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RESEARCH ON THE GELLING AND EMULSIFYING CAPACITY OF PECTIN OBTAINED BY SEMISYNTHESIS AND USED IN THE FOOD INDUSTRY

Camelia HODOSAN[1], Lucica NISTOR[1], Paula POSAN[1], Daniela IANITCHI[1], Sorin BARBUICA[1], Anca BORDIANU[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2] University of Medicine And Pharmacy Carol Davila, Bucharest - Bagdasar-Arseni Emergency Hospital, Bucharest, Romania

Corresponding author email: lucia_mamina@yahoo.com

Abstract

The chemical composition and nutritional value of food products are determined both by the raw materials from which they come and by the contribution of auxiliary materials. These substances, for the most part, are added in order to improve some properties of the products. Respecting the legislation regarding the allowed limits of additives in food products, it is necessary to pay special attention in order to maintain the safety and health of the population. The generic term pectin includes polygalacturonic acids (acid polysaccharide) whose carboxylic groups are esterified in varying proportions with methyl alcohol and partially neutralized with calcium or magnesium ions. The specific properties of pectic substances, due to which they have uses in the food industry, are the gelling capacity and the stabilizing capacity of emulsions. Also, the gel does not show the phenomenon of syneresis, does not absorb moisture from the external environment and is resistant to sugaring. In our country, pectin is obtained industrially from apple pomace, a by-product resulting from obtaining juices and has multiple uses. The functional properties are largely determined by the degree of methoxylation of polygalacturonic acid, which determine the degree of esterification of this polysaccharic acid.

Key words*: emulsifying, food industry, gelling, pectin.*

INTRODUCTION

The generic term pectin includes polygalacturonic acids (polysaccharide) whose carboxylic groups are esterified in varying proportions with methyl alcohol and partially neutralized with calcium or magnesium ions (Mohnen, 2008).
The specific properties of pectic substances due to which they are used in the food industry are: the ability to gel and the ability to stabilize emulsions. Also, the celtic gel does not show the phenomenon of synerese, does not absorb moisture from the outside environment and is resistant to sugaring.
Pectin is used in the manufacture of jams, marmalades, jellies, as a stabilizer of emulsions, in the manufacture of margarine, mayonnaise and ice cream, in the preparation of aspic for meat or fish products, in addition to prolonging the shelf life of fresh pastries, at obtaining edible protective coatings for cold-preserved products, as thickeners for creams, in obtaining various preparations for diabetics (Laurent & Boulenguer, 2003; Liu et al., 2003).
The functional properties are largely determined by the degree of methoxylation of polygalactouronic acid (Dergal et al., 2006).
Totally esterified pectin lacks the ability to gel. Pectins with a high degree of esterification, with a methoxyl group content over 9% are obtained from apple pomace or citrus peel (albedo). A normal gelation in the case of these pectins is obtained for a concentration of 65% sucrose in the gel and a pH = 3 (Pilgrim et al., 1991).
Low esterification pectins (less than 60%) containing less than 7% methoxyl groups are extracted from sugar beet borer. In these pectins, gelling occurs for a lower percentage of sucrose (less than 35% sucrose in the gel) or even in the absence of sugar, but in the presence of calcium ions and in a wide pH range between 2.5-6. Calcium ions make the

connection between carboxyl groups of polygalactouronic acid molecules (BeMiller, 1986).
The fruit processing industry uses exclusively highly esterified pectin. These pectins are partially desesterified in a controlled manner, using various demethoxylation processes (Willats, 2001).
Pectins have industrial uses as gelling and stabilizing agents for food and cosmetics. They have been used in the synthesis of biofilms, adhesives, paper substitutes and medical products for implants or drug carriers (Thakur, 1997).
Many studies indicate its benefits for human health, as it has been shown to help lower blood cholesterol and glucose levels, in addition to boosting the immune system.
Pectin as a finished product can be obtained in different forms:
- Pectic extract, which is obtained by extracting hot apple pomace, followed by concentration. The extract contains 10-12% soluble dry matter and 3-4% soluble pectin;
- Pectin powder, obtained by drying pectic extracts or by precipitating aqueous extract, followed by conditioning and then drying;
- Pharmaceutical pectin, characterized by an advanced purity (Thakur, 1997).
Because the manufacture of pectin powder requires complex installations and higher fuel consumption, pectic extracts can be used for short-term use after manufacture.
The technological scheme for the manufacture of apple pectin is shown in Figure 1.
Fresh apple pulp contains 0.7-1.2% pectic substances and 60-70% water. Therefore, it cannot be stored as such, in addition, in order to ensure the use of pectin for several months, it is necessary to preserve the pomace. Two methods can be used: chemical preservation (with 0.15-0.20% SO_2 - for short duration) and drying (up to 5% humidity in drum dryer - the most commonly used method for long-term preservation).
In the absence of drying facilities, pomace can also be preserved by adding SO_2 (5% solution in proportion of 10% compared to pomace), by keeping it cold (more expensive).

Washing the pomace is practiced to remove the accompanying substances of pectic substances (sugars, acids, tannins, dyes, mineral salts) with water at a maximum temperature of 40°C.

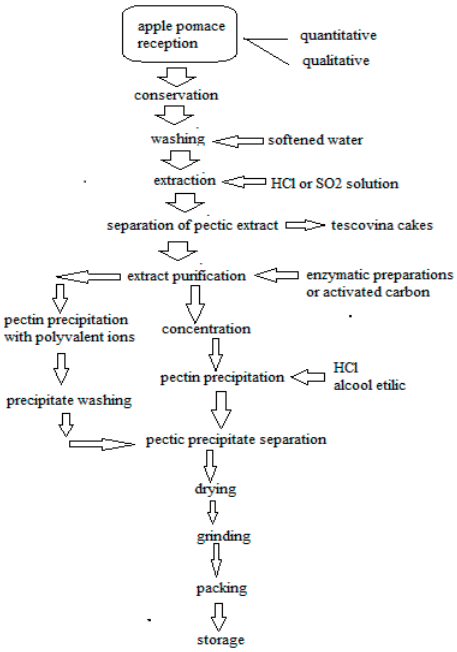

Figure 1. Technological scheme for the manufacture of apple pectin

It is used a volume of washing water of 5 to 6 times larger than the amount of pomace, the duration of the operation being 1-3 hours. The soaking and washing time depends on how the pomace was preserved (12 hours for dry pomace).

The soaking and washing process is performed in an extractor, in which the pectin is subsequently extracted, with the possibilities of stirring and heating the mixture. Sugar-rich wash water can be used.

The extraction of pectin consists in the transformation of the existing protopectin into apple cider vinegar and its transition into solution. The most advantageous hydrolysis takes place with acidified, softened hot water. The added water is 5-6 times higher than the amount of fresh pomace and 10-12 times the amount of dehydrated. It is recommended that the extractive water be added in three stages. The acidification to pH = 2-3 is done with

hydrochloric acid or sulfuric acid. In the last case, which allows to obtain a light pectin, the working parameters are: temperature (86°C), duration (2.5-3 hours) and in the last phase water without acid is added. The extractive process must be conducted in such a way that the efficiency of the operation is as good as possible and the pectin is not degraded. The three solutions of pectic extract, obtained by draining, are cooled to 40°C and mixed with the liquid obtained from pressing the pomace (Adetunji et al., 2017).

Purification of pectic extracts, an operation necessary especially for the processing of insufficiently ripe apple pomace, can be done by two methods: treatment with enzymatic preparations (from *Aspergillus oryzae*) or absorption on activated carbon. The first method is based on the hydrolyzed action of enzymatic preparations added proportion of 0.03-0.05%, at 40-50°C, for 30-40 min, on starch and proteins. In some cases, in order to avoid the hydrolysis of pectin, the pectic extracts are purified by adding 0.1% active carbines and 0.3% kieselgur, followed by filtration (Robledo & Vázquez, 2018).

Concentration of pectic extracts. The purified extracts have a low pectin content, between 0.15 and 0.4%, therefore they are concentrated in vacuum installations at a maximum temperature of 60°C, until the volume is reduced by 8 to10 times, reaching 12% soluble dry matter.

Pectin precipitation. Two methods can be used to separate pectin from solution:

- Alcohol precipitation of the concentrated extract with approximately 3% pectin; the extract is mixed with 1.5% concentrated hydrochloric acid (d = 1.18) and then with 1-1.2 parts of 95% ethyl alcohol, the pectin precipitating as a fibrous mass which separates by filtration. Pectin is washed with ethyl alcohol in a ratio of 1:1. The alcohol used for precipitation and washing is recovered by distillation and reintroduced into the circuit, the method being the most used.
- Precipitation with polyvalent ions, which is done directly on unconcentrated pectic extracts, at pH = 3.6-3.7 and at temperatures of 30°C. The obtained curd is washed with a mixture of alcohol and hydrochloric acid for the complete removal of the metal ions from the curd. The washing liquids are separated from the curd by centrifugation, and the alcohol is recovered. The best results were obtained using anhydrous aluminum chloride.

Pectin drying. The pectic precipitate is dried in vacuum drying instalations at a maximum temperature of 75-80°C, up to a product humidity of 4-5%.

The pectin grindind is done to a maximum size of 2 mm, with the help of hammer-mills. To obtain a product with the same degree of gelling, pectin powder is mixed with powdered sugar in varying amounts.

Pectin packaging is done in shipping containers of different capacities, made of materials intended for dehydrated and hygroscopic products (for example, polyethylene bags or sacks).

Storage will be done in warehouses where temperatures should not exceed 25°C (Shalini R. and Gupta D.K., 2010)

MATERIALS AND METHODS

This paper presents three laboratory methods of pectin semisynthesis.

Method I

Principle of the method:

A method for obtaining pectin from apple pomace is presented, using the process of transforming insoluble protopectin into soluble pectin. This process is carried out with the help of sulfuric acid, followed by the extraction, concentration and purification of the obtained semisynthetic substance.

Procedure:

In a 250 ml flask, provided with ascending refrigerant, thermometer and mechanical stirrer, a quantity of 20 g of dried apple cider vinegar and approximately 150-200 ml of water heated to 45°C is introduced. The contents of the flask are stirred for 30 minutes, after which the liquid containing soluble ballast substances (sugars, mineral salts, tannin, and corrosive substances) is removed.

After removing the liquid, 150 ml of distilled water, heated to 90°C, is introduced into the flask, the pH is corrected to the value of 2 by adding sulphuric acid. The reagent mass is stirred for one hour at 90°C.

The resulting pectin extract is drained and stored, after which two more extractions are

successfully performed, working in conditions identical to those used in the first extract (150 ml distilled water, temperature 90°C, pH = 2 by adding sulphuric acid, stirred for one hour).
The three extracts obtained are mixed and cooled to a temperature of 45°C, after which the gross suspensions are removed by centrifugation.
To clarify the liquid, it is filtered through a kieselgur filter, after which it is concentrated in vacuo at 65°C to a content of 12% dry matter.
The concentrated extract is vigorously mixed with concentrated HCl (d=1.18) in a proportion of 1.5% compared to the obtained solution and then with an equal part of ethanol.
The curd formed is separated by filtration and washed in two steps with ethanol of 95% concentration in a ratio of 2:1 to the precipitate.
The purified pectin is crushed, dehydrated by drying at a temperature of 75-80°C, cooled, grinded and conditioned by the addition of powdered sugar.

Method II

Principle of the method:
A method of obtaining pectin from apple pomace or orange peel (albedo) is proposed. A combined process of acid hydrolysis with enzymatic treatment is used.
An amylolytic preparation obtained from *Aspergillus oryzae* is used.
Procedure:
A quantity of 20 g of apple pomace or dried orange peel, finely chopped, are placed in a 250 ml flask provided with an ascending refrigerant, a thermometer and a mechanical stirrer.
Sulfuric acid solution is added until the pH of the reagent mass is 2.5-3.5.
Pectin extraction takes place at a temperature of 85-92°C for one hour.
The obtained extract is separated from the solid residues by filtration, then the pH is corrected to the value of 4.5-5 by the addition of Na_2CO_3.
The crude pectyl extract is further treated with 0.5% amylolytic preparation (*Aspergillus oryzae* mycelium, grown on wheat bran), at a temperature of 40-50°C for 30-60 minutes. At the end, the temperature of the reagent mass is raised to 80°C to inactivate the enzymes.
For clarification and classification of the extract, it is mixed with 0.02% kieselgur (relative to the amount of extract) after which it is filtered.
The clear filtrate is concentrated under reduced pressure at 50-60°C to a content of about 3% pectin (15 degrees refractometric).
The concentrated extract is mixed with 95% ethanol, in a ratio of 1:1.2 and acidified with concentrated HCl in a proportion of 1.5%. A pectin clot is formed which separates by filtration. The curd is washed on a filter with 95% ethanol. The purified product, having the consistency of a thick paste, is dehydrated by drying at a temperature of 60-70°C.

Method III

Principle of the method:
Pectin is obtained from sugar beet borer using the acid hydrolysis (HCl) reaction to convert insoluble protopectin to soluble pectin. The precipitation of soluble pectin is done by treating the pectin extract with aluminium sulphate.
Procedure:
In a 250 ml flask equipped with an ascending refrigerant, thermometer and mechanical stirrer, a quantity of 15 g of sugar beet marc (with a humidity of 1%), 4.5 ml of 35% HCl solution and 16 ml of water are introduced.
The contents of the flask are heated with continuous stirring at 70°C for two hours. The resulting extract is passed through a centrifugal separator to separate the pulp particles.
The clear solution obtained is partially discoloured and deodorized by mixing with activated charcoal in a proportion of 0.5% carbine compared to the extract, for 25-30 min. under stirring, after which it is filtered. To the clear solution of pectin there was added a 10% ammonia solution to pH = 4 and then with continuous stirring, aluminium sulphate in a proportion of 3 g and aluminium sulphate 9% concentration. Pectin changes into aluminium pectinate, which precipitates.
A new pH correction is made at the value of 4 by adding ammonia.
The precipitated pectin is separated by filtration, after which, together with a solution of citric acid of 56% concentration (in the ratio of curd/citric acid of 4:1), it is introduced into a flask provided with a stirrer and mixed at room temperature until obtain a homogeneous viscous fluid. By adding water, the

concentration is corrected to about 10% dry matter. 0.12% SO_2 or 0.15% sodium benzoate is added to preserve the solution.

RESULTS AND DISCUSSIONS

The gelling capacity of pectin was checked as follows: a mixture of 0.26 g pectin, 17.5 ml water and 3 g sugar flour, put in a Berzelius glass and boil for about 2 minutes, until the pectin is completely dissolved. 22.7 g of caster sugar were added to the obtained solution and heated and stirring for 7-8 minutes. Because pectin is difficult to dissolve in concentrated sugar solutions, not all sugar is added from the beginning.

After the boiling was stopped, the solution was left to stand for one minute, after which 1 ml of tartaric acid solution was poured over the obtained solution (tartaric acid solution contains 488 g of tartaric acid/11 g solution).

The resulting product was allowed to stand again at 25°C until a gel was obtained. The gelling power of pectin was expressed in US-SAG degrees.

In Tables 1, 2 and 3 there are presented pectin characteristics and pectin LM, LMC and LMA gelling conditions.

Table 1. Pectin characteristics

Parameter	Pectine HM	Pectine LM-LMC	Pectin LM-LMA
DE	58-85	25-50	23-50
DA	0	0	≤ 25
pH	2.8-3.5	3.2-4.7	3.5-4.7
MM	140000-190000	70000-140000	70000-140000

DE- degree of esterification, DA- degree of acetylation, MM-molas mass
Pectine HM - High methoxylated pectins
Pectine LM - Low methoxylated pectins
LMC - Low calorie methoxylate
LMA - Amidated low methoxyl

Table 2. Pectin LM gelling conditions

Parameter	Pectine LM		
DE	40 - 37	33 - 30	27 – 24
COO^-	+	+ +	+ + +
Ca^{2+} sensitivity	Low	Medium	High
Reaction speed	Low	Medium	Fast
Ca^{2+} requirement	High (20)	Medium (12)	Low (7)

COO^- - Carboxylate ion
Ca^{2+} - Calcium (mg/g)

Table 3. Gelling conditions for CML and AML pectins

Parameter	Pectine LM-LMC	Pectin LM-LMA
DE	30	30
DA	0	17
COO^-	70	53
$COOCH_3$	30	30
$CONH_2$	0	17
Ca^{2+} requirement	High	High

$COOCH_3$ - methyl acetate
$CONH_2$ - amide group

Figure 2 shows the strength of the gel formed with LM as a function of Brix and the addition of Ca^{2+} in mg/g of pectin.

The mechanism of pectin gel formation involves the presence of calcium ions, structurally the gel having the configuration of the egg shell (Figure 3).

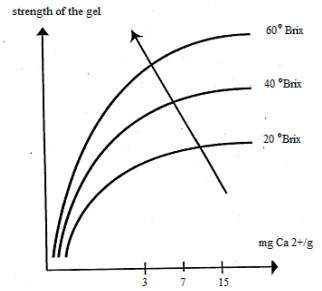

Figure 2. The strength of the gel formed, depending on the Brix and the addition of Ca^{2+}.

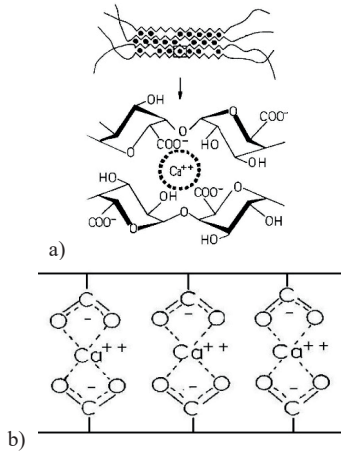

Figure 3. Mechanism of gel formation with low methoxylated pectins:
a) "egg formwork" mechanism;
b) Ca^{2+} intervention in the binding of two polygalacturonic chains

The mechanism of gel formation with highly methoxylated pectins involves the formation of hydrogen bonds and hydrophobic interactions, which support the formation of the three-dimensional network of the gel.
The conditions for the formation of the gel are: total soluble substance, minimum 60° Brix, sugar having the role of reducing the acidity, the acidity of the medium must ensure a pH<3.5.
The acidity decreases the electrostatic repulsion between the HM pectin chains. HM pectin gel is irreversible and its strength depends on pH value (Figure 4) and Brix value (Figure 5).

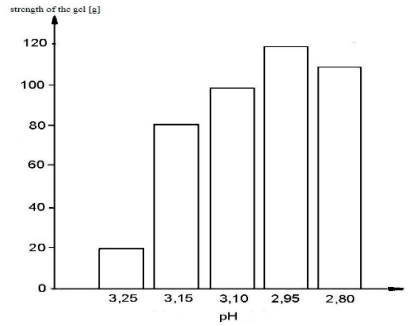

Figure 4. HM pectin gel strength depending on pH

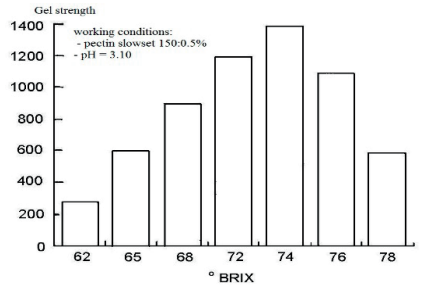

Figure 5. HM pectin gel strength depending on °Brix

CONCLUSIONS

Pectins are an important source of fiber that is present in a large number of vegetables and fruits consumed daily by humans, as it is a structural part of the cell walls of most green plants.
Due to their composition, pectins are very soluble in water molecules, which is why they have multiple applications, especially in the food industry.
Pectins can be used as gelling, stabilizing or thickening agents for many dishes, especially jellies and jams, yogurt drinks, milk and fruit milkshakes and ice cream.
Apple pectin is pectin that is derived from apples and is usually sold in powder form. It can be used as a gelling and thickening agent, as well as a food stabilizer. It is also used in medicine, as a supplement, in chewers such as throat lozenges or as a laxative additive for its natural purgative qualities. Apple pectin is full of healthy carbohydrates, dietary fiber, sodium, manganese, copper and zinc.
NH pectin is an apple pectin commonly used for fruit glazes and fillings. It is a modified type of LM pectin. NH pectin needs calcium to gel, like any other type of LM pectin. It is also thermally reversible, which means it can be melted, set, remelted and then reset again.

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