"LUCIAN BLAGA" UNIVERSITY OF SIBIU
FACULTY OF AGRICULTURAL SCIENCE, FOOD INDUSTRY AND ENVIRONMENTAL PROTECTION

DOCTORAL THESIS SUMMARY

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“LUCIAN BLAGA” UNIVERSITY OF SIBIU
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Obtaining, characterization and testing of bioactive films with applications in food industry, having corn starch as a base biopolymer

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Sibiu 2015

Keywords: Starch based films, Boactivity, food films.

CONTENTS

FOREWORD

The *paper "Preparation, characterization and testing of bioactive films with applications in the food industry based on corn starch biopolymer"* prezent a series of Infirmation regarding the food films based on corn starch.

The research is divided into two parts, namely Part I of the thesis wich concerns about bibliographic research, and Part II presents the experimental results obtained in the research.

Part I is structured in four chapters that focus on themes of interest in the thesis field. The second part is divided into three chapters describing the materials and methods, results obtained and the conclusions of the research.

In the section on results and discussions were approached four research directions, namely:

- The first subchapter present the results of the factorial design;
- In the second subchapter are presented the results of the most suitable film in terms of features taken from a factorial design wich was selected;
- The next five subchapter presents the results from acetylated corn starch combination with other six biopolymers (sodium alginate, sodium caseinate, xanthan gum, chitosan, carboxymethyl cellulose and k-carrageenan);
- The last two subchapters present the results of organoleptic analysis and microbiological examination carried out on samples of meat and cheese wrapped in four films selected in previous research (film 100% starch acetylated, film composed of 25% sodium alginate combined with 75% acetylated starch, film composed of 50% carboxymethyl cellulose combined with 50% acetylated starch and film composed of 75% k-carrageenan combined with 25% acetylated starch).

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INTRODUCTION

In recent decades,we often ask questions about the future (unfortunately increasingly darker) of our planet, given that there is continuity of this linear path to self-destruction. In parallel with the development of burgeoning technologies and increased wealth, held an intensification of human pressure with large negative effects, unwanted in the whole ecosphere with implications in kind by disrupting natural cycles, leading eventually to real ecological disasters.

A major category of waste is plastic (plastic bottles, packaging), whose production has recorded a massive increase, especially in recent years. It is well known that plastic does not degrade due to its nature that gives resistance to microbial attacks and falling oil in its composition represents a danger to marine animals when plastics end up in the oceans. Also for printing of plastic bags is being used a highly toxic metal: cadmium, which is released in the air by burning them. Under certain conditions, plastics that have reached nature can be broken into smaller fragments, making possible their ingestion by animals. Due tot his major problem nowadays has been put agreat emphasis on finding solutions for pollution prevention or remediation of the effects on the ecological systems. Therefore, the current trend is the production of biodegradable materials to replace conventional synthetic plastics.

An innovation of this is making food based films from biodegradable modified starch, and the production costs are very low. On the entire planet, starch is a continuous source which can also be recovered from waste water in the processing industry.

Current research on improving the properties of food based films from modified starch, showed that the structure can be incorporated into various antibacterial compounds which maintain the integrity of the food by protecting against moisture, oxygen and other foreign matter, thus ensuring longer life of the product. The desire to use biopolymers in all domains is given by their derivation from renewable and fossil fuel depletion.

SCIENTIFIC OBJECTIVES OF THE THESIS

The general objective of the thesis was “***Obtaining, characterization and testing of bioactive films with applications in food industry, having corn starch as a base biopolymer*”.**

In order to achieve the general objective have been proposed following specific objectives:

1. Optimization in obtaining food films based on corn starch.

2. Testing of physico-chemical, rheological and antimicrobial properties of obtained films
3. Optimization in obtaining starch based films combined with other biopolymers.

4. Testing by microscopic procedures the uniformity and miscibility of the components used in the production of films.
5. Testing the thermal behavior of the films obtained.

6. Verification of the preservation capacity of food products packaged in films.

7. Testing the biodegradability of the obtained films.

STRUCTURE OF THE THESIS

The PhD thesis entitled *"Preparation, characterization and testing of bioactive films with applications in the food industry based on corn starch biopolymer"* contains 198 pages, 73 figures and charts, 25 tables and 244 references.

This doctoral thesis is structured into two distinct parts, namely: literature review and experimental research.

- Documentary study which present:
  - starch modifying, properties and application, starch based food films;
  - biopolymers with application in food industry;
  - Theoretical Aspects regarding pathogenic microorganisms and lactic;
  - Theoretical aspects regarding essential oils;
- Own research that present:
  - Research organization with a clear description of motivation topic, research objectives, the factors included in the study and research organization;
  - Materials and methods with detailed description of the material to research and analytical methods used;
  - experimental result: results, discussions and conclusions;
  - final conclusions and perspectives for further research describing contributions;
  - bibliography and appendixes.

ACHIEVEMENT PLAN OF THE RESEARCH

I. OPTIMIZATION OF FILMS MADE OF 100% STARCH

Corn starch emulsion+ glycerol + acetic acid

- Factorial design of 2^2 type

Optimized starchy films

Physico-chemical and rheological analysis

- Rheometry
- Dry matter
- Ash
- Acidity
- Water activity

Biodegradation

II. SELECTION OF FILMS WITH THE BEST PROPERTIES

Film surface analysis

- SEM
- AFM

Incorporating essential oils in film

- Tea tree
- Basil
- Cedar
- Thyme
- Fennel
- Juniper
- Peppermint
- Cinnamon
- Grape seed
- Pine

Analysis of starch film bioactivity

- Lactic acid bacteria
- Mesophilic lactic acid bacteria
- *Aspergillus niger*
- *Bacillus cereus*
- *Penicillium crysogenum*
- *Salmonella anatum*
- *Staphyloccocus aureus*

III. OPTIMIZATION OF OBTAINING STARCH MIXTURES COMBINED WITH OTHER BIOPOLYMERS

Combining starch with other polymers in proportion of: *25:75, 50:50, 75:25,*

- Sodium caseinate
- Sodium alginate
- Carboxymethyl cellulose
- Xanthan
- K-Carrageenan
- Chitosan

Physico-chemical and rheological analysis

- Rheologic behavior
- Dry matter
- Ash
- Acidity
- Water activity

Testing uniformity by microscopic ways

- Macroscopic
- Electronic microscope (SEM)

Testing thermal behavior of obtained films

- Thermic stability
- Temperature resistance
- Melting point

Biodegrability

IV. PRESERVATION CAPACITY OF PACKAGED PRODUCTS IN FILMS

Selected films with optimal properties

Testing preservation capacity of food (meat and cheese) at thr temperature of 4°C and -15°C

- Sensory analysis
- Microbiological analysis

The influence of the type of packaging on sensory characteristics

- ANOVA Statistical analysis

6. MATERIALS AND METHODS

6.1. OBTAINING FILMS MADE OF STARCH 100%

Materials used in order to realize films composed of starch in different proportions were:

- Corn starch (Sigma Aldrich);
- Glycerol 99.8% (Kynta S.R.L.);
- Acetic acid 1N.

The two main features of starch film deemed important for film quality are related to the rheological behavior, together with its biodegradability. The main factors considered important (showing influence) on the characteristics of the starch film are the amounts of glycerol and acetic acid used in the production of the starch film.

To optimize the composition of starch films has been used a factorial experimental design. There were selected two variables: the amount of glycerol and the amount of acetic acid used for producing the starch film. In order to study their influence on the starch film quality has been used a factorial experimental plan of the order of 2^2, which is described in Table 2.

Table 2. Experimental design used to optimize starch film's composition

		DESCRIPTION			**LEVEL**		
					-1	0	+1
Entry sizes	variables	x_1	Amount of glycerol	%	2.5	5	..5
		x_2	Amount of acetic acid	%	2.5	5	7.5
	constants	X_3	Temperature	^oC	90		
		x_4	Amount of starch	%	5		
Response sizes		y_1	Starch gel viscosity	Pas			
		y_2	Biodegradation degree	%			
		y_3	Water activity				
				x_1		x_2	
Sample abbreviations			Sample 1	7.5	%	7.5	%
			Sample 2	7.5	%	2.5	%
			Sample 3	2.5	%	7.5	%
			Sample 4	2.5	%	2.5	%
			Sample 5	5	%	5	%

6.2. Obtaining films made of corn starch combined with other biopolymers

The polymers used were as follows:

- Corn starch (Sigma Aldrich origin)
- Sodium caseinate (Supremia Grup origin)
- Chitosan (Sigma Aldrich origin)
- Xanthan (Sigma Aldrich origin)
- Sodium alginate (Sigma Aldrich origin)
- K-carrageenan (Sigma Aldrich şi Supremia Grup origin)
- Carboxymethyl cellulose (Supremia Grup origin).

The share of the starch and the polymer used to make the films is shown in Table 4.

Tabelul 4. Share of starch and polymer

No.	Polymer	Corn starch		
		25%	**50%**	**75%**
1.	Carboxymethyl cellulose	75%	50%	25%
2.	Xanthan	75%	50%	25%
3.	Chitosan	75%	50%	25%
4.	Sodium alginate	75%	50%	25%
5.	Sodium caseinate	75%	50%	25%
6.	K-Carrageenan	75%	50%	25%

Starch, acetic acid (5%), glycerol (5%) and other biopolymers used (Sodium alginate, Sodium caseinate, xanthan, k-carragernan, chitosan şi carboxymethyl cellulose) were disolved separately and were heated for 1.5 hours, after which the solutions were combined, mixed and heated to 90°C for another 30 minutes. This timeframe is important because it provides starch gelation and homogeneous mixing of solutes. The next stage is to obtain films by pouring the mixture into Petri dishes which are left to dry for 48-72 hours in order to evaporate water and solvents.

6.3. Rheological behavior analysis

The rheological behavior of fluids can be analyzed if subjected to a constant shear rate. The behavior of a fluid can be judged on the basis of experimental data, plotting the variation of shear effort and shear rate. This curve represents the mathematical model of rheological behavior of the product (Mironescu, 2014).

It analyzed the rheological behavior of gels based on corn starch combined with various biopolymers (sodium alginate, sodium caseinate, xanthan, K-carrageenan, chitosan, and carboxymethyl cellulose) using two devices: rheometer rotation type Yield YR1 (Brookfield) and rheometer mixing type Viscotester VT 550 (Haake).

6.4 Physico-chemical characterization of corn starch based films

6.4.1 Determination of dry matter by weighing

Represents the total solid content substances that remain after evaporation of water in the product at a temperature of 110°C.

The dry matter content is calculated from the relation:

$$w = \frac{g_1 - g_2}{g_1 - g_0} \cdot 100$$

Where:

g_0= empty vial mass, in grams;

g_1= the vial’s mass prior to drying with the sample in grams;

g_2= the vial’s mass after drying with the sample in grams. (Mironescu, 2000)

6.4.2. Determination of starch film acidity

The acidity is given by the presence of acids in the raw material used and the acid added during the technological process of obtaining a film starch (acetic acid). In the treatment of starch with an acid, acid hydrolysis of the glycoside acting on the starch molecules, the starch molecules become smaller.

The relationship is determined by the degree of acidity of a product:

$$A = \frac{10 \cdot V \cdot K}{g}$$

Where: A - acidity expressed in degrees of acidity, in ml/100g;
V - the volume of sodium hydroxide solution consumed in the titration, in ml;
K - factor of titrating solution used in;
g - the analyzed sample weight in grams;

6.4.3.Determination of total ash content

The ash content refers to the residue obtained after calcination of the samples. When ignited, the organic substances are transformed into volatile substances and minerals remains as ash. Analysis of the ash component may be a means of verifying working conditions (Mironescu and Mironescu, 2000).

Total ash contentof product by mass is calculated with the relation:

$$C = \frac{g_1 - g_0}{g} \cdot 100$$

Unde g_1= ash crucible mass in grams;
g_0= empty crucible weight in grams;
g= mass of the sample taken in grams;

6.4.4. Determination of water activity

Water activity is a measure of the durability of a product in relation to certain types of damage. The free water in the product is crucial to the growth of microorganisms and toxin production.

$$a_w = \frac{p}{p_0} = n_w + n_s$$

Where: p = the vapor pressure of water at temperatures T;
p_0 = the vapor pressure of water in the atmosphere at temperatures T;
t_w = the equilibrium temperature of the system;
n_w = the number of moles of water;
n_s = the number of moles of substance.

6.5. Analysis of the film composition and topography of modified starch selected from the application of factorial design

Results obtained by using the device provides valuable information on the uniformity of structure, surface roughness and proof. It used electronic microscope (SEM) and atomic force microscope (AFM).

6.6. Structrulal analysis of acetylated starch based films combined with other biopolymers

In the sample preparation has been used a Sputter Coater device, Agar. Three deposits were made of gold, each with a thickness of 4 nm. The samples for examination in SEM (Scanning Electron Microscope Quanta 250, Fei) are mounted on conductive aluminum holders using double-sided adhesive strips carbon.

6.7. Determination of the antimicrobial action for the film selected following a factorial design

For the set of experiments in which to watch the action on blends of essential oils in solid lactic bacteria was used MRS agar culture medium (the abbreviation comes from its inventors: Man, Rogosa and Sharpe).

Microorganisms used in order to study the action of essential oils on lactic bacteria cultures are represented by:

- Mix lactic bacteria for the production of cheese
- Mix lactic bacteria for the production of mesophilic cheese
- Microbiological material (pure culture of *Bacillus cereus* - ATCC 14579, *Aspergillus niger* - ATCC 1015, *Staphylococcus aureus* - ATCC 12600, *Salmonella anatum*- ATCC 9270).

Pencillium crysogenum culture isolated in the laboratory (Moza el al., 2012).

Natural compounds used in the two experiences were 10 oils namely:

1. Tea tree (*Melaleuca alternifolia*)
2. Basil (*Ocimum basilicum*)
3. Cedar (*Cedrus deodara*)
4. Thyme (*Thymus vulgaris*)
5. Fennel (*Foeniculum vulgare*)
6. Juniper (*Juniperus communis*)
7. Peppermint (*Mentha piperita*)
8. Cinnamon *(Cinnamomum verum)*
9. Grape seed (*Vitis vinifera*)
10. Pine (*Pinus sylvestris*)

In order to observe the activity of biofilms impregnated with essential oils was used aromatogram method.

6.8. The analysis of the thermal stability of films based on acetylated corn starch in combination with other biopolymers

The procedure was as follows:

- Sample is weighed (1-6 mg);
- The aluminum crucible is sealed with a press;
- The crucible cap is punctured in order to avoid the formation of internal pressure;
- The sample is placed into the machine (Maia 200);
- Is being run analysis software (software Netzsch) in which previously have been set the operating parameters (temperature: 30-300 °C, heating rate: 10 °C/minute and the gas used is nitrogen with pressure of 40ml/min);
- Thermograms obtained are recorded/saved.

6.9. Sensory analysis of samples of meat and cheese wrapped in acetylated corn starch films

Following the achievement determinations, were previously selected four types of films that were tested for capacity in terms of preserving meat and cheese. The films selected to achieve sensory and microbiological analysis were:

- Film consisting of 100% acetylated corn starch;
- Film consisting of 25% sodium alginate combined with 75% acetilated corn starch;
- Film consisting of 50% carboxymethyl cellulose combined with 50% acetilated corn starch;
- Film consisting of 75% k-carragrenan combined with 25% acetilated corn starch.

6.9.1. Description and preparation, packaging and coding samples

Samples of meat (pork) and cheese were purchased from SC Metro Cash & Carry Romania S.R.L., having on the label their shelf life. After purchase samples were stored at 4°C according to the manufacturer's specification.

Samples of meat and cheese were portioned and packaged in films. All film samples were sterilized in advance using a UV lamp in order to eliminate existing microorganisms on the surface to contaminate meat and cheese samples.

Coding samples was conducted taking into account: sample type (meat, cheese), the type of film used, the temperature at which the sample was deposited and storability.

6.9.2. Heat treatment (refrigeration / freezing)

Cheese meat samples that were stored for a period of 16 days in an incubator ICP model 800 with cooling to -15ºC respectively 4ºC. Plateau was monitored by Celsius software. The research was conducted over 16 days during which to draw from four to four days samples which were subjected to sensory analysis. Optical observations are given in Annex 1.

6.9.3. Sensory analysis of meat

Sensory analysis of the meat was made in accordance with STAS 7585-75 which refers to sensory examination of meat from butchers animals (cattle, swine, sheep, goats), cut into specialized units and under veterinary supervision. The index evaluates each sensory quality using the scoring scale of 0 to 5 points.

Sensory examination is done on pieces of meat and consists of appreciation of the following features:

- aspect
- color
- consistency
- smell

6.9.4. Sensory analysis of Cheese

Sensory analysis of the cheese was made in accordance with SR 6345-1995 which refers to the sensory analysis of dairy products.

The evaluation of each sensory characteristics as described in this standard, by comparison with scales score from 0 to 5 points. The samples are brought to a temperature of 18°C ± 2°C.

Compact solid products are in the form of whole piece or sector, to assess appearance. The samples were subsequently cut into equal pieces and then evaluated.

6.10. Determining the type of packaging influence on sensory characteristics

ANOVA (ANalysis Of VAriance) is a comparison test, but has the advantage of simultaneously comparing more averages lots.

- H_0: Media samples tested from which parameters are equal.
- H_1: At least one of the media samples from which the parameters differ from one another.
- Result *p*, of the assay, provided by the program, has the same meaning as in the other tests, namely:
- If $p>0,05$ is accepted H_0 are not significant differences in materiality level of 95%.
- If $p<0,05$ is rejected H_0 are significant in level of 95%. At least two medias are significant.
- If $p<0,01$ is rejected H_0 in level of 99%. The difference is highly significant.
- If $p<0,001$ se respinge H_0. The difference is very highly significant.

6.11. Microbiological analysis of meat and cheese samples packaged in acetylated corn starch films

When determining the total number of germs (NTG) in this study was used incorporating adapted sowing method (Stancu et al., 2013). The culture medium was used for NTG was PCA (Plate Count Agar) and for determining the number of fungus and yeasts was Sabouraud. After an incubation period of 16 days, each sample was analyzed, yielding the microorganisms on its surface for sowing, in order to highlight differences in the growth of fungi and bacteria in the presence of different types of films tested on each different substrate and to verify the action for each type of film used on samples of meat and cheese.

6.12. Starch biodegradability of the film combined with other biopolymers

To achieve the objectives of this research is needed to determine the extent and conditions under which experimental film degrades under conditions as close to those found in nature.

This was achieved through an experiment conducted in standard conditions ISO 16929:2002 "Plastics – determination of the degree of disintegration of plastic material sunder defined composting conditions in a pilot-scale test", adapted to conditions provided in the laboratory.

7. RESULTS AND DISCUSSION

7.1. Optimizing the composition of starch films and their characterization

To optimize the composition of the film, it was aimed to identify the optimum content of acetic acid (as the modifying agent of starch) and glycerol (as a plasticizer) to obtain a uniform, stable film, with characteristics of rheological and physico-chemical good properties, low water content and high capacity of biodegradation.

7.1.1. The rheological behavior of gels consisting of acetylated starch

The regression equation obtained is in the form:

$$y_1 = 8{,}715 - 2{,}47 \cdot x_2 + 1{,}695 \cdot x_1 \cdot x_2$$

where y_1 is yield strength, x_1 is glycerol and x_2 is acetic acid.

The function obtained indicates the major influence of free variable (x_o), other than the analyzed variables. Of the two variables studied, the acetic acid has a negative influence on the flow, and the combined action of acetic acid and glycerol positively influences the rheological behavior of gels; glycerol does not have significant influence on these characteristics.

7.1.2. Biodegradation of films composed of acetylated corn starch

The regression equation obtained is in the form:

$$y_2 = 43{,}525 + 2{,}655 \cdot x_1 - 5{,}575 \cdot x_2 + 13{,}905 \cdot x_1 \cdot x_2$$

where y_2 is yield strength, x_1 is glycerol and x_2 is acetic acid.

Also in this case, the function obtained indicates the major influence of free variable (x_o), other than the analyzed variables. Of the two variables studied, glycerol positively influences and acetic acid has negative influence on the biodegradation of modified starch film; the combined action of acetic acid and glycerol strong positive influence mass decrease of films.

The decline of mass in most films is that given by samples made using intermediate values of glycerol and acetic acid.

7.1.3. The influence of acetic acid and the glycerol on water activity

Most of the samples have a low quantity of free water (a_w<40%), below the microorganisms needed for growth and multiplication. A low free water content in product,(a_w<0,60) confirms that the samples are microbiologically safe. The low content of free water does not permit the activation of microorganisms to produce altered starch film.

The regression equation obtained is in the form:

$$y_3=0{,}422-0{,}076 \cdot x_1+0{,}089 \cdot x_2-0{,}046 \cdot x_1 \cdot x_2$$

where: y_3 is the degree of water binding, x_1 is the amount of glycerol used, x_2 is the amount of acetic acid used. Both variables chosen are significant; in this case, glycerol has a negative influence characteristics analyzed.

7.1.4. The influence of the glycerol and acetic acid on the dry matter

A high amount of dry matter affects the elasticity and prevent plasticization of starch film.

The regression equation obtained is in the form:

$$y_4=87{,}925-1{,}825x_1+0{,}550x_2+1{,}300x_1x_2$$

where: y_4 is the amount of dry matter, x_1 is the amount of glycerol, x_2 is the amount of acetic acid. Also for this model, both variables chosen are significant; in this case, glycerol has a negative influence on analyzed characteristics, i.e. the dry matter content.

7.1.5. The influence of the glycerol and acetic acid on the acidity of the starch film

The regression equation obtained is in the form:

$$y_5=2,500+0,750\cdot x_1-0,250\cdot x_2+0,500\cdot x_1\cdot x_2$$

where: y_5 is acidity, x_1 is the amount of glycerol, x_2 is the amount of acetic acid.

Also in this case, both variables chosen are significant but glycerol has positive influence on acidity. Interestingly, the model obtained shows that acetic acid has negative influence on acidity of films.

7.1.6. The influence of acetic acid, glycerol on the ash of film

All samples had small amounts of minerals in their composition less likely in the sample in which has been used a large amount of acetic acid and a small amount of glycerol. Glycerol is a good plasticizer of starch, but low concentrations were unable to sufficiently plasticize the sample.

7.1.6. Conclusions

Factorial experiments aimed to identify the optimal composition of glycerol and acetic acid in the ranges chosen for the two variables, de 2,5 – 7,5%.

In conclusion, an appropriate quantity of glycerol and acetic acid (5%) results in formation of a harder, which is poured, spread well, allows the formation of a uniform film, stable, biodegradable, which can be stored as such for a long time as it is not attacked by microorganisms (very low water activity). Further in this research, for obtaining modified starch films were used glycerol and acetic acid in the concentrations of 5% on the total weight of the 5% suspension of starch.

7.2. Analysis of surface, topography and bioactivity of film composed of acetylated corn starch selected following a factorial design on lactic acid bacteria and pathogens

7.2.1. Surface analysis of modified starch-based films

The sample being analyzed (the film formed by the acetylated starch) showed a homogeneous structure from the point of view of the distribution of the components (starch, glycerol and acetic acid).

The sample exhibits little uneven areas which are incompletely gelatinized starch residues.

7.2.2. Analysis of topography of films from acetylated corn starch

Modified starch film shows relatively uniform and continuous surface. Analysis was performed in contact mode which shows some areas with sharp projections. These projections increase film roughness and thus appearance. The highest areas on the film surface were analyzed by 1.26 μm.

7.2.3. The action of essential oils on lactic acid bacteria used to obtain cheeses

On cultures of lactic bacteria used to obtain cheese, volatile oils showed different influences, some having an inhibitory action, other stimulatory effect. There were also volatile oils that have had no activity against these microorganisms.

The degree of inactivation of essential oils on the culture of lactic bacteria used to obtain cheeses can be reported as follows: cinnamon oil > thyme oil. The use of tea tree essential oil, basil essential oil, fennel essential oil, grape seeds essential oil was stimulatory.

7.2.4. The action of essential oils on mesophilic lactic acid bacteria used to obtain cheeses

Essential oils that have favored the development of mesophilic lactic acid bacteria were: tea tree, basil, cedar, fennel, juniper, mint and pine.

The essential oil which inhibited the growth of mesophilic lactic bacteria was the cinnamon.

Essential oils that do not have any action on the development of mesophilic lactic acid bacteria were the thyme and the grape seed.

7.2.5. Influence of peppermint essential oil incorporated into modified starch based film on pathogens

Peppermint oil had a medium inhibitory action on microorganisms:

Bacillus cereus (RI%=23%), Penicillum crysogenum (RI%=100%) și Staphylococcus aureus (RI%=23%).

On the development of the microorganism *Salmonella anatum* and *Aspergillus niger* peppermint oil had no effect.

7.2.6. Influence of cinnamon essential oil incorporated into modified starch based film on pathogens

Cinnamon essential oil inhibited all four tested microorganisms. *Aspergillus niger* și *Penicillium crysogenum au fost inhibați în proprție de 100%.*

In the case of *Bacillus cereus, Salmonella anatum* și *Staphiloccocus aureus* the medium rates of inhibition were: 58%, 47% și 23%.

7.2.7. The influence of fennel essential oil incorporated into modified starch based film on pathogens

Fennel essential oil showed an average rate of 100% inhibition on microorganisms: *Aspergillus niger* și *Penicillium crysogenum.*

On *Staphylococcus aureus* the medium rate of inhibition was 22%. *Salmonella anatum* throughout the testing period (21 days) was favored by this oil on the starch film.

7.2.8. The influence of thyme essential oil incorporated into modified starch based film on pathogens

The average inhibition of the thyme essential oil was the following on pathogens:

- 89% on *Aspergillus niger;*
- 33% on *Bacillus cereus;*
- 100% on *Penicillium crysogenum;*
- 39% on *Salmonellei anatum;*

- 44% on *Staphylococcus aureus.*

7.2.9. The influence of juniper essential oil incorporated into modified starch based film on pathogens

Juniper essential oil showed inhibitory action (RI% medie 100%) only on *Penicillium crysogenum.* For the other four microorganisms had no effect.

7.2.10. The influence of tea tree essential oil incorporated into modified starch based film on pathogens

The only totally inhibited microorganism in the presence of tea tree oil was *Penicillium crysogenum.* On the microorganisms *Aspergillus niger* and *Staphylococcus aureus* tea tree oil had no effect.

The films that was incorporated tea tree oil had an average RI% of 21% on the microorganism *Bacillus cereus* and on *Salmonella anatum* tea tree oil had a RI% of 10%.

7.2.11. The influence of pine essential oil incorporated into modified starch based film on pathogens

The microorganism *Penicillium crysogenum* was totally inhibited (RI%=100%) in the presence of pine oil.

The microorganism *Aspergillus niger* showed no change of growth and development in the presence of this essential oil, while *Salmonella anatum* was favoured. The average rate of inhibition obtained for the pathogen *Bacillus cereus* was 32% and on *Staphylococcus aureus* was 26% during the three observations.

7.2.12. The influence of cedar volatile oil incorporated into modified starch based film on pathogens

Development of selected microorganisms in the presence of the cedar essential oil was completely stopped on the pathogen *Penicillium crysogenum.*

On *Staphylococcus aureus* was observed an average inhibition of 22% during the analysis period. *Aspergillus niger* and *Bacillus cereus* were developed in the presence of the essential oil of cedar, while *Salmonella anatum* was favoured by its presence.

7.2.13. Influence of basil essential oil incorporated into modified starch based film on pathogens

The essential oil of basil in starchy films presented a total inhibitory action on the organism *Penicillium crysogenum.*

The medium rate of inhibition on pathogen *Staphylococcus aureus* was 12%and on *Bacillus cereus* only 17%. On *Aspergillus niger* was not observed any change. Only *Salmonella anatum* was favoured by the presence of basil essential oil.

7.2.14. Influence of grape seed essential oil incorporated into modified starch based film on pathogens

Grape seed essential oil showed an inhibitory action on microorganisms *Bacillus cereus* and *Penicillum crysogenum.*

On pathogen *Salmonella anatum* and on *Staphylococcus aureus* this oil has favoring action. On microorganism *Aspergillus niger* had no action.

7.2.14. Conclusions

The microorganism *Aspergillus niger* was inhibited by essential oils of cinnamon, fennel and thyme.

The microorganism *Bacillus cereus* was inhibited by essential oils of cinnamon, thyme, tea tree, pine and basil.

The microorganism *Penicillium crysogenum* was inhibited by all tested essential oils.

The microorganism *Salmonella anatum* was inhibited by essential oils of cinnamon, thyme and tea tree.

The microorganism *Staphiloccocus aureus* was inhibited by essential oils of cinnamon, fennel, thyme, pine, cedar and basil.

7.3. Determination of the rheological behavior of acetylated corn starch gels combined with other biopolymers

7.3.1. Determination of the rheological behavior of the gel consisting of 100% acetylated corn starch

The function obtained by the analysis of the rheological behavior of the gel consisting of 100% acetylated corn starch is:

$$y = 0{,}4777 \cdot x^{0{,}6577}$$

This feature indicates that the sample consists of 100% acetylated starch presented a pseudoplastic rheological behavior. Values of n less than 1 indicates that a rheological behavior of pseudoplastic type; The value of n is close to 0, is more pronounced pseudoplastic behavior (Steffe, 1996).

7.3.2. Determination of the rheological behavior of the gel formed by corn starch and sodium alginate

Functions Type Power Law obtained from analyzing rheological behavior of the gel consists of acetylated corn starch with alginate are:

$$y_1 = 0{,}1443 \cdot x^{0{,}9343}$$

$$y_2 = 0{,}1282 \cdot x^{0{,}8847}$$

$$y_3 = 0{,}0128 \cdot x^{1{,}0869}$$

Samples composed of 25% and 75% of sodium alginate showed weak pseudoplastic behavior and the sample composed of 50% sodium alginate had poor dilated behavior.

7.3.3. Determination of the rheological behavior of the gel formed by corn starch and sodium caseinate

Functions Type Power Law obtained from analyzing rheological behavior of the gel consists of acetylated corn starch with sodium caseinate are:

$$y_1 = 0{,}2104 \cdot x^{0{,}7613}$$

$$y_2 = 0{,}1597 \cdot x^{0{,}7193}$$

$$y_3 = 0{,}085 \cdot x^{1{,}0845}$$

Samples composed of 25% and 50% of sodium alginate showed weak pseudoplastic behavior and the sample composed of 75% sodium caseinate had poor dilated behavior.

7.3.4. Determination of the rheological behavior of the gel formed by corn starch and xanthan

The flow limit effort for the sample consisting of 25% xanthan sample was 40.46 Pa, and after application of the material forces, it began to run. The sample consisting of 50% xanthan mixed with 50% acetylated starch corn has a flow effort limit of 22 Pa.

Where 75% xanthan sample was combined with 25% acetylated corn starch presented different behaviors from the other two tests, namely, was a flow limit effort of 132.6 Pa.

7.3.5. Determination of the rheological behavior of the gel formed by corn starch and k-carrageenan

Maximum effort that stood the test consisted of 25% K-carrageenan and was 4.05 Pa and the sample composed of 50% k-carrageenan can withstand a drop of 11.04 flow limit effort Pa. The sample in which 75% k-carrageenan was combined with 25% acetylated corn starch presented different behaviors from the other two tests, namely, resisted an flow limit effort of 195.74 Pa.

7.3.6. Determination of the rheological behavior of the gel formed by corn starch and chitosan

The flow limit effort of the sample composed of 25% chitosan was 4.76 Pa and the sample composed of 50% chitosan was 34.08 Pa. The sample in which 75% chitosan was combined with 25% acetylated corn starch presented a similar behavior with the sample composed of 50% chitosan, with an effort of 44.28 Pa.

7.3.7. Determination of the rheological behavior of the gel formed by corn starch and carboxymethyl cellulose

The flow limit effort of the sample made of 25% carboxymethyl cellulose was 37.32 Pa. The sample consists of 50% carboxymethyl cellulose mixed with 50% acetylated corn starch was in a flow limit effort of 199.84 Pa, effort is very close to that of the sample consisting of 75% carboxymethyl cellulose (199.02 Pa).

7.3.8. Conclusions

The film composed of 100% acetylated starch showed a pseudoplastic behavior.

The gel consisting of sodium alginate showed weak pseudoplastic behavior (25% and 50% of sodium alginate) alginate gel composed of 75% showed a dilated poor behavior.

The gel consisting of sodium caseinate presented pseudoplastic behavior (25% and 50% samples) sample consists of 75% sodium caseinate presented a different behavior, namely poor behavior dilated.

When 75% xanthan sample was combined with 25% acetylated corn starch presented a flow effort limit of 132.6 Pa.

The sample in which 75% k-carrageenan was combined with 25% acetylated corn starch a flow effort limit of 195.74 Pa.

Of 50% chitosan gel mixed with 50% acetylated corn starch presented a maximum a flow effort limit of 34.08 Pa.

The sample consists of 50% carboxymethyl cellulose blended with 50% acetylated corn starch presented a flow effort limit of 199.84 Pa.

7.4. The analysis of films on the surface and structure of acetylated corn starch combined with different polymers

7.4.1. Film consisting in 100% acetylated corn starch

From a microscopic point of view, the surface is rough on the upper side of the 100% acetylated corn starch film. The lower face, the one that is in contact with the support is smooth, without visible roughness. The 100% starch film section is arranged in layers.

7.4.2. Film consisting in sodium alginate combined in various proportions with acetylated corn starch

The film consisting in sodium alginate combined in various proportions to acetylated corn starch is continuous and uniform from a macroscopic viewpoint. From the point of view of microscope samples made up of 25% and 75% have cracks in their structure. The film consisting in 50% of sodium alginate is continuous and uniform.

7.4.3. Film consisting in sodium caseinate combined in various proportions with acetylated corn starch

From macroscopic point of view sodium caseinate combined in various proportions with acetylated corn starch has formed a film because the structure has not kept when handled. From a microscopic point of view, films consisting of 25% and 50% sodium caseinate are smooth but have areas in which polymers have not blended, and polymers sections are arranged in layers. The film consists of 75% sodium caseinate was a rough and uneven.

7.4.4. Film consisting in xanthan combined in various proportions with acetylated corn starch

As for the films obtained showed microscopic air bubbles embedded in the polymer matrix after drying were sticky and difficult to detach from the surface of the support.

The sample composed of 25% xanthan combined with 75% acetylated starch is rough and non-uniform in all the three phases (the upper side, the lower side and in section), on a microscopic level.

The sample composed of 50% xanthan have small cracks in the surface which were encountered on the lower side. Instead the upper surface of the sample made up of 75% xanthan is more smooth and free of visible cracks.

7.4.5. Film consisting in k-carrageenan combined in various proportions with acetylated corn starch

Optical observations have revealed that the film obtained by combining k-carrageenan 25% with 75% corn starch, presented an increased fragility. This brittleness has been reduced in the sample obtained in 75% k-carrageenan combined with 25% corn starch.

Microscopic observations revealed the roughness and the uniformity of the film made from 25% k-carrageenan in all the three phases (the upper side, the lower side and in section). The same roughness can be seen in the section of the sample consisting of 50% K-carrageenan. Samples consisting of 50% or 75% k-carrageenan showed similar upper surface, smooth and continuous.

7.4.6. Film consisting in chitosan combined in various proportions with acetylated corn starch

From macroscopic point of view all three films obtained have shown in their structure areas where the chitosan has not completely dissolved.

Films consisting of 50% or 75% chitosan showed microscopic smooth and continuous surfaces. Instead sample composed of 25% chitosan embedded air in its structure. Section composed of 25% chitosan sample is not homogeneous, showing uneven areas. The other two films composed of 50% and 75% chitosan, the section showed that the layers are overlaid.

7.4.7. Film consisting in carboxymethyl cellulose combined in various proportions with acetylated corn starch

Macroscopic analysis shows that films made from 50% and 75% carboxymethyl cellulose have embedded air greatly in the polymer matrix, instead film composed of 25% carboxymethyl cellulose had a continuous and relatively uniform appearance.

Microscopic analysis of the films consisting of 75% carboxymethyl cellulose exhibited a smooth surface with cracks while the sample consisting of 50% carboxymethyl cellulose exhibited a poor mixing of the two polymers and the sample with 25% of carboxymethyl cellulose had large cracks on its surface.

In the section of the three films can be observed a relatively compact and uniform structure.

7.4.8. Conclusions

The analysis of the film surface consisting of 100% acetylated corn starch showed a continuous, uniform and compact film.

From the point of view of uniformity and continuity of the films it can be said that the film obtained by mixing 25% of sodium alginate and 75% starch is superior to the other two films analyzed and tested.

Following these observations it can be said that the film consisting of sodium caseinate combined with acetylated starch corn is not suitable for applications in the food industry.

The film consists of 75% K-carrageenan and 25% acetylated starch corn presents the best features compared to the other two films (25% and 50%) analyzed.

According to observation of chitosan film composed of acetylated corn starch and can highlight the high quality of the film composed of 50% chitosan combined with 50% acetylated corn starch.

Following these comments can highlight the quality of the film composed of 75% carboxymethyl cellulose combined with 25% acetylated corn starch.

Following this microscopic analysis may conclude that samples consisting of 25% xanthan and 75% acetylated corn starch had probably the smoothest surface, continuous and with the least air embedded in the polymer matrix.

7.5. Physicochemical characterization of obtained films

Humidity of the nineteen films ranged from 17.6467% for the sample composed of 75% sodium caseinate combined with 25% acetylated corn starch, and 30.1600% for the sample composed of 75% chitosan combined with 25% acetylated corn starch.

The quantity of ash obtained from the analysis ranged between 0.0433% for the film made up of 100% acetylated corn starch and 9.7752% for the film obtained from 75% sodium alginate mixed with 25% acetylated corn starch.

From the point of view of the acidity of the nineteen samples showed values between 0.6800 degree of acidity (film with a composition of 25% chitosan and 75% acetylated corn starch) and 8.2962 degrees of acidity (film with a composition of 25% xanthan and 75% acetylated corn starch).

From the point of view of water activity, samples showed values between 0.393 for the film consisting of 25% k-carrageenan combined with 75% acetylated corn starch, and 0.559 for the film obtained from a combination of 75% of sodium alginate with 25 % acetylated corn starch. In all nineteen samples the amount of free water is very small, which means that micro-organisms can not grow on the surface.

7.6. Thermal behavior of films based on corn starch combined with various biopolymers

7.6.1. Thermal behavior of the film consisting in 100% acetylated corn starch

Thermal changes of the film of starch begin at 35°C and the gelatinization starts at a temperature of 90°C, where the sample supports the heat (endothermic reactions occur), and is complete at 130°C. The next thermal process (crystallization) is carried out at a temperature of 148°C, then at 219°C the sample is completely melted (Naiaretti et al., 2016).

7.6.2. Thermal behavior of the films formed by acetylated corn starch and sodium alginate in various proportions

The process of gelatinisation of the three starch films is in a temperature range of 70-130°C.

The sample consisted of 25% of sodium alginate has recorded the highest crystallization temperature, namely 193°C, the melting temperature was 238°C. The sample 50% alginate had the most low crystallization temperature, namely 138°C, and the melting point was 236°C. The last sample composed of 75% of sodium alginate showed a crystallization temperature of 151°C and a melting temperature of 244°C (Naiaretti et al., 2016).

7.6.3. Thermal behavior of the films formed by acetylated corn starch and sodium caseinate in various proportions

The process of starch gelatinisation of the three films is in temperature range of 70-120°C.

The sample consisted of 25% of sodium caseinate had the most low crystallization temperature, namely 153°C, and the melting point was recorded at 237°C. The sample consisting of 50% sodium caseinate showed a crystallization temperature of 156°C and a melting temperature of 267°C. The sample consisting of 75% sodium caseinate combined with 25% acetylated corn starch showed a crystallization temperature of 160°C and the melting point was 270°C (Naiaretti et al., 2016).

7.6.4. Thermal behavior of the films formed by acetylated corn starch and xanthan in various proportions

The process of starch gelatinisation of the three films is in temperature range of 70-130°C.

The sample consisted of 25% xanthan combined with 75% acetylated corn starch and 50% xanthan combined with 50% acetylated corn starch showed the highest crystallization temperature, namely 160°C and melt temperatures were 268°C in the sample consisting of 25% xanthan and 255°C the sample consisting of 75% xanthan. The sample consisted of 75% xanthan combined with 25% acetylated corn starch showed a crystallization temperature of 149°C and a melting temperature of 236°C (Naiaretti et al., 2016).

7.6.5. Thermal behavior of the films formed by acetylated corn starch and chitosan in various proportions

The process of starch gelatinisation of the three films is in temperature range of 70-130°C.

The sample consisted of 25% chitosan combined with 75% acetylated corn starch recorded the highest crystallization temperature, namely 160°C and a melting temperature of 267°C. The sample consisted of 75% chitosan combined with 25% acetylated corn starch showed a crystallization temperature of 149°C and a melting temperature of 267°C (Naiaretti et al., 2016).

7.6.6. Thermal behavior of the films formed by acetylated corn starch and k-carrageenan in various proportions

The process of gelatinization of starch and processes of crystallization of the three films were not captured in these measurements due to the large amount of heat acceptance by the samples.

The sample consisting of 25% k-carrageenan showed a melting temperature of 110°C, the sample consisting of 50% of k-carrageenan has completely melted at 112°C and the film composed of 75% k-carrageenan at 109°C (Naiaretti et al., 2016).

7.6.7. Thermal behavior of the films formed by acetylated corn starch and carboxymethyl cellulose in various proportions

The process of starch gelatinisation of the three films is in temperature range of 70-130°C.

The sample consisting of 25% carboxymethyl cellulose combined with 75% acetylated corn starch recorded the highest crystallization temperature, namely 165°C and a melting temperature of 272°C. The sample consisting of 50% carboxymethyl cellulose has made in a crystalline transition temperature of 160°C and a melting temperature by 278°C. The sample consisting of 75% carboxymethyl cellulose showed a crystallization temperature of 146°C and a melting temperature by 269°C (Naiaretti et al., 2016).

7.6.8. Conclusions

As a result of DSC analysis of sodium alginate can be concluded that:

- The higher stability in terms of thermal behavior of the sample was obtained in the sample consisting of 25% of sodium alginate combined with 75% acetylated corn starch.
- The highest melting point (244°C) was recorded in the sample composed of 75% sodium alginate combined with 25% acetylated corn starch.

As a result of DSC analysis of sodium caseinate can be concluded that:

- The sample consisting of 75% sodium caseinate combined with 25% corn starch acetylated showed the best stability (160°C) and the highest melting point (270°C) from the point of view of thermal behavior.

As a result of DSC analysis of xanthan can be concluded that:

- The highest stability (160°C) from the point of view of the thermal behavior of the sample was obtained consisting of 25% and 50% of sodium alginate combined with 75% and 50% acetylated corn starch.

- The highest melting point (268°C) was recorded on the sample composed of 25% xanthan combined with 75% acetylated corn starch.

As a result of DSC analysis of chitosan can be concluded that:

- The highest stability (160°C) from the point of view of the thermal behavior of the sample was obtained combining of 25% chitosan combined with 75% acetylated corn starch.
- The highest melting point (267°C) was recorded at sample composed of 75% chitosan combined with 25% acetylated corn starch.

As a result of DSC analysis of k-carrageenan can be concluded that:

- The highest melting point (112°C) was recorded at the sample composed of 50% k-carrageenan combined with 50% acetylated corn starch.

As a result of DSC analysis of carboxymethyl cellulose can be concluded that:

- The highest stability (165°C) from the point of view of the thermal behavior of the sample was obtained on the sample consisting of 25% carboxymethyl cellulose combined with 75% acetylated corn starch.
- The highest melting point (278°C) was recorded at the sample composed of 50% carboxymethylcellulose combined with 50% acetylated corn starch.

7.7. Biodegradation of acetylated corn starch films in combination with other polymers

7.7.1. Biodegradation evolution of films composed of 100% acetylated corn starch over the 21 days of observations

Over the test period (21 days) film consisting of 100% acetylated starch has degraded at a rate of 81% compared to the original table, that indicates an accelerated decomposition.

7.7.2. Biodegradation evolution of films composed of sodium alginate combined with acetylated corn starch over the 21 days of observations

The tendency films consisting of 25% or 75% is to increase its weight within 7 days after starting the decomposition process. This trend has not had a film composed of 50% sodium alginate and has lost approximately 46% of mass after 7 days of test, and after another seven days has degraded completely.

The sample composed of 25% sodium alginate looses 65% of mass and the sample composed of 75% only about 29% after 21 days of observation.

7.7.3. Biodegradation evolution of films composed of sodium caseinate combined with acetylated corn starch over the 21 days of observations

All three films (25%, 50% and 75%) regardless of the ratio of starch and sodium caseinate were completely degraded within 7 days.

7.7.4. Biodegradation evolution of films composed of xanthan combined with acetylated corn starch over the 21 days of observations

All three films (25%, 50% and 75%), regardless of the ratio between the starch and xanthan were hydrolyzed to the gel state during the first 7 days of testing. This made it impossible to quantify the samples.

7.7.5. Biodegradation evolution of films composed of chitosan combined with acetylated corn starch over the 21 days of observations

The film composed of 25% chitosan lost 90% of the initial weight, the film composed of 50% chitosan has lost about 80% of the initial mass and the 75% chitosan film only about 31% after 21 days of testing.

7.7.6. Biodegradation evolution of films composed of k-carrageenan combined with acetylated corn starch over the 21 days of observations

The film composed of 25% k-carrageenan lost 75% of the initial mass and the film made of 50% k-carrageenan lost about 8% over a period of 21 days.

The film made up of 75% k-carrageenan increased its weight by about 60% compared to the initial mass after 21 days of testing.

7.7.7. Biodegradation evolution of films composed of carboxymethyl cellulose combined with acetylated corn starch over the 21 days of observations

None of the tested combinations of modified starch and carboxymethyl cellulose (25%, 50% and 75%) do not retain their structural integrity after 14 days of testing the biodegradability.

7.7.8. Conclusions

Films that were completely decomposed after seven days of testing were as follows: 75% carboxymethyl cellulose, xanthan and consisting of sodium caseinate, all samples.

Films that were completely degraded after fourteen days of testing were as follows: 50% of sodium alginate and 25% carboxymethyl cellulose.

Films that were split in a ratio of 50% or more within 21 days were 100% acetylated starch, 25% sodium alginate, 25% to 50% chitosan and 25% k-carrageenan.

Films that were split in a ratio of less than 50% within 21 days were 50% k-carrageenan, 75% chitosan and 75% sodium alginate.

The only evidence that increased their weight after 21 days of test was composed of 75% K-carrageenan.

7.8. Sensory analysis of samples of meat and cheese wrapped in acetylated corn starch films selected from analyzes conducted

7.8.1. Sensory analysis of meat samples during the test period

7.8.1.1. Sensory analysis of the control samples of meat over the test period at 4ºC and -15ºC

Meat samples stored at 4ºC showed degradation pursued sensory characteristics (appearance, color, consistency and odor) during the 16 days of test, process observed immediately after the first 4 days of storage.

For the meat samples stored at -15°C the sensory characteristics showed a deterioration in the quality from the 12th day of test.

7.8.1.2. Sensory analysis of samples of meat packaged in the corn starch film throughout the testing period at 4ºC and -15ºC

Samples of the meat packaged in the film comprises 100% starch and stored at 4°C have had quality deterioration of the product after 4 days of storage. The meat samples stored at -15°C followed sensory characteristics showed a deterioration in the quality from the 8th day of test.

7.8.1.3. Sensory analysis of samples of meat packaged in the sodium alginate starch film throughout the testing period at 4ºC and -15ºC

In the case of samples of meat packaged in the film composed of 25% of sodium alginate combined with 75% starch and stored at 4°C pursued sensory characteristics showed a degradation of the quality of the product after 4 days of storage.

If meat samples selected movie packed and stored at -15ºC pursued sensory characteristics (appearance, color, consistency and smell) remained satisfactory for 12 days.

7.8.1.4. Sensory analysis of samples of meat packaged in the k-carrageenan film throughout the testing period at 4ºC and -15ºC

Samples of the meat packaged in the film composed of 75% k-carrageenan combined with 25% starch and stored at 4°C and maintained sensory characteristics pursued 8 days of storage.

Samples of the meat packaged in the film composed of 75% k-carrageenan combined with 25% corn starch and stored at -15°C had good sensory characteristics over the entire test period (16 days).

7.8.1.5. Sensory analysis of samples of meat packaged in the carboxymethyl cellulose film throughout the testing period at 4ºC and -15ºC

Samples of the meat packaged in the film composed of 50% carboxymethyl cellulose combined with 50% starch and stored at 4°C and maintained only 4 days the sensory characteristics during storage.

Samples of the meat packaged in the film composed of 50% carboxymethyl cellulose combined with 50% corn starch and stored at -15°C have had good sensory characteristics in most of the test period (12 days).

7.8.2. Sensory analysis of the samples of cheese over the test period

7.8.2.1. Sensory analysis of the control samples of cheese over the test period at 4ºC and -15ºC

Control samples of cheese stored at 4ºC maintained good sensory characteristics only 8 days storage period, after which began the process of degradation.

Control samples of cheese stored at -15ºC showed very good characteristics on almost all sensory testing period (16 days), the look has changed just in the last analysis.

7.8.2.2. Sensory analysis of cheese samples packaged in the corn starch film throughout the testing period at 4ºC and -15ºC

Cheese samples packaged in film composed of 100% starch and stored at 4ºC maintained all the sensory characteristics only 8 days storage period, after which began the process of degradation in terms of appearance.

Cheese samples packaged in film composed of 100% starch and stored at -15ºC maintained their sensory characteristics only 4 days, after which only the color has not changed throughout the testing period.

7.8.2.3. Sensory analysis of cheese samples packaged in the sodium alginate film throughout the testing period at 4ºC and -15ºC

Samples of cheese packed in the film composed of 25% sodium alginate combined with 75% starch and stored at 4°C maintained all the sensory characteristics only 8 days of the storage period, after which it started the process of degradation in the appearance and odor.

Samples of cheese packed in the film composed of 25% of sodium alginate and stored at -15°C maintained their sensory characteristics of only 4 days, after which all entered into a downward process of degradation over the test period.

7.8.2.4. Sensory analysis of cheese samples packaged in the carboxymethyl cellulose film throughout the testing period at 4ºC and -15ºC

Samples of cheese packed in the film composed of 50% carboxymethyl cellulose combined with 50% starch and stored at 4°C maintained all the sensory characteristics within 12 days of the storage period, after which it started the process of degradation in the appearance.

Samples of cheese packed in the film composed of 50% carboxymethyl cellulose and stored at -15°C maintained their sensory characteristics 12 days, after which only the odor and appearance deteriorated.

7.8.2.5. Sensory analysis of cheese samples packaged in the k-carrageenan film throughout the testing period at 4ºC and -15ºC

Samples of cheese packed in the film composed of 75% carrageenan combined to 25% starch, and stored at 4°C maintained all the sensory characteristics during the entire period of storage.

Cheese samples packaged in film composed of 75% K-carrageenan and stored at -15ºC maintained all of the sensory characteristics throughout testing period (16 days).

7.8.2.6. Conclusions

Following this sensory research it can be concluded that almost all films tested are suitable for use as wrapping meat and cheese.

The film composed of 100% acetylated corn starch showed the weakest sensory characteristics of the test.

As for the score obtained after the sensory analysis the film composed of 25% sodium alginate combined with 75% acetylated corn starch it has ranked third in terms of their score.

A film with superior properties to those composed of 100% acetylated corn starch was 25% of sodium alginate combined with 75% acetylated corn starch and 50% carboxymethyl cellulose combined with 50% acetylated corn starch.

Ranked first in terms of the preservation of meat and cheese products stood the film composed of 75% K-carrageenan combined with 25% acetylated corn starch.

7.8.3. Influence of package type used on the sensory characteristics of meat and cheese samples

7.8.3.1. Influence of the type of packaging used on the appearance of meat and cheese samples

The greatest influence on appearance of meat and cheese samples in terms of the type of packaging used to preserve has the film composed of 75% K-carrageenan (F=12,812).

The level of significance is 0.00 that is below 0.01, so **appearance** is influenced significantly by the type of packaging used.

7.8.3.2. Influence of the type of packaging used on the color of meat and cheese samples

The greatest influence on the color of meat and cheese samples in terms of the type of packaging used to preserve has the film composed of 75% K-carrageenan (F=2,183).

The significance level for **color** is 0.079 which is more than 0.05, thus color is not significantly influenced by the type of packaging used.

7.8.3.3. Influence of package type used on the consistency of meat and cheese samples

The greatest influence on the consistency of meat and cheese samples in terms of the type of packaging used to preserve has the film composed of 75% K-carrageenan (F=2,170)

The significance level for consistency is 0.080 which is more than 0.05, thus **consistency** is not significantly influenced by the type of packaging used.

7.8.3.4. Influence of package type used on the smell of meat and cheese samples

The greatest influence on the smell of meat and cheese samples in terms of the type of packaging used has the film composed of 75% K-carrageenan (F=6,045)

The significance level for smell is 0.00 that is below 0.01, so the **smell** is significantly influenced by the type of packaging used.

7.8.3.5. Conclusions

ANOVA statistical analysis revealed that appearance, color, consistency and smell are influenced by the type of the package used for meat products and cheese.

From the point of view of the appearance of films made of 100% starch, 25% sodium alginate combined with 75% corn starch and 50% carboxymethyl cellulose combined with 50% acetylated corn starch, have a negative influence on samples of meat and cheese.

As for of color all four films tested resulted positive influence on meat and cheese samples throughout the testing period.

As for of consistency the only film that presented a positive influence was the one composed of 75% K-carrageenan combined with 25% acetylated corn starch.

As for of positive influence on the smell of meat and cheese samples only the film composed of 75% K-carrageenan combined with 25% acetylated starch presented these features.

7.9. Microbiological analysis of meat and cheese samples packaged in selected films

7.9.1. Determining the total number of germs (NTG)

In the case of meat samples packaged in the starch based films stored at -15°C and 4ºC the maximum number of bacteria has been found in samples packaged in the film composed of 25% of sodium alginate, followed by the film composed of 100% acetylated starch.

In the case of cheese samples packaged in the starch based films and stored at 4ºC the maximum number of germs has been found in samples packaged in the film composed of 100% acetylated starch followed by the film composed of 50% carboxymethyl cellulose. At -15°C most germs were found in the film sample composed of 100% acetylated starch followed by the film composed of 25% sodium alginate.

7.9.2. Determining the number of fungus and yeasts

Cheese samples stored at 4°C and -15°C packaged in the acetylated starch films combined with other biopolymers have developed a much smaller number of yeasts and molds on their surface compared with the control sample which was packed in film composed of 100% acetylated corn starch, which indicates a good protection from the microbiological point of view.

Only sample composed of 25% sodium alginate has developed a large number of yeasts and molds

Samples of meat and cheese wrapped in the film composed of 75% K-carrageenan combined with 25% acetylated starch corn did not develop at all yeasts and molds.

7.9.3. Conclusions

Samples of meat and cheese wrapped in the film made up of 100% acetylated starch had the highest total number of germs (132 CFU) for sample cheese stored at 4°C. This has remained the same for the number of yeasts and molds, we found a number of 50 CFU.

Samples of the meat and cheese packed in film consisting of 25% of sodium alginate 75% combined with acetylated starch had a total plate count of 80 CFU for meat sample stored at -15°C. In the case of the number of yeasts and molds, the highest value was obtained for the sample of the cheese stored at 4°C.

Samples of the meat and cheese packed in film made up of 50% carboxymethyl cellulose in combination with 50% acetylated starch the maximum number of bacteria (90 CFU) occurred in the cheese sample stored at 4°C. In the samples in which were determined the number of yeasts and molds were found a number 10 CFU in the sample of meat stored at -15°C.

Samples of meat and cheese packed in film made up of 75% k-carrageenan have developed a total number of germs in the amount of 28 CFU in the sample of meat stored at 4°C. In the case of the total number of yeasts and molds the highest value (20 CFU) was found in the sample of meat stored at 4°C.

8. FINAL CONCLUSIONS

1. Following optimization of the addition of acetic acid and glycerol in the composite film it was proved that the best properties in the composition is the one in which have been used acetic acid and glycerol in average amounts, namely 5%.
2. The research found that the strongest partial inhibiting action on lactic bacteria used to obtain the cheese is shown by the presence of the cinnamon essential oil, followed by thyme essential oil. The basil, juniper and pine essential oils, presented on all types of lactic bacteria a strong action favoring culture development.
3. As for of the action of essential oils tested in research on pathogenic microorganisms can conclude that the highest inhibitory activity has been met for cinnamon and thyme oil.
4. The research was carried out using six types of samples, each sample consisting of three sub-samples representing the films consisting of concentrated different biopolymers using (25%; 50%; and 75% biopolymer by weight of the film), using as a reference sample the 100% acetylated corn starch film.

From the point of view of rheological behavior can be concluded that:

- The film formed from 100% corn starch acetylated shows pseudoplastic behavior,
- The film composed of various concentrations of sodium alginate presented pseudoplastic behavior (50% and 75%) and dilator (25%),
- The film composed of various concentrations of sodium caseinate has presented a dilator behavior (50% and 75%) and pseudoplastic (25%),
- When 75% xanthan sample was combined with 25% acetylated corn starch presented a flow effort limit of 132.6 Pa.

- The sample in which 75% k-carrageenan was combined with 25% acetylated corn starch a flow effort limit of 195.74 Pa.
- Of 50% chitosan gel mixed with 50% acetylated corn starch presented a maximum a flow effort limit of 34.08 Pa.
- The sample consists of 50% carboxymethyl cellulose blended with 50% acetylated corn starch presented a flow effort limit of 199.84 Pa.

5. From the point of view of uniformity and structure of the film consisting of various proportions of acetylated corn starch in combination with other biopolymers can be concluded that the films consisting of sodium alginate, carboxymethyl cellulose and k-carrageenan showed the best uniformity.
6. As for physicochemical can say that all films fit in quality standards for measurements performed.
7. Analysis of the thermal stability showed the deconstructing and restructuring of the polymer - and highlighted the starch polymers from the mixture’s compatibility through inflection represented by DSC thermograms. Compatibilization was highlighted by gelatinisation and melting endothermic (peaks) which showed a high degree of morphological change of polymers, aspect indicated by the values of endothermic peaks obtained according time and temperature.
8. Upon researching the degree of decomposition it was shown that the majority of acetylated starch and biopolymers combinations with different concentrations, tend relatively similar degradation. Mass growth trend in the first 7 days, followed by a mass loss in the next 14 days.
9. The most efficient film tested in terms of preservation capacity and structural integrity of the fresh meat and cheese at 4°C and -15°C was represented by the film composed of 75% k-carrageenan combined with 25 % acetylated corn starch.
10. In terms of total microbiological germs that have developed on the surface of meat and cheese samples packaged in four films tested was between 132 CFU (cheese packed in film composed of 100% acetylated starch and stored at 4 °C) and 2 CFU (cheese packed in the film consists of 75% k-carrageenan and stored at -15 °C). From the point of view of the number of yeasts and molds maximum 70 CFU met the test cheese packed in the film 25% of sodium alginate and stored at 4 °C, the sample of the cheese stored at 4 °C did not develop any micro-organism.

9. PERSONAL CONTRIBUTIONS AND PERSPECTIVES FOR FURTHER RESEARCH

Personal contributions:

- Optimization of obtaining bioactive acetylated corn starch-based films;
- Characterization of obtained films in terms of rheology, physico-chemical and structural;
- The influence of different essential oils on lactic bacteria used in obtaining cheese and on pathogens;
- The characterization of the rheological point of view, physico-chemical, structural and thermal performance of films obtained from the combination with acetylated starch, sodium alginate, sodium caseinate, xanthan, K-carrageenan, chitosan and carboxymethyl cellulose;
- Making biodegradability analysis for films obtained;

- Testing the preservation capacity of meat and cheese products;
- ANOVA analysis study on the influence of films on the sensory characteristics.

Perspectives for further research:

- Incorporating essential oils or other bioactive natural compounds in the films that presented the best features to be tested and used as bioactive packaging.
- Testing the transferring of compounds.
- Testing of food films in other food groups.
- Testing elasticity, breaking strength and deformation.
- Effort resistance test under the action of temperature.
- Improving films properties using cold plasma treatment.
- The approach of other polymer proportions involved in obtaining corn starch based food films.
- The approach of other polymers with properties compatible with biodegradable, biocompatible, and bioactive plastic.
- Testing biodegradability of films also in aquatic environment.

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