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Bioactive Compounds and Antioxidant Capacity of Moringa Leaves Grown in Spain Versus 28 Leaves Commonly Consumed in Pre-Packaged Salads

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Abstract: Total antioxidant capacity (TAC) evaluated by ferric ion reducing antioxidant power (FRAP) assay, ABTS, DPPH, and Oxygen radical absorbance capacity (ORAC) assay, and total polyphenol content (TPC) by Folin–Ciocalteu were determined in *Moringa oleifera* leaves (MO) grown in Spain, and compared with 28 different vegetable leaves pre-packaged for consumption as a salad. Total carotenoids, flavonoids, and chlorophylls were also determined in the samples with highest TAC. Two different extraction procedures were applied to obtain the methanolic fraction and the lipophilic and hydrophilic fractions. The highest TAC and TPC contents were found in MO. High values were also found in red chicory, "lollo rosso", and oak lettuce. The lowest TAC and TPC values were obtained in iceberg lettuce. The correlations between the extraction procedures and methods assayed were high and statistically significant. In the light of these results, we suggest the addition of MO to the existing range of fresh-cut salad foods would increase their antioxidant content by up to six times.

Keywords: *moringa oleifera*; antioxidant capacity; carotenoids; chlorophylls; phenolic compounds; salad

1. Introduction

Moringa oleifera (MO) is a tree from the sub-Himalayan regions (India, Pakistan, Bangladesh, and Afghanistan) and is also widely present in eastern, western and central Africa, Arabia, south-eastern Asia, the Pacific, the Caribbean, and South America [1]. Many parts of this tree (leaves, flowers, fruits, and immature pods) are used in traditional food formulations and medicines and for industrial purposes. The leaves are consumed fresh or cooked in soups or porridges [2]. Fresh and dried moringa leaves are part of the diet in African countries such as Ethiopia, Ghana, and Nigeria, and are used like spinach [1].

Moringa leaves are characterized by a high content of various phenolic compounds (mainly flavonoids) [2], together with other antioxidant compounds such as ascorbic acid and carotenoids. However, the bioactive composition of these plants depends on several factors such as their physiological stage, pedoclimatic conditions, and geographic origin [3–5]. In addition, the extraction methods used and cultivation conditions play an important role. According to Yang et al. [6], MO leaves have a significantly higher antioxidant content than fruits such as strawberries which are known for this property. Epidemiological studies have shown that foods rich in antioxidants provide protection against degenerative diseases including cancer, coronary heart disease, and Alzheimer's [7,8]. Therefore, it is

considered important to increase the intake of antioxidants from dietary sources [9]. The leaves of MO could serve as a supplementary dietary resource, facilitating the achievement of nutritional objectives.

Total antioxidant capacity (TAC) describes the cumulative ability of all antioxidants present in food to scavenge free radicals. It is considered an effective marker of the antioxidant quality of the diet and is also used to monitor the protective effect of plant foods in epidemiological studies [10].

The analysis of TAC is a straightforward, effective means of estimating the health potential of antioxidant-rich products. For this purpose, various databases of antioxidant activities have been published [11]. The methods used to assess TAC can be classified in several ways. In one approach, the classification is based on the mechanism of reaction between antioxidant compounds and the oxidant species. Hence, methods based on single electron transfer (SET) can be distinguished from those based on hydrogen atom transfer (HAT). Direct competition methods, on the other hand, focus on the fact that natural antioxidants compete for the radical with respect to the free-radical scavenger, while indirect methods monitor the decay of a free radical following the addition of the antioxidant-containing simple radical [10,12].

In Spain, moringa leaves are mainly produced in Andalusia, where several companies transform this product. However, to our knowledge no data have been published on the antioxidant capacity of the moringa leaves grown in this region.

Our study aim is to determine the total free antioxidant capacity of moringa leaves grown in Andalusia, extracted using two different solvents, and to compare this TAC with that of other leafy vegetables used directly in fourth-range (pre-packaged, ready to eat) salads and which make a notable contribution to the diet in this respect.

2. Materials and Methods

2.1. Chemical

2,2′-Azino-bis(3-ethylbenz-thiazoline-6-sulphonic acid) (ABTS), 2,2′-Azobis (2-methyl-propionamidine) dihydrochloride (AAPH), 2,4,6-Tris(2-Pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), β-carotene, acetic acid, acetone, catechin hydrate, dichloromethane, fluorescein sodium salt, gallic acid, hexane, hydrochloric acid 37%, iron (II) sulphate-7-hydrate, iron (III) chloride hexahydrate, aluminium chloride, sodium acetate, sodium carbonate, sodium hydroxide, and sodium nitrite were purchased from Sigma-Aldrich (Madrid, Spain). Potassium persulphate, sodium di-hydrogen phosphate anhydrous, and methanol were purchased from Panreac (Barcelona, Spain). Folin–Ciocalteu phenol reagent was purchased from Merck. All chemicals used were analytical grade, and water was obtained in situ using the Millipore Milli-Q water system.

2.2. Vegetable Samples

Twenty-eight vegetables were obtained from various local markets in Granada (Spain) and moringa was obtained from a farm in Cajiz (Málaga, Spain) in June 2015. The stated origin was Spain for all of the vegetables except the red chicory leaves, which were source from Italy.

2.3. Sample Preparation

Each sample (100 g) was dried in the laboratory at room temperature for 48 h, and then chopped and triturated for 10 s using an Oster blender. The samples were then homogenized in an ice bath using an IKA 725 digital Ultraturrax homogenizer. The moisture content was determined (in dry and fresh samples) according to AOAC method 934.06 [13]. Finally, the samples were stored at −80 °C until needed for analysis.

The vegetable extracts were obtained using two different methods, resulting in the methanolic extract (ME) on the one hand [12], and the hydrophilic extract (HE) and the lipophilic extract (LE) on the other [14]. To obtain the methanolic extract, 1 g sample was placed in a capped centrifuge tube

to which 5 mL methanol–water (80:20, *v*/*v*) was added. The tube was vortexed for 2 min in a vortex mixer (Cleaver Scientific Ltd., Rugby, UK) and then shaken at room temperature in a shaker (P-Selecta Rotaterm, Barcelona, Spain) for 15 min. Subsequently, the tube was centrifuged at 9000 rpm/10 min (centrifuge Sigma 2–16 Pk Sartorius) at 4 °C and the supernatant was recovered. This process was repeated once. The two supernatants were then combined in a 10 mL volumetric flask to which methanol–water was added up to a final volume of 10 mL. Finally, the methanolic extract was stored at −80 °C until needed for analysis. To obtain the hydrophilic and lipophilic extracts, 1 g sample was placed in a capped centrifuge tube to which 10 mL hexane–dichloromethane (50:50, *v*/*v*) were added. The tube was then vortexed for 2 min in a vortex mixer (Cleaver Scientific Ltd., Rugby, UK) and shaken at room temperature in a shaker (P-Selecta Rotaterm, Barcelona, Spain) for 5 min. The tube was then centrifuged at 9000 rpm for 10 min in a Sigma 2–16 Pk Sartorius centrifuge at 4 °C. The supernatant was recovered, and the process was repeated. The two supernatants containing the lipophilic extract were combined and evaporated in a rotavapor (Büchi R-114 with Waterbath B-480, Flawil, Switzerland). Finally, 10 mL acetone were added, and the lipophilic extract was stored in a topaz glass flask at −80 °C until analysis. To obtain the hydrophilic extract, 10 mL AWA (70% acetone, 27.5% water, 2.5% acetic acid) were added to the residue of the second centrifugation. The tube was then vortexed for 2 min, shaken for 5 min, and centrifuged at 9000 rpm for 10 min at 4 °C. The supernatant was separated and transferred to a 25 mL volumetric flask and AWA was added to reach a final volume of 25 mL, which was stored at −80 °C until needed for analysis.

2.4. Antioxidant Assays

Trolox equivalent antioxidant capacity (TEAC) assay: The TEAC assay was carried out according to the method described by Re et al. [15]. ABTS stock solution was prepared from 7 mM ABTS and 2.45 mM potassium persulphate in a volume ratio 1:1, and then incubated in the dark for 16 h at room temperature. The radical $ABTS^{\bullet+}$ solution was obtained by diluting ABTS stock solution with phosphate buffer 5 mM at pH = 7.4 to obtain an absorbance of 0.7 ± 0.02 at 730 nm. 30 µL test sample, diluted appropriately with water, or Trolox standard or blank (distilled water) were placed in each well of a 96-well polystyrene microplate, after which 270 µL radical $ABTS^{\bullet+}$ were added. The plate was stored at 30 °C for 30 min, and then the absorbance was measured at 730 nm using a Fluostart omega microplate reader (BMG Labtech, GmbH, Ortenberg, Germany). Aqueous solutions of Trolox concentrations (20–200 µM) were used for calibration. The results are expressed as micromoles of Trolox equivalent (TE) per 100 g fresh weight (µmol TE/100 g FW).

DPPH antioxidant assay: The DPPH (1,1-Diphenyl-2-picryl-hydrazyl) assay was carried out following the procedure described by Sharma and Bhat [16]. The working DPPH solution was obtained by dissolving DPPH powder in methanol to obtain an absorbance of 0.7 ± 0.02 at 517 nm. 20 µL test sample, diluted appropriately with water, or Trolox standard or blank (distilled water) were placed in each well of a 96-well polystyrene microplate, after which 280 µL working DPPH solution were added. After 30 min at 30 °C, the absorbance was measured at 517 nm using a Fluostart omega microplate reader (BMG Labtech, GmbH, Ortenberg, Germany). Aqueous solutions of Trolox concentrations (50–500 µM) were used for calibration and the results are expressed as micromoles of Trolox equivalent (TE) per 100 g fresh weight (µmol TE/100 g FW).

Ferric Ion Reducing Antioxidant Power (FRAP) assay: The FRAP assay was performed as previously described by Benzie and Strain [17]. 20 µL test sample, diluted appropriately with water, or Trolox standard, or ferrous sulphate standard or blank (distilled water) were placed in each well of a 96-well polystyrene microplate, after which 280 µL FRAP reagent (containing TPTZ, $FeCl_3$, and acetate buffer) freshly prepared and warmed at 37 °C were added. The absorbance values at 595 nm after 30 min were measured using a Fluostart omega microplate reader (BMG Labtech, GmbH, Ortenberg, Germany) thermostatted at 37 °C. The standard curves were constructed using $FeSO_4$ (115–1150 µM) and Trolox solutions (20–400 µM) and the results are expressed as micromoles of Trolox equivalent (TE) per 100 g fresh weight (µmol TE/100 g FW). In the FRAP assay, the relation between Trolox ($y = 0.0027x + 0.0876$;

r^2 = 0.9978) and $FeSO_4$ (y = 0.0014x + 0.0965; r^2 = 0.9968) standard curves was determined. A relation of 1.87 was obtained between the values expressed as $FeSO_4$ and as Trolox equivalent.

Oxygen Radical Absorbance Capacity (ORAC) assay: The ORAC assay was carried out according to Ou et al. [18] with some modifications. 75 µL test sample, diluted appropriately with water, or Trolox standard or blank (phosphate buffer 75 mM, pH 7.4) were placed in each well of a black 96-well polystyrene microplate, after which 75 µL 70 nM fluorescein and 37.5 µL AAPH 300 mM were added. Fluorescence values were measured at 490 nm excitation and 545 nm emission each 210 s for 31 cycles at 37 °C using a Fluostart omega microplate reader (BMG Labtech, GmbH, Ortenberg, Germany). Phosphate buffer solutions of Trolox concentrations (25–200 µM) were used for calibration and the results are expressed as micromoles of Trolox equivalent (TE) per 100 g fresh weight (µmol TE/100 g FW).

2.5. Total Phenolic Content (TPC)

The total phenolic content was determined using the Folin–Ciocalteu assay [19]. 190 µL distilled water were placed in each well of a 96-well polystyrene microplate, to which 30 µL test sample diluted appropriately with water, or gallic acid standard or blank (distilled water) were added. Finally, 15 µL Folin–Ciocalteu reagent and 60 µL 10% sodium carbonate solution were added quickly. The absorbance was measured at 725 nm after 60 min using a Fluostart omega microplate reader (BMG Labtech, GmbH, Ortenberg, Germany) at 30 °C. Aqueous solutions of gallic acid (10–100 mg/L) were used for calibration and the results are expressed as mg of gallic acid equivalent (GAE) per 100 g fresh weight (mg GAE/100 g FW).

2.6. Total Carotenoid Content

The absorbance of methanolic extract samples, appropriately diluted, was measured at 455 nm in a spectrophotometer (Perkin-Elmer Lambda 25 UV/VIS, MA, USA). Four readings were taken for each sample. Methanolic solutions of β-carotene (40.5–405 mg/L) were used for calibration and the results are expressed as mg β-carotene equivalent per 100 g of fresh weight (mg β-carotene equivalent/100 g FW).

2.7. Total Flavonoid Content (TFC)

The TFC was determined as previously described for a colorimetric assay [4] with slight modifications. Briefly, 1 mL methanolic extract from each sample was appropriately diluted and added to a 10 mL volumetric flask containing 4 mL H_2O. Then 0.3 mL 5% $NaNO_2$, 0.3 mL 10% $AlCl_3$ at 5 min, and 2 mL 1 M NaOH at 6 min were added. Immediately, the content of each flask was diluted with 2.4 mL H_2O and mixed. The absorbance values were determined at 510 nm in a spectrophotometer (Perkin-Elmer Lambda 25 UV/VIS. Six readings were taken for each sample and the mean value calculated. Methanolic solutions of catechin (30–300 mg/L) were used for calibration and the results are expressed as catechin equivalent per 100 g fresh weight (mg cat equivalent/100 g FW).

2.8. Chlorophyll Pigments (a and b)

The content of chlorophyll pigments *a* and *b* was determined following the procedure described by Lichtenthaler and Wellburn [20]. The absorbance of lipophilic extract samples, appropriately diluted, was measured at 645 nm and 662 nm in a spectrophotometer (Perkin-Elmer Lambda 25 UV/VIS). Four readings were taken for each sample. The results, expressed as µg of chlorophyll pigments *a* and *b* per 100 g fresh weight, were calculated using the equations:

$$\text{Chlorophyll } a\text{: } 11.75 \times A_{662} - 2.35 \times A_{645}$$

$$\text{Chlorophyll } b\text{: } 18.61 \times A_{645} - 3.96 \times A_{662}$$

2.9. Statistical Analysis

The extraction assays were carried out in duplicate, and three aliquots of each extract were analyzed. The results are expressed as mean ± standard deviation (SD). The normality of the variables

analyzed (Shapiro–Wilk test), the Spearman correlation coefficients ($p < 0.05$) and principal component analysis (PCA) were evaluated using Statistica 8.0 software (2007, StatSoft, Tulsa, OK, USA).

3. Results

The leaves of MO and of a wide range of other plants commonly consumed in pre-packaged salads in Spain were analyzed to determine TAC and TPC, using two extraction procedures, four antioxidant assays and the Folin–Ciocalteu method. The results obtained are summarized in Table 1.

3.1. Total Antioxidant Capacity (TAC) in Methanol Extract (ME)

The values obtained by each method are shown in Table 1.

3.1.1. TEAC

The TEAC values obtained ranged from 43.8 to 6208 µmol TE/100 g FW (iceberg lettuce and moringa, respectively), with a mean value of 789 µmol TE/100 g FW. In 39.3% of cases, the value exceeded 500 µmol TE/100 g FW, in 39.3% it was between 200 and 500 µmol TE/100 g FW and in 21.4% of them it was less than 200 µmol TE/100 g FW.

3.1.2. DPPH

The DPPH values ranged from 42.4 to 3055 µmol TE/100 g FW (green cabbage and moringa, respectively), with a mean value of 490 µmol TE/100 g FW. In 31.0% of cases, the value exceeded 500 µmol TE/100 g FW, in 20.7% it was between 200 and 500 µmol TE/100 g FW, and in 48.3% it was less than 200 µmol TE/100 g FW.

3.1.3. FRAP

By FRAP, the antioxidant values ranged from 22.9 µmol TE/100 g FW for iceberg lettuce to 3962 µmol TE/100 g FW for moringa, with a mean value of 719 µmol TE/100 g FW. In 41.4% of cases, the value exceeded 500 µmol TE/100 g FW, 17.2% it was between 200 and 500 µmol TE/100 g FW, and 41.4% it was less than 200 µmol TE/100 g FW.

3.1.4. ORAC

By ORAC, the antioxidant values ranged from 192 µmol TE/100 g FW for iceberg lettuce to 10,805 µmol TE/100 g FW for moringa, with a mean value of 2717 µmol TE/100 g FW. In 39.9% of cases the value exceeded 2500 µmol TE/100 g FW, in 24.2% it was between 2500 and 1000 µmol TE/100 g FW, and in 37.9% it was less than 1000 µmol TE/100 g FW.

3.2. Total Phenolic Content (TPC) in Methanol Extract (ME)

TPC values ranged from 8.4 to 504 mg GAE/100 g FW (iceberg lettuce and moringa, respectively) with a mean value of 98.1 mg GAE/100 g FW. In 31.0% of cases, the value exceeded 100 mg GAE/100 g FW, in 31.0% it was between 40 and 100 mg GAE/100 g FW, and in 38.0% it was less than 40 mg GAE/100 g FW (Table 1).

3.3. Total Antioxidant Capacity (TAC) in Hydrophilic (HE) and Lipophilic Extracts (LE)

Tables 2 and 3 show the results obtained in these extracts by each of the methods assayed.

The hydrophilic fraction was 10 times greater than the lipophilic fraction. The values obtained from the sum of the two fractions were 2.4 times lower than those obtained from the methanol extract.

Table 1. Vegetables analyzed, moisture content, mean values of total antioxidant capacity, and total polyphenol content determined by the methanol–water extraction method (ME).

Vegetables	Scientific Name	Moisture (%)	TEAC μmol TE/100 g FW		DPPH μmol TE/100 g FW		FRAP μmol TE/100 g FW		ORAC μmol TE/100 g FW		TPC mg GAE/100 g FW		Tabart Index
			Values	Rank *	Values	Rank *	Values	Rank *	Values	Rank *	Values	Rank *	
Batavia green lettuce plants	*Lactuca sativa*	94.4	200 ± 7	22	252 ± 7	12	101 ± 4	21	712 ± 23	23	36.8 ± 1.8	20	0.0102
Batavia red lettuce plants	*Lactuca sativa*	95.4	513 ± 1	11	536 ± 10	8	159 ± 0	18	1786 ± 15	15	70.2 ± 2.7	13	0.0228
Beet leaves	*Beta vulgaris* L.	89.8	1991 ± 146	4	529 ± 39	9	808 ± 40	9	3550 ± 311	8	201.3 ± 9.6	5	0.0528
Chard	*Beta vulgaris* L.	93.1	268 ± 6	15	104 ± 2	22	260 ± 21	16	2072 ± 97	13	44.5 ± 2.4	18	0.0145
Chinese cabbage	*Brassica rapa*	94.7	214 ± 11	18	62.1 ± 2.4	25	152 ± 12	19	729 ± 57	22	19.4 ± 1.5	25	0.0076
Curly green cabbage (green leaves)	*Brassica oleracea*	86.4	604 ± 34	10	235 ± 15	13	591 ± 13	11	1934 ± 142	14	75.8 ± 2.7	10	0.0242
Curly green cabbage (white leaves)	*Brassica oleracea*	90.1	342 ± 22	14	139 ± 10	21	247 ± 17	17	822 ± 48	20	34.4 ± 2.7	21	0.0119
Endive	*Cichorium intybus* L.	96.4	246 ± 12	16	234 ± 8	14	349 ± 19	13	1421 ± 52	16	49.0 ± 3.8	15	0.0156
Escarole	*Cichorium endivia*	94.9	111 ± 2	25	175 ± 6	17	79.2 ± 3.3	23	615 ± 16	24	23.6 ± 0.2	23	0.0072
Green cabbage	*Brassica oleracea*	93.4	70.4 ± 1.5	27	42.4 ± 2.1	29	42.8 ± 0.6	27	336 ± 12	28	13.1 ± 0.1	27	0.0031
Green lettuce plants	*Lactuca sativa*	95.7	205 ± 9	20	189 ± 15	16	266 ± 17	15	917 ± 18	19	48.4 ± 1.9	16	0.0118
Green sprout lettuce	*Lactuca sativa*	95.2	180 ± 11	23	81.3 ± 0.7	23	93.5 ± 6.0	22	541 ± 21	25	21.1 ± 1.5	24	0.0063
Iceberg lettuce plants	*Lactuca sativa*	97.0	43.8 ± 0.9	28	51.5 ± 1.7	26	22.9 ± 0.0	29	192 ± 7	29	8.40 ± 0.25	29	0.0023
Kale	*Brassica oleracea*	88.0	829 ± 12	7	308 ± 25	10	1206 ± 78	8	4448 ± 357	5	121 ± 10	7	0.0434
Lamb's lettuce	*Valerianella locusta* L.	93.8	207 ± 12	19	294 ± 12	11	104 ± 4	20	1393 ± 22	17	54.5 ± 1.8	14	0.0132
Lollo rosso lettuce	*Lactuca sativa*	91.0	2253 ± 203	2	1393 ± 74	4	2060 ± 97	3	7848 ± 621	3	296 ± 8	3	0.0996
Mix of rocket salad. watercress. lamb's lettuce and lollo rosso lettuce	-	92.2	1302 ± 64	5	1080 ± 87	5	1374 ± 76	7	4275 ± 251	6	206 ± 5	4	0.0638
Moringa	*Moringa oleifera*	72.2	6208 ± 206	1	3055 ± 140.0	1	3962 ± 297	1	10,805 ± 690	1	504 ± 28	1	0.2058
Oak leaf lettuce	*Lactuca sativa*	92.5	2080 ± 146	3	1400 ± 110	3	2133 ± 111	2	7671 ± 610	4	197 ± 11	6	0.0981
Red cabbage	*Brassica oleracea*	90.5	1081 ± 22	6	540 ± 39	7	1435 ± 105	6	3585 ± 237	7	105 ± 3	9	0.0503
Red chicory leaves	*Cichorium intybus*	89.7	-	-	2009 ± 75	2	1917 ± 130	4	9081 ± 633	2	319 ± 14	2	0.1162
Red lettuce plants	*Lactuca sativa*	94.8	812 ± 52	8	665 ± 53	6	1701 ± 129	5	3086 ± 30	10	114 ± 8	8	0.0511

Table 1. *Cont.*

Vegetables	Scientific Name	Moisture (%)	TEAC µmol TE/100 g FW		DPPH µmol TE/100 g FW		FRAP µmol TE/100 g FW		ORAC µmol TE/100 g FW		TPC mg GAE/100 g FW		Tabart Index
			Values	Rank *	Values	Rank *	Values	Rank *	Values	Rank *	Values	Rank *	
Red lettuce sprouts	*Lactuca sativa*	95.0	201 ± 17	21	71.8 ± 5.5	24	73.6 ± 5.3	24	404 ± 32	27	11.4 ± 0.9	28	0.0057
Rocket salad	*Eruca vesicaria cav.*	93.7	215 ± 2	17	42.8 ± 1.4	28	54.2 ± 1.9	25	1058 ± 39	18	37.2 ± 1.1	19	0.0072
Roman lettuce plants	*Lactuca sativa*	95.1	97.6 ± 2.6	26	150 ± 1	20	49.3 ± 1.7	26	417 ± 12	26	19.0 ± 0.3	26	0.0057
Spinach	*Spinacia oleracea*	93.2	156 ± 1	24	45.9 ± 1.0	27	26.7 ± 1.1	28	810 ± 22	21	29.0 ± 0.9	22	0.0055
Spinach sprouts	*Spinacia oleracea*	89.9	405 ± 14	13	156 ± 12	18	685 ± 28	10	3280 ± 194	9	72.3 ± 4.0	11	0.0259
Turnip greens (turnip tops)	*Brassica napus*	93.6	791 ± 49	9	206 ± 10	15	308 ± 19	14	2174 ± 61	12	44.6 ± 2.8	17	0.0232
Watercress	*Nasturtium officinale*	90.7	467 ± 6	12	153 ± 11	19	583 ± 19	12	2830 ± 160	11	70.3 ± 1.6	12	0.0239

* Rank: The values obtained are ranked from highest (1) to lowest (29).

Table 2. Mean values of total antioxidant capacity and total polyphenol content of hydrophilic extracts (HE) by Prior et al. 2003 [14] extraction's method.

Vegetables	TEAC µmol TE/100 g FW		DPPH µmol TE/100 g FW		FRAP µmol TE/100 g FW		ORAC µmol TE/100 g FW		TPC mg GAE/100 g FW		Tabart Index
	Values	Rank *	Values	Rank *	Values	Rank *	Values	Rank *	Values	Rank *	
Batavia green lettuce plants	41.1 ± 0.1	12	64.6 ± 1.3	11	66.7 ± 3.3	13	212 ± 10	18	11.4 ± 0.3	14	0.0133
Batavia red lettuce plants	143 ± 3	8	117 ± 4	8	176 ± 1	9	839 ± 18	7	20.4 ± 0.7	9	0.0353
Beet leaves	20.9 ± 0.2	20	28.6 ± 2.2	18	50.7 ± 4.0	18	149 ± 2	23	16.7 ± 0.6	11	0.0079
Chard	17.1 ± 0.2	21	20.9 ± 0.1	24	31.1 ± 1.9	23	416 ± 32	15	9.70 ± 0.50	20	0.0088
Chinese cabbage	4.20 ± 0.30	27	6.30 ± 0.36	28	22.5 ± 1.2	25	35.9 ± 0.6	28	2.50 ± 0.20	28	0.0024
Curly green cabbage (green leaves)	12.1 ± 0.2	25	15.2 ± 1.2	25	21.1 ± 1.7	26	40.0 ± 2.6	26	10.0 ± 0.3	19	0.0035
Curly green cabbage (white leaves)	24.3 ± 1.9	19	23.9 ± 1.4	20	49.6 ± 3.3	19	105 ± 6	24	10.8 ± 0.5	17	0.0071
Endive	15.9 ± 0.4	23	28.4 ± 2.0	19	31.8 ± 2.2	22	186 ± 4	20	6.40 ± 0.50	24	0.0069
Escarole	129 ± 4	9	141 ± 1	7	178 ± 1	8	581 ± 8	10	33.2 ± 0.1	7	0.0340
Green cabbage	27.0 ± 0.3	17	35.6 ± 1.0	17	62.0 ± 0.0	14	201 ± 7	19	6.27 ± 0.14	25	0.0099
Green lettuce plants	9.30 ± 0.61	26	11.8 ± 1.0	26	18.6 ± 0.4	27	42.4 ± 3.1	25	6.60 ± 0.37	23	0.0029
Green sprout lettuce	2.80 ± 0.19	28	6.40 ± 0.50	27	4.30 ± 0.30	29	12.7 ± 1.0	29	1.70 ± 0.09	29	0.0010
Iceberg lettuce plants	15.1 ± 0.3	24	22.9 ± 0.3	21	26.1 ± 0.3	24	162 ± 1	22	5.36 ± 0.14	26	0.0058

Table 2. *Cont.*

Vegetables	TEAC µmol TE/100 g FW		DPPH µmol TE/100 g FW		FRAP µmol TE/100 g FW		ORAC µmol TE/100 g FW		TPC mg GAE/100 g FW		Tabart Index
	Values	Rank *	Values	Rank *	Values	Rank *	Values	Rank *	Values	Rank *	
Kale	621 ± 48	5	155 ± 8	6	409 ± 23	4	2025 ± 83	4	62.7 ± 3.2	5	0.0864
Lamb's lettuce	87.7 ± 0.1	10	92.9 ± 0.8	10	125 ± 3	10	593 ± 8	9	30.3 ± 0.0	8	0.0253
Lollo rosso lettuce	675 ± 55	3	251 ± 3	4	463 ± 26	3	2348 ± 37	2	85.5 ± 4.0	2	0.1033
Mix of rocket salad. watercress. lamb's lettuce and lollo rosso lettuce	538 ± 7	6	224 ± 4	5	320 ± 17	6	1312 ± 38	6	60.3 ± 4.6	6	0.0753
Moringa	1426 ± 72	1	854 ± 68	1	635 ± 22	1	6683 ± 204	1	145 ± 6	1	0.2423
Oak leaf lettuce	625 ± 55	4	311 ± 20	2	341 ± 6	5	2277 ± 180	3	67.9 ± 5.6	4	0.0978
Red cabbage	80.4 ± 3.7	11	64.0 ± 1.3	12	124 ± 2	11	230 ± 18	16	11.1 ± 0.9	16	0.0186
Red chicory leaves	691 ± 49	2	307 ± 17	3	495 ± 10	2	1782 ± 91	5	82.5 ± 5.8	3	0.1042
Red lettuce plants	209 ± 17	7	102 ± 7	9	195 ± 16	7	641 ± 49	8	26.4 ± 1.6	9	0.0361
Red lettuce sprouts	37.8 ± 2.9	15	48.3 ± 0.7	13	54.5 ± 4.2	16	430 ± 32	13	10.6 ± 0.6	18	0.0135
Rocket salad	37.9 ± 0.3	14	40.7 ± 1.7	15	57.8 ± 0.2	15	454 ± 11	12	16.7 ± 0.7	12	0.0133
Roman lettuce plants	16.9 ± 0.3	22	35.7 ± 0.2	16	40.6 ± 1.1	21	182 ± 6	21	7.26 ± 0.08	22	0.0080
Spinach	39.3 ± 0.5	13	22.9 ± 0.3	22	44.0 ± 0.4	20	429 ± 13	14	16.4 ± 0.3	13	0.0109
Spinach sprouts	25.0 ± 0.7	18	22.6 ± 1.8	23	54.3 ± 4.2	17	229.5 ± 16.3	17	8.50 ± 0.65	21	0.0086
Turnip greens (turnip tops)	36.6 ± 0.8	16	44.7 ± 2.5	14	77.2 ± 4.9	12	544 ± 17	11	11.3 ± 0.9	15	0.0157
Watercress	2.60 ± 0.25	29	6.10 ± 0.42	29	15.6 ± 0.9	28	39.2 ± 2.7	27	3.10 ± 0.18	27	0.0020

* Rank: The values obtained are ranked from highest (1) to lowest (29).

Table 3. Mean values of total antioxidant capacity and total polyphenol content of lipophilic extract (LE) according to the extraction method described by Prior et al., 2003 [14].

Vegetables	TEAC µmol TE/100 g FW		DPPH µmol TE/100 g FW		FRAP µmol TE/100 g FW		ORAC µmol TE/100 g FW		TPC mg GAE/100 g FW		Tabart Index
	Values	Rank *	Values	Rank *	Values	Rank *	Values	Rank *	Values	Rank *	
Batavia green lettuce plants	19.5 ± 0.2	22	10.5 ± 0.5	10	14.1 ± 0.6	23	11.4 ± 0.3	24	3.50 ± 0.02	20	0.0140
Batavia red lettuce plants	21.0 ± 0.7	20	7.60 ± 0.01	14	7.80 ± 0.14	26	9.50 ± 0.30	25	3.10 ± 0.03	21	0.0111
Beet leaves	154 ± 12	2	27.2 ± 0.5	2	180 ± 8	4	53.6 ± 3.4	6	27.9 ± 1.2	2	0.0712
Chard	59.6 ± 3.8	11	8.30 ± 0.60	12	62.8 ± 1.9	12	37.3 ± 2.5	9	8.50 ± 0.65	13	0.0293
Chinese cabbage	15.6 ± 0.5	23	<ld		14.7 ± 0.5	20	35.0 ± 1.2	11	2.90 ± 0.23	23	0.0161
Curly green cabbage (green leaves)	97.5 ± 3.9	9	13.0 ± 0.5	9	113 ± 5	8	61.7 ± 3.6	5	15.1 ± 0.9	8	0.0488
Curly green cabbage (white leaves)	36.4 ± 0.2	17	<ld		20.6 ± 1.6	17	12.0 ± 0.6	23	3.00 ± 0.22	22	0.0132
Endive	11.7 ± 0.9	26	<ld		8.60 ± 0.74	25	6.90 ± 0.27	29	1.10 ± 0.08	27	0.0055
Escarole	15.4 ± 0.6	24	7.40 ± 0.29	16	4.90 ± 0.89	28	16.4 ± 0.1	19	2.90 ± 0.11	24	0.0116
Green cabbage	<ld		<ld		9.80 ± 0.23	24	15.5 ± 0.5	20	0.20 ± 0.01	29	0.0096
Green lettuce plants	34.7 ± 2.0	18	<ld		38.8 ± 1.8	14	30.8 ± 1.8	12	6.80 ± 0.28	14	0.0215
Green sprout lettuce	15.3 ± 1.1	25	<ld		16.6 ± 1.1	19	23.5 ± 1.2	16	4.00 ± 0.10	18	0.0126
Iceberg lettuce plants	<ld		5.50 ± 0.02	17	1.30 ± 0.03	29	14.7 ± 0.3	21	0.50 ± 0.02	28	0.0099
Kale	103 ± 8	7	<ld		100 ± 4	9	83.0 ± 3.4	2	11.7 ± 0.9	10	0.0592
Lamb's lettuce	41.8 ± 0.8	15	23.1 ± 1.5	5	29.5 ± 0.2	16	26.1 ± 0.2	15	11.6 ± 0.4	11	0.0308
Lollo rosso lettuce	54.7 ± 4.3	13	10.3 ± 0.8	11	62.1 ± 4.8	13	28.5 ± 1.8	14	6.30 ± 0.24	16	0.0278
Mix of rocket salad. watercress. lamb's lettuce and lollo rosso lettuce	141 ± 1	3	25.9 ± 1.8	4	201 ± 16	2	65.1 ± 4.8	4	15.3 ± 1.3	7	0.0736
Moringa	341 ± 16	1	125 ± 10	1	580 ± 50	1	201 ± 11	1	63.5 ± 4.9	1	0.2416
Oak leaf lettuce	107 ± 7	6	<ld		117 ± 3	7	30.1 ± 1.7	13	18.6 ± 1.4	4	0.0452
Red cabbage	45.6 ± 2.7	14	7.50 ± 0.28	15	14.3 ± 1.0	22	7.80 ± 0.58	29	2.20 ± 0.18	25	0.0145
Red chicory leaves	57.6 ± 1.3	12	<ld		36.3 ± 2.7	15	41.3 ± 1.6	8	6.40 ± 0.50	15	0.0285
Red lettuce plants	93.0 ± 7.5	10	14.0 ± 1.1	8	99.5 ± 8.7	10	18.1 ± 1.1	18	14.0 ± 1.1	9	0.0369
Red lettuce sprouts	28.5 ± 1.6	19	<ld		20.4 ± 1.6	18	22.1 ± 1.3	17	4.70 ± 0.34	17	0.0150
Rocket salad	20.4 ± 0.6	21	26.5 ± 4.2	3	63.0 ± 0.5	11	13.7 ± 5.8	22	3.80 ± 0.03	19	0.0310
Roman lettuce plants	10.2 ± 0.2	27	5.30 ± 0.12	18	7.50 ± 0.05	27	8.40 ± 0.38	27	1.80 ± 0.04	26	0.0078
Spinach	37.1 ± 1.2	16	8.10 ± 0.40	13	14.3 ± 1.2	21	8.50 ± 0.10	26	8.60 ± 0.12	12	0.0140
Spinach sprouts	136 ± 2	4	22.5 ± 1.6	6	185 ± 9	3	75.0 ± 3.7	3	18.7 ± 0.9	3	0.0712
Turnip greens (turnip tops)	101 ± 6	8	16.5 ± 1.3	7	155 ± 9	5	36.6 ± 1.5	10	15.6 ± 1.2	6	0.0500
Watercress	113 ± 6	5	<ld		131 ± 8	6	45.5 ± 3.1	7	15.8 ± 0.9	5	0.0532

<ld: below the detection limit. * Rank: The values obtained are ranked from highest (1) to lowest (29).

3.4. Total Phenolic Content (TPC) in Hydrophilic (HE) and Lipophilic Extracts (LE)

Tables 2 and 3 show the results obtained in these extracts by the TPC assay. The hydrophilic fraction was approximately five times greater than the lipophilic fraction. The values obtained from the sum of the two fractions were 2.8 times lower than those obtained from the methanol extract.

3.5. Correlations between the Methods and Principal Component Analysis

The correlations between the extraction procedures, for the same measurement methods, with and without the moringa values, are shown in Table 4. All correlations were positive, except the correlation between the hydrophilic and lipophilic extracts. When the MO values were excluded, the correlations were lower.

Table 4. Correlations between values obtained by different extraction procedures in similar methods of analysis with, and without, *Moringa oleifera* (MO) (* $p < 0.001$; ** $p < 0.01$; *** $p < 0.05$).

Extract	Method	r	r without MO
ME versus HE	TEAC	0.495 **	0.437 ***
	DPPH	0.714 *	0.682 *
	FRAP	0.519 **	0.465 ***
	ORAC	0.610 *	0.567 **
	TPC	0.679 *	0.643 *
ME versus HE + LE	TEAC	0.822*	0.802 *
	DPPH	0.731 *	0.701 *
	FRAP	0.769 *	0.743 *
	ORAC	0.647 *	0.608 *
	TPC	0.801 *	0.779 *
HE versus LE	TEAC	0.335	0.255
	DPPH	0.175	0.020
	FRAP	0.232	0.146
	ORAC	0.174	0.083
	TPC	0.412 ***	0.347
HE versus HE + LE	TEAC	0.790 *	0.766 *
	DPPH	0.971 *	0.968 *
	FRAP	0.825 *	0.806 *
	ORAC	0.994 *	0.993 *
	TPC	0.892 *	0.880 *

The correlations were higher when the sum of HE and LE was included, which indicates that the methanol–water mixture also extracts fat-soluble compounds. The highest correlation was obtained by the TEAC assay.

Table 5 shows the statistically significant correlations obtained between the different antioxidant methods assayed for the same extract, with and without MO. In general, high correlations ($r > 0.6$) were obtained, with the highest for HE and the lowest for LE. When moringa values were taken into account, the correlations were slightly higher.

The higher correlation obtained for HE shows that this extraction method is more selective than ME when applied to antioxidant compounds.

TEAC and TPC obtained better correlations than the other methods.

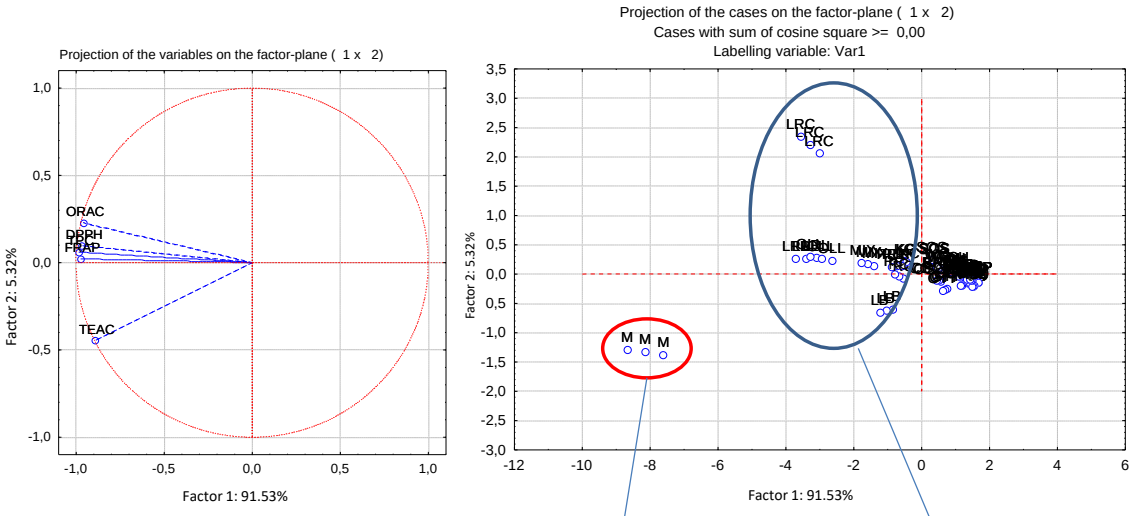
Following Tabart, Kevers, Pincemail, Defraigne, and Dommes [21], the weighted average of the results obtained by the different assays was calculated, as an overall impression of the antioxidant potential of the samples (Tables 1–3). Therefore, the antioxidant capacity obtained by each specific assay (TEAC, DPPH, FRAP, and ORAC) was divided by the average activity of all samples according to the same assay. The values obtained by each assay were then summed and the total was divided

by the number of assays used. By this procedure, the TEAC assay produced the highest correlation (r = 0.9771) with the weighted average antioxidant capacity.

Table 5. Correlations between values obtained by different methods of analysis in similar extraction procedures with, and without, MO (* $p < 0.001$; ** $p < 0.01$).

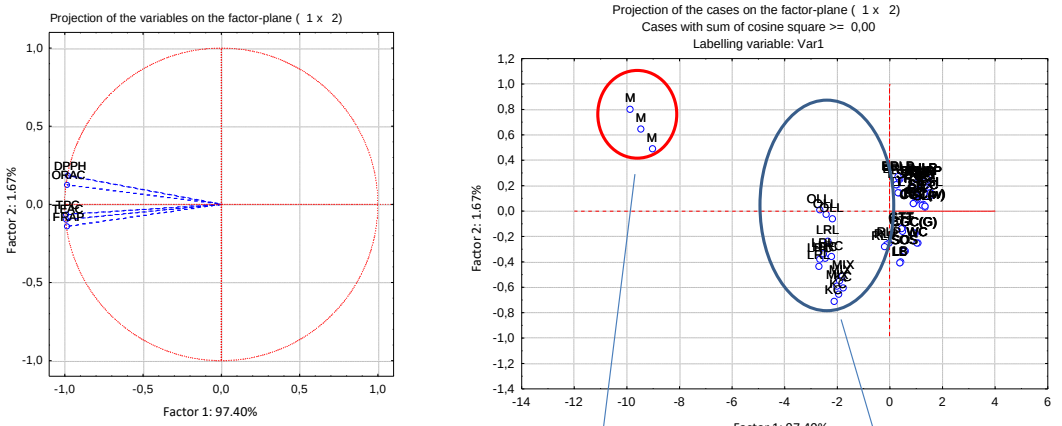
	Extract					
	ME		HE		LE	
Method						
	r	**r without MO**	**r**	**r without MO**	**r**	**r without MO**
TEAC versus DPPH	0.802 *	0.780 *	0.951 *	0.948 *	0.696 **	0.635 **
TEAC versus FRAP	0.934 *	0.926 *	0.975 *	0.973 *	0.902 *	0.890 *
TEAC versus ORAC	0.940 *	0.933 *	0.949 *	0.943 *	0.777 *	0.750 *
TEAC versus TPC	0.923 *	0.914 *	0.934 *	0.926 *	0.903 *	0.892 *
DPPH versus FRAP	0.857 *	0.841 *	0.964 *	0.960 *	0.864 *	0.839 *
DPPH versus ORAC	0.810 *	0.789 *	0.911 *	0.901 *	0.688 **	0.630 **
DPPH versus TPC	0.882 *	0.869 *	0.896 *	0.884 *	0.843 *	0.814 *
FRAP versus ORAC	0.922 *	0.914 *	0.929 *	0.921 *	0.816 *	0.796 *
FRAP versus TPC	0.929 *	0.921 *	0.904 *	0.893 *	0.909 *	0.899 *
ORAC versus TPC	0.953 *	0.948 *	0.894 *	0.883 *	0.771 *	0.745 *

Principal component analysis (PCA) was applied to the samples and methods to determine their distribution. The two first principal components accounted for 96.85% of total system variability in the methanolic extract and for 99.07% of total system variability in the total extract (hydrophilic and lipophilic) (Figures 1 and 2, respectively).



	PC1	*PC2*
TEAC	-0.893786	-0.445726
DPPH	-0.968782	0.098922
FRAP	-0.975021	0.021265
ORAC	-0.959317	0.230420
TPC	-0.983888	0.061765

Figure 1. Loadings (**left**) and Scores (**right**) for plots obtained by principal component analysis of methanol extract from the vegetable samples.



	PC1	*PC2*
TEAC	-0.991631	-0.096306
DPPH	-0.978588	0.184311
FRAP	-0.986458	-0.142747
ORAC	-0.985763	0.124304
TPC	-0.991973	-0.067124

Figure 2. Loadings (**left**) and Scores (**right**) for plots obtained by principal component analysis of hydrophilic + lipophilic extract from the vegetable samples.

In Figures 1 and 2, the data for the moringa samples are shown separately.

3.6. Carotenoid, Flavonoid and Chlorophyll Contents

The samples with the highest TAC and TPC were used to perform a more detailed analysis of the antioxidant compounds, i.e., carotenoids, flavonoids, and chlorophylls. Twenty samples of vegetables were analyzed. Flavonoids and carotenoids were analyzed in the methanol extract and carotenoids and chlorophylls in the lipophilic extract. The results (Table 6) were calculated for 100 g fresh product and are expressed as mg catechin (+), mg β-carotene and mg chlorophyll *a* and *b*.

3.6.1. Carotenoids

The carotenoid content in the methanolic extract ranged from 8.7 mg β-carotene/100 g FW endive to 369 mg β-carotene/100 g FW beet with a mean value of 106 mg β-carotene/100 g FW. In the lipophilic extract, the β-carotene contents ranged from 0.1 to 35.1 (endive and moringa, respectively) with a mean value of 7.8 mg β-carotene/100 g FW. The correlation between the β-carotene contents in the two extracts was r = 0.827 ($p < 0.001$). The order obtained was similar to that found in the different extracts and methods for TAC.

The correlation obtained between the carotenoids in the methanolic extract and the antioxidant capacity for the same extract was r = 0.697 ($p < 0.001$) for TEAC, r = 0.496 ($p < 0.05$) for DPPH, r = 0.571 ($p < 0.01$) for FRAP and r = 0.624 ($p < 0.01$) for ORAC.

In the lipophilic extract, the correlations were, r = 0.929 for TEAC, r = 0.939 for DPPH, r = 0.981 for FRAP, r = 0.733 for ORAC and r = 0.967 for TPC. In every case, the values were statistically significant ($p < 0.001$). The antioxidant capacity of the lipophilic extract is probably due to the carotenoids, but the carotenoid fraction is only a small percentage of the TAC.

3.6.2. Flavonoids

The flavonoid content ranged from 3.8 mg cat (+)/100 g FW Chinese cabbage to 372 mg cat (+)/100 g FW MO, with a mean value of 68 mg cat (+)/100 g FW.

The correlations obtained between flavonoids for the methanolic extract and the antioxidant capacity for the same extract were r = 0.805 for TEAC, r = 0.922 for DPPH, r = 0.892 for FRAP, and r = 0.892 for ORAC, and were statistically significant ($p < 0.01$). As expected, a high correlation was also obtained with TPC (r = 0.919). The flavonoids probably make a significant contribution to the antioxidant capacity in the methanolic extract.

Table 6. Mean values of carotenoids, flavonoids and chlorophyll *a* and *b* obtained by methanolic extract (1) and lipophilic extract (2). All values are expressed as mg/100 g fresh weight (FW). In parentheses, the rank order of the vegetables according to the values obtained, from highest to lowest.

Vegetable	β-Carotene (1)	β-Carotene (2)	Cat (+) (1)	Chlorophyll *a* (2)	Chlorophyll *b* (2)
Beet leaves	368.5 ± 8.3 (1)	13.9 ± 0.8 (4)	92.9 ± 6.6 (6)	185.2 ± 5.9 (6)	63.6 ± 1.7 (5)
Chard	62.1 ± 4.8 (12)	5.0 ± 0.4 (11)	12.6 ± 0.5 (15)	127.8 ± 10.3 (9)	34.0 ± 1.7 (9)
Chinese cabbage	9.8 ± 0.9 (19)	0.2 ± 0.0 (19)	3.8 ± 1.2 (20)	3.3 ± 0.9 (17)	1.1 ± 0.4 (17)
Curly green cabbage (green leaves)	133.3 ± 3.5 (6)	8.6 ± 0.5 (9)	9.4 ± 1.7 (17)	62.3 ± 3.1 (11)	17.4 ± 5.2 (11)
Curly green cabbage (white leaves)	32.8 ± 0.3 (15)	0.3 ± 0.1 (17)	5.6 ± 0.3 (18)	2.2 ± 0.5 (19)	0.7 ± 0.2 (20)
Endive	8.7 ± 1.5 (20)	0.1 ± 0.0 (20)	42.4 ± 2.7 (8)	2.1 ± 0.2 (20)	1.0 ± 0.4 (18)
Green lettuce plants	48.2 ± 5.9 (14)	2.9 ± 0.0 (12)	25.0 ± 5.3 (11)	83.0 ± 0.0 (10)	34.8 ± 0.0 (8)
Green lettuce sprouts	15.0 ± 0.4 (18)	0.5 ± 0.0 (16)	9.9 ± 0.4 (16)	8.4 ± 2.0 (15)	3.9 ± 0.0 (15)
Kale	173.3 ± 13.6 (4)	7.4 ± 0.3 (10)	30.2 ± 0.3 (9)	43.1 ± 0.2 (12)	13.2 ± 0.6 (13)
Lollo rosso lettuce	87.5 ± 11.0 (9)	2.7 ± 0.1 (13)	175.9 ± 12.0 (3)	33.2 ± 6.1 (13)	9.5 ± 2.2 (14)
Mix of rocket, watercress, lamb's lettuce and lollo rosso lettuce	133.9 ± 7.5 (5)	16.1 ± 3.0 (3)	115.2 ± 23.9 (5)	334.6 ± 66.6 (1)	110.3 ± 26.4 (2)
Moringa	353.9 ± 17.1 (2)	35.1 ± 0.9 (1)	327.2 ± 13.8 (1)	210.4 ± 3.9 (5)	33.8 ± 1.0 (10)
Oak leaf lettuce	121.7 ± 5.8 (8)	9.6 ± 0.6 (7)	172.9 ± 12.3 (4)	183.8 ± 9.7 (7)	60.5 ± 0.5 (6)
Red cabbage	23.2 ± 1.8 (16)	0.3 ± 0.0 (18)	27.8 ± 1.8 (10)	2.6 ± 0.3 (18)	0.9 ± 0.2 (19)
Red chicory leaves	61.3 ± 6.3 (13)	0.6 ± 0.0 (15)	191.2 ± 14.8 (2)	4.1 ± 0.1 (16)	1.3 ± 0.1 (16)
Red lettuce plants	69.5 ± 6.7 (11)	8.7 ± 0.2 (8)	70.0 ± 18.2 (7)	218.3 ± 33.6 (4)	79.7 ± 12.1 (3)
Red lettuce sprouts	22.3 ± 0.2 (17)	1.5 ± 0.1 (14)	5.4 ± 1.3 (19)	24.0 ± 0.8 (14)	14.6 ± 2.5 (12)
Spinach	174.6 ± 8.9 (3)	18.2 ± 0.9 (2)	16.1 ± 4.8 (13)	269.5 ± 38.5 (3)	72.2 ± 18.4 (4)
Turnip greens	85.5 ± 9.2 (10)	13.4 ± 1.8 (5)	13.8 ± 4.8 (14)	330.5 ± 85.3 (2)	116.7 ± 30.9 (1)
Watercress	129.7 ± 4.5 (7)	10.1 ± 0.6 (6)	19.0 ± 1.5 (12)	135.6 ± 2.9 (8)	45.0 ± 8.2 (7)

3.6.3. Chlorophylls

The content of chlorophyll *a* ranged from 2.1 mg/100 g FW endive to 335 mg/100 g FW mixed salad, and 331 mg/100 g FW turnip greens, with a mean value of 112 mg/100 g FW. The chlorophyll *b* content ranged from 0.7 mg/100 g FW to 117 mg/100 g FW (curly green cabbage (white leaves) and turnip greens, respectively) with a mean value of 65 mg/100 g FW. The relation between these chlorophyll *a* and *b* values was statistically significant (r = 0.964, $p < 0.001$). Interestingly, MO did not present the highest values for chlorophyll content, but was only in the 5th and 10th positions, for chlorophyll *a* and *b* respectively.

The correlations obtained between chlorophyll *a* in the lipophilic extract and the antioxidant capacities for the same extract were: r = 0.803 ($p < 0.001$) for TEAC, r = 0.661 ($p < 0.05$) for DPPH, r = 0.901 ($p < 0.001$) for FRAP and r = 0.593 ($p < 0.01$) for ORAC. The correlation obtained between chlorophyll *a* and carotenoids was r = 0.941 ($p < 0.001$). The correlations obtained with chlorophyll *b* were lower for all methods. This value was not obtained for FRAP.

4. Discussion

4.1. Total Antioxidant Capacity (TAC) and Total Phenolic Content (TPC)

Three antioxidant assays (FRAP, TEAC and DPPH) based on single electron transfer and one based on hydrogen atom transfer (ORAC) were applied to evaluate antioxidant activities. This compound approach was adopted because no universal assay can accurately reflect all of the antioxidants in a complex system [22].

Among the vegetables assayed, the moringa leaves presented the highest TAC and TPC values for all the methods and extracts assayed, the lower water content of moringa might explain its greater

antioxidant capacity. Very high values of TAC and TPC were obtained for red chicory, lollo rosso lettuce, and oak leaf lettuce in both extracts (ME and HE). The lowest TAC and TPC values were obtained for iceberg lettuce, green cabbage, and roman lettuce plants in ME and for watercress, green sprout lettuce, and Chinese cabbage in HE.

In the lipophilic extract (LE), MO also presented the highest values of TAC and TPC, by all methods, followed by beet and the mix of rocket salad, watercress, lamb's lettuce, and lollo rosso lettuce. The lowest values were obtained for endive, iceberg lettuce and roman lettuce, which also had the highest water content. The correlation between water content and TAC was negative (r = 0.85–0.90), even when the moringa value was excluded (r = 0.52–0.67).

The average TAC of the hydrophilic fraction (HE) of the total extract (HE + LE) ranged from 58.2% for TEAC to 86–87% for the ORAC and DPPH methods. Three of the 29 samples presented higher antioxidant activity in the lipophilic than the hydrophilic extracts for the ORAC and DPPH methods, and also in nine of the 29 samples for the TEAC and FRAP methods. The highest activity in the lipophilic extract was obtained for curly green cabbage (green leaves), green sprout lettuce, and watercress, in all methods assayed.

With respect to other vegetables, the TAC and TPC has been examined in many studies. For example, Carlsen et al. [23] examined the antioxidant capacity of 3100 foods (including 303 vegetables), generating a large database. According to these authors, the antioxidant content of vegetables measured by FRAP in water/methanol ranges from 0.01 mmol Fe^{2+}/100g in lettuce to 48.07 mmol Fe^{2+}/100g in leaves from the African baobab tree. To compare the latter with our results, we examined the values for ten samples. The values obtained by these authors were statistically different from ours, but there was a very high correlation between them (r = 0.768, $p < 0.01$).

Pellegrini et al. [10] studied the antioxidant capacity of 104 foods (34 vegetables). For comparison with our results, we examined the reported values for six samples extracted in water/acetone and analyzed by FRAP and TEAC. The values of the samples compared ranged from 494 µmol Fe^{2+}/100 g FW in green lettuce to 2694 µmol Fe^{2+}/100 g FW in spinach by FRAP and from 0.1 mmol TE/100g FW to 0.8 mmol TE/100g FW by TEAC for the same samples. These results differed significantly from ours and there was no correlation between them.

The United States Department of Agriculture Agricultural Research Service Database [24] includes the TAC (determined by ORAC) and the TPC (determined by Folin–Ciocalteu) of 326 foods, 98 of which are vegetables. The values of the nine samples compared ranged from 406 µmol TE/100 g FW in iceberg lettuce to 2476 µmol TE/100 g FW in red cabbage, by HE-ORAC, and 9 to 162 µmol TE/100 g FW in beet and roman lettuce, respectively, by LE-ORAC. For TPC, the values ranged from 11 mg GAE/100 g FW in red lettuce to 231 mg GAE/100 g FW in red cabbage. Our results were different and there was no correlation. However, parameters such as the location of the samples, the time of collection, the maturity, processing, and storage could all account for these differences.

Deng, Lin, Xu, Gao, Xie, and Li [25] analyzed the antioxidant capacity of 56 vegetables by FRAP, TEAC and TPC assays. Tetrahydrofuran for the lipophilic fraction and a methanol–acetic acid–water mixture (50:3.7:46.3, *v*/*v*/*v*) for the hydrophilic fraction were used as solvents. Twenty five of the samples were leaves and four were similar to those analyzed in the present study (Chinese cabbage, green cabbage, green lettuce, and spinach). The values obtained by these authors were higher than ours. The geographic origin (China), the variety of vegetable or differences in the extraction procedure may account for these differences. However, as in our study, the correlations between TEAC- FRAP, TPC-FRAP, and TPC-TEAC were very high and statistically significant.

As is the case with common vegetables, the TAC and TPC of MO have been widely studied, but the results obtained vary widely according to the analysis method employed, the extraction procedure and the origin and time of collection of the moringa leaves. Thus, in their analysis of moringa leaves from India, Nicaragua, and Niger, Siddhuraju and Becker [3] measured higher TPC values when 80% methanol was used versus 70% ethanol. The TPC for the plants from Nicaragua was 1.5 times higher than that for the plants from India.

Iqbal and Bhanger [4] analyzed moringa leaves from different regions of Pakistan, obtained at various times, using methanol 80% as the solvent. The TAC values for the plants from the Chakwal region were about 24 times lower than those for the plants collected elsewhere. Pakade et al. [5] in a study of moringa leaves conducted in South Africa measured TPC values 1.6 times higher in the Limpopo region than in Atteridgeville, using an 80:20 acetone–water mixture as the solvent. These TPC values were about 2.5 times and 2 times higher than those for cabbage and spinach, respectively.

Rodriguez-Perez, Quirantes-Piné, Fernández-Gutiérrez, and Segura-Carretero [26] studied the effects on the TPC content of moringa leaves collected in Madagascar, using either acetone, methanol, or an ethanol–water mixture in various proportions. The best procedures were found to be ethanol and ethanol–water 50%. Rodriguez-Perez et al. [27] used pressurized liquid and microwave-assisted extraction methods and reported variations of up to nine times in the TEAC and TPC contents between the lowest and highest values obtained.

Principal component analysis was performed to discriminate the samples. As shown in Figure 1, PC1 explains 91.5% of total variation and was related to antioxidant activity and TPC; in brief, PC1 discriminates the samples with high TPC content (>197 mg GAE/100 g FW) from the others. PC2 explains 5.3% of the variation and appears to be correlated to TEAC. With respect to the sum of hydrophilic and lipophilic extracts, as shown in Figure 2, PC1 explains 97.4% of the variance and, in particular, discriminates the samples with high TPC content (>74 mg GAE/100 g FW).

4.2. Carotenoids, Flavonoids and Chlorophylls

4.2.1. Carotenoids

In the present study, the carotenoid content of the samples was determined in the methanolic and lipophilic extracts. However, most previous studies in this field have been conducted using an apolar solvent (lipophilic extract). To our knowledge, the only exception is the study by Saini, Shetty, Prakash, and Giridhar [28], who analyzed moringa leaves in an extract of acetone using a spectrophotometric measurement method. These authors reported values of 68.81 mg/100g FW, lower than ours but within the same range.

The USDA database [24] contains analyzes of sixteen vegetables similar to those examined in the present study. The USDA values range from 0.67 mg β-carotene/100 g FW in curly green cabbage to 14.7 mg β-carotene/100 g FW in chard, with a mean value of 5.93 mg β-carotene/100 g FW. The value reported for MO is 6.6 mg β-carotene/100 g FW. There is no significant correlation with our data.

The CESNID food composition table [29] contains data on four vegetables coinciding with the samples examined in our study (lipophilic extract). These values range from 0.1 to 2.9 mg β-carotene/100 g FW in endive and watercress, respectively, with a mean value of 1.1 mg β-carotene/100 g FW. The correlation with our results is high (r = 0.9960) and significant ($p < 0.05$).

Seven of our samples were compared with those described in the Mataix food composition table [30]. The values shown in this table range from 0.011 to 6 mg β-carotene/100 g FW in red cabbage and turnip greens, respectively, with a mean value of 2.16 mg β-carotene/100 g FW. Although these values are lower than ours, the correlation is statistically significant (r = 0.795 $p < 0.05$).

4.2.2. Flavonoids

Of all the vegetables analyzed in the present study, moringa leaves have the highest flavonoid content (327.2 mg cat (+)/100 g FW), about five times higher than the mean value of the other vegetables (68.3 mg cat (+)/100 g FW).

In the USDA database [24] there are 16 vegetables that coincide with those we analyze, with flavonoid contents ranging from 0 to 210 mg cat (+)/100 g FW in turnip greens and red cabbage, respectively, with an average value of 39.73 mg cat (+)/100 g FW. The USDA value for moringa leaves is high (23 mg/100 g FW) but a higher content was measured in another four vegetables. No statistically relationship was obtained between the USDA values and our data.

Flavonoids, which are secondary metabolites that perform various metabolic functions, could be considered the main phenolic group in moringa plants (comprising quercetin, kaempferol, apigenin, luteolin, and myricetin glycosides) [26,31], but the content is variable, depending on the origin and on the extraction method used. Siddhuraju and Becker [3] determined the total flavonoid content in moringa leaves collected in Nicaragua, India, and Niger, using three solvents (water, 80% methanol, and 80% ethanol). The values obtained ranged from 3.26 g/100 g rutin DM for water extract in the plants collected in India, to 14.07 g rutin DM for those obtained by methanol extract and collected in Nicaragua.

Nobossé, Fombang, and Mbofung [32] analyzed the effect of age and solvent extraction (methanol, ethanol, and water) on antioxidant activity (ABTS, FRAP), total polyphenols (TPC), total flavonoids (TFC) and chlorophyll in moringa leaves harvested at 30, 45, and 60 days. TPC and TFC increased with maturity, except in water extract. The thirty-day-old leaves contained larger quantities of chlorophyll and carotenoids and presented greatest ABTS activity. Methanol was the best extraction solvent for TPC (4.6 g GAE/100 g DM) while ethanol was the best with respect to flavonoids (1.8 g CE/100 g DM).

4.2.3. Chlorophylls

Saini et al. [28], using a similar methodology, recorded values similar to ours, with 166.29 mg/100 g chlorophyll *a* and 49.58 mg/100 g chlorophyll *b* in fresh moringa leaves analyzed in acetone extract. Nobossé et al. [32] measured a lower chlorophyll content (0.28 mg/g DM) in moringa leaves, using methanol and ethanol, together with strong positive correlations ($r \geq 0.8$; $p < 0.05$) between chlorophyll content and DPPH and ABTS in moringa leaves, which suggests that chlorophyll is an important contributor to the antioxidant capacity of moringa leaves.

4.3. TAC and TPC Values for Fourth-Range Vegetable Packs, with or without, Moringa Leaves

TAC and TPC values were determined for three IV-gamma salads selected because in each case the percentage content of each ingredient is stated on the label: Vital salad 150 g (escarole 45%, red cabbage 25%, spinach sprouts 10%, lollo rosso lettuce 10%, and rocket 10%), Capriccio salad 100 g (green lettuce sprouts 40%, red lettuce sprouts 40%, and rocket 20%) and Gourmet salad mix 350 g (escarole 60%, red chicory leaves 20%, and lamb's lettuce 20%).

The antioxidant capacity of these salads was estimated for the total extract (hydrophilic and lipophilic) by the TEAC and TPC methods, which presented the best correlation with the Tabart Index ($r^2 = 0.98084$ TEAC-Tabart and $r^2 = 0.98092$ TPC-Tabart).

The TEAC values were 191 µmol TE/100 g FW for the Vital salad, 60 µmol TE/100 g FW for Capriccio, and 262 µmol TE/100 g FW for the Gourmet salad mix. The TPC values were 33.5, 16.8, and 47.8 mg GAE/100g FW for Vital salad, Capriccio salad, and Gourmet salad mix, respectively.

When half of the ingredients presenting the lowest TEAC and TPC values were replaced by the same amount of moringa leaves, the antioxidant content increased sharply, by 145% to 686% for TEAC and by 128% to 340% for TPC (Figure 3a,b). When the ingredient with the lowest antioxidant content was replaced by MO, the content rose by 1273% for TEAC and by 580% for TPC, in both cases for the Capriccio salad (Figure 3c,d).

The use of the leaves of MO as a food fortificant is common practice in African countries such as Ghana, Nigeria, and Ethiopia [33]. They are also used as a source of antioxidants when added to other foods, like mayonnaise and bulk sunflower oil [34]. Moreover, moringa leaves have good functional properties for use in ready-to-eat food products or snacks, such as ribbon-shaped toasted products [35]. Due to its high oil absorption capacity, raw moringa leaf flour can be used in bakery food formulations, while alkali-pre-treated moringa leaf flour could be more suitable for making low-fat snack products [36].

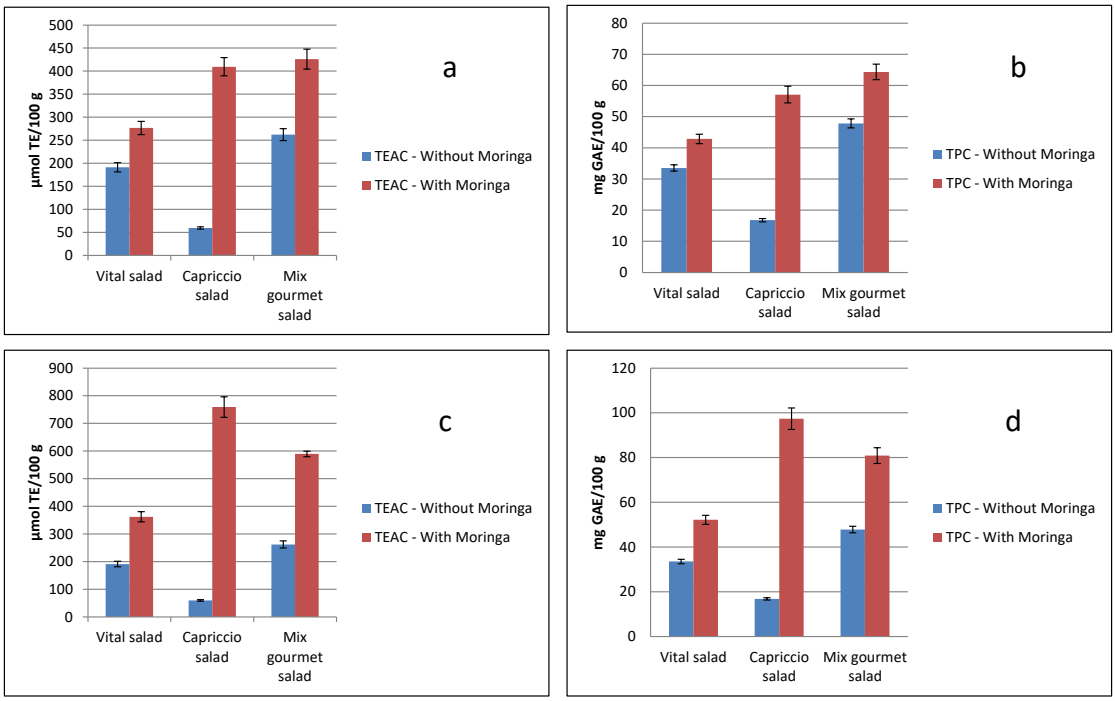


Figure 3. Increase in Trolox equivalent antioxidant capacity (TEAC) and total polyphenol content (TPC) in salad by adding MO (**a**,**b**) or by replacing an ingredient with MO (**c**,**d**).

4.4. Antioxidant Capacity Provided by the Consumption of Vegetables, in the Spanish Diet

In Spain, according to the Ministry of Agriculture, Fisheries and Food [37], the per capita annual consumption of vegetables is 1.35 kg of cabbage and similar, 3.41 kg of lettuce/escarole/endive and similar, 1.24 kg of leafy vegetables, 6.7 kg of fourth-range vegetables, and 10.13 kg of other vegetables.

All the vegetables analyzed in the present study except MO and the rocket salad mix were included in the above four groups. For each group, the TEAC and TPC values and the corresponding percentages were calculated using the data obtained for the methanol extract. As shown in Figure 4, the fourth-range vegetables contributed most to the antioxidant capacity of all vegetables, representing 50% and 63% of the total for TEAC and TPC, respectively.

In view of the high TEAC and TPC values obtained for MO, its use as a fortifier of fourth-range salads might contribute significantly to the antioxidant capacity of the Spanish diet.

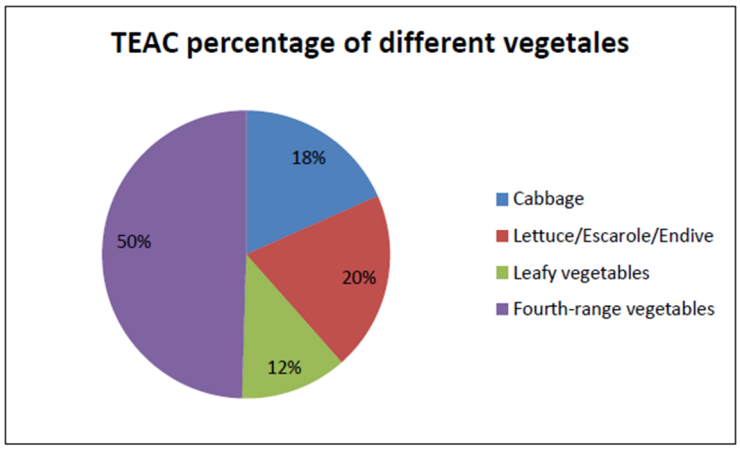


Figure 4. *Cont.*

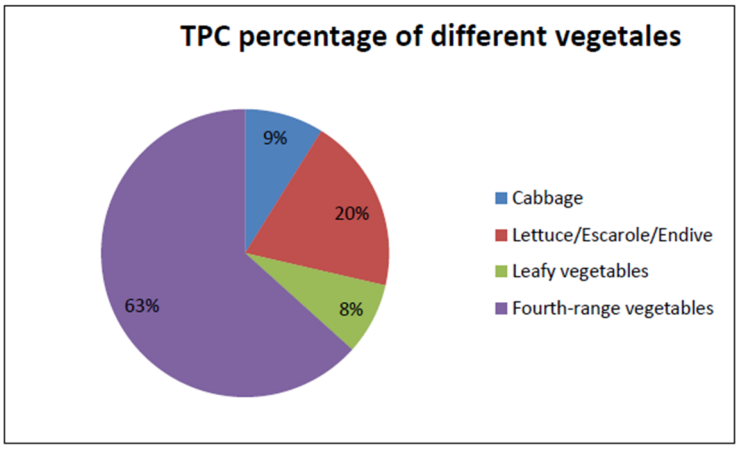


Figure 4. Percentage contribution to TEAC and TPC of the different vegetables consumed in Spain.

5. Conclusions

The antioxidant capacity of the moringa leaves grown in Andalusia (Spain) is much higher than that of the other 28 leafy vegetables analyzed. This difference is apparent in all of the extracts considered and with all of the methods employed. Furthermore, the leaves are rich in total phenolic compounds, carotenoids, total flavonoids, and chlorophylls. In consequence, its inclusion as an ingredient in fourth-range (fresh-cut pre-packaged food) would significantly increase the theoretical antioxidant capacity of this commercial product, which currently provides over 50% of the antioxidant capacity of all vegetables consumed in Spain.

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