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Ultrasound Assisted Extraction of Lutein from Citrus Fruit Peels

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Abstract—*This study was conducted to determine the lutein content in citrus fruit peels and effect of different extraction methods such as maceration and ultrasonication on the yield of lutein. Simultaneous extraction along with saponification, identification and quantification of lutein was carried out from citrus peels extract after separation with diethyl ether. Total phenols, flavonoids, tannins and antioxidant potential (DPPH) was determined from peels of Citrus reticulata & Citrus limetta. The results showed the ability of citrus peels as a potential source for the extraction of lutein which can be used as coloring and antioxidant agents in food industry.*

Keywords*: Lutein, Ultrasonication, Extraction, High Performance Liquid Chromatography, Citrus peels.*

1. INTRODUCTION

Globally, India has fifth rank in citrus fruit production and it is one of the important and widely cultivated fruit tree after the banana and mango. According to the National Horticulture Board 2016-17, the production of kinnow (*Citrus reticulata*) and sweet lime (*Citrus limetta*) were 44.38 lakh MT and 32.09 lakh MT respectively. In total quantity, approximately 30% of it is used for processing, out of which 50-60% waste is generated in the form of peel, seed and rags. These wastes constitute various bioactive compounds such as carotenoids, which have different health beneficial properties. Carotenoids constitute more than 600 naturally occurring tetraterpenoid organic pigments synthesized by plants, algae, and photosynthetic bacteria. These molecules are the source of several colors like yellow, orange, and red colors in many plants [1]. These compounds are classified into two classes, carotene and xanthophylls.

Lutein [(3R,3'R,6'R)-beta, epsilon-carotene-3,3'-diol], are one of the important carotenoids owing to various health benefits. These are abundantly found in leafy green vegetables like spinach, lettuce and kale [2, 3]. It is insoluble in water but soluble in fat as well as lipophilic solvents [4] and has different solubility, stability and absorptivity in various organic solvents [5]. Environmental factors such as temperature and light intensity have a profound influence on the concentration of major carotenoids such as β-carotene in summer and lutein in other seasons [6].

Lutein cannot be synthesized in the human body; it must be supplied through diet only According to the USDA, the average daily intake of lutein is 1.7 mg/day by Americans (National Institute of Medicine, 2001) but Europeans consume approximately 2.2 mg/day [7], these values are below the proposed levels to reduce the age-related macular degeneration [8, 9].

Extraction of lutein can be carried out using different solvents such as acetone, petroleum ether, methanol, hexane either alone or in different combinations [10-12]. Extraction is generally carried out at 0°C in order to avoid degradation and/or isomerization or any other undesirable changes. [13] To avoid these, different methods such as the use of nitrogen atmosphere, addition of di tert-butyl-methylphenol to the solvents and working under the subdued or ultra-violet filtered light has been proposed [11, 14].

Keeping in view of the above, the present study was conducted to extract the lutein from different citrus peels using two methods (maceration and ultrasonication), and determination of their different antioxidant properties.

2. MATERIALS AND METHODS

2.1. Procurement of Citrus Peels and Chemicals

Peels of fresh citrus fruits (*Citrus reticulata and Citrus limetta)* were taken from the local market in Longowal, Sangrur (Punjab). Citrus peels were dried, ground in fine powder and stored at -20°C. Chemicals used in the present study, such as Sodium carbonate, 1,1-diphenyl-2-picrylhdrazl

(DPPH), vanillin, Folin-Ciocalteu regent, ferric chloride, aluminum chloride hexahydrate, ascorbic acid, sodium acetate, lutein and gallic acid were purchased from Sigma Chemicals, Germany.

2.2. Extraction

Ten grams powder of the dried citrus peels were taken and mixed with methanol in the ratio of 1:3. The extraction was carried out by maceration and ultrasonication at 37±1°C for 30 minutes, after which the extract was filtered and evaporated by rotary evaporator [15].

Further, saponification of the extract was carried out subsequently, with methanolic solutions of KOH and NaOH at different concentrations of 10, 15, 20, 25 and 30%, Apart from this, simultaneous extraction and saponification of citrus peel powder was carried out using ultrasonication.

Figure 1 Extraction of Lutein from Citrus Peels by Maceration

2.3. Determination of Different Antioxidant Properties

2.3.1. Free radical scavenging activity

The DPPH radical scavenging activity of the acetone extract of citrus peels was measured by Yi et al [16] with minor modifications. The DPPH radical scavenging activity was calculated by:

$$\text{DPPH radical scavenging activity} = \frac{A^{o}-A1}{A^{o}} \times 100$$

2.3.2. Total phenolic content

Total phenolic content of the citrus peel was estimated by using Folic –Ciocalteu regent [17] with some modifications. The total phenolic content was calculated from a calibration curve with pure gallic acid as standard and results were expressed as gallic acid equivalents (GAE), in mg/g dry extract.

2.3.3. Total flavonoids content

Total flavonoids in the sample extracts were determined by using aluminium chloride method described by Liu et al [18] and results were expressed as mg catechin equivalent per gram dry weight of the samples.

2.3.4. Total tannins content

Total tannins content was estimated by the vanillin-HCL method [19] and results were expressed in mg catechin equivalent per gram dry weight of the samples.

2.4. Quantification of Lutein in Citrus Peel Extract

Estimation of lutein in the extract was determined by HPLC following the method of Craft [20]. Samples were analyzed by HPLC using UV-Vis detector at 450 nm using acetronitrile (100 mL), methanol (900 mL) and triethylamine (1 mL) at the flow rate of 1ml/min.

3. RESULTS AND DISCUSSION

Extraction of lutein was carried out using two techniques; maceration and ultrasonication. The results of the extraction techniques and its antioxidant properties have been discussed below.

3.1. Estimation of Different Anti-Oxidant Properties

The various anti-oxidant properties of lutein extract in terms of DPPH activity, total phenolic content, total flavonoid, and tannic content have been shown in Table 2. The total phenolics in the kinnow peel extract obtained were 25 mg GAE/g DW and 38 mg GAE/g DW by UAE as well as maceration method, respectively as compared to sweet lime peels. This indicates that kinnow peel have significant amount of phenolic compounds and can be used in applications of different sectors. Total flavonoids obtained by both the methods were in the range of 0.98 mg catechin/g DW to 4.40 mg/g DW However, the amount of total tannins varied in the range of 0.43 mg catechin/g DW to 0.58 mg/g DW. Similar results have been obtained from [21].

Table 1: Different Antioxidant Properties of Citrus Peels

Extract of Peels	Percentage Yield (%)	DPPH inhibition (%)	Total Phenolics (mgGAE/g DW)	Total Flavonoids (mg catechin/ g DW)	Total Tannins (mg catechin /g DW)
Maceration					
Citrus reticulata (Kinnow)	5.20	75.23	25	3.75	0.54
Citrus limetta (Sweet Lime)	12.20	22.46	20	2.07	0.43
Ultrasound Assisted Extraction (Ultrasonication)					
Citrus reticulata (Kinnow)	5.85	42.96	38	4.40	0.58
Citrus limetta (Sweet Lime)	11.95	69.73	31	0.98	0.52

3.2. Effects of various extraction methods on lutein yield

The yield of lutein extract from kinnow and sweet lime peels obtained by the ultrasound assisted extraction and maceration

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technique is depicted in Figure 2. It was observed that high lutein content was obtained from ultrasound assisted extraction (UAE) followed by maceration.

In contrast to maceration, UAE is advantageous in terms of reduced extraction time, less solvent-peel ratio, lower temperature and high yield without degradation or damage in phenolic compounds [22]. UAE is based on the phenomena of acoustic cavitaion, which may lead to high yield due to increase in permeability of the cell wall, faster kinetics and strong impact on the surface of the commodity [22-24].

3.3. Effect of different concentrations of KOH and NaOH on lutein yield

Effect of the different concentrations of KOH and NaOH on lutein yield from various citrus peels was investigated. When compared with NaOH, KOH resulted in maximum lutein yield from both citrus peels using both UAE and maceration technique. Using UAE, the maximum yield (8.20 µg/g) was obtained at 20% (w/v) KOH; however, 30% (w/v) KOH was found to be optimum concentration during extraction of lutein by maceration (Table 1).

A decrease in the lutein concentration at higher alkali concentration was observed, which may be due to the degradation of carotenoids [25].

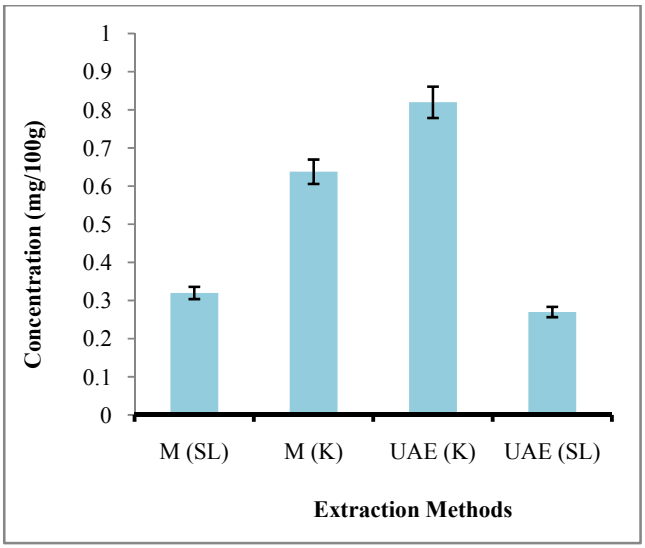
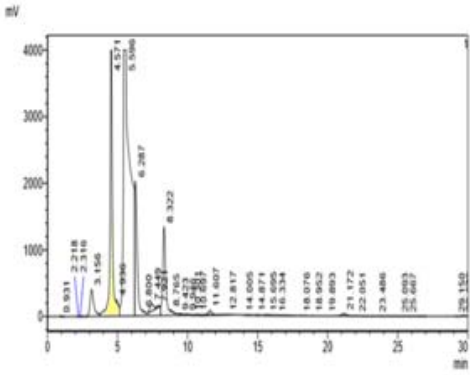


Figure 2: Simultaneous extraction and saponification of Lutein with Methanol

SL: Sweet Lime; K: Kinnow; M: Maceration; UAE: Ultrasound assisted extraction

Saponification is used to get free lutein from the intact chlorophyll and xanthophyll esters along with the removal or separation of degraded, undesired lipids and interfering substances [26, 27].

Figure 4 and 5, shows the chromatographical analysis (HPLC) of lutein in kinnow and sweet lime peel after simultaneous extraction and saponification with saturated methanolic solution of KOH and NaOH, respectively.



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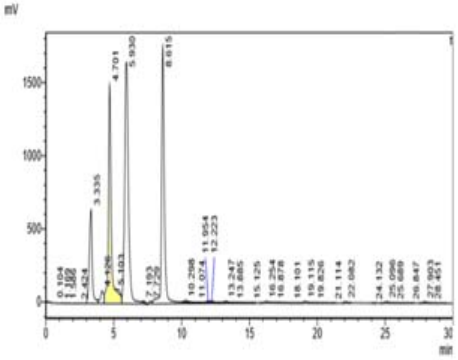
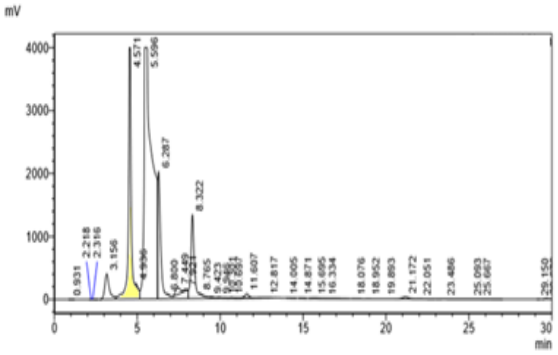


(b)

Figure 3: Chromatographically analysis (HPLC) of Lutein in *Citrus reticulata* (a) and *Citrus limetta* (b) peels after simultaneous extraction and saponification with saturated methanolic solution of KOH



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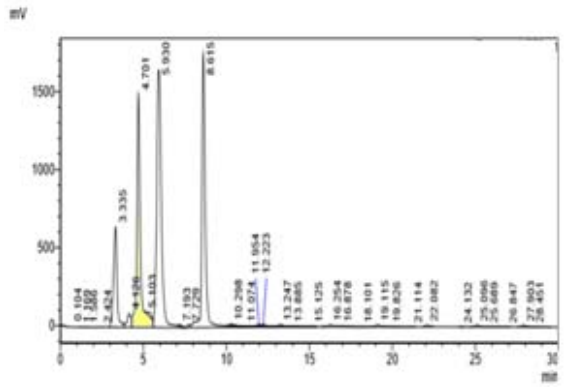

(b)

Figure 4: Chromatographically analysis (HPLC) of Lutein in *Citrus limetta* (a) and *Citrus reticulata* (b) peels after simultaneous extraction and saponification with saturated methanolic solution of NaOH

Table 2: Optimized parameters for the extraction of lutein from *Citrus reticulata* peels

Extraction techniques	Extraction temperature (°C)	Extraction time (min, h)	Methanolic KOH solution (%)	Solvent to dried peel ratio (ml/g)	Amplitude (%)	Extraction Yield (µg/g DW)
Maceration	57	40	30	12:1	-	6.38
UAE	50	60	20	3:1	30	8.20

4. CONCLUSION

Lutein, one of the carotenoid generally present in all green leafy vegetables and citrus fruit peels. Among two methods, lutein from sweet lime and kinnow peels was estimated and it was found that ultrasound extraction technique was efficient and resulted in maximum lutein yield. Moreover, KOH showed the maximum yield than NaOH, when used as a saponifying agent in both the techniques. Citrus peels extract from kinnow peels using both technologies showed high antioxidant properties as compared to sweet lime peels.

Therefore, high antioxidant properties of kinnow peels has significant potential in food sector and can be used in different food products for the development of functional foods. Since, ultrasonication is one of the safest, easiest and cost effective techniques that can be used to extract various bioactive compounds from natural plant sources and so it can be used at the industrial scale. Further research can be carried out on the other potential sources of bioactive compounds and various extraction techniques.

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