysis of variance (Table 5). Analysis of variance revealed that SDS contributed 71.2% to SME input, 71.9% to whiteness and 58.5% to consolidation. This confirmed the observations made during extrusion where extruding formulations with no SDS resulted in poorly consolidated samples, which were powdery, had many surface defects and were easy to break. In some cases consolidation reached a plateau at 3 pph_{BM} SDS which suggested that this amount of SDS was sufficient to reduce hydrophobic interactions allowing the protein chains to interact and reform new stabilising interactions so the plastic could consolidate.

Table 5: Percentage contribution of SDS, water and TEG to SME, extrudate whiteness and consolidation.

	SME (%)	Extrudate Whiteness (%)	Consolidation (%)
SDS	71.2	71.9	58.5
Water	1.8	3.5	9.1
TEG	12.9	9.6	17.4
SDS*Water	1.1	2.0	0.6
SDS*TEG	6.4	6.1	2.8
Water*TEG	3.4	2.2	2.5
SDS*Water*TEG	3.2	4.6	9.2

SDS was required to detangle the protein chains in the bloodmeal aggregates by reducing the hydrophobic interactions. The detangled proteins were then able to re-entangle and form new stabilising interactions during extrusion resulting in consolidation. Previous studies have suggested that when proteins are detangled or unwound the amount of surface area for new stabilising interactions and entanglements increases during processing (Mo and Sun, 2000). If this was true then at 0 pph_{BM} SDS the bloodmeal protein would not be detangled sufficiently resulting in low amounts of entanglement and few molecular interactions due to the low contact area between protein chains. This could explain the poor consolidation at 0 pph_{BM} SDS. At 3 and 6 pph_{BM} the protein was unwound enough to allow sufficient entanglements and the formation of new stabilising interactions during extrusion resulting in good consolidation.

When biopolymers entangle and form new stabilising interactions during extrusion the viscosity increases and causes an increase in required SME input. At 0 pph_{BM} SDS, low SME input was required during extrusion. This was probably due to low chain entanglements and chain interactions as the protein was not detangled sufficiently enough to allow a high amount of these to new entanglements and stabilising interactions to form.

At 3 pph_{BM} SDS, the protein was detangled sufficiently and the chains were able to entangle and interact during extrusion resulting in a high SME input. No increase in SME occurred at 6 pph_{BM} SDS and in some cases SME decreased slightly. This suggested that 3 pph_{BM} SDS was sufficient to detangle enough of the protein chains and as SDS content increased the excess SDS could have been acting as a plasticiser as has been shown previously in other studies where SDS was used at high concentrations (Mo and Sun, 2000).

CONCLUSIONS

Attempts to extrude 3-5% PAA decoloured bloodmeal powders using the existing NTP formulation were unsuccessful. Using additives to increase chain interactions were also unsuccessful and some caused the extrudate to darken, possibly due to increased interactions causing an increase in shear stresses which caused the protein to degrade.

Extrusion was a difficult scouting technique to use due to its wide range of parameters and limited success was achieved. As a result, compression moulding was utilised to investigate if consolidation was possible using 3-5% PAA decoloured bloodmeal powders. Compression moulding with water, SDS and TEG showed consolidation was possible using 3-5% PAA treated powders even without urea and sodium sulphite. After returning to extrusion with water, SDS and TEG, better quality extrudates were obtained using 4% PAA treated bloodmeal.

SDS was found to be the most important factor for consolidation during extrusion and SME due to it unwinding the aggregated protein and allowing new protein entanglements and new protein interactions to form. The best extruded sample based on consolidation and whiteness contained 40 pph_{BM} water, 10 TEG pph_{BM} and 6 pph_{BM} SDS. This produced a 98% consolidated and 49% white sample.

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