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Value addition of kinnow juice processing industry by-products using green solvents

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Abstract
Processing of citrus fruits yields a significant amount of by-products as waste but bitterness of these by-products for their subsequent use in production of food products needs considerable attention. This study has been focused on the debittering of Kinnow juice industry by-products such as kinnow pulp residue by using green solvent method and the optimization of process parameters.In solvent genesis method, compounds responsible for bitterness such as naringin and limonin got solubilized in acetone with sample: solvent ratio 1:5 at ambient temperature for 3hr that has been quantified using HPLC method. Bitterness causing compounds such as naringin and limonin content in acetone treated kinnow pulp residue has been reduced considerably which indicates debittering of kinnow industry by-products. Acetone treated kinnow pulp residue can be used as a raw material for development of different food products at commercial scale. This process is not only an effective utilization of agro-industrial by-product but also a solution to the environmental pollution caused by kinnow juice industry.

Keywords: Kinnow pulp residue, green solvents, solvent genesis, bitterness causing compounds, FTIR, SEM

Introduction

Citrus fruits belonging to the family rutacae have been most important commercial food crops grown all over the world. Among different citrus fruits such as tangerines, mandarins, clementine, satsuma, lemon, lime and grape fruits etc, orange group is widely grown as it occupies almost 70% of citrus crop output [1]. More than 40 million tons of Agro-industrial waste has been generated worldwide. Citrus waste (pulp residue, peel and seed) comprises almost 50% of whole fruit mass [2]. Citrus fruits are rich source of vitamin C (ascorbic acid) and polyphenols (such as hesperidin naringin and Limonin), which have anti-inflammatory and anticancer activity [3-5]. Citrus pulp and peel contains various active constituents and essential oils [6]. Industrial processing of citrus fruits generate large amount of peel waste (19 Million tonnes annually) which can be used as bio-refinery raw material [7]. Dumping of byproducts of orange juice processing industry has created various disposal and environmental problems and also resulted in loss of nutrients as these peels were rich source of cellulose, pectin, hemicelluloses, lignin, essential oils and phenolic compounds [8].

“Kinnow,” a hybrid between king and willow mandarins (*Citrus nobilis Lour* × *C. deliciosa Tenore*) is one of the major citrus fruit crops in India, especially in Punjab, Haryana, Rajasthan and Himachal Pradesh [9]. India ranks fourth in citrus fruit production. Its annual production in India is more than 1.0 MMT. Kinnow peel and pulp are the major by-products of the kinnow juice processing industry, which accounts for 55-60% of the fresh fruit weight [10], whereas around 30% of the produce of citrus fruits is processed to make juice. The main problem with the kinnow pulp is that it rapidly deteriorates due to the high moisture and sugar content, thereby being facile to quick growth of moulds and yeast. While drying the fresh pulp is a cost intensive step, heat treatment volatilises its essential/fragrant oils [11]. Consumers demand food products having traditional nutritional aspects as well as additional health benefits in spite of its regular ingestion. These foods are known as functional or nutraceutical food [12].

Materials and Methods

Procurement of Raw Material

The Raw material, pulp residue (leftover after juice clarification) was procured from kinnow juice processing Industry, which has been obtained as by-product during clarification of jui
ce was procured from Punjab Agro Juices Limited Village Alamgarh, Near Abohar, District Ferozepur (Punjab), India.

Physico-chemical Characterization of Kinnow Juice Processing By-product (Pulp Residue)

Physico-chemical study of kinnow juice industrial by-product (pulp residue) was carried out for its different constituents/nutritional and functional properties such as moisture using moisture analyser, (MA35, Saritorius), Protein [13], crude fat [14]. Pectin, Dietary fiber, total phenols and ash has been done according to standards of association of analytical communities [15]. Total phenolic content has been determined by Folin- Ciocalteu method [16], DPPH activity according to method proposed by [17].

Debittering of Kinnow Pulp Residue

Various methods such as food grade green solvent treatment have been employed for debittering of kinnow pulp residue. Kinnow juice industrial by-product (pulp residue) was subjected to maceration technique as prescribed by [18] with minor modifications. Experiments were performed using different food grade solvents such as acetone, hexane, ethanol and ethyl acetate with the different ratios of sample: solvent (1:5, 1:10 and 1:15) at 25ºC. These solvent mixtures were homogenised for 1, 2, 3, 4 and 5 hr at 2000 rpm. Extracts were centrifuged (Eppendorf Centrifuge 5810 R) at 8000 rpm for 3 minutes at ambient temperature. Supernatant has been collected and the solvent was recovered using rotary evaporator (IKA, HB 10 Control) system under vacuum at temperature of 45ºC which was then stored for further reuse. Debittered residue obtained was air dried for overnight (12 hrs) and ground for further use.

Sensory Evaluation

Sensory analysis of debittered kinnow pulp residue has been performed according to [19]. Before the sample was tasted, the mouth was fully rinsed with distilled water. The sample was tested in the mouth for 10 s, and the taste of each sample was averaged. Lyophilized pulp, which was bitter, has been used as the reference.

Analytical Techniques for Quantification of bitterness causing Soluble Components

Estimation of bitterness causing compounds that were extracted by solvent genesis were filtered through 0.45-µm membrane filter and then injected into Waters Acquit UPLC H-Class (WAT-176015007) (Milford, MA, USA) equipped with quaternary pump (Waters Quaternary Solvent Manager), injector (Waters Sample Manager-FTN (Flow Through Needle)), column compartment with Eclipse RP C18 column (150 × 4.6 mm; 5 µm), Waters 2998 PDA (Photodiode Array) detector and interfaced to mass detector (Waters TQ (Tandem Quadrupole, WAT-176001263). 0.1% Ortho-phosphoric acid in water (v/v) (solvent A) and acetonitrile (solvent B) were used as mobile phase [20]. The temperature of column was kept at 30°C using a thermostat. The wavelength of detector scan range was set between 200 and 400 nm. Flow rate of 1ml/min has been induced for analysis having sample volume 20µl with UV detector set at 254 nm for phenolic acids.

Results and discussion

Physico-Chemical Characterization of Kinnow Pulp Residue

Physico-chemical characterization of kinnow juice industrial by-product (pulp residue) has been shown in Table 1. The major content is dietary fiber in kinnow pulp residue was 60.11% after moisture 89.11%. This shows that it can be used as fiber source as well in different food products. The protein content in kinnow pulp residue was 10.9%. Total carbohydrates in kinnow pulp were 73.84 mg/g and ash content in kinnow pulp residue was 0.50%. Pectin content was 3.02% in pulp. The chemical composition of kinnow residues are in similar pattern as mentioned in previous literature [10]. Total Phenolic Content was found to be 1.95 mg/g and 3.45 mg/g in acetone treated pulp residue and pomace. DPPH activity was found to be 23.15 % and 42.45 % in acetone treated pulp residue and pomace [21].

Table 1: Chemical characterization of kinnow juice industrial waste on dry weight basis

Experiment	Kinnow pulp residue (%)
Moisture	89.11 ± 1.76
Ash	0.50± 0.02
Crude fat	4. 21± 0.08
Hemicellulose	1.01 ± 0.49
Total phenols	1.83± 0.35
Dietary Fiber	60.11± 0.51
Pectin	10.06± 1.43
Protein	10.91± 1.03

Debittering of kinnow pulp residue

Solvent genesis Method

Various food grade solvents such as hexane, acetone, ethyl acetate and ethanol have been used for debittering the kinnow residue. It was observed that the acetone treatment was more effective as maximum bitterness causing compounds got solubilised in it as compared to other solvents (Fig.1). Extractives containing bitterness causing compounds in kinnow pulp residue got solubilised to 35% (w/w) of initial dry biomass after acetone treatment consisting of 331. 25 mg/g sugars, 1.83 mg/g phenolic compounds. Debittered Insoluble pulp residue got settled down at bottom was recovered by filtration.

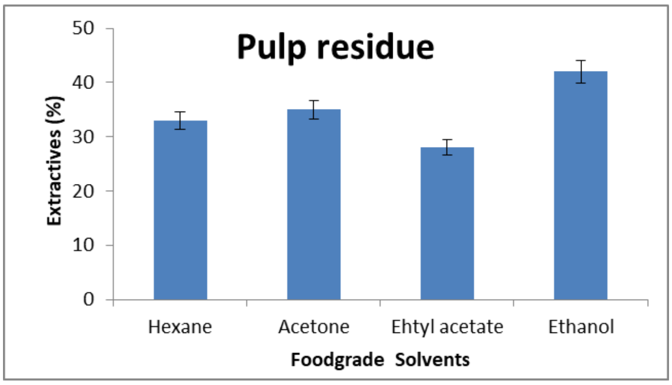


Fig 1: Percentage of bitterness causing compounds extracted after treatment with different food grade solvents

Marketability and consumer response depends upon sensory evaluation. Panelists analyzed it for appearance, taste color, aroma, body/texture, flavor, astringency and overall acceptability. The original flavor of kinnow without bitterness was observed in acetone treated pulp (Debittered, 5) and acetone treated pulp was also obtained (4) hedonic scale

reading. Sensory analysis of other solvents such as hexane, ethyl acetate and ethanol give sour and bitter taste. The acetone treated fibers are rich in antioxidants as majority of bitterness causing naringin, limonin and hesperidin got solubilized [22].
It has been found that amount of solubilized naringin and limonene compounds responsible for bitterness were in accordance to previous literature [23] but due to influence of environment, climatic change, geographical area, method used for analysis, so variation in results have been found. It has been found that naringin, limonene and hesperidin, were present in 7.8, 5.497 and 31. 22 mg/g in kinnow pulp residue extract after acetone treatment (Table 2). So, the acetone treatment process was further optimized for sample: solvent ratio and time. According to [24] amount of hesperidin extracted from pre-treated and dried peels ranges between 3.7% and 4.5% respectively.

Table 2: Analysis of extracted bitterness causing compounds obtained after solvent treatment of kinnow pulp residue

Kinnow	Debittering Process	Naringin (mg/g)	Limonin (mg/g)	Hesperidin (mg/g)
Pulp residue	Ethanol	2.33±0.46	3.62±0.16	31.91±0.12
	Ethyl Acetate	1.88±0.06	4.44±0.13	4. 28±0.09
	Acetone	7.88±0.12	5.97±0.10	31. 22±0.07
	Hexane	0.43±0.08	3.01±0.12	2.41±0.09

Effect of Solvent Concentration on Extractives

The effect of solid-solvent ratio on maximum solubilisation of bitterness causing compounds has been shown in Fig. 2. The results showed that with increase in concentration of solvent, maximum compounds got solubilised and after that equilibrium stage has been reached at 1:5 solid solvent ratio, when maximum extraction of bitterness causing compounds-naringin and hesperidin has been achieved at ambient temperature. Maximum yield of bitterness causing compounds has been observed after solvent treatment in pulp residue after time period of 3hr which was 34% at sample to solvent ratio of 1:5. Whereas there is no significant increase in yield of extract which contains bitterness causing compounds.

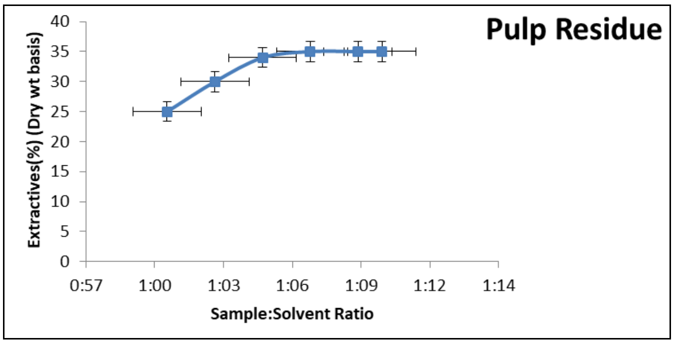


Fig 2: Effect of solvent ratio on solubilisation of bitterness causing compounds extracted after acetone treatment on kinnow industry by-products at constant pH

Effect of Extraction Time

The effect of extraction time indicated that (Fig. 3) that maximum extraction of soluble solids containing bittering compounds got extracted up to 3 hr after that there has been no significant increase in the naringin and hesperidin. This shows that the extraction process is sensitive to extraction time and solid-sample ratio [25]. It was observed from the data that after 3 hrs, soluble solids in case of kinnow pulp residue has been found to be 34.78%. After 3 hrs of treatment time, no significant change in increase of soluble solids has been found both in case of pulp.

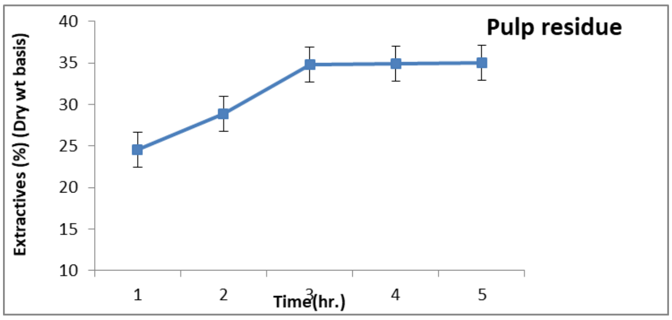
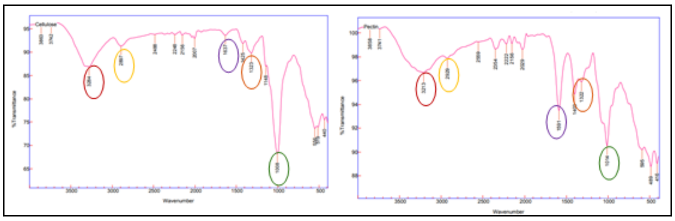


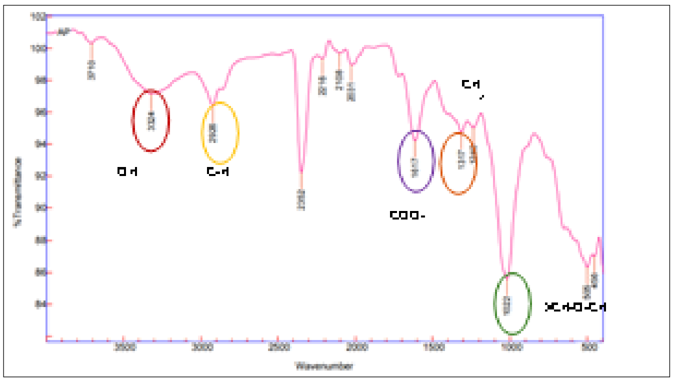
Fig 3: Effect of time on extraction of bitterness causing compounds after acetone treatment at different time intervals on kinnow industry by-products at constant pH and sample: solvent ratio



A

Wavenumber	Groups
3200-3600 cm-1	-OH
2926-2929cm-1	-CH
1500-1700 cm-1	COO-
1300-1400 cm-1	$-CH_2$
1000-1022cm-1	CH-O-CH stretching

B



C

Fig 4: FTIR of treated kinnow pulp residue A) Cellulose standard, B) Pectin standard and C) Acetone treated kinnow pulp residue.

FTIR spectra of acetone treated kinnow juice processing residue were compared with standard cellulose and pectin. The absorption wavelengths at range of 1500- 1700 cm −1 observed in the spectra are attributed to the COO- group that also present in standards. The noticeable peaks of the cellulose and pectin along with in sample due to CH_2 group occurred between 1300- 1400 cm $^{-1}$ wavelength. The sharp peaks in range of 1000- 1022 cm $^{-1}$ wavelength are due to the CH-O-CH stretching. The acetone treated kinnow processing residue has majorly pecto-cellulose fibers.

Conclusions

The major content of dietary fiber in kinnow pulp residue was 16.94. Among various solvents used Acetone-treated kinnow pulp residue extract shows hesperidin, naringin and limonene as 1.19, 3.40 and 5.97 mg/g respectively. The original flavor of kinnow without bitterness has been observed in acetone treated pulp can be used for kinnow flavour food products.

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