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Effect of Drying on Leutin Concentration of Noni (*Morinda citrifolia* L.) Leaves

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ABSTRACT: Leutin is a plant-derived compound and non-vitamin A carotenoid. Leutin cannot be synthesised by humans and hence obtained by the ingestion of foods *viz.*, fruits and vegetables. It possesses multiple protective properties like anti-cancer, anti-inflammation, and hepatoprotection and also helps in the prevention of age-related maculopathy, cardiovascular diseases, diabetes etc. Leutin content in fresh noni leaves and the leaves dried after three different treatments *viz* plain dried without treatment, steam blanched and hot water blanched were assessed. For this study, availability and procurement of noni leaves was challenging since only tender leaves were used. Leutin concentration could vary due to several factors like variety, stage of maturity, growing climatic conditions etc. In this study, two different solvents *viz* acetone and butanol were used for extraction. The study shows drying has a positive effect on the leutin concentration of noni leaves specifically in acetone than butanol extraction. the leutin concentration increased in acetone extraction from 0.067 µg/g to 0.096 µg/g from zeroth hour to ninth hour respectively than other treatments.

Keywords: Leutin, noni leaves, fresh, Pretreatment, dried, acetone, butanol.

INTRODUCTION

Lutein (β, ε-Carotene- 3, 3' diol) is a naturally occurring oxygenated derivative of hydrocarbon carotenoids (Tsao *et al.,* 2004). It is a fat-soluble carotenoid pigment, present in egg yolk, fruits and vegetables (Sujak *et al.,* 1999). Lutein belongs to the family of hydrophobic carotenoids found in abundance in dark green leafy vegetables. Lutein has restricted use since it has low solubility in water and is easily affected by oxygen, light, or heat (Boon *et al.*, 2010; Qv *et al.,* 2011). Leutin possesses many therapeutic properties such as hepatoprotection, anti-inflammatory, neuroprotection, cardioprotection, osteoarthritis, anti-cancer, anti-cataract, anti-diabetic retinopathy etc, which is due to their ability to act as scavengers for reactive oxygen species and to bind with physiological proteins in humans (Snodderly, 1995). Leutin in immune tissues gets depleted during inflammation and the depletion level depends on the dietary leutin levels (Koutsos *et al.,* 2006). It has been suggested that 0.6-1.05 mol/l of regulating plasma concentration of leutin is required to be safe for humans and exert the anticipated beneficial effects (Granado *et al.,* 2003). Lutein induces apoptosis of gastric cancer cells by increasing NADPH oxidase-mediated ROS production (Eom *et al.,* 2022). Leutin is deemed an effective bioactive compound with considerable functional properties that aid human health. It could be exploited in the pharmaceutical, supplement, and food industries. Leutin has low bioavailability due to its lipophilic nature resulting in poor aqueous solubility before it reaches the circulatory system and targeted organs (Fuad *et al.,* 2020). Carotenoids also have low stability for processing, so new developments are currently under study to improve carotenoids' stability. The microencapsulation method introduced by Rigon and Noreña (2016) can alleviate the oxidation of bioactive compounds in foods and allow better powder dispersal in water. Other techniques suggested by Brum *et al.* (2017) used to stabilize lutein include nanoemulsion and nanocapsules, where the particle size smaller than 1 µm can significantly facilitate lutein's solubility, thus improving its bioavailability and stability. Hence, this study was conducted to uncover leutin potential in noni leaves which would help to accelerate its development for future study.

MATERIALS AND METHODS

Collection of sample. The top three leaves of noni (*Morinda citrifolia* L.) plant were selected for the conduct of this study. The tender leaves were procured from the Thomsons organic farm, in Angamaly, Ernakulam, Kerala, India.

Treatment of noni leaves. Noni leaf powder was developed from tender noni leaves using a cabinet drier adopting three different treatments mentioned below in Table: 1 and fresh leaves were taken as control.

Table 1: Treatment for the development of noni leaf powder by cabinet drying.

Treatments	Method	Drying Température (°C) and Time (Hours)
LT_1 - Plain leaves without blanching	Dried leaves	60°C for 6 hrs
LT_2 - Steam blanched leaves	Steamed at 90°C for 3 minutes	60°C for 6 hrs
LT_3 - Hot water blanched leaves	Blanched at 85°C for 2 minutes	60°C for 6 hrs
LT_4 - Fresh leaves	No treatment	No drying

Estimation of Leutin concentration. To estimate the Leutin concentration, fresh noni leaves and dried leaves of different treatment was taken for the study. LT_1 - Plain leaves without blanching, LT_2 - Steam blanched leaves, LT_3 - Hot water blanched leaves and LT_4 (Control) . fresh leaves were taken to know the effect of drying on Leutin concentration in two different solvents i.e acetone and butanol.

Extraction. The samples (10 g each) were ground with a mortar and pestle and mixed with a solvent. Two different solvents (40 mL) i.e. acetone and butanol were used. The solution was filtered using Whatman No. 1 filter paper. The filtrate was centrifuged at 10,000 rpm for 10 minutes. The aqueous phase was collected and stored at 4◦C.

Measurement of Absorbance. Absorbance was measured using a spectrophotometer at a wavelength of 446 nm at the 0th hour and at intervals of every 3 hours standing time till 9 hours for both standard and samples and was recorded.

Calculation. The concentration of lutein was calculated using the following formula:

The concentration of lutein (µg/g of sample) = A × V (ml) X dilution factor/ € × W (g)

Where, A = Absorbance at 446 nm, V = Volume of extract in ml, €= Absorption coefficient (2589), W= Dry weight of the sample.

Volume=4ml, €= 2589, W=1g

RESULTS AND DISCUSSION

The study was conducted to find the effect of drying with different treatments of noni leaves on leutin concentration when compared to the leutin concentration in fresh leaves. It was also to find out the most suitable solvent for the extraction of leutin.

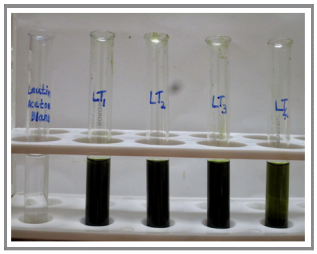

Fig. 1. Leutin extracted samples using acetone.

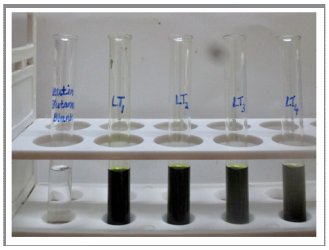

Fig. 2. Leutin extracted samples using butanol.

Table 2: Concentration of leutin in noni leaves with different solvents.

Sr. No.	Standing time (hours)	Treatment	Acetone	Butanol
			Conc. of Lutein in µg/g of sample	Conc. of Lutein in µg/g of sample
1.	0th hour	LT_1	0.042^{b}	0.033^{a}
		LT_2	0.067^{a}	0.036^{a}
		LT_3	0.037^{b}	0.036^{a}
		LT_4	0.011^{c}	0.012^{b}
2.	3rd hour	LT_1	0.044^{b}	0.040^{ab}
		LT_2	0.077^{a}	0.050^{a}
		LT_3	0.048^{b}	0.037^{b}
		LT_4	0.026^{c}	0.024^{c}
3.	6th hour	LT_1	0.060^{b}	0.050
		LT_2	0.089^{a}	0.053
		LT_3	0.052^{c}	0.053
		LT_4	0.057^{bc}	0.046
4.	9th hour	LT_1	0.071^{b}	0.063^{a}
		LT_2	0.096^{a}	0.060^{a}
		LT_3	0.063^{b}	0.060^{a}
		LT_4	0.067^{b}	0.050^{b}

Results showed the presence of leutin in all the samples. The relative amount of leutin was measured using a spectrophotometer at a wavelength of 446 nm. The absorbability and concentration of leutin present in noni leaves extracted using two solvents i.e acetone and butanolat different standing times are mentioned in Table 2.

Fig. 3-6 shows the concentration of leutin in noni leaves at the zeroth hour, after 3 hours, 6 hours and 9 hours of standing time respectively in the given two solvents. During the standing time of this study, it was observed that leutin concentration increased over the increase in standing time. It could be due to the longer duration that allowed for the dispersion to take place (Hajare *et al.,* 2013).

Among all the treatments, LT_2 (steam blanched) showed higher concentration of leutin in both the solvents. When the leutin concentration of noni leaves were compared, LT_1 (plain dried), LT_2 (steam blanched), and LT_3 (hotwater blanched), showed higher concentration of leutin than LT_4 (fresh noni leaves). The results showed significant ($p \leq 0.05$) difference among the treatments at all the hours of standing time except 6th hour of butanol extraction. This proves that drying has significant effect on leutin concentration. In case of LT_2 (steam blanched), the leutin concentration increased in acetone extraction from 0.067 µg/g to 0.096 µg/g from zeroth hour to ninth hour respectively. Comparing the solvents used for extraction, acetone extracted higher leutin than butanol in all the samples, it is because acetone has lower polarity than that of butanol (Hajare *et al.,* 2013). The findings in the study correlated with Athira *et al.* (2022) the highest yield of total carotenoids was obtained by maceration using acetone than hexane. Maiani *et al.* (2009); Valduga *et al.* (2009) have reported that thermal processing might enhance the extractability and bioavailability of carotenoids by disruption of food matrices. In the present study, though fresh leaves have lower leutin concentration, LT_1 (plain dried), and LT_3 (hotwater blanched) also have significant difference from LT_2 (steam blanched). In the study conducted by Agamou *et al.* (2015); Djuikwo *et al.* (2011), the carotenoids content of fresh Moringa leaves are in the range of mean values that increased with blanching. This could be due to the hydrothermal treatments such as blanching that has been ascribed to the breakdown of the cellulosic structure and to the thermal disruption of the non-covalent associations between carotenoids and proteins, which allows a more effective extraction of carotenoids (Ferracane *et al.,* 2008). On the other hand, Pellegrini *et al.* (2010) found that carotenoids content decreased in cooked Brassica vegetables. It thus shows that the response of carotenoids to hydrothermal treatments may be influenced by the type of treatment.

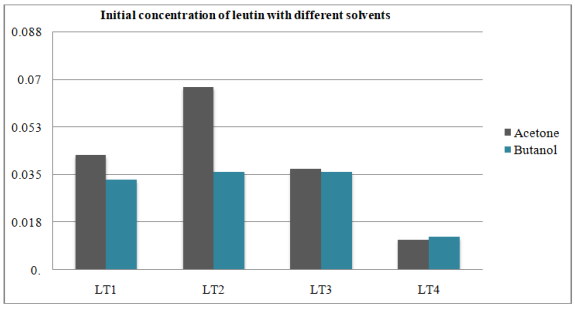

Fig. 3. Concentration of leutin at zeroth hour of standing time in two different solvents acetone and butanol**.**

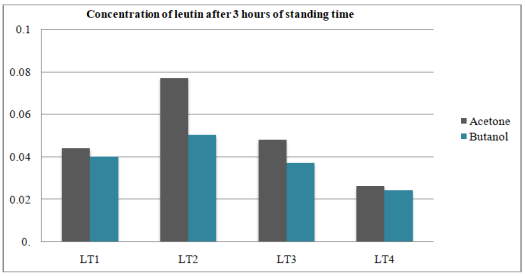

Fig. 4. Concentration of leutin after three hours of standing time.

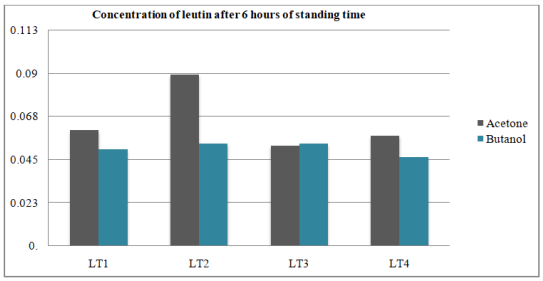

Fig. 5. Concentration of leutin after six hours of standing time.

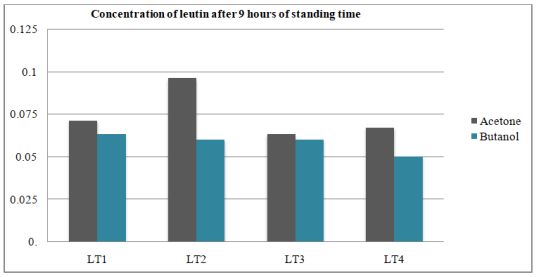

Fig. 6. Concentration of leutin after nine hours of standing time.

CONCLUSIONS

The study concludes that drying of noni leaves by different prior treatment, particularly steam blanched has positive effect on the leutin concentration than that of other treatments and fresh noni leaves. The solvent used for extraction plays a major role in the yield obtained. Hence it is suggested that the selection of appropriate method of treatment and solvent for extraction is important for further study of this compound.

FUTURE SCOPE

Since bioavailability of leutin is low, novel delivery systems needs further study for the effective utilization of available leutin in foods.

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Conflict of Interest. None.

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