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PECTIN EXTRACTION AND CHARACTERIZATION FROM LOCAL WHITE PUMPKIN (*LAGENARIA SICERARIA*)

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ABSTRACT:

Pectin, a naturally occurring heteropolysaccharide, has in recent years grown increasingly in importance. In this present study, white pumpkin or bottle gourd was chosen as a representative of the Cucurbitaceae family to extract powder pectin. At initial and final times, treatment (T_1) and treatment (T_2), the amount of pectin in calcium pectate in fresh pumpkin ranges from 3.7% to 5.8%. Standardized water bath heating was used to extract pectin from white pumpkins by boiling the fruit at atmospheric pressure for five minutes at 95 ºC. Distilled water was also tested for pectin isolation from white pumpkins as an extraction solvent. The weight increased by 844.98% at T_1 and decreased to 675.67% at T_6, following which was precipitation with 95% ethanol yielding maximum on the above during the extraction of pectin by water bath at varied period durations. Finally, the degree of esterification was the rate 68.76% at T_1 to 59.87% at T_6, with the methoxyl content having a value 9.45% at T_1 to 6.82% at T_6 and the anhydrouronic acid content (AUA%) being 76.66% at T_1 to 64.77% at T_6. Moisture, equivalent weight, degree of esterification, and methoxyl concentration all reduced as extraction time increased. However, many indicators, like ash content, acetyl value, and jelly grade, exhibited an early rise in value that later declined as extraction time increased. Thus, the optimal method for pectin isolation involves boiling the pumpkin in water at atmospheric pressure for 5 to 30 minutes. In addition, among different times of extraction pectin that was obtained from 30 minutes had the highest lightness 82.07 while the lowest redness 2.03 and yellowness 5.14 of pectin was recorded at this time. This pectin met the criteria for food additive use, indicating its potential as a commercially viable alternative source.

KEYWORDS: Extraction condition, IR-spectroscopy, Equivalent weight, Methoxyl content, AUA acid, Degree of esterification

1. INTRODUCTION

White pumpkin or bottle gourd and in some countries is named Calabash is a member of the genus (*Lagenaria siceraria* (Molina) Standley) of the family *Cucurbitaceae* and is the fruit that usually grows in tropical and practically in frost-free areas (Rahman, 2003). Bottle gourd is an important source of Vitamin-B complex, fibers, and polyphenols also the fruit has been reported to have a rich source of water and minerals (Kumar et al., 2012). In addition, the pharmaceutically used to treatment of headache, help liver function, and also helps contest constipation (Minocha, S., 2015). Pectin, as defined by (Dranca and Oroian, 2018), is a complex macromolecular heteropolysaccharide consisting of α-D (1→4) galacturonic acid units that is typically found in the primary cell wall of dicotyledonous and middle lamella of higher plant tissues. Pectin functions as a hydrating agent and facilitates the joining of the cellulose network. It is further distinguished by a high concentration of galacturonic acid (GalA), with a minimum of 65% of GalA. (Willats et al., 2006). Due to its ability to alter during plant separation, storage, and processing, pectin's structure is highly elusive (Zouambia et al., 2017). The (1,2)-linked side-chains of pectin, which include L-rhamnose residues and some other neutral sugars like L-arabinose and D-galactose, partially disrupt the linear structure of the protein (Yoo et al., 2012).

Pectin has been used in food and beverage productions for many years, according to the international numbering system pectin is very well defined as a food additive with E-number no. 440 (Sengar et al., 2020). Pectin provides an important source of dietary fiber and is widely employed as a gelling agent, thickener, stabilizer, and emulsifier in the food industry sector (Sandarani, 2017). Pectin exists in various forms, one of which is homogalacturonan (HG), which is the most prevalent polysaccharide. It makes up around 65% of the total pectin and is made up of α-D-(1,4) linked galacturonic acid (GalA) units. In 2015, (Cameron et al., 2015) According to (Yang et al, 2019), the branching rhamnogalacturonan I (RGI) region makes up 20–35% of pectin and is composed of several disaccharide units such as galactan, arabinan, and arabinogalactan that are linked at carbon no. 4 of the L-rhamnosyl residues. Furthermore, less than 10% of the minor component is the rhamnogalacturonan II (RGII) region (Harholt et al., 2010). Pectin can be classified into two categories based on the amount of methylation (DM): high methoxy pectin, which has DM of more than 50%, and low methoxy pectin, which has DM of less than 50% (Kliemann et al., 2009). Industrial

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pectin is often extracted from a variety of fruits, including citrus and apple fruits. According to multiple publications, the dry basis contents of citrus peels and apple pomace are respectively 15% and 20%. The main objective of this work was to determine the effect of the temperature of drying white pumpkin on pectin characteristics.

2. MATERIALS AND METHODS

2.1 Raw Material

A fresh white pumpkin was purchased from the farms that close to Akre district in the Kurdistan Region/Iraq in July of 2023, samples were collected randomlly. After removing all dirt, the pumpkins were cleaned and sliced, then dried in oven at 60˚C in a Daihan Labtech Co., Ltd. oven with air circulation until it reached a consistent moisture level, then it was ground with an electric blender. After grinding the white pumpkin, it was sieved by a 60 mesh 250um, sieve to eliminate any uncrushed particles then it was then kept between 4 and 10˚C in storage. Before being used, the white pumpkin powder was sealed in a polyethylene bag and kept dry (Sharma et al., 2014).

2.2 Extraction Procedure:

2.2.1 Pectin Extraction

Pectin extraction from white pumpkins was conducted according to the techniques that reported by (Sayah et al., 2014). In order to perform the extraction, 500 mL of deionized water that had been acidified to 1.5 level of pH used by 1N of sulfuric acid, along with 50 mL of the previously prepared solvent and 1.75g of dry white pumpkin then were stirred for approximately five minutes, or until all of the dry powder had dissolved and the mixture had become homogenized completely. Subsequently, the mixture was placed in a water bath and heated to 95˚C period times, stirring continuously every 30 minutes. The extract was heated for 5, 10, 15, 20, 25 and 30 minutes in Table 1, at different period times per minutes. The extract was then filtered with cheesecloth five times by a vacuum pump and pressed to remove all extract. The extract pulp was then filtered with filter paper Whatman No. 3 (second filtration). The pectin was fallen in the filtrate by adding concentrated ethanol 95% in the ratio of one-part extract to two parts ethanol 1:2 and allowed it to settle at room temperature the next day. The filtrate was then filtered via filter paper Whatman No. 4 (third filtration). Following that, the precipitate was washed over filter paper using the Buchner funnel with 75%, 85%, and 95% ethanol [v/v] to purify the extracted pectin. Ultimately, a gel was produced, dried for around 10 hours at 45°C, pulverized into a fine powder in a mortar, and then put through additional testing. Figure 1 shows the specific steps needed to extract pectin from white pumpkin. The dry weight of the extracted pectin divided by the starting weight of the powdered white pumpkin utilized for extraction (2) was used to assess the pectin yield. This is how the % yield was computed:

$$\textbf{Pectin yield (\%)} = \frac{\text{Extracted pectin (g)}}{\text{Dried white pumpkins powder (g)}} * 100$$

Table 1: Specifications for the water-based solvent extraction process of pectin from pumpkins

Treatment (T)	Heating method	Treatment Details	
		Temperature (ºC)	Time (min.)
T_1	Bath heating	95	5
T_2			10
T_3			15
T_4			20
T_5			25
T_6			30

Figure 1: Flow diagram for pectin extraction from white pumpkin by acid method.

2.3 Characterization of Pectin

The obtained pectin was characterized by as per the several methods at the General Direction of Industrial Development – Directorate of Quality Control, Kurdistan Region-Erbil. In terms of pectin yield, equivalent weight, methoxyl content, anhydrogalacturonic acid, and degree of esterification, the pectin was isolated.

2.3.1 Equivalent Weight

The equivalent weight of pectin from white pumpkin was calculated using the method that reported by Ranganna, (1986). 0.5g of dried powder pectin was added to a 250mL glass flask, and 5mL of ethanol 95% was added and then 100mL of deionized water were added. Six drops of red phenol indicator were added after 1g of sodium chloride NaCL was added to improve the endpoint. The resulting mixture was rapidly stirred to dissolve all of the pectin substances; at last, the titration process was carried out slowly with 0.1N sodium hydroxide NaOH until the pink color appeared or the indicator's pH reached 7.5.

$$\textbf{Pectin Eq. weight} = \frac{\text{Weigh of Sample (g)} * 1000}{\text{Vol.of alkali (ml)} * \text{Normality of alkali}}$$

2.3.2 Methoxyl Content Determination

Methoxyl content was measured according to Khamsucharit et al. (2018). By adding 25mL of 0.25N sodium hydroxide to the neutralized solution that was obtained from the equivalent weight determination, the methoxyl content of pectin from white pumpkins was determined. After thoroughly stirring the final mixture in a stoppered flask, it was allowed to stand at room temperature for 30 minutes. Subsequently, 25mL of 0.25N hydrochloric acid (HCl) was added to the mixture and titrated until the titrant's color turned purple or to the same endpoint as previously. The following formula was used to determine the methoxyl content:

$$\textbf{Methoxyl Content \%} = \frac{\text{Vol.of alkali (ml)} * \text{Normality of alkali} * 31}{\text{Weight of Sample (g)} * 1000} * 100$$

Molecular weight of methoxyl (CH_3O) = 31

2.3.3 Anhydrouronic Acid (AUA) Determination

Determining the purity, degree of esterification (DE), and some physical characteristics of the extracted pectin from white pumpkins requires an estimation of the AUA concentration of the pectin. Using the resulting volumes that were titrated from the determination of equivalent weight and methoxyl content, the anhydrouronic acid of extracted pectin was computed (Salma et al., 2012). Pectin's AUA was computed as follows:

$$\textbf{AUA \%} = \frac{176 * 0.1z * 100}{W * 1000} + \frac{176 * 0.1\,y * 100}{W * 1000}$$

Where:

Molecular unit of AUA [1unit] =176

mL of sodium hydroxide from equivalent weight = z

mL of sodium hydroxide from methoxyl content = y

weight of sample [g] = w

2.3.4 Degree of Esterification Determination

The ratio of esterified galacturonic acid (GalA) groups to the total quantity of (GalA) groups present in pectin is known as the degree of esterification (DE) (Owens, 1952). The following formula was used to determine how much of the pectin from white pumpkin had been esterified:

$$\textbf{DE \%} = \frac{176 * \text{Methoxyl content \%}}{31 * \text{An hydrouronic acid \%}} * 100$$

2.4 IR Spectroscopic Method

The pectin that was extracted from white pumpkin was desiccated in a vacuum jar containing blue silica gel prior to IR analysis. IR spectra of sample was obtained using a single reflectance IR spectrophotometer with a DTGS detector (SHIMADZU, CO. LTD.) (Singthong et al., 2004).

2.5 Colour Measurement

The color parameters of the extracted pectin were determined by using a Hunter Lab colorimeter by measuring L*, a* and b* values in the CIE system (Mesbahi et al., 2005).

Statistical analysis

All experiments were carried out at least in triplicate. Data were interpreted by one-way analysis of variance (ANOVA) using SPSS 21 software. Where significant results were found, comparison among means was made by Duncan's multiple range test with a p value of 0.05.

3. RESULTS AND DISCUSSION

3.1 Pectin Yield

In Table 1 displays the yield of pectin extracted from bottle gourds or white pumpkins. In the current investigation, the pectin yield was "6.3%". "Figure 2 aligns with the findings of Yoo et al. (2012), who reported a pectin yield from pumpkin at pH 1.5 from 3.7% to 6.3%. However, the yield of pectin from various sources was significantly variable; the maximum yield of pectin was recovered from banana peel "24.08%" while the lowest yield of pectin was obtained from dried orange peel 2.2% (Liu et al., 2006). Previous research has shown that during the extraction procedure, dried fruit peels contain more pectin than fresh fruit peels. Furthermore, a number of studies have demonstrated that as fruit ripeness increases, pectin output falls (Ramachandran et al., 2017).

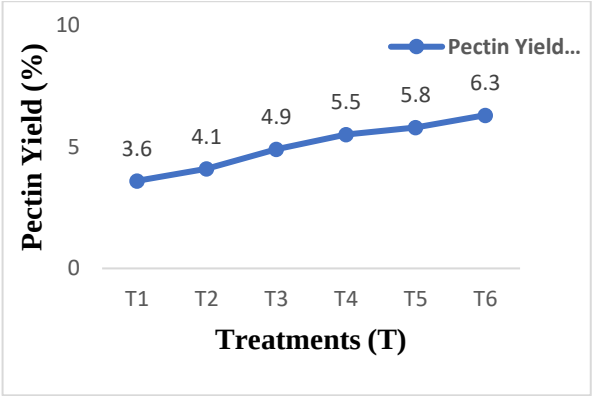

Figure 2**:** Impact of the heating time (T_1: 5, T_2: 10, T_3: 15, T_4: 20, T_5: 25 and T_6: 30 minutes at (95 ºC in a bath) on the pectin output from white pumpkin at various intervals.

3.2 Pectin Characterization

3.2.1 Equivalent Weight (Eq.w)

According to (Ramachandran et al., 2017), the equivalent weight of pectin extracted from white pumpkin ranged from 844.98 to 675.67 at T_1 to T_6 respectively as showed in the Table 2. This indicates that the pectin recovered from different pumpkins at different periods has varying equivalent weights. The equivalent weight of pectin, which was successfully extracted using the bath heating method at 95 °C, was also reported in earlier studies. In addition to the comparative equal weight of certain fruits, such as lemon pomace from 386, 1175 and 1632 correspondingly, as reported by Azad et al. (2014) and Bawer (2021). There was also an equivalent weight of pectin produced from six different apple types, ranging from 691.53 to 1136.36. A pectin extraction process that uses a method may cause an equivalent weight to increase or decrease depending on the total free acid that is typically present in the pectin substance (Nazaruddin Ramli and Asmawati, 2011).

3.2.2 Methoxyl Content

Since pectin is categorized as having a high or low methoxyl content, and its ability to form gels under specific conditions changes accordingly, the methoxyl content is another characteristic that indicates the functioning of the extracted pectin (O'shea et al., 2015). Table 2. indicates that the methoxyl content of the separated pectin from bottle gourds or white pumpkin ranged from 9.45 to 6.82%, with the lowest rate at T_6 and the highest rate at T_1. And this outcome is consistent with (Ramachandran et al., 2017). Hamed (2015) found a similar outcome for pectin derived from pumpkin peels, noting that the methoxyl content of the extracted pectin was 8.16, 8.65, 9.32, 9.41, 9.46, and 9.55% in each case. Additionally, according to (Ismail et al., 2012), the pectin extracted from dragon fruit had the lowest methoxyl concentration 2.98 to 4.34% and the highest methoxyl content 10.25%.

3.2.3 Anhydrouronic Acid Content (AUA)

Table 2, illustrates that the AUA content ranged from 76.66 to 64.77% in pectin, with T_1 through T_6 exhibiting the greatest and lowest contents, respectively. The aforementioned result agreed with the values of 59.52 to 70.50%. Similar findings were observed in the pectin of apple pomace and dragon fruit, as reported by Ptitchkina et al. (1994) and Kumar et al. (2012), who discussed the maximum anhydrouronic acid (AUA) results extracted from three sets of pumpkin. According to the purity characteristics criteria established by the European Commission (EC) and the Joint FAO/WHO Expert Committee on Food

Additives, pectin must contain less than 65% galacturonic acid to meet the required standards (Müller-Maatsch et al., 2016). The extracted pectin from white pumpkin may not be sufficiently pure, resulting in a significant concentration of protein, starch, and certain sugars, according to the AUA results that obtained by (Khamsucharit et al., 2018).

3.2.4 Degree of Esterification (DE)

Table 2 showed the degree of esterification of pectin from bottle gourds or white pumpkins, which was 68.76 to 59.87 % at the T_1 to T_6 respectively. This figure is lower than 51.01 to 51.24%, which was found for grapefruit peel pectin (Koubala et al. 2008; Hamed 2015). However, it is higher than the determined degree of esterification for pumpkin pectin, which was 67.64% (Monsoor, and Proctor, 2001). Notably, pectin from pumpkin extract has a lot of potential for usage as a thickening and gelling agent in jam and jellies.

Table 2: The impact of the bath heating procedure on the properties of the pectin that is extracted from pumpkin at different times.

Characteristics	Bath heating at 95ºC (minutes)					
	5	10	15	20	25	30
Eqt. weight	844.98 ± 0.01^{a}	823.12 ± 0.11^{a}	796.44 ± 0.21^{b}	755.67 ± 0.28^{b}	748.45 ± 0.11^{bc}	675.67 ± 0.15^{c}
Meo content (%)	9.45 ± 0.00 ^{a}	9.32 ± 0.01^{a}	8.88 ± 0.21^{ab}	8.66 ± 0.37^{b}	7.87 ± 0.33^{c}	6.82 ± 0.12^{d}
AUA (%)	76.66 ± 1.12^{a}	80.11 ± 1.09^{b}	80.78 ± 0.96^{b}	81.02 ± 0.89^{b}	79.56 ± 0.99^{b}	64.77 ± 0.79^{c}
DE (%)	68.76 ± 0.12^{a}	65.51 ± 0.11^{a}	66.11 ± 0.22^{a}	62.45 ± 0.93^{a}	60.76 ± 1.00^{a}	59.87 ± 0.87^{ab}

3.3 IR Spectroscopic

The chemical structure of extracted pectin from white pumpkin in 30 minutes was characterized by IR and their spectra are showed in Figure 3. A board peak which occurs at 3383.14-3329.14 cm-1 attributed to O-H stretching vibration. One of the characteristic bands in the spectra of the extracted pectin as polymeric carboxylic acids results from the out of plane bending of bonded O-H. The band occur near 920 cm^{-1} (Silverstein and Bassler, 1962). The O-H bending of COOH group occurs at 1232.5 cm^{-1}. Increasing ring strain moves the C-H stretching bands progressively to high frequencies. The CH2 and CH groups in the ring absorb in the region of 3100-2990 cm^{-1} (Pavia et al., 2014). The band area of the carbonyl free carboxylic acid is larger than of the carboxylate stretching band. This confirm that the obtained DE results indicates extracted pectin heteropolysaccharide is low methoxylated (Manrique and Lajolo, 2002).

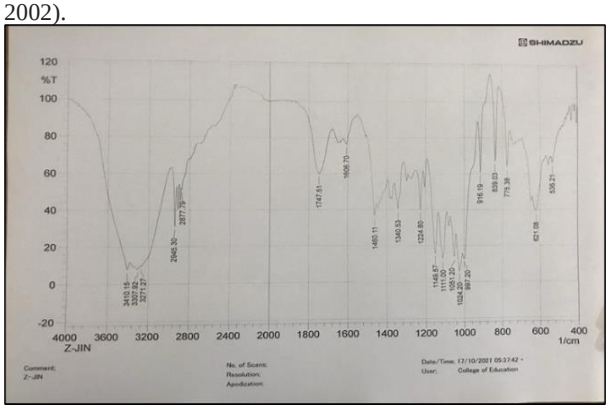

Figure 3: IR spectrum of extracted pectin of white pumpkin in 30 minutes

3.4 Color of Pectin

Color parameters of pectin isolated from white pumpkin at temperature 95 ºC in different minutes were determined using a Hunter Lab colorimeter and the obtained value are displayed in Table 3. The lightness (L*) of extracted white pumpkin pectins in different times are ranged from 65.32 to 78.38 with significant difference (p\0.05). In general, pectins extracted from white pumpkin in 30 minutes presented higher lightness than others time. While the redness (a*) and yellowness (b*) of the white pumpkin pectins from 5 to 20 minutes were greater than 30 minutes. The obtained results are in agreement with the study that was carried out on the five types of banana peels (Khamsucharit et al., 2018).

Table 3: Color parameter of white pumpkin at 95ºC in different times

Extraction condition at 95ºC (minutes)	Color parameter		
	L*	a*	b*
5	65.32 ± 0.12	5.07 ± 2.33	9.65 ± 0.05
10	64.77 ± 0.12	3.69 ± 2.74	10.22 ± 0.03
15	67.99 ± 1.87	3.84 ± 2.45	8.21 ± 0.04
20	73.11 ± 0.98	2.94 ± 2.09	8.02 ± 0.04
25	78.38 ± 0.11	2.31 ± 0.98	6.99 ± 0.06
30	82.07 ± 0.12	2.03 ± 0.77	5.14 ± 0.06

CONCLUSION

Pectin was successfully isolated from bottle gourds or white pumpkins in the current investigation. Pumpkin pectin has a unique structure that can determine its physical qualities, setting it apart from other types of pectin. When compared to other pectin sources, the pectin obtained using deionized water showed a high yield and good color. The lowest purity of pectin is indicated by high anhydrogalacturonic acid, which is present in white pumpkin. This suggests that white pumpkin pectin has a highly branched structure made up of neutral sugars and rhamnogalacturonan I. The resulting pectin is classified as high methoxyl pectin based on its methoxyl concentration and degree of esterification. As well as the obtained results indicated that pectin that was extracted in 30 minutes has a good color compared to another times of extraction. Finally, we can say that we have the chance to use the leftover white pumpkin and byproducts from this investigation, which could cause environmental issues and be useful.

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