Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XVIII, Issue 1 - 2019, pag. 5 - 12

FOOD AND ENVIRONMENT SAFETY

NEW TRENDS IN FOOD PACKAGING TECHNOLOGY: BIOPOLYMERS BASED MATERIALS ENRICHED WITH *STEVIA REBAUDIANA*

*** Roxana PUSCASELU[1], Sonia AMARIEI[1]**
[1]*Faculty of Food Engineering, Stefan cel Mare University,*
Suceava, Romania, roxana.puscaselu@usm.com,
*Corresponding author
Received 15th February 2019, accepted 28th March 2019

Abstract: *The purpose of this paper is the development of fully biodegradable and edible biopolymer materials used for the packaging of powdered food products. Stevia is a natural sweetener used in the food and beverage industry. The films were obtained through cast method, from agar and sodium alginate, plasticized with glycerol. Stevia was added in proportion of 1,25% of the total ingredients used. The films made with stevia added were fine, soft, transparent, odorless, but sweet, resilient and elastic. Their surface was free of cracks and, where there were pores, they did not pierce the material. Microbiological determinations and the absence of tested microorganisms indicate the safety of consumption of these membranes. The results indicate the possibility of using these materials to the detriment of the synthetic ones, as well as their transposition on an industrial scale.*

Keywords: agar, sodium alginate, biofilm, powdered product

1. Introduction

Nowadays, population growth and increased food consumption have led to the need to pack products in order to keep them for a longer time. But with this necessity, the industry has created and developed plastic packaging that is extremely damaging to the environment. [1-3] Studies have shown that even the first piece of plastic ever thrown can be found in nature. Besides the environmental problems and the impossibility of selective waste recycling, plastics can interact with the constituent compounds of the products they contain. A viable alternative to plastic packaging is the use of bio-packages entirely made of biopolymers. [4-7] They have advantages in use; are completely biodegradable and compostable, obtained from renewable resources, at low cost, are edible and can be enriched with various additives (essential oils [8], antioxidant substances, medicinal herbs, spices, natural dyes or flavorings, etc.). Edible films and coatings made from biopolymers have become a real alternative to conventional packaging materials. The importance of the use of films and coatings derives, in particular, from the advantages of their use: they extend the shelf life, improve the quality of fresh products, prevent the loss of nutrients, volatile compounds, maintain or even improve the appearance of the products they protect [9]; there are a selective barrier to moisture transfer, prevents access to oxygen and, implicitly, to lipid oxidation, but also to harmful microorganisms. In addition, the replacement of conventional packaging with edible or completely biodegradable

packaging has the effect of reducing environmental pollution. [10]
Polysaccharides are most commonly used to develop edible films because of their outstanding film forming capability. Polyols, such as glycerol or sorbitol, are especially used to plastify the films. Glycerol is often used as it improves physical properties and solubility, but modifies the mechanical properties of hydrophilic films; prevents the destruction of the films during handling or storage. It is a non-volatile substance that reduces the hydrogen bonding between polymeric chains, increases molecular volume, and improving film flexibility. [11]
Stevia (*Stevia Rebaudiana*) is a natural sweetener used in the food and beverage industry. Due to its chemical composition, it is considered to be the best substitute for sugar, especially for patients with diabetes [12-14], due to the chemical and nutritional composition; this is a good source of carbohydrates, proteins, fibers, minerals, amino acids, sterols, chlorophyll, organic acids and inorganic salts. [15], [16] The most important bioactive compounds are tannins and phenolic compounds alkaloids, flavonoids, that are able to improve and prevent many diseases. [17-20] Because of these benefits, stevia is the ideal substitute for sugar.
The purpose of the research was to obtain edible films from bioplymers, enriched with stevia. They can be used for packing instant drinks such as soluble coffee, capuccino (when replacing sugar in their composition), or for coating powdered mixtures of the type of dehydrated vegetables. They can also be eaten by adults, children, elderly people or special-purpose individuals. In this way, they successfully replace conventional, multi-component, composite packaging made from plastic and metal, difficult to select and recycle when they become waste.

2. Materials and methods

The biomaterials were obtained from agar, sodium alginate, and glycerol in variable proportions; the mass of the stevie remained constant (1,25% of total ingredients). Water was used to solubilize the constituents and to plasticize the biofilms. Except for agar (which was made available by B & V The agar company, Italy), the sodium alginate and glycerol were purchased from Sigma Aldrich Company. Stevia was obtained from local certified manufacturers. In this way, 30 samples were obtained by the cast method.
The main objectove of this survey is to obtain an intelligent material tested for use as edible packaging for dehydrated food.

2.1. Determination of microstructure, physical and optical properties; texture profile analysis evaluation.

In order to determinate the physical characteristics and microstructre of each membrane, a number of determinations were made, such taste or smell, adhesion to the drying surface, thickness, retraction ratio, and transmitance. Film transmission was identified with the Ocean Optics HR 4000 CG-UV-NIR spectrometer at 660 nm wavelength. An important property of the material intended for the packaging of products that may be affected by light is transmitance. To establish the roughness and observe the microstructure, the films were tested with the Mar Surf CWM 100 microscope (MahrSurf Company) and the images obtained were processed with the Mountain Map software. The texture profile analysis (TPA) - the adesivness, elasticity and rupure point were made with the Perten TVT 6700 textrometer (Perten Instruments of Australia). Specific test accessories, probe, perforated device, and 10 kg loading cell were used. The determinations were performed according to the standard method for gelatin sheets described in the user manual. [21]

2.2. The evaluation of mechanical properties.

The samples were tested for tensile strength and elongation at break by using ESM 301 - Mark 10 texturometer and the grips for thin films and films as attachments. For determination purposes, STAS ASTM D882 (Standard Test Method for Tensile Properties of Thin Plastic Sheeting) was used. [22] Tests were performed at ambient temperature of 24.4°C.

2.3. Solubility assesment.

To establish the suitable film for the development of a completely soluble and edible material, determinations were made in order to characterize the material from this point of view: moisture, water solubility, and water activity index. For moisture determination, film samples (3x3 cm) were weighed and maintained for 24 hours at 110°C. They were then reweighed, and the results were noted in the moisture calculation formula. Solubility in water implied the use of the same type of sample; thus, 3x3 cm pieces were cut, weighed, immersed in water for 8 hours (22°C temperature), dried in an oven for 24 hours at 110°C and reweighed. [23]

The water activity index (a_w) was determined using the AquaLab equipment at 22.8°C ±1.5°C. The value recorded represents the sum of five determinations.

2.4. Determining antimicrobial safety.

In order to be safe for consumption, the material must also be tested from a microbiological point of view. Thus, both the obtained films and the ingredients used were tested for the identification of coliforms, enterobacteria, *E. coli*, *Staphylococcus aureus*, and yeasts and molds. For this purpose, specific culture media Compact Dry type were used.

All determinations were made in triplicate.

3. Results and discussion

3.1. Characterization of the materials obtained.

The films obtained were soft, fine, thin, flexible, without pores or cracks into structure, with intense gloss, no odor, but sweet taste; exhibited low adhesion to the silicone support used for drying, had regular edges, and were pleasing to touch. From the point of view of physical characteristics, they can successfully compete with conventional packaging.

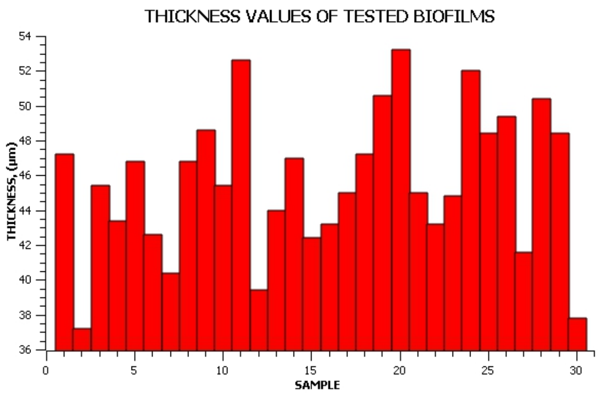

Fig.1. Values obtained for thickness determination

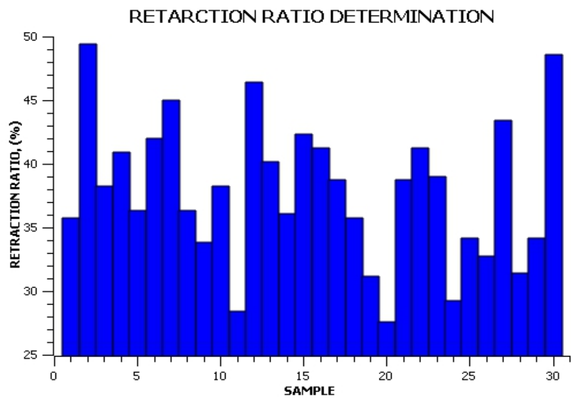

Fig.2. Values obtained for retraction ratio

Thickness was reduced, sample **S30**, with high glycerol content (1,50 g) into composition showed a value of 37,80 µm (fig. 1). The thickest film was **S24** (0.75 g glycerol), with a value of 52,00 µm. The retraction ratio (fig. 2) is an important parameter when the production recipe is used at industrial level so the results indicate the possibility of controlling the final thickness of the membrane (e.g., if a film with the ingredients and sample thickness **S1** is desired and the value of the withdrawal ratio is known - 35,78 μm in this case - then the manufacturer must pour the film-forming solution and level it so that it has an initial thickness of approximately 73,15 μm).

Sample **S2** (without agar into composition) was a very thin film, smooth, glossy and transparent, with no pores or cracks, no non-solubilized particles, regular and well defined edges, no odor, low sweet taste, low solubility, medium mechanical properties. It can be successfully used for packing whole or sliced meat products, cheeses, cut fruits and vegetables or other high moisture products. **S9** (with 1,25 g agar and 1,70 g sodium alginate) is a film with low transparency and gloss, but uniform, homogeneous, with regular edges, without pores or cracks in the structure. With good mechanical properties and high solubilitycan be used for packaging products with medium (reduced) moisture content or powdered products. **S12** (without agar into composition and 0,50 g glycerol) showed intense gloss, was transparent, soft, without pores or cracks, with regular edges, allows multiple bending, very flexible, odorless, slightly perceptible sweet taste, rapid total solubility. In terms of solubility, is best suited for packaging pulverulent products. Although the **S20** (high amount of agar) film is thick, unlike the other films, the film is flexible but with low gloss and transparency. High solubility promotes it to pack instant drinks, but requires improvements (increasing alginate content could be a solution). Medium mechanical properties. The **S24** sample (0,75 g glycerol) was a touchy, lightweight film with medium gloss, flexible, moderate sweet taste, odorless, very low solubility as opposed to other samples, reduced mechanical resistance but elasticity above average; can be used for packaging high-moisture or high-fat products or light-sensitive compounds (dyes, antioxidants, etc.). The images and microstructre of these films can be observed in fig. 5.

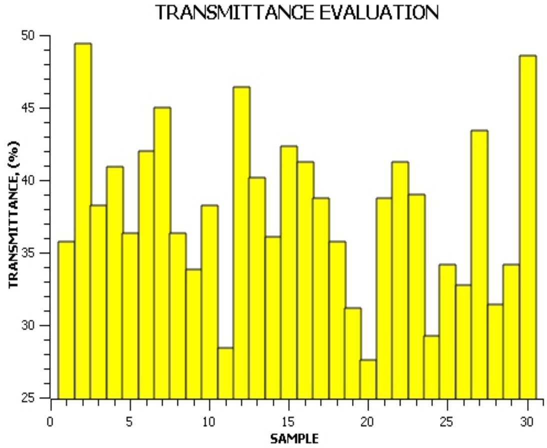

Fig.3. Values obtained for transmittance determination

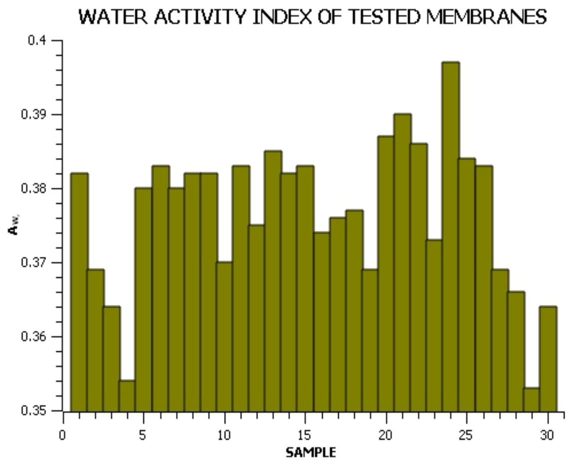

Fig. 4. Water activity index values for biofilms

Film transmittance is an important parameter when the packaged product can be degraded due to light radiation. Sample **S10, S20, S24**, and even **S28,** due to their low transmittance values, can be used as packaging materials for food products with high lipid concentration into composition (fig. 4). For the other biofilms**, S2, S12, S30**, with high vales of transmittance, the films can be improved by the addition of substances that can lower the values obtained, such as natural food dyes, for example. The table 1 shows the correlations that can be established between the thickness and the mechanical properties and texture profile of the membranes with stevia. Considerable positive correlations between thickness, breaking strength and rupture point are noted. Elasticity correlates negatively with roughness and adhesion. From the data obtained, it is not possible to establish clear connections between thickness and elasticity. Roughness correlates negatively with the break point, which is absolutely normal.

Table 1.
Pearson correlation of stevia-added biofilms

	T	TS	E	R	RP_{TPA}	A_{TPA}	E_{TPA}
T	1	0.90	0.10	0.35	0.70	-0.04	0.09
TS		1	0.34	0.24	0.20	0.12	-0.11
E			1	-0.76	0.04	-0.19	0.13
R				1	-0.15	0.14	0.20
RP_{TPA}					1	0.13	-0.18
A_{TPA}						1	-0.24
E_{TPA}							1

T – thickness, (μm), RT – tensile strength, (MPa), E-elongation, (%), R-roughness, (nm), RP_{TPA}- rupture point (TPA determination), A_{TPA} – adhesiveness (TPA determination), E_{TPA} – elasticity (TPA determination).

The complete solubility of samples **S2, S12, S26** and **S27** highlights the ability of sodium alginate to produce biofilm that is completely dissolved. Thus, rewinding was impossible, hence the lack of values in table 2. The other samples with agar in the composition retained their integrity even after 20 minutes immersion in water at ambient temperature (21 ± 2°C). According to the results obtained, we can conclude that sodium alginate has the ability to form films with higher solubility than agar (which prevents water absorption), although biofilms obtained from equal or relatively equal amounts of biopolymers (**S17, S18, S29, S30**) showed high solubility.

The moisture content increased directly in proportion to the increase in the amount of sodium alginate in the composition - **S2, S3, S19** (table 2). However, the maximum moisture content of sample S10, obtained from approximately equal amounts of agar, sodium alginate and glycerol, can be observed.

Table 2.
Solubility determinations of membranes with stevia into composition

Sample	Moisture (%)	Water solubility, (%)
S1	11.09 ± 0.08	40.24 ± 0.48
S2	13.70 ± 0.65	solubilization
S3	12.72 ± 0.57	62.85 ± 0.66
S4	8.00 ± 0.64	43.73 ± 0.41
S5	8.36 ± 0.81	41.24 ± 0.23
S6	11.53 ± 0.56	69.07 ± 0.33
S7	10.32 ± 0.70	48.70 ± 0.85
S8	10.89 ± 0.25	38.30 ± 0.50
S9	7.01 ± 0.30	61.13 ± 0.21
S10	15.96 ± 0.16	58.03 ± 0.18
S11	8.46 ± 0.36	54.61 ± 0.74
S12	11.12 ± 0.50	solubilization
S13	9.31 ± 0.34	51.60 ± 0.59
S14	7.56 ± 0.12	59.50 ± 0.75
S15	8.76 ± 0.36	65.01 ± 0.31
S16	8.51 ± 0.54	72.26 ± 0.96
S17	6.89 ± 0.46	86.60 ± 0.51
S18	8.33 ± 0.95	76.63 ± 0.48
S19	12.85 ± 0.70	63.12 ± 0.94
S20	9.71 ± 0.17	87.03 ± 0.64
S21	10.87 ± 0.91	91.00 ± 0.17
S22	7.22 ± 1.09	13.12 ± 0.80
S23	8.16 ± 0.38	12.83 ± 0.38
S24	8.47 ± 0.58	12.19 ± 0.92
S25	9.03 ± 0.81	11.93 ± 0.10
S26	11.67 ± 0.70	solubilization
S27	11.13 ± 0.43	solubilization
S28	8.94 ± 0.50	71.00 ± 0.41
S29	9.52 ± 0.91	73.81 ± 0.33
S30	7.11 ± 0.33	96.00 ± 0.92

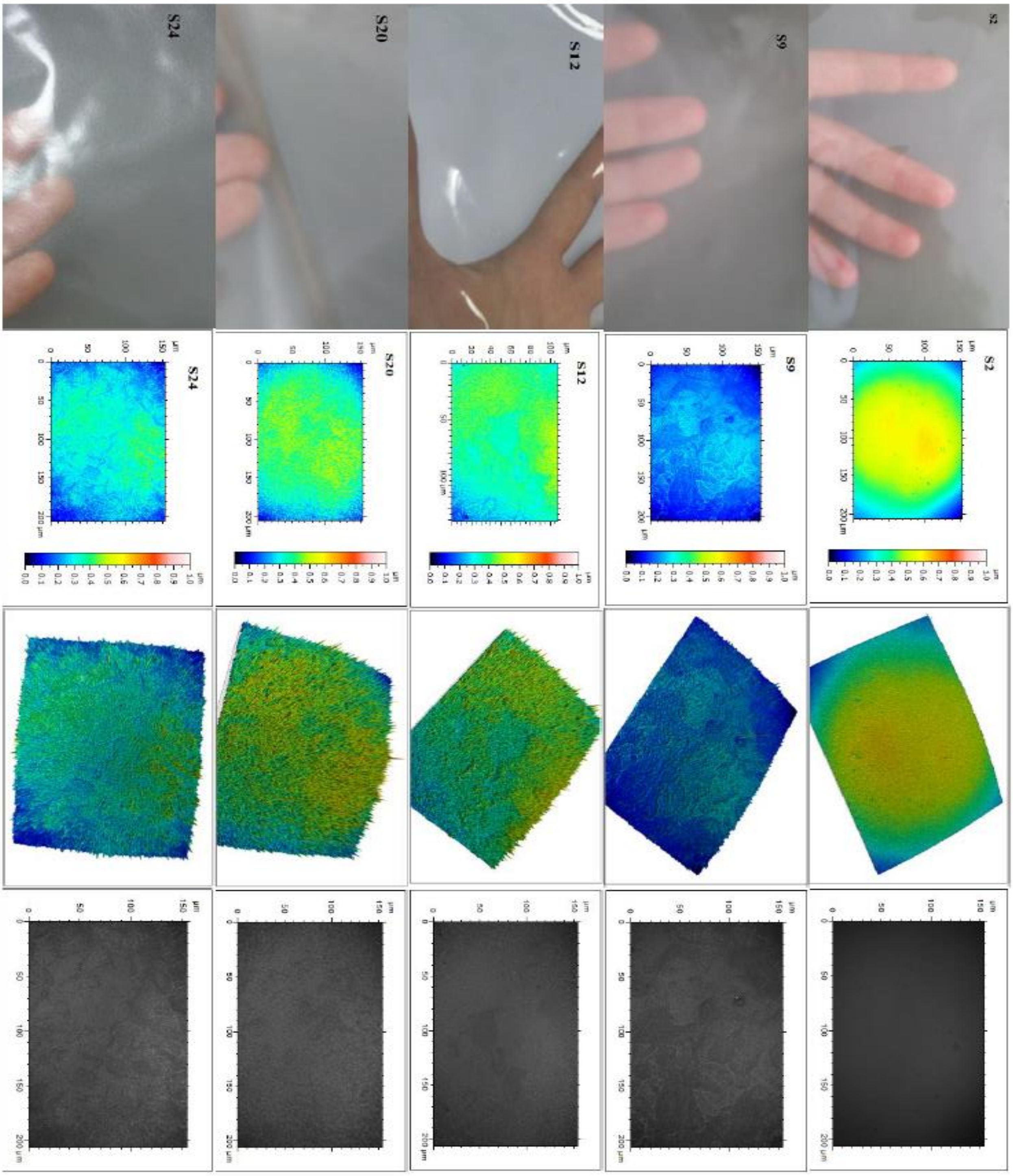

Fig. 5. Images and microstructures of stevia-added films

After microbiological testing, no microorganisms have been developed on the culture media. Thus, the consumption of this material is safe from the point of view of the incidence of coliforms, *Staphylococcus Aureus*, *Escherichia Coli*, yeasts and molds.
Reduced values of the water activity index (fig. 5) indicate the probability that on the surface of the membranes will not develop microorganisms on the entire shelf life.

4. Conclusion

Stevia is considered the best substitute for sugar, the benefits of consumption referring to the nutritional and biochemical composition. The addition of stevie has improved the physical characteristics of the material, which was transparent, fine, very glossy, with no odor, but sweet taste, with homogeneous microstructure, without pores or cracks. The presence of pores was identified on the higher agar-containing membrane in the composition; but it should be noted that the pores are at the surface of the material that has not been pierced. At the same time, solubility has increased, the desired aspect in this case. Though added in small quantities, stevia has moved from the relatively sweetener threshold to the ingredient ingredient of materials that can be used successfully in the food industry and other adjacent industries.
In conclusion we can remark the possibility of using stevia as an ingredient in biofilm production.

5. References

[1] MOORE, C.J., et al., A comparasion of Plastic and Plankton in the North Pacific Central Gyre. *Marine Pollution Bulletin* 42(12): 1927-1300, (2001);
[2] THAKUR, S., et al., Sustainability of bioplastics: opportunities and changes. *Current Option in Green and Sustainability Chemistry*, 13:68-75; (2018), https://doi.org/10.1016/j.cogsc.2018.04.013;
[3] WROBLEWSKA-KREPSZTUL, J., et al., Recent progress in biodegradable polymers and nanocomposite-based packaging materials for sustenaible environment. *International Journal of Polymer Analysis and Characterization*, 23(4):383-395; (2018), DOI: 10.1080/1023666X.2018.1455382;
[4] MAIZURA, M, et al., Antibacterial activity and mechanical properties of partially hydrolized sago starch – alginate edible film containing lemongrass oil. *Journal of Food Science*, 72(6): C324-C330, (2007), doi: 10.1111/j.1750-3841.2007.00427.x;
[5] CAGRI, A, USTUNOL, Z, RYSER, E., Antimicrobial films and coatings. Review. *Journal of Food Protection*, 67(4):833-848, (2004);
[6] YADAV, A., et al., Biopolymers as packaging material in food and allied industry. *International Journal of Chemical Studies*, 6(2):2411-2418, (2018);
[7] ELSABEE, MZ, ABDOU, E.S., Chitosan based edible films and coatings: A review. *Material Science Engineering*, C 33: 1819–1841, (2013); doi: 10.1016/j.msec.2013.01.010;
[8] PUSCASELU, R., AMARIEI, S., The antibacterial properties of seaweed biopolymer based films incorporated with essential oils, *Journal of Agroalimentary Processes and Technologies*, 23(3):157-163, (2017);
[9] ROJAS-GRAU, M.A., et al., Effects of plant essential oils and oil compound on mechanical, barrier and antimicrobial properties of alginate-apple puree edible films. *Journal of Food Engineering*, 81: 634-641, (2007); doi: 10.1021/jf061717u;
[10] RUIZ-NAVAJAS, Y., et al., In vitro antibacterial and antioxidant properties of chitosan edible films incorporated with Thymus moroderi and Thymus piperella essential oils. *Food Control*, 30:386-392, (2013);
[11] BORGES, J.A. et al., Influence of different starch sources and plasticizers on properties of biodegradable films. *International Food Research Journal* 22(6): 2346-2351, (2015);
[12] HOSSAIN, M.F. et al., Cultivation and uses of Stevia: a Review. *Afr.J.Food Agr. Nutr Dev*, 17(4): 12745-12757, (2017).
[13] PARKER, M., et al., Consumer acceptance of natural sweeteners in protein beverages. *Journal of Dairy Science*, 101(10): 8875-8889, 2018; https://doi.org/10.3168/jds.2018-14707;
[14] SURESH, V., et al., Uses of Stevia. *Journal of Medicinal Plant Studies*. 6(2):247-248, 2018;
[15] SAMUEL, P., et al., Stevia Leaf to Stevia sweetener: exploring its science, benefits, and

future potential. *The Journal of Nutrition*, 148(7):1186S-1205S, (2018), https://doi.org/10.1093/jn/nxy102;

[16] CIRIMINNA, R., et al., A bioeconomy perspective for natural sweetener Stevia. *Biofuels, Bioproducts & Biorefining*, (2018); https://doi.org/10.1002/bbb.1968;

[17] AMCHRA, F. et al., Effect of *Stevia rebaudiana*, sucrose and aspartame on human health: A comprehensive review. *Journal of Medical Plants Studies*, 6(1): 102-108, (2018);

[18] GHANDI, S., et al., Natural sweeteners: health benefits of Stevia. *Foods and raw materials.* 6(2):392-402, (2018), http://doi.org/10.21603/2308-4057-2018-2-392-402;

[19] RIZWAN, F., et al., Preliminary analysis of the effect of Stevia (*Stevia rebaudiana*) in patients with chronic kidney disease (stage I to stage III). *Contemporary Clinical Trials Communications*, 12:17-25, (2018); https://doi.org/10.1016/j.conctc.2018.08.007;

[20] UCAR, A., et al., A research on the genotoxicity of Stevia in human lymphocytes. *Drug and Chemical Toxicology*, 41(2): 221-224, (2018); https://doi.org/10.1080/01480545.2017.1349135

[21] Gelatin Leaf Burst Strength by Puncture, Perten Instruments Method Description TVT Method 71-04.02;

[22] STAS ASTM D882 -Standard Test Method for Tensile Properties of Thin Plastic Sheeting

[23] PUSCASELU, R., AMARIEI, S., The application of the Peleg model in order to obtain completely soluble materials for food product packaging. *Journal of Applied Packaging Research*, 10(1):98-106, (2018).