

Comparison of physicochemical parameters of pectic polysaccharides from different plant materials

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Physicochemical parameters – degree of methoxylation, purity, molecular weight, foaming, emulsifying and stabilizing properties of pectins derived through consecutive fractional extraction from waste rose petals were investigated and compared with properties of eight commercial and non-commercial pectins. All of the investigated pectins were high molecular weight with molecular masses in the range $1.32 \times 10^4 \div 4.08 \times 10^5$ Da. The polyuronic acid content (purity) was above 50 % except for acid extracted rose pectin. It was found that all of the investigated pectins except the one derived from celery had relatively low foaming and foam-stabilizing properties. Emulsions obtained with acid extracted pectin (Rose A) showed higher transmittance index but it was relatively unstable when mechanically treated. The most stable emulsion was obtained using celery pectin. The present data show that waste rose petals could be used as source for obtaining of pectins with physicochemical properties similar to other well-known pectins.

Key words: pectins, waste rose petals, foams, emulsions.

INTRODUCTION

Pectins are complex structural heteropolysaccharides build mainly by 1,4-linked α -D-galacturonic acid units interrupted by single (1 \rightarrow 2) linked α -L-rhamnose residues [1]. They are commonly found in the middle lamellae of higher plants [1, 2]. Pectins were used successfully for many years in the food and beverage industry as a thickener, emulsifier, texturizer, a colloidal stabilizer and as gelling agent in preparation of jams and jellies [1, 3]. Pectin is usually added in fruit juices, fruit drink concentrates, desserts, baking fruit preparations and dairy products [1].

Apple pomace and citrus peels are the raw materials traditionally used for industrial extraction of pectins [4] because they contain high amounts of pectic substances and are available in abundant supply as residues from fruit juice production [5]. Other sources of pectin were also explored as alternative sources – sugar beet pomace [6], sunflower head residues, olive pomace [1] and mango waste [7]. Pectins were also extracted from potato pulp [6], peach pulp [8], pumpkin pulp [9] and linseed residues [10]. In previous experiments Slavov *et al.* [11] investigated the possibility to extract water soluble pectin from waste rose petals.

The aim of the current research was to investigate and compare the degree of methoxylation, purity, molecular weight, foaming, emulsifying and stabilizing properties of pectins obtained through consecutive fractional extraction

of waste rose petals with pectic polysaccharides derived from well-known commercial (apple pomace, citrus peels) and non-commercial sources (sunflower head residues, celery tubers, carrots and grapefruits).

MATERIALS AND METHODS

Materials

The following pectins were used: from waste rose petals (W-water, C-chelate, A-acid extracted; prepared as described [12]), sunflower heads, apple pomace, carrots, citrus peels, grapefruit peels and celery tubers – prepared by acid extraction according to [7]. Three of the investigated pectins were commercial – two apple (obtained from Obipectin, Switzerland; №6 – low-methoxyl pectin and №8 – high-methoxyl pectin; numbering according to Table 1) and one citrus pectin (obtained from CP Kelco, Germany). All solutions were prepared with deionized water from Milli-Q system. Electrolyte 0.15 M NaCl (Merck) was used for preparation of all the pectin solutions.

Methods

1. Physicochemical characterization

1.1. Degree of methoxylation

The degree of methoxylation (DM) of the pectins was determined according to Kratchanova *et al.* [13].

1.2. Intrinsic viscosity and determination of molecular weight

The viscosimetric measurements of pectic solutions were performed as described by Panchev *et al.* [14].

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2. Foam and emulsifying properties

2.1. Model foam systems

2.1.1. Foam preparation

In order to eliminate the influence of the protein part co-extracted with pectic polysaccharides, pectins were deproteinized by the Sevage method [15]. Pectins were dissolved (0.6 wt %) by stirring at 45 °C. Foam ability and stability were studied by a stirring/shaking method whipping out 20 ml pectin solution for 60 s in a graduated cylinder. The initial foam volume and the subsequent foam decrease during 60 minutes were monitored. The foam ability was characterized by the volume of the trapped air.

The total volume of pectin solution before (V_1) and after (V_2) whipping, the volume of the formed foam ($V_{\text{foam}0}$) were measured immediately after shaking (at $t = 0$). The foam stability was characterized by the volume of entrapped air, still remaining in the foam after a certain period of time, $t > 0$ (V_{foam}) changed with the time t . All foam tests were performed thrice and the data presented as mean values.

2.1.2. Evaluation of foam capacity

Foam capacity was determined as described by Diniz *et al.* [16] with some modification. Foam capacity (FC) was determined by volume increase (%) immediately after whipping and was calculated by the formula:

$$\text{FC}\% = (V_2 - V_1/V_1) \times 100,$$

where V_2 is the volume of pectin solution after whipping and V_1 is the volume of solution before whipping.

2.1.3. Evaluation of foam stability

Foam stability was determined according to Marinova *et al.* [17] with modification. Foam stability was expressed by the parameter percentage volumetric foam stability, FS % which is defined as:

$$\text{FS}\% = (V_{\text{foam}}/V_{\text{foam}0}) \times 100,$$

where $V_{\text{foam}0}$ was the volume of the formed foam; V_{foam} was the volume of the foam changed with time t . Stability of the foams over time was assessed by measuring the foam volume at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30 and 60 min after stirring.

2.2. Model emulsion systems

2.2.1. Emulsion preparation

Pectins were dissolved (0.6 wt %) by stirring at 45 °C. 20 ml solution (6 mg/ml) was homogenized with 20 ml sunflower oil for 5 min at 50 s⁻¹ using homogenizer (Ultra Turrax IKA T18 Basic, Germany). The dispersity was determined measuring the translucency index (T %) after 10 times dilution, on a Camspec-M 107 spectrophotometer at $\lambda=540$ nm.

2.2.2. Evaluation of emulsifying activity and emulsion stability

Emulsifying activity and emulsion stability of the model systems of the studied pectins were determined as described by Kuncheva *et al.* [18].

Microscopic Test – The microstructure of the emulsions was evaluated by microscope system (microscope CarlZeiss, Germany, equipped with a camera connected to a personal computer) at 100 magnification immediately after preparation.

Centrifugal Test – The emulsion stability was evaluated by centrifugation of 10 ml emulsion at 3000×g (Hettich EBA 20, Germany) for 20 min. The height of the emulsified layer and separated phases were recorded. The emulsifying stability was calculated as a ratio of the height of the emulsified layer and the height of the total content of the tube and multiplied by 100.

Temperature Test – 5 ml of each emulsion was placed in tubes and stored at four temperatures: -18 °C (frozen); 4°C (refrigeration conditions); 25°C (room temperature) and 50°C for 48 hours. After that the height of the emulsified layer and separated phases were recorded. The emulsion stability was calculated as a ratio of the height of the emulsified layer and the height of the total content of the tube and multiplied by 100.

RESULTS AND DISCUSSION

1. Physicochemical characterization of the pectins

Important parameters of pectic polysaccharides which determine their possible application in food industry are their degree of methoxylation, purity and molecular weight. For this reason we analyzed the pectic polysaccharides and the results are presented in Table 1.

From the eleven samples two were high-metoxyl, one was medium-metoxyl and the rest – low-metoxyl pectins. All the pectins had polyuronic acid content (purity) above 50% except the pectic polysaccharides obtained by acid extraction from waste rose petals. The ash content of the pectins was in the range from 0.40 to 5.54 %.

2. Characterization of model foam systems

Foams are important two-phase systems present in numerous colloidal products. Food foams very often contain surface-active agent (usually proteins) which improves the foam-forming and foam-stabilizing properties.

In certain cases food polysaccharides could be used as foaming agents or could be added in order to significantly improve physico-chemical properties of the foams [19, 20]. In this regard we examined the foam-forming abilities of the investigated pectins (Fig. 1).

Table 1. Physico-chemical characteristics of the pectins

№	Type of pectin	DM, %	Purity, %	Viscosity $[\eta]$, dm ³ /g	Molecular weight, Da	Ash content, %
1	Rose W	61.70 ± 0.8	53.97 ± 1.3	0.0975	1.03 x 10 ⁵	5.06
2	Rose C	33.20 ± 0.7	68.72 ± 1.6	0.0362	2.65 x 10 ⁴	4.29
3	Rose A	49.50 ± 0.9	46.22 ± 1.1	0.0683	6.32 x 10 ⁴	5.54
4	Carrot	64.13 ± 0.5	62.85 ± 0.5	0.0416	3.20 x 10 ⁴	4.6
5	Sunflower	28.28 ± 0.4	88.94 ± 0.8	0.0218	1.32 x 10 ⁴	3.31
6	Apple*	34.49 ± 0.7	50.78 ± 1.2	0.1755	2.31 x 10 ⁵	1.77
7	Apple	35.06 ± 1.2	56.23 ± 0.6	0.0515	4.29 x 10 ⁴	3.25
8	Apple*	56.26 ± 0.6	71.29 ± 0.9	0.2663	4.08 x 10 ⁵	1.01
9	Citrus*	14.29 ± 1.3	54.14 ± 1.2	0.0449	3.56 x 10 ⁴	1.62
10	Grapefruit	14.00 ± 0.5	78.33 ± 0.8	0.0253	1.62 x 10 ⁴	0.40
11	Celery	36.13 ± 0.9	56.28 ± 0.7	0.1170	1.32 x 10 ⁵	1.90

* - commercial pectins.

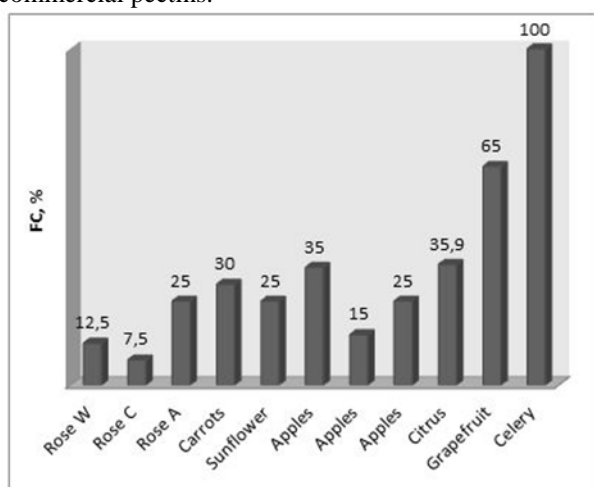


Fig. 1. Foam-forming abilities of the pectins

From the results it could be concluded that with the highest foam-forming ability (> 100 %) is characterized celery pectin, followed by grapefruit – 65 % and citrus pectins (commercial) – 35.9 %. The pectins obtained from waste rose petals showed relatively low foam-forming ability – the one obtained by acid extraction had 25 %, followed by chelate-extracted pectin – 12.5 %. The lowest foam-forming ability showed pectin obtained by aqueous extraction – 7.5 %.

In further experiments, the ability of the polysaccharides to stabilize the resulting foams was investigated and the results for the 30th and 60th minutes are summarized on Fig. 2.

All the investigated pectins showed less than 45 % retained foam volumes after 60 minutes.

The most stable foams were obtained using pectins extracted through consecutive fractional extraction of waste rose petals (Rose W and Rose A samples) – 44.0% after 30 minutes and 1 hour. Nevertheless these results should be interpreted with caution since the initial values of foam ability for these two pectin samples were very low. As a conclusion from the results obtained for the foam

stability it could be said that some of the pectins were able to produce foams but they were not stable over time.

3. Characterization of model emulsion systems

Emulsions are two (or more) phase dispersed colloidal systems. Many foodstuffs exist in the form of emulsions – milk, butter, margarine, mayonnaise etc. In order to avoid the separation between two immiscible phases an emulsifying agent is added. The emulsifying agents have amphiphilic nature (i.e. having both hydrophilic as well as lipophilic moieties) and thus will migrate to, and organize, at the interphase. In practice the most used substances are lecithins, mono- and diglycerides, sucrose esters and polyglycerol esters. Certain biopolymers also possess emulsifying properties – proteins [21], polysaccharides [18] etc. Therefore it was of interest to investigate and compare the emulsifying properties of the pectic polysaccharides from different sources.

Until now there were no data presented about the emulsifying properties of pectins obtained from waste rose petals. Emulsion systems type oil/water (O/W – 1:1) were prepared. The microstructure of the emulsions was monitored by direct observation and comparing the structures of all emulsions it was found that the celery pectin gave the best quality emulsion system (equally dispersed droplets) – Fig. 3.

In the images the oily dispersed phase can be seen as areas of irregular shape (Fig. 3a, b and c) or spheres (Fig. 3d) which suggest that the emulsion prepared with celery pectin is the most stable. Generally emulsions prepared using rose pectins are not stable, with large droplets (Rose A), irregular shapes and even for Rose W pectin with distinct phase separation. Of the three types rose pectins the most stable emulsion is the one made with Rose A pectin.

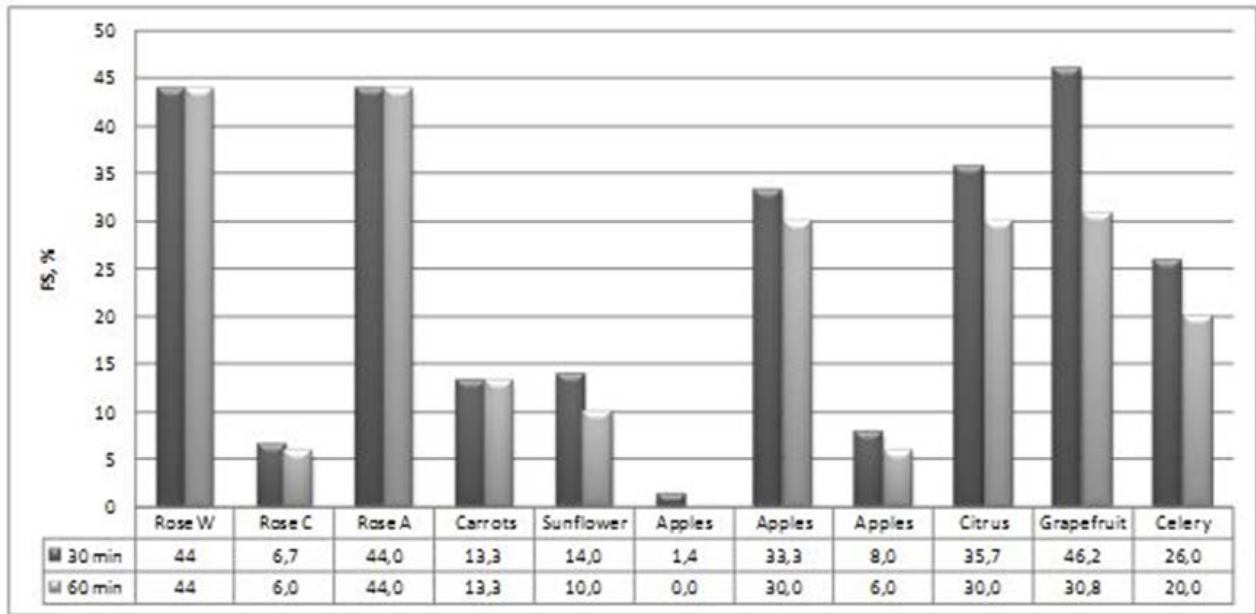


Fig. 2. Foam stabilities

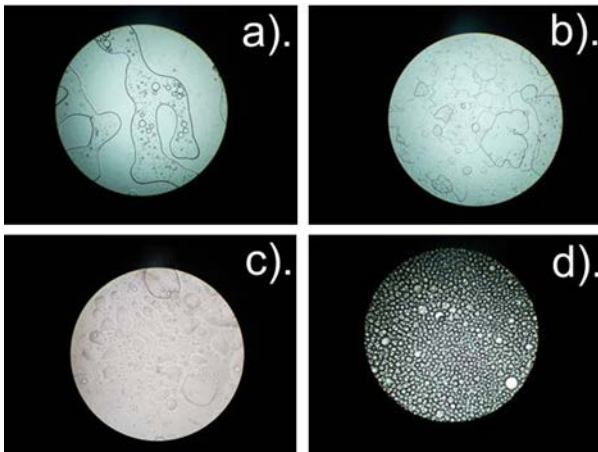


Fig. 3. Images of the structures of model O/W emulsions prepared with 0.6% pectin solutions: a). Rose W; b). Rose C; c). Rose A and d). Celery

Table 2. Emulsion stability and light transmittance of 50 % O/W emulsions

№	Type of pectin	Separated phases, %			T, %
		Oil	Water	Emulsion	
1	Rose W	2.0 ± 0.1	44.5 ± 1.2	53.0 ± 0.6	33.6 ± 1.2
2	Rose C	8.0 ± 0.2	36.5 ± 1.3	55.0 ± 0.8	48.9 ± 2.3
3	Rose A	3.8 ± 0.1	24.0 ± 0.8	72.1 ± 1.1	5.8 ± 0.9
4	Carrot	8.2 ± 0.3	39.8 ± 1.5	52.0 ± 0.6	26.9 ± 2.8
5	Sunflower	2.0 ± 0.1	46.0 ± 0.7	52.0 ± 1.1	98.0 ± 1.9
6	Apple	2.3 ± 0.2	44.4 ± 1.1	53.3 ± 0.9	78.0 ± 2.6
7	Apple	6.1 ± 0.1	40.8 ± 0.9	53.1 ± 1.1	28.6 ± 1.2
8	Apple	4.7 ± 0.3	44.2 ± 1.5	51.2 ± 0.8	19.8 ± 1.3
9	Citrus	4.3 ± 0.2	47.9 ± 1.1	47.9 ± 0.7	76.8 ± 2.4
10	Grapefruit	5.4 ± 0.1	59.5 ± 0.8	35.1 ± 1.3	51.7 ± 1.8
11	Celery	4.9 ± 0.2	42.6 ± 0.7	52.5 ± 0.9	19.6 ± 0.8

Furthermore the quality and stability of the emulsions was examined by measuring the light transmittance index (T, %) and carrying out centrifugal test. The results obtained are presented in Table 2.

All the emulsions subjected to mechanical treatment showed separation to a certain extent and formation of oil and water phases.

The emulsion with the highest retain O/W phase was obtained using Rose A (72.1 %) but from the microscopic observations it is seen that the droplets are not as fine as the emulsion obtained with the celery pectin. The lowest separated oil phase had emulsion with sunflower pectin but it shows also formation of high amount of water phase. This emulsion has the highest light transmittance (98.0%), meaning quickly disrupting emulsion. The emulsion obtained from Rose A pectin has the lowest light transmittance (5.8 %).

An important parameter influencing the stability of emulsions is the temperature of their storage. For this reason they were stored 48 hours (Table 3) at four temperatures (-18° C, 5 °C, 20 °C and 50 °C) which were chosen because they are similar to temperatures usually used for storage of foodstuffs. Storing the emulsions under refrigeration (5 °C) and ambient temperature (20 °C) results in the smallest degree of separation (largest % saved O/W emulsion). When the emulsions were frozen (-18 °C.) and subsequently thawed this often caused phase reversion (O/W in a W/O emulsion) or increased the percentage of water and oil phases. This kind of inversion was observed for emulsions obtained with apple (sample № 7 and 8), citrus and grapefruit pectins. Increase of temperature to 50 °C led in most cases to small increase of disruption of emulsions compared to 20 °C.

Stabilities of emulsions prepared with pectins from waste rose petals and celery were compared. At -18°C the emulsions were the least stable and were disrupted except for the celery pectin emulsion which showed equal stability (over 80%

retained O/W phase). The smallest changes of the investigated rose pectins the highest emulsion emulsions were observed under ambient (20 °C) stability was observed with rose C pectin. and refrigeration (5 °C) conditions. From the three

Table 3. Temperature test of emulsions stored for 48 hours at -18 °C, 5 °C, 20 °C and 50 °C.

Pectin	t_s °C	Water	O/W emulsion	W/O emulsion	Oil
1. Rose W	-18 °C	44.9 ± 1.3	8.2 ± 1.1	0.0	46.9 ± 1.0
	5 °C	33.3 ± 0.9	66.7 ± 0.7	0.0	0.0
	20 °C	30.6 ± 0.8	67.3 ± 0.9	0.0	2.0 ± 0.3
	50 °C	34.7 ± 1.2	65.3 ± 0.6	0.0	0.0
2. Rose C	-18 °C	76.0 ± 1.5	24.0 ± 1.5	0.0	0.0
	5 °C	22.4 ± 1.0	77.6 ± 0.8	0.0	0.0
	20 °C	20.0 ± 0.8	80.0 ± 0.9	0.0	0.0
	50 °C	37.5 ± 0.7	62.5 ± 0.4	0.0	0.0
3. Rose A	-18 °C	37.7 ± 1.5	54.7 ± 1.7	0.0	7.5 ± 0.9
	5 °C	22.0 ± 1.1	78.0 ± 0.6	0.0	0.0
	20 °C	25.5 ± 0.5	74.5 ± 1.1	0.0	0.0
	50 °C	40.4 ± 0.7	59.6 ± 0.7	0.0	0.0
4. Carrot	-18 °C	47.9 ± 1.3	52.1 ± 1.1	0.0	0.0
	5 °C	33.3 ± 0.9	66.7 ± 0.6	0.0	0.0
	20 °C	29.2 ± 0.6	70.8 ± 1.8	0.0	0.0
	50 °C	36.0 ± 0.9	60.0 ± 0.7	0.0	4.0 ± 0.6
5. Sunflower	-18 °C	42.3 ± 1.2	44.2 ± 1.1	0.0	13.5 ± 1.1
	5 °C	40.4 ± 0.6	44.7 ± 1.3	0.0	14.9 ± 0.7
	20 °C	44.0 ± 0.7	50.0 ± 0.8	0.0	6.0 ± 0.8
	50 °C	46.0 ± 1.0	34.0 ± 0.9	0.0	20.0 ± 0.7
6. Apple	-18 °C	48.0 ± 1.4	40.0 ± 1.4	0.0	12.0 ± 1.3
	5 °C	43.1 ± 0.9	52.9 ± 0.7	0.0	3.9 ± 0.9
	20 °C	45.1 ± 1.1	54.9 ± 0.6	0.0	0.0
	50 °C	46.9 ± 1.1	46.9 ± 0.9	0.0	6.1 ± 0.4
7. Apple	-18 °C	40.0 ± 1.3	0.0	54.0 ± 1.2	6.0 ± 1.6
	5 °C	34.7 ± 0.9	57.1 ± 1.0	0.0	8.2 ± 0.7
	20 °C	34.0 ± 1.2	58.0 ± 0.9	0.0	8.0 ± 0.8
	50 °C	43.1 ± 0.8	51.0 ± 1.2	0.0	5.9 ± 0.6
8. Apple	-18 °C	38.0 ± 1.0	0.0	22.0 ± 1.0	40.0 ± 1.5
	5 °C	8.0 ± 0.7	92.0 ± 1.6	0.0	0.0
	20 °C	29.2 ± 0.6	70.8 ± 0.7	0.0	0.0
	50 °C	28.6 ± 1.1	71.4 ± 0.8	0.0	0.0
9. Citrus	-18 °C	41.5 ± 1.4	58.5 ± 1.4	0.0	0.0
	5 °C	44.2 ± 0.6	3.8 ± 0.9	44.2 ± 0.9	7.7 ± 0.8
	20 °C	45.1 ± 0.8	49.0 ± 0.8	0.0	5.9 ± 0.8
	50 °C	46.0 ± 0.5	54.0 ± 0.9	0.0	0.0
10. Grapefruit	-18 °C	45.1 ± 1.3	51.0 ± 1.4	0.0	3.9 ± 1.0
	5 °C	34.6 ± 0.4	0.0	57.7 ± 0.4	7.7 ± 0.5
	20 °C	37.3 ± 0.6	0.0	58.8 ± 0.9	3.9 ± 0.9
	50 °C	47.1 ± 1.0	0.0	52.9 ± 1.0	0.0
11. Celery	-18 °C	7.8 ± 0.8	92.2 ± 1.4	0.0	0.0
	5 °C	18.0 ± 1.1	82.0 ± 1.2	0.0	0.0
	20 °C	20.0 ± 0.8	80.0 ± 0.8	0.0	0.0
	50 °C	17.6 ± 0.7	82.4 ± 0.9	0.0	0.0

CONCLUSIONS

For the first time to our knowledge we investigated physicochemical properties of pectins derived through consecutive sequential extraction of waste rose petals and compared them with the properties of known commercial and non-commercial pectins. The results from the

experiments showed that rose pectins have (as most of the investigated pectins except for celery pectin) slight foaming and foam-stabilizing abilities. Concerning the emulsifying properties the highest quality emulsion was obtained with celery pectin. From rose-derived pectins the highest emulsifying ability showed acid extracted pectin (Rose A) but its emulsions were relatively unstable. The highest

emulsion stability was observed using celery pectin. The present data showed that waste rose petals are promising source for obtaining of pectins with physico-chemical properties similar to other commercial and non-commercial pectins.

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Сравнително изследване на физико-химични параметри на пектинови полизахариди от различни растителни източници

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РЕЗЮМЕ

Физико-химичните параметри – степен на метоксилиране, чистота, молекулна маса, пенообразуващи, емулгиращи и стабилизиращи свойства на пектини, получени чрез последователна фракционна екстракция от отдестилирани розови цветове са изследвани и сравнени със свойствата на осем комерсиални и некомерсиални пектинови полизахариди. Всички изследвани проби са високомолекулни полизахариди с молекулни маси от 1.32×10^4 до 4.08×10^5 Da. Полиуронидното съдържание (чистотата) е над 50% с изключение на киселинно-екстрахиран пектин от отпадъчен розов цвят. Намерено е, че всички изследвани пектини с изключение на пектин от целина, имат относително ниски пенообразуващи и пеностабилизиращи свойства. Емулсия, получена с киселинно-екстрахиран пектин от отпадъчен розов цвят, има най-висок индекс на светлопропускливост, но е нестабилна при механични въздействия. Най-стабилни емулсии се получават с пектин от целина. Получените резултати показват, че отпадъчния розов цвят е добър източник за получаване на пектинови полизахариди, чиито физико-химични свойства са сравними с тези на други добре изучени пектини.

Ключови думи: пектини, отпадъчен розов цвят, пени, емулсии.