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Weight loss and antioxidants degradation in spears of five asparagus cultivars during cold and freeze storage

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Abstract

Asparagus (*Asparagus officinalis* L) is considered as a vegetable rich in antioxidants. The present study investigated and compared the weight loss and degradation of antioxidants including ascorbic acid (ASC), glutathione (GSH) and cysteine (CYS) in spears of five asparagus cultivars (Bonlim, Eposs, Mars, Thielim and Vulkan). Spears were stored either under cold storage at 4 °C for 12 days or were deep frozen at - 20 °C for 12 months. The results showed that there are significant differences among asparagus cultivars with respect to their weight loss and antioxidants content of ASC, GSH and CYS. Weight loss and degradation of antioxidants were increased with increasing storage duration. At the end of 12 days period of cold storage, the cultivar Eposs showed the highest weight loss with 3.14%, while for the cultivar Vulkan, the lowest weight loss with 2.14% was recorded. When spears were deep frozen, the weight loss was lower than that recorded under cold storage. After 6 months of storage, the weight loss of all cultivars was below 2% except that for Mars and Vulkan. Under cold storage, The ASC and GSH content of spears decreased by 15 – 40% and 13 – 42%, respectively, in all cultivars after 8 days while 15 - 35% of the CYS content was lost over 4 days. Under conditions of deep freezing, the ASC, GSH and CYS content decreased by 12 - 31%, 11 – 21% and 17 -25% respectively, in all cultivars after 6 months. The cultivar Vulkan showed the highest degradation of antioxidants while the cultivar Mars showed the lowest values. The results revealed that cultivar, storage condition and period of storage are important factors influencing the quality of asparagus spears. Deep freezing at – 20 °C proved to be a superior method to cold storage at 4 °C for preserving asparagus content of antioxidants.

Keyword: Ascorbic acid, Glutathione, Systeine, White asparagus.
Abbreviations: ASC_ascorbic acid; CYS_cysteine; GSH_glutathione; HPLC_High performance liquid chromatography.

Introduction

Asparagus (*Asparagus officinalis* L.) is a high-priced premium vegetable with organoleptic features and beneficial nutritional characteristics. Asparagus is a good source of essential nutrients, vitamins and dietary fibers (Shalaby et al., 2004; Gebczynski, 2007; Sun et al., 2007). In addition, asparagus is one of richest vegetables with a view to total quantity and quality of antioxidants (Vinson et al., 1998; Pellegrini et al., 2003). With regard to an increasing consumer awareness for a balanced diet, these characteristics may contribute to enhancing its cultivation and consumption. However, asparagus is also one of the most perishable which needs to be consumed rapidly within 2 - 5 days after harvesting. It deteriorates fast because of its highly active metabolism as reflected by its high respiration rate.Postharvest deterioration of asparagus goes along with physiological changes such as an increased reduction of vitamin C, total solides, soluble carbohydrates, water content (Chang, 1987) and protein and amino acids (Lill et al., 1990; Sun et al., 2007). Consequently, these modifications will unfold a great influence on the quality of the commercialized fresh product. Consumers are increasingly interested in vegetables with a high content of health promoting compounds and some amino acids are attributed anti-oxidative properties (Martinez-Tome et al., 2001; Katayama and Mine, 2007).

Asparagus has a relatively short harvest time (60 – 65 days) while the consumer demand continues all year round so that storage would greatly alleviate peak production problems (Drake and Lambert, 1985). Thus, storage of asparagus during peak harvest times for later distribution to the fresh and processing markets is a major interest of the asparagus processing industry. Time in refrigerated storage usually accounts for the time that is required for delivering asparagus to either fresh or processing markets. Deep freezing has been reported to preserve food quality, too (Feher, 1994). Quality of asparagus must be maintained over this period of time if the consumers are to be satisfied with the product. The present study reports on weight loss and degradation of important antioxidants such as ascorbic acid (ASC), glutathione (L-glutamyl-L-cysteinyl-glycine, GSH) and cysteine ([(2*R*)-2-amino-3-sulphanyl-propanoic acid], CYS) in five asparagus cultivars as affected by storage duration and

Procedure

Results and discussion

Weight loss

Asparagus spears lost weight continuously with storage time at a rate dependent on cultivar and storage condition (Table 1). Under cold storage, a sharp increase of the weight loss was recorded after 10 days of storage for all cultivars tested. At the end of 12 days period of storage, the cultivar Eposs showed the highest weight loss with 3.14%, while for the cultivar Vulkan, the lowest weight loss with 2.14% was

Table 1. Relative weight loss (%) in five asparagus cultivars under cold and freeze storage. [Z] Asparagus spears were stored under cold storage at 4 °C for 12 days or deep freezing at - 20 °C for 12 months. [W]Relative weight loss (%) was calculated on basis of the fresh weight at harvest (day 0). [X] Mean separation within columns by Duncan's multiple range test at 5% level. [y] NS, *, **, *** not significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively.

Storage method	Cultivar[W]	Period of storage[Z]					
		2	4	6	8	10	12
Cold	Bonlim	1.28 ab[X]	1.44 b	1.60 abc	1.80 b	2.05 b	2.28 bc
	Eposs	0.79 e	1.06 f	1.14 d	1.29 c	1.52 de	3.14 ab
	Mars	1.07 bcd	1.14 def	1.30 bcd	1.64 b	1.88 bc	2.16 bc
	Thielim	0.90 de	1.19 cdef	1.41 bcd	1.70 b	1.80 bcd	2.64 abc
	Vulkan	0.78 e	1.11 ef	1.24 cd	1.32 c	1.66 cd	2.14 bc
Freeze	Bonlim	1.11 bc	1.49 b	1.51 bc	1.54 bc	1.55 de	1.58 c
	Eposs	1.27 ab	1.30 c	1.53 abc	1.66 b	1.80 bcd	1.85 c
	Mars	1.37 a	1.79 a	1.87 a	2.16 a	2.71 a	3.05 ab
	Thielim	1.05 cd	1.24 cde	1.30 bcd	1.32 c	1.29 e	1.53 c
	Vulkan	1.25 ab	1.26 c	1.62 ab	2.11 a	2.55 a	3.43 a
Significance [y]							
Storage method (SM)		***	***	**	**	**	NS
Cultivar (C)		**	***	NS	***	***	NS
SM × C		***	***	*	***	***	**

recorded. When spears were deep frozen, the weight loss was lower than that recorded under cold storage. After 6 months of storage, the weight loss of all cultivars was below 2% except that for Mars and Vulkan. Consequently, after 12 months of freezing for the cultivars Mars and Vulkans, the highest weight loss values of 3.05% and 3.43%, respectively, was determined. The weight loss of asparagus spears after harvest is mainly caused by a loss of moisture. It has a significant effect on the spear quality as it strongly influences its appearance (King et al., 1988). According to Robinson et al. (1975), a weight loss of 8% makes spears unmarketable. In addition, the shelf-life of asparagus has been shown to decrease linearly with the increase of storage period and accumulated heat-units between harvest and post-transport handling (Lill, 1980; King et al., 1988). Weight loss could be reduced by maintaining higher humidity during storage whereby a sufficiently high humidity of 98 – 100% will be required in conventional cooling chambers. In the present study, the storage temperature significantly influenced the weight loss of asparagus spears. It has been reported that temperature influences many deteriorative processes (Esteve et al., 1995; Barry-Ryan and O'Beirne, 1999). The relative weight loss proved to be dependent on cultivar and period of storage. The latter two factors were also reported to affect the marketable shelf-life of asparagus significantly (King et al., 1986).

Degradation of ascorbic acid (ASC)

ASC was gradually degraded during the period of storage in relation to cultivar and storage method (Table 2). Under cold storage, the cultivars Eposs and Vulkan recorded the highest degradation of about 50%. The ASC content decreased by15 – 40% in all cultivars after 8 days. Under conditions of deep freezing, the ASC content of spears decreased by 12 - 31% after 6 months and 45 – 57% by the end of 12 months in relation to the cultivar. It is interesting to note that degradation processes were lowest in the cultivar Mars during the entire storage period under conditions of cold storage and deep freezing when compared to other cultivars. .In contrast, ASC was degraded most strongly in the cultivar Eposs.ASC is an essential vitamin for plants, animals and humans and the ASC content of vegetables one quality criterion. ASC is labile and its preservation is regularly investigated when evaluating postharvest storage effects on the nutritional quality of vegetables (Barth et al., 1993). Substantial loses of ASC could be nutritionally relevant in particular for vegetarians who consume large amounts of vegetables. In general, the loss of ASC is useful as an index of oxidative deterioration. It has been reported that asparagus spears lost 55% of their ASC content after 6 weeks at 6 ˚C (Lill, 1980) and up to 64% after 21 days of storage at 2 ˚C (Drake and Lambert, 1984). In the present study, cultivar and storage method significantly influenced the degradation of ASC. A maximum loss of 50% was recorded by Eposs and Vulkan under cold storage and 57.18% recorded by Bonlim under deep freezing.

Degradation of glutathione (GSH) and cysteine (CYS)

The degradation of the thiols GSH and CYS in asparagus were significantly influenced by storage conditions and cultivar. After 12 days of cold storage, the GSH content was reduced by 50.53 and 47.95, respectively in the cultivars Vulkan and Bonlim , while the corresponding values for Mars was as low as 20.8%. Under freeze storage, only 35% of the GSH was degraded after 8 months. Subsequently, a sharp increase in GSH degradation of another 30% was recorded after 12 months (Table 3).

After 12 days cold storage, Vulkan and Thielim showed the highest CYS degradation of 68.82% and 66.45%, respectively, while Mars had the lowest value at 51.53%. Under freeze storage, Thielim also recorded the highest CYS degradation of 49.82% while Eposs and Mars recorded the lowest values of 32.22% and 32.97%, respectively. The results showed that 35% of the CYS content was lost over 4 days of cold storage which indicates that CYS is quickly degraded. The cultivar Theilim showed the highest degradation rate while Mars showed the lowest under cold storage and freeze storage (Table 4). Thiols, such as GSH and CYS, are highly important antioxidants that protect cells from oxidative damage (Sen and Packer, 2000; Wlodek, 2002). The significance of GSH for the maintenance of good health, detoxification, and cancer prevention in the human body has been addressed more frequently in recent years. Extensive reviews have been written about GSH and its multiple functions (Mullineaux and Rausch, 2005; Yeung et

al., 2005; Dewir and Paek, 2011). Its physiological roles include storage and transport of CYS, plus a coenzymatic role in several reactions with foreign compounds (Meister and Anderson, 1983). CYS and GSH delivery compounds have been used to protect normal cells from antitumor agents and radiation (Neal et al., 2003). CYS is generally recognized

Table 2. Relative degradation (%) of the ascorbic acid content in five asparagus cultivars under cold and freeze storage. [Z] Asparagus spears were stored under cold storage at 4 °C for 12 days or deep freezing at - 20 °C for 12 months.[W]Ascorbic acid content of Bonlim, Eposs, Mars, Thielim and Vulkan at harvest (day 0) was 15.4, 18.0, 12.3, 15.0 and 15.6 µmol g-1 FW, respectively. [X] Mean separation within columns by Duncan's multiple range test at 5% level. [y] NS, *, **, *** not significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively.

Storage method	Cultivar[W]	Period of storage[Z]					
		2	4	6	8	10	12
Cold	Bonlim	10.54 de[X]	16.21 d	17.99 cd	20.12 ef	29.08 e	41.37 d
	Eposs	28.77 a	29.53 a	34.53 a	40.53 b	44.28 bc	50.05 b
	Mars	3.32 f	5.48 e	12.80 e	15.78 f	26.69 e	30.93 e
	Thielim	16.35 cd	23.17 bc	26.63 b	30.29 d	31.74 de	40.73 d
	Vulkan	22.35 d	25.84 ab	30.83 ab	33.40 cd	43.42 c	50.06 b
Freeze	Bonlim	13.29 de	19.15 cd	19.93 c	25.10 e	49.79 abc	57.18 a
	Eposs	24.09 ab	25.75 ab	31.35 ab	52.47 a	50.84 ab	56.43 a
	Mars	4.09 f	4.99 e	14.66 de	36.63 bc	36.22 d	45.08 c
	Thielim	8.47 ef	10.48 e	12.10 e	35.93 bc	49.19 abc	50.40 b
	Vulkan	19.88 bc	25.56 ab	26.98 b	38.32 bc	51.44 a	54.63 a
Significance [y]							
Storage method (SM)		NS	*	**	***	***	***
Cultivar (C)		***	***	***	***	***	***
SM × C		NS	**	***	***	*	***

Table 3. Relative degradation (%)of the glutathione content in five asparagus cultivars under cold and freeze storage. [Z] Asparagus spears were stored under cold storage at 4 °C for 12 days or deep freezing at - 20 °C for 12 months. [W] Glutathione content of Bonlim, Eposs, Mars, Thielim and Vulkan at harvest (day 0) was 20.4, 12.8, 15.3, 17.1 and 22.1 µmol g-1 FW, respectively. [X] Mean separation within columns by Duncan's multiple range test at 5% level. [y] *, **, *** significant at $P \leq 0.05$, 0.01 and 0.001, respectively.

Storage method	Cultivar[W]	Period of storage[Z]					
		2	4	6	8	10	12
Cold	Bonlim	16.38 b[X]	26.76 b	31.46 b	39.97 ab	46.74 d	47.95 c
	Eposs	8.03 cd	12.79 c	13.47 cd	22.95 cde	33.75 e	35.71 d
	Mars	2.15 d	11.04 c	13.91 cd	15.35 de	18.61 g	20.82 f
	Thielim	6.47 cd	8.32 c	14.28 cd	19.42 de	27.29 f	29.49 e
	Vulkan	33.70 a	37.58 a	42.13 a	45.63 a	49.22 cd	50.53 c
Freeze	Bonlim	3.28 cd	7.18 c	15.6 cd	33.2 bc	62.2 a	66.5 a
	Eposs	3.37 cd	8.54 c	11.2 d	13.3 e	58.2 ab	61.5 b
	Mars	7.34 cd	13.6 c	16.5 cd	22.1 de	29.4 ef	32.7 de
	Thielim	3.95 cd	12.6 c	20.6 c	26.1 cd	53.4 bc	57.8 b
	Vulkan	11.1 bc	14.3 c	20.2 c	22.1 de	58.6 ab	60.6 b
Significance [y]							
Storage method (SM)		***	***	***	*	***	***
Cultivar (C)		***	***	***	***	***	***
SM × C		***	***	***	**	***	***

as an important precursor to sulphur-containing flavour compounds in food products and a source of sulphur in the production of natural and process flavours. The thiol group in CYS is nucleophilic, easily oxidized and forms the reaction centre. According to Garlick and Reeds (1993), cystine, oxidative product of CYS, is one of the most sensitive amino acids to damage during the processing or storage of food products. Asparagus is considered a vegetable which is rich in GSH and CYS. Among 32 vegetables, asparagus showed the second highest GSH and the eighth highest CYS content (Mills et al., 1997). The dietatry intake of GSH and CYS by vegetables is rated as being more efficient and less costly than oral supplements. The high levels of thiols in asparagus make this vegetable a suitable source for a fortified supplement with GSH and CYS thus taking full advantage of the protective and therapeutic effect of these constituents.

Materials and Methods

Plant material and sample preparation

Asparagus (*Asparagus officinalis* L) spears of five cultivars (Bonlim, Eposs, Mars, Thielim and Vulkan) were harvest manually and trimmed to a uniform length of 18 cm. Only straight undamaged spears with closed bracts and with no obvious symptoms of disease were used. Spears were hydro-cooled one hour after their harvest through immersion in a bath of iced water for 10 min until the temperature of the spears dropped to 1°C. These spears were stored flat either under cold storage at 4 °C for 12 days or deep freezing at - 20 °C for 12 months. Stored spears were sampled in triplicates every 2 days from cold storage and every 2 months from deep freezing. Relative weight losses were determined and then

samples were frozen in liquid nitrogen and stored at – 70 °C for analysis of ASC, GSH and CYS.

Table 4. Relative degradation (%)of the cystein content in five asparagus cultivars under cold and freeze storage. [Z] Asparagus spears were stored under cold storage at 4 °C for 12 days or deep freezing at - 20 °C for 12 months. [W]Cystein content of Bonlim, Eposs, Mars, Thielim and Vulkan at harvest (day 0) was 5.9, 7.0, 10.2, 12.3 and 5.7µmol g-1 FW, respectively.[X] Mean separation within columns by Duncan's multiple range test at 5% level. [y] NS, *, **, *** not significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively.

Storage method	Cultivar[W]	Period of storage[Z]					
		2	4	6	8	10	12
Cold	Bonlim	2.67 d[X]	15.16 b	26.72 b	38.81 b	54.96 ab	59.62 abc
	Eposs	10.86 cd	17.23 b	19.64 bc	38.95 b	55.10 ab	58.42 bc
	Mars	6.00 cd	16.09 b	22.02 bc	23.80 d	49.15 bc	51.53 cd
	Thielim	19.58 ab	34.03 a	40.81 a	55.02 a	64.54 a	66.45 ab
	Vulkan	24.58 a	31.28 a	38.12 a	57.57 a	64.10 a	68.82 a
Freeze	Bonlim	14.63 bc	15.31 b	23.25 bc	26.76 cd	35.42 de	39.07 ef
	Eposs	7.469 cd	13.52 b	17.22 c	22.5 d	27.92 e	32.22 f
	Mars	11.46 bcd	16.85 b	22.11 bc	27.48 cd	28.74 e	32.97 f
	Thielim	6.536 cd	17.51 b	23.78 bc	30.41 c	40.54 cd	49.82 cd
	Vulkan	8.602 cd	15.67 b	24.79 b	37.51 b	42.43 cd	44.00 de
Significance [y]							
Storage method (SM)		NS	***	***	***	***	***
Cultivar (C)		*	***	***	***	***	***
SM × C		***	**	**	***	NS	NS

Quantification of ascorbic acid (ASC)

One hundred gram of spears was blended with 100 ml oxalic acid (6%) for 5 min. using an electric mixer. Afterwards, the mixture was filtered and 20 ml of the filtrate were taken and made up to 100 ml with 3% oxalic acid. Finally, ASC (mg 100 g^{-1}) was determined employing the 2, 6-dichlorophenol indophenol visual titration method according to AOAC (1970).

HPLC quantification of glutathione (GSH) and cysteine (CYS)

GSH and CYS were extracted and determined according to Hell and Bergmann (1990). Thirty milligram of fine ground freeze-dried plant material were dissolved with 1 ml of 0.1 M HCl containing 4% PVP (Polyvidon-25) and homogenized in 1.5 ml Eppendorf tube. The samples were homogenized 3 - 4 times and kept in a freezer for 15 min. Then, they were centrifuged twice for 5 and 3 min at 14000 rpm at 4 °C and the supernatant was transferred into Eppendorf tubes. DTT (DL-dithiothreitol) was added as a reducing agent prior to adding the fluorescence Bromobimane (Sigma No. B - 4380). Sample preparation for HPLC analysis was conducted in tinted Eppendorf cups (2 ml ambra) to prevent light oxidation of the samples. HPLC was equipped with a fluorescence detector (480 nm). The total sample volume was 270 µl. Instrumental settings of HPLC were as follows: column (Nova-Pak C18, 4µm, 60Å, 4, 6 x 250 mm), flow rate (1.0 ml/min), detection (fluorescence detection, excitation wavelength = 380 nm, emission wavelength = 480 nm), retention time (between 15 and 22 minutes), loop (20 µl) and column heater at 28°C.

Statistical analysis

Experiments were set up in a completely randomized design with three replicates. Data were subjected to ANOVA and Duncan's multiple range test using SAS program (Version 6.12, SAS Institute Inc., Cary, USA).

Conclusion

The results of the present study showed that changes in the quality of asparagus in terms of weight loss and antioxidants content during storage is closely related to cultivar and storage temperature. Generally, the cultivars Vulkan and Eposs showed the highest weight loss and the highest degradation of antioxidants while the cultivar Mars showed the lowest values. Deep freezing proved to be a superior method to cold storage for preserving the antioxidants content in asparagus spears.

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