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Optimization of Corn Starch- Natural Deep Eutectic Solvent based Novel Edible Film Formulation Using RSM

Esther Magdalene Sharon M.* and Geetha P.
Assistant Professor, College of Food and Dairy Technology,
Tamil Nadu Veterinary and Animal Sciences University, Chennai (Tamil Nadu), India.

(Corresponding author: Esther Magdalene Sharon M.)*
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ABSTRACT: Packaging protects and preserves food from the time it is packaged to consumption. Manufacturers often prefer synthetic, non-biodegradable polymer materials for packaging due to their strength, durability, and water vapor barrier properties. However, these conventional plastics, like polyethylene and polystyrene, resist biological degradation, leading to environmental concerns. Therefore, there is a growing need for eco-friendly, biodegradable alternatives. Biodegradable packaging made from natural biopolymers such as polysaccharides, proteins, and lipids can meet packaging requirements while being renewable, recyclable, and environmentally friendly. In particular, edible films and coatings serve as barriers to gas and moisture, extending food shelf life to reduce quality deterioration. This study focuses on optimizing the inclusion level of corn starch (10% - 14%) along with Natural Deep Eutectic Solvents (NADES) at different pH ranges (2.3 - 2.8) in edible film formulation using Response Surface Methodology (RSM). The films were evaluated based on physical and mechanical properties, such as thickness, tensile strength, grammage, water absorption, and water vapor permeability. The optimum formulation of edible films using NADES (Lactic acid – Glucose) for meeting the set criteria of response functions was corn starch concentration of 14% and a pH 3.

Keywords: Edible film, Corn starch, NADES, Lactic acid – Glucose.

INTRODUCTION

The main aim of food packaging is to protect the food's quality and other safety factors from the manufacturing stage to when it is consumed by the consumer. Moreover, the packing should also prevent any harm caused to the goods due to impact, chemical agents, or multiple biological factors. In order to achieve those goals, the common alternatives are synthetic materials like polyethylene or other plastic materials derived from petrochemicals due to their low cost, flexibility, and availability. The improper disposal or recycling of waste materials is one of the primary disadvantages of the conventional alternatives. However, little plastic waste is collected and put back into productive use, the greater part of it is littered. The materials made from synthetic polymers do not decompose for ages if ever. Therefore, these forms of packing substances when used in soils and dumpsites cause environmental pollution. There is significant research interest in using cost-effective, biodegradable, and recyclable natural sources for food packaging.

Interest in employing edible films has increased due to their potential in the food industry and their environmentally friendly impact. The food-grade edible packaging materials are composed of naturally occurring polymers like starch, cellulose, chitosan, proteins (either animal or plant based) and lipids (also of animal or plant origins), and microbial-based polymers (Krochta and Mulder-Johnston 1997). Biodegradable packaging materials are being formulated according to requirements and these can be consumed along with the food. "An edible film or coating is defined as a thin continuous layer of edible material formed or placed on or between the layer of foods or food components" (Torres, 1994).

Corn starch. Edible films developed from starch blend are isotropic, odorless, tasteless, colorless, non toxic and biologically degradable. Films from starch have good flexibility and low water permeability, decomposability, recyclability, superior oxygen barrier properties indicating potential application as edible films (Guilbert *et al.*, 1996; Parra *et al.*, 2004).

But films prepared from pure starch are brittle. Hence, the films properties such as sensory, functional and mechanical properties could be customized by the incorporation of additives in smaller amounts. The addition of plasticizer in the starch based film formulation is essential to fabricate good flexible starch films without any brittleness. The addition of an appropriate plasticizing agent to edible films reduces their brittleness and improves the flexibility and extensibility by acting as spacers between polymer chains and decreasing the intermolecular forces between adjacent polymeric chains.

NADES. The term "Natural Deep Eutectic Solvents" (NADES) was coined by Choi *et al.* (2011). Deep eutectic solvents are frequently defined as binary or ternary mixtures of compounds that are able to associate mainly via hydrogen bonds. Combining these compounds at a certain molar ratio result in a eutectic mixture (Zhang *et al.*, 2012). NADES can be made from primary metabolites such as organic acids, amino acids, sugars, polyols, and choline derivatives (Dai *et al.,* 2013). Besides, water can also be part of natural deep eutectic solvents' composition. NADES has attracted scientific interest not only because of their favourable physicochemical properties (e.g., liquid state over a wide temperature range, low volatility, chemical and thermal stability, non-flammability, and non-toxicity of component ingredients), but also because of their long-term "green" properties (Vandana and Sharon 2023). NADES compounds are mostly comprised of nontoxic substances found naturally in food (Pereira *et al*. 2015), that can be directly incorporated into food formulations without further purification steps, which is a unique advantage over conventional solvents (Savi *et al.,* 2019). NADES appear to be excellent natural products with good biological characteristics when manufactured from low-cost raw materials (Souza *et al.,* 2018). Whey Protein Concentrate-Natural Deep Eutectic Solvents Based Edible Coating of Paneer has been carried using lactic acid: fructose (LF), lactic acid: glucose (LG), citric acid: fructose (CF) and citric acid: glucose (CF) to extend its shelf life (Vandana *et al.,* 2023). Here, the application of natural deep eutectic solvents (NADES) as alternative plasticizers or solvents for corn starch based edible film formulation is explored.

Scope of the study. The scope of this study encompasses the development and evaluation of biodegradable edible films as an eco-friendly alternative to conventional plastic packaging. The focus here is to create edible films using corn starch at varying concentrations (10%-14%) combined with an alternative plasticizer Natural Deep Eutectic Solvents (NADES) Lactic acid-Glucose (LG) in a 5:1 ratio in different pH range (2.3 - 2.8). The investigation included comprehensive testing of the films' physical and mechanical properties, including thickness, tensile strength, grammage, water absorption, and water vapor permeability, to assess their potential as sustainable food packaging materials. Through this systematic evaluation, the study aims to identify the optimal formulation for producing effective biodegradable packaging solutions.

MATERIALS AND METHODS

Raw materials. Corn starch, Lactic acid, Glucose and Sodium Hydroxide were sourced from the local vendor and used for the preparation of edible film.

Experimental design and statistical analysis

Response Surface Methodology (RSM) was used to generate the experimental designs, statistical analysis and regression model with the help of Design Expert Software Version 13 (Statease Inc.). The Central Composite Design (CCD) with a quadratic model was employed,with corn starch concentration and pH as independent variables. Each independent variable had 3 levels. The response variables measured were Film thickness, Tensile strength, Water Vapour permeability, Water absorption capacity and Grammage of the edible film. 3D surface maps were generated using statistical analysis of regression coefficients from the regression models. Optimization and validation of the empirical models were carried out.

Preparation of Corn starch - NADES Edible Film Solution. The NADES (plasticizer) solution, Lactic acid: glucose (5:1) were prepared according to the protocol prescribed by Vandana *et al.* (2022). The flow chart for preparation of edible films from corn starch and NADES is presented in Fig. 1. Film-casting was done by pouring the solutions on to glass petri dish. Teflon sheet was applied to the base of the glass plates to reduce the friction and to facilitate the film removal. The solution was spread uniformly over the plate by tilting it slightly, if required. In each plate, the quantity of biopolymer and volume of film-forming solution were kept constant to get films of uniform thickness. The plates were placed on levelled trays in an incubator and dried at 25- 30°C for 72-96 h. After drying, the films were peeled off from the plate surface and tested for various film properties.

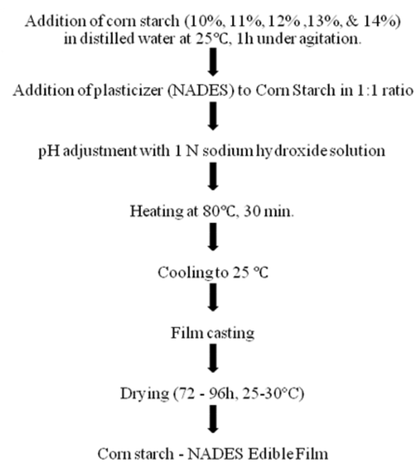

Fig. 1. Flowchart for the Preparation of corn starch - NADES Edible film.

Properties of corn starch - NADES Edible film

Film thickness. The thicknesses of corn starch films, plasticized independently with glucose and lactic acid, were measured using a digital micro-meter (Mitutoyo, Co., Japan) at ten different places randomly.

Grammage. To determine the Grammage of a specific sample, a standardized procedure is followed: a circular sample is cut using a specialized cutter with sharp blades, any creases are removed, the sample's weight is measured using a digital balance, and the Grammage in gsm is the ratio of weight in grams to the area in square meters.

Tensile strength. Tensile strength, also referred to as ultimate tensile strength and is calculated by dividing the peak tension force the sample withstands by its cross- sectional area. Tearing strength tester calculates the tensile force which is required to tear the edible film. A load cell is fitted to the tensile tester to measure tensile force. Cutting knife in the tester is used to create a slit in the sample which ends 43 mm from the far edge of the sample. The pendulum is released to propagate the slit through the remaining 43 mm. The energy loss by the pendulum, measured by the machine, is used to calculate an average tearing force.

Water Vapour Permeability (WVP). Water Vapor Permeability (WVP) was determined gravimetrically using a modified ASTM E96-95 method. Circular film specimens (8 cm × 8 cm) were cut and their thickness measured. The specimens were mounted on polycarbonate cups filled with distilled water, ensuring a 1 cm gap between the film and the water surface. The cups were then placed in an environmental chamber maintained at 25°C and 50% relative humidity. Weight loss was recorded at 2-hour intervals for up to 10 hours. The steady-state portion of the weight loss versus time curve was used to calculate the Water Vapor Transmission Rate (WVTR). Finally, the WVP was calculated using the WVTR and the film thickness. The WVP of the films is expressed in (g /m^2 h Pa).

Water Absorption Capacity. Water holding capacity quantifies a material's ability to absorb liquid within a specific timeframe. This measurement is crucial for assessing the liquid absorptiveness or resilience of diverse materials, including treated and untreated papers, boards, fabrics, and edible films. Excessive moisture absorption can compromise the strength, shape, and function of edible films. For packaging materials like corrugated board, this test helps determine the optimal moisture level to retain their structural integrity and protective properties. The test involves exposing a specimen of known area to a liquid source for a specified duration. The index of water penetration is calculated by measuring the weight of water absorbed per square centimetre of surface area.

RESULTS AND DISCUSSION

The properties of corn starch - NADES Edible film with respect to the independent variables, Corn starch concentration and pH fitted in Central Composite Experimental Design is presented in Table 1.

Table 1: The Central Composite Experimental Design for Preparation of Corn starch - NADES Edible Film.

Sr. No.	Independent variables		Dependent variables				
	Factors		Responses				
	A	B	T (mm)	TS (MPa)	GSM (g/ m^2)	WA (%)	WVP (g/ m^2 Pa)
1.	-1	0	0.14	6.36	82.00	11.07	4.10
2.	-1	+1	0.151	6.12	80.00	10.11	4.70
3.	-1	+1	0.155	6.10	80.00	9.94	4.70
4.	0	+1	0.170	6.25	81.00	9.70	4.40
5.	+1	0	0.198	7.76	82.00	11.26	4.00
6.	0	-1	0.18	6.76	83.00	11.54	3.80
7.	+1	+1	0.19	7.58	80.00	10.19	4.50
8.	1	+1	0.195	7.71	79.00	10.15	4.60
9.	0	-1	0.185	6.62	83.00	11.35	3.80
10.	-1	-1	0.160	6.58	83.00	11.78	3.90
11.	-1	-1	0.165	6.79	84.00	11.83	3.80
12.	-1	0	0.145	6.42	82.00	11.76	3.90
13.	0	+1	0.175	6.35	81.00	9.65	4.50
14.	+1	0	0.195	7.84	82.00	11.07	4.00
15.	+1	-1	0.203	8.00	83.00	12.24	3.80
16.	+1	-1	0.207	8.12	84.00	12.10	3.70

Independent variables: A - Corn starch concentration (10-14%), B - pH (3-7).

Responses: T - Film thickness (mm), TS - Tensile strength (MPa), GSM - Grammage (g/m^2), WA - Water absorption (%), WVP - Water Vapour Permeability (g/metre sq. h Pa).

Empirical model for prediction of edible film properties

The empirical model was developed by fitting the experimental data obtained from CCD into quadratic equations (1-5).

$$\text{Film thickness} = 0.16875 + 0.0226667\ A - 0.00533333\ B - 0.00075\ AB + 0.00075\ A^2 + 0.00875 B^2 \quad (1)$$

$$\text{Tensile strength} = 6.465 + 0.72\ A - 0.23\ B + 0.04\ AB + 0.63\ A^2 + 0.03\ B^2 \quad (2)$$

$$\text{Grammage} = 82.375 - 0.0833333\ A - 1.58333\ B - 0.125\ AB - 0.375\ A^2 - 0.375\ B^2 \quad (3)$$

$$\text{Water absorption Capacity} = 10.8075 + 0.0433333\ A - 0.925\ B - 0.055\ AB + 0.4825\ A^2 - 0.2475\ B^2 \quad (4)$$

$$\text{Water Vapour Permeability} = 3.9125 - 0.0416667\ A + 0.383333\ B - 0.0125\ AB + 0.0875 A^2 + 0.2125 B^2 \quad (5)$$

where, A – Corn starch (10-14%) and B – pH (3- 7)

The relationship between independent variables and response variables from the developed mathematical model equations 3D contour plots were constructed between two independent variables and one dependent variable and presented in Fig. 2-6.

Effect of Corn starch concentration and pH on thickness of edible film. The thickness of an edible film is a crucial factor influencing its performance and functionality as a food packaging material. It significantly impacts barrier properties, mechanical properties, optical properties, and cost of production. The effect of corn starch concentration and pH on the thickness of the edible film is presented in Fig. 2.

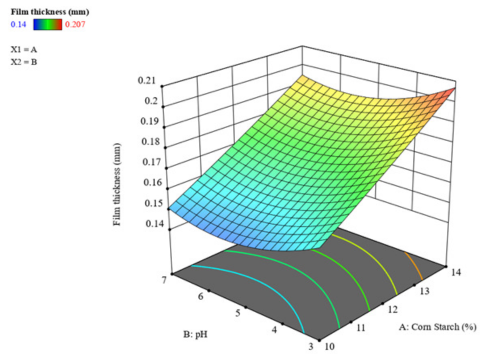

Fig. 2. Effect of Corn starch concentration and pH on thickness of edible film

As the corn starch percentage increases, the film thickness generally decreases. As the pH increases, the film thickness generally increases. Cho and Rhee (2004) observed that as soy protein isolate concentration increases, the thickness of film also increases at constant plasticizer level. At lower pH values, increasing the corn starch percentage has a more significant impact on reducing film thickness compared to higher pH values. This might be due to the influence of pH on the degree of cross-linking between polymer chains in the film; higher pH might promote more cross-linking, leading to a thicker film.

Effect of Corn starch concentration and pH on Grammage of edible film. Grammage, refers to the weight of a specific area of material, typically expressed in grams per square meter (g/m²). In the context of edible films, grammage is a crucial parameter influencing various properties and functionalities. The effect of corn starch concentration and pH on the thickness of the edible film is presented in Fig. 3.

The grammage increases as the pH increases and decreases as the corn starch percentage increases. This is likely due to corn starch acting as a plasticizer, which can reduce the density of the film. There seems to be an interaction between corn starch percentage and pH. This means that the effect of one variable on grammage depends on the level of the other variable. At lower pH values, increasing the corn starch percentage has a more significant impact on reducing grammage compared to higher pH values (Thakur *et al.,* 2007).

Effect of Corn starch concentration and pH on Tensile strength of edible film. Tensile strength is a critical mechanical property of edible films that significantly impacts their performance as food packaging materials. It measures the maximum stress a material can withstand before breaking or tearing. A higher tensile strength indicates a stronger and more durable film. The effect of corn starch concentration and pH on Tensile strength of the edible film is presented in Fig. 4.

As the corn starch percentage increases, the tensile strength generally decreases. As the pH increases, the tensile strength generally increases. At lower pH values, increasing the corn starch percentage has a more significant impact on reducing tensile strength compared to higher pH values. This might be due to the fact that as the corn starch percentage increases, the film becomes more flexible, leading to a decrease in tensile strength. Similar results were noted by Nandane and Jain (2015) soy protein based edible film.

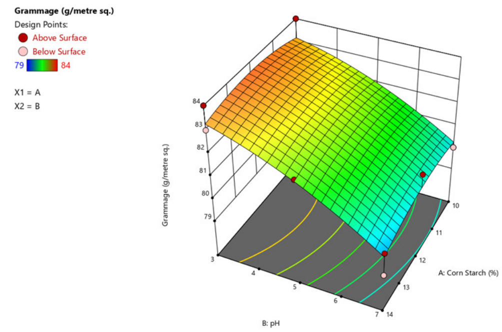

Fig. 3. Effect of Corn Starch concentration and pH on Grammage of edible film.

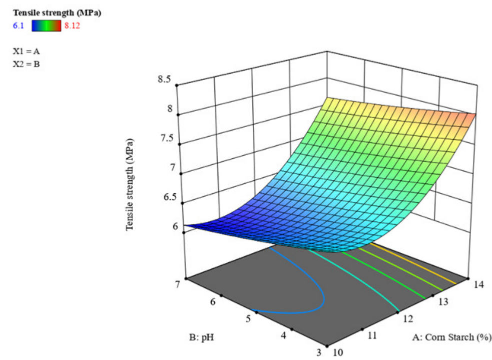

Fig. 4. Effect of Corn starch concentration and pH on Tensile strength of edible film.

Effect of Corn starch concentration and pH on Water Vapour Permeability of edible film. Water Vapor Permeability (WVP) is a critical property of edible films that measures the rate at which water vapor can pass through the film. A lower WVP indicates better barrier properties, which is essential for preserving the quality and extending the shelf life of food products. The effect of corn starch concentration and pH on WVP of the edible film is presented in Fig. 5.

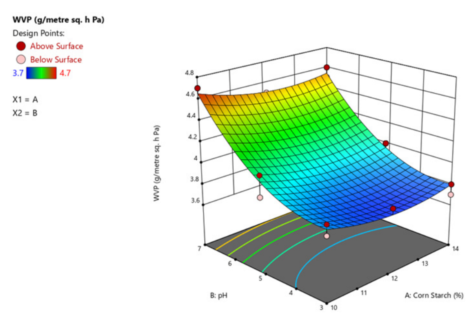

Fig. 5. Effect of Corn starch concentration and pH on Water Vapour Permeability(WVP) of edible film.

The WVP generally decreases as the pH increases and decreases as the corn starch percentage increases. This is likely due to corn starch acting as a plasticizer, which can reduce the permeability of the film. Similar results were observed by Thakur *et al.* (2007) where increased concentration of pea starch enhanced the WVP rate of chitosan novel edible film formulation.

At lower pH values, increasing the corn starch percentage has a more significant impact on reducing WVP compared to higher pH values. As the corn starch percentage increases, the film becomes more flexible due to its plasticizing effect. However, increased flexibility can also lead to a denser film structure, which can hinder the diffusion of water vapour.

Effect of Corn starch concentration and pH on Water Absorption Capacity of edible film. Water Absorption Capacity is a crucial property of edible films that measures the amount of water a film can absorb under specific conditions. A lower water absorption capacity is generally desirable for food packaging applications, as it helps to maintain the quality and freshness of the packaged food. The effect of corn starch concentration and pH on Water Absorption Capacity of the edible film is presented in Fig. 6.

The water absorption capacity generally decreases as the pH increases. The water absorption capacity generally decreases as the corn starch percentage increases. At lower pH values, increasing the corn starch percentage has a more significant impact on reducing water absorption capacity compared to higher pH values. At higher pH values, the cross-linking effect might be more pronounced, reducing the impact of corn starch on water absorption capacity (Nandane and Jain 2015).

Optimization and validation of Corn Starch - NADES edible film. The best formulation of edible film was obtained through optimisation of the concentration of corn starch and the pH for the desired edible film characteristics. RSM was employed for the optimization of the edible film formulation. In view of the effect of the independent variables (concentration of corn starch and pH) on the response variables (properties of the edible film), it was determined that the optimal conditions of film formulation should be Corn Starch 14% and pH 3.0%. In order to confirm the validity of these predicted conditions, this formulation was experimented in triplicate and the results obtained confirmed that the values for the physical and mechanical property measured as actual were closely similar to the predicted values (Table 3). This outcome indicates that this formulation can be used to prepare Corn Starch - NADES edible film with excellent film characteristics suitable for further investigations.

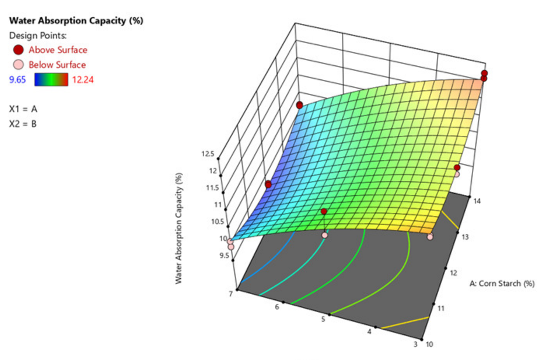

Fig. 6. Effect of Corn starch concentration and pH on Water absorption of edible film.

Table 2: Validation of predicted values for properties of edible film.

Response Variables	Predicted value	Experimental value (n = 3)
Film thickness (mm)	0.207 ± 0.004	0.213 ± 0.003
Tensile strength (MPa)	8.035 ± 0.081	8.021 ± 0.059
Grammage (g/m^2)	83.250 ± 0.598	83.250 ± 0.598
Water absorption (%)	12.060 ± 0.219	12.060 ± 0.219
Water Vapour Permeability (g/m Pa)	3.80 ± 0.084	3.80 ± 0.084

CONCLUSIONS

This study investigated the influence of corn starch concentration and pH on the properties of edible films derived from NADES. The results demonstrated that both factors significantly impacted the film's thickness, grammage, tensile strength, water vapor permeability, and water absorption capacity. An increase in corn starch concentration generally reduced film thickness, grammage, tensile strength, and water vapor permeability. Conversely, higher pH values tended to increase film thickness and tensile strength while decreasing water vapor permeability and water absorption capacity.

The interaction between corn starch concentration and pH was also evident. At lower pH levels, increasing corn starch concentration had a more pronounced effect on reducing film properties. At higher pH levels, the cross-linking effect dominated, mitigating the impact of corn starch. Based on these findings, an optimal formulation was identified as 14% corn starch concentration at pH 3.0with the desired properties of thickness, strength, and permeability. This formulation resulted in a film with desirable properties, including suitable thickness, adequate mechanical strength, and good barrier properties.

FUTURE SCOPE

Further research could explore the incorporation of additional functional components to enhance the properties of these edible films and expand their potential applications in food packaging.

Conflict of Interest. None.

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