ISSN : 0974 - 7435 Volume 12 Issue 1

BioTechnology
An Indian Journal

FULL PAPER

BTAIJ, 12(1), 2016 [053-058]

Effects of storage conditions on peroxidase isoenzyme-activities, antioxidant-capacity and chlorophyll-content of white cabbage

Éva Csajbók-Csobod, Barbara Biró, Zsolt Hatvany, Noémi Hegedüs, Csaba Orbán*, Adrienn Lichthammer, Katalin Tátrai-Németh

Department of Dietetics and Nutrition Sciences, Faculty of Health Sciences, Semmelweis University, Vas street 17, H-1088, Budapest, (HUNGARY)

E-mail: orban.csaba@se-etk.hu

ABSTRACT

White cabbage is a popular leafy vegetable in many culture's diet. Although lots of study aimed to investigate the nutritional value changes during food processing, we are in a lack of knowledge about the alterations occurred during short term storage on different temperatures of the minimally processed vegetable. In our current study, we aimed to assess the changes of wane, chlorophyll-content, antioxidant-capacity and peroxidase-isoform-activities of minimally processed white cabbage samples packed to folpack and modified atmosphere packages (MAP) stored on 6°C, 12°C, 20°C for 3, 6, 9 days. Our results indicate that wain and antioxidant-capacity values were altered less rapidly in the case of MAP samples. The cell wall bound peroxidase enzyme form proved to be more stable. Between packaging methods, differences only manifested in the fresh and 3 days stored samples. The MAP proved to be a better method for preservation of the minimal chlorophyll content as well. Correlation analysis refuted our hypothesis that peroxidases are good indicators of stress factors of white cabbage. We can conclude, that white cabbage is characterized by fast changes in it's nutritional values, hence it has got crucial role to keep the optimal storage conditions, even in the case of MAP samples.

KEYWORDS

White cabbage;
Antioxidant-capacity;
Modified atmosphere package;
Peroxidase-activity;
Chlorophyll-content.

INTRODUCTION

White cabbage (B. oleracea L. var. capitata) is a popular vegetable in the gastronomy of many nations. It has got meaningful vitamin C (36mg/100g), and folic acid content (43μg/100g). It's dietary fiber content of approximately 2,5 g/100g also helps to improve the human health[1]. From it's minerals, the relatively high calcium (40mg/100g) and potassium (170mg/100g) contents can be enlisted as the most important ones for health promotion[1]. Besides the vitamins and minerals, glucosinolates can be also found in white cabbage[2], which have got important role in mutagenesis, and hence cancer preventions[3]. Many antioxidant type substances also can be found in this leafy vegetable[4] e.g. phenolic compounds,

FULL PAPER

carotenoids and tocopherols. One of the most relevant substances are the chlorophylls[5], which are affected by many environmental factors[6]. They have got crucial role, as many of the non-communicable diseases were connected to free radical related alterations of biologically active molecules, e.g. DNA, or functional proteins[7]. Antioxidant type molecules proved to be effective to eliminate the reactive substances, hence they often called the guardians of the appropriate oxidative status of human cells[8, 9]. In food science, researchers often measure the total capacity of a foodstuff to eliminate the free radicals instead of determinate the exact concentration of the different substances, hence they obtain a more complex picture. This often measured parameter called antioxidant-capacity[10].

While there are many studies that investigated the alterations of vitamin content, and antioxidant-capacity of the white cabbage during fermentation[11], or under other food technology processes[12], there are only limited information available from the changes occurred during short term storage of the minimally processed plant on different temperatures. We neither have data about the role of different packaging technologies. It is a huge deficiency, as in salad mixes, or during other food preparation steps, the leafy vegetables stored on different conditions until the utilization. One of the most commonly applied preserving storage method is the modified atmosphere package (MAP)[13], which means altered ratio of the gases of the air inside the plastic cover. In most of the cases, the level of nitrogen increased, while the oxygen level decreased. It helps to improve the storability of the different vegetables[14].

Our pervious study on corn salad also indicated meaningful alterations occurred during short term storage on different temperatures[15].

We are also in lack of knowledge from the factors that affects the nutritional values of the minimally processed white cabbage during storage, although these factors means potential biological target points for plant breeding to develop less sensitive varieties[16]. Others found that enzymes involved in plant metabolism pathways may serve as sensitive indicators of the stress factors in fruits and vegetables[17, 18]. One of the most commonly studied enzymes are the members of peroxidase (POx) family. These enzymes have got many functions, described in other's article[19]. While most of the studies measured the total POx-activity, there are new data that highlight the distinct role of soluble, and cell wall bound POx-isoforms[20, 21]. Indeed, the soluble enzyme form, which located in the cytosol, mainly modify the redox homeostasis of the cells, while the ionically cell wall bound form alters the composition of the cell wall. It is also an interesting observation that cell wall bound form can dissociate from cell wall, and become the part of soluble enzyme form regime[22]. Meaningful POx-activity changes mainly occurred as a respond to environmental changes e.g. chilling injure[23], infection or other oxidative stress factors[18], hence the alteration patterns can serve as a hallmark of the plant tissue condition, thus can help us to find the optimal storage parameters.

Because of the afore mentioned reasons, we aimed to investigate the chlorophyll-content, antioxidant-capacity and POx-isoenzyme-activity alterations of white cabbage during short term storage on different temperatures packed with folpack and MAP.

EXPERIMENTAL

Raw, and modified atmosphere packed white cabbage (B. oleracea L. var. capitata L. f. alba) samples were purchased from commercial trade, hence they represent the vegetable available for consumers.

Raw samples were cleaned, and sliced into 1cm x 5-7cm pieces, similar that can be found in the modified atmosphere products. These freshly cutted samples were divided into 200 gram vials, and covered by folpack. The folpack and modified atmosphere samples were stored in household fridges on 6°C as the declared ideal storage temperature, 12°C, which represents a wrong transfer chain temperature, and 20°C, as an extremely wrong condition for the vegetables. Measurements were carried out from fresh samples, and on the 3rd, 6th, 9th days of storage.

Each time of the measurements, following the removal of surface water, weights of samples were

assets, and wane values were calculated.

Measurements of antioxidant-capacity were performed by the DPPH-assay as described by Brand-Williams et al. The homogenized samples were destructed with 96% ethanol contains 10% H_2SO_4, at 70°C for 20 min, than completed to 50 cm^3 with 96% ethanol. After filtration trough Whatman No.1 filter paper, 100 µl of this extract was added to 3,9 ml of $6x10^{-5}$M DPPH (2,2-diphenyl-1-picrylhydrazyl) solution, than kept at dark for 20 min. Absorbance of the pure DPPH solution and the reaction mixture after the incubation time was read at λ=515nm, and inhibition % (I%) was calculated[24].

To determinate chlorophyll content, 5g sample was homogenized with pure acetone. After shaking on 180 RPM for 10minutes, samples were filtrated trough Whatman No.1 filter paper, and filled to 20 cm^3 with acetone. Absorbances of this extracts were recorded at λ=661.6 and λ=644.8 nm, and chlorophyll-a and -b contents were calculated by the Lichtenthaler formula[25].

Measurements of the activity of soluble and cell wall bounded PO_X enzymes, were executed based on the method described by Tijskens et al[22]. 3g sample were homogenized with 6ml pH8.8 50mM TrisMes buffer contained 1% PVPP, than spined at 2000xg for 15 min. Upper layers was collected, and the activity of soluble form was measured from this supernatant. After a washing cycle, pellet was resuspended with pH8.8 TrisMes buffer contains 0,4M $CaCl_2$, than spined 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 of pH5.5 50mM TrisMes buffer with 50 µl o-phenilendiamine, and 100 µL 1% H_2O_2 as hydrogen donor. Absorbance were recorded for 3 min at λ=420 nm. One unit of enzyme-activity was defined as the change in absorption per minute occurred by 1g sample.

Each of the laboratory measurements were carried out in triplicates.

To analyze the differences of the measured parameters by the storage time and temperature, two-way ANOVA with Bonferroni post hoc test were performed. Comparison of the folpack covered and the modified atmosphere samples values, the Mann-Whitney test was utilized, as the sample size was relatively small (lower than 13), respectively.

For correlation analysis, the Spearman rank test was used as a test of normality (performed according to Kolmogorov–Smirnoff) indicated non normal distribution of data. All statistical tests, and data visualization were performed at 5% significance level (p=0,05) using the Statistica 10 software (StatSoft Inc, Tulsa, OK, USA).

RESULTS AND DISCUSSION

Results of wane calculations

Our results (TABLE 1.) indicate that on higher temperature the wane percentages showed higher values. Between folpack and MAP samples meaningful differences manifested as MAP packages could preserve the water content of white cabbage better. These result are in line with other's observations and the reasons behind the application of MAP[13, 14].

Results of POx-isoenzyme activity measurements

POx-enzyme activities brought the results (TABLE 1.) that both in folpack and MAP samples, the soluble-isoform had got higher activity compare to cell wall bound-isoform's. The only exception is the fresh MAP sample, where the 748,3 U/g value of soluble-isoform was lower than the 21418,7 U/g value of bound-isoform, but from the 3rd day, in this packaging mode the soluble-isoform became dominant as well, respectively. The predominance of soluble-isoform in the investigated samples did not alter during the storage trial, only the difference had been decreasing. The high initial value of the folpack covered white cabbage increased to the 3rd day of storage at 6°C, but later on a continuous decreasing could be observed until the end of storage trial (1184,8 U/g). The outstanding soluble values of MAP samples at the 3rd day stored on 6°C and 12°C compared to pervious time point samples and to the bound-isoform indicated that these samples may responded to some stress factors, e.g. start of pathogen infection[18]. The results that cell wall bound-isoform more stable than the soluble-isoform are in agreement with the result of Tijskens and co-work-

FULL PAPER

TABLE 1 : Wane and peroxidase-activity alterations of samples during storage

Storage conditions		Wane (%)		POx-Soluble isoform (U/g)		POx-Membrane bound isoform (U/g)	
		Folpack	Modified athmosphere	Folpack	Modified athmosphere	Folpack	Modified athmosphere
Time of storage	Temperature of storage			mean±SD	mean±SD	mean±SD	mean±SD
Fresh		0	0	5875,00±68,43	748,27±32,18	475,50±132,87	21418,67±2069,08^{e}
	6°C	51,2	3,44	7143,67±618,51^{a}	811846,22±20644,77^{a,e}	427,49±25,18	242,85±15,15^{a}
3rd day	12 °C	61,16	3,62	5563,33±429,68^{c}	784787,96±3996,54^{a,c,e}	316,58±28,76^{a}	247,41±24,81^{a}
	20 °C	84,30	4,56	2306,96±337,62^{a,c,d}	233,64±28,32^{c,d}	403,49±6,89^{d}	44,86±0,00^{a}
	6°C	76,30	3,50	2605,04±65,36^{a,b}	2122,42±197,04^{b}	288,25±15,03^{a,b}	229,97±3,24^{a}
6th day	12 °C	84,81	5,62	1143,65±26,45^{a,b,c}	1825,21±208,73^{b}	129,60±7,71^{a,b,c}	163,37±8,83^{a}
	20 °C	90,40	19,70	1004,15±31,80^{a,b,c}	278,21±30,32	368,30±8,74^{a}	64,08±5,26^{a}
	6°C	89,4	3,48	1184,85±20,51^{a,b}	3184,72±370,90	151,37±5,80^{a,b}	187,97±7,81^{a}
9th day	12 °C	88,45	10,67	4566,63±25,32^{a,b,c}	1397,68±128,72	507,76±2,34^{b,c}	123,16±8,08^{a}
	20 °C	90,50	21,62	2019,12±3,74^{a,b,c,d}	267,47±12,99	412,04±4,13^{c}	55,87±1,08^{a}

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

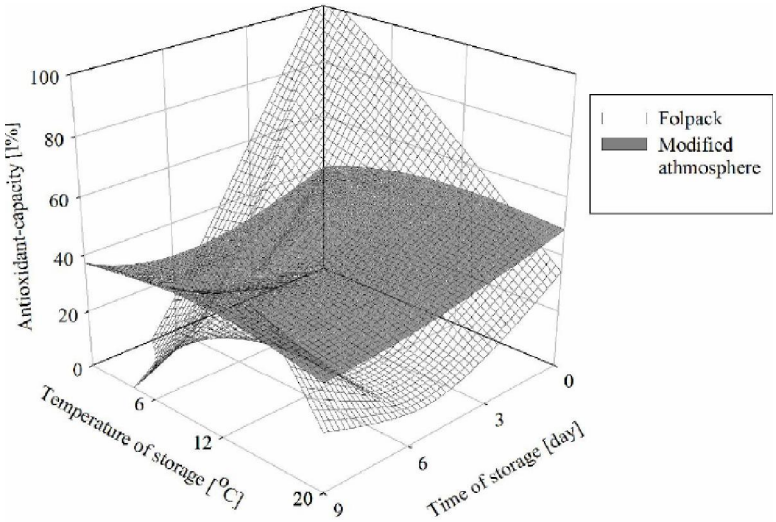

Figure 1 : Distinct antioxidant-capacity changes of folpack and MAP packed samples during the storage

ers, who also reported that bound POx-isoform is less sensitive to heath treatment[22]. Our pervious study with corn salad that applied the same experimental design indicated the higher sensitivity of soluble-isoform as well[15]. The high rate of alterations imply that the enzyme-activity changes are reactions from the plant tissues to the stress[16, 18]. Significant differences between the packing methods only manifested in fresh and 3rd day of storage.

Results of antioxidant-capacity determinations

Our results shown that the alteration patterns of antioxidant-capacity differed between folpack and MAP packed samples (TABLE2.). While the manually chopped white cabbage sample had got the highest value in the dataset (105,03 I%), the MAP sample only had got (40,38 I%) free radical eliminating capacity. But while the MAP samples could maintain this moderate value during the whole storage trial, even in the case of samples stored on 20°C, the folpack packed samples lost their antioxidant molecule regime rapidly (Figure 1.) to only the 20% of the initial value. On higher temperature, the decreases were greater in folpack samples. These results are in line with our pervious results of corn salad[15], and with other's findings with different

TABLE 2 : Chlorophyll-content and antioxidant-capacity changes of samples during storage

Storage conditions		Antioxidant-capacity (I%)		Chlorophyll a (µg/g)		Chlorophyll b (µg/g)	
		Folpack	Modified athmosphere	Folpack	Modified athmosphere	Folpack	Modified athmosphere
Time of storage	Temperature of storage	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD
Fresh		105,03±2,04	40,38±3,13^{e}	0,75±0,12	0,09±0,09^{e}	1,17±0,23	0,22±0,13^{e}
	6°C	50,92±1,61^{a}	40,84±2,27^{e}	0,10±0,03^{a}	1,35±0,03^{a,e}	0,08±0,07^{a}	2,51±0,06^{a,e}
3rd day	12 °C	39,35±1,26^{a,c}	42,11±5,35^{c}	0,10±0,01^{a}	1,58±0,13^{a,e}	0,14±0,02^{a}	2,91±0,25^{a,c,e}
	20 °C	17,29±2,41^{a,c,d}	42,79±1,74^{e}	0,05±0,02^{a}	2,64±0,55^{a,c,d,e}	0,08±0,03^{a}	4,25±0,05^{a,c,d,e}
	6°C	27,47±1,60^{a,b}	27,30±0,27^{a,b}	0,21±0,11^{a}	1,71±0,04^{a,b,e}	0,35±0,18^{a}	3,13±0,07^{a,b,e}
6th day	12 °C	16,81±1,79^{a,b,c}	38,69±1,31^{e}	0,18±0,10^{a}	2,81±0,32^{a,b,c,e}	0,32±0,20^{a}	5,27±0,55^{a,b,c,e}
	20 °C	11,17±3,65^{a,c}	37,97±1,44^{b,c,e}	0,19±0,09^{a}	2,06±0,04^{a,b,c,d,e}	0,35±0,16^{a}	3,78±0,07^{a,b,c,d,e}
	6°C	12,66±15,7^{1a,b}	40,96±1,26^{b,e}	0,18±0,03^{a}	0,87±0,04^{a,b,e}	0,33±0,06^{a}	1,66±0,07^{a,b,e}
9th day	12 °C	49,52±0,91^{a,b,c}	41,72±1,35^{,e}	3,08±0,23^{a,b,c}	1,43±0,01^{a,b,c,e}	5,69±0,43^{a,b,c}	2,69±0,03^{a,b,c,e}
	20 °C	19,98±0,98^{a,b,d}	35,91±3,29^{a,c,d,e}	0,49±0,01^{a,b,c,d}	1,42±0,07^{a,b,c,e}	0,91±0,01^{b,c,d}	2,62±0,12^{a,b,c,e}

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

TABLE 3 : Spearman correlation matrix of the investigated parameters

	Spearman p-values						
	Time of storage [day]	Temperature of storage [°C]	Chlorophyll-a [µg/mg]	Chlorophyll-b [µg/mg]	POx-Soluble isoform [U/g]	POx-Membrane bound isoform [U/g]	Antioxidan capacity [I%]
Time of storage [day]	1	0,206	0,155	0,172	0,227	0,100	0,141
Temperature of storage [°C]	0,294	1	0,356	0,342	0,038	0,109	0,152
Chlorophyll-a [µg/mg]	0,329	0,216	1	< 0,0001	0,700	0,063	0,124
Chlorophyll-b [µg/mg]	0,317	0,223	0,989	1	0,547	0,067	0,180
POx-Soluble isoform [U/g]	-0,282	-0,470	-0,092	-0,143	1	0,020	0,053
POx-Membrane bound isoform [U/g]	-0,379	-0,370	-0,426	-0,420	0,522	1	0,405
Antioxidant-capacity [I%]	-0,342	-0,332	0,355	0,311	0,441	0,195	1

fruits[26], and highlights the sensitivity of antioxidant-type molecules to environmental factors.

Results of chlorophyll-content alterations

Our results (TABLE 2.) indicated that even in fresh samples, only minimal amount of chlorophyll-a, and –b can be found. It is reasonable, as the white color of this vegetable comes from the lack of green pigments. Compare to other leafy vegetables, white cabbage can not enrolled among the good chlorophyll sources[27].

Folpack and MAP samples are distinct in their alteration patterns, as folpack samples were low in their chlorophyll-a, and -b contents, while MAP samples shown increased contents, mainly in the –b molecule form, on the 3rd and 6th days of storage. It is maybe due to loss of water. Even on higher temperature, the amounts of green pigments were lower. It is in agreement with the sensitive characteristics of chlorophylls[6]. Although the difference is small, but we can conclude that MAP could preserve the chlorophyll content in a more effective manner.

Results of correlation analysis

Spearman rank correlation analysis indicated

FULL PAPER

(TABLE 3.) that from storage conditions, the temperature had got significant correlation with the soluble POx-isoenzyme-activity. Interestingly the time of storage do not affects statistically any of the laboratory parameters. The soluble and cell wall bound POx-isoform-activity, and the chlorophyll-a and chlorophyll-b content also correlated. We could not demonstrate any correlation between the investigated parameters and the antioxidant-capacity. It is in contrast with our pervious findings[15], and maybe due to the different expression rate of the enzymes, and the distinct sensitivity of the different species to the same storage conditions.

CONCLUSION

In conclusion, we can state, that the high antioxidant-capacity, and low chlorophyll-content of white cabbage alters meaningfully during the short term storage. These alterations have got temperature dependency, and they are the lowest at the storage temperature of 6°C. Although POx-isoenzyme-activities were sensitive to the storage, but they were did not correlate well neither with chlorophyll-contents, nor antioxidant-capacity in the case of white cabbage, hence we can not declare them as good indicators. Comparison of our current results with the pervious findings and with others data, we can suspect that each of the vegetable species has got unique alteration characteristic patterns of the investigated parameters.

REFERENCES

[1] T.N.A.Library, Release, 27 **(2015)**.
[2] H.E.CiskaKozłowska; European Food Research and Technology, **212**, 5 **(2001)**.
[3] W.M.Jongen; Proceedings of the Nutrition Society, **55**, 1B **(1996)**.
[4] N.Hounsome, B.Hounsome, D.Tomos, Edwards G.Jones; Postharvest Biology and Technology, **52**, 2 **(2009)**.
[5] U.M.Lanfer-Marquez, R.M.Barros, P.Sinnecker; Food Research International, **38**, 8 **(2005)**.
[6] A.Gossauer N.Engel; Journal of Photochemistry and Photobiology B: Biology, **32**, 3 **(1996)**.
[7] T.Devasagayam, J.Tilak, K.Boloor, K.S.Sane, S.S.Ghaskadbi, R.Lele, Japi; **52**, 794804 **(2004)**.
[8] B.Halliwell; The lancet, **344**, 8924 **(1994)**.
[9] M.Valko, D.Leibfritz, J.Moncol, M.T.Cronin, M.Mazur, J.Telser; The international journal of biochemistry & cell biology, **39**, 1 **(2007)**.
[10] D.Huang, B.Ou, R.L.Prior; Journal of agricultural and food chemistry, **53**, 6 **(2005)**.
[11] B.Kusznierewicz, A.Śmiechowska, A.Bartoszek, J.Namieśnik; Food Chemistry, **108**, 3 **(2008)**.
[12] A.Ismail W.Lee; Asia Pacific Journal of Clinical Nutrition, 13, **(2004)**.
[13] R.Ahvenainen; Trends in Food Science & Technology, **7**, 6 **(1996)**.
[14] A.A.Kader, D.Zagory, E.L.Kerbel,C.Y.Wang; Critical Reviews in Food Science & Nutrition, **28**, 1 **(1989)**.
[15] C.Orbán, R.Csajbók, N.Hegedüs, P.Borbély; **(2015)**.
[16] S.D.Tanksley; Plant Molecular Biology Reporter, **1**, 1 **(1983)**.
[17] T.Keller; European Journal of Forest Pathology, **4**, 1 **(1974)**.
[18] J.S.Venisse, M.A.Barny, J.P.Paulin, M.N.Brisset; FEBS letters, **537**, 1 **(2003)**.
[19] F.Passardi, C.Cosio, C.Penel, C.Dunand; Plant cell reports, **24**, 5 **(2005)**.
[20] T.M.Lee, Y.H.Lin; Plant Science, **106**, 1 **(1995)**.
[21] C.C.Lin, C.H.Kao; Plant Science, **160**, 2 **(2001)**.
[22] L.Tijskens, P.Rodis, M.Hertog, K.Waldron, L.Ingham, N.Proxenia, Van C.Dijk; Journal of food engineering, **34**, 4 **(1997)**.
[23] C.Qian, Z.He, Y.Zhao, H.Mi, X.Chen, L.Mao; Journal of the Science of Food and Agriculture, **93**, 3 **(2013)**.
[24] W.Brand Williams, M.Cuvelier, C.Berset; LWT-Food Science and Technology, **28**, 1 **(1995)**.
[25] H.K.Lichtenthaler, C.Buschmann; Current protocols in food analytical chemistry, **(2001)**.
[26] O.Patthamakanokporn, P.Puwastien, A.Nitithamyong, P.P.Sirichakwal; Journal of Food Composition and Analysis, **21**, 3 **(2008)**.
[27] M.V.Agüero, M.V.Barg, A.Yommi, A.Camelo, S.I.Roura; Journal of Food Science, **73**, 1 **(2008)**.