Use of Biopolymers in Designing Edible Packaging Materials for Food Industry

Development of statistical models

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This study aims to develop biodegradable and edible materials based on hydrocolloids and to analyze their physical and mechanical properties for use as food packaging. Edible packings were obtained from a hydrocolloid matrix (agar, starch, sodium alginate), plasticized with glycerol in different proportions (33.33% and 16.7% from the total amount of polysaccharides used). The physical and mechanical properties were analyzed. The best results were obtained with agar- sodium alginate packing, plasticized with the highest amount of glycerol.

Keywords: hydrocolloids, ecosystem, hardness, eco packaging

Lately, there has been an increased interest in biopolymer packaging due to their benefits in use. In contrast to the conventional materials, they present broad spreading, regenerability, low cost, biodegradability and biocompostability, avoiding the environmental pollution (wastage of non-renewable natural resources, global warming or other problems due to the usage of petroleum based plastic packaging) as well [1-3] Due to consumer and industry demand for better quality food, less processed and preserved, with longer shelf-life, in recent years innovative food packaging material has emerged [4-7]. Consumers have become aware of the importance of the a safe diet (inadequately packed healthy food becomes a health hazard, especially if we take into account the additives from synthetic packaging used in order to improve the physical and chemical properties; these adjuvants contain dangerous compounds for humans and environment) [8, 9].

These intelligent packaging are able to extend the shelf-life and mantain the microbiological quality (the addition of inhibitory substances can prevent spores or proliferation of pathogens) [10-15], can improve the sensorial characteristics (by adding natural flavors or dyes), and prevent qualitative and quantitative losses (by adding antioxidants that prevent browning, and maintain freshness and texture) [16, 17]; they are a selective barrier to moisture transfer and are able to prevent lipid oxidation and oxygen access [18-20].

The present study sought to obtain biopackaging based on starch, agar, sodium alginate, plasticized with glycerol. In order to use these membranes as edible food packaging material for instant beverages, a range of physical and mechanical properties have been evaluated; the obtained results provides information about thier potential applications in food industry.

Experimental part

Agar powder was made available by the *B&V. The agar company* from Italy. Wheat starch, sodium alginate, and glycerol were purchased from Sigma Aldrich company. All products used for determinations are of high purity.

Membranes were obtained throught casting method, from mixtures of 3 g hydrocolloids, glycerol and water. A sillicone support was used to dry the membranes; the films were kept at ambiental temperature until complete drying. The thickness was measured using the Mitutoyo micrometer, and the result was expressed as an arithmetic mean of at least five readings in different areas of the film. To determine the retraction ratio was used the initial film thickness and the dry film thickness, according to Phan The et al. [21] The color's evaluation was made by CieLAB method, using the CROMA METER CR 400 colorimeter (Minolta, Tokyo, Japan). The result was expressed as the arithmetic mean at least five readings taken in different areas of biofilms.

For microstructure analysis, VEGA II LMU Electron Scanning Microscope was used. This equipment and Alicona software were used for roughness determination, as well. The roughness is an important parameter when films are intented to be use as packaging material.

Mechanical properties, tensile strenght, elastic modulus and hardness of the biofilms were determined. For the evaluation of the tensile strenght and elongation was used ESM 301 Mark-10 Texture Analyser; for Martens hardness evaluation was used DUH-211S Shimadzu microhardness tester. At present, one of the most advanced and fast material characterization in term of important mechanical strenght characteristics is the Martens instrumental hardness test. For this type of hardness, it can be determinated some important mechanical properties, such as: Martens hardness, elasticity modulus, deformation energy, plastic deformation, creep, but also cold work degree. For measurement of the indentation depth, the loading of force must be kept constant over time [22, 23, 31].

In order to be used at industrial level, these membranes must posses some characteristics. The hardness is an important mechanical feature in the subsequent mechanical processing of its shape and size if we take into account the product path, and, implicitly, the packaging from the manufacturer to the consumer. The main factor that influences the hardness of a mixture of hidrocolloids is the composition; in this case, the material is made from agar, starch, sodium alginate, and glycerol, in different proportions (33.3 and 16.7% of the total amount). The components of the hidrocolloids mixture are: agar (M_{ag}), starch (M_{am}), sodium alginate (M_{al}), glycerol (M_{gl}), and the *HMV* hardness value as a purpose function, (table 1).

For the elaboration of the empirical model of the investigated process we used the programming of the experiment in the factorial space, replacing the influence factors of actual values with encoded equivalent values.

SAMPLE	$m_{AGAR} (M_{ag})$, (g)	m_{STARCH} (M_{am}), (g)	$m_{SODIUM\ ALGINATE}$ (M_{al}), (g)	$m_{GLYCEROL}$, (M_g), (g)	HMV (N/mm²)
S₁	1	1	1	1	1.65
S₂	2	0	1	1	0.64
S₃	2	1	0	1	3.77
S₄	0	2	1	1	0.75
S₅	1	2	0	1	1.55
S₆	1	0	2	1	0.385
S₇	0	1	2	1	11.61
S₈	1.5	1	1	0.5	16.01
S₉	1	1.5	1	0.5	8.94
S₁₀	1	1	1.5	0.5	8.63

Table 1
FACTORS OF INFLUENCE USED FOR THE MATHEMATICAL MODELING

SAMPLE	AGAR $(M_{ag}=x_1)$	STARCH $(M_{am}=x_2)$	SODIUM ALGINATE $(M_{al}=x_3)$	GLYCEROL $(M_{gl}=x_4)$	HMV (N/mm²)
S₁	0	0	0	1	1.65
S₂	1	-1	0	1	0.64
S₃	1	0	-1	1	3.77
S₄	-1	1	0	1	0.75
S₅	0	1	-1	1	1.55
S₆	0	-1	1	1	0.385
S₇	-1	0	1	1	11.61
S₈	0.5	0	0	-1	16.01
S₉	0	0.5	0	-1	8.94
S₁₀	0	0	0.5	-1	8.63

Table 2
ENCODING THE INFLUENCE FACTORS USED TO COMPUTE THE MATHEMATICAL MODEL

In table 2, after coding (values between *-1* and *+1*), the minimum values of the membrane constituents are noted with *-1* (lower level), maximum values are noted with *+1* (higher level), and the arithmetic mean of the minumim and maximum values is the *0* level interval.

The type of the mathematical model

To explain the behavior of the studied system, it was used a first-order polynomial (rel. 1) in order to obtain the mathematical prediction model of hardness according to the mixture components [24].

$$P = a_0 + \sum_{i=1}^{k} a_i x_i + \sum_{\substack{i=1 \\ j=1 \\ i \neq j}}^{k} a_{ij} x_i x_{ij} \quad (1)$$

where *P* represent the hardness response value for each type of membrane, a_0 represent the intersection term, a_i represent the coefficients of linear and a_{ij} the coefficients of the interaction terms of the $x_1, x_2, x_3, x_4, x_5, ... x_{11}$ variables.

The domains and the levels of the independent variables used to obtain a predictive model of hardness are shown in table 3.

The calculation of mathematical model coefficients

The regression coefficients of the empirical model are calculated using equation (2) [25]:

$$a = (X^T \cdot X)^{-1} \cdot X^T \cdot Y \quad (2)$$

where *a* represent matrix regresion coefficients, *X* is matrix of encoded variables, X^T represent *X* matrix transposed, *Y* represent response values matrix.

All determinations were made in triplicate.

Results and discussions
Physical properties

The thickness of the membranes varied with the composition of the film-forming solution (table 4). Assays with high content in agar and starch (S1-S4, 2 g agar and 2 g starch) shows close values of thickness: 29.4 μm - 30.6 μm. The addition of sodium alginate (2 g) resulted in an increase in the film thickness: 35 and 40.6 μm, respectively. Regarding S8, S9, S10 samples, the decrease of the glycerol mass from 1 g to 0.5 g resulted in films with high thickness values, normal aspect if we take into consideration the literature: glycerol is a compound that improves film elasticity and flexibility, but decreases its thickness and strength.

Regarding the reatractio ratio, the highest value was identified for S3 (2 g agar and 1 g starch in the composition) - 96.18%, but similar values were observed at other samples, with no major changes. The retraction ratio is an important determination for the industry, as the final thickness of the membrane can be appreciated by the original thickness; the thickness of the final packing can be controlled, as well. Studies have shown that the films with agar in the composition have high rehydration ratios due to the agar gel syneresis over time. [26] Using the casting methods in order to obtain biofilms, the retraction ratio influences the final thickness of this membranes, and the other physical properties, as well (table 4).

The color varied depending on the ingredients used, so that S2, S3 and S8, with high agar content in the composition, showed a higher intensity of yellow color. The same trend is observed for samples with low glycerol content into composition (S8, S9, S10). Therefore, samples with 2 g and 1.5 g agar - 33.33% and 16.7% (S2, S3, S8) also have lower brightness compared to high starch samples (S4, S5). The high sodium alginate sample in the matrix (S7) showed the best brightness, intermediate values for *a** and reduced for *b**.

Mechanical properties

The highest roughness value was recorded for the high agar and non-starch sample (S2 - 55.17 μm, table 5). For the uniform solubilization of all ingredients, it is considered

Independent variables	Code	The variables level -1	The variables level 0	The variables level +1	Domain Δ = 2
AGAR (M_{ag})	x₁	0	1	2	2
STARCH (M_{am})	x₂	0	1	2	2
SODIUM ALGINATE (M_{al})	x₃	0	1	2	2
GLYCEROL (M_{gl})	x₄	0	0.5	1	1

Table 3
THE EXPERIMENTAL DOMAIN AND LEVELS OF INDEPENDENT VARIABLES

SAMPLE	RETRACTION RATIO (%)	THICKNESS (µm)	COLOR		
			L*	a*	b*
S1	95.72±0.22	33 ± 0.54	91.6±0.05	-5.45±0.03	15.4±0.2
S2	96.16±0.28	29.6 ± 0.82	90.2±0.78	-5.32±0.13	16.5±1.48
S3	96.18 ±0.19	29.4 ± 0.51	90.8±0.76	-5.16±0.11	16 ±0.98
S4	96.03±0.19	30.6 ± 0.62	92.3±0.38	-5.7±0.02	12.91±0.77
S5	96.11±0.24	30 ± 0.87	92.6±0.46	-5.55±0.04	13.47±0.56
S6	94.73 ±0.25	40.6 ± 0.94	90.6±0.67	-5.3±0.075	12.52±1.11
S7	95.46±0.2	35± 0.58	91.86±0.25	-5.5±0.02	14.02±0.27
S8	95.51±0.19	34.6 ±0.71	90.7±0.32	-5.33±0.03	16.7±0.45
S9	95.17 ±0.21	37.2 ± 0.64	91.25±0.55	-5.21±0.11	15.7±1.02
S10	94.80± 0.47	41.6 ± 0.67	90.42±1.07	-5.2±0.15	16.75±1.32

Table 4
DATA OBTAINED FOR THE EVALUATION OF THE PHYSICAL PROPERTIES OF THE FILMS
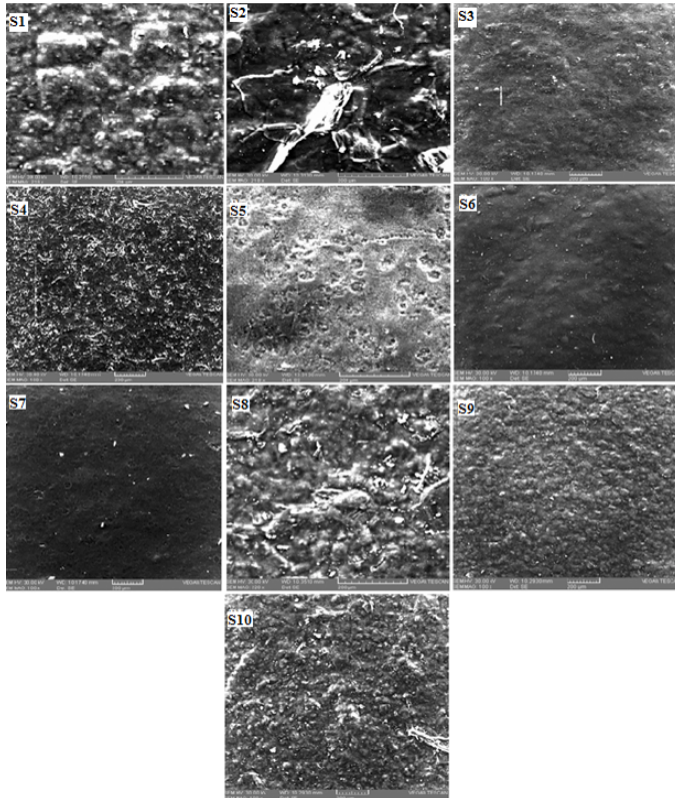
Fig.1. Images of the microstructure of samples, obtained using the TESCAN electronic microscope

necessary to use a high hydrophillic starch type, such as that obtained from potatoes. [27] From the images obtained (fig. 1), less glycerol amount samples showed a less regular matrix (S8, S9, S10), normal aspect if we consider the ability of the plasticizer to facilitate uniform, well-bonded membranes, aspect related throughout the literature (hence the necessity of using plasticizers).

Table 5 shows the values obtained and used for the mechanical characterization when the membranes were tested with both the Texturometer (TS, E) and the DUH-211S microhardness (HMV, E_{IT}). Both methods clearly establish the superior net resistance of S7 film obtained

with a high amount of sodium alginate (60% of total hydrocolloids) - 12.32 MPa and 11.61 N / mm², as also seen in the literature. [28, 29] The low-glycerol film (S8) indicates the strongest packing, a normal aspect if we take into account that the polyol, although improving its physical properties, has a negative effect on the mechanical properties.

As can be seen from SEM images, pronounced roughness occurs in samples with high agar content (S2); the smallest of those with higher amounts of sodium alginate (S6 and S7), but also those with 50% less plasticizer in the composition. A hidrocolloids mixture with a high amount of sodium alginate could be considered to produce uniform packings, as well as the average values obtained in the case of Rz (table 5).

However, differences appear to determine the elasticity, where the test performed with the texturometer indicates a higher value of it, unlike the one made with the microhardness equipment. Both determinations indicate S6 as the most elastic sample. Very good elasticity presents membranes with high agar content into the composition, but low strength. We can appreciate that the combination of agar with sodium alginate is suitable for the development of resistant and elastic packings due to their synergy.

It can be observed in figure 2, samples S2 (high agar content), S4 (high starch content) and S6 (high sodium alginate content) have the highest indentation depth. In figure 2, S3 (agar-starch), S7 (alginate-starch), S10 (high starch concentration) are the most resistant packings. We can note, however, that the only bond between these membranes is that they are composed of two hydrocolloids and a plasticizer. We can appreciate that, as added in the 1: 2 ratio, both agar and starch or sodium alginate result in the production of resistant packings, regardless of the combinations made. Biomembranes obtained from the mixture of three hydrocolloids losses this property (S8, S9, S10 - membranes made from starch-agar-sodium alginate have the lowest penetration depth value).

If we take into account the composition of S7 (2 g of sodium alginate and 1 g of starch), we can conclude that the sodium alginate-agar mixture produces films with higher elasticity, and good resistance as well.

| SAMPLE	TS (MPa)	E (%)	h_{max} (mm)	HMV (N/mm²)	E_{IT} (N/mm²)	ROUGHNESS R_Z (µm)
S1	1.14 ± 0.008	18.26 ± 0.11	3.3529	1.65	2.227e+001*	36.46 ± 0.85
S2	0.40 ±0.01	22.13 ± 0.41	5.3418	0.64	9.163e+000*	55.17 ± 0.94
S3	1.81±0.012	38.12 ± 0.02	2.2565	3.77	1.195e+002*	40.01 ± 0.32
S4	1.04 ± 0.01	8.26 ± 0.01	4.9739	0.75	1.076e+001*	47.09± 0.42
S5	1.21±0.001	10.61 ± 0.01	3.4456	1.55	2.815e+001*	40.47 ± 0.17
S6	0.60 ± 0.001	13.21 ± 0.05	6.8081	0.385	5.008e+000*	46.89 ± 0.35
S7	12.32 ± 0.01	11.57 ± 0.01	1.2127	11.61	1.738e+002*	39.19 ± 0.35
S8	13.35 ± 0.05	15.4 ± 0.3	1.077	16.01	2.333e+002	33.37 ± 0.74
S9	9.1 ± 0.01	6.87 ±0.02	1.3936	8.94	1.278e+002*	44.87 ± 0.13
S10	7.04 ± 0.01	25.81 ± 0.07	1.4717	8.63	1.220e+002*	44.91 ± 0.32

Table 5
MECHANICAL PROPERTIES OF TESTED SAMPLES
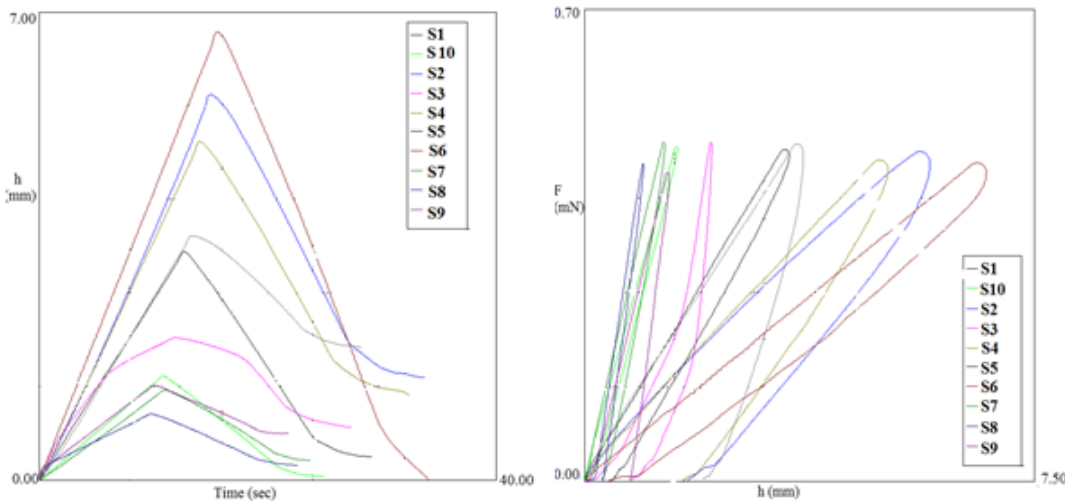
Fig 2. Graphic representation depth-time/ force-depth for tested packings

Statistical models

The testing of the model signifiance

The signifiance of the regression model coefficients is tested by comparing the absolute value of the coefficients with the confidence interval (calculated with Student test).

After calculating the Student test, the effects x_4 and x_3x_4 coefficients are insignificant and have been excluded from the mathematical model so that it becomes:

$$P = 1.65 + 20.12x_1 + 13.82x_2 + 13.96x_3 + 0.95x_1x_2 - 6.04x_1x_3 - 8.60x_1x_4 + 0.68x_2x_3 - 0.76x_2x_4 \qquad (3)$$

Testing the adequacy of the mathematical model

The testing of the adequacy of the mathematical model is made with Fischer test, which consist in reporting the data dispersion versus the regression model (the adequances dispersion, s_{conc}^2) to the dispersion of the experimental data versus the average (dispersion of data reproductibility, s_0^2). [30] The results of model adequacy testing are shown in table 6.

Fischer test was made for a $\alpha = 0.05$ (5%) level of trust and $\nu_1 = 10$ and $\nu_2 = 4$ freedom degrees. The calculated Fischer test value ($F_c = 0.0034$) has been compared with tabled Fischer test value ($F_{T(i1,i2)} = 5.912$). Because $F_c < F_T$, the mathematical model is adequate and can be used in optimization processes.

In figure 3a graphic we present a comparison of the hardness values of analyzed films, obtained experimentally, (blue color, first column) with those calculated using the regression model (red color, second column). At the same time, through the two graphical representations (fig.3.a,b), it can be noticed that the hardness values obtained with the mathematical model do not significantly differ from the values obtained by the experimental measurements with the DUH 211 Shimadzu microdurimeter.

These graphical representations and the small differences between the calculated hardness values with the mathematical model and the experimental values of hardness obtained by measuring with the microdurimeter demonstrates that the mathematical model obtained can be used to predict the hardness of the membranes made from a mixture of hidrocolloids, depending on the composition.

Further, the individual and the cumulative effects of the proposed variables using the graphical representations of the regression model resulting from the coefficient significance testing, called The Response Area (RSM) also are described.

The influences of membrane components on their hardness using the proposed mathematical model

According to the graph from figure 4, the membranes' hardness increases liniarly with both the increase in the *agar* and *starch* contents. The maximum hardness values are obtained to the maximum values of the *agar* (*2 g*) and *starch* contents (*2 g*), respectively. At the same time, the hardness is higher with the increase in the *agar* content, as shown in the graph.

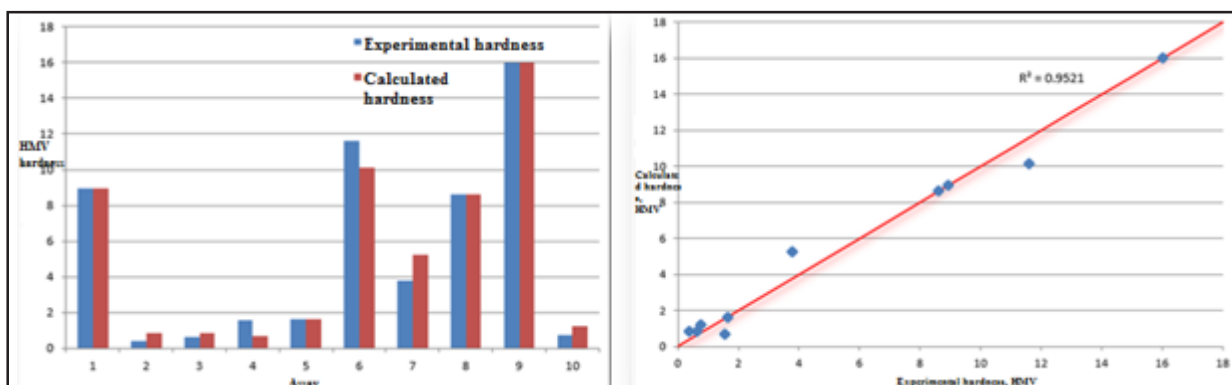| s_0^2	s_{conc}^2	Level of trust (α)	Degrees of freedom		Fischer test value	
			ν_1	ν_2	F_C	F_T
62.015	0.214	0.05	10	4	0.0034	5.91

Table 6
THE RESULTS OF MODEL ADEQUACY TESTING

Fig. 3.a/b - The comparison of the hardness values of analyzed films, obtained experimentally, with those calculated using the regression model
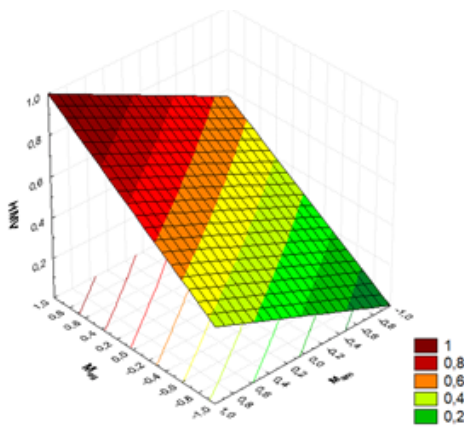
Fig. 4. The variation of the packing hardness obtained with the regression model according to the interactions between agar and starc. On X axis -1 value represent 0 g agar, and +1 value represent 2 g agar; on Y axis -1 value represent 0 g starch, and +1 value represent 2g starch; on Z axis 0 value represent 0,64 HMV and +1 value represent 16,014 HMV. The others parameters remains constant at the midpoint of the range (-1,1), and level 0, respectively (tab. 2)
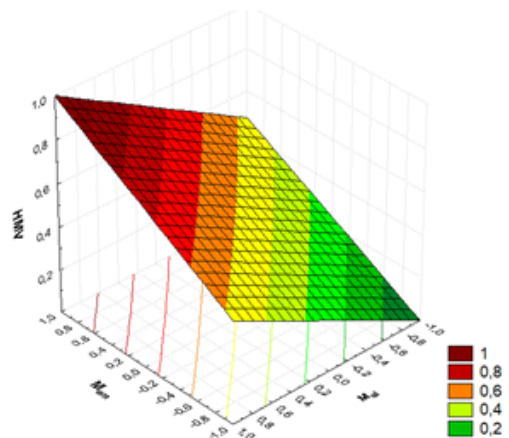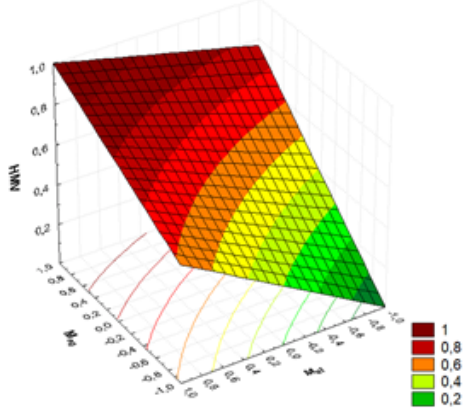

Fig. 7. The variation of the packing hardness obtained with the regression model according to the interactions between starch and sodium alginate. On X axis -1 value represent 0 g starch, and +1 value represent 2 g starch; on Y axis -1 value represent 0 g sodium algiante, and +1 value represent 2g sodium alginate; on Z axis 0 value represent 0.64 HMV and +1 value represent 16.014 HMV. The others parameters remains constant at the midpoint of the range (-1,1), and level 0, respectively (tab. 2).

Fig. 5. The variation of the packing hardness obtained with the regression model according to the interactions between agar and sodium alginate. On X axis -1 value represent 0 g agar, and +1 value represent 2 g agar; on Y axis -1 value represent 0 g sodium alginate, and +1 value represent 2g sodium alginate ; on Z axis 0 value represent 0.64 HMV and +1 value represent 16.014 HMV. The others parameters remains constant at the midpoint of the range (-1,1), and level 0, respectively (tab. 2)
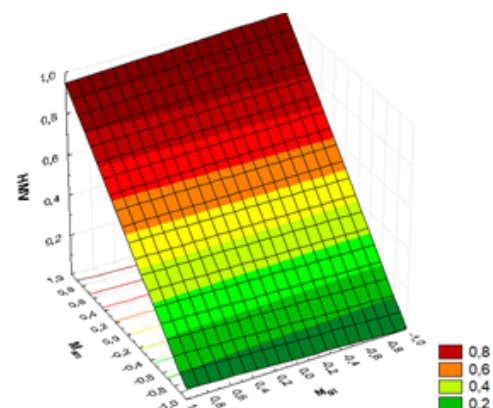

Fig. 8. The variation of the packing hardness obtained with the regression model according to the interactions between sodium alginate and glycerol. On X axis -1 value represent 0 g sodium alginate, and +1 value represent 2 g sodium algnate; on Y axis -1 value represent 0.5 g glycerol, and +1 value represent 1 g glycerol; on Z axis 0 value represent 0.64 HMV and +1 value represent 16.014 HMV. The others parameters remains constant at the midpoint of the range (-1,1), and level 0, respectively (tab. 2)

The packing hardness increases with the increase of *agar* and *sodium alginate* contents, (fig. 5). The maximum membrane hardness values are obtained to the maximum levels of *agar* (*2 g*) and *sodium alginate* (*2 g*).

A significant increase occurs when the *sodium alginate* concentration increases to maximum of *2 g* and the *agar* content is maintained at a minumum value of *0 g*. By increasing the *agar* content, the hardness value has a less significant increase compared to the increase in the *sodium alginate*. The membrane hardness increases with the increase in the *agar* content and decreases with increasing *glycerol* content (fig. 6). The maximum hardness values are obtained to the maximum levels of *agar* (*2 g*) and minimum *glycerol* content (*0.5 g*). The packing hardness increases liniarly with both the increase in *starch* content and *sodium alginate* content, (fig. 7). The maximum hardness values are obtained at the maximum *starch* content (*2 g*) and *sodium alginate* content (*2 g*), respectively.

At the same time, as shown in the graph, the increase in hardness is as significant as the increase in *starch* and
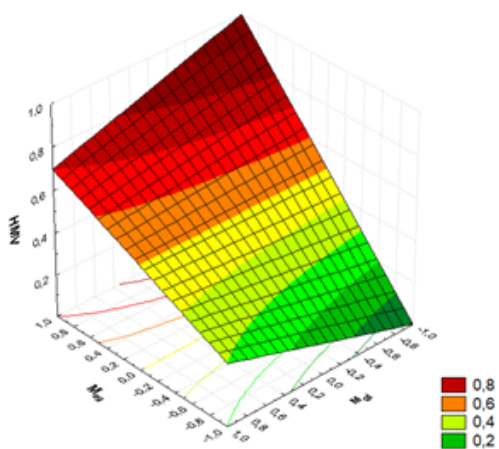

Fig. 6. The variation of the packing hardness obtained with the regression model according to the interactions between agar and glycerol. On X axis -1 value represent 0 g agar, and +1 value represent 2 g agar; on Y axis -1 value represent 0.5 g glycerol, and +1 value represent 1 g glycerol; on Z axis 0 value represent 0.64 HMV and +1 value represent 16.014 HMV. The others parameters remains constant at the midpoint of the range (-1,1), and level 0, respectively (tab. 2).

sodium alginate content. The packing hardness increases with the increase in the *sodium alginate* content and decreases insignificant with *glycerol* content, (fig. 8). The maximum hardness values are obtained to the maximum levels of *sodium alginate* (*2 g*) and minimum *glycerol* content (*0.5 g*). Furthermore, it can be observed an insignificant increases when the *sodium alginate* content is minimal (*0 g*) and the *glycerol* concentration has a maximum value (*1 g*).

Conclusions

The aim of this study was to test the membranes obtained from hydrocolloids in order to use them as edible packaging materials for instant beverages. Samples have shown that membranes containing a high amount of sodium alginate (S7) are more resistant, uniform, with medium roughness values. But they are less elastic. Samples obtained from mixtures of two hydrocolloids (ratio 1: 2) - S2, S4, S6 - showed good characteristics both in terms of physical and mechanical properties: uniform color, medium thickness, strength and elasticity, with the exception of S8 membrane obtained from equal mixtures of starch and sodium alginate, but 50% amount of the plasticizer - 0.5 g, unlike the other samples where 1 g of glycerol was used. The microstructure revealed a uniform membrane with low roughness in the case of packings obtained from equal or relatively equal amounts of hydrocolloids - S1, S8. Biomembranes made from mixtures of three hydrocolloids exhibit weaker characteristics, unlike those conceived from combinations of two hydrocolloids (agar-starch, agar- sodium alginate, starch-sodium alginate), which have good properties for use as packaging material, thus replacing conventional synthetic packaging.

The results obtained from the hardness research of packings made from a mixture of hydrocolloids using the mathematical model obtained were plotted according to their composition. These membranes' analysis highlights the significance increase in the membranes' hardness with the increase in agar, starch, and sodium alginate contents, and decreases with increasing glycerol concentration.

From the theoretical and experimental researches, it can be concluded that the hardness of the packings, which is an important mechanical feature in the subsequent mechanical processing of thier shape and dimensions, can be predicted with 95% probability using the mathematical model, starting with the concentrations of constituents.

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