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Effect of lactic acid bacteria on the textural properties of an edible film based on whey, inulin and gelatin

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This study was aimed to evaluate the effects of different components and the addition of probiotic bacteria of lactic acid bacteria (LAB, *Lactobacillus casei*) on the physicochemical and textural characteristics of edible films using a response surface Box-Behnken design. The edible films were made of the following components: Whey (8%), glycerol (6%), inulin (0 to 4%), gelatin (2 to 5%) and probiotics (0 to 2%). The addition of LAB significantly affected the pH, viscosity, luminosity, hardness and Young's modulus of the films. However, all formulations presented physicochemical and textural characteristics suitable for the survival of lactic acid bacteria during storage. Formulas 10 and 3 with a composition of 2% inulin, 3.5% gelatin and 1% probiotic, demonstrated higher responses to pH, viscosity, luminosity, hardness and Young's modulus, whereas formulas 14 and 12 exhibited lower responses to the above-mentioned features but proved the most resilient. Therefore, edible films with added probiotic bacteria could be applied to a variety of foods and to increase consumer choice.

Key words: Edible film, lactic acid bacteria, inulin, gelatin, whey.

INTRODUCTION

Some studies indicated that probiotics have favorable effects on many aspects of human health including digestion, the immune system as well as preventing certain cancers (Cornelius et al., 2002; De las Caigigas, 2002; Corrales et al., 2003; Sungsoo and Finocchiaro, 2010). Therefore, products that provide this type of bacteria should be a part of the healthy diet. The International Dairy Federation (IDF) indicates that the recommended minimum intake should be more than 10^6 CFU/g. With regard to the Mexican population, consumption of probiotic bacteria is often limited due to factors for example food culture, ignorance of probiotic

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Abbreviations: EF, Edible films; **FFS,** film-forming solution; **LAB,** lactic acid bacteria; **MRS,** De Man-Rogosa Sharpe.

bacteria and a limited access to dairy products (Röble et al., 2010; Olaiz-Fernández, 2012), thus, incorporating incorporating probiotics as a part of healthy diet. Current research suggests that various applications of edible films, including functional foods, could be an alternative source for probiotic bacteria, helping to increase consumption and provide major health benefits (Röble et al., 2010; Sungsoo and Finocchiaro, 2010; Olaiz-Fernández, 2012).

The collection and application of edible films (EF) have been central themes for scientific research (Villagómez-Zavala, 2008; Rodríguez and Schöbitz, 2009; Peres et al., 2012) as alternatives for protecting and managing perishable foods primarily because of their ability to extend shelf life by acting as barriers to moisture, oxygen and solutes between the food and the environment (Bosquez and Vernosn-Carter, 2005; Chillo et al., 2008; Escobar et al., 2008; Krochta, 2009).

Previous investigations have reported the application of EF on foods such as minimally processed fruits and vegetables (Rojas-Graü et al., 2009; Valencia-Chamorro, 2011), melon (Ruiz-Cruz et al., 2010), tomato (Galietta et al., 2005; Amaya et al., 2009), loquat (Márquez et al., 2009), strawberry (Ribeiro et al., 2007) and avocado (Aguilar-Mendez, 2005). These research provided results that describe improved shelf life.

Furthermore, EF has been under investigation because of its ability as a vehicle for various active substances including nutraceuticals, antioxidants, flavours, colours, essential oils, extracts and antimicrobials. Some studies (Rodríguez and Schöbitz, 2009; Guiga et al., 2010; Shakeri et al., 2011), related the use of antimicrobials as a component of the EF and its effect as a biopreservant inhibiting the growth of various microorganisms. This application can extend the utility of the films in foods according to their physicochemical characteristics.

Quantity is one of the EF components that must be considered to directly determine the application and functionality of EF on foods (Bosquez and Vernon-Carter, 2005; Villagómez-Zavala et al., 2008). Because each component has its own physicochemical characteristics that determine the structure of the intermolecular bonds or the tri-dimensional network of the film, understanding the manner in which these components interact is critical to designing an EF with specific structural characteristics for food applications.

Although research using probiotic bacteria such as EF components is ongoing (Tapia et al., 2007; Rojas-Graü et al., 2009; Ramírez et al., 2012), the addition of LAB, the EF could be an alternative to the consumption of probiotics as described by Tapia et al. (2007). Considering the need to understand the interaction between the components and the LAB, the objective of this study was to analyse the effect of the addition of LAB into an edible film based on 4 components and to determine the physicochemical characteristics, texture and the survival time for the LAB during storage.

MATERIALS AND METHODS

Sample preparation

The production process of EF was performed under sterile conditions under a laminar flow hood (Class II Biosafety Cabinet Purifier, Labconco, Kansas City, Missouri, USA). Sterile distilled water was used with five components in the following proportions: Whey of 8% milk (extra dry grade prepared from pasteurised sweet whey, Darigold Inc., Seattle, WA, USA), 6% glycerol (J.T. Baker Analyzed™, ACS Mexico), 2 to 5% gelatin (Duche 12/1), 0 to 4% inulin (Frutafit IQ, VA Mexico SA CV), and *Lactobacillus casei* Shirota, which was used as a probiotic and obtained by centrifuging a fermented drink called Yakult® (0 to 2%) at 3500 rpm (5810 R Eppendorf AG 22331 centrifuge, Hamburg, Germany) and kept at 4°C for 30 min. For the formation of the EF, the method of Escobar et al. (2008) was used with some modifications. Distilled water was heated to 70°C, and the following components were added one-by-one in order under constant agitation for 30 min until completely dissolved: gelatin, inulin, whey and glycerol. The mixture was allowed to stand until cooled to 25°C. Then, the LAB was added at the concentrations indicated in Table 1, while maintaining constant stirring until they became completely incorporated into the solution. This mixture was referred to the film-forming solution (FFS). For the EF, the FFS was placed in 6 mL aliquots on 6 cm diameter plastic plates and kept under a controlled atmosphere in an incubator (Model 131 Felisa, Mexico) at 25°C for 10 days. The thickness of all the obtained films was measured using the Vernier Micrometer (Central Tool Co. Cranston, R.I., USA) with an average value of 5 EF.

Physicochemical tests

pH

A 90 mL sample of the as-prepared FFS was tested at 25°C. The methodology was approved by the American Society for Testing and Materials (ASTM) D 1293-84 (1990) and utilised a benchtop potentiometer, model pH 210 (Woonsocket-USA, Romania), that had been previously calibrated with buffer solutions of pH 4.01 and 7.00.

Viscosity

The methodology was approved by the A.S.T.M. D1439 - 03 (1990) using a Brookfield RVT model 203015 from Engineering Laboratories (Middleboro, MA 02346 USA). From the recently developed FFS, 90 samples were used at a temperature of 20°C, a needle (spin) number of 4 and at a spin rate of 100 rpm over 30 s. The results indicated on the dial were corrected by the factor provided in the tables to determine the viscosity in centipoise (cps).

Colourimetry

A Konica Minolta model CR-400 Chroma Meter, Sesing, Inc., Japan was used. To calibrate the colourimeter, calibration plate number 126633047 was used. EF samples of 6 cm in diameter were used within 48 h of being made. The previously calibrated colourimeter was placed on the EF and a shot was fired to record values in the Hunter L scale, a* b*, in which L 100% = white, L 0% = black; +a> 0 = red,−a <0 = green, +b> 0 = yellow, −b <0 = blue.

Table 1. Edible films composition from Box-Behnken response surface.

Codify values				Real values		
Formula	**Factor A**	**Factor B**	**Factor C**	**% Inulin**	**% Gelatin**	**% LAB**
1	1	1	0	4	5	1
2	-1	0	-1	0	3.5	0
3	0	0	0	2	3.5	1
4	1	-1	0	4	2	1
5	0	1	-1	2	5	0
6	0	1	1	2	5	2
7	-1	-1	0	0	2	1
8	1	0	-1	4	3.5	0
9	-1	0	1	0	3.5	2
10	0	0	0	2	3.5	1
11	0	0	0	2	3.5	1
12	0	-1	1	2	2	2
13	0	-1	-1	2	2	0
14	-1	1	0	0	5	1
15	1	0	1	4	3.5	2

Factor A, Inulin; factor B, gelatin; factor C, LAB; 1.0, minimum level of component; 1.0, maximum level of component; 0, middle level of component. The whey protein and glycerol levels were constant in all SFP (8 and 6% respectively).

Texture tests for edible film

A TA-XT2 Instron Universal Texturometer was used for the hardness determination. Samples of edible film of 6 cm diameter and 0.17 mm average thickness were placed between two metal plates with a central hole with leaving exposed of 2.5 cm which introduced a punch of spherical tip of 6.5 mm diameter. The test conditions were: rate of 2 mm and a distance of tension from 5 mm up to achieve the breakdown of the film and pass it entirely. The resulting graph determined the maximum (N) force to rupture, the total area under the curve, and the area under the curve up to the breaking point for the film. Young's moduli (%) were considered the slope of the curve of tensile strength, from the beginning to the breaking point of the film, and elongation (%) was considered as the distance from the start of the test to the point of breaking.

Survival of lactic acid bacteria (LAB)

The technique for producing a casting plate, according to NOM 110 and 092 SSA 1994, was used on 1 cm^2 EF samples within 0, 5 and 10 days of preparation. The samples were placed in a Man-Rogosa Sharpe (MRS) broth and incubated for 48 h. Then, the mixtures were diluted. Aliquots (1 mL) of the last three dilutions were placed in sterile Petri dishes. MRS agar was added and allowed to incubated for 48 h at 30°C. Colony counting was performed (Solbat® scientific apparatus S de RL, Mexico).

Film morphology

Scanning electron microscopy (SEM) was used to study the morphology of the films using JSM-5510 (SEM, JEOL Ltd., Tokyo, Japan) at 5.0 kV. Film samples were cut into appropriate-sized samples and mounted on stubs using double-sided adhesive tape. Prior to analysis, films were coated with gold to make the samples conductive. Subsequently, samples were observed at 1000× magnification.

Experimental design

A Box-Behnken design was used for response surface modelling that involved three centres under a block design. 15 treatments were obtained, 11 of which included the addition of probiotic (LAB). All physicochemical and textural determinations were performed in triplicate and the readings were recorded and analysed using the Statgraphics Plus version 4.1 database. Table 1 shows the compositions of the different EF as determined by the response surface model.

RESULTS AND DISCUSSION

Circular sheets of 6 cm in diameter and 0.17 mm in thickness were obtained. These sheets were an opaque light yellow, flexible and soft to the touch. The mean values of the responses for the physicochemical characteristics and the textural EF formulations are presented in Table 2. The results indicate that the samples were homogeneous with each other in pH, viscosity and luminosity. However, their textural characteristics varied more with the composition of the EF. The pH values were generally observed within the range of 5.79 to 6.15 in which the maximum difference was only 0.36. The viscosity was found in the range from 10.5 to 30.5 cps, and a relationship was observed between the viscosity and the hardness of the EF derived from the combination of the different components in the

Table 2. Mean values for the physicochemical variables and textures of the different formulations of edible film.

Film	pH		Viscosity (cps)		Luminosity (%)		a*		b*		Hardness (N)		Distance (N)		Young's modulus		Elongation (%)	
	μ	S	μ	S	μ	S	μ	S	μ	S	μ	S	μ	S	μ	S	μ	S
1	5.87	± 0.04	21	± 1.15	71.06	± 0.43	-2.51	± 0.034	11.97	± 1.11	12.18	± 3.02	20.51	± 2.23	0.36	± 0.16	0.84	± 0.15
2	5.81	± 0.09	22	± 0	71.32	± 0.45	-1.52	± 0.208	8.03	± 1.11	22.19	± 3.8	20.18	± 0.40	0.73	± 0.07	0.86	± 0.03
3	5.83	± 0.10	30.5	± 1	71.56	± 0.72	-1.54	± 0.608	8.01	± 0.68	19.59	± 1.84	18.16	± 1.80	0.78	± 0.12	0.75	± 0.19
4	5.79	± 0.01	21.5	± 1.9	73.44	± 0.75	-2.29	± 0.088	7.58	± 0.59	8.31	± 0.41	20.38	± 0.55	0.3	± 0.03	0.89	± 0.04
5	5.92	± 0.09	11	± 1.2	73.94	± 0.28	-1.81	± 0.162	6.48	± 0.98	3.06	± 0.32	13.05	± 0.61	0.22	± 0.004	0.44	± 0.03
6	5.8	± 0.13	20	± 0	70.08	± 1.61	-2.86	± 0.716	17.96	± 5.79	8.92	± 3.25	16.92	± 2.66	0.45	± 0.08	0.59	± 0.17
7	6.07	± 0.11	20	± 1.63	73.38	± 0.31	-2.68	± 0.536	11.17	± 1.98	5.31	± 0.74	15.16	± 0.74	0.29	± 0.04	0.57	± 0.04
8	5.93	± 0.01	11	± 1.15	75.94	± 0.86	-2.45	± 0.388	9.92	± 1.46	0.65	± 0.08	6.76	± 0.62	0.11	± 0.01	0.13	± 0.02
9	5.89	± 0.01	16	± 1.63	74.24	± 3.68	-1.72	± 0.130	7.6	± 1.0	6.96	± 1.43	16.23	± 0.34	0.31	± 0.04	0.62	± 0.02
10	6.15	± 0.22	19.5	± 1	70.96	± 1.66	-2.77	± 0.410	12.6	± 3.59	15.38	± 2.17	18.93	± 0.83	0.57	± 0.07	0.82	± 0.05
11	5.82	± 0.08	19.5	± 1	71.71	± 0.99	-1.72	± 0.752	7.07	± 2.89	10.58	± 2.39	19.32	± 0.51	0.37	± 0.07	0.83	± 0.03
12	5.95	± 0.04	11.5	± 1.9	76.05	± 1.34	-2.87	± 0.245	10.75	± 1.18	1.25	± 0.03	11.38	± 0.53	0.13	± 0.01	0.36	± 0.03
13	6.08	± 0.05	20.5	± 1	72.9	± 0.56	-2.03	± 0.498	7.27	± 1.71	9.38	± 1.19	17.84	± 0.86	0.37	± 0.03	0.71	± 0.05
14	5.91	± 0.08	10.5	± 1	70.37	± 2.91	-1.78	± 1.061	16.7	± 3.49	3.45	± 0.87	14.37	± 1.30	0.21	± 0.03	0.57	± 0.08
15	5.93	± 0.14	18.5	± 1	71.66	± 1.07	-2.57	± 0.879	12.99	± 4.69	8.27	± 2.51	16.79	± 0.75	0.38	± 0.09	0.67	± 0.05

formula. The luminosity of the EF was found to range from 70 to 76%, that is, the colored films were characterised as negative a* and a trend was observed toward positive b* from green to yellow; showed a final light yellow colour. The hardness of the EF was found to range from 0.66 to 22.18 N, and the higher viscosity formulations proved the strongest. The following section refers to the physicochemical and textural characteristics found during the analysis of the variance and the regression coefficients.

Physicochemical characteristics

pH

The pH of the EF was significantly affected ($p<0.05$) by the addition of gelatine, gelatin–inulin and the inulin–LAB interactions. As observed in the regression coefficients of the model fit (Table 3), the pH increased with the addition of inulin and LAB, while the gelatin–LAB interaction caused a decrease in the pH (Figure 1). This result can be attributed to the direct interaction between the different components (inulin 5 to 7 and 4 to 4.7 gelatin) and the LAB that can increase or decrease the FFS pH (Kip et al., 2005; Marigal and Songronis, 2007).

Viscosity

Viscosity was affected ($p<0.05$) by the gelatin and gelatin–inulin interaction; the addition of inulin increased the viscosity, while the gelatin and gelatin–LAB interaction decreased it (Figure 2). Gómez-Ocampo et al. (2007) reported that the molecular weight of the gelatin has a direct relationship to the viscosity. Madrigal et al. (2007) mentions that the viscosity of inulin is a key factor in the formation of gels because thickening causes synergy with other gelling agents such as gelatin, which is consistent with the results obtained in this investigation.

Colorimetry

The luminosity was found to decrease ($p<0.05$) with the addition of LAB because the inulin–LAB and gelatin–LAB interactions caused a decrease in the L value of the EF (Figure 3), which can be directly attributed to the colouration of the product used to obtain the LAB. Reduced luminosity was

Table 3. Coefficients of regression for the models adjusting the physicochemical and textural variables of the edible film.

Parameter	pH	Viscosity	Luminosity	a*	b*	Hardness	Distance	Young's modulus
K	5.87	17.153	72.577	-2.51	10.95	6.537	16.54	0.273
A	0.086							
B		-4.75		-2.241	1.391	-5.706		-0.175
C	0.049			0.227				
AA		3.855				4.677		0.182
AB		-4.75						
AC			-1.643	-0.305	2.956	3.006		0.104
BB				0.565	-2.91			
BC	-0.072		-1.715	0.35	0			
CC	0.089	-1.894			1.903		-0.277	
R^2	46.6	77.4	33.3	49.6	44.7	72.6	0.67	71.39
SE	0.09	0.1	1.9	0.41	2.9	6.23	4.08	0.1

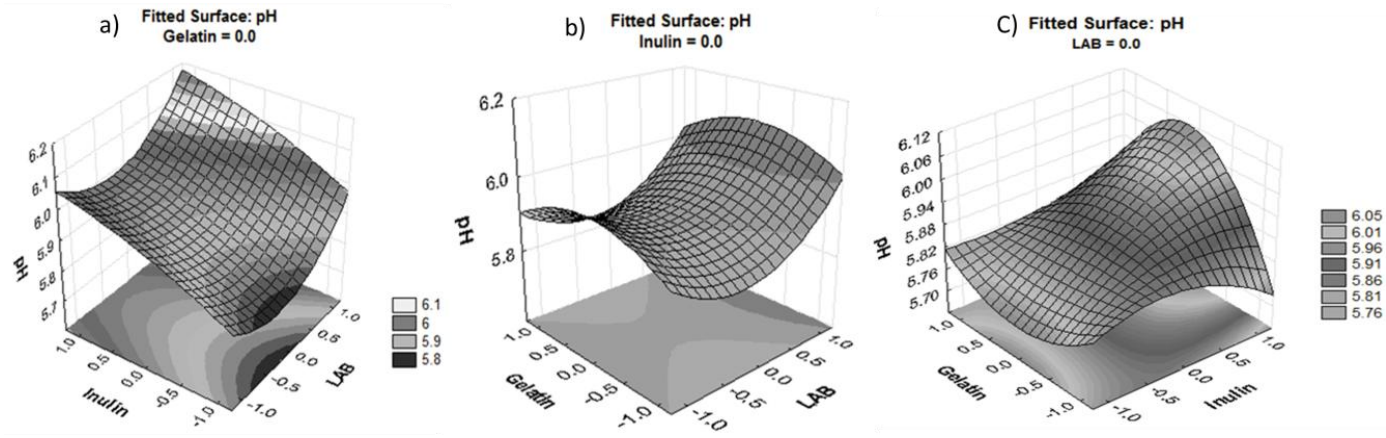

Figure 1. The effect of (a) Gelatin, (b) inulin and (c) LAB on the pH of edible films; significant at p< 0.05. 1.0, Minimum level of component; 1.0, maximum level of component; 0, mid-level of component.

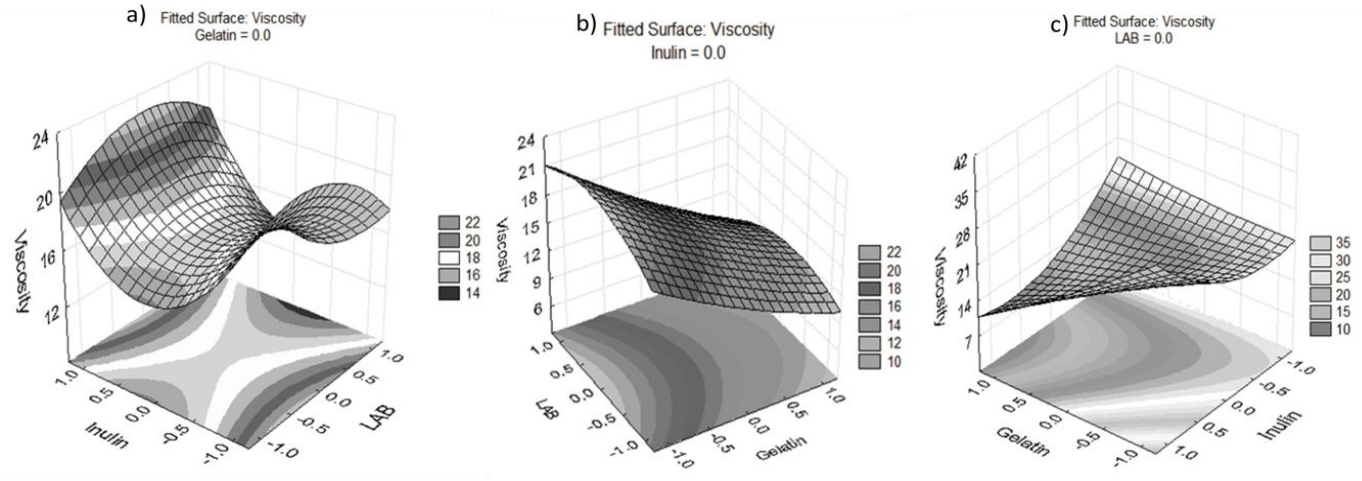

Figure 2. The effect of (a) Gelatin, (b) inulin and (c) LAB on the viscosity of edible films, significant at p< 0.05.-0.1, Minimum level of component; 1.0, maximum level of component; 0, mid-level of component.

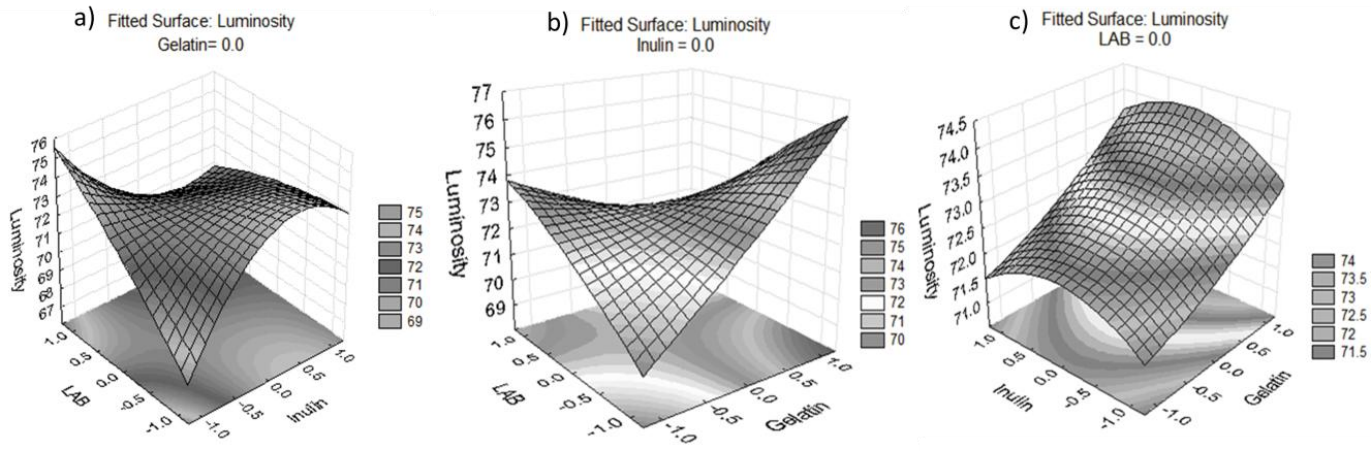

Figure 3. The effect of (a) Gelatin, (b) inulin and (c) LAB on the luminosity of edible films, significant at p< 0.05. -1.0, Minimum level of component; 1.0, maximum level of component; 0, mid-level of component.

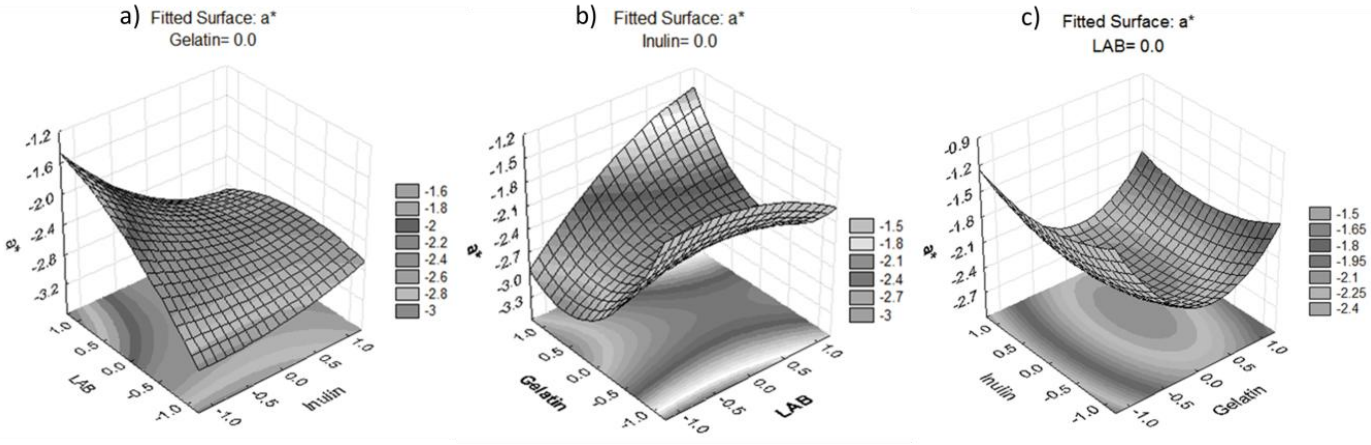

Figure 4. The effect of (a) Gelatin, (b) inulin and (c) LAB on the a* of edible films, significant at p<0.05. -1.0, Minimum level of component; 1.0, maximum level of component; 0, mid-level of component.

also found in the formulas with higher gelatin content. The value of a* was affected by the presence of gelatin, LAB and inulin–LAB–LAB gelatin interaction and was also found to be negative in all cases (that is, tending toward the green with a range of −2.87 to −1.52 (Figure 4). In contrast, b* was significantly affected by the presence of gelatin and the inulin–LAB interaction, generating a positive effect (Figure 5) in the yellow direction with a range of 6.4 to 17.9.

These may be due to the interactions between different components that contribute a particular colouration to the FFS (Won-Seok and Jung, 2001; Escobar et al., 2008), resulting in a yellowing of the EF analysed in this study and could be relevant to food applications (Delmoro et al., 2010).

Texture tests for edible films (EF)

The gelatin and inulin were found to have an obvious effect (*p<0.05*) on the hardness of the EF, while the addition of LAB had a negative effect on the hardness of different EF formulations, making them weaker. In addition, the presence of inulin was found to strengthen the EF structure (Figure 6).The formulas that could withstand the greatest force also exhibited the highest viscosity and vice versa. This result, which contributes to the overall structural hardness, may be due to the ability of the inulin and the gelatin to form gels and to their physicochemical properties that create synergy among the components to enable the formation of three-dimensional networks to create EF (Kip et al., 2005;

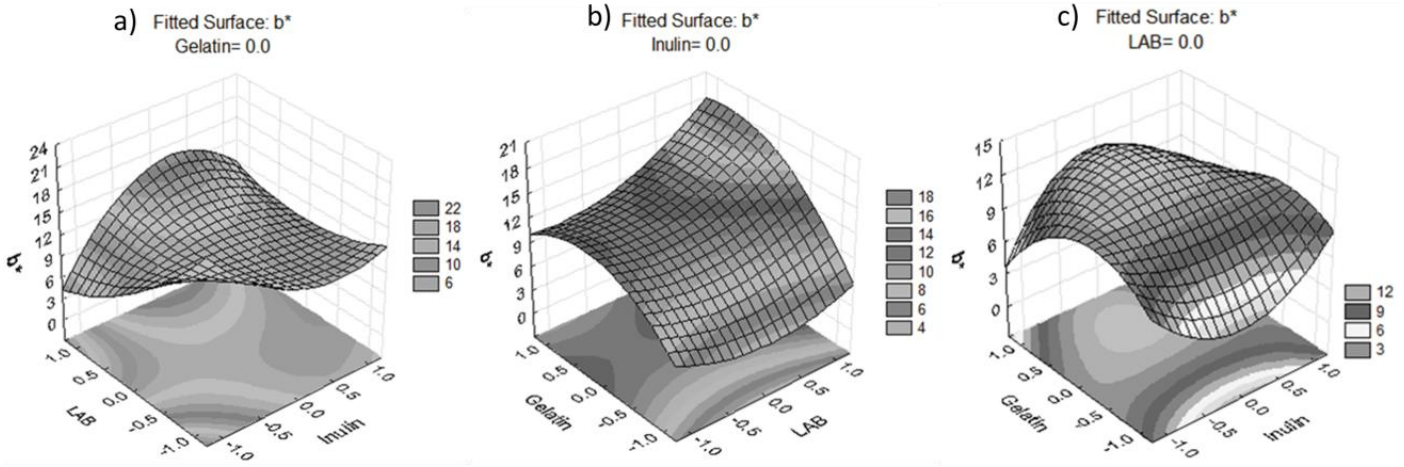

Figure 5. The effect of (a) Gelatin, (b) inulin and (c) LAB on the b* of edible films significant at p<0.05. -1.0, Minimum level of component; 1.0, maximum level of component; 0, mid-level of component.

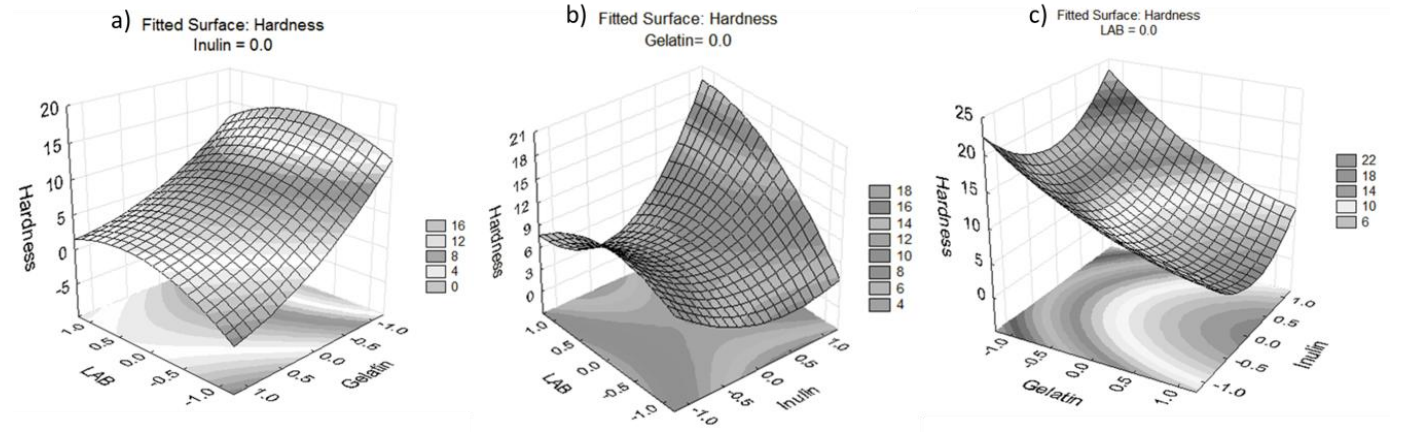

Figure 6. The effect of (a) Gelatin, (b) inulin and (c) LAB on the hardness of edible films significant at p<0.05. -1.0, Minimum level of component; 1.0, maximum level of component; 0, mid-level of component.

Gómez-Ocampo et al., 2007; Marigal and Songronis, 2007). The addition of LAB can weaken the structure due to the interactions between the protein chains that reduce flexibility and increase the fragility of the EF. Villagomez-Zavala et al. (2008) reported that these characteristics may be related to the type of structures involved in the gelation of multicomponent films.

The Young's modulus were also influenced (*P<0.05*) by the presence of gelatin and the inulin–LAB interaction (Table 4). The elasticity was found to increase with the addition of inulin (Figure 7), and a lower Young's modulus was recorded for the less-strong formulations characterised by higher elasticity. This result agrees with the findings of Villagomez-Zavala et al. (2008), which relate the decrease in Young's modulus to an increase in the film elasticity. The strongest EF was found to have an increased Young's modulus, that is, it is less elastic. This has a correlation factor of r^2 = 0.93, which is crucial in making decisions for implementing the EF on food (Figure 8). In addition, the different components, including the addition of LAB, were found to have no significant effect (*P> 0.05*) on the textural characteristics, the distance or the elongation of different EF formulations.

The EF formulas with higher responses in the physicochemical and textural characteristics were 10, 3 and 2. Those identified with the lowest responses were 14, 12 and 5 (Table 5). The compositions of formulas 10 and 3 were found to be identical (inulin 2%, 3.5% gelatine and 1% LAB), while Formula 2 was composed of 3.5%

Table 4. Analysis of the variance in the physicochemical and textural variables of different formulas of edible film.

Parameter	pH		Viscosity		Luminosity		a*		b*		Hardness		Distance		Young's Modulus		Elongation	
	SSC	p-value	SSC	p-value	SSC	p-value	SSC	p-value	SSC	p-value	SSC	p-value	SSC	p-value	SSC	p-value	SSC	p-value
A:Inulin	0.24	0.0006	0.0028	0.844	0.287	0.85	0.084	0.575	4.118	0.484	4.65	0.74	35.50	0.433	0.002	0.78	0.14	0.378
B:Gelatin	0.00045	0.82	1.8	0.0009	19.95	0.14	1.87	0.025	61.97	0.0215	1041.89	0.0008	132.93	0.15	0.99	0.0005	0.49	0.12
C:LAB	0.07	0.015	0.025	0.56	3.81	0.49	1.66	0.032	3.75	0.503	41.5	0.33	10.57	0.66	0.02	0.43	0.035	0.65
AA	0.004	0.47	0.56	0.021	3.09	0.54	0.353	0.265	17.95	0.164	314.61	0.022	69.69	0.281	0.49	0.0043	0.2	0.29
AB	0.01	0.26	0.902	0.0066	0.066	0.93	0.242	0.35	1.17	0.706	7.01	0.68	28.15	0.48	0.01	0.54	0.11	0.44
AC	0.000025	0.96	0.03	0.52	43.23	0.043	1.49	0.039	139.83	0.003	144.58	0.089	19.84	0.55	0.17	0.046	0.08	0.49
BB	0.043	0.052	0.01	0.71	0.27	0.85	4.72	0.002	132.64	0.003	39.77	0.341	17.53	0.58	0.02	0.38	0.046	0.6
BC	0.084	0.013	0.01	0.71	47.06	0.036	1.96	0.02	33.03	0.071	11.59	0.59	19.98	0.55	0.005	0.68	0.07	0.53
CC	0.106	0.007	0.127	0.21	1.05	0.72	0.35	0.27	48.83	0.035	92.86	0.16	0.029	0.98	0.045	0.26	0.006	0.85
Blocks	0.001	0.969	0.023	0.95	10.07	0.72	1.605	0.17	60.49	0.121	17.73	0.92	4.75	0.99	0.033	0.79	0.019	0.99
Lack-of-fit	0.477	0.288	0.21	1	112.71	0.98	10.5	0.485	439.86	0.291	344.31	0.99	122.43	1	0.31	0.998	0.49	1

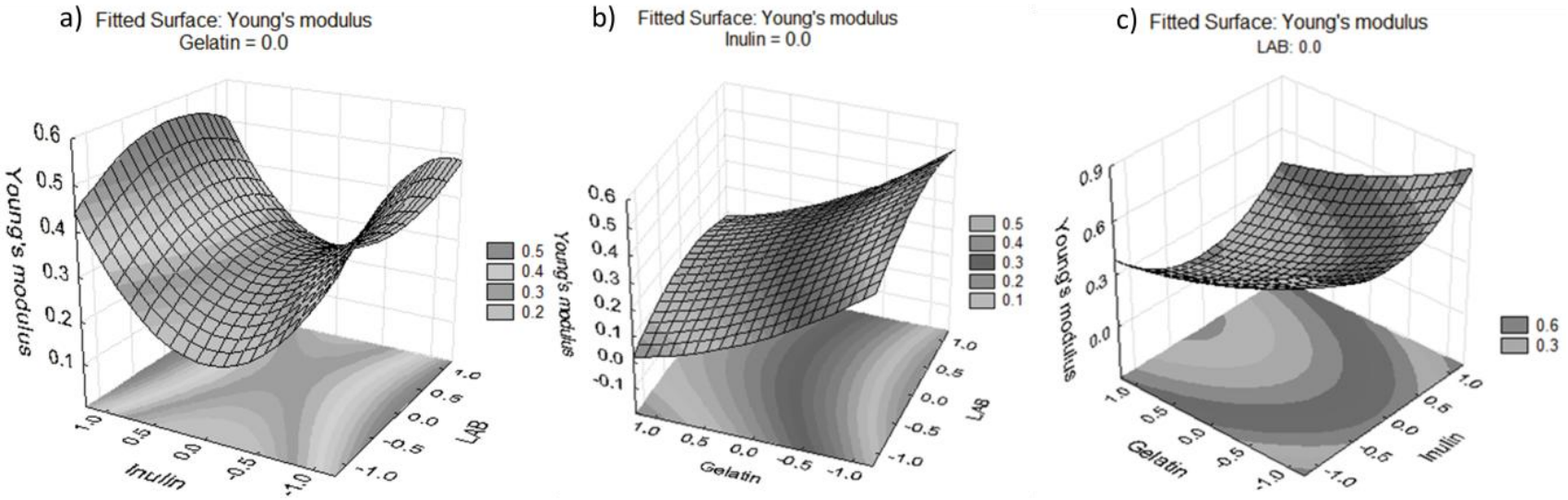

Figure 7. The effect of (a) Gelatin, (b) inulin and (c) LAB on the Young's modulus of edible films significant at $p<0.05$. -1.0, Minimum level of component; 1.0, maximum level of component; 0, mid-level of component.

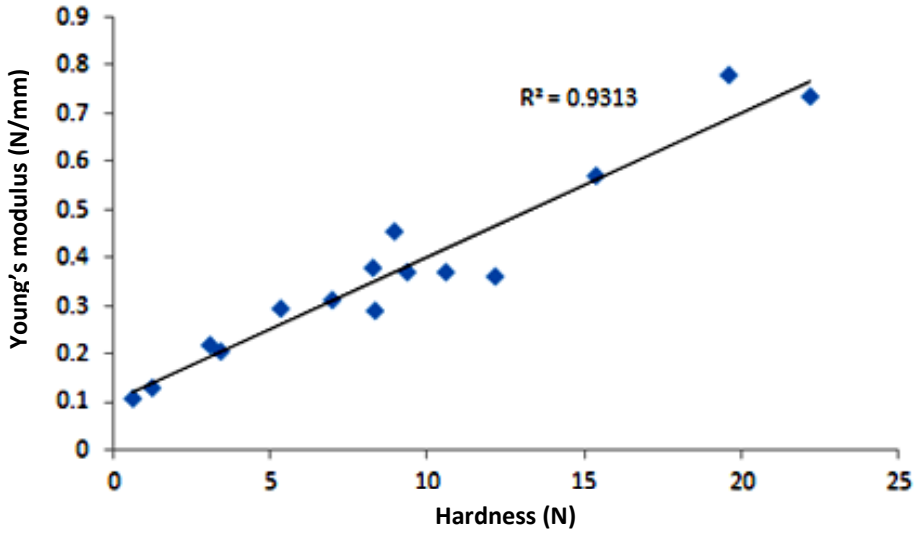

Figure 8. Relationship between Young's modulus and the hardness of edible films.

Table 5. Edible film formulas with higher and lower physicochemical and textural responses.

Physicochemical and textural response	EF (higher response)	EF (lower response)
pH	10,13,7,12	4,6,2,11
Viscosity	3,2,4,1	14,12,8,5
Luminosity	12,8,9,5	6,14,10,1
a*	2,3,11,9	12,6,10,7
b*	6,14,15,10	5, 11,13,4
Hardness	2,3,10,1	8,12,5,14
Young'smoldulus	2,3,10,6	8,12,14,5

EF, Edible films.

gelatin and 0% inulin and LAB. Formula 10 was found to have the highest pH (6.15), formula 3 was the most viscous (30.5 cps) and formula 2 the strongest (22.19 N). The composition of formula 14 was 0% inulin, 5% gelatin and 1% LAB; in formula 12, the percentage of each component was 2%. Formula 5 contained 2% inulin, 5% gelatin and 0% LAB. Reduced stresses were registered on formula 12 (76%).The physicochemical properties and observed textures for these formulations indicated 6 different molecular structures. The molecular structures interact as expected to form three-dimensional networks that can be used as the basis of an edible film (Villagómez-Zavala et al., 2008).The physicochemical and textural characteristics of formulas 10 and 3 spiked with LAB (as derived from this research) could be used to coat foods such as meat, sausages, cereals, etc. EF 12, 14 and 5 whose structures were weaker but more elastic could be applied to fruits and vegetables, considering that the physicochemical characteristics of the foods are not altered beyond the point of consumer acceptance.

Survival of lactic acid bacteria (LAB)

Studies designed to determine the survivability of LAB in films during storage, indicated the presence of countless numbers of LAB. At a dilution of 106, the combination of different components (inulin, gelatin, whey and glycerol) makes it possible for LAB to remain present throughout the storage period, indicating that the edible film with the above-mentioned composition could provide an alternative vehicle for this type of bacteria and could be applied to non-dairy foods as an alternative source of probiotics for mass consumption. Sandoval (2012) noted that when using EF made from whey (10%), gelatin (1%), glycerol (3%) and inulin (10%) with added LAB as a coating on white bread, the textural properties of the food are maintained. In addition, the sensory characteristics,

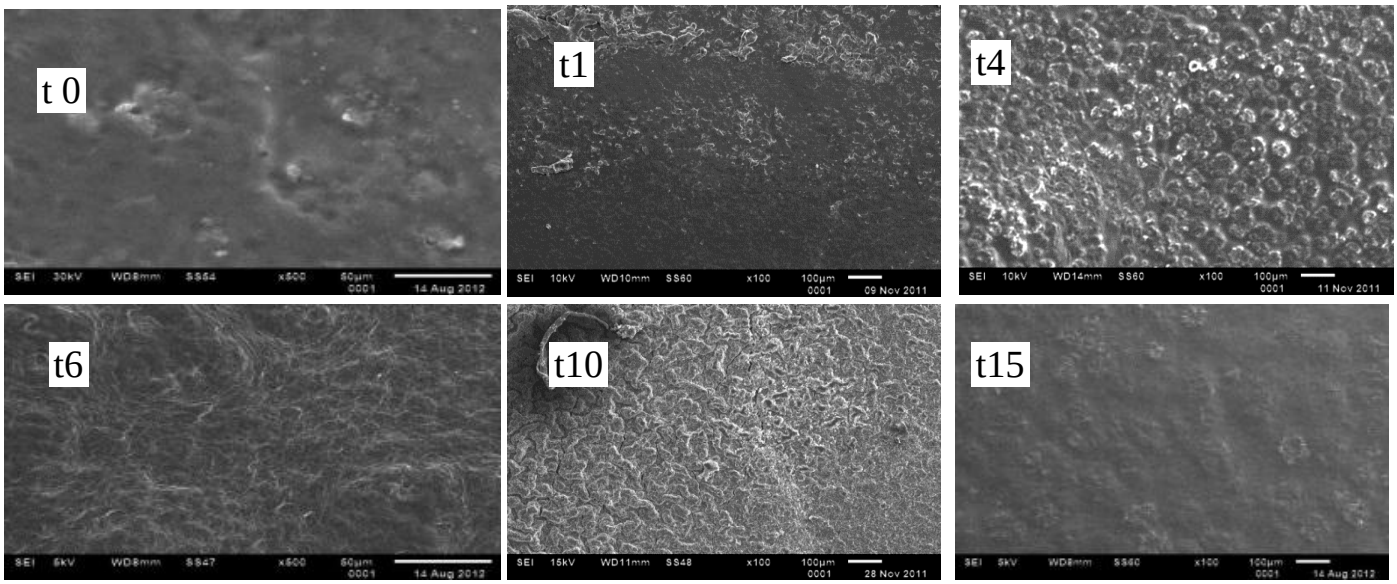

Figure 9. SEM microphotographs for EF formulas.

including product appearance, are improved when a quantity of 10^6 CFU/g LAB is maintained on top of the product during its life. Furthermore, Alvarez (2012) observed that a film with a similar composition (inulin, milk whey and glycerol) achieved a high microorganism count on broccoli over 15 days of storage, maintaining the textural characteristics of the broccoli during its shelf life. Tapia et al. (2007) reported the use of probiotics in an alginate-based EF applied to fresh fruit, and obtained an increase greater than 10^6 CFU/g after 10 days of storage. Thus, this type of film has been shown to function as a good vehicle for LAB. The optimum formulation from experimental designs was 3.96% inulin, 2% gelatin and 1.3% LAB.

Film morphology

Some preliminary determinations of the microstructure of films containing gelatin, inulin and LAB by SEM were taken in order to study the uniformity of the films (Figure 9). The surfaces of all films were smooth and uniform. Control films showed a continuous and homogeneous microstructure, while the addition of the inulin caused discontinuities associated with the presence of the 2 phases in the matrix. It is important to note that it was not observed any structural gradation which confirms the good dispersion of the inulin and gelatin which appearing to act in the formation of the network; LAB did not alter the formation of this one. Zamudio et al. (2006) showed that the compatibility of components provides best features of the EF barrier which contributes to a better ability to protect food against environmental degradation, increasing its shelf life.

Conclusions

Stable edible films were developed with the combination of whey, gelatin, inulin and glycerol, and have been demonstrated to allow the formation of EF with adequate characteristics to maintain the survival of LAB during 10 days of storage. Clearly, the components exert different effects on the physicochemical and textural properties of the EF studied in this research. Adding the LAB itself significantly affects the pH, viscosity, luminosity, hardness and elasticity of the EF, features that are relevant to applications in the food industry. The results suggest that EF spiked with LAB could be applied to various types of food and provides a viable source for probiotics. The strong and elastic films may be used as packaging, while the weaker elastic coatings could become targets for consumption as films and coatings directly applied to foods. However, these coatings depend on the characteristics of the food itself. Considering the nutritional value in this type of bacteria, the consumption of probiotics via EF spiked with LAB may have important implications in preventing various health problems and could provide a LAB alternative in non-dairy foods. In addition, the EF spiked with LAB could provide products with increased shelf lives. This research must be extended to determine the effects of long-term storage on EF and the potential risks for contamination by other microorganisms that can cause spoilage to ensure a product with nutritional and functional properties suitable for human consumption.

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