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Effect of Ulraviolet Light, pH and Temperature on the Thickening Property of Pectin Extracted from Banana, Orange and Lime Peels

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ABSTRACT: Pectin is a polysaccharide used as rheology modifier in products such as food and beverages. This work assessed the effect of UV light, pH and temperature on the thickening property of pectin extracted from banana, orange and lime peels. Pectin was extracted using alcohol precipitation method and was analyzed using FT – IR spectrometer and GCMS. pH and UV light degradation/depolymerization of pectin solutions were carried out using viscometric and statistical methods. Increase in temperature negatively affects the viscosity of the samples. The viscosity of the banana, orange and lime pectin samples decreased on exposure to UV light for both 30 and 60 days. The observed decrease in the kinematic viscosity of the samples might be attributable to depolymerization which might have occurred with the samples on exposure to the UV light. The viscosity of the samples remained the same at the pH of 4, 7 and 10. Analysis of variance (one way) indicated significant difference in the kinematic viscosity measured to determine the effect of ultraviolet light and temperature ($p < 0.05$). No significant difference in viscosity was observed in the effect of pH ($p > 0.05$). Therefore, exposure to sunlight of pectin containing food drinks can cause decline in the quality of the product.

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Pectin is a family of complex variable polysaccharides extracted from the primary cell wall of higher plants (Canteri-Schemin et al., 2005, Castillo-Israel *et al*., 2015 and Joel et al., 2018**).** It is primarily located in the middle lamella between cells in the tissues of higher plants. Structurally, pectin consists mainly of a chain of 1-4 α- D-galacturonic acid residues with various degree of methyl esterification (Kar and Arslan, 1999). Galacturonic acid makes up 60% of native pectins while commercial pectins contain up to 100% galacturonic acid (Voragen *et al.,* 2009). Pectin is classified commercially according to the degree of esterification (DE) into three: HM (high ester); LMC (low ester conventional) and LMA (low ester amidated). (Canteri-Schemin *et al*., 2005**).** Pectin is used as a thickening agent, gelling agent and a colloidal stabilizer in the food and beverage industry, Their wide use as clouding agents in drinks, in jams and jellies, fruit preparations, fruit drink concentrates, fruit juices, desserts and fermented dairy products to achieve desired firmness or consistency in food and confectionery industries is due to their gelating (Aina, et al., 2012; Bagde, *et al*.,. 2017). Effect of temperature and concentration on the viscosity of orange peel pectin solutions was studied by Kar and Arslan, 1999 and found that viscosity decreases with increase in temperature due to increase in thermal energy and intermolecular distances arising from thermal expansion. Complex heterogeneous polysaccharides that comprises of sodium alginate (a polysaccharide utilized in medicine, pharmacy, basic science and foods) and pectin were reported to be partially depolymerized by a photochemical reaction using ultra-violet light in the presence of titanium oxide catalyst within the period of 6 hours at pH 7, it resulted in a total decrease in molecular weight which in turn reduces thickening property of the pectin, influence of pH on pectin degradation was also studied (Burana-osot, et al., 2009 and 2010). Guimarães et al., 2009 studied the influence of pH, temperature and concentration on the viscosity of pectin were it was reported that there was a direct relation between density of pectin and concentration and an inverse relation with temperature at pH values of 3.0, 4.0, 5.0, and 6.0 and temperature 303.1K, 308.1K, 313.1K and 318.1K respectively. pH is an important gelation factor. Since pectin is an anionic polysaccharide, lowering pH protonates hydroxyl group in pectin, reducing electrostatic repulsion along and between pectin chains, at lower pH non-dissociated carboxylic group form inter and intra hydrogen bonds with secondary alcohol groups (Chan and Choo 2013: Chan et al., 2016). When pH of pectin solution is lowered ionization of the carboxilate group is suppressed and this result in reduction in hydration of the carboxylic acid group, due to the reduced ionization the pectin

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molecule will associate and form gel and this is more prone to low methoxyl pectin, high methoxyl pectin gels at higher pH (Sriamornsak, 2003: Yang et al., 2017).

The objective of this work was to assess the effect of ultraviolet light, pH and temperature on the thickening property of banana, orange and lime peels pectin.

MATERIALS AND METHODS

Sample Collection: The materials used for the extraction of pectin are banana, orange and lime peels, Fresh lime and orange fruits were obtained from 'Yan Lemo Fruits Market, Kano, Nigeria. They were examined to ensure they are in good and sound condition.

Preparation of Sample: Each of the fruits was cut into four parts and the peel (a soft white substance inside the skin of citrus fruits) was removed. The peels were cut into smaller pieces for easy drying, and then weighed with a digital weighing balance and air dried at room temperature. (Aina, et al., 2012; Bagde, et al. 2017). Fresh unripe banana was also obtained from 'Yan Lemo Fruits Market, Kano, Nigeria. The peels were removed, cut into smaller pieces and oven dried at 55^0C for 24 hours. The dried peels were then cooled at ambient temperature and ground into powder using mortar and pestle. The powdered peels were then stored in air tight containers (Castillo-Israel et al., 2015).

Proximate analysis on the peels: Proximate analysis was carried out on the banana, orange and lime peels to determine their protein, carbohydrate, moisture, ash content, crude fat content and crude fibre composition using standard methods (AOAC 2006: Chinyere, et al., 2014). Ash content was determined by measuring the residue remaining after incinerating the sample overnight in a muffle furnace at 600^OC, Protein content was determined by Kjeldahl method, crude fat was determined using gravimetric method after extraction from petroleum ether, crude fibre was determined by boiling the sample in150ml of 1.25% H_2SO_4 for 30 minutes under reflux, total carbohydrate was determined by differences (AOAC 1990: Chinyere, et al., 2014).

Carbohydrate % = 100 – (moisture % + protein % + ash % + lipid % + crude fiber %)

Extraction of pectin from orange and lime peels: 50 g each of dried orange and lime peels was measured using digital weighing balance. Twice of 500 cm^3 of distilled water was also measured and transferred into each of two 1000 cm^3 beakers, 2.5 $cm^3$of concentrated HCl was added to give a pH of 2.2, the measured peels was transferred into the beakers, it was then heated separately for 45 minutes. Thereafter the peels were removed and allowed to cool and then filtered through an ordinary screen with 1 mm mesh size with two layer cheese cloth. The filtrate was collected in each 100 $cm^3$of the filtrate, 100 $cm^3$of 95% ethanol was added with thorough stirring to precipitate the pectin, the precipitate was filtered through miracloth, the gelatinous pectin was air dried and weighed. Percentage yield for each of orange and lime pectin was calculated using the relation below (Aina, *et al*., 2012; Bagde, *et al*.,. 2017).:

$$\% \text{ PY} = \frac{\text{Extracted pectin in gram}}{\text{Weight of samplein gram}} \text{ x } 100$$

Where PY = Pectin Yield

Pectin Extraction from Banana Peels: 100g of banana peel powder was added to 500 cm^3 distilled water which was previously adjusted to a pH of 1.5 with 0.5 N HCl, this was then heated with continuous stirring for 4 hours. The solution was cooled and filtered through an ordinary screen with 1mm mesh size with two layer cheesecloth. The filtrate was collected and then added with twice its volume of absolute ethanol (ratio 1: 2). The precipitate was filtered through miracloth. The pectin yield for banana was calculated using the equation: (Castillo-Israel et al., 2015).

$$\% \text{ PY} = \frac{\text{Extracted pectin in gram}}{\text{Weight of sample in gram}} \text{x } 100$$

*Measurement of Kinematic Viscosity***:** Pectin solution (50 mg/5 cm^3) was prepared in distilled water for each of banana, orange, and lime and commercial pectin. About 5 ml was placed in a screw capped test tube and then kinematic viscosity of the samples was measure using ostward capillary viscometer before 30 days, after 30 and 60 days UV light exposure in a black box equipped with UV light source to determine whether UV light has effect on the kinematic viscosity of pectin solutions.

12 mg/5 cm^3 of banana, orange and lime peels pectin was prepared and the kinematic viscosities of the pectin solutions were measured at 30, 50, 70, 90 and 110^0C respectively. This is to determine whether temperature has some impact on the kinematic viscosity of pectin

Following the method of Burana-osot et al., 2010 the effect of pH on the viscosity of pectin can be determined by measuring an aqueous 1% (w/v) pectin solution for each of banana, orange and lime pectin at pH 4 was made by adjusting the pH with 10 mM hydrochloric acid using mechanical stirrer. Pectins with pH 7 were made by adjusting with 10 mM

ammonium hydroxide. Pectins with pH 10 were made by adjusting with 100 mM ammonium hydroxide. Kinematic viscosities of the pectin solutions above with the pH stated will be measured to ascertain the effect of pH on the thickening property of pectin solutions.

RESULTS AND DISCUSSION

The percentage compositions of moisture, ash, lipid, protein, fibre and carbohydrate are all substantial in the banana, orange and lime peels. Higher percentages of moisture, lipid, fibre and carbohydrate were present in the orange peel, compared of those in the lime and banana peels. Moisture content in the orange peel (15.16±0.16), banana peels (14.64±0.16), and lime peel (13.65±0.36) respectively, these results agrees with the findings of Adewole et al., (2014) and Anhwage et al., (2014).

All the samples of peel had as the carbohydrate as the content with highest percentage and agrees with the findings of Romelle et al., (2016) in the study of the proximate composition of eight different peels (orange, water melon, apple, banana, pomegranate, pineapple, pawpaw and mango). Romelle et al., (2016) showed that the percentage lipid, protein, ash, crude fibre and carbohydrate in the fruit peel were in the ranges: lipid 3.36 ± 0.37 to 12.61 ± 0.63%, protein 2.80 ± 0.17 to 18.96 ± 0.92%, ash 1.39 ± 0.14 to 12.45 ± 0.38%, crude fibre11.81 ± 0.06 to 26.31 ± 0.01% and carbohydrate 32.16 ± 1.22 to 63.80 ± 0.16%. Carbohydrate content in this research was: orange peel (67.80±0.57), banana peel (52.53±0.21), lime peel (61.90±0.13). Lipid content is presented in table 4.1 in orange peel (11.40±0.28), banana peels (10.05±0.21), and lime peels (10.15±0.64). Orange and lime peels (67.80±0.57 and 61.90±0.13) to have higher content of carbohydrate, which indicates that they are good source of pectin carried out in this research. Banana peels (52.53±0.21) has the least carbohydrate content but can substantially yield.

Table 1, Proximate Analysis Results Obtained from Banana, Orange and Lime Peels

S/N	Components	% Moisture	% Ash	% Lipid	% Protein	% Fibre	% CHO
1	Banana peels	14.64±0.16	11.37±0.10	10.05±0.21	11.42±0.06	12.11±0.01	52.53±0.21
2	Orange peels	15.16±0.16	2.78±0.04	11.40±0.28	2.87±0.09	22.78±0.32	67.80±0.57
3	Lime peels	13.65±0.36	7.23±0.04	10.15±0.64	7.09±0.12	18.07±0.10	61.90±0.13

Table 2. Characterization of Pectin Extracted From Banana, Orange and Lime Peel

S/N	Parameters	Banana pectin	Orange pectin (%)	Lime pectin (%)	Commercial citrus pectin (%)
1	Yield (%)	11.21	8.98	3.38	
2	Equivalent Weight (mg/ml)	487.8	431.03	694.40	595
3	Methoxyl Content (%)	14.52	5.10	3.25	5.43
4	Moisture Content (%)	12.15	11.12	10.14	10.03
5	Ash Content (%)	12.10	8.10	10.30	9.1
6	pH	4.60	4.30	3.91	4.10

Table 3. FTIR Characteristic Peaks and Functional Groups

Functional Groups	Banana pectin peaks	Orange pectin peaks	Lime pectin peaks	Commercial pectin peaks
O-H of carboxylic group	3235.3 cm^{-1}	3257.6 cm^{-1}	3276.3 cm^{-1}	3231.6 cm^{-1}
C-H of methylene group	2922.2 cm^{-1} and 2851.0 cm^{-1}	2922.2 cm^{-1} and 2855.1 cm^{-1}	2922.2 cm^{-1} and 2855.1 cm^{-1}	2967 cm^{-1} and 2937.0 cm^{-1}
C=O esterified free carboxyl group	1740.7 cm^{-1} and 1613 cm^{-1}	1740.0 cm^{-1} and 1625.1 cm^{-1}	1736.9 cm^{-1} and 1617.7cm-^{1}	1722.0 cm^{-1} and 1423.1cm^{-1}
C-O of ester group	1013.8 cm^{-1}	1010.1 cm^{-1}	1010.1 cm^{-1}	1010.1 cm^{-}
OH-C of alcohol	1099.6 cm^{-1}	1092.1 cm^{-1}	1073.5 cm^{-1}	1092.1 cm^{-1}
Isopropyl occurring at the pectin molecule	1312.0 cm^{-1} and 1379.1cm^{-1}	1329.7 cm^{-1} and 1367.7 cm^{-1}	1319.5 cm^{-1} and 1367.7 cm^{-1}	1330.7 cm^{-1} and 1371.7 cm^{-1}

Qualitative Test on Pectin Extracted from Banana, Orange and Lime Pectin: Showed that the pectin obtained from orange and lime peels is brown in colour, while banana peels pectin is dark brown in color, the brown colour of the pectin extracted from the three sources corresponds with that of commercial pectin (standard) which is also brown in colour. But the brownish colour of the extracted pectin are slightly darker than that of commercial pectin, methanol is the best solvent that dissolves BP, OP, LP and CP on gentle heating. BP is completely soluble in hot water, OP and LP forms partially soluble suspension but on heating to 100^0C forms yellow precipitate. CP is completely soluble forming clear solution this was consistent with the findings of Aina et al., 2012, Castillo-israel et al., 2015 and Joel et al., 2018. The equivalent weight of banana, orange, lime and commercial pectin in mg/ml was found to be 487.8, 431.03, 694.40 and 595.0 respectively. While the methoxyl content for the three samples and the

commercial pectin (standard) are 14.52, 5.10, 3.25, and 5.43 respectively. The pectin from all the three sources and the commercial pectin are low methoxyl pectin (LM) because all the values obtained experimentally for the degree of methylation are less than 50%. The percentage moisture content obtained from the three samples and the commercial pectin (standard) is 12.15, 11.12, 10.14, and 10.03% respectively. The percentage yield of the three extracted pectin was calculated to be 11.21% (banana pectin), 8.98% (orange pectin), and 3.38% respectively.

FT-IR analysis of the pectin extract: The pectin samples obtained from banana, orange and lime peels was analyzed using FT-IR spectrometer to ascertain the functional group present and compare with commercial pectin as standard. The FT-IR spectra of banana, orange, lime and commercial pectin (standard) corresponds to the characteristics functional groups present in the pectin structure,

Table 5.3 above presents the characteristic peaks of pectin extracted from banana, orange lime and commercial pectin as standard which shows 3235.3 cm-1, 3257.6 cm-1, 3276.3 cm-1 ,3231.6 cm-1, for O-H stretching for carboxylic group. 2922.2 cm-1 and 2851.0 cm-1, 2922.2 cm-1 and 2855.1 cm-1, 2922.2 cm-1 and 2855.1 cm-1, 2967 cm-1 and 2937.0 cm-1 for sp3 C-H stretching vibration. 1740.7 cm-1 and 1613 cm-1, 1740.0 cm-1 and 1625.1 cm-1, 1736.9 cm-1 and 1617.7cm-1, 1722.0 cm-1 and 1423.1cm-1 for C=O esterified free carboxyl group. 1013.8 cm-1, 1010.1 cm-1, 1010.1 cm-1and 1010.1 cm- 1 for C-O for ester group. 1099.6 cm-1 , 1092.1 cm-1, 1073.5 cm-1, and 1092.1 cm-1 for OH-C of alcohol group. And lastly 1312.0 cm-1 and 1379.1cm-1, 1329.7 cm-1 and 1367.7 cm-1, 1319.5 cm-1 and 1367.7 cm-1 and 1330.7 cm-1 and 1371.7 cm-1 for isopropyl occurring at the pectin molecule.

These findings are in consistent with the findings of Kanmani et al., 2014 which extracted pectin from orange peels (citrus limon) and reported that the peaks for the FT-IR result produce 3595.31 cm-1 for O-H stretching for carboxylic group, 2931.80 cm-1 and 2862.30 cm-1 for C-H stretch, 1728.22 cm-1 for unsaturated ester group respectively (Kanmani et al., 2014). The above findings are consistent with the FTIR results obtained on pectin extracted from dried and wet azanza garkeana fruits which produced 3415.19cm-1 and 3418.18 for O-H stretching, 2936.69 cm-1 and 2935.40 cm-1 is for sp3 C-H stretching, 2380.32 cm-1 and 2362.31cm-1 is for C=C stretching, 1533.85 cm-1 and 1532.86 cm-1 is for N-H bending of amine, 1380.87 cm-1 and 1380.66 cm-1 is for sp3 CH2 of methylene bridge, 1059.13 cm-1 and 1058.44 is for C-O stretching. The pair of peaks is for wet and dry azanza garkeana fruit (Joel et al., 2018). Similar analysis was done on pectin extracted from dried eleven samples of apple pomace using HNO3 as precipitating agent (Sato *et al*., 20)

Table 4. Viscometric Measurement for the Effect of Ultraviolet Light

S/N	Sample	Kinematic Viscosity (m^2s^{-1}) at 0 day UV Light exposure	Kinematic Viscosity (m^2s^{-1}) after 30 days exposure to UV Light	Kinematic Viscosity (m^2s^{-1}) after 60 days exposure to UV Light
1	Banana peel pectin 50 mg/5 ml distilled water	0.48	0.23	0.02
2	0range peel pectin 50 mg/5 ml distilled water	1.66	1.40	0.89
3	Lime peel pectin 50 mg/5 ml distilled water	1.63	1.35	1.09
4	Commercial pectin 50 mg/5 ml distilled water	1.23	1.98	1.69

Table 5, Viscometric Measurement for the Effect of Temperature

S/N	Experimental Sample	Kinematic viscosity (m^2s-1) at 30^0C	Kinematic viscosity (m^2s-1) at 50^0C	Kinematic viscosity (m^2s-1) at 70^0C	Kinematic viscosity (m2s-1) at 90^0C	Kinematic viscosity(m^2s-1) at 110^0C
1	Banana peel pectin 12 mg/5 ml distilled water	0.48	0.44	0.38	0.31	0.24
2	Orange peel pectin 12 mg/5 ml distilled water	1.66	1.42	1.17	0.89	0.66
3	Lime peel pectin 12 mg/5 ml distilled water	1.52	1.34	1.14	0.93	0.73
4	Commercial pectin 12 mg/5 ml distilled water	1.19	1.02	0.87	0.69	0.54

Table 6: Viscometric Measurement for the Effect of pH

S/N	Experimental sample	Kinematic viscosity (m^2s^{-1}) at pH 4	Kinematic viscosity (m^2s^{-1}) at pH 7	Kinematic viscosity (m^2s^{-1}) at pH 10
1	Banana peel pectin 12 mg/5 ml distilled water	0.33	0.81	1.30
2	Orange peel pectin 12 mg/5 ml distilled water	0.89	1.74	2.22
3	Lime peel pectin 12 mg/5 ml distilled water	1.00	1.44	1.87
4	Commercial citrus pectin 12 mg/5 ml distilled water	1.23	1.71	2.14

The result in the table above shows a decrease in viscosity of the banana orange and lime pectin samples on exposure to UV light for both 30 and 60 days. A regular decrease of the viscosity was observed on exposure for the 30 and 60 days periods and almost regular. The UV light was observed to have lead to the decrease in the viscosity by a relatively similar amount in the three samples and the reference sample. The decrease in the viscosity of the sample might be due to depolymerization which might have occurred with the samples on exposure to the UV light. A regular decrease in the kinematic viscosity (the measure of the inherent resistance of fluid to flow when no external force was exerted except gravity) with increase in temperature for all the samples (BP, OP, LP and CP) at 30^0C, 50^0C, 70^0C, 90^0C and 110^0C respectively. This result is consistent with findings of Morris et al., 2001 and Roeck et al., 2009. Analysis of variance (one way) was carried out to determine whether there is significant difference between the mean kinematic viscosity measurement for the effect of UV light, temperature and pH respectively, the results indicated that there is significant difference for the kinematic viscosity measured to determine the effect of UV – light ($p = 0.0036$) and temperature ($p = 0.00072$). No significant difference was observed for the effect of pH ($p = 0.231$) at 95% confidence limit.

*Conclusion***:** The viscosity of the banana, orange and lime pectin samples decreased on exposure to UV light for both 30 and 60 days. This can be attributed to depolymerization which might have occurred with the samples on exposure to UV light. Analysis of variance (one way) indicated significant difference in the kinematic viscosity of the pectin as a function of UV – light and temperature ($p < 0.05$). No significant difference was observed due to the effect of pH ($p > 0.05$). Therefore, long-term exposure of foods containing pectin to sunlight and temperature beyond 60 days tend to affect the quality of the food products.

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