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INCREASING ANTIOXIDANT CONTENT OF BROCCOLI SPROUTS USING ESSENTIAL OILS DURING COLD STORAGE

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Broccoli sprouts are natural functional foods for cancer prevention because of their high content of glucosinolate and antioxidant. Sprouts and mature broccoli are of potential importance in devising chemoprotective strategies in humans.The aim of the investigation was to study the effect of essential oils on broccoli seed germination, increase their antioxidant content and determine the glucosinolate concentration and other phytochemical parameters in 3-day -old sprouts during cold storage at 4°C and 95% RH for 15 days. The results showed that all treatments of essential oils increased germination index, seed germination percentage, seedling length, seedling vigour index, yield and the antioxidant content of broccoli sprout and reduced the microbial load compared to the control. Fortunately, the coliform bacteria was not detected in all treatments. Different essential oils of fennel, caraway, basil, thyme and sage were tested. The thyme oil was the best treatment, which increased the accumulation of the phenolic compounds and glucosinolate compared to the control at different storage periods. In the sprouts treated with thyme oil treatment and the control, at the end of cold storage, 1.98% and 28.06% of total phenolic content, 1.90% and 20.28% of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity, 1.39% and 58.33% of flavonoids, 1.93% and 36.25% of vitamin C, 2.95% and 22.02% of anthocyanin and 2.18% and 49.12% of glucosinolate were lost, respectively. A slight reduction differences in all detected compound concentrations occurred between the initial content and the end of storage period because of the application of thyme oil compared to the control. Therefore, the total glucosinolate level in the sprout (27.02 µg/g F.W.) was higher than that in the florets (7.37 µg/g F.W.). Glucoraphanin was the most abundant aliphatic glucosinolate present in the sprout and reached the highest value (16.24 µg/g F.W.) followed by glucoerucin (5.9 µg/g F.W.) and glucoiberm(1.2 µg/g F.W.).

Key words: broccoli, sprout, antioxidant, polyphonic compounds, flavonoids, essential oils

Broccoli sprouts are considered as a functional food. Essential nutrient content provides diverse secondary metabolites and phytochemicals (Villarreal -García *et al*. 2016). The phenolic compounds, especially flavonoids and anthocyanin, show a great ability to capture free radical that lead to oxidative

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stress; therefore, these compounds are attributed a beneficial effect in the prevention of cardiovascular diseases, circulatory problems, neurological disorders and cancer (Baenas *et al*. 2014). Broccoli has been identified as a vegetable with potential anticancer activity because of high levels of glucosinolates that are divided into three major categories: aliphatic, indole and aromatic glucosinolates (Yan & Chen 2007). The high content of the aliphatic glucosinolates in broccoli sprouts is mainly attributed to glucoraphanin. The degradation products of aliphatic glucosinolates are considered to have the higher phase 2 detoxification enzyme inducer ability than the other two groups, which is effective in blocking chemical carcinogenesis; therefore, they are thought to be the main contributor to the protection against carcinogenesis. The glucoraphanin can be hydrolysed tosulphoraphane (Figure 1). Sulphoraphane is a naturally occurring isothiocyanate in cruciferous vegetables and has a high capacity to induce phase 2 enzymes (López-Cervantes *et al*. 2013). This compound plays an important role in controlling, preventing or blocking any of the multiple stages of the carcinogenic process (Parnaud *et al*. 2004). Moreover, broccoli sprouts have 20–50 times higher concentration of glucoraphaninand 30–50 times higher concentration of sulphoraphane when compared to mature plants. Level of myrosinaseenzyme, which protects against carcinogens, in broccoli sprouts is 10–100 times higher than that in mature broccoli heads (Fahey *et al*. 1997). Use of sprouts in the daily nutrition maintains the nutritional value, protects from diseases and eliminates spoilage and pathogenic bacteria.

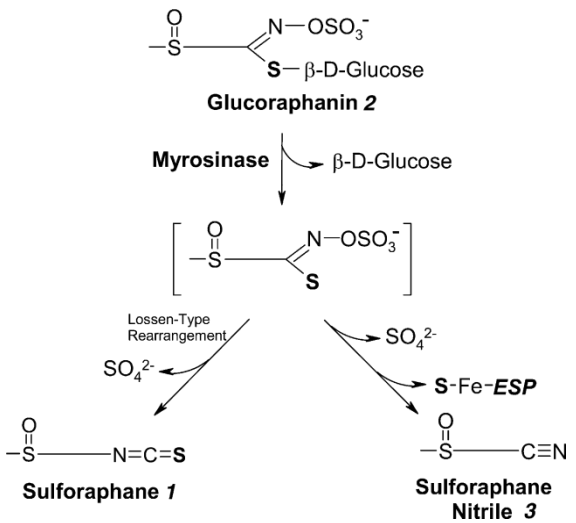

Figure 1. Formation mechanism of sulphoraphane and sulphoraphane nitrile in broccoli sprouts (Matusheski *et al*. 2004)

The use of treatments with essential oil rich in antioxidant to stimulate broccoli seed germination should be considered. Application of 4% thyme and basil oils reduced the pathogenic fungi from seed to seedling and had a positive effect on the seed germination of infected seeds (Nguefack *et al*. 2005). Aromatic plants, especially essential oils, are well known for their antioxidant and antimicrobial properties that prevent food degradation and alteration (Justesen & Knuthsen 2001), as they are rich in phenolic substances, usually referred to as polyphenols, which are ubiquitous components of plants and herbs.

Campas-Baypoli *et al*. (2010) indicate that 30 g of broccoli sprouts may have the amount of antioxidant present in 600 g of mature broccoli. Furthermore, broccoli sprouts are sold in sealed plastic containers and destined to be stored in domestic refrigerators for consumption. Although the concentration of bioactive compounds may be high at the time of harvest, there is no accurate data that document the stability of these phytochemicals during the storage (West *et al*. 2004). Several researches have shown that the temperature and storage time are the main factors responsible for the changes in the concentration of diverse compounds, such as glucosinolates (Fiskaa *et al*. 2009). Therefore, it is necessary to know the stability of glucosinolates in the food matrix under refrigeration storage.

The aim of this investigation was to use the essential oils during cold storage to produce healthy broccoli sprout with high nutritive values, quality and antioxidants content with low microbial load. After choosing the best oil treatment, the glucosinolate concentration, total phenolic content, flavonoids, vitamin C and DPPH radical scavenging capacity content in sprouts were determined during cold storage.

MATERIAL AND METHODS

1. Plant material and germination condition

The study was conducted in the Laboratory of Mansoura Horticultural Research Station, Horti-

culture Research Institute, Egypt, during the period from 2012 to 2014 seasons. Broccoli seeds (*Brassica oleracea* L. var. *italica* and the variety name is F1 Hybrid Sakura) from Tokita Seeds CO., LTD (Saitama, Japan) was used. Germination of broccoli seeds was carried out according to Pérez-Balibrea *et al*. (2011). The seeds (1,000 seeds, nearly 5 g) for each treatment were soaked in 0.5% v/v sodium hypochlorite solution for 15 min, then immersed in 50 ml of distilled water for 5½ hours with shaking for every 30 min and washed with distilled and sterilised water. On September 15, the seeds were sown broadcast over absorbent medical cotton in sprouting plastic containers (220 mm × 110 mm). The cotton was treated with emulsion of different natural essential oils at 0.05% concentration and emulsified using 1.5 ml/l of Tween 80, and the containers were closed immediately. Germination conditions were maintained at 25±2°C with a cycle of 16 h light and 8 h darkness, a relative humidity (RH) between 80% and 90% and light intensity of 7.4 lmol/m^2/s. After 3 days, sprouts were cut from their root mats, and 20 g of sprouts from each was weighed and placed in a container. The container was stored and placed at 4°C and 95% RH in the dark simulating a domestic refrigerator for 15 days for the best treatment and control.

2. Characterisation of essential oils

The major compounds of essential oil of caraway, *Carum carvi,* were carvone (57%) and limonene (30%). The other compounds were oleic acid (C 18:1), which accounted for 52.28%, and linoleic acid (C 18:2), which accounted for 30.84%. *Carum carvi* essential oil was characterised by the predominance of oxygenated monoterpenes (79.79%). In essential oil of fennel, *Foeniculum vulgare*, the most abundant component was trans-anethole (83.80%), then limonene (9.34%), fenchone (4.84%) and methyl chavicol (1.36%). The other compounds were monoterpenes (15.04%) and phenylpropanoids (84.79%). According to the results of the chemical analysis of thyme essential oil, *Thymus vulgaris*, 25 compounds were identified, which represented 94.53% of the oil content. The highest percentage of compounds includes three classes: monoterpene hydrocarbons, aromatic hydrocarbons and oxidised monoterpenes. More than half of the total compounds of thyme essential oil include six dominant compounds. Amongst them, the most dominant is the oxidised monoterpene thymol 40.12% and the monoterpene hydrocarbon p-cymene (21.15%). Other dominant components of this essential oil are the following: carvacrol (14.34%) and linalool (4.54%). The major constituents detected in our samples of basil, *Ocimum basilicum*, include eugenol (39.51%), linalool (27.24%), 1,8 cineole (17.88%) and ß-bisabolene (15.37%). The dominant constituent in essential oils of sage, *Salvia officinalis*, is thujone (41.33%). 1,8 cineole is also present in high amount (39.5%).

3. Essential oils extraction methods

Seeds of fennel, caraway and herbs of basil, thyme and sage (200 g from each one) were used for oil extraction by hydro-distillation for 2–3 h according to Charles and Simon (1990). After extraction, essential oils were separated and their basic constituents were identified using gas liquid chromatography (GLC).

4. Experimental design for essential oils treatments

A completely randomised design with three replicates was used. The experiment included seven treatments of the above-mentioned essential oils in addition to hot water (36°C) treatment and tap water as a positive control.

Mature winter grown broccoli was sown on 18 November in both seasons and was harvested by hand on 28 January from the field of the Baramoon Experimental Farm. The variety used was the same as mentioned previously. The other agricultural practices were carried out as commonly followed in the district. Broccoli was harvested when the diameter of floret was about 0.35–0.40 cm. On 26 January, the germination of broccoli seeds has been done in incubator chamber at 20°C and 60% RH for 3 days. After germination, the samples of sprout were taken for chemical analysis to compare with samples of florets.

5. Recorded data

Vegetative characters of broccoli sprout

Germination [%] = Total number of normal seedlings / Total number of seeds (1)

Germination index (GI) was calculated according to the following formula:

$$GI = \Sigma T_i \times N_i/S \quad (2)$$

where: T_i is the number of days after planting, N_i is the number of seeds germinated on day I and S is the total number of planted seeds.

Seedling vigour index = Germination [%] ×Seedling length [cm] (3)

Germination index and seedling vigour index were calculated by the above equation of International Seed Testing Association (2010). At the end of germination (3 days), 10 seedlings from each treatment were taken randomly for the determination of sprout length [cm] and fresh weight of sprout [g].

Methanolic extracts for phytochemical determination

Extraction was performed under dark conditions using the solvent methanol/water at a ratio 8:2; specifically weighed 0.3 g of fresh broccoli sprouts were added to 5 ml of 80% v/v methanol, homogenised for 30 s, vortexed and sonicated for 5 min. The mixture was filtered using Whatman no. 41 paper (Du *et al*. 2009).

Total phenolic and total flavonoid contents measurements

Total phenolic content was determined using the Folin–Ciocalteu method. In an Eppendorf tube, 7.9 ml of distilled water, 100 µl of broccoli sprout extract and 500 µl of Folin–Ciocalteu reagent (1:1 with water) were added and mixed. Exactly after 1 min, 1,500 µl of sodium carbonate (20 g/100 ml) was added and the mixture was mixed and allowed to stand at room temperature in the dark for 2 h. The absorbance was read at 765 nm by spectrophotometer. Gallic acid was used for calibration curve. Results were expressed as mg GAE/100 g F.W. (Du *et al*. 2009).

In order to determine the total flavonoid content, 150 µl of broccoli sprout extract, 1,700 µl of 30% ethanol, 150 µl of 0.5 mol/l $NaNO_2$ and 150 µl of 0.3 mol/l $AlCl_3.6H_2O$ were added and mixed. After 5 min, 1 ml of 1 mol/l NaOH was added, and the mixture was measured spectrophotometrically at 506 nm. Results were expressed as mg/100 g F.W. of flavonoids content in sprouts (Du *et al*. 2009).

Vitamin C and Anthocyanin

Vitamin C was determined by titration of fruit juices with indophenol method and indicated as mg ascorbic acid per 100 ml (AOAC 2000).

Total anthocyanin content was measured using a spectrophotometric differential pH method following the procedure of Yuan *et al*. (2010). Frozen samples (100 mg) were crushed into powder and extracted separately with 2 ml of pH 1.0 buffer containing 50 mM KCl and 150 mM HCl as well as 2 ml of pH 4.5 buffer containing 400 mM sodium acetate and 240 mM HCl. The mixtures were centrifuged at 12,000 g for 15 min at 4°C. Supernatants were collected and diluted for direct measurement of absorbance at 510 nm. Total anthocyanin content was calculated using the following equation:

$$\text{Amount (mg/g F.W.)} = (A_{510\,nm} \text{ at pH } 1.0 - A_{510\,nm} \text{ at pH } 4.5) \times 484.8/24.825 \times \text{Dilution factor}$$

The molecular mass of cyanidin-3-glucoside-chloride is 484.8 and its molar absorptivity (e) at 510 nm is 24.825. Each sample was analysed in triplicate, and the results were expressed as the average of ± SD.

Total chlorophyll

Total chlorophyll content of broccoli was determined by using a spectrophotometry (Sabir & Agar 2011). One gram of blended broccoli portions was homogenised with 10 ml of chloroform–methanol mixture (2:1 v/v) for 1 min. Extracts was filtered with filter paper. The residue was resuspended in 10 ml of chloroform–methanol mixture and then filtered. All the filtrates were combined and solutions were supplemented with chloroform–methanol mixture to 25 ml final volume. Total chlorophyll was determined by measuring absorbance of the solution in UV spectrophotometer at 663 and 645 nm against chloroform–methanol blank. The total chlorophyll was estimated by the following formula:

$$\text{Total chlorophyll [(mg 100/g)]} = 8.02 \times (A663) + 20.02 \times (A645)$$

Measurement of DPPH radical scavenging capacity

2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity was determined by Brand-Williams *et al*. (1995). A working solution of 0.1 mM DPPH radical was prepared in 80% methanol,

which shows an absorbance of 1.237 at 515 nm. The DPPH radical scavenging capacity of the sample was expressed as mM Trolox equivalent (TE) per 100 g F.W.

Extraction and Desulphation of Glucosinolates

Desulphoglucosinolate contents were determined according to Kiddle *et al.* (2001). Each sample (20 µl) was analysed on a Merck-Hitachi HPLC system (Merck-Hitachi Ltd., Tokyo, Japan) consisting of a variable UV detector set at 227 nm and a Lichosphere RP-18 column (Merck, Darmstdat, Germany) (RP18, 25 cm × 0.4 cm, 5 µm particle size). The mobile phase was a mixture of water (A) and acetonitrile (B). Desulphoglucosinolates were eluted off the column in 28 min with a linear gradient starting with 1% B and reaching 20% B at 28 min and 90% B at 30 min. The flow rate was 1.5 ml/min. Extraction and desulphation were done according to Vallejo *et al.* (2002).

Microbial evaluation in the stored broccoli sprouts

The pour plate method was used for the enumeration of the microbial community in broccoli sprouts through the 15-day storage period, with 5-day interval. The total bacterial, yeast and fungal counts were determined using the media of Collins and Lyne (1985) and Marshall (1992), after 2, 3 and 5 days of incubation at 30°C. The enumeration of coliform bacteria was performed according to Lorenz *et al*. (1982). The counts of the different groups were expressed as colony forming unit (CFU) per gram of fresh sprouts.

6. Statistical Analysis

Data were analysed using analysis of variance technique, and the differences between individual pairs of treatment means were compared using Duncan's multiple range test at 5% according to Snedecor and Cochran (1989).

RESULTS

1. *Vegetative characters of broccoli sprout*

The treatments with essential oil rich in antioxidant stimulates broccoli seed germination. All different essential oils had significant effects on germination index [%], germination [%], seedling length [cm], seedling vigour index and yield [g] compared with the control during the two seasons (Table 1). The fennel, caraway and thyme oils increased the seed germination index of the seeds by 171.43%, 170.29% and 148.02% (an average of the two seasons), respectively, compared with the control (100%). The increases in seed germination percentage over the control (tap water) reached to 12.73%, 13.74% and 15.82% for the most effec-

Table 1

Vegetative characters of broccoli seeds treated with different essential oils before cold storage

Treatment		Germination index [%]		Seed germination [%]		Seedling length [cm]		Seedling vigour index [cm]		Yield [g] container / 242 cm^2	
		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
1	Water (control)	13.36^{e}	12.96^{d}	86.67^{c}	86.00^{a}	4.67^{c}	4.00^{b}	4.03^{c}	3.44^{c}	36.40^{e}	34.20^{d}
2	Hot water	14.61^{de}	13.02^{d}	93.78^{b}	90.44^{bc}	5.00^{c}	4.80^{b}	4.71^{c}	4.33^{c}	40.88^{de}	37.21^{d}
3	Fennel oil	22.01^{a}	23.01^{a}	97.33^{ab}	97.33^{a}	7.33^{ab}	7.67^{a}	7.13^{ab}	7.47^{ab}	56.90^{b}	49.17^{c}
4	Caraway oil	21.94^{a}	22.88^{a}	97.33^{ab}	99.00^{a}	8.00^{ab}	8.33^{a}	7.79^{a}	8.25^{a}	54.97^{bc}	67.75^{a}
5	Basil oil	20.22^{ab}	21.82^{ab}	94.67^{b}	92.33^{b}	7.00^{b}	7.67^{a}	6.63^{b}	7.07^{b}	64.87^{a}	68.17^{a}
6	Thyme oil	18.81^{bc}	20.14^{b}	100.00^{a}	100.00^{a}	8.20^{a}	8.30^{a}	8.20^{a}	8.30^{a}	66.54^{a}	67.75^{a}
7	Sage oil	16.91^{cd}	17.76^{c}	100.00^{a}	100.00^{a}	7.83^{ab}	7.83^{a}	7.83^{a}	7.83^{ab}	47.83^{cd}	49.17^{c}

Means followed by the same letter (s) within each column do not significantly differ using Duncan's multiple range test at the level of 5%

tive treatments, respectively. Thyme, caraway and fennel oils had significant increases in the seedling vigour index and yield over the control to 50.25%, 73.82% and 90.22%, respectively (means of the two seasons).

2. *Phytochemical characters*

All treatments significantly surpassed over the control in broccoli sprout bio-constituents, that is, total phenolic acid, total flavonoid content, anthocyanin and ascorbic acid, whilst the control treatment gave the highest DPPH radical scavenging

T a b l e 2

Phytochemical screening by GLC for 3-day-old broccoli sprouts produced from seeds treated with essential oils before cold storage (0 time)

Treatment		Total phenolic acid [mg/100 g F.W.]		Total flavonoids [mg/100 g F.W.]		Anthocyanin [mg/100 g F.W.]		Ascorbic acid [mg/100 g F.W.]		DPPH [Mmol TE/g F.W.]	
		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
1	Water (control)	83.33^{d}	84.11^{e}	91.99^{d}	95.18^{e}	7.13^{d}	7.70^{d}	70.58^{e}	81.23^{d}	23.66^{a}	24.66^{a}
2	Hot water	88.71^{c}	88.56^{c}	100.95^{c}	101.03^{d}	8.62^{c}	8.77^{c}	86.81^{c}	86.81^{c}	23.54^{b}	23.66^{a}
3	Fennel oil	88.46^{c}	88.90^{c}	107.66^{b}	107.72^{c}	8.86^{c}	8.87^{bc}	87.66^{c}	88.00^{c}	21.98^{d}	21.98^{c}
4	Caraway oil	87.90^{c}	88.13^{cd}	104.66^{b}	104.73^{c}	9.84^{bc}	9.84^{bc}	77.33^{d}	85.80^{c}	21.96^{de}	21.96^{c}
5	Basil oil	122.06^{b}	122.29^{b}	113.00^{a}	113.00^{b}	11.71^{a}	12.05^{a}	94.67^{b}	94.67^{b}	21.94^{de}	21.94^{c}
6	Thyme oil	131.66^{a}	131.60^{a}	115.66^{a}	116.24^{a}	12.09^{a}	12.14^{a}	102.33^{a}	103.33^{a}	21.86^{e}	20.03^{d}
7	Sage oil	87.90^{c}	84.74^{de}	104.33^{bc}	104.59^{c}	10.38^{b}	10.38^{b}	82.33^{cd}	86.69^{c}	22.79^{c}	22.79^{bc}

Means followed by the same letter (s) within each column do not significantly differ using Duncan's multiple range test at the level of 5%

T a b l e 3

Phytochemicals of sprouts treated with essential oils after 15 days of cold storage at 4°C

Treatment		Total phenolic acid [mg/100 g F.W.]		Total flavonoids [mg/100 g F.W.]		Anthocyanin [mg/100 g F.W.]		Ascorbic acid [mg/100 g F.W.]		DPPH [Mmol TE/g F.W.]	
		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
1	Water (control)	60.33^{d}	59.81^{e}	39.19^{d}	40.28^{e}	5.34^{d}	5.87^{d}	43.75^{e}	46.28^{d}	28.06^{a}	27.85^{a}
2	Hot water	87.27^{c}	86.45^{c}	98.59^{c}	99.23^{d}	7.92^{c}	8.07^{c}	82.08^{c}	82.51^{c}	24.15^{b}	24.86^{a}
3	Fennel oil	86.54^{c}	87.19^{c}	105.76^{b}	105.17^{c}	8.16^{c}	8.17^{bc}	85.16^{c}	86.00^{c}	22.08^{d}	22.00^{c}
4	Caraway oil	85.23^{c}	86.11^{cd}	102.96^{b}	102.57^{c}	9.04^{bc}	9.24^{bc}	76.83^{d}	81.90^{c}	21.98^{de}	22.00^{c}
5	Basil oil	120.86^{b}	119.99^{b}	110.60^{a}	111.00^{b}	11.21^{a}	11.75^{a}	92.66^{b}	92.16^{b}	22.03^{de}	21.99^{c}
6	Thyme oil	130.33^{a}	130.50^{a}	113.16^{a}	114.02^{a}	11.92^{a}	12.84^{a}	101.93^{a}	102.81^{a}	21.96^{e}	20.33^{d}
7	Sage oil	86.05^{c}	84.48^{de}	100.33^{bc}	102.25^{c}	10.08^{b}	10.03^{b}	82.00^{cd}	86.06^{c}	22.94^{c}	22.29^{bc}

Means followed by the same letter (s) within each column do not significantly differ using Duncan's multiple range test at the level of 5%

capacity, in both the seasons (Table 2). The thyme oil treatment produced significant increases in total phenolic content, total flavonoid content, anthocyanin content and ascorbic acid. Thyme and basil oils decreased significantly the DPPH radical scavenging capacity. Accordingly, this treatment (thyme oil) has been chosen to study the storage behaviour, in addition to control treatment. After 15 days of cold storage, all treatments had significant effects on all phytochemical characters compared to the control, in the two seasons (Table 3). The control decreased than the initial time (0 time, Table 2) in all studied traits. Thyme oil treatment gave the best results. So, it was chosen to compare with the control during storage.

3. *Antioxidant activity during cold storage*

3.1. *Total phenolic content and DPPH radical scavenging capacity*

A gradual increase in the total phenolic content reached a maximum value at day 5 and 10 (132.67 and 135.04 mg GAE/100 g F.W.) compared to the initial value but this concentration decreased to 129.03 mg at day15 because of the application of thyme oil (Figure 2). Keeping in view that the control treatment decreased to 73.84 GAE/100 g F.W. at day 5.

On the 15 days-old sprout from storage, the DPPH radical scavenging capacity in the control was reduced to 28.57% when compared to thyme oil treatment (1.98%). The DPPH radical scavenging capacity in control increased significantly until day 10 (29.43 mg /100 g F.W.) and finally decreased (28.46 mg/100 g F.W.) at day 15, the loss increased to 20.28% compared with the initial value (Figure 3). However, some authors confirm that low storage temperature causes an accumulation of total polyphenols (Policegoudra & Aradya 2007).

3.2. *Total flavonoid contents*

Total flavonoid content (Figure 4) was found in a higher concentration in 3-day-old sprouts of thyme oil treatment, with values of 115.95 mg/100 g F.W.; after 5 and 10 days of storage, it slightly decreased to 0.021% and 0.086%, respectively, when compared with the initial value and, finally, reduced by 1.39%. The high loss of flavonoids reached to 10.59% and 47.89%, after 5 and 10 days, respectively, and at 15 days, the loss increased to 58.33% for the control treatment (average two seasons).

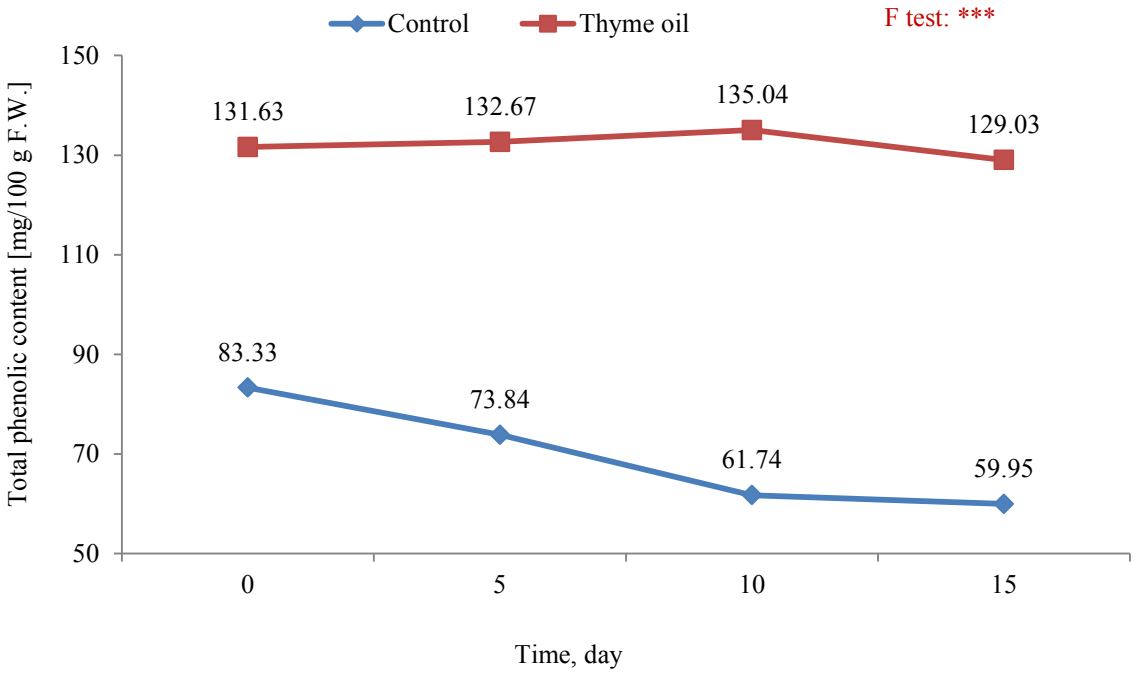

Figure 2. Total phenolic content as affected by the thyme oil compared to control treatment at different storage period

3.3. *Ascorbic acid*

Thyme oil application slightly decreased vitamin C content (0.37% loss) when compared with the initial value after 5 days of storage; the respective losses at the end of cold storage were 1.93%. At day 5 of storage, the vitamin C content in control treatment was decreased by 37.71%. Finally, the losses reached to 36.25% (average two seasons, Figure 5). This behaviour was clearly in contrast to that found for phenolic compounds and glucosinolates.

3.4. *Total chlorophyll*

Changes in chlorophyll amount of broccoli sprouts are illustrated in Figure 6. During the 15 days of storage, no significant change was observed in broccoli sprouts in samples treated with thyme oil compared to the control.

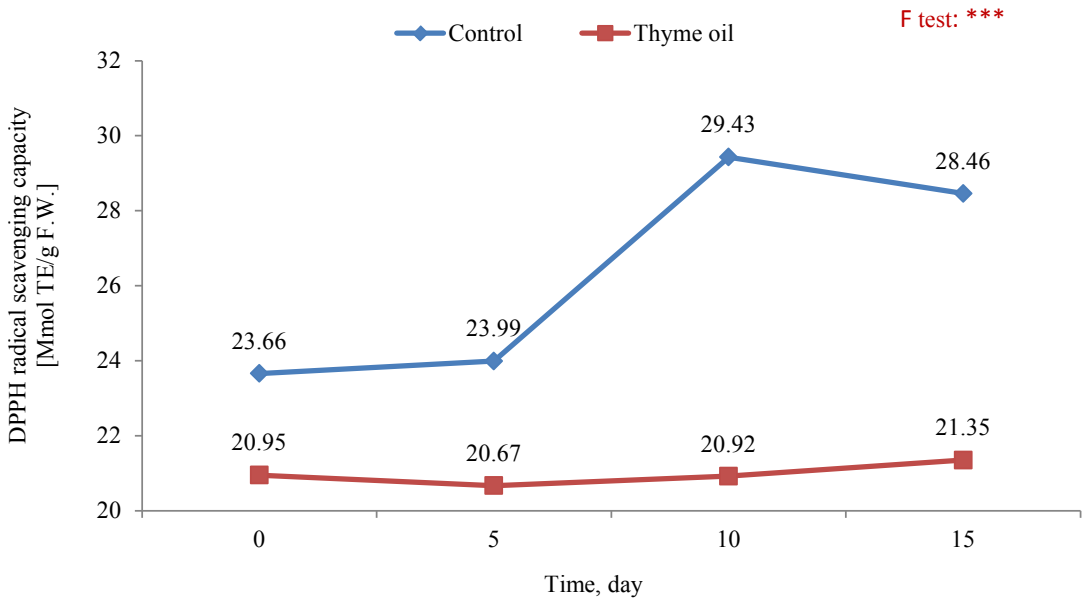

Figure 3. DPPH radical scavenging capacity as affected by the thyme oil treatment compared to the control treatment at different storage period

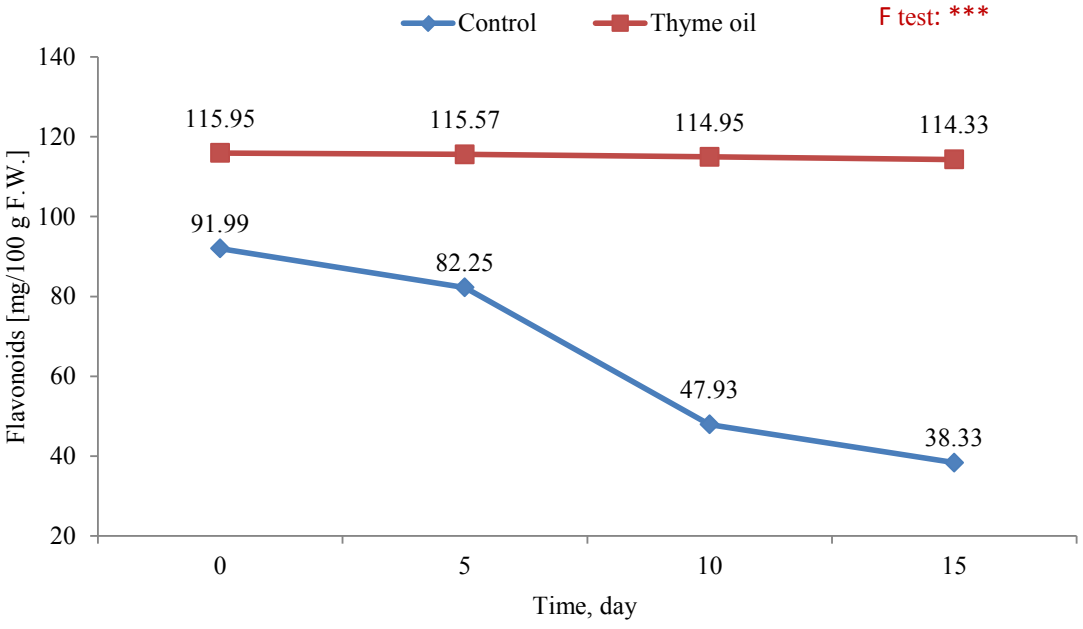

Figure 4. Total flavonoids content as affected by the thyme oil compared to the control treatment at different storage period

3.5. *Total anthocyanin*

Thyme oil treatment in 3-day-old sprout broccoli increased the level of anthocyanin compared with the control. A slight decrease in anthocyanin content was observed (2.95%) at the end of storage periods, compared with the initial value. A gradual decrease was noticed in control treatment and the changes reached about 22.019% at the end of storage period (average two seasons, Figure 7).

3.6. *Glucosinolates content*

The results of the samples analysed showed significant differences with respect to storage time. Thyme oil application increased glucosinolates content in 3-day-old sprouts compared to control treatments (Figure 8). Thyme oil had a high value of glucosinolates (27.02 µg/g F.W.), and it slightly decreased up to a concentration of 26.43 µg/g F.W. on day 15. The change percentage decreased to about 2.18% at the end of storage. The reduction in total glucosinolate content was observed in control treatment, in which the change percentage reached about 49.12% at the end of storage.

3.7. *Microbial population in the stored broccoli sprouts*

The microbial populations were presented in terms of bacterial (total and coliform), fungal and yeast counts (Table 4).

Marked variations were detected amongst the different essential oil treatments from one side and the different microbial groups in the other side. Regarding bacteria, it recorded the highest numbers amongst the groups, along the storage period; fortunately, the coliform bacterial count was not detected at all, indicating the suitability of such preparation for the nutritional aspects. Coliform bacteria are described and grouped based on their common origin or characteristics, as either total or faecal coliform.

T a b l e 4

Count (C.F.U.) of microbial groups on the sprouts of broccoli as a function of cold storage

Microbial group	Treatment	0 day	5 days	10 days	15 days
Bacteria*	Water (control)	110	8,000	19,700	200,500
	Hot water	100	10,000	18,850	21,800
	Fennel oil	0	330	6,530	15,670
	Caraway oil	0	670	3,670	4,670
	Basil oil	0	1,650	2,760	2,950
	Thyme oil	0	500	500	550
	Sage oil	20	980	1,750	2,009
Fungi	Water (control)	0	0	10	40
	Hot water	0	0	0	10
	Fennel oil	0	0	0	0
	Caraway oil	0	0	0	0
	Basil oil	0	0	5	10
	Thyme oil	0	0	0	0
	Sage oil	0	0	0	0
Yeast	Water (control)	0	20	40	50
	Hot water	0	0	0	10
	Fennel oil	0	0	0	10
	Caraway oil	0	0	0	10
	Basil oil	0	0	10	20
	Thyme oil	0	0	0	0
	Sage oil	0	0	20	30

*No coliform bacteria were detected; C.F.U. – colony forming unit

The total group includes faecal coliform bacteria that exist in the intestines of warm-blooded animals and humans and are found in bodily waste, in animal droppings and naturally in soil. Most of the faecal coliform in faecal material (faeces) is known to cause serious human illness. Oppositely, fungi group was the lowest detected groups in the tested treatments. Yeast count came in moderate numbers. However, the numbers of such groups are low enough to indicate the high quality of broccoli sprouts. Thyme oil followed by sage oil recorded the lowest bacterial load. On the other hand, the tested oils except basil oil completely inhibited any fungal growth on broccoli sprouts, along the storage period. Regarding the yeast group, thyme oil was the best. Generally, the microbial load of broccoli sprouts as results of the tested oils was reasonably accepted especially with the absence of any form of coliform bacteria and relatively low microbial load as a whole, representing that there is no any restriction for broccoli sprouts for human consumption.

4. *Mature versus sprout broccoli glucosinolate content*

The total glucosinolate level in sprout (27.02 µg/g F.W.) is higher than that in florets (7.37µg/g F.W.) (Figures 9 and 10). Glucoraphanin is the most abundant aliphatic glucosinolate present in sprout and reached the highest (16.24 µg/g F.W.) followed by glucoerucin (5.9 µg/g F.W.) and glucoiberm (1.2 µg/g F.W.). However, the florets contain the highest level of aromatic/indolylglucosinolates and neoglucobrassicin (2.11 µg/g F.W.) followed by glucobrassicin (1.67 µg/g F.W.). Our results are in agreement with those obtained by Fahey *et al*. 1997.

DISCUSSION

The essential oils are frequently known to induce stimulatory or inhibitory effects on the germination of seeds and other physiological processes depending on their basic component, concentration, allelochemicals interaction and selectivity due to the site of application and plant species. The lower doses of essential oil showed a stimulatory activity (Leth 2002).

The obtained results of current study revealed that the used essential oils improved the germination of broccoli seeds. However, thyme oil gave 100% of sprouts after germination (Table 1). The

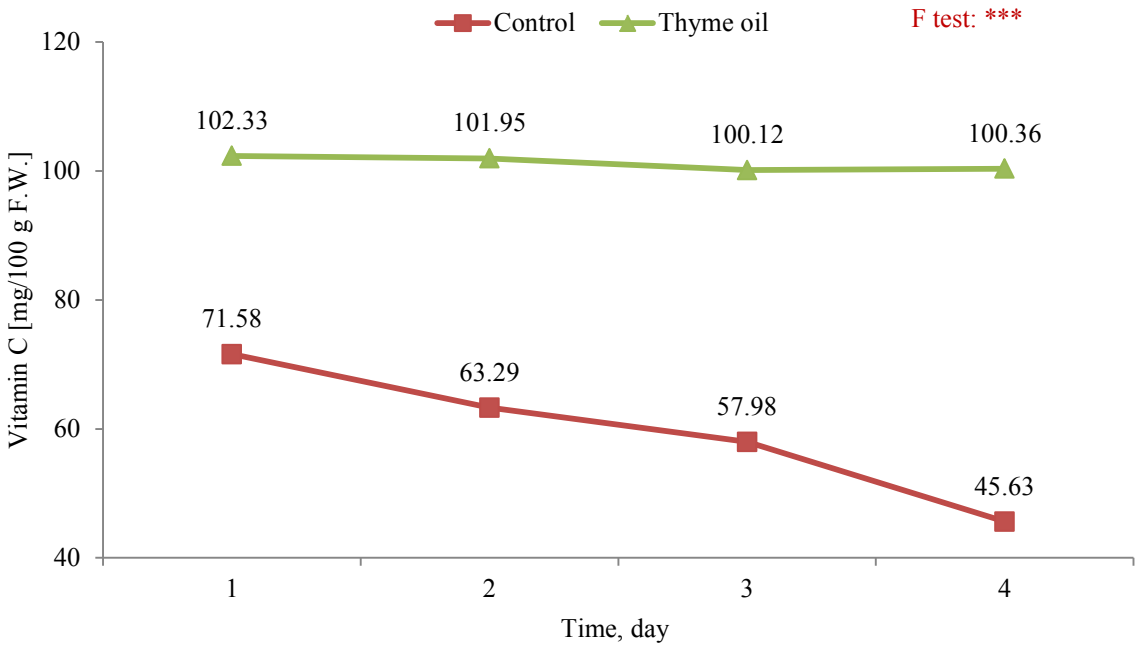

Figure 5.Vitamin C content as affected by the thyme oil compared to the control treatment at different storage period

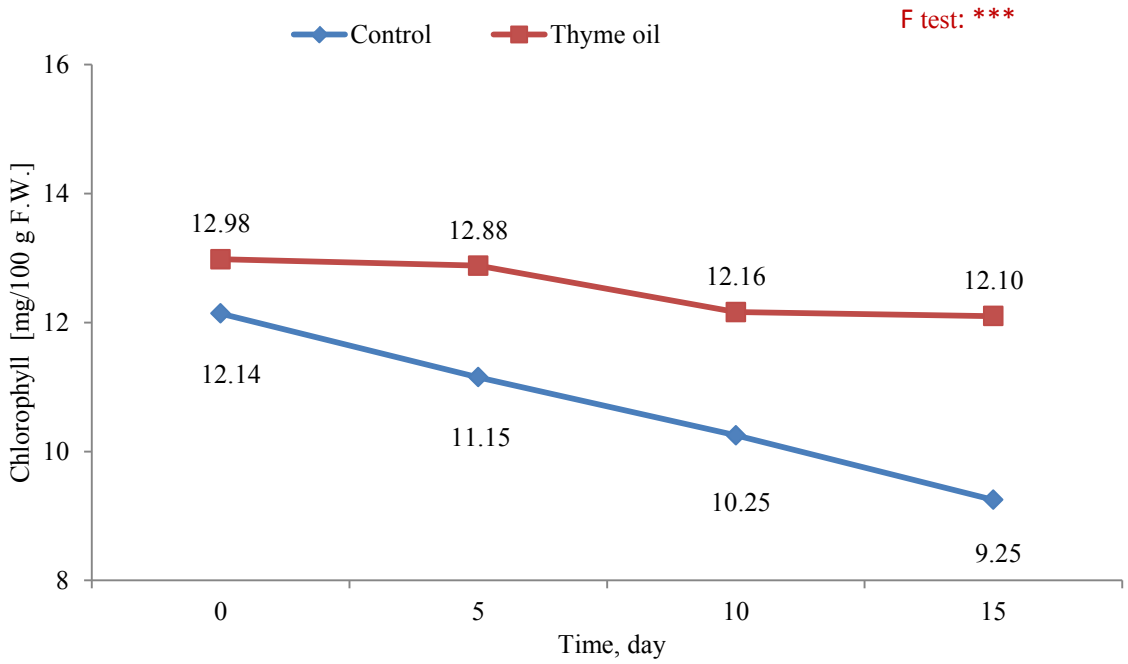

Figure 6. Total chlorophyll content as affected by the thyme oil compared to the control treatment at different storage period

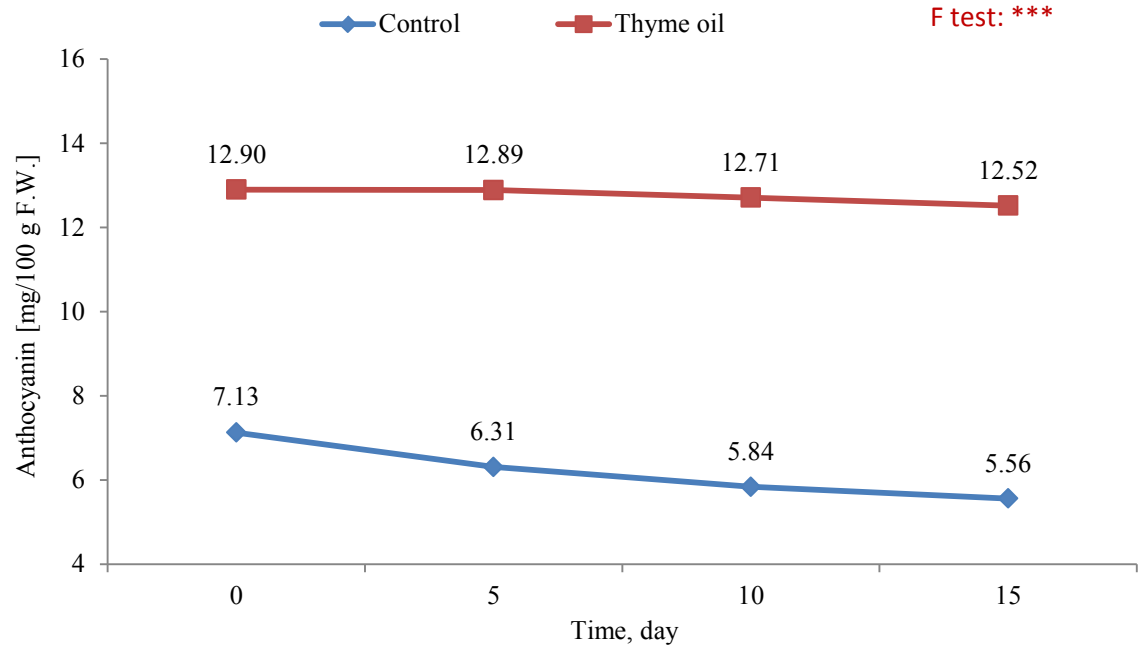

Figure 7. Total anthocyanin content as affected by the thyme oil compared to the control treatment at different storage period

impact of essential oils on seed germination of other plant species was reported as 24 out of 47 tested terpenoids enhanced the seed germination of *Lactuca sativa* (Vokou *et al*. 2003). Also, the positive impact of thyme oil on broccoli seeds could be due to its active ingredients. Kulisic *et al*. (2004) found that the phenolic compound containing thymol and carvacrol as major components exhibited strong antioxidant activity. The essential oils play an important role in the reduction of time need for broccoli germination, where it gives full germination on 3 days compared to normal conditions (7 days). So, we can produce two growing and production cycle in 7 days.

The thyme oil treatment had significant increases in total phenolic content, total flavonoid content, anthocyanin content and ascorbic acid (Table 2). The majority of the antioxidant activity attributes to phenolic compounds, flavonoids and ascorbic acid in essential oils (Heim *et al*. 2002). Moreover, the antioxidant effect was due to the presence of hydroxyl groups in their chemical structure. Milos *et al*. (2000) found that the oregano essential oil inhibited hydroperoxide formation and that the CHO fraction showed the highest antioxidative activity.

The thyme oil showed very poor radical scavenging capacity (Table 2). All other antioxidants showed high and almost the same DPPH radical scavenging capacity effect. It was described that radical scavenging abilities of some compounds can be influenced by their different kinetic behaviour (Kulisic *et al*. 2004). For slow-reacting compounds, the influence was attributed to the complex reacting mechanism. In our study, probably, the constituents from thyme essential oil involved one or more secondary reactions, which result in the slower reduction of DPPH solutions (Kulisic *et al*. 2004). After 15 days of storage (Table 3), application of thyme oil may help in the maintenance of the stored sprouts reserves, keeping the internal biochemical enzymatic activities in minimum level and in more stable case, thereby prolonged their shelf life. Also, this treatment was highly effective in the protection of sprouts against the known degradable effects of higher free radicals during storage conditions.

Application of 4% thyme and basil oils reduced the pathogenic fungi from seed to seedling (Table

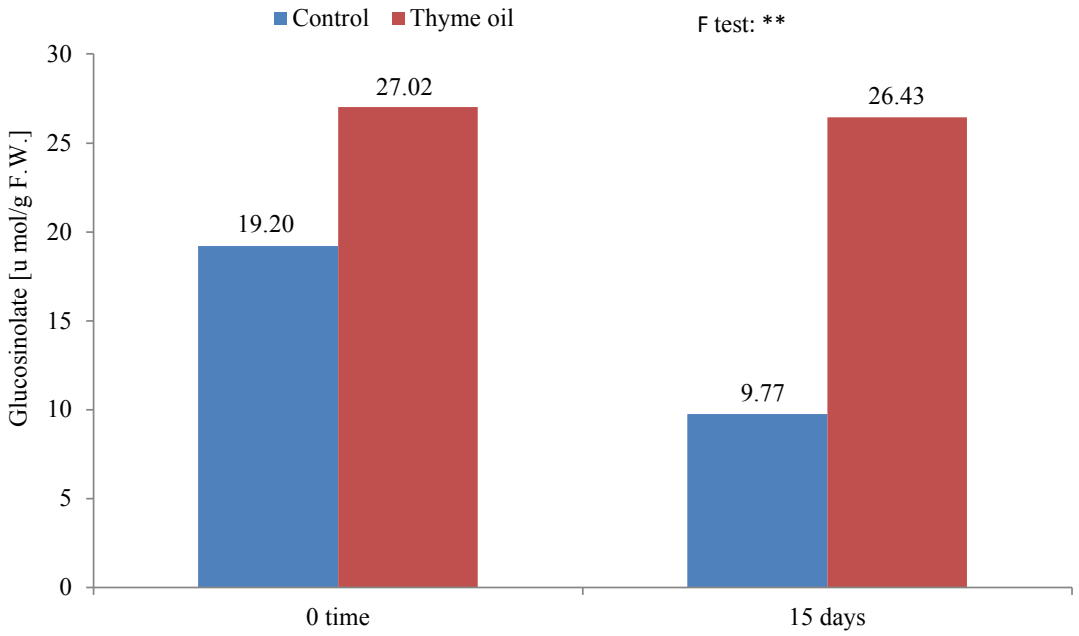

Figure 8. Total glucosinolate content as affected by the thyme oil compared to the control treatment at 0 time and 15 DAS

4) and had a positive effect on the seed germination of infected seeds, and basic constituents (monoterpenes and antioxidants) tended to slow down the activity of carbohydrates and protein breakdown associated enzymatic systems as well as enzyme involved in respiration and energy metabolism (Ngue-fack *et al*. 2005).

Application of thyme oil increases phenolic compound content especially in 10 days compared to that in the initial period of cold storage (Figure 2). The results obtained in this study are in agreement with the phenolic profile reported by Pająk *et al*. (2014). However, some authors confirmed that low storage temperature causes an accumulation of total polyphenols (Villarreal-García *et al*. 2016).

During cold storage (Figure 3), at day 15 the DPPH in control was reduced to 28.57% compared to that in sprouts treated with thyme oil (1.98%) Nath *et al*. (2011) observed a steady decrease in the DPPH radical scavenging capacity for 144 h of storage of broccoli inflorescences. The above behaviour may be due to the constant changes in plant metabolism during storage as a result of oxidative stress, which may include structural changes in synthesis or antioxidant compounds (Xiao *et al*. 2014).

In our study, thyme oil treatments possibly affected the environmental stresses of sprout broccoli; however, in contrast with control treatment, total flavonoid content decreased to 58.33% compared to that measured at initial period (Figure 4). An explanation for this could be the very high respiratory rate of broccoli (Izumi *et al*. 1996) that could increase the metabolism and, therefore, the degradation of the phenolic compounds.

During cold storage, broccoli's potential for maintaining the stability of vitamin C levels found in the fresh product was due to thyme oil application (Figure 5). Thus, according to previous report (Davey *et al*. 2000), broccoli retain its vitamin C levels because of the protection of other oxygen scavengers.

Total chlorophyll and total anthocyanin contents slightly decreased at 4°C because thyme oil application prevented the degradation of anthocyanin and chlorophyll (Figures 6 and 7), but this might be lost at the control, which may be associated with water loss (Haminiuk *et al*. 2012; Sabir 2012).

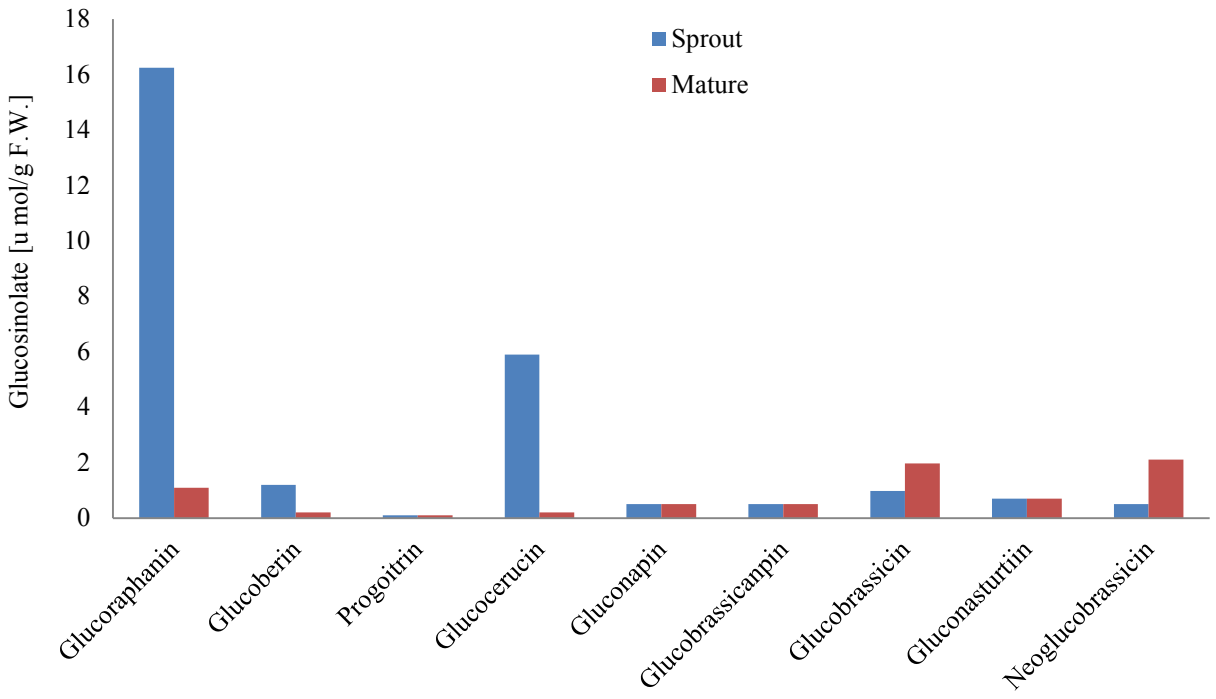

Figure 9. Total and individual levels of aliphatic, aromatic and indole glucosinolates in 3-day-old broccoli sprout and mature at harvest

Figure 10.(a) Mature plant and (b) sprout of broccoli

Thyme oil increased glucosinolates content in 3-day-old sprouts, compared to control treatments (Figure 8). The highest decrease in total glucosinolate content was observed in control treatment at the end of storage. This behaviour is consistent with experiments conducted by Howard *et al*. (1997), who reported a decrease of 50% on day 14 of storage at 4°C in broccoli sprouts. Cultural practices, handling and storage conditions, as well as the vegetable preparation, have a potential impact on the glucosinolates content, causing a change in the rate of formation of sulphoraphane (Jeffery & Araya 2009). However, the total glucosinolate content of the seedling was highest at both 4 and 7 days. Glucosinolates are plant defence compounds and, consistent with this function, are accumulated preferentially in the organs that contribute most to plant fitness at a particular moment in the growth cycle (Halkier & Gershenzon 2006).

The total glucosinolate content in sprout is higher than that in florets (Figures 9 and 10). Glucosinolates are divided into three major categories: aliphatic, indole and aromatic glucosinolates (Yan & Chen 2007). The high content of the aliphatic glucosinolates in broccoli sprouts is mainly attributed to glucoraphanin (16.24 µg/g F.W.). The glucoraphanin can be hydrolysed to form sulphoraphane. This compound plays an important role in controlling, preventing or blocking any of the multiple stages of the carcinogenic process (Parnaud *et al*. 2004). The florets contain the highest level of aromatic/indolylglucosinolates and neoglucobrassicin (2.11 µg/g F.W.) followed by glucobrassicin (1.67). Indole-3-carbinol (C_9H_9NO) is produced by the breakdown of the glucobrassicin. Indole-3-carbinol is a powerful strategy for achieving protection against carcinogenesis, mutagenesis and other forms of toxicity.

The impact of harvest and storage techniques on phytochemical has only recently begun to be explored. In general, phenolic compounds are considered to be relatively stable at cool temperature storage.

CONCLUSIONS

The results of this study show that the content of phytochemicals such as phenolic compounds, total flavonoids, anthocyanin, chlorophyll, ascorbic acid and glucosinolate in broccoli sprouts is stable during storage after 15 days at 4°C because of the application of essential oils. Thyme oil gave the best results on the content of phytochemicals and had a highest content of the glucosinolate and reduced the microbial load compared to the control. Fortunately, the coliform bacteria was not detected in all treatments. The results also indicate that the total glucosinolate content in sprout is higher than that in florets. The sprouts had significant values of glucoraphanin that can be hydrolysed to sulphoraphane. This compound plays an important role in controlling, preventing or blocking any of the multiple stages of the carcinogenic process. Therefore, the consumption of this food can play an important role in the prevention of related diseases with free radical generation, considering broccoli sprouts as a functional food.

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