

# Sustainable Bioplastics from Amyloid Fibril-Biodegradable Polymer Blends

Mohammad Peydayesh,<sup>§</sup> Massimo Bagnani,<sup>§</sup> and Raffaele Mezzenga\*

Cite This: https://doi.org/10.1021/acssuschemeng.1c03937



**ABSTRACT:** Plastics waste production is a global challenging problem because its accumulation in the environment causes devastating effects on the planet's ecosystem. Sustainable and green solutions are urgently needed, and this pairs with increasingly stronger regulations combined with improved ecological awareness. This study proposes a simple, scalable, and water-based process to produce free-standing, transparent, and flexible bioplastics films by combining amyloid fibrils with biodegradable polymers as two main building blocks. Amyloid fibrils can be obtained through denaturation and self-assembly from a broad class of food proteins found in milk, soy, and egg, for example. Whey is used here as a model protein because it is the major byproduct of dairy industries, and its valorization creates a valuable



opportunity to produce sustainable, biodegradable, and environmentally friendly bioplastics perfectly integrated within a circular economy. Against this background, we highlight the sustainability superiority of these bioplastics over common plastics and bioplastics *via* a detailed life cycle assessment, anticipating an important role of this new class of bioplastics in mitigating the pressing plastics pollution challenge.

KEYWORDS: bioplastics, amyloid fibrils, sustainability, waste valorization, circular economy

# 1. INTRODUCTION

Plastics is one of the most abundant man-made materials, and although its widespread use started only 70 years ago, an estimate of 9 billion metric tons (Mt) of plastics has been produced until 2021.<sup>1,2</sup> The intensive utilization of single-use containers drastically accelerated plastics production, and packaging is now plastics' largest market.<sup>3</sup> Approximately 183 Mt of solid plastics, corresponding to half of the annual global production, is thrown away each year worldwide.<sup>4</sup> Most polymers used to produce plastics are derived from fossil hydrocarbons, posing a threat to fossil fuel sources. Additionally, most common plastics are not biodegradable and, if not permanently destroyed by proper thermal treatment, accumulate in the natural environment or landfills, causing devastating effects to the planet's ecosystem. Up to 80% of the global plastics waste ends up contaminating the environment, and 4-12 Mt of plastics enters the oceans each year.<sup>5</sup> Plastics debris can be found in all the ocean basins<sup>6</sup> and are so abundant in the environment that they can be used as a geological indicator of the Anthropocene era.<sup>7</sup> Although recycling is suggested as a possible solution to mitigate some of these problems, this process remains limited to less than 9% of global plastics waste, because it is costly and time-consuming, it cannot be applied to many polymeric materials, and the quality of the polymers obtained is low.<sup>4</sup>

Thanks to the growing awareness about the environmental issues related to the accumulation and disposal of traditional plastics, stringent regulations are being implemented worldwide, pushing the plastics market toward a transition to more sustainable products and processes. The production of bioplastics increased dramatically in the past few years, and it is expected to grow even more substantially in the future.<sup>8–10</sup>

Protein-based bioplastics are attracting tremendous attention due to their broad availability, fast biodegradation rates, and food-grade nature resulting in films that can even be classified as edible.<sup>11–14</sup> The main drawbacks of protein-based bioplastics derive from the intrinsic nature of native protein monomers, which are often globular, hydrophilic, and watersoluble and result in difficulties to process films, show poor mechanical and barrier properties, and are very sensitive to water and humidity.

In the dairy industries, for producing 1 kg of cheese, 8–9 kg of whey are produced as a byproduct. It represents the main byproduct of the dairy industries, where each year, approximately 120 million tons of whey are produced globally. Because only half of the whey produced is transformed into valuable products such as human or animal feed<sup>15</sup> (Figure 1),

Received: June 11, 2021 Revised: August 10, 2021





**Figure 1.** Schematic representation of the bioplastics preparation protocol. Each year in Switzerland, 1.3 Mt of whey is produced, of which only half of it is valorized efficiently.<sup>15</sup> WPI, glycerol, and PVA are dispersed in water, and the solution is heated at 90 °C for 5 h to allow  $\beta$ -lactoglobulin monomers to self-assemble into amyloid fibrils. The solution is then cast to obtain the bioplastics film through solvent evaporation.

the disposal of surplus whey represents a crucial issue for the dairy industries and causes environmental concerns due to the high biological oxygen demand of the byproduct.<sup>16–18</sup> In fact, whey contains a high load of organic matter, which is mainly composed of lactose (0.18–60 kg/m<sup>3</sup>), proteins (1.4–33.5 kg/m<sup>3</sup>), and fats (0.08–10.58 kg/m<sup>3</sup>).<sup>17,19</sup> Whey is, therefore, a rich source of proteins, of which the most abundant is  $\beta$ -lactoglobulin,<sup>20,21</sup> a globular protein that can easily self-assemble into amyloid fibrils.<sup>22–24</sup>

Amyloid fibrils play critical functional roles in various biological processes in multiple organisms, ranging from bacteria to humans. Thanks to their promising biophysical properties and mechanical and chemical stabilities, many applications have been proposed.<sup>25–28</sup> Amyloid fibrils can also self-assemble *in vitro* from various proteins in milk, egg, and soy through denaturation and hydrolysis under proper conditions, typically involving low pH and high temperatures.<sup>29–31</sup> A broad range of functionalities characterizes the fibrils obtained from these food proteins, and thanks to their remarkable properties that are far superior to those of single monomers, such as high stiffness and aspect ratios, they have been used as building blocks for developing suspensions, emulsions, membranes, and gels with high performances.<sup>27,28,31,32</sup>

This study focuses on developing bioplastics composed of amyloid fibrils and showcases them as ideal candidates to produce hybrid films. In particular, we show that amyloid fibrils can be used as building blocks for engineering novel bioplastics with targeted characteristics, further tuned by blending different functional additives such as biopolymers and plasticizers to improve the performances of the resulting films. We present various hybrid bioplastics that can be biodegradable [polyvinyl alcohol (PVA) as an additive] and compostable (cellulose as an additive) depending on the composition. Other important film properties such as water stability, hydrophobicity, and antioxidant activity can be tuned by chemical treatment or coatings. These novel bioplastics are characterized by a wide range of properties that can be achieved and tuned without requiring the use of nonbiodegradable or toxic compounds. Moreover, these bioplastics show great potential for commercialization with economic viability thanks to the meager cost of the protein used, which are mainly obtained by waste products of the food industry, and the cheap, scalable, and environmentally friendly waterbased production protocol for film formation. Ultimately, producing bioplastics using food wastes helps the industry in two ways: not only it improves their production in terms of sustainability, waste management, and valorization, but it also directly improves their circular economy.

# 2. RESULTS AND DISCUSSION

**2.1. Bioplastics Films.** The bioplastics presented here can be obtained by a simple, scalable, and water-based protocol, which is schematically described in Figure 1 and in detail elaborated in the experimental section. The WPI (whey protein isolate)—here taken as a model protein available from food processing byproducts—is dispersed in acidic water together with a plasticizer and a water-soluble polymer. After the fibrillization process in the presence of other ingredients, free-standing homogeneous and transparent films (see Figure 1) can be obtained by casting the solution onto suitable substrates, followed by solvent evaporation.

**2.2. Microstructure.** To verify that amyloid fibril selfassembly is not inhibited by the other compounds in solution (glycerol and PVA and other compounds contained in the WPI), atomic force microscopy (AFM) has been used to image the solution resulting after the heat treatment. Figure 2a shows amyloid fibrils obtained from pure  $\beta$ -lactoglobulin dispersed in pH 2 water. As a comparison, Figure 2b shows the amyloid fibrils formed from the WPI and in the presence of PVA and glycerol, confirming that the other compounds do not inhibit the self-assembly of  $\beta$ -lactoglobulin into amyloid fibrils in solution. The main difference between the fibrils obtained



**Figure 2.** Characterization of amyloid fibrils and film surfaces. (a) AFM of amyloid fibrils, (b) AFM of the mixture of amyloid fibrils, glycerol, and PVA, (c) SEM of the hybrid amyloid film top surface, and (d) SEM of the hybrid monomer film top surface.



Figure 3. (a) Water contact angle of the films after different cross-linking treatments, (b) mass change, and (c) water absorption.

appears to be their contour length distribution, which decreases when amyloids are produced in the presence of other compounds. This difference is derived from the fact that a very high viscosity characterizes the solution used to produce bioplastics: to avoid gelation, vigorous stirring has to be applied during the heat treatment. In fact, these mechanical stresses are known to induce shortening of amyloid aggregates.<sup>33</sup>

In Figure 2c,d, the scanning electron microscopy (SEM) images of the surface of the hybrid amyloid and monomer films are presented. As shown in the figure, the amyloid-based film surface is smooth, homogenous, and without cracks. However, the surface of the films obtained with WPI monomers shows multiple cracks spanning several micrometers. These cracks increase the oxygen and vapor transport through the films, resulting in films that might not be suitable for food packaging. Whey has already been proposed as the right candidate to obtain edible films,<sup>34–36</sup> thanks to the low cost and broad availability of this byproduct. However, the film resulting from the whey protein in their native or partially hydrolyzed state results in low mechanical properties and water stability.<sup>34–36</sup>

On the contrary, amyloid-based bioplastics result in a film characterized by a highly homogeneous surface, thanks to the high degree of interactions between amyloid fibrils, the plasticizer (glycerol), and the polymer chains (PVA), favored by the very high aspect ratio of amyloid fibrils combined with the numerous functional groups on their surface.

On the outer surface of amyloid fibrils, there are many amino acids containing various functional group terminals that can bind with PVA hydroxyl groups, leading to the strong hydrogen bond interaction inside the film matrix.<sup>37</sup>

2.3. Surface Contact Angle. One of the most significant weaknesses of bioplastics is their inherent low water stability, especially compared to petrol-based plastics.<sup>13,38-40</sup> Producing films that are not degraded by vapor or water exposure is essential for their real-world application. We performed static water contact angle analysis to determine the hydrophobicity of amyloid-based films (Figure S1). As depicted in Figure 3a, the contact angle varies highly depending on the composition and the chemical treatment. In particular, the contact angle of the film formed by native WPI monomers is the smallest one, confirming the hydrophilic nature of this species. However, the contact angle measured on amyloid fibril-based films resulted in a more hydrophobic nature than the monomer-based ones. Most importantly, the chemical cross-linking treatment using FAS resulted in films with a surface characterized by a superhydrophobic nature, with contact angles above 90°. The films containing citric acid (CA) showed the lowest contact angle measured even when compared to protein monomers.

**2.4. Water Stability.** To further investigate the interaction of the films with water, we performed two additional experiments, the water absorption and the mass release tests. As shown in Figure 3b, a substantial mass release of about 50% has been measured on all the films after 2 h of water immersion. However, after this first water immersion, the mass loss measured in films containing amyloid fibrils decreased drastically, leaving the mass essentially stable up to 24 h. The films' main component released in water is assumed to be primarily the plasticizer due to the high water solubility of this compound. This is further confirmed by the fact that the dried films appeared much more brittle than the same films before water immersion. At the same time, the films composed of

С



Figure 4. Mechanical properties of films. (a) stress-strain plot, (b) maximum stress, (c) maximum strain, and (d) toughness.

WPI native monomers showed a continuous mass loss during all the time tested up to complete film dissolution (not shown in the figure).

The water adsorption measurements resulted in a very similar behavior in the films. As shown in Figure 3c, amyloid-based films absorbed around 225% of water after 24 h of immersion. The swelling, however, increased significantly in the films obtained by WPI monomers, reaching water adsorption values of around 325%. The low performances of monomer-based films are supposed to be related not only to the hydrophilic nature of the protein native state but also to the surface properties of these films. As already shown in Figures 2d and 3a, monomer-based films are characterized by a hydrophilic, inhomogeneous, and fractured surface, confirming the highest performances of amyloid fibrils compared to native monomers.

2.5. Mechanical Properties. The mechanical properties of the hybrid whey amyloid, monomer, amyloid FAS-coated, and CA-contained films are depicted in Figure 4. As observed in stress-strain curves (Figure 4a), fibrillization, combination, and coating directly affect the mechanical properties of the films. The maximum stress at break, ultimate elongation, and toughness values are presented in Figure 4b-d, respectively (for Young's modulus, see Figure S2). The maximum stress of the pure amyloid hybrid film is 17 MPa, similar to the value for monomer hybrid films. However, as shown in Figure 4c, the elongation of hybrid amyloid films is improved at least a factor of 2 compared to hybrid monomer films. It was speculated that the better elongation of the amyloid hybrid films was attributed to the good alignment of nanofibril chains, allowing for interfibrillar molecular rearrangement during deformation without undergoing fracture.<sup>41</sup> The excellent elongation at break of hybrid films is a very important property for food

packaging applications. However, this property leads to a lower Young's modulus for hybrid amyloid fibril films than monomer hybrid films (Figure S2).

Furthermore, it appeared that although either coating or hybridizing the films with FAS or CA improves the maximum stress and Young's modulus, it decreases the film strain, resulting in rigid and less flexible films. This phenomenon is due to the cross-linking effect and limited mobility of whey amyloid fibril chains after the addition of CA or coating with FAS.<sup>42</sup> Finally, the toughness of different hybrid films was measured as the area under the stress–strain curves, and the results are summarized in Figure 4d. As observed, all the hybrid amyloid-based films are characterized by higher toughness, which are up to two times higher than hybrid monomer ones.

**2.6. Optical Properties.** The optical properties of the films were analyzed using UV–vis spectroscopy, and the resulting transmittance values in the visible spectrum (660 nm) and the UV range (280 nm) are summarized in Table 1. All the films tested are characterized by a high degree of transparency in the visible spectra with transmittance values above 95%. The films showed good UV-screening ability in the UV range with transmittance values dropping below 60%. This property is

#### Table 1. Optical Properties of the Prepared Films

	% transmittance (660 nm)	% transmittance (280 nm)
hybrid amyloid	$95.9 \pm 0.4$	57 ± 7
hybrid monomer	$96.0 \pm 0.0$	$53 \pm 6$
hybrid amyloid FAS	$97.4 \pm 0.1$	$58 \pm 2$
hybrid amyloid CA	$97.2 \pm 0.1$	56 ± 2

pubs.acs.org/journal/ascecg



Figure 5. Film characterization. (a) WVP. (b) Antioxidant activity. (c) Food migration analysis with Tenax.

desirable in food-packaging materials because UV irradiation increases the oxidation rates of food even at low temperatures.<sup>43</sup> At the same time, high transparency in the visible spectra is an important physical property, providing a seethrough on the wrapped items. Hence, hybrid amyloid films exhibit ideal optical properties for packaging applications.

**2.7. Water Vapor Permeability.** The barrier properties of the films against water vapor have been measured for 24 h, and the results are summarized in Figure 5a. Amyloid-based films showed the best performances, and the water vapor permeability (WVP) remained stable over the 24 h sampling. The hybrid films produced with WPI monomers showed WVP values higher than the hybrid amyloid films, and WVP drastically increased with time. Interestingly, the amyloid-based film showed extremely low WVP, and close to zero mass change was measured during the first 4 h of the test. On average, the hybrid monomer films showed a WVP value of  $2.05 \times 10^{-6}$  g m m<sup>-2</sup> day<sup>-1</sup> Pa<sup>-1</sup>, which is 1.5 times higher than the hybrid amyloid ones  $(1.65 \times 10^{-6}$  g m m<sup>-2</sup> day<sup>-1</sup> Pa<sup>-1</sup>).

**2.8. Antioxidant Activity.** The antioxidant properties of the different hybrid films were analyzed with ABTS radical scavenging activity, and the results are presented in Figure 5b. The untreated hybrid amyloid films and FAS-treated amyloid films showed higher antioxidant performance when compared to hybrid WPI monomer films. Amyloid films treated with CA showed antioxidant properties such as those produced with WPI monomers. The antioxidant properties of the films are derived from the amino acids such as cysteine, tyrosine, tryptophan, and histidine, which are strong free radical scavengers.<sup>44</sup> Importantly, this activity is enhanced for amyloid fibrils due to their significantly higher surface-to-volume ratio than protein monomers.

**2.9. Food Migration.** To evaluate the performance of films as food packaging materials, the migration of components from the films to food was tested using Tenax, a standard dry food simulant. As shown in Figure 5c, in hybrid amyloid films, a migration intensity of  $3.6 \text{ mg/dm}^2$  is found, a value which is well below the limit ( $10 \text{ mg/dm}^2$ ) set by European Union legislation. Hybrid WPI monomer films, however, resulted in a migration rate of  $35.7 \text{ mg/dm}^2$ , that is, well above the acceptable threshold.

**2.10. Generality, Scalability, and Benchmark to Other Plastics.** To explore the generality of the approach for fabricating different hybrid amyloid fibrils, we replaced PVA with methylcellulose (MC). As observed in Figure 6a, the resulting HAm–MC film was smooth, flexible, and transparent. While both PVA and MC are biodegradable polymers, MC has the advantage of being from biosources and more sustainable. However, the water stability of native MC hybrid amyloid fibril films was lower than the ones with PVA. Next, we demonstrate the scalability of the hybrid whey amyloid fibril bioplastics by their production on a larger scale. As shown in Figure 6b, the bioplastics film with the size of  $1.5 \text{ m} \times 1.5 \text{ m}$  can be produced successfully *via* a simple solution-casting process.

Additionally, to exhibit these novel bioplastics' potential applications, we develop different product prototypes using hybrid amyloid–PVA bioplastics films. As shown in Figure 6c, the bioplastics are transparent and robust and can be easily transformed into different packaging products.

The mechanical properties of the hybrid amyloid fibril films with PVA and MC were compared to different engineering, thermosets, rubbers, and biodegradable plastics<sup>45</sup> (Figure 7a). Although both bioplastics in this work have similar elastic modulus, HAm–PVA has a higher elongation value compared to HAm–MC, reaching the value of 750%. This excellent



**Figure 6.** Generality, scalability, and application of hybrid bioplastics. (a) Visual appearance of the hybrid bioplastics based on amyloid fibrils and MC. (b) Scalability example of hybrid amyloid fibril bioplastics. (c) Packaging application of hybrid amyloid PVA bioplastics.

strain value places the HAm–PVA film among the best elastic plastics ever produced. Even though the young modulus of bioplastics in this work was in the order of polytetrafluoroethylene (PTFE), polybutylene succinate (PBS), poly( $\varepsilon$ caprolactone) (PCL), low-density polyethylene (LDPE), and ethylene propylene diene monomer (EPDM), their elastic modulus was lower than the bioplastics such as PBS and polylactic acid (PLA). However, precisely owing to such a high elastic modulus and a low elongation rate, it is not easy to make applicable bioplastics with PBS and PLA, and typically, they need to be blended with more flexible plastics for market applications.<sup>46</sup> This fact can be reflected in the toughness values shown in Figure 7b. Most biodegradable plastics suffer from low toughness values, resulting in products that are not ductile and are fragile. In the case of amyloid-based films, this property is significantly enhanced, resulting in toughness values several orders of magnitude superior to most of the commonly used bioplastics.

2.11. Life Cycle Assessment. Life cycle assessment (LCA) of HAm-PVA compared to PLA and polyvinyl fluoride (PVF), including the exact amount of each category impact, is summarized in Table 2. Furthermore, the normalized environmental impact profile of production of the three plastics, comprising all 18 impacts, is presented in Figure 8a. Across all the impact categories, HAm-PVA bioplastics have the lowest environmental impact compared to PLA and PVF. This superiority is due to the HAm–PVA bioplastics' greener and more straightforward production process. More importantly, the raw material for HAm-PVA bioplastics production is a waste from the dairy industry, which adds an additional value to sustainability and the circular economy. Combined with their excellent properties and degradability, the bioplastics presented in this work could lead to further environmental benefits not included in this LCA.

As observed, freshwater and marine ecotoxicities, human toxicity, and freshwater eutrophication have the highest relative contribution to the environmental impacts. The LCA results reveal that the leading cause for the high values in these categories comes from the required energy for plastics production. To shed light on energy demand and correlate it with the other environmental impacts, we further performed cumulative energy demand (CED) analysis. The energy use breaks down into the categories of nonrenewable (fossil, nuclear, and biomass) and renewable (biomass, solar/wind/ geothermal, and hydro) energy.<sup>52</sup> As shown in Figure 8b, PVF and PLA have four and three times higher energy consumption, respectively, than HAm-PVA bioplastics. Figure 8c shows the impact of the three plastics production on climate change. While 1 kg of PVF and PLA production approximately results in around 17 and 9 kg of  $CO_2$  eq, production of 1 kg HAm-PVA bioplastics only emits about 4 kg of CO<sub>2</sub> eq. Marine ecotoxicity is one of the most acute destructive effects



**Figure 7.** Benchmark to other plastics. (a) Mechanical properties of different hybrid amyloid fibril bioplastics and other plastics.<sup>45</sup> (b) Toughness values of bioplastics based on starch,<sup>47,48</sup> PHB,<sup>49</sup> PHA,<sup>50</sup> PLA,<sup>49</sup> cellulose,<sup>51</sup> and hybrid amyloid films.

impact category	unit	HAm-PVA	PLA	PVF
climate change	kg CO <sub>2</sub> eq	4.260198	9.220808	16.65613
ozone depletion	kg CFC-11 eq	$5.58 \times 10^{-7}$	$4.76 \times 10^{-7}$	$1.14 \times 10^{-6}$
terrestrial acidification	kg SO <sub>2</sub> eq	0.020939	0.048895	0.078856
freshwater eutrophication	kg P eq	0.001091	0.004544	0.007002
marine eutrophication	kg N eq	0.001531	0.007004	0.002663
human toxicity	kg 1,4-DB eq	0.934583	2.914094	6.327723
photochemical oxidant formation	kg NMVOC	0.010556	0.027956	0.037809
particulate matter formation	kg PM10 eq	0.007353	0.029297	0.042293
terrestrial ecotoxicity	kg 1,4-DB eq	0.000881	0.008153	0.000708
freshwater ecotoxicity	kg 1,4-DB eq	0.03041	0.079411	0.174013
marine ecotoxicity	kg 1,4-DB eq	0.02145	0.070634	0.163069
ionising radiation	kBq U235 eq	0.065584	1.161086	1.593614
agricultural land occupation	m²a	0.071638	1.518946	0.576349
urban land occupation	m²a	0.009264	0.102883	0.103839
natural land transformation	$m^2$	0.000828	0.001176	0.002372
water depletion	m <sup>3</sup>	0.070116	0.322912	0.21498
metal depletion	kg Fe eq	0.028109	0.115734	0.662357
fossil depletion	kg oil eq	1.468327	2.324439	3.864043

#### Table 2. LCA Impact Assessment Based on the ReCiPe Midpoint Method

of plastics on the environment because 10% of the plastics produced every year ends up in the oceans.<sup>53</sup> As observed in Figure 8d, the results for marine ecotoxicity showed the same trend as the climate change impact, where HAm–PVA bioplastics showed a minimal effect (0.02 kg 1,4-DB eq) compared to PLA (0.07 kg 1,4-DB eq) and PVF (0.16 kg 1,4-DB eq). Furthermore, as shown in Figure 8e, compared to the other two plastics, PLA has the highest impact on water resources, mainly due to water irrigation during crop production and high energy demand. Altogether, the LCA results demonstrate the superiority of HAm–PVA bioplastics unambiguously in terms of sustainability and environmental compatibility compared to other typical plastics and bioplastics.

## 3. CONCLUSIONS

In this work, we introduced amyloid fibrils as a suitable building block for developing hybrid bioplastics. To this end, in situ fibrillization of the whey monomer, selected here as a model protein from food processing waste, took place in the presence of a plasticizer and biodegradable plastics such as PVA and MC. The resultant films were transparent, robust, flexible, and tough and exhibited acceptable water stabilities and good barrier properties for food packaging applications. The films can be prepared from low-priced bio-based and biodegradable sources, highlighting their affordability and environment friendliness for a broad range of applications. Accordingly, LCA performed on these new bioplastics and two biodegradable polymers as benchmarks revealed a superior performance of the present bioplastics in all the normalized environmental impact indicators. Additionally, this new class of bioplastics adds value to the circular economy by valorizing whey as a byproduct of the dairy industry. These results demonstrated the potential of hybrid whey amyloid fibril bioplastics as an efficient, sustainable, and inexpensive solution for alleviating the global plastics production and pollution issue.

#### 4. EXPERIMENTAL SECTION/METHODS

**4.1. Materials.** The WPI was supplied from Fonterra, New Zealand. PVA (fully hydrolyzed,  $M_w$  approx. 200,000) and hydro-

chloric acid (36%) were purchased from Merck. MC (viscosity: 400 cP), glycerol ( $\geq$ 99.5%), CA ( $\geq$ 99.5%), 1H,1H,2H,2H-perfluorooctyl-triethoxysilane (FAS), 2,2'-azinobis(ethyl-2,3-dihydrobenzothiazo-line-6-sulfonic acid) diammonium salt (ABTS), and Tenax porous polymer adsorbent (60–80 mesh) were obtained from Sigma-Aldrich.

4.2. Bioplastics Films Formation. For fabricating hybrid amyloid fibril films, 4 g of the WPI first were dissolved in 100 mL of water. Then, the pH of the solution was adjusted to 2, and 3 g of glycerol, as a plasticizer, was dispersed in the solution. For hybrid amyloid fibril films with PVA and MC, 3 and 2 g of each compound, respectively, were added to the solution. To convert the WPI monomers to amyloid fibrils and dissolve the biodegradable polymer, the solution was stirred and heated at 90 °C for 5 h. After the incubation, the solution was quenched and cast immediately on a Petri dish to dry at room temperature. For the study of the effect of CA in film properties, 1.5 g was added to solutions before the fibrillization process. Moreover, for fabricating more hydrophobic films, they were placed in an ethanol solution containing FAS (0.5 wt %) for 1 h. Subsequently, the films were dried under room temperature to obtain the FAS-coated hybrid amyloid films. The details of composition and treatment are listed in Table 3.

**4.3. Characterization.** *4.3.1. Atomic Force Microscopy.* AFM and SEM were used to characterize the morphologies of amyloid fibrils and their hybrid films, respectively. For AFM, the solutions were dried onto cleaved mica and analyzed by applying the tapping mode. A Hitachi SUS000 scanning electron microscope characterized the structure and properties of hybrid bioplastics films. Small pieces of films were attached to stubs with paste and sputter-coated with 5 nm of platinum/palladium under planetary rotational movement (Safematic, CCU-10, Switzerland) before imaging.

4.3.2. Mechanical Properties. The mechanical properties of films were evaluated by measuring tensile strength and elongation using a Z010 (Zwick) equipped with a 100 N load cell. The stress ( $\sigma$ )-strain ( $\varepsilon$ ) curves were obtained at room temperature. The Young's modulus was calculated from the stress-strain curves. All experiments were performed in triplicate.

4.3.3. Water Contact Angle. The water contact angle of the films was recorded by the Nikon D300 digital camera at 25  $^{\circ}$ C and a relative humidity of 50%. To shed light on the interaction of films with water, their weight loss and water absorption after immersing in water at different time intervals were measured.

**4.3.4.** Water Absorption. For the water absorption evaluation of films, approximately 150 mg of each film was immersed in water for 1 h, and the weight changes due to water absorption were measured before and after immersion.



**Figure 8.** LCA of HAm–PVA compared to PLA and PVF. (a) Normalized environmental impact profile of HAm–PVA compared to PLA and PVF, comprising all 18 impact categories of the ReciPe method (CC: climate change, OD: ozone depletion, TA: terrestrial acidification, FE: freshwater eutrophication, ME: marine eutrophication, HTOX: human toxicity, POF: photochemical oxidant formation, PMF: particulate matter formation, TTOX: terrestrial ecotoxicity, FTOX: freshwater ecotoxicity, MTOX: marine ecotoxicity, IR: ionizing radiation, ALO: agricultural land occupation, ULO: urban land occupation, NLT: natural land transformation, WAT: water depletion, MET: metal depletion, and FOS: fossil depletion). (b) LCA result comparison based on the CED impact. (c) LCA result comparison based on the climate change impact. (d) LCA result comparison based on the water depletion impact.

# Table 3. Bioplastics Film Composition

bioplastics	whey amyloid (wt %)	whey monomer (wt %)	PVA (wt %)	MC (wt %)	Gly (wt %)	FAS treatment
hybrid amyloid	40		30		30	no
hybrid monomer		40	30		30	no
hybrid amyloid FAS	40		30		30	yes
hybrid amyloid CA	40		30		30	no
hybrid amyloid MC	45			23	32	no

4.3.5. Antioxidant Activity. The antioxidant activity of the film was determined by the spectrophotometric method described by

Kusznierewicz *et al.*<sup>54</sup> Briefly, the stock solution of ABTS with the concentration of 7 mM was diluted with water to display the

$$Sc_{ABTS} = \frac{V_{ABTS} \times (A_0 - A_f) \times 1000}{\varepsilon \times l \times m}$$
(1)

where,  $Sc_{ABTS}$  is the amount of scavenged ABTS ( $\mu$ mol),  $V_{ABTS}$  is the volume of stock solution of ABTS added to the film (mL),  $A_0$  is the absorbance of the initial ABTS solution;  $A_f$  is the absorbance of the radical solution after reaction time;  $\varepsilon$  is the ABTS molar extinction coefficient (16,000 M<sup>-1</sup> cm<sup>-1</sup> at 734 nm), l is the optical path of the cuvette (1 cm), and *m* is the film mass (g).<sup>54</sup>

4.3.6. Food-Contact Migration. The food-contact migration properties of the bioplastics films were evaluated based on EU technical guidelines for compliance testing in the framework of the plastics FCM Regulation (EU) no. 10/2011.<sup>55</sup> To assess the possible migration of molecules from the films to the food, Tenax was used as a dry food simulant. In a clean glass Petri dish, a square-shaped film with a dimension of 1 cm was placed between two layers of Tenax powder (15 mg below and 15 mg above the sample) and stored in the oven for 2 h at 70 °C. The overall food migration was calculated by the mass difference of Tenax before and after the treatment.

**4.3.7.** Water Vapor Permeability. The WVP of the different hybrid films has been characterized using the modified cup method.<sup>56</sup> Briefly, glass vials with an inner diameter of 1.5 cm and a height of 6 cm were filled with 15 mL of water. The films were mounted on top of the vial using the Parafilm to block air leakages. The weights of the vials have been measured after 1, 2, 4, 8, and 24 h, and the WVP was estimated for each time step using the following formula

$$WVP\left(g\frac{m}{m^2 \text{ day Pa}}\right) = \frac{W \cdot t}{A \cdot T \cdot \Delta P}$$
(2)

where W (g) is the weight decrease, t (m) is the film thickness, A (m<sup>2</sup>) is the film surface area exposed to air, T (day) is the time, and  $\Delta P$  is the difference in water vapor pressure between the inside and the outside of the vial (assumed 3173 Pa).

4.3.8. Life Cycle Assessment. The environmental impact of HAm– PVA bioplastics was compared via LCA with one typical plastics film PVF and one common bioplastics PLA. The LCA was attributional and prospective of an emerging product and performed according to the protocol of the (ISO) 14040/44 standard.<sup>57,58</sup> The LCA assesses cradle-to-use life cycle impacts of producing 1 kg of these plastics. The life cycle inventory (LCI) for all plastics is summarized in Table S1. For HAm–PVA bioplastics, the process data were provided by our laboratory experiments. For PLA, the inventory data for Ingeo polylactide production technology from corn were used based on the LCA assessed by Suwanmanee *et al.*<sup>59</sup> Life cycle models were built using SimaPro v. 8.3 and based on Ecoinvent 3 database. The ReCiPe midpoint (H) method was used to evaluate the impact of the LCI over a broad range of impact categories, 18 in this case. Additionally, CED was used for energy use calculations.

#### ASSOCIATED CONTENT

#### **Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.1c03937.

LCI data, photographs of water contact angle on the film surface, and Young's modulus values for different films (PDF)

# AUTHOR INFORMATION

# **Corresponding Author**

Raffaele Mezzenga – Department of Health Sciences and Technology, ETH Zurich, 8092 Zurich, Switzerland; Department of Materials, ETH Zurich, 8093 Zurich, Switzerland; orcid.org/0000-0002-5739-2610; Email: raffaele.mezzenga@hest.ethz.ch

# Authors

Mohammad Peydayesh – Department of Health Sciences and Technology, ETH Zurich, 8092 Zurich, Switzerland; orcid.org/0000-0002-6265-3811

Massimo Bagnani – Department of Health Sciences and Technology, ETH Zurich, 8092 Zurich, Switzerland; orcid.org/0000-0002-1326-1600

Complete contact information is available at: https://pubs.acs.org/10.1021/acssuschemeng.1c03937

### **Author Contributions**

<sup>§</sup>M.P and M.B. contributed equally to this work.

#### Notes

The authors declare the following competing financial interest(s): A patent was filed, as duly disclosed in the manuscript final section.

#### REFERENCES

(1) Yuan, L.; Buzoglu Kurnaz, L.; Tang, C. Alternative Plastics. *Nat. Sustain.* **2021**, 2021, 1–2.

(2) Global plastic production 1950-2019. https://www.statista.com/ statistics/282732/global-production-of-plastics-since-1950/ (accessed Aug 2, 2021).

(3) Geyer, R.; Jambeck, J. R.; Law, K. L. Production, Use, and Fate of All Plastics Ever Made. *Sci. Adv.* **2017**, *3*, No. e1700782.

(4) Garcia, J. M.; Robertson, M. L. The Future of Plastics Recycling. *Science* **201**7, 358, 870–872.

(5) Brooks, A. L.; Wang, S.; Jambeck, J. R. The Chinese Import Ban and Its Impact on Global Plastic Waste Trade. *Sci. Adv.* **2018**, *4*, No. eaat0131.

(6) Barnes, D. K. A.; Galgani, F.; Thompson, R. C.; Barlaz, M. Accumulation and Fragmentation of Plastic Debris in Global Environments. *Philos. Trans. R. Soc., B* **2009**, *364*, 1985–1998.

(7) Zalasiewicz, J.; Waters, C. N.; Ivar do Sul, J. A.; Corcoran, P. L.; Barnosky, A. D.; Cearreta, A.; Edgeworth, M.; Gałuszka, A.; Jeandel, C.; Leinfelder, R.; McNeill, J. R.; Steffen, W.; Summerhayes, C.; Wagreich, M.; Williams, M.; Wolfe, A. P.; Yonan, Y. The Geological Cycle of Plastics and Their Use as a Stratigraphic Indicator of the Anthropocene. *Anthropocene* **2016**, *13*, 4–17.

(8) 8-Bioplastics from agricultural wastelElsevier Enhanced Reader. https://reader.elsevier.com/reader/sd/pii/ B 9 7 8 0 1 2 8 1 9 9 5 3 4 0 0 0 0 5 7 ? to k e n =

5 D B 1 6 8 C 9 A 1 F 0 5 C F 5 8 9 C 6 C 3 5 0 9 3 2 6 5 4 1 2 D B235589465A1979E7C4B80EE600D776ECF43F36B5BAAD74 BE0767032E95D9AC (accessed Mar 22, 2021).

(9) Iles, A.; Martin, A. N. Expanding Bioplastics Production: Sustainable Business Innovation in the Chemical Industry. *J. Clean. Prod.* **2013**, *45*, 38–49.

(10) Arikan, E. B.; Ozsoy, H. D. A Review: Investigation of Bioplastics. J. Civ. Eng. Architect. 2015, 9, 188–192.

(11) Arvanitoyannis, I.; Psomiadou, E.; Nakayama, A.; Aiba, S.; Yamamoto, N. Edible Films Made from Gelatin, Soluble Starch and Polyols, Part 3. *Food Chem.* **1997**, *60*, 593–604.

(12) Guilbert, S.; Cuq, B.; Gontard, N. Recent Innovations in Edible and/or Biodegradable Packaging Materials. *Food Addit. Contam.* **1997**, *14*, 741.

(13) Jerez, A.; Partal, P.; Martínez, I.; Gallegos, C.; Guerrero, A. Protein-Based Bioplastics: Effect of Thermo-Mechanical Processing. *Rheol. Acta* **2007**, *46*, 711–720.

(14) Pommet, M.; Redl, A.; Morel, M.-H.; Domenek, S.; Guilbert, S. Thermoplastic Processing of Protein-Based Bioplastics: Chemical Engineering Aspects of Mixing, Extrusion and Hot Molding. *Macromol. Symp.* **2003**, *197*, 207–218.

(15) Kopf-Bolanz, K.; Bisig, W.; Jungbluth, N.; Denkel, C. Potential of whey as a food constituent in Switzerland. https://www.agrarforschungschweiz.ch/en/2015/06/potential-of-whey-as-a-food-constituent-in-switzerland/ (accessed 25 May 2021).

(16) Nikodinovic-Runic, J.; Guzik, M.; Kenny, S. T.; Babu, R.; Werker, A.; O Connor, K. E. Carbon-Rich Wastes as Feedstocks for Biodegradable Polymer (Polyhydroxyalkanoate) Production Using Bacteria. *Adv. Appl. Microbiol.* **2013**, *84*, 139–200.

(17) Prazeres, A. R.; Carvalho, F.; Rivas, J. Cheese Whey Management: A Review. J. Environ. Manage. 2012, 110, 48-68.

(18) Amaro, T. M. M. M.; Rosa, D.; Comi, G.; Iacumin, L. Prospects for the Use of Whey for Polyhydroxyalkanoate (PHA) Production. *Front. Microbiol.* **2019**, *10*, 992.

(19) Chandrapala, J.; Duke, M. C.; Gray, S. R.; Weeks, M.; Palmer, M.; Vasiljevic, T. Nanofiltration and Nanodiafiltration of Acid Whey as a Function of PH and Temperature. *Sep. Purif. Technol.* **2016**, *160*, 18–27.

(20) Kontopidis, G.; Holt, C.; Sawyer, L. Invited Review:  $\beta$ -Lactoglobulin: Binding Properties, Structure, and Function. *J. Dairy Sci.* 2004, 87, 785–796.

(21) Rocha-Mendoza, D.; Kosmerl, E.; Krentz, A.; Zhang, L.; Badiger, S.; Miyagusuku-Cruzado, G.; Mayta-Apaza, A.; Giusti, M.; Jiménez-Flores, R.; García-Cano, I. Invited Review: Acid Whey Trends and Health Benefits. *J. Dairy Sci.* **2021**, *104*, 1262–1275.

(22) Lara, C.; Adamcik, J.; Jordens, S.; Mezzenga, R. General Self-Assembly Mechanism Converting Hydrolyzed Globular Proteins into Giant Multistranded Amyloid Ribbons. *Biomacromolecules* **2011**, *12*, 1868–1875.

(23) Mezzenga, R.; Fischer, P. The Self-Assembly, Aggregation and Phase Transitions of Food Protein Systems in One, Two and Three Dimensions. *Rep. Prog. Phys.* **2013**, *76*, 046601.

(24) Adamcik, J.; Mezzenga, R. Proteins Fibrils from a Polymer Physics Perspective. *Macromolecules* **2012**, *45*, 1137–1150.

(25) Cao, Y.; Mezzenga, R. Food Protein Amyloid Fibrils: Origin, Structure, Formation, Characterization, Applications and Health Implications. *Adv. Colloid Interface Sci.* **2019**, *269*, 334–356.

(26) Knowles, T. P. J.; Mezzenga, R. Amyloid Fibrils as Building Blocks for Natural and Artificial Functional Materials. *Adv. Mater.* **2016**, *28*, 6546–6561.

(27) Shen, Y.; Posavec, L.; Bolisetty, S.; Hilty, F. M.; Nyström, G.; Kohlbrecher, J.; Hilbe, M.; Rossi, A.; Baumgartner, J.; Zimmermann, M. B.; Mezzenga, R. Amyloid Fibril Systems Reduce, Stabilize and Deliver Bioavailable Nanosized Iron. *Nat. Nanotechnol.* **2017**, *12*, 642–647.

(28) Bolisetty, S.; Mezzenga, R. Amyloid-Carbon Hybrid Membranes for Universal Water Purification. *Nat. Nanotechnol.* **2016**, *11*, 365–371.

(29) Akkermans, C.; Van Der Goot, A. J.; Venema, P.; Gruppen, H.; Vereijken, J. M.; Van Der Linden, E.; Boom, R. M. Micrometer-Sized Fibrillar Protein Aggregates from Soy Glycinin and Soy Protein Isolate. J. Agric. Food Chem. **2007**, 55, 9877–9882.

(30) Kavanagh, G. M.; Clark, A. H.; Ross-Murphy, S. B. Heat-Induced Gelation of Globular Proteins: Part 3. Molecular Studies on Low PH  $\beta$ -Lactoglobulin Gels. *Int. J. Biol. Macromol.* **2000**, 28, 41– 50.

(31) Cao, Y.; Mezzenga, R. Food Protein Amyloid Fibrils: Origin, Structure, Formation, Characterization, Applications and Health Implications. *Adv. Colloid Interface Sci.* **2019**, *269*, 334–356.

(32) Nyström, G.; Fernández-Ronco, M. P.; Bolisetty, S.; Mazzotti, M.; Mezzenga, R. Amyloid Templated Gold Aerogels. *Adv. Mater.* **2016**, *28*, 472–478.

(33) Bagnani, M.; Nyström, G.; De Michele, C.; Mezzenga, R. Amyloid Fibrils Length Controls Shape and Structure of Nematic and Cholesteric Tactoids. *ACS Nano* **2019**, *13*, 591–600.

(34) Sabato, S. F.; Ouattara, B.; Yu, H.; D'aprano, G.; Le Tien, C.; Mateescu, M. A.; Lacroix, M. Mechanical and Barrier Properties of Cross-Linked Soy and Whey Protein Based Films. *J. Agric. Food Chem.* **2001**, *49*, 1397.

(35) Cinelli, P.; Schmid, M.; Bugnicourt, E.; Wildner, J.; Bazzichi, A.; Anguillesi, I.; Lazzeri, A. Whey Protein Layer Applied on Biodegradable Packaging Film to Improve Barrier Properties While Maintaining Biodegradability. *Polym. Degrad. Stab.* **2014**, *108*, 151–157.

(36) Sothornvit, R.; Krochta, J. M. Oxygen Permeability and Mechanical Properties of Films from Hydrolyzed Whey Protein. *J. Agric. Food Chem.* **2000**, *48*, 3913–3916.

(37) Guo, Y.; Lu, H.; Zhao, F.; Zhou, X.; Shi, W.; Yu, G. Biomass-Derived Hybrid Hydrogel Evaporators for Cost-Effective Solar Water Purification. *Adv. Mater.* **2020**, *32*, 1907061.

(38) Bilo, F.; Pandini, S.; Sartore, L.; Depero, L. E.; Gargiulo, G.; Bonassi, A.; Federici, S.; Bontempi, E. A Sustainable Bioplastic Obtained from Rice Straw. *J. Clean. Prod.* **2018**, *200*, 357–368.

(39) Liu, M.; Arshadi, M.; Javi, F.; Lawrence, P.; Davachi, S. M.; Abbaspourrad, A. Green and Facile Preparation of Hydrophobic Bioplastics from Tea Waste. *J. Clean. Prod.* **2020**, *276*, 123353.

(40) Trujillo-de Santiago, G.; Rojas-de Gante, C.; García-Lara, S.; Verdolotti, L.; Di Maio, E.; Iannace, S. Thermoplastic Processing of Blue Maize and White Sorghum Flours to Produce Bioplastics. *J. Polym. Environ.* **2015**, *23*, 72–82.

(41) Zhang, F.; You, X.; Dou, H.; Liu, Z.; Zuo, B.; Zhang, X. Facile Fabrication of Robust Silk Nanofibril Films via Direct Dissolution of Silk in CaCl2-Formic Acid Solution. *ACS Appl. Mater. Interfaces* **2015**, 7, 3352–3361.

(42) Wilpiszewska, K.; Antosik, A. K.; Zdanowicz, M. The Effect of Citric Acid on Physicochemical Properties of Hydrophilic Carboxymethyl Starch-Based Films. *J. Polym. Environ.* **2019**, *27*, 1379–1387.

(43) Rahma Khoirunnisa, A.; Made Joni, I.; Panatarani, C.; Rochima, E.; Praseptiangga, D. UV-Screening, Transparency and Water Barrier Properties of Semi Refined Iota Carrageenan Packaging Film Incorporated with ZnO Nanoparticles. *AIP Conf. Proc.* **2018**, *1927*, 30041.

(44) Berlett, B. S.; Stadtman, E. R. Protein Oxidation in Aging, Disease, and Oxidative Stress. J. Biol. Chem. 1997, 272, 20313.

(45) Luzi, F.; Torre, L.; Kenny, J.; Puglia, D. Bio- and Fossil-Based Polymeric Blends and Nanocomposites for Packaging: Structure– Property Relationship. *Materials* **2019**, *12*, 471.

(46) Sindhu, R.; Ammu, B.; Binod, P.; Deepthi, S. K.; Ramachandran, K. B.; Soccol, C. R.; Pandey, A. Production and Characterization of Poly-3-Hydroxybutyrate from Crude Glycerol by Bacillus Sphaericus NII 0838 and Improving Its Thermal Properties by Blending with Other Polymers. *Braz. Arch. Biol. Technol.* 2011, *54*, 783–794.

(47) Ghosh Dastidar, T.; Netravali, A. Cross-Linked Waxy Maize Starch-Based "Green" Composites. ACS Sustain. Chem. Eng. 2013, 1, 1537–1544.

(48) Shi, A.-m.; Wang, L.-j.; Li, D.; Adhikari, B. Characterization of Starch Films Containing Starch Nanoparticles Part 1: Physical and Mechanical Properties. *Carbohydr. Polym.* **2013**, *96*, 593–601.

(49) Chee Chuan Yeo, J.; Muiruri, J. K.; Hoon Tan, B.; Thitsartarn, W.; Kong, J.; Zhang, X.; Li, Z.; He, C. Biodegradable PHB-Rubber Copolymer Toughened PLA Green Composites with Ultrahigh Extensibility. *ACS Sustainable Chem. Eng.* **2018**, *6*, 15517.

(50) Thellen, C.; Coyne, M.; Froio, D.; Auerbach, M.; Wirsen, C.; Ratto, J. A. A Processing, Characterization and Marine Biodegradation Study of Melt-Extruded Polyhydroxyalkanoate (PHA) Films. *J. Polym. Environ.* **2008**, *16*, 1–11.

(51) Wei, P.; Huang, J.; Lu, Y.; Zhong, Y.; Men, Y.; Zhang, L.; Cai, J. Unique Stress Whitening and High-Toughness Double-Cross-Linked Cellulose Films. *ACS Sustain. Chem. Eng.* **2019**, *7*, 1707–1717.

(52) Li, Q.; McGinnis, S.; Sydnor, C.; Wong, A.; Renneckar, S. Nanocellulose Life Cycle Assessment. ACS Sustain. Chem. Eng. 2013, 1, 919–928.

(53) Mendenhall, E. Oceans of Plastic: A Research Agenda to Propel Policy Development. *Mar. Pol.* **2018**, *96*, 291–298.

(54) Kusznierewicz, B.; Staroszczyk, H.; Malinowska-Pańczyk, E.; Parchem, K.; Bartoszek, A. Novel ABTS-Dot-Blot Method for the Assessment of Antioxidant Properties of Food Packaging. *Food Packag. Shelf Life* **2020**, *24*, 100478.

(55) Perotto, G.; Ceseracciu, L.; Simonutti, R.; Paul, U. C.; Guzman-Puyol, S.; Tran, T.-N.; Bayer, I. S.; Athanassiou, A. Bioplastics from Vegetable Waste: Via an Eco-Friendly Water-Based Process. *Green Chem.* **2018**, *20*, 894–902.

(56) Han, J.; Shin, S.-H.; Park, K.-M.; Kim, K. M. Characterization of Physical, Mechanical, and Antioxidant Properties of Soy Protein-Based Bioplastic Films Containing Carboxymethylcellulose and Catechin. *Food Sci. Biotechnol.* **2015**, *24*, 939–945.

(57) Arvidsson, R.; Kushnir, D.; Sandén, B. A.; Molander, S. Prospective Life Cycle Assessment of Graphene Production by Ultrasonication and Chemical Reduction. *Environ. Sci. Technol.* **2014**, 48, 4529–4536.

(58) Walser, T.; Demou, E.; Lang, D. J.; Hellweg, S. Prospective Environmental Life Cycle Assessment of Nanosilver T-Shirts. *Environ. Sci. Technol.* **2011**, *45*, 4570–4578.

(59) Suwanmanee, U.; Varabuntoonvit, V.; Chaiwutthinan, P.; Tajan, M.; Mungcharoen, T.; Leejarkpai, T. Life Cycle Assessment of Single Use Thermoform Boxes Made from Polystyrene (PS), Polylactic Acid, (PLA), and PLA/Starch: Cradle to Consumer Gate. *Int. J. Life Cycle Assess.* **2013**, *18*, 401–417.