

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/



Nutritional Value, Antioxidant and Anticancer Activities of Some Nano Fruit Wastes

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Abstract

Food production, processing and consumption generate enormous volumes of wastes - byproducts that cause environmental and economical negative effects. These wastes are considered an inexpensive source of bioactive components that can be used as functional ingredients in the food and dairy sectors. This study aimed to evaluate the nutritional value, antioxidant and anticancer activities of apricot (*Prunus* sp.) and peach (*Prunus persica* L. *Batsch*) kernels (AK & PK), watermelon (*Citrullus lanatus*) rind (WMR) and banana (*Musa* spp.) peel (BP) in native and nano-powder form. The results revealed that AK and PK are rich sources of fat and protein while WMR and BP had the highest carbohydrate, ash and dietary fiber content. All tested wastes had variable concentrations of major and minor elements. AK and PK had significant amounts of oleic and linoleic acids, while WMR and BP had significant amounts of palmitic, stearic, linoleic, and linolenic acids. All samples contained high concentrations of aspartic and glutamic acids, arginine, leucine and lysine.

BP and WMR are characterized by their higher phenolic and flavonoids contents, antioxidant effect and high cytotoxic effect against human liver cancer HepG2. Nano-powder of all the tested wastes had significantly higher solubility index (%), antioxidant and anticancer effects.

Keywords: Apricot kernels; Peach kernels; Banana peel; Watermelon rind; Nano-powder; Antioxidant and anticancer activities.

1. Introduction

The food production, processing and consumption generate enormous volumes of trash and byproducts (food wastes or food loss) that negatively affect the environment and create major economic losses. Furthermore, the land filling of food waste results in greenhouse gas emissions leading to environmental pollution and climate change. Food wastes can be attributed to agriculture, industrial processing, retail and ultimate consumption, which represent 11-23, 17-19, 8-17, and approximately 50% of food wastes, respectively [1]. The development of value-added goods for potential uses in the food, pharmaceutical, and cosmetic sectors through the sustainable use and valuation of agri-food wastes and/or by-products is a significant global issue [2]. However, food wastes are thought to be a cheap source of bioactive components such as fibers, proteins, polysaccharides, and phytonutrients, which can be utilized as functional

components in pharmaceutical and food products [3,4]. These bioactive components have a range of physiological characteristics and health effects such as their role in the prevention of diabetes, immunological disorders, and neurogenerative diseases as well as their effect as anti-allergenic, antianti-microbial, atherogenic, anti-inflammatory, antioxidant, anti-thrombotic, cardio protective, and anti-cancer properties [5,6].Nowadays, there is a growing interest in using vegetable and fruit processing wastes as functional ingredients in food and dairy products to improve their nutritional value and functional properties [7,8,9]. Although the residues of many fruits have been studied and used to fortify many foods, some residues have not received sufficient attention. Bananas (Musa spp.) are the second most common tropical fruit consumed globally. Bananas are valued for their high nutritional properties as well as their digestibility. Banana peel (BP) accounts for around 1/3 of the weight of this foodstuff and is commonly discarded as waste,

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Received date 20 December 2023; revised date 22 January 2024; accepted date 09 February 2024e DOI: 10.21608/EJCHEM.2024.256873.9008

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resulting in approximately 39.9 million tons of BP waste per year worldwide [10].

Bioactive compounds of BP possess several nutritional, biological and antimicrobial activities [11,12]. Many studies were conducted on the use of BPs in various food industries including meat, baked goods, biscuits and bread [12]. Watermelon is a member of the gourd family (cucurbitaceous). According to FAO, the global production of watermelon is approximately 118 million tons. Watermelon rind (WMR) has been recognized for its nutritional and biological properties [13,14]. WMR represents about 30 - 40% of the total weight of watermelon fruit which is often discarded as waste [15].

It is a rich source of bioactive components that have been reported to exert several nutritional benefits and pharmacological effects [16]. Also, it might relax blood vessels which can aid in cancer and cardiovascular diseases [17]. Due to the nutritional and health benefits of WMR, it was incorporated in many foods i.e., fruit butter cookies, dehydrated candy. reduced-fat mayonnaise and novel biscuits[18]. Apricot (stone fruit) belongs to genus Prunus. It is a carbohydrate-rich commodity and a good source of fiber, minerals and vitamins. Nutritional, phytochemical, antioxidant, antimicrobial and pharmacological activities of apricot and its kernel have been reported [19,20]. Peach (Prunus persica L. Batsch) fruit is considerably rich in antioxidants, vitamins A, B, and C, carotenoids and phenolic compounds which increase its nutritional and health value [21]. Only 10% of the production of apricot is used as fresh product, while the rest is stored and submitted to different processes resulting in the generation of a large amount of residues (stones containing the shell and the kernel) [22,23].

Both of apricot and peach kernels (AK & PK) are considered unconventional potential sources of micro and macro minerals as well as many bioactive components, which provide the human with nutrients and essential elements, however, they haven't been extensively studied yet [22,24]. Their role in nutrition and as a substance with medicinal properties make them a target for anti-cancer drugs [25]. However, the use of both in the food industry was still poorly studied. In the pharmaceutical industry, the bioconversion of agricultural wastes into bioactive compounds, organic fertilizers, composites, and

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biofuels, as well as nutraceuticals, food items, and products, all depend on the nanotechnology. Due to its several potential applications, it is increasingly used in the food and healthcare sectors to enhance the bioavailability and levels of bioactive compounds, and boost the capability for administration of these compounds to specific organs or tissues [26]. Lignocellulosic materials (residues of agro-wastes) require physical (grinding), chemical, and biological treatments to reduce their complexity by transforming the complex molecular structures into simpler monomers. These steps are necessary pretreatment to modify agro-waste to be more useful and effective [27]. Therefore, this study aimed to evaluate the nutritional value, antioxidant and anticancer effects of AK, PK, WMR, and BP in a nano-powder form, as a novel source of various nutrients that have not received enough attention to facilitate its usage and application then after.

2. Materials and Methods

2.1. Materials

Debittered apricot (*Prunus* sp.) kernel powder was purchased from Hebei Seven Fruit Trade Co., Ltd, China. According to the manufacturer, this powder was made by spray drying technology from peeled apricot kernels. Pure peeled peach (*Prunus persica* L. *Batsch*) kernel powder (Tao Ren powder) was purchased from Mountain Herbs Co., China. According to the manufacturer, the peach seeds were naturally sun dried in continuous working temperature ranged from 15 - 38 °C for 7 days then milled using a Dade DF60 grinding machine.

Watermelon (Citrullus lanatus) rinds were separated after washing the fresh fruit peels then cut into small pieces, sliced by a high-power slicer, rinsed with tap water then dried at 50 °C for 24 hours using a hot air oven (DRTH, Dreieich, West Germany). The resultant dried rinds were grounded in a laboratory mill (KENWOOD BL480, China) to fine powder followed by sieving through 50 mesh screens as described by Al-Sayed and Ahmed [17].To produce banana peel (Musa spp.) powder, the banana peels were removed from the fruit and washed with tap water. The peels were soaked in acetic acid solution (5% v/v) for 10 min to minimize the enzymatic browning, then drained and dried using a microwave oven (Sharp, model R-750MR) at power 960 W for 6 minutes with pleating the peels every 30 seconds to prevent their burning through drying as

recommended by Vu *et al.* [28] and Amini Khoozani *et al.* [29]. After drying, the peels were grinded and sifted as mentioned above. To increase the solubility and efficiency of the tested materials, all the tested samples were milled using a ball milling apparatus (ph-BML911model, Photon Scientific Company, Egypt) for 5 h /9 days at a constant speed of 500 rpm at 25 °C, The ratio of balls weight to powder (20:1). The apparatus was equipped with an aircooling system to balance the over-heating generated during milling process.

2.1.1. Chemicals and reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxyl toluene (BHT), dimethylsulfoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2Htetrazolium bromide (MTT) were purchased from Sigma-Aldrich co. (St. Louis, MO, USA). Folin-Ciocalteu's reagent was purchased from Loba Chemie PVT. LTD. (India). The human liver cancer cell line (HepG2) was obtained from VACSERA (Egypt) and grown in Roswell Park Memorial Institute medium (RPMI) for preparing the cell line for cytotoxicity test. All laboratory chemicals and solvents used were of analytical grade.

2.2. Methods of analysis

2.2.1 Particle size determination of the powders

The particle size of the nano-powders was determined before and after milling by transmission electron microscope (TEM) (Model JEM-1400HC, JEOL, Tokyo, Japan) operating at an accelerating voltage of 80 kV. The preparation of the sample was performed as described by Akbari *et al.* [30].

2.2.2. Chemical analysis of the tested powders

The moisture, fat, protein, and ash contents were determined according to George and Latimer [31]. Protein content was determined by the Kjeldahl method, multiplying the calculated total nitrogen by a factor of 6.25. Total carbohydrates were calculated by subtracting all the determined components from the dry weight of the sample. Crude fiber was determined by filter bag technique (ANKOM technology - AOCS Approved Procedure Ba 6a-05). Total, soluble, and insoluble dietary fibers were determined by Enzymatic-Gravimetric method using α -amylase, protease amyloglycosidase and as described by George and Latimer [31].

2.2.3. Minerals content, fatty and amino acids profile

Atomic absorption spectroscopy (Thermo Scientific iCE 3300 Atomic Absorption Spectrometer, Germany) was used the for determination of the mineral elements [32]. To determine the fatty acids profile, the fat of each sample was extracted by biphasic 2:1chloroform/methanol (v/v) using the Folch extraction method as the lower chloroform layer was evaporated at 40 °C. According to ISO 12966-2:2017 [33], fatty acid methyl esters (FAME) were injected in GC (model 7890B from Agilent Technologies) equipped with a flame ionization detector (FID). The separation was carried out using a Zebron ZB-FAME column (60 m x 0.25 mm internal diameter x 0.25 µm film thickness). Analyses were performed using hydrogen as the carrier gas at a flow rate of 1.8 ml/min at a split-1:50 mode, injection volume of 1 µl and the following temperature program was 100 °C for 3 min; rising at 2.5 °C /min to 240 °C and kept for 10 min. The injector and detector were held at 250 °C and 285 °C, respectively. The amino acids profile of the tested samples was determined by mixing 0.1 g of each sample with 2.5 ml distilled water and 2.5 ml of 6 M HCl, then the mixture was heated at 100 °C for 24 h and filtered. Finally, 1 ml of the filtrate was dried and resuspended in 0.1 M HCl and injected into HPLC (Agilent 1260, USA). The separation was carried out using Eclipse Plus C18 column (4.6 mm x 250 mm, Inner Diameter 5 µm) and a mobile phase consisted of buffer (sodium phosphate dibasic and sodium borate), pH 8.2 (A), and acetonitrile: MeOH: H₂O (45:45:10) (B) at a flow rate of 1.5 ml/min [34].

2.2.4. Determination of solubility index

In a centrifuge tube, 1 g of each powder (native and milled) was mixed with 10 ml distilled water and heated to 90 °C for 30 minutes in a water bath with continuous shaking. The tube was removed from the water bath, wiped dry, cooled to room temperature and centrifuged for 15 minutes at 2200 rpm (Hermle Labor Technik GmbH – Z 323 K, Germany). The supernatant was removed, and the residue was dried and weighed. The solubility index was calculated according to Mohan *et al.* [35] as follows:

Solubility index = (weight of dry sample in supernatant) \times 100 / (weight of original sample) *Weight of dry sample in supernatant = weight of original sample – weight of dry sample in the residue

2.2.5. Determination of total phenols and flavonoids

5 g of each powder (native and milled) was extracted using 100 ml of methanol 80%, with constant shaking for 24 hours at room temperature $(25 \pm 2 \ ^{\circ}C)$ as mentioned by Zeyada *et al.* [36].The extracts were filtered with Whatman No.1 filter paper. The filtered material was re-extracted to

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maximize the yielded extract. Each extract obtained was evaporated under vacuum in a rotary evaporator (Model N-1000 Tokyo Rikakikai Co., LTD., Japan) at 45°C and the resultant extracts were stored at – 20°C until further analysis.

To determine the total phenolic contents of the extracts, Folin-Ciocalteu's method was used [37]. Total phenolic content was measured at 750 nm using gallic acid as standard. The obtained results are expressed as mg gallic acid equivalents/g sample. The total flavonoid content of the extracts was determined using the aluminum chloride method according to Zhishen *et al.* [38] and expressed as mg quercetin equivalents/g sample.

2.2.6. DPPH radical scavenging activity

To determine the antioxidant activity of the previously obtained extracts of the tested materials, DPPH assay was used [39]. The antioxidant activity of each extract was expressed as IC_{50} (mg/ml). BHT was used as standard (200 µg/ml) with serial dilutions. The radical scavenging effect was calculated as follows:

DPPH scavenging effect (Inhibition %) = [(Ac – As / Ac) \times 100]

Where: Ac is the absorbance of the control (reagent without the extracts) and As is the absorbance of the extract samples.

2.2.7.Phenolic and flavonoid compounds identification using HPLC

The phenolic compounds of each sample were identified by HPLC as reported by Kim et al. [40] using Agilent Technologies 1100 series liquid chromatograph equipped with an autosampler and a diode-array detector (DAD). The analytical column was an EclipseXDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was held at 0.8 ml/min for a total duration of 70 min. The injection volume was 50 µl and peaks were monitored simultaneously at 280, 320 and 360 nm for the benzoic acid derivatives, cinnamic acid derivatives and flavonoids, respectively. Before injection, all samples were filtered using a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI). Peaks were identified by congruent retention times and UV spectra, and compared with those of the standards.

2.2.8. Cytotoxicity effect against HepG2 cells

MTT assay was used to determine the viability of the human liver cancer cell line according to Van de

Loosdrecht *et al.* [41]. Briefly, the well tissue culture plate was inoculated with 1 X 10^5 cancer cells/ml (100 µl/ well) in the presence of RPMI with 2 mM glutamine as growth medium and incubated at 37° C for 24 hours to develop a complete monolayer sheet. Growth medium was decanted after confluent sheet of cells was formed. The cell monolayer was washed twice with phosphate buffered saline, (PBS).

Two-fold dilutions of the tested sample were made in RPMI medium with 2% fetal calf serum as maintenance medium. 0.1 ml of each dilution was tested in different wells leaving 3 wells as control, receiving only maintenance medium. After incubation at 37°C, the plates were examined for any physical indicators of toxicity, such as partial or total loss of the monolayer, rounding, shrinkage, or granulation of the cells. To each well, 20 µl of MTT solution (5 mg/ml in PBS) was added. The plates were shakenat 150 rpm for 5 min. then incubated (37 °C, 5% CO₂) for 4 hours to allow the MTT to be metabolized. Formazan (MTT metabolic product) was resuspended in 200 µl DMSO. Optical density was measured at 560 nm and background was subtracted at 620 nm. The cell amount is directly correlated with the optical density.

2.3. Statistical analysis

The obtained data (three replications) were statistically analyzed using a randomized complete block design with two factors. The treatments means were compared by least significant difference (L.S.D.) test as given by Snedecor and Cochran [42]using Assistat program [43].

3. Results and discussion

3.1. Chemical composition of the tested wastes

As shown in Table (1), both of AK and PK had the lowest moisture content (4.35 and 7.30%) compared to WMR and BP with significant differences (10.37 and 13.90%).On the contrary, both AK and PK had the highest and significant fat and protein contents being 51.79, 45.19% fat and 22.70, 23.00% protein compared to WMR and BP (2.92, 7.41 and 11.80, 8.60%), respectively.Concerning the carbohydrate content of the studied wastes, Table (1) also revealedthat both of WMR and BP represent a rich source of carbohydrates (45.81, 43.28%)while AK and PK contained lower concentration(13.90, 4.63%). Ash content behaved in a similar trend as carbohydrate and moisture content. WMR and BP powders had the highest ash content compared to AK

Dietary fibers are carbohydrate polymers that provide the cell walls of plants with their structural stiffness. Based on its solubility in water, it was divided into two groups: soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). Dietary fiber is regarded as an essential component of a healthy human diet. It can lower the risk of several diseases, including diabetes and cardiovascular diseases, lower the incidence of hypertension, obesity, stroke, and a few gastrointestinal illnesses as well as improve the immune function. Dietary fiber extracted from vegetables and fruits wastes and by-products can be a rich source of polyphenols, flavonoids, and carotenoids and can constitute as an antioxidant dietary fiber that can exhibit physiological effects [44,45].

Data in Table (1) indicated that PK had the highest crude fiber content (18.28%) while AK had the lowest content (5.54%)with significant differences.Both WMR and BP had almost similar (13.85 crude fiber content and 13.88%, respectively). Concerning the insoluble dietary fibers, it behaved in a similar trend as the crude fibers. AK had the lowest content with significant differences between PK, WMR and BP. As for the soluble dietary fibers content, WMR had the highest content (15.50%) followed by BP (12.60%) with non-significant differences. Both AK and PK had almost similar content (10.55 and 10.05%). Also, it is noticeable that WMR had the

highest content of total dietary fibers (52.80%) followed by BP (48.40%),PK (47.60%)with nonsignificant differences, and lastly AK (36.45%).From the foregoing results, it is clear that WMR, BP and PK are considered a rich source of dietary fibers which is in agreement with Naknaen *et al.*[46].

Data in Table (2) illustrate the mineral content of the tested samples. WMR had higher content of the major minerals (Ca, Mg, Na, K and P) as compared to BP, AK and PK. For minor minerals, BP is considered the richest source of Fe being 152.80 mg/kg followed by PK (92.46 mg/kg). AK had the highest Zn content (24.77 mg/kg) while WMR had the lowest content (5.03 mg/kg). Also, PK had the highest Cu content (0.85 mg/kg) followed by AK (0.66 mg/kg).

Data in Table (3) show the fatty acids profile of the tested wastes. WMR had the highest saturated fatty acids (C4 – C24) being 66.78% followed by BP (51.61%) while both AK and PK had almost the same low values (5.69 and 5.81%). On the contrary, PK and AK had the highest concentrations of monounsaturated fatty acids (69.90 and 67.18%), followed by WMR (11.01%) and lastly BP (4.36%).

AK had the highest concentration of mono and polyunsaturated fatty acids (94.31%) followed by PK (94.19%), BP (48.37%), and lastly WMR (33.21%). These results are in accordance with Fratianni *et al.* [47] who mentioned that kernel oil of both PK and AK contains some biologically active substances, which provide nutritional value and functional properties of the extracted oil.

Components	Apricot kernel	Peach kernel	Watermelon rind	Banana peel	LSD 0.05
Moisture	$4.35\pm0.08^{\rm d}$	$7.30\pm0.54^{\circ}$	$10.37\pm0.54^{\text{b}}$	$13.90\pm0.41^{\rm a}$	0.6952
Fat	51.79 ± 0.85^{a}	45.19 ± 0.13^{b}	$2.92\pm0.08^{\text{d}}$	$7.41\pm0.30^{\rm c}$	0.4967
Protein	$22.70\pm0.11^{\text{b}}$	$23.00\pm0.10^{\rm a}$	$11.80\pm0.08^{\rm c}$	$8.60 \pm 0.06^{\rm d}$	0.0341
Carbohydrates	$13.90\pm0.31^{\circ}$	$4.63 \pm 0.01^{\text{d}}$	$45.81\pm0.89^{\rm a}$	$43.28\pm0.14^{\rm b}$	0.9889
Ash	$1.72\pm0.14^{\rm c}$	$1.60 \pm 0.13^{\rm d}$	$15.25\pm0.06^{\rm a}$	$12.93\pm0.04^{\rm b}$	0.1188
Crude fiber	$5.54\pm0.11^{\rm c}$	$18.28\pm0.17^{\rm a}$	13.85 ± 0.13^{b}	$13.88\pm0.10^{\rm b}$	0.0432
Insoluble dietary fiber	25.90 ± 2.70^{b}	37.55 ± 1.15^{a}	37.30 ± 1.90^{a}	$35.80\pm3.70^{\mathrm{a}}$	1.9785
Soluble dietaryfiber	10.55 ± 1.75^{b}	$10.05 \pm 1.25^{\text{b}}$	$15.50\pm1.90^{\rm a}$	12.60 ± 1.70^{ab}	3.7551
Total dietaryfiber	$36.45\pm4.40^{\rm c}$	47.60 ± 0.10^{b}	$52.80\pm2.70^{\rm a}$	48.40 ± 1.70^{ab}	4.9768

 Table 1. Chemical composition of the nano-powder of different tested wastes (% of dry matter).

AK: Apricot kernel, PK: Peach kernel, WMR: Watermelon rind, BP: Banana peel

Carbohydrates were calculated by subtracting the sum of components (Moisture, ash, fat, protein and crude fiber) from the dry weight of sample (100 g).

Data are expressed as mean \pm SD of three replications per treatment.

Means with different superscripts in the same row are significantly different at $p \le 0.05$.

	1						
Minerals	AK	РК	WMR	BP			
Major minerals(g/100 g)							
Calcium (Ca)	0.02	0.02	0.21	0.21			
Magnesium (Mg)	0.14	0.04	0.19	0.06			
Sodium (Na)	0.34	0.46	0.46	0.33			
Potassium (K)	0.13	0.21	8.38	8.02			
Phosphorus (P)	0.22	0.18	0.20	0.24			
Minor minerals(mg/Kg)							
Iron (Fe)	33.67	92.46	28.53	152.80			
Manganese (Mn)	12.06	12.17	14.57	15.45			
Copper (Cu)	0.66	0.85	0.24	0.31			
Zinc (Zn)	24.77	13.89	5.03	8.37			

Table 2. Mineral contents of the nano-powder of the tested wastes

AK: Apricot kernel, PK: Peach kernel, WMR: Watermelon rind, BP: Banana peel

 Table 3. Fatty acids profile of the nano-powder of the tested wastes (Area sum %).

Fatty acids	AK	PK	WMR	BP
Saturated fatty acids				
Butyric acid (C4:0)	0.03	0.03		
Caproic acid (C6:0)			9.62	1.03
Undecanoic acid (C11:0)	0.01			
Lauric acid (C12:0)				1.02
Tridecanoic acid (C13:0)	0.01			
Myristic acid (C14:0)	0.01		0.88	1.48
Pentadecanoic acid (C15:0)		0.01	0.67	0.62
cis-10-pentadecenoic acid (C15:0)			0.72	
Palmitic acid (C16:0)	3.99	4.2	40.32	34.98
Margaric acid (C17:0)	0.02		0.48	0.56
cis-10-Heptadecenoic acid (C17:0)		0.01		
Stearic acid (C18:0)	1.53	1.51	12.47	8.58
Arachidic acid (C20:0)	0.07		0.4	0.98
Behenic acid (C22:0)			0.44	1.09
Tricosanoic acid (C23:0)			0.2	0.32
Lignoceric acid (C24:0)	0.02	0.05	0.58	0.95
Total saturated fatty acids (%)	5.69	5.81	66.78	51.61
Monounsaturated fatty acids				
Myristoleic acid (C14:1n-5)			0.17	0.08
Palmitoleic acid (C16:1n-7)	0.62	0.65	1.89	0.36
Elaidic acid (C18:1)	0.63	0.48		
Oleic acid (C18:1)	65.85	68.73	8.9	3.88
cis-11-Eicosenoic acid (C20:1)	0.08	0.04	0.02	0.02
(Gondoic acid)	0.00	0.04	0.02	0.02
Erucic acid (C22:1)			0.03	0.02
Total monounsaturated fatty acids (%)	67.18	69.90	11.01	4.36
Polyunsaturated fatty acids				
Linolelaidic acid (C18:2)	0.01	0.01	0.43	0.52
Linoleic acid (C18:2)	27.04	24.25	13.92	26.87
Linolenic acid (C18:3)	0.08	0.01	7.36	16.25
Homo-γ-linolenic acid (18:3n-6)		0.01	0.04	
cis-11,14-Eicosadienoic acid (C20:2)			0.44	0.34
cis-11,14,17-Eicosatrienoic acid (C20:3)				0.03
Eicosapentaenoic acid (C20:5n-3)		0.01	0.01	
Total polyunsaturated fatty acids (%)	27.13	24.29	22.20	44.01

AK: Apricot kernel, PK: Peach kernel, WMR: Watermelon rind, BP: Banana peel

It is worthy to mention here that essential fatty acids, such as mono and polyunsaturated fatty acids, have several human health effects including lowering lowdensity lipoprotein (LDL), enhancing brain function and cell growth, lowering triglycerides and lowering the risk of heart disease, controlling blood pressure, and lowering the risk of diabetes [48].

The amino acids profile of the tested wastes is shown in Table (4). It is noticeable that PK had the highest amino acids either essential or non-essential (6.84, 13.23 g/ 100 g sample) followed by AK while the lowest content was recorded in WMR. Also, both WMR and BPhad the smallest content of methionine while cystine was not detected in these wastes. Concerning the branched amino acids (isoleucine, leucine and valine), PK had the highest content followed by AK, BP and lastly WMR being 3.45, 2.98, 0.99 and 0.68 g/100 g sample.

3.2. Particle size and solubility index

Reducing the particle size for the nanoscale of plant materials or bioactive components became one of the successful valorization strategies and techniques for improving their stability, solubility, bioavailability, and delivery, hence enhancing their functional activity [49].

Data in Fig. (1A) indicate that the particle size of the native forms ranged from 98.22 to 173.93 nm with significant differences between all samples. The grinding process minimized the particle size with significant differences ranging from 13.75 to 32.09 nm and as a result the solubility index increased. The solubility index of the native powders ranged from 31.93 to 52.32% with significant differences while it increased in the nano-powders to reach values among 39.55 to 56.12% with significant differences between both AK & WMR and PK & BP (Fig. 1B).

It is worth mentioning that although the solubility index of AK is significantly higher than WMR (native form), there is no significant difference between both in the case of nano state. By calculating the % of solubility increment due to the grinding process, it is clear that the highest increment was recorded with WMR (61.1%) followed by BP (23.86%) then PK and AK which recorded almost the same value being 7.89 and 7.26%.







3.3. Phytochemical profile

The results of the phytochemical profile of the native and nano-tested materials (total phenols and flavonoids) are presented in Fig. (2). The total phenolic content (Fig. 2A) increased in the nano form as compared with the native one [50,51]. BP (native form) had the highest and significant phenolic content (95.15 mg gallic acid/ g of dry matter) followed by WMR (17.6), while both PK and AK had nonsignificant differences being 6.22 and 4.48 mg gallic acid/ g of dry matter, respectively. However, BP recorded the lowest rate of increment by nanonization (10.28%). On the other hand, WMR (either in native or nano form) recorded the highest rate of increment (264.83%) followed by AK (168.30%) and PK (137.46%).

The total flavonoid content (Fig. 2B) in both the native and nano forms behaved in the same trend as the highest value was recorded for BP followed by

WMR, PK and lastly AK. However, BP recorded the lowest % of flavonoids increment (5.22%) followed by WMR (61.14%), PK (111.75%) and lastly AK which recorded the highest rate of increment (130.82%).

Table 4. Amino acids profile of the nano-powder of the tested wastes.

	Concentration (g/100 g of sample)				
Amino acids —	AK	РК	WMR	BP	
Essential amino acids					
Histidine	0.45	0.53	0.12	0.14	
Methionine	0.15	0.23	0.03	0.05	
Phenylalanine	0.94	1.04	0.17	0.25	
Threonine	0.57	0.63	0.10	0.27	
Lysine	0.96	0.96	0.84	1.34	
Isoleucine*	0.77	0.89	0.22	0.26	
Leucine*	1.41	1.65	0.23	0.40	
Valine*	0.80	0.91	0.23	0.33	
Total essential amino acids	6.05	6.84	1.94	3.04	
Non-essential amino acids					
Aspartic	1.84	1.96	0.37	0.56	
Glutamic	4.56	5.06	0.52	0.60	
Serine	0.74	0.80	0.22	0.29	
Glycine	0.91	0.96	0.16	0.29	
Alanine	0.80	0.90	0.22	0.32	
Tyrosine	0.45	0.39	0.12	0.14	
Cystine	0.30	0.30	ND	ND	
Proline	0.89	1.13	0.27	0.22	
Arginine	1.60	1.73	0.38	0.25	
Total non-essential amino acids	12.09	13.23	2.26	2.67	

AK: Apricot kernel, PK: Peach kernel, WMR: Watermelon rind, BP: Banana peel *Branched amino acids.

ND: Not detected



Figure 2. Total phenols (Å) and flavonoids (B) content of native and nano-powders of apricot kernel, peach kernel, watermelon rind and banana peel.

3.4. Phenolic acids profile of the tested wastes

The identified phenolic and flavonoid by HPLC in AK, PK, WMR and BP (μ g/g) were presented in Table (5). AK is characterized by its higher content of catechin (37.556) and chlorogenic acid (5.343) as compared to the other wastes, while BP is considered a rich source of ferulic acid (235.919), p-coumaric acid (9.055), rutin (4.188), quercetin (2.264), sinapic acid (4.045) and catechin (17.566). It is worth to mention that gallic acid and rutin are detected only in BP (2.329, 4.188). As for WMR, it

is a good source of protocatechuic acid (12.691), phydroxybenzoic acid (9.673), chlorogenic acid (4.851), caffeic acid (4.915), syringic acid (12.103), ferulic acid (12.278) and cinnamic acid (2.577). It is also clear that PK lacks many phenolic and flavonoid compounds compared to the other wastes. It contained protocatechuic acid (0.510), phydroxybenzoic acid (2.525), catechin (12.669), vanillic acid (1.142), ferulic acid (2.711), sinapic acid (0.624) and cinnamic acid (0.591).

Table 5. Phenolic and flavonoid components profile of the tested nano-wastes powder by HPLC ($\mu g/g$).

Compounds	AK	РК	WMR	BP	
Phenolic compounds					
Gallic acid	ND	ND	ND	2.329	
Protocatechuic acid	0.927	0.510	12.691	2.241	
p-hydroxybenzoic acid	5.856	2.525	9.673	3.689	
Chlorogenic acid	5.343	ND	4.851	ND	
Caffeic acid	1.227	ND	4.915	3.087	
Syringic acid	0.658	ND	12.103	ND	
Vanillic acid	0.285	1.142	0.516	0.608	
Ferulic acid	0.371	2.711	12.278	235.919	
Sinapic acid	ND	0.624	0.632	4.045	
p-coumaric acid	0.659	ND	ND	9.055	
Cinnamic acid	0.520	0.591	2.577	0.476	
Flavonoid compounds					
Quercetin	ND	ND	0.901	2.264	
Catechin	37.556	12.669	2.039	17.566	
Rutin	ND	ND	ND	4.188	

AK: Apricot kernel, PK: Peach kernel, WMR: Watermelon rind, BP: Banana peel

ND: Not detected

3.5. Antioxidant activity (DPPH scavenging activity %)

Several methods are used to evaluate the effectiveness of antioxidants in scavenging free radicals. The most used one is the DPPH method, which is fast, easy to use, dependable and doesn't require a particular reaction or apparatus [52]. As shown in Table (6), the antioxidant activity is significantly increased as the concentration of the tested materials increased either in native or nano form especially for WMR and BP. Also, the milling process had a remarkable effect as the antioxidant effect is significantly increased as compared to the native form [50,51].

Also, BP had the highest and most significant antioxidant activity as compared to BHT or the tested wastes. The DPPH scavenging activity % can be

the concentration of phenolic and flavonoid components either in native or nano form. The extracts' ability to scavenge radicals was shown to be positively correlated with their total amount of phenolic compounds which is consistent with other published results [52,53]. Additionally, utilizing several antioxidant modelsin vitro, Kumar *et al.* [54] discovered a strong link between antioxidant activity and the overall phenolic content. IC₅₀ value is a commonly used parameter to assess the

antioxidant activity of the examined materials. It is defined as the concentration of an antioxidantcontaining material needed to scavenge 50% of the original DPPH radicals. The lower the IC₅₀ value, the more potent is the substance for scavenging DPPH and this implies a higher antioxidant activity [55]. The

arranged as follows: BP > WMR > PK and lastly AK at all concentrations. This may be due to the type and

obtained results, as shown in Table (6) depict that BP is more potent than standard compounds (BHT).

 IC_{50} for BP is 1.01 and 0.83 mg/ml for the native and nano forms while for BHT (synthetic standard antioxidant) is 1.09 mg/ml with non-significant differences. Also, significant differences were

observed between all the tested materials. BP is more efficient compared to other wastes either in native or nano form. According to IC_{50} , the tested materials can be descendingly arranged as follows: BP > BHT > WMR > PK and lastly AK (Fig. 3A).

Table 6.DPPH scavenging activity % of the methanolic extracts of the tested native and nano-wastes powder at different concentrations.

Extracts	% Inhibition at different concentrations (mg/ml)							
	1.25	2.5	5	7.5	10			
Native powder								
AK	$3.64\pm0.45^{\rm q}$	3.87 ± 0.33^{pq}	4.11 ± 0.23^{pq}	4.35 ± 0.33^{p}	$5.69\pm0.23^{\rm o}$			
РК	5.83 ± 0.33^{no}	6.38 ± 0.23^n	7.57 ± 0.56^{m}	$11.16\pm0.90^{\rm l}$	$15.27\pm0.45^{\rm k}$			
WMR	$18.03 \pm 1.01^{\rm j}$	$21.11\pm0.56^{\rm i}$	$23.96\pm0.67^{\rm h}$	$33.44 \pm 1.12^{\text{g}}$	$34.55\pm0.78^{\rm f}$			
BP	$67.00 \pm 1.23^{\text{e}}$	78.50 ± 0.78^{d}	$79.29\pm0.45^{\rm c}$	80.08 ± 0.89^{b}	$80.95 \pm 1.00^{\rm a}$			
LSD 0.05 for concentrations = 0.3144 LSD 0.05 for treatments = 0.2812 LSD 0.05 for interaction = 0.6288								
Nano-powder								
AK	$3.66\pm0.11^{\rm o}$	$3.93\pm0.11^{\rm o}$	4.89 ± 0.04^{no}	6.98 ± 0.60^{mn}	$8.09\pm0.19^{\rm lm}$			
РК	7.72 ± 0.11^{lm}	8.41 ± 0.20^{lm}	$9.69\pm0.35^{\rm l}$	$14.06\pm0.74^{\rm k}$	21.66 ± 0.87^{j}			
WMR	$49.34\pm1.26^{\rm i}$	$53.39\pm0.20^{\rm h}$	$59.05\pm0.81^{\text{g}}$	$69.44\pm0.85^{\text{e}}$	$71.76\pm2.39^{\text{e}}$			
BP	$75.61\pm0.11^{\rm d}$	$79.11 \pm 1.00^{\circ}$	79.72 ± 0.27^{bc}	81.98 ± 1.23^{ab}	83.33 ± 0.96^{a}			
BHT as standard	$57.70\pm5.27^{\rm g}$	$65.80 \pm 1.80^{\rm f}$	$76.41 \pm 1.06^{\rm d}$	80.30 ± 0.97^{bc}	80.40 ± 0.53^{bc}			

 $LSD_{0.05} \text{ for concentrations} = 1.1846 \quad LSD_{0.05} \text{ for treatments} = 1.1846 \quad LSD_{0.05} \text{ for interaction} = 2.6488 \quad LSD_{0.05} \text{ for interact$

AK: Apricot kernel, PK: Peach kernel, WMR: Watermelon rind, BP: Banana peel

Data are expressed as mean \pm SD of three replications per treatment.

Means with different superscripts in the same column and row are significantly different at $p \le 0.05$.



Figure 3. Antioxidant (A) and anti-cancer (B) activities (IC₅₀) of native and nano-powders of apricot kernel, peach kernel, watermelon rind and banana peel.

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3.6. Effect of the tested materials on liver cancer HepG2

Of all liver cancers, up to 90% are hepatocellular carcinomas. Liver cancer is the sixth most common cancer worldwide. It ranks ninth among cancers in women and fifth among cancers in males. In 2020, there were about 900,000 new cases of liver cancer. Over 700,000 deaths globally are attributed to liver cancer each year [56].

According to WHO (2020) reports, 24,512 fatalities in Egypt were due to liver cancer, accounting for 4.57% of all deaths [57]. Hepatocellular carcinoma (HCC) represents a major public health concern in Egypt, where it accounts for 13.54% of all malignancies in women and 33.63% of all cancers in men [58].

Data in Fig. (3B) and Fig. (4) illustrate the anticancer activity (IC₅₀) and cytotoxic effect (%) of the tested materials against human liver cancer cells (HepG2). The cytotoxic effect increased significantly as the sample's concentration increased either in native or nano form. Also, the milling process led to minimizing the particles size and as a result increased significantly the cytotoxic effect (%) which is in accordance with Kumar et al. [59]. This effect was observed in all the tested wastes with significant differences. IC50 decreased from 362.34 to 323.87 µg/ml for AK, 232.69 to 229.06 for PK, 376.07 to 224.33 for WMR while it decreased dramatically for BP from 346.78 to 67.65 which reflects its powerful effect due to its phenolic content (quantitative and qualitative).

4. Conclusion

The food production, processing and consumption generate enormous volumes of wastes and byproducts that negatively affect the environment and create major economic losses. Food wastes are considered an inexpensive source of bioactive components and could be used as functional ingredients in food and dairy sectors. In the current study, the nutritional value, antioxidant and anticancer effects of apricot (Prunus sp.) and peach (Prunus persica L. Batsch) kernels, watermelon (Citrullus lanatus) rind and banana (Musa spp.) peel in native and nano-powder form were evaluated. The obtained results revealed that both of BP and WMR had the highest carbohydrate, ash, dietary fiber, phenolic and flavonoid contents. These wastes demonstrated significant antioxidant and anticancer properties, making them suitable for utilizing in the food and pharmaceutical industries, followed by AK and PK. Further studies are needed to investigate more functional characteristics of these wastes.



Figure 4. Cytotoxicity effectof native and nano-powders of apricot kernel (AK), peach kernel (PK), watermelon rind(WMR) and banana peel (BP) against human liver cancer (HepG2) cell line.

Declarations

Competing interests

The authors declare that there are no competing interests.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding declaration

The authors did not receive support from any organization for conducting this study.

Data availability information

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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