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Radiation drying effects on biological characteristics of peel and leaves of "Maltaise demi-sanguine" oranges

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Abstract— Infrared and microwave drying changes in terms of chemical characteristics and antioxidant properties of "Maltaise demi-sanguine" orange peel and leaves extracts were studied.

When comparing the dried samples with the fresh one, it was shown that both infrared and microwave treatments led to improvement of the leaves extracts chemical composition by increasing contents of all components (total phenols, chlorophylls a and carotenoids), except chlorophylls b which disappeared. In the case of the peel, both chlorophylls a and b were degraded after radioactive drying. However, an increase in total phenol and carotenoids content was registered at some temperatures and powers of treatment.

After radioactive drying, maximum of DPPH radical scavenging activity of peel and leaves extracts was reached respectively at 50 and 60 $^{\circ}\text{C}$ and at 450 and 180 W (an increase of respectively 8, 61, 69 and 53% with regard to the fresh state). The reducing power decreased considerably in leaves extracts. Concerning the ferrous ion-chelating ability of extracts, it was improved by infrared and microwave treatments especially at 60 $^{\circ}\text{C}$ for peel and at 50 $^{\circ}\text{C}$ and 300 W for leaves.

Keywords— Infrared and microwave drying, chemical characteristics, antioxidant properties, orange peel extracts, leaves extracts.

I. INTRODUCTION

Citrus by-products have high contents of bioactive compounds such as flavonoids and terpenes which exhibit interesting antioxidant properties and some authors have claimed that certain parts of what is considered as dietary fiber might also exert antioxidant effects [1]. Due to their antioxidant properties, bioactive components obtained from Citrus by-products might decrease risk of cancer and cardiovascular diseases and prevent atherosclerosis and cataracts [2]. However, Citrus by-products (like peels and leaves), because of their high moisture content (1.509 kg water/kg db in leaves; 2.970 kg water/kg db in peels) [3], are highly perishable and so require reduction of their moisture content to a level at which microbial spoilage and

deterioration reactions are minimized, thus increasing product shelf-life.

Dehydration has become a widely used food preservation process for the most agro-food products. There are several drying processes such as natural sun drying, convective hot air drying. However, high temperatures and long drying periods usually reduce the quality of the final product [4]. Microwave (MW) and infrared (IR) treatments have gained popularity as alternative drying methods for a variety of food products such as fruit, vegetable, snack food and dairy product. MW and IR drying is rapid, energy efficient and produces a high-quality end product compared to conventional methods ([5], [6]).

The aim of this work was to determine the influence of MW and IR drying on biological characteristics of "Maltaise demi-sanguine" orange peel and leaves in terms of chemical composition and antioxidant properties of their extracts.

II. MATERIALS AND METHODS

A. Materials

Fresh oranges (*C. sinensis*) and leaves of the "Maltaise demi-sanguine" variety were picked from Manzel Bouzalfa (Nabeul, Tunisia) in an advanced stage of ripeness. The whole oranges and the leaves were stored at 0-4 °C until processing.

B. Methods

- 1) Radiation Drying: MW drying experiments of oranges peels and leaves were performed in a domestic microwave oven (TDS: Triple Distribution System, M 1714, Korea) at seven MW output powers (100-850 W). IR drying experiments were conducted by the means of an IR moisture analyser (Sartorius MA40) at different temperatures (40, 50, 60 and 70 °C). Drying was applied until obtaining a constant weight of the samples.
- 2) Chemical Characterisation: Dried and fresh samples were grinded and extracted by percolation with ethanol 95° at room temperature. All extracts were concentrated over a

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rotary vacuum evaporator until a solid extract sample was obtained. The resulting crude extract was freeze-dried.

Total phenol content (TPC) was determined by the Folin-Ciocalteau method [7]. Results were expressed as mg gallic acid equivalent (GAE) per gram of dry weight (DW) (mg GAE/g DW).

Carotenoids and chlorophylls (a and b) were determined according to the method described by [8]. Spectroscopic measurements of methanolic solution of each extract were performed at recommended wavelengths corresponding to maximal absorbance (A) of chlorophyll a, chlorophyll b and carotenoids (carotenes and xanthophylls). Respective concentrations of these pigments were given by the following equations:

$$C_a(\mu g/ml) = 16.72 A_{665} - 9.16 A_{652.4}$$
 (1)

$$C_b(\mu g/ml) = 34.09 A_{652.4} - 15.28 A_{665.2}$$
 (2)

$$C_{x+c}(\mu g/ml) = (1000 A_{470} - 1.63 C_a - 104.96 C_b)/221$$
 (3)

3) Antioxidant Activity: The 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) scavenging activity fresh and infrared dried orange peel and leaves extracts was measured using the method described by [9]. BHA was used as standard control. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

The ferrous ion-chelating activity was carried out using [10] method. EDTA was used as positive control. EC_{50} value ($\mu g/ml$) is the concentration at which the chelating activity was 50%.

Reducing power was determined according to the method of described by [11]. The extract concentration providing 0.5 of absorbance (EC₅₀) was calculated from the graph of absorbance registrated at 700 nm against the correspondent extract concentration. BHA was used as standard control.

C. Statistical Analyses

All measurements were carried out in triplicate. ANOVA test was performed in order to examine the effect of radiation drying on chemical characteristics and antioxidant properties. The SPS® version 11.0 (Statistical Package for Social Science) was used for statistical investigations. For all statistical analysis, the level of significance is fixed at 95%. Each factor having a p value < 0.05 was considered significant.

III. RESULTS AND DISCUSSION

A. Effect of Infrared Drying on Biological Characteristics of Orange Peel and Leaves Extracts

1) Effect of Infrared Drying on Chemical Characteristics: Table 1 presents the effect of IR drying $(40-70\,^{\circ}\text{C})$ on total phenols, chlorophylls a and b and carotenoids in "Maltaise demi-sanguine" orange peel and leaves extracts. The applied IR temperatures affected significantly all chemical characteristics of both peel and leaves extracts (p<0.05).

TABLE I EFFECT OF INFRARED DRYING (40 - 70 °C) ON TOTAL PHENOLS, CHLOROPHYLLS A AND B AND CAROTENOIDS IN "MALTAISE DEMI-SANGUINE" ORANGE PEEL AND LEAVES EXTRACTS.

	Total phenols (mg GAE/g DW)	Chlorophylls a (mg/g DW)	Chlorophylls b (mg/g DW)	Carotenoids (mg/g DW)
Peel				
FM	24.680 ± 0.792	0.080 ± 0.003	0.300 ± 0.011	0.136 ± 0.005
40 °C	24.700 ± 0.198	0	0	0.055 ± 0.002
50 °C	37.780 ± 0.877	0	0	0.053 ± 0.002
60 °C	22.120 ± 0.962	0	0	0.052 ± 0.002
70 °C	18.560 ± 0.453	0	0	0.099 ± 0.002
Leaves				
FM	32.370 ± 0.971	0	33.755 ± 1.350	0
40 °C	40.210 ± 1.598	14.185 ± 0.120	0.260 ± 0.010	1.545 ± 0.068
50 °C	47.320 ± 1.420	16.255 ± 0.629	0.115 ± 0.004	1.895 ± 0.021
60 °C	64.720 ± 2.150	18.865 ± 0.629	0	2.235 ± 0.021
70 °C	42.320 ± 1.270	11.135 ± 0.262	0	1.265 ± 0.056

All the given values are the means of three determinations ± standard deviation FM: fresh matter; DW: dry weight; GAE: gallic acid equivalent

When comparing the IR-dried samples with the fresh one, it was shown that IR treatment led to improvement of the leaves extracts chemical composition by increasing the contents of all components, except the chlorophylls b which disappeared. Maximum values were obtained at 60 °C for total phenols $(64.720 \pm 2.150 \text{ mg GAE/g DW})$, carotenoids $(2.235 \pm 0.021 \text{ mg/g DW})$ and chlorophylls a $(18.865 \pm 0.629 \text{ mg/g DW})$. In the case of the peel, both chlorophylls a and b were degraded after IR drying and a reduction of 27 (at 70 °C) to 62% (60 °C) in carotenoids was noted. However, an increase of 53% in

total phenols contents was registered respectively at 50 °C.

Improvement of the chemical characteristics after drying processes could be explained by damage of cell structures making easier extraction of antioxidant components from the plant material. [12] also dehydrated olive leaves (*Olea europaea* L.) by the same process and registered an increase of 9.6 % at 40 °C to 132.7 % at 70 °C in phenolic compounds, by comparison with the fresh state. Lee et al 2006 evaluated TPC in peanut hulls (*Arachis hypogaea* L.) after IR radiation at 150 °C and found an increase of about 94 % with regard to the fresh product. [13] showed previously that IR drying may have capability to cleave covalent bonds and liberate antioxidants such as flavonoids, carotene, tannin, ascorbate, flavoprotein or polyphenols. Furthermore, application of high temperatures could inactivate degradative enzymes (such as polyphenolxidases) and so avoid loss of phenolic compounds.

2) Effect of Infrared Drying on Antioxidant Properties: Table 2 presents the effect of IR drying (40 – 70 °C) on antioxidant activities of "Maltaise demi-sanguine" orange peel and leaves extracts. Statistical analyses showed that IR temperatures affected significantly all the activities of the extracts (p<0.05).

TABLE III

EFFECT OF INFRARED DRYING (40 - 70 °C) ON ANTIOXIDANT ACTIVITIES OF
"MALTAISE DEMI-SANGUINE" ORANGE PEEL AND LEAVES EXTRACTS.

	IC ₅₀ values of DPPH RSA	EC ₅₀ values of RP	EC ₅₀ values of FICA		
	(µg/ml)	(µg/ml)	(µg/ml)		
Peel					
FM	819.630 ±	319.910 ±	-		
r IVI	27.048	10.557			
40 °C	880.700 ±	988.400 ±	2365.790 ±		
40 °C	34.347	38.548	10.165		
50 °C	751.200 ±	590.700 ±	2740.900 ±		
30 °C	33.053	25,991	32.891		
60 °C	1094.400 ±	760.500 ±	1194.770 ±		
00 °C	51.437	35.744	26.285		
70 °C	916.000 ±	441.900 ±	-		
70 C	32.976	15.908			
Leaves					
FM	326.420 ±	297.815 ±	677.670 ±		
r IVI	9.140	8.339	10.165		
40 °C	430.900 ±	3882.500 ±	$728.720 \pm$		
40 C	14.220	128.123	10.931		
50 °C	215.150 ±	1407.300 ±	465.459 ±		
30 C	8.391	54.885	7.913		
60 °C	128.300 ±	1692.500 ±	479.006 ±		
00 C	5.645	60.930	9.101		
70 °C	236.350 ±	873.850 ±	729.820 ±		
70 C	11.108	24.468	8.758		

FM: fresh matter; DW: dry weight; DPPH RSA: DPPH radical scavenging activity; RP: reducing power; FICA: ferrous ion-chelating ability

Compared with the peel, fresh leaves exhibited the strongest antioxidant potential. In fact, IC $_{50}$ values of DPPH radical scavenging activity, EC $_{50}$ values of reducing power and EC $_{50}$ values of ferrous ion-chelating ability were revealed lower than those obtained with fresh peel extracts (326.420 \pm 9.140 $\mu g/ml$, 297.815 \pm 8.339 $\mu g/ml$ and 685.000 \pm 14.385 $\mu g/ml$ respectively for leaves extracts, against 819.630 \pm 36.064 $\mu g/ml$, 351.500 \pm 15.466 $\mu g/ml$ and 0 respectively for peel extracts).

After drying, maximum of DPPH radical scavenging activity of peel and leaves extracts was reached respectively at 50 and 60 °C (an increase of respectively 8 and 61% with regard to the fresh state): the respective IC $_{50}$ values calculated were 751.200 \pm 33.053 and 128.300 \pm 5.645 $\mu g/ml$. The reducing power decreased considerably after IR treatment in extracts of both peel and leaves. The lowest EC $_{50}$ values were obtained at 70 °C (441.900 \pm 15.908 $\mu g/ml$ for peel; 873.850 \pm 24.468 $\mu g/ml$ for leaves). Concerning the ferrous ion-chelating ability of extracts, it was improved by IR drying especially at 60 °C for peel and 50 °C for leaves.

The increase of antioxidant activities of peel and leaves extracts at certain applied IR temperatures may be explained by the formation, during drying process, of new compounds with antioxidant activity. [12] and [14] also reported an enhancement of the antioxidant activity of IR dried olive oil and peanut hulls. According to [15], it is likely that the drying favour the non-enzymatic browning or "Maillard reactions". These reactions provided high antioxidant capacity generally associated to the formation of brown melanoidins [16].

The relationship between chemical composition and antioxidant activities was statistically investigated. The correlation coefficient was found very variable indicating that antioxidant capacities was provided not only by the determined components, but also by different generated antioxidant compounds having varying degree of antioxidant activity developing antagonistic, synergic or additional effects with themselves or with the other constituents of peel and leaves extracts [17].

B. Effect of Microwave Drying on Biological Characteristics of Orange Peel and Leaves Extracts

1) Effect of Microwave Drying on Chemical Characteristics: Table 3 presents the influence of MW drying (100 – 850 W) on total phenols, chlorophylls a and b and carotenoids contained in "Maltaise demi-sanguine" orange peel and leaves extracts. All chemical characteristics of both peel and leaves extracts were found significantly affected by the applied MW powers (p<0.05).

When comparing the MW dried samples with the fresh one, it was shown that MW treatment led to improvement of the leaves extracts chemical composition by increasing the contents of all components, except the chlorophylls b which disappeared. Maximum values were obtained at 180 W for total phenols (137.040 \pm 6.222 mg GAE/g DW) and chlorophylls a (21.095 \pm 0.290 mg/g DW) and at 600 W for carotenoids (2.010 \pm 0.090 mg/g DW). In the case of the peel, both chlorophylls a and b were degraded after MW drying. However, a maximum increase of 57% in total phenol content and of 360% in carotenoids was registered respectively at 700 W and 100 W.

Drying process would generally result in a depletion of naturally occurring antioxidants in raw materials from plants. But in some cases, processing causes little or no change, significant losses, or enhancement to the content of certain components. [12] showed and confirmed the increase of TPC in olive leaves (Olea europaea L.) extracts after MW drying. In fact, compared to the fresh state, improvement percentage of TPC in dehydrated leaves was found of about 17% at 100 W and 92% at 300 W. MW heating, brought about by absorption of MW energy by water molecules, could inactivate oxidative enzymes (such as polyphenolxidases) and so prevent the degradation of phenolic compounds [18]. Increase in antioxidants content following MW treatment could also be attributed to the release of bound phenolic compounds brought about by the breakdown of cellular constituents [19].

TABLE IIIII

EFFECT OF MICROWAVE DRYING (100 - 850 W) on total phenols, chlorophylls a and b and carotenoids in "Maltaise demi-sanguine" orange peel and leaves extracts.

	Total phenols (mg GAE/g DW)	Chlorophylls a (mg/g DW)	Chlorophylls b (mg/g DW)	Carotenoids (mg/g DW)
Peel	•			
FM	24.680 ± 0.792	0.080 ± 0.003	0.300 ± 0.011	0.136 ± 0.005
100 W	14.170 ± 0.382	0.080 ± 0.003	0.300 ± 0.011	0.136 ± 0.005
180 W	19.215 ± 0.576	0	0	0.625 ± 0.028
300 W	22.870 ± 0.686	0	0	0.226 ± 0.010
450 W	36.240 ± 0.453	0	0	0.170 ± 0.007
600 W	35.040 ± 0.113	0	0	0.300 ± 0.011
700 W	38.640 ± 0.792	0	0	0.126 ± 0.006
850 W	22.480 ± 1.018	0	0	0.248 ± 0.011
Leaves		I	l	l
FM	32.370 ± 0.971	0	33.755 ± 1.350	0
100 W	124.720 ± 2.828	14.995 ± 0.488	8.000 ± 0.297	0
180 W	137.040 ± 6.222	21.095 ± 0.290	0	1.795 ± 0.021
300 W	129.440 ± 5.431	13.880 ± 0.625	0	1.625 ± 0.073
450 W	98.720 ± 1.131	14.970 ± 0.198	0	1.190 ± 0.028
600 W	94.475 ± 1.237	11.085 ± 0.332	0	2.010 ± 0.090
700 W	94.745 ± 1.167	17.160 ± 0.772	0	0.750 ± 0.014
850 W	78.760 ± 3.267	12.825 ± 0.544	0	1.895 ± 0.064

All the given values are the means of three determinations \pm standard deviation

FM: fresh matter; DW: dry weight; GAE: gallic acid equivalent

2) Effect of Microwave Drying on Antioxidant Properties: Table 4 presents the effect of MW drying on antioxidant activities of "Maltaise demi-sanguine" orange peel and leaves extracts. Statistical analyses showed that MW powers affected significantly all the activities of the extracts (p<0.05).

Compared with the peel, fresh leaves exhibited the strongest antioxidant potential. In fact, IC_{50} values of DPPH radical scavenging activity, EC_{50} values of reducing power and EC_{50} values of ferrous ion-chelating ability were revealed lower than those obtained with fresh peel extracts (326.42 \pm

9.140 µg/ml, 297.815 \pm 8.339 µg/ml and 685.000 \pm 14.385 µg/ml respectively for leaves extracts, against 819.630 \pm 36.064 µg/ml, 351.500 \pm 15.466 µg/ml and 0 respectively for peel extracts).

After drying, maximum of DPPH radical scavenging activity of peel and leaves extracts was reached respectively at 450 and 180 W (an increase of respectively 69 and 53% with regard to the fresh state): the respective IC50 values calculated were 253.190 \pm 9.115 and 152.750 \pm 5.499 $\mu g/ml$. The reducing power of leaves extracts decreased considerably after MW treatment when peel extracts revealed their highest activity at 700 W: the lowest EC50 value was 139.210 \pm 4.594 $\mu g/ml$. MW drying also improved the ferrous ion-chelating ability of leaves extracts especially at 300 W (the correspondent EC50 was 437.300 \pm 6.560 $\mu g/ml$). For the peel, the EC50 values of the ferrous ion-chelating ability were calculated only at 300, 450, 700 and 850 W with a maximum of 494.220 \pm 9.390 $\mu g/ml$ at 700 W.

The increase of antioxidant activities of peel and leaves extracts at certain applied MW powers may be explained by the formation, during drying process, of new compounds with antioxidant activity. [20] noticed an enhancement of the antioxidant activity after MW drying of grapes. According to these authors, it is likely that the drying favour the non-enzymatic browning or "Maillard reactions". These reactions provided high antioxidant capacity generally associated to the formation of brown melanoidins [16].

The relationship between chemical composition and antioxidant activities was statistically investigated. The correlation coefficient was found very variable indicating that antioxidant capacities was provided not only by the determined components, but also by different generated antioxidant compounds having varying degree of antioxidant activity developing antagonistic, synergic or additional effects with themselves or with the other constituents of peel and leaves extracts [17].

TABLE IVV

EFFECT OF MICROWAVE DRYING (100 - 850 W) ON ANTIOXIDANT ACTIVITIES
OF "MALTAISE DEMI-SANGUINE" ORANGE PEEL AND LEAVES EXTRACTS.

	IC ₅₀ values of	EC ₅₀ values	EC ₅₀ values
	DPPH RSA	of RP	of FICA
	(µg/ml)	(µg/ml)	(µg/ml)
Peel			
FM	819.630 ±	319.910 ±	-
r IVI	27.048	10.557	
100 W	1257.700 ±	436.420 ±	-
100 W	49.050	17.020	
180 W	$786.700 \pm$	$370.520 \pm$	-
100 W	34.615	16.303	
300 W	342.500 ±	$218.540 \pm$	4512.800 ±
300 W	16.098	10.271	54.154
450 W	253.190 ±	191.440 ±	3560.600 ±
430 W	9.115	6.892	60.53
600 W	343.500 ±	235.930 ±	-
000 W	9.618	6.606	
700 W	296.600 ±	$139.210 \pm$	494.220 ±
700 11	9.788	4.594	9.390
850 W	900.500 ±	$587.800 \pm$	1775.900 ±
	35.120	22.924	37.294
Leaves			
FM	326.420 ±	$297.815 \pm$	677.670 ±
I IVI	9.140	8.339	10.165
100 W	199.600 ±	$971.100 \pm$	612.750 ±
100 W	9.381	45.6417	7.353
180 W	152.750 ±	$1411.400 \pm$	1226.400 ±
100 11	5.499	50.810	26.981
300 W	197.100 ±	$980.500 \pm$	437.300 ±
300 W	5.519	27.454	6.560
450 W	219.100 ±	$1712.000 \pm$	440.400 ±
430 11	7.230	56.496	7.487
600 W	$275.850 \pm$	$605.000 \pm$	441.100 ±
000 11	10.758	23.595	9.263
700 W	181.500 ±	1095.300 ±	445.400 ±
700 W	7.986	48.193	10.165
850 W	200.800 ±	$712.500 \pm$	439.400 ±
030 11	9.438	33.488	5.273

FM: fresh matter; DW: dry weight; DPPH RSA: DPPH radical scavenging activity; RP: reducing power; FICA: ferrous ion-chelating ability

IV. CONCLUSIONS

The multiple antioxidant activities of "Maltaise demisanguine" orange peel and leaves extracts demonstrated in this study clearly indicates the potential application value of these by-products. Further studies are needed on the isolation and characterization of individual compounds to elucidate their different antioxidant mechanisms and the existence of possible synergism, if any, among the compounds.

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