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Optimization of Flaxseed Cake Pectin Extraction and Shelf-Life Prediction Model for Pear Fruit Preserved by Pectin Edible Coating

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Abstract

The aim of this study was the possibility of pectin flaxseed cake extraction by using organic and mineral acids, and improves the edible coating formation from flaxseeds pectin and sodium alginate (1%) for pear fruit. Pectin characterization (Equivalent Weight, Methoxyl Content, Total anhydrouronic acid content and Degree of Esterification) was estimated, also characterized according to their fingerprint from the FT-IR spectroscopy whether flaxseed pectin extracted by citric and hydrochloric acid (FPE1 and FPE2, respectively). The substance used in these experimented were the pear fruit coated with pectin extraction by citric and hydrochloric acid (CEP1 and CPE2, respectively) compared with uncoated fruit. The effect of edible coating on quality attributes of fruit samples storage at 4±1°C was determined. The shelf-life of pear fruit was determined by using different mathematical analyses such as statistical and kinetics models. The results of this study demonstrated that the beast edible coating of pear fruit was CEP2 followed by samples CEP1 as compared with uncoated fruit samples. The fruit quality evaluation revealed that the coating treatments were more effective in retention of the weight, antioxidant activity and total anthocyanins content in pear fruit during storage periods. Pear fruit coated with pectin extraction two (CEP2) retains an acceptability consumption for a longer period than other tested fruit samples. Finally, it was evident from results the incorporation of flaxseed pectin with an edible coating solution improved the quality of fruit samples and antioxidant stability.

Keywords: Flaxseed; Pectin Characterization; FT-IR spectra; Edible Coating; Food Quality Evaluation; Statistical and Kinetics Models.

1. Introduction

Flaxseed or linseed (*Linum usitatissimum* L.) is one of the family of linaceae, commonly known as "Alsi", which is cultivated for the production of textile fiber, seed and flaxseed (linseed) oil. Flaxseed is pressed to produce oil for various industrial developments. After the recovery of the oil, the residual are by-product primarily used as a protein-rich cattle feed despite its potentially rich nutrient composition and may be suitable for application in human foods [1,2].

Flaxseed is a source of functional ingredients like polysaccharides (other than starch), alpha-linolenic acid (ALA) and fibre. There are two types of fibre, soluble and insoluble: insoluble fibre is made up of substances like lignin, cellulose and hemicellulose. In the presence of water, soluble fibre forms a gel, and this complex incorporates sugars, gums and pectin which form mucilage. Recent studies of the flaxseed mucilage demonstrated the presence arabinoxylan (AX) as the major component (75%). Also, flaxseed contains some pectin [3,4].

Pectin is polygalacturonic acids with varying degrees of methyl esterification and are widely used to create gels). Pectin is used in food, pharmaceuticals, and cosmetics because of its thickening and emulsifying properties, as well as its ability to solidify into a gel. Because of its natural abundance, low cost, and sustainable nature, pectin has been identified as one of the major raw materials

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used to produce edible coatings and films. [5]. Edible coatings have long been used in food preservation, such as wax coatings for fruit, which have been around since the 12th century, chocolate coatings for the confectionery and lipid films for meat products. In their modern version, edible coatings are applied in a very thin layer to avoid affecting the product's appearance and to prolong its shelf life, which can be eaten together or removed before eating. Edible coatings can improve food product quality by preventing microbiological chemical and physical degradation such as oxidation, moisture loss and enzymatic browning reactions, as well as optimize particle clustering and possibly improving visual and tactile properties of food product surfaces. [6-8].

Fresh-cut fruits and vegetables are very critical to be coated due to their high free moisture content and high respiration rate. Therefore, edible coatings tend to act as a protective layer around the surface of fruits and vegetables, limiting respiration and thereby delaying spoilage. [9,10]. The application of pectin-based edible coating for persimmon [11], tomatoes [12], apricot [13] and potato chips [14], recently published. The study's main goal is to extract pectin from flaxseed cake (by-product) and add value to it by applying flaxseed pectin in an edible coating as a natural barrier for moisture in order to extend the shelf life of fresh cut pear fruit.

2. Experimental:

2.1. Materials: Flaxseed by-product (cake) was obtained from Squeezing and Extracting Natural Nils - Oil unit - National Research Centre. Flaxseed cake was washed by distilled water at 40–50°C for removing the dirt and dust. The residue was dried in a hot air oven at 50 °C for 24 h and were pulverized using a coffee grinder. The resulting powder was stored at -18 °C in polyethylene bag until use in the extraction process. Pear fruit samples were purchased from a local market in Cairo, Egypt. The fruit were selected taking into account their size maturity, visual absence of microorganisms and damage on the skin. Pear fruit samples were disinfected with a solution of calcium hypochlorite (0.2 g/L) for 5 min then, dried at room temperature until coated by dipping methods.

2.2. Methods

2.2.1. Pectin extraction

The flaxseed cake powder was defatted using Soxhlet extraction equipment with petroleum ether for complete purification of residual oils. After oil elimination this powder was dried, then subsequently extracted two times with ethanol at 80°C in order to remove free monosaccharides.

Pectin extraction from the flaxseed cake powder was carried out through two acid types: Flaxseed Pectin Extraction one (FPE1) using organic acid (citric acid) and Flaxseed Pectin Extraction two (FPE2) using mineral acid (Hydrochloric acid) using microwave extraction method [15] with a slight modification. Flaxseed cake powder (10 g) was suspended in 400 g of citric acid (20%) or HCl (0.05M). The mixture was homogenized at 15,000 rpm for 1 min and heated in a microwave oven (SHARP microwave oven R-340R, SHARP Corporation, Thailand) with an output power of 1100W for 15 min. To avoid spilling of sample, the process was made intermittent (ton/toff = 1/3 min). The extract solution was filtrated then the flaxseed cake residue was extracted again under the same conditions. After that, the collected pectin solution was homogenized again at 15,000 rpm for 1 min., and Centrifuged at 5000 rpm for 10 minutes. To the supernatant, ethanol (1:2 v/v) was added and kept at room temperature (25 ± 5 °C) for 12 h to precipitate pectin. After vacuum filtration, the pectin was washed 3 times with absolute ethanol to remove other components and then finally washed with acetone. The pectin was left until there was no odor of acetone, dried at 50°C using a hot air oven and crushed to powder using a mortar (Figure 1). The Flaxseed Pectin (FP) yield was calculated using the Equation (1):

$$\textbf{Pectin yield}\ (\%) = \frac{\text{extracted pectin (g)}}{\text{initial sample (g)}} x100 \quad (1)$$

Fig. 1. Flaxseed; FPE1: Flaxseed pectin extracted by citric acid; FPE2: Flaxseed pectin extracted by hydrochloric acid

2.2.2.Pectin Characterization

The Equivalent Weight (EW), Methoxyl Content (MeO), Total anhydrouronic acid content (AUA) and

Degree of Esterification (DE) were determined according to the method of Owens [16].

Equivalent Weight (EW): EW is used for calculating the anhydrouronic acid content and the degree of esterification. Weighing 0.5 g FP in a 250 ml conical flask and moistening it with 5 ml of ethanol. One gram of sodium chloride was added to sharpen the end point. Distilled water (100 ml) and six drops of phenol red indicator were added. The mixture was then stirred rapidly to ensure that all the pectic NaOH until the color of the indicator changed to pink (pH 7.5) and persisted for at least 30 s. The neutralized solution was used for the methoxyl determination. The following Equation (2) was used to calculate the EW:

$$\textbf{Equivalent Weight} = \frac{weight\ of\ sample\ x\ 100}{ml\ of\ alkali\ x\ Normality\ of\ alkali} \quad (2)$$

Methoxyl Content: The methoxyl (MeO) content was performed by adding 25 ml of 0.25 N NaOH to the neutralized solution which was shaken thoroughly and allowed to stand for 30 min at room temperature in a Stoppard flask. Twenty-five milliliters of 0.25 N HCl will be added and titrated to the same endpoint pink as before. The following Equation (3) was used to calculate the methoxyl content:

$$\textbf{Methoxyle content} = \frac{\text{ml of alkali x normality of alkali x 31 x } 100}{weight\ of\ sample} \quad (3)$$

Where: 31 is the molecular weight of the methoxyl group.

Degree of Esterification (DE): Fifty milligram of the FP powder was moistened with 65% isopropanol and dissolve in 10 ml of distilled water. Then, the resulting pectin was titrated with 0.1 N NaOH solutions (a ml to pH 7.5. The solution was added with 30 ml of 0.1 N NaOH and keep for 30 min, followed by the addition of 30 ml of 0.1 N HCl. The pectin solution was then titrated again with 0.1 N NaOH (b ml) to pH 7.5. DE of the each extracted pectin was calculated by using Equation (4)

$$\textbf{DE}\ \% = \left(\frac{\mathbf{b}}{\mathbf{a}} + \mathbf{b}\right)\mathbf{x100} \quad (\mathbf{4})$$

Total anhydrouronic acid content: Estimation of anhydrouronic acid (AUA) content was essential to determine the purity and the degree of esterification (DE), by using EW and methoxyl content value. Total AUA of pectin was obtained by the following Equation (5):

$$\textbf{AUA}\% = \left(\frac{\mathbf{176\ x\ 0.1z\ x100}}{\mathbf{W\ x\ 1000}}\right) + \left(\frac{\mathbf{176\ x\ 0.1y\ x100}}{\mathbf{W\ x\ 1000}}\right) \quad (\mathbf{5})$$

Where:
molecular unit of AUA (1 U) = 176 g,
z = ml of NaOH from equivalent weight determination,
y = ml of NaOH from methoxyl content determination,
w = weight of sample.

FT-IR spectra: Flaxseed Pectin was also characterized according to their fingerprint from the FT-IR spectroscopy, using a Fourier-Transform Infrared Spectrophotometer (JASCO FT/IR-300E Fourier Transform Infrared Spectrometer). The characteristic spectra of the pectin samples were recorded in the range 4,000–400 cm-1 at a resolution of 4cm-1.

2.2.3. Pectin coating treatment

Preparation of dipping solution: Pectin (PE1 or PE2) (1%) and sodium alginate (1%) with the glycerol (1.25%) were dissolved in the distilled water using the magnetic stirrer. After all the components were completely dissolved, the solution was cooled to room temperature. For the retarding browning and inhibited yeast growth, calcium chloride solution (2% w/v) containing ascorbic acid (0.5% w/v) and cysteine (0.75%w/v) was prepared [17-18].

Pear fruit coating: The clean fruit were cut into wedges and immersion into coating solutions then, dried at the room temperature until the solidification of edible coating. This process was repeated one more time for final coated time 120s.

The excess of coating material was allowed to drip off for 1 min before submerging the fruit again for 2 min., in the calcium chloride solution containing ascorbic acid and cysteine. The uncoated fruit samples, which just treated with distilled water. After drying, uncoated and both coated treatments fruit were packed in cartoon boxes and stored for 8 days at refrigerated temperature 4 ±1°C at three groups as follows: uncoated samples were used as the control treatment; CPE1 pear fruit Coated with Pectin Extraction one; CPE2 pear fruit Coated with Pectin Extraction two (Figure 2). The quality evaluation tests of uncoated and both coated fruit were carried out on regular interval of time (2,4,6 and 8 days).

Fig. 2. Control (uncoated pear fruit); CPE1: pear fruit coated with pectin extraction one; CPE2: pear fruit coated with pectin extraction two

2.2.4. Fruit quality evaluation

Weight loss: The weight loss was determined gravimetrically using analytical balance (ADAM PW124, UK). For each treatment, the weight loss was calculated based on the comparison of the weight of the sample at its initial condition and its final condition [19]. The percentage of weight loss was calculated by Equation (6)

$$\textbf{weight loss} = \frac{\textbf{initial weight} - \textbf{final weight}}{\textbf{initial weight}} \textbf{x100} \quad (6)$$

Total soluble solids (TSS): Pulp (20g) from fruit was homogenized in a grinder and then centrifuged at 15,000g for 20min. The supernatant phase was collected, drop of this juice was placed onto the plate surface of the hand
refractrometer and the reading was taken directly as ^{o}Brix [20].

Titratable acidity and pH: cut fruit (10 g) was homogenized in a blender and then centrifuge, and collected the clear liquid for analysis. 5 g juice up to 50 ml with distilled water and titrated with 0.1N NaOH to an endpoint of pH 8.1. TA was expressed as percentage of citric acid. pH of each groups fruit determined by digital pH meter.

Total anthocyanin content: Pear fruit (10 g) extracted with 200 ml of 1% HCl–methanol for 2 hr. the extracts were filtered. Buffer solutions at pH 1.0 (0.025 M potassium chloride) and at pH 4.5 (0.4 M sodium acetate) were prepared. Each sample was diluted with the buffers to give an absorbance reading between 0.2 and 1.4 AU. Absorbance was measured at 520 and 700 nm with a spectrophotometer (T80 UV-VIS spectrophotometer, PG Instruments Ltd). Total anthocyanins content (mg/ L) was expressed as cyaniding-3-glucosideaccording to the following Equation (7) and was converted to mg anthocyanin / 100 g sample [21].

$$\textbf{Anthcyanin (mg/100g)} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1} \quad (7)$$

where:

$A = (A_{520nm} - A7_{00nm})$ pH 1.0 – $(A_{520nm} - A_{700nm})$ pH 4.5

MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside

DF = dilution factor

ε is the molar absorptivity of cyanidin-3-glucoside (26,900)

1 = cell path length in (1cm)

10^3 = factor for conversion from g to mg

*Antioxidant activity:*1,1-Diphenyl-2-picryl-hydrazyl hydrate (DPPH) method was used to HCl–methanol was used to determine antioxidant activity [22]. Two milliliters of 0.15 mM DPPH solved in methanol was added to 1 mL of fruit extracts and mixed well. Absorbance (Abs_{sample}) was measured at 517 nm after 30 min. Absorbance of a blank without extract was recorded (Abs_{blank}), and the antioxidant activity was calculated, and results were expressed as percentage (%) according to Equation (8):

$$\textbf{\textit{Antioxidant activity}} = \frac{Abs_{blank} - Abs_{sample}}{Abs_{blank}} x100 \quad (8)$$

2.2.5. Shelf-life estimation

Shelf-life determination of pear fruit was done by choosing parameters that have the highest correlation coefficient (R2). Moreover, the Reaction rate constants were determined by fitting the experimental data to zero and first order kinetic models, (Equation 9) and (Equation 10). respectively, [23].

$$C = C_0 + k_0 t \quad (9)$$

$$C = C_0 e^{k1t} \quad (10)$$

Where:

C is the studied parameter (pH, TSS, DPPH, Total anthocyanins) at any given reaction time, C_0 are initial values of untreated samples and k_0, k_1 are rate constants.

Shelf-life of quality attributes for pear fruit at zero order kinetics were obtained according to the Equation (11):

$$Ae = Ao + kt_s \quad (11)$$

Where:

Ao = A value at the beginning of shelf-life; Ae = A value at end of shelf-life; t_s = shelf-life; k = constant rate.

Half-life period explains the degradation of 50% of quality parameters from its original value. Both shelf-

life (t_s) and half-life ($t_{½}$) for a quality attributes at first order kinetics were calculated using Eq (12,13).

$$\text{Ln}\ \frac{A0}{Ae} = Kt_s \quad (12)$$

Where:
A0 A value at the beginning of shelf life
Ae A value at end of shelf life
k the rate constant of the reaction
t_s Shelf life

$$t_{1/2} = \frac{\ln(2)}{K} \quad (13)$$

Where:
k the rate constant of the reaction
$t_{½}$ the half-life of the reaction

2.2.6. Statistical analysis

All data were subjected to statistical analysis using the SPSS 20 statistical analysis packaged software and means were compared by Duncan's Multiple Pearson's correlation test on data sets. range test at the 5 % level of probability. Analysis of relationships between variables was carried out sing Pearson's correlation test on data sets. range test at the 5 % level of probability. Analysis of relationships between variables was carried out using

3. Results and Discussion:

3.1. Pectin Characterization

Pectin yield: The yield of pectin generally depends on the extraction process's conditions, like extraction time, extraction solvent, temperature and drying method [24,25]. The yield of Flaxseed pectin extracted with citric acid and hydrochloric acid was shown in Table 1. Hydrochloric acid extracting was obtained the highest yield (11.605 %), while citric acid extraction yielded the lowest yield (5.555 %). Similar results were observed in the extraction of pectin from the lime peel by hydrochloric and citric acid [26]. On the other hand, Maric et al., [27] Preferred use organic acid because it is less depolymerizing of pectin and has a low capacity to hydrolyze as well as more environmentally friendly. In addition, ammonium oxalate can be used to extract pectin from defatted flaxseeds with a maximum yield 12.88% [28].

Equivalent Weight: The EW of pectin extracted from FCP using citric acid and hydrochloric acid were 438.056 and 380.784, respectively. Pectin extracted from papaya fruit by HCl and citric acids has an equivalent weight of 912.17 and 455.1, respectively [29]. Small particles of pectin could be result from using strong acid due to an increased partial degradation and hydrolysis of pectin during the long extraction process and higher temperature [30].

Methoxyl Content: Low-methoxyl pectins are often used as edible coatings because encouraging higher firmness and structural integrity while decreasing water vapour permeability in addition to their ability to form firm gels in the presence of calcium cations [31]. Flaxseed pectin extracted by using citric acid resulted a lower MeO (5.440 %) when compared to hyrcochloric acid (6.136 %). This result was in agreement with Chodijah et al.,[32] who reported that MeO content of pectin extracted from banana peel using citric acid was 3.906% (< 7%) with good edible film characterization.

Degree of Esterification (DE): DE can be used to pectin classify, low methoxyl pectin (DE ≤50%) and high methoxyl pectin (DE >50%). Low-methoxyl pectin can be used as a gelling agent, thickening agent and stabilizer. Based on DE values, PE1 and PE2 pectin can be classified as low methoxyl pectin as DE ≤ 50%. Furthermore, there was no significant difference ($p > 0.05$) between pectin extracted with citric acid and hydrochloric acid in DE (Table 1). These results were in accordance with observations reported in pectin extracted from black carrot pomace using a microwave-assisted method [33]. Likewise, Rodsamran and Sothornvit [15] who reported that DE of pectin extracted from the pineapple peel was in the range of 35.69- 39.39 %, and it has been used to produce good edible films.

Total anhydrouronic acid content: The AUA content of PE1 and PE2 was 72.866 and 80.999 %, respectively. The anhydrouronic acid content must not less than 65 percent in pure pectin based on the Food Chemical Codex (1996). According to AUA content for PE1 and PE2 it can be considered both FP high purity pectin. The anhydrouronic acid content of pectin extracted from gooseberries and strawberries pomace [34], tomato peel [35], apple pomace [36] and custard apple peel [37] was found 19.25%, 22.88%, 42.67%, 64.32% and 70.24%. respectively, and the wide range of the AUA could be depending on the method extraction and the nature of the fruit used. The high purity pectin from lemon peel was extracted using a microwave-assisted extraction process. Microwave techniques, as opposed to other extraction methods, may be attributed to improving increase the degree of acid penetration into the fruit tissue and inhibit pectinases more rapidly. [38].

FT-IR spectra: In this study, we examined functional groups FP from both acid extractions using FT-IR spectroscopy. Figure1 shows the FTIR spectra of PE1 and PE2. The two characteristic absorption bands were detected in PE1at 3452 cm−1 and 2926 cm−1, which are attributed to O–H asymmetric stretching

vibration and C–H symmetric stretching vibration, respectively.

Table 1. Characterization of flaxseed pectin using microwave-assisted extraction by two acid type

Parameters (%)	PE1*	PE2**
Yield	5.555 ± 0.61	11.605 ± 1.58
Equivalent Weight	438.056 ±3.780	380.784 ±4.48
Methoxyl Content	5.440 ± 0.046	6.138 ±0.072
Degree of Esterification	42.666±1.527	43.333±1.54
Anhydrouronic acid	72.866 ± 2.931	80.999±1.710

*PE1: using organic acid (citric acid)

**PE2: using mineral acid (Hydrochloric acid)

The peaks at 1625 cm−1 and 1450 cm−1 were attributed C=O and CH2, respectively. The peak at 1049 cm−1 was assigned to C = C stretching mode. These results indicate that the extracted PE1 is pectin compound [39]. In case of PE2 spectrum (Figure 1), the O–H asymmetric stretching vibration and C–H symmetric stretching vibration are appeared at 3446 cm−1 and 2927 cm−1, respectively. The peak observed at 1750 cm−1 can be assigned to carboxymethyl (−COOCH3). The peaks at 1633 cm−1, 1447 cm−1 and 1396 cm−1 could be assigned to C=O, CH2 and –COO groups. The presence of these function groups confirm that the extracted PE2 has pectin structure as previously reported [40].

3.2. Fruit Quality Evaluation

Effect on the Weight: Fresh fruit and vegetable weight loss results in a loss of quality and freshness, and subsequently economic loss. In addition, mass losing plays an important role in shortening the shelf life of fresh produce [41]. Figure 4 illustrated the effect of pectin edible coating on the weight loss of pear fruit during eight days of storage at 4 ±1° C. The weight loss % increased during storage and this reduction was more rapid in uncoated samples (control). Similar results were found [42], who reported that coating treatment of pear slices was more effective in maintained weight loss. Edible coatings for fruit and vegetables have the ability to control the mass loss and providing other functions [43]. Weight loss was slower in CPE2 samples than in other tested fruit. Generally, pectin-based edible coating (CPE1 and CPE2) was observed the reduced rate of weight loss throughout the storage period. Also, low methoxyl pectin coating for fresh-cut melon was effective in improved preventing dehydration and maintaining the initial firmness during storage (15 days at 4 °C) [44].

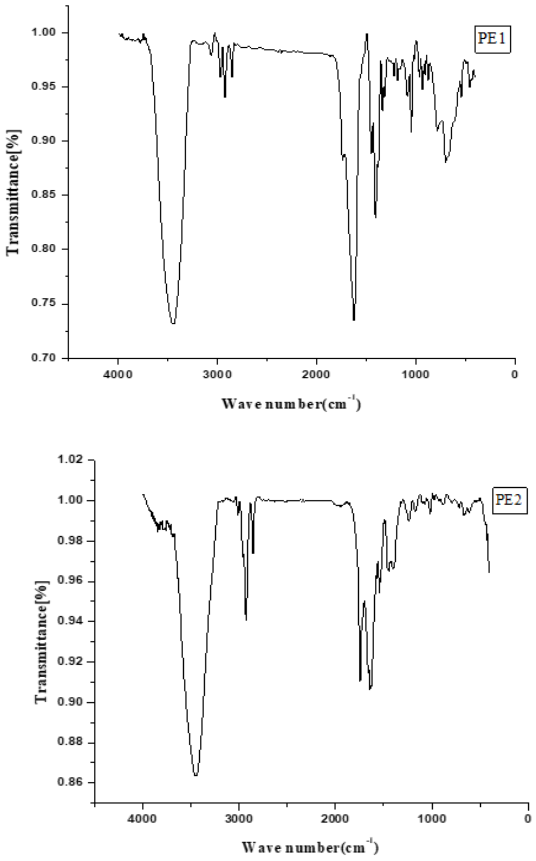

Fig. 3. FTIR spectra of PE1: pectin powder extracted using citric acid; PE2: pectin powder extracted using hydrochloric acid

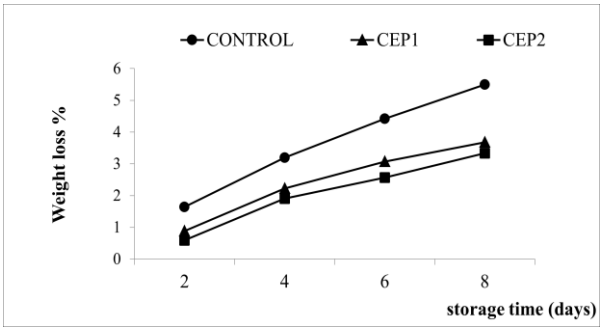

Fig. 4. Weight loss of control, CPE1, CPE2 pear fruit during 8 days at 4±1°C

Effect on the pH: The initial pH values of uncoated, CPE1 and CPE2 pear fruit samples on average were 4.27, 4.31 and 4.46 respectively, they increased progressively in the storage period can be seen in Figure 5(a) The pH values of uncoated fruit samples

as a control sample and both coated fruit samples showed significant differences ($p \leq 0.05$) throughout refrigerated storage periods. Likewise, at the end of cold storage (day 8), the pH of uncoated fruit samples were 4.74, while the pH of CPE1 and CPE2 coated fruit samples were 4.40 and 4.59, respectively. These results agree with Saucedo-Pompa et al. [45] who noted that pH values increased of coated and uncoated avocado fruit during the period of storage at 5°C. The pear fruit samples coated with CPE1 showed a low rate of increase in the pH values when compared to that of CPE2 and uncoated samples through the initial storage periods Figure 5(b). The increasing in pH value for coated fruit consequence of the breakdown of the nutrient compounds present in fruit such as sugars into organic and hence causes accumulationof OH-ions [46].

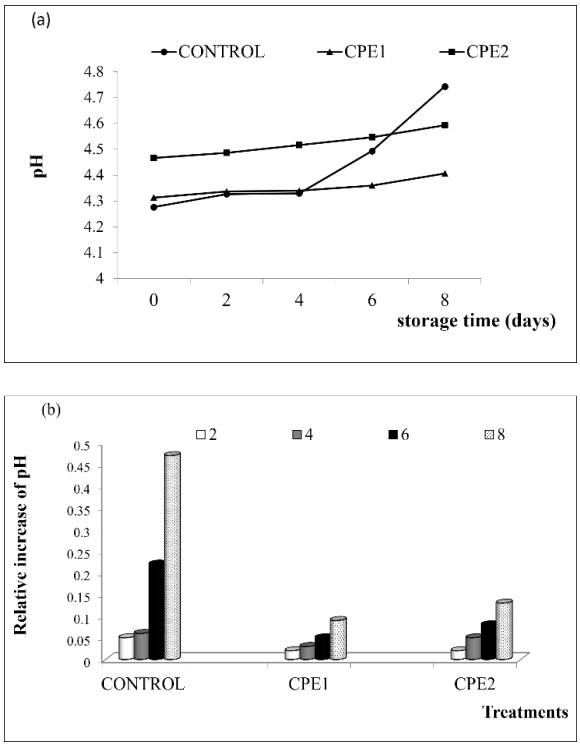

Fig. 5. (a) pH means values; (b) Relative increase of control, CPE1, CPE2 pear fruit during 8 days at 4±1°C

Effect on the Total Soluble Solids: Figure 6 (a), (b) shows the changes in TSS mean values and relative increase of fresh-cut pears during cold storage. The TSS gradually increases with a storage period for all fruit studied and the highest increase rate was observed in the control samples. The TSS of pear fruit samples were significantly ($P \leq 0.05$) affected by the incorporated effect of storage period and coatings. Uncoated fruit had the highest TSS (17.8 °Birx) at the end of the storage period (8 days). While, after 8 days of cold storage, the CPE2 showed the least rise in TSS (12.0 °Birx). These results agree with Mona et al., [47] who mentioned the gelatin coating of fresh cut kiwi fruit reduced the increase in the TSS values at refrigerated storage when compared to control kiwi fruit. The TSS values increase during fruit storage because of respiration and ripening process and the application of edible coating decelerates the rate of respiration and metabolic processes [48]. The complex carbohydrates in the fruit transform to simple sugar molecules during storage, resultingin an increase in TSS values. [49]. Otherwise, Yossef et al [50] stated that the total soluble solid value of strawberry fruit coated with pectin edible films had no significant change during the 16 days of storage when compared to control samples which observed the highest increase rate in the TSS value over time.

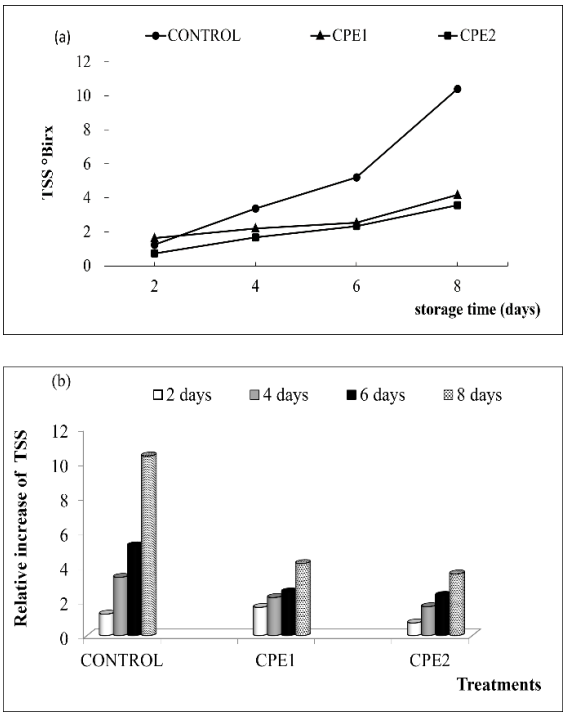

Fig. 6. (a)Total soluble solid (TSS) mean values; (b) Relative increase of control, CPE1, CPE2 pear fruit during 8 days at 4±1°C

Effect on the Titratable acidity: Titratable acidity (TA) was influenced by the storage time for all tested pear samples. The changes in TA were slower within the early days of storage ($p \leq 0.05$). The coated fruit showed a decrease in acidity percentage during the first periods of storage, and then there was a slight increase and returns to decline again at the end of the storage period. The same trend was observed in previous studies [51,52]. The percentage of TA for uncoated samples was ranged from 0.55 to 0.29 , while CPE1 and CPE2 coated samples was range from 0.57 to 0.44 and from 0.55 to 0.42 respectively,

as shown in Figure7. There were almost no significant differences in acidity percentage for pears coated with CPE1 and CPE2. Barrazueta-Rojas [51] who demonstrate that the decrease in acidity was accompanied by a rise in pH, which was due to the use of organic acids as an energy source for the senescence process of fruit. The coatings form a protective layer on the fruit, slowing the senescence process and keeping volatile compounds, allowing organic acids to remain stable for longer [53].

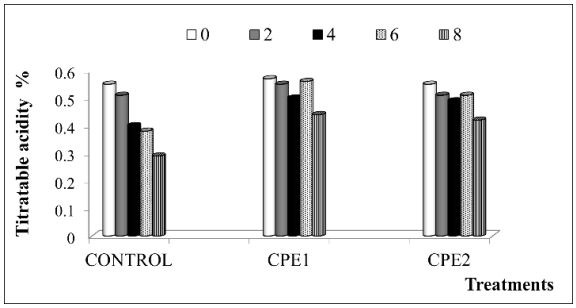

Fig. 7. Titratable acidity mean values of control, CPE1, CPE2 pear fruit during 8 days at 4±1°C

Effect on the antioxidant activity: DPPH radical scavenging activity for uncoated and coated samples during 8 days of refrigerated storage was illustrated in Figure 8(a). The pear fruit of each group were evaluated for their antioxidant activity, the higher percentage of the DPPH assay indicated higher free radical scavenging activity. Within eight days of storage, the antioxidant activity (AA) of all tested samples (coated and uncoated) decreased incrementally, and the highly losing was showed in uncoated samples. Coated samples (CPE1 and CPE2) had higher antioxidant activity compared to uncoated samples. The percentage of DPPH radical inhibition (47.934%) was observed in uncoated samples at 2 days of storage while CPE1 and CPE2 coated samples were observed (47.585 % and 48.715 %. respectively,) at 6 days of storage. Similar results for the effect of pectin-based edible coating on antioxidant activity has been reported for plum fruit [54] and fresh persimmon [55]. Conversely, Sakooei-Vayghan et al, [56] stated that the antioxidant activity of apricot cubes uncoated and coated with pectin was not significantly diff erent ($P > 0.05$).

The relative degradation of DPPH% inhibition of uncoated and coated samples was presented in Figure 8 (b). Among all tested fruit, CPE2 samples have the lowest relative degradation (15.04 %), whereas uncoated samples had the highest relative degradation (57 %) at the end of the storage period. In this case, the PE2 coating treatment can be improving the fruit's ability to retain phytochemical compounds. The positive impact of pectin coatings on antioxidant activity could be associated with the protective effect on phytochemicals active compounds in fruit that possess strong antioxidant activity, in addition to attempting to keep respiratory rates at a low level [57].

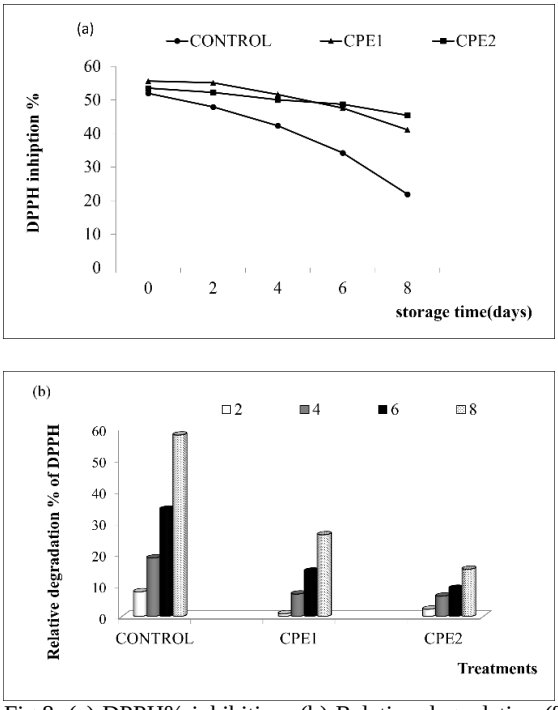

Fig.8. (a) DPPH% inhibition; (b) Relative degradation (%) of control, CPE1, CPE2 pear fruit during 8 days at 4±1°C

Effect on the total Anthocyanins: During the refrigerated storage, there was a decrease in total anthocyanins in all tested samples uncoated and coated pear fruit Figure 9(b). In this context, the uncoated samples illustrated a higher rate of decrease in the total anthocyanins when compared to the coated samples (CPE1 and CPE2). Total anthocyanins content in the uncoated fruit decreased during storage from zero to eight days, ranging from 7.553 to 4.703 mg/100g. While the coated pears (CPE1 and CPE2) showed the total anthocyanins in the range of 7.830 to 5.686 mg/100g and 7.606 to 5.496 mg/100g, respectively.

The total anthocyanins content in the uncoated and both coated samples were significantly different ($p \leq 0.05$). More recently, a similar effect was observed in fresh blackberries and plum fruits for using pectin-based edible coatings [57-58]. The decrease of anthocyanins was referred to as the sensitivity of the pigment during storage and processing [59]. Otherwise, strawberry coated with chitosan showed the opposite trend in anthocyanins content there was increasing over time, attributed to anthocyanin

synthesis during the fruit's physiological processes [60].

Among all fruit inspected, uncoated samples had the highest relative degradation of total anthocyanins (47.814%), whereas CPE1 and CPE2 samples had 27.458 and 27.763 % relative degradation, respectively, at the end of storage days. The total anthocyanin content of both pectin-based edible coating (CPE1 and CPE2) fruits did not show significant relative degradation with storage time Figure 9(a).

The decrease in total anthocyanins content coincides with antioxidant activity decreased in all fruit samples. On the other hand, the coating application reduced the loss in the contents of the phytochemical compounds represented in the estimation of anthocyanins and antioxidant activity compared to the control sample.

The reason for high antioxidant activity in coated samples is obviously due to phytochemical compounds such as an anthocyanin improve the antioxidative capacity and activity of antioxidant enzymes, as well as prevent the generation of free radicals and then contribute to human health in a helpful way [61].

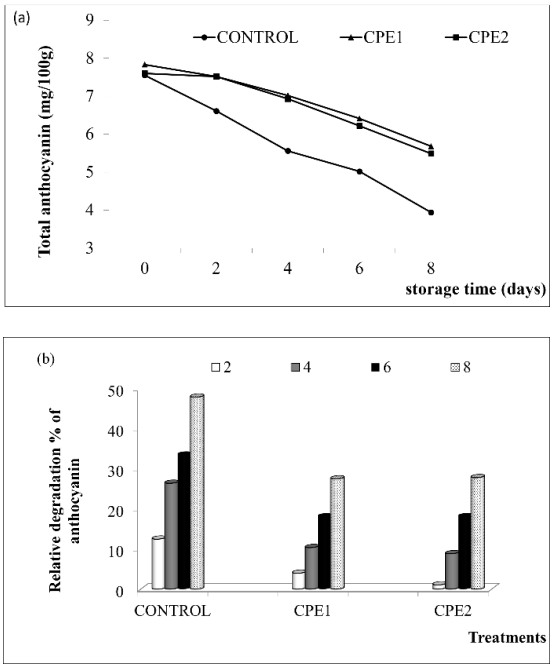

Fig. 9. (a)Total anthocyanins content (mg/100g); (b) Relative degradation (%) of control, CPE1, CPE2 pear fruit during 8 days at 4±1°C

3.3. Shelf-life estimation

The effect of coating treatment on pear fruit shelf-life was applied in different mathematical analysis and was interpreted by statistical and kinetics models represented in Table 2. The choice of the reaction order of kinetics model (zero or first-order) for fruit quality parameters is dependent on the value of R2 in each linear regression equation at the same treatment. The TSS and pH of pear fruit samples were based on zero order reaction with a shelf-life of almost 9 days for CEP1 and 8 days for CEP2. Rate constant of TSS for control, CEP1 and CEP2 were 1.238, 0.462 and 0.445 $days^{-1}$, respectively. The lower the reaction rate constant, lesser the degradation rate and vice versa [62]. Whereas antioxidant activity (DPPH) and total anthocyanins content were based on first order reaction. Shelf-life of DPPH for control, CEP1and CEP2 were 12.30, 28.75 and 52.65 days, respectively. Likewise, Table 2 also presented the half-life of DPPH for control, CEP1 and CEP2 were 6.7, 18.43 and 35.00 days .respectively, the shelf-life and half-life also follows the similar trend. The same table can also be explained by shelf life of the CEP1 and CEP2 it will take 29.55 and 28.36 days for the anthocyanins degradation to cause the product to become unacceptable. Based on the results of shelf-life estimation, The data suggested that the CEP2 had the best treatment, which was attributed to slower degradation of quality parameters.

Table 3,4,5 shows the correlation matrix and the relation with the fruit quality parameters for control, CEP1 and CEP2, strong correlation coefficient (r) of DPPH with anthocyanins content (r = 0.91), (r = 0.99) and (r = 0.99).respectively, were observed. Regarding the Pearson's correlation coefficients test, it can be stated that the relative degradation of anthocyanin content coordinates is related to the decrease in antioxidant activity.

4. Conclusions

This study aiming to flaxseed cake pectin extraction by two types of acids and their characterizations was evaluated. The chemical properties of flaxseed pectin including WE, MeO, AUA and DE revealed that flaxseed pectin could be categorized as low-methoxyl pectin. The FP owing to its lower methoxyl content less than 7% can be used as a good ingredient in dipping coating solutions for fruit. Pectin was used in the dipping solution for pear fruit to extend their shelf life. The fruit quality evaluation such as weight loss, TSS, pH, antioxidant activity and total anthocyanins content was investigated at refrigerated storage for 8 days. With the progression of the storage period, all coated tested pears showed a better trend in antioxidant activity (DPPH) and total anthocyanins content than control samples (uncoated). The shelf-life of the coated pear fruit based on antioxidant activity was 28.75 and 52.65 days for CEP1 and CEP2, respectively. From the previous results, the pectin extracted from flaxseed can be considered a good edible coating ingredient and it has a good effect on improving quality parameters and shelf life of pear fruit.

Table 2. kinetic and Statistical parameters of the fruit quality Parameters during cold storage. Data were combined for the assessment of shelf-life (0, 2,4,6 and 8 days) at cold storage

samples	Parameters	kinetic parameters					Statistical parameters	
		Zero-order model		First-order model				
		k (days^{-1})	t_s(days)	k (days^{-1})	t_s(days)	$t_{1/2}$ (days)	R^2	p-value*
Control	TSS	1.238	8.26				0.923	< 0.05
	pH	0.055	8.49				0.827	< 0.05
	DPPH			-0.1033	12.39	6.71	0.892	< 0.05
	total anthocyanins			-0.0612	21.35	11.32	0.975	< 0.05
CPE1	TSS	0.462	9.03				0.931	< 0.05
	pH	0.0105	8.86				0.895	< 0.05
	DPPH			-0.0376	28.75	18.43	0.901	< 0.05
	total anthocyanins			-0.0401	29.55	17.28	0.964	< 0.05
CPE2	TSS	0.0445	8.54				0.929	< 0.05
	pH	0.0157	8.06				0.978	< 0.05
	DPPH			-0.0198	52.65	35.00	0.964	< 0.05
	total anthocyanins			-0.042	28.36	16.50	0.934	< 0.05

*** p = probability level in relation to the time studied.**

Table 3. Pearson's correlation coefficients amongst 5 fruit quality parameters in the uncoated pear treatments

	pH	***TSS***	***TA***	***DPPH***	***total anthocyanins***
pH	*1*				
TSS	*0.975969*	*1*			
TA	*-0.88298*	*-0.96078*	*1*		
DPPH	*-0.97474*	*-0.99507*	*0.96344*	*1*	
total anthocyanins	*-0.80285*	*-0.88916*	*0.967349*	*0.911615*	*1*

Correlations significant at p < 0.05.

Table 4. Pearson's correlation coefficients amongst 5 fruit quality parameters in the CPE1 pear treatments

	pH	***TSS***	***TA***	***DPPH***	***total anthocyanins***
pH	1				
TSS	0.962369	1			
TA	-0.81526	-0.82512	1		
DPPH	-0.96932	-0.92029	0.775436	1	
total anthocyanins	-0.97089	-0.96553	0.812208	0.986361	1

Correlations significant at p < 0.05.

Table 5. Pearson's correlation coefficients amongst 5 fruit quality parameters in the CPE2 pear treatments

	pH	***TSS***	***TA***	***DPPH***	***total anthocyanins***
pH	1				
TSS	0.961478	1			
TA	-0.89117	-0.88809	1		
DPPH	-0.9991	-0.95621	0.871994	1	
total anthocyanins	-0.99162	-0.92388	0.840441	0.99498	1

Correlations significant at p < 0.05.

5. Conflicts of interest

The authors declare no conflict of interest.

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