Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 5683 - 5693
Received 25 April 2021; Accepted 08 May 2021.

Production of Natural Antioxidant Rich Food Products (Bread, Tofu & Curd) – An Alternate to Synthetic Food Products

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ABSTRACT

Antioxidants are important compounds that are used in the cosmetics and food industries. They are used in food processing industries as additives to inhibit oxidation, to enhance the shelf life of the food products, smell, flavour, etc. There are different types of antioxidants. Recent studies show that synthetic antioxidants like BHA, BHT and propyl gallate which are used commonly in food processing industries can have side effects on human health. Most fruits and vegetables are rich in antioxidants which are beneficial for our health. The objective of this review work is therefore to use natural antioxidants from fruits and vegetables instead of synthetic antioxidants to enhance the shelf-life of food products. In the study, apple peels, beetroot peels and green coffee beans were used as natural sources of antioxidants. Among the three extracts, apple peels exhibited the highest percentage of antioxidant yield of 16.4%, followed by beetroot peels (12.5%) and green coffee beans had the lowest yield (8.4%). Phenolic and flavonoid content was also highest in apple peels (71.56 mg/mL and 54.80 mg/mL respectively). Green coffee beans had higher phenol and flavonoid content than beetroot peels (62.97 mg/mL > 51.17 mg/mL and 12.22 mg/mL >4.70 mg/mL respectively). The natural extracts were then used in tofu, curd and bread for enhancing their shelf-life by preventing and decreasing peroxide formation. Thus, natural antioxidants can be used to enhance shelf-life of food products and may impart health benefits when consumed in comparison with synthetic antioxidants.

Keywords: Antioxidant, DPPH, hydrogen peroxide

INTRODUCTION

Antioxidants are the substances that protect the cells from being damaged by free radicals in such a way that they inhibit the occurrence of oxidation in the organisms. Oxidation is a chemical process that produces free radicals. Free radicals when present at a high level in the body can cause harm. They are linked to many diseases like lung diseases, cancer, diabetes, heart diseases etc., They are caused by exposure to toxic chemicals and

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pesticides, fried foods, alcohol, smoking, ionizing radiation, UV light, inflammation and many more. Antioxidants are scavenging radicals and act by inhibiting initiation and breaking of chain reaction, suppressing the formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, and quenching superoxide and singlet oxygen [1].

Antioxidants are of different types – natural antioxidants, synthetic antioxidants, endogenous antioxidants, exogenous antioxidants and enzymatic antioxidants. Synthetic antioxidants like Butylated hydroxyacetic acid (E-320), tert-butylated hydroxyquinone (E-321) and propyl gallate (E-312) are the most commonly incorporated ones in food industries. Coffee has been claimed as a functional beverage being an important source of antioxidants in the human diet, especially due to the high amounts of phenolic compounds and their derivatives (such as chlorogenic acids), alkaloids, diterpenoid alcohols (such as cafestol and kahweol), carbohydrates, lipids, and volatile and heterocyclic compounds [2]. Coffee beans are generally roasted before consuming which changes the colour, flavour and odour. However, the roasting process introduced causes 8–10% chlorogenic acid degradation and 11% to 45% polyphenol degradation [3].

Beetroot *(Beta vulgaris* L.*)* is considered to have more antioxidants in comparison with other vegetables. The phenolic antioxidants in beetroot mainly consist of phenolic acids, flavonoids and catechins. Beetroot is a potential source of valuable water-soluble nitrogenous pigments, called betalains, and these compounds are widely used as additives in the food industry because of their natural colourant properties and absence of toxicity [4].

Apples *(Malus domestica*) are consumed and used as large contributors of phenolic compounds. It is known that the concentration of total phenolic compounds is much greater in the peel of apples than in the flesh [5]. The addition of antioxidants in food products has many advantages. They are easy to incorporate into foods, can be added at low levels, and are cost-effective. According to the required period of the shelf-life for the packaging, long distribution and storage conditions, antioxidants added to the foods may differ. Tofu and curd are Indian traditionally coagulated products, which are consumed all over India and bread is also consumed not only in India but throughout the world. Nature is always the best supplier and rich source of countless ingredients and compounds that are suitable for health. Therefore there is a trend towards replacing synthetic antioxidants with natural ones. The present study aims to use natural antioxidants for the preparation of food products which will be cost-effective and boost the overall health of the consumer.

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MATERIALS AND METHODS

Materials

Chemicals: Ethanol, sodium hydroxide, Ferric chloride, Folin-Ciocalteau reagent, Sodium carbonate, Gallic acid, Hydrogen peroxide, Phosphate buffer, DPPH (Diphenyl Picryl Hydrazyl), Ascorbic acid, Potassium iodide, Glacial acetic acid, Chloroform, Sodium thiosulphate, Aluminium chloride.

Raw materials: Peels of apples and beetroots, Green coffee beans

Experimental procedure:

Extraction of antioxidants: 10 grams of the dried powdered samples were soaked in 100 ml of ethanol, plugged with cotton and kept in a shaker at 120 revolutions per minute for 2 days. The extract was filtered using Whatman filter paper for removal of unwanted peel particles and it was kept in a hot air oven for evaporation of solvent at 40ºC.

Determination of yield of extraction:

The extracts obtained after the filtration were weighed to determine the yield of the extraction. Extraction yield (%) = (Weight of the residue)/(Total weight of the powdered samples taken) x 100

Qualitative analysis

Determination of flavonoid: Alkaline Reagent Test has used. 2 ml of 2.0% Sodium hydroxide was added to 1 ml of extract.

Determination of phenol: Ferric Chloride Test was done. To 2 ml of sample extracts, 1 ml of ferric chloride was added.

Quantitative analysis

Determination of total flavonoid content: The flavonoid content was determined according to the modified aluminium chloride colorimetric method. 1 ml of the extracts were taken in test tubes and 4 ml of distilled water was added to each test tubes. Then, 0.3 ml of 5% sodium nitrate was added. After 5 minutes, 0.3 ml of 10% Aluminium chloride was added. 2 ml of 1M Sodium hydroxide was added after 2 minutes and the content was diluted with 10 ml of distilled water. It was then kept in the dark for 30 minutes and measured at 510 nm (6).

Determination of total phenol content: 0.2 ml of samples were taken in test tubes. 0.8 ml of Folin-Ciocalteau reagent was added to the test tubes. 2 ml of 7.5% sodium carbonate was then added. The total content was diluted to 7 ml with distilled water and then left in the dark

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for 2 hours. After this, it was measured at 765nm using a colorimeter. Gallic was used as a standard [7].

Determination of Hydrogen peroxide radical scavenging activity of antioxidant extracts: A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (100 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). The absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of the extracts and standard compounds were calculated:

% Scavenged [H2O2] = [(AC – AS)/AC] x 100

Where AC is the absorbance of the control and AS is the absorbance in the presence of the sample extracts and standards.

Determination of DPPH Radical scavenging activity of antioxidant extract: The antioxidant activity of each sample was calculated from the graph after plotting inhibition percentage against extract concentration. DPPH assay was carried out after making some modifications in the standard protocol [8]. 1.5 ml of 0.1mM DPPH solution was mixed with 1.5ml of various concentrations (10 to 500µg/ml) of peel extract. The mixture was shaken vigorously and incubated at room temperature for 30 minutes in the dark. The reduction of the DPPH free radical was measured by reading the absorbance at 517nm by a Spectrophotometer. The solution without any extract and with DPPH and methanol was used as control. Ascorbic acid was used as positive controls. Inhibition of DPPH free radical in percentage was calculated by the formula:

$$\textbf{Inhibition (\%)} = [(A_{control} - A_{test}) / A_{control}] \times 100$$

Where A control is the absorbance of the control (L-Ascorbic acid) and A test is the absorbance of reaction mixture samples (in the presence of sample).

Preparation of curd, tofu and bread with antioxidant extracts: Fresh curd and tofu were prepared and fresh bread was bought from the bakery. 10g of each of the food products were taken and 5% of the antioxidant extracts were added to each of the food products. Controls were prepared without antioxidant extracts addition [9].

Determination of peroxide value of the food products: Accelerated oxidation test or Schall Oven's test was performed to accelerate lipid oxidation in the food products. Lipid oxidation was determined as a change in peroxide value. Peroxide value titration was performed by the

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following steps: A clean dry boiling tube was used to measure 10 g of the food sample. 2.0 g of powdered potassium iodide was added and then 20 ml of solvent (2 vol. glacial acetic acid + 1 vol. chloroform) was added into the tube. The tube was placed in boiling water such that the mixture boils within 30 seconds and then allowed to boil vigorously for more than 30 seconds before it is poured quickly into a flask containing 25 ml of water and the mixture in the flask was titrated against with 0.01 N sodium thiosulphate solution using starch as an indicator. The blank was performed at the same time.

The experiment was repeated and calculation was done as below

$$\textbf{Peroxide value}=((S-B)\times N\times 100)/W$$

Where, S, B, N and W are the volume of titrant of the sample, blank, normality of sodium thiosulphate and weight of sample respectively. The food products were stored for 8 days in glass bottles. The peroxide value was determined for each food products (curd, tofu and bread) with the antioxidant extracts of apple peels, beetroot peels and green coffee beans on the first day of storage and the eight-day of storage. Control was also performed at the same time for each food products. Commercial products of tofu, curd and bread were also stored for 8 days and the peroxide values were taken for each product following the same protocol.

RESULTS AND DISCUSSION

Extraction of antioxidants: Table 1 shows the percentage of extraction yield in each sample. Apple peels showed the highest extraction yield of antioxidants of 16.4%, followed by beetroot peels of 12.5% and green coffee beans showed the lowest extraction yield of 8.4%. Ethanol was the solvent used for the extraction of the antioxidants. The cold extraction method was used as it is cheaper than non-conventional methods. Extraction is an important step to isolate bioactive compounds from the samples. Different solvents can be used for the extraction of antioxidants. The extraction yield also varies depending on the solvents. Ethanol and methanol can extract higher yield compared to distilled water, chloroform, dichloromethane, and acetone extracts because the polarity of the solvents has an influence on the extraction of natural ingredients with antioxidant activity [10]. However, ethanol was particularly used because it is a highly polar solvent which can extract the bioactive compounds in higher yield and is safe for human.

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Table 1: Results of the calculated percentages of extraction yield of each sample

Sl. No.	SAMPLES	WEIGHT OF THE RESIDUES (in gm)	EXTRACTION YIELD (%)
1	Apple peels	1.64	16.4
2	Beetroot peels	1.25	12.5
3	Green coffee beans	0.84	8.4

Qualitative analysis of phenol and flavonoid: The analysis showed that flavonoid and phenol are present in all three samples by showing yellowish colour (indicating the presence of flavonoid) and dark blackish colour (indicating the presence of phenol).

The dark blackish colour was due to the presence of a phenol group in each sample. When ferric chloride was added, phenols present in the samples form a complex with ferric ions, this complex has an intense colour which may vary depending upon the nature of phenol. When flavonoids containing samples were treated with sodium hydroxide, a yellow colour developed. Samples containing more flavonoids showed a more intense yellowish colour.

Quantitative analysis of total phenol and flavonoid content: The total phenolic content was found to be highest in apple peels (71.56 mg/mL) which was followed by Green coffee beans (62.97 mg/mL) and Beetroot peels had the lowest phenolic content (51.17 mg/mL). Apples are a good source of phenolic compounds [11]. It has a high content of two polyphenols – Procyanidin B2 and Epicatechin.

The aluminium chloride colorimetric method showed that apple peels had the highest concentration of flavonoid (54.80 mg/mL) than green coffee beans (12.22 mg/mL) and beetroot peels (4.70 mg/mL).

Apple peels possess all of the additional flavonoids not found in the beetroots and green coffee beans such as quercetin glycosides and therefore contain the highest content of flavonoid. Of the catechins, only (+)-catechin and (-) – epicatechin are present in appreciable amounts in apples, with epicatechin being approximately twice as concentrated as catechin in the peels.

Total antioxidant activity by Hydrogen peroxide radical scavenging assay: The hydrogen peroxide radical scavenging activity of the three samples (apple peels, beetroot peels and green coffee beans) were determined by calculating the percentage inhibitions and is shown

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in the figure. The antioxidant extracts of each sample were added in two different concentrations – 10 µl (Concentration A) and 20 µl (Concentration B). As the concentration increases, the OD value decreases and the inhibition percentage increases which indicates that the activity of hydrogen peroxide was inhibited or lowered by the antioxidants. Results show that the scavenging activity of apple peels was the highest in the order of Apple peels > beetroot peels > green coffee beans.

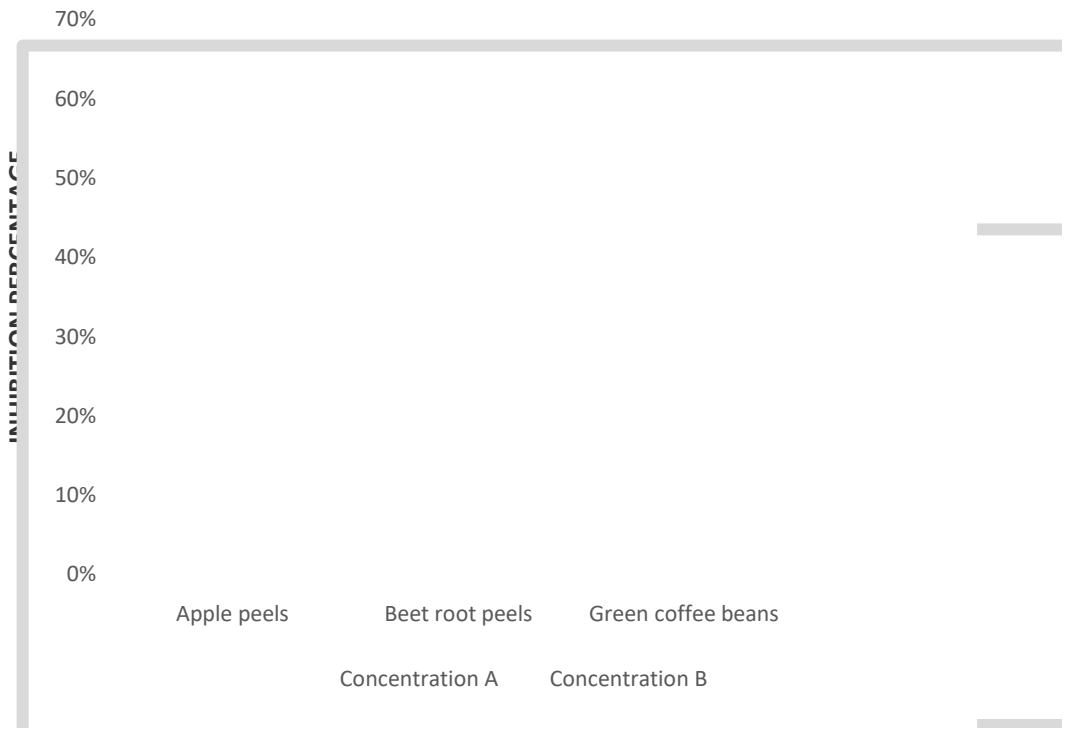

Figure 1: Result of Hydrogen peroxide radical scavenging assay by each sample

Total antioxidant activity by DPPH assay: Figure 2 shows the DPPH radical scavenging activity of the three samples. The extracts were capable of inhibiting the DPPH activity and the inhibiting percentage was evaluated. The result showed that Apple peels had the maximum antioxidant activity of 63% which was followed by Beetroot peels of 62%. Green coffee beans had the lowest antioxidant activity of 46%. The scavenging ability of each sample was due to the presence of flavonoid and phenols. A similar effect of antioxidant activity was already identified in the leaves of *Psidium guajava* [12, 13].

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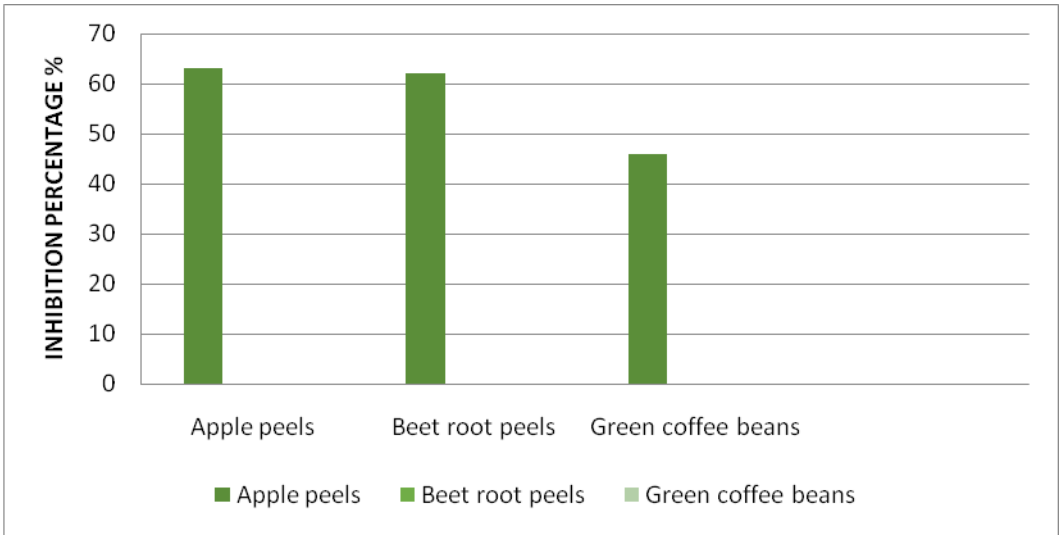

Figure 2: Result showing DPPH assay of each sample

Preparation of curd, tofu and bread with antioxidant extracts: Fresh curd, tofu and bread were prepared. Singh and Emmanuel [7] study, added the antioxidant extracts at different concentrations (1%, 2% and 3%). In order to get better results in this study, higher concentrations of antioxidant extracts (5%) were added to each of the food samples. Control was also prepared at the same time without antioxidant extract.

Effect of antioxidants on the peroxide value of food products: Accelerated oxidation test or Schall Oven test was performed [7]. The food products were stored for 8 days and the peroxide value was determined by the hydrogen peroxide titration method. Peroxide values were taken on the 1st day and 8th day of storage. Table 2, 3, and 4 show the evaluated peroxide values of bread, tofu and curd containing the natural antioxidants obtained from apple peels, beetroot peels and green coffee beans and their comparison with commercially available products of the same foods.

The peroxide value of each sample increases with an increasing period of storage. The 1st day of storage generally shows the same peroxide value in all the samples including the control samples. However, the 8th day of storage shows a significant difference in the values. The difference in the values with changes in the storage time indicates that the antioxidant activity decreases with increasing period of time.

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Table 2: Results of the effect of antioxidant extracts at the level of 5% on peroxide value of tofu compared with the peroxide value of commercial tofu

TOFU WITH NATURAL ANTIOXIDANTS				COMMERCIAL TOFU	
Sl. No.	**SAMPLES**	**1ST DAY**	**8TH DAY**	**1ST DAY**	**8TH DAY**
1	Control	0.05	2.46	0.06	1.45
2	Apple peels	0.07	1.42		
3	Beetroot peels	0.06	1.51		
4	Green coffee beans	0.05	1.80		

Table 3: Results of the effect of antioxidant extracts at the level of 5% on peroxide value of curd compared with the peroxide value of commercial curd

CURD WITH NATURAL ANTIOXIDANTS				COMMERCIAL CURD	
Sl. No.	**SAMPLES**	**1ST DAY**	**8TH DAY**	**1ST DAY**	**8TH DAY**
1	Control	0.04	2.34	0.06	1.58
2	Apple peels	0.06	1.44		
3	Beetroot peels	0.06	1.56		
4	Green coffee beans	0.06	1.65		

Table 4: Results of the effect of antioxidant extracts at the level of 5% on peroxide value of bread compared with the peroxide value of commercial bread

BREAD				COMMERCIAL BREAD	
Sl. No.	**SAMPLES**	**1st DAY**	**8thDAY**	**1ST DAY**	**8TH DAY**
1	Control	0.05	1.80	0.5	1.43
2	Apple peels	0.05	1.33		
3	Beetroot peels	0.05	1.49		
4	Green coffee beans	0.05	1.71		

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From the above tables, it is shown that apple peels had the maximum ability to enhance the shelf life of the food products, followed by beetroot peels and green coffee beans had the lowest potential. In comparison with the commercially available products containing synthetic antioxidants, the peroxide values of the food products containing natural antioxidants were almost the same or average (Apple peels in bread 1.33 > commercial bread 1.43 > beetroot peels in bread 1.49 > green coffee beans in bread 1.71).

When the food products (curd, tofu and bread) containing natural antioxidants were compared with commercially available products of the same food items in their ability, it can be concluded that apple peels and beetroot peels can be used as alternatives to synthetic antioxidants to decrease lipid oxidation and enhancing the shelf-life of the food products. While Green coffee bean has lower potential than the rest of the samples, it can still be used with greater amount or concentration. In addition, natural antioxidants are safer, nutritious and good for the health of consumers.

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