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Subject

**Development and characterization of active/intelligent starch
edible films based on local natural extract**

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Dedication

This work is dedicated to:

My mother and my father, who never stop giving of themselves in
countless ways.

To my adorable sister: Assala” My right Hand”

To all my brothers.

To my dear friend: Linda

To my buddy in this work: Meriem

Chahinez

Dedication

This work is dedicated to:

*My mother and my father, who never stop giving of themselves in
countless ways.*

To my adorable sister: Sara” my right Hand”

To My brothers: Bilal & Yacine

To my angel: M’hamed Djaoued

To my dear friend: Yasmine

To my buddy in this work: Chahinez.

Meriem

Abstract

Plastic materials are the typical example of the anti-nature pollutants that daily human need remains unavoidable at all levels, which abolishes any attempt to exclude them or eradicate their use, and leads to think out the box to substitute them cleverly with natural innovative alternatives. This work targeted the confection of biodegradable intelligent starch films, becoming pH-sensitive through addition of beetroot betalains to unveil food alteration. The betalains extracted was formed mostly by betacyanins moieties. The combination of starch with betalains gave more thickness, density and humidity to the films. Also, these films were more permeable to water vapor passage and had a higher transparency but nearly an even water solubility to control films, against a lesser surface wettability. Moreover, the colored films had narrowly an increased degradation time of three days. Betalains release into food simulants indicated their matching with aqueous low-fat foods. The issued smart pH-sensor patches were seemingly very effective in pointing out, in real time, the spoilage of stored chicken at different temperature conditions, through color-shifting (red to dark purple) as a result of monitoring the emission of ammonia vapor.

Keywords: Betalains, smart, packaging, patch, pH-response.

Résumé

Les matières plastiques sont l'exemple type des polluants anti-nature que les besoins quotidiens de l'homme rendent inévitables à tous les niveaux, ce qui abolit toute tentative de les exclure ou d'éradiquer leur utilisation, et conduit à réfléchir à les remplacer astucieusement par des alternatives naturelles innovantes. Ce travail a porté sur la confection de films d'amidon intelligents biodégradables, devenant sensibles au pH grâce à l'ajout de bétalaïnes de betterave pour dévoiler l'altération des aliments. Les bétalaïnes extraites étaient principalement formées par des bétacyanines. La combinaison de l'amidon et des bétalaïnes a permis d'augmenter l'épaisseur, la densité et l'humidité des films. De plus, ces films étaient plus perméables au passage de la vapeur d'eau et avaient plus de transparence, contre une hydro-solubilité presque égale à celle des films contrôles et une surface moins mouillable. De plus, les films colorés avaient un temps de dégradation de trois jours à peine plus long. La libération des bétalaïnes dans les simulants d'aliments a indiqué leur compatibilité avec les aliments aqueux à faible teneur en matières grasses. Les patches intelligents pH-sensibles étaient très efficaces pour signaler, en temps réel, la détérioration du poulet stocké dans différentes conditions de température, grâce au changement de couleur (du rouge au violet foncé) résultant de la surveillance de l'émission de vapeur d'ammoniaque.

Mots clés: Bétalaïnes, intelligent, emballage, patch, pH-sensible.

ملخص

يمثل البلاستيك للأسف احد النماذج الملوثة للطبيعة التي تجعلها الاحتياجات اليومية للإنسان حتمية على جميع المستويات، مما يلغي أي محاولة لاستبعادها أو القضاء على استخدامها، ما يدفعنا لإيجاد بدائل أخرى طبيعية مبتكرة وعاجلة. ركز هذا العمل على صنع أغلفة ذكية نشوية قابلة للتحلل، تصبح حساسة لدرجة الحموضة من خلال إضافة بيتالان الشمندر للكشف عن تلف الطعام. يتشكل البيتالان المستخرج بشكل رئيسي من البيتاسيانين. أدى الجمع بين النشا والبيتالان الى زيادة سماكة وكثافة ورطوبة الأغشية، بالإضافة إلى جعلها أكثر نفاذاً لبخار الماء وأكثر شفافية، مع تميزها بدوبانية مائية مشابهة لأغشية التحكم و سطح أقل قابلية للبلل. بينت النتائج ان الأفلام الملونة تتحلل في وقت أطول قليلاً (ثلاثة أيام) مقارنة بالشواهد، كما اشارت تجربة تحرر البيتالان في محاكيات الطعام إلى توافقها مع الأطعمة المائية قليلة الدسم. في الأخير، ابانت هذه الافلام الملونة عن فعالية كبيرة في الكشف عن تلف الدجاج المخزن تحت ظروف درجات حرارة مختلفة في الوقت الفعلي، وذلك بفضل تغير لونها (من الأحمر إلى الأرجواني الداكن) تبعاً لانبعاث بخار الأمونيا.

الكلمات المفتاحية: بيتالان، ذكي، تغليف، ملصق، حساس لدرجة الحموضة.

List of abbreviations

%: pourcentage

°C: degree Celsius

Cm: centimeter

HCL: hydrogen chloride

KCL: potassium chloride

Na₂HP₄: sodium hydrogen phosphate

NaOH: sodium hydroxide

BC: betalain content

WVTR: water vapor transmission rate

g: gram

mL: milliliter

CaCl₂: Calcium chloride

h: Hour

WS: Water solubility

A₆₀₀: Absorbance at 600

Bc: betacyanin

Bx: betaxanthin

W/W: weight in weight

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Introduction

Introduction

Plastic packaging consumption is increasingly accompanied by serious and negative environmental problems. Currently, the existing waste and recycling infrastructure cannot keep up with the growing volume of end-of-life plastic used especially in consumer (end) products including, in particular, single-use plastic packaging, bags and bottles, all of which are most commonly found in global waste streams (**Beaumont et al., 2019; Bergmann et al., 2019; Carpenter and Wolverton, 2017; Wright and Kelly, 2017; Jambeck et al., 2015; Marsh and Bugusu, 2007**). Single-use plastic packaging has a short lifespan and it is then, typically, discarded carelessly by consumers (**Hahladakis and Iacovidou, 2018; Mutha et al., 2006**).

Interest in the use of active and intelligent packaging systems for meat and meat products has increased in recent years. Active packaging refers to the incorporation of additives into packaging systems with the aim of maintaining or extending meat product quality and shelf-life. Active packaging systems include oxygen scavengers, carbon dioxide scavengers and emitters, moisture control agents and antimicrobial packaging technologies. Meanwhile, intelligent packaging systems are those monitoring the condition of packaged foods to give information regarding the quality of the packaged food during transport and storage. Recognition of the benefits of active and intelligent packaging technologies by the food industry, development of economically viable packaging systems and increased consumer acceptance is necessary for commercial realization of these packaging technologies (**Kerry, O’Grady and Hogan, 2006**).

The smart packaging films have attracted consumer’s interest since they facilitate their life due to their attribute of food quality of freshness detection especially during their storage for long term. In particular, there is a focus on the creation of active packaging materials from natural polymer ingredients, especially plant-based ones. The film matrix is typically constructed from film-forming food components, such as proteins, polysaccharides and lipids (**Bao et al., 2022**).

Starch is the most popular plant polysaccharides, which has been widely used for the development of edible coating films because of its abundance, cost-effectiveness, and excellent film-forming abilities. Starch-based films have good optical, organoleptic and gas barrier properties, however, they have poor mechanical properties (**Rahul Thakur et al., 2019**).

Natural food additives as pigments; such as antioxidants and antimicrobials, are often directly added to foods to reduce their spoilage due to oxidation reactions or microbial infection (**Kumar et al. 2015**). Betalains are water soluble nitrogen phyto-pigments accumulated in flowers, fruits and vegetables. These pH-Sensing pigments could be split into red-violet betacyanins and yellow betaxanthin. Recognized for being safe, nontoxic and harmless natural colorants with high antioxidant capacities, they have been employed in food and pharmaceutical industries for decades. Whereas, their utility as indicators for tracking spoilage and deterioration of packed food knew an unprecedented thrust (**Stintzing and Reinhold, 2004; Lee et al.2002; Escibano et al.1998**).

This study aims to make an original intelligent film/packaging material endowed with pH-responsive ability, by adding a betalains rich-extract obtained from local beetroot legumes on a starch matrix-based. The designed film will undergo a variety of physicochemical, mechanical and optical tests targeting its broad characterization. Thereafter, the above-mentioned film will be examined for its competence as pH-monitoring patch on stored food.

Chapter I

Materials and methods

I.1. Objectives

- Extraction and characterization of beetroot betalains.
- Preparation and description of the starch/betalains intelligent film.
- Assessment of the applicability of the preformed film as a pH-sensible patch on food packaging.

I.2. Materials

I.2.1. Raw material

The beetroots used in this study were purchased from the local market of the Wilaya of Tissemsilt in February 2023, than cold-conserved until their use. The starch powder was bought from a local grocery in the same period.

I.2. Laboratory material

Table 1 : Material and chemicals used

Instrument	Chemicals	Glassware
- Analytical balance	- Acetic acid (CH ₃ COOH)	- Cuvette
- Desiccator	- Ammonia (NH ₄ OH)	- Adjustable micropipette
- Magnetic stirrer	- Ascorbic acid (C ₆ H ₈ O ₆)	- Airtight bags
- Micrometer	- Calcium chloride (CaCl ₂)	- Beakers
- Oven	- Citric acid (C ₆ H ₈ O ₇)	- Erlenmeyer flasks
- PH meter (HANNA instruments)	- Distilled water	- Filter paper
- Refrigerator	- Ethanol (C ₂ H ₆ O)	- Magnetic bars
- Rotavapor (Bushi R_200)	- Glycerol (C ₃ H ₈ O ₃)	- Measuring flask
- Stick blender	- Hydrochloric acid (HCl)	- Spatula
- Stirrer	- Potassium chloride (KCl)	- Test tubes
- Vacuum filter (KNF)	- Sodium hydroxide (NaOH)	- Watch glass
- Water bath		
- Spectrophotometer UV-VIS (JENWAY 7305)		

I.3. Experimental protocol

The diagram below schematizes the entire methodology followed in this study in which each step was replicated three times:

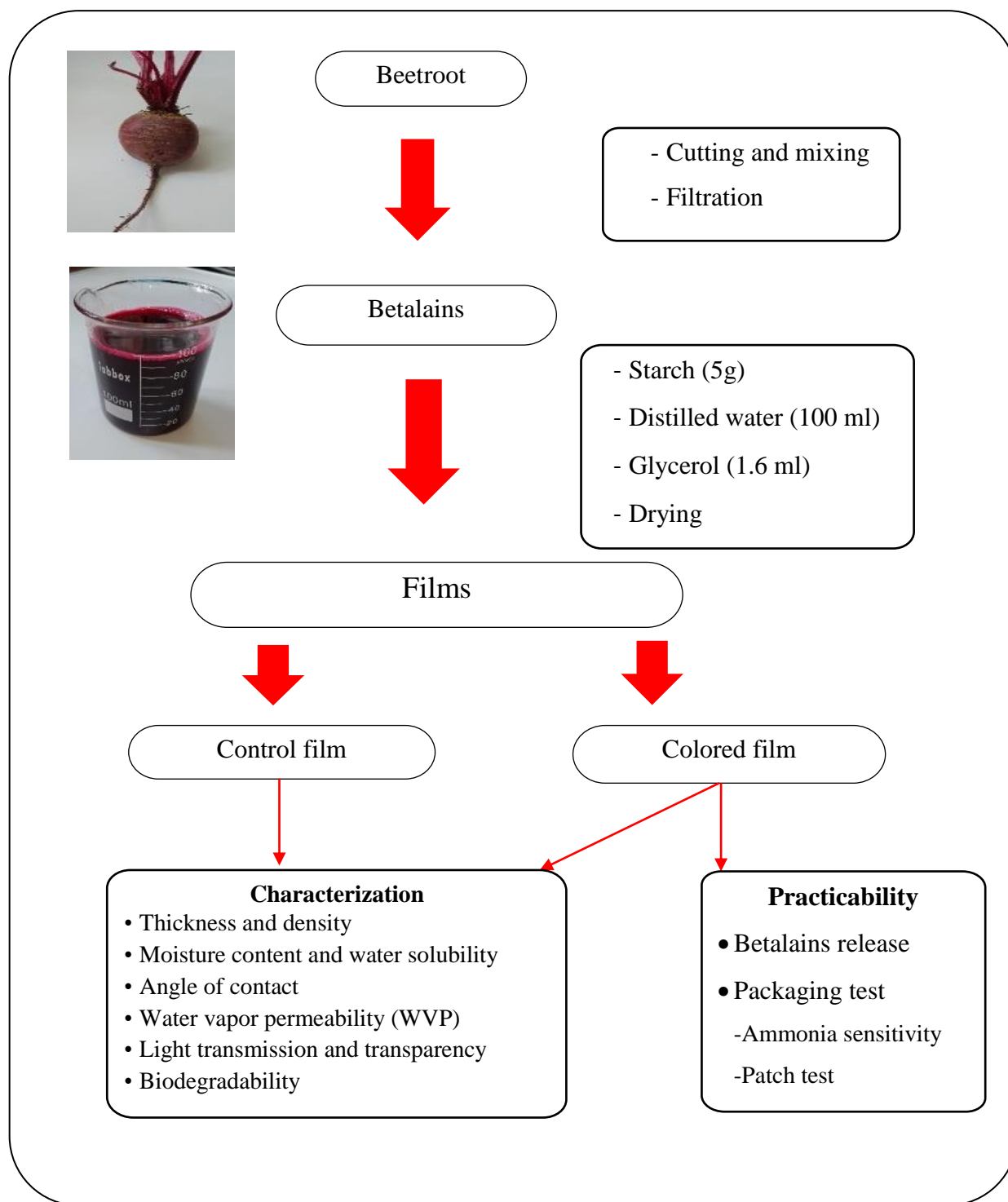


Figure 1: Diagram of the experimental process

I.3.1. Betalains

I.3.1.1. Extraction

According to **Sigwela et al. (2021)** with some modifications, 100 g of peeled beetroot was grated and added by 100 ml of distilled water. After that, the solution was mixed using a blender then filtered in double time through cotton cloth and filter paper consequently. 1 g of ascorbic acid as preservation additive was put to the filtered mixture.

I.3.1.2. Spectrophotometric quantitation

The stock solution of betalains was diluted to 1/10 before measuring its absorbance at 480nm for betaxanthin and 538 nm for betacyanin. Betalain content was calculated as follow (**Zin et al., 2021**).

$$\text{BC (mg / L)} = \frac{A * \text{DF} * \text{MW} * 1000}{\epsilon \times l}$$

Where:

A: Absorbance, DF: Dilution factor and l: Optical path length (1cm).

For the quantification of betacyanins and betaxanthantins; the molecular weight (MW) and the molar extinction coefficient (ϵ) are respectively:

For betanin (MW=550 g/mol; ϵ = 60,000 L/mol.cm; λ =538 nm)

For indaxanthin (MW = 308 g / mol; ϵ = 48,000 L / mol.cm; λ = 480 nm).

I.3.1.3. pH-response

2 drops of betalains extract were poured into 12 tubes containing 7 ml of buffer solutions expanding from pH 1 to 12. HCl/Kcl buffer was prepared to get pH=1 while citrate-phosphate (Na_2HPO_4) solution was adjusted to pH= 2 – 8. The pH range (9-12) was attained by mixing NaOH (0.1M) in appropriate proportion the previous citrate-phosphate pH=8 buffer. Photos of color alteration in the tubes were taken after 5 minutes of stabilization (**Rawdkuen et al., 2020**)

I.3.2. Films

I.3.2.1. Formulation

Based on the modified method published by **Araujo-Farro et al. (2010)**, 5g of starch was dissolved and stirred for 10 min in 100 ml of distilled water including 20 ml of beetroot betalains. Right after, the filmogenic solution was heated at 95 C°/20 min in water bath until gelatinization, and glycerol was added to the mixture as a plasticizer at 40% (w/w) based on starch weight. A final magnetic stirring was applied for 3 min before pouring the mixture into anti-adhesive mold at a proportion of 0.16mL/cm². The films were left for 24 h at 25 C° to dryness, then peeled and cooled in a silica-gel desiccator for 24h until their stabilization. Ultimately, formed films were stored in sealed food containers until their use. The film shaped without betalains extract, in the same conditions, represents the uncolored control.

I.3.2.2. Characterization

I.3.2.2.1. Thickness and density

The thickness of films was determined by a digital comparator in 10 different points of each film (**Gheribi et al., 2018**).

Density (d) was calculated by reporting the weight (m) on the volume of film bands of 2 cm² (s) with a known thickness (e) as follows (Li et al., 2020)

$$d = \frac{m}{s \cdot e}$$

I.3.2.2.2. Humidity content and water solubility

Humidity content of films was evaluated according the method of (**Jouki et al., 2013**). 1cm×1cm size films fragments were weighed (m_i) then dried for 24 h at 90 °C, before being weighed again (m_f).

Humidity was calculated following the equation (**Gheribi et al., 2018**):

$$HR = \frac{m_i - m_f}{m_i} \times 100$$

Afterwards, water solubility of films was performed by dipping, 1 cm² priorly dried (90°C/24h) films pieces (m_i), in 50 ml distilled water for 30 min. The remaining undissolved parts of films were redried and reweighed (m_f) (Jouki et al., 2013). The equation of hydrosolubility was given by (Gheribi et al. 2018):

$$WS = \frac{m_i - m_f}{m_i} \times 100$$

I.3.2.2.3. Contact angle

A droplet (4 μ L) of distilled water was deposited on the film's surface. The angle formed between the baseline of the droplet and the tangent line at the contact point was provided by image software J. (Joonas, 2021 64-bit Java 8) (Gheribi et al., 2018).

I.3.2.2.4. Water Vapor Permeability (WVP)

The film was placed upon a crucible containing CaCl₂ and the total weight was noted. To know the weight gain, the crucible placed in food container including 500 ml of distilled water at room temperature was weighed for 6 h at one hour intervals. Water vapor permeability is calculated using equation (Zhang et al., 2016; Gonzalez et al., 2019).

$$WVP = \frac{WVTR}{A} \frac{e}{\Delta P v}$$

Where:

WVTR: water vapor transmission rate (g.s⁻¹), or the slope of the regression linear weight gain = f (time), calculated by drawing the final weight minus the initial weight of sample ($W_f - W_0$) as a function of time (t); **e**: the average film thickness (m); **A**: the transfer area (m²),

$\Delta p v$: the difference in water vapor pressure between cacl2 atmosphere and the room atmosphere (2337 pa)

I.3.2.2.5. Light transmission rate and film transparency

The film's transparency was measured by placing film strips (0.5cm x 4.0cm) in quartz cell at 600 nm. It was calculated as cited by (Gonzalez et al., 2019):

$$Transparency = \frac{A600}{S} = \frac{-Log T600}{S}$$

A600 and T600: absorbance and transmittance at 600 nm, respectively; S: film thickness.

I.3.2.2.6. Release of betalains in food simulants

Colorful film pieces of (2cm×2cm) were immersed in 25 ml of different food simulants (water, 25%, 50% and 95% ethanol solutions). Thus, absorbance of 2 ml of each solution was measured at 480 and 538 nm within a time of (30, 60, 90, 120, 180 and 240 min) (Alizadeh-Sani et al., 2021).

I.3.2.2.7. Biodegradability test

3cm×3cm films with a defined weight were put at approximately 6 cm from the surface of plastic cups full of horticultural soil daily watered; the evolvement of weight loss was surveilled each 3 days counting from first 24 h (mi), and ended at the 15th day. (Shanmathy et al., 2021).

I.3.2.3. Activity of films

I.3.2.3.1. Ammonia sensitivity test

Square film parts (3cm×3cm) were hanged at 1 cm from the free surface of ammonia solution (8 mM, 80 mL). The color change of the samples was photographed every 5 min for 30 min (Alizadeh-Sani et al., 2021). The R, G, and B values of the film were measured using the Pixie program for Windows, and the sensitivity of the film to volatile ammonia vapor was calculated as follows:

$$SRGB(\%) = \frac{(Ri - Rf) + (Gi - Gf) + (Bi - Bf)}{Ri + Gi + Bi} \times 100$$

Where:

SRGB: Sensitivity Red Green Bleu

Ri, Gi, Bi and Rf, Gf, Bf were the initial and final values of the red, green, and blue values.

I.3.2.3.2. Smart patch efficiency test

Sample films having dimensions (1cm×2 cm) were stuck at the interior face of packaging box transparent lids, covering 50 g of ground chicken. The boxes were stored discriminately at room temperature (25°C) or at refrigerator (4°C) for 72h, with taking pictures of the film change periodically (every 6 h) (Qin et al., 2020).

Chapter II

Results and discussion

II.1. Betalains

II.1.1. Betalains concentration

The spectral method was used routinely to quantify the betalains content in plants, by exploiting the wavelengths 538 and 480nm to measure betacyanins and betaxanthins, respectively (Zin et al.2021).

Table 2: Betalains content in beetroot extract

	Betacyanins	Betaxanthins
Content (mg/L extract)	119.16 ±0.59	43.63±0.26
Content (mg/g beetroot)	0.11 ±0.007	0.043±0.004

The results showed supremacy of betacyanins over betaxanthin, expressed as percentages of 73.22% versus 26.80%, in accordance with the predominance of the first class up to 75-95% of red pigment extracted from beetroot as stipulated by the work of (Von Elbe et al. 1972).

Otherwise, these results had a similar weighting of the ratio betacyanin/betaxanthin but were lower than those of Sawicki et al. (2016) for the same vegetable (10.26-17.15mg betacyanin/g; 7.20-17mg betaxanthin/g). However, the opposite result contradicted those of the current study with (16.33 mg betacyanins/g against 30.78 mg betaxanthins/g) (Georgiev et al. 2010). According to Sawicki et al. (2016) explained that these differences in betalains content and proportions were dependent to the part of root, its own fingerprint and the extraction conditions.

II.1.2. Betalains pH-response

The pH plays a highly significant role on beetroot pigment color degradation, which could utilized as an indication of acidic, neutral or basic state of the ambiance (Mohamed Arif et al., 2021). The figure 02 showed that the color modification range was divided to three main shades; red-pink for the acidic pH array 1 to 6, purple for the neutral to basic range 7 to 10 and orange-yellow for the intensively basic interval 11 to 13.

Speaking of the shift from red to purple color, translating the passage from acidic to neutral slightly alkaline pH, (Nemzer et al. 2011) correlated this effect to betacyanins expressing primordially, in an imposing way, their color to the solution. The betacyanins are conjugated by

cyclo-dihydroxyphenylalanine (cyclo-dopa) which corresponded to the color from red to violet (Izabela et al 2021).



Figure 2: pH-response of beetroot betalains

On the other side, the fluctuation from purple to orange-yellow in the pH field 11 to 13, affirmed the dominantly expression of betaxanthins; conjugated by amino acids or amines (Kay et al. 1993).

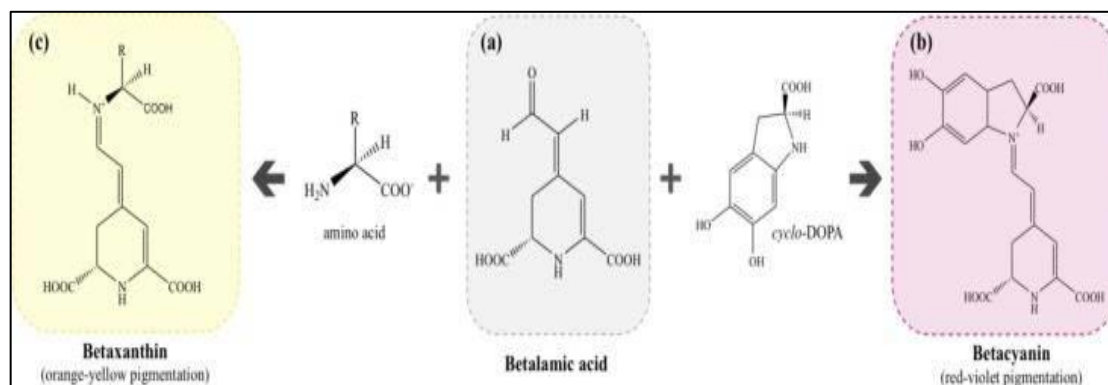


Figure 3: Relation between the biochemical structure and the apparent color of betalains.

II.2. Smart films

II.2.1. Characterization of smart films

The figure 04 showed the colored and uncolored films. The last was smoother and more homogeneous, easier to peel and required a lesser time to dry as well. So, the presence of betalains increased the adhesiveness of starch films and made the step of immediate desiccator stabilization mandatory. Likewise, it has been noticed the clear effect of these beetroot pigments on gaining elasticity of films (Esra Tekin et al., 2022) .



Figure 4: The colored and uncolored films

The table 03, illustrates the physicochemical properties of betalains colored films and uncolored films:

Table 3: Physicochemical properties of colored and uncolored films

Properties	Films	
	Colored	Uncolored
Thickness (mm)	0.0784 ±0.002	0.066 ±0.002
Density (g/ml)	0.19 ±0.007	0.15 ±0.007
Humidity (%)	16 ±1.41	10 ±0.70
Water solubility (%)	38.14 ±0.6	37.14 ±0.27
Contact angle (°)	74.44 ±0.14	70.80 ±0.39
Water vapor permeability (WVP) (g·m ⁻¹ ·s ⁻¹ ·Pa ⁻¹)	5.23 x10 ⁻⁹ ±0.374 x10 ⁻⁹	4.06 x10 ⁻⁹ ±0.263 x10 ⁻⁹
Light Transmission rate and transparency (%)	11.8 ±0.33	4.63 ±0.13

II. 2.2. Thickness and density

The thickness is a crucial parameter that affects biological, physical and mechanical characteristics of the film (**Ajansson et al., 2004**).

The results disclosed that the colored film was thicker than the uncolored one with rates of $0.0784 \pm 0.002 \text{ mm}$ compared to $0.0662 \pm 0.002 \text{ mm}$, respectively. Therefore, it could be suggested an increase of the thickness of films due to the addition of betalains pigments. According to **Gutierrez et al. (2015)**, adding other ingredients to the base matrix will relatively raise the thickness of the film.

Also, the starch type had a genuine impact on the film thickness. As a comparison, the starch/betalains or only starch films viewed in this work, were thicker than films of potato starch (0.055 mm) tested by **Dandan Li et al. (2022)**, but thinner than those of cassava starch (0.103 mm) and rice starch (0.145 mm) analyzed by **Zuzanna et al. (2021)**.

In this context, **Luan Ramos da Silva et al. (2022)** saw the concentration of additives, the starch mass and its concentration as direct influencers on film thickness. The higher concentration you have the thickener film you obtain. Also, the thickness of the starch based film increased with the increase of amylose content (**Wang et al., 2007**).

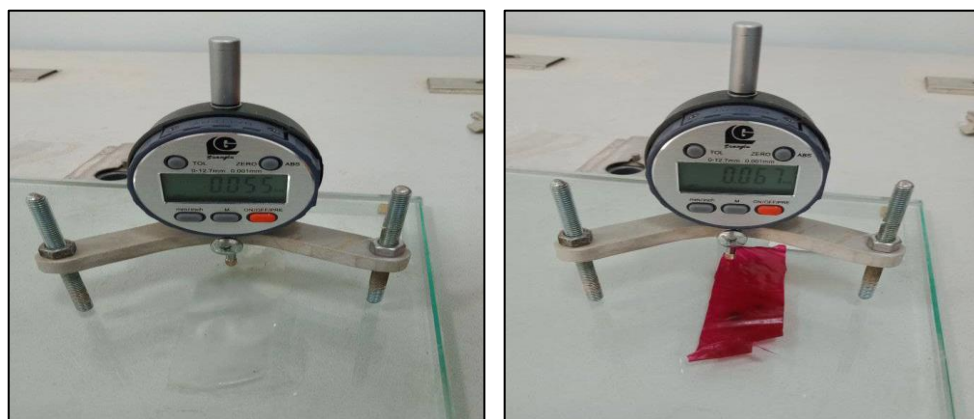


Figure 5: Measurement of colored and uncolored film thickness.

Besides, the density of the colored film was also higher than the density of uncolored one with a gap of 0.04 g/ml . These results are significantly consensual with the work of **Jessica et al. (2018)**, in which authors related the addition of natural pigment extract to the increase of chitosan/starch film density.

II.2.3. Humidity content and water solubility

This test was carried out to evaluate the weight loss percentage according to water waste of films during the drying (**Galus et al., 2016**). According to **Chiou et al. (2009)**, the test of humidity gauged the mechanical properties of the film.

From table 03, the humidity of betalains films was higher than control films ($16\pm 1.41\%$ vs $10\pm 0.70\%$), which is in agreement with the results of **Paul et al. (2016)**, showing $13.10\pm 2.10\%$ starch film with betalains against $8.3\pm 1.7\%$ for starch films without them.

These researchers explained that with a possible interaction between beetroot extract and starch film matrix leading to an increase its moisture. In addition, moisture was the property the most touched by plasticizers, for that glycerol. The films containing 33% glycerol have a lower moisture absorption capacity compared to the films with higher glycerol content (**Ewelina Basiak et al, 2018**).

As it concerns water solubility, this trait give information about the integrity of films in aqueous environment. A higher solubility indicated a lower resistance of water (**Granasambandan et al.1997; Handa A et al 1999**). The uncolored film was bit more hydro-soluble than betalains film with a slight difference of $1.0\pm 0.33\%$.

The addition of different plasticizers shows improvement in water resistance; film plasticized with glycerol had the lowest water absorption property (**Abdurrahman et al., 2022**).

II. 2.4. Contact angle

This test is a measure of the water's ability to wet the surface of the film, the shape of the drop on the surface depends on the angle of contact. For that, the contact angle is a very important parameter for measuring the wettability or surface hydrophobicity (**Qin et al.2019**). The hydrophobicity of films depended on their contact angle; the higher angle found, the most hydrophobic will be the film (**jouki et al. 2013**).

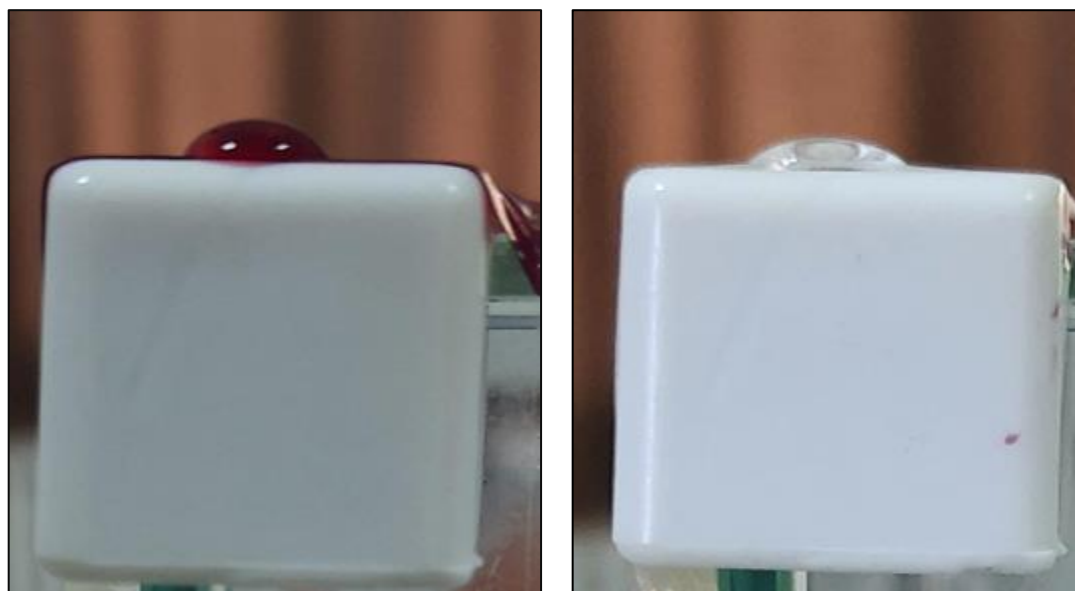


Figure 6: Water droplet appearance on A) Betalain film, B) Control film.

As it is shown in table 04, the colored film showed a higher angle equal to $74.44 \pm 0.14^\circ$, overtaking the uncolored film with a result up to $70 \pm 0.39^\circ$. The starch film was more hydrophobic when it was pigmented by betalains.

The same observation was made by **Carlos et al. (2022)** for cassava starch film with beetroots and cassava starch films only (13.6° and 2.6° , respectively). The microparticles of betalains rich-extract decreased the spread ability of water droplet and its contact with the surface as a result; thus it increased its hydrophobicity. This effect could be associated to the change of structure because of the interaction of beetroot pigment with the starch surface (**Carlos et al., 2022**).

II. 2.5. Water vapor permeability

This test measured the quantity of water diffusion through the film and it is related to the thickness and moisture of the film (**Cazon et al., 2022**). Water vapor permeability determines the amount of the permeating of water in the film matrix, but also the suitable polymeric film for each kind of food; thus, the WVP should be as low as possible (**Patriciab et al., 2022**).

Herein, the colored film was more permeating to water than the uncolored one with a small difference in results ($1.17 \times 10^{-9} \pm 0.111 \times 10^{-9} \text{ g} \cdot \text{m}^{-1} \cdot \text{s}^{-1} \cdot \text{Pa}^{-1}$). This result could be ascribed to the presence of many hydroxyl groups hydroxyl groups and their position of attachment or H-donating groups, and glycosylation. These groups lead to the formation of hydrogen bonds responsible for this hydrophilicity of Betalains (**Fathordoobady et al., 2016**).

II.2.6. Light transmission rate and film transparency

The transparency defines the transmittance passed through the film at certain wavelength and it's proportional to it (**Ligot et al., 2015**). Paradoxically, betalain films were more transparent than control (11.8 ± 0.33 against $4.63 \pm 0.13\%$). In the opposite, corn starch films added with other ingredients like chitosan was had more opacity than control. Chitosan worked as a coating and covered light passage (**Gutierrez et al., 2017**).

It is possible to suggest a fluctuating effect of the ingredient co-mixed with the starch on the opacity/transparency outcome. A similar conclusion was cited by (**halis and Hassouni, 2021**) using polyphenolic extract on natural film. The difference of structure between ingredients and the interaction between them can lead to variable actions on light transmittance through films (**Gorgieva et Kokol, 2011**).

II.2.7. Release of betalains

A release pigment test aimed to evaluate the entire interactions between them and film matrix by conducting the rate of pigment in each food simulant (**Alizadah et al., 2020**). Figure 7 represented the betacyanin and betaxanthin liberation in each food simulant.

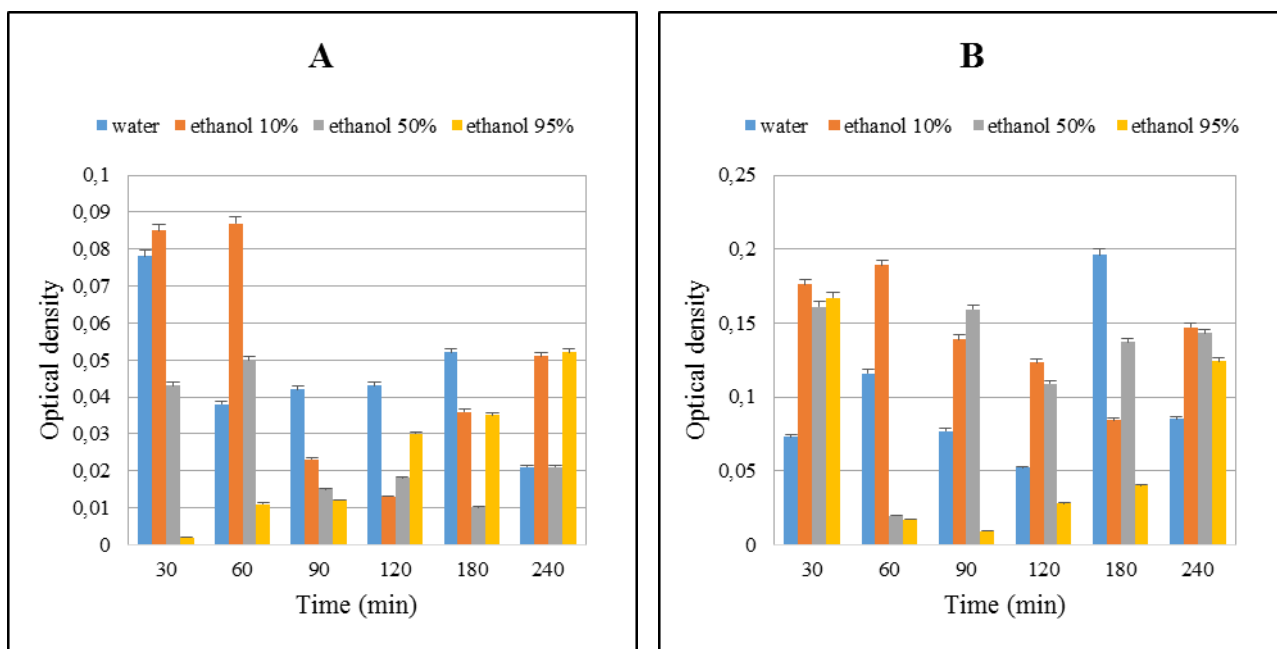


Figure 07 : Release of (A) betacyanins and (B) betaxanthins from the colored film in different food simulants

The both classes were denoted by a higher rate of release in the solution 10% ethanol and water, followed orderly by 50 and 95% ethanol solutions. Overall, the first hour of contact with the

four solutions met the summit release of pigments. The water-soluble nature of nitrogen beetroot dyes named betalains explained this behavior. **Alizadeh et al. (2020)** shaded the light on the direct link between pigment release from films and their nature besides the nature the simulant food type.

It is plausible to propose probably a better solubilization of beetroot betalains in aqueous low-fat foods.

II.2.8. Biodegradability test

Biodegradability studies the film breakdown ability in the environment caused by the activity of soil (**Martucci et al., 2009**). the table 04 represents the weight mass loss of colored and uncolored films during 15 days of soil burying. It is logical to say that uncolored films degraded quicker than control films, taking into consideration the respective time taken by each type to be totally vanished (12 and 15 days).

According to **Jaranillo et al. (2016)**, test of biodegradability counting the daily watering, imitated the degradation caused by soil bacterial microflora. The degradation of starch film can be related to the presence of hydrolysable and oxidizable groups of polymeric chains which facilitated their assimilation by microorganism (**Warscheid et al., 2000**).

The presence of betalains may slowdown this phenomenon regarding their antimicrobial activity. In both cases, the two films are alike in the matter of being environment friendly.

Table 4. Weight loss of colored and uncolored films (biodegradability test)

Days	Weight loss percentage (%)	
	Control film	Colored film
3	20±0.70	6.84±0.10
6	30.76±0.16	21.42±0.40
9	41.66±0.23	32.23±0.54
12	100	39.39±0.42
15	/	100

II.3. Activity of films

II.3.1. Ammonia sensitivity test

This test aimed to identify the response of starch/betalain films to volatile ammonia vapor assigned to food nitrogen compounds degradation. The changing of film color is an indicator for the consumability of food, since it marked its freshness loss status due to ammonia vapor emitted during the spoilage phase. Color change equaled food expiration using digital camera coupled with RGB colour analysis (Wells et al 2019).

Figure 08 represents the curve of film sensitivity to ammonia vapor monitored by color changing. At the end of 30 min, the film turned from red to dark purple, simultaneously to an increase in sensitivity to ammonia vapor (SRGB) going from 3.51% at 5 min to 51.75% at 15 min, then 70.35% at 30 min. As seen previously, this color corresponded to the alkaline pH range of 7-10, which lead to the dominant expression of betacyanins. The FTIR analysis of the same red purple extract done by Shiv et al. (2012) showed that the presence of amine and carbonyl functional groups attributed the red purple color to the betacyanin pigments.

Accordingly to the pH of the diluted ammonia solution, the non-reaching of high pH values, over 10 units, inhibit the expression of the yellow color allocated to betaxanthin dyes. Comparing to that, Shahab et al. (2021) explained the obtaining of purple to yellow colors in presence of ammonia vapor to the presence of the large pH 2-13 range where each betalain class was apparent in a specific part of the range.

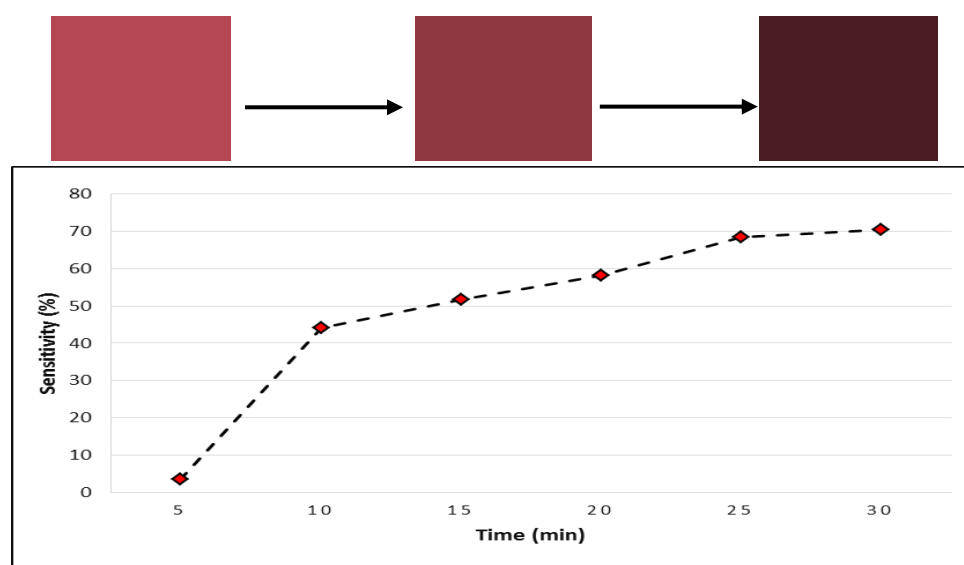


Figure 8: Sensitivity of smart film to ammonia vapor.

II.3.2. Smart patch efficiency test









The freshness of chicken meat reduces with time. The main cause of chicken spoilage due to the activity of microorganism increasing protein degradation which automatically increase the volatile nitrogen and ammonia and pH concomitantly inside the packaging (Zhang et al.2021).

The evolvement of patch color changing on the packaging of ground chicken stored at 25 or 4°C is in the table 5.

At 25°C, the initial clear red –pink color shifted to dark purple by the end of the 72 hours, parallely to the increase of pH during chicken spoilage. It is worthy to cite the consolidation of this result by the anterior ammonia test. This change from red to dark purple visually viewed is the result of betacyanin attributes modifying their color at pH array of 8-10, following the cyclo-dopa conjugation (**Nazeeruddin et al. 2011; Nemzar et al., 2011**).

For the storage at 4°C, the film didn't show any color changing until 48 hours, where the clear red color turned to a darkest shade of it. This result showed that the pH of the container inner atmosphere remained in the acidic array. Comparing these results to the work of **Carlos et al. (2022)** on fish storage at 4°C, the Cassava starch with beetroot indicator film didn't show any results of changing until the 8th day by turning from red to brown. This results may due to the time spoilage of each food material and the different process addition to the methods of packaging and film preparation.

Table 05. Evolution of color change of packaging smart films.

Time (h)	Smart film color change	
	Stored at room temperature (25°C)	Stored in refrigerator (4°C)
6h		
12h		
18h		
24h		

30h



36h



42h



48h



54h



60h



66h



72h



Conclusion

Conclusion

Intelligent films as packaging systems are capable of carrying out intelligent functions such as responding in a particular manner when there is some change in the quality, safety, or maturity of a packaged food, or to provide an indication of these changes. As a result, these smart packaging materials can play an important role in improving food quality and safety management (**Alizadeh et al.2020; Bijik et al., 2011; Kuswandi et al., 2011**).

Recently, the role of packaging has increased beyond its basic function with changing consumer preferences and expectations. Now, it is also contributing towards extending the shelf life and acts as a quality indicator of the packed food products. The focus is also on the development of more and more interactive packaging systems, which are classified as active and intelligent packaging simultaneously (**Dobrucka & Cierpiszewski, 2014**).

The conception of starch films integrated with natural betalains from the abundantly available beetroot attempted to achieve a non-easy technological compromise in the recent field of smart packaging by getting, at low-cost, a high efficient and simple pH-sensitive patch, assuring on the top of that a decent standard of eco-friendship. The addition of betalains dyes composed for the most part of betacyanins, into starch-based films made them thicker, denser and damper. As well, pigmented films were more transparent with a higher permeation to water vapor penetration regardless their very close hydrosolubility to control films. Even though, their surface in presence of beetroot betalains was more hydrophobic, so less wettable.

Otherwise, betalains liberation from film matrix fitted perfectly with aqueous low-fat foods, and the degradation of this colored films in soil take barely three more days than their equals control films. The tendency of applying composite films starch/betalains as pH-sensor sticker on containers of ground chicken, conducted successfully to visible color shift going from clear red to dark purple passing by intermediate violet shades, conferring a reliable monitoring of the ammonia vapor emission and mirroring the progressive chicken spoilage. The occurrence of this color shift was simultaneous to that observed in vapor ammonia assay.

Enhancing the whole performance an especially the mechanical resilience of the designed smart patch, all along with the epitomizing of a marketable prototype by overcoming its technological flaws, abide to be envisaged as imminent insights for a possible large scale production.

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الجمهورية الجزائرية الديمقراطية الشعبية

وزارة التعليم العالي و البحث العلمي

جامعة تيسمسيلت

عنوان المشروع:

ملصق النشا/بيتالين الذكي لمراقبة صلاحية الدجاج المبرد المعطب

**Smart starch/betalains patch for “packed
refrigerated chicken” shelf life monitoring**

مشروع لنيل شهادة مؤسسة ناشئة في اطار القرار الوزاري 1275

صورة العلامة التجارية



الاسم التجاري

TIS-PATCH

السنة الجامعية

2023 _ 2022

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المحور الرابع: خطة الإنتاج والتنظيم

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المحور السادس : النموذج الاولي التجريبي

المحور الأول: تقديم المشروع

ملصق النشا/بيتالين الذكي لمراقبة صلاحية الدجاج المبرد المعب

Smart starch/betalains patch for “packed refrigerated chicken” shelf life monitoring

يمهد تطوير نظام تغليف مبتكر للمواد الغذائية المسمى بالتغليف الذكي لمراقبة التغييرات التي تحدث داخل المنتج الغذائي بدقة عن طريق تغير لونه تبعاً لدرجة فساد الطعام خاصة للحوم و الدجاج، مما يجذب اهتمام المستهلك لأنها تسهل حياته نظراً لخاصية اكتشاف درجة صلاحية وجودة الطعام أثناء تخزينها بشكل بسيط وفعال. بالإضافة لذلك، فالجدير بالذكر ان هذه المواد يمكنها المساهمة في حل الكثير من المشاكل البيئية المتعلقة باستعمال البلاستيك، باعتبار امكانية الحصول عليها من مواد طبيعية متوفرة، بأسعار غير مكلفة و بآليات جد سهلة مقارنة بالتغليف الكلاسيكي.

فكرة المشروع (الحل المقترح)

يهدف هذا المشروع الى تطوير ملصق استشعاري ذكي قادر على الكشف على تلف الاطعمة المحفوظة عن طريق تغير لونه، يعتمد في تكوينه على مواد اولية طبيعية موجودة بوفرة وبأثمان جد معقولة، كما انها قابلة للتحلل.

القيم المقترحة

يوفر هذا المشروع عديد الحلول في مختلف الجوانب:

1. علاج مشكل تلوث البيئة بالبلاستيك بتوفير بدائل طبيعية قابلة للانحلال في الطبيعة.
2. توفير تكاليف و اعباء التصنيع التقليدية للمشتقات البترولية البلاستيكية.
3. تسهيل مراقبة صلاحية الأغذية بطريقة عملية من طرف المستهلك ومساعدته في اختيار الاطعمة المناسبة.
4. تطوير شعبة التغليف الغذائي باستعمال هذه التقنيات الحديثة.
5. تسهيل تطبيق مفهوم المراقبة الذاتية للمصنعين والباعة.
6. منح خاصيتي التدخل المسبق-التتبع لمصالح مراقبة النوعية مع اضافة عامل الشفافية.

1. فريق العمل

يضم فريق العمل طالبي الماجستير 2 غاني شهيناز وعائيد مريم المتخصصتين في البيوكيمياء التطبيقية، تحت تأطير الاستاذ الرئيسي د. موساوي بدر الدين، استاذ محاضر -أ- المتخصص في التغذية والصحة والتكنولوجيا الغذائية واستاذين مساعدين، حناني طاهر طالب دكتوراه في تخصص التكنولوجيا الزراعية الغذائية ومراقبة النوعية و الدكتور قمو العيد استاذ محاضر -ب- المتخصص في علوم الطبيعة والحياة.

2. أهداف المشروع

تصنيع ملصقات ذكية تكون على الجهة الداخلية لبلاستيك علب حفظ الاطعمة "اللحوم والدواجن بالخصوص"، تتميز بالقدرة على استشعار تغير درجة الحموضة pH الناتج عن تلف هذه الاطعمة مما يدل على عدم صلاحيتها. تتم عملية التعرف هذه من خلال ملاحظة لون الملصق ثم مقارنته مع المؤشر (المفتاح) المرافق له والذي يمكن من اتخاذ القرار بشأن استهلاك الغذاء من عدمه.

يمكن هذا المشروع بالتالي من استثمار المنتوجات النباتية المحلية، حتى الملقاة منها على شكل نفايات ، بشكل ثوري ومبتكر، مع منح القيمة المضافة المرجوة وتوفير محتمل لمناصب شغل.

3. جدول زمني لتحقيق المشروع :

هذا المشروع هو محل بحث مستمر لغاية الساعة ويهدف الى الحصول على براءة اختراع كمرحلة اولية قبل الانطلاق في عملية التحجيم ودراسة السوق من اجل الوقوف على قابلية انتاجه الصناعي وتسويقه على مستوى واسع.

الزمن المقدر للحصول على 27 ملصق هو 48 ساعة باعتبار:

- الزمن المقدر لاستخلاص لتر واحد من المستخلص الملون الطبيعي (البيتالين) ساعتين.
- الزمن المقدر تحضير السائل المكون للفيلم الذكي 35 دقيقة.
- الزمن المقدر تجفيف الفيلم في الهواء الطلق 24 ساعة.
- الزمن المقدر لاستقرار الفيلم ساعة واحدة.
- الزمن المقدر لتشكيل الملصقات المستشعرة 20 دقيقة.

المحور الثاني: الجوانب الابتكارية

يملك هذا المشروع العديد من الجوانب المبتكرة القابلة للتجسيد بشكل مباشر ويسير:

- فتح مجال جديد في ميدان التعليب والتغليف خالق للثروة ومناصب الشغل ومانح للقيمة المضافة في ميدان الصناعة الزراعية الغذائية.
- الانطلاق في استعمال اللاصقات الذكية، الغير موجود تماما في الوقت الحالي في السوق الوطني، وتعميم العمل بها كطريقة معترف بها في مجال تعليم ووسم الاطعمة الى جانب بطاقة المعلومات المفروضة سابقا من طرف السلطات المعنية.
- الاستعانة بهذا النوع من الطرق الحديثة والجد بسيطة المعتمدة على مقارنة الالوان في الكشف عن الاطعمة التالفة ، مما يمكن جميع فئات وشرائح المجتمع، بما فيها الاطفال ومحدودي المستوى، من تحديد صلاحية المعلبات بشكل دقيق مما يجنب التسممات الجماعية.
- يمكن تطوير هذه التقنية لا لتشمل المزيد من المنتجات فقط(الحليب ومشتقاته، الخضار والفواكه، المواد المحولة)، بل لتتعدى ذلك الى باقي المجالات حتى الصناعية منها.

المحور الثالث: التحليل الاستراتيجي للسوق

على ضوء تحليل السوق تم تحديد مايلي:

- السوق المستهدف : الشركات والمصانع بما فيها الصغيرة والمتوسطة، الخاصة بتغليف وتعليب الدجاج واللحوم الطازجة المبردة، كما يمكن ان تشمل زبائن خواص حسب الطلب.
- القطاع المستهدف: يعتبر قطاع تحويل الاغذية بشكل عام الهدف الاساسي لهذا المنتج، بحيث يكون الانطلاق من شعبة اللحوم والدواجن ليتوسع بعدها تدريجيا لبقية الشعب كشعبة الحليب ومشتقاته تماشيا وتطوير المنتج.
- وقت التسويق: تماشيا والقطاع المستهدف، فان المنتج يسوق طول السنة مع امكانية زيادة في الانتاج خلال مواسم الذروة (رمضان الكريم، فصل الصيف...).
- الميزانية: سيكون سعر المنتج جد معقول وفي متناول المؤسسات المستهلكة ولن يمثل الا تكلفة اضافية بسيطة في الثمن الاجمالي للتعليب.
- المنافسون : لا توجد فئة منافسة لهذا المنتج باعتباره فكرة جديدة و مبتكرة كليا في السوق الجزائري ولا توجد له أي منتجات مشابهة على المستوى الوطني.

عنوان المشروع : ملصق النشا/بيتالين الذكي لمراقبة صلاحية الدجاج المبرد المعب

- استراتيجية المنتج: سوف تمنح هذه الملصقات افضلية تسويقية لمستعملها مع زبائنهم لإضافتها المزيد من الشفافية لمنتجاتها مع سهولة التعرف على تاريخ الصلاحية لكل الفئات بالاعتماد على مقارنة الالوان ببساطة.
- التسعير: سيعتمد السعر على اساس التكلفة الثابتة والمتغيرة للملصق مع زيادة هامش الربح
- الترويج: يمكن الاعتماد بشكل اولي على الاتصال المباشر مع المؤسسات لإقناعها بفعالية المنتج، كما يمكن الاستناد بمنصات التواصل الاجتماعي التي تعتبر من بين الطرق الجدة مؤثرة في الاعلان والوصول لأكبر قدر من المستهلكين.
- التوزيع: نظرا لطبيعة المنتج (وزن جد خفيف، عدم الحاجة للتبريد، سهولة التوضيب...)، فان عملية التوزيع ستكون في المتناول ولن تحتاج مركبات ثقيلة او خاصة.

المحور الرابع: خطة الإنتاج والتنظيم

تشتمل الخطة التنظيمية للإنتاج على النقاط المتسلسلة التالية:

- الحصول على براءة الاختراع كأولوية استعجالية
- تطوير وتحسين الملصق الذكي بتصحيح الاخطاء التقنية الممكن وجودها والبحث عن بدائل اكثر كفاءة
- الحصول على محل يمثل مقرا من اجل بداية عملية الانتاج على المستوى التجاري
- الحصول على آلة صناعية لهرس الشمندر واستخلاص مركز البيتالين
- الحصول على آلة صناعية لتحضير المزيج القابل لتكوين الفيلم
- الحصول على صينيات غير لاصقة مع نظام تجفيف هوائي لتسريع وتيرة الحصول على الفيلم الجاهز
- توفير مموين للمواد الاولية بشكل دائم يضمن استمرارية الانتاج الى غاية توفير مخازن لها

عنوان المشروع : ملصق النشا/بيتالين الذكي لمراقبة صلاحية الدجاج المبرد المعلب

المحور الخامس: الخطة المالية PLAN FINANCIER

مع الاخذ بالحسبان الاعباء التالية:

- ثمن 1 كغ من الشمندر الاحمر 60 دينار جزائري، علما ان كل كغ سيمنح 3 لتر من المستخلص الملون المركز. باستعمال الصيغة التجريبية 0.16 مل/سم²، فان 1ل يمنح 0.625م²، بما يعادل 1.875م² لكل 3ل (1كغ شمندر)
- ثمن 1 كغ من حمض الاسكوريك 480 دينار جزائري، 3 لتر من المستخلص الملون المركز تحتاج 30 غ من حمض الاسكوريك مقدرة ب 14.4 دينار جزائري.
- ثمن 1 كغ من النشا 200 دينار جزائري، مع الاخذ بعين الاعتبار ان 3 لتر من المستخلص الملون المركز تستهلك 150 غ فقط من النشا بسعر 30 دينار جزائري .
- ثمن 1 كغ من الغليسيرول 680 دينار جزائري، باحتساب ان 3 لتر من المستخلص الملون المركز تحتاج 60 غ بسعر 40.8 دينار جزائري.
- ثمن 1م² من الورق القابل للصق 353 دينار جزائري، أي 662 دينار جزائري لكل 1.875م²
- ثمن الطاقة المستهلكة لتحويل 3ل الى فيلم نهائي يعادل 3 ساعات استهلاك بما يساوي 12.5 دينار جزائري (معدل 100 دينار جزائري/اليوم)

يصبح السعر الاجمالي 162 دينار جزائري :

- كل ملصق يستعمل قطع فيلم بأبعاد $3\text{cm} \times 4\text{cm} = 12\text{cm}^2$
- الفيلم المشكل الكلي يسمح بتكوين 1562 وحدة ذات 12cm^2

وبالتالي فان الثمن المقدر للملصق المستشعر الواحد هو 0.1 دينار جزائري

المحور السادس : النموذج الاولي التجريبي

يتكون النموذج التجريبي للملصق الاستشعاري من مربع ورقي مضاعف 2سم X 2 سم يحوي الفيلم البيتاليني النشوي بشكل بيئي محاط بالطبقتين الورقيتين من الاعلى والاسفل، مع جهة ورقية قابلة للصق تثبت الملصق على الجهة الداخلية لغلاف التعليب. يجب أن يحتوي المربع على فتحة

عنوان المشروع : ملصق النشا/بيتالين الذكي لمراقبة صلاحية الدجاج المبرد المعب

وسطية تكشف الفيلم الذكي للعيان، محاط بدائرة لونية سميكة مطبوعة على الجهة الظاهرة للطبقة الورقية العلوية.

باعتبار ان العمر الافتراضي للدجاج الطازج المبرد المعب هو ثلاث ايام في درجة حرارة 4م، فان الدائرة المفتاحية المحيطة بالفيلم ستقسم الى ثلاث اجزاء بالوان مختلفة:

- الجزء الاول بلون الفيلم النظر الغير مستعمل (الوردي)، يطبع بجانبه كلمة " fresh طازج" للدلالة على تواجده بالفترة المثلى للاستهلاك.
- الجزء الثاني بلون الفيلم اثناء بداية التعفن الغذائي (البنفسجي الفاتح)، يطبع بجانبه كلمة " استهلك الآن eat now" للدلالة على تواجده بالفترة الضرورية للاستهلاك.
- الجزء الثالث بلون الفيلم في نهاية صلاحية المنتج (البنفسجي الداكن)، يطبع بجانبه كلمة " فاسد inedible" للدلالة على عدم صلاحيته للاستهلاك. يجب التخلص منه فورا.

الملحق رقم 04: نموذج العمل التجاري

