Journal of Food and Nutrition Research, 2017, Vol. 5, No. 3, 197-204
Available online at http://pubs.sciepub.com/jfnr/5/3/10

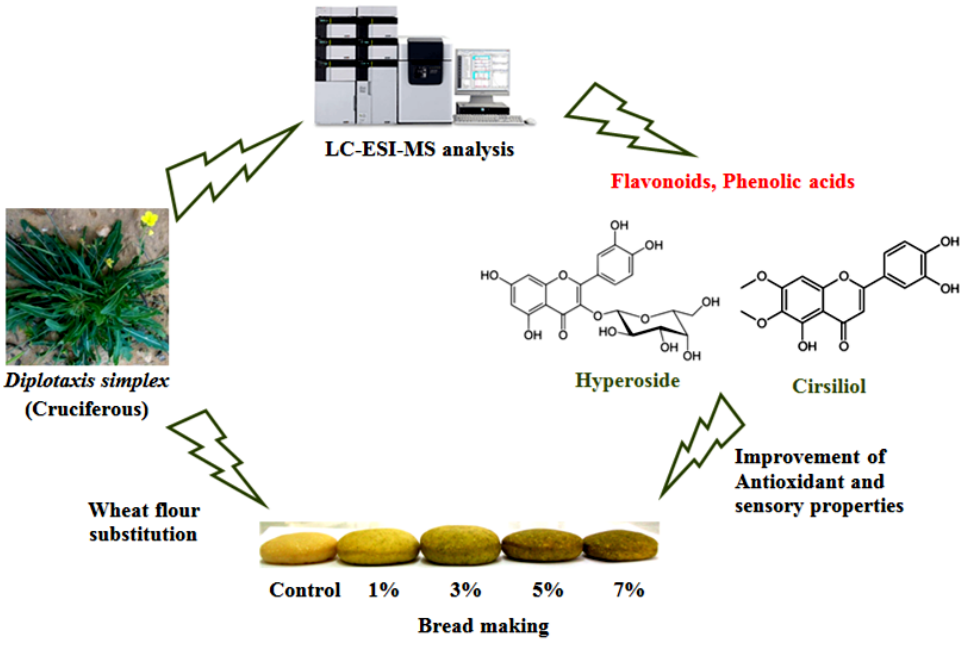
DOI:10.12691/jfnr-5-3-10

The Rocket, *Diplotaxis simplex*, as a Functional Ingredient: LC-ESI-MS Analysis and Its Effect on Antioxidant and Physical Properties of Bread

Hamida Jdir[1], Mourad Jridi[2], Mahmoud Mabrouk[3], M.A. Ayadi[4], Moncef Nasri[2], Nacim Zouari[5,*], Nahed Fakhfakh[2]

[1]Laboratory of Enzyme Engineering of Lipases and Biochemistry, Engineering National School of Sfax (ENIS), University of Sfax, Sfax, Tunisia
[2]Laboratory of Enzyme Engineering and Microbiology, ENIS, University of Sfax, Sfax, Tunisia
[3]Central Laboratory of the Institute of Arid Areas of Medenine, Medenine, Tunisia
[4]Laboratory of Food Analysis, ENIS, University of Sfax, Sfax, Tunisia
[5]High Institute of Applied Biology of Medenine (ISBAM), University of Gabes, Medenine, Tunisia
*Corresponding author: znacim2002@yahoo.fr

Abstract The rocket, *Diplotaxis simplex*, is an edible cruciferous that possesses interesting biological properties. The effect of rocket leaves powder substitution to wheat flour on alveographic properties of dough, and physical, antioxidant and sensory characteristics of wheat bread were examined. The rocket powder contained relatively high levels of dietary fibers and total phenolics. The liquid chromatography-electrospray ionization-tandem mass spectrometry analysis of rocket leaves allowed the identification of 10 flavonoids and 5 phenolics acids. The flavonoids constituted the largest group accounting for 88.26% of the total identified compounds, among which the hyperoside (quercetin-3-*O*-galactoside) was found to be the major compound. The (P/L) ratio of the dough increased with the increment of rocket powder level, whereas the deformation energy (W) decreased. A 3% supplementation resulted in an increase of the bread specific volume by 46% accompanied by a decrease in the hardness. Interestingly, the bread containing rocket powder showed enhanced total phenolics content, DPPH• scavenging activity and Fe^{3+} reducing power as compared to the control. At 3% substitution level, the cruciferous *D. simplex* may be useful in improving nutraceutical quality of bread without altering its sensory and physical properties.



Keywords: *Rocket D. simplex, LC-ESI-MS, antioxidants, hyperoside, dough, bread quality*

Cite This Article: Hamida Jdir, Mourad Jridi, Mahmoud Mabrouk, M.A. Ayadi, Moncef Nasri, Nacim Zouari, and Nahed Fakhfakh, "The Rocket, *Diplotaxis simplex*, as a Functional Ingredient: LC-ESI-MS Analysis and Its Effect on Antioxidant and Physical Properties of Bread." *Journal of Food and Nutrition Research*, vol. 5, no. 3 (2017): 197-204. doi: 10.12691/jfnr-5-3-10.

1. Introduction

Epidemiological studies strongly suggest that diet plays an important role in the prevention of many diseases. In recent years, there has been a global trend towards the use of natural substances as a source of functional ingredients. In particular, natural antioxidants present in food have received considerable attention for their beneficial effects on specific biological functions in the body. In fact, they contribute to improve the general state of health and/or to reduce the risk of some diseases of oxidative stress origin [1]. Thus, it is considered that consumption of antioxidant-rich food, in the context of a balanced diet, is associated with the prevention of many degenerative diseases of modern society characterized by physical inactivity, stress, smoking, among others [2]. For this reason, both industrials and researchers are involved in optimizing food production technology to improve the functionality of traditional foods such as bakery products that play an important role in human nutrition. Bread made from refined wheat flour is characterized by a low antioxidant potential and would be therefore an interesting support requiring the incorporation of functional supplements to improve its health benefits [3]. Currently, there are some successful trials concerning the enhancement of antioxidant activity of wheat bread by the addition of plant materials rich in natural antioxidants. In fact, wheat bread can be enriched with many agro-resources, such as buckwheat, spices and waste plant materials [4-9]. However, there is little information about enriching bread with the green parts of vegetables and many studies did not take into consideration the consumer acceptance of fortified breads [3].

The popularity and consumption of vegetables from the Brassicaceae (cruciferous) family is increasing for their beneficial effects on human health in terms of their nutritional value and their potential to reduce the risk of chronic diseases including cardiovascular diseases and cancer [10]. As a matter of fact, the cruciferous vegetables are very nutritive providing nutrients and health-promoting phytochemicals such as vitamins, fibers, soluble sugars, minerals and phenolic compounds [11]. Moreover, they are unique because they are rich source of glucosinolates, which are known by their cancer chemoprotective attributes [10]. The Diplotaxis simplex (Viv.) Spreng (Brassicaceae) is an herbaceous plant indigenous to North Africa. This rocket is consumed raw or cooked in salads and soups and is highly appreciated for its strong pungent flavor, which is due to its richness in organosulphur compounds [12]. In previous works, we reported that this species suppresses postprandial hyperglycemia in mice, and shows an anti-inflammatory potential as well as an interesting anti-proliferative activity against the human colon cancer Caco-2 cells [13,14].

In the present work, *D. simplex* leaves were used as a new functional ingredient for enhancing phytochemical content as well as physical quality of wheat bread. Rocket leaves were characterized in terms of phytochemical and functional characteristics. The objective of the study was to evaluate the effects of rocket leaves powder substitution to wheat flour on dough alveographic properties, and physical, antioxidant and sensory characteristics of bread.

2. Materials and methods

2.1. Chemicals and Standards

HPLC grade methanol, formic acid, ethanol and 2,2-diphenyl-1-picrylhydrazyl (DPPH•) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Water was of HPLC grade and was obtained from Scharlau (Barcelona, Spain). Chemical standards (quinic acid, gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, *trans*-ferulic acid, *o*-coumaric acid, *trans*-cinnamic acid, 4-*o*-caffeoylquinic acid, 1,3-di-*o*-caffeoylquinic acid, 3,4-di-*o*-caffeoylquinic acid, 4,5-di-*o*-caffeoylquinic acid, rosmarinic acid, salvianolic acid, (+)-catechin, epicatechin, acacetin, apigenin-7-*O*-glucoside, apigenin, cirsilineol, cirsiliol, hyperoside (quercetin-3-*O*-galactoside), luteolin-7-*O*-glucoside, luteolin, naringenin, naringin, quercitrin (quercetin-3-*O*-rhamonoside), quercetin, rutin and silymarin) at the purity > 98% were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2. Plant Material and Rocket Powder Preparation

The *D. simplex* was collected, in February 2015, from the area of Sfax (Tunisia). After harvest, the leaves were separated and shade-dried for 20 days. Then, they were ground in a spice grinder (Black & Decker CBG100S Smartgrind, Maryland, USA), sieved through 250 µm sieve and the obtained powder was stored at 4°C until use.

2.3. Chemical Analysis and Functional Characteristics of Rocket Powder

The dietary fibers and total chlorophyll contents were determined as previously described [15,16]. The water holding capacity (WHC) expressed as g of water bound per 100 g rocket powder, fat absorption capacity (FAC) expressed as g of oil bound per 100 g rocket powder and swelling capacity (volume/g rocket powder) were measured as previously reported by Ayadi et al. [16].

2.4. Antioxidant Activity and LC-ESI-MS Analysis of Rocket Powder Extract

2.4.1. Ethanolic Extract Preparation

The rocket powder (25 g) was extracted by maceration using 250 ml of ethanol during 24 h. The solvent was then evaporated under vacuum and the residual solvent was removed by flushing with nitrogen.

2.4.2. Total Phenolics, Flavonoids and Antioxidant Activity

The total phenolics and flavonoids contents were measured in the ethanolic extract of rocket powder as previously described [17]. The total phenolics content was

expressed as mg gallic acid equivalent (GAE)/100 g rocket powder and flavonoids content was expressed as mg quercetin equivalent (QE)/100 g rocket powder. The DPPH• radical-scavenging activity and reducing power of the ethanolic extract were measured as previously described [18]. The results of DPPH• radical-scavenging was presented by IC_{50} value, which was defined as the extract concentration needed to scavenge 50% of DPPH•. Lower IC_{50} values reflected better antioxidant activity. In the reducing power assay, the presence of antioxidants in the sample would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Then, an amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue $Fe_4[Fe(CN)_6]_3$ at 700 nm. The increase in absorbance at 700 nm indicated an increase in reductive ability.

2.4.3. Liquid Chromatography-electrospray Ionization-Tandem Mass Spectrometry (LC-ESI-MS) Analysis

The rocket extract was dissolved in ethanol and the resulted solution (4 mg/ml) was filtered through a 0.45 µm membrane filter before injection into the HPLC system. LC-ESI-MS analysis was performed using a LCMS-2020 quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an electrospray ionisation source (ESI) and operated in negative ionization mode. Mass spectrometer was coupled online with an ultra-fast liquid chromatography system consisted of a LC-20AD XR binary pump system, SIL-20AC XR autosampler, CTO-20AC column oven and DGU-20A 3R degasser (Shimadzu, Kyoto, Japan). An Aquasil C18 column (Thermo Electron, Dreieich, Germany) (150 mm × 3 mm, 3 µm) preceded by an Aquasil C18 guard column (10 mm × 3 mm, 3 µm, Thermo Electron) were applied for analysis. The mobile phase was composed of A (0.1% formic acid in H_2O, v/v) and B (0.1% formic acid in methanol, v/v) with a linear gradient elution: 0-45 min, 10-100% B; 45-55 min, 100% B. Re-equilibration duration was 5 min between individual runs. The flow rate of the mobile phase was 0.4 ml/min, the column temperature was maintained at 40°C and the injection volume was 5 µl. Spectra were monitored in mode SIM (Selected Ion Monitoring) and processed using Shimadzu LabSolutions LC-MS software. High-purity nitrogen was used as the nebulizer and auxiliary gas. The mass spectrometer was operated in negative ion mode with a capillary voltage of -3.5 V, a nebulizing gas flow of 1.5 l/min, a dry gas flow rate of 12 l/min, a DL (dissolving line) temperature of 250°C, a block source temperature of 400°C, a voltage detector of 1.2 V and the full scan spectra from 50 to 2000 Da.

2.5. Dough Alveographic Properties

The dough alveographic properties were measured by an alveograph (Chopin alveograph MA 82, Tripette et 142 Renaud, Villeneuve La Garenne, France) following the standard method [19]. The studied samples were wheat flour (control) and blends containing a mixture of wheat flour and rocket powder in the ratios (m/m): 99/1 (formulation 1: F1), 97/3 (formulation 2: F2), 95/5 (formulation 3: F3), and 93/7 (formulation 4: F4). The following alveographic parameters (P, L and W) were automatically recorded by a computer software program. The maximum overpressure (P) needed to blow the dough bubble indicated the dough tenacity. This parameter was related to the quality and quantity of gluten as well as to their ability to absorb water. The average abscissa (L) at bubble rupture indicated the dough extensibility or the ability of the gluten to hold the gas. The configuration ratio (P/L) indicated the balance between the tenacity and the dough extensibility. The deformation energy (W) represented an index of dough strength.

2.6. Bread Preparation

Bread was prepared in a local pastry industry (Société Patisserie-Masmoudi, Sfax, Tunisia). The standard bread formulation consisted of: 1 kg wheat flour, 433 g water, 100 g olive oil, 33.33 g whole egg, 23.33 g sodium chloride, 8.33 g instant dry yeast and 1.66 g sodium bicarbonate. The chemical composition of wheat flour (g/100 g) was as follows: starch, 71.22; water, 13.55; proteins, 10.43; total fiber, 0.76 and fat, 0.65 as determined by a multipurpose analyzer (MPA) spectrometer (Bruker Optics, Wissembourg, France). Breads with variable levels of rocket powder were made from wheat flour (control) and blends containing 1 g, 3 g, 5 g and 7 g of rocket powder per 100 g wheat flour substitution basis (F1, F2, F3 and F4). The yeast was dissolved in warm water (35°C) and the resulted solution was added to the dry ingredients and finally the olive oil was added. The mixture was blended manually for 10 min and the resulting dough was fermented for 90 min at 30°C. The dough circles of 50 mm diameter and 5 mm thin were shaped and placed on proofing trays for 2 h before baking, which was conducted at 180°C for 10 min. Finally, the flat breads were cooled to room temperature and then stored at −18°C in plastic bags. Prior to analysis, the breads were thawed for 24 h at 4°C and then equilibrated to room temperature for 4 h.

2.7. Physical Properties, Total Phenolics and Antioxidant Activity of Bread

Hardness (N), springiness (mm), chewiness (N × mm) and cohesiveness of bread was measured using a texturometer (Lloyd Instruments Ltd., West Sussex, UK) as previously described by Ayadi et al. [16]. Bread volume (cm^3) was determined by the rapeseed displacement method. Bread specific volume (cm^3/g) was measured as bread volume divided by bread mass. Total phenolics (mg gallic acid equivalents/100 g bread), DPPH• radical-scavenging activity and Fe^{3+} reducing power were determined in ethanol extract of bread [17,18]. The bread was dried overnight at 40°C and then converted into fine powder by using pestle and mortar. After that, 10 g of powdered sample were homogenized with 100 ml ethanol for 24 h at ambient temperature using an orbital shaker at stirring speed of 200 rpm. After filtration, the solvent was evaporated under vacuum and the residual solvent was removed by flushing with nitrogen. Finally, the obtained extract was recovered, dissolved in ethanol and kept in the dark at 4°C until further analysis. DPPH• radical-scavenging activity was

presented as IC_{50} values (mg/ml) and the reducing power (A_{700}, absorbance at 700 nm) was determined at 3 mg/ml of bread extract.

2.8. Sensory Evaluation

The sensory properties (color, odor, taste, texture and overall acceptability) of fresh prepared breads were evaluated according to the method of Murray et al. [20] by sixty panelists. A seven-point hedonic scale was used, where 7: like very much, 6: like moderately, 5: like slightly, 4: neither like nor dislike, 3: dislike slightly, 2: dislike moderately and 1: dislike very much for each attribute.

2.9. Statistical Analysis

All analytical determinations were performed in triplicate. One-way analysis of variance was conducted using the SPSS software for Windows™ (version 17, SPSS Inc., Chicago, IL, USA). Duncan's multiple range test ($p < 0.05$) was used to compare the average responses between treatments.

3. Results and Discussion

3.1. Rocket Powder Characteristics

The results for the chemical and functional characteristics of the rocket powder were presented in Table 1. The insoluble and soluble dietary fibers were found to be 32.50 and 7.0 g/100 g, respectively, which were in the range of the values reported for some cereals, vegetables and fruit processing by-products [21]. It is well known that dietary fibers play an important role in many physiological processes and in the prevention of some diseases. Besides, fibers-rich agro-resources may serve as functional ingredients that can improve food textural characteristics. Therefore, water holding capacity (WHC), swelling capacity and fat absorption capacity (FAC) were measured (Table 1). Rocket powder showed swelling capacity (6.5 cm^3/g rocket powder) similar to the finding of Ayadi et al. [16] for prickly pear cladodes. In addition, WHC of rocket powder was found to be 383 g water/100 g rocket powder. In this context, apple, pea, wheat, sugar beet and carrot fibers presented WHC values ranging from 250 to 1000 g water/100 g dry sample [22]. As shown in Table 1, FAC of rocket powder (201 g oil/100 g rocket powder) was comparable to the values reported for prickly pear cladodes or fibers from some vegetables [16,22].

Table 1. Chemical and functional characteristics of *D. simplex* leaves powder

Parameters	
Moisture[a]	8.20 ± 0.10
Insoluble dietary fibers[a]	32.50 ± 0.50
Soluble dietary fibers[a]	7.0 ± 1.0
Total chlorophyll[b]	106.24 ± 0.80
Total phenolics[c]	796.16 ± 31.84
Flavonoids[d]	931.51 ± 34.50
DPPH• scavenging activity (IC_{50}, mg/ml)	0.18 ± 0.02
Reducing power (EC_{50}, mg/ml)	0.17 ± 0.01
Water holding capacity[a]	383.0 ± 17.0
Fat absorption capacity[a]	201.0 ± 21.0
Swelling capacity (cm^3/g)	6.50 ± 0.50

Data presented as the mean ± standard deviation (n = 3); [a] g/100 g rocket powder; [b] mg/100 g rocket powder; [c] mg gallic acid equivalents/100 g rocket powder; [d] mg quercetin equivalents/100 g rocket powder.

Table 2. LC-ESI-MS analysis of the *D. simplex* leaves ethanolic extract and literature review of the DPPH• radical-scavenging presented as extract concentration needed to scavenge 50% of DPPH• (IC_{50}) values

No[a]	Compounds[b]	Molecular formula	Molecular mass	$[M-H]^-$ m/z	Retention time (min)	Content (µg/g extract)	IC_{50} (µg/ml)	References
1	Protocatechuic acid	$C_7H_6O_4$	154	153	7.279	1.2	0.89	[26]
2	Epicatechin	$C_{15}H_{14}O_6$	290	289	12.725	0.4	18.34	[27]
3	Syringic acid	$C_9H_{10}O_5$	198	197	13.612	15.4	0.50	[27]
4	p-Coumaric acid	$C_9H_8O_3$	164	163	16.120	16.9	105.3	[28]
5	*trans*-Ferulic acid	$C_{10}H_{10}O_4$	194	193	17.468	14.8	3.34	[29]
6	Luteolin-7-*O*-glucoside	$C_{21}H_{20}O_{11}$	448	447	20.965	5.5	4.45	[30]
7	Salvianolic acid	$C_{36}H_{30}O_{16}$	718	717	21.026	26.3	94	[31]
8	Hyperoside (quercetin-3-*O*-galactoside)	$C_{21}H_{20}O_{12}$	464	463	21.049	299.4	5.19	[32]
9	Quercetrin (quercetin-3-*O*-rhamonoside)	$C_{21}H_{20}O_{11}$	448	447	22.820	27	12.50	[33]
10	Naringenin	$C_{15}H_{12}O_5$	272	271	24.717	0.7	282	[33]
11	Luteolin	$C_{15}H_{10}O_6$	286	285	28.404	3.6	5.09	[30]
12	Apigenin	$C_{15}H_{10}O_5$	270	269	30.565	1.8	> 135	[30]
13	Cirsiliol	$C_{17}H_{14}O_7$	330	329	30.576	55.6	2.34	[30]
14	Cirsilineol	$C_{18}H_{16}O_7$	344	343	32.922	26.3	> 172	[30]
15	Acacetin	$C_{16}H_{11}O_5$	284	283	36.192	48.4	> 142	[30]

[a]The numbering refers to elution order of compounds from an Aquasil C18 column. [b]Identification was confirmed using 32 authentic commercial standards.

Phenolic compounds such as flavonoids are mainly responsible for the antioxidant properties and several studies were devoted to find natural antioxidants in cheap raw materials. Therefore, the amounts of total phenolics and flavonoids contents in rocket powder were determined. As compared to other *Brassica* vegetables [11], the *D. simplex* leaves contained high level of total phenolics (796.16 mg GAE/100 g rocket powder) and flavonoids (931.51 mg QE/100 g rocket powder), which correlate with their appreciable antioxidant potential in 2,2-diphenyl-1-picrylhydrazyl DPPH• radical-scavenging (IC_{50}: 0.18 mg/ml) and Fe^{3+} reducing (EC_{50}: 0.17 mg/ml) assays (Table 1). It's well known that dietary antioxidants from vegetables contribute to the defense system against oxidative stress. As a result, they protect cells against oxidative damage and may therefore prevent chronic diseases, such as cancer, diabetes, neurodegenerative disorders, and cardiovascular and anti-inflammatory diseases [1,14]. Therefore, it's important to identify the compounds that contribute to good health in the diet. To the best of our knowledge, there are no studies about the identification of phenolic compounds in *D. simplex* leaves. High-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC-ESI-MS) analysis of *D. simplex* leaves extract resulted in the identification of 15 phenolic compounds that were divided into 5 phenolic acids and 10 flavonoids (Table 2). The compounds identification was carried out by comparing retention times and mass spectra with those of the authentic standards. Flavonoids constituted the largest group accounting for 88.26% of the total identified compounds, among which the hyperoside (quercetin-3-*O*-galactoside) was found to be the major compound (299.4 µg/g extract). A survey of the literature shows that the majority of the identified compounds had potent antioxidant potential with IC_{50} values less than 20 µg/ml (Table 2). Thus, the consumption of food products supplemented with rocket leaves would potentially provide antioxidant potential and consequently health benefits.

3.2. Dough Alveographic Characteristics

The rocket powder contained relatively important dietary fibers content, so that its incorporation in wheat flour may modify the dough rheological parameters and consequently the bread physical properties. The dough rheological characteristics were determined by the alveographic method (Figure 1). The obtained results showed that a partial substitution of wheat flour by the rocket powder induced important modifications on alveograph characteristics. Indeed, the dough tenacity (P) increased as the substitution level rose. The control sample has a (P) value of 65 mm H_2O and it increased to 86 mm H_2O at 7% substitution level (F4), which might be a consequence of poor gluten hydration. By contrast, a decrease in the extensibility (L) and the deformation energy (W) was observed, indicating that high substitution level prevented the dough lifting. By comparing the control sample and formulation 4, (L) and (W) values decreased from 101 mm to 43 mm and from 213 10^{-4} J to 128 10^{-4} J, respectively. The resulting effect on (P) and (L) parameters became evident in the (P/L) ratio, which gave information about the elastic resistance and dough extensibility balance. In fact, 7% substitution level resulted in the highest (P/L) ratio (2 *versus* 0.64 in the control), which also indicated inextensible dough. Our findings recalls those reported by Wang et al. [23] and Borchani et al. [24], who showed an increase in the (P/L) ratio and a decrease in the deformation energy (W) of the dough containing pea and date flesh fibers, respectively. However, the influence on the deformation energy (W) depended on the nature of the added fibers. In fact, other studies showed that carob fibers or inulin resulted in an increase in the (P/L) ratio as well as in the deformation energy (W) of the composite dough, which led to an improvement of wheat protein behavior [23]. The obtained results could be mainly explained by the important water retention capacity of rocket powder that could prevent an optimal gluten hydration. Moreover, fibrous material might interact with gluten proteins and affect their structure. Therefore, gluten proteins were less likely to be hydrated and associated in order to form a viscoelastic network able to retain the fermentation gas.

3.3. Enriched Bread Quality

3.3.1. Physical Properties

Table 3 shows a significant ($p < 0.05$) increase in the bread volume as well as in the specific volume that reached maximum values in F2 formulation (Figure 2A). The specific volume of control bread was 1.98 cm^3/g that increased to 2.9 cm^3/g for bread made with 3% rocket powder (F2) and then it was reduced to 1.62 cm^3/g at 7% substitution level (F4). The reduction of bread specific volume observed at high substitution level (F4) was expected since the dough extensibility (L) was reduced (Figure 1). The obtained results for the formulation 4 were comparable to the studies on breads enriched by turmeric powder or lemon fibers that reported a significant ($p < 0.05$) decline in bread specific volume [4,8]. In fact, Lim et al. [4] demonstrated that 8% substitution of wheat flour by turmeric powder reduced the bread specific volume by 23%. Besides, Fu et al. [8] noted a decline of the specific volume by 44% at 9% substitution of wheat flour by lemon fiber. However, they did not report an increase of specific volume at low substitution level as was found for the present study. The different effects of agro-resources enrichment on bread volume could be explained by the different techno-functional properties of their fibers and consequently their interactions with the dough components.

Table 3 also shows that the increase in bread volume observed for formulation 2 (F2) was accompanied by a significant ($p < 0.05$) increase in the springiness and cohesiveness, and a decrease in the hardness. Nevertheless, at high substitution level (F4) an increase in the hardness and chewiness as well as a decrease in the springiness and cohesiveness were observed, which indicated that gluten network was weakened and became less cohesive. The increase in the hardness and the decrease in the springiness and cohesiveness were also observed for lemon fibers-enriched bread [8]. Thus, the densification of these breads could be explained by the fibers-gluten interaction. In fact, dietary fibers contained in rocket powder could alter the formation of a continuous gluten network and exhibited a destabilizing effect at the interfaces of the dough gas cells [8,25].

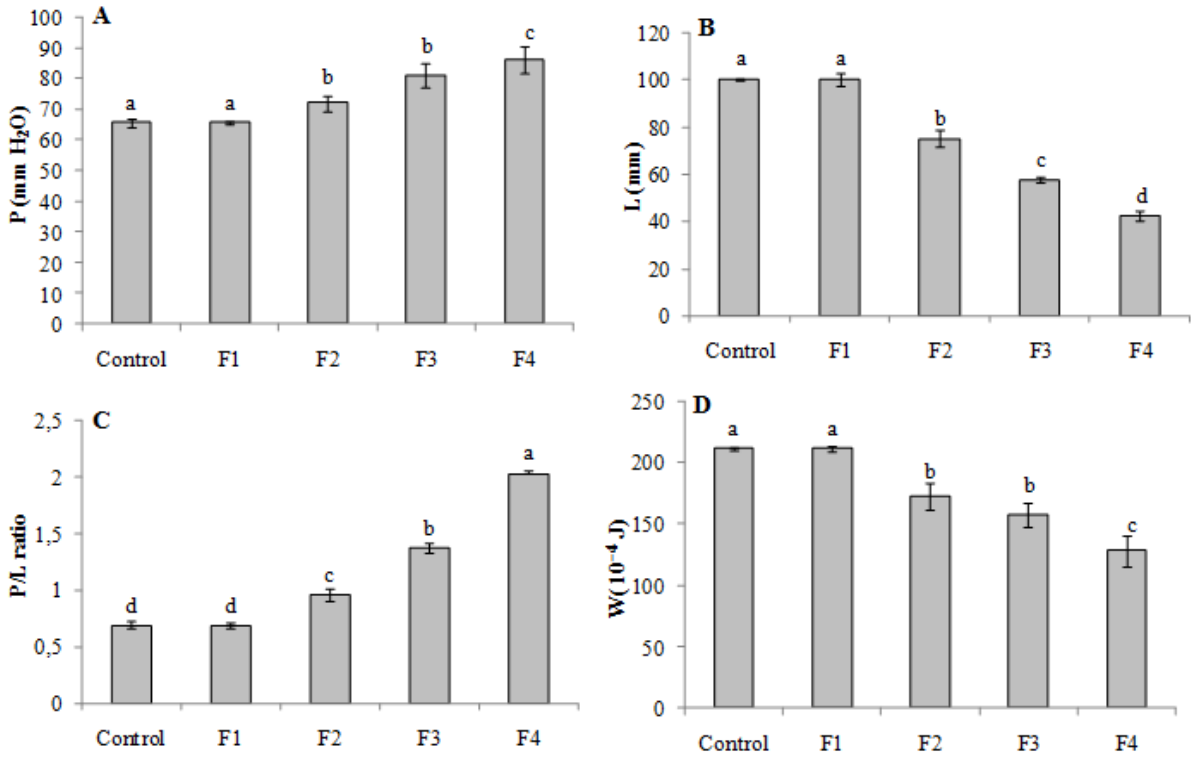


Figure 1. Effect of *D. simplex* leaves on the tenacity P (A), extensibility L (B), P/L ratio (C) and deformation energy W (D) of dough. [a,b,c,d] Different letters above the bars indicate significant differences ($p < 0.05$)

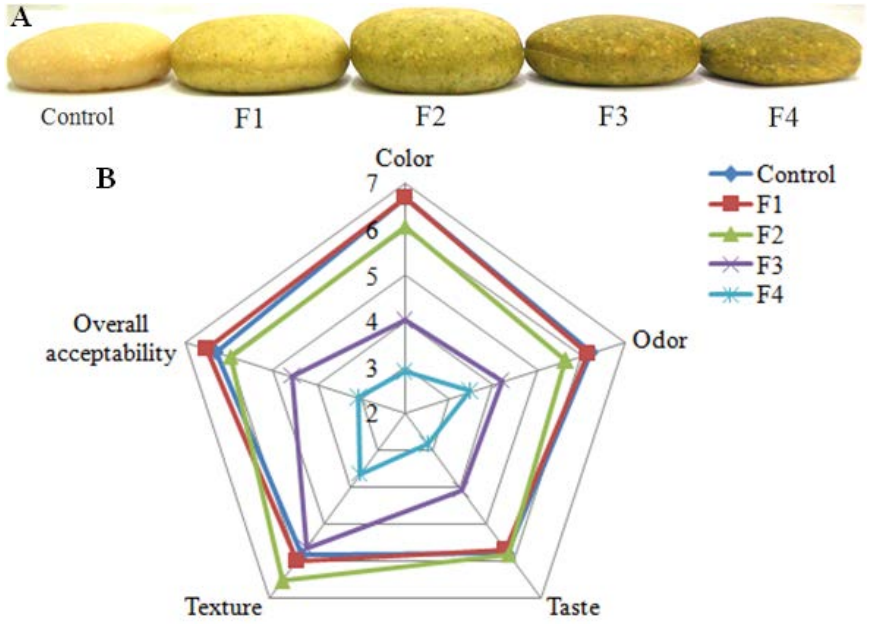


Figure 2. (A) Breads prepared with 1% (F1), 3% (F2), 5% (F3) and 7% (F4) of *D. simplex* leaves. The control represented the product without enrichment; (B) Sensory evaluation of formulated breads using a seven-point hedonic scale, where 7: like very much, 6: like moderately, 5: like slightly, 4: neither like nor dislike, 3: dislike slightly, 2: dislike moderately and 1: dislike very much

Table 3. Physical properties of bread enriched with *D. simplex* leaves

Substitution level (g/100 g of wheat flour)	Volume (cm^3)	Specific volume (cm^3/g)	Hardness (N)	Springiness (mm)	Cohesiveness	Chewiness (N × mm)
0 (control)	15 ± 1.0^{c}	1.98 ± 0.03^{b}	6.15 ± 0.26^{b}	3.72 ± 0.13^{b}	0.39 ± 0.03^{c}	8.48 ± 0.65^{c}
1 (F1)	17 ± 1.5^{b}	2.12 ± 0.04^{b}	5.24 ± 0.25^{c}	3.85 ± 0.05^{b}	0.42 ± 0.02^{b}	8.54 ± 0.30^{c}
3 (F2)	24 ± 2.0^{a}	2.90 ± 0.02^{a}	4.81 ± 0.14^{d}	4.05 ± 0.07^{a}	0.49 ± 0.01^{a}	8.47 ± 0.15^{c}
5 (F3)	14 ± 1.0^{c}	1.79 ± 0.03^{b}	6.24 ± 0.31^{b}	3.74 ± 0.15^{b}	0.37 ± 0.08^{c}	9.93 ± 1.96^{bc}
7 (F4)	13 ± 1.0^{d}	1.62 ± 0.03^{c}	9.35 ± 0.47^{a}	3.38 ± 0.12^{c}	0.37 ± 0.01^{c}	13.27 ± 1.65^{a}

[a,b,c,d] Values with same superscript letters in the same column are non-significant at $p < 0.05$.

Table 4. Total phenolics and antioxidant activity of bread enriched with *D. simplex* leaves

Substitution level (g/100 g of wheat flour)	Total phenolics	Scavenging activity	Reducing power
0 (control)	2.65 ± 0.11^{e}	23.50 ± 0.05^{b}	0.12 ± 0.01^{e}
1 (F1)	4.82 ± 0.07^{d}	15.75 ± 0.07^{a}	0.22 ± 0.01^{d}
3 (F2)	6.49 ± 0.04^{c}	12.85 ± 0.05^{c}	0.43 ± 0.02^{c}
5 (F3)	8.90 ± 0.08^{b}	8.90 ± 0.08^{d}	0.70 ± 0.01^{b}
7 (F4)	10.68 ± 0.04^{a}	4.90 ± 0.07^{e}	1.03 ± 0.04^{a}

^{a,b,c,d,e} Values with same superscript letters in the same column are non-significant at $p < 0.05$. DPPH• radical-scavenging activity is presented as IC_{50} values (mg/ml); Reducing power (A_{700}, absorbance at 700 nm) is determined at 3 mg/ml of bread extract; Total phenolics are expressed as mg gallic acid equivalents/100 g bread.

3.3.2. Total Phenolics Content and Antioxidant Activity

Nowadays, much interest has been focused on health-promoting food enriched with bioactive compounds, generally, derived from plants thanks to their safety and effectiveness in the prevention and/or treatment of human diseases. Therefore, in order to evaluate the rocket powder contribution to the antioxidant properties of the resulting bread, total phenolics content, DPPH• radical-scavenging activity and Fe^{3+} reducing power were determined (Table 4). Control bread was attributed to the lowest phenolics content (2.65 mg GAE/100 g bread), DPPH• radical-scavenging activity (IC_{50}: 23.5 mg/ml) and Fe^{3+} reducing power (A_{700}: 0.12). Interestingly, at 7% level of rocket powder supplementation, bread exhibited the highest phenolics amount (10.68 mg GAE/100 g bread), DPPH• radical-scavenging activity (IC_{50}: 4.9 mg/ml) and reducing power (A_{700}: 1.03). The phenolic acids and flavonoids identified in the *D. simplex* leaves (Table 2) may contribute individually or synergistically to the antioxidant activity observed in the rocket-enriched bread. It was reported that phenolics retain their antioxidant activity after the baking process, which has potential health benefits for consumers [3]. The obtained results suggest that the antioxidant potential of rocket-supplemented bread reinforces their nutritional quality. Thus, bread fortification with rocket seems to be a very easy and cheap way for improving food quality. However, a compromise between nutritional value and sensory quality should be established, since taste, odor and texture strongly influenced consumer preferences towards cereal products.

3.3.3. Sensory Properties

Texture is an important quality affecting consumer's acceptability, since its change has become one of the most common forms of dysphagia, and it is widely considered as an important factor for promoting safe and efficient swallowing. To determine sensory profile of fresh prepared breads, color, odor, taste, texture and overall acceptability were studied using an hedonic test (Figure 2B). Obtained results indicated that control and tested products showed detectable differences in their sensory parameters. Textural hardness is an important characteristic of bread; nevertheless, a too hard structure could have a negative effect on the product sensory qualities. The sensory analysis highlighted the important effect of the rocket supplementation in improving bread texture. In fact, Figure 2B reveals that F2 (3% substitution), which had the highest specific volume, presented the highest texture score as compared to the control product. A decline in texture score of F4 (7% substitution) was expected as was shown by the alveographic and the instrumental texture analyses. The bread crust color is an important parameter to determine its acceptability. Obtained results showed that color scores decreased as the incorporation of rocket powder increased. As shown in Figure 2, rocket powder gave greenish color characteristic to finished products. This could be explained by the richness of rocket powder in chlorophyll, whose content was found to be 106.24 mg/100 g rocket powder (Table 1). Rocket possessed a pungent flavor, which is due to its richness in organosulfur compounds. Interestingly, obtained results indicated that odor and taste scores of F1 and F2 were comparable to the standard product, and then there was a declining trend of the scores at high supplementation levels. In terms of overall acceptability of the enriched bread, consumer score was slightly reduced at 3% substitution level (F3). The product containing 5% of rocket powder remained acceptable since the obtained mean score for the overall acceptability was 4.55 (Figure 2B). A survey of the literature showed that bread enrichment up to 3-5% of functional ingredients (eg. onion skin, and turmeric powder) gave satisfactory consumer acceptability [3].

4. Conclusions

Bread played an important role in human nutrition and it could be considered an interesting vehicle for functional supplements. The *D. simplex* leaves showed richness in functional bio-molecules such as dietary fibers and phenolic compounds, which constituted a valuable supplement for developing bread with enhanced sensory and nutraceutical properties. At high substitution level (7%), rocket powder substantially affected dough rheology and bread physical quality. The obtained results suggest that dietary fibers were related to the modifications of the gluten network structure and water plasticizing capacity. From the current study, it seems worthy to say that 3% supplementation with rocket was optimal for improving total phenolics content and antioxidant potential of bread without altering its sensory acceptability.

Statement of Competing Interests

The authors have no competing interests.

References

[1] Ferrari, C. K. B., & Torres, E. A. F. S. (2003). Biochemical pharmacology of functional foods and prevention of chronic diseases of aging. Biomed Pharmacother 57, 251-260.

[2] Koechlin-ramonatxo, C. (2006). Oxygene, stress oxydant et supplémentations antioxydantes ou un aspect différent de la nutrition dans les maladies respiratoires. Nutr Clin Metabol 20, 165-177.

[3] Dziki, D., Rózyło, R., Gawlik-dziki, U., & Świeca, M. (2014). Current trends in the enhancement of antioxidant activity of wheat bread by the addition of plant materials rich in phenolic compounds. Trends Food Sci Tech 40, 48-61.

[4] Lim, S, Park, S. H., Ghafoor, K., Hwang, S. Y., & Park, J. (2011). Quality and antioxidant property of bread containing turmeric (*Curcuma longa* L.) cultivated in South Korea. Food Chem 112, 157-1582.

[5] Fakhfakh, N., Jdir, H., Jridi, M., Rateb, M., Belbahri, L., Ayadi, M. A., Nasri, M., & Zouari, N. (2017). The mallow, *Malva aegyptiaca* L. (Malvaceae): phytochemistry analysis and effects on wheat dough performance and bread quality. LWT-Food Sci Technol 75, 656-662.

[6] Msaddak, L., Abdelhedi, O., Kridene, A., Rateb, M., Belbahri, L., Ammar, E., Nasri, M., & Zouari, N. (2017). *Opuntia ficus-indica* cladodes as a functional ingredient: bioactive compounds profile and their effect on antioxidant quality of bread. Lipids Health Dis 16: 32.

[7] Gawlik-Dziki, U., Świeca, M., Dziki, D., Baraniak, B., Tomiło, J., & Czyż, J. (2013). Quality and antioxidant properties of breads enriched with dry onion (*Allium cepa* L.) skin. Food Chem 138, 1621-1628.

[8] Fu, J. T., Chang, Y. H., & Shiau, S. Y. (2015). Rheological, antioxidative and sensory properties of dough and Mantou (steamed bread) enriched with lemon fiber. Lwt-Food Sci Technol 61, 56-62.

[9] Ziobro, R., Gumul, D., Korus, J., & Korus, A. (2016). Starch bread with a share of non-wheat flours as a source of bioactive compounds in gluten-free diet. J Food Nutr Res 55, 11-21.

[10] Cartea, M. E., Sotelo, T., Velasco, P., & Soengas, P. (2016). Biological functionality of cruciferous vegetables. In D. A. Vattem & V. Maitin (Eds.), Functional foods, nutraceuticals and natural products: concepts and applications (pp. 643-673). USA: DEStech publications Inc.

[11] Podsedek, A. (2007). Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. LWT- Food Sci Technol 40, 1-11.

[12] Zouari, N. (2015). Eating wild edible plants can be a good alternative? Med Aromat Plants S1:e001.

[13] Jdir, H., Khemakham, B., Chakroun, M., Zouari, S., Ben Ali, Y., & Zouari, N. (2015). *Diplotaxis simplex* suppresses postprandial hyperglycemia in mice by inhibiting key-enzymes linked to type 2 diabetes. Rev Bras Farmacogn 25, 152-157.

[14] Jdir, H., Khemakham, B., Najjaa, H., Chakroun, M., Jridi, M., Ben Arfa, A., Ben Ali, Y., & Zouari, N. (2016). Anti-inflammatory and anti-proliferative activities of the wild edible cruciferous: *Diplotaxis simplex*. Pharm Biol 26, 1-8.

[15] Prosky, L., Asp, N. G., Schweizer, T. F., DeVries, J. W., & Furda, I. (1988). Determination of insoluble, soluble, and total dietary fiber in foods and food products: interlaboratory study. J Assoc off Anal Chem 71, 1017-1023.

[16] Ayadi, M. A., Abdelmaksoud, W., Ennouri, M., & Attia, H. (2009). Cladodes from *Opuntia ficus indica* as a source of dietary fiber: effect on dough characteristics and cake making. Ind Crop Prod 30, 40-47.

[17] Dewanto, V., Wu, X., Adom, K. K., & Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J Agric Food Chem 50, 3010-3014.

[18] Yildirim, A., Mavi, A., & Kara, A. A. (2001). Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. J Agric Food Chem 49, 4083-4089.

[19] A. A. C. C. (2000). Approved methods of the American Association of Cereal Chemists, method 54-30 (alveogrpahic analysis) (10th ed.). St. Paul, Minnesota: American Association of Cereal Chemists.

[20] Murray, J. M., Delahunty, C. M., & Baxter, I. A. (2001). Descriptive sensory analysis: past, present and future. Food Res Int 34, 461-471.

[21] Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., & Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. Food Chem 124, 411-421.

[22] Thebaudin, J. Y., Lefebvre, A. C., Harrington, M., & Bourgeois, C. M. (1997). Dietary fibres: nutritional and technological interest. Trends Food Sci Techno 8, 41-48.

[23] Wang, J., Rosell, C.M., & Benedito de Barber, C. (2002). Effect of the addition of different fibers on wheat dough performance and bread quality. Food Chem 79, 221-226.

[24] Borchani, C., Masmoudi, M., Besbes, S., Attia, H., Deroanne, C., & Blecker, C. (2011). Effect of date flesh fiber concentrate addition on dough performance and bread quality. J Text Stud 42, 300-308.

[25] Noort, M. W. J., Van Haaster, D., Hemery, Y., Schols, H. A., & Hamer, R. J. (2010). The effect of particle size of wheat bran fractions on bread quality - Evidence for fibre-protein interactions. J Cereal Sci 52, 59-64.

[26] Kakkar S., & Bais S. (2014). A review on protocatechuic acid and its pharmacological potential. ISRN Pharmacol.

[27] Dias, S. A., Cardoso, F. P., Santin, S. M. O., da Costa, W. F., Vidotti, G. J., de Souza, M. C., & Sarragiotto MH. (2009). Free radical scavenging activity and chemical constituents of *Urvillea ulmaceae*. Pharma Biol 47, 717-720.

[28] Ayranci, E., & Erkan, N. (2013). Radical scavenging capacity of methanolic *Phillyrea latifolia* L. extract: anthocyanin and phenolic acids composition of fruits. Molecules, 18, 1798-1810.

[29] Mishra, K., Ojha, H., & Chaudhury, N. K. (2012). Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. Food Chem 130, 1036-1043.

[30] Yokozawa, T., Chen, C. P., Dong, E., Tanaka, T., Nonaka, G. I., & Nishioka, I. (1998). Study on the inhibitory effect of tannins and flavonoids against 1.,1-diphenyl-2-picrylhydrazyl radical. Biochem Pharmacol 56, 213-222.

[31] Zhao, G. R., Zhang, H. M., Ye, T. X., Xiang, Z. J., Yuan, Y. J., Guo, Z. X., & Zhao, L. B. (2008). Characterization of the radical scavenging and antioxidant activities of danshensu and salvianolic acid B. Food Chem Toxicol 46, 73-81.

[32] Zhao, Y., Dou, J., Wu, T., & Aisa, H. A. (2013). Investigating the antioxidant and acetylcholinesterase inhibition activities of *Gossypium herbaceam*. Molecules 18, 951-962.

[33] Khanduja, K. L., & Bhardwaj, A. (2003). Stable free radical scavenging and antiperoxidative properties of reservetrol compared in vitro with some other bioflavonoids. Ind J Biochem Biophys 40, 416-422.