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Effect of process parameters on quality of dried nettle leaves

Khan Chand and Anupama Singh

Abstract
Stinging nettle (*Urtica dioica*. L.) is a wild, unique herbaceous perennial flowering plant with stinging hairs. It has long history of use as a food source as a soup or curries, and also used as a fiber as well as medicinal herb. The current aim of present study was to analyze the effect of temperature and loading density on protein content and colour of dried nettle leaves. The present study was undertaken with different combinations of temperatures (55, 65 and 75^{0}C) and loading density (0.5, 1 and 1.5 kg/m^2). The experimental data of quality parameters were fitted into polynomial model developed using Multilevel Categoric Design and checked the adequacy of model by calculating R^2 and Fisher values. The colour change (ΔE) was more evident as the temperature was increased but the protein content was found higher at lower temperature and high loading density. For getting the best results, the optimum values of temperature and loading density were obtained 55^{0}C and 1.5 kg/m^2. At these optimum values of process parameters, the colour change (ΔE) was found minimum (2.12) indicates that no deterioration in colour was observed while the protein content also was found maximum (2.78 mg/ml) which retained in dried nettle leaves.

Keywords: nettle leaves, temperature, loading density, protein and colour

1. Introduction
Stinging nettle (*Urticadioica L.*, *Urticaceae*) is a weed plant widespread in the world, predominantly in wasteland areas with unpleasant stinging hair on the stems and leaves (Kavalali, 2003) [7]. Its genus name *Urtica* is derived from *uro*, to burn, or *urere*, meaning to sting given by Grieve, (1931) [6]. The stinging nettles species name *dioica* is Latin for "two houses", from the Greek word *oikia*, meaning house, and refers to the plant's dioecious nature, bearing male and female flowers on separate plants. Nettle leaf is a micronutrient dense, nutritious food; however, it should be steamed or cooked before ingestion to destroy the stinging hairs, which contain histamine, formic acid, acetylcholine, acetic acid, butyric acid, leukotrienes, 5-hydroxytryptamine, and other irritants (Wagner *et al.*, 1994 and Emmelin, 1949) [14, 4]. When contact with the hairs leads to a mildly painful sting, development of an erythematous macule, and itching or numbness for a period lasting from minutes to days. Medicinal extracts of nettle do not cause this reaction as the hairs are destroyed in processing. Stinging nettle is a powerhouse of nutrients, contains on average 22% protein, 4% fats, 37% non-nitrogen extracts, 9- 21% fibre, and 19-29% ash. The leaves contain about 4.8 mg chlorophyll per gram of dry leaves, depending on whether the plant was grown in the sun or shade. Surprisingly, more chlorophyll and carotenoids are found in plants that are grown in the shade, and dried nettle leaves contains 40% protein (Vance, 2017; Umberto, 2012) [13, 12].
Nowadays, in form of leaves and roots extracts, stinging nettle is used as supportive therapy to help relieve rheumatic complaints and seasonal allergy symptoms in reducing difficulties in urination associated with early stages of benign prostatic hyperplasia (Roschek *et al.*, 2009; Tanzil *et al.*, 2002a) [10, 11]. Antimicrobial and antioxidant activities (Kukrić *et al.*, 2012) [8], the possibilities for decreasing of cardiovascular risks (Alisi *et al.*, 2008) [2] and investigations of chemo preventive properties of stinging nettle extracts in breast cancer cells are still researched (Güler, 2003) [5]. Another research indicates household remedy usage in Moorish, stalk, and leaves of nettle used in treatment of diabetes, hypertension, astringent, anti-rheumatic, diuretic, antidiuretic, and cholagogue (Ziyyat *et al.*, 1997) [15].
The study was focused on the effect of temperature and loading density to the quality of dried nettle leaves because dried leaves are processed for teas, tablets and capsules, and other preparations, and also results in retention significant quantity of proteins and other nutritional

components as reported by Adhikari *et al.,* (2015) [3]. High quality nettle herb has a rich deep green colour with few stems present and those present are small in size. Faded or darkened colouration or presence of large stems is an indication of poor quality material.

2. Material and Methods

2.1 Raw material collection and preparation

Fresh nettle leaves were procured from the farmer of Village Jawahar Nagar, Tehsil-Kichha, District: U.S.Nagar, Uttarakhand in May 2017 and the gloves were used in hand to avoid irritation, and to cut, sort the material for the experiment purpose. After it, leaves were brought to laboratory in polybags and kept it in refrigerator at 4 ^{0}C for quality analysis. Stinging nettle leaves were cleaned (washed) so that the foreign particles were removed**.** Then the fresh nettle leaves at different loading density (0.5, 1, 1.5kg/m^2) and temperature (55, 65 and 75℃) were dried in tray dryer at constant air velocity 0.6m/s. The dried leaves were analyzed for quality parameters such as protein and colour.

2.2 Quality analysis of dried nettle leaves

2.2.1 Colour

Photograph of all fresh and dried nettle leaves were under a uniform light source with the help of a digital camera. The samples were placed under the source of light at minimum distance, and the intensity of light over the sample was uniform for good quality colour. Digital camera was used to capture the image of sample before drying and after drying. The colour brightness coordinates L measure the whiteness value of a colour and ranges from black at 0 to white at 100. The chromaticity coordinate a measures red when positive and green when negative, and the chromaticity coordinate b measures yellow when positive and blue when negative (Alibas, 2008). The values of L*, a* and b* were calculated for colour of fresh as well as dried nettle leaves. The value of L* represents the lightness or darkness of the sample. Hunter a* represents redness (+) or greenness (-). Hunter b* represents yellowness (+) or blueness (-). These values can be calculated as:

$L^* = (L/250) \times 100$, $a^* = (240a/255) - 120$ and $b^* = (240b/255) - 120$

The above values of colour indices were used to calculate ΔE* for all the samples of fresh as well as dried nettle leaves using following expression

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad 1$$

Where,

$\Delta L^* = L^* - L_f^*$ difference in lightness of sample, $\Delta a^* = a^* - a_{f*}$ difference in greenness and $\Delta b^* = b^* - bf^*$ difference in yellowness of the sample

The values of L*, a* and b* are the colour coordinates of the dried samples and $L_f^* a_f^*$ and b_f^* are the colour coordinates of the fresh sample.

2.2.2 Protein

The analysis of protein content is accomplished by modified Lowry's method (Lowry *et al*., 1951) [9]. The assay utilizes Folin-Ciocalteu's phenol reagent which is a mixture of phosphomolybdic and phosphotungstic acid. The peptides of nitrogen react with copper (II) ions in alkaline medium. Subsequently reduction of Folin-Ciocalteu's reagent takes place to form a blue colored complex, heteropolymolybdenum blue, the concentration of which can be identified by spectrophotometric analysis at 660 nm. The protein determination procedure is initiated by mixing 50 ml of 2% Na_2CO_3 with 50 mL of 1NaOH. To this solution 1 ml each of 1% copper suphate ($CuSO_4.5H_2O$) and 2% sodium potassium tartarate ($KNa\text{-}C_4H_4O_6.4H_2O$) is added to form copper-tartarate-carbonate complex. This solution is marked as reagent A. The protein content of all samples was calculated using spectrophotometer.

2.3 Data Analysis

Data analysis and optimization of temperature and loading density were done using Multi Level Categoric Design. The second order mathematical regression equations were developed for fitting the data of quality parameters.

3. Results and Discussions

3.1 Assessment of quality of dried nettle leaves

The adequacy of the model of dried nettle leave was tested using coefficient of determination (R^2) and Fisher's F-test to interpret the effect of Temperature and Loading Density and both the variables were also optimized for getting best results of protein and colour of dried nettle leaves. The analysis of variance was used to analyze both models of dried nettle leaves. The sign and magnitude of the coefficients explain the nature of the effect. Negative sign at linear level means decrease in the response when the level of the independent variables was increased while positive sign indicate increase in the response. Negative interaction suggest that the level of one of the independent variable can be increased while that of other decrease for constant value of response.

Table 1: Experimental result of responses

Std	Temperature (^{0}C)	Loading density (kg/m^2)	Protein content (mg/ml)	Change in colour (ΔE)
1	55	0.5	2.13	2.05
2	65	0.5	1.76	3.40
3	75	0.5	1.04*	4.32
4	55	1	2.23	1.95*
5	65	1	1.91	3.23
6	75	1	1.36	4.27
7	55	1.5	2.86**	2.13
8	65	1.5	2.28	3.43
9	75	1.5	1.77	4.48**

*&** indicates minimum and maximum values

3.2 Statistical analysis of protein

The data presented in Table 1 depicted the maximum protein content was obtained to be 2.86mg/ml for the experiment no.7 with the combination of temperature (55 ^{0}C) and loading density (1.5kg/m^2) and minimum protein content obtained to

be 1.04mg/ml for experiment no.3 with the combination of temperature (75 ^{0}C) and loading density (0.5kg/m^2) due the high temperature reduced the protein in the dried nettle leaves. The ANOVA is where the descriptive statistics and statistical tests are presented in Table 2. The p-value of model was found 0.0055 which implies that the model was found highly significant (p<0.01). The effect of both temperature and loading density on protein at linear level was found highly significant at 1% level of significance means that levels of both variables increases with increased the protein in dried leaves. Quadratic term and interactive term were found insignificant.

Table 2: ANOVA for protein of dried nettle leaves

Source	Sum of Square	df	Mean Square	P-value
Model	2.25	5	0.45	0.0055*
Temperature (A)	1.54	1	1.54	0.0012*
Loading Density (B)	0.66	1	0.66	0.0042*
AB	2.500E-005	1	2.500E-005	0.9642
A^2	0.015	1	0.015	0.3183
B^2	0.038	1	0.038	0.1528
Residual	0.032	3	0.011	
Cor total	2.29	8		
R^2 (0.9862)	Adj R^2 (0.9631)		Pred R^2 (0.8330)	

* & ** indicates 1 and 5% level of significance

Regression analysis was performed to fit the data of protein. The regression coefficients for the second order polynomial equations and their corresponding p-value for the linear, quadratic and interactive terms were presented in Table 2. The statistical analysis indicates that the proposed model was adequate, possessing significant fit and very satisfactory values of R2 for protein. The coefficient of determination (R2) for the regression model for protein was obtained as 0.9861 which was closer to the value of R2 to the unity, the better the empirical model fit into the actual data. The "Pred R-Squared" of 0.8330 is in reasonable agreement with the "Adj R-Squared" of 0.9631 i.e. the difference of both is less than 0.2. The second order polynomial equation was developed which shows the empirical relationship between the protein and independent variables given below:

$$\text{Protein} = 1.380278 + 0.0625A - 0.41083B - 0.0005AB - 0.00087A^2 + 0.553333B^2 \quad \ldots 2$$

Where

A=Temperature (^{0}C); and B=Loading density (kg/m^2)

3.2.1 Graphical interpretation

The Figure 1 revealed that the effect of temperature on protein of dried nettle leaves at optimum point of loading density (1.5kg/m^2). It shows that the drying temperature was increased from 55 to 75 ^{0}C then the protein content decreased from 2.2 to 0.5mg/ml due to the high loading density of leaves and low temperature (55 ^{0}C). From Figure 2 at optimum point of temperature (55 ^{0}C) the loading density was increasing gradually from 0.5 to 1.5kg/m^2 the protein content also increased from 1.55 to 2mg/ml in the dried nettle leaves, it was due low temperature and high loading density of nettle leaves

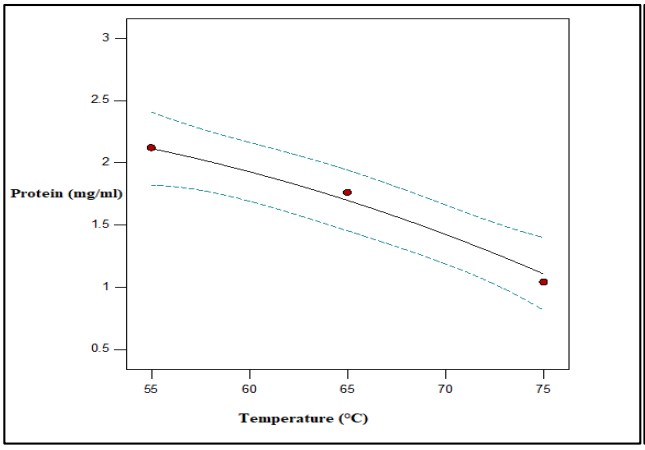

Fig 1: Effect of temperature on protein

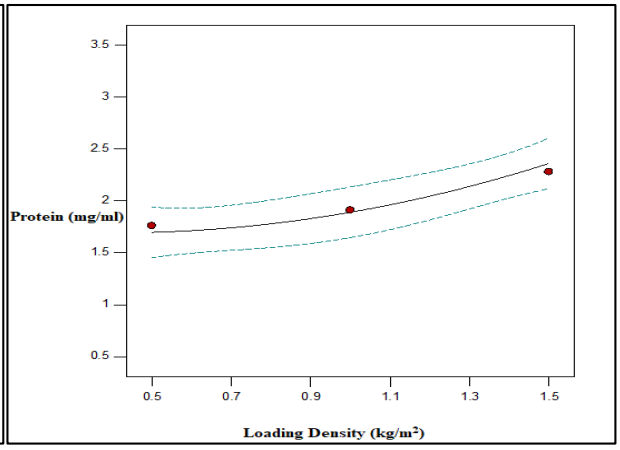

Fig 2: Effect of loading density on protein

3.3 Statistical analysis of Colour

The data presented in Table 1 depicted the maximum value of colour was obtained to be 4.48 for the experiment no.9 with the combination of temperature (75 ^{0}C) and loading density (1.5kg/m2) more variation in ΔE was due to high temperature and minimum value of change in colour of dried nettle leaves obtained to be 1.95 for experiment no.4 with the combination of temperature (55 ^{0}C) and loading density (1.0 kg/m2), due to low temperature and at center point of loading density better colour of dried leaves observed. The ANOVA of colour are presented in Table 3 to evaluate significant effect of variables. The "Pred R-Squared" of 0.9949 is in reasonable agreement with the "Adj R-Squared" of 0.9987; i.e. the difference is less than 0.2 means the model is fitting the data and can reliably be used to interpolate. The p-value of model was found 0.0001 which implies that the model was found highly significant (p<0.01) model could be analyzed for colour. The effect of temperature on colour at linear as well as quadratic level was found highly significant at 1% level of significance while no effect at interactive level. But loading density (p<0.1).

Table 3: ANOVA for colour of dried nettle leaves

Source	Sum of squares	df	Mean squares	P-value
Model	8.13	5	1.63	< 0.0001*
A-Temperature (^{0}C)	8.03	1	8.03	< 0.0001*
B-Loading density (kg/m^2)	0.012	1	0.012	0.0542**
AB	1.600E-003	1	1.600E-003	0.3453
A^2	0.047	1	0.047	0.0090*
B^2	0.046	1	0.046	0.0093*
Residual	3.844E-003	3	1.281E-003	
Cor Total	8.14	8		
R^2 (0.9995)			Adj R^2 (0.9987)	
Pred R^2 (0.9949)				

* & ** indicates 1 and 10% level of significance

The regression coefficients for the second order polynomial equations and their corresponding p-value for the linear, quadratic and interactive terms were presented in Table 3. The statistical analysis indicates that the proposed model was adequate, possessing significant fit and very satisfactory values of R^2 for colour of dried nettle leaves. The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space. The second order polynomial equation was developed which shows the empirical relationship between the dependent and independent variables shown below:

Colour = -9.96778 + 0.311A - 1.38B + 0.004AB - 0.0015A^2 + 0.607B^2 …3

3.3.1 Graphical interpretation

Figure 3 intepretates **t**he effect of temperature on colour of dried nettle leaves at optimum values of loading density (1.5kg/m^2). It was clear from figure the colour is changing continuously by increasing level of temperature from 55 to 75 ^{0}C. The best colour of dried nettle leaves were found at 55 ^{0}C due less heat supplied to the product, and least variation in ΔE means closer to the colour of fresh leaves. From Figure 4 It was concluded that as the loading density is increasing from 0.5 to 1.5kg/m^2, the change in colour decreased after that it again increased means best results were obtained at center point.

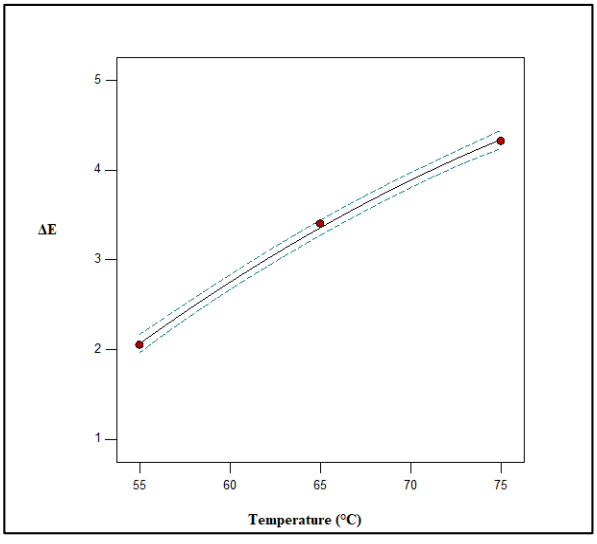

Fig 3: Effect of temperature on colour change in colour (ΔE)

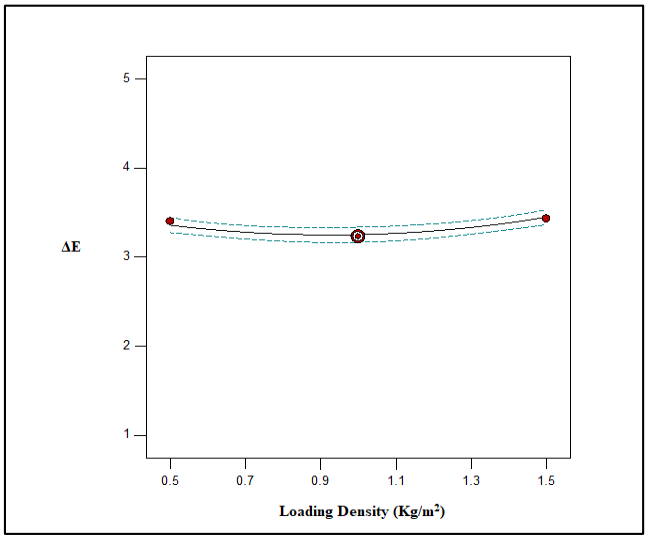

Fig 4: Effect of loading density on colour change in colour (ΔE)

Optimization of parameters for dried nettle leaves

The most suitable values obtained after optimization of process parameters given in Table 4 with respect to optimum values of change in colour and protein were 2.12 and 2.78mg/ml.

Table 4: Optimum values of process parameter for dried nettle leaves

Parameters	Actual values
Temperature (A, ^{0}C)	55
Loading Density (B, kg/m^2)	1.5

4. Conclusion

Nettle is a plant easy to grow. It is rich of chemical components and composition, and is widely used from cosmetic to food. It was concluded that the protein are basic building block of tissue, and are part of enzymes and hormones regulating many important life process so that dried nettle leaves is good source of protein used as a tea and other way in diets. The colour change ΔE in dried nettle leaves varied from 1.95 to 4.48 which are closer to colour of fresh leave. The protein varied from 1.04 mg/ml to 2.86 mg/ml.

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