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Evaluation of β-Carotene Content and Antioxidant Activity of Banana Peels and Banana Peel Extracted Insoluble Dietary Fibres

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ABSTRACT

The present study explores the potential antioxidant activity and β-Carotene content of banana peel and banana peel extracted insoluble dietary fibres. Grand Nain cultivar of bananas was selected. Two stages of ripened banana peel powder mainly stage 3 and 4 were compared along with the extracted insoluble dietary fibre for their β-Carotene and antioxidant activity. β-Carotene content in stage 3 banana peel powder and banana peel extracted insoluble dietary fibre powder varied from 42.8 to 29.9 mg/100g and in stage 4 banana peel powder and banana peel extracted insoluble dietary fibre powder varied from 26.8 to 11.8 mg/100g respectively. Antioxidant activity varied from 61.41±1.46% to 61.08±9.46 in stage 3 banana peel powder and banana peel extracted insoluble dietary fibre powder respectively, whereas, in stage 4 banana peel powder and banana peel extracted insoluble dietary fibre powder it varied from 47.46±7.32% to 43.44±8.54% respectively.

Highlights

- It highlights the process of extraction of dietary fibres (Insoluble and soluble) from banana peel biomass.
- It highlights the presence of bioactive compound beta-carotene in banana peel and the extracted insoluble dietary fibre.
- It shows the variation in the content of beta-carotene and antioxidant activity according to the stages of growth.
- It highlights the importance of biomass as a rich source of nutrients and its possible utilization in food products.

Keywords: Banana, banana peels, insoluble dietary fibre, β-Carotene, antioxidant activity

Fruits and vegetable wastes and by-products, which are formed in great amounts during industrial processing, represent a serious problem, as they exert an influence on environment and need to be managed and/or utilized. These plant based by-products especially peels contain several compounds and nutrients that are important for human nutrition as they are considered to provide various health benefits. Efforts are being made to improve methods and ways of re-using fruits and vegetable wastes (Chodak and Tarko 2007). Recently, many studies have been oriented towards improving methods and efficiency of recovery from different fruit industry wastes. Chitturi *et al.* (2013) evaluated the protein levels and antioxidant potential of air-dried medicinally significant domestic fruit peels Gooseberry (*Phyllanthus emblica*), apple (*Malus domestica*), red banana (*Musa acuminata*), jujube (*Ziziphus zizyphus*), papaya (*Carica*), guava (*Psidium*), avocado (*Persea americana*), watermelon (*Citrullus lamatus*), muskmelon (*Cucumis melo*), kiwi fruit (*Actinidia delicosia*), orange (*Citrus limetta*), pineapple (*Ananas comosus*), mango (*Mangifera*), pomegranate (*Punica granatum*), sapota (*Manilkara zapota*) and

their extracts. Highest antioxidant concentration was observed in the peel of gooseberry (2.39%). Based on various recent studies, the peels and seeds of some fruits contain high amount of phenolic compounds, carotenoids and antioxidant potency (Ibrahim *et al.* 2016). Also, dietary fibre can be used in various functional foods like bakery, drinks, beverages and meat products (Dhingra *et al.* 2012).

Carotenoids are pigments found in plants and some microorganisms, but not synthesized in animals. The importance of carotenoids in food goes beyond as natural pigments. Biological functions and actions are now being increasingly attributed to these pigments. Carotenoids are present intra-cellularly and their actions involve in the regulation of gene expression or effect cell functions like inhibition of monocyte adhesion and platelet activation (Sharma *et al.* 2012). They also exhibit biological activities as antioxidants and affect immune response. Fewer than 10% of the carotenoids can function as vitamin A precursor in mammals. Carotenoids in tissues reflect food choices. Higher carotenoid intakes and tissue concentrations epidemiologically, have been associated with reduced cancer and cardiovascular disease risk, although results from clinical trials do not support this evidence. Continued research in this area is likely to stimulate better intervention strategies with clinical and public health applications (Rock 1997). Carotenoids show antioxidant activity, through deactivation of free radicals and singlet oxygen quenching (Fiedor and Burda 2014).

Banana peel is the main by-product of banana processing industry, representing approximately 30% by weight of fruit. Majorly, banana peels are used as a feed for livestock (Onwuka *et al.* 1997). Practical utilisation of banana by-products includes the production of biomass, protein, ethanol, methane, pectins and enzymes (Clarke *et al.* 2008). Besides, banana peels also show antioxidant scavenging activity which is relative to the presence of β-Carotene. The antioxidant scavenging activity is considered to suppress oxidative stress thereby, considered to have a preventive role towards various diseases. Although, the controversial issue surrounding the benefits of dietary antioxidants for health promotion is the lack of clinical evidence and specific molecular markers able to measure the impact of dietary antioxidants, not only on oxidative stress status, but on health status (Dalle-Donne *et al.* 2006). The present study was undertaken in order to determine the antioxidant activity present in banana peel biomass and effect of stages of growth in bioactive compounds like β-Carotene. Grand Naine variety of banana peels was investigated and banana peel insoluble dietary fibres were also extracted from the peels for studying them under same criterion. Two ripening stages of banana peels i.e. stage 3 (more green than yellow) and stage 4 (more yellow than green) were selected for analysis. Novelty in the present study lies in the fact that there are several underutilized, nutritionally rich biomass which could be alternative source of extraction or production of food based products or natural food additives. Dietary fibres are regularly studied for their health benefits. Therefore, banana peels at various growth stages could be one of those alternative nutritionally rich source.

MATERIALS AND METHODS

Plant material

Stage 1 and 2 of bananas are mainly plantain or green unripe bananas which require cooking before consumption. They are used for the production of edible products, like banana chips mostly in Southern states of India. They are numerous times considered as vegetables rather than a fruits. Therefore, for the current study Grand Nain variety of bananas (*Musa acuminate*) were purchased from the local market of Mohali (Punjab), India, at stage 3 (more green than yellow) and 4 (more yellow than green) of ripeness, the stages at which the fruit pulp is edible raw and does not require cooking. These bananas were the cultivars of the southern state of India, Andhra Pradesh. Chemicals were procured from Sigma-Aldrich or Merk (St. Louis, MO, USA).

Processing of banana peels

Banana peels were thoroughly cleaned to remove dust, dirt and other foreign materials. Banana peels were washed under running water and then dried in oven at 55^{0}C for 12 hrs. The fully dried peels were subjected to grinding in hammer mill (Polymix Px-MFC 90D by Kinematica). The mesh size of sieve was 1.5 mm.

Extraction of dietary fibre

Gravimetric method with water as solvent was used

for the extraction of dietary fibre. Banana peels were washed thoroughly and water was added to the sample. Supplementary fig. 1, 2 and 3 indicates the whole process. The entire mixture was then placed in water bath at 90 °C for 4 hrs with constant stirring. The mixture was then strained with the help of a muslin cloth. The residue consisted of insoluble dietary fibres while the supernatant was used for the precipitation of soluble dietary fibres with the help of ethanol.

Approximately, 42.5g/100g (DW) of insoluble dietary fibre was extracted from banana peels. Insoluble dietary fibre offers many benefits to intestinal health, including a reduction in the risk and occurrence of hemorrhoids and constipation. Over the past decade, the importance of 'intrinsic intact dietary fibre from plant tissue', and questions concerning the importance of separately determining 'soluble' and 'insoluble' dietary fibre components has increased (McCleary 2003). Chau and Huang (2003) isolated the fibre rich fractions (FRFs) including soluble and insoluble dietary fibres (SDF and IDF) from the peel of orange (*Citrus sinensis*). They concluded that the consumption of these peel insoluble FRFs have desired phytochemical properties and can be used as a source of food fibres or low-calorie bulk ingredients in food applications requiring oil and moisture retention. According to (Slavin and Lloyd (2012), cooking may increase the fibre content of a product if water is driven out in the cooking process. Baking or other heat treatments (e.g., extruding) used in food processing also increase the fibre content of the product, either by concentrating the fibre by removal of water or producing Millard products that are captured as fibre in gravimetric methods**.** Cereals, seeds, beans, many fruits and vegetables, bran and whole grain are food sources of insoluble fibre (Marlett *et al.* 2002 and Derek and Slavin 2008). World Health Organisation recommends a daily intake of 30 g of dietary fibre.

β-Carotene analysis

β-Carotene was evaluated by "Reversed phased HPLC system", the method of (Ahamad *et al.* 2007) with some modifications. Five grams of sample was homogenized in 30ml of acetone and then 0.1% (BHT) was added as an antioxidant. The resulting extract was filtered through Buchnar's funnel. The residue was washed twice with acetone till it became colourless. The residue was discarded and the filtrate was combined with 20gm of anhydrous sodium sulphate as a dehydrating agent. The anhydrous sodium sulphate was removed through filtration. The extract was transferred quantitatively to 50ml volumetric flask. β-Carotene was detected using Ultra Performance Liquid Chromatography (UPLC). Precautions were taken in order to perform all the operations under reduced light and at 4 °C.

Standard Preparation of β-Carotene

Standard of β-Carotene (5g enclosed in vial) was obtained from Sigma-Aldrich or Merck. Stock solution of β-Carotene was prepared by taking 1mg in 10ml n-hexane. The concentration of stock solution was equal to 100 ppm. The stock solution was diluted to different known concentrations i.e. 0.5, 1, 2.5, 5 and 10 ppm, dilutions were obtained in 5 ml of n-hexane solution. The standards along with the samples were analysed using Waters UPLC programme, having C18 guard column, connected with PDA detector. Peak identification and quantification was made by "Empower TM ^{3} Software" for UPLC system. UPLC was calibrated by running mobile phase (Acetonitrile, dichloromethane and methanol by the ratio of 70:20:10, respectively) at the rate of 1ml per minute. Wave length was fixed at 452 nm. Each standard solution (10µl) of beta carotene was injected when the injector was in load mode. The standard beta carotene peak was achieved at the retention time of 5.4 minutes (Rt = 5.4). The concentrations of the beta carotene standards were plotted against the peak area to obtain a straight line.

Sample Assay

Each sample of beta carotene extract in acetone was used for UPLC assay like standard; each banana peel and banana sample (10µl) was injected using micro litre syringe. The sample before injection into the UPLC system was filtered through 20 mm syringe filter. The peak was automatically identified and quantified by comparing the retention time of the sample with the standard retention time.

Antioxidant activity analysis

The sample of banana peels (stage 3 and stage 4) for antioxidant activity was prepared according

to the method described by Zhang and Hamauzu (2004) with some modifications. Two-gram sample was homogenized with 6 ml of 80% methanol for two hours at 30 ˚C. The homogenate was filtered through four layers of cheesecloth. The filtrate was centrifuged at 4000 rpm for 10 minutes. The supernatant of methanol extract was collected. The extract was used for total antioxidant activity determination. Antioxidant activity was determined by the 1, 1- diphenyl-2-picryl-hydrazyl (DPPH) method of Brand-William *et al.* (1995) with some modifications. A 0.1mM solution of DPPH in methanol was prepared and 4 ml of this solution was treated with 4.5 µl of extract. The mixture was left to stand for 30 minutes in dark and the absorbance was measured at 517 nm. Antioxidant activity was expressed as the percentage of DPPH decrease using the equation:

$$AA\% = \frac{\text{Control Absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Statistical Analysis

Paired t-test was applied to calculate the statistical differences between the β-carotene and antioxidant activity of the sample using Microsoft Excel Programme package 2010.

RESULTS AND DISCUSSION

β-Carotene detection

β-Carotene compound was detected in the samples using UPLC and the peak area was used for calculating the concentration of β-carotene in the sample. Supplementary fig. 4 indicates the difference. Significant difference ($p<0.05$) was found between the β-Carotene content of banana peels of stage 3 and 4 with values equivalent to 42.8 and 26.8 mg/100g respectively. Similarly, significant difference ($p<0.05$) was observed between the β-Carotene content of banana peel insoluble dietary fibres (stage 3 and 4) with values equal to 29.9 and 11.8 mg/100g respectively. Non-significant difference ($p>0.05$) was observed between the β-Carotene content of fresh banana (6mg/100g) and fresh banana peels (6.5mg/100g). Amount of β-carotene present in dried and powdered banana peels was found to be significantly higher than that of fresh banana peels. This could be attributed to the compound getting concentrated due to heat and drying process of the sample. β-Carotene content may vary according to the solvent, time and temperature conditions put forth for extraction. Kaur *et al.* (2017) studied Nendran and Rasthali cultivars were studied for changes in carotenoids in fruit-peel as collected at unripe and ripe stages. A higher amount of β-Carotene was present in banana peels of unripe (2500 µg/100g) Nendran variety as compared to ripe stage (500 µg/100g) whereas, in case of Rasthali variety β-Carotene present in ripe peels was found to be more. Arora *et al.* (2008) estimated the β-Carotene content in two varieties of banana peels and fruit of banana cultivars i.e. Karpooravalli and Red banana. β-carotene content in the banana peels of Karpooravalli variety was 143.12 µg/ 100g and in the peel and pulp of Red banana variety was 241.91 µg/ 100g peel and 4 µg g^{-1} respectively. Banana peel and fibre therefore, are a good source of β-carotene.

Table 1: Paired t-Test table (without replicates)

	Banana peel	Banana peel insoluble dietary fibre
Stage 3	42.8	29.9
Stage 4	26.8	11.8

p>0.05 or t stat>t crit (Significant difference).

Table 2: Paired t-Test table

	Banana peel	Banana peel insoluble dietary fibre
Stage 3	61.41±1.46	61.08±9.46
Stage 4	47.47±7.32	43.44±8.54

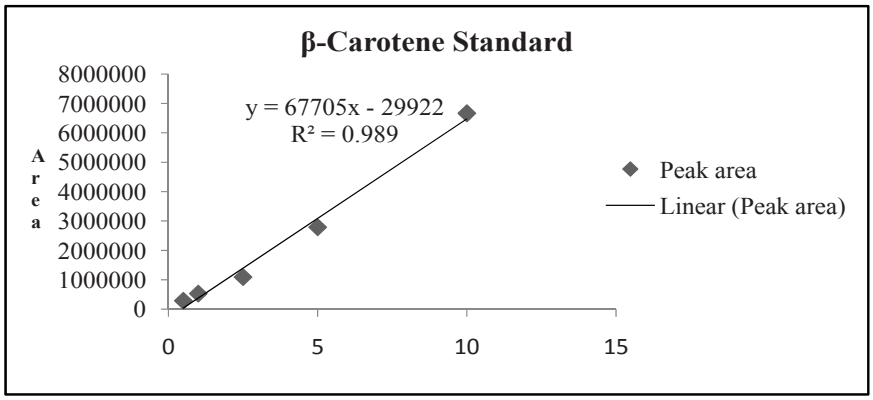
P<0.05 or t stat<t crit (Non-significant difference).

Antioxidant activity analysis

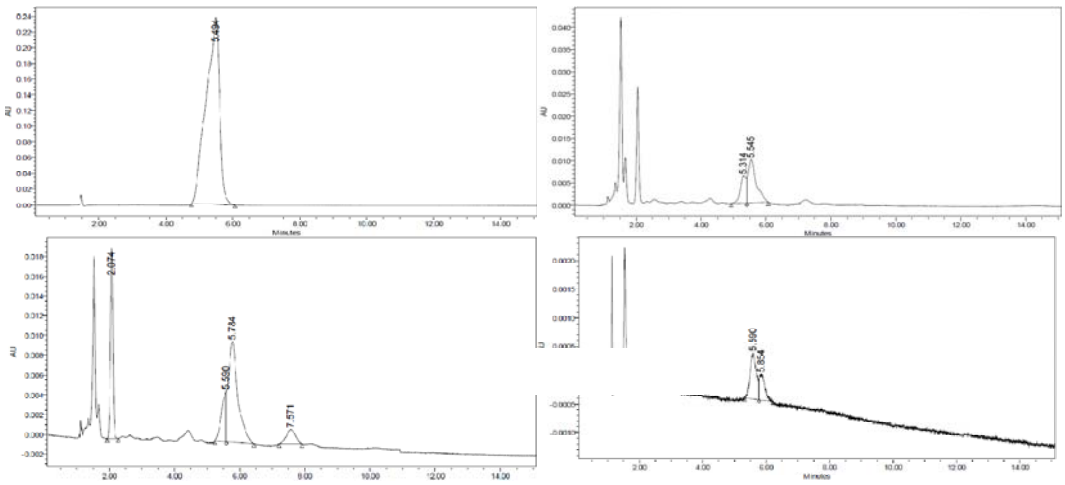
There was a non-significant difference between the antioxidant activity of banana peels stage 3 and 4 with values equivalent to 61.41±1.46% and 47.46±7.32% respectively. Supplementary fig. 5 indicates the data. Antioxidant activity in banana peel insoluble dietary fibres of stage 3 and 4 were 61.08±9.46 and 43.44±8.54% respectively. Increasing the concentration of the banana peel dietary fibre extract in relation to banana peel extract showed

SUPPLEMENTARY FILE

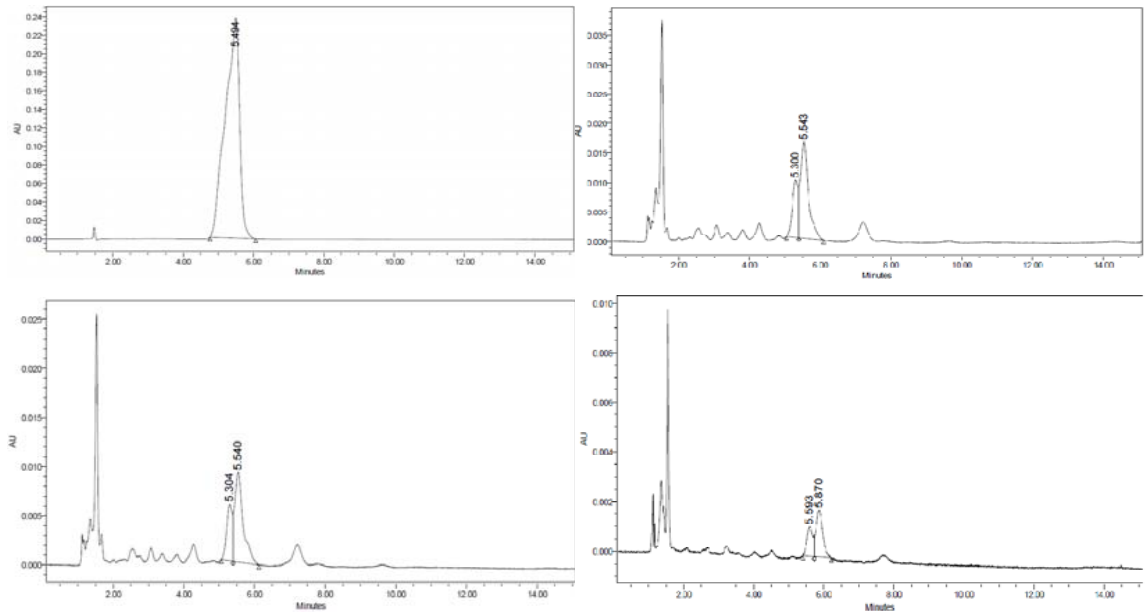
Calibration curve



Concentration (ppm)	Peak area
10	6666770
2.5	1092198
5	2790389
1	529911
0.5	288633



(a) Standard βcarotene peak at 452nm **(b)** Stage 3 banana peelinsoluble dietary fibre βcarotene peak **(c)** Stage 4 banana peel insoluble dietary fibre βcarotene peak **(d)** Fresh banana fruit βcarotene peak



(a) Standard βcarotene peak at 452nm **(b)** Stage 3 banana peel βcarotene peak **(c)** Stage 4 banana peel peel βcarotene peak **(d)** Fresh banana peel βcarotene peak

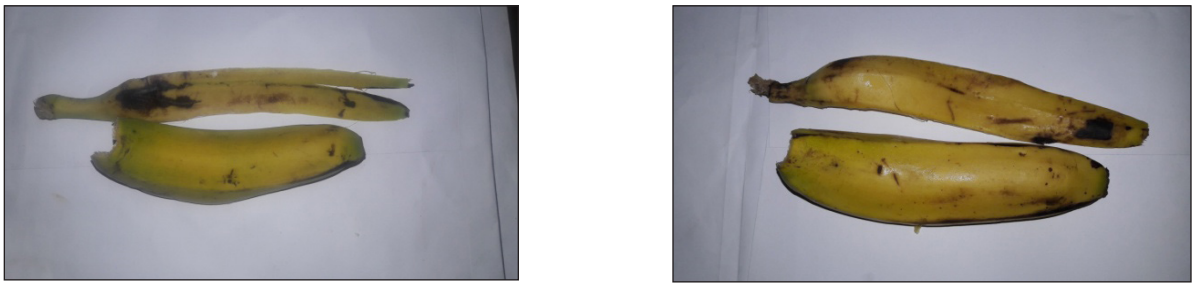


Fig. 1: Stage 3 and 4 ripened banana peels

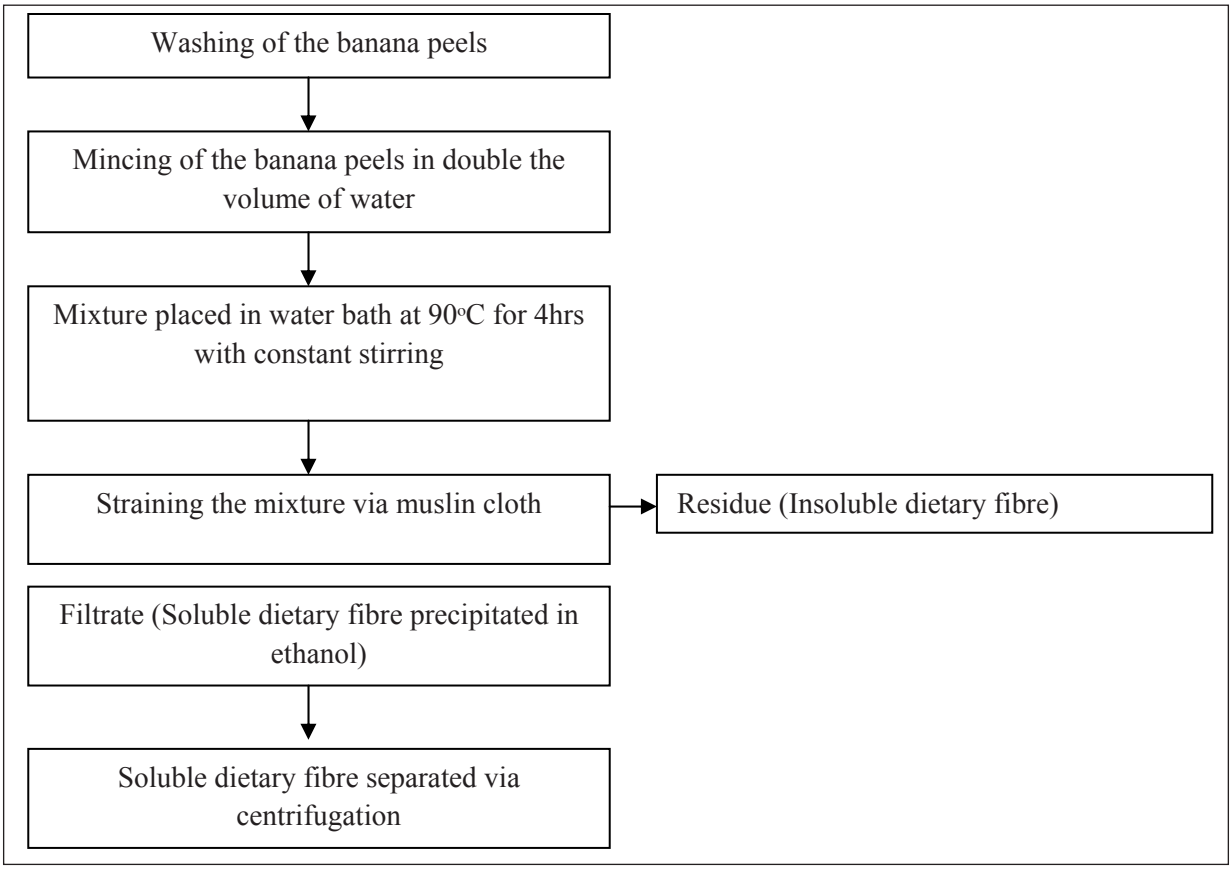


Fig. 2: Flow diagram showing the extraction of dietary fibres from banana peels

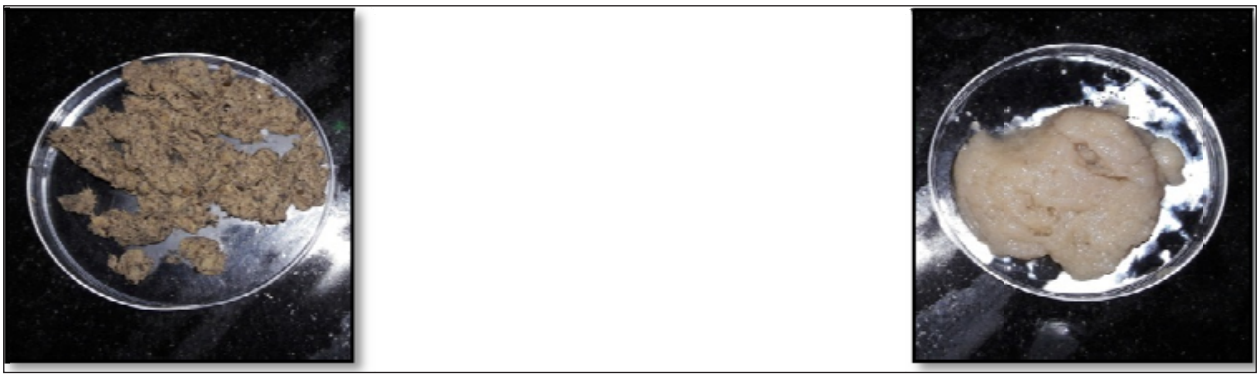


Fig. 3: Banana peel insoluble dietary fibre and soluble dietary fibre

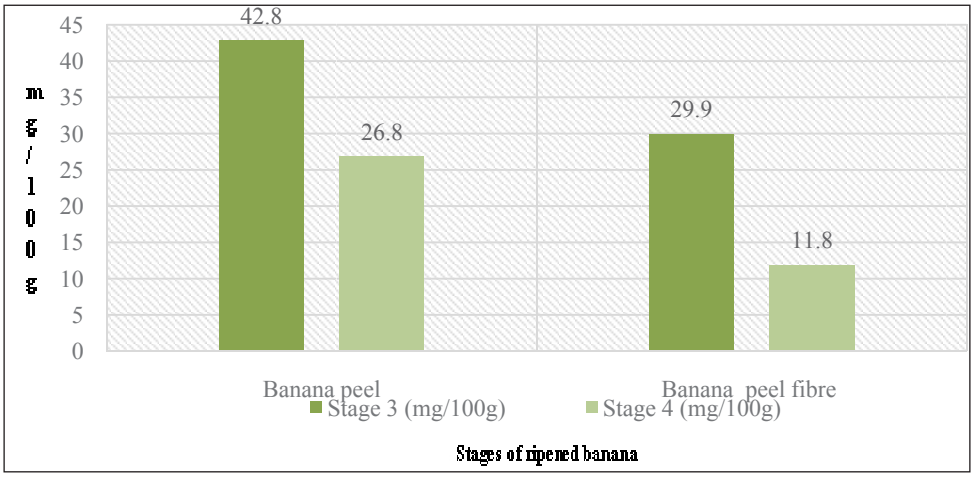


Fig. 4: β-Carotene content in banana peel and banana peel insoluble fibre

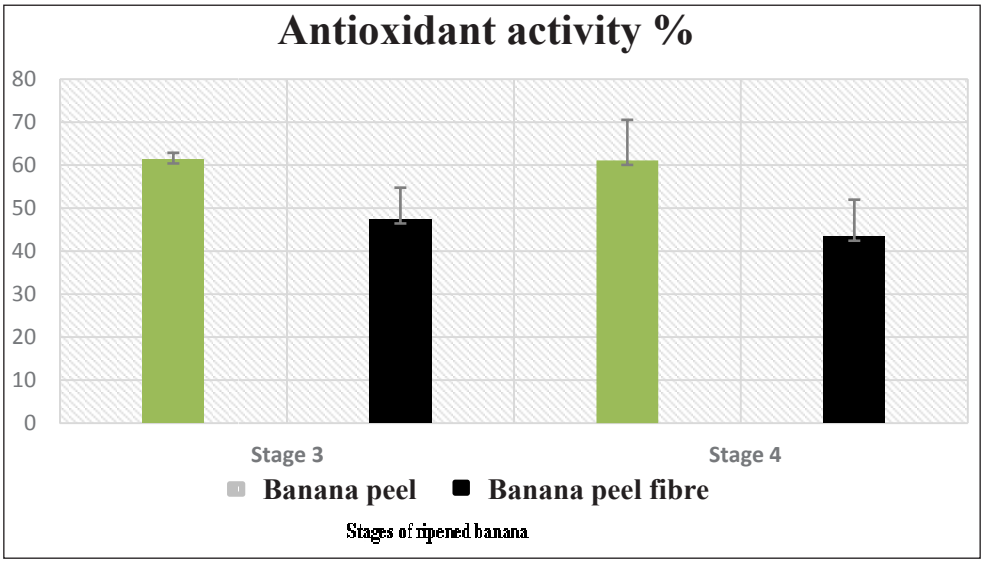


Fig. 5: Percentage antioxidant activity in banana peels and banana peel fibre

some anti-oxidant activity. Antioxidant activity may vary according to the solvent, time and temperature conditions put forth for extraction.

Gonzalez-Montelongo *et al.* (2010) analysed the antioxidant capacity (mg Trolox equivalents/100g DW) of banana peels of Grand Naine variety using various solvents, temperature and time. According to them the solvent system of acetone: water (1:1) at 55 ˚C, 1 min showed the highest antioxidant capacity i.e. 133±3 mg Trolox equivalents/100g DW. In case of methanol (104±1 mg Trolox equivalents/100g DW) as solvent highest extraction time and temperature was 120 min and 55 ˚C. Baskar *et al.* (2011) studied the antioxidant activity of various banana peel varieties. Pachainadan variety showed highest activity in the range of 5.85 mMg^{-1} in comparison to other varieties of banana peel, whereas the ethanolic extract of Nendran showed least activity. The total antioxidant activity of three varieties of banana peel samples in water extract ranged from (74080 – 94803 µmoles) followed by methanol (48577- 66727 µmoles) and ethanol (44558 – 47670 µmoles) extracts (Nagarajaiah and Prakash 2011). Antioxidants are substances which are both nutrients, viz. vitamins E, C, β-Carotene, selenium and non-nutrients, viz. plant phenols, flavonoids, coumarins, benzyl isothiocyanates, caffeic, ferrulic, gallic and ellagic acids, some enzymes like, catalase, superoxides mutase. It is well established that vegetables and fruits, legumes and spices and beverages such

as tea and wine and cereals are excellent sources of antioxidant, some scientific evidence for their protective role is available only for vegetables and fruits in several chronic disorders (National Institute of Nutrition (2010).

CONCLUSION

Banana peels and banana peel extracted insoluble dietary fibres are high in β-Carotene content. They also show potential antioxidant activity. This may also vary according to the variety and the form in which the substance is measured i.e. the dried powdered form will show higher concentration of β-Carotene content as compared to the fresh form. Therefore, banana peels especially dietary fibres extracted from them can be utilized as a fortificant in beverages and other food products. Further studies exploring this aspect can be conducted.

Conflict of interest: There is no conflict of interest.

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