PHYSICOCHEMICAL AND RHEOLOGICAL CHARACTERIZATION OF PECTIN FROM PUMPKIN (CUCURBITA MAXIMA) PULP SOLUBILIZED WITH AMMONIUM OXALATE

Baississe Salima[a*], Fahloul Djamel[a], Zitouni Bariza[a]
[a]Laboratory of Food Science (LSA), Department of Food Technology, Institute of Veterinary and Agricultural Sciences University of Batna 1Hadj Lakhdar, Biskra Avenue, Batna, 05005, Algeria
[*]s.baississe@gmail.com

Abstract
Pectin was extracted from pumpkin (Cucurbita maxima) pulp at 82°C and pH 4.6, in the presence of ammonium oxalate at 0.25%. Soluble pectin was recovered as pectinates aluminium by precipitation, in the presence of aluminium sulphate. Extracted pectin contains uronic acids and neutral sugar with a percentage of 58.43±0.10% and 21.11% respectively. The studied pectin was highly methylated (DE 77.61±0.04%). Three equations were used: Huggins, Kraemer and Solomon, Cituâ and Morris to measure the intrinsic viscosity. The latter ranged from 9.00±0.40 to 10.86±0.60 dl/g. Its molecular weight depends on the intrinsic viscosity of pectin solution, as expressed by Mark-Houwink-Sakurada equation. The obtained values varied between 224.40±9.50 KDa and 266.64±20.34 KDa. The effect of pH on rheological behavior of pectin solution was investigated. At pH 4, pectin showed a non-Newtonian rheological behavior at low viscosities. The increase of pectin concentration led to the increases in viscosity and the rheological behavior becomes rheo-thickening. At pH 7, the viscosity of pectin solution was rather low to develop a non-Newtonian behavior or modify the Newtonian behaviour.The viscoelastic behavior of pectin studied at different concentrations. Solid behavior can be observed for relatively low values of G '(of a few Pa), indicating the existence of a three-dimensional structure.

Keywords: Pumpkin, Pectin, Intrinsic viscosity, Rheological behavior, viscoelasticity

Received: 08.01.2018 Reviewed: 02.05.2018 Accepted: 12.05.2018

1. INTRODUCTION

Pectin is a polysaccharide present in the wall of plant cell. It is composed of many saccharide chains more or less esterified. It is considered as an important food additive because of its gelling, emulsifying, stabilizing and thickening properties.

Pectin is usually extracted from plants of economic value such as apple marcs, citrus peel, sugar beet (Košťálová et al., 2010; Košťálová et al., 2013a) and apricot (Kurz et al., 2008). Researches on pectin are currently interested to other plant sources, such as pumpkin, where few studies have been performed. Pumpkin is largely consumed as vegetable. Its consumption is related to its nutritional value and therapeutic properties (Adams et al., 2011; Jacobo-Valenzuela et al., 2011).

The most common extraction techniques of pectin include extraction by fractionation, ultrasound, high-pressure and microwave. These techniques greatly affect pectin macromolecules physicochemical and functional characteristics (Wang et al., 2007; Guo et al., 2014).

Functional properties of macromolecules are based on their behavior in aqueous solution, in particular shape and hydrodynamic volume. The intrinsic viscosity measurement is used to characterize pectin solutions as a hydrocolloid substance. In water, pectin usually forms a colloidal solution. Pectin solubility is a critical property for its use. Hydration ratio is a function of its structure, molecular weight and solvent physicochemical characteristics (e.g. including pH, ionic strength, ions nature, dielectric constant and temperature).

Several studies have been conducted on pectin physicochemical properties including rheological characteristics (Evageliou et al., 2005; Fissore et al., 2013) and electric charge (Lutz et al., 2009;Guo et al., 2014).

Researchers conducted on pectin, isolated from pumpkin by enzymatic extraction, have been oriented towards its structural characteristics (Kurz et al., 2008; Ptichkina et al., 2008).
The objective of this work is focusing on extraction, and evaluation of physicochemical characteristics, rheological and electrophoretic charge properties of pectin extracted from pulp squash variety of Cucurbita maxima grown in Algeria, using ammonium oxalate.

2. MATERIALS AND METHODS

1.1. Pectin extraction

Pumpkin pulp variety (Cucurbita maxima) cultivated in south of Algeria were used for pectin extraction. Protopectin hydrolysis is made with ammonium oxalate at 0.25% in a water bath IKA-HEIZBAD HB-250 type. Temperature varied from 80 to 82°C, at pH 4.6 with continuous stirring by IKA provided overhead stirrer RW 28 basic for 60 min. pH was measured using a pH meter WTW inoLab type.
After solubilization, insoluble materials were removed by pressing and by filtration under pressure. Resulting pectin juice was cooled to 20°C and stored at 4°C for 24h. Juice concentration was obtained in a vacuum rotary Heidolph® LABOROTA4010 Digital Evaporators type at a velocity 150 rpm/min and a temperature value of 35°C for 25min. Centrifugation achieved at 2000 rpm/min for 20 min. Finally, filtration held through a Whatman paper (150 mm ø, Cat # 589). The clarified filtrate was treated with aluminum sulphate which precipitates a fibrous coagulum, pectinates aluminum. For one milliliter juice, fifteen milliliters of aluminum sulphate at 25% was added. pH was adjusted between 4 and 4.2 by ammonia. Then, the fibrous pectin was washed, pressed, dried in a freeze drier PHYWE Christ type and stored in glass bottles (Figure 1).

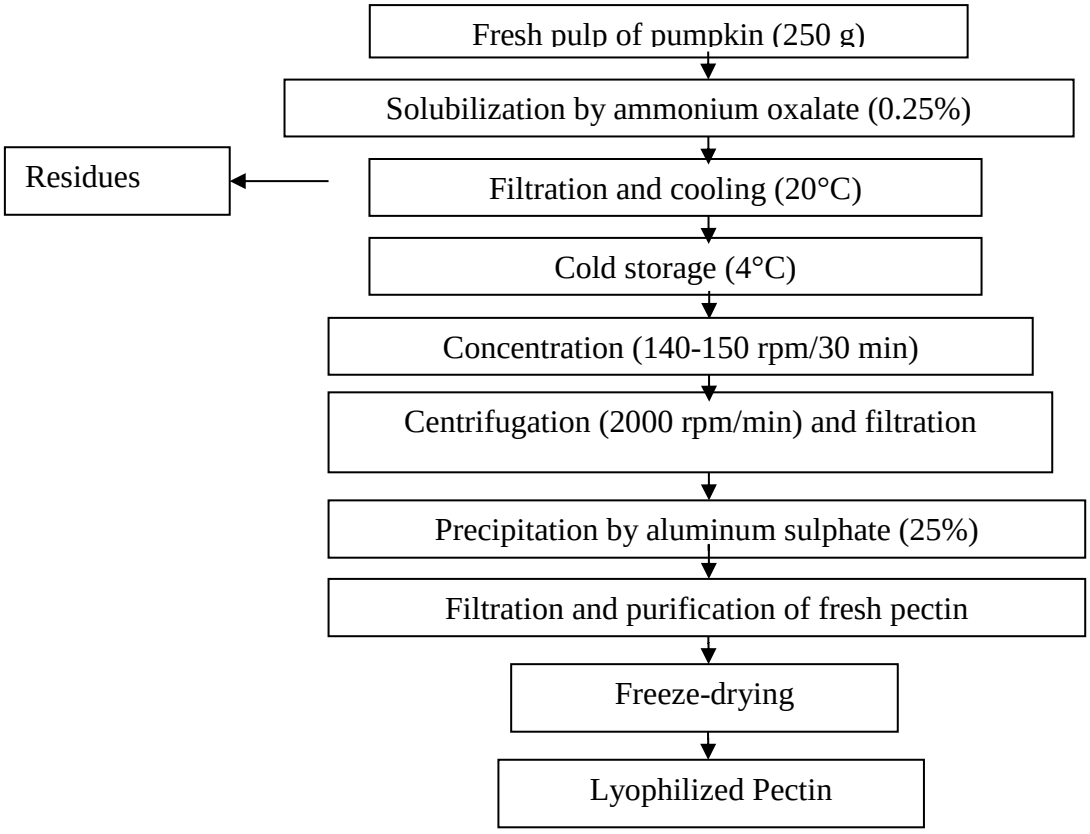

Fig. 1. Extraction process of pumpkin pectin

1.2. Pectin analysis

Galacturonic acid (GalA) content was determined using Blumenkrantz and Asboe-Hansen method (Blumenkrantz & Asboe-hansen, 1973). Total sugars were determined by Dubois et al. (1956) method. Proteins were estimated using Kjeldahl method. Pectin conversion coefficient is 6.25 (AOAC, 1984). Ash content was determined after organic matter incineration in a muffle furnace at 600°C (AOAC, 1984). Methyl group's content was determined according to Waldron and Selvendran (1990) recommended method (Barros et al., 2002). Degree of esterification (DE) was determined according to the formula given by Schultz (1962) cited by Hwang and Kokini (1992):

$$DE(\%)=\frac{176*\text{methoxyl content }(\%(w/w))}{31*\text{GalA content }(\%(w/w))}*100 \quad (1)$$

Where, the values 31 and 176 are the molecular weights of - OCH3 and GalA, respectively.

1.3.1. Solubility

Pectin's solubility conditions were optimized according to Masuelli (2011). Briefly, an amount of 0.6 g of freeze-dried pectin weighed by Mettler AE240 balance type was dissolved in 15 ml of buffer solution citric acid/sodium citrate to 0.2M. The mixture was heated to 40°C for 2 hours, with continuous stirring 1400 rpm/min in an agitator Heidolph® MR Hei-Tec Magnetic Hot Plate Stirrers, Brinkmann® type. A solution of 4% was obtained at pH 4.5.

1.3.2. Intrinsic viscosity and molecular weight

Pectin's solution apparent viscosity was measured at 20°C using a rheometer of Anton Paar MCR-301 type with double gap geometry. The flow curve was drawn between 10 and 1000 s-1. Pectin's intrinsic viscosity was determined from apparent viscosity using Huggins, Kraemer and Solomon-Ciutâ equations (Morris et al., 2008; Morris et al., 2014):

- Huggins equation:

$$\frac{\eta_{sp}}{C}=[\eta](1+K_H[\eta]C) \quad (2)$$

- Kraemer equation:

$$\frac{\ln(\eta_{rel})}{C}=[\eta](1-K_K[\eta]C) \quad (3)$$

- Solomon-Ciutâ equation:

$$[\eta]=\frac{2\eta_{sp}-2\text{In}(\eta_{rel})^{1/2}}{C} \quad (4)$$

where C: concentration (g/ml), η_{rel}: relative viscosity, η_{sp}: specific viscosity, [η]: intrinsic viscosity (dl/g), K_H: Huggins constant, K_K: Kraemer constant.

Equations (2) and (3) form is: Y = m x + b. The intrinsic viscosity is the viscosity when 'c' tends to zero. According to Liet et al. (2013), specific relative viscosity is defined by the following formulas:

$$\eta_{rel}=\frac{\eta}{\eta_s} \quad (5)$$

$$\eta_{sp}=\frac{\eta-\eta_s}{\eta_s}=\eta_{rel}-1 \quad (6)$$

where η: apparent viscosity, ηs: solvent viscosity.

According to Masuelli (2011), the relationship between intrinsic viscosity and molecular weight (M_W) is given by Mark-Houwink- Sakurada (M-H-S) equation:

$$[\eta] = K M_w^a \quad (7)$$

Calculating M-H-S parameters are performed using the following equation:

$$\ln [\eta] = \ln K + a \ln Mw \quad (8)$$

Where 'K' and 'a' are M-H-S constants, which depend on polymer type, solvent and temperature. The constant 'a' is a function of polymer geometry and its value varies from 0.5 to 2. In the present study, the following values were considered: $k = 9.55 \times 10^{-2}$ and a = 0.73 (Anger& Berth, 1986).The overlap parameter (C [η]) in log/log representation is determined by plotting viscosity curve as a function of concentration. The critical concentration (C*) representing total macromolecules occupation volume is determined by the intersection of the two line segments with the main curve (Morris et al., 1981; Hwang & Kokini, 1992).

1.3.3. Rheological properties

Flow properties were characterized with "Anton Paar rheometer MCR-301" equipment with rough surface geometry. Flow curves were measured with shear rate range 0.01-1000s-1 at 20°C.Viscoelastic behavior is studied using parallel disk geometry or plane-plane. The plan - plan system allows precise measurements of normal stresses.

The obtained data were statistical evaluated by the Tukey test analysis of variance (One way ANOVA) using a Sigma Plot V.11 software. Mean of different treatments were considered statistically significant at p-values < 0.05.

3. RESULTS AND DISCUSSION

3.1. Chemical composition

The chemical composition of the extracted pulp pectin is presented in Table 1. As clearly seen, the total sugar content and total uronic acids are 73.57±0.09 g and 58.43±0.10 g per 100g respectively.

Similar values were found by NosáĺOvá et al. (2011) ranging from 51.1±1.8% to 77.0±1.4% for total sugars and from 39.5±2.1% to 75.3±2.1% for uronic acids, and by Košťálová et al. (2013a) ranging from 57.1±1.8% to 77.7±0.4% for total sugars and from 6.1±0.8 g/100g to 63.1±3.0g / 100g for uronic acids, for pectin extracted from Cucurbita pepo L. var. styriaca. However, lower values were obtained by Junet al. (2006) ranging from 17.78±2.14 to 24.78±1.39g/100g for total sugars and from 4.89±2.54 to 46.07±5.96 g/100g of total uronic acids for pectin extracted from Cucurbita moschata Duch.

Table 1 Chemical composition and DE of pectin extracted from pumpkin pulp

Chemical composition	Pectin extracted from pulp[(a)]
Total sugars (g/100g)	73.57±0.09
Methyl groups (g/100)	7.44±1.20
Protein (g/100g)	0.13±0.01
Ash (g/100)	5.63±0.40
DE (%)	77.61±0.04

[(a)] Values are given in g/100g of dry matter and are means of triplicate measurements

Moreover, galacturonic acid amount of the polysaccharides isolated from the pulp was determined and found to be 52.46±0.14 g/100g of lyophilized pectin (dry basis). The result shows a strong correlation between GalA and uronic acids (R2 = 0.94). This could be explained by the fact that the studied pectin is rich in GalA. Similar values of GalA were obtained in literature, varying from 39 to 78g/100g for Cucurbita moschata Duch (Fissore et al., 2009) and from 10.4 to 95.1 (mol%) for Cucurbita pepo (Košť álová et al., 2013a).

In addition, neutral sugar content was found 15.14±0.8g/100g of dry pectin. Similar values ranging from 162±6 to 243±3(mg/g) and from 9.7±0.5 to 17.8±0.9 g/100g of pectin extracted from mango and lime solubilized in ammonium oxalate (Koubala et al., 2007) and from passion fruit, respectively, were reported (Yapo, 2009a). However, Košťálová et al. (2010) found very low values (2.0±0.06 to 7.2±0.10%) for pectin extracted from Cucurbita pepo L. var. styriaca. Several studies confirmed the effect of extraction conditions, vegetable origin, variety and geographic region on chemical composition, particularly on GalA and neutral sugar contents. As shown in Table 1, pectin extracted from Pumpkin pulp are highly methylated (DE greater than 50%). The degree of methyl esterification was found

77.61±0.04%. Similar studies have extracted high methylated pectin from mango and lime, solubilized in ammonium oxalate (DE ranging from 64±1.4 to 86±1.8% (Koubala et al., 2007) and from lime and ambarella solubilized in ammonium oxalate (DE= 58±1.4%) (Koubala et al., 2008).

On the other hand, pectin's protein content was very low (0.13±0.01 g/100 g dry sample) (Table 1). This could be attributed to leaching fractions during extraction under solvent effect. These values are similar to those found by Nosáĺováet al. (2011) and Košťálová et al. (2013b), for pectin extracted from Cucurbita pepo L. var. styriaca, varying from 0 to 3.2%. In addition, the extracted pectin was found rich in ash (5.63±0.4 g/100g dry sample) (Table 1). Several studies have shown that pectin precipitated with aluminum salts has high ash content.

3.2. Physicochemical and rheological properties

3.2.1. Intrinsic viscosity and molecular weight determination

Figure 2 (a, b) shows the changing in reduced and inherent viscosities as a function of pectin concentration.

They are evaluated based on the experimental viscosity measured at acidic and neutral conditions (pH 4 and 7) at room temperature. By applying Huggins and Kraemer equations, linear relationships of the general mathematical formula Y = mx + b were obtained. The linear correlation coefficient (R2) ranges from 0.86±0.16 to 0.96±0.04 and from 0.905±0.02 to 0.927±0.001 for Huggins and Kraemer equations respectively at pH 4 and 7 (Figure 2a and Table 2).

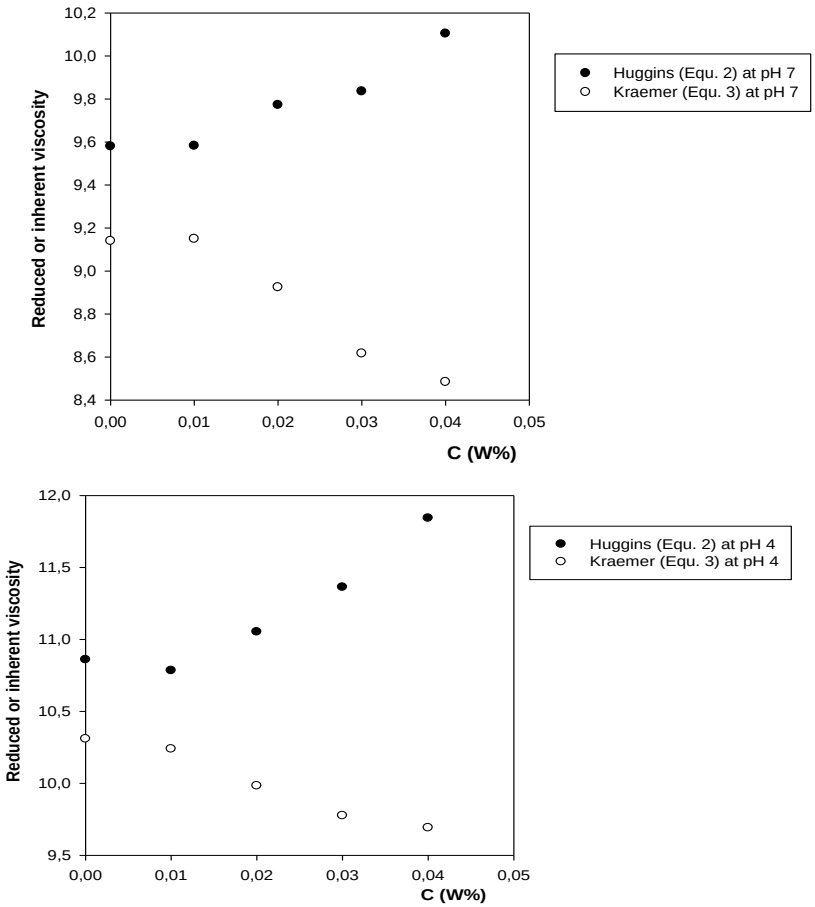

Fig. 2. Reduced and inherent viscosities of pectin extracted from pumpkin pulp dissolved in citric acid/sodium citrate 0.2M at 20°C, (a): pH 4, (b): pH 7

The parameter [η] is defined as viscosity when the solution concentration tends to zero. The intrinsic viscosity [η] calculated by the three equations (2, 3 and 4) is shown in Table 2.

Table 2 Physicochemical parameters of pectin extracted from pumpkin pulp dissolved in citric acid/sodium citrate 0.2 M, at pH 4 and 7, at room temperature, using different mathematical models

Model	Property	Pectin solution at pH 4	Pectin solution at pH 7
Huggins equation	$[\eta]_H$ (dl/g)	10.86±0.60	9.58±0.50
	K_H	0.39±0.04	0.27±0.002
	R^2_H	0.86±0.16	0.905±0.02
	M_{WH} (kDa)	266.64±20.34	239.11±11.84
Kaermer equation	$[\eta]_K$ (dl/g)	10.31±0.54	9.14±0.43
	K_K	-0.10±0.03	-0.23±0.03
	R^2_K	0.96±0.04	0.927±0.001
	M_{WK} (kDa)	257.29±13.45	228.13±10.75
	K_H-K_K	0.49±0.07	0.50±0.03
Solomon-Ciutâ equation	$[\eta]_{S\ et\ C}$ (dl/g)	10.36±0.41	9.00±0.40
	$M_{WS\ et\ C}$ (kDa)	259.72±10.21	224.40±9.50

Values are means of triplicate measurements

The inherent viscosity obtained by Kraemer and Solomon-Ciutâ equations, show an inverse relationship between [η] and pH value. It is clear that [η] value increased by shifting the pH of the medium from neutral (pH 7) to acidic (pH 4) from 9.14±0.43 to 10.31±0.54 dl/g (Kraemer equation) and from 9.00±0.40 to 10.36±0.41 dl/g (Solomon-Ciutâ equation), respectively. The inherent viscosity values of the same order were found by Koubala et al. (2007) at the same conditions, from 886±9 to 1346±10 ml/g for pectin extracted from mango and lime solubilized in ammonium oxalate, by Koubala et al. (2008) from 480±4 to 757±8 ml/g for pectin extracted from ambarella and lime solubilized in the ammonium oxalate and by Morris et al. (2008) from 325±10 to 600±30 ml/g for pectin extracted from citrus. However, lower values were obtained by Li et al. (2013) ranging between 2.89 and 4.98 dl/g for apple pectin solubilized in NaNO3 (0.2M). The inherent viscosity of pectin increases with increasing dissociation of its macromolecules. It corresponds to the hydrodynamic specific volume occupied by polymer mass unit in the reference solvent system (Diaz et al., 2007).

Statistically significant difference in the effect of pH on the intrinsic viscosity determined by Equation 4, were found by statistical analysis using the Tukey test, with p 0.050, p = 0.039; while no significant difference between the effect of pH on intrinsic viscosity using equation 2 or 3 with P> 0.05, p= 0,136.

Huggins "K_H" and Kraemer "K_K" constants are shown in Table 2. Values of "K_H" oscillate between 0.39±0.04 and 0.27±0.002, while, the values of "K_K" range between -0.10±0.03 and -0.23±0.03 for pectin solutions prepared at pH 4 and 7 respectively. These results are in the range values found by Li et al. (2013) from 0.44 to 6.09 for "K_H" and -2.73 to 0.13for "K_K".

Huggins constant "K_H" is a polymer - polymer index. The value of "K_H" is related to the structures of polymers. It enables an estimate of [η] to be made from a single determination of η_{sp}=C. It contains information about hydrodynamic and thermodynamic interactions between coils in solution. Its value ranges from 0.20 to 0.80 for flexible strings. Its value is 0.3 in the best solvent and up to ~ 2 for uncharged spheres (Diaz et al., 2007). The K_H + $K_K$result is ≈ 0.50. According to Li et al. (2013) there is a

close relationship between pH values and Huggins constant "kH". At pH <3.5, its value is almost constant and greater than one.

Moreover, molecular weight (M_W) results of the extracted pectin, calculated using the three equations (2, 3 and 4) at different pH values (4 and 7), are shown in Table 2. At pH 4, MW of the extracted pectin is 257.29±13.45kDa; 259.72±10.21 and 266.64±20.34 kDa respectively. At pH 7, M_W values are lower compared to those measured at pH 4, they vary between 224.40±9.50 and 239.11±11.84 kDa. The decrease of M_W values could be explained by the fact that increasing pH may cause intense depolymerization. The latter is a characterization of molecular weight distribution of soluble pectin. This depolymerization results in the fragmentation of pectin macromolecules into oligomers. Proteins are characterized by their susceptibility to degradation by β-elimination in neutral to slightly acidic medium. This sensitivity to β-elimination is caused by high degrees of methylation. Indeed, the obtained pectin has an esterification degree of 77.61±0.04%.

Diaz et al. (2007) reported that chemical β-elimination is one of non-enzymatic degradation mechanisms in pectin. The rate of this reaction is accelerated with increasing degrees of methylation, temperature, and pH (4-6). The critical pH of β-elimination reaction is greater than 6. These authors showed thatβ-elimination reactions caused significant reduction in relative viscosity and consequently molecular weight.

Fissore et al. (2013) extracted highly polymerized pectin from some pumpkin varieties with MW results ranging from 136.0 to 1309.0 kDa. Lower values were found by Ptichkina et al. (2008) for pectin extracted by enzymatic and chemical methods from *Volzhskaya Grey* pumpkin variety (from 26 to70 kDa) and from 70±2 to 95±1 kDa for pectin extracted from passion fruit (Koubala et al., 2007; Kontogiorgos et al., 2012). Indeed, M_W is greatly affected by extraction conditions and plant age. Fissore et al. (2013) reported that pectin has a high heterogeneity of chemical structure and M_W. In general, pectin molecular weight value extracted from different vegetable sources varies from 104 to105 Da.

3.2.2. Rheological behavior of pectin in solution

Rheological characterization obtained from pectin solutions and prepared at different concentrations at two pH values (4 and 7) are illustrated in Figure 3 (a, b). Rheological characterization was obtained by measuring shear stress as a function of shear rate. Pectin solutions could have three different rheological behaviors: thinning, thickening and Newtonian. The rheological behavior of food hydrocolloids such as pectin in solution is significantly influenced by its M_W, shape and rigidity.

From Figure 3a, it could be observed that at pH 4 and at low concentrations, pectin has a thickening orpseudo-plastic behavior. In this case an inverse relationship is observed between viscosity and flow rate. This behavior changes gradually to a Newtonian behavior at high concentrations.

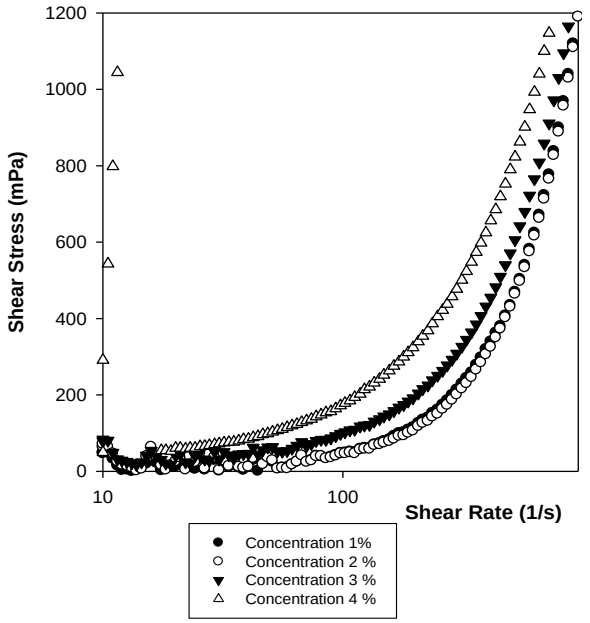

Fig. 3a. Rheological characterization of pectin extracted from pumpkin pulp solubilized at various concentrations in 0.2M citric acid/sodium citrate at 20°C, (a): pH 4 (representation in logarithmic coordinates)

On the other hand, at pH 7, pectin exhibits a Newtonian behavior regardless of concentration (Figure 3b). In this case, the viscosity increases with shear rate. At pH 7, the viscosity is very low to develop a non-Newtonian behavior or modify its behavior. Similar observations were reported by Fissore et al. (2009) and Evageliou et al. (2005) for some pumpkin varieties.

Most biopolymers used in the food industry present a thinning behavior in solutions.

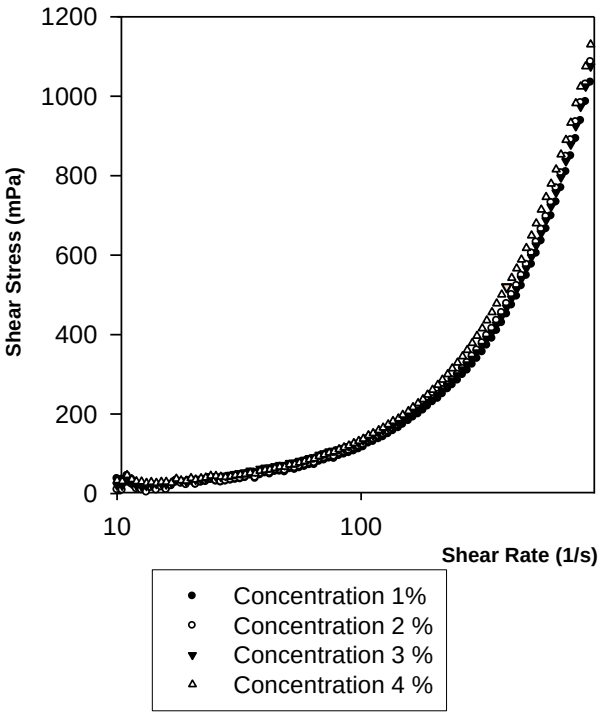

Fig. 3b. Rheological characterization of pectin extracted from pumpkin pulp solubilized at various concentrations in 0.2M citric acid/sodium citrate at 20°C (b): pH 7 (representation in logarithmic coordinates)

Figure 4 shows the viscoelastic behavior of pectin studied at different concentrations. Solid behavior can be observed for relatively low values of G ' (of a few Pa), indicating the existence of a three-dimensional structure.

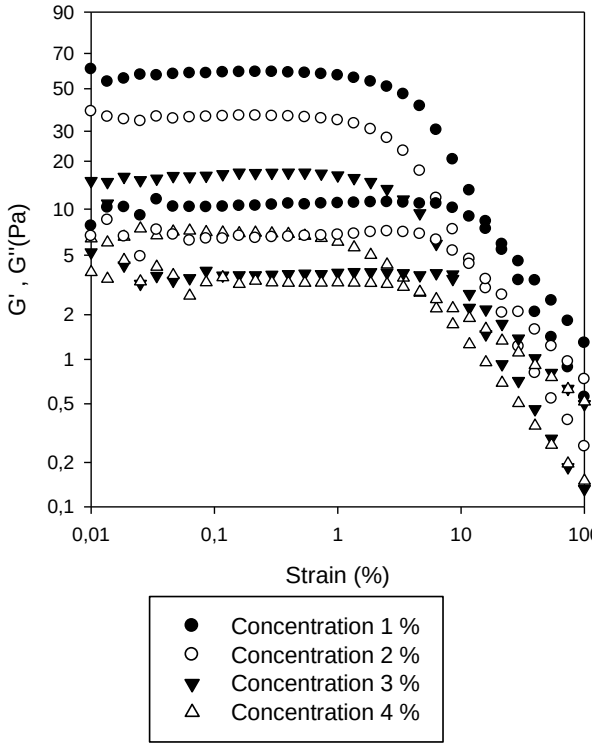

Fig. 4. Viscoelastic behavior of pectin extracted from pumpkin pulp solubilized at various concentrations in 0.2M citric acid/sodium citrate at 20°C (representation in logarithmic coordinates)

4. CONCLUSION

Study of pumpkin pectin solubilized in ammonium oxalate showed that this pectin is rich in AGA, highly methylated and has a high molecular weight. Viscosity is one of the parameters which greatly influence rheological behavior of pectin in solution. Viscosity and molecular weight decrease with increasing pH. At pH 7, pectin exhibits Newtonian behavior because of its low viscosity, whereas at pH 4, it shows a thickening behavior. From these results, it could be concluded that pumpkin is an important source of high quality pectin, which could be used as a thickening, stabilizing and emulsifying additive in the preparation of foods such as ice creams, mayonnaise and sauces.

5. REFERENCES

[1]. Adams, G. G., Imran, S., Wang, S., Mohammad,

A., Kok, S., Gray, D. A., Channell, G.A., Morris, G. A., & Harding, S. E. (2011). The hypoglycaemic effect of pumpkins as anti-diabetic and functional medicines. *Food Research International*, *44*, 862–867.

[2]. Anger, H., & Berth, G. (1986). Gel permeation chromatography and the Mark-Houwink relation for pectins with different degrees of esterification. *Carbohydrate Polymers*, *6*, 193-202.

[3]. AOAC., (1984). Official methods of analysis, 14th ed.; Association of official analytical chemists: Washington, DC. B.

[4]. Blumenkrantz, N., & Asboe-hansen, G. (1973). New Method for quantitative determination of uranic acids. *Analytical biochemistry*, *54*, 484-489.

[5]. Diaz, J. V., Anthon, G. E., & Barrett, D. M. (2007). Nonenzymatic degradation of citrus pectin and pectate during prolonged heating: effects of pH, temperature, and degree of methyl esterification. *Journal of Agricultural and Food Chemistry*, 55(13), 5131-5136.

[6]. Doublier, J. L., & Wood, P. J. (1995). Rheological properties of aqueous solutions of (1→3)(1→4)-β-D-glucan from oats (Avena sativa L.). *Cereal Chemistry*, *72* (4), 335-340.

[7]. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *23* (3), 350-356.

[8]. Evageliou, V., Ptitchkina, N. M., & Morris, E. R. (2005). Solution viscosity and structural modification of pumpkin biopectin. *Food Hydrocolloïds*, *19*, 1032–1036.

[9]. Fissore, E.N., Matkovic, L., Wider, E, Rojas, A.M., & L.N. Gerschenson, L.N. (2009). Rheological properties of pectin-enriched products isolated from butternut (Cucurbita moschata Duch ex Poiret). *LWT - Food Science and Technology*, *42*, 1413–1421.

[10]. Fissore, E. N., Rojas, A. M., Lía, N., Gerschenson, L. N., & Williams, P. A. (2013). Butternut and beetroot pectins: Characterization and functional properties. *Food Hydrocolloïds*, *31*,172-182.

[11]. Guo, X., Zhao, W., Pang, X., Liao, X., Hu, X., & Wu, J. (2014).Emulsion stabilizing properties of pectins extracted by high hydrostatic pressure, high-speed shearing homogenization and traditional thermal methods: A comparative study. *Food Hydrocolloïds*, *35*, 217-225.

[12]. Hwang J., & Kokini J. L. (1992). Contribution of the side branches to rheological properties of pectins. *Carbohydrate Polymers*, *19*, 41-50.

[13]. Jacobo-Valenzuela, N., Maróstica-Junior, M. R., Zazueta-Morales, J. J., & Gallegos-Infante,J. A. (2011). Physicochemical, technological properties and health-benefits of Cucurbita moschata Duchense vs. Cehualca. *Food Research International*, *44*, 2587-2593.

[14]. Jun, H-II., Lee, C.-H., Song, G.-S., & Kim, Y-S. (2006). Characterization of the pectic polysaccharides from pumpkin peel. *LWT - Food Science and Technology, 39*, 554-561.

[15]. Kontogiorgos, V., Margelou, I., Georgiadis, N., & Ritzoulis, C. (2012). Rheological characterization of okra pectins. *Food Hydrocolloïds*, *29*, 356-362.