Electronic Supplementary Material (ESI) for Green Chemistry.

Electronic Supplementary Information

Thermo-compression molding of chitosan with deep eutectic mixture for biofilms development

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ESI 1. Materials ad Methods

ESI 1.1 Optimization strategy

The Box-Behnken design (BBD) with response surface methodology (RSM) were used to find the conditions of eutectic mixture amount (X_1; %), compression load (X_2; kN) and compression time (X_3; min) that ensured films with lower energy requirements and best mechanical properties consisted in 12 experimental runs (runs 1-12 in Table S1) with three levels for each factor (high, intermediate and low values coded as +1, 0 and -1, respectively) plus 3 replications at the center point (all factors at 0 level; runs 13-15 in Table S1).[17]

The tensile strength, *TS* (Y_1; MPa) and elongation at break, *E* (Y_2; %) of the chitosan films obtained in each experimental run (Table S1) were fitted to second order models to obtain the optimal conditions for film production by multifactor variance analysis (ANOVA) and inspection of 3D response surface plots. Statistical significance was determined by student's *t*-test and *p*-values for a 95% confidence level ($p<0.05$). All analyses were performed using the *Statistica 8.0* software (StatSoft, Tulsa, OK, USA).

ESI 1.2 Wide Angle X-ray diffraction (WAXD)

WAXD spectra of optimized films samples were acquired at room temperature with a Bruker D8 Discover diffractometer (lambda source of 0.154 nm). Film samples were scanned at diffraction angles (2θ) from 5 to 35o using 0.04° steps. Chitosan powder with no further preparation was also analyzed with the same procedure (Fig S2).

ESI 2. Results and discussion

ESI 2.1 Optimal conditions for film production

The experimental domain and independent variables affecting the TS and E of the films were chosen after preliminary single-factor experiments (data not shown). The use of BBD avoided getting experimental points at extreme values of the design space[17] and this was important for the eutectic mixture amount and applied load which were very hard to use outside the ranges listed in Table S1 (20-40% and 176.5-215.7 kN, respectively).

As desired, the *TS* and *E* regression models reached high statistical significance ($p<0.003$) while their lack of fit was not significant ($p>0.05$; Table S2). The significant effect of the factors on the studied responses was confirmed by the high F-values obtained for both models (12.62 and 10.34, respectively; Table S2). The correlation (R^2) and adjusted correlation (R^2-adj) coefficients of the models were high and as close as 0.9044 and 0.8327 for *TS* and 0.8858 and 0.8002 for *E* confirming a good agreement between predicted and observed data. Based on this information both regression equations were considered adequate[17] and optimal conditions for film production were found by ANOVA analysis and 3D surface plots inspection (Figs. S1a, b).

The amount of eutectic mixture in the film (X1) was the most influential effect ($p<0.0002$; Table S2) for TS. Films with less eutectic mixture (20%) showed higher mechanical strength than those prepared with more (40%; respectively, runs 1 vs 2, 3 vs 4, 5 vs 6 and 7 vs 8 in Table S1). Improved mechanical strength was found when using 20% eutectics and high or low compression loads (215.7 or 176.5 kN; not shown) as well as long or short compression times (10 or 20 min; Fig. S1a). This agreed well with the strong curvature affecting the TS surface profile imposed by the applied load (X22; $p<0.05$) and compression time (X32; $p<0.002$). Considering energy and time savings, best possible set of conditions for maximum TS could be (set1): 20% of plasticizer, 176.5 kN of applied load and 15 min of compression time. Experimental runs producing the most resistant films (TS ~ 46-48 MPa; Table S1), i.e. run 9 (set2: 30% eutectic mixture, 176.5 kN, 10 min) and run 10 (set3: 30% eutectic mixture, 215.7 kN, 10 min) were also tested and no significant differences ($p> 0.05$) were found between the three sets of conditions after means comparison using the Student's t-test.

The compression time was not an influential parameter to *E* ($p>0.05$; Table S2) contrarily to the applied load, X_2 ($p<0.005$; Table S2), and the quadratic effect of the eutectic mixture amount, X_1^2 ($p<0.0002$). Films prepared using low compression load (176.5 kN) showed higher *E* (Fig. S1b) (e.g. runs 9 and 11; Table S1). Formulations with 30% eutectic mixture processed at 176.5 kN led to films with higher *E* than those prepared with 40% (Fig. S1b). This agreed well with a previous study where an increase in plasticizer content led to a drastic reduction in the mechanical properties, *TS* and E, of compression molded chitosan films.[16] The authors attributed that phenomenon to a phase separation that can occur between the polyol and the chitosan polysaccharide. Unfortunately, in our case the phase separation hypothesis was not fully supported by structural information.

Our highest *E* was obtained in run 9 (~4.19%; Table S1) set of conditions (set2: 30% plasticizer, 176.5 kN, 10 min) was chosen as optimal for film production.

Table S1 Real and coded values for the three-level-three-factor Box-Behnken design (X_1 – % of eutectic mixture; X_2 – applied load; X_3 – time) and experimental results for the response variables, tensile strength, *TS* ($Y_{1;}$ MPa) and elongation at break, *E* ($Y_{2;}$ %). Values of Y_1 and Y_2 for each run represent a mean of three replicates

	Real values			Response	
Run	X_1 (%)	X_2 (kN)	X_3 (min)	Y_1 (MPa)	Y_2 (%)
1	20 (-)	176.5 (-)	15 (0)	39.6	1.87
2	40 (+)	176.5 (-)	15 (0)	30.0	1.30
3	20 (-)	215.7 (+)	15 (0)	42.0	0.89
4	40 (+)	215.7 (+)	15 (0)	21.9	1.23
5	20 (-)	196.1 (0)	10 (-)	43.8	1.38
6	40 (+)	196.1 (0)	10 (-)	26.7	1.24
7	20 (-)	196.1 (0)	20 (+)	43.5	1.09
8	40 (+)	196.1 (0)	20 (+)	32.2	1.56
9	30 (0)	176.5 (-)	10 (-)	46.4	4.19
10	30 (0)	215.7 (+)	10 (-)	48.6	2.03
11	30 (0)	176.5 (-)	20 (+)	38.8	3.69
12	30 (0)	215.7 (+)	20 (+)	43.5	2.25
13	30 (0)	196.1 (0)	15 (0)	39.7	2.12
14	30 (0)	196.1 (0)	15 (0)	36.0	2.69
15	30 (0)	196.1 (0)	15 (0)	34.7	2.92

Table S2 Analysis of variance (ANOVA) for the regression models excluding non-significant interactions X_iX_j.

Response	Source	SS	DF	MS	F-value	P
TS (Y_1; MPa)	Model	847.8	6	141.3	12.62	0.00107[a]
	X_1	468.2	1		41.80	<0.0002[a]
	X_2	1.36	1	1.36	0.1215	0.7364[b]
	X_3	7.03	1		0.6278	0.4510[b]
	X_1^2	46.17	1		4.033	0.0795[b]
	X_2^2	67.6	1	67.6	6.03	0.0396[a]
	X_3^2	249.9	1	249.9	22.32	<0.00149[a]
	Residual	89.60	8	11.20		
	Lack of fit	83.87	6	13.98	4.875	0.1799[b]
	Pure error	5.73	2	2.87	49.28	0.02001[a]
	Total	937.4	14			
	R^2	0.9044				
	R^2-adj	0.8327				
E (Y_2; %)	Model	11.714	6	1.9523	10.34	0.00211[a]
	X_1	0.02531	1	0.02531	0.1314	0.7237[b]
	X_2	2.856	1	2.856	15.13	0.00461[a]
	X_3	0.01361	1	0.01361	0.07212	0.7951[b]
	X_1^2	7.974	1	7.974	42.25	0.000188[a]
	X_2^2	0.2955	1	0.2955	1.566	0.2462[b]
	X_3^2	0.1202	1	0.1202	0.6368	0.4479[b]
	Residual	1.50997	8	0.18875		
	Lack of fit	1.1707	6	0.19512	1.15022	0.5339[b]
	Pure error	0.3393	2	0.1696	11.509	0.08209[b]
	Total	13.224				
	R^2	0.8858				
	R^2-adj	0.8002				

SS= Sum of squares; DF= Degree of freedom; MS= Mean square; R^2= quadratic correlation coefficient; R^2-adj= adjusted quadratic correlation coefficient; [a] significant ($p<0.05$); [b] not significant ($p>0.05$)

Figure S1 a) 3D response surface plot of tensile strength (*TS*) of chitosan films (Y_1) as a function of eutectic mixture % (X_1) and time (X_3) (compression load (X_2) = 196.1 kN).

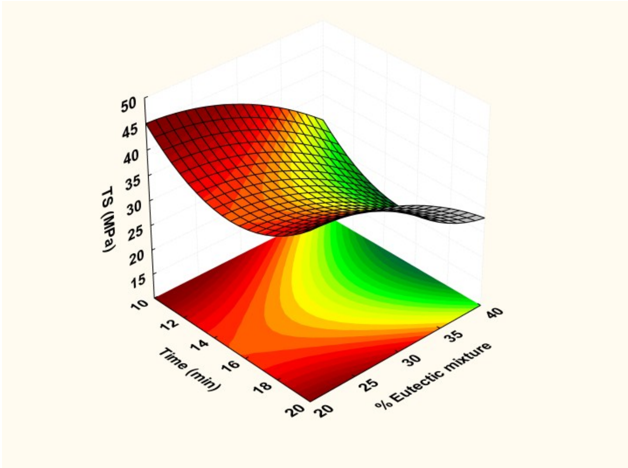

Figure S1 b) 3D response surface plot of elongation at break, (*E*) of chitosan films (Y_2) as a function of eutectic mixture % (X_1) and compression load (X_2) (time (X_3) = 15 min).

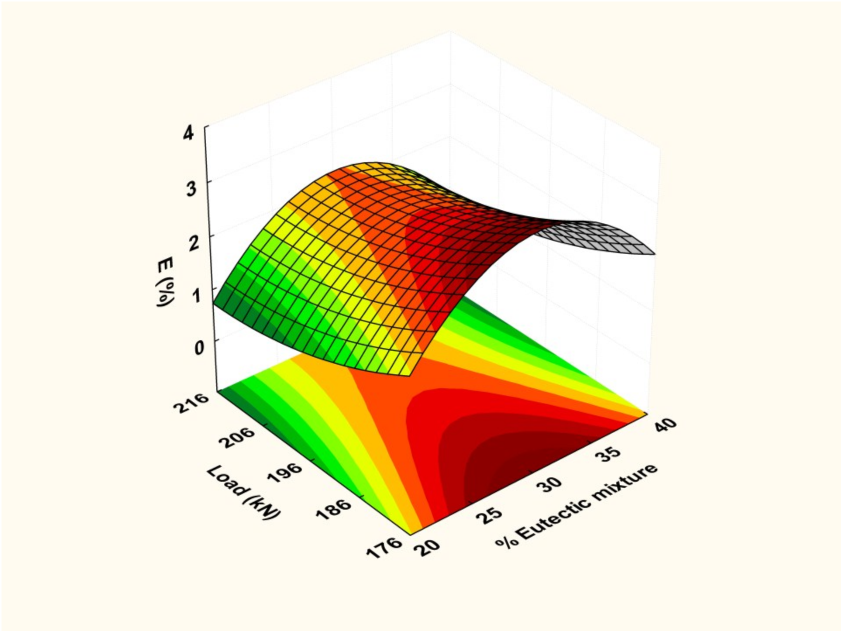

Fig. S2 XRD spectra of chitosan powder, and thermo-compressed Chit-CA and Chit-ChCl-CA films.