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Changes in Polyamine, Total Protein and Total Carbohydrate Content and Peroxidase Activity during the Lifetime of Chrysanthemum 'Faroe'

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ABSTRACT

The aim of the present work was to evaluate the changes in polyamine (PA) content, peroxidase (POX) activity and levels of total protein and total soluble carbohydrates throughout the lifetime of leaves and inflorescences of chrysanthemum 'Faroe' treated with gibberellic acid (GA_3) (used in production practices) and kept at room temperature and cold storage. The treatments were composed of four doses of GA_3 (0, 15, 30 and 45 mg L^{-1}) applied at the beginning of flower bud formation (28 days after transplanting of seedlings). After harvesting, the stems (95% of the expanded ligule) were stored at 10°C and 95% relative humidity for 48 hrs, or kept at room temperature. For biochemical analysis samples of leaves and inflorescences were collected at the 4th, 8th, 12th and 16th day after harvest. The application of GA_3 in the field and cold storage increased the content of PAs. There was an increase in POX activity in leaves and inflorescences during postharvest and this increase was related to oxidation of the PAs studied. The amount of proteins and carbohydrates in chrysanthemum 'Faroe' decreased during storage at 25°C and under cold conditions.

Keywords: biochemistry, *Dendranthema grandiflora*, senescence, plant growth regulator, postharvest flowers

INTRODUCTION

Studies in the literature show that gibberellins may mediate the effect of other hormones and cold storage can induce modification of a plant's lifetime, certainly by modifying cell metabolism (Valle *et al*. 1989; Nowak and Rudnick 1990; Galston and Kaur-Sawhney 1994; Meng *et al*. 2009).

The deterioration of newly harvested plant products is the result of physiological changes and the maintenance of plant materials at low temperature after harvest tends to alter the production of substances involved in senescence, such as ethylene. The response and sensitivity of a tissue to ethylene is dependent on plant development and variety and is differently perceived by different plant organs (Ciardi and Klee 2001).

Ethylene appears from the precursor S-adenosylmethionine (SAM), which is common to the synthesis of the polyamines (PAs), spermidine (Spd) and spermine (Spm) (Bouchereau *et al*. 1999). Among PAs, putrescine (Put), Spd and Spm are substances related to cellular division and growth (Crozier *et al*. 2001). PAs inhibit ethylene production by regulating the activity of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and oxidase (Lee *et al*. 1997), while ethylene alters the formation of PAs by reducing the activity of arginine decarboxylase (ADC) and SAM decarboxylase (SAMDC) (Roustan *et al*. 1993). The concentration of PAs may vary depending on the plant organ, degree of ripeness and postharvest treatment (reviewed by Teixeira da Silva 2006; Kuznetsov and Shevyakova 2007; Pang *et al*. 2007). Production of PAs under water or low-temperature stress has also been related to protection against oxidative stress (Nayyar and Chander 2004).

Peroxidases (POX; EC 1.11.1.7) are enzymes that are also related to plant senescence and different types of biotic and abiotic stresses (Lima *et al*. 1999). This group of enzymes, hemeproteins, catalyzes oxidation-reduction reactions, using hydrogen peroxide (H_2O_2) as an electron acceptor, to catalyze different oxidative reactions on organic substrates (Blokhina *et al*. 2003). The activity of this enzyme is altered with senescence, due to the disintegration of cell and organelle membranes, leading to H_2O_2 formation from NAD(P)H and O_2 by oxidases (Costa *et al*. 2005; Hossain *et al*. 2006). This makes this enzyme useful as a biochemical marker of oxidative physiological events.

Other substances may also be related to senescence in plants, such as carbohydrates, whose function is to provide substrates for respiration and cell wall synthesis (Norikoshi *et al*. 2008) and proteins (Azeez *et al*. 2007).

Increased shelf-life of vegetables and horticultural commodities is extremely important, and much research has been undertaken to determine the factors that influence the

Fig. 1 (**A**) Greenhouses used for the cultivation of chrysanthemum (*Dendranthema grandiflora* Tzvelev) cv. 'Faroe'; (**B**) post-harvest storage of chrysanthemum cv. 'Faroe'.

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biochemical processes. One way to increase shelf-life is to use low temperature storage with the application of plant growth regulators (PGRs), such as gibberellins, particularly gibberellic acid (GA_3). This can be clearly observed in lily cv. 'Snow Queen' (*Lilium longiflorum* Thunb) where the application of 500 mg L^{-1} GA_3 and benzyladenine can significantly slow down yellowing and the respiration rate (Franco and Han 1997). According to Takahashi *et al.* (1986), this hormone has been used to modify plant growth and development, working as a regulator of cell division and enlargement and delay of senescence. However, the effects of PGRs on flowers are quite unknown and contradictory. Brackmann *et al.* (2005), evaluating the effect of GA_3 in three varieties of chrysanthemum, reported the stimulation of senescence in both flowers and leaves.

In Brazil, chrysanthemum is one of the most marketed flowers in all the main sale markets, and cv. 'Faroe' is one of the most used (see **Fig. 1**).

This work aimed to evaluate the change in PA content, POX activity and the levels of total protein and soluble carbohydrates occurring during the life-time of leaves and flowers of chrysanthemum 'Faroe' treated with GA_3 when kept under room temperature and cold storage.

MATERIALS AND METHODS

Experiment

The experiment was conducted in plastic greenhouses in Cordeirópolis, São Paulo, Brazil (22° 28' 55" S, 47° 27' 24" W). Medium sized seedlings of chrysanthemum (*Dendranthema grandiflora* Tzvelev) cv. 'Faroe' were used, characterized by dichotomous leaves with alternate disposition on the stem, a globular inflorescence, formed by small white petals and not visible internal disk flowers. In the cultivation conditions of Cordeirópolis, plants were cultivated for 7½ weeks for flower induction, in greenhouses at 22 ± 2 °C, under natural light.

In order to induce vegetative development after trasplantation, plants were subjected to prolonged light exposure for 4 weeks. Continuous incandescent light bulbs were used to illuminate plants (one 100 W lamp each 2 m^2) from 4 to 8 P.M.

During the reproductive phase (7½ weeks) light exposure was reduced to 10 hrs by using dark plastic sheets on the greenhouse walls.

Application of gibberellic acid

Treatments were composed of four doses (0, 15, 30 and 45 mg L^{-1}) of GA_3 (Pro-Gibb® - 10%, Valent, Canada). GA_3 was applied at the beginning of flower bud formation (28 days after transplanting the seedlings. Plants (stems and leaves) were sprayed in the morning with 100 mL of each concentration for each treatment. Experiments were carried out on 40 plants for each treatment (cultivated at a density of 64 plants m^{-2}) with 4 replicates. Plants on the borders were discarded. In all the treatments 30 mL 100 L^{-1} of a non-ionic surfactant (Extravon®, Syngenta Agro S/A), to improve wetting and spray distribution, was added. The apparatus used for GA_3 application was a CO_2 backpack tank, equipped with a sprayer nozzle-shaped fan.

Harvest and postharvest

Stems were harvested with approximately 95% of the ligule expanded (53 days after application) and standardized to 75 cm in length, eliminating leaves 15 cm from the base of the stem. For each GA_3 treatment, 16 stems were used, totaling 128 stems in the two post-harvest treatments.

Stems were placed under two conditions: 1) Storage in a cold chamber at 10°C and 95% relative humidity in 5-L plastic containers with water for 48 hrs and then placed under identical conditions as treatment 2 for 16 days; 2) room temperature (i.e., 25°C) in plastic containers, containing water, which was changed every two days for 16 days.

Biochemical analysis

The biochemical analyses were carried out in pre-harvest on leaves, positioned in the middle of the stem, on plants subjected to GA_3 treatment. Samples of leaves and flowers were collected in the morning, at 7–9 A.M., at 4, 8, 12 and 16 days after harvest. The same post-harvest analyses were carried out on stems and flowers kept at room temperature and under cold storage.

Determination of polyamines

Polyamines (μg Put/Spd and Spm g^{-1} fresh weight) were determined by thin layer chromatography following the method described by Flores and Galston (1982), adapted by Lima *et al.* (2008). The fresh material was homogenized for 1 min in 5% (v/v) cold perchloric acid (Merck), using a food homogenizer. After centrifugation for 20 min at 4°C, dansyl chloride (Sigma, 95%) and saturated sodium carbonate were added to the supernatant. Proline (100 mg mL^{-1}) (Sigma, min. 99%) was added after 1 h to stop the reaction and the solution was brought to 60°C. The mixture was maintained in the dark for 30 min, at room temperature. Toluene was used to extract the dansylated PAs and aliquots were applied onto thin layer chromatography plates (glass plates coated with 60G silica Gel – Merck; 20 × 20 cm). Separation was carried out in laboratory bowls containing chloroform: triethylamine (Merck) (10: 1). Put (Sigma, min. 98%), Spd (Sigma, min. 98%) and Spm (Sigma, min. 95%) standards were subjected to the same process. The entire procedure was monitored under UV light (254 nm). PAs were quantified by comparison against standards, which were also applied onto the plates, by fluorescence emission spectroscopy (excitation at 350 nm and emission measurement at 495 nm), in a Video Documentation System, using the Image Master version 2.0 software program.

Determination of peroxidase activity

The activity of peroxidase (POX, EC 1.11.1.7); μmol of H_2O_2 decomposed min^{-1} g^{-1} fresh weight) was determined by the method described by Lima *et al*. (1999). Fresh material (leaves and flowers) was collected, weighted in 50-mg aliquots and dissolved in 5 mL of 0.2 M potassium phosphate, pH 6.7, on ice. Samples were then centrifuged at 10000 × *g* for 10 min at 4°C and 1 mL of the supernatant was used as the source of enzyme. Hydrogen peroxide solution (0.5 mL of 20 mM H_2O_2 by Merck) was added to 0.5 mL of 0.2 M potassium phosphate, pH 6.7, containing 4 mM 4-aminoantipyrine (4-amino-1,5-dimethyl-2-phenyl-4-pyrazolin-3-one; Sigma) and 10 mM dichlorophenol (Sigma). Samples were incubated for 5 min at 30°C and then the reaction was stopped with 2 mL of absolute ethanol (Merck). The spectrophotometric readings, corrected for blanks, were carried out at 505 nm.

Determination of total carbohydrate and total protein content

Whole flowers and young and old leaf samples were analyzed for non-structural carbohydrates. They were freeze-dried and ground in a Wiley mill to pass a 40-mesh filter. After sonication of a tissue sample (20 mg), soluble carbohydrates were extracted four times with hot 80% ethanol. After each extraction, homogenates were centrifuged at 1000 × *g* for 5 min. The supernatant was used for analysis of soluble sugars. Sugar was assayed colorimetrically by the phenol and sulfuric acid technique (Dubois *et al.* 1956). For each set of samples, a standard curve was prepared with commercial glucose (Sigma).

Dried and finely ground samples (treated as for carbohydrate analysis) were analyzed for crude protein (Kjeldahl nitrogen × 6.25), following the procedures reported by AOAC (AOAC 1997).

Statistical analysis

The experimental design was completely randomized, and consisted of seven replicates. Analysis of variance was performed to detect differences between treatment means, which were separated by Tukey's test ($P < 0.05$) using SAS/STAT software (2008).

RESULTS AND DISCUSSION

The highest dose of GA_3 applied during pre-harvest caused an increase in the content of Put, Spd and Spm in leaves, 4 days after harvest. This effect was also noticed for Put 8 days after harvest (**Table 1**). The same dose induced an increase in Spm content in inflorescences (**Table 2**) 4 days after harvest, while no significant effect was observed in the other PAs. The results in this experiment are consistent with those of Valle *et al*. (1989), who reported that gibberellins are able to act as secondary messengers capable of mediating the effects of other hormones. According to Taiz and Zeiger (2004), the role of gibberellins in the phase change control is complex, varying among species and involving interactions with other factors. In this work, even observing changes in PA concentrations, GA_3 alone were not sufficient to prolong the lifetime of chrysanthemum 'Faroe'.

In relation to the effect of cold storage on leaves (**Table 3**) and inflorescences (**Table 4**), Spd and Spm appeared at higher concentrations than Put. In leaves, there was a clear trend, i.e., higher levels of Put in refrigerated samples. Accumulation of Put was also noted in pepper, cucumber, zucchini and citrus (orange and lemon) during cold exposure (Martínez-Romero *et al*. 2003).

Generally, senescence or stresses lead to an increased rate of ethylene synthesis, reducing the levels of Spd and Spm and, sometimes, to the accumulation of Put (Bouchereau *et al*. 1999; Capell *et al*. 2004). Many studies showed that when the Put/(Spd + Spm) ratio is low, it can be related to a protective effect on plants against some kind of stress (Capell *et al*. 2004). The results reported in **Tables 3** and **4** show that the Put/(Spd + Spm) ratio is higher in leaves kept under refrigeration, while no differences were observed in flowers. These results are consistent with another study (Yamaguchi *et al*. 2007) reporting that changes in the levels of PAs may be a consequence of stress and/or environmental factors, pointing to their possible role as biochemical markers of metabolic events.

Several studies have shown that there is a decrease in the levels of PAs with senescence, attributed to the competition between ethylene and S-adenosylmethionine (SAM) to form Spd and Spm (Bouchereau *et al*. 1999). Changes in the levels of PAs and ethylene have been reported during senescence in some plants, such as plums (De Dios *et al*. 2006). Studies also showed that degradation can occur by oxidases of PAs, generating H_2O_2, which is metabolized by POX, inducing an increased activity of this enzyme (Bouchereau *et al*. 1999; Cona *et al*. 2006). The oxidation products cannot be converted back to PAs, and the role of PA oxidase in plant cell physiology may be a simple involvement with the terminal catabolism of PAs (Cervelli *et al*. 2000).

POX activity increased with the storage time of leaves and inflorescences in all GA_3 treatments, independently of the concentration used (**Tables 1, 2**). Pre-harvest treatments with gibberellins alter the metabolism of postharvest senes-

Table 1 Post-harvest putrescine, spermidine, spermine, total soluble carbohydrate, total protein content and peroxidase activity in leaves of C*hrysanthemum* 'Faroe' during and following the pre-harvest application of different doses of GA_3.

(mg L^{-1} GA_3)	Chrysanthemum leaves, post-harvest (days)			
	4	8	12	16
	Putrescine (μg g^{-1} fresh weight)			
0	39.09 aB	28.46 bB	17.46 cA	10.11 cA
15	37.61 aB	26.44 bB	13.66 cA	12.59 cA
30	37.98 aB	26.16 bB	15.30 cA	8.83 cA
45	58.06 aA	37.19 bA	16.55 cA	11.42 cA
	CV (%) = 6.54			
	Spermidine (μg g^{-1} fresh weight)			
0	82.28 aB	70.53 bA	46.92 cA	34.11 dA
15	80.43 aB	68.66 bA	43.50 cA	38.24 cA
30	81.88 aB	69.74 bA	45.06 cA	34.22 dA
45	97.86 aA	67.09 bA	40.19 cA	35.83 cA
	CV (%) = 5.30			
	Spermine (μg g^{-1} fresh weight)			
0	129.51 aB	130.63 aA	127.86 aA	102.39 bA
15	131.53 aB	134.91 aA	123.43 bA	104.11 cA
30	129.03 aB	132.71 aA	125.27 aA	106.68 bA
45	145.87 aA	134.34 bA	120.01 cA	106.40 dA
	CV (%) = 6.12			
	Peroxidase (μmol H_2O_2 decomposed min^{-1}. g^{-1} fresh weight)			
0	1.8 10^{-3} dA	2.5 10^{-3} cA	3.7 10^{-3} bA	5.9 10^{-3} aA
15	1.9 10^{-3} dA	2.3 10^{-3} cA	3.6 10^{-3} bA	5.7 10^{-3} aA
30	1.8 10^{-3} dA	2.3 10^{-3} cA	3.8 10^{-3} bA	5.8 10^{-3} aA
45	1.8 10^{-3} dA	2.4 10^{-3} cA	3.6 10^{-3} bA	6.0 10^{-3} aA
	CV (%) = 7.86			
	Total soluble carbohydrate (%)			
0	18.53 aA	18.32 aA	15.96 bA	12.94 cA
15	18.55 aA	18.38 aA	15.91 bA	12.82 cA
30	18.56 aA	18.35 aA	15.78 bA	12.81 cA
45	18.51 aA	18.33 aA	15.83 bA	12.77 cA
	CV (%) = 6.81			
	Total protein (%)			
0	8.65 aA	8.60 aA	6.86 bA	5.75 bA
15	8.54 aA	8.53 aA	6.74 bA	5.58 bA
30	8.43 aA	8.46 aA	6.87 bA	5.46 bA
45	8.95 aA	8.48 bA	6.83 bA	5.73 bA
	CV (%) = 5.18			

Means followed by the same lowercase letters on the same line and letters in the same column do not differ by Tukey's test (α = 0.05).

Table 2 Post-harvest putrescine, spermidine, spermine, total soluble carbohydrate, total protein content and peroxidase activity in flowers of *Chrysanthemum* 'Faroe' following the pre-harvest application of different doses of GA_3.

(mg L^{-1} GA_3)	Chrysanthemum flowers, post-harvest (days)			
	4	8	12	16
	Putrescine (μg g^{-1} fresh weight)			
0	36.46 aA	26.03 bA	11.76 cA	11.93 cA
15	43.52 aA	31.61 bA	6,22 cA	8.19 cA
30	38.83 aA	27.57 bA	16.35 cA	10.17 cA
45	37.40 aA	26.41 aA	15.43 bA	10.28 bA
	CV (%) = 3.22			
	Spermidine (μg g^{-1} fresh weight)			
0	92.45 aA	73.60 bA	45.16 cA	33.25 dA
15	94.15 aA	73.39 bA	47.61 cA	36.14 dA
30	91.38 aA	69.57 bA	45.18 cA	32.69 dA
45	91.76 aA	75.04 bA	41.83 cA	33.74 dA
	CV (%) = 7.53			
	Spermine (μg g^{-1} fresh weight)			
0	164.18 aB	136.83 bA	83.51 cA	78.86 cA
15	163.53 aB	135.16 bA	88.24 cA	84.37 cA
30	161.62 aB	129.60 bA	87.93 cA	82.88 cA
45	190.20 aA	134.10 bA	86.05 cA	83.19 cA
	CV (%) = 5.44			
	Peroxidase (μmol H_2O_2 decomposed min^{-1} g^{-1} fresh weight)			
0	1.6 10^{-3} dA	2.1 10^{-3} cA	3.5 10^{-3} bA	5.9 10^{-3} aA
15	1.7 10^{-3} dA	2.1 10^{-3} cA	3.6 10^{-3} bA	6.0 10^{-3} aA
30	1.6 10^{-3} dA	2.1 10^{-3} cA	3.6 10^{-3} bA	5.8 10^{-3} aA
45	1.5 10^{-3} dA	2.2 10^{-3} cA	3.5 10^{-3} bA	5.9 10^{-3} aA
	CV (%) = 6.37			
	Total soluble carbohydrate (%)			
0	24.86 aA	22.91 bA	19.61 cA	17.43 dA
15	25.01 aA	22.78 bA	19.55 cA	17.43 dA
30	24.95 aA	21.97 bA	19.58 cA	17.32 dA
45	24.79 aA	22.86 bA	19.73 cA	17.44 dA
	CV (%) = 7.09			
	Total protein (%)			
0	11.55 aA	11.46 aA	9.91 bA	9.28 bA
15	11.50 aA	11.47 aA	9.83 bA	9.04 bA
30	11.48 aA	11.46 aA	9.85 bA	8.96 bA
45	11.54 aA	11.49 aA	9.80 bA	9.31 bA
	CV (%) = 8.44			

Means followed by the same lowercase letters on the same line and letters in the same column do not differ by Tukey's test (α = 0.05).

Table 3 Post-harvest putrescine, spermidine, spermine, total soluble carbohydrate, total protein content and peroxidase activity in leaves of *Chrysanthemum* 'Faroe', as a function of thermal conditioning.

Conditioning	Chrysanthemum leaves, post-harvest (days)			
	4	8	12	16
	Putrescine (μg g^{-1} fresh weight)			
Environment (25.2°C)	33.35 aB	20.14 bB	17.81 bB	7.87 cB
Refrigerated (10°C)	50.18 aA	31.02 bA	30.44 bA	26.74 bA
	CV (%) = 4.06			
	Spermidine (μg g^{-1} fresh weight)			
Environment (25.2°C)	85.46 aA	78.51 aA	40.27 bB	35.83 bB
Refrigerated (10°C)	69.56 aB	54.56 bB	52.97 bA	48.90 bA
	CV (%) = 5.26			
	Spermine (μg g^{-1} fresh weight)			
Environment (25.2°C)	130.80 aA	134.09 aA	120.55 bA	104.97 cA
Refrigerated (10°C)	124.05 aA	100.16 bB	88.16 cB	80.83 cB
	CV (%) = 7.73			
	Peroxidase (μmol H_2O_2 decomposed. min^{-1}. g^{-1} fresh weight)			
Environment (25.2°C)	1.9 10^{-3} dA	2.6 10^{-3} cA	3.9 10^{-3} bB	6.1 10^{-3} aA
Refrigerated (10°C)	2.1 10^{-3} cA	2.2 10^{-3} cB	4.3 10^{-3} bA	5.7 10^{-3} aB
	CV (%) = 6.94			
	Total soluble carbohydrate (%)			
Environment (25.2°C)	18.59 aA	18.21 aA	15.76 bA	12.87 cA
Refrigerated (10°C)	18.70 aA	18.55 aA	15.90 bA	13.00 cA
	CV (%) = 2.89			
	Total protein (%)			
Environment (25.2°C)	8.54 aA	8.48 aA	6.54 bA	5.67 bA
Refrigerated (10°C)	8.66 aA	8.50 aA	7.00 bA	5.52 cA
	CV (%) = 3.40			

Means followed by the same lowercase letters on the same line and letters in the same column do not differ by Tukey's test (α = 0.05).

Table 4 Post-harvest putrescine, spermidine, spermine, total soluble carbohydrate, total protein content and peroxidase activity in flowers of *Chrysanthemum* 'Faroe', as a function of thermal conditioning.

Conditioning	Chrysanthemum flowers, post-harvest (days)			
	4	8	12	16
	Putrescine (μg g^{-1} fresh weight)			
Environment (25.2°C)	51.71 aA	49.00 aA	38.23 bA	41.46 bA
Refrigerated (10°C)	53.59 aA	31.02 bB	37.99 bA	32.57 bB
	CV (%) = 3.12			
	Spermidine (μg g^{-1} fresh weight)			
Environment (25.2°C)	94.89 aA	78.61 bA	40.27 cA	35.59 cB
Refrigerated (10°C)	100.79 aA	39.48 bB	21.21 cB	31.49 bA
	CV (%) = 5.91			
	Spermine (μg g^{-1} fresh weight)			
Environment (25.2°C)	168.98 aA	138.09 bA	89.79 cB	84.41 cB
Refrigerated (10°C)	163.07 aA	141.22 bA	106.21 cA	101.52 cA
	CV (%) = 6.23			
	Peroxidase (μmol H_2O_2 decomposed min^{-1} g^{-1} fresh weight)			
Environment (25.2°C)	1.6 10^{-3} dA	2.2 10^{-3} cA	3.6 10^{-3} bA	5.7 10^{-3} aB
Refrigerated (10°C)	1.8 10^{-3} dA	2.1 10^{-3} cA	3.8 10^{-3} bA	6.3 10^{-3} aA
	CV (%) = 4.88			
	Total soluble carbohydrate (%)			
Environment (25.2°C)	24.54 aA	22.63 bA	19.22 cA	15.93 dA
Refrigerated (10°C)	24.77 aA	22.75 bA	19.43 cA	16.15 dA
	CV (%) = 3.55			
	Total protein (%)			
Environment (25.2°C)	11.42 aA	11.08 aA	9.64 bA	8.27 bA
Refrigerated (10°C)	11.51 aA	11.23 aA	10.06 aA	6.83 bB
	CV (%) = 5.63			

Means followed by the same lowercase letters on the same line and letters in the same column do not differ by Tukey's test (α = 0.05).

cence, inducing changes in POX activity (Khader *et al*. 1998), but this did not occur in the present work. Senescence was observed in all leaves and inflorescences treated with GA_3, independently of its concentration. Increased POX activity in *Chrysanthemum morifolium* petals during senescence was related to a defense mechanism against oxidizing molecules that can promote membrane damage (Bartoli *et al*. 1997). During the senescence of gladiolus, Hossain *et al*. (2006) reported that increased levels of H_2O_2 could represent a programmed regulation of the activity of POX enzymes, which seems to be a prerequisite for the onset of senescence of petals. In the present work we observed a decrease in the levels of PAs with sample storage time, which could be due to the action of PA oxidases concomitantly producing H_2O_2. This last substance is then reduced to water by POX (Cona *et al*. 2006). The production of H_2O_2 by PAs oxidation can stimulate the observed POX activity. According to Costa *et al*. (2005), the increase in POX activity could be an aim to eliminate H_2O_2, thereby decreasing the formation of hydroxyl radicals, thereby preventing possible cellular damage.

Significant differences in POX activity in leaves and flowers were noticed, this parameter continuously increasing during the lifetime of both organs. The results showed an increase in enzyme activity after harvest, becoming more pronounced with the onset of senescence (12 days) (**Tables 3, 4**). POX activity was not enhanced at room temperature or cold storage. In the same period, no symptoms of chilling injury occurred on inflorescences. Significant differences were noted only in the last days of observation in refrigerated samples. During the storage period a significant increase of POX activity was observed.

Regarding the total soluble carbohydrate content, no effect of GA_3 application during the pre-harvest period was noted, and a general decrease in total carbohydrate content with the lifetime of leaves and flowers was observed (**Tables 1, 2**). No significant differences attributable to cold storage conditions were noticed, considering the postharvest storage time of chrysanthemum leaves and flowers (**Tables 3, 4**).

Considering the sink-source concept, leaves are generally considered the source of assimilates, and the sink any other part of the plant, such as flowers (inflorescences). The re-distribution of sugars has been reported in some crops. Generally, the carbohydrate content in excised flowers is limited, and competition for these substances during flower development may occur.

The decrease in total carbohydrates, regardless of the treatment used, can also be attributed to plant respiration, increasing during senescence, to produce energy for maintaining living cells (Siedow and Day 2001).

Furthermore, the data in **Tables 1** and **2** show no significant influence of GA_3 treatment on pre-harvest levels of total protein in chrysanthemum. Only a decrease in protein content with sample lifetime can be noticed.

Probably, spraying gibberellin directly into the vase or onto the inflorescences (Kim and Miller 2008) could have prolonged the plant lifetime. Similar results can be described for stems subjected to low temperature treatment (**Tables 3, 4**). Also in this case, no effect of temperature on protein content was observed. This parameter decreased with senescence and there was significant variation in inflorescences only in the last days of observation. These results confirm the data of Sood *et al*. (2006), who noted that the protein content of roses was higher in young plants. In petals of *Dendrobium* cv. 'Khao Sanan' (Lerslerwong *et al*. 2009) there was a decrease in protein content during senescence. The decrease in protein content can be attributed to senescence of organelles and degradation of macromolecules (Hörtensteiner 2006).

CONCLUSIONS

Some conclusions can be drawn from the results obtained during the present work. The application of GA_3 in the field did not reduce or retard the senescence process in chrysanthemum 'Faroe' inflorescences, independently of the postharvest temperature treatment. Stems stored at room temperature showed a decrease in protein and carbohydrate con-

tent both in leaves and flowers. Furthermore, an increase of POX activity in stems was observed during storage. Interestingly, application of GA_3 alters the levels of PAs. The content of Spd and Spm were higher than that of Put and their concentrations were even higher in leaves than in inflorescences.

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