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(RESEARCH ARTICLE)

Extraction and determination of vitamins from pectin obtained from four different citrus fruit peels

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Abstract

Pectin is a polysaccharide with wider applications in foods, pharmaceuticals, and several of other industries. The extraction and determination of vitamins from pectin obtained from citrus fruit peels were studied. The pectin from citrus peels was extracted using ethanol. It was observed that pectin from orange peels gave the highest yield (28.31±2.85%) followed by red grape (14.72±1.83%), lime (12.10±0.97%), and the least in lime (10.15±0.61%).The level of vitamin A (mg/g) was high in pectin from red grape (1483.637±10.420) followed by orange peels (1156.301±8.840), lime peels (475.099±5.510), and the least in white grape (283.228±3.770). There was a significant difference ($p \leq 0.05$) in the level of vitamin A content in all the extracted pectin. The vitamin B_2 (mg/g) content was highest in white grape (0.183±0.050) followed by orange (0.150±0.040), red grape (0.110±0.030), and the least value in lime (0.083±0.020) respectively. There was no significant difference ($p \leq 0.05$) in the level of vitamin B_2 pectin samples. The vitamin B_{12} (mg/g) content was highest in white grape (3.300±1.130) followed by lime (2.931±1.100), orange (1.900±0.930), and the least value with Red grape (0.888±0.870). There was no significant difference in the level of vitamin B_{12} between pectin from lime and white grape. The increasing order of vitamin C (mg/g) content in the extracted pectins was as follows: white grape (11.706±1.150) <orange (16.940±2.100) < lime (17.404±2.130) <red grape pectin (17.678±2.150). There was no significant difference in vitamin C content of extracted pectin for lime, red grape, and orange peels. The level of vitamin E (mg/g) content was highest in orange (0.233±0.480) followed by white grape (0.179±0.710), lime (0.141±0.860), and the least red grape (0.080±0.630). The vitamin E content from pectin had no significant difference ($p \leq 0.05$) in lime and white grape peels. The vitamin K (mg/g) content was highest in orange peel (0.042±0.070) followed by lime (0.035±0.050), white grape peel (0.016±0.030), and the least red grape (0.006±0.010). There was no significant difference ($p \leq 0.05$) in orange and lime in vitamin K.

Keywords: Pectin; Fruit peels; Yield; Vitamins; Citrus

1. Introduction

Pectin, a major component in fruit which is responsible for the formation of a gel after heating and addition of sugars. Pectin is a water-soluble pectinic acids with various methyl ester contents capable of forming gels when exposed to the correct conditions [1]. Due to its outstanding gelling ability, pectin is commonly used as food ingredient. It is made up of α-(1,4) linked D-galacturonic acid units linked in a linear fashion and rhamnogalacturonan, a neutral sugar, which is responsible for splitting and causing kinks in the galacturonic acid chain [1]. Protopectin is a substance found in plant cell walls from which pectin is created [2]. Pectin is present in all plants but the content and composition varies depending on the species, variety, and maturity of the plant, plant part, tissue, and growing condition. The processes used during preparation and subsequent treatments also determine pectin properties [3]. It is a natural prophylactic substance used as detoxification agent. Pectin is a natural additive for foods, therefore it is considered for a number of

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applications beyond the traditional jams and jellies. Pectin is now used as thickeners, water binders, and stabilizers. It is used as a stabilizer in drinkable yogurts and blends of milk and orange juices [4]. Pectin is used as a texturizing fat replacer to mimic the mouth-feel of lipids in low-calorie foods and shorter chain galacturonic acids have been considered as clarification agents in orange juices. It has been investigated for its usefulness in the pharmaceutical industry. Among other uses it has been considered in the class of dietary fibers known to have a positive effect on digestive processes and to help lower cholesterol [5]. Also, it is used to stabilize liquid pharmaceutical emulsions and suspensions [6].

Vitamins are organic nutrients that are essential for life. Our bodies need vitamins to function properly. Most of these vitamins cannot be produced by ourselves, at least not in sufficient quantities to meet our needs [7]. Therefore, they have to be obtained through the food we eat. A variety of foods is therefore vital to meet the body's vitamin and mineral requirements. Of the known vitamins, four types of vitamins that are fat-soluble, which means that fat or oil must be consumed for the vitamins to be absorbed by the body. These fat-soluble vitamins are A, D, E, and K. The others are water-soluble: these are vitamin C and the B-complex, consisting of vitamins B_1, B_2, B_6, B_{12}, niacin, folic acid, biotin, pantothenic acid, and choline. To meet our body's demand for macro elements we need to consume sufficient and varied foods. The trace minerals are so named because they are present in relatively small amounts in the body. If we were to pool the requirements for trace minerals, they would produce only a bit of dust, hardly enough to fill a teaspoon. Yet they are no less important than the macrominerals or any of the other nutrients. The trace mineral contents of foods depend on soil and characteristics of water and the methods used in food processing determines the level of trace mineral in food. The dietary requirement for a micronutrient is defined as an intake level that meets specified criteria for adequacy, thereby reducing the level of nutrient deficit [8].

Citrus is a genus of flowering trees and shrubs that belongs to the rue family (Rutaceae). Plant in the genus includes oranges, lemons, lime, grapefruits, and pomelos. Sweet orange (*Citrus sinensis*) produces is a small evergreen tree 7.5 to 15 m high. Orange trees produces leathery and evergreen leaves of different shapes ranging from elliptical to oblong to oval. The leaves height ranges from 6.5-15 cm long and the width ranges from 2.5-9.5 cm and which often bearing narrow wings on the petioles. The tree bears fragrant white flowers either singly or in whorls of 6, about 5 cm wide, with 5 petals and 20-25 yellow stamens [9]. Orange trees are widely grown in tropical and subtropical climates for their sweet fruit. The fruit of orange tree can be eaten fresh or refined to get their juice or fragrant peel. Orange trees grow moderately at a temperature between 15.5 ^{o}C and 29 ^{o}C (59.9 and 84.2 ^{o}F). It requires considerable amount of sunshine and water [10], [9].

Lime (*Citrus avrantiifolia*) is a citrus fruit which is round and green in colour. It has a diameter of 3 to 6 cm (1.2 to 2.4 inches) and it contains acidic juice vesicles. The tree of lime grows more than 5 m (16ft) high and is shrub-like, its branches spread and they are irregular with short stiff twigs, small leaves, and small sharp thorns. The evergreen leaves are pale green with small white flowers which are usually borne in clusters. The fruit is about 3 to 4 cm (1 to 1.5 inches) in diameter. It has an oval shape and often with a small apical nipple and the peel is thin and greenish yellow when the fruit is ripe. Lime juice is very useful for treating some illnesses and can also be added to herbs [11].

Grape (*Citrus paradisi)* trees mature largely and high ranges from 4.5 to 6 m (15 to 20 ft.). It has dense leafage, glabrous, dark leaves, and smooth green. The flowers come out singly in the axils of the leaves. The fruit ranges from 100 to 150 mm (4 to 5 inches) in diameter, the size of the fruits depends on the specie and growing conditions. Its pulp is light yellowish and tender with very full of juice. The grapefruits are usually consumed as food or juice which have white and red grape varieties [12].

This work is aimed at extracting and determining the yield of pectin from citrus fruits (orange, lime, white grape and red grape) as well as quantifying the vitamins contained in each extracted pectin obtained from the varieties of citrus fruit peels.

2. Material and methods

2.1. Materials

Fresh lime,white grape, and red grape fruits were gotten from Oja Oba, Akure, and orange fruits were gotten from Adeyemi market, Ondo City, Ondo State, Nigeria. All chemicals used for the experiment were of analytical grade gotten from Chelab Nigeria Limited Akure, Ondo State, Nigeria.

2.2. Methods

2.2.1. Material preparation

The fruit samples were rinsed with water. Peels of lime, orange, white grape, and red grape were separated from the fruits and seeds using a sterile knife to prevent contamination. These peels were air-dried for 3 days at 30 °C and 45% relative humidity. The isolated lime, orange, white grape, and red grape peels were separately ground with a Marlexel electrical blender. The powdered samples were separately kept in an air-tight container and stored before the extraction process [6]. Figure 1- 4 shows the peels for each sample.

Figure 1 Red grapefruit peel

Figure 2.2 White grapefruit peel

Figure 3 Lime fruit peel

Figure 4 Orange peel

2.2.2. Extraction of pectin

Extraction of pectin from the citrus samples was performed under acidic condition using the methods of [6] with little modification. 50 g of each of the sample peels of lime, orange, white, and red grape wsa weighed and each weighed sample was placed into 1000 mL beakers. 500 mL of acidified water (0.4% $^{v}/_{v}$ HNO_3) was added to each of the samples and stirred. It was then placed in the water bath at 100 °C for 1 h.The sample mixtures was filtered using a Cheesecloth. Ethanol (98%) was added to the filtrate immediately before cooling and a pectin precipitate was formed. The precipitated pectins were separated by filtration and washed three times with ethanol. The isolated pectins were dissolved in ethanol (98%), re-precipitated (for complete removal of impurities), filtered, washed with distilled water, and oven-dried at 50 °C. The pectins were dried to a constant weight in an oven at 50 °C for about 12 h. The isolated dried pectins were then cooled in a desiccator, broken up, ground, and sieved to a fine powder. The pectin yield was determined using Equation 1. The dried pectin samples were then stored in airtight containers at room temperature. Figures 2.5-2.8 shows the pectin extracted from the fruit peels.

$$\text{Percentage yield of pectin} = \frac{\text{Weight of pectin extracted}}{\text{Weight of samples peeled}} \times 100 \text{ ....1}$$

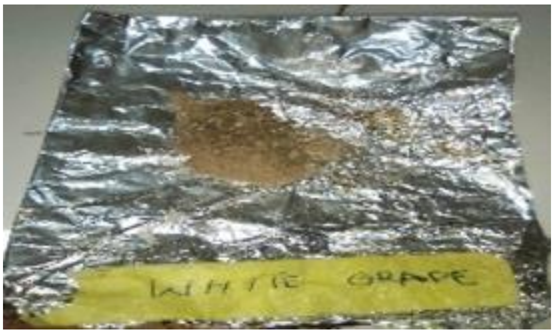

Figure 5 Pectin from whitegrape fruit peels

Figure 6 Pectin from red grapefruit peels

Figure 7 Pectin from orange fruit peels

Figure 8 Pectin from lime fruit peels

2.2.3. Determination of vitamins on the extracted pectins

Determination of vitamin A

1.0 g each of the extracted pectin samples was mixed with 30 mL absolute ethanol, 3 mL of 5% potassium hydroxide, and boiled gently under reflux for 30 minutes in a stream of oxygen-free nitrogen. It was allowed to cool rapidly and 30 mL of water was added and transferred to a centrifuge where it was separated into different layers for 15 minutes. After complete separation, the lower layer was discarded andthe upper layer was washed with 50 mL ether and four times with 50 mL water, during the first two washes, the formation of the emulsion was avoided cautiously. The evaporated sample was washed down to about 5 mL. The remaining ether in the sample was removed in a stream of nitrogen at room temperature. The residue was dissolved in sufficient isopropyl alcohol to give a solution containing 9-15 units per mL and measure the extinctions at 300 nm, 310 nm, 325 nm and 334 nm and the wavelength of maximum absorption 350 nm [13].

Determination of vitamin B_2 (Riboflavin)

5.0 g of the sample was extracted with 100 mL of 50% ethanol and shaken for one hour. This was filtered into a 100 mL flask. 10 mL of the extract was pipetted into a 50 mL volumetric flask. 10mL of 5% potassium permanganate and 10 mL of 30% H_2O_2 was added and allowed to stand over water bath for 30 minutes, 2 mL of 40% sodium sulphate was added. This was made up to 50 mL mark and the absorbance measured at 510 nm using spectrophotometer [13].

Determination of vitamin B_{12}

50 mg of the sample was weighed and 10 mL of the extraction buffer was added with vortex mixing. After standing for 30 minutes, the samples were autoclaved at 121 ^{o}C for 25 minutes at 15 psi and then cooled to ambient temperature in a water bath. Extracts were transferred quantitatively to a 25 mL test tube and diluted to volume with extraction buffer. 50 µL of the reagent and theimmobilized solution was added to 200 µL of the extract, after 1 h 100 µL of HBS-EP buffer and c-Cbl (Casitas B-cell Lympoma) binding-protein, and regeneration solutions were added, thereafter absorbance was read at 480 nm [13].

Determination of vitamin C

The vitamin C content was determined using the ascorbic acid as the reference standard. 200 µL of the extract was pipetted and mixed with 300 µL of 13.3% of trichloroacetic acid (TCA) and 75 µl of 2,4- dinitrophenylhydrazine (DNPH).

The mixture was incubated at 37 °C for 3 h and 500 µL of 65% H_2SO_4 was added and the absorbance was read at 520 nm [14].

Determination of vitamin E

1.0 g of the pectin sample was weighed in a 100 mL flask fitted with a reflux condenser, then 10 mL of absolute alcohol and 20 mL of 1M alcoholic sulphuric acid were added.It was then refluxed for 45 minutes and cool. 50 mL of water was added and transferred to a separating funnel of low actinic glass with further addition of 50 mL of water. The unsaponifiable matter was extracted with 30 mL diethyl ether five times. The combined ether extract was washed (free from acid) and dried over anhydrous sodium sulfate. The extract was evaporated at a low temperature to protect it from being denatured. The residue was dissolved in 10 mL absolute ethanol and both the standard and the sample were transferred to a 20 mL volumetric flask, 5 mL of absolute ethanol was added, followed by 1 mL concentrated nitric acid. The flask was placed in a water bath at 90 °C for 3 minutes and passed under running water to cool. It was then made up to the volume 20 mL with absolute ethanol. The absorbance was read at 470 nm against a blank containing absolute ethanol [13].

Determination of vitamin K

1.0 g pectin sample was placed in a flask and sodium pentacyanoamino ferrate reagent was added. The solution was stirred and then allowed to stand for 15 minutes to allow maximum colour development. When the blue colour was developed, the absorption of the solution was measured by means of a spectrophotometer at 650 nm with standard vitamin K solution. The standard vitamin K solution was prepared by dissolving 5 mg of crystalline vitamin K in water and diluted to 100 milliliters. This solution was stable for 4 to 6 h. The absorption of the solution was read on a spectrophotometer at 650 nm against a reagent blank [15].

3. Results and discussion

3.1. Extraction and percentage yield of pectin obtained from citrus fruit peels.

The percentage yield of extracted pectin from citrus fruit peels is shown in Table 1. The optimum percentage yield of pectin were 12.10±0.97%, 28.31±2.85% 14.72±1.83% and 10.15±0.61% for lime (*C. avrantiifolia*), orange (*C.sinensis*), red grape (*C. paradisi*) and white grape (*C. Paradisi*) respectively. The percentage pectin yield in lime and red grape peel was not significantly different ($p \leq 0.05$) while there was a significant difference ($p \leq 0.05$) in the percentage yield of pectin obtained from peels of orange and white grape. *C. Sinensis* has the highest pectin yield which may be due to the composition of the fruits. Research has shown that citrus fruit has about 60-70% pectin yield [16]. This deviation could have been brought about by factors such as surface contamination, environmental factors, and types of fruits used (the species). Also, this could have been brought about by the amount of alcohol used for precipitation and purification during the experiment [16].It was reported that the yield of papaya peel pectin expressed as the dry weight of extract varied from 2.8% to 16% but when citric acid was used asacidified water, the yield varies from 1.9% - 9.9%. Consequentially, extraction of pectin is influenced by several variables such as extraction time, pH, temperature, solid/solvent ratio [3], irradiation time and irradiation power [17], [18]. Previous studies had shown that pectin yield varies with time and temperature, which were shown from the studies of *T. cacao, C. milleni,* and *I. Gabonensis* that the higher the temperature and time the more the pectin yield generated. From these recent studies the time (1 h) and the temperature (100 °C) were kept constant. At one hour, the yield of pectin was high. If the time of extraction were to be varied from two hours upward, more pectin yield would have been gotten [6].

Table 1 Percentage yield of Extracted Pectin from citrus fruit peels (%)

Pectin Samples	*Percentage (%) yield
Lime	$12.10^{b}\pm 0.97$
Red Grape	$14.72^{b}\pm1.83$
White Grape	$10.15^{c}\pm0.61$
Orange	$28.31^{a}\pm2.85$

* Results values are expressed in mean± standard deviation of triplicate determination., Means that follows the same superscripts in the same column are not significantly different at $p \leq 0.05$.

3.2. Qualitative analysis of extracted pectinsobtained from citrus fruit peels

The qualitative analysis of extracted pectins obtained from citrus fruit peels are presented in Table 2. The extracted pectins from orange peels showed a brown colour while others showed golden yellow. The pectin samples were soluble in ethanol, nitric acid (HNO_3) and hot water but partially soluble in cold water. These observations confirmed the qualitative properties of pectins obtained from plant extracts [6].

Table 2 Qualitative analysis of extracted pectin obtained from citrus fruit peels.

Parameter	Extracted pectin			
	Orange fruit peel	**Lime fruit peel**	**White grape fruit peel**	**Red grapefruit peel**
Colour	Brown	Golden yellow	Golden yellow	Golden yellow
Pectin in cold water	partially soluble	partially soluble	partially soluble	partially soluble
Pectin in nitric acid	Soluble	Soluble	Soluble	Soluble
Pectin in hot water	Soluble	Soluble	Soluble	Soluble
Pectin in ethanol	Soluble	Soluble	Soluble	Soluble

3.3. Vitamin contents in extracted pectins obtained from citrus fruit peels

The vitamin contents in extracted pectins obtained from citrus fruit peelsis depicted in Table 3. It was observed that vitamin A was present in appreciable quantity in all the extracted pectins (lime, orange, red grape and white grape fruit). The vitamin A content (mg/g) in pectin obtained from red grape fruit peel (1483.637 ± 10.420) was higher than the rest, although the least value (283.228 ± 3.770) was obtained in white grape fruits peels. The vitamin A content of pectin gotten from the citrus fruit peels was significantly different at $p \le 0.05$. Each fruit peel contained adequate vitamin A in their pectins which suggested that they could be added to food as supplement to reduce eye deficiency problem.

The level of vitamin B_2 (mg/g) in pectin obtained from white grape, orange, red grape and lime peels were 0.183 ± 0.050, 0.150 ± 0.040, 0.110 ± 0.030 and 0.083 ± 0.02 respectively. The vitamin B_2 content of pectin obtained from white grape, orange and red grape peels have no significant differences at $p \le 0.05$.

The level of vitamin B_{12} (mg/g) was highest in extracted pectin obtained from white grape peels (3.300 ± 1.130) than the others and lowest (0.888 ± 0.870) in red grape fruit peel. The vitamin B_{12} content from lime and white grape peel pectins had no significant difference at $p \le 0.05$. This indicated that all the extracted pectins can be used to perform the purpose of growth and reproduction in any areas of their applications most especially in growing children.

The vitamin C content (mg/g) of pectins obtained from red grape fruit peels (17.678 ± 2.150) was the highest while the lowest (11.706 ± 1.15) was from white grape fruit peel. The vitamin C content in pectins from red grape, lime and orange had no significant difference at $p \le 0.05$. The amount of vitamin C present in the extracted pectins makes it useful in fighting infection in the body and also in enhancing the white blood cell.

The vitamin E content (mg/g) obtained from pectin in the orange peels (0.233 ± 0.480) was the highest and the least value (0.080 ± 0.630) from the red grape fruit peel. The vitamin E content had no significant difference at $p \le 0.05$ in pectin obtained from lime and white grape peels while there was a significant difference at $p \le 0.05$ in pectin obtained from orange and red grape peels. It shows that it can be used to improve/boost the immune system of the body system.

The vitamin K content (mg/g) in the extracted pectin obtained from orange peels (0.042 ± 0.07) was highest and the least value (0.006 ± 0.06) was from red grape peel. The vitamin K content has no significant difference at $p \le 0.05$ in pectin obtained from lime and orange peels while there is no significant difference at $p \le 0.05$ from pectin obtained from white grape and red grape peels. The presence of the appreciable amount of vitamin K in the extracted pectins obtained from the citrus fruit peels will prevent the clotting of blood in both human beings and animals.

Table 3 Vitamin contents in extracted pectins obtained from citrus fruit peels**.**

Samples	*VitaminA (mg/g)	*VitaminB2(mg/g)	*Vitamin B12(mg/g)	*Vitamin C (mg/g)	*Vitamin E (mg/g)	*Vitamin K (mg/g)
Lime	475.099c±5.510	0.083b±0.020	2.931a±1.100	17.404a±2.130	0.141b±0.860	0.035a±0.050
Orange	1156.301b±8.840	0.150a±0.040	1.900b±0.930	16.940a±2.100	0.233a±0.480	0.042a±0.070
White Grape	283.228d±3.770	0.183a±0.050	3.300a±1.130	11.706b±1.150	0.179b±0.710	0.016b±0.030
Red Grape	1483.637a±10.420	0.110a±0.030	0.8882c±0.870	17.678a±2.150	0.080c±0.630	0.006c±0.010

*Result values are expressed in mean± standard deviation of triplicate determination; Means that follows the same superscripts in the same column are not significantly different at p≤ 0.05.

4. Conclusion

This research work has confirmed that pectin extracted from orange peels gave the highest yield when compared with pectin from other citrus peels. Also, it has been observed that Vitamin A, B_2, B_{12}, C, E and K are present in the pectin extracted from the four fruit peels (orange, white grape, red grape and lime) which means that the pectins can be used for various purposes like formulation of eye supplements drugs in adults to help improve vision. These extracted pectins can be used in the fortification of children's foods to boost their immunity (snacks, sweets, beverages, yoghurt and drinks). Due to the appreciable quantity of vitamins present in the citrus fruit peels, it can be used directly in compounding animal feeds instead of throwing them away, thereby turning waste to wealth. The pectin from all the citrus peel samples shows a moderate level of vitamin content which make them potentials in food formulation and supplements in drug production. Finally, the study also provides alternative uses of agricultural waste as a useful industrial products.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declared no conflict of interest.

Author contributions

Oloye, M. T., Arawande, J.O and Borokini, F.B. design the study concept. Preparation of sample materials, data collection, and data analysis were carried out by Lawal, T.P. First draft of manuscript was done by Lawal, T.P. All authors commented on the first version of the manuscript; the revised and final version of the manuscript was prepared by Oloye, M. T, Oderemi, Y. O. and vetted by Arawande J. O. All authors read and approved the final manuscript.

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