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Isolation and Characterization of Insoluble Dietary Fibres from Kinnow Mandarine (*Citrus nobilis* + *Citrus deliciosa*) Peel

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Dietary fibre consumption offers numerous health beneficial effects. In the present study dietary fibre was isolated from kinnow peel using enzymatic gravimetric method with 47 ± 0.23% yield. Various physiochemical properties of kinnow peel and the isolated dietary fibre samples were studied. The water holding capacity (WHC) and oil holding capacity were 6.92 g and 1.26 g per g of the fibre sample respectively. Elemental analysis was also done for kinnow peel powder (0.74% nitrogen, 29.60% carbon, 6.70% hydrogen and 62.97% oxygen) and for the extracted fibres (41.78% carbon, 6.42% hydrogen and 51.80% oxygen). SEM and FTIR tests were used for examining the changes in the structures of dietary fibres. From the results of elemental analysis and FTIR, it was clear that the extracted fibre does not contain proteins. Rheology of the extracted dietary fibres exhibit non-Newtonian behavior and shear thinning properties.

Keyword: Composition analysis, Elemental analysis, Gravimetric extraction, Rheology enzymatic, SEM

Introduction

Kinnow is a high yield hybrid variety crossbreed of King Mandarin (*Citrus nobilis*) and Willow Leaf Mandarin (*Citrus deliciosa*). It is extensively grown during winter broadly in the Punjab region of India and Pakistan. The fruit is instant source of energy due to the presence of glucose, fructose, sucrose, minerals, salts and other constituent of several nutrients such as Vitamin B complex, Vitamin C, iron, lime, phosphorus and the peel is rich in dietary fibre. The fruit enhances metabolism by alleviating acidity, enhances appetite and pancreas functions, anti-poisonous and known as miraculous fruit for recurrent fever therefore it is an effective medicine for stomach. It provides relief from typhoid, chronic constipation and improves skin health.[1] Incorporation of High amount of dietary fibre in diet reduces the risk of hypertension, obesity, diabetes, stroke, coronary heart disease, and certain gastrointestinal diseases significantly, also reduce blood pressure and serum cholesterol levels.

Dietary fibres are either water soluble or insoluble. Both are beneficial for health.[2] As dietary fibre has good water holding properties, gelling ability, fat mimic, anti-sticking, anti-clumping, texturising and thickening effects, it modifies sensory properties, enhances shelf life, improves texture when added in food. It is used as bulking agent in low calorie products, fat replacer to maintain moisture balance, coloring agent, and as natural antioxidant. Peel is the by-product of juice processing industry containing large amount of dietary fibre about 50% of mandarin varieties of citrus fruits. Therefore the present study was planned for extraction and characterization of the dietary fibre from kinnow peel.

Materials and Methods

Materials

Kinnow was procured from the local market, peeled by hand, cleaned with water and then cut into 2.5 cm × 2.5 cm size. They were dried in hot air oven at 60ºC until constant weight. The pieces were then grinded, homogenized and sieved to get particle size of 1–2 mm. All the chemicals used were of analytical grade. Enzymes were gifted by Centre of Innovation and Applied Bioprocessing (CIAB), Mohali, India.

Fibre Extraction and Characterization

The key steps in fibre extraction by gravimetric method is enzymatic treatments to remove starch and protein followed by precipitation of soluble dietary fibre components using aqueous ethanol then dietary fibre residues are separated by filtration.[3] A flow diagram for the extraction was shown in Fig. 1. Moisture, ash, fat, protein, dietary fibre (DF) were calculated following AOAC method.[4] Mineral

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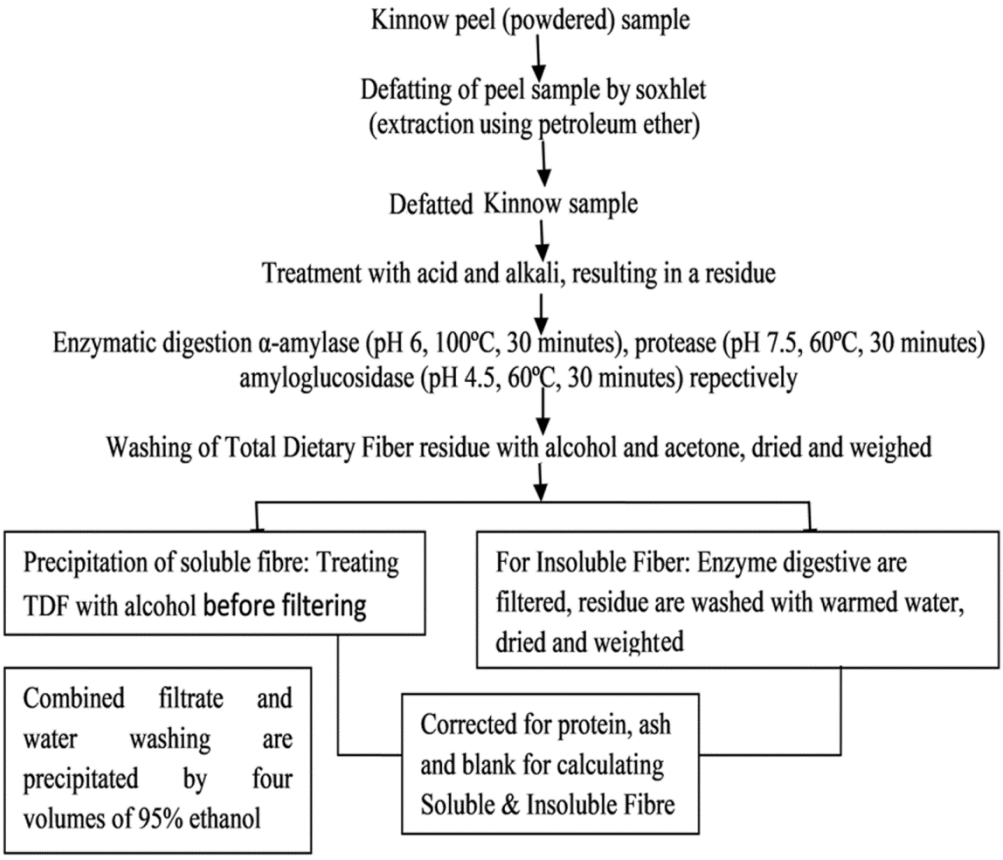

Fig. 1 — Flow chart for the Extraction of Dietary Fibres

contents were determined using Atomic Absorption Spectrometer (Thermo Fisher Scientific Inc., MA, USA). Sugar content was determined by Miller's method using 50–100 μg/mL D-glucose standard solution and absorbance was measured at 600 nm in UV-visible spectrophotometer (UV 2401 PC, Shimazdu, Kyoto, Japan).[5] Particle size of the dry powder of extracted fibre was determined using Zetasizer (Nano ZS-90 model, Malvern, UK). Water activity was measured using water activity meter (Aqualab, Decagon, USA). Bulk density of the dried fibre was determined by freely pouring the dried powder fibre sample in a 50 mL graduated measuring cylinder taking 25g of powder fibre sample. Water holding capacity (WHC) and oil holding capacity (OHC) were measured following the method of Ahmed *et al.* (2013)[(6)] expressed as g of water adsorbed/g sample of fibre and g oil absorbed/g sample respectively. Rheology is a study of flow of matter**.** The rheological measurements of (3%, 4%, 5% w/v) of dried fibre powder were done using cup and bob (CC27) assembly of dynamic rheometer (MCR 102 Anton Paar, Austria). The rheological parameter shear stress (Pa) was measured in duplicate with linearly increasing shear rate till 250 s^{-1} at 25ºC. Carbon, hydrogen, nitrogen and sulfur (CHNS) analysis were performed on a Flash 2000 Organic Elemental Analyzer (Thermo Fisher Scientific Inc., MA, USA). Sample amounting 4 mg was undergone combustion at 950ºC in presence of pure oxygen and helium as the carrier gas followed by analyzing through gas chromatography comparing with the standard material. Visual color of the extracted dietary fibre was measured using a Hunter colorimeter ColorFlex model (Hunter Associates Laboratory, Reston, VA) in terms of L* (lightness), a* (redness and greenness) and b* (yellowness and blueness) as described earlier by Ghoshal *et al*., 2013.[7]

Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM)

The dry powdered fibre samples were characterized by FTIR (Perkin-Elmer IR spectroscope, Waltham, Massachusetts, USA) and the spectra were recorded on a Perkin Elmer Spectrum software (Version 10.03.08) (Thakur *et al*., 2016).[8] SEM was done to determine micro and nano-phase structures of the peel

and the fibres in Scanning Electron Microscope (Ultra-high Resolution Scanning Electron Microscope JSM-6100, Digital SEM) following the instrumental protocol.[8]

Statistical Analysis

The outcomes were expressed as mean ± SD. Statistical analysis was done using Microsoft excel, $p>0.05$ stands for not significant.

Results and Discussion

Yield of total dietary fibre obtained from the kinnow peel was 47.14 g per 100 g of the extracted dietary fibres (DF) (Table 1) which resembles the value of orange peel (49.78 g/ 100 g) and grapefruit peel (44.2 g/ 100 g).[9] High DF intake significantly lowers the risk of diabetes, cardio vascular diseases, stroke, hypertension, obesity, gastrointestinal diseases.

Chemical Composition

The moisture content of kinnow peel (powdered sample) and extracted dietary fibres (DF) were found to be 4.9±0.1 g and 5.3±0.15 g per 100 g of sample respectively, which is lower than that of dietary fibre rich orange by-products (DFROBP), i.e., 9.9 g per 100 g.[9] Ash content of kinnow peel and extracted dietary fibres (DF) was found to be 2.4 ± 0.01 g and 1.9 ± 0.15 g per 100 g of sample respectively, which is almost same as DFROBP and grapefruit peel, i.e., 2.6 g and 2.99 g per 100 g but lower than in lemon peel, i.e., 6.7 g per 100 g[9] and orange peel, i.e., 3.3 g per 100 g. Fat content of kinnow peel was found to be 1.74 g per 100 g of the sample which resembles to that of orange peel (1.64 g/ 100 g) and lower than grapefruit peel (2.01 g/ 100 g)[9]. The crude fibre content of kinnow (powdered) peel sample was 14.87 ± 0.2 g per 100 g of sample (Table 1). Kinnow fiber is rich in protein contents shown in Table 1. Several authors are reported presence of high protein (5.95 to 16.45 g/100 g) content in fruit fibers.[2,9,10] In some cases too high protein content can be elucidated by the existence of a part of protein that attach strongly to cell wall's fibre components[11] and another portion that is insoluble in water. CHNS method is faster, very sensitive to low concentrations of nitrogen, less susceptible to interferences (thus yielding results as accurate as 0.3 g/100 g), and it requires only small amount of sample.[12] The crude protein content determined by the combustion method is in the range 10.63–18.14 g/100 g sample for peel and fibers. These results are higher compared to crude proteins estimated by Kjeldahl method.[12] According to Ahmed *et al*. (2013)[(6)] the Kjeldahl method is a more reliable indicator of the true protein content than the total nitrogen value (Table 1). From the D-glucose standard curve the relation between absorbance and concentration (μg/mL) was determined and straight line equation y = 0.708x + 0.084 was obtained for the estimation of reducing sugar content (Table 1). As good amount of P, Ca, Cu, Mn, Fe, Zn are present (Table 1) so the extracted fibre is a good source of minerals and can be incorporated in processed foods. The average hydrodynamic diameter of the kinnow peel powder was found in the range between 1130 nm to 1160 nm and average particle size was 1145 nm and dried dietry fiber powder particles varied between 1016 and 1040 nm and average hydrodynamic diameter of dry kinnow peel was 1028 nm (Table 1). Particle size was found little bigger compared to other fruit peel powder. Water holding capacity of extracted dietary fibres (DF) was found to be 6.92 g water per g of the sample (Table 1) which was found to be lower than the value reported for the orange dietary fibre concentrate and lemon peel, i.e., 7.3 and 7 g water/g of sample respectively.[2] Oil holding capacity of

Table 1 — Characterization of peel sample and fibre sample

Parameter	Peel powder	Dietary Fibre
Moisture (g/100g)	4.9 ± 0.1	5.5 ± 0.15
Ash (g/100g)	2.4 ± 0.01	1.9 ± 0.15
Fat (g/100g)	1.96 ± 0.11	1.74 ± 0.14
Protein (Kjaldhal method) (g/100g)	13.32 ± 0.75	8.54 ± 0.18
Protein (CHNS method) (g/100g)	18.14 ± 0.03	—
Crude fibre (g/100g)	14.7 ± 1.05	31.3 ± 0.85
Water activity (a_w)	0.34 ± 0.05	0.18 ± 0.08
Bulk density (kg/m^3)	200 ± 1.50	250 ± 1.25
Average particle diameter (nm)	1145	1028
Reducing Sugar	5.32	0.805 ± 0.865
Non reducing Sugar	2.02	—
Total Sugar	7.34	0.805
P, ppm	—	1.25
Ca, ppm	—	8.5
Cu, ppm	—	0.085
Mn, ppm	—	0.125
Fe, ppm	—	1.27
Zn, ppm	—	0.84
Water holding capacity (g water per g of fibre)	—	6.92
Oil holding capacity (g of oil per g of fibre)	—	1.26
Color (L*)	25 ± 0.54	30 ± 0.67
(a*)	2.56 ± 0.23	0.87 ± 0.09
(b*)	9.45 ± 0.11	4.56 ± 0.24

extracted dietary fibres (DF) was found to be 1.26 g oil per g of the sample (Table 1) which resembles the value reported for the orange dietary fibre concentrate, i.e., 1.27 g oil per g of the sample and lower than the value reported for the lemon peel, i.e., 6.7 g oil per g of the sample[2]. The elemental analysis (CHNS test) for kinnow peel (powdered) sample showed in Table 1. Nitrogen (protein) is absent in the extracted fibres from kinnow peels. Mineral content was reported in Table 1. The flow behavior in terms of shear stress vs. shear rate of extracted dietary fibre at 3%, 4% and 5% concentrations were shown in Fig. 2a where shear stress increased with increasing shear rate which exhibit pseudo-plastic behavior. The flow behavior in terms of viscosity vs. shear rate of extracted dietary fibre at 3%, 4% and 5% concentrations were shown in Fig. 2b. Viscosity decreased with increase in shear rate which shows the extracted dietary fibre (DF) at 3%, 4% and 5% concentration exhibit the shear thinning property. Color values of fibre in terms of L*, a* and b* were reported in Table 1. Extracted fibre samples were more brighter and less yellowish and reddish compare to powdered peel sample.

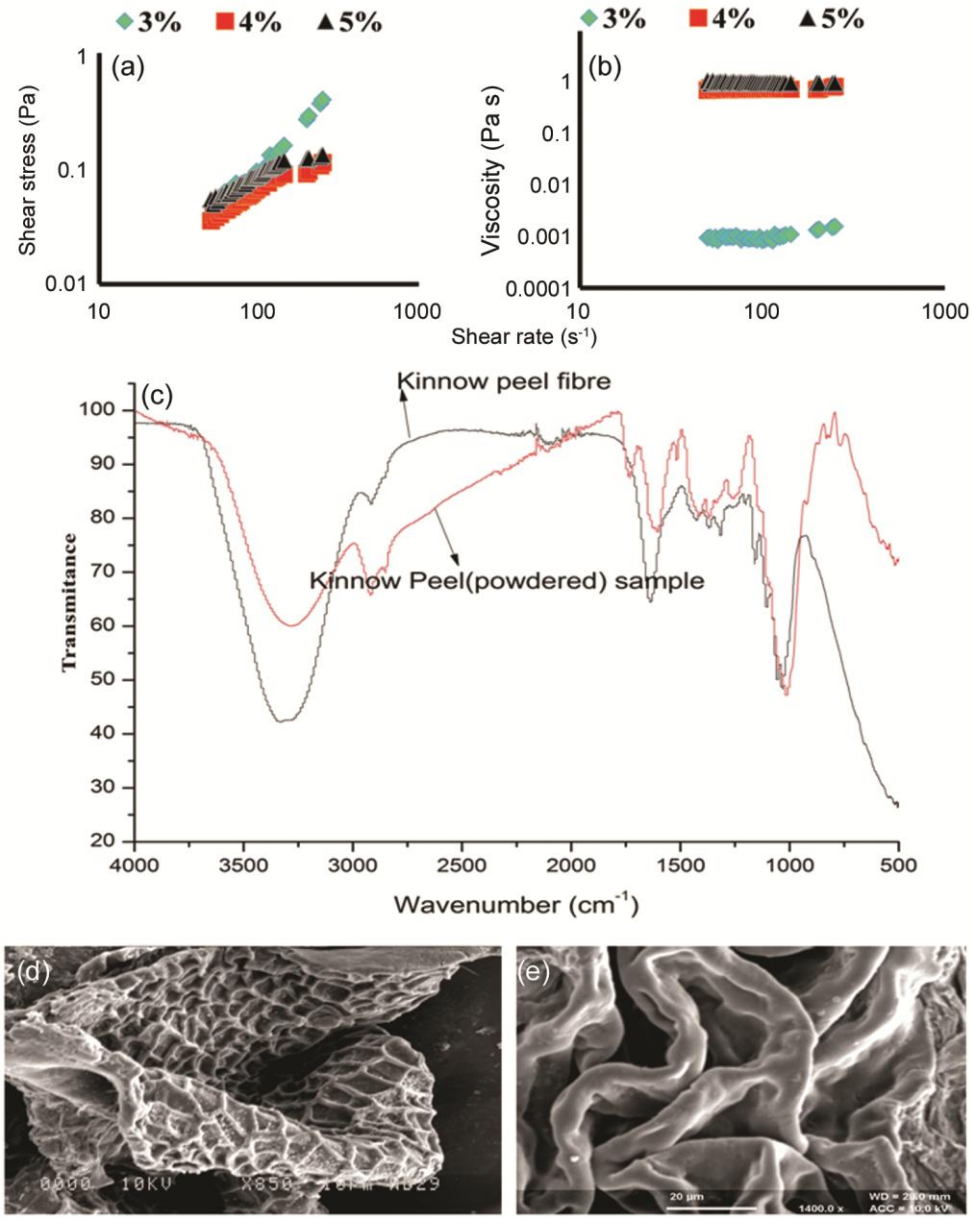

Fig. 2 — (a) Comparative flow curve; (b) Apparent viscosity curve of extracted DF; (c) FTIR of peel and fibre; (d) SEM of peel; (e) SEM of extracted fibre

FTIR and SEM Analysis of Kinnow Peel (Powdered) and Extracted Fibre Sample

The comparative FTIR graph of kinnow peel (powdered) sample and the extracted fibres is shown in Fig 2c shows. The main difference between both the graphs is that the FTIR graph for the extracted fibres does not show any characteristic peak which indicates the presence of the nitrogen (-CN, -NH, etc.). This implies that extracted fibre is purely carbohydrates. SEM pictures of kinnow peel (powdered) sample at high magnification is shown in Fig. 2d. The picture showed large number of regularly homoginized raw fibres on the particle's surface. It also showed that the powdered peel particles are well dispersed and exfoliated. The particle size ranged from 80–100 micrometer (µm) showing uniformly dispersed particles. The SEM image of the extracted fibres is shown in Fig. 2e. The average length of the fibre thread range from 103.20–106.35 µm. From the given figure, the shape of the fibres can be very clearly seen. It is very clear that the shape of the fibres are thread like. The fibres here are forming bundles, fibres showed the longitudinal regular repeated pattern with parallel orientations. The intercellular gaps, in the form of shallow longitudinal cavities, can be clearly marked as the fibres were exposed. The intercellular space is filled up by the binder lignin or by fatty substances that hold the fibres firmly.

Conclusions

Yield of dietary fibre obtained from kinnow peel was found to be 47.14 g per 100 g of the extracted dietary fibres, the value was similar to orange peel but higher than the grapefruit peel. It is clear that extracted dietary fibres (DF) behave Non-Newtonian and showed shear thinning property. FTIR study confirms that kinnow peel (powder) exhibit characteristic peak of aromatic amine at 1258 cm^{-1} which indicates presence of protein content in the kinnow peels. While there is no characteristic peak in the FTIR graph of the extracted fibres which showed that the fibre extracted are purely carbohydrates. The particle size ranged from 80–100 micrometer (µm) showing uniformly dispersed particles. Scanning Electron Micrographs of the extracted fibres shows that length of the fibre thread range from 103.20–106.35 micrometer (µm) and shape of the fibres are thread like. As good amount of mineral is present and its oil holding capacity is favorably higher therefore the extracted fibre from kinnow peel can be used as a micronutrient rich additive having cholesterol lowering capacity in food products.

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