INFLUENCE OF PLASTICIZER CONTENT ON ORGANOLEPTIC, PHYSICO-CHEMICAL AND STRENGTH CHARACTERISTICS OF APPLE SAUCE-BASED EDIBLE FILM

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Received April 07, 2017; Accepted in revised form May 26, 2017; Published December 26, 2017

Abstract: Biodegradable edible films are developed as alternative packaging materials. Due to their unique chemical composition, apples can act as a raw material for the production of edible films. The aim of the work is to create an edible film from apple raw materials using the following plasticizers: agar, carrageenan or xanthan gum. Edible films have a yellowish color, characteristic for apple sauce. The film with the addition of xanthan gum as a plasticizer has the most acceptable flavor properties. Microscopic examination of edible film samples was carried out using a conventional and laser microscope. The structure of the edible film with an increase in the proportion of fiber becomes more homogeneous. IR spectra with a Fourier resolution of the analyzed edible film samples were obtained, which make it possible to isolate the presence of free hydroxyl groups. For edible films based on apple sauce with the addition of agar and xanthan gum as a plasticizer, there is a tendency to increase the tensile strength with increasing the amount of plasticizer (from 1.32 to 1.70 MPa for agar and from 1.68 to 3.50 MPa for xanthan gum). With a longer time of exposure to water and a higher water temperature, the edible film samples are destroyed.

Keywords: Edible film, apple sauce, agar, carrageenan, xanthan gum

DOI 10.21603/2308-4057-2017-2-5-14 *Foods and Raw Materials*, 2017, vol. 5, no. 2, pp. 5–14.

INTRODUCTION

Currently, packaging films are widely used to preserve perishable products such as meat, fish, confectionery, cheeses, pasta, fats, etc. Polyethylene films made from synthetic polymers are increasingly used for food packaging because of their low cost, ease of molding and high mechanical and barrier properties [1]. However, they do not decompose and do not recycled that causes serious environmental problems due to waste and the accumulation of environmental pollution [2]. Interest in environmental problems is increasing all over the world and it is especially relevant for the Russian Federation, because for a variety of reasons, 2017 has been declared a year of ecology in Russia. Biodegradable food films are developed as alternative packaging materials and are of great interest to many researchers. Over the past 10 years, the production of biodegradable films has increased tenfold. Research of scientists in this area also increased manyfold. Food films are known, made of polysaccharides, such as cellulose, chitosan and starch derivatives; proteins such as gelatin, corn zein, wheat gluten and soy protein; and lipids, such as paraffins, fatty acids and resins [3–7].

Currently, active research of the chemical composition and physical and chemical properties of apples is underway, as they are a widely consumed fruit, as well as a good source of insoluble (cellulose, xyloglucan) and soluble dietary fibers (mainly pectic polysaccharides) [8].

Apples have a number of advantages as a raw material for the production of edible films:

(1) wide raw material base within the Volga region and the whole territory of the Russian Federation;

(2) presence in the chemical composition of substances necessary for the quality of edible films: cellulose, hemicellulose, pectin, etc.;

(3) developed technologies of processing into various semi-finished products;

(4) ability to vary the chemical composition depending on the variety of apple;

(5) presence of special physical properties of semi-finished products from apples important for the production of edible films: fluidity, pumpability, moldability.

In addition, apples are an important source of phytonutrients with biological activity and health effects, such as vitamin C, potassium, dietary fiber. Due to these components, the content of saturated fats, cholesterol, and sodium in living systems decreases. Among medical research, the most relevant at the moment are studies on the effect of apple components on the development of cancerous diseases. In the study [9] of the effectiveness of reducing the occurrence of breast cancer cells by the example of apples of different varieties, important results were obtained: apples of Red Delicious and Golden Delicious varieties prevent the studied disease. Brazilian researchers conducted [10] studies in 2003 on 33 women over 45 years of age and found a correlation in the diet of apples, melons, tomatoes and the risk of breast cancer.

Due to their unique chemical composition, apples can act as a raw material for the production of edible films. As a plasticizer for apple raw materials, such food raw materials as agar, carrageenan and xanthan gum can be used.

Agar-agar (agar) is obtained from marine red algae of *Cracolaria, Gelidium, Ahnfeltia* species growing in the White Sea and the Pacific Ocean. Depending on the type of algae, the composition of the isolated polysaccharides can vary (mixture of agarose and agaropectin) [11]. The agar dissolves slightly in cold water and swells in it. In hot water, it forms a colloidal solution, which upon cooling gives a good, durable gel with a vitreous fracture. In agar, this process is carried out by the formation of double helices and their association, regardless of the content of cations, sugars or acids. Gel-forming ability of agar is 10 times higher than that of gelatin. When heated in the presence of acid, the gel-forming ability is reduced. Gels are stable at pH above 4.5 and thermo-reversible. Agar is used in the production of confectionery products (jelly marmalade, pastille, marshmallow), meat and fish jellies, various jellies and puddings, and also for clarifying juices. All of the above allows to recommend agar-agar as a component of edible films.

The term "carrageenans" refers to a group or family of sulfonated galactans extracted from red algae of *Rhodophyta* species by dilute alkali solutions, usually producing the sodium salt of carrageenan [12]. They are linear chains consisting of D-galactopyranosyl units linked by alternating (1→3)-α-*D*- и (1→4)-β-*D*-glycosidic bonds, most of the saccharide units having one or two sulfate half-ester groups are esterified at the C_2 or C_4 atom to hydroxyl groups. Galactcopyrase units often contain 3,6-anhydride rings. The content of sulfates in such compounds is 15–40%. In this case, the sulphate group giving the chain a negative charge at all pH values is ionized, so that the molecules do not precipitate at low pH values. In modern food products, the stabilizing and gelling properties of carrageenan are used. It is found not only in ham, sausages, poultry products, but also used to make the glaze of cakes and pastries. Carrageenan forms good elastic gels, which allows it to be used in edible films.

By chemical nature, xanthan gum is a heteropolysaccharide consisting of the residues of D-glucose, D-mannose and D-glucuronic acid, and is obtained by fermentation using the bacterium *Xanthomonas campestris*. Films of xanthan gum have the properties of pseudoplastics with rheological behavior in an aqueous medium. This property is favorable for films, since xanthan gum is easily dispersed in cold or hot water, but all this has little effect on its viscosity, regardless of temperature and acidity [13]. Films of gelatin saturated with xanthan gum are transparent with excellent resistance to ultraviolet light, low vapor permeability, improved mechanical properties and thermal stability [14]. Xanthan gum is characterized by formation of high-viscosity stable food systems and it also makes it possible to use it in edible films.

[15] describes the production of biodegradable films from agar-agar, carrageenan and hydroxypropylmethylcellulose and glycerol, which were used as a plasticizer. Various compositions from biodegradable films based on natural polysaccharides were analyzed for their rheological properties and physico-mechanical characteristics, as well as for preservation and ecotoxicity index. It was found that these polymer films are biodegradable and relatively bio-safe. Strength tests showed that the film compositions with carrageenan have a higher strength than polymers containing agar-agar only. Also, biopolymers containing carrageenan have an increased chemical resistance (prolonged time of dissolution in hydrochloric acid).

The use of hydrocolloids of vegetable origin such as, starch, pectin, carrageenan, and agar-agar is promising because of their structural and mechanical properties and micromorphological features [16]. It has been found that with respect to strength and suitability for use in films, the plasticizers can be arranged in ascending order as follows: starch, carrageenan, pectin, agar-agar.

Although edible films are not so widely known to the conventional consumer as synthetic polymeric films, however, a number of scientific papers in recent years have as their object of study the creation, formulation, production technology, the study of physical properties and mechanical strength for edible films, both on the basis of vegetable, and animal raw materials [17].

French scientists produced an edible film based on the hydrocolloid matrix of iota-carrageenan with the addition of glycerol and 30, 60, 90% glycerin monostearate by drying at 100, 150, 200°C [18]. The structure of the film was studied by electron microscopic examination. The data show that it was possible to obtain a two-layer film.

For biscuit and gel from agar, an intermediate edible film of acetylated monoglycerides is proposed [19]. The film was obtained by melting, sputtering or pouring. The larger the film thickness, the better its barrier properties.

In the literature, examples were found on the use of edible films with various plasticizers as packaging materials. For beef cutlets, several types of edible coatings were used from wheat gluten, soy protein, carrageenan, chitosan [20]. In cutlets, moisture loss, oxidation level (thiobarbit ratio, hexanal content) were monitored during storage for 3 days at 4°C. As a control, a polyvinyl chloride film was used. Based on the results obtained, the authors conclude that edible films do not protect against oxidation.

d-limonene and n-hexanal were encapsulated in an edible film of iota-carrageenan with or without lipids [21]. Such film samples were tested for wetting ability at temperature 25 and 35°C of medium composition (water and 0.9% NaCl solution). All the factors considered influence the level of retention of aromatic substances in the film.

Thus, the prospects for creating edible films are obvious.

The purpose of this work is to create an edible film from apple raw material using agar, carrageenan and xanthan gum as plasticizers, to compare the organoleptic, physico-chemical and mechanical characteristics, the water-absorbing capacity of the edible films, depending on the nature and amount of the plasticizer introduced.

OBJECTS AND METHODS OF STUDY

Experimental work was conducted at the Department of Technology and Organization of Public Catering of the Samara State Technical University.

The apples used in this study grow on the territory of the Samara Region, Russia (53 12' N and 50 06' E) of the 2016 crop. Apples of varieties Semerenko, Sinap and Antonovka were used. These varieties were chosen in terms of yield and chemical composition.

Chemicals and reagents. All plasticizers used have "chemically pure" degree of purity: agar, carrageenan, xanthan gum (Sigma Aldrich, Germany).

Preparation an edible film. Edible films from apple raw material using as a plasticizer xanthan gum, carrageenan and agar-agar were prepared as follows: apples were cleared from inedible parts, treated with water vapor, ground to a sauce state, the sauce was concentrated, then a plasticizer (2–4% by weight of apple sauce) was added to the resulting mass and a sheet of film was made by rolling. The resulting sheet of edible film with a thickness of 1–3 mm was dried at 55–70°C for 1–3 hours, and then cooled to room temperature.

Organoleptic analysis of edible film. The organoleptic analysis of edible films was carried out in accordance with GOST R ISO 8586. The analysis involved 14 people, whose average age was from 18 to 50 years old, trained according to GOST ISO 8586-1-2011. The general rules of organoleptic analysis corresponded to GOST ISO 11037–2013. For the analysis, samples of edible films 10x10 cm were taken. Color, taste, flavor, and consistence were used as the evaluated parameters for organoleptic analysis of edible films [22–25]. Table 1 shows the distribution of scores for different indicators. The results of the organoleptic evaluation were treated statistically.

Table 1. Organoleptic properties of edible films from apple raw material with the addition of plasticizers

Parameters	Test results	Number of points
Edible film using 2 agar		
Color	Pale yellow	5
Flavor	Weak apple	5
Taste	Sourish apple	5
Consistence	Resilient	4
Chewing ability	Good	4
Edible film with 4 agar		
Color	Pale yellow	5
Flavor	Weak apple	5
Taste	Apple	4
Consistence	Resilient elastic	5
Chewing ability	Medium	4
Edible film with 2 carrageenan		
Color	Pale yellow	5
Flavor	Weak apple	5
Taste	Sourish apple	5
Consistence	Resilient	4
Chewing ability	Good	4
Edible film with 4 carrageenan		
Color	Pale yellow	5
Flavor	Weak apple	5
Taste	Apple	5
Consistence	Resilient elastic	5
Chewing ability	Medium	4
Edible film with 2 xanthan gum		
Color	Pale yellow	5
Flavor	Weak apple	5
Taste	Sourish apple	5
Consistence	Resilient	4
Chewing ability	Good	5
Edible film with 4 xanthan gum		
Color	Pale yellow	5
Flavor	Weak apple	5
Taste	Apple	5
Consistence	Resilient elastic	5
Chewing ability	Medium	4

Photographing samples of edible film. Photographs of edible film samples were made using a camera Sony Alpha ILCE–6000 Kit: film samples having a size of 5 × 5 cm were placed on a sheet of black velvet paper and photographed in daylight.

Microscopic examination of film samples. The film structure was studied using a 400x, Celestron biological laboratory microscope, and JEOL–6390A scanning microscope with a resolution of up to 3 nm and magnification of 30x, 100x and 500x; accelerating voltage 20 kV. A thin section of the edible film was placed on the center of the slide and covered with a cover glass on top.

IR spectroscopy with Fourier resolution. IR spectra were recorded on a Shimadzu IRAffinity-1 instrument in a thin layer with a Fourier transform.

Water-absorbing capacity. The effect of the introduced plasticizer on the water-absorbing capacity of the film is determined. The water-absorbing capacity was determined for all films by the method of Gialamas H. with amendments [20]. Samples of films were placed in distilled water and kept at 23°C for 30, 60, 90 minutes and at 90°C for 30, 60, 90 minutes in the TSO–1/80 thermostat to produce films with different moisture content. The degree of water absorption was determined as the ratio of the film weight after the experiment to the film weight before the experiment in percent. After three consecutive measurements, the average of the arithmetic mean was determined.

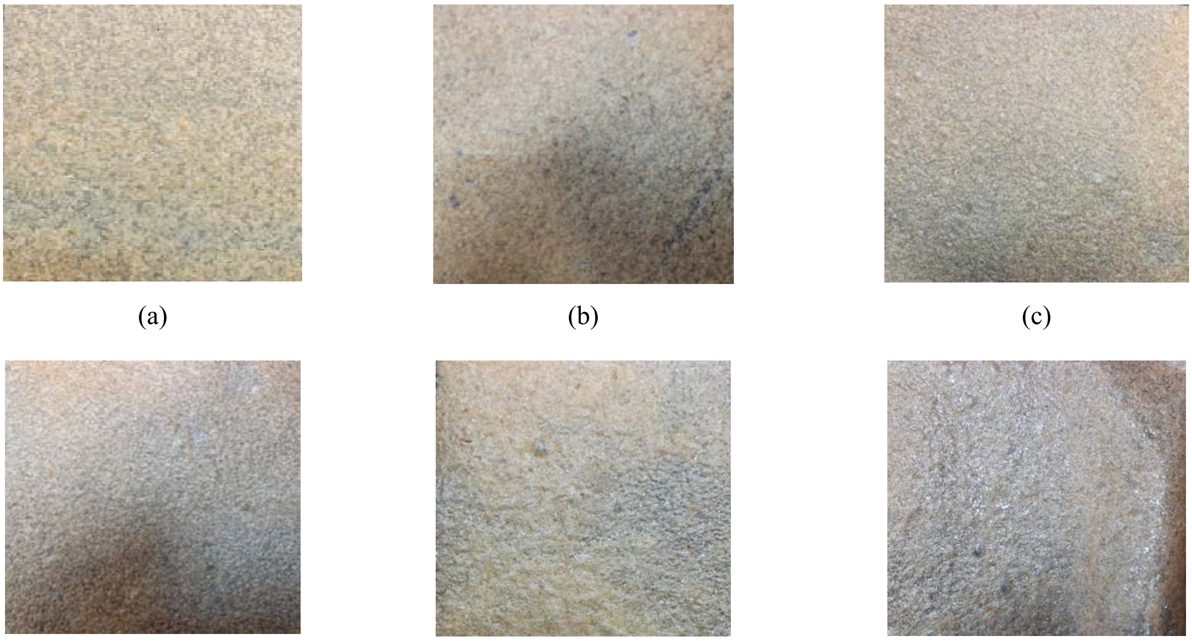
Film thickness. The film thickness was measured with a digital micrometer FIT 19909. Five measurements were made for each film: one in the center of the sample and in different parts of the perimeter. The average thickness value w calculated (Table 2).

Tensile tests. Tensile tests of film materials were carried out on a laboratory test complex including a tensile machine INSTRON-5988 (Testing Laboratory for Determining Mechanical Properties and Chemical Composition of Structural Materials, Samara State Technical University, research scientist A.E. Gorbunov) with the rate of application of the load in large ranges from 0.001 mm/min to 508 mm 0.001 mm/min. Samples with a width of 10 mm were tested at a distance between the clamps of 150 mm. Determination of deformation properties of materials with obtaining "load-displacement", "stress-displacement" dependence diagrams and mathematical processing of the results was carried out using the Bluehill 3 software (GOST R 53226-2008).

RESULTS AND DISCUSSION

According to the technology developed by us, six samples of edible film with different types and contents of plasticizers were made. For the prepared film samples, organoleptic characteristics, structure, strength properties and water-absorbing capacity were studied.

Photographs were obtained for all six film samples. Fig. 1 shows that all the films have a yellowish color characteristic for apple sauce. The color shades of the film differ slightly. The structure of all the film samples is dense. For a film with the addition of agar, a decrease in the number of inclusions of air bubbles with an increase in the amount of the additive is observed. The film with the addition of xanthan gum practically does not have inclusions of air bubbles. The surface of the films is smooth and shiny.



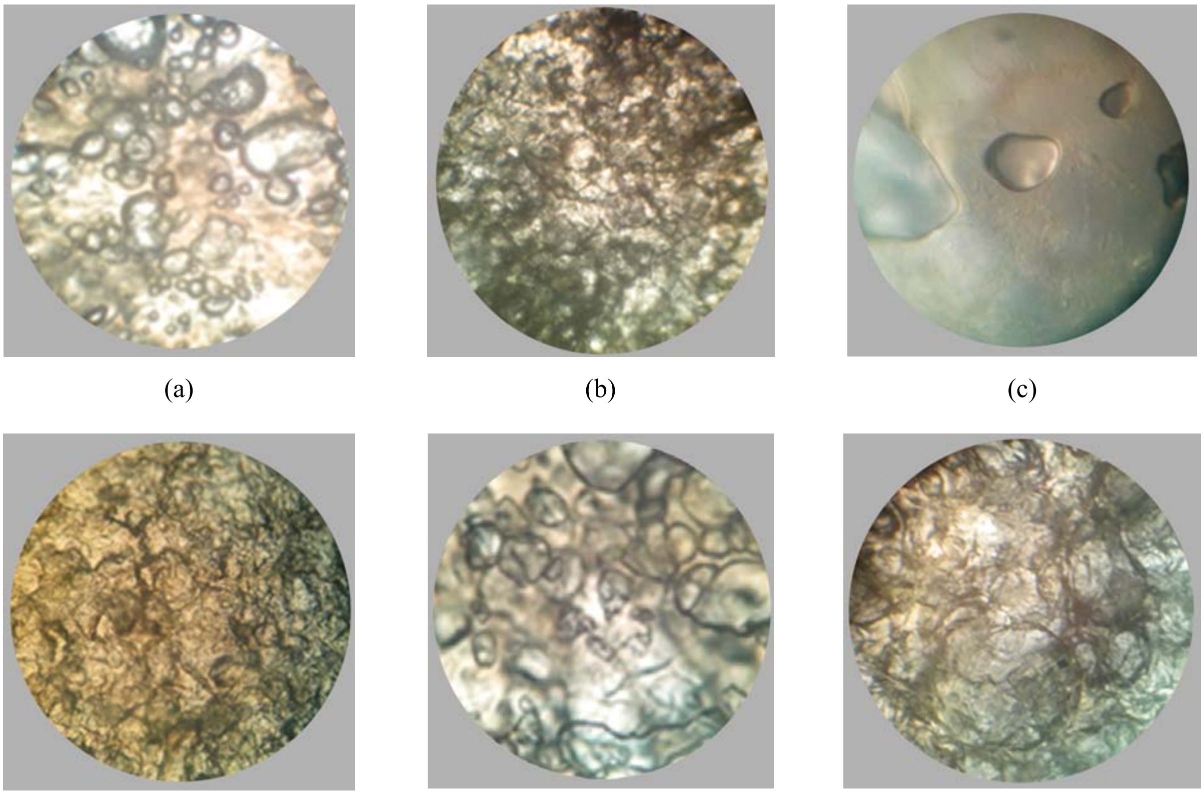
(a) (b) (c) (d) (e) (f)

Fig. 1. Appearance of edible film from apple raw material with the addition of plasticizers: (a) 2% agar, (b) 4% agar, (c) 2% carrageenan, (d) 4% carrageenan, (e) 2% xanthan gum, (f) 4% xanthan gum.

For the conventional packaging of food products, appearance and flavor of the material are important. However, the edible film is a very specific type of packaging, for which, in addition to the appearance and flavor, taste and consistency of the edible film are important as well. Therefore, organoleptic testing in the case of edible film has a particularly important role. With all the positive properties, an edible film with poor organoleptic properties will not find its consumer. All films have a weak taste of apple sauce. All films have an attractive natural pale-yellow color. Films have apple flavor, corresponding to the natural flavor of apples. The taste of all films is associated with apples. They have a sourish pleasant taste. A film of xanthan gum has the most acceptable taste properties. All edible films have resilience and elasticity. This is especially pronounced in the presence of plasticizers in the film composition in an amount of 4%. However, with an increase in the amount of plasticizer for all types of films, the chewing ability of films decreases. In general, it should be noted that, despite the fact that all the edible films obtained have an attractive appearance associated with natural fruits – apples, samples of films with a lower content of plasticizers are more appealing in terms of taste and chewing ability. Increasing the proportion of plasticizer to 4% leads to the presence of a "rubber-like" structure. For edible films, criteria for organoleptic tests have been developed and the results are given in Table 1.

For packaging materials, structural properties are extremely important, since the structure largely determines the qualitative characteristics and physical properties. The structure of the film was studied using a biological laboratory microscope – 400x, Celestron and the results are shown in Fig. 2. The mechanical properties of the edible film are determined by the presence of a homogeneous structure. For an edible film made from apple sauce with the addition of agar and an increase in the proportion of plasticizer, the density of the structure increases and the number of air bubbles decreases (Fig. 2a, 2b). A similar dependence is observed for an apple sauce-based edible film with the addition of xanthan gum (Fig. 2e, 2f). Increasing the proportion of plasticizer, although somewhat degrades the eating qualities of edible films, but improves their structure. In this series of experiments, it can be stated that agar, carrageenan and xanthan gum exhibit stabilizing properties, making the film structure more homogeneous.

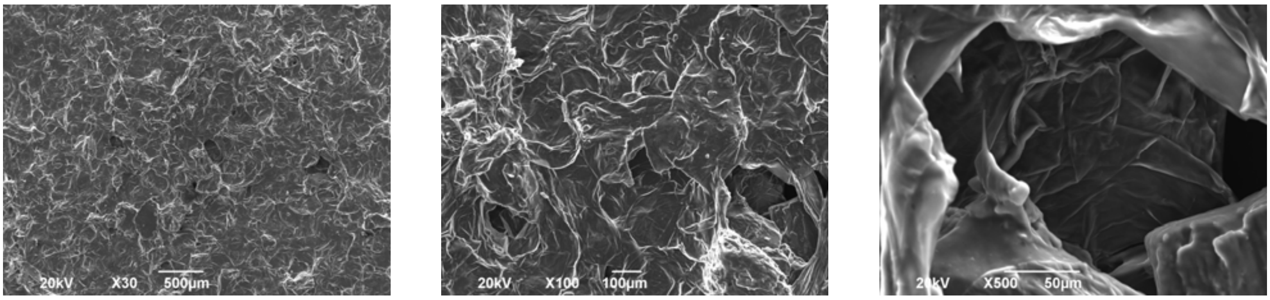
More detailed data on the structure of the film can be obtained by electron microscopic examination. Fig. 3–5 shows photographs of film samples made with the JEOL-6390A scanning electronic microscope. Studies carried out with the help of an electronic microscope make it possible to obtain more extensive data on the structure of edible films. Interestingly, edible films with carrageenan have a more ordered structure than other film samples studied.



(a) (b) (c)

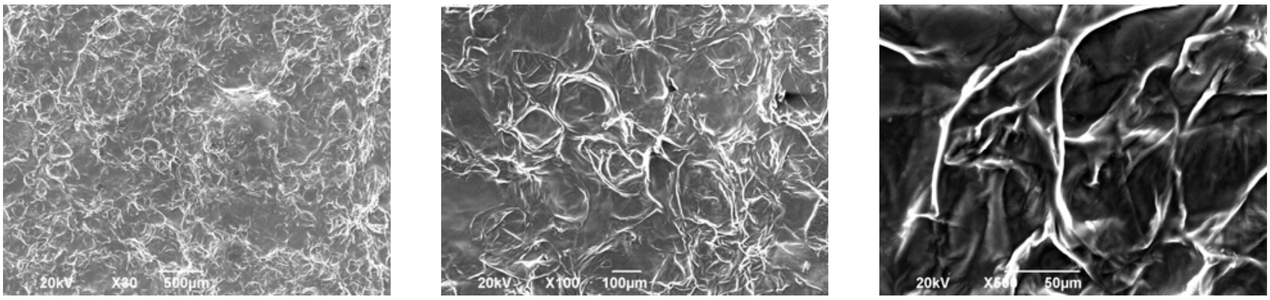
(d) (e) (f)

Fig. 2. Microscopic examination of edible films based on apple raw material with the addition of a plasticizer: (a) 2% agar, (b) 4% agar, (c) 2% carrageenan, (d) 4% carrageenan, (e) 2% xanthan gum, (f) 4% xanthan gum.



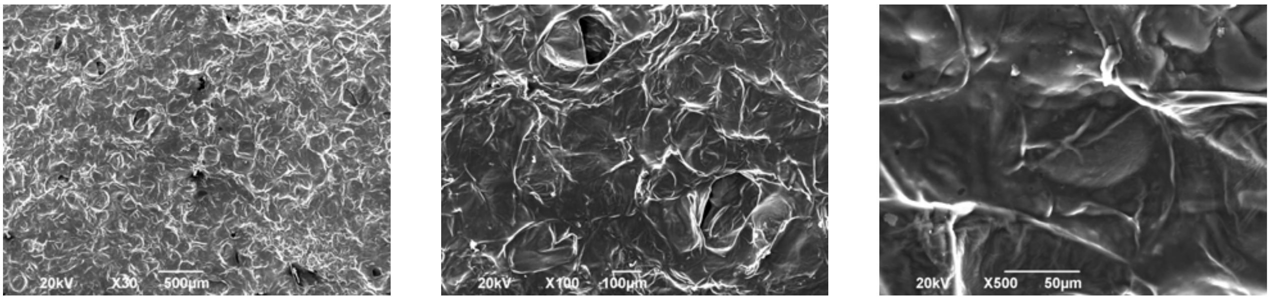
(a) (b) (c)

Fig. 3. Electron microscopic examination of edible films with the addition of 2% agar with magnification of: (a) 30x, (b) 100x, and (c) 500x.



(a) (b) (c)

Fig. 4. Electron microscopic examination of edible films with the addition of 2% carrageenan with magnification of: (a) 30x, (b) 100x, and (c) 500x.

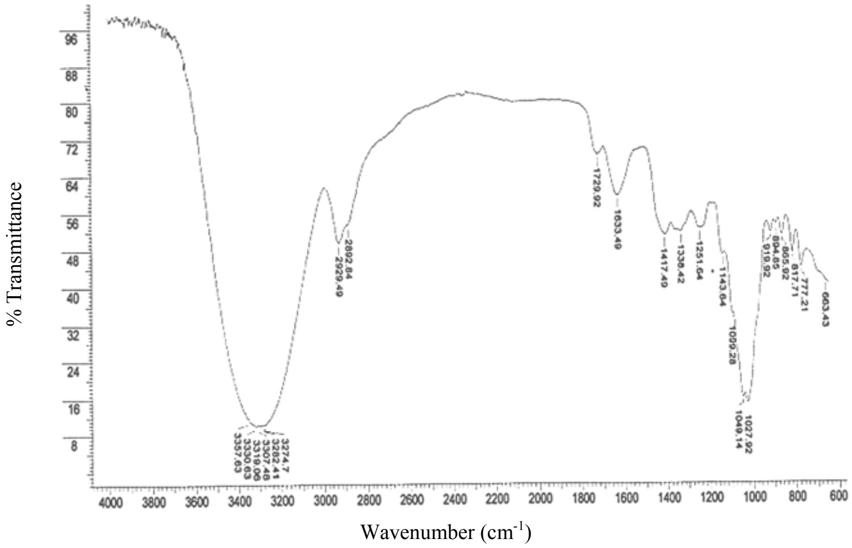


(a) (b) (c)

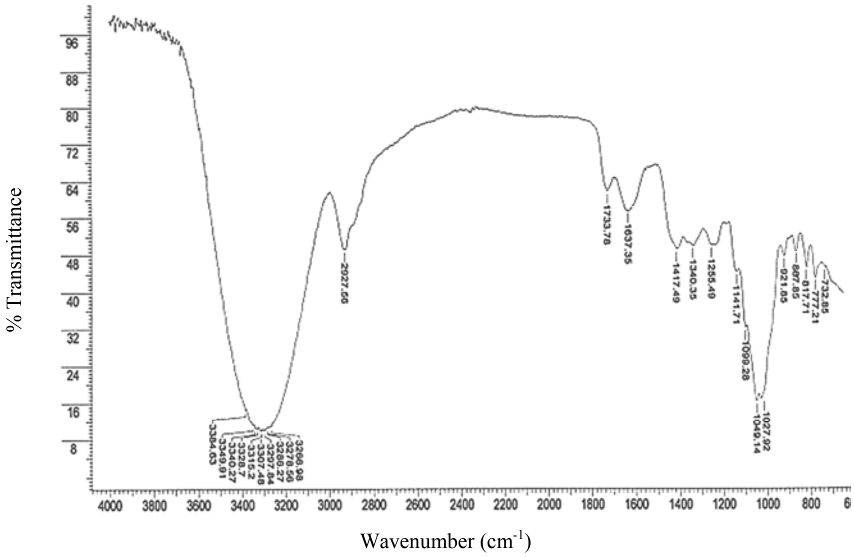
Fig. 5. Electron microscopic examination of edible films with the addition of 2% xanthan gum with magnification of: (a) 30x, (b) 100x, and (c) 500x.

IR-spectroscopy is a method that allows to characterize the chemical composition of the edible film. The IR spectrum of an apple sauce-based edible film with agar, carrageenan, xanthan gum as a plasticizer, is characterized by the presence of a wide absorption band in the region of 3274–3357 cm^{-1}, relating to the stretching vibrations of the hydroxyl group (υ_{OH}); deformation vibrations of the hydroxyl group are observed in the region of 1338 and 1417 cm^{-1} (Fig. 6). A weak peak of the valence vibrations of the carbonyl group in the region of 1729 cm^{-1} describes the presence of agar plasticizer in the film. In the IR spectrum of the apple sauce-based edible film with the addition of carrageenan, there is a broad band in the region of 3266–3384 cm^{-1}, which refer to the valence vibrations of the hydroxyl group (υ_{OH}). The band has a high intensity, because the group participates in the formation of intermolecular bonds. The deformation vibrations δ_{OH} of this group are detected by a peak of 1340, 1417 cm^{-1}. The plasticizer xanthan gum on the spectrum can be described by a weak signal of valence vibrations of the C=O group (1733 cm^{-1}).

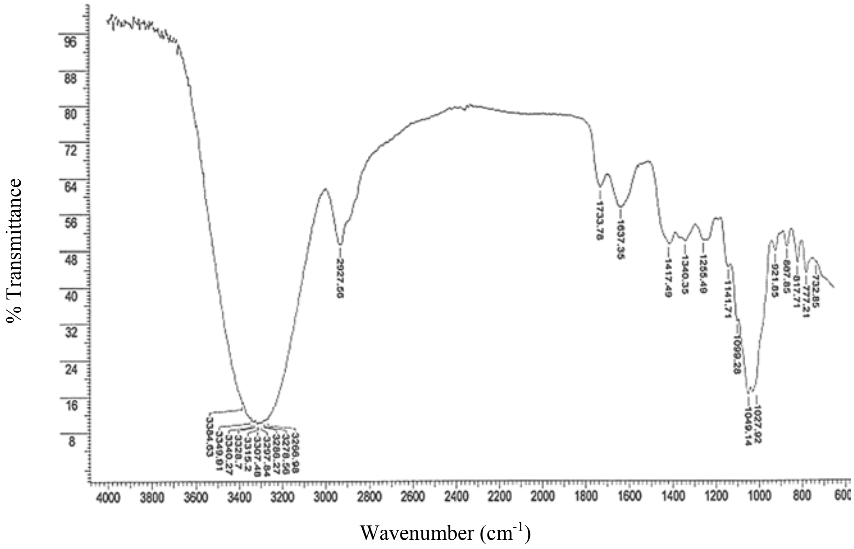
Tests of the water-absorbing capacity of the edible film (Table 2) were carried out at two different temperatures: 23 and 40°C, and three time intervals: 30, 60 and 90 min.



(a)



(b)



(c)

Fig. 6. IR spectra of edible film samples based on apple sauce with the addition of: (a) agar, (b) carrageenan, (c) xanthan gum.

Table 2. Water absorption capacity of edible films based on apple sauce with the addition of a plasticizer

Conditions (t, τ)	Water absorption capacity, %					
	Agar-agar, 2%	Agar-agar, 4%	Carrageenan, 2%	Carrageenan, 4%	Xanthan gum, 2%	Xanthan gum, 4%
23°C, 30 min	1084.21	895.24	351.43	402.04	894.59	984.38
23°C, 60 min	Sample is dissolved	Sample is dissolved	428.57	477.56	1108.11	1143.75
23°C, 90 min	Sample is dissolved	Sample is dissolved	511.43	473.47	Sample is dissolved	Sample is dissolved
40°C, 30 min	797.14	1016	Sample is dissolved	Sample is dissolved	962.50	826.00
40°C, 60 min	Sample is dissolved	Sample is dissolved	Sample is dissolved	Sample is dissolved	1167.50	916.00
40ºC, 90 min	Sample is dissolved	Sample is dissolved	Sample is dissolved	Sample is dissolved	Sample is dissolved	Sample is dissolved

The characterization of the water absorption capacity is very important, since water absorption on one side characterizes the ability of the edible film to split in the human gastrointestinal tract, and on the other hand describes the barrier properties of the edible film with respect to water. For all films, the general trend is: a higher temperature (40°C) and a longer time of contact with water (60 and 90 min) promotes dissolution of a film. In terms of water solubility, edible films with the addition of plasticizers can be arranged in a row in descending order: films with agar> films with carrageenan> films with xanthan gum.

Strength characteristics of edible films determine primarily the ability of the film to act as a barrier to mechanical influences. But with respect to edible films, another aspect should be considered: The too high strength of the edible film causes a low chewing ability of the film. As for the edible film, one should find the most optimal strength with good chewing ability. The results of tests of thickness, strength characteristics of film edible materials are summarized in Table 3. For edible films based on apple sauce with the addition of agar and xanthan gum as a plasticizer, there is a tendency to increase the tensile strength with an increase in the amount of plasticizer, however, for a film with the addition of carrageenan, the value of the tensile strength varies in the same range, regardless of the amount of plasticizer. This may be due to the fact that the edible films of agar and xanthan gum with the increase of the plasticizing additive change the structure with a tendency to regularity, while the number of air bubbles decreases. Films become more homogeneous and well-ordered.

Undoubtedly, edible films with xanthan gum and carrageenan are more durable. At the same time, their results are more than 2 times higher than the results of edible films with agar. It should be noted that studying the structure of the edible film with a laser microscope shows that the structure of the film with carrageenan is more homogeneous. In this case, films with carrageenan and xanthan gum are more resistant to water absorption. If we summarize these data with the results of an organoleptic analysis of edible films, we can choose two types of films: with carrageenan and xanthan gum as the most preferred.

Thus, on the basis of the data obtained, one can state the effect of the type and amount of plasticizer added on the organoleptic and physico-chemical properties of apple sauce-based edible films. The most durable is a film with the addition of 4% xanthan gum.

The complex of studies of single-layer edible films performed during this work allows us to draw a number of conclusions:

(1) For edible films with addition of 2 and 4% of agar, carrageenan, xanthan gum plasticizers the film with a lower number of plasticizers and have more attractive organoleptic properties;

(2) Electron microscopic examination of edible films allows to choose carrageenan as a plasticizer with a high stabilizing ability;

(3) At room temperature films with carrageenan are more resistant to water, but when the temperature is raised to 40°C, edible films with xanthan gum can be distinguished as resistant to water for a longer period of time;

(4) The strength characteristics of the films also depend on the nature and content of the plasticizer. Films with carrageenan and xanthan gum are more durable;

(5) On the totality of the studied properties and characteristics, films with carrageenan and xanthan gum are promising.

Table 3. The thickness of the edible film based on apple raw material with the addition of plasticizers and its tensile test

Plasticizer added to film		Film thickness, mm	Tensile strength, MPa	Tensile strength load, N
Agar	2%	0.30	1.32	3.95
	4%	0.28	1.70	5.03
Carrageenan	2%	0.24	3.17	6.34
	4%	0.28	3.26	9.07
Xanthan gum	2%	0.31	1.68	5.04
	4%	0.33	3.50	10.48

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Please cite this article in press as: Bykov D.E., Eremeeva N.B., Makarova N.V., Bakharev V.V., Demidova A.V., and Bykova T.O. Influence of Plasticizer Content on Organoleptic, Physico-Chemical and Strength Characteristics of Apple Sauce-Based Edible Film. *Foods and Raw Materials*, 2017, vol. 5, no. 2, pp. 5–14. DOI: 10.21603/2308-4057-2017-2-5-14.