

Seed Coating Increase Broccoli Nutrient Content and Availability after Cooking

Micaela Carvajal¹, M. Carmen Martinez-Ballesta¹, Diego A. Moreno², Javier Bernabeu³
& Cristina García-Viguera²

¹ Plant Nutrition Department, CEBAS-CSIC, Espinardo, Murcia, Spain

² Food Science Technology Department, CEBAS-CSIC, Espinardo, Murcia, Spain

³ Sakata Seed Iberica, Pl. Poeta Vicenta Gaos, Valencia, Spain

Correspondence: Micaela Carvajal, Plant Nutrition Department, CEBAS-CSIC, Espinardo, 30100 Murcia, Spain.
E-mail: mcarvaja@cebas.csic.es

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Abstract

Broccoli is a rich source of minerals which play an important role in health. From farm to food there are many processes that affect the mineral nutritional quality. As the quality of the seeds is the basis for the successful production, the protection of seeds by coating is studied to improve the seed quality and maintenance during storage. Also, the studies about the implication in the development of the plants are carried out. For that, the influence of seed capsulation on yield, mineral content, CO₂ fixation and gas exchange was determined. The results showed that capsulation treatment improves germination and vegetative growth and production. This may be due to greater absorption of N and Fe and higher C fixation. When inflorescences were cooked, there was a greater loss of mineral nutrients with high pressure and microwave, and lower loss with steaming.

Keywords: broccoli, cooking methods, CO₂ fixation, human health, mineral nutrition, seeds coating

1. Introduction

Nowadays one of the most important challenges for the agriculture is to enhance the food production and providing almost all the essential minerals and organic nutrients to humans in order to maintain health and organ functions. Humans need more than 22 mineral elements, some of them are required in large amounts, but others, such as Fe, Zn, Cu, I and Se, are required in trace amounts because higher concentrations can be harmful (Welch & Graham, 2004; Grusak & Cakmak, 2005). Although vegetables constitute the main source of minerals for the human diet, vegetables do not always contain enough amounts of these essential nutrients to meet dietary requirements (Welch et al., 1997).

Broccoli, contains high amounts of bioactive compounds and vitamins but also high content of minerals that can be an important supplement in nutrient-incomplete diets (Agte et al., 2000). The main mineral elements (Na, K, Ca, Mg, Cl, S, N and P) present in broccoli are essential for human beings in amounts > 50 mg/day, while trace elements (Fe, Zn, Cu, Mn, I, F, Se, Cr, Mo, Co, Ni) are essential in concentrations of < 50 mg day⁻¹. These main and trace elements have very varied functions, e.g., as electrolytes, as enzymes constituents and as building materials, e.g. in bones and teeth.

Magnesium (Mg) acts as a Ca antagonist on vascular smooth muscle tone and on post-receptor insulin signaling and it has been related with muscles and nerves excitability (Huskisson et al., 2007). Potassium (K) and sodium (Na) play important role in the maintenance of the balance of physical fluids system (Sobotka et al., 2008). The major function for iron (Fe) is related to the synthesis of haemoglobin and myoglobin. Therefore, a severe iron deficiency results in hypochromic anaemia (Huskisson et al., 2007). Copper (Cu) is necessary for the development of connective tissue and nerve coverings (myelin sheath) and also participates in the Fe metabolism (Huskisson et al., 2007; Shenkin, 2008). The main physiological function of manganese (Mn) is related with metabolism of carbohydrates and gluconeogenesis (Shenkin, 2008), while zinc (Zn) is required for glucose use and insulin secretion (Lukaski, 2004).

The level of minerals in vegetables depends on a number of factors including genetic properties of the crop species, climatic conditions, soils characteristics and the degree of maturity of plant at the moment of harvesting.

New technologies, like the use of coating seed technology with soluble polymers is starting to be proposed due to the demand of improving the interaction soil-plant system. This technology has been implemented to be used as antibacterial or antifungal protection against diseases (Someya et al., 2007). However, the coating itself could affect the efficiency of the germination and the primary developing process (Almeida & Rocha, 2008) with later effect on adult plant.

Broccoli, as other *cole crops*, is rich in the previously mentioned minerals anions and cations, but the effect of capsulation of seed on the mineral composition of this vegetable has not been studied neither the effect of cooking on the final mineral concentration in food. The mineral content might fluctuate depending on genetic factors, climatic conditions, agricultural procedures, composition of the soil, etc, but also changes occur in the processing of the raw material. In this aspect, broccoli is frequently consumed as a cooked vegetable and some previous studies with other phytochemicals have demonstrated that domestic procedures (cooking, storage, etc.) play an important role in final food quality (Vallejo et al., 2002, 2003a, 2003b).

Therefore, the aim of this study is determining the growth, carbon fixation, mineral composition and gas exchange parameters of broccoli grown from coating seeds, and the effect of different domestic cooking procedures (steaming, high pressure boiling, and microwaving) on mineral content.

2. Materials and Methods

2.1 Plant Material and Growth Conditions

The experiment was carried out with *Brassica oleraceavar. italica*, cv Parthenon seedlings obtained from a commercial nursery for horticultural plants in Murcia (Spain). All the seeds were commercial and provided by SAKATA. The experiment was conducted during the spring period in soil of the CEBAS-CSIC “La Matanza” Experimental Farm (Santomera, Murcia, SE Spain), under a semi-arid Mediterranean climate.

Daily mean temperature and relative humidity were recorded every 10 min using dataloggers (AFORA SA, Barloworld Scientific, Murcia, Spain). The humidity was 50/80% (day/night) and the temperature varied from 21/9 °C (day/night). A total of 360 plants, 180 per treatment (control and capsule) were planted in a 300 m² plot with randomized block design. All plants were drip irrigated with a diluted (1:4) Hoagland nutrient solution (2 dS m⁻¹): KNO₃ (6 mmol L⁻¹), Ca(NO₃)₂ (4 mmol L⁻¹), NH₄H₂PO₄ (1 mmol L⁻¹), MgSO₄ (1 mmol L⁻¹), KCl (50 μmol L⁻¹), H₃BO₃ (25 μmol L⁻¹), MnSO₄ (2 μmol L⁻¹), ZnSO₄ (2 μmol L⁻¹), CuSO₄ (0.5 μmol L⁻¹), (NH₄)₂MoO₄ (0.5 μmol L⁻¹) and Fe-EDDHA (20 μmol L⁻¹).

The plants were harvested 90 days after transplanting the seedlings. The inflorescences were separated from the rest of the plant, which was divided into leaves, stems, and roots and weighed.

2.2 Mineral Elements

The concentrations of Ca, Mg, K and Na were analyzed on samples of plant material which had been ground finely in a mill grinder after drying at 65 °C to constant weight. The samples were digested in a microwave (CEM Mars Xpress, North Carolina, USA), reaching 200 °C in 20 min and holding at this temperature for 2 h, using 5ml of concentrated HNO₃, 17ml of H₂O, and 3 ml of H₂O₂. The concentrations of the elements were measured by inductively coupled plasma spectrometry (Iris Intrepid II, Thermo Scientific, Waltham, MA, USA). The solution to analyse is conducted by a peristaltic pump through a nebulizer into a spray chamber, leading into argon plasma. As a consequence an alternate magnetic field is induced that accelerate electrons into a circular trajectory. Due to collision between the argon atom and the electrons ionisation occurs, giving rise to stable plasma. Therefore, atomisation and ionisation of the sample takes place. Due to the thermic energy taken up by the electrons, they reach a higher excited state that is liberated when the electrons drop back to ground level energy. Each element has an own characteristic emission spectrum. The emitted photons in different frequencies are captured simultaneously on a CCD chip (Charged Coupled Device).

2.3 Carbon Analysis

The plants were dried into an oven at 65 °C until constant weight, to determine the dry weight and to dry the samples of the different plant parts before grinding them in a laboratory analytical mill (IKA A10 model).

The total carbon contents were analyzed in leaves, stems, fruits, and roots using a CN analyzer (Thermo-Finnigan 1112 EA elemental analyzer; Thermo-Finnigan, Milan, Italy). Oxygen combusts solid samples when ignited using helium as carrier. The first furnace, set to 950 °C, holds a chromium(III) oxide column which oxidizes carbon into carbon dioxide. The carbon dioxide gas flows through a magnesium perchlorate column which removes water before the gas enter the gas chromatograph column at 50 °C to be separated by retention rates. The gases are sensed by the thermal conductivity detector (TCD). The curves are integrated and used to

calculate the weight percent of carbon in the sample.

The results % C data were used for calculating g of CO₂ fixed per plant. For calculation of g of CO₂ fixed per square meter of each crop, plant distance (P.D.) was taken into account (P.D._{broccoli} = 3.5 plants m⁻²).

2.4 Leaf Gas Exchange Parameters

Net assimilation of CO₂ (A_{CO₂}) and stomatal conductance (G_s), were measured using a portable photosynthesis system (model LCA-4, ADC Bioscientific Ltd., Hoddesdon, UK) and a PLC-4N leaf chamber (11.35 cm²), configured to an open system. The abaxial stomatal conductance was measured on the most-recent, fully-expanded leaves. The measurements were carried out at 09:00 to 12:00 h, to avoid the high temperatures and low humidity of the afternoon, and only on clear days, to minimize the impact of variations in light intensity.

2.5 Cooking Experiments

For analytical purposes a total of 4 inflorescences were randomly selected, comprising four replicates for each treatment. The edible portions were washed and cut (~3 cm diameter and ~1 cm stalk).

In order to compare the possible influence of mineral composition of tap water different cooking procedures were done with deionised water. Boiling and steaming (S) were applied using a pressure cooker (Rapid-express, Fagor, Guipuzcoa, Spain) containing 50 g of freshly cut raw broccoli and 50 ml of deionised water, using high pressure cooking (HPD). The inflorescences were fully dipped in the water and cooked for 2 min, whilst they were suspended above the boiling water for 2 min for steaming. A microwave oven (Samsung, Cleveland, UK) operating at full power (1000 W) for 2 min was used for microwaving (MW), with the same amount of broccoli and cooking water as above. Prior to the cooking processes, time/pressure relationships were established as in Vallejo et al. (2003a, 2003b).

2.6 Mineral Content in Cooked Inflorescences

Cooked samples were dried at 65 °C for 5 days and ground. Digestions and measurements were done as for fresh material

2.7 Statistical Analysis

Data were statistically analyzed using the SPSS 7.5 software package, by analysis of variance (ANOVA) and by Tukey's Multiple Range Significant differences were determined at $p < 0.05$.

3. Results

The germination rate was recorded daily and it was similar in both treatments control and capsulated (data not shown). However, the final level of germination after 15 days reached the 84% in control while in capsulated was 90%.

The growth expressed in fresh and dry weight (Table 1) was higher in plants from capsulated seeds. The increase in both fresh and dry weight was due to the higher values in leaves and inflorescence. The humidity was higher in stems, leaves and inflorescence than in roots, but there were no significant differences in % humidity between control and capsulated.

Table 1. Results of fresh and dry weight, % humidity and CO₂ fixation of broccoli, cv Parthenon grown from control and capsulated seeds. Data are mean (n = 10) ± SE

Control	Fresh Weigh (g plant ⁻¹)	Dry Weigh (g plant ⁻¹)	Humidity (%)	% C	Total C (g plant ⁻¹)	Total CO ₂ Fixation (g plant ⁻¹)
<i>Plant (Inputs)</i>						
Roots	457.0 ±26.3	85.4±5.3	81.3±6.2	41.5±1.6	35.4±2.6	129.9±8.3
Stems	600.9±35.9	63.0±7.5	89.5±4.3	41.5±1.5	26.1±1.7	95.9±5.6
Leaves	207.9±14.8	22.1±1.5	89.4±2.5	42.0±2.2	9.3±0.3	34.1±2.7
Inflorescence	229.4±12.4	24.6±1.6	89.3±3.8	42.7±2.0	10.5±0.5	38.5±2.5
Total	1495.1±48.6	195.1±13.4			81.4±7.6	298.3±20.7
Capsulated	Fresh Weigh (g plant ⁻¹)	Dry Weigh (g plant ⁻¹)	Humidity (%)	% C	Total C (g plant ⁻¹)	Total CO ₂ Fixation (g plant ⁻¹)
<i>Plant (Inputs)</i>						
Roots	460.0±28.6	86.4±6.1	81.2±4.2	42.5±2.2	36.7±1.7	134.6±2.5
Stems	608.7±30.2	62.3±2.5	89.1±3.8	42.6±2.0	26.8±1.3	98.4±5.6
Leaves	231.4±15.9	24.5±0.8	88.9±5.3	44.5±1.5	10.9±0.3	40.0±1.3
Inflorescence	278.6±8.6	29.1±1.3	89.6±2.7	45.1±1.3	13.1±0.4	48.0±2.0
Total	1570.9±56.2	202.9±12.7			87.6±2.6	321.0±16.7

The % C (Table 1) was very stable comparing each organ of the plant in control treatment. However, in plants from capsulated seeds, there were an increase in leaves and inflorescence in comparison to roots and stems. Therefore, when total carbon or total CO₂ fixation was calculated, a significant increase was observed in capsulated due to the increase in both growth and C composition in leaves and inflorescence comparing to no capsulated plants.

The gas exchange was measured at sunrise and midday (Figure 1). The net assimilation of CO₂ (A_{CO₂}) and stomatal conductance (G_s) showed only slight differences between the two times of measurement. However, both parameters were higher in capsulated plants than in control.

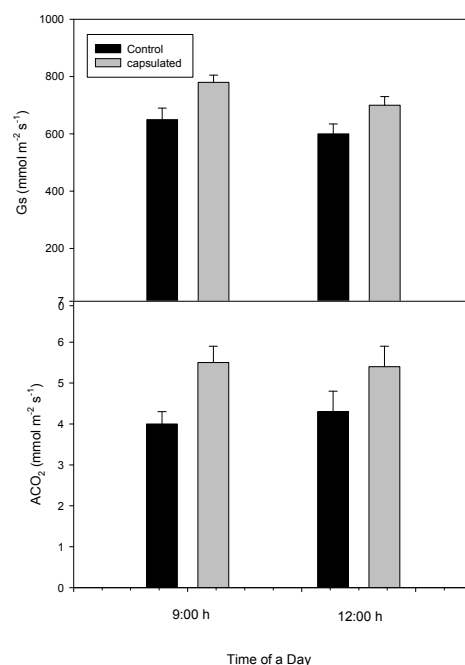


Figure 1. Net assimilation of CO₂ (A_{CO₂}) and stomatal conductance (G_s) in of broccoli, cv Parthenon grown from control and capsulated seeds. Data are means of five plants ± SE

The yield (Figure 2) was measured as number of inflorescence by harvesting date (Figure 2a) and as the weight of the inflorescences (Figure 2b). As it can be observed in Figure 2a, the development of the inflorescences from control plants was earlier than from capsulated during the whole harvesting period. When the total weight of the inflorescences harvested by date was recorded (Figure 2b), it can be observed a slight increase of control inflorescences during the first dates but a higher increase in capsulated inflorescences at the final dates. All that gave a higher final weight for capsulated.

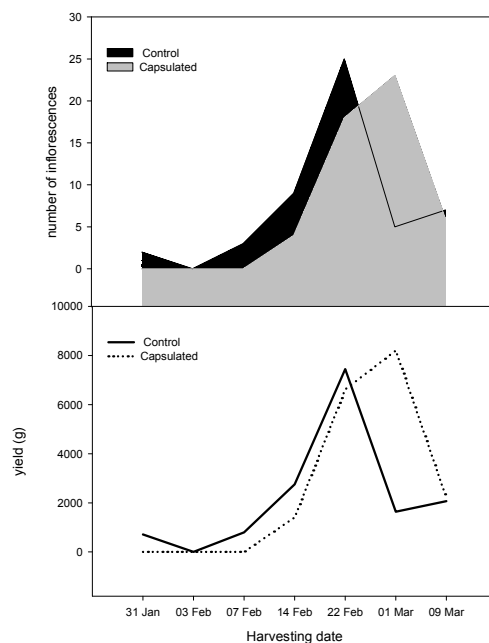


Figure 2. Number of inflorescences and total yield (g) of broccoli, cv Parthenon grown from control and capsulated seeds. Data are obtained from 51 plants

When the mineral composition of the inflorescences was determined (Table 2), it can be observed changes only in some of the elements: N, C and Ca (macroelements), Fe and Zn (microelements). In general, except for the Ca, the capsulation of the seeds produced an increase of the referred elements, with no different changes for the rest (Na, K, Mg, Cu and Mn).

Table 2. Mineral composition of inflorescence (d.w) control and from plants grown from capsulated seeds

	Control	Capsulated
N (g/100g)	1.71 ± 0.11*	2.54 ± 0.14*
C (g/100g)	42.78 ± 0.61*	45.07 ± 0.34*
Na (g/100g)	0.05 ± 0.001	0.06 ± 0.002
K (g/100g)	1.87 ± 0.25	1.95 ± 0.30
Ca (g/100g)	2.93 ± 1.01*	1.88 ± 0.20*
Mg (g/100g)	0.13 ± 0.01	0.11 ± 0.02
Fe (mg/Kg)	24.12 ± 1.51*	33.04 ± 1.65*
Zn (mg/Kg)	12.01 ± 0.12*	23.40 ± 1.62*
Cu (mg/Kg)	1.97 ± 0.23	2.02 ± 0.17
Mn (mg/Kg)	104.26 ± 13.88	92.42 ± 19.11

Only the results with significant changes are shown. Data are mean (n=10) ± SE. Values with * differ significantly according to Tukey's test (p = 0.05).

In the experiments when broccoli is cooked (Table 3), it can be observed that a daily serving (200 g) of this vegetable (raw) could provide the total requirement of K (requirement 782 mg day⁻¹), over 80% of Mn (requirement 2-5 mg day⁻¹), between 15% and 30% of daily requirements of Ca (requirement 800-1500 mg day⁻¹), Mg (requirement 300-400 mg day⁻¹), Fe (requirement 15 mg day⁻¹), and Cu (requirement 1-1.5 mg day⁻¹), about 10-15% of Zn and less than 3% of Na (Belizt et al., 2004). As broccoli is eaten cooked, these percentages would vary when the vegetable is cooked under different conditions. Due to the less availability of Ca in plants from capsulated seeds and higher availability of Fe and Zn, the % of the requirements per day were altered. The analysis of the main mineral cations in cooked samples, Na, K, Ca and Mg (Table 3) showed a marked decrease in those broccoli samples cooked under high pressure boiling procedure (around 20%) and lower decrease in samples cooked with microwave (around 12%). There was an exception with Na content, which decrease was similar in all the boiling procedure. Steaming cooking produced no significant differences with control with only a slight decrease in Ca content.

Table 3. Mineral content in 200 g of fresh broccoli (Con = Control and Cap = capsulated in those minerals that showed difference. In the rest, an average is shown) with the percentage of retention after each type of cooking

		Precooking (Con/Cap)	High pressure (Con/Cap)	Vapour (Con/Cap)	MicroW (Con/Cap)
Na	% retention	100	78	100	75
	% RDA	2.7	2.1	2.7	2.2
K	% retention	100	76	104	86
	% RDA	115	88	119	98
Ca	% retention	100	78	92	88
	% RDA	21/13	16/10	19/12	19/12
Mg	% retention	100	82	100	88
	% RDA	22	18	22	19
Fe	% retention	100	63	97	97
	% RDA	23/31	15/21	23/31	23/31
Zn	% retention	100	77	92	85
	% RDA	16/31	12/23	15/29	14/27
Cu	% retention	100	70	45	76
	% RDA	33	23	15	25
Mn	% retention	100	56	72	72
	% RDA	90	50	65	65

The values are also expressed as the 'Recommended Daily Allowance', RDA (Belizt et al., 2004).

Concerning trace cation Fe, Zn, Cu and Mn (Table 3), a high decrease was observed in the case of Fe for pressure cooking but no changes were observed in steaming and microwave. Zn decreased for all cooking methods ranking from pressure to microwave to steaming with the lower lost. A general decrease was observed for Cu. It is very interesting that the highest decrease resulted in the steamcooking (55%). However, there was a high reduction in Mn content with high pressure 44% while the decrease was less (28%) for steaming and microwave.

4. Discussion

Seed coating or capsulation refers to application of layers of solid or semisolid compounds covering the natural seed coat. It usually includes pelting and many seed treatments that adhere around the seed. They are generally used for improve the seed-soil interchange and it usually affect the physiology of the adult plant (Scott, 1989). The effect of the seed coating or capsulation depends on the material used and the germination conditions: soil, moisture, and nutrients (Halmer, 2008). In our experiments, the capsule material (peptides) could slow the speed of hydration or simply protect the seed in the early germination process having a potential effect on metabolism

and then yield (Figure 2). This could be a similar effect than priming or seed invigoration with the related growth and yield enhancement reported (Farooq et al., 2012). However, the exact mechanism for improving growth and yield by the capsulation should be investigated.

The percentage of carbon content in broccoli plants showed a significant increase in leaves, inflorescence and whole plants from capsulated seeds (Table 1). These results together with the high growth, provided a high capacity for carbon fixation in plants from capsulated seed. Obviously, when plant CO₂ fixation (determined per plant) was studied, there was an increase in capsulated due to the higher biomass (Mota-Cadenas et al., 2010). Therefore, besides the higher growth and yield production per plant that capsulated provided, the ecological benefits of a sustainable agriculture is an important factor. The fact that every plant population can sequester CO₂ and thus counteract the Greenhouse Effect is becoming an aspect to consider (Geissler et al., 2009).

The rate of photosynthetic CO₂ assimilation is generally related to C content (Brugnoli & Lauteri, 1990). The results obtained in our plants showed lower values of ACO₂ and G_s in control plants compared with capsulated. The photosynthetic CO₂ assimilation has been related to CO₂ diffusion to the chloroplasts (Yang & Lu, 2005; Wilson et al., 2006). In this sense, the changes observed in ACO₂ have been reported to be due partly to alterations of G_s and the consequent changes of the availability of CO₂ for carboxylation (Flexas et al., 2008). The fact that in our plants there were a consistent relationship between ACO₂ and G_s, could be related to the fact that stomatal opening is dominating in partial CO₂ pressure in the leaves and inducing higher assimilation of more CO₂. Thereby this could make the C signatures of the newly-formed plant tissue higher (Groenigen & Van Kessel, 2002).

Different *Brassica* vegetables such as cauliflower (*B. oleracea* var. *botrytis*), bok choy (*B. rapa*), broccoli (*B. oleracea* var. *italica*) and kale (*B. oleracea* var. *acephala*) are reported to have a high mineral content (Puupponen-Pimia et al., 2003). In general, *Brassica* plants have been found to be rich in many minerals including calcium and iron (Miyazawa et al., 2005). When the results obtained in the present work (Tables 2 and 3) are compared with those reported for different fresh vegetables, such as watercress, endive, peas, kale, spinach, carrots, potatoes, cabbage, cauliflower, Brussels sprouts, Chinese cabbage, white cabbage, etc (Osler et al., 2001; Kawashima & Valente-Soares, 2003; Belizt et al., 2004; Koplik et al., 2004; Kmiecik et al., 2007), it could be considered that broccoli is a very good source of these essential minerals for humans beings.

Our results indicated that the studied broccoli presented a relatively high level of calcium (2.93 g 100 g⁻¹), higher than those given for other popular vegetables such as chicory (0.50 g 100 g⁻¹), green peas (0.43 g 100 g⁻¹), beans (0.37 g 100 g⁻¹), cabbage (0.44 g 100 g⁻¹) or Brussels sprouts (0.36 g 100 g⁻¹) (Kawashima & Valente-Soares, 2003; Rickman et al., 2007). Even after capsulation, were slight decrease is observed, broccoli can be regarded as a good source of available calcium and their consumption in the diet may contribute to improve calcium nutrition (Lucarini et al., 1999).

In relation to other minor cations (Table 2), our results revealed high amounts of Fe (24.12 mg Kg⁻¹), Zn (12.01 mg Kg⁻¹), Mn (104.26 mg Kg⁻¹) and Cu (1.97 mg Kg⁻¹) in comparison to other vegetable sources as kale or cauliflower that have up to three times less amounts of these minerals (Kawashima & Valente-Soares, 2003; Kmiecik et al., 2007). It is remarkable that the concentration obtained for Fe was higher than in lentils, a traditional vegetable source for iron nutrition. In fact, in this study iron levels were two times higher than those found in some lentils varieties (Wang et al., 2009). Furthermore, in our results can be observed that concentration of Fe and Zn are significantly higher in inflorescences from capsulated than from control.

In our results, a general decrease of mineral content was observed when broccoli samples were cooked under high pressure. Similar results were showed by Kmiecik et al. (2007) in broccoli and cauliflower. Moreover, also a slight decrease of mineral content occurred when broccoli samples were cooked in a microwave oven showing a very similar behaviour than that obtained by López-Berenguer et al. (2007). In general, Na, K, Ca, Mg and Zn showed a small percentage of losses. Iron losses depended of the cooking process and Cu and Mn showed lower values of these minerals after any type of cooking. On the other hand, steaming was the treatment that maintained the higher values in mineral content in almost all cases followed by microwaving. However, high pressure showed the lower mineral content for almost all the minerals analysed after cooking. All these results indicate that the retention of minerals in broccoli florets were higher in steaming than in microwave and high-pressure.

The results showed that encapsulation of seed improve germination and vegetative growth and final production. This may be due to greater absorption of N and Fe and higher fixation C that could be conferred to plant in the very early stage of germination. The higher C plant concentration in capsulated plants was related to high ACO₂ and high G_s according to an increase in gas diffusion through stomata. In other side, broccoli has been revealed

as a good source of the main mineral elements, such as Na, K, Ca, and Mg, and trace elements, such as Fe, Zn, Mn and Cu, essential for human's beings. Significant higher amounts of Fe and Zn have been observed in inflorescences of plants coming from capsulated seed with the increase in the nutritional value. Cooking method have a high influence in retaining or losing cations showing that streaming give better results in retention than microwave and high pressure probably due to maintenance of tissue structures.

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