CHAPTER

6

Modification of Protein Rich Algal-Biomass to Form Bioplastics and Odor Removal

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1 INTRODUCTION

Proteins are by-products from the agricultural and horticultural industries (Verbeek and van den Berg, 2010). Because proteins are naturally occurring polymers and do not require polymer synthesis, they can become value-added products when converted into plastics. However, unlike synthetic polymers that contain identical monomers, proteins have 20 different amino acids that form their polypeptide chain. The protein is folded into secondary, tertiary, and quaternary structures and is stabilized by intramolecular interactions, such as hydrogen bonding, electrostatic interactions, and hydrophobic interactions. The amino acid sequence of the protein will define its final properties as a polymer (Rouilly and Rigal, 2002; Swain et al., 2004; Verbeek and van den Berg, 2010). Plant proteins used for plastic production include corn zein, wheat gluten, peanut protein, and soy protein (Swain et al., 2004). Animal proteins, such as keratin, gelatin, collagen, and whey protein, have also been used to produce plastics (Mekonnen et al., 2013).

To improve their functional properties, it is necessary to modify the proteins. Protein modification includes the denaturation of proteins. Proteins are denatured either by thermal or chemical treatment. Denaturation allows the protein to unfold and realign in a new three-dimensional configuration that is stabilized by new intramolecular interactions (Verbeek and van den Berg, 2010). Depending on their primary structure, denaturation can cause proteins to become available to form cross-links.

Protein Byproducts
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Soy protein was used to make the first biopolymers, and this set the precedent for making polymers based on agricultural materials. For example, in the 1930s, Henry Ford manufactured automobile body parts from a mixture of soy protein and phenol-formaldehyde resin (Swain et al., 2004). Soy protein is isolated from the soybean through processing. Soy protein products include soy flour, soy concentration, and soy isolate. Soy isolates have the highest concentration of protein (more than 90% dry weight) (Swain et al., 2004).

Soy protein plastics have moderate strength and good biodegradability, but they are also typically brittle (Zhang et al., 2003; Mo and Xiuzhi, 2001). Denaturation through chemical modification to the soy protein could improve its ability to form plastics with better mechanical properties. Urea is a common chemical used for the denaturation of protein. In one study, authors Mo and Sun showed that the tensile strength and Young's modulus increased with elevated urea concentration for urea-modified soy protein isolate plastics. Furthermore, compatibilizers or cross-linking agents can be added to protein bioplastics to improve the mechanical properties of protein bioplastics (Mo and Xiuzhi, 2001). Park et al. (2000) showed that soy isolate protein cross-linked with glutaraldehyde significantly improved the tensile strength and elongation at break with increasing concentrations of glutaraldehyde.

The primary disadvantage of using soy protein and other terrestrial proteins for the production of bioplastics is the feedstock. Also plastic production competes with the food industry for its food applications. It becomes advantageous to invest in other agricultural coproducts and waste products as sources of bioplastic proteins. For example, feather meal protein, a by-product of rendering or recycling of animals and waste, becomes a value-added product when considered for bioplastic conversion. Sharma et al. (2008) reported a 9.2 MPa stress at break, 1.40% strain at break, and 2.20 GPa Young's modulus for compression-molded feather meal–protein plastic. Feather meal–protein plastic has high stiffness and low extensibility and is comparable to other bioplastics made from unplasticized proteins. Modification to feather meal protein using thermal denaturation requires pressure and temperature. Differential scanning calorimetry (DSC) analysis indicated a peak denaturation temperature of feather meal protein occurring at 134°C (Sharma et al., 2008).

Algae offers an alternative for bioplastic production because of their protein content, high biomass yield, ability to be cultivated in their natural environment, potential cost effectiveness, and minimized effect on the food chain (Rajendran et al., 2012; Becker 2007). Typically 10–47% of green algae's dry weight is protein (Fleurence, 1999). *Euglena gracilis* has a protein content of 39–61% of dry matter (Becker, 2007). Unlike soy protein isolate or feather meal protein, it is not economical or technically feasible to isolate the protein from the alga biomass. Therefore, harvested and dried algae (refined to a particle size approximately <150 μm) are added whole in bioplastic and thermoplastic blend formulations.

Zeller et al. (2013) investigated bioplastics and thermoplastic blends from spirulina and chlorella microalgae. Spirulina and chlorella microalgae went through modification by denaturation and thermoplastic blending. DSC indicated that spirulina had a peak denaturation at 100°C and chlorella had a peak denaturation at 110°C. Dynamic mechanical analysis (DMA) helped in optimizing and/or identifying that glycerol-plasticized spirulina and chlorella bioplastics (20% glycerol by weight) exhibited satisfactory viscoelastic properties. Tensile testing indicated that spirulina bioplastics and chlorella bioplastics had low extension and high modulus. The mechanical properties of algae bioplastics were comparable to soy protein isolate, feather meal, and duckweed. The authors also studied the influence of blending with polyethylene on mechanical performance spirulina- and chlorella-based thermoplastic blends (Zeller et al., 2013).

Zeller et al. (2013) proved that algae are a feasible alternative for bioplastic and thermoplastic production. Although they investigated chlorella and spirulina, the study in this chapter investigated the protein modification of catfish algae (planktonic algae)—considered waste and a nuisance for catfish farms—and *Nannochloropsis* (microalgae). The latter is a protein-rich coproduct and has been scaled commercially because of its ability to accumulate high levels of polyunsaturated fatty acids. Algae bioplastics and their thermoplastic blends were developed through thermomechanical processing and were evaluated for their thermal and dynamic mechanical properties. Additionally, one of the drawbacks to the protein modification of algae is odor, which either occurs naturally within the algae or is generated through thermoplastic processing because of heat and pressure. To commercialize these algae bioplastics, the odor-causing volatiles must be removed. A systematic design of experiment approach was undertaken to determine the influence of the factors, such as types of algae, scavenger materials (adsorbents), synthetic resin, and compatibilizer on the odor of plastics.

2 EXPERIMENTAL

2.1 Materials

The Solix microalgae and the catfish algae dry powder received from Algix, Inc. (Meridian, Mississippi), were used in this research. Solix microalgae is *Nannochloropsis*, which is mostly found in marine environments, but also occurs in fresh and brackish water (Fawley and Fawley, 2007). The algae of the *Nannochloropsis* genus have a diameter of about 2–3 μm and a very simple ultrastructure with reduced structural elements compared to neighboring taxa (Kandilian et al., 2013). The catfish algae are planktonic algae that grow in fishponds and are usually eaten by catfish. They are microscopic free-floating plants that are usually suspended in the top few feet of water where there is enough sunlight for their photosynthesis. These algae are mainly composed of green algae, blue–green algae, diatoms, and euglenas. The ultrahigh molecular weight polyethylene (UHMW-PE) powder and syntactic polypropylene granules were received from Sigma-Aldrich (St. Louis, Missouri). The polyethylene powder had particle sizes of 53–75 μm, and the polypropylene was processed via cryogenic grinding to similar particle sizes.

2.2 Bioplastics Processing

For preparing the algae bioplastic, thermomechanical compression molding of the samples was performed using a 24-ton benchtop press (Carver Model 3850, Wabash, Indiana) with electrically heated and water-cooled platens. The stainless steel molds can form two rectangular flex bars for DMA at one time. Each different weight ratio formulation was thoroughly hand mixed. Compression molding of samples used a 20-min cook time at ±150°C followed by a 10-min cooling period, and both were performed under pressure greater than 24,000 Pa.

2.3 Thermal Properties Analysis

Thermal analysis can provide information about the changes of the samples through the heating process. Thermal gravimetric analysis (TGA) was performed using a Mettler Toledo TGA/SDTA851e. DSC was performed using a Mettler Toledo DSC821e. TGA was performed from 25 to 500°C under N_2 environment with a heating rate of 10°C/min. DSC was performed from –50 to 250°C under N_2 environment with a heating rate of 20°C/min. All samples were prepared with sample weights between 3 and 8 mg. For the plastic samples, fine pieces were cut from DMA flex bars before running on TGA and DSC.

TABLE 6.1 Algae Bioplastics Odor Testing Factors and Level

	Levels	
Factor	**–**	**+**
A = Algae	Catfish algae	Solix microalgae
B = Scavenger	Activated carbon	Zeolite
C = Resin	Polyethylene	Polypropylene
D = Compatibilizer	Absent	Present

2.4 Mechanical Properties Analysis

DMA was used to characterize the viscoelastic behavior of the algae bioplastics. DMA was performed using a dual-cantilever setup at a frequency of 1 Hz on a DMA 8000 instrument from PerkinElmer, Inc. (Waltham, Massachusetts) for specimens with dimensions in millimeters: 9 (width) × 2.5 (thickness) × 12.5 (length). All samples were run with a displacement of 0.05 mm from room temperature to 160°C at a temperature ramp of 2°C/min. All samples were run in duplicate.

2.5 Odor Panel Test

The algae bioplastics odor testing factors are listed in Table 6.1. The percentage of algae was kept constant at 50% by weight. If either scavenger or compatibilizer or both of the two were present, 5% of each was added to the formulations, and consequently, the percentage of resin was decreased by 5 or 10%, respectively. All the samples for odor testing were conditioned at 25°C and 55% relative humidity for 24 h to be assessed by the panelists.

3 RESULTS AND DISCUSSION

3.1 Thermal Analysis of Algae Powder

Through TGA, the changes in physical and chemical properties of materials were measured as a function of increasing temperature (with constant heating rate). The TGA results of Solix microalgae and catfish algae powder showed two-step degradation (Fig. 6.1). The first one around 50–100°C represents the bound water

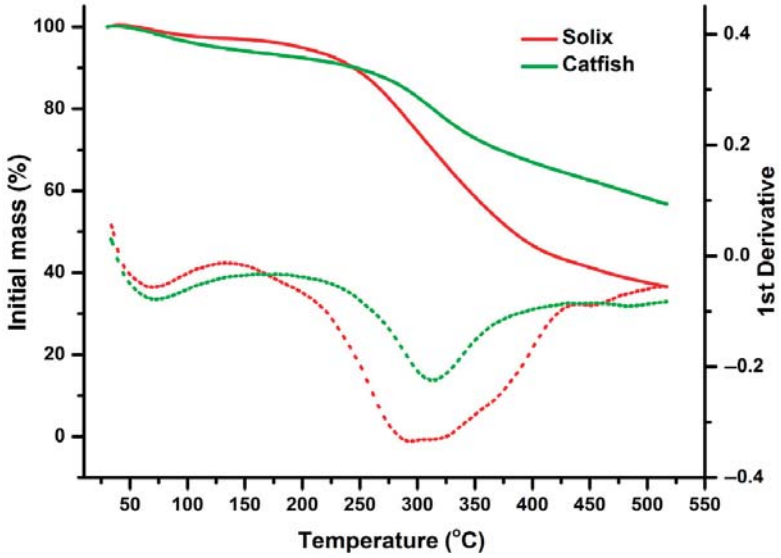

FIGURE 6.1 **TGA of Solix and catfish algae powder.**

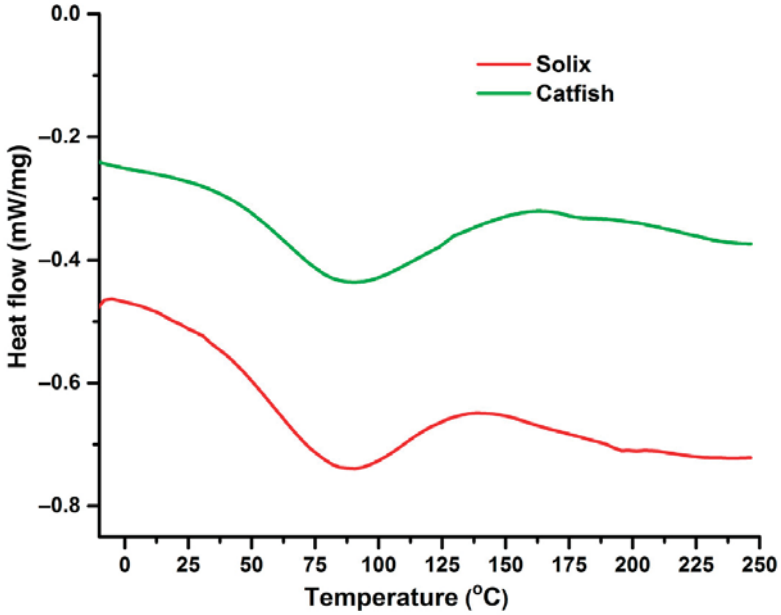

FIGURE 6.2 **DSC of Solix and catfish algae powder.**

and low volatiles loss. The second degradation occurring from 175 to 375°C and peaking at 300°C represents carbohydrate and protein burning because it occurs in the range where carbohydrates (eg, hemicelluloses, cellulose, and starch) and protein are typically degraded (Sharma et al., 2008). Solix microalgae has a greater and broader degradation peak, which may be caused by its higher content of lipids than the catfish algae.

DSC reveals the difference in the amount of heat required in increasing the temperature of a sample and reference. For the DSC results, a strong denaturing peak begins at around 20°C and ends at about 150°C (Fig. 6.2) for both Solix and catfish algae powder. The peak centers around 90°C. At 150°C, both the algae proteins are maximally denatured. The TGA result indicates that degradation can occur from around 175°C, whereas the DSC result indicates that proteins are maximally denatured at 150°C. Therefore, both kinds of algae powder are best processed at 150°C, which do not risk their degradation at higher temperatures, yet yielding their maximum denaturation.

3.2 Thermal and Mechanical Analysis of Algae Bioplastics

Figs. 6.3 and 6.4 show the TGA of algae bioplastics made of Solix and catfish algae and their thermoplastic blends (50/50 w/w) with UHMW-PE and isotactic polypropylene (PP). Both the Solix-based bioplastic and the catfish-based bioplastic showed three-step degradation in the TGA thermograph. The first degradation, which happened at 25–100°C, was caused by bound water loss and volatiles. The second degradation, which took place around 300°C, again represents the degradation of the two kinds of algae, with Solix-based bioplastic showing a broader peak. The third degradation peaks occur above 400°C because of the PP or polyethylene (PE) degradation.

Fig. 6.5 shows the DSC of Solix- and catfish-based bioplastics. The endothermic peak at around 75°C represents the algae denaturing.

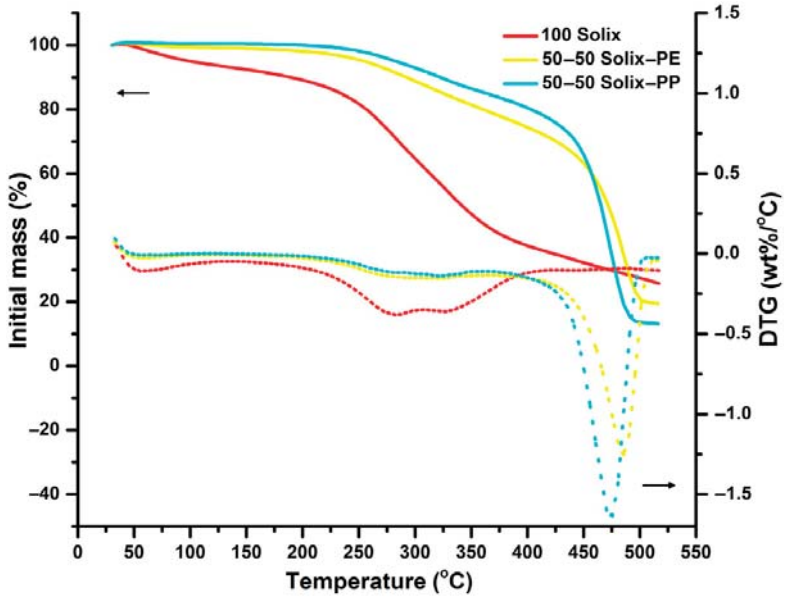

FIGURE 6.3 **TGA of Solix algae based bioplastics and their thermoplastic blends.**

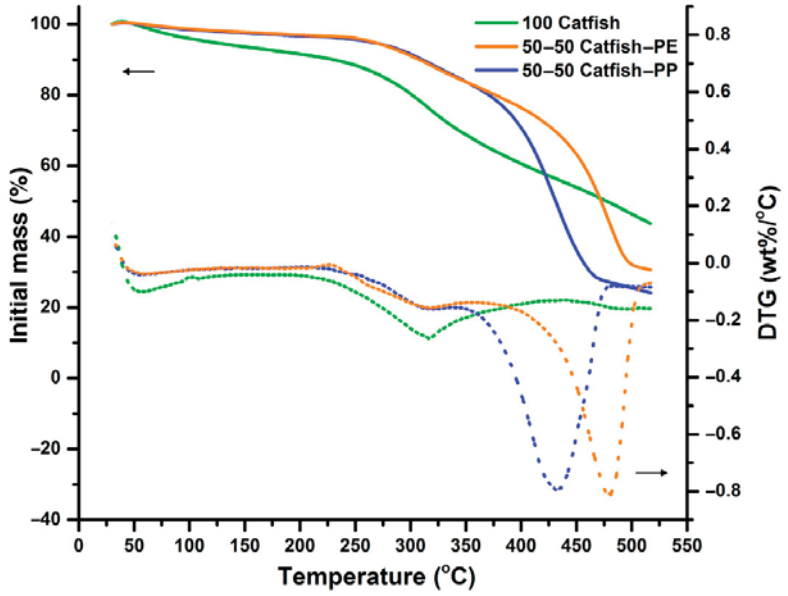

FIGURE 6.4 **TGA of catfish algae based bioplastics and their thermoplastic blends.**

The melting point of PE can be observed at around 130°C whereas the melting point of PP is near 165°C.

Figs. 6.6 and 6.7 show DMA results of viscoelastic properties of pure algae bioplastics compared to PE and PP, and PE/PP thermoplastic algae blends. From Fig. 6.6, we can see that the 100% Solix microalgae bioplastic has the lowest modulus and highest tan delta, meaning it is soft and flexible, whereas the

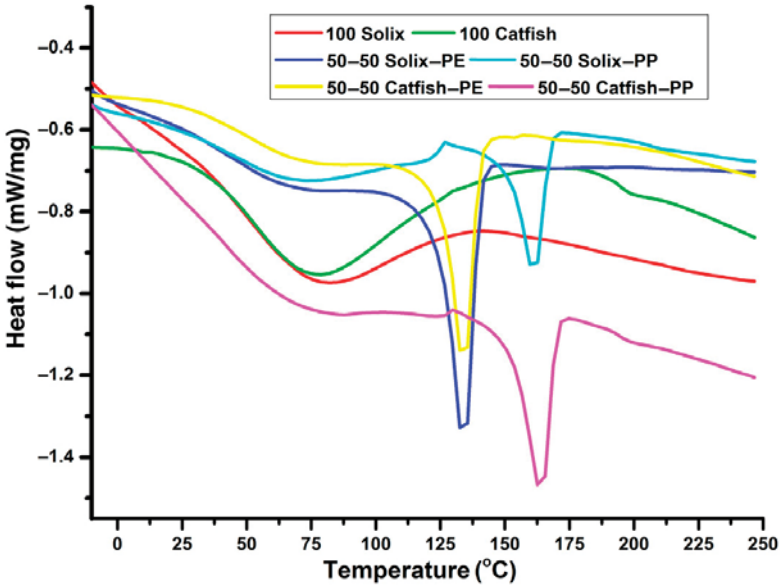

FIGURE 6.5 **DSC of Solix and catfish algae based bioplastics and their thermoplastic blends.**

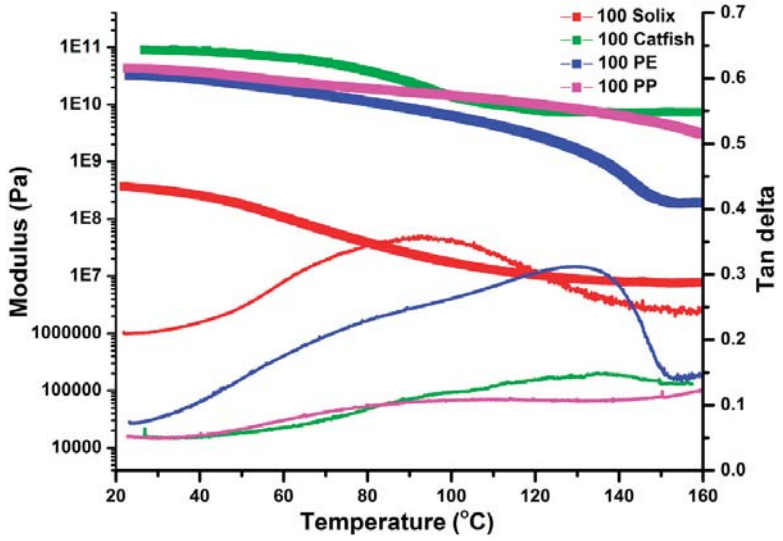

FIGURE 6.6 **DMA of pure algae bioplastics compared to PE and PP.**

100% catfish algae bioplastic has the highest modulus and lowest tan delta, meaning it is hard and stiff. PP behaves stiffer than polyethylene. The DMA of PE/PP blended algae bioplastics again show that the combination of the two relatively stiff materials, catfish algae and PP, leads to the highest modulus and lowest tan delta. However, the blend of the two flexible materials, Solix and PE, shows the lowest modulus and highest tan delta. The 50–50 catfish-PE and 50–50 Solix-PP formulations have medium modulus and tan delta. Between

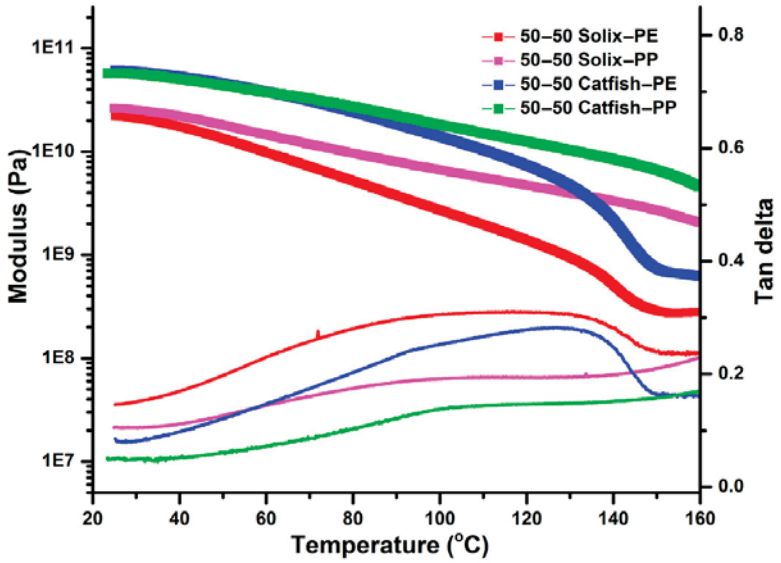

FIGURE 6.7 DMA of PE/PP blended algae bioplastics.

these two, catfish-PE is better because its modulus and tan delta values are both higher than those of Solix-PP blend.

3.3 Odor of Algae Bioplastics

The experimental design and panelist responses are provided in Table 6.2. The design used is a resolution *IV* half fraction of 2^4 full factorial design, given by D = ABC. The response was categorical in nature—1: almost no odor; 2: less odor; 3: medium odor; 4: more odor; and 5: serious odor.

Let π_{ij} denote the probability that bioplastic samples in the *i*th experimental setting (*i*th row of Table 6.2) will have odor in category *j*. Here *j* can be 1 (no odor) to 5 (strong odor), that is, $j = 1, 2, \ldots, J = 5$. Clearly, we have $\pi_{i1+}\ \pi_{i2+}\ \pi_{i3+}\ \pi_{i4} + \pi_{i5} = 1$ as the odor of the bioplastic sample must take a value between 1 and 5. Let y_{ij} be the number of the observations falling into category *j* for experimental setting *i*, and let $n_i = \sum_{j=1}^{J} y_{ij}$ be the total number of bioplastic samples taken in the *i*th experimental setting. Clearly, in our study, $n_i = 5$ for all *i*. Then we have Eq. 6.1:

$$(Y_{i1}, Y_{i2}, \ldots, Y_{iJ}) \sim \text{Multinomial}(n_i; \pi_{i1}, \pi_{i2}, \ldots, \pi_{ij})$$

We consider ordinal multinomial data where there is a natural order to the categories. An ordered response can be conveniently modeled with the cumulative probabilities:

$$\gamma_{ij} = \pi_{i1} + \pi_{i2} + \cdots + \pi_{ij} \tag{6.1}$$

From now on, we will denote our covariates A, B, C, and D by the vector *x*. The cumulative probabilities of Eq. 6.1 are linked to the covariates by the link function $g(\bullet)$ as follows:

$$g(\gamma_{ij}) = \theta_j - x_i^T \beta \tag{6.2}$$

Note that the vector $\boldsymbol{x}_i$ does not include an intercept term, and the coefficient vector β is same for all groups $j = 1, \ldots, 5$, so that the predictors have same effects on the response categories. This model is called a *cumulative link model* (Agresti, 2012). There are several standard choices of the link function $g(\bullet)$ and logit and probit are most popular among them. The breakpoints (θ_j) have a nice interpretation in terms of an unobserved continuous variable that might be thought of as the real underlying latent response. Simply put, it can be thought that the observed bioplastic sample will fall in category 1 if the underlying latent variable

TABLE 6.2 Algae Bioplastics Odor Panel Testing Results

Exp.	A	B	C	D	Rating	Rating scales	Response					Total
							y_{i1}	y_{i2}	y_{i3}	y_{i4}	y_{i5}	n_i
1	+	+	+	+	3,4,3,3,3	1: Almost no odor	0	0	4	1	0	5
2	+	+	−	−	5,4,3,4,5		0	0	1	2	2	5
3	+	−	+	−	3,5,2,2,3	2: Mild odor	0	2	2	0	1	5
4	+	−	−	+	4,4,2,3,4	3: Medium odor	0	1	1	3	0	5
5	−	+	+	−	2,2,1,2,1		2	3	0	0	0	5
6	−	+	−	+	2,2,3,3,2	4: Strong odor	0	3	2	0	0	5
7	−	−	+	+	1,1,1,2,1	5: Serious odor	4	1	0	0	0	5
8	−	−	−	−	2,2,2,2,2		0	5	0	0	0	5

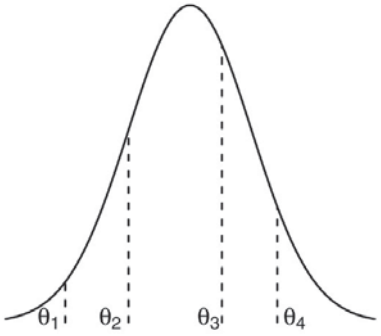

FIGURE 6.8 Latent variable (Z_i) view of an ordered multinomial response (grid moves as x changes).

is less than θ_1, in category 2 if the underlying latent variable is more than θ_1 but less than θ_2, and so forth. As x changes, these cutpoints will move together to change the relative probabilities of the five response categories. This latent variable explanation for the model is displayed in Fig. 6.8 and different cutpoints for the latent variable Z_i are also plotted. Here, assume that the distribution of $Z_i - x_i^T\beta$ is normal, or in other words, consider the probit link for Eq. 6.2. Five categories of response is possible, depending on the position of Z relative to the cutpoints θ_j. As x_i changes, the cutpoints will move together to change the relative probabilities of the five responses.

Here we consider logit links such that,

$$g(\mu) = \log\left(\frac{\mu}{1-\mu}\right) \tag{6.4}$$

$$\text{which implies } \gamma_{ij} = \frac{\exp\left\{\theta_j - x_i^T\beta\right\}}{1+\exp\left\{\theta_j - x_i^T\beta\right\}} \tag{6.5}$$

for $j = 1, \ldots, J-1$ and $\gamma_{ij} = 1$. As there are five categories of responses, from no odor to serious odor, we have $J = 5$. Here the log-likelihood is proportional to

$$l = \sum_{i=1}^{4}\sum_{j=1}^{3} y_{ij}\log(\pi_{ij}) \tag{6.6}$$

We used the statistical software R to fit the model and the output is given in Tables 6.3 and 6.4:

TABLE 6.3 Coefficients of Statistical Model

Factors	Estimate	Std. error	z value	Pr(>|z|)
A	2.89085	0.64019	4.516	6.31E-06
B	0.84123	0.35653	2.360	0.018299
C	–1.47642	0.42566	–3.469	0.000523
D	–0.02378	0.34984	–0.068	0.945812

TABLE 6.4 Threshold Coefficients of Statistical Model

Parameters	Estimate	Std. error	*z* value
θ_1	–4.2758	0.9773	–4.375
θ_2	0.3620	0.5410	0.669
θ_3	3.3091	0.8462	3.911
θ_4	5.4514	1.0816	5.040

We can see that the *p* values corresponding to the factors A and C are very small. We can safely conclude that these two factors are statistically significant. Factor B is marginally significant and factor D does not have any effect at all.

Table 6.5 gives the estimated probabilities for all possible combinations. From there, we can make recommendations for future experiments.

The results indicate that factor A (algae) and C (resin) are significant in affecting algae plastic odors. It is obvious that Solix microalgae, which has high levels of polyunsaturated fatty acids, has more serious odor than the catfish algae. It seems that blending with PP creates less odor than blending with PE. Factor B, types of absorbent, has some effect on removing the odor. Activated carbon is more effective than zeolite in absorbing odorous volatile compounds and alleviating the smell. Factor D, the absence of compatibilizer, does not have much influence on odor removal.

4 CONCLUSIONS

The thermal analysis of algae powder and bioplastics and its thermoplastic blends helped in determining processing conditions. TGA results showed that the Solix and catfish algae degradation happened from 175 to 375°C and

TABLE 6.5 Estimated Probabilities

Setup				Estimated probabilities				
A	*B*	*C*	*D*	No odor	Mild odor	Medium odor	Strong odor	Serious odor
−1	−1	−1	−1	0.1147	0.8158	0.0656	0.0034	0.0005
−1	−1	−1	+1	0.1196	0.8139	0.0628	0.0033	0.0004
−1	−1	+1	−1	0.7128	0.2833	0.0037	0.0002	0.0000
−1	−1	+1	+1	0.7224	0.2739	0.0035	0.0002	0.0000
−1	+1	−1	−1	0.0235	0.6898	0.2660	0.0182	0.0025
−1	+1	−1	+1	0.0246	0.6983	0.2574	0.0174	0.0024
−1	+1	+1	−1	0.3157	0.6637	0.0194	0.0010	0.0001
−1	+1	+1	+1	0.3261	0.6543	0.0186	0.0009	0.0001
+1	−1	−1	−1	0.0004	0.0392	0.4005	0.4299	0.1299
+1	−1	−1	+1	0.0004	0.0411	0.4104	0.4235	0.1247
+1	−1	+1	−1	0.0076	0.4339	0.4962	0.0545	0.0077
+1	−1	+1	+1	0.0080	0.4453	0.4872	0.0522	0.0074
+1	+1	−1	−1	0.0001	0.0075	0.1199	0.4270	0.4455
+1	+1	−1	+1	0.0001	0.0079	0.1249	0.4334	0.4337
+1	+1	+1	−1	0.0014	0.1267	0.6087	0.2229	0.0402
+1	+1	+1	+1	0.0015	0.1321	0.6124	0.2156	0.0384

peaked at 300°C, representing carbohydrate and protein burning. Additionally, DSC results indicated that proteins were maximally denatured at 150°C, suggesting the best processing temperature at 150°C to yield maximum denaturation. DMA concluded that the 100% Solix microalgae bioplastic was soft and flexible whereas the 100% catfish algae bioplastic was hard and stiff. Moreover, by blending with PE or PP, the modified thermoplastic blends using algae could lead to a range of mechanical properties for targeted applications. The odor panel sensory study is a great tool to gauge human perception to determine suitability of algae-based plastics in applications such as packaging and other consumer products. The headspace solid-phase microextraction (SPME) and GC/MS procedures could be used to determine which products produce objectionable odors and to address their remediation.

Acknowledgments

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