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Citrus peel can make anti-oxidant rich food with free radical scavenging property: Development, acceptability and evaluation

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Abstract
Formation of free radical is normal part of the body's stress response, but they can damage healthy cells and are most likely to attack the fats that provide structure to the cell membrane. Antioxidants are the compound which neutralizes the effects of free radicals. Fruits and vegetables are most important part of our diet because they provide several vital vitamins and phytochemicals. In fruit processing industry, large volumes of peels were thrown as waste material, which have very high antioxidant nutrients. This study was aimed to develop wholesome, nutritious, savoury cake with inclusion of citrus peels (Citrus limon peel and Citrus limetta peel), which is rich with antioxidant. Many variation of savory cake was made with different concentration of citrus peels. Chemical evaluations were also performed using acceptable products. The result showed that developed product was very rich in ascorbic acid, beta –carotene and total poly phenol and have high free radical scavenging property.

Keywords: antioxidant, phytochemicals, citrus peels

1. Introduction
Antioxidant is a molecule which can decrease the oxidative damage directly via reacting with free radicals or indirectly by inhibiting the activity or expression of free radical generating enzymes or enhancing the activity or expression of intracellular antioxidant enzymes. Antioxidants act as radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agents [1]. Almost all organisms possess antioxidant defense mechanism and repair systems, but these systems are insufficient to prevent the damage entirely. For this reason, dietary supplementation is necessary to strengthen the intrinsic protection systems. There are growing interests in using natural resources as antioxidants for preventive and therapeutic properties. It has been postulated that a network of antioxidants with different chemical properties may work in a synergistic way, protecting cells from damage. Without the necessary intake of antioxidants, free radicals can spread and eventually lead to stroke, heart attack, arthritis, vision problems, Parkinson's disease, Alzheimer's disease and various types of cancer. [2]
Citrus belonging to the largest genus in the family *Rutaceae* is the most traded horticultural product in the world. Citrus contain a variety of vitamins, minerals, fiber, and phytochemicals such as carotenoids, flavonoids, and limonoids, which appear to have biological activities and health benefits. The main industrial transformation of citrus is focused on juice production which results in the accumulation of huge amounts of by-products that account for about the half of the fruit weight. The industrial by-products contain peels, seeds and pulp membrane residues. Citrus peel, which is generally thrown away, contains a wide variety of secondary components with substantial antioxidant activity in comparison with other parts of the fruit [3]. Not only that Citrus peels are packed with immune-boosting vitamin C, bone-building calcium and anti-inflammatory, antioxidant bioflavonoids. They also provide potassium, which helps keep blood pressure in check, and limonene, a phytochemical that may have anti-cancer effects and can help with heart burn.

2. Materials and Methods
2.1 Materials
Sample collection - Fresh citrus fruits (*Citrus limetta* and *Citrus limon*) were purchased from local market, Kolkata, India. Citrus peels were finely grated from the fresh citrus fruits.
Reagents required - Bovine Serum Albumin (BSA), Biuret reagent, Anthrone reagent, petroleum ether, 2,6-dichloro indophenol dye, metaphosphoric acid, Folin-Ciocalteau reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent.

2.2 Product Development
Savoury cake was chosen for product development. Basic ingradients and recipe is presented in table1. Different variations were tried by incorporating two different citrus peels, i.e., *Citrus limetta* peel and *Citrus limon* peel with varying amounts of 5 gram, 10 gram, 15 gram, 20 gram, and 25 gram individually as well as in a combination (equal amounts) in the standard recipe of savoury cake (Table 2)

Table 1: Basic recipe/basic ingredient of savory/cake

Ingredients	Amount (gram)
Semolina	100
Bengal gram flour	50
Curd	80
Carrot	40
French beans	40
Red bell pepper	40
Onion	40
Ginger	1.25
Garlic	1.25
Green chilli	2.5
Mustard seeds	2.5
Sesame seeds	15
Honey	45
Baking powder	7.5
Red chilli powder	2.5
Turmeric powder	1.25
Coriander powder	5
Salt	15
Oil	20

Table 2: Different variation of Savory cake (variation was done with only by addition of two different citrus peels, i.e., *Citrus limetta* peel and *Citrus limon* peel)

Product code	Variations
Product A	Basic Recipe
Product B	5 gram *Citrus limetta* peel
Product C	10 gram *Citrus limetta* peel
Product D	15 gram *Citrus limetta* peel
Product E	20 gram *Citrus limetta* peel
Product F	5 gram *Citrus limon* peel
Product G	10 gram *Citrus limon* peel
Product H	15 gram *Citrus limon* peel
Product I	20 gram *Citrus limon* pee
Product J	2.5 gram *Citrus limetta* peel + 2.5 gram *Citrus limon* peel
Product K	5 gram *Citrus limetta* peel + 5 gram *Citrus limon* peel
Product L	7.5 gram *Citrus limetta* peel + 7.5 gram *Citrus limon* peel
Product M	10 gram *Citrus limetta* peel + 10 gram *Citrus limon* peel

2.3 Sensory Evaluation
The standard product and all the variations was evaluated organoleptically for different quality attributes (colour, appearance, texture, taste, and odour) and overall acceptability by 20 panel members using 9 point hedonic scale [4].

2.4 Chemical Analysis
The standard and approved products as chosen by the panel members were chemically analysed for their moisture content, total ash, protein, fat, total carbohydrate, vitamin C, beta carotene, phenolic compounds, and DPPH radical scavenging activity.

2.4.1 Determination of moisture and ash content
Moisture content of the products was determined by drying sample in a pre-weighed crucible in a hot air oven at 130°C for two hours. Ash content of the products was determined by placing the measured amount of sample in a pre-weighed crucible in a muffle furnace at 600°C for 3 hours [5].

2.4.2 Determination of macronutrient content
Protein content of the products was estimated by Biuret method [6]. Fat content of the products was measured by using Soxhlet method [7]. Total carbohydrate of the products was estimated by the Anthrone method [8].

2.4.3 Determination of ascorbic acid content
The ascorbic acid content of the products was measured by indophenol dye method. In a conical flask, equal amount of metaphosphoric acid and standard ascorbic acid was filtered through cotton. In a test tube, 1 ml of 2, 6-dichlorophenol indophenol dye was taken which was titrated with the filtered solution of metaphosphoric-ascorbic acid until colourless. The readings were noted in triplicates. Similarly, test samples were prepared by filtration of equal amounts of metaphosphoric acid-sample solution which was used to titrate the dye until colourless and the readings were noted in triplicates. The concentration of the ascorbic acid in the test samples was calculated [9].

2.4.4 Determination of beta-carotene content
Five gram of sample was taken, crushed in 10-15 ml of acetone with the help of pestle and mortar and few crystals of anhydrous sodium sulphate were added. The solution was centrifuged at 3000 rpm for 3-4 minutes. The supernatant was transferred to a separating funnel, 10-15 ml of petroleum ether was added and mixed thoroughly. Two layers separated out on standing. The lower layer was discarded and upper layer was collected in 100 ml volumetric flask. The volume was made to 100 ml with petroleum ether and optical density was recorded at 452 nm using petroleum ether as blank [10].

2.4.5 Determination of Total Phenolic content
Total phenolic content was measured by Folin-Ciocalteau's method. Firstly, 0.1 mL of extract was made up to 5 mL with distilled water in a 10-mL volumetric flask, followed by addition of 0.5 mL 2 N Folin-Ciocalteau's phenol reagent. About 1 mL of saturated (35% w/v) sodium carbonate solution was added into the mixture after three minutes. The mixture was made up to 10 mL with water. After 1 hour, the mixture was measured spectrophotometrically at 725 nm against the reagent blank. Gallic acid within the concentration range of 0-400 µg/mL assay solution was used as the standard curve for the total phenolic acids content. The reaction between the Folin-Ciocalteu reagent and phenolic compounds in alkaline medium results in the formation of a blue chromophore constituted by a phosphotungstic/phosphomolybdenum complex that absorbs radiation and allows quantification. Results were expressed as mg gallic acid equivalents (GAE)/100 g of fresh weight. [11]

2.4.6 DPPH Free Radical Scavenging Activity
0.4 mM solution of DPPH in ethanol was prepared. 1 ml of DPPH solution was added to 3 ml of ethanolic test sample extract. A control was made by adding 1 ml of DPPH solution to 3 ml of ethanol. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min then absorbance was measured at 517 nm by using

spectrophotometer [12].

3. Results and Discussion

The results of chemical analysis were expressed as mean ± standard deviation of triplicate analyses while mean of sensory scores for each attribute was based on twenty judgements.

The standard and approved products as chosen by the panel members were chemically analysed for their moisture content, total ash, protein, fat, total carbohydrate, vitamin C, beta carotene, phenolic compounds, and DPPH radical scavenging activity.

3.1 Sensory Evaluation

Acceptability of variation product/ recipe including basic were evaluated from the ratings obtained through the score card using 9 point hedonic scale during the sensory evaluation and comparative study between the products was done.(Table 3). Standard product was labelled as 'A'. Product B to Product E contains different concentrations of *Citrus limetta* peel; Product F to Product I contains different concentrations of *Citrus limon* peel; and Product J to Product M contains combination of the peels with varying amounts of 5, 10, 15, 20 gram for all the three categories. From each section most accepted product were chosen (D, H, L) and chemical analysis were performed,

Table 3: Sensory evaluation of different variations

Product code	Appearance	Colour	Texture	Taste	Odour	Overall rating
A	7.80 ± 0.59	7.90 ± 0.69	7.90 ± 0.63	7.45 ± 0.87	7.55 ± 0.59	7.85 ± 0.66
B	7.70 ± 0.54	7.65 ± 0.52	7.80 ± 0.60	7.30 ± 0.56	7.50 ± 0.50	7.35 ± 0.48
C	7.65 ± 0.48	7.70 ± 0.53	7.80 ± 0.60	7.30 ± 0.64	7.40 ± 0.49	7.40 ± 0.49
D	7.70 ± 0.56	7.75 ± 0.62	7.75 ± 0.54	7.40 ± 0.81	7.50 ± 0.67	7.50 ± 0.50
E	7.70 ± 0.64	7.65 ± 0.58	7.75 ± 0.43	5.75 ± 0.95	7.15 ± 0.80	7.00 ± 0.55
F	7.65 ± 0.73	7.60 ± 0.53	7.75 ± 0.77	7.65 ± 0.66	7.50 ± 0.67	7.65 ± 0.73
G	7.70 ± 0.56	7.70 ± 0.62	7.75 ± 0.70	7.70 ± 0.72	7.55 ± 0.50	7.70 ± 0.72
H	7.85 ± 0.66	7.75 ± 0.57	7.85 ± 0.57	7.75 ± 0.77	7.60 ± 0.74	7.75 ± 0.83
I	7.70 ± 0.85	7.70 ± 0.55	7.75 ± 0.53	6.50 ± 0.50	7.60 ± 0.67	7.50 ± 0.59
J	7.60 ± 0.74	7.70 ± 0.55	7.80 ± 0.68	7.45 ± 0.59	7.60 ± 0.67	7.45 ± 0.67
K	7.70 ± 0.72	7.75 ± 0.62	7.75 ± 0.77	7.50 ± 0.67	7.50 ± 0.59	7.50 ± 0.67
L	7.75 ± 0.63	7.85 ± 0.58	7.75 ± 0.70	7.75 ± 0.70	7.75 ± 0.63	7.80 ± 0.68
M	7.70 ± 0.64	7.70 ± 0.55	7.75 ± 0.77	6.30 ± 0.56	7.45 ± 0.50	7.10 ± 0.63

3.2 Chemical analysis

3.2.1 Total moisture and ash content

Total moisture content and ash of the products were estimated. Moisture content of the standard product and citrus peel supplemented approved product did not showed any changes. But the ash content of the citrus peel supplemented product showed higher value compared to the standard product. (Table: 4).

Table 4: Moisture and ash content of acceptable variations of product

	Standard	Product D	Product H	Product L
Moisture content (%)	39.4 ± 6.6	39.6 ± 7.5	39.7 ± 7.0	39.6 ± 6.3
Ash Content (%)	12.6 ± 2.5	15.1 ± 3.2	14.9 ± 3.5	15.2 ± 2.8

3.2.2 Total Macronutrient content

Protein, carbohydrate and fat content of the standard and citrus peel supplemented acceptable products ware estimated. It was found that the protein, carbohydrate and fat content of the standard and most acceptable product did not show much changes. Protein, carbohydrate and fat content (gm) per 100 gm of products were represented in Fig 1.

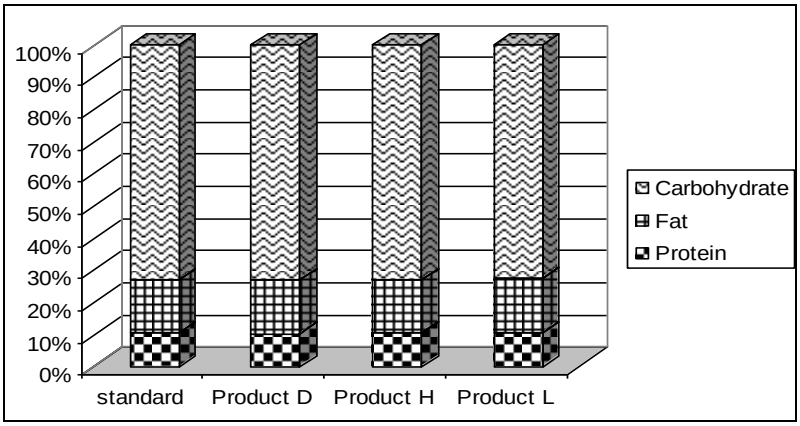

Fig 1: Graphical presentation of the amount of protein, carbohydrate and fat present in different variations of product

3.2.3 Beta-carotene content
Beta- carotene content of the citrus peel supplemented acceptable products were much higher compared to the standard product. Product D, Product H and Product L showed 16%, 11% and 18% increase of beta-carotene level compared to the standard product. (Table: 5).

3.2 4 Ascorbic acid content
Ascorbic acid content of the citrus peel supplemented acceptable products were much higher compared to the standard product. Product D, Product H and Product L showed 100%, 78 % and 107 % increase of ascorbic acid level compared to the standard product. (Table: 5).

3.2.5 Total Phenolic content
Total Phenolic content of the citrus peel supplemented acceptable products were much higher compared to the standard product. Product D, Product H and Product L showed 100%, 104 % and 112 % increase of total phenolic level compared to the standard product. (Table: 5).

Table 4: Amount of beta-carotene, ascorbic acid and total phenol content of different variations of product

Parameters	Standard	Product D	Product H	Product L
Beta- Carotene (µg/ 100 gm product)	496.4 ± 35.6	575.8 ± 40.6	546.4 ± 42.8	585.7 ± 46.7
Ascorbic acid (mg/100 gm product)	14 ± 2.6	28 ± 3.5	25 ± 4.2	29.5 ± 4.5
Total Phenol (mg GAE/ 100 gm product)	125.7 ± 20.2	255 ± 27.8	260 ± 30.3	270 ± 32.3

3.2.6 DPPH free radical scavenging activity
DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging ability is frequently used in the determination of free radical scavenging ability. Total Scavenging activity of citrus peel supplemented acceptable products was much higher compared to the standard product. Product D, Product H and Product L showed much higher scavenging activity compared to the standard product. (Fig: 2).

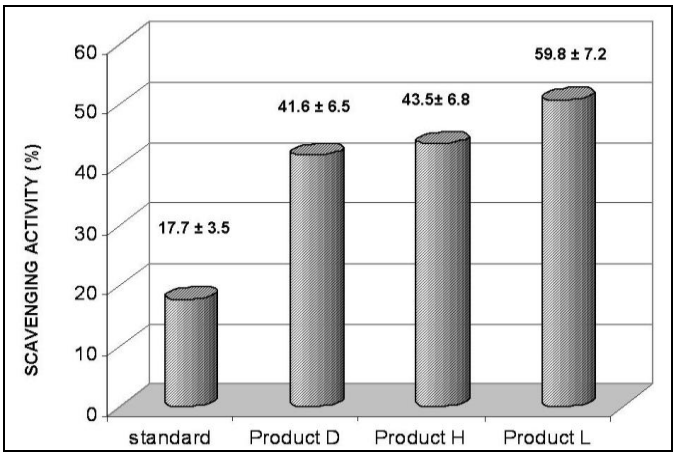

Fig 2: Free radical scavenging activity of different variations of product.

4. Conclusion
From the above observation it can be concluded that addition of citrus peels to the savoury cake significantly increased ascorbic acid, beta-carotene, phenolic content, and also exhibited higher DPPH free radical scavenging activity. Vitamin C being an important dietary antioxidant, significantly decreases the adverse effect of reactive oxygen and nitrogen species that can cause oxidative damage to macromolecules such as lipids, DNA and proteins which are implicated in chronic diseases including cardiovascular disease, stroke, cancer, and neurodegenerative diseases [13]. β-carotene acts as an antioxidant by quenching singlet molecular oxygen and scavenging reactive oxygen species, especially peroxyl radicals [14]. Phenolics are capable of scavenging free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals, and inhibit oxidases and helps in the management of degenerative diseases, such as diabetes and hypertension [15]. Increased DPPH scavenging activity indicated higher antioxidant activity of the product due to addition of citrus peels.

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