

Biodegradability of wheat gluten based bioplastics

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Abstract

A large variety of wheat gluten based bioplastics, which were plasticized with glycerol, were subjected to biodegradation. The materials covered the total range available for the biochemical control parameter F_i , which expresses the percentage of aggregated proteins. This quantity can be related to the density of covalent crosslinks in the wheat gluten network, which are induced by technological treatments. The biodegradability tests were performed in liquid medium (modified Sturm test) and in farmland soil. All gluten materials were fully degraded after 36 days in aerobic fermentation and within 50 days in farmland soil. No significant differences were observed between the samples. The mineralization half-life time of 3.8 days in the modified Sturm test situated gluten materials among fast degrading polymers. The tests of microbial inhibition experiments revealed no toxic effects of the modified gluten or of its metabolites. Thus, the protein bulk of wheat gluten materials is non-toxic and fully biodegradable, whatever the technological process applied.

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1. Introduction

The durable properties of plastics (e.g. polyethylene, PVC) make them an ideal material for a large number of applications. As a consequence of their use in throw-away products, they constitute nowadays an important part of the waste stream. Moreover, they tend to accumulate in ecosystems (e.g. soil, fresh water, marine habitats), because of their resistance to microbial degradation. The limitation of the waste deposit sites in Europe, the discussion about renewable versus fossil resources, new environmental regulations, and the introduction of composting infrastructures in the waste management have led to a considerable research effort in

the field of biodegradable materials. As a consequence, over the last decade there has also been a renewed interest in the development of recyclable, and/or biodegradable materials formed with raw materials from agricultural origin. Wheat gluten, which constitutes the protein by-product of the starch fabrication, is an interesting raw material for the development of biopolymers, because it is readily available in large quantities and at low prices.

Proteins are, in contrary to most other biodegradable polymers, heteropolymers. The different amino acids which constitute the polymer offer a large spectrum of chemical functionalities, which may give rise to a big variety of polymer network structures (Guilbert and Cuq, in press). One of the outstanding features of wheat gluten among other proteins are its unique viscoelastic properties, which in the past have been mainly investigated for the purposes of the baking industry. Its viscoelastic and flow properties have already been subject

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Nomenclature

SDS	sodium dodecyl sulfate	$y_{36 \text{ days}}$	normalized percentage of wheat gluten material mineralization after 36 days
DTE	dithioerythriol	r_{max}	maximal degradation rate
F_i	percentage of sodium dodecyl sulfate-insoluble wheat gluten protein	$t_{1/2}$	half-life time of biodegradation
E	Young's modulus	y_{max}, k, n	parameters of the Hill model respectively
σ_{max}	tensile strength at break		maximal degradation percentage, half-life time, and curvature
ε_{max}	elongation at break		
ΔDOC	dissolved organic carbon		

to large investigations. Plasticized with glycerol gluten forms a malleable phase, which resembles a structured viscoelastic solid with pseudo-plastic behavior (Guilbert and Cuq, in press). A study of the plasticized gluten's rheological properties showed that the principles of time/temperature superposition can be applied (Redl et al., 1999a). The temperature range of its validity is restricted, however, because the polymer becomes reactive at temperatures higher than 60 °C, so that cross-linking reactions occur (Weegels and Hamer, 1998; Lefebvre et al., 2000; Domenek et al., 2002). Redl et al. (1999b) performed studies on the extrusion of plasticized wheat gluten. They simulated successfully the flow properties of gluten during extrusion despite to the just mentioned limitations. Its thermoplastic properties and its high capacity for chemical modification may offer the possibility to develop a range of materials finding their application in the non-food sector, e.g. composite materials, films for agricultural uses, or molded objects (Guilbert and Cuq, in press).

Homogeneous, transparent, and strong water-resistant films can be obtained by the casting technique or thermal film forming processes (Gontard and Guilbert, 1998). The casting process is generally suitable for the production of coated materials (e.g. paper coatings), or the direct spreading of the film forming solution on seeds, or foodstuffs. Cast wheat gluten films have impressive and selective gas barrier properties especially against O₂, provided they are not moist (Gontard et al., 1995). The development of packaging leading to a modified atmosphere around fresh vegetables and fruits is a promising way due to the selectivity of the gas barrier properties of gluten films.

The fabrication of such materials with wheat gluten involves a wide range of technological treatments. The most important modifying factors of the polymer structure are temperature and shear stress. They will lead to a large restructuring within the protein material, due to unraveling of polymer chains, their scission due to mechanical stress, and their reaction due to increased temperature. Little is known about the consequences of those important modifications either on the degradation

properties of plasticized gluten materials or on the eventual formation of toxic products. A biochemical control parameter of the changes in the gluten polymer network is the percentage of protein, which is insoluble in non-ionic detergents, such as sodium dodecyl sulfate (SDS). The percentage of SDS-insoluble protein is a well established quantity, which reflects the covalent cross-linking degree of the protein network (Dachkevitch and Autran, 1989; Weegels and Hamer, 1998; Redl et al., 1999a,b; Domenek et al., 2002; Morel et al., 2002). The aim of this study was to investigate the effects of technological treatments of wheat gluten bioplastics on their biodegradation and on the formation of possible toxic products. To this end cast, hot-molded, and mixed gluten materials were investigated with a biodegradation test in liquid culture and in farmland soil.

2. Materials and methods

The Amylum Group (Aalst, Belgium) graciously provided non-processed wheat gluten. Protein, starch, lipid, and ash quantities were 76.5%, 11.8%, 5.0%, and 0.8% of dry mass, respectively. Moisture content was 7.2% (wet mass basis). Chemicals, unless specified separately, were purchased from Sigma Aldrich (L'Isle d'Abbeau Chesnes, France) in p.a. quality. Anhydrous glycerol was obtained from Fluka Chemie (Buchs, Switzerland).

2.1. Preparation of wheat gluten materials

Cast wheat gluten films were prepared as previously described by Gontard et al. (1993). The film forming solution was prepared using wheat gluten at a concentration of 0.2 g ml⁻¹ in 1% acetic acid, 0.0004 g ml⁻¹ Na₂SO₄, and 0.04 g ml⁻¹ glycerol. All components were vigorously mixed with a homogenizer (Ultra Turrax T 25 basic, Ika, Germany). The film forming solution was then allowed to settle in order to separate the foam formed (30 min). Films were subsequently spread onto a plexiglas surface using a thin-layer applicator and dried

at 60 °C for 30 min in a ventilated oven (Memmert 600, Schwabach, Germany).

For the preparation of hot-molded wheat gluten films, gluten and glycerol (35 w/w%) were hand-mixed in a mortar and rested for 20 min. The blend (3 g) was then hot-molded in a heating press (TechmoPL 10T, Nazzelles-Negron, France) at 150 bar. Pressing time and temperature were varied in order to produce film samples with different crosslinking degrees. The exact fabrication conditions together with the crosslinking degree are given in Table 1.

In order to produce mixed gluten blends, the wheat gluten powder was mixed with glycerol (35 w/w%) in a two-blade counterrotating measuring mixer (Plasticorder PL 2000, Brabender, Germany) turning at 3:2 differential speed, which is a model system for an extrusion process. The mixing chamber (50 ml) was filled with 50 g material and thermoregulated at chosen temperature. The torque and the product temperature were continuously recorded during the mixing process. The sample preparation parameters are given in Table 1.

2.2. Characterization of wheat gluten materials

The biochemical characterization of the gluten materials was done by the determination of the percentage of aggregated, SDS-insoluble protein with size-exclusion chromatography (SEC) according to Domenek et al. (2002). Wheat gluten materials were ground under liquid nitrogen in a laboratory ball mill and then extracted with a 0.1 mol/l phosphate buffer (pH 6.9) containing 1% SDS. The SDS-insoluble protein fraction (F_i) was extracted with SDS-phosphate buffer containing 20 mmol/l dithioerythriol (DTE) and tip sonicated to bring the gluten proteins into solution. Both extracts, the SDS-soluble gluten proteins and the SDS-insoluble protein

fraction (F_i), were quantified with SEC. SEC analysis was carried out on a TSK-G 4000 SW XL (TosoHaas) size-exclusion analytical column (7.5×300 mm) preceded by a TSK 3000-SW XL (TosoHaas) guard column (7.5×75 mm). The columns were eluted at room temperature with a 0.1 mol/l sodium phosphate buffer (pH 6.9) containing 0.1% SDS. The flow rate was 0.7 ml/min and proteins were detected at 214 nm. The column was calibrated with protein standards according to Dachkevitch and Autran (1989). The F_i can be related to the degree of covalent crosslinking between wheat gluten proteins, which is ensured through disulfide bonds (Domenek et al., 2002; Morel et al., 2002).

The sample contents in carbon and nitrogen were measured with an elementary analyzer (Thermoquest NC2100-soil analyzer). The sulfur content was measured after mineralization of the sample in presence of $Mg(NO)_2$. The sulfate content was measured as Ba_2SO_4 by turbidimetry at 420 nm.

The rheological measurement of the wheat gluten materials was performed with a dynamic mechanical thermal analyzer (DMTA IV, Rheometric Scientific, Piscataway, USA). The measuring chamber was thermoregulated at 30 °C with a continuous air flow at 60% relative humidity (water content of gluten–glycerol films $20.3 \pm 0.9\%$). The rectangular film samples (5 mm×20 mm) were equilibrated 1 h in the measuring device. The mechanical spectrum of the samples was recorded applying a sinusoidal strain amplitude of 0.05% (linear domain of the samples) in a frequency range of 0.001–100 Hz. A constant force of 1 g was applied to the samples during the measurement. The storage modulus E' , the loss modulus E'' and the $\tan \alpha (= E'/E'')$ were recorded and plotted against the frequency. The values are averages of four samples, each analyzed in duplicate. The elongation and tensile strength at break were

Table 1
Preparation data and biochemical and rheological properties of the wheat gluten materials subjected to biodegradation

Material	Preparation	F_i [%]	h [μ m]	E [MPa]	σ_{\max} [MPa]	ϵ_{\max}
1	Casting	0	110 (10)	4.7 (1.3)	0.92 (0.02)	544 (99)
2	Mixing 100 min ^{-1a}	64.16	1257 (706)	n.d. ^a	n.d.	n.d.
3	Film 100 °C 2'	27.80	351 (40)	0.65 (0.18)	0.550 (0.079)	111 (14)
4	Film 100 °C 60'	48.92	320 (35)	1.46 (0.30)	1.24 (0.13)	189 (34)
5	Film 120 °C 35'	84.27	239 (38)	2.13 (0.86)	1.50 (0.17)	240 (13)
6	Mixing 10 min ^{-1b}	4.10	356 (60)	0.16 (0.06)	0.275 (0.036)	286 (91)
7	Film 150 °C 20'	60.77	246 (25)	2.39 (0.30)	1.48 (0.24)	224 (31)

Values in brackets represent the standard deviation.

F_i , percentage of SDS-insoluble protein [%].

h , thickness of the material film [μ m].

E , Young's modulus [MPa] measured at 0.02 mm s⁻¹.

σ_{\max} , tensile strength at break [MPa].

ϵ_{\max} , elongation at break.

^a The material was mixed for 30 min at 107 °C. Due to the high crosslinking density the material could not be shaped into a film without altering its properties. The Young modulus was therefore not determined.

^b The material was mixed for 86 min at 37 °C.

measured in the plateau zone at 0.02 mm s^{-1} speed (strain rate of 0.00667 s^{-1}). The determined values are averages of seven measurements minimum.

The method chosen to evaluate the biodegradability of the wheat gluten materials in liquid medium was the international standard ISO 14852, which measures the ultimate aerobic biodegradability of packaging materials. It is based on the modified Sturm test (Sturm, 1973). The test equipment was previously described by Calmon et al. (2000). The sample materials were ground for under liquid nitrogen (3 min for 4 g) in a laboratory ball mill (Prolabo, France). The test vessel was inoculated with 15 ml of the supernatant of a sample taken from the supernatant in the activated sludge tank of the waste water treatment plant in St. Clement la Riviere (France). The mineralization of the sample, which constituted the only source of carbon, was followed by the quantity of CO_2 formed during the test. In order to measure the CO_2 production, the degradation vessel was flushed in regularly time intervals with CO_2 -free air. The entrapped quantity of CO_2 was recorded by a infrared detector. The theoretical CO_2 content (ThCO_2) produced by total oxidation of the material is calculated from

$$\text{ThCO}_2 = C \cdot \frac{44}{12} \quad (1)$$

The degree of mineralization is then expressed by

$$\text{Mineralization} = \frac{[\text{CO}_2]_{\text{Material}} - [\text{CO}_2]_{\text{blank}}}{\text{ThCO}_2} \quad (2)$$

where $[\text{CO}_2]_{\text{Material}}$ is the amount of CO_2 [mg] measured in the test vessel, and $[\text{CO}_2]_{\text{blank}}$ the quantity in the negative control vessel, which contained no carbon source. In order to check the carbon mass balance of the different experiments the introduced carbon mass was corrected by the carbon content of the inoculum and the concentration of dissolved carbon in the degradation medium. Each material was measured in duplicate. Each series of biodegradation included three controls. Cellulose was chosen to be the positive reference because of its well-known degradation characteristics. It was measured in duplicate. The negative control was a test run without addition of a carbon source.

In order to investigate, if toxic metabolites will be formed during the degradation process, a test of microbial inhibition was carried out. For that, the material powder was mixed with pure cellulose and the mixture was subjected to the modified Sturm test. The rates and the final mineralization percentage were compared to the pure cellulose. Inhibition should result in diminished rates and/or decreased final conversion.

The degradation of the samples in farmland soil was simulated on a laboratory scale. The soil was taken from an experimental field of the CEMAGREF in Montpellier (France). It included 239 g/kg clay, 396 g/kg lime,

342 g/kg sand, and 23.2 g/kg organic matter. The ratio C/N was 9.48, and the pH 7.8. Filter paper with an ash content <0.1% (Whatman) was used as the positive reference material. Three samples (approximately 0.5 g dry weight each) were dug in 1.2 kg soil contained in glass vessels, which were stored at 20°C . The fill height of the glass vessels was between 25 and 30 cm. The sample film thickness was measured with a hand held micrometer (Braive Instruments, Checy, France). The water content of the soil was kept constant at 75% of its capacity of water retention ($\approx 300 \text{ g/kg}$ soil). The dug out samples were washed under a gentle water stream in order to clean them from soil particles. However, fungi adhered strongly on the surface of the samples, so that their mycel held back a large quantity of soil particles. In order to estimate the weight loss, the ash content of the degraded samples was determined after ISO 2171-1980. The weight loss of the recuperated gluten–soil mixture corrected by the weight loss of the soil under the same conditions was assigned to the organic matter of the material and the fungi.

3. Results and discussion

The different technological treatments applied to the gluten–glycerol blend resulted in materials, which covered a large range of the degree of protein aggregation (F_i) which can be attained with technological modifications. The preparation conditions and the corresponding F_i -values are given in Table 1. The F_i of the native gluten used in this work was 13.67%. Gluten materials having a F_i smaller than this value are depolymerized compared to the native protein. The depolymerization was reached by two different ways; on the one hand disulfide bonds between gluten molecules were reduced chemically with Na_2SO_4 during the casting process (material 1), and on the other hand chemical bonds were broken mechanically due to shear stresses in the cold mixing process (material 6). Such a depolymerization of wheat gluten is well-known from the bread baking process (Feillet, 2000). The polymerization of the samples is governed by the heat-treatment of wheat gluten (Domenek et al., 2002). Increasing severity of the heat-treatment causes the F_i to rise (Weegels and Hamer, 1998; Domenek et al., 2002). For a very severe heat-treatment, however, a F_i -decrease is observed (material 7, pressed at 150°C). This result may be attributed to network degradation due to high-temperature. The sample pressed at 120°C may therefore be close to the maximum F_i , which can be obtained with gluten–glycerol blends. Evidence for this behavior has been found during kinetic studies (Domenek et al., in press).

The mechanical spectra of the different gluten materials were recorded in frequency sweep experiments at 30°C and at a relative humidity of 60%. Due to the

hygroscopic character of glycerol and the sample shape (between 100 and 400 μm thickness) the water content of the sample depends strongly on the ambient relative humidity. Because water is an excellent plasticizer of gluten, it influences strongly the mechanical properties of gluten materials (Cuq et al., 1998; Micard et al., 2000). The frequency sweep curves of the materials are presented in Fig. 1. We observe two distinct behaviors. Samples, which have a F_i higher than the native value show a rubbery plateau in the lower frequency range, whereas samples which were depolymerized during the processing show a continuous decrease in the storage modulus E' . The rubbery plateau can be related to the crosslinking status of the network (Ferry, 1980). A clear rubbery plateau can be assigned to a crosslinked polymer with a high density of covalent bonds. Materials with a transient rubbery plateau are typically lightly crosslinked, having a permanent and a temporary entangled network. The plateau modulus increases with increasing F_i (Table 1), which evidences the relationship between the biochemically and the rheologically determined crosslinking degree (Domenek et al., in press). The cast gluten film showed no distinct rubbery plateau, which evidences that its network structure is dominated by transient entanglements. The decrease of the storage

modulus with the frequency of the mechanically depolymerized mixed material 6 had a diminished slope. For easier comparison, the Young modulus, the tensile strength at break and the elongation at break (0.02 mm s^{-1}) are given in Table 1.

3.1. Biodegradability in liquid medium

The Sturm test was carried out on five different gluten samples, having undergone different heat- and shear-treatments. In order to facilitate the comparison of the different degradation series (samples 1–3, and samples 4–5) all data were normalized. For that cellulose was chosen to be the reference material. The mineralization of the samples was expressed in percent of the maximal cellulose mineralization of the corresponding series which was set to 100%. The carbon balance of all materials is given in Table 2. If we compare the quantity of carbon measured as CO_2 ($\text{C}(\text{CO}_2)$) with the quantity of carbon introduced, we find a percentage of mineralization around 85% for all samples. The remaining 15% of carbon contribute to the biomass increase during the fermentation and to the rising quantity of soluble metabolites in the fermentation broth.

Fig. 2 shows the results obtained for the different samples in the Sturm test. As can be read off, the degradation of all materials was faster than the degradation of the reference material cellulose. After a short lag-phase, which was typically smaller than 1 day, the degradation rate rose sharply to its maximum after approximately 2 days. Then, a short phase of degradation slow-down was observed (after 8 days), which ended with a second phase of accelerated mineralization between 15 and 20 days until the available carbon was fully depleted. This development can be followed easily in Fig. 3, where the differentiated degradation curves are displayed. The second accelerated phase might be due to changes in the culture medium, such as variations in the pH. In absence of further data, no explication can be given for this behavior.

There are obviously no detectable differences between the different samples. In order to confirm this and to quantify the degradation behavior of gluten materials, the curves were modeled with the recently proposed Hill model (Calmon et al., 1999):

$$y = y_{\max} \cdot \frac{t^n}{k^n + t^n} \quad (3)$$

Here, y is the percentage of degradation [%] at time t [days], y_{\max} [%] the percentage of degradation at infinite time, k [days] the half-life time and n the curve radius of the sigmoidal function. In order to apply the model to our data two approximations were made. The small plateau in the sample mineralization (between 8 and 15 days) and the observed lag-times of the degradation

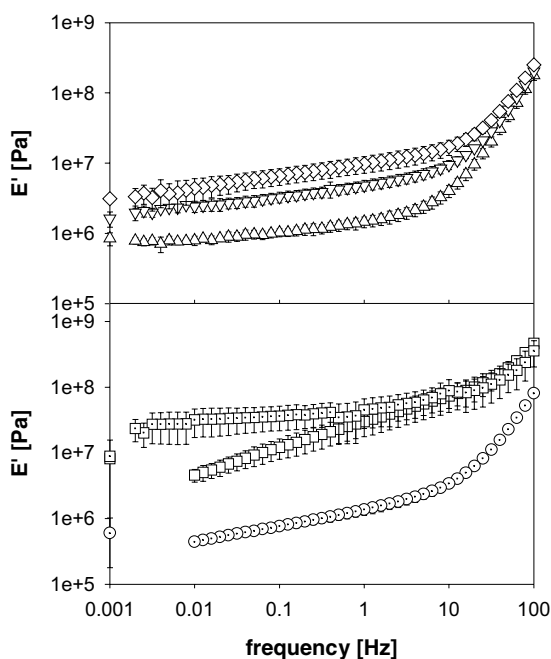


Fig. 1. Mechanical spectra of wheat gluten materials. In the upper graph is shown (from top to bottom): material 5 (\diamond), material 4 (∇), and material 3 (\triangle). In the lower graph is shown (from top to bottom): material 7 (\square), material 1 (\square), and material 6 (\odot).

Table 2

Total carbon measured for the different gluten materials in two tests of biodegradation in the modified Sturm test

	Material				
	1	2	3	4	5
Carbon introduced C_i [mg]	50.97	51.02	50.82	48.06	47.99
Δ DOC [mg]	1.9	1.7	1.7	2.4	2.7
$C(\text{CO}_2)$ [mg]	45.64	46.56	45.50	44.35	44.67
ΔC (biomass) [mg]	4.90	11.61	7.60	13.25	8.37
Residual material [mg]	0	0	0	0	0
$C_{\text{fin}} [\Sigma \text{mg}]$	52.45	59.87	54.80	60.00	56.44
Carbon balance C_{fin}/C_i [%]	102.90	117.34	107.83	123.91	116.75
Standard deviation	3.94	14.24	6.50	19.36	–
	Cel 1	Cel 2		Inhib 4	Inhib 5
Carbon introduced C_i [mg]	51.27	48.10		47.86	48.22
Δ DOC [mg]	1.6	2.3		2.5	2.8
$C(\text{CO}_2)$ [mg]	47.22	42.87		44.44	44.19
ΔC (biomass) [mg]	2.67	12.23		9.42	8.07
Residual material [mg]	0	0		0	0
$C_{\text{fin}} [\Sigma \text{mg}]$	51.48	57.40		56.35	55.06
Carbon balance C_{fin}/C_i [%]	100.42	118.45		116.88	113.35
Standard deviation	12.75			–	–

 Δ DOC—dissolved organic carbon. $C(\text{CO}_2)$ —measured carbon as CO_2 . ΔC (biomass)—carbon from biomass.

–: single measurement.

Cel 1, 2—cellulose.

Inhib 4, 5—inhibition test carried out on materials 4 and 5.

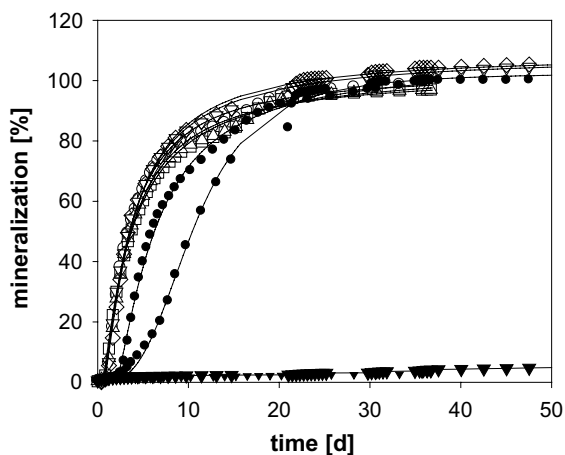


Fig. 2. Degradation of wheat gluten materials in the Sturm test and the degradation behavior of cellulose: material 1 (\square), material 2 (\circ), material 3 (\triangle), material 4 (∇), material 5 (\diamond), cellulose (positive reference, \bullet), and negative reference (\blacktriangledown). The solid lines show the degradation curve, which was calculated with the Hill model.

were not taken into account during the fitting procedure. The obtained fitting curves are shown together with the experimental data in Fig. 2. The fitting parameters and

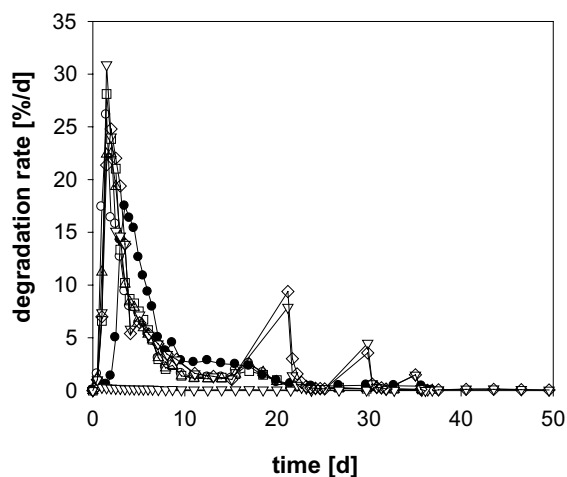


Fig. 3. Degradation rate of wheat gluten materials in the Sturm test and of cellulose: material 1 (\square), material 2 (\circ), material 3 (\triangle), material 4 (∇), material 5 (\diamond), cellulose (positive reference, \bullet), negative reference (\blacktriangledown).

the calculated half-life times, as well as the maximal degradation rates, are given in Table 3. The calculated values of y_{max} are higher than the observed ones, which comes from the fact that the model calculates the de-

Table 3
Hill parameters of the degradation of wheat gluten materials in the modified Sturm test

Material	Time [days]	$y_{36 \text{ days}}$ [%]	r_{max} [%/days]	y_{max} [days]	k [days]	n	$t_{1/2}$ [days]	χ^2	R^2
1	1.41	86.27	29.88	104.47	3.39	1.12	3.99	12.94	0.9984
2	1.41	88.02	30.43	102.95	2.94	1.20	3.64	21.39	0.9981
3	1.41	86.25	26.55	102.13	3.08	1.18	3.78	15.40	0.9976
4	1.66	82.72	32.53	108.43	3.22	1.20	3.92	17.49	0.9992
5	1.66	83.66	26.00	108.56	3.30	1.28	4.00	23.71	0.9984
Inhib 4	1.83	84.23	12.30	117.63	7.80	1.45	8.50	55.89	0.9962
Inhib 5	2.99	82.32	11.24	113.44	8.15	1.56	8.75	30.50	0.9982
Cellulose 1	3.58	88.88	19.04	105.81	4.62	1.33	6.62	24.12	0.9984
Cellulose 2	8.59	80.33	10.92	103.83	8.56	2.46	10.56	15.13	0.9986

Inhib 4, 5—degradation parameters of the inhibition experiments carried out on wheat gluten samples 4 and 5 in the modified Sturm test.

$y_{36 \text{ days}}$ —normalized percentage of mineralization after 36 days.

Time—time at maximal degradation rate.

r_{max} —maximal degradation rate.

y_{max} —calculated maximal degradation percentage.

k, n —Hill parameters half-life and curvature.

$t_{1/2}$ —half-life time.

χ^2, R^2 —sum of residuals and correlation coefficient of the Hill model.

gradation at infinite time, whereas the experimental value was taken after 36 days. No significant differences were observed between the gluten materials, despite the varied technological fabrication processes. Calmon et al. (1999) calculated the kinetic parameters of the Hill model for a large variety of biodegradable polymers which are commercially available to date. The gluten degradation is comparable with fish protein materials, which had a half-life time of approximately 6 days and the curve radius of the fish protein-based polymer ($n = 1.67$) compared well with our findings. Gluten materials degraded considerably faster than well-known materials like paper ($t_{1/2} = 10$ days) or uncoated cellophane ($t_{1/2} = 30$ days). New materials based on starch/polycaprolactone mixtures, e.g., degrade in times more comparable to cellophane ($t_{1/2} = 36$ days). A recent study of the degradation of the starch based material Mater-Bi in a modified Sturm test showed half-life times around 30 days, depending on the fungi strain they used (Kim et al., 2000). Comparisons are, however, difficult to make, because in this study the authors worked exclusively with fungi at elevated temperature (27 °C). In conclusion, although the technological treatment of gluten materials had a substantial impact on their rheological properties, the degradation behavior was merely affected. The increased crosslinking degree of the protein network seemed not to restrict its accessibility to proteolytic enzymes.

3.2. Inhibition of microbial growth

The introduction of new covalent bonds in the protein network changes considerably its biochemical

structure. The investigation of a potential inhibition of microbial growth was carried out on a material, which was situated in the middle of the F_i -range (sample 4) and the material, which showed the highest F_i of our series (sample 5, Table 1). The results are shown in Fig. 4. As can be read off, no inhibition of the degradation was found. The maximal degradation rate was still faster than the degradation of cellulose. The values for the maximal degradation rate and the final mineralization

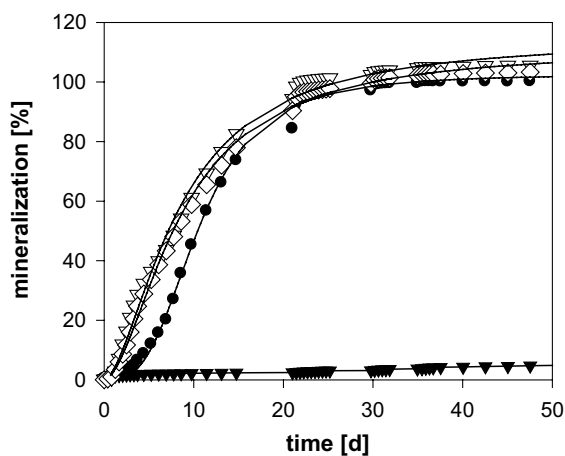


Fig. 4. Degradation curves of wheat gluten materials in the modified Sturm test in order to probe microbial inhibition compared to the degradation behavior of cellulose: material 4 (∇), material 5 (\diamond), cellulose (positive reference, \bullet), negative reference (\blacktriangledown). The solid lines show the degradation curve, which was calculated with the Hill model.

percentage together with the Hill parameters are given in Table 3. In conclusion, the protein bulk of gluten materials even strongly modified technologically shows no toxic effect on the microorganisms in the test vessel during the degradation process.

3.3. Degradation in soil

In parallel to the tests in the liquid medium a laboratory-scale screening test of the degradation of wheat gluten materials in farmland soil was carried out. This test may give an estimate of the degradation times of the materials which is closer to practical circumstances. Materials 2–5 were investigated with both methods in order to probe the correlation between the two test methods. The results are given in Fig. 5, and the corresponding Hill parameters in Table 4. The experiments showed the total degradability of wheat gluten samples

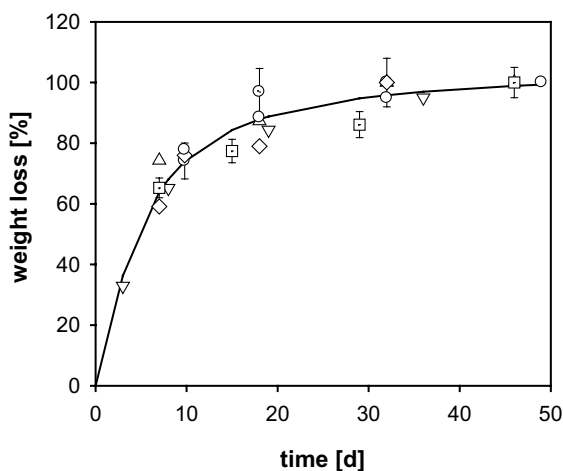


Fig. 5. Degradation curves of wheat gluten materials in farmland soil. Due to the absence of significant differences all materials are confounded to a single curve. The solid line shows the degradation curve, which was calculated for all materials with the Hill model. Materials: 2 (○), 3 (△), 4 (▽), 5 (◇), 6 (◊), and 7 (□).

Table 4

Hill parameters of the wheat gluten material degradation in farmland soil

Material	y_{\max} [days]	k [days]	n	χ^2	R^2
All samples	104.54	4.91	1.28	6.8097	0.9564

y_{\max} —calculated maximal degradation percentage.

k, n —Hill parameters half-life ($= t_{1/2}$) and curvature.

χ^2, R^2 —sum of residuals and correlation coefficient of the Hill model.

in soil and confirmed that, within the errors of the experiment, samples show no significant differences. After 50 days all materials had completely vanished. This time frame compares well with the mineralization duration of the samples in the Sturm test. The findings are in concordance with the results of Park et al. (2000), who reported a weight loss of approximately 80% of gluten–glycerol–soy protein films after 30 days. Mater-Bi, for comparison, reached a plateau of 18% mineralization after 120 days in a laboratory test (Solaro et al., 1998).

4. Conclusion

The protein fraction of wheat gluten remains highly degradable, even when it is subjected to severe technological treatments. The degradation rates are situated among the rates of fast degrading polymers. The results of the modified Sturm test correlate well with the findings of the degradation in farmland soil. When buried, gluten vanishes within 50 days completely. Furthermore, no toxic effects of the proteins or their metabolites on the microorganisms were found during the biodegradation. Its degradation features together with its unique viscoelastic and gas barrier properties make the wheat gluten polymer an ideal precursor for the development of new biodegradable polymers.

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