Studii şi Cercetări Ştiinţifice
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

2019, **20** (2), pp. 265 – 275

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry

ISSN 1582-540X

ORIGINAL RESEARCH PAPER

CAROB POWDER AND ITS POTENTIAL APPLICATIONS IN EDIBLE BIOPOLYMERS - BASED FILMS FOR FOOD INDUSTRY PACKAGING

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Received: January, 11, 2019
Accepted: June, 14, 2019

Abstract: The aim of the present research was to develop a new packaging material for powdered food products, such as instant beverages or dried vegetables, enriched with carob powder. Due to its proprieties can successfully replace cacao, and can be consumed by both adults (with or without special needs), but also by children. Packaging made from biopolymers is completely edible and can be consumed with the product which contain due to its full solubility in hot water (30 seconds maintaining in water at 80 ºC). The addition of carob into the film composition has improved the physical and mechanical properties of the control samples (without carob powder). The obtained results successfully qualify these materials for use as food packaging material and not only.

Keywords: *hydrocolloids, Ceratonia Siliqua L., material, edible, ecosystem*

INTRODUCTION

Hydrocolloids are an important part of the daily diet, being found in various products due to their functional properties. The main feature is the ability to bind the water molecule, thereby changing the properties of the food ingredients; changing rheological features also helps improve sensory properties [1, 2]. They are special matrices capable of retaining into the structure many other substances that can enrich or attribute new properties to the materials [3, 4]. In addition to these attributes, hydrocolloids are successfully used due to their high fiber content. According to the latest definition of food fibers, presented in the Codex Alimentarius (ALINORM 09 /32 / A), most food hydrocolloids can be categorized as food fibers.

The carob tree (*Ceratonia siliqua L*), also called locust, belongs to the *Fabaceae* family and grows in multiple areas of the Mediterranean region. Bean-like carob fruits are milled, similar to flour, commonly found in stores and supermarkets [5]. The chemical composition of this product has been extensively studied, especially due to the increasing demand. It has increased sugar content (over 50 %), of which over 75 % is sucrose. It also contains an appreciable amount of fiber (over 40 %), proteins (2 - 7 %), and mineral substances such as potassium, calcium, phosphorus and magnesium, but low amounts of fat [6, 7]. Thus, the carob is a natural flavor and chocolate-like sweetener. This is very important if we take into account that, unlike chocolate, the carob is devoid of caffeine and theobromine (stimulants) but also oxalic acid (a potential source of kidney stones) [8]. Low fat and sodium content promotes it as a healthy food ingredient. Lately, research has turned to use this product as a source of bioactive natural compounds. Carob fiber and polyphenols have many benefits for human health: reducing LDL cholesterol in patients with hypercholesterolemia, showing blood glucose regulating effect, beneficial effect on body weight, and improving digestion [9 – 12].

This study aimed at designing an edible packaging made entirely of biopolymers, enriched by the addition of carob powder, used to pack instant drinks for adults and children. Carob-containing foods maintain satiety (important for reducing obesity), lower cholesterol, reduce cardiovascular disease and diabetes [13] and are also for cocoa allergy sufferers [14]. Moreover, the research has shown that the addition of carob leads to the extension of the shelf life, showing the antimicrobial activity against *Listeria monocytogenes*; its ability to retain water helps increase shelf life (it can hold up to 3 times the volume of water, which reduces the proliferation of microorganisms due to free water.) [15].

Besides other aspects, this type of edible packaging encourages the reduction of household waste. The amount of waste should be reduced to protect scarce resources and to ensure as much food as possible for the whole of humanity [16, 17].

Last but not least, this is an attractive package for the entire population, an important aspect for the producer / trader and the consumer [18, 19].

MATERIALS AND METHODS

Biofilms were made using sodium alginate, wheat starch and glycerol purchased from Sigma Aldrich Company, and agar, which was made available by the company "*B & V*

The agar company", Italy. The carob powder was purchased from local traders. The coatings were obtained by the cast method from various proportions of biopolymers, glycerol and carob (10 and 20 % respectively), in a total mass of 9 g and a volume of 350 mL of distilled water. The solution thus obtained was maintained at 90 °C for 30 minutes under vigorous stirring. The next step was pouring it onto the silicone support surface and maintaining it at ambient temperature until complete drying (about 48 hours). The recipe for obtaining is described in the following Table 1.

***Table.1**. The ingredients used to design edible films*

SAMPLE	m_{AGAR} [g]	m_{STARCH} [g]	$m_{SODIUM\ ALGINATE}$ [g]	m_{CAROB} [g]	$m_{GLYCEROL}$ [g]	m_{WATER} [mL]
A1C	-	2	4	-	2	350
A1R	-	2	4	1	2	
A2R	-	2	4	2	2	
A3R	-	2	4	1.5	2	
A4C	1	2	3	-	2	
A4R	1	2	3	1	2	
A5R	1	2	3	1	2	
A6R	1	2	3	1.5	2	

The physical properties of biofilms

Their adhesion to the support surface, the external appearance, the taste, and the smell were evaluated. The color was determined by the CieLAB method using the Chroma Meter CR 400 colorimeter (Minolta, Tokyo, Japan). The thickness of the films was evaluated using the Mitutoyo digital micrometer. Both color and thickness were established as arithmetic mean of at least five different areas. The microstructure of the films was observed using the Motic Microscopes digital microscope (X40).

Determination of solubility

The evaluation is important when the film is used to make packaging materials, as its destination can be appreciated: for products with high / low moisture values or for completely water-soluble packaging as they are interested in this paper. For determination of solubility and hydration capacity, determinations such as moisture content, swelling ratio, water solubility, and rehydration ratio were performed. The results obtained provide useful information on the products that can be packaged and consumed together with the protective film.

Swelling ratio index (SR)

For this determination, the films were immersed in water at 22 °C, over a period of 30 seconds - 10 minutes. The film pieces were weighed before and after immersion, and the value of the ratio was determined using the formula (1):

$$SR\ [\%] = [(W_t - W_0)/W_0] \times 100 \qquad (1)$$

where W_t represents the mass of the film at time t, and W_0 the initial mass [g], respectively [20]. The values obtained are of interest as it highlights the hydration capacity of the biofilms, but also their complete solubility.

Water solubility (WS)

Determination was made by weighing film samples before and after immersion in water (50 mL distilled water recipient at room temperature) and mild homogenization. The initial film sample was the dry sample according to the method described by *Rhim, Park & Ha* [12], it was immersed in distilled water for 8 hours, after which it was lightly buffered with filter paper to remove excess water and underwent drying for 24 hours at 110 °C in a hot air oven; the water solubility value was determined with the following equation (2):

$$\text{WS [\%]} = (W_0 - W_f)/W_0 \times 100 \tag{2}$$

where W_0 represents the sample mass before immersion and W_f the film mass after immersion and drying, [g], [20].

Mechanical properties evaluation

In order to evaluate the film in terms of mechanical strength, the study focused on the determination of tensile strength and elongation resistance. For this, ESM 301-Mark 10 has been used. The films have been prepared according to STAS ASTM D882 (Standard Test Method for Tensile Properties of Thin Plastic Sheeting) [18]. Testing was performed at an ambient temperature of 28.4 °C.

Microbiological tests

When discussing about food or other product that is consumed, it must be free of pathogenic microorganisms. Thus, both the films and the carob powder were tested for the identification of coliforms, Enterobacteriaceae, *E. coli*, *Staphylococcus aureus*, but also for yeasts and molds. For this purpose, specific culture media were used.
Due to the fact that these edible films are obtained in order to be used as food packaging material and the products are marketed over time, a number of determinations were repeated after a period of 24 weeks.
All determinations were made in triplicate.

RESULTS AND DISCUSSIONS

All of the samples presented low adhesion to the drying surface, *i.e.* the silicone film. The films were easily removed from the support and completely dried after 48 hours at room temperature (~ 22 °C). Films containing 10 % carob powder (A1R, A2R, A3R) in the composition showed light brown color, uneven distribution of carob particles, observed with the naked eye, no obvious pores or cracks, fine odor of the carob and specific taste, slightly bitter. They were smooth, pleasant to touch and flexible. Except for the specific taste, which has disappeared since the second week, the other attributes have remained unchanged.
The control sample (A1C) did not show any taste or odor. Unlike these, the A4R, A5R and A6R films, containing 20 % of the carob powder in the composition, showed dark brown and bitter taste. As in the previous sections, the distribution of carob particles can be observed; samples were smooth and pleasant to touch, but not so flexible.
The control sample (A2C) was more rough and less flexible (this may be due to the addition of agar in the composition but also to higher starch content and lower alginate as opposed to A1C) - Figure 1.

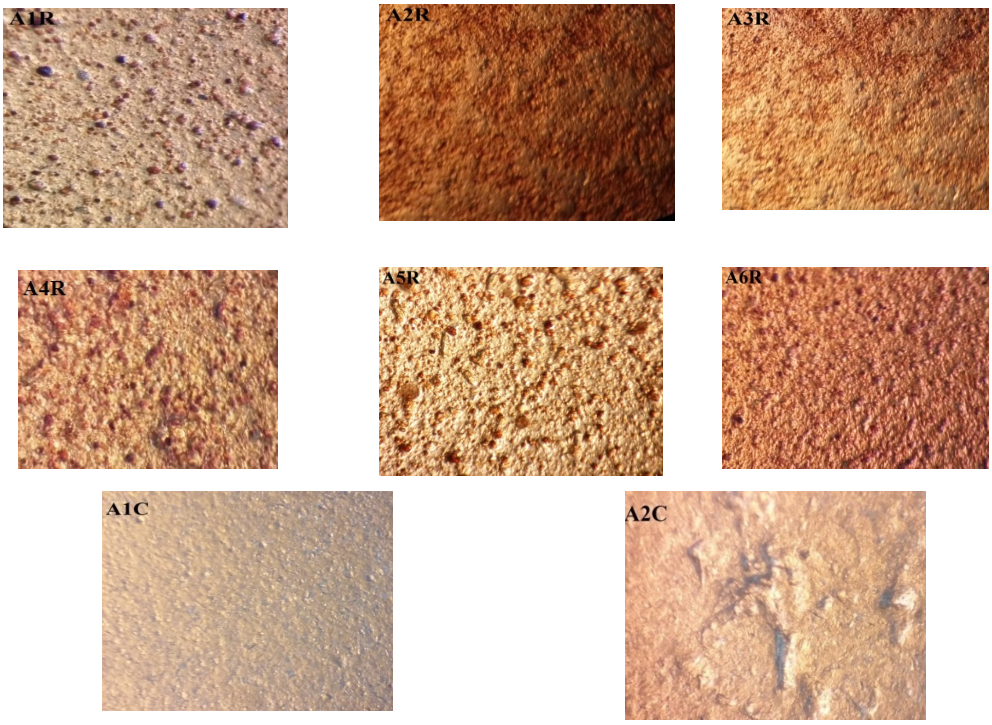

Figure 1. *The microstructures of the edible films observed by microscope*

Both in the second and the third week, the characteristics remained unchanged, including the bitter taste. Thus, we can appreciate that 20 % of the addition of carob powder allows for the preservation of taste and smell properties over time. It is also possible that addition of 15 % carob flour will produce a film that can retain its taste, but this will be less obvious.

As for color, in the case of the first sample, it has intensified, aspect evidenced by the decrease of the brightness values and the increase of the values of $a*$ and $b*$ (Table 2).

Table 2. *The color evaluation during the test period*

Sample	*L**			*a**			*b**		
	W1	W2	W3	W1	W2	W3	W1	W2	W3
A1C	91.77 ± 0.21	94.42 ± 0.33	91.6 ± 0.3	-5.42 ± 0.03	-5.19 ± 0.02	-5.21 ± 0.01	14.2 ± 0.3	14.8 ± 0.67	14.61 ± 0.83
A2C	90.45 ± 0.43	90.11 ± 0.37	90.28 ± 0.42	-5.46 ± 0.02	-5.06 ± 0.04	-5.08 ± 0.06	14.74 ± 1.17	17.2 ± 0.9	17.07 ± 0.75
A1R	70.78 ± 1.14	69.48 ± 0.21	68.77 ± 1.13	2.87 ± 1.3	4.21 ± 0.6	4.18 ± 0.64	29.28 ± 0.98	30.37 ± 0.9	30.42 ± 0.93
A2R	64.41 ± 0.84	64.05 ± 0.9	70.03 ± 1.39	15.8 ± 1.1	15.02 ± 0.85	13.95 ± 2.24	44.57 ± 0.91	39.54 ± 0.21	44.91 ± 0.26
A3R	70.72 ± 0.91	78.5 ± 1.48	77.37 ± 1.47	11.95 ± 0.25	9.34 ± 1	9.88 ± 0.95	43.47 ± 0.55	43.9 ± 0.64	43.73 ± 0.92
A4R	55.25 ± 1.57	53.22 ± 1.6	52.6 ± 1.74	9.24 ± 0.86	10.58 ± 1.06	10.83 ± 1.08	32.76 ± 0.64	33.4 ± 0.44	32.98 ± 0.45
A5R	83.34 ± 1.05	83.53 ± 0.83	84.7 ± 0.77	6.17 ± 0.86	5.8 ± 0.24	5.4 ± 3	41.76 ±.95	41 ± 0.3	41.06 ± 0.33
A6R	74.22 ± 0.98	75.03 ± 0.58	74.14 ± 0.94	11.06 ± 0.63	10.64 ± 0.3	11.05 ± 0.77	45.33 ±1.15	45.42 ± 0.1	45.04 ± 0.32

W1 – week 1, W2 - week 2, W3 - week 3

The films with 20 % content of carob (A4R, A5R, A6R) suffered minor changes, so they were more stable in terms of color, not just smell and taste: L-52.25 in the first week and 52.59 in week 3; *b** - 32.76 in the first week and 32.98 at the end of the test period for A4R (Table 2).
The A2C sample, as well as those with the addition of 20 % carob flour into the composition, also maintained their relatively constant thickness, as opposed to samples with low added carob flour and high alginate content (Figure 2).

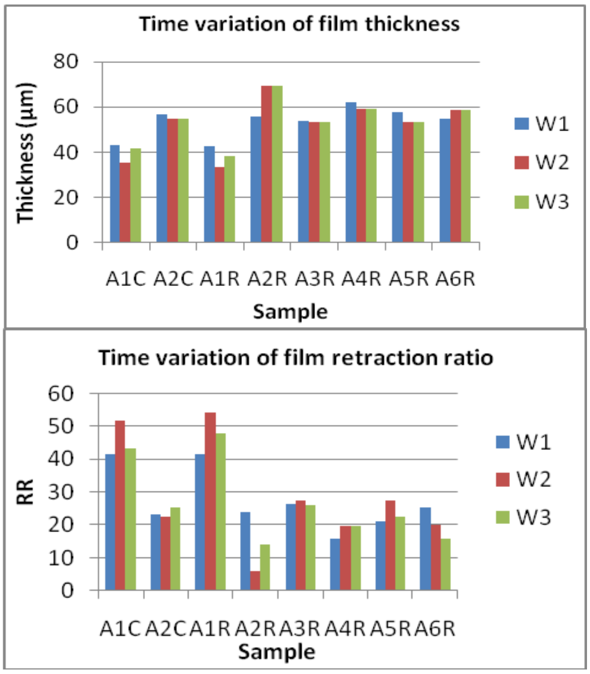

Figure 2. *The evaluation of film thickness (A) and the retraction ratio (B) over a three week test period*

Thickness is not so much influenced by the addition and / or increase in the amount of carob flour as the composition in biopolymers (A4R - A6R films are obtained from higher wheat starch and lower alginate content, but also contain agar).
The film retraction ratio presented the same trends (Figure 2). It can be observed that, although with a less uniform appearance, the membranes with agar into composition retained their physical properties through the test period.
When considering making a film for the packaging of powdered products (cappuccino, instant soluble coffee, baby cocoa) and consumed with them, an important attribute is solubility.
Thus, from Table 3, it can be seen that the addition of carob flour reduces the solubility of the membranes (if A1C, A2R, A3R are completely soluble, A2R indicates the lowest values). This is normal if we also refer to the thickness of A1R film ~ 38 µm and A4R ~ 60 µm.

***Table 3.** Tested films characteristics*

Sample	Water solubility, WS [%]				Moisture content, MC [%]			
	W1	**W2**	**W3**	**W24**	**W1**	**W2**	**W3**	**W24**
A1C	complete solubilization				17.57 ±0.21	16.55 ±0.03	19.07 ±0.02	14.20 ±0.07
A2C	54.32 ±0.02	56.35 ±0.05	54.07 ±0.04	50.11 ±0.03	21.66 ±0.03	19.50 ± 0.04	16.26 ±0.12	15.67 ±0.23
A1R	63.71 ±0.01	62.34 ±0.04	76.05 ±0.02	60.87 ±0.1	16.16 ±0.21	17.55 ±0.05	16.00 ±0.40	20.30 ±0.05
A2R	complete solubilization				22.75 ± 0.2	16.80 ±0.14	14.51 ±0.08	11.67 ±0.01
A3R	complete solubilization				21.87 ±0.83	14.10 ±0.20	14.90 ±0.06	30.42 ±0.26
A4R	55.08 ±0.07	55.07 ±0.07	61.05 ±0.07	50.06 ±0.03	19.22 ±0.06	16.95 ± 0.05	17.47 ±0.02	17.85 ±0.10
A5R	50.61 ±0.03	55.70 ±0.23	58.00 ±0.11	54.17 ±0.07	29.55 ±0.27	14.84 ±0.18	14.56 ±0.10	16.60 ±0.05
A6R	53.40 ±0.10	53.83 ±0.08	47.50 ±0.23	51.66 ±0.05	29.73 ±0.17	13.83 ±0.14	13.70 ±0.15	15.80 ±0.10

W1 – week 1, W2 - week 2, W3 - week 3, W24 - week 24

However, in hot water (80 °C), all films with carob were solubilized after holding for 30 seconds (Figure 3). Thus, the membranes can be used for packaging instant drinks as their preparation requires hot water and in this way the packaging becomes completely soluble and edible with the beverage. Unlike films with other additions, solubilization occurs quite quickly (compared to those with added beet, solubilized in hot water after 40 - 100 seconds of maintenance [2].

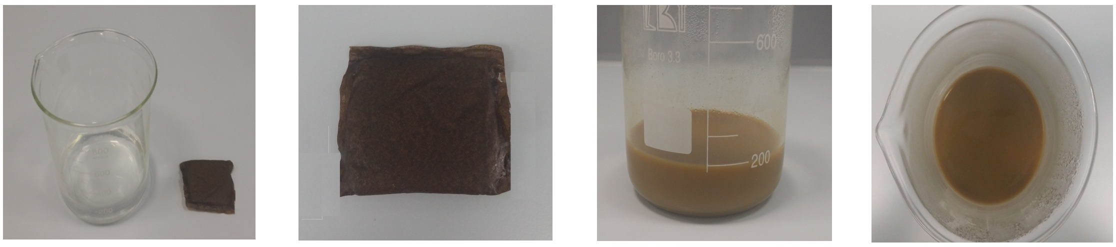

***Figure 3.** Complete solubilization of the film with carob powder into composition (A4R) after 30 " maintenance in hot water (80 °C)*

It can be seen (Figure 4 - 7) the absence of A2R and A3R samples due to their extremely rapid solubility. If, after immersing the samples for 30 seconds or 1 minute, the samples disintegrated and it was impossible to re-weight, after 3 minutes of immersion these were completely solubilized.

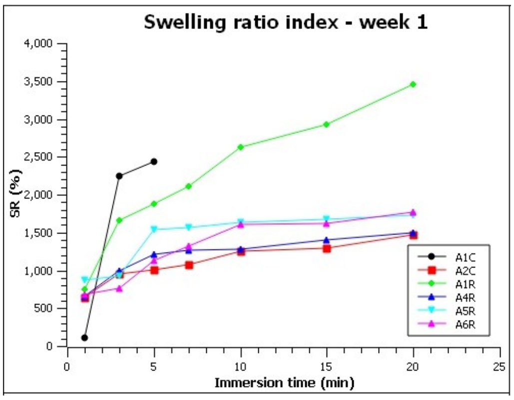

Figure 4. *Swelling ratio index values for first week of testing*

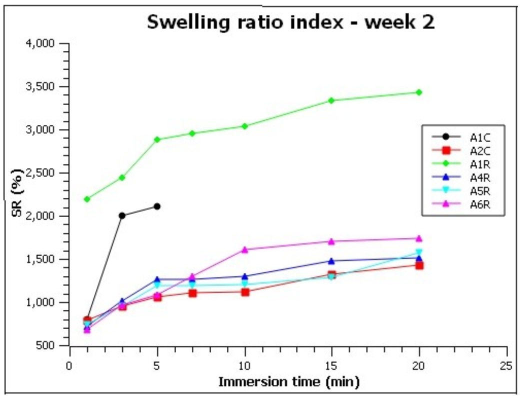

Figure 5. *Swelling ratio index values - second week of testing*

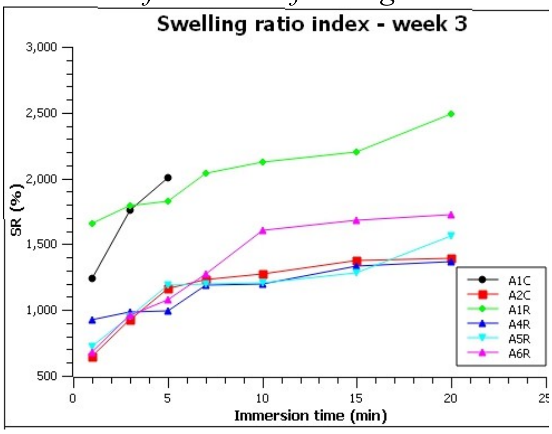

Figure 6. *Swelling ratio index determination (week 3)*

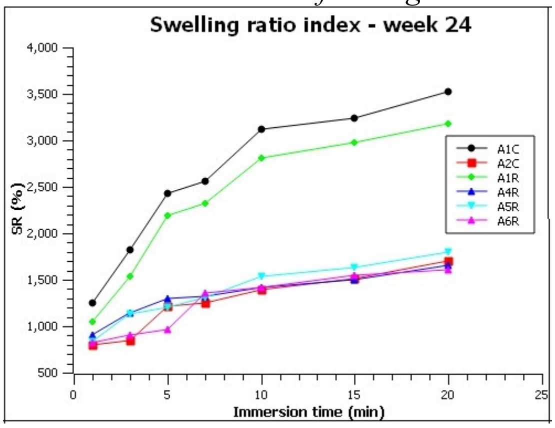

Figure 7. *Swelling ratio index (week 24)*

The same behavior was noted even in the 24 weeks after membrane production (Figure 7) when the A2R and A3R samples were solubilized after 1 minute holding in water at 23.4 °C.

Differences occur in the case of swelling index values, which are higher than those obtained for 1 - 3 weeks. It can also be seen that the A1R film retains its integrity even after 20 minutes of immersion, as opposed to the determinations made in weeks 1 - 3, when it has completely solubilized after 5 minutes of maintenance in water (ambient temperature) - Figures 4 - 6. It was highlighted that the high alginate composition of the A4R sample retained its integrity very well, even after 20 minutes of immersion, but also after drying the wet sample. Such a material can be used to package sliced meat or high moisture content products, but for the production of edible packaging for foods that do not require high temperature solubility; for example, films containing medicaments or various substances to be dissolved in the oral cavity, as well.

Another important aspect when using films as a material for packaging food is represented by its ability to allow the transfer of light radiation. Therefore, the values obtained in determining transmittance are of interest.

Thus, the addition of carob powder prevents the transfer of light through the material (Tables 3 and 4): If the control samples have a transmittance value of 60 and 32 % respectively, those with the carob addition reach 5 %. To note that the control sample with high starch content into the matrix shows much lower values than the one with smaller starch quantity.

Table 4*. Physical properties of the tested films*

Sample	Water activity index, a_w			Transmittance, T [%]		
	W1	W2	W3	W1	W2	W3
A1C	0.37±0.03	0.33±0.07	0.31±0.90	61.96±0.08	60.20±0.75	58.00±0.20
A2C	0.38±0.01	0.33±0.52	0.29±0.47	32.58±0.27	31.90±0.80	30.60±0.05
A1R	0.37±0.15	0.35±0.97	0.34±0.02	56.87±0.48	53.21±0.03	52.90±0.14
A2R	0.38±0.02	0.32±0.05	0.30±0.65	50.47±0.38	50.12±0.23	50.16±0.97
A3R	0.37±0.01	0.33±0.07	0.31±0.45	53.54±0.02	51.80±0.18	48.14±0.02
A4R	0.38±0.57	0.34±0.60	0.33±0.98	9.83±0.32	8.14±0.03	6.74±0.07
A5R	0.37±0.11	0.33±0.14	0.32±0.78	7.06±0.17	6.92±0.21	5.50±0.11
A6R	0.36±0.01	0.32±0.58	0.30±0.05	3.60±0.06	3.20±0.08	3.30±1.02

W1 – week 1, W2 - week 2, W3 - week 3

The same algorithm applies to samples with the addition of carob powder (A1R, A2R, A3R - reduced starch content in the composition, higher transmittance values ~ 50 %, unlike A4R, A5R and A6R - doubling of starch and values much reduced transmittance: from 9 to 3 %). The values did not change considerably during the test period (Table 4). The addition of agar and the reduction in the amount of alginate (correlated with the increase in wheat starch) influenced the elasticity and strength of the films over the three weeks (Table 5).

Table 5*. Mechanical properties modifications during the tested period*

Sample	TS [MPa]			E [%]		
	W1	W2	W3	W1	W2	W3
A1C	1.23± 0.01	1.108± 0.01	1.22± 0.016	39.00± 0.17	36.60±0.41	33.40± 0.42
A2C	0.80± 0.12	0.52± 0.07	0.30±0.70	37.04± 0.05	36.00± 0.11	23.20± 0.07
A1R	0.37± 0.01	0.22± 0.01	0.43± 0.01	36.20± 0.01	35.40± 0.02	28.60± 0.17
A2R	0.28± 0.54	0.14±0.50	0.12±0.67	47.40± 0.08	44.20± 0.54	38.70± 0.78
A3R	0.57±0.68	0.35± 0.46	0.29± 0.72	77.80± 0.23	77.00± 0.13	72.60± 0.43
A4R	0.90±0.23	0.68± 0.30	1.33± 0.01	37.60± 0.30	39.00± 0.62	27.80± 0.13
A5R	0.58±0.36	0.38± 0.56	0.39±0.64	109.80± 0.20	106.90± 0.67	103.45± 0.89
A6R	0.63± 0.34	0.58±0.12	0.51±0.47	28.20± 0.63	27.80± 0.20	25.00± 0.82

W1 – week 1, W2 - week 2, W3 - week 3

Changes have also been made, but not so notable. Thus, A2C, with 37.04 % elongation in the first week, showed a reduced value of about 15 units. For A4R sample, which has retained its elasticity but with variations in tensile strength, it can be consider that the addition of carob flour had a positive impact from this point of view (taking into account that the only difference is the carob from the composition). The same trend can be seen in other films with the addition of 20 % carob powder in the composition, but especially at those with agar in the matrices - A5R, A6R. However, there have been no significant changes, so films can ensure the durability of packaging, at least within three weeks. This period can be prolonged by keeping single-use packs in cartons (as is currently the case), which could help maintain mechanical and physical properties.
Microbiological determinations have shown the safety of using these films as edible packaging material. The tested microorganisms have not grown on culture media:

coliforms, Enterobacteriaceae, *E. coli*, *Staphylococcus aureus*, yeasts and molds, so that these films are safe for consumption.

CONCLUSIONS

The results indicate the possibility of the use of carob powder and biopolymers for the production of edible, completely water-soluble packaging materials. Obtained in relatively plentiful supply, at low cost, these materials represent the future of food packaging and not only. Due to the addition of carob, the resulting films have superior net nutritional qualities, ultimately resulting not only in a completely edible, 100 % biodegradable, environmentally and ecosystem-friendly packaging material but, above all, a product with real health benefits to the consumer, regardless of whether it is an adult or a child, or whether they represent people at risk (allergic or suffering from various diseases involving personalized diet).

These films are applicable in the food, pharmaceutical, cosmetic, etc. industries.

The study showed that the addition of carob in the composition of the films had a positive effect on their matrix; the obtained membranes preserved their physical and mechanical properties during the tested period.

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