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Study of influence of various drying methods on chlorophyll degradation, decreased color quality and their correlation in the Keluss medicinal plant (*Kelussia Odoratissima* Mozaff)

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Abstract

This study was performed with the purpose of studying and comparing various drying methods on physical quality of plant's color, chlorophyll and their correlation in Keluss medicinal plant employing two different microwave including 150 and 300w, three different oven temperatures including 40°C, 50°C and 60°C and the natural method (Shade and sun). Sample drying in all methods continued until their humidity content reached to 0.1% based on dry weight (or 10% based on wet weight). This research was conducted as a quite experimental fully random project with four replications and 7 treatments. Spectrophotometer was employed for chlorophyll assessments and physical color quality of plants was performed via visual appraisal and correlation was computed using spearman correlation coefficients. Results revealed that there is a significant relation between various drying methods and the assessed traits. The highest and the lowest chlorophyll a and total chlorophyll were those of the shade treatment and 60°C oven. The highest chlorophyll b content (21mg/dry matter) was seen in 40°C oven. The highest ratio of chlorophyll a to b (35.2) was that of the 40°C oven and its least amount was that obtained using sun method and 300w microwave. The highest color quality (4) was that of the shade method for green color which statistically has no significant difference with the sun method and the lowest quality score was relevant to the 60°C oven with score 1. Generally, to obtain the highest color quality and decreased chlorophyll in Keluss plant, using a temperature of 40°C can be recommended.

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Introduction

Keluss (*Kelussia odoratissima* M.) belongs to the umbellifereae (apiaceae) family and is known by its local name of keluss in Iran. It is a perennial plant and is considered as one of the important and valuable food, medicinal, and forage endemic plants of the Iranian pastures growing on Zagross mountain chains and is one of the medicinal plants the existence of which has not been so far reported from other parts of the world (Mozafariyan, 1997). Drying is one of the oldest methods employed for post-harvest preservation of agricultural crops. This process includes humidite elimination via evaporation to the point of a particular should so that crop can be stored for a long period during which its enzymatic microorganisms and yeast activities are stopped (Azizzi *et al.*, 1999). Selection of appropriate drying procedure for drying plant organs is one of the crucial post-harvest operations. Application of inappropriate method can lead to loss of plant organs or wasting of all their active ingredients (Beiguy, 2009). Color plays an important role in acceptability of medicinal plants. When the plant is used as tea or spice, the natural green color of leaf as a quality measure enjoys high importance for the consumers. In drying plants using hot air, due to loss of chlorophyll, the light green color of the leaf is changed to a clark color and sometimes it becomes brown (Minica *et al.*, 2004). In a research conducted in 1999 by Mahan om *et al.* on 8 types of medicinal plants like mint, celery and some of the endemic plants of the region, drying methods in a 50°C oven for 9 hours and in 70°C oven for 5 hours and freeze drying were employed for assessing chlorophyll, carotenoid, niacin, flavonoid and ascorbic acid, it was found that drying via freeze drying causes less loss of such substances compared with the oven method and in the 50°C oven for 9 hours the highest amount of these substances were lost. Some phenolic materials, ascorbic acid and pigment responsible for color of fruits and vegetables like carotenoids and chlorophyll show the highest level of sensitivity to change in processing leading to changed color of products (Diplock *et al.* 1998, Suzeto *et al.*, 2002). Impact of

drying via hot air current of microwave on chlorophyll content and color in six medicinal plants was studied by sald and Viroi-Rogert in 2012. Obtained results revealed chlorophyll degradation and color change in plants. Such changes, were dependent on type of plants and plants of one family showed identical reaction. Plants of the umbellifereae family bore the highest level of chlorophyll and color quality loss compared with other species and a severe correlation was seen between the gloss degree and plant color and chlorophyll a content and the obtained result shows that chlorophyll a degradation has led to color modification in other species is not solely due to their pigment content, and they have become brown probably as a result of enzymatic or non-enzymatic reactions (witrowa- Rajchert, 2009) or it may be due to the degree of carotenoid degradation at the time of drying (Polak *et al.* 2009). In 2008 Erge *et al.* studied the effect of heat on chlorophyll and decrease of color in garden pea. They reported that the green color in garden pea in the form of blue-green tint is due to presence of yellowish green chlorophylls a and b in that plant. Chlorophyll a, compared with chlorophyll b in reaction to heat is more readily destroyed therefore, through increase of heat the ration of chlorophyll a to chlorophyll decreases and green color of garden pea tends to become yellow. The aim of this research was conducted as a quite experimental fully random project with four replications and 7 treatments. Spectrophotometer was employed for chlorophyll assessments and physical color quality of plants was performed via visual appraisal and correlation was computed using spearman correlation coefficients.

Materials and methods

Study and sampling method

To conduct this project, Keluss was obtained from Fereydoonshahr situated in Isfahan and was identified by experts at the Bureau of Natural Resources of this city. This test was done in the framework of a complete random project with 7 treatments and four replications. To determine original humidity content of Keluss plant, four 50gr

samples were dried up in an oven at 105 °C for a period of 24 hours. Then they were weighed via a digital balance with a 1/1000 precision and the results were inserted in relations 1 and 2. Original humidity content of Keluss twigs was about 83.26% based on wet-weight or 4.97 based on dry weight.

1. Humidity level based on wet weight = Humidity weight/(Humidity+ weight of dry matter)

2. Humidity level based on dry weight = Humidity weight/weight of dry matter.

Drying of samples methods

Drying of samples was performed employing three different methods. 1. Natural method consisting of drying in shade with a mean temperature of 18 °C and sun-drying with a mean temperature of 17 °C. 2. Three different oven temperatures included 40 °C, 50 °C and 60 °C and 3. A domestic Delonghi microwave oven made in Italy, with maximum electric output of 1000 w and dimensions of 380×320×550 mm equipped with a tray and digital power and time adjustment was used. In this research, two various 150w and 300w microwave powers with equal volume of Keluss twigs were used. Hundred gram Keluss samples were dispersed on the tray to absorb microwave energy uniformly. In all methods sample drying was continued to the point when humidity content of samples based on dry weight reached to 10% (or 10% based on wet weight) to assess the impact of drying methods on apparent quality of samples following drying, visual assessment (Ghan and Azzizi, 2009) was performed and samples were scored 1 to 4 indicating the following qualitative characteristics.

Indicating the following qualitative characteristics

Score 4. Very good, without causing bad color in the sample. Score 3. Good, slight color modification of the sample. Score 2. Moderate, partially affected by the drying method. Score 1. Weak, drying has left undesirable effect on apparent color of the sample. Chlorophyll assessment was conducted via the

Lichtenthaler procedure (1987). To extract this pigment, 0.02gr of dry tissue of plant was grinded in mortar, fully mixed with 80% acetone and then filtered and their absorbance's were read at 645 and 663 wavelengths for chlorophyll a and chlorophyll b respectively via the Shimadzu spectrophotometer model UV-120-01 made in Japan. To adjust the device, 80% acetone was used. Then the chlorophyll amount was computed based on mg/g of dry weight.

Amounts of chlorophylls a and b were computed according the following equations respectively.

$$(4-3) \text{ Mg chlorophyll a/gr dry weight} = \{12/7(A663) - 2/69(A645)\} \times V/W \times 1000$$

$$(5-3) \text{ Mg chlorophyll b/gr dry weight} = \{22/9(A645) - 2/68(A663)\} \times V/W \times 1000$$

$$(6-3) \text{ Mg total chlorophyll/ gr dry weight} = \{20/2(A645) + 8/02(A663)\} \times V/W \times 1000$$

W=Weight of dried tissue (gr), V=Volume of chlorophyll solution (ml), A=Light absorbance of extract.

Software for modeling

Obtained data was analyzed employing the SAS software and to compare means the Duncan test at the 5 percent probability was used. To draw the diagrams, the EXEL program was employed. And to compute the correlation coefficients between chlorophyll and color quality of the plant, the Spearman correlation coefficients were used.

Amount of Chlorophyll-a

According to the results of variance analysis table, drying methods were effective on the amount of chlorophyll and were significant at the 1 percent statistical level. Regarding the diagram of the mean comparison diagram (Fig. 1), the highest chlorophyll a level was seen in those samples dried in the shade (0.88 mg/g of dry tissue and it least level was obtained via the 60 °C oven method (0.29 mg/g of dry

tissue. The level of chlorophyll a in 60 °C oven was nearly three times less than the level of chlorophyll a

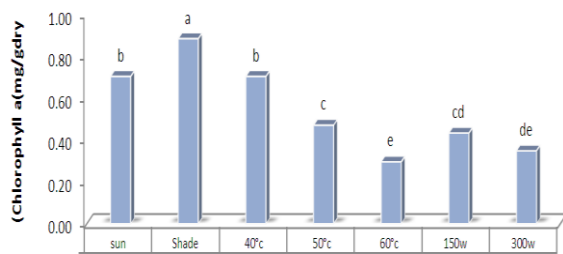
obtained from the shade treatment.

Results and discussions

Table 1. Results of variance analysis of impact of treatments on chlorophyll and its components.

Source variation	(df)	(MS)				
		chlorophyll a	chlorophyll b	Total chlorophyll	Chlorophyll a/b	Color quality
Treatment	6	***0.19	0.02***	***0.27	537.89***	***4.354
Error	21	0.007	0.0001	0.006	2.06	0.033
(%) CV		14.84	11.37	11.72	14.71	6.30

*** Indicates significance at the statistical level of 0.001.



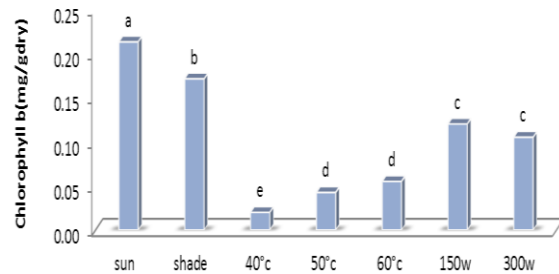
Natural drying, different oven temperatures and microwave powers

Fig. 1. Effect of imposed treatments on chlorophyll a level. Means with identical letters have no significant differences at the statistical 1% level.

Chlorophyll-b level

Based on results obtained of variance analysis table, drying method was effective on the chlorophyll b level and was significant at the 1% probability. Regarding the mean comparison diagram (Fig. 2), the highest chlorophyll b level was obtained from the sun-drying method (0.02 mg/g of dry tissue) and its least level was achieved using 40°C oven (0.02 mg/g of dry tissue). In the sun treatment the amount of obtained chlorophyll b was 10.5 times more than the amount obtained from the 40 °C oven method. Through increased temperature in the oven, the chlorophyll b level increased as well however, between the 50 °C and 60 °C oven procedures, in spite of amount, no statistically difference was seen.

From the view point of chlorophyll b level, microwave powers shoed no statistical difference.



Natural drying, different oven temperatures and microwave powers

Fig. 2. Effect of performed treatments on the chlorophyll b level. Means with identical letters have no significant difference at the 1 percent statistical level.

Total chlorophyll level in the dried plant

According to table 1, results of variance analysis of total chlorophyll level was influence by various drying methods and was significant at the 1% statistical level. Its highest amount was obtained from the shade-drying method (1.05 mg/g of dry tissue) and its least level was relevant to the 60 °C oven method (0.33mg/g of dry tissue) which had no significant difference with what was resulted from the 300w microwave procedure. In fact its amount is equal to one third of the chlorophyll obtained from the shade treatment. At oven temperatures, along with increased temperature, the chlorophyll level decreased. At microwave powers, along with power increase total chlorophyll level decreased however, statistically it was not significant. Total chlorophyll level has been depicted in fig. 3.

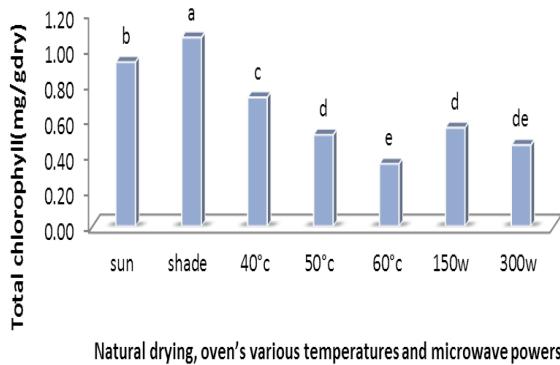


Fig. 3. Effect of performed treatments on total chlorophyll level. Means with identical letters showed no statistical difference at 1 percent level.

Ratio of Chlorophyll-a to Chlorophyll-b

According to the results depicted in table 1 of variance analysis, various drying methods were effective on chlorophyll a to chlorophyll b ratio at 1% significance.

According to the fig., regarding obtained means, the highest chlorophyll a to chlorophyll b was achieved from the 40 °C oven treatment (32.5) and its least amount was obtained by the 300w microwave (3.26) which statistically had no significant difference with the sun drying treatment.

In other words, this ratio at 40 °C oven is 11 times more than the ratio in 300 w microwave. In various oven temperatures, through increased temperature this ratio decreased. Ratio of chlorophyll a to chlorophyll b between shade and 60 °C oven treatments showed no significant difference.

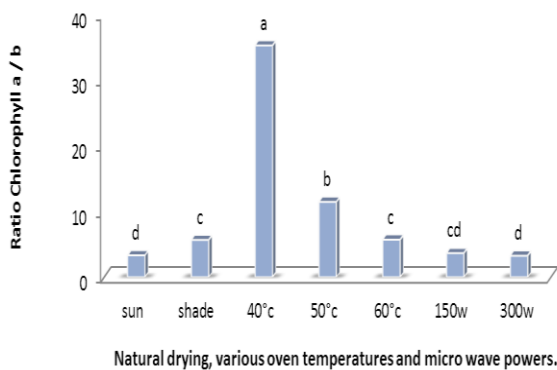


Fig. 4. Effect of conducted treatments on chlorophyll a/b ratio.

Means with identical letters show no significant difference at the statistical 1 percent level.

Color quality of the dried plant

Based on results obtained of variance analysis, drying method significantly influenced the plant color quality and were significant at the 1% probability level. Based on the mean comparison diagram (Fig. 4), the highest color quality score was relevant to shade method (Score 4) of green color which statistically has no significant difference with the sun drying method and the least quality score was relevant to the drying in the 60 °C oven (60 °C, with score '1' in olive green color. Various microwave powers had no significant difference. The obtained color from these methods was light green. At oven temperatures, along with increased temperature, color quality level decreased and was changed from green to olive green.

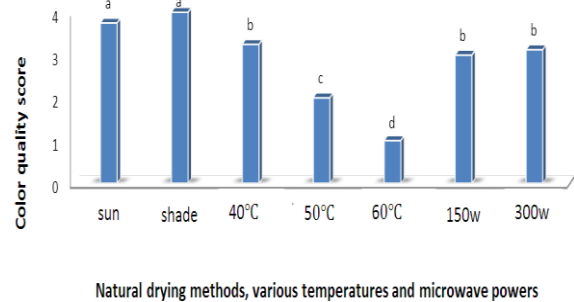


Fig. 5. Effect of performed treatments on the color quality means with identical letters show no significant difference at the 1 percent statistical level.

Spearman correlation coefficients between chlorophylls and plant color quality correlation coefficients between chlorophylls and color quality of the Keluss plant was significant at 1% probability level.

Results revealed that, the highest correlation exists between the plant color quality (evaluated visually) and total chlorophyll. It is worth mentioning that between total chlorophyll and chlorophylls a and b, the highest correlation was found between chlorophyll a and the total chlorophyll.

The correlation between the color quality of the plant and the chlorophyll a to b was negative.

Table 2. (Spearman) correlation coefficients between chlorophyll content and color quality of the plant.

	Color quality plant	Chlorophyll a	Chlorophyll b	Chlorophyll Total	Chlorophyll a/b
Color quality plant	1				
Chlorophyll a	**0.80	1			
Chlorophyll b	0.62**	0.30	1		
Chlorophyll Total	0.88**	0.97**	0.48**	1	
Chlorophyll a/b	-0.19	0.32	-0.72**	0.11	1

** Indicates significance at the 0.01 statistical level (n=28).

Chlorophyll-a

Results obtained on influence on drying methods on chlorophyll a rate indicated that drying the keluss plant in the shade has provided the highest rate of chlorophyll a. and the lowest rate was seen in the 60 °C oven and sun treatments.

In the other words, the rate of chlorophyll a achieved in the shade drying method, was 3 times more than that of chlorophyll a obtained by the 60 °C oven method. On the other hand, along with increase of temperature from 40 °C to 50 °C, chlorophyll a rate decreased. Also in the microwave treatment along with increased power we witness decrease of this pigment. In the sun treatment a higher rate of chlorophyll compared with microwave powers was observed. This influence might be due to chlorophyll sensitivity to increased temperature in the oven and augmented power of microwave that in higher temperatures has led to higher degradation of this pigment. In some researches it has been reported that chlorophyll is highly sensitive to increased temperature and that it stability decreases (Ali bas, 2010; Arsallan *et al.* 2010; Witrowa *et al.* 2009, Di Cesare *et al.* 2003 and 2004).

Chlorophyll-b

Influence of drying methods on the rate of chlorophyll a showed that its highest rate based on mg/g of dry matter was obtained in the sun drying method while the least rate was observed using the 40 °C oven. Also, through increased temperature from 40°C to 60

°C the trend of increased of chlorophyll b is observed. This result indicates that the color of the sun-dried plant is yellowish green while that of the plant dried in a 40 °C oven is dark green. The reason might be loss of chlorophyll a influenced by increased activity of chlorophylase enzyme in high temperature in the 60 °C oven and also insensitivity of chlorophyll b to high temperatures and its stability in high temperature and power in the sun and microwave drying treatments. Results obtained of this research corresponds with those reported by Szeto and Witrowa on garden pea.

Total chlorophyll

Results obtained on influence of drying methods on total chlorophyll rate in the shade drying method like that of the chlorophyll a was at the highest level which did not have a significant difference with the sun-drying method and the least rate was observed in the 60 °C oven method the rate obtained in which procedure had no significant difference with that achieved using the 300 w microwave. The level of chlorophyll obtained in the shade was almost 2.7 time more than the rate obtained by employing the 60 °C oven. Through increase of temperature, the total chlorophyll rate decreased as well and in the microwave method too, along with increased power this augmentation was observed but it was not significant.

The reason for this increase and reduction might be attributed to the influence of temperature on total

chlorophyll. In the sun-drying treatment, the total chlorophyll rate is higher than the 60 °C oven. Probably the influence of temperature on chlorophyll degradation has been more than the effect of solar rays, or the type of chlorophyll reaction to these two factors is different (Arab-Hosseini *et al.* 2006; 2009 and Salaz and Witrowa-Rajchert 2012).

Chlorophyll a/b ratio

Influence of various drying treatment methods on the chlorophyll a/b ratio revealed that, the highest ratio of chlorophyll a/b was obtained employing the 40 °C oven treatment and its least rate was registered for the sun treatment. By increase of temperature in the oven, this ratio decreased. In the natural method, this rate was higher using the shade compared with sun method. Through increased microwave power as well, this ratio decreased however they had no significant differences. The reason might be increased temperature in the oven which along with increased temperature, the chlorophyll a/b ratio decreases. In the shade treatment method, increased drying period might be considered the culprit for this ration reduction. Results obtained of this research corresponded with results reported by Erge *et al.* 2008.

Color quality of the dried plant

The color quality of the dried plant (assessed visually) was different in various treatments. The best color quality of the Keluss plant was relevant to the shade treatment n green and the least color quality was relevant to 60 °C oven dark olive green. Along with increased temperature in the oven, the dried plant color decreased as well and from green changed to dark olive green. Regarding microwave powers, increased power led to production of a green color with little tendency to yellow however, statistically no significant differences were observed. The reason might be the negative influence of temperature on loss of plant color. Reports of Diplock *et al.* (1998), Sortz *et al.* (2002) indicate color modifications in fruits and vegetables in thermal process.

The highest correlation was seen between total chlorophyll and the plant's color quality. It is worth mentioning that between total chlorophyll and chlorophylls a and b the highest correlation coefficient was observed between chlorophyll a and the total chlorophyll. To determine the dried plant color, probably the rate of total chlorophyll or chlorophyll a can be assessed. Results of this research corresponds with part of the results reported by Seldz and Witrow-Rajchert in 2012 on six types of herbs.

Conclusion

Drying is one of the most important post-harvest processes of some of the agricultural crops, especially medicinal plants and in most cases lead to concentration of some of the effective substances. Regarding results obtained of this research, it can be pointed to the following points. In this study, increased temperature negatively influenced chlorophyll rate and the plants color quality. As a whole, regarding the results obtained from the 60 °C oven treatment in this test it can be said that this temperature is not appropriate for the Keluss herb. For a more careful color evaluation in the Keluss herb, instead of visual assessment which is prone to error and influence of personal viewpoint, perform the grading using total chlorophyll assessment for grading the dried plant. For drying the Keluss, employment of 40°C oven can be recommended as the best procedure.

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