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Determination of Pectin Yield and Their Characters of Some Local Apple Varieties in Kurdistan Region

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Abstract

Pectin, a naturally occurring heteropolysaccharide, has in recent years gained increasingly in importance. The benefits of natural pectin are also more and more appreciated by scientists and consumer due to its biodegradability. Industrial pectins are extracted from by-products of the fruit juice industry apple pomace or citrus peels. They are extracted in acidic conditions and chemically modified to give High Methoxy (HM) pectins and Low Methoxy (LM) pectins. The properties of these two main types of differ widely. In this study, six variety of apple was selected as a representative of the apple fruit family to extract pectin. After removing seeds, the sliced pieces were dried at 65◦C for about 20hrs until the weight of samples become constant. Then the dry apple samples were crushed by an electric grinded. The grinder apple was sieved to remove the uncrushed particles, in a sieve of 60 mesh 250um and stored at a temperature 6-10˚C. Pectin was extracted from sieved apple samples of each sample (A-F), the extraction was performed by using 500ml of hot deionized water which was acidified with 1N hydrochloric acid to pH value 1.8. 45ml of acidified water solvent was added to 1.5g of dry apple powder in conical flask and stirred for about 2 min until the homogenize mixture was appeared. Then the conical flask was transferred to the hot water bath and heated to 90˚C for 2hrs with continuous stirring for 30min. The pectin yield and its equivalent weight, methoxyl contents, anhydrouronic acid (AUA) and degree of esterification (DE) of extracted pectin of each apple fruit samples (A-F) were determined.

Keywords: *Apple pomace; Pectin, Yield; Methoxyl Content; Anhydrouronic acid; Degree of esterification*

1 INTRODUCTION

Apple is most important fruit preferred for taste and nutritive value and they are an extremely adaptable crop. The cultivation range spreads from the extreme cold environs. At present, more than 60 countries produce over 1000 or more metric tons of apple varieties (FAO, 2004). In Iraqi Kurdistan region apple varieties, among the excellent varieties well altered to the local environment condition were Star king, Red-Lebanon and Green-yellow-Lebanon cultivars from Barwari Bala, these cultivars have a great demand by the consumer. Nutritionally, apple is an excellent source of sugars, minerals, dietary fiber and functional compounds such as ascorbic acid and phenolic compound (Bondonno et al. 2017). Apples have displayed beneficial effects on the health (Hyson, 2011). Pectin is a natural macromolecular heteropolysaccharide substance that are widely present in the primary cell walls and the middle lamella of apple, citrus fruits, banana, watermelon and vegetable. Pectin is introduced as a food additive such as used as emulsifiers, gelling agents, stabilizers, and thickeners (Santos et al., 2013). Pectin has a color from light yellow to light brown powder. According to the numerical system used by the Codex Alimentarius Commission, pectin and its derivatives are listed as a natural food additive as emulsifier, stabilizer thickeners and gelling agents by the European Union (EU) legislation product E440 (Efsa, 2017). Pectin is a natural component of all omnivorous diet and an essential source of dietary fiber. Pectin is used in large number of food products such as ice creams, low fat spreads, yoghurts, margarine, salad dressings, salty spreads, bakery products, and many other creamy sauces, to keep

them in stable emulsion (Ngouemazong et al., 2015). Also, pectin is used in food products as a gelling agent in the preparation of jams, jellies, and marmalades (Lofgren et al., 2005). Also, Pectin is one of the most important renewable natural polymers which are used in food packaging usually in the preparation of edible films and coatings (Bourtoom, 2008). Furthermore, pectin plays an important role in the pharmaceutical industry, like its effects on reducing cholesterol levels in blood (Sriamornsak, 2001). Also, pectin is widely used in combination with colloids for the treatment of diarrheal diseases, especially in infants and children (Naggar et al., 1992). This research study is aimed to determine pectin yield with its characteristics in local apple fruit samples.

2 MATERIALS AND METHODS

2.1 Samples Collection and Description

Among different variety of apple, six species were selected for present study and collected from several local places in Kurdistan Region at October and November, 2020. The samples were harvested directly from tree then stored in the refrigerator at 4°C. The sample varieties are included: Red Delicious apple from Haje Omran-Erbil type (A), this cultivate can be grown in different areas such Halabja in Sulaymaniyah and Nahla in Akre, its average weight is about 170g, and its color is dark red. Red-Yellow apple from Haje Omran-Erbil type (B), the origin of this type from France, that is cultivated in Amedi in Duhok also someone is named Balak, approximately the average weight 178g. This variety is distinguished by more order and sweetest taste. Yellow or Golden Delicious apple from Haje Omran-Erbil type (C), which is the most abundant in Kurdistan and its size medium to large with the average weight 150-200g, its color yellow to green. Star king apple from Brwari Bala-Duhok type (D), this type has dark red color and a point on the skin of apple, it has medium size and average weight 100g. Red Delicious apple the origin of this variety from Lebanon but is grown in Brwari Bala Duhok type (E), the wholly ripe fruit color is red- yellowish and has medium size with average weight 120g. Green-Yellow Delicious apple also it originated from Lebanon but planted in Brwari Bala-Duhok type (F), the color is white in fully ripen then become yellow.

2.2 Pectin yield

The percentage yield of extracted pectin was estimated by dividing the weight of pectin after drying to the weight of dry apple powder which was utilized for extraction (Grassino *et al.*, 2016), and the yield was calculated according to the following equation:

$$\text{Pectin yield\%} = \frac{\text{Weight of dried pectin (g)}}{\text{Apple powder used (g)}} * 100$$

2.3 Equivalent Weight

0.5 g of dried pectin was taken and placed into a 250 ml conical flask and 5 ml of ethanol 95% was added to it. After addition of 1.0 g of sodium chloride and 100 ml of deionized water to conical flask content, six drop of phenol red indicator were dissolved and titrated against 0.1 N of sodium hydroxide until the dark pink color was appeared (Ranganna, 1995). The equivalent weight was calculated for each apple samples (A-F) by the following equation:

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

2.4 Methoxyl Content Determination

25 ml of 0.25 N of NaOH was added to the neutralized solution which obtained after titration for equivalent weight determination and the solution was stirred very well and let to stand for about 30 min at room temperature in a stoppered conical flask. Then 25 ml of 0.25 N of HCl was added to the solution, afterward six drops of red phenol indicator were dissolved and titrated with 0.1 N of NaOH until the color of titrant changed to purple (Lopez-Palacios *et al.*, 2012). The methoxy content was estimated by following equation:

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

31 = Molecular weight of methoxyl (CH_3O)

2.5 Anhydrogalacturonic Acid Estimation

Estimating the anhydrogalacturonic acid (AGA) content is essential for determining the purity of powder pectin. AGA was calculated by applying the pectin equivalent weight and methoxyl content in the below formula (Suhaila and Zahariah, 1995).

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

Where:

176 = molecular unit of AGA (1 unit)

Z = ml of NaOH from equivalent weight

y = ml of NaOH from methoxyl content

w = weight of sample (g)

2.6 Degree of Esterification (DE) Determination

Degree of esterification was measured from the value that observed methoxyl content and anhydrogalacturonic acid content and DE was calculated for apple samples (A-F) by the following equation (Franchi *et al.*, 2014). The degree of esterification is the amount of esterified Galacturonic acid (GalA) groups to the total amount of GalA groups that present in pectin. DE is a significant factor that determines the formation of gel in pectin (Ngouemazong *et al.*, 2012).

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

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2.7 Statistical analysis

Throughout the research, all the results obtained were as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used for the statistical analysis of the data using Statistical Package for Social Sciences (SPSS) software version 26, (2019). Duncan test was used to find the significant differences at α = 0.01 between the treatment. All of the experiments were performed in triplicate and mean treatments was calculated and compared with Completely randomized design (CRD) method.

3 RESULTS AND DISCUSSION

3.1 Pectin Yield

As seen in Figure 1, generally the yield of pectin from apple fruit samples (A-F) at the ripen stage was significantly different, sample A gave highest amount 7.011% while sample D contains lowest quantity of 5.453%. the obtained results were among the yield obtained from the study of (Sharma et al., 2014) who stated that the yield of pectin pomace for some apple was ranged between 3.4 to 10.5%. Also, the range of pectin for apple pomace were stated in other study which ranged from 1.68 to 9.18% (Dranca and Oroian, 2019). Whereas Venkatanagaraju et al., (2019) enumerated the yield of pectin from (12.9- 20.9%) the result was higher than that of current study. It is important to understand that inside the medium of cell, pectin is present in the form of protopectin and the process of isolation causes its suspension into the solvent as pectin. Thus, the extraction depending on the rate of pectin suspension which is made by the high temperature and breakdown of cell wall matrix (Xu et al., 2014).

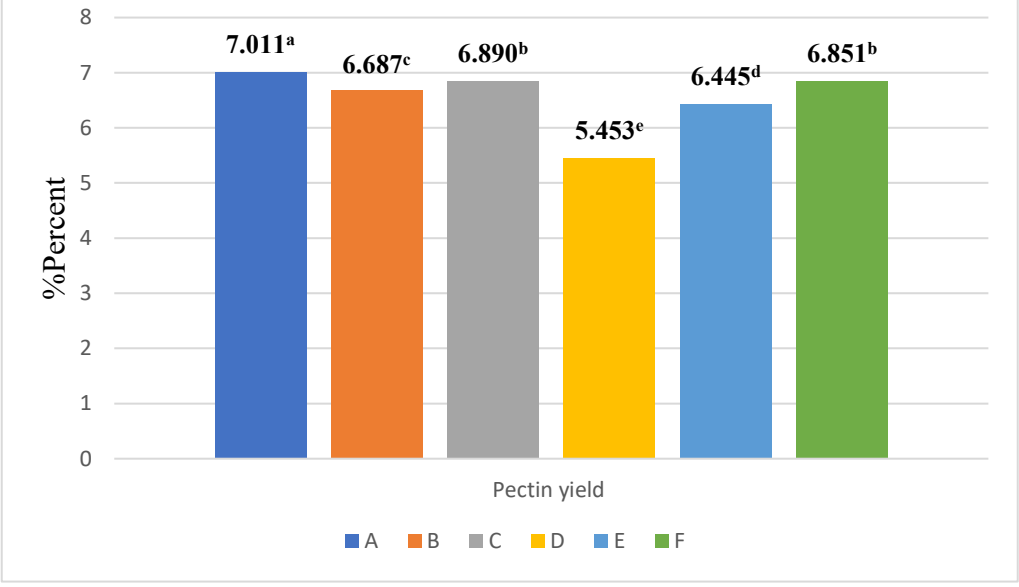

Fig. 1 The yield of pectin in apple samples (A-F)

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3.2 Equivalent Weight

As indicated in Table 4.9. The equivalent weight of extracted pectin from apple samples (A-F) was ranged from 693.920 to 1135.680, where sample B recorded higher score of 1135.680 and sample F scored the lowest value of 693.920, whereas sample A and C recorded similar number of equivalent weights. The obtained

results are in agreement with the study Kumar and Chauhan, (2010) who stated that the equivalent weight of apples pectin ranged from 714.29 to 1666.30. The higher equivalent weight could be due to lower partial degradation of pectin. Also, the increased or decreased of the degree of equivalent weight might be dependent upon the amount of free acid which present in pectin substance or the procedure used in the extraction which effect on the yield and other properties of extracted pectin (Ramli, 2011). The obtained results indicated that sample has higher equivalent weight than the others, this property is a good for formation of gel. Equivalent weight is defined as the weight of the substance which contains or reacts with 1g of hydrogen or 8 g of oxygen, or 35.45 g of chlorine (Rattanapitikon, 2010).

3.3 Methoxyl Content

The methoxyl content is an important factor in controlling the time sitting of pectin and determining the capacity of the pectin for the formation of gel (Constenla and Lozano, 2003). The Table 4.9 displayed that the methoxyl content of almost apple samples (A-F) was significantly different, sample E showed lower methoxyl content 3.959% while, sample A recorded higher content 8.184%. It was found that the methoxyl content in pectin for two apples variety was 8.73 and 7.4% respectively (Jain *et al.*, 1984). Further, Virk and Sogi, (2004) were reported the methoxyl content of apple pomace was 3.7%. Quality spreading and sugar binding ability of pectin are increased with raising methoxyl content and when the methoxyl content is high lead to formation of high solid pectin gel (Madhav and Pushpalatha, 2006).

3.4 Anhydrogalactouric Acid (AGA) content

The AGA content for extracted pectin of apple samples (A-F) was significantly different, as seen in Table 4.9 the highest AGA content was present in sample F 66.175%, whereas the lowest content of AGA was found in sample E 43.647%. The mentioned results were in agreement with the values that stated by (Kumar and Chauhan, 2010). Generally, all values of AUA content which obtained from apple samples were less than 65% except sample A that means the extracted pectin might not be pure and have a high quantity of protein, starch and some sugar like galactose, arabinose and rhamnose (Ismail *et al.*, 2012). The anhydrogalactouric acid content is an important parameter which determines the purity of extracted pectin and indicates the suitability of extracted pectin for its use in food processing such as jams, jellies etc. Also, is suggested to be not less than 65% (Food Chemicals Codex, 2016).

3.5 Degree of Esterification (DE)

As seen in Table 4.9, the degree of esterification in apple samples was ranged from 51.611 to 71.738% sample A noted significantly differ than sample E. The obtained values from apple pectin samples (A-F) are comparable to the results reported by Wikiera *et al.*, (2016) who illustrated that the DE of five apple variety ranged from 56.1 to 73.4%. Depending on the obtained results from current study the extracted pectin was high methoxyl pectin, this is a good property for the preparation of food products like jams and jellies. Reliant on DE pectin is classified into two type which are low methoxyl pectin (LMP) with ≤ 50% DE and high methoxyl pectin (HMP) with >50% DE. Also, the degree of esterification effects physical properties such as emulsification abilities, surface tension and formation of gel (Yoo *et al.*, 2006; Lutz *et al.*, 2009).

Table 3.1 Characterization of extracted pectin from apple samples

Samples	Gram	%	%	%
	Equivalent weight	Methoxy content	AUA	DE
A	960.515±1.43[c]	8.184±0.00[a]	64.766±0.00[b]	71.738±0.00[a]
B	1135.680±0.96[a]	5.702±0.00[f]	47.871±0.00[e]	67.646±0.00[b]
C	960.010±2.14[c]	6.446±0.00[c]	54.911±0.00[c]	66.660±0.00[b]
D	1040.640±1.44[b]	5.948±0.00[d]	50.687±0.00[d]	66.650±0.00[b]
E	831.830±2.12[d]	3.959±0.01[e]	43.647±0.00[f]	51.611±0.00[d]
F	693.920±0.73[e]	7.191±0.00[b]	66.175±0.00[a]	61.202±0.70[c]
*Different letters indicate presence of statical differences at the level of $\rho \leq 0.01$ and Values are means± SD of three replicates				

CONCLUSIONS

In conclusion, Pectin was successfully isolated from local apple in the current investigation. Apple pectin has a unique structure that can determine its physical qualities, setting it apart from other types of pectin. all local apple samples appeared to be most appropriate for health benefits since of high content of total phenol and minerals. Whereas the extracted pectin from local apple samples seems to be high methoxy pectin, it means has high quality and become good source for such pectin to applied in food products.

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