ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 10, Issue, 09, pp.10242-10246, September, 2019

RESEARCH ARTICLE

EXTRACTED FOOD PROCESSING WASTE UTILIZE AS NATURAL ANTIOXIDENT FOR OXIDATIVE STABILITY OF GHEE

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ARTICLE INFO

Article History:
Received 12th June, 2019
Received in revised form
15th July, 2019
Accepted 18th August, 2019
Published online 30st September, 2019

Key words:
Antioxidant, Rancidity,
Phenolic compound,
Stability.

ABSTRACT

The search for cost-efficient natural antioxidants has led to the exploration with raw materials of residual origin. The present introduction of lipid oxidation and the antioxidant mechanisms, as well as the most recent research on the recovery and utilization of food processing wastes. A majority of these natural materials contains phenolic acids and flavonoids, the levels of actives in the wastes are usually found to be higher than the actual products. Gaps in the area of by-products research as well as constraints of waste exploitation. Bioactive compounds found in granules, pallets and powder (peels of banana, apple, pomegranate, orange and tulsi leaves) were extracted using ethanol (80%), ethyl acetate and n-hexane. Total phenolic compounds (as gallic acid equivalent) ranged between 0.89 and 16.6, 1.83 and 261, and 1.56 and 124 mg gallic acid/g extract for granules, pallets and powder, respectively. Ethanol extracts of different by-products were added to ghee at concentrations of 200, 400 and 600 ppm, respectively. BHA was also added to ghee at a concentration of 200 ppm for comparison. All samples were incubated at 63 C for 6 months. Ethanol extracts of granules, pallets and powder gave good antioxidant activity during accelerated oxidative incubation of ghee. The results revealed that ethanolic extracts under study, at a concentration of 200 ppm, could be used to retard fat auto-oxidation.

Citation: Prerna Srivastava and Neetu Singh. **2019.** "Extracted food processing waste utilize as natural antioxident for oxidative stability of ghee", *Asian Journal of Science and Technology*, 10, (09), 10242-10246.

INTRODUCTION

Food processing industry (e.g. juice production) is produced in large quantities worldwide and contains high levels of lingo cellulose. These processing wastes are produced in state of solid and liquid. The solid waste is the portion of the starting material that cannot be utilized in the production of the intended products, such as the skins, pulps and fibers of fruit which are removed in the production of juice. To some extent, value-added products are extracted from this waste, but the majority of the waste is currently an utilized and discarded. Energy generation from this waste has been investigated in the form of production of biogas, hydrogen and bio ethanol. Efficient bio ethanol production requires the enzymatic hydrolysis of the total polysaccharides within this waste into monomer sugars for further fermentation into ethanol. Factors limiting this process are the complexity of the lingo cellulose, its recalcitrance and insolubility and the number of enzymes required to degrade it. Obtaining complete enzymatic hydrolysis of these substrates requires an understanding of the composition of the polysaccharides and their associations within the overall substrate. This will allow appropriate selection of enzymes. It has also been established from work on other lingo cellulose substrates that the associations between polysaccharides pose an obstacle to their hydrolysis and cooperative enzyme interaction is required to overcome these obstacles. Organic wastes and wastewater from producing operations are usually treated in a "pre-treatment" process before being discharged into the sewer. This is done to reduce sewer charges by lowering the BOD of the waste water. BOD stands for "Biological Oxygen Demand", and is a measurement of the amount of organic material in the waste water. The research and exploration of natural antioxidants has been rising in recent years. This increased attention is driven by several trends in the food industry. First of all, the reformulations of food products using healthier ingredients have imposed a negative impact on product shelf life. For example, whole grain ingredients are increasingly used instead of refined flour. Major food manufacturers have gone far beyond sliced breads to incorporate whole grains into a variety of shelf stable products. The germ in the whole grain contains high levels of naturally occurring fat composed of polyunsaturated fatty acids, which are susceptible to oxidation.

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Therefore, whole grain reformulation calls for additional protection from antioxidants to maintain high product quality. Omega-3 enrichment has been another continuing trend. Rancidity occurs when oils or oil-containing foods become oxidized. Autoxidation is the major mechanism of oil oxidation at ambient condition. Autoxidation leads to the generation of off-odors and off-flavors that render foods unacceptable. Oxidation of lipid and lipid containing foods reduces food quality, shortens shelf life, and compromise their nutrition value. Antioxidants inhibit lipid oxidation under different mechanisms. Free radical scavenging is the most common mechanism; these antioxidants quench radicals in lipid or food by donating a hydrogen molecule. The resulting antioxidant molecules are more stable than the lipid radical, thus intercept further free radical reactions. Antioxidants may also slow down oxidation by chelating transition metals, which is a major catalyst for oil degradation. Some antioxidants exhibit more than one mechanism of activity. In addition, usually more than one antioxidant is present in a complex food system. Each antioxidant may work with each other and provide synergistic effects.

Ghee is anhydrous milk fat, occupies a prominent place in the Indian diet. Chemically ghee is a complex lipid of mixed glycerides together with a small amount of free fatty acids, phospholipids, sterols and their esters, fat soluble vitamins (A, D, E and K), carotenoids, carbonyl compounds, hydrocarbons, charred casein, moisture and traces of trace elements like copper and iron. Oxidative rancidity is the major pathway by which ghee undergoes deterioration. This is referred to as autoxidation because the rate of oxidation increases as the reaction proceeds under usual processing and storage conditions. Several workers have done exhaustive work to improve the stability of ghee against autoxidation through feeding specific feed to milch animals (Tandon, 1977; Hagrass et al., 1983), altering processing parameters (Singh et al., 1979), using proper packaging materials and storage conditions (Chauhan and Wadhwa, 1987; Amr, 1990a), adding synthetic antioxidants, incorporating natural antioxidants from edible plant materials, spices and condiments, aromatic herbs etc. Pomegranate is a good example for these types of fruits where in their peels constitutes approximately 40% of the whole fruit and are rich in ellagic acid derivatives (Cerda et al., 2003; Seeram et al., 2005).

Sharma (1997) isolated the antioxidant principles of Tulsi(Ocimum sanctumLinn.) leaves via a pre-extraction. The anti-oxygenic compounds of Tulsi leaves were extracted into methanol and then vacuum dried. The dried materials were further fractionated into water insoluble fraction which was then treated with mixture of silica gel and charcoal and designated at SCF. Addition of SCF pre-extract at the level of 0.6 per cent (w/v) was found to be more effective than the addition of BHA at the level of 0.02 per cent. The phenolic compounds appeared to be the main scontributory factors in enhancing the oxidative stability of ghee. Apples are commonly eaten and are large contributors of phenolic compounds in European and North American diets. The peels of apples, in particular, are high in phenolics. During applesauce and canned apple manufacture, the antioxidant-rich peels of apples are discarded. To determine if a useful source of antioxidants is being wasted, the phytochemical content, antioxidant activity, and anti proliferative activity.

Citrus peel extract as a natural source of antioxidant was evaluated during 6 months storage of refined corn oil at 25 and 45 °C. Extracts of citrus peel were prepared by refluxing the dried ground peel with ethanol, methanol, acetone, hexane, diethyl ether and dichloromethane. Maximum amount of citrus peel extract was obtained with methanol. Antioxidant activity of methanolic extract was assessed by measuring free fatty acid (FFA) content peroxide value (POV) and iodine value (IV) during 6 months storage of refined corn oil at 25 and 45 °C. After 6 months of storage at 45 °C. Banana (*Musa acuminata* Colla AAA) peel extracts obtained in this work had a high capacity to scavenge 2,2-diphenyl-1-picrylhydrazyl ($DPPH_{\bullet}$) and 2,2′-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid ($ABTS_{\bullet}^{+}$) free radicals, and they were also good lipid peroxidation inhibitors. Phenolic compounds exhibit a wide range of physiological properties such as anti-allergenic, antiatherogenic, anti-inflammatory, anti-microbial, antioxidant,anti-thermobiotic, cardio protective and vasodilatory effects (Balasundran et al., 2006). Phenolics could be extracted by water or solvents and the extraction conditions need to be optimized with respect to solvent polarity and physical conditions (Nepote et al., 2005). In addition, research has indicated that natural phenolic compounds can be extracted from raw materials or waste products of food industry (Peschel et al., 2006). Objective- To examin phenolic substance in its and standardization the granules, pallets and powder sample. Studies were conducted to investigate antioxidant properties of Pomegranate, Tulsi leaves, Banana, Apple and Oranges has been used extensively in the folk medicine of many cultures and its consumption has grown tremendously especially in the last decades (Li et al., 2006; Cam et al., 2009). The peels of some fruits have higher antioxidant activity than pulps (Guo et al., 2003; Fuhrman et al., 2005).

MATERIALS AND METHODS

Determination of total phenolic compounds (TPC): The concentration of TPC in different extracts was measured using UV spectrophotometer (Jenway-UV–VIS Spectrophotometer), based on a colorimetric oxidation/reduction reaction, as described by S˘kerget et al. (2005) using Folin–Ciocalteu reagent. Specifically, 0.5 mL of diluted extract (10 mg in 10 mL solvent) was mixed with 2.5 mL of Folin–Ciocalteu reagent (diluted 10 times with distilled water) and 2 mL of Na2CO3 (75 g/1 L). The sample was incubated for 5 min at 50C then cooled. For a control sample, 0.5 mL of distilled water was used. The absorbance was measured at 760 nm. Total phenolic content expressed as gallic acid equivalent (GAE) was calculated, and the results were expressed as amg GAE g1extract.

Identification of phenolic acids using HPLC: Phenolic acids of the dried extracts were identified according to the method described by Mattila et al. (2000). HPLC (Hewllet Packard series 1050, USA) equipped with autos ampling, injector, solvent degasser, UV detector set at 330 nm and quarter HP pump (series 1050) was used. Column (C18 hypersil BDS) with particle size 5lm was used. The separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 mL/min. The column temperature was performed at room temperature (25C) throughout the experiment. Identification and quantification were carried out based on calibrations of the standards prepared from phenolic acids dissolved in a mobile phase.

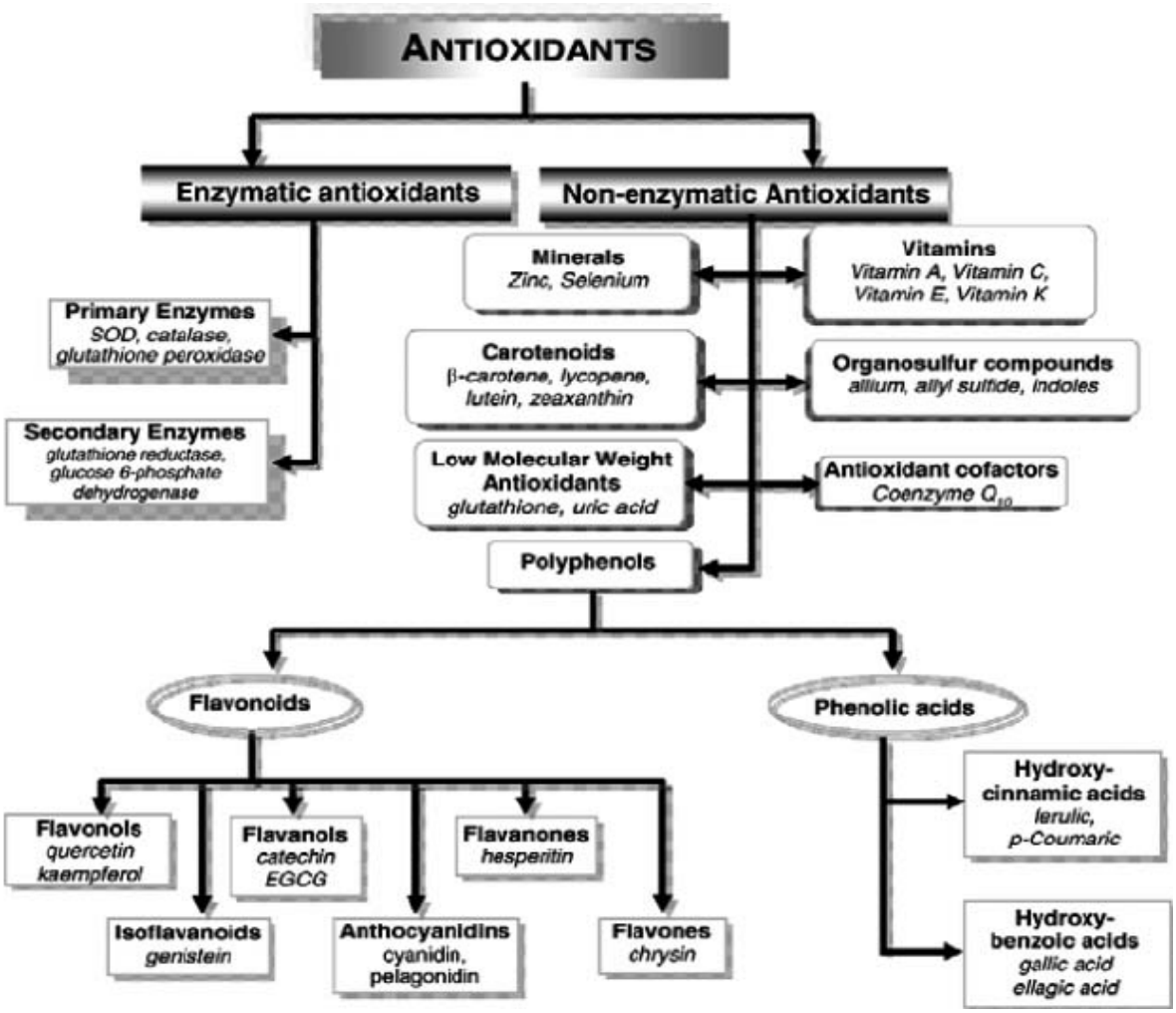


Fig. 1. Classification of antioxidants (Carocho & Ferreira, 2013)

Table 1 . Changes in the analytical characteristics of ghee during development of rancidity-

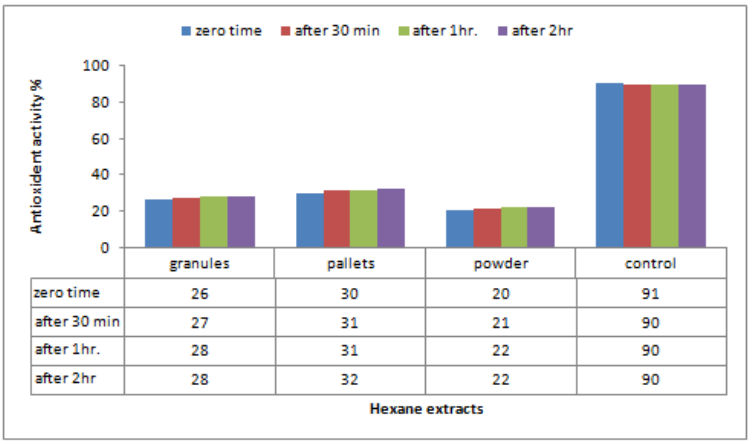
Characteristic	Original samples	
	Average	Range
Acid value	Negligiable	Negligiable
Saponification value	223.9 - 239.3	223.9
Iodine value	25.7 – 31.1	28.9
Butyrorefractometer reading (40degree celcious)	39.2 – 43.1	41.9
Reichert value	25.7 – 39.1	32.1
Polenske value	1.1 – 2.7	1.7
Peroxide value	Negligible	Negligiable

Table 2. Total phenolic compounds (mg gallic acid/g extract) in different extracts

By- product	Concentration(mg gallic acid/ g extract)		
	Hexane	Ethyl acetate	Ethanol 80%
Granules	16.63	0.89	12.23
Pellets	1.56	12.49	124.23
Powder	1.83	5.69	261.69

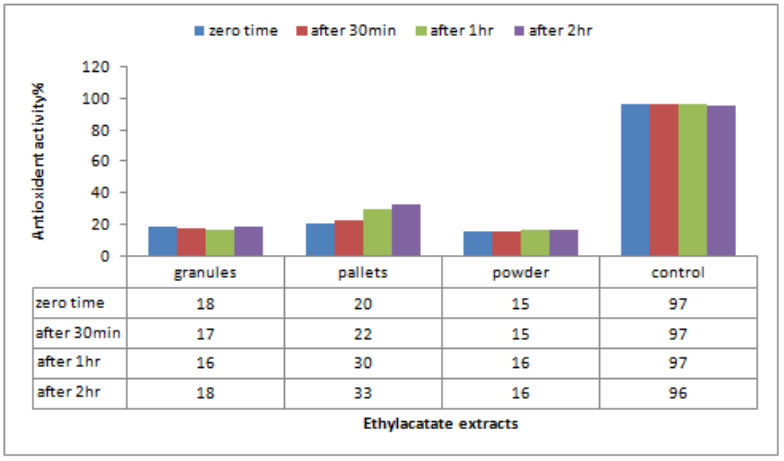
Table 3. Concentration of identified phenolic compounds (mg/g dry matter) in food processing waste extracts as determined by HPLC

	Granules	Pallets	Powder
Gallic	0.07	0.01	0.0
Pyrogallol	0.90	0.27	12.64
Chlorogenic	2.58	0.06	0.84
Protocatechuic	5.07	1.0	1.86
Caffeine	0.69	0.04	0.21
Ellagic	1.40	0.46	5.90
Catachin	10.64	0.0	0.0
Vanillic	0.19	0.04	0.34



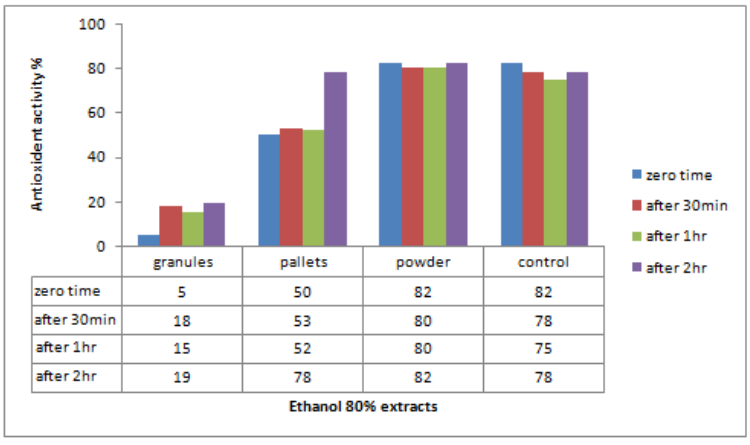
	granules	pallets	powder	control
zero time	26	30	20	91
after 30 min	27	31	21	90
after 1hr.	28	31	22	90
after 2hr	28	32	22	90

Hexane extracts



	granules	pallets	powder	control
zero time	18	20	15	97
after 30min	17	22	15	97
after 1hr	16	30	16	97
after 2hr	18	33	16	96

Ethylacatate extracts



	granules	pallets	powder	control
zero time	5	50	82	82
after 30min	18	53	80	78
after 1hr	15	52	80	75
after 2hr	19	78	82	78

Ethanol 80% extracts

Fig. 2. Radical scavenging activity (RAS) of hexan (A), ethyl acetate (B) and ethanol 80% (C) waste extract in DPPH and standardization of ghee for different time interval

Retention time and peak area were used for calculation of phenolic acid compounds by the data analysis of Hewllet Packared Software. The products were in excellent condition when originally analyzed, but, when the present study was undertaken, had been maintained in storage under the conditions mentioned earlier for 3-4 years. The characteristics were determined in the usual way: acidities are expressed as acid values (mg. KOH/g. fat); peroxide values as ml. 0-002w-Na2S203/g. fat; and refractive indices in terms of butyrorefractometer degrees at 400.

Oxidation stability test of ghee using Rancimat equipment: Determination of an oxidative stability of ghee by Rancimat equipment, was based on volatile acids from oxidation reaction passed through deionized water, in which conductivity values were detected. Heating block was held constant at130 C. A rate of air flow through liquid butter oil (ghee) was 10 l/h.

RESULTS AND CONCLUSION

Determination of total phenolic compounds (TPC): TPC of different food processing waste extracts were determine do the basis that the Folin–Ciocalteu method measures the reduction of the reagent by phenolic compounds via the formation of a blue complex that can be measured at 760 nm against GAE as a standard. The amount of TPC varied in the different extracts, ranging from 0.89 to 261.69 mg GAE g 1 extract (Table 5). In general, the results stated that ethanol 80% and ethyl acetate were better than hexane in extracting phenolics from Granules and Pallets owing to their higher polarity and good solubility

(Siddhu raju and Becker, 2003; Kequan and Liangli, 2004). On the other side, hexane extracted the highest amount of phenolics from Powder followed by ethanol 80% as shown in Table -2.

Identified phenolic compounds of food processing wastes: Table-3 - shows the percentage of identified phenolic compounds in Granules, Pallets and Powder. There was a great variation among the components identified in each waste by-product. Phenolic compounds identified in Granules were pyrogallol, protocatechuic, catachin and ellagic acid with amounts ranging from 0.07 to 10.64 mg/g. The main phenolics identified in powder were pyrogallol, gallic acid, vanillic acid, protocatechuic, ellagic acid with amount ranging from 0.01 to 1.0 mg/g. The major phenolic compounds identified in PP were pyrogallol, gallic acid, coumaric acid, caffeine with amount ranging from 0.05 to 12.64 mg/g.Balasundran et al. (2006)stated that the antioxidant activity of phenolic compounds depends on the structure, in particular the number and positions of the hydroxyl groups of the nature of substitution on the aromatic rings. Moure et al. (2001) reported that the antioxidant compounds from residual sources could be used for increasing the stability of foods by preventing lipid peroxidation and also for protecting oxidative damage in living systems by scavenging oxygen radicals. It is well known that total antioxidant activity of waste extracts was lineearly proportional to the concentration of total phenolics.

RSA (Radical scavenging activity) against DPPH (2,2-diphenyl-1-picrlhdrazyl): The results of RSA of various extracts are represented in Fig.18. The results clearly indicated that all extracts exhibited antioxidant activity. The extracts that contained high amount of TPC (Table-2) showed high RSA. In general, ethanolic extracts followed by hexane then ethyl acetate extracts showed RSA as strong as that of control (Fig.2). It has been proved that the antioxidant activity of plant extracts is mainly ascribable to the concentration of phenolic compounds.

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