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Enhancing the quality of Havarti cheese: Chitosan films with nettle *Urtica dioica* L. extract as slice separators to retard lipid oxidation

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ABSTRACT

The aim of this study was to develop an active slice separator based on a chitosan film with nettle extract (Ch_NE) for vacuum-packed Havarti cheese and to evaluate the changes during storage for 45 days at 5 °C. The active properties of the developed slice separator film were evaluated by measuring antioxidant and optical properties of the cheese and the film, as well as lipid oxidation of the cheese. The addition of nettle extract to chitosan films caused a decrease in mechanical properties such as tensile strength from 58.02 to 1.81 MPa and Young's Modulus from 1535.92 to 4.43 MPa, making them easy to apply and handle as slicing separators. Furthermore, the thermal properties of the films indicated their stability at temperatures commonly encountered during food heat processing. After 45 days of refrigerated storage, the Ch_NE slice separator demonstrated a significant reduction in thiobarbituric acid reactive substances in cheese samples, indicating a 56% decrease in lipid oxidation compared to the control group. In conclusion, an environmentally friendly alternative to synthetic and non-biodegradable slice separators have been successfully developed using chitosan, a by-product of the fish industry, enriched with an extract from nettle, a widely distributed wild plant.

1. Introduction

It is well known that plants are a great source of active compounds with antimicrobial and antioxidant properties. Thus, by using aqueous extracts from wild plants that are widely distributed throughout the world, it is possible to obtain powerful antioxidant films with acceptable transparency using biodegradable polymers from renewable sources. However, the obtainment of these compounds is influenced by a variety of factors, including the extraction method, solvent, agroclimatic conditions and plant parts (Valdés et al., 2015). Polyphenols are present in most plants and they are secondary metabolites that promote protection against ultraviolet radiation, pathogens, parasites and predators (Vieira et al., 2022). They are responsible for the biological properties of the plants, generating great interest due to their antioxidant capacity (Stagos, 2019). The ability to donate hydrogen atoms from their hydroxyl groups and increase the interaction with peroxyl radicals allows the formation of stable phenoxyl radicals, and this way stop the chain reactions of lipid peroxidation (Barbosa-Pereira et al., 2013; Hughes, Thomas, Byun, & Whiteside, 2012). Hence, a wide variety of plant extracts have been studied in relation to their effects of the techno-functional characteristics of food packaging such as *Pistacia terebinthus* (Kaya, Khadem, et al., 2018), *Santalum album* (Flórez, Cazón, & Vázquez, 2022a) or *Nephelium lappaceum* (Chollakup, Pongburoos, Boonsong, & Khanoonkon, 2020).

The use of natural antioxidants like polyphenols obtained from plants and agricultural by-products is one of the current topics in food packaging research (Valdés et al., 2015). Nettle, scientifically known as *Urtica*, is a plant from the *Urticaceae* family recognized for its distinct serrated leaves (Mzid et al., 2017). These leaves have a lipid profile composed of fatty acids such as α-linolenic acid, palmitic acid and *cis*-9, 12-linoleic acid. Additionally, they contain smaller proportions of n-3 and n-6 fatty acids. Furthermore, nettle leaves possess a mineral profile comprising both micro and macroelements, including Na, K, Ca, Mg, Fe and Mn (Đurović et al., 2017; Simopoulos, 2003). Nettle extracts are a useful natural source of polyphenols, pigments, and bioactive chemicals (Repajić et al., 2020). Several species of nettle are known: *Urtica dioica* L., *Urtica membranacea* Poir and *Urtica urens* L. Nevertheless, the *Urtica dioica* L. species has the highest concentration of polyphenols (Rita et al., 2017). Polyphenols such as chlorogenic, ferulic and chicoric acid; and flavonoids such as luteolin or quercetin-3-glucoside are identified in the extracts obtained from nettle (Repajić et al., 2020). The antioxidant properties of this extract depended on the time, extraction method

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solvent used, as well as the time of harvesting, the sun exposure, or the soil in which it has grown (Đurović et al., 2017; Flórez, Cazón, & Vázquez, 2022b).

Extensive research has been conducted on a diverse range of biopolymers including polysaccharides with the aim of developing food packaging films. These biopolymers exhibit compatibility with plasticizers, plant extracts, and other materials, leading to the presence of hydroxyl, amino, carbonyl, and polar groups in the resulting film. The formation of a hydrogen bond network structure among these polar groups contributes to the film's favorable mechanical and barrier properties (Dehghani, Hosseini, & Regenstein, 2018). Notably, chitosan-based films have gained popularity as an environmentally conscious technique for extending the shelf life of perishable foods (Elsabee & Abdou, 2013; Flórez, Guerra-Rodríguez, Cazón, & Vázquez, 2022). These films have the potential to serve as active packaging by incorporating compounds that enhance antioxidant and/or antimicrobial effects (Cazón & Vázquez, 2019; Lunkov, Ilyina, & Varlamov, 2018; Mujtaba et al., 2019).

Guaranteeing the protection of food requires the development of packaging systems that have antioxidant capacities. The presence of oxygen in the surrounding environment is associated with textural alterations, discoloration, and the emergence of undesirable flavours (Flores, Pelegrín, Ramos, Jiménez, & Garrigós, 2021). For this reason, the packaging of fatty foods such as cheese is problematic because of the oxidation of the lipids that make up the food. Foods with a high fatty acid content are more prone to oxidative deterioration, which results in the development of toxic aldehydes. In this way, the food becomes unsuitable for human consumption because of its loss of nutritional quality (García, Beltrán Sanahuja, & Garrigós Selva, 2013; Sanchez-Bel et al., 2011).

Havarti is a ripened firm/semi-hard cheese in conformity with the General Standard for Cheese. The colour of the body ranges from nearly white or ivory to light yellow. This cheese is characterised by the presence of abundant, irregular, and thick holes with the size of a rice seed. Havarti-type cheeses typically contain 30–45 % of fat (Codex Standard for Havarti, 267–1966).

The oxidation of Havarti cheese poses a significant challenge in terms of maintaining its quality and shelf life. Havarti cheese, known for its smooth and creamy texture, is susceptible to oxidation due to its high fat content. When exposed to oxygen, the unsaturated fatty acids present in Havarti cheese can undergo oxidation reactions, leading to the formation of off-flavours, textural changes, and a decrease in overall product quality. Additionally, oxidation can result in the loss of nutritional value and the development of rancidity in the cheese. Therefore, addressing the problem of cheese oxidation in Havarti is crucial to ensure its sensory attributes, nutritional integrity, and extended shelf life for consumer satisfaction.

Employing active packaging systems with antioxidant properties presents a promising alternative for enhancing the stability of oxidation-sensitive foods such as Havarti cheeses. This approach involves the development and utilization of packaging systems that actively counteract the effects of oxidation (Valdés et al., 2015). In addition, due to the large number of holes characteristic of this cheese due to its production process, it is common to use separators in the sliced version, to facilitate its handling and to be able to easily remove the slices. The most common separators are based on non-biodegradable polymers such as polyamide/polyethylene, making it necessary to search for biodegradable alternatives that cause less environmental impact.

According to the literature reviewed, chitosan-based films enriched with nettle extract have not been studied yet. Nettle is a highly available and low-cost plant worldwide. The objective of this study was to develop an active chitosan-based film with Nettle (Ch_NE) as slice separator. The effect of nettle extracts at different ratio on its mechanical properties were analysed. Simultaneous thermal analysis (TGA/DSC) were carried out to evaluate the thermostability of the films. The novel Ch_NE film has been tested as slice separator for vacuum-packed Havarti cheese. The active properties of the developed slice separator were evaluated by measuring the lipid oxidation, antioxidant and optical properties of the packaged cheese and the film used every 15 days up to 45 days of storage.

2. Material and methods

2.1. Materials

Chitosan, with a molecular weight range of 100,000–300,000 and CAS number 9012-76-4 was obtained from Acros Organics (Geel, Belgium). The chitosan film-forming solution was prepared using acetic acid (CAS number 64-19-7) and glycerol (CAS number 56-81-5) obtained from Scharlau Microbiology (Barcelona, Spain). To establish a control, polyamide/polyethylene (PA/PE) films from Plastinal S.L. (Arrubal, La Rioja, Spain) were employed. *Urtica dioica* was harvested from Luarca, Asturias, Spain (43°32′51.659″N 6°31′17.548″W) during the period of September to October 2022. The Havarti cheese utilized for this study was purchased from IFA RETAIL SA (Madrid, Spain). Homogeneous samples were obtained by selecting cheeses from the same batch.

For the analysis of the antioxidant capacity, 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH•) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) were provided by Alfa Aesar (Haverhill, MA, USA). Folin-Ciocalteu phenol reagent was provided by Panreac (Barcelona, Spain).

2.2. Preparation of plant extracts

To obtain uniform and healthy nettle leaves, the nettle plant underwent a cleaning process and defoliation. Subsequently, the leaves were dehydrated at 45 °C for 24 h to eliminate moisture in a Model 3500 dehydrator (Excalibur® Food, Sacramento, CA, USA). The dried leaves were then crushed and ground into a fine powder using an electric grinder. The resulting nettle powder was carefully stored in airtight jars at room temperature (20 ± 5 °C) to maintain its quality.

The aqueous nettle extracts was obtained following optimized conditions that were previously studied and described elsewhere (Flórez et al., 2022b). Briefly, 1 g of dried nettle powder was weighed, transferred to a screw-capped tube, and diluted with 15 mL of distilled water. The tube was placed in an ultrasonic bath at 65 °C for 3.15 h (Flórez et al., 2022b). Thereupon, the nettle extracts were filtered with Whatman nº1 filter paper using a funnel to remove any solid residues present in the aqueous extract. The extract was refrigerated for 24 h to precipitate any residue present. Afterwards, the extraction was filtered with a funnel and finally centrifuged (9000 rpm, 20 min, 5 °C). The extract was stored in darkness and refrigerated at 5 °C until use.

2.3. Preparation of chitosan films

Chitosan films were prepared by dissolving 20 g/L chitosan in an aqueous solution containing 10 g/L acetic acid. The resulting stock solution was continuously stirred overnight at room temperature (20 ± 5 °C) using a magnetic stirrer. Glycerol was added as plasticizer at concentrations of 0 g/L, 2.5 g/L, and 5 g/L. Subsequently, nettle extract was gradually introduced into the chitosan solution until achieving final nettle extract concentrations of 0 g/L, 125 mL/L, and 250 mL/L, while maintaining a constant chitosan concentration of 10 g/L. The mixture was homogenized at 15000 rpm for 2 min (Ultra Turrax®, IKA, Staufen, Germany).

Chitosan films with 250 mL/L nettle extract were manufactured from an initial solution of 20 g/L chitosan and another of nettle extract diluted 50% with distilled water (nettle extract:distilled water 1:1 v/v). Then, the 20 g/L chitosan solution was slowly diluted with the 50% nettle extract under vigorous agitation, until a final volume ratio 1:1. Hence, a filmogenic solution with a final concentration of 10 g/L

chitosan and 250 mL/L nettle extract was obtained.

The same process as described above was followed to obtain chitosan-based films with 125 mL/L nettle extract. In this case, a 20 g/L chitosan solution was carefully diluted (1:1 vol ratio) with a solution of 250 mL/L nettle extract, to reach a final concentration of 10 g/L chitosan and 125 mL/L nettle extract in the filmogenic solution. Subsequently, glycerol was added as plasticizer at concentrations of 0 g/L, 125 mL/L and 250 mL/L.

The bubbles of the filmogenic solution were removed by ultrasonic bath for 15 min. It was evaluated 9 formulations: Ch_Gly0, Ch_Gly0_NE125, Ch_Gly0_NE250, Ch_Gly2.5, Ch_Gly2.5_NE125, Ch_Gly2.5_NE250, Ch_Gly5, Ch_Gly5_NE125, and Ch_Gly05_NE250. Where Ch is chitosan 10 g/L in acetic acid 1 g/L; Gly is glycerol; NE is nettle extract. The number in the film sample shows the concentration in g/L for glycerol and mL/L for nettle extract.

Polystyrene petri plates with a diameter of 210 mm were used, and 40 mL of the film-forming solution was poured into them. The films were dried at 25 °C for 48 h. The dried films were cut to predetermined sizes based on the specific test requirements. Subsequently, the films were conditioned for 5 days under controlled conditions. The thickness in mm was measured at five random locations using a Thickness Meter ET115S (Etari GmbH, Stuttgart, Germany).

2.4. Mechanical properties analysis

The films were subjected to tensile and puncture tests using a texturometer (TA-XT plus, Stable Micro System, UK) and the accessories recommended for each test. The tensile test was used to calculate the tensile strength (TS, MPa), percentage of elongation at break (%E, %) and Young's Modulus (YM, MPa). Using the ASTM standard method D-882 (https://www.astm.org/d0882-18.html), the samples (15 × 100 mm) were clamped between the texturometer's grips with an initial separation of 40 mm and the load rate set to 1 mm/s. For each batch, seven replicates were examined (Cazón, Vázquez, & Velazquez, 2018).

In the puncture test, burst strength (BS) or puncture force and distance to burst (DB) or puncture deformation were measured. A film holder (Reference HDP/FSR, Stable Micro System, UK) was used to hold the sample (30 × 30 mm) during the test. A cylindrical probe with a diameter of 3 mm and moving at a speed of 1 mm/s was used to measure the force (g) and strain (mm) up to rupture. For each batch, the test was carried out by triplicate.

2.5. Application of films for the separation of Havarti cheese slices

Havarti cheese slices (10 x 10 × 0.2 cm) were packed in vacuum bags using a vacuum heat-sealer to create an anaerobic environment. Three types of film were tested as cheese slice separators: 1) polyamide/polyethylene (PA/PE) as control, 2) pure chitosan 10 g/L and 3) chitosan 10 g/L enriched with nettle extract (181 g/L) and glycerol (1 g/L). The nettle extract and glycerol concentration of Ch_NE separators were established based on the optimization of the film composition. The optimization was performed using the statistical program Design Expert 11® and the mechanical data obtained previously in the tensile and puncture tests. By prioritizing the maximization of the deformation capacity and breaking strength of the samples, the concentration of nettle extract and glycerol was determined by the software Design Expert 11®. Hence, a film formulation (181 g/L NE and 1 g/L glycerol) with the best mechanical properties based on the established criteria, data and within the analysed concentrations were obtained to test as separator for cheese slices. For each type of analysed film, 4 packages were prepared with 5 slices of cheese separated by the corresponding film to be tested and stored at 5 °C. Each package corresponded to an analysis time (0, 15, 30 and 45 days). The analyses were performed by triplicate. Time and temperature were selected based on previous studies analysing cheese properties after 45 days of refrigerated storage (Küçük, Çelik, Mazi, & Türe, 2020; Modzelewska-Kapituła, Kłębukowska, & Kornacki, 2007).

2,2-Diphenyl-1-picrylhydrazyl radicals (DPPH•) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•$^{+}$) free radical scavenging parameters in the cheese samples packaged with the different separator formulations and the films themselves were analysed at 0, 15, 30 and 45 days of storage. The film and cheese extracts were prepared taking 1 g of film in 24 mL of methanol or 2 g of Havarti cheese in 9 mL of distilled. The mixtures were left under stirring overnight in the dark. The mixtures were homogenized in vortex for 1.5 min and then they were filtered through Whatman nº1 filter paper (Bottesini et al., 2013). The radical scavenging on DPPH was examined using a UV–Vis spectrophotometer V-670 (Jasco Inc, Japan) at 515 nm, the radical cation scavenging activity of ABTS^{+} was examined at 734 nm. The results were expressed as % for DPPH and ABTS^{+}. Tests were carried out by triplicate for each formulation.

TBARS values in the cheese samples were determined by a spectrophotometric method (Lee, Yang, & Song, 2016). Cheese samples (2 g) were mixed with 10 mL of 75 g/L trichloroacetic acid. After filtration trough Whatman nº1 filter paper, 5 ml of thiobarbituric acid (TBA) 0.02 M was added to 5 mL of the mixture. The solution was incubated at 95 °C in a hot water bath for 45 min and then cooled. After the centrifugation, the absorbance was measured at 539 nm. The analysis was carried out by triplicate. The TBARS value was represented as mg malondialdehyde (MDA)/kg cheese.

Color analysis (measuring L*, a*, and b* values) was performed on cheese samples and film separators at 0, 15, 30, and 45 days of storage at 5 °C. ColorStriker colorimeter (Mathai, Hannover, Germany) was used, and two measurements were performed for each film formulation and cheese sample.

2.6. Thermal (TGA/DSC) properties of films

The thermal stability behaviour of the films was examined using differential scanning calorimetry and TGA/DSC thermogravimetry equipment (Metter Toledo, Switzerland). The test involved heating the samples at a rate of 10 °C/min from 50 to 400 °C in a nitrogen environment (50 mL/min). The samples were placed in hermetic aluminium pans.

2.7. Statistical analysis

Statistical analysis of the results was conducted using Microsoft Excel® software through one-way analysis of variance (ANOVA). To identify differences between the results with a certain level of confidence, the Tukey Post Hoc test was performed. The significance level was set at $p < 0.05$, indicating a statistically significant difference. Additionally, the obtained results were further analysed using Design Expert 11® software (Stat-Ease, Minneapolis, MN, USA). The experimental design involved the independent variables of glycerol and nettle extract content, denoted as A and B, respectively. The impact of glycerol and nettle extract content on the dependent variables was calculated and evaluated using a complete factorial design.

3. Results and discussion

3.1. Evaluation of the mechanical properties of the slice separators

The incorporation of extracts into chitosan matrices or other polysaccharides often exhibits a significant influence on the mechanical properties of the resulting films. The specific impact of these extracts is contingent upon their composition, the final concentration achieved in the chitosan solution, and the particular interactions transpiring within the polymer matrix (Flórez et al., 2022). In this case, nettle extract may influence the mechanical properties of the films due to the interaction with proteins and lipids present in the extracts (J. Li, Miao, Wu, Chen, & Zhang, 2014; Tan, Lim, Tay, Lee, & Thian, 2015). Nettle is a plant characterised by a contribution mainly of carbohydrates (74.9 g/kg),

followed by proteins (27.1 g/kg) and fats (1.1 g/kg) (Jan, zarafshan, & Singh, 2017). The analysis of variance determined that nettle extract and glycerol had a significant effect ($p < 0.05$) on the tensile parameters analysed (Table S1).

The tensile test for Ch_NE film showed values in the range 1.81–58.02 MPa for TS (Table 1). Data fitted well to a quadratic model. The F-value of 78.15 and *p*-value of 0.0022 indicated that the model was significant. The p-values showed that only the quadratic effect of glycerol (A^2) had a significant effect on TS with a F-value of 30.04. The linear effect of the glycerol and nettle extract concentration, followed by the quadratic effect of nettle extract had a negligible effect on the TS, as shown by their p-values and F-values. The fit statistics values indicated a r^2 of 0.99. The predicted r^2 value of 0.93 and the adjusted r^2 value of 0.98 were in reasonable agreement and the adequate precision was 23.88 (Table S2).

Equation (1) forecasts the TS values of the chitosan films as a function of the glycerol and nettle extract concentrations. Fig. 1A shows the prediction of the model for TS given by equation (1).

$$TS = 60.09 - 19.904 \cdot Glycerol\ (g/L) - 0.062 \cdot NE\ (mL/L) + 0.0141 \cdot Glycerol\ (g/L) \cdot NE\ (mL/L) + 1.8776 \cdot Glycerol^2\ (g/L) - 0.0001 \cdot NE^2\ (mL/L) \quad (1)$$

Fig. 1A shows the strong decrease in the TS values by the addition of glycerol. However, the content of nettle extract in the matrix did not cause a great difference between the samples with glycerol. When glycerol is not presented, TS is reduced by the increase in the nettle extract concentration. The behaviour of the films could be related to the reduction of the intermolecular interactions between the chitosan chains when nettle extract is present (Mir, Dar, Wani, & Shah, 2018). The presence of flavonoids and phenolic acid in the extract produced interactions that lead to weakening of the intermolecular interactions between the chitosan chains (Corrales, Han, & Tauscher, 2009; Đurović et al., 2017).

The analysis of the elongation of the films showed %E values ranged from 3.15 to 25.22 %. Data fitted well to a linear model (Table S1). The F-value of the model was 8.17 and the *p*-value was 0.0193, meaning that the model was significant. Regarding the terms of the model, only the glycerol concentration (*p*-value = 0.0070) had a statistically significant effect. According to the mathematical predictive model, nettle extract did not affect to the elongation of the films. The fit statistics values were r^2 of 0.73, predicted r^2 of 0.53 and adjusted r^2 of 0.64. The values of predicted and adjusted r^2 were in reasonable agreement, whereas the adequate precision (6.48) indicated and adequate signal (Table S2). The elongation of the Ch_NE films can be predicted using Equation (2). The prediction of the model is shown in Fig. 1B.

$$\%E = 4.66 + 3.674 \cdot Glycerol\ (g/L) + 0.010 \cdot NE\ (mL/L) \quad (2)$$

Table 1
Tensile properties of chitosan films. Ch is chitosan; Gly is glycerol; NE is nettle extract; TS is tensile strength; %E is elongation at break and YM is Young's modulus.

Film samples	TS	%E	YM
	MPa	%	MPa
Ch_Gly0	58.02 ± 7.26 ^{ab}	3.43 ± 0.90 ^{a}	1535.92 ± 121.43 ^{a}
Ch_Gly0_NE125	52.99 ± 1.84 ^{a}	3.15 ± 0.19 ^{a}	1445.90 ± 158.28 ^{a}
Ch_Gly0_NE250	35.13 ± 4.07 ^{b}	4.90 ± 2.25 ^{a}	948.68 ± 106.63 ^{b}
Ch_Gly02.5	24.66 ± 0.99 ^{c}	11.71 ± 1.50 ^{b}	810.81 ± 121.22 ^{c}
Ch_Gly2.5_NE125	13.93 ± 1.23 ^{d}	25.20 ± 4.31 ^{bc}	140.62 ± 41.51 ^{d}
Ch_Gly2.5_NE250	6.76 ± 0.84 ^{e}	21.64 ± 4.44 ^{c}	26.24 ± 5.10 ^{d}
Ch_Gly5	6.98 ± 1.91 ^{e}	25.22 ± 5.73 ^{c}	18.92 ± 1.38 ^{d}
Ch_Gly5_ NE125	6.18 ± 0.43 ^{e}	19.57 ± 1.55 ^{c}	51.08 ± 9.49 ^{d}
Ch_Gly5_ NE250	1.81 ± 0.09 ^{e}	21.81 ± 0.99 ^{c}	4.43 ± 0.67 ^{d}

Ch is chitosan 10 g/L in acetic acid 1 g/L; Gly is glycerol; NE is nettle extract. The number in the film sample shows the concentration in g/L for glycerol and mL/L for nettle extract. Values are expressed as mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).

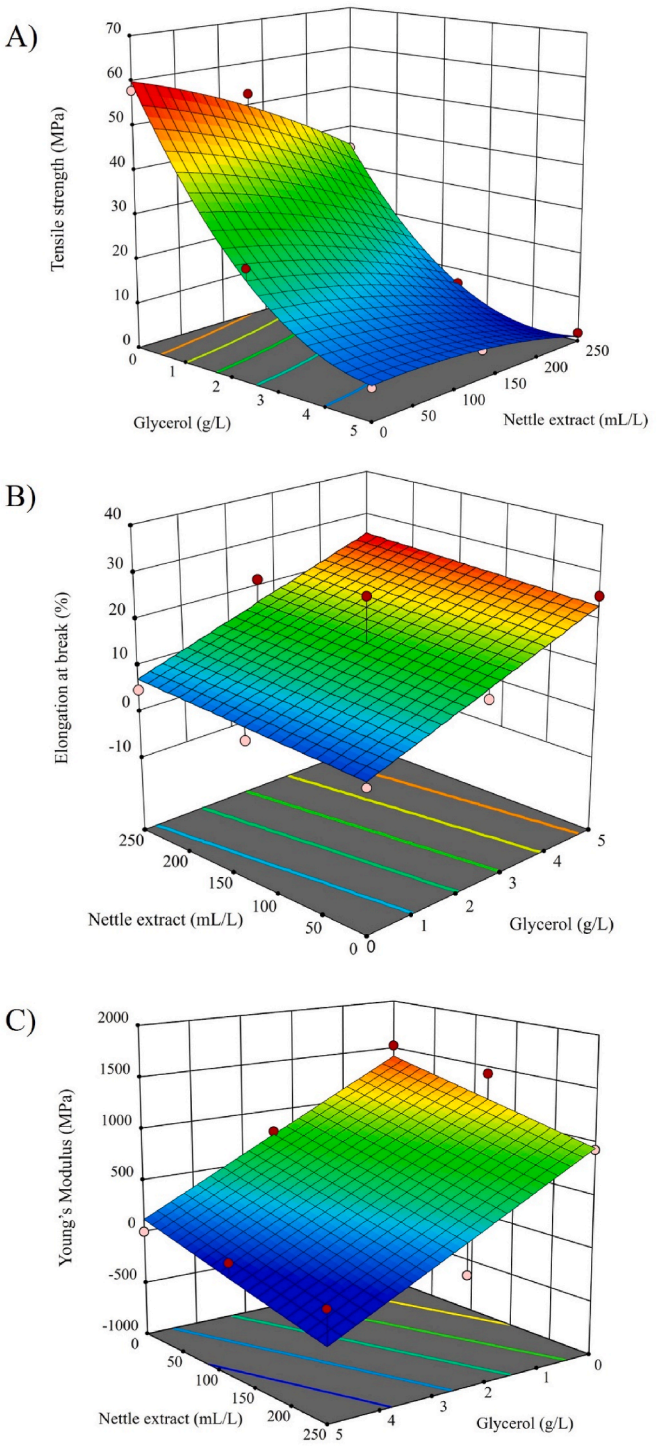

Fig. 1. Prediction of the models for: A) Tensile strength (MPa), B) Elongation at break (%), C) Young's Modulus (MPa).

The response surface revealed that the increase in the glycerol concentration increased the elongation of the films. However, at low glycerol concentrations, the plasticizing effect of glycerol was enhanced by the presence of nettle extract, doubling the %E values of the compound. Note that this way, the glycerol-nettle extract interaction resulted in similar %E values than those for composites with 5 g/L glycerol. When considering the TS and %E values together, it can be inferred that at low glycerol concentrations, the presence of polyphenols (such as luteolin, gallic acid, gentisic acid) and polysaccharides like pectin from nettle extract reduces polymer chain interactions. This leads to a decrease in

TS values and an increase in %E values. However, at higher glycerol concentrations, the film's resistance to breakdown significantly diminishes, resulting in limitations to the %E values (Cegledi, Garofulić, Zorić, Roje, & Dragović-Uzelac, 2022; Jan et al., 2017).

The calculated Young's modulus (YM) values of the films ranged from 4.43 to 1535.92 MPa. Data fitted well using a natural logarithmic equation, as recommended the Box-Cox plot. The statistical analysis indicated that the model had an F-value of 23.59 and a *p*-value of 0.0014, implying the lineal model was significant. Regarding to the p-values, the glycerol and the nettle extract concentration were significant. The F-values showed that the effect of glycerol content (F-value = 40.40) was higher that the effect of nettle extract on the YM of chitosan films. Equation (3) predicts the YM values, and the prediction of the model is shown in Fig. 1C.

$$\text{Ln (YM)} = 3.49\text{–}0.379 \cdot \textit{Glycerol}\ \text{(g/L)} - 0.003 \cdot \textit{NE}\ \text{(mL/L)} \quad (3)$$

Based on these results, it can be determined that glycerol manifested a normal plasticizer effect with a drastic reduction of TS and YM and the increase of %E (Dicharry et al., 2006). Ch_NE films acquire a less rigid structure by reducing the cohesion of the forces that constitute the polymeric network (Flórez et al., 2022a). This plasticizing effect has been slightly enhanced by the presence of nettle extract, being more prominent at low glycerol concentrations and especially in the properties of TS and YM. Similar behaviour were observed in chitosan films with the addition of grapefruit seed extract (Tan et al., 2015) and *Berberis crataegina* fruit extract (Kaya, Khadem, et al., 2018).

Previously, other extracts with polyphenols showed similar plasticizing behaviour on chitosan films. For instance, extracts of *Pistacia terebinthus* (Kaya, Khadem, et al., 2018), extracts from purple and black rice (Yong et al., 2019), *Santalum album* essential oil (Flórez et al., 2022a) or anthocyanins from purple tomato (Y. Li, Wu, Wang, & Li, 2021) showed a plasticizing effect on chitosan-based films, resulting in films with higher %E and lower TS and YM values.

The resistance and elongation of the films under a puncture force deformation are revealed by the BS and DB parameters (Vázquez, Velazquez, & Cazón, 2021). Table 2 shows the results of mechanical properties obtained from the puncture tests. BS values ranged from 849 to 3465.61 g. Data fitted well to a quadratic model. The analysis of variance of the model (Table S3) showed a F-value of 2721.56 and p-value <0.0001. In this case, all the terms implied were significant terms ($p < 0.05$). Mainly, the glycerol concentration (F-value = 846.17) was the term that promoted alterations on the BS response, followed by interaction between the glycerol and the nettle extract content (F-value = 714.25). The r^2 value was 0.99 and the predicted r^2 (0.99) was in reasonable agreement with the adjusted r^2 (0.99). The ratio of the adequate precision (154.74) was very high, indicating an adequate signal (Table S4). Equation (4) predicts the BS parameter as a function of the glycerol and nettle extract concentrations on the chitosan films.

$$\text{BS} = 3457.82\text{–}202.631 \cdot \textit{Glycerol}\ \text{(g/L)} - 3.700 \cdot \textit{NE}\ \text{(mL/L)} + 0.8818 \cdot \textit{Glycerol}\ \text{(g/L)} \cdot \textit{NE}\ \text{(mL/L)} - 23.9584 \cdot \textit{Glycerol}^2\ \text{(g/L)} - 0.0187 \cdot \text{NE2}\ \textit{(mL/L)} \quad (4)$$

Fig. 2A shows the prediction of the model for BS. Both glycerol and nettle extract resulted in a decrease in BS values. The chitosan pure films (without glycerol or nettle extract) exhibited the highest BS value of 3465.61 g.

The DB values of the film samples under puncture forces ranged from 3.21 to 5.78 mm (Table 2). The F-value of the model was 9.99 and the r^2 was 0.04, which means that the model was significant. The p-values showed that only the quadratic term of glycerol (A^2) had a significant effect on DB. The value of r^2 was 0.94, the predicted r^2 was 0.34 and the adjusted r^2 was 0.84, therefore the values were not as close as might be expected and it is considered that there is no reasonable agreement between them. However, the ratio of adequate precision (8.57) indicated an adequate signal. Equation (5) predicts the DB response of the chitosan films as a function of the glycerol and nettle extract concentration.

Table 2
Puncture properties of chitosan films. Ch is chitosan; Gly is glycerol; NE is nettle extract; BS is burst strength and DB is distance to burst.

Film samples	BS	DB
	g	mm
Ch_Gly0	3465.61 ± 1564.82 ^{a}	3.21 ± 1.00 ^{a}
Ch_Gly0_NE125	2704.73 ± 428.40 ^{bc}	4.07 ± 0.19 ^{ab}
Ch_Gly0_NE250	1351.99 ± 338.48 ^{abc}	3.74 ± 0.89 ^{abc}
Ch_Gly02.5	2779.13 ± 838.50 ^{ab}	4.96 ± 0.73 ^{bcd}
Ch_Gly2.5_NE125	2331.10 ± 298.02 ^{bc}	5.78 ± 0.23 ^{cd}
Ch_Gly2.5_NE250	1269.89 ± 397.87 ^{abc}	5.59 ± 0.65 ^{d}
Ch_Gly5	1860.29 ± 228.03 ^{abc}	5.46 ± 0.26 ^{cd}
Ch_Gly5_ NE125	1630.18 ± 375.40 ^{c}	5.06 ± 0.16 ^{bcd}
Ch_Gly5_ NE250	849.00 ± 121.14 ^{abc}	5.07 ± 0.10 ^{bcd}

Ch is chitosan 10 g/L in acetic acid 1 g/L; Gly is glycerol; NE is nettle extract. The number in the film sample shows the concentration in g/L for glycerol and mL/L for nettle extract. Values are expressed as mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).

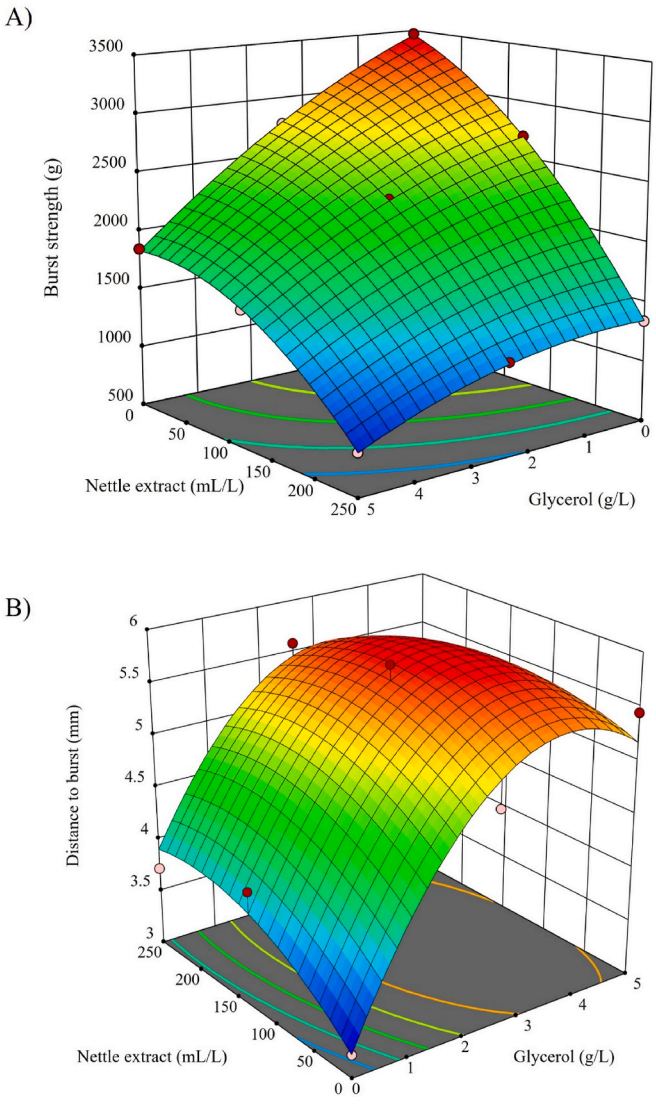

Fig. 2. Prediction of the models for puncture parameters: A) Burst strength (g) and B) Distance to burst (mm).

$$DB = 3.21 + 1.203 \cdot Glycerol\ (g/L) + 0.007 \cdot NE\ (mL/L) - 0.0007 \cdot Glycerol\ (g/L) \cdot NE\ (mL/L) - 0.1613 \cdot Glycerol^2\ (g/L) \quad (5)$$

Fig. 2B shows the prediction of the model for DB. The addition of glycerol to the matrix clearly increased the DB values. It was 3.21 mm for films without glycerol and increased to 5.46 mm for films with the highest concentration of glycerol (5 g/L). In general, the incorporation of glycerol and nettle extract resulted in a decrease in BS and an increase in DB. The impact and trend observed in the tensile test parameters were also observed in the puncture test, demonstrating a significant effect ($p < 0.05$). Glycerol at the concentrations evaluated showed a clear

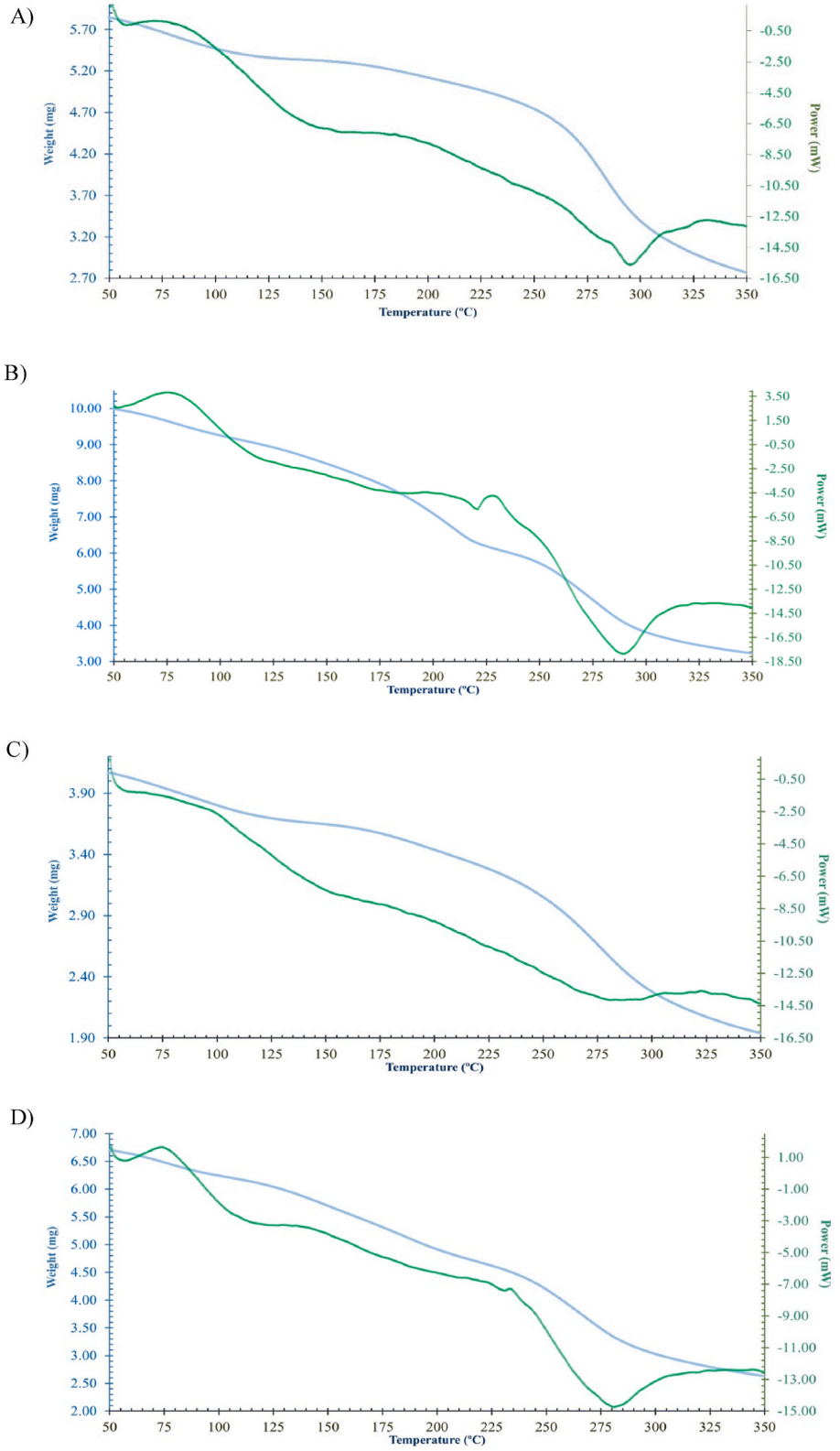

Fig. 3. Thermogravimetry and differential scanning calorimetry of chitosan films at different concentration of glycerol and nettle extract: A) 10 g/L chitosan; B) 10 g/L chitosan and 5 g/L glycerol; C) 10 g/L chitosan and 250 mL/L nettle extract; D) 10 g/L chitosan, 250 mL/L nettle extract and 5 g/L glycerol.

plasticizing behaviour, well known in combination with other polysaccharides (Cazon, Vázquez, & Velazquez, 2019; Leceta, Guerrero, & De La Caba, 2013; Rivero, Damonte, Garcia, & Pinotti, 2016). Glycerol acted as an internal lubricant, easing the mobility of the polymeric chains by reducing friction forces between chitosan chains. Besides, like glycerol, the presence of the nettle extract reduced the chitosan-chitosan interactions, to form new, probably weaker interactions or disrupted the interactions between the polymer, resulting in a reduction of BS and increase of DB values.

3.2. Evaluation of the thermal properties (TGA/DSC)

The Ch_NE films were evaluated by TGA/DSC for study the effects of the nettle extract and glycerol on the thermal physical property. Fig. 3 shows the thermograms of chitosan films, pure or enriched with nettle extract up to 250 mL/L and with glycerol from 0 g/L to 5 g/L.

Thermogravimetric curves showed the degradation in three main steps. The first step was observed at temperatures between 50 and 150 °C, where the mass loss can be attributed to the water evaporation (10–15% weight loss for each film) (Kaya et al., 2018a). The second step, which was between 150 and 285 °C, it is attributed to a weight loss related to the evaporation of glycerol (Dou, Dupont, Williams, Chen, & Ding, 2009). Mass loss in this stage was close to the weight percentage of glycerol in the composition. For this reason, the curve related to loss mass was more pronounced in those films with a glycerol concentration of 5 g/L. The greatest weight loss occurred between 240 and 350 °C due to the denaturation of the polymeric organization of the chitosan and nettle extract (Zawadzki & Kaczmarek, 2010).

As can be seen in Fig. 3, the weight loss followed a very similar trend in both pure chitosan films and those with nettle extract added to the composition. At 275 °C, the total weight losses of the samples increased with the glycerol concentration, being 25.90% for Ch_Gly0, 51.28% for Ch_Gly5, 32.98% for Ch_Gly0_NE250 and 46.59% for Ch_Gly5_NE250 samples. Similar behaviour was observed in a previous report on the production of chitosan films with grapefruit seed extract where no alterations in the thermal stability of the film were reported (Tan et al., 2015).

Chitosan films exhibited endothermic peaks between 75 and 295 °C. All the films samples have two peaks at 75 °C and 140-150 °C, respectively, indicating the volatilization of gas substances and the structure loosing of chitosan chains (Zhang, Lian, Shi, Meng, & Peng, 2020). This temperature is lower than the value of 205 °C for pure chitosan previously reported by Sakurai, Maegawa, and Takahashi (2000). This was attributed to the effects of plasticizer in the films (Mathew, Brahmakumar, & Abraham, 2007). The 295 °C endothermic peak corresponds to the phase inversion temperature for the breakdown of chitosan. However, the phase inversion temperature was reduced with the addition of nettle extract, dropping to 275 °C, which leads to a slightly decrease in thermal stability (Park & Zhao, 2004).

From the results obtained, it can be determined that the interactions generated between chitosan and nettle extract (from 0 to 250 mL/L) slightly altered the thermal stability of the films. Moreover, the stability of the films was not altered at 120 °C, which allows their application in thermal food processes carried out at sterilization temperatures. Similar trend was observed in chitosan-based films enriched with other extracts with polyphenols such as green or black tea extracts (Peng, Wu, & Li, 2013) or *Santalum albu*m essential oil (Flórez et al., 2022a) at low concentrations.

3.3. Antioxidant stability of Havarti cheese with active slice separators

Havarti-type cheeses typically contain 300–450 g/kg fat. The fat content of the cheese used for this study was 380 g/kg. The antioxidant properties of the Ch_NE films as slice separators for Havarti cheese slices were tested for 45 days. Fig. 4 shows the cheese samples sealed in vacuum PA/PE bags using different film formulations as slice separators in the first day of storage.

The DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ values of the developed slice separators was measured throughout the test time (45 days) to evaluate the stability of the antioxidant capacity of the films (Table 3).

The films used as control (PA/PE films), obtained residual DPPH$^{\bullet}$ values from 4.13 to 7.63 %, and ABTS$^{\bullet+}$ values from 4.63 to 10.10 %. The PA/PE film could not be considered to have a relevant antioxidant capacity since the very low values observed. Similar results were found for pure chitosan films, showing DPPH$^{\bullet}$ values ranging from 2.84 to 7.85 %, and ABTS$^{\bullet+}$ values from 7.01 to 14.40 %. However, chitosan films with 181 g/L of nettle extract showed an extraordinary antioxidant capacity over time. After 45 days, Ch_NE samples showed a DPPH$^{\bullet}$ radical scavenging ability of 93.22% and 95.40% for ABTS$^{\bullet+}$ radical. A significant increase ($p < 0.05$) was observed in both parameters comparing with PA/PE or chitosan films. Hence, results indicated the stability of the antioxidant capacity of the films over time for the 45-day test period.

On the other hand, the antioxidant capacity of the cheese samples was evaluated to determine a potential migration of the antioxidant compounds from the polymeric matrix to the food. The cheese packaged with PA/PE films as slice separators showed non-significant differences in terms of DPPH$^{\bullet}$ values ranging from 4.32 to 6.76%. However, ABTS$^{\bullet+}$ values ranged from 8.23 to 11.70% showed significant statistically differences ($p < 0.05$) between the value of the initial time and the rest of the measurements. However, considering the results, note that the values for the control sample and the pure chitosan sample remained in the same range, being very low values with an antioxidant capacity that can be negligible. These values may be due to the variations or test conditions that occur due to the indirect method used.

However, the cheese samples separated with Ch_NE films showed higher antioxidant values compared to samples in contact with control or chitosan films. DPPH$^{\bullet}$ values ranged from 11.79 to 12.55 %, while ABTS$^{\bullet+}$ values showed a significant difference ($p < 0.05$) ranging from

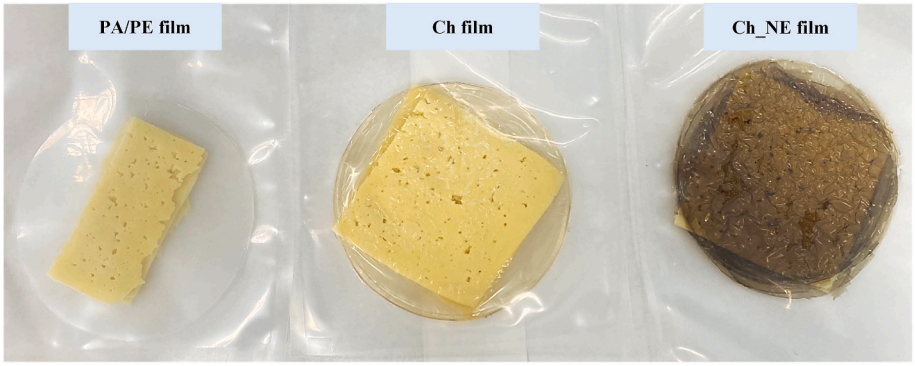

Fig. 4. Havarti cheese with slice separator of polyamide/polyethylene (PA/PE) film, pure chitosan (Ch) film (10 g/L) and chitosan films enriched with nettle extract (181 mL/L) and glycerol (1 g/L) (Ch_NE), sealed in vacuum PA/PE bags and stored at 5 °C for 45 days.

Table 3
Antioxidant properties measured in the films used as slice separators and the packaged Havarti cheese samples for the storage at 5 °C. PA/PE is polyamide/polyethylene, Ch is pure chitosan (1% w/w) and Ch_NE is chitosan (10 g/L) enriched with nettle extract (181 mL/L) and glycerol (1% g/L).

	Storage time (days)	Film samples		
		PA/PE	Ch	Ch_NE
DPPH•	0	4.65 ± 1.60 ^{a}	3.49 ± 0.29 ^{a}	84.05 ± 0.25 ^{a}
	15	4.13 ± 1.54 ^{a}	7.85 ± 6.89 ^{a}	93.93 ± 0.00 ^{b}
	30	7.63 ± 0.36 ^{a}	6.60 ± 1.55 ^{a}	93.00 ± 0.14 ^{c}
	45	4.96 ± 2.88 ^{a}	2.84 ± 1.23 ^{a}	93.22 ± 0.49 ^{c}
ABTS•+ (%)	0	4.63 ± 0.76 ^{a}	7.01 ± 0.59 ^{a}	98.05 ± 1.04 ^{a}
	15	5.58 ± 0.93 ^{a}	13.82 ± 0.18 ^{b}	99.39 ± 0.10 ^{ab}
	30	9.10 ± 0.37 ^{b}	14.18 ± 0.65 ^{b}	99.52 ± 0.10 ^{b}
	45	10.10 ± 1.79 ^{b}	14.40 ± 3.30 ^{b}	95.40 ± 0.38 ^{c}
	Storage time (days)	**Cheese samples**		
		PA/PE	Ch	Ch_NE
DPPH• (%)	0	4.32 ± 1.00 ^{a}	5.73 ± 3.44 ^{a}	11.79 ± 1.56 ^{a}
	15	5.67 ± 2.04 ^{a}	7.77 ± 1.58 ^{a}	12.55 ± 5.17 ^{a}
	30	6.76 ± 0.54 ^{a}	7.94 ± 1.52 ^{a}	12.34 ± 0.72 ^{a}
	45	6.54 ± 0.55 ^{a}	7.72 ± 1.52 ^{a}	12.14 ± 0.72 ^{a}
ABTS•+ (%)	0	11.70 ± 1.28 ^{a}	12.55 ± 8.14 ^{a}	23.52 ± 0.64 ^{a}
	15	8.67 ± 0.21 ^{b}	12.85 ± 0.52 ^{a}	19.52 ± 4.40 ^{b}
	30	9.16 ± 0.54 ^{b}	9.87 ± 0.31 ^{a}	27.05 ± 0.68 ^{c}
	45	8.23 ± 0.46 ^{b}	9.74 ± 0.69 ^{a}	26.80 ± 0.28 ^{c}

PA/PE – Polyamide/Polyethylene.
Values are expressed as mean ± standard deviation (SD).
Different letters in the same column and antioxidant method indicate significant differences ($p < 0.05$).

19.52 to 27.05 %, increasing with the time. This could be attributed to the fact that part of the antioxidant compounds migrated from the film to the food. This outcome aligns with prior studies that demonstrated an enhancement in the antioxidant capacity of Kareish cheese when packaged with a chitosan/gelatin coating and enriched with papaya leaves and thyme (Hassan, Korany, Zeinhom, Mohamed, & Abdel-Atty, 2022).

3.4. Lipid oxidation of Havarti cheese packaged with active slice separators

The TBARS method is used to measure and quantify lipid peroxidation in a food or other biological sample. Lipid peroxidation occurs when lipids like fatty acids are degraded due to the presence of reactive oxygen species such as free radicals. The degradation of these lipidic compound may have a significant effect on the quality and safety of a food product. TBARS methos is one of the most widespread methods for determining lipid peroxidation because it is considered a reliable and accurate method. The analysis quantifies a substance known as malonaldehyde (MDA), a breakdown product of endoperoxides resulting from the oxidation of unsaturated fatty acids (Lyn et al., 2021), giving a ratio of lipid oxidation as mg MDA per kg of sample analysed. Fig. 5 shows the measured mg MDA/kg of Havarti cheese over 45 days under refrigeration as a function of the separators: PA/PE, pure chitosan or Ch_NE films. TBARS values increased in samples with pure chitosan and Ch_NE films as separators until day 15 of storage time while samples with slice separators of PA/PE increased until day 30. The increase in these values during storage may be an indicator that lipids are continuously being oxidized, producing oxidative by-products as a result (Abdou, Galhoum, & Mohamed, 2018). TBARS values for Havarti cheese separated with PA/PE films ranged from 5.87 to 11.57 mg MDA/kg while cheese samples separated with pure chitosan films showed values from 7.50 to 15.39 mg MDA/kg cheese at day 15. These variations throughout the study may be due to the fact that the films do not provide a complete barrier against lipid oxidation.

Regarding the Ch_NE film, the TBARS values achieved a maximum of 12.05 mg MDA/kg cheese at 15 day and then achieved the lowest TBARS value (3.38 mg MDA/kg cheese) at the end of the study (45 day). This implied a decrease of a 56% in the lipid oxidation using the slice separators of Ch_NE films compared with slice separators of PA/PE or pure chitosan films.

Note that TBARS assay has certain limitations since it only measures substances reactive to TBA, which may be degraded during the lipid

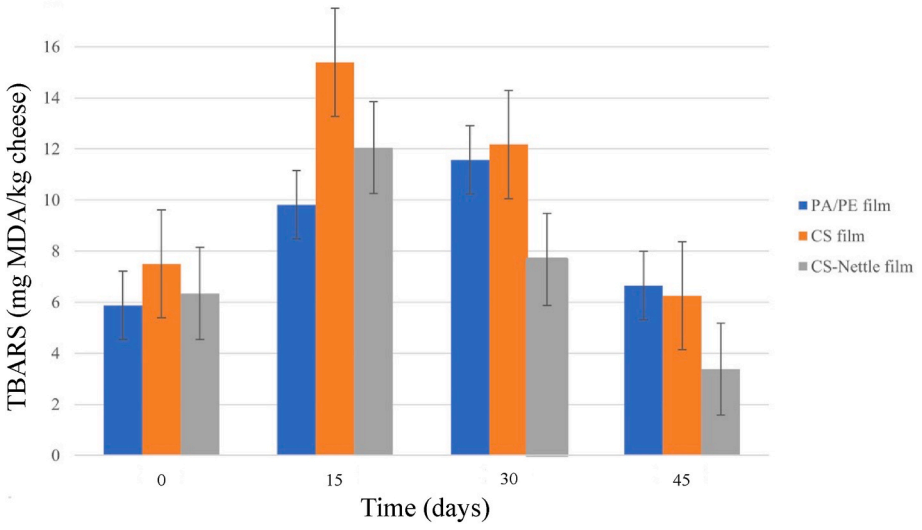

Fig. 5. Variations in TBARS values with the time for Havarti cheese with slice separators of different film compositions: polyamide/polyethylene (PA/PE) film, pure chitosan (Ch) film (10 g/L) and chitosan films enriched with nettle extract (181 mL/L) and glycerol (1% g/L) (Ch_NE).

oxidation process. The increase of MDA content in the packaged cheese slices at 15 days suggested that the oxidative state of the slices reached the peak of oxidation (Andrade, Ribeiro-Santos, Guerra, & Sanches-Silva, 2019).

On the other hand, cheese separated by pure chitosan films showed a lower antioxidant activity compared to Ch_NE films. Hence, the addition of antioxidant compounds to the chitosan solution significantly reduced the oxidation of lipids present in the food (Farsanipour, Khodanazary, & Hosseini, 2020). Based on these results, Havarti cheese slices' TBARS values were impacted by storage and packaging material. Similar results were observed when a gelatin-based film enriched with *Lepidium Sativum* extract showed lower TBARS values compared to the unpackaged Ricotta cheese sample (Salem et al., 2021). Likewise, white cheese was packaged with foxtail millet starch and clover leaf oil. In this case, the TBARS values of the cheese samples were the lowest in comparison with control film. It was attributed to the fact that phenolic compounds present in the matrix can inhibit peroxide production by scavenging free radicals from the cheese (Yang, Cao, Kim, Beak, & Song, 2018).

3.5. Colour properties of Havarti cheese and films used as separators

The colour of the films and the packaged cheese were evaluated during the test time through the CIE coordinates (Table 4). Therefore, the films colour variations by the storage time and the cheese colour alteration due to possible migration of coloured components from the polymer matrix to the cheese were analysed.

Significant differences ($p < 0.05$) were observed for all values and samples analysed. In the case of the PA/PE film, the lightness (L*) ranged from 77.16 to 83.13, while the values of a* (redness parameter) ranged from 1.29 to −0.48. The findings suggest that there was an inherent proclivity towards darker tones, while the film exhibited a gradual propensity towards a slightly greener colour palette as the time progressed. Regarding the b* values (yellowness parameter), the film started with a value of 5.58 and decreased to 1.39 on the last day of the study. This means that there was a slight decrease in the yellowing of the film throughout the study, probably attributed to the presence of traces of cheese and fat in the film.

This behaviour was similar for the chitosan films. L* ranged from 81.97 to 84.38, while a* values ranged from 1.10 to −0.45. The b* values as well decreased with time, ranging from 8.26 to 3.92. In the case of the Ch_NE films, the L* values were lower than in the other films, ranging from 63.42 to 70.44. This was consistent with the fact that film was much darker than the other films used as separators. The values of a* and b* decreased over time, ranging from 4.71 to 1.08 for a* values and from 21.02 to 10.89 for b* values. This implied that the presence of the nettle extract showed an impact on the film colour, leading to a discernible shift towards a subtle greenish tint, accompanied by a reduction in the yellowing process observed throughout the duration of the test.

Regarding the chromatic parameters of the cheese (Table 5), the samples packaged with PA/PE separators presented values at day 0 of L*, a* and b* of 80.03, −0.43 and 22.95, respectively. Over time, significant differences ($p < 0.05$) were observed in the cheese, mainly in the L* and a* coordinates being 79.64 and −1.10, respectively on the last day of the study (day 45). These chromatic changes resulted in the darkening of the cheese. The cheese samples packaged with chitosan pure film used as separators showed in the first day L* value of 74.69, a* value of −1.27 and b* value of 25.31. In the last day of the study, the L* value increased to 79.13, a* values decreased slightly to −1.16 and b* values decreased to 20.89. Hence, the cheese samples turned greyish (increased clarity and decreased redness) as the storage period increased. Probably, the lipid oxidation together with other degradations that the cheese underwent during the storage time, resulted in the alterations of the colour of the sample (Siripatrawan & Noipha, 2012).

On the other hand, cheese samples that were separated by Ch_NE films suffered a significant decrease ($p < 0.05$) in all coordinates. The brightness varied from 84.41 to 71.12. The a* value decreased very slightly from −1.53 to −1.18, which means that there is a slight decrease in the cheese sample with respect to greenness with the time. Whereas the b* values ranged from 31.42 to 18.54, this means that the samples lose yellowish colour, intimately related to the ability of the film to darken the sample.

4. Conclusions

In this work, an active biodegradable film based on chitosan and nettle extract has been successfully developed and tested as a separator for sliced cheeses. For the first time, nettle extract with potent antioxidant properties has been incorporated into a biodegradable matrix to extend the shelf life of high-fat foods by retarding lipid oxidation. The results of the TBARS analysis concluded that the application of slide separators developed with chitosan and nettle extract decreased a 56% the lipid oxidation of Havarti cheese. The films enriched with nettle

Table 4
Color parameters of the chitosan-based films used as slice separators throughout the storage study at 5 °C for 45 days. PA/PE is polyamide/polyethylene, Ch is pure chitosan (1% w/w) and Ch_NE is chitosan (10 g/L) enriched with nettle extract (181 mL/L) and glycerol (1% g/L).

	Storage time (days)	Film samples		
		PA/PE	Ch	Ch_NE
L*	0	81.50 ± 0.01 ^{a}	82.90 ± 0.03 ^{a}	67.11 ± 2.89 ^{a}
	15	83.13 ± 0.03 ^{a}	84.38 ± 0.03 ^{b}	63.42 ± 0.36 ^{a}
	30	77.16 ± 0.01 ^{b}	82.49 ± 0.08 ^{ac}	64.38 ± 0.18 ^{a}
	45	81.97 ± 1.32 ^{ac}	81.97 ± 0.64 ^{ac}	70.44 ± 0.08 ^{ab}
a*	0	1.29 ± 0.04 ^{a}	1.10 ± 0.01 ^{a}	4.72 ± 0.66 ^{a}
	15	−0.48 ± 0.04 ^{b}	−0.37 ± 0.06 ^{b}	2.53 ± 0.08 ^{b}
	30	−0.61 ± 0.24 ^{bc}	−0.45 ± 0.09 ^{bc}	1.93 ± 0.09 ^{bc}
	45	−0.26 ± 0.21 ^{bc}	−0.36 ± 0.03 ^{bc}	1.08 ± 0.03 ^{bd}
b*	0	5.58 ± 0.01 ^{a}	8.26 ± 0.01 ^{a}	21.02 ± 3.44 ^{a}
	15	1.93 ± 0.03 ^{b}	4.72 ± 0.05 ^{b}	16.98 ± 0.18 ^{a}
	30	2.84 ± 0.06 ^{c}	5.81 ± 0.10 ^{c}	16.99 ± 0.17 ^{a}
	45	1.39 ± 0.21 ^{d}	3.92 ± 0.20 ^{d}	10.89 ± 0.09 ^{bc}

L*. Lightness: black = 0 and white = 100; a*. Green = -a* and red = +a*); b*. Blue = -b* and yellow = +b*.
Values are expressed as mean ± standard deviation (SD).
Different letters in the same column indicate significant differences ($p < 0.05$).

Table 5
Color parameters of the cheese samples throughout the storage study at 5 °C for 45 days. PA/PE is polyamide/polyethylene, Ch is pure chitosan (1% w/w) and Ch_NE is chitosan (10 g/L) enriched with nettle extract (181 mL/L) and glycerol (1% g/L).

	Storage time (days)	Cheese samples		
		PA/PE	Ch	Ch_NE
L*	0	80.03 ± 0.10 ^{a}	74.69 ± 0.26 ^{a}	84.41 ± 0.13 ^{a}
	15	80.59 ± 0.19 ^{b}	81.95 ± 0.78 ^{b}	75.80 ± 0.57 ^{b}
	30	80.26 ± 0.01 ^{a}	80.77 ± 0.06 ^{bc}	76.84 ± 0.07 ^{bc}
	45	79.64 ± 0.04 ^{ac}	79.13 ± 0.13 ^{bd}	71.12 ± 0.03 ^{d}
a*	0	−0.43 ± 0.04 ^{a}	−1.27 ± 0.10 ^{a}	−1.53 ± 0.18 ^{a}
	15	−1.38 ± 0.25 ^{b}	−1.52 ± 0.03 ^{a}	−0.98 ± 0.03 ^{b}
	30	−0.87 ± 0.06 ^{a}	−1.96 ± 0.02 ^{b}	−1.18 ± 0.12 ^{a}
	45	−1.10 ± 0.05 ^{bc}	−1.16 ± 0.11 ^{abc}	−1.18 ± 0.12 ^{a}
b*	0	22.95 ± 0.78 ^{ab}	25.31 ± 0.16 ^{a}	31.42 ± 0.31 ^{a}
	15	22.30 ± 0.14 ^{a}	26.68 ± 0.02 ^{b}	30.40 ± 0.28 ^{b}
	30	23.74 ± 0.03 ^{ab}	25.20 ± 0.11 ^{a}	27.43 ± 0.03 ^{c}
	45	24.05 ± 0.06 ^{b}	20.89 ± 0.08 ^{c}	18.54 ± 0.02 ^{d}

L*. Lightness: black = 0 and white = 100; a*. Green = -a* and red = +a*); b*. Blue = -b* and yellow = +b*.
Values are expressed as mean ± standard deviation (SD).
Different letters in the same column indicate significant differences ($p < 0.05$).

extract demonstrated consistent high antioxidant capacity even after 45 days of storage. Additionally, these films exhibited favorable properties such as tensile strength, puncture resistance, and elongation, making them easy to apply and handle as slicing separators. This can be attributed to the presence of glycerol as a plasticizer. Furthermore, the thermal properties of the films indicated their stability at temperatures commonly encountered during food heat processing. As a result, an environmentally friendly alternative to synthetic and non-biodegradable slicing separators has been successfully developed using chitosan, a by-product of the fish industry, and enriched with an extract from nettle, a widely distributed wild plant.

Ethical statement

Not Applicable. This research work does not carry out human or animal trials experiment.

CRediT authorship contribution statement

María Flórez: Investigation, Writing – original draft. **Manuel Vázquez:** Conceptualization, Methodology, Writing – review & editing. **Patricia Cazón:** Investigation, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.115504.

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