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Alteration of peroxidase-activity, chlorophyll content and antioxidant-capacity of corn salad (*Valerianella locusta*) during storage

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ABSTRACT

Although many data can be found about the health promoting effects, and nutritional values of leafy vegetables, corn salad (*Valerianellalocusta*) still remained out of interest of food analytic researches. Because it is become more and more popular among the consumers, we aimed to determinate chlorophyll content, antioxidant-capacity changes of intact corn salad leafs during storage at 6°C, 9°C, 12°C for 3, 6, 9 days, and determinate the activities of soluble-, and ionically bound peroxidase enzyme forms as possibly good indicators of plant metabolism. Our results indicate that corn salad possess high amount of chlorophyll, and high antioxidant-capacity, which both decrease by the ongoing storage, and this pattern became more drastic on higher temperatures. Peroxidase-activities show similar trends. Activity of both soluble, and ionically bound form decrease, although soluble form seems to be more sensitive. POX-isoenzyme-activity seems to be good indicator of the plant metabolism rate, and can help to improve storage conditions. From our result the 6°C storage temperature seems to be the best for the unprocessed corn salad leafs to keep it's beneficial values for the consumers.

KEYWORDS

Corn salad;
Chlorophyll;
Antioxidant-activity;
Soluble POX;
Bound POX.

INTRODUCTION

Leafy vegetables are basic components of the human nutrition[1,2]. Their vitamin, dietary fibre[3,4] and element content helps to prolong health in all age[5-7]. Group of leafy vegetables contains many botanically different kind of plant, eg. lettuce (*Lactucasativa*), cabbage (*Brassica oleracea*), rucola (Erucasativa) etc. Many article can be found about their composition and positive effects on human health. Among these scientific papers, there are several one focusing on agricultural aspects e.g. optional condition of breeding and storage of the different species[8-10]. Although corn salad (*ValerianellaLocusta*) also belongs to leafy vegetables, there are only limited information about it, and most of them also discuss production parameters[11-13], and there are very few article about the antioxidant-activity or chlorophyll content changes during the storage. Ferrante

et al. investigated the chlorophyll, total carotenoid and phenolic compound changes during the storage of intact and fresh cut corn salad, and found that the parameters of intact leafs don't change at 4 °C during a 8 day storage, while in cutted leafs chlorophyll and carotenoid contents start to decrease at the 5th day of trial[14]. They also found that the storage temperature has got crucial role on decreasing process, because at 10°C decrease is much more characteristic than at 4°C. They also observed that changes occurred at the 7th day independently from the storage temperature[15].

Chlorophylls, one of the most studied components of vegetables, are antioxidant molecules[16], which can be found in every green plant tissue, and they have got intensive metabolism, which is affected by many environmental factors[17]. Beside chlorophylls, many different molecules give the antioxidant-capacity of plant foods, which parameter express the efficiency of the sample against free radicals[18]. Although there are many type of antioxidant molecules, common property of them is the sensitivity for the environmental factors, thus find optional post harvest circumstances have crucial importance to keep the health promoting property of vegetables. It is a complex task, because plant tissues continue their metabolism after harvesting, hence the anabolic and catabolic reactions of bioactive molecules proceed as well[19], thus instead of searching for the optional circumstances for the bioactive molecules we should rather find the best environmental settings for the still living plant tissue. To determinate the ideal storage conditions, there is needed to find an indicator for the plant metabolism rate, which can show the reaction of the plant to the changes of environmental factors. There are many article discuss this question, and most of these researches use some of the sensitive plant enzyme-activity changes to monitoring the alteration of plant biochemical pathways[20-23]. Peroxidases (POX) became to the focus because they have got many functions during the life process of plants, inter alia protect cells from stress[24]. There are several method to measure peroxidase-activity, and these methods differ from each other quite a lot. Most of them only measure total POX-activity, although there are clear evidence that the soluble enzyme form, which located in the cytosol, preliminary modify the redox homeostasis of the cells, while the ionically cell wall bound form modify the composition of the cell wall itself, hence isoenzymes have got distinct role during tissue development[25,26]. It is interesting, that bound form can dissociate from cell wall, and become soluble enzyme form[27]. Generally such POX-activity changes occurred as a respond to environmental changes e.g. chilling injure[28], infection or other oxidative stress factor[23]. Because of these, monitoring the POX-isoenzyme-activity, chlorophyll content, and antioxidant-capacity changes under different storage condition, can give more information about the ideal storage circumstance of intact corn salad leafs.

EXPERIMENTAL

Plant materials

Corn salad (*Valerianellalocusta*var.*olitoria*L.) samples were brought from a small producer from hydroponic growing system, at the day of harvest. Processing of the plants were executed immediately. Fresh samples were measured, and intact plant leafs for storage trial were put into thermostats at 6-, 12-, 20°C for 3-, 6-, 9 days without any processing, covered with perforated plastic foil. Each time of the measurements, weights of samples were assets, and wane values were calculated.

Measurement of antioxidant power

For the measurements of antioxidant-capacity, DPPH-assay were used as described by Brand-Williams et al.[29]. Homogenized sample was destructed with 96% ethanol contains 20% H_2SO_4, at 70°C for 20 min, than completed to 50 cm^3 with 96% ethanol. 100 µl of this extract were added to 3,9 ml of DPPH (2,2-diphenyl-1-picrylhydrazyl) solution, than kept at dark for 30 min. Absorbance of the pure DPPH solution and the reaction mixture after the incubation time were read at λ=515nm, and inhibition % was calculated.

Measurement of chlorophyll content

For the measurement of Chlorophyll content, 5g sample was homogenised with pure acetone, and absorbance at λ=661.6 and λ=644.8 nm were measured., and chlorophyll content were calculated by the Lichtenthaler formula[30].

Measurements of POX-isoenzyme-activities

Measurements of the activity of soluble and cell wall

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bounded POX enzymes, were executed based on the method described by Tijskens et al[27]. 3g sample were homogenized with 6ml pH8.8 50mM TrisMes buffer contained 1% PVPP, than centrifuged at 2000xg for 15 min. Supernatants were collected, and the activity of soluble form were measured from the supernatant. After a washing cycle, pellet was resuspended with pH8.8 TrisMes buffer contains 0,4M $CaCl_2$, than centrifuged again. Supernatant obtained from this step was used to the measurements of cell wall bound POX-isoenzyme-activity. 50 µl of supernatants were added to 2,7ml pH5.5 50mM TrisMes buffer with 50 µl o-phenilendiamine, and 100µL 1% H_2O_2 as hydrogen donor. Absorbance were recorded for 3 min at λ=420 nm. One unit of enzyme-activity wasdefined as the change in absorption per minute occurred by 1g sample.

Statistical analysis

To analyse the differences of the measured parameters by the storage time and temperature, two-way ANOVA with Bonferronipost test were performed. To analyse that POX-isoenzymes as plant metabolism indicators are capable of predict the antioxidant quality of corn salad or not, we used Pearson correlation analysisas a test of normality (performed according to Kolmogorov–Smirnoff) indicated normal distribution of data. All statistical test were performed at 5% significance level (p=0,05) usingGraphPadPrism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

RESULTS AND DISCUSSION

POX-isoenzyme-activity changes

In the case of POX activity, highest values were detected in fresh corn salad leafs, both in the case of soluble (87 U/g) and bound isoenzyme forms (165.5 U/g). These high values decrease as the storage trial ongoing. Although in fresh samples the cell wall bound enzyme form posses the higher activity, later on the soluble form became dominant. Higher the storage temperature, the higher the decrease of the enzyme activity in the case of both isoenzyme forms. It is correlate with the wane changes (TABLE 1), in which case highest wane could be observed in samples stored at 20°C, while lowest change at the case of 6°C storage. POX-activity results are also in line with others findings, which studies indicate that POX-activity has got crucial role in the accommodation process to stress factors[24,28]. It could be also determinated, that soluble form shows higher rate of change, while the cell wall bound form only show moderate alteration. These results particular match with the results of Tijskens et al., who also found that the Soluble form alter on a higher scale, while bound form much more stable[27].

Results of chlorophyll content measurements

Chlorophyll content changes (Figure 1) show similar patterns as POX-activity alteration. Fresh samples contains ~50µg/mg total chlorophyll content, but as the time passes, leafs start to lose from their chlorophyll

TABLE 1 : Results of different temperatures storage trial of intact corn salad leafs

Storage conditions		Wane (%)	POX-activity (U/g)		Chlorophyll content (µg/mg)		Antioxidant activity (I%) mean ± SD
Time (day)	Temperature (°C)		soluble mean ± SD	Bound mean ± SD	Cl*a* mean ± SD	Cl*b* mean ± SD	
Fresh	-	100.00	87.00 ± 5.20	165.50 ± 28.48	34.26 ± 0.89	13.12 ± 0.11	103.54 ± 2.74
	6	67.16	29.72 ± 1.53 [a,]	22.16 ± 3.59 [a]	24.09 ± 0.92 [a]	9.87 ± 0.09 [a]	75.49 ± 1.91 [a]
3 day	12	61.47	26.74 ± 5.79 [a,]	10.91 ± 3.73 [a,c]	24.41 ± 0.96 [a]	11.27 ± 0.08 [a,c]	64.53 ± 9.25 [a,c]
	20	40.79	15.29 ± 3.83 [a,c,d]	5.71 ± 0.69 [a,c]	15.68 ± 1.00 [a,c,d]	7.14 ± 0.06 [a,c,d]	48.91 ± 3.78 [a,c,d]
	6	53.16	39.39 ± 1.17 [a,b]	16.08 ± 1.16 [a,b]	19.59 ± 1.03 [a,b]	8.23 ± 0.04 [a,b]	63.45 ± 0.75 [a,b]
6 day	12	49.03	16.63 ± 1.31 [a,b,c]	23.54 ± 2.22 [a,b]	15.94 ± 1.07 [a,b,c]	6.36 ± 0.02 [a,b,c]	58.90 ± 6.01 [a]
	20	18.38	7.54 ± 0.49 [a,b,c,d]	15.21 ± 0.72 [a,b]	6.42 ± 1.10 [a,b,c,d]	3.04 ± 0.03 [a,b,c,d]	21.71 ± 8.33 [a,b,c,d]
	6	38.66	29.84 ± 1.21 [a,b]	38.08 ± 0.89 [a,b]	9.66 ± 1.14 [a,b]	3.70 ± 0.02 [a,b]	49.53 ± 2.86 [a,b]
9 day	12	35.83	27.68 ± 1.68 [a,b]	30.37 ± 1.34 [a,b,c]	14.21 ± 1.18 [a,c]	6.93 ± 0.04 [a,c]	50.67 ± 2.02 [a,b]
	20	8.05	14.50 ± 0.17 [a,b,c,d]	23.44 ± 0.50 [a,b,c]	7.36 ± 1.21 [a,d]	4.17 ± 0.06 [a,c,d]	22.81 ± 2.10 [a,c,d]

[a]: p<0,05 vs. fresh sample; [b]: p<0,05 vs. same temperature, pervious time point sample; [c]: p<0,05 vs. same time point 6 °C sample; [d]:p<0,05 vs. same time point 12 °C sample

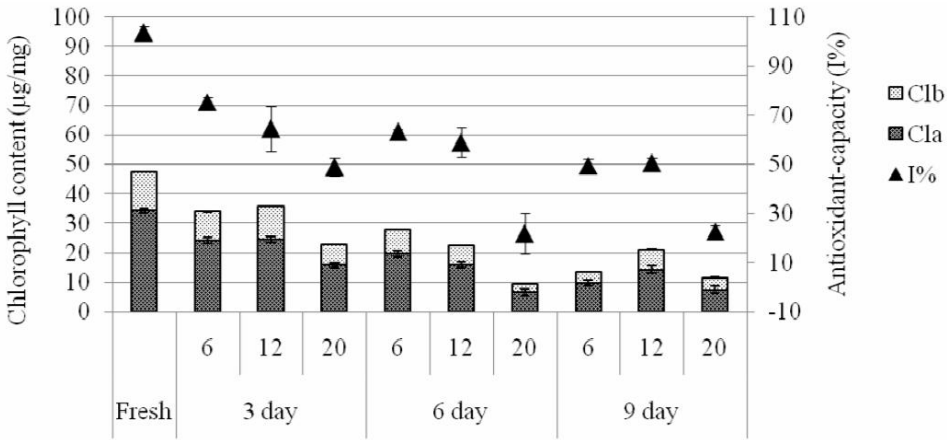

Figure 1 : Chlorophyll content and antioxidant-capacity changes during storage (mean, SD, n=5)

pool, and this deficit ongoing continuously, however higher the temperature, the faster the losing process. It is in agreement with the plant physiological property of chlorophylls[17], and the higher wane values of samples stored at higher temperature, and also is in line with the results of Ferrante et al., with one exception. They also find this change, but in the first part of their experiment there were not any meaningful change, although their experiments were executed on 4°C and 10°C, so distinct results can came from this different experiment settings[14,15]. In our results significant difference can be found between the storage temperatures as well as the storage time. The highest decrease manifested on the 9 day 20 °C storage condition, while the lowest slope of decrease can be described in the case of 6°C storage.

Results of antioxidant-capacity

Antioxidant-capacity show tendentious changes (Figure 1). Highest free radical scavenger values can be found in fresh samples. This initial 103,54 % inhibition decrease rapidly as the storage time increase, and even in the case of the 6°C storage, to the 9th day, it decrease lower than the half of the initial value. At higher temperature this change became more drastic, and 20°C storage time brings 5 fold decrease in this parameter. These results are in agreement with others, who also found that antioxidant-capacity decrease during storage in the case of some vegetable[31,32].

POX-isoenzyme-activities as indicators

To determinate the potential plant metabolism activity rate indicator role of POX-isoenzyme-activities, we analysed the soluble and bound form activity and antioxidant-capacity results with Pearson correlation (Figure 2.). We found that both POX-isoenzyme forms are correlate significantly with the results of DPPH-assay, although soluble form seems to be better ($p=0.0017$), than bound form ($p=0.0309$). It is maybe due to the higher scale of alteration of it's activity during storage. It is in agreement with the results of Tijskens et al.[27], who also observed superior soluble activity change upon bound form in response to blanching treatment.

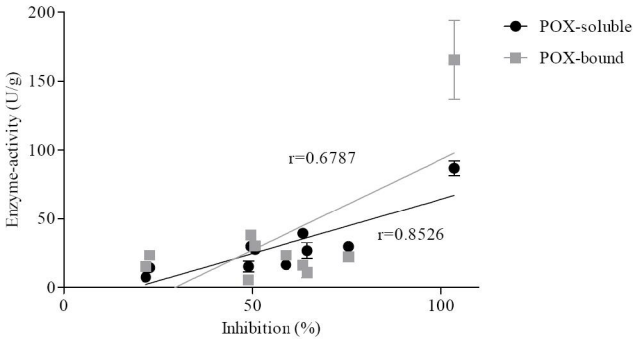

Figure 2 :Correlation of POX-isoenzyme-activities and antioxidant-capacity

CONCLUSION

Our results confirm others data which show high chlorophyll content and antioxidant-capacity in corn salad, as well as drastic changes of these parameters during storage. We also found that higher storage temperature makes the decreasing process faster, and 6°C seems to be the best temperature for the optional circumstance for the intact corn salad leafs. In our experiment, POX-isoenzyme-activities, especially the soluble form proved to be good indicators of plant metabolism rate, and hence the antioxidant-capacity.

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