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Effect of processing on color and antioxidants of *Malva parviflora* leaves

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Abstract - The aim of this work was to establish a comparative investigation of the effects of microwave drying (160, 350, 500, 650, 750 W), air drying (50, 75°C), infrared drying (40, 50, 60, 70°C) and combined air-microwave drying (50°C-160 W; 50°C-350 W, 75°C-160 W; 75°C-350 W) on color, phenol content and antioxidant activity of mallow leaves (*Malva parviflora*). Drying time of the mallow leaves sharply decreased with increasing drying temperature from 40 to 70°C and/or applied microwave power level from 160 to 750 W. Microwave drying and combined air-microwave drying allowed obtaining short drying times and high drying rates. Page Model described adequately all drying kinetics of mallow leaves (R^2>0.9911). Whatever the drying process, the value of colorimetric parameters (a*: greenness/redness and b*: yellowness / blueness) decreased if compared to those of fresh leaves. Total phenolic and flavonoid contents of dried leaves significantly increased (except for air drying at 50°C and combined air-microwave drying at 350 W-50°C and at 350 W-75°C) if compared to fresh ones. The maximal phenolic contents are recorded for leaves dried by microwave drying. The mallow leaves extract concentration providing 50% inhibition (IC50) of 2, 2-Diphenyl-1-picrylhydrazyl (DDPH) varies from 2.574±0.098 to 3.100±0.115 mg /ml.

Key words: *Malva parviflora*, phenol, flavonoid, IC50, color parameters, drying.

1. Introduction

Mallow (*Malva sylvestris* L.) is a species of the Mallow genus *Malva* in the family of Malvaceae (Zohra et al. 2013). It is a natural plant growing in Europe, North Africa and South-west Asia (Prudente et al. 2013). Mallow is commonly used as vegetable. Young Mallow leaves are eaten raw in salads, leaves and shoots are consumed in soups and as boiled vegetables (Samavati and Manoochehrizade 2013). Mallow leaves are rich in carbohydrates (71.46 g/100g DM), ash (13.53 g/100g DM), proteins (12.25 g/100g DM), reducing sugars (6.22 g/100g DM), fat (2.76 g/100g DM) and provide energy (359.72 g/100g DM) (Barros et al. 2010). The biological activity of mallow leaves may be attributed to antioxidants, such as phenols (386.5 mg/g extract), vitamin C (0.17 mg/g extract), vitamin E (106.51 mg/100g extract), carotenoids (0.19 mg/g extract) (Barros et al., 2010) and others substances such as terpenoids, coumarins, mucilage and pigments (Gasparetto et al. 2012).
Traditionally, mallow is also used in medicinal applications to treat specified disorders of several systems of the body, such as the digestive system, the respiratory, the genitourinary, the muscular and skeletal systems, as well as skin disorders and injuries (Barros et al. 2010; Marouane et al. 2011). The leaves, flowers and aerial parts of *M. sylvestris* are known worldwide due to their anti-inflammatory properties, mainly against gingivitis, abscesses and tooth pain (Conforti et al. 2008; Idolo et al. 2010). Additionally, the leaves and flowers have ample potential for use in the treatment of urological problems, insect bites, burns, furuncles and ulcerous wounds (Lardos 2006; Cornara et al. 2009).

Because of their high moisture content (>75 %), fresh mallow leaves are highly perishable. Drying can extend the consumption period of mallow leaves while maintaining their nutrition content by evaporating the moisture in the product up to a certain threshold value (Alibas 2007). Convective air drying methods can cause adverse changes in the taste, color and nutrient content of the dry product as a result of the applied long drying period and high temperature (Drouzas et al. 1999). Microwave drying has become common because it prevents the alteration of the products sensorial properties and ensures the rapid and efficient distribution of heat within the product (Li et al. 2009; Dong et al. 2011). Moreover, microwave drying reduces the drying time (Balbay et al. 2011). Infrared drying was also used to obtain high quality foodstuffs, including fruits, vegetables and grains (Zhu et al. 2002). The advantages of infrared drying are higher drying rate, energy saving, uniform temperature distribution and giving a better quality of dried product (Nowak and Lewicki 2004; Alibas and Köksal 2014).
The aims of this study were (i) to evaluate the efficacy of microwave drying, infrared drying, air drying and combined air-microwave drying for mallow leaves stabilization, (ii) to examine the changes in color, total phenol- and flavonoid contents and antioxidant activity of dried leaves; (iii) and to determine the optimum drying method for drying mallow leaves considering the total phenols, total flavonoids, antioxidant activity and drying period.

2 Material and methods

2.1. Raw material

Fresh mallow leaves (*Malva parviflora*) were collected from plants in the governorate of Beja, (Tunisia), in floral physiological stage during February and March 2013.

2.2. Drying processes

Fresh leaves were uniformly spread in a thin layer before drying. A programmable domestic combined air -microwave oven (Whirpool®, France), with a maximum output of 750 W at 2450 MHz was used for microwave and combined air-microwave drying experiments. The microwave drying was performed according to a preset power (160, 350, 500, 650, 750 W). Air drying experiments were carried out at 50 and 75°C. Combined air-microwave drying was investigated (50°C/160 W; 50°C/350 W, 75°C/160 W; 75°C/350 W). Infrared drying experiments were conducted at 40, 50, 60 and 70°C by the means of an infrared moisture analyzer (Sartorius MA 40).
Drying processes of fresh mallow leaves continued until reaching a constant weight. The moisture content of the leaves was determined during drying by recording the sample weight.

2.3. Mathematical treatment

Product weight, initial moisture content (X_0) and dry matter content of the mallow leaves were used to calculate the moisture content of the leaves (X_t) obtained at any drying time (t) and the corresponding dimensionless moisture content (Eq. 1):

$$X=\frac{X_t-X_f}{X_0-X_f}, \qquad (Eq.1)$$

Where X_f is the final moisture content (kg / kg DM). The experimental moisture contents data obtained for drying experiments of mallow leaves were converted to dimensionless moisture content X and were fitted by the thin layer drying Page model (Eq. 2) (Page,1949) by using Matlab software®. Correlation coefficient (R^2) and standard error (SE) were retained to determine the goodness of the fit:
Page model: $X = \exp(-kt^n)$ (*Eq.2*)
Where k and n are the model constants.

2.4. Colorimetric parameters

The colorometric parameters of mallow leaves were evaluated by using a colorimeter (Maselli LC, Beijing). The latter measures the spectrum of the reflected light and converts it into a color coordinate set the CIE Lab L* value is the value of the brightness from 0 (black) to 100 (white); the a* value is from -100 (green) to 100 (redness) and b* ranges from -100 (blue) to +100 (yellow). The total color difference (ΔE) was determined by using the following equation (Eq. 3) where the subscript "0" in equation refers to the color of fresh leaves:

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \qquad (Eq.\ 3)$$

2.5. Conventional solvent extraction

Dried mallow leaves samples were ground using a coffee grinder (Moulinex®, France). 2g of mallow leaves powder were extracted with 30 ml of ethanol. The mixture was shaken and then placed in darkness for 24h at room temperature. The crude extract was centrifuged and the supernatant was filtered. The filtrate was evaporated. The extracts were collected and stored at 4°C for further measurements. Each measurement was performed three times.

2.6. Total phenols and total flavonoids

Total phenols were determined by Folin-Cioccalteu method according to Wong et al. (2006). 125 µl of extract were added to Folin-Cioccalteu reagent and Na_2CO_3 solution. The tubes were agitated for 15s and allowed to stand for 1h30 min at dark before spectrophotometric analysis (Biochrom Libra S22, Cambridge). Absorbance was then measured at 765 nm. Gallic acid was used to calculate the standard curve. The results were expressed as mg Gallic acid equivalent/g DM ± standard deviation for three triplicates.

Total flavonoids were determined following the modified procedure of Djeridane et al. (2006). 250 µl of extract was placed in a 2.5 ml volumetric flask and then 75 µl of 5% $NaNO_2$ was added. After 5 min, 150 µl of 10% $AlCl_3$ were added. 5 min later, 500 µl of 1M NaOH were added and the volume made up with distilled water. The solution was mixed and absorbance was measured at 510 nm using a spectrophotometer (Biochrom Libra S22, Cambridge). Total flavonoids amounts were expressed as mg Quercetin equivalent /g DM ± standard deviation for three triplicates.

2.7. Determination of antioxidant activity by DPPH assay

Antioxidant activity of mallow leaves extracts was determined by DPPH assay (Ba et al. 2009) with minor modifications. A 63.4 µM of 1.1-diphenyl-2-picrylhydrazyl (DPPH) was prepared by dilution of 2.5 mg of DPPH with 100 ml of ethanol. 2 ml of sample extract was added to 250 µl of DPPH solution and incubated in dark for 30 min. The reduction of the DPPH radical was determined by measuring the absorption at 520 nm. The percentage of DPPH radical scavenging activity was calculated between A_0 and A_t, according to the following equation (Eq.4) with A_0 as initial optical density and A_t as final optical density. Appropriate solvent blanks were run in each assay.

$$\text{Percentage of inhibition} = \frac{(A_0 - A_t)}{A_0} \times 100 \qquad (Eq.4)$$

The extract concentration providing 50% of radicals scavenging activity (IC50) was calculated from the graph of percentage of inhibition against extract concentration.

2.8.Statistical analysis

Statistical analysis was carried out using the software package IBM. SPSS 20.0 and the comparison of averages of each treatment were based on the analysis of variance (ANOVA) at significance level 5%. Values followed by the same letter are not statistically significant according to Duncan's multiple range test at significance level $p < 0.05$.

3. Result and discussion

3.1. Drying kinetics of mallow leaves

Variations of moisture contents of mallow leaves obtained for different drying modes and conditions (microwave, convective, infrared and combined air- microwave drying) were presented in Figure 1. Microwave drying kinetics show a significant reduction in drying time with the increase in microwave power level from 160 to 750W. In fact drying time corresponds to 58, 24, 14, 11 and 10 min for microwave drying at 160, 350, 500, 650 and 750 W, respectively.

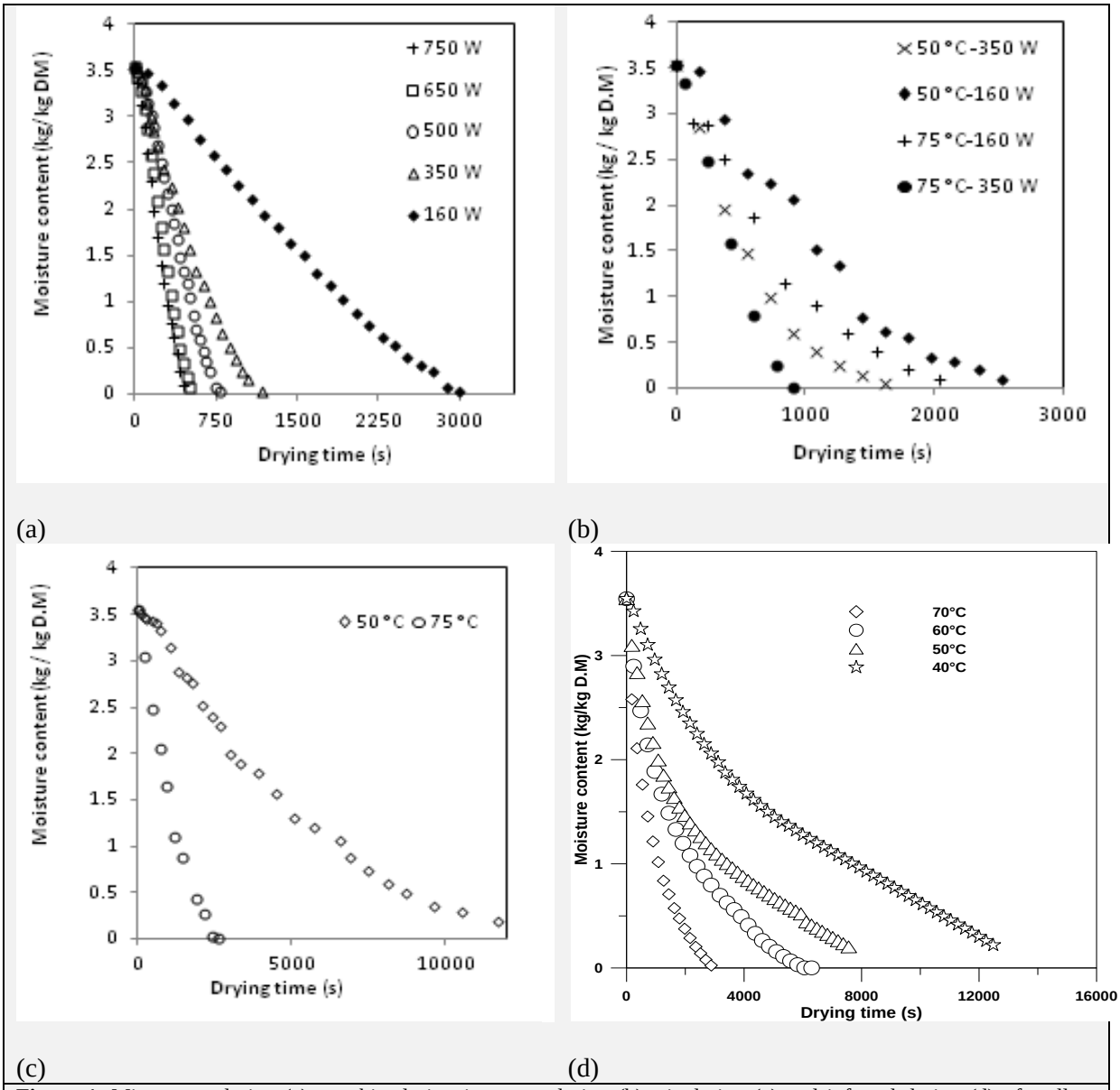

Figure 1. Microwave drying (a), combined air-microwave drying (b), air drying (c) and infrared drying (d) of mallow leaves

The drying time obtained for 160 W was 5.80 times longer than that obtained at 750 W. The drying times of the mallow leaves were 208, 126, 106 and 65 min for the infrared drying temperatures of 40, 50, 60 and 70°C. The infrared drying time of mallow leaves obtained at 70°C was shortened by 3.20, 1.94 and 1.63 times compared with the infrared drying conducted at 40, 50 and 60°C, respectively. For a fixed microwave power (160 or 350 W), a marked decline of drying time was observed with increasing temperature from 50 to 75°C. Time required time for air drying of mallow leaves at 50°C was equal to 195 min and it was reduced to 44 min for air drying at 75°C.

Alibas and Köksal (2014) compared the drying kinetics of mallow leaves obtained for three drying methods: microwave (500, 650, 750, 850 W), convective drying (50, 75, 100 and 125°C) and vacuum drying (3, 7 kPa at 50 and 75°C). The authors reported that the drying times ranged from 6-10, 26-150 and 38-130 min for microwave, convective and vacuum drying, respectively.

Table 1 presents Page model constants and statistical parameters obtained for fitting of experimental drying kinetics of mallow leaves. Page Model described adequately the drying kinetics of mallow leaves ($R^2 > 0.9911$ and SEE<0.1007).

Table 1. Page model and statistical parameters obtained for fitting of experimental mallow leaves drying kinetics.

Drying process	Drying condition	Page model parameters		Statistical parameters	
		k	n	R^2	SEE*
Microwave drying	160 W	5.851e-006	1.648	0.9918	0.0200
	350 W	8.701e-005	1.49	0.994	0.01497
	500 W	9.098e-006	1.927	0.9934	0.01667
	650 W	2.255e-005	1.891	0.9956	0.008885
	750 W	0.0001005	1.677	0.9955	0.007528
Infrared drying	40 °C	0.000363	0.9556	0.9927	0.1007
	50 °C	0.001991	0.8148	0.9923	0.0536
	60 °C	0.001406	0.884	0.9911	0.05793
	70 °C	0.00273	0.8845	0.9934	0.02091
Air drying	50°C	1.409e-005	1.32	0.9964	0.01161
	75°C	6.518e-005	1.374	0.9925	0.01273
Combined air- microwave drying	160 W-50°C	1.31e-005	1.598	0.9883	0.0201
	160 W-75°C	0.0002406	1.254	0.9897	0.01327
	350 W-50°C	0.0002753	1.293	0.9983	0.001868
	350 W-75°C	2.723e-005	1.715	0.9962	0.003819

*SEE (±), standard error of estimate; R^2, coefficients of determination; k, drying rate constant, min^{-1}; n, drying exponent.

3.2. Effect of drying on color of mallow leaves

The color parameters (*L**, *a**, *b**) and the global color difference of fresh mallow leaves and those obtained after microwave, convective, infrared and combined air-microwave drying are presented in Table 2. All drying processes induce decreasing of the value of a* (greenness/redness) and b* (yellowness / blueness) if compared to those of fresh leaves. The greenness (a*) of the leaves decreased from -19.18±0.426 for fresh leaves to -11.58 ± 0.159 for air drying at 75°C. According to the global color difference (ΔE) determined according to fresh leaves, the most color preservation was obtained for microwave drying, combined air-microwave drying and infrared drying at 40 and 50°C.

Table 2. Effect of different drying modes and conditions on the colorimetric parameters of mallow leaves.

		L*	a*	b*	ΔE
Fresh leaves	-	35.79 ± 0.426^{def}	-19.18 ± 0.923^{a}	12.49 ± 1.718^{a}	0
Air drying	50 °C	38.76 ± 0.613^{ab}	-13.46 ± 0.170^{cdef}	9.57 ± 0.260^{def}	7.078
	75 °C	34.74 ± 0.635^{g}	-11.58 ± 0.159^{f}	9.12 ± 0.250^{ef}	8.375
Infrared drying	40 °C	34.50 ± 0.635^{g}	-16.96 ± 0.062^{ab}	9.48 ± 0.255^{def}	3.953
	50 °C	32.65 ± 0.726^{h}	-17.42 ± 0.895^{ab}	9.45 ± 0.091^{def}	4.714
	60 °C	33.31 ± 0.688^{h}	-12.54 ± 5.899^{ef}	6.72 ± 0.157^{g}	9.142
	70 °C	22.95 ± 0.708^{i}	-12.83 ± 0.581^{def}	10.77 ± 2.026^{bcd}	14.431
Microwave drying	750 W	37.94 ± 0.164^{bc}	-17.01 ± 0.095^{ab}	11.26 ± 0.241^{abc}	3.289
	650 W	36.03 ± 0.707^{f}	-16.25 ± 0.552^{abc}	10.35 ± 0.378^{bcde}	3.631
	500 W	36.29 ± 0.408^{ef}	-15.54 ± 0.430^{bcd}	9.99 ± 0.422^{bcdef}	4.439
	350 W	37.32 ± 0.460^{cde}	-17.02 ± 0.466^{ab}	11.55 ± 0.365^{ab}	2.804
	160 W	36.94 ± 0.301^{cdef}	-14.87 ± 0.247^{bcde}	10.45 ± 0.170^{bcde}	4.900
Combined air- microwave drying	350 W-75°C	36.49 ± 0.150^{def}	-15.8 ± 0.123^{bc}	9.01 ± 0.121^{f}	4.902
	350W-50 °C	37.67 ± 0.230^{c}	-16.39 ± 0.453^{abc}	9.54 ± 0.345^{def}	4.473
	160W-75°C	37.46 ± 0.223^{cd}	-15.81 ± 0.185^{bc}	9.40 ± 0.176^{def}	4.865
	160W-50°C	39.15 ± 0.123^{a}	-16.34 ± 0.345^{abc}	9.9 ± 0.234^{cdef}	5.102

L*: brightness, a*: redness/greenness; b*: yellowness/blueness, ΔE: global color difference between fresh and dried leave. Values with the same letter are not significantly different at $p < 0.05$.

Alibas and Köksal (2014) reported that among all the drying trials (microwave, convective and vacuum drying), the most similar colorimetric parameters to those of fresh leaves were obtained for microwave drying at 750 W, while the least similar colorimetric parameters to those of fresh leaves were noted for convective air drying at 125°C. Alibas and Koksal, (2014) reported that microwave drying (750 W, 6.5 min) gives the best color of mallow leaves if compared to the fresh ones.

3.3. Effect of drying on phenolics and antioxidant activity of mallow leaves

Total phenols and flavonoids contents and antioxidant activity of mallow leaves dried by microwave drying, air drying, infrared drying and combined air-microwave drying were presented in Table 3. Total phenols and flavonoids contents of fresh leaves are about 211.585± 0.968 mg GAE/g DM for total phenolics and 112.959 ±1.000 mg quercetin/g DM for total flavonoids. Phenolics of dried leaves significantly increased compared to fresh ones except of those air dried at 50°C and dried by combined air -microwave-air drying at 350 W-50°C and at 350 W-75°C.

The maximal phenols content are obtained for leaves dried by microwave drying at 350 W (292.548±0.833 mg GAE/g DM). Whereas, the maximal flavonoids content is obtained for leaves dried by microwave drying at 160 W (227.116±0.129 mg quercetin/g DM). This result can be explained by the fact that dehydration involves removal of water from the leaves and facilitates the liberation of bound phenols from the matrix.

Drying increases the porosity of the vegetable matrix, thereby increasing the solvent diffusion rate and consequently, the extraction of phenols is enhanced (Londoño-Londoño et al. 2010). Similar results were obtained by Bahloul et al. (2010) for olive leaves.

Table 3. Effect of different drying modes and conditions on the phenolics and antioxidant activity of mallow leaves.

		Total flavonoids (mg Quercetin equivalent/ g DM)	**Total phenols** (mg Gallic acid equivalent/ g DM)	**IC50** (mg/ml)
Fresh leaves	-	112.959 ±1.00^{k}	211.585± 0.968^{l}	2.733±0.150^{ab}
Air drying	50 °C	68.147±0.175^{l}	182.749±0.867^{m}	2.641±0.125^{ab}
	75 °C	180.679±0.542^{e}	259.282±1.607^{h}	2.623±0.134^{ab}
Infrared drying	40 °C	140.440±0.226^{i}	240.930±1.374^{k}	2.673±0.156^{ab}
	50 °C	156.403±0.308^{g}	256.989±0.373^{i}	2.701±0.143^{ab}
	60 °C	191.406±0.106^{c}	289.620±0.208^{b}	2.622±0.120^{ab}
	70 °C	137.899±0.262^{j}	265.704±0.325^{g}	2.580±0.105^{b}
Microwave drying	750 W	174.520±0.220^{f}	276.916±0.169^{e}	2.574±0.098^{b}
	650 W	184.177±0.166^{d}	279.175±1.565^{d}	2.626±0.123^{ab}
	500 W	179.957±0.435^{e}	286.866±1.029^{c}	2.605±0.106^{ab}
	350 W	215.037±0.405^{b}	292.548±0.833^{a}	2.613±0.894^{ab}
	160 W	227.116±0.129^{a}	275.305±0.303^{f}	2.680±0.115^{ab}
Combined air- microwave drying	350 W-75°C	55.127±1.014^{m}	141.164±1.222^{o}	2.850±0.167^{ab}
	350W-50°C	67.144±0.267^{l}	161.143±0.581^{n}	3.100±0.115^{a}
	160W-75°C	142.109±0.295^{h}	259.031±0.429^{h}	3.010±0.097^{ab}
	160W-50°C	142.004±0.732^{h}	251.214±0.238^{j}	2.963±0.105^{ab}

Values with the same letter are not significantly different at p <0.05.

Moreover, it appears that the increase of microwave power from 350 to 750 W leads to a decrease of phenols and flavonoids contents (from 292.548±0.833 to 276.916±0.169 mg GAE/g DM, and from 215.037±0.405 174.520±0.220 mg quercetin/g DM), but values remain superior to those of fresh leaves. This observation could be explained by the fact that high microwave power induces a drastic heating of the leaves which improves the phenol extraction and/or induces the development of antioxidant compounds (by polymerization or Maillard reaction) until a temperature level for which phenols and other antioxidants were degraded. So, the appropriate microwave power for obtaining the highest content of total phenols and total flavonoids seem to be 350 W.

Application of high microwave power (> 350W) for drying of mallow leaves leads to the degradation of phenolics. Jawad and Langrish (2012) reported that high microwave power causes the trigger of the Maillard reaction which leads to the formation of the Maillard reaction by-products (MRPs) named as melanoidins. These products were characterized by their high antioxidant activity (Wagner et al. 2002).

The leave extract concentration providing 50% inhibition (IC50) of 2, 2-Diphenyl-1-picrylhydrazyl (DDPH) show small differences according to applied drying mode and/or conditions. It varies from varies from 2.574±0.098 to 3.100±0.115 mg /ml for the whole investigated operating conditions.

4. Conclusion

Mallow leaves were dried by using four different drying methods: microwave (160, 350, 500, 650, 750 W), convective (50, 75°C), infrared (40, 50, 60, 70°C), and combined air-microwave drying (50°C-160 W; 50°C-350 W, 75 °C-160 W; 75°C-350 W). Drying time decreases with increasing microwave power and /or air temperature. The lowest total phenols and flavonoids content were found for combined microwave drying at 350 W-75°C. The highest total phenols and flavonoids content were obtained at 350 W and 160 W, respectively. The appropriate air drying temperature in terms of total phenols and flavonoids content was 75°C. Moreover the most suitable drying condition in terms of phenolic contents was 160 W-75°C for combined air-microwave drying and 60°C for infrared drying.

The most effective drying method of leaves with regard to phenolics content, color parameters and antioxidant activity and drying time was microwave drying at 350 W. The increase of antioxidants in dried leaves can be explained by the fact that heat treatment improves their release from the product, and subsequently facilitates their extraction.

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