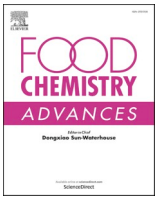
Food Chemistry Advances 3 (2023) 100530

Contents lists available at ScienceDirect

Food Chemistry Advances

journal homepage: www.elsevier.com/locate/focha



Physicochemical and functional properties of pulp and pectin from agro-waste of three Cucurbitaceae species

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ARTICLE INFO

Keywords:
Cucurbitaceae agro-waste
Cucurbitaceae pectin
Antioxidant characterization

ABSTRACT

Cucurbita pepo L., *Citrullus lanatus* T. and *Cucumis melo* L. belong to the family Cucurbitaceae and are cultivated for their seeds. The fruit pulp and peel are discarded as agro-waste after seed removal. This study evaluated the physicochemical and functional properties of the pulp and pectin extracts from the Cucurbitaceae species. Proximate, antioxidant, total polyphenol and flavonoid contents were determined. The purity, structural and sugar constituents of the extracted pectin were analysed using spectrophotometric techniques (NMR, FTIR and LCMS). Results showed that the pulp wastes had high carbohydrate content (28.4 to 69.7%) and demonstrated high antioxidant activities (40 to 50%, 63 to 85% and 0.2 to 0.4 mg mL^{-1} for DPPH, ABTS and FRAP respectively). *Cucurbita pepo* L. had the highest pectin yield (13.9%), and the purity of the pectin extracts ranged from 60.4 to 75.2% for total carbohydrate and 3.3 to 4.4 % for protein. The pectin extracts showed structural similarities. LCMS fingerprints of the pectin extracts showed that the monosaccharides comprised of mannose, rhamnose, galacturonic acid, glucose, galactose, xylose, arabinose and fucose. The polymers demonstrated high water absorption capacities (309 g/100 g to 604 g/100 g) and can be applied as thickeners in food systems.

Introduction

Globally fruits and vegetables account for the highest amount (45%) of food wastes (Dias et al., 2020). The agricultural sector, and fruit/-vegetable processing industries significantly contribute to fruit waste in the form of pulps, peels, seeds and kernels (del Pilar Sanchez-Camargo et al., 2019). Improper management of fruit wastes and increased disposal of these wastes result in environmental pollution (Sulaiman et al., 2022; Wei et al., 2022). *Cucurbita pepo* L., *Citrullus lanatus* T. and *Cucumis melo* L. belong to the family Cucurbitaceae and are cultivated for their seeds which find limited utilization in stew/soups as protein supplements. After seed removal from the fruits, the peel and pulp which become agricultural by-products are either discarded or left on the farm for microbial decomposition. The biomass is known to comprise 90% of the fresh fruit (Košťálová et al., 2013). The discarded fruit pulp however is a potential source of bioactive compounds and functional polysaccharides.

Different members of the Cucurbitaceae family are rich sources of nutrients, antioxidants, polyphenol compounds, carotenoids, peptides, sterols, dietary fiber and minerals (Li et al., 2021). Antioxidants are compounds that help delay macromolecule oxidation and protect the integrity of cellular structures from damage caused by free radicals (Fukumoto & Mazza, 2000). Plant polysaccharide on the other hand comprises of varied sugars and has found useful applications as functional ingredients in both food and pharmaceutical products (Kpodo et al., 2017). The bioactive activities of polysaccharides which include anti-oxidative, anti-diabetic, anti-glycation, anti-tumor, anti-coagulant, anti-proliferative and immunomodulatory properties is of current interest (Kpodo et al., 2018).

Few studies have systematically characterized different members of the Cucurbitaceae family (Denman & Morris, 2015). Furthermore, the changing nature of polysaccharide composition coupled with the need for new sources of functional ingredients had presented a critical need to characterize extracts from new plant sources. Depending on the source of a plant, variety and extraction method used, research had established differences in macromolecular properties which subsequently influence

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https://doi.org/10.1016/j.focha.2023.100530
Received 19 September 2023; Received in revised form 11 November 2023; Accepted 12 November 2023
Available online 13 November 2023

polymer functional characteristics (Kpodo et al., 2020).

In this current study, the agro-waste from three different Cucurbitaceae species were characterized in terms of their proximate, antioxidant, phenolic and polysaccharide properties for potential application in food and non-food formulations. The effective management of Cucurbitaceae agro-waste and its subsequent utilization would reduce environmental pollution and provide new sources of cheaper functional ingredients for the food industry.

Materials and methods

Sample preparation

Agro-waste from the three Cucurbitaceae species (*Citrullus lanatus* T., *Cucumis melo* L. and *Cucurbita pepo* L.) were obtained from a farm in Tsito in the Volta Region of Ghana (Fig. 1). The fruit pulp was cut into pieces and then dried in an air oven at a temperature of 55 °C for 6 h. The dried samples were milled to a particle size of 450 μm using a hammer mill. The milled samples were kept in zip locked bags and stored in the freezer (Protech PRCF-500, China) at −18 °C prior to raw material characterization and pectin extraction.

Chemical characterization of fruit pulps

Proximate analysis

The proximate analysis of each sample was carried out in duplicate using standard methods as outlined by the Association of Official Analytical Chemists (AOAC, 2005). Eq. (1) was used to calculate the proportion of carbohydrates:

$$\%\text{Carbohydrate} = 100 - (\%\,\text{moisture} + \%\,\text{protein} + \%\,\text{fat} + \%\,\text{ash} + \%\,\text{fiber}). \quad (1)$$

Extraction of samples for antioxidants and phenolic analysis

The milled samples were weighed (15 g each; oven dried), transferred into a conical flask and 75 mL of methanol was added (Darko et al., 2022). The samples were covered with aluminum foil to prevent evaporation, and allowed to stand for 24 h at 25 °C away from light. After 24 h, the samples were filtered using a filter paper. The filtrates were transferred into pre-weighed petri dishes and evaporated at 45 °C using an oven (Binder Heating and Drying Oven, Tuttlingen, Germany) to remove the methanol. The extracts obtained after drying were used for total phenol and antioxidant determinations (Obeng et al., 2020).

Antioxidant activity

1, 1-diphenyl–2 picrylhydrazyl (DPPH) radical scavenging activity assay. The extract (20 μL of 2 $mgmL^{-1}$) and 980 μL of 0.1 mM DPPH solution were mixed. The mixture was incubated at 4 °C for 30 min and absorbance measured at 516 nm (Osei et al., 2023). The decrease in absorbance was proportional to antioxidant activity (AA) of extracts, calculated according to Eq. (2).

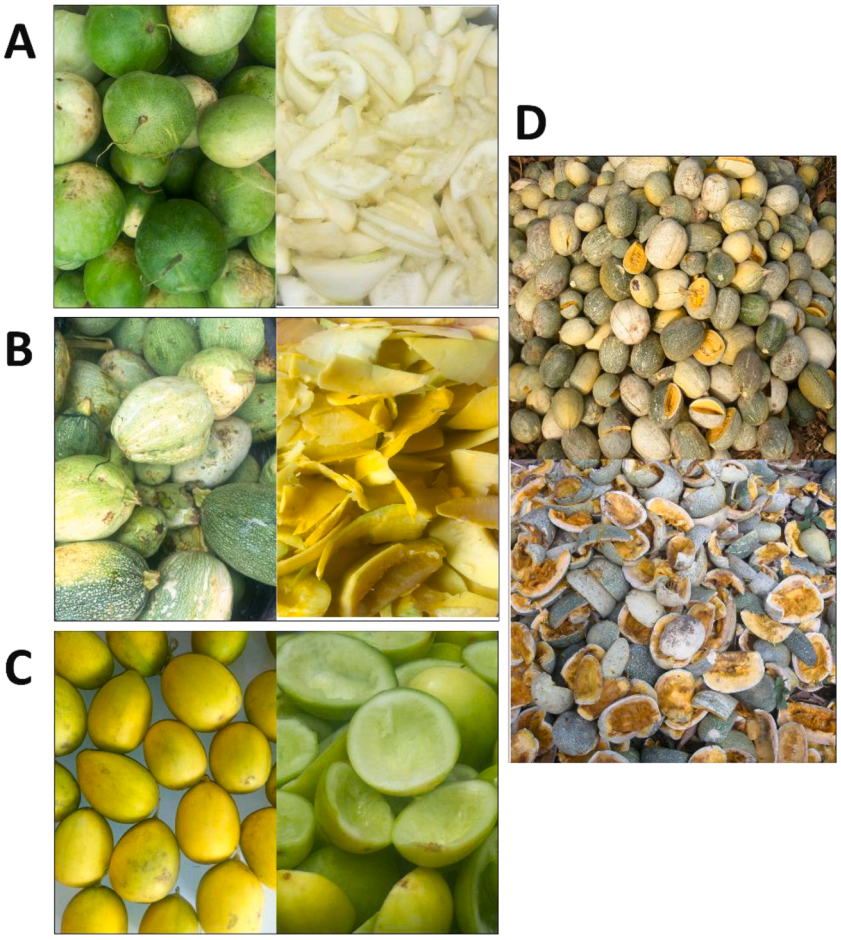

Fig. 1. Raw materials (A) *Citrullus lanatus* T. fruit and pulp, (B) *Cucurbita pepo* L. fruit and pulp, (C) *Cucumis melo* L. fruit and pulp, (D) Agro-waste generated from *Cucurbita pepo* L. after seed removal.

$$\%\ Antioxidant\ activity = \frac{absorbance\ of\ control - absorbance\ of\ sample}{absorbance\ of\ control} \times 100 \quad (2)$$

2 2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic) (ABTS) acid scavenging activity. According to methods described previously (Darko et al., 2022) with some modification, ABTS stock solution (7 mM) and potassium persulfate (2.4 mM) were mixed according to 1:1 ratio to generate ABTS free radicals after 16 h incubation in the dark. A working solution of 0.1 mM ABTS solution was obtained from the ABTS radical solution. Vitamin C (50 μL) and 50 μL of extracts each were added to 150 μL of ABTS in a 96 well microtiter plate. Samples were incubated at room temperature for 30 min. The absorbance of the samples were read at 734 nm. The radical scavenging activity was calculated as percentage of the control sample (Eq. (3)):

$$\%\ Antioxidant\ activity = \frac{absorbance\ of\ control - absorbance\ of\ sample}{absorbance\ of\ control} \times 100 \quad (3)$$

Ferric reducing antioxidant potential (FRAP) analysis. FRAP reagent was prepared by mixing 30 mL of acetate buffer (pH 3.68), 3 mL of 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 3 mL of 20 mM ferric chloride and 40 mM of HCl (Osei et al., 2023). The freshly prepared solution (0.3 mL) was placed in a test tube and 30 μL of each sample was added. The resulting mixture was vortexed and incubated for 30 min at 37 °C and absorbance determined at 593 nm. The value for FRAP was calculated by Eq. (4):

$$\text{FRAP value}\ \left(\text{mg mL}^{-1}\right) = \frac{absorbance\ of\ sample}{absorbance\ of\ vitamin\ C} \quad (4)$$

Determination of total polyphenolic content

Total polyphenol content was estimated using the Folin Ciocalteu method (Osei et al., 2023; Darko et al., 2021). Folin reagent (10%) and sodium carbonate (20%) were freshly prepared. Samples (100 μL) were placed in test tubes and 500 μL of distilled water was added, followed with 100 μL of Folin – Ciocalteu's reagent. The mixture was vortexed and incubated for 6 min. Sodium carbonate (20%) and additional 500 μL of distilled water was added, and the resulting solution was incubated for 90 min at 25 °C. The mixture (200 μL) was placed in a 96 wells microtiter plate and absorbance (750 nm) read spectrophotometrically. Total polyphenol content of each sample was determined in Gallic Acid Equivalent (GAE) mgg^{-1} on dry weight basis.

Total flavonoids content

Total flavonoid was determined using 10% aluminum chloride, 5% sodium nitrite and 1 M of sodium hydroxide. 100 μL of the sample, vitamin C (standard) and standard catechin (0.0625, 0.25, 0.5, 1 and 2 μg/mL) was placed in a 96 wells microplate, 100 μL of distilled water and 30 μL of 5% sodium nitrite was added. After 5 min, 30 μL of aluminum chloride was added. After 6 min, 100 μL of sodium hydroxide was added to the reaction mixture. Orange yellowish color was observed. The absorbance was determined at 510 nm.

Pectin extraction from Cucurbitaceae pulp

Alcohol insoluble residue (A.I.R.) was prepared according to the method as described previously (Nyarko et al., 2021). The milled dried Cucurbitaceae pulp samples were treated with 95% ethanol (1:2 w/v), followed by acetone (1:2 w/v) for 48 h. The samples were then decanted and the insoluble fraction was oven dried at 35 °C for 48 h. The dried A.I.R. was kept in zip locked bags and stored in a dry keeper. The pectin extraction was done as described by Denman and Morris (2015) with slight modification. The alcohol insoluble residue was weighed and dissolved in HCl (1.5 g: 45 mL; pH 1). The mixture was then heated on a hotplate at 80 °C with magnetic stirring continuously for 4 h. The solution was then cooled and the pH was adjusted to 4.5 with 2 M NaOH solution. The solution was then centrifuge at 2500 rpm for 15 min. Equal volume of ethanol (95% v/v) was added to the supernatant to precipitate the pectin out of solution. The pectin extract was washed with 95% ethanol and freeze dried (at −47 °C to −55 °C, 0.002 to 2.7 Torr) for 72 h using the vacuum freeze dryer (model: YK-118–50, Taiwan) for further analysis.

Physicochemical analysis

Pectin yield and purity

The percentage pectin yield was calculated based on the amount of the milled dried Cucurbitaceae pulp sample used for the extraction process (Eq. (5)).

$$\%\ \text{Pectin yield} = \frac{\text{Weight of freeze} - \text{dried pectin extract}}{\text{Weight of dried Cucurbitaceae pulp powder}} \times 100 \quad (5)$$

Total carbohydrate was estimated using the phenol-sulphuric acid assay (DuBois et al., 1956). The Bradford protein assay was used in the determination of the protein content of the pectin (Bradford, 1976).

Liquid chromatography–mass spectrometry (LCMS)

LC-MS analysis was carried out using a UHPLC Dionex Ultimate 3000 RS LC System with a C18 (2.1 × 100 mm, 2.2 μm) column using 2 μL injection volume, 0.5 mL/min flow rate and a gradient binary mobile phase of 0.1 % formic acid in water (A) and 0.1% formic acid in acetonitrile containing (B): t_{0min} 2 % B, t_{2min} 8 % B, t_{11min} 18 % B, t_{15min} 35 % B, t_{20}–22 min 100 % B, t_{23}–25 min 2 % B

The samples were first dissolved in 20 % acetonitrile on ultrasonic bath for 5 min and centrifuged at 6020 × *g* for 5 min. Detection was on a Dionex Ultimate DAD-3000 RS (λ 200 - 400 nm) and Bruker Daltonics micrOTOF-QII time-of-flight mass spectrometer (Bruker, Germany) using Apollo electrospray ionization source (+ mode) at 5 Hz. *m/z* was acquired with 5 bar nitrogen nebulizer gas in a range of 50–2000 at 9 L/min, at 220 °C: capillary voltage of 4500 V. Each analysis was done using an internal dataset calibrated in an HPC mode of the 10 mM sodium fumarate in 50% isopropanol infused during LC re-equilibration via divert valve with 20 μL sample loop.

Fourier-transform infrared (FTIR) and nmr spectroscopy

Fourier transform infra-red (FTIR) spectra were obtained between 400 and 4000 cm^{-1} for all pectin samples in Attenuated Total Reflection (ATR) mode at a resolution of 4 cm^{-1} using 128 scans (Nicolet 380, Thermo Scientific, UK). Spectral smoothing was applied using instrument software (OMNIC 3.1) (Kpodo et al., 2017). For all the samples ^{1}H NMR was conducted at 70 °C with D_2O as the solvent using the Bruker Avance (500 MHz) and 125.76 MHz for ^{13}C. The samples (2 mg) were dispersed in 2 mL deuterium oxide (D_2O) and then freeze dried. The freeze-dried samples were subsequently again dissolved in 600 μL D_2O prior to performing the NMR spectroscopy.^{1}H NMR spectra were recorded with 64 scans at the same temperature (Kpodo et al., 2019).

Pectin functional properties

The water absorption, oil absorption and emulsion capacities of the different Cucubitaceae pectin were determined as described by Nyarko et al. (2021).

Statistical analysis

All experimental results were obtained from duplicate separate experiments and presented as means ± standard deviations. Statgraphics (Graphics Software System, STCC, Inc. USA) was used to analyze the

data. Comparisons between the different samples were done using one-way analysis of variance (ANOVA) and differences between means were determined with LSD. A probability value of $p \leq 0.05$ was considered to be statistically significant for the tests carried out.

Results and discussion

Physicochemical properties of Cucurbitaceae pulps

The chemical compositions of the different Cucurbitaceae pulps were assessed in this study prior to pectin isolation to provide useful information on the chemical nature of the raw materials. The proximate composition of the pulp of *Cucurbita pepo* L., *Cucumis melo* L. and *Citrullus lanatus* T. is presented in Table 1. The carbohydrate content was significantly higher ($p<0.05$) in *Cucurbita pepo* L. (69.7%) compared to *Cucumis melo* L. (28.4%) and *Citrullus lanatus* T. (42.8%). Carbohydrates values obtained, were lower than that reported for the flesh of *Cucurbita maxima* (74.01%) (Jahan et al., 2023). Crude protein content were relatively lower (6.9 – 8.2%) and not significantly different ($p>0.05$) in the fruit pulps studied. Hence, the agro-wastes from the different Cucurbitaceae pulp samples can be preferentially utilized for the extraction of complex carbohydrates, whereas seeds of Cucurbitaceae are good sources of proteins. *Cucumis melo* L. demonstrated a significantly higher ($p<0.05$) moisture, ash, crude fat and fiber content than *Cucurbita pepo* L. and *Citrullus lanatus* T. The high ash and fiber content of the fruit pulp from *Cucumis melo* L. will be advantageous when processed into fruit flours and utilized in diet formulations to increase the mineral and fiber content. This will ultimately improve the total nutritional value of potential substituted food products. Foods with high dietary fiber decrease the incidence of constipation, type II diabetes, obesity and colon cancer.

ABTS, DPPH, and FRAP tests were used to profile the antioxidant potentials of the different Cucurbitaceae pulps (Table 1). *Cucumis melo* L. pulp showed the highest antioxidant activity (DPPH and ABTS assays). Results obtained for the ABTS assay (63–85%) were significantly different ($p<0.05$) and also relatively higher than the reported value for *Citrullus lanatus* (41.5%) (Neglo et al., 2021), but lower than findings on watermelon fruit peel extracts (91%) (Neglo et al., 2021). Fruits exhibit heterogeneity in antioxidant potential and TPC composition depending on factors such as fruit type, source, variety, fruit part, stage of maturity and storage. Different fruits show differences in their ability to scavenge free radicals and this is attributable to variations in the composition of antioxidant compounds, and the interaction of the antioxidant constituents with other fruit components. Hence, the different antioxidant compounds present in each fruit studied influenced the levels of antioxidant activity of the extracts. The ferric-reducing ability of *Cucurbita pepo* L. and *Citrullus lanatus* T. did not differ significantly ($p>0.05$) as compared to *Cucumis melo* L. which recorded the highest value. The FRAP values of the Cucurbitaceae pulps ranged from 0.2 to 0.4 mg mL^{-1} and were lower than that reported for undefatted white melon seed flour (1.0 mg mL^{-1}) and defatted white melon seed flour (1.5 mg mL^{-1}) (Ijarotimi et al., 2022). Studies have associated plant polyphenolic content with antioxidant activity, most likely due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Chang et al., 2001). The total polyphenolic content of the Cucurbitaceae extracts varied from 6.6 to 15.5 mg GAE/100 g (Table 1). The total polyphenolic content of the different extracts were significantly different ($p<0.05$), and *Cucumis melo* L. demonstrated higher total phenolic composition than the other pulp samples studied. This possibly explains the high antioxidant potential of the *Cucumis melo* L. pulp sample relative to extracts from *Cucurbita pepo* L. and *Citrullus lanatus* T. However, on the whole values obtained are generally lower than reported values for muskmelon (16.7 mg GAE/100 g) and watermelon (29.3 mg GAE/100 g) (Shofian et al., 2011). Flavonoids are also known to possess biological activities such as anti-carcinogen, anti-inflammatory and antioxidant activities (Maseko et al., 2019; Tapas et al., 2008). The flavonoid content of *Cucurbita pepo* L. was comparable ($p>0.05$) to that of *Cucumis melo* L. (Table 1). Results obtained were lower than that obtained for the pulp of *C. moschata* (0.3 mg CE/mg) (Enneb et al., 2020). Processing of the different Cucurbitaceae fruits for their seeds generate a large amount of agriculture pulp waste with significant amounts of functional ingredients (carbohydrates, fiber, antioxidant and phenolic compounds) that can be recovered and used in food, cosmetic and pharmaceutical products.

Table 1
Physicochemical properties of Cucurbitaceae pulp and pectin.

Physicochemical Properties	*Cucurbita pepo* L.	*Cucumis melo* L.	*Citrullus lanatus* T.
Moisture (%)	5.4 ± 0.1^{a}	15.4 ± 0.4^{b}	15.0 ± 0.5^{b}
Carbohydrate (%)	69.7 ± 0.2^{c}	28.4 ± 0.3^{a}	42.8 ± 0.1^{b}
Crude fiber (%)	5.0 ± 0.1^{a}	24.7 ± 0.3^{c}	12.8 ± 0.2^{b}
Protein (%)	8.2 ± 0.6^{a}	6.9 ± 0.3^{a}	7.9 ± 0.5^{a}
Total Ash (%)	5.6 ± 0.4^{a}	8.6 ± 0.1^{c}	6.7 ± 0.2^{b}
Crude fat (%)	6.2 ± 1.0^{a}	15.9 ± 0.7^{b}	14.6 ± 0.1^{b}
DPPH (%)	40.0 ± 0.3^{a}	50.0 ± 0.2^{c}	43.0 ± 0.2^{b}
ABTS (%)	63.0 ± 0.2^{a}	85.0 ± 0.3^{c}	75.0 ± 0.1^{b}
FRAP (mg mL^{-1})	0.2 ± 0.0^{a}	0.4 ± 0.0^{b}	0.2 ± 0.0^{a}
Total polyphenols (mg GAE/ 100 g)	8.7 ± 0.1^{b}	15.5 ± 0.1^{c}	6.6 ± 0.1^{a}
Flavonoids (mg CE/mg)	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	ND
Pectin yield (%)	13.9 ± 1.3^{c}	5.2 ± 0.6^{a}	7.9 ± 0.2^{b}
Pectin protein (%)	3.3 ± 0.3^{a}	4.4 ± 0.5^{c}	3.9 ± 0.5^{b}
Pectin total carbohydrate (%)	62.9 ± 0.3^{b}	75.2 ± 3.3^{c}	60.4 ± 0.1^{a}

Values are means ± standard deviations of duplicate determinations. Values in the same row with the same superscript letters are not significantly different ($p>0.05$). ND-Not determined.

Pectin yield, protein and carbohydrate content

The yields of pectin obtained from the various pulp samples are presented in Table 1. Percentage pectin yield was significantly different ($p<0.05$) and ranged from 5.2 to 13.9% with *Cucurbita pepo* L. recording the highest yield. The yields obtained were lower than those for pectic polysaccharides from pumpkin (1.3–5%) (Zhao et al., 2017) and kiwifruit (1.4–4.4%) (Yuliarti et al., 2015). The protein and carbohydrate content of the samples were significantly dependent on the Cucurbitaceae species used. The pectin protein content varied between 3.3 and 4.4% whereas carbohydrate content varied between 60.4 and 75.2%. *Cucumis melo* L. recorded the highest carbohydrate and protein content. The RG-1 region of pectin has side chains which contain hydrophobic protein moieties contributing to its complex structure and influencing the unique emulsification functionality of pectin. Higher protein content (7.1–18.4 %) have been previously reported for pumpkin samples (Bai et al., 2020), however analysis of muskmelon pectin revealed comparable amount of protein (4.05%) (Nyarko et al., 2021). Differences in yield, protein and carbohydrate content of samples can be attributed to Cucurbitaceae specie uniqueness.

Fourier-transform infrared spectroscopy (FT-IR) analysis

The typical FTIR spectra of pectin from the various Cucurbitaceae species are depicted in Fig. 2. Similar absorption bands were detected for all the pectin from the different Cucurbitaceae species. The broad peak within the range of 3500 to 3000 cm^{-1} corresponds to the O—H stretching vibration band. This band occurrence is attributable to inter- and intra- molecular hydrogen bonding of the d-GalA backbone (Alba et al., 2015). The occurrence of this peak agreed with previous reports of extracted polysaccharides (Sims et al., 2018). The peak observed at 2914 cm^{-1} was recognized as a C—H bend in CH_2 groups (Wu et al., 2021). The primary peaks around 1727 cm^{-1} and 1603 cm^{-1} respectively corresponds to the C = O of methyl esterified groups and COOH, and the stretching vibration of the carboxylate anion (COO^{-}) (Pan et al., 2022).

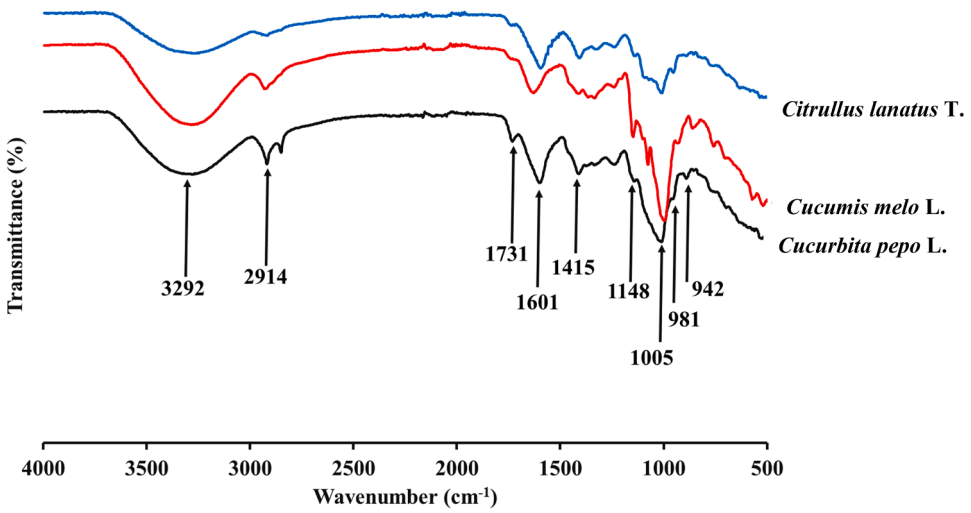

Fig. 2. FTIR of Cucurbitaceae pectin.

These two critical peak areas are used in the determination of degree of esterification values. The characteristic carbohydrate finger print region was also observed between 1200 cm^{-1} and 900 cm^{-1}. The peak at 1415 cm^{-1} which was clearly distinct in *Cucurbita pepo* L. and *Citrullus lanatus* T. pectin samples have been attributed to the C—H bending vibration. The peaks between 1148 cm^{-1} and 1005 cm^{-1} caused by C—OH, C—O-C and C—C stretching vibrations is indicative of the presence of a pyranose in the pectin structures (Li et al., 2021), and had also been previously reported to occur between 1152 cm^{-1} and 1020 cm^{-1} in pumpkin pectin (Pan et al., 2022). The absorption bands at 981 cm^{-1} and 942 cm^{-1} signify the presence of d-glucopyranosyl groups and α-linkage between GalA units respectively (Pan et al., 2022).

NMR spectroscopy

A comparison of the ^{1}H NMR spectra of the pectin extracts from the three different Cucurbitaceae species (Figs. 3A-C) showed similar structural similarity although *Cucumis melo* L. demonstrated sample specific chemical shift uniqueness. Proton signals in the low field region around 5.00 ppm was attributed to protons originating from anomeric sugars. All the pectin extracts showed signals in the region between 4.01–4.18 ppm ascribed to the presence of methyl groups connecting to the carboxyl group of d-GalA (Kpodo et al., 2019). Signals between 2.07–2.42 ppm for pectin samples from *Cucurbita pepo* L. and *Citrullus lanatus* T., and 2.58–2.70 ppm for *Cucumis melo* L. were assigned to

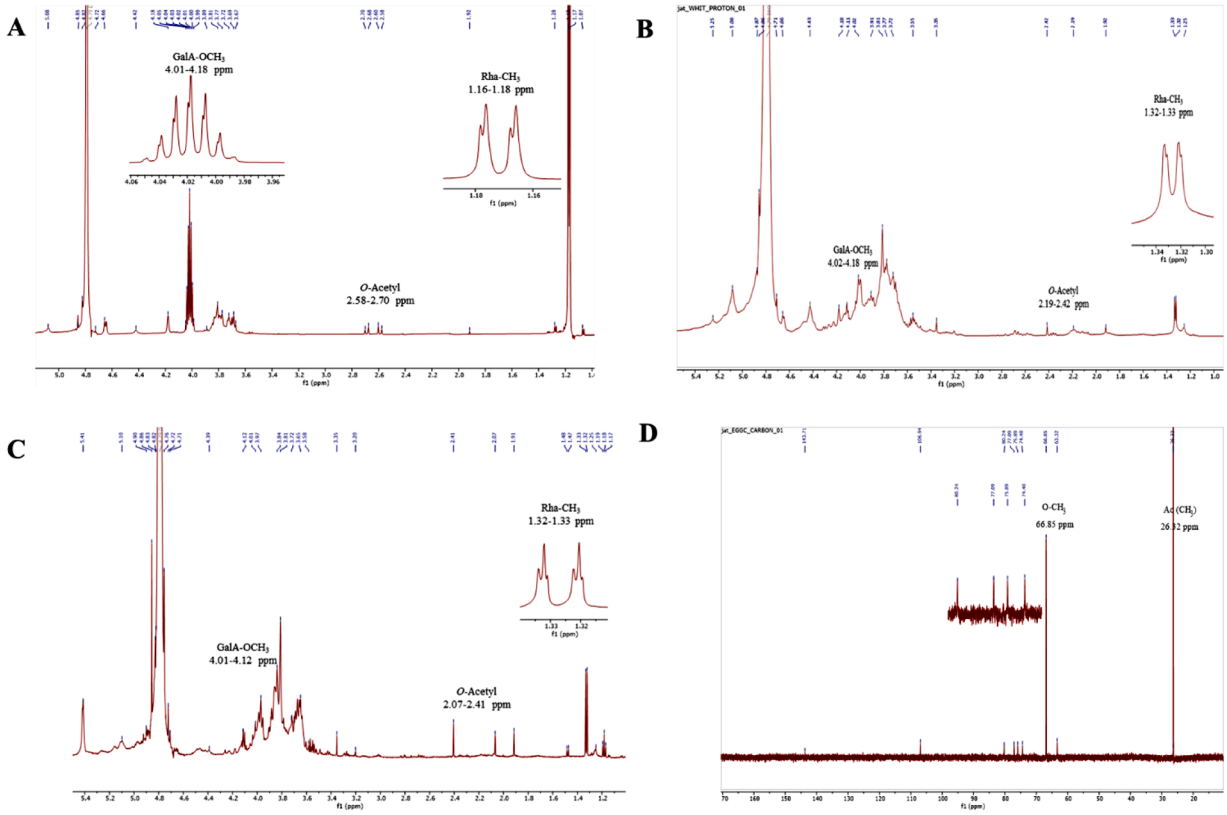

Fig. 3. ^{1}H NMR spectra of pectin from *Cucumis melo* L. (A), *Citrullus lanatus* T. (B), *Cucurbita pepo* L. (C), ^{13}C NMR spectra of *Cucumis melo* L. (D).

o-acetyl groups (Alba et al., 2015). The methyl groups of unbranched α (1→2) – linked and branched α (1→2) and α (1→4) linked rhamnose were observed between 1.32–1.33 ppm for *Cucurbita pepo* L. and *Citrullus lanatus* T., and between 1.16–1.18 ppm for *Cucumis melo* L.

The ^{13}C NMR (Fig. 3D) showed a signal at 26.32 ppm which was attributed to the O-methyl as an acetyl group (Xia et al., 2020). The chemical shift at 66.85 ppm confirmed the presence of a methyl group esterified to the carboxyl group of GalA. Xia et al. (2020) observed this signal in *Acanthopanax senticosus* leaves at 52.9 ppm. The chemical shifts at 80.2, 77.1, 75.9, and 74.4 ppm are assigned to the C1-C4 of (1→2)-α-Rhap respectively (Xia et al., 2020). In addition the downfield carbon signal at 106.9 ppm was ascribed to the C1 in α−1,5-linked Araf (Xia et al., 2020).

LC-MS characterization

UHPLC-MS is popular method for determining the monosaccharide composition of glycan polymers because it potentially determine acidic, basic and neutral sugars (Liu et al., 2020). In this study the monosaccharide constituents in three Cucurbitaceae species were determined using UHPLC-MS. The chromatograms of the pectin samples are shown in Fig. 4. The monosaccharide composition analysis demonstrated that the samples contained mannose, rhamnose, galacturonic acid, glucose, galactose, xylose, arabinose and fucose, hence the polymers were heteropolysaccharides. Pectins are complex polysaccharides with three main constituents which include homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II) (Bai et al., 2020; Ghori et al., 2014). Homogalacturonan is a homopolymer composed of α(1→4) linked α-d-galacturonic acid (GalA) residues containing methyl esterified carboxyl groups and partially acetylated hydroxyl groups (Ghori et al., 2014; Maxwell et al., 2012). The occurrence of galactose demonstrates the presence of side chains of the RG-I fraction. Bai et al. (2020) previously reported that galactose combines with arabinose (arabinogalactan) in various ways to form the side chains of the RG-I component. The RG-I are highly branched structures with neutral sugars (mainly α-l-arabinose and β-d-galactose) side chains (arabinans, galactans and arabinogalactans) attached to the rhamnose residue (Maxwell et al., 2012). The branches are repetition of α(1→4) linked α-d-galacturonic acid and of α(1→2) linked α-l-rhamnose units (Ghori et al., 2014; Vincken et al., 2003). The de-oxy hexose sugar fucose was also detected in all three species demonstrating the possible presence of RG-II fraction in the pectin polymers. Fucose has also been previously reported in three pectin fractions from *Cucumis melo* L. (Pan et al., 2022).

The RG-II segment comprises of a diverse group of monomer sugar units, hence its structure is complex (Ghori et al., 2014). Pectin extracts can also contain xylogalacturonan (XG) and rhamnogalacturonan (RGH). The Cucurbitaceae samples studied also showed the presence of a xylose peak indicative of the possible presence of xylogalacturonan unit in the pectin structure.

Water absorption capacity (WAC), oil absorption capacity (OAC) and emulsion capacity

The physical and chemical properties of pectin extracts influence

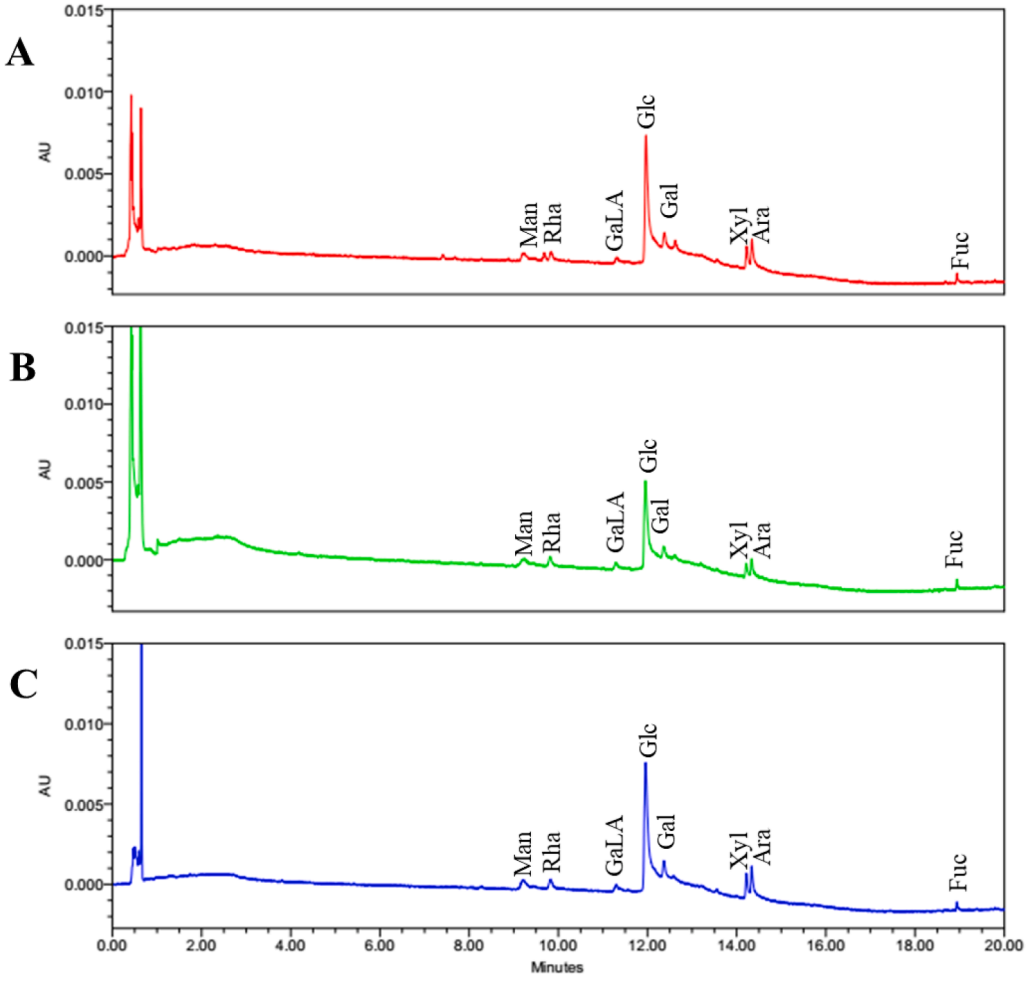

Fig. 4. UHPLC-MS base peak chromatograms of three Cucurbitaceae pectin extracts. Monosaccharide composition of *Cucumis melo* L. (A), *Citrullus lanatus* T. (B) and *Cucurbita pepo* L. (C).

their behaviors in food systems. Polysaccharides because of their hydrophilic character are useful as water binders in food systems. Pectin is used in food and non-food systems mainly due to its gelling and thickening abilities. It is applied as a gelling agent in jam and jellies, confectionary products and bakery filling. Aside these food applications, pectin extracts can also be used as stabilizers and thickeners in acidified milk and yoghurt products. The WAC of the pectin extracts studied ranged from 309 g/100 g to 604 g/100 g (Fig. 5), and were significantly different ($p<0.05$). *Citrullus lanatus* T. demonstrated the highest water absorption capacity (WAC) whereas *Cucurbita pepo* L. had the least WAC. The high WAC reported for the pectin extracts in this study can be attributed to widespread distribution of hydroxyl groups which suitably adapt the polymer to specific functionality as thickening agents in a water-based food environment. Generally values recorded for WAC in this study were higher than reported values for oven dried and solar dried musk melon samples (208 g/100 g - 269 g/100 g) (Nyarko et al., 2021) and lower than values for okra pectin (800–963 g/100 g (Kpodo, 2018). Oil absorption capacity ranged from 128 to 135 g/100 g (Fig. 5) and were not significantly different ($p>0.05$). The values recorded were lower than that reported for muskmelon pectin (237 and 152 g/100 g) (Nyarko et al., 2021). The OAC of the Cucurbitaceae pectins were appreciably high, but considerably and comparatively lower than their corresponding WAC. This observation was due to the fact that traditionally pectin is considered as an effective water binder due to their high hydrophilicity. The presence of hydrophilic groups and galacturonic acid residues in the pectin contributed to their high water absorption and binding properties in solution. The emulsion capacities recorded in this study ranged from 43.5 to 48.0% with no significant differences ($p>0.05$). The hydrophobicity of the pectin extracts can be attributed to the presence of protein moieties in the side chains (Kpodo et al., 2018). Also, the presence of acetyl groups in the pectin extracts increased the polymer emulsifying potential. When pectin is used as an emulsifier, as observed in the case of okra and sugar beet pectin, acetyl groups adsorb at surfaces and increase emulsifying activity (Alba et al., 2015; Kpodo et al., 2018). Additionally pectin incorporation increases the viscosity of the continuous aqueous phase of emulsions and prevent oil droplet coalescence.

Conclusion

The agro-waste from the different Cucurbitaceae species exhibited antioxidant potentials with the pulp wastes particularly from *Cucumis melo* L. showing the highest antioxidant activity and TPC content as compared to *Cucurbita pepo* L. and *Citrullus lanatus* T. Pectin yield increased in the order *Cucumis melo* L. < *Citrullus lanatus* T. < *Cucurbita pepo* L. Cucurbitaceae fruit wastes are thus good sources of natural antioxidants and pectin. Although the pectin extracts from the fruit wastes showed similar macromolecular properties, they all demonstrated high water absorption capacities and can be employed as functional thickening agents in food systems.

Ethical statement

The authors declare that no experiments were performed on animals or humans.

CRediT authorship contribution statement

Fidelis M. Kpodo: Conceptualization, Funding acquisition, Project administration, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Jonathan Jato:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. **Clementina Naa Adjeley Adjei:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Azi Walter:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Jacob K. Agbenorhevi:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing. **Joyce Duah:** Methodology, Resources, Supervision, Writing – review & editing. **Peter Nuro-Ameyaw:** Conceptualization, Resources, Supervision, Writing – review & editing.

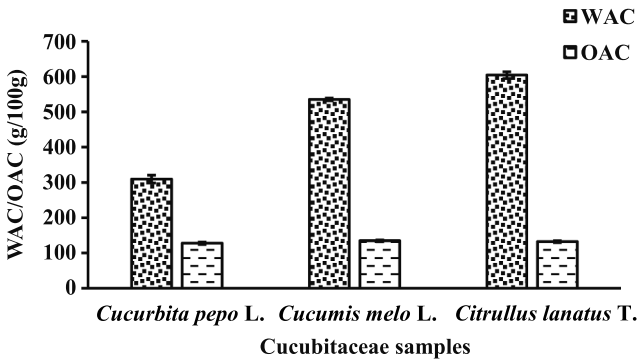

Fig. 5. Water absorption capacity (WAC) and oil absorption capacity (OAC) of pectin.

Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare no conflicts of interests with respect to the research, authorship, and/or publication.

Data availability

Data will be made available on request.

Acknowledgement

The authors gratefully acknowledge the financial support from the UHAS Research Fund.

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