Obtaining the Edible Films with Natural Polymeric Matrix and Biologically Active Constituents Extracted from Plants

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The aim of this study was to obtain an edible film polymer matrix naturally extracted from tubers of Helianthus tuberosus L and biologically active components extracted from the roasted seeds of Vitis vinifera, in order to protect the active components from oxidation reactions during food conservation. The film was prepared by filling technique. It was evaluated the antioxidant activity of the film by means of DPPH (98.7%) technique, solubility water solubility (20.4%) and Isothermal sorption of water by GAB (Guggenheim, Anderson, de Boer) technique resulting a value of water activity of 0.167 and a value monolayer of 0.026 g water/g film. Through these values the resulting film ensure a sound management of the evolution of biological systems from the point of view of their complexity, especially food whose degradation can impact on their safety.

Keywords: edible films, biopolymers, bioactive compounds

Excessive consumption of plastics derived from petroleum and delayed degradation leads to an accumulation of waste massive negative impact on the environment pollutants are classified as extreme [1,2]. To avoid this negative impact the European Union promotes different strategies to deal with this problem.

A very important strategy is the growing interest in obtaining organic polymeric materials made from raw and auxiliary materials from renewable natural, agricultural, marine and animal sources (fig. 1) [3,4].

The use of natural polymers for the production of biodegradable packaging has emerged as an alternative to face this problem. Edible films made from natural polymers contribute to waste disposal by partial replacement of non-biodegradable plastic packaging. There are various technical and legal definition for the concept of biodegradable packaging. In accordance to the European standard EN 13432, these are materials that are degraded after use into low molecular weight substances (carbon dioxide, water and biomass) during the combination of physical, chemical agents and microorganisms. After the ASTM (American Society for Testing and Materials) and ISO (International Standards Organization) these biodegradable packaging undergo a significant change in their chemical structure under specific environmental conditions; they naturally degrade under the action of microorganisms such as bacteria, fungi and algae; they degrade under the influence of natural light, oxidation and hydrolysis reactions and biological processes during composting, producting carbon dioxide, water, inorganic compounds, and biomass and leaves no toxic or visible residues [5].

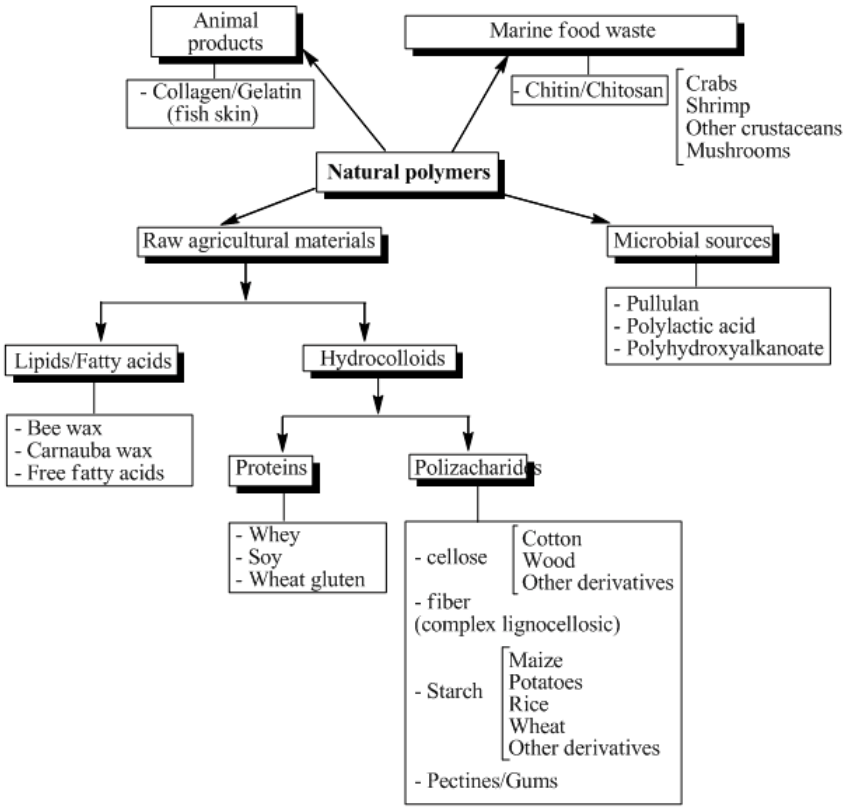

Fig. 1. Sources of natural biopolymers

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Biodegradable polymers used in the production of biodegradable packaging can be classified according to their source of origin:

- polymers extracted directly from biomass polymers (polysaccharides, proteins, polypeptides, polynucleotides);
- polymers using monomers manufactured by chemical synthesis, biological or renewable sources and oil mixed biomass (bio-polyester or polylactic acid);
- polymers produced from genetically modified organisms or bacteria (polyhydroxybutyrate, bacterial cellulose, xanthan curdian, pulluan).

The diversity of materials used for packaging products is very high. From a technical point of view, the packaging of goods consists of a set of materials designed to protect the quality and integrity of products, and facilitate their manipulation. Also, product quality is influenced by packaging e.g. improper packaging, may lead to the impairment of the product, and contributes to reducing its quality.

The use of biodegradable packaging films or coatings in the form of complete biodegradability has a very important role in preserving security and basic nutritional properties of food. They help delay or prevent physical processes chemical, biochemical and microbiological food that can occur during storage, keeping quality breakneck with increasing duration of preservation. They can be applied directly on food by dipping, spraying, brushing or form films to prevent water loss, gas exchange and provide a modified atmosphere around the food [6,7].

Edible films or this cover is generally defined as a thin layer of material that can be consumed by consumers as part of food [8] and enhance the appearance of food [7, 9,10].

Of a particular interest in obtaining biodegradable films is the incorporation of active natural compounds that act as antioxidants, antimicrobial agents, crosslinking etc., by changing some functional properties, physical and chemical properties of the film [7,11,12]. The purpose of this study was to obtain a film based on poliglucide extracted from tubers of *Helianthus tuberosus* L to which a built-in antioxidant compound extracted from roasted *Vitis vinifera* seeds was added.

Experimental part

Materials and methods

Tubers of *Helianthus tuberosus* L were purchased from a private household with a moisture content of 78 and 13.9% in inulin.

Vitis vinifera seed purchased from the Research Station of the University of Craiova teaching-Romania having the following characteristics: *the appearance*: the dry surface without any trace of mold; *the color*: brown or dark red, various shades, characteristic for the variety of provenance; *smell:* is specific from the seed of *Vitis vinifera*, but it is not allowed the smell of mould; *seeds altered*: less than 5%; *impurities (pieces of the bunch, skin):* below 3%; *the moisture*; 35-40%; *density*: 1.1-1.3g/cm^3; *higroscopicity*: 7-15 *mL/100 g* [13].

Extraction of polysaccharides from tubers of Helianthus tuberosus L.

Samples of tubers of *Helianthus tuberosus* L were washed with tap water to remove dust and other unwanted materials and grounded in a vertical blender Braun MR 404 Plus.

In a reactor with a capacity of 2 L were introduced 800 mL tubers and 400g distilled water (1 ratio 2). The mixture was heated at 80°C and permanently stirred for 3 h. The obtained extract was filtered through a filter paper. To the filtrate was added 95% ethanol a ratio of 1:1 to give a precipitate which was twice washed with 95% ethanol. The precipitate obtained was placed in the Memmert type oven and dried at 40°C after which it was ground to give a fine powder.

Extraction of total polyphenols contained in Vitis vinifera roasted seeds

The seed roasting was done in a Rombat type fryer for 15 min at 220°C and their grinding was made in a Viacenza-type machine 200 adjusted so that to obtain a 1-1.25 mm particle size [14,15].

The extraction of polyphenols from *Vitis vinifera* roasted seeds was performed in a spherical reactor with a capacity of 1 L in which, to the obtained powder was added 100 g of *Vitis vinifera* seeds and 300 mL of distilled water. The mixture was heated at 80°C for 2 h. The obtained extract was filtered through filter paper and stored in a refrigerator [15].

Determination of total polyphenol content in Vitis vinifera seeds

The total polyphenol content was measured using the Folin Ciocaletau colorimetric method. To 800vL of deionised water, 50µL of Folin Ciocalteu's phenol reagent and a volume of sample ranging from 10 to 50 µL were added and accurately mixed. After 1 min, 100µL of 20% sodium carbonate solution was added and mixed. Deionised water was then added up to a volume of 1 mL. The solution was carefully mixed and total phenol content was spectrophotometrically estimated at 765 nm (Cary 50) after 2 h incubation Quantification was based on the standard curve generated with gallic acid (fig. 2). All determinations were carried out in triplicates [16].

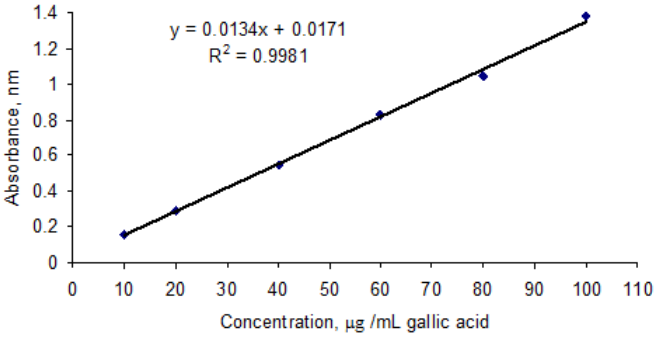

Fig. 2. Standard curve gallic acid

Preparation

In a 200 mL reactor were introduced 125 mg of polysaccharide powder, 100 mL of the aqueous extract obtained from the *Vitis vinifera* roasted seeds, 10 mg of sodium alginate and 0.2 mL of glycerol in order to obtain a flexible and elastic film. The mixture was heated in a water bath at 60°C for 60 min and then degassed under vacuum.

Coating the film

There are several processes of forming biopolimere films such as pouring, spraying, extrusion and thermoforming. The most common method used in the laboratory is pouring. In this case, the film-forming solution, was poured on a non-sticking plate (glass) and left in atmosphere for 24 h to evaporate the solvent and form the film [17-19]. The film thus formed is immersed in a calcium chloride solution with a concentration of 50 mg/L for 2-3 min after which it is introduced for 48 h in a desiccator containing $MgCl_2$ for 48 h.

Determination of the Film Properties

One mL of the extract in methanol was added to 4 ml of 0.1 mmol/L methanolic solution of DPPH. A blank probe was obtained by mixing 4 ml of 0.1 mmol/L methanolic solution of DPPH and 200 µL of deionized distilled water (ddH_2O). After 30 min. of incubation in the dark at room temperature, the absorbance was read at 517 nm against the prepared blank. Inhibition of free radicals by DPPH in percent (I %) was calculated using this formula [20]:

$$I\% = (A_{blanc} - A_{sample})/A_{blanc} \times 100$$

where A_{blanc} is the absorbance of the control reaction containing all reagens except the test compound and A is the absorbance of the test compound.

Determination of Solubility in Water of the Polymer Film

The solubility of the polymer film represents the dissolved dry matter content after a 24 h immersion in water. The initial content of dry matter of the film was determined using a MX-50 thermogravimetric balance. Discs (2 cm diameter) were cut, weighed (Mi), and placed in a volume of 50 mL distilled water. After 24 h of immersion the samples were removed and dried in a Memmert oven at 105°C to constant weight (Mf) to determine the weight of dried material which has not been solubilized in water. The solubility of the film was determined by the formula [2]:

$$Water\ solubility\ \% = (M_i - M_f)/M_i \times 100$$

where M_i - the initial mass of the film; M_f - final table of film.

Determination of the Water Sorption Isotherm of the Polymer Film

The graphical representation of the balance between hard moisture and water activity is called water sorption isotherm.

Calculation of the equilibrium moisture content *m* of the film samples with moisture *zero* was carried out by placing them in desiccators containing saturated salt solutions. At a constant temperature (25°C) saturated salt solutions used (table 1) have a water activity between 0.1 and 0.9.

Table 1

ACTIVITY OF SATURATED SALT WATER SOLUTIONS AT 25°C

No	Formula	Water activity, t = 25°C
1.	LiCl	0.112
2.	$MgCl_2$	0.326
3.	K_2CO_3	0.431
4.	$Mg(NO_3)_2$	0.527
5.	NaCl	0.754
6.	KCl	0.843

Obtaining *zero* moisture film samples was carried out in an oven at 60°C under vacuum, for 4 h followed by storage for 7 days in a desiccator containing a drying agent P_2O_5. Check of *zero* humidity for film samples stored in desiccators containing saturated solutions of salts was carried out a MX 50 analyzer. It was determined a humidity value of 0.042% in the film samples. Samples of the polymer were introduced into desiccators with satured solutions in order to obtain a liquid crystal layer with a thickness of 2 mm above them. To avoid contamination of vials containing samples of film it was used a layer of glass wool plate placed over a desiccant support. Its preservation as samples in the desiccator at 25°C was for 21 days. Weighing the samples was done with an accuracy of 0.001g using a PRECISA XB 120 type A balance, before and after introducing the samples in desiccator.

The equilibrium moisture was calculated using the formula:

$$m = (w_2 - w_1)/w_1 \ \ g\ water/g\ dry\ film$$

where:

w_2 - film sample weight after 21 days, g;

w_1 - original film sample, g.

The data were graphically processed using a special program for obtaining water sorption isotherms (AWRPLOT), adapted equation GAB (Guggenheim-Anderson-duBoer) [21,22]:

$$m = \frac{m_0 . K_b . C . a_w}{(1 - K_b . a_w)(1 - K_b . a_w + K_b . C . a_w)}$$

where:

m - humidity balance, g water/g film;

m_0 - optimal humidity (monolayer), g water/g film;

a_w - water activity;

K_b - constant correction, 0.9395;

C - constant Guggenheim, 61.6091.

Results and discussions

Achievements of edible films with natural polymer matrix and biologically active components extracted from plants is a central goal of chemical research, due to progress in all areas. Adding active compounds, such as antioxidants, these films can increase their functional properties and make them applicable in food preservation [23]. Controlling oxidation is essential to manage the evolution of biological systems in their complexity, especially for food products whose degradation can have an impact on food safety [24].

The literature presents a series of researches to include in biopolymers films certain antioxidants derived from plant extracts [25-27], essential oils [28,29], and other components with antioxidant activity, such as α-tocopherol [30], ascorbic acid [31-33] or citric acid [34,35]. This paper presents a research concerninh the insertion of a polyphenol extract from the seeds of *Vitis vinifera* in a matrix that due to their content in polyphenols (gallic acid, monomers, flavan-3-ol: catechin, epicatechin, galocatechine, epicatechin 3-O gallate, dimers, trimers and polymers of procyanidins) makes the antioxidant activity 20 times greater than that C vitamin and 50 times stronger than that E vitamin [14,36,37]. By using the polyphenol extract of *Vitis vinifera* seeds whose concentration in polyphenols was 750 mg GA/100g seed powder *Vitis vinifera*, it was obtained a film whose antioxidant activity was determined by the DPPH method to be of 89.7%.

In most cases, the antioxidant capacity of the films obtained are proportional to the concentration of the active compound in the film without a noticeable loss of activity during the formation of this coat [38,39].

High antioxidant activity of the film helps to lower the rate constant for the oxidation reaction thus protecting the active ingredients in food from oxygen.

The water solubility of the film is one of the major problems of the film carbohydrates it is determined through various methods: sorption and water activity, solubility, moisture content, permeability etc. [2,40]. The film solubility in water can also provide an insight about the behavior of a film in aqueous media, a measure of its

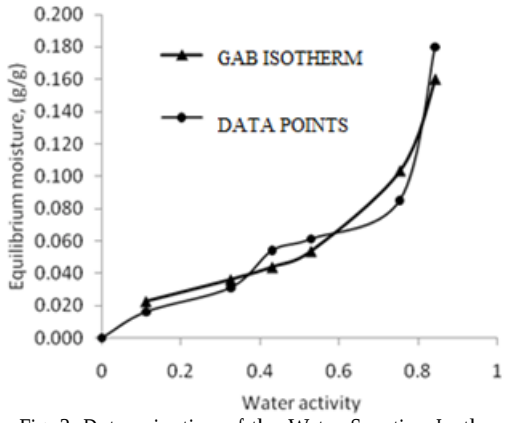

Fig. 3. Determination of the Water Sorption Isotherm

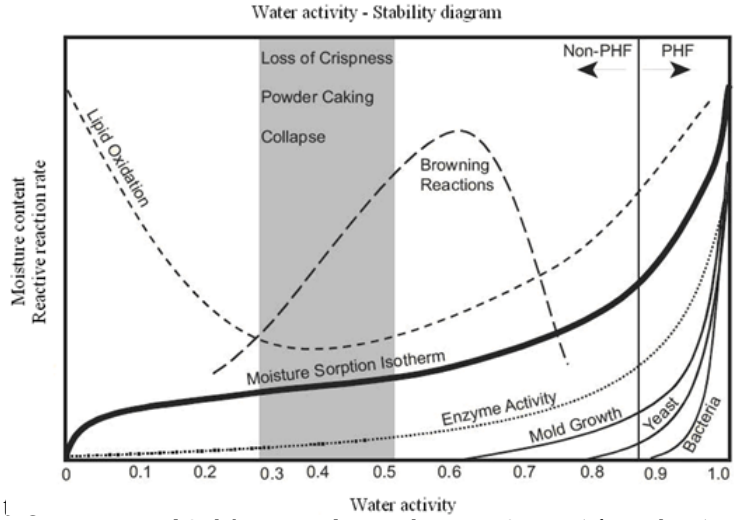

Fig.4. Food Stability Depending on the Water Activity (after Labuza)

resistance to water, and thus about the hydrophily of t related material. The moisture content is the parameter related to the total void volume occupied by water molecules in the film microstructure network [2,41]. It is also an important factor when determining the film biodegradability when they are used as packaging materials [2,42]. Sometimes, it is desired a high solubility in water. This is when the film or coating is consumed as food.

The water solubility value of film was established to be 20.4%.

After determining the water sorption isotherm of the obtained film, (fig. 3) a, resulted a water activity of 0.167 and a value of the monolayer, m_0 of 0.026 g water/g film.

A value of the monolayer of 0.026g water/g film is the optimum value at which the film has the maximum stability [43]. From the stability figure (fig. 4), it is observed the influence of water on the various chemical reactions that occur during storage in food [43].

At a water activity value of 0.167 obtained for the film it is noted that the enzymatic and oxidation reactions are those that can affect the film. According to figure 4 it is shown a small influence on the film. This influence is diminished by the antioxidant role of polyphenols content film.

Conclusions

The film obtained constitutes a basic element of edible coatings in order to improve life and food quality by providing an effective barrier against oxygen action, reducing enzymatic reactions, microbiological contamination and increasing the validity of food products. Use of *Vitis vinifera* seeds to obtain polyphenolic extractsas well as *Helianthus tuberosus L.* tubers contribute to a more efficient use of these agricultural products.

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