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Effect of the Pasteurization Process on the Contents of Ascorbigen, Indole-3-carbinol, Indole-3-acetonitrile, and 3,3′-Diindolylmethane in Fermented Cabbage

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ABSTRACT: The aim of the study was to investigate the effect of the pasteurization process on the content of ascorbigen, indole-3-carbinol, indole-3-acetonitrile, and 3,3′-diindolylmethane in fermented cabbage. Pasteurization was run at a temperature of 80 °C for 5−30 min. Significant changes were only observed in contents of ascorbigen and 3,3′-diindolylmethane. The total content of the compounds analyzed in cabbage pasteurized for 10−30 min was found to be decreased by ca. 20%, and the losses were due to thermal degradation of the predominating ascorbigen. Pasteurization was found not to exert any considerable effect on contents of indole-3-acetonitrile and indole-3-carbinol in cabbage nor did it affect contents of the compounds analyzed in juice.

KEYWORDS: *Fermented cabbage, sauerkraut, pasteurization, glucobrassicin breakdown products, ascorbigen, indole-3-carbinol, indole-3-acetonitrile, 3,3′-diindolilmethane*

INTRODUCTION

Brassica vegetables, including cabbage, broccoli, cauliflower, or Chinese cabbage, are a natural source of aliphatic aryl and indole thioglycosides, referred to as glucosinolates. Damage of the structure of plant tissue leads to hydrolysis of glucosinolates and the release of a variety of biologically active products with a diverse effect on human health. The type of the released products is affected, among other things, by medium pH, as well as the presence of Fe^{2+} ions and epithio-specific protein (ESP),[1−4] but, most of all, is determined by the chemical structure of maternal glucosinolate. Glucobrassicin, representing indole thioglycosides, is one of the major and often prevailing glucosinolates in *Brassica* vegetables. It was found that direct or secondary degradation products of glucobrassicin, such as indole-3-acetonitrile, indole-3-carbinol, 3,3′-diindolylmethane, ascorbigen, and indolo[3,2-*b*]carbazole, were likely to act as anticarcinogens by decreased carcinogen activation through inhibition of the phase I enzymes, increased detoxification by induction of the phase II enzymes that affect the xenobiotic transformations, as well as inhibition of tumor cell growth and stimulation of apoptosis.[5−10]

Brassica vegetables are consumed either fresh or processed. Their processing involves such treatments as, e.g., peeling, cutting, cooking, blanching, freezing, and, less common, fermentation.

The effect of various conditions of vegetable processing on the content of glucosinolates in the end product has been addressed in several studies. Data are missing, however, on changes in the contents of degradation products in processed vegetables. Although vegetables are mainly consumed after hydrothermal treatment, investigations of boiled, blanched, or pasteurized vegetables have been focused on changes in the contents of non-hydrolyzed glucosinolates.

Results of those investigations have shown that the most popular treatments or processes, i.e., storage, cutting, hydrothermal processing, and freezing, evoke partial reduction of glucosinolates in the end product.[11−14] In turn, fermentation leads to complete degradation of glucosinolates as early as in the initial phase of the process.[15]

Fermentation is usually applied to white cabbage, which, along with most of the *Brassica* vegetables, is characterized by a high content of indole glucosinolates, glucobrassicin in particular.[16] Similar to other glucosinolates occurring in fresh cabbage, glucobrassicin is subject to complete degradation as soon as the initial phase of fermentation, spanning for ca. 10 days.[15] Fermented cabbage contains direct products of glucobrassicin hydrolysis, including indole-3-carbinol, indole-3-acetonitrile, as well as ascorbigen, formed in the reaction of indole-3-carbinol and ascorbic acid,[15,17] and 3,3′-diindolylmethane, a product of acidic condensation of indole-3-carbinol.[18] Apart from the indole compounds, fermented cabbage has been demonstrated to contain nitriles and isothiocyanates released during hydrolysis of aliphatic glucosinolates.[15,19] Studies have shown that the main product of glucosinolate hydrolysis in fermented cabbage is ascorbigen.[15] Its content reaches ca. 14 μmol/100 g of fresh weight (FW), which constitutes ca. 40% of the glucobrassicin content of shredded cabbage used for fermentation. Concentrations of the other compounds is usually lower than 2 μmol.

Fermented cabbage is usually consumed fresh in vegetable salads or is cooked prior to consumption. In our previous study, we have demonstrated that boiling cabbage resulted in a decrease of the total content of the glucobrassicin degradation products.[18] The changes were mainly caused by leaching of ascorbigen predominating in cabbage into cooking water and by its thermal hydrolysis. Ascorbigen losses resulting from thermal hydrolysis accounted for 30% after 10 min of boiling

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Table 1. Content (μmol/100 g of FW) of Glucobrassicin Degradation Products in Non-pasteurized and Pasteurized Fermented Cabbage and Fermented Cabbage Juice

	ascorbigen		indole-3-carbinol		indole-3-acetonitril		3,3′-diindolylmethane		total	
pasteurization time (min)	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
					Fermented Cabbage					
0	9.44[a]		0.141		0.065		0.0105		9.71	
5	8.34[b]	0.22	0.134	0.010	0.061	0.001	0.0088	0.001	8.54	0.23
10	7.94	0.23	0.116	0.008	0.059	0.005	0.0077	0.001	8.08	0.22
15	7.66	0.26	0.120	0.020	0.061	0.001	0.0077	0.001	7.77	0.24
20	7.42	0.62	0.128	0.028	0.063	0.001	0.0076	0.001	7.80	0.67
25	7.90	0.30	0.140	0.012	0.059	0.001	0.0113	0.003	8.11	0.30
30	7.36	0.36	0.140	0.024	0.062	0.002	0.0133	0.005	7.58	0.38
LSD[c]	0.58		0.030		0.005		0.0020		0.60	
					Fermented Cabbage Juice					
0	1.49		0.016		0.014		tr[d]		1.52	
5	1.57	0.23	0.016	0.003	0.015	0.002	tr		1.62	0.24
10	1.60	0.21	0.017	0.002	0.015	0.001	tr		1.65	0.21
15	1.55	0.13	0.016	0.003	0.014	0.000	tr		1.59	0.14
20	1.52	0.09	0.017	0.004	0.014	0.001	tr		1.57	0.09
25	1.37	0.10	0.016	0.001	0.013	0.000	tr		1.42	0.10
30	1.14	0.03	0.016	0.002	0.013	0.001	tr		1.18	0.03
LSD	0.25		0.004		0.004				0.27	

[a]Values are means from three average samples taken from one batch of fermented cabbage. [b]Values are means from three independent experiments (n = 3). [c]Least significant differences at $p \leq 0.05$. [d]Trace, mean of three samples <0.001 μmol/100 g of FW.

and 90% after 60 min of boiling. The decrease in ascorbigen content was accompanied by a drastic increase in the content of 3,3′-diindolylmethane, a condensation product of indole-3-carbinol. After 40 min of boiling, the total content of 3,3′-diindolylmethane in cabbage and cooking water was 6-fold higher than in the non-cooked cabbage.

Fermentation is a traditional method of preserving cabbage, which enables its storage as long as until spring months. The properly conducted fermentation process assures preserving a good quality of cabbage over long-term storage. In practice, however, many commercial producers use pasteurization to further increase its shelf life. Usually, pasteurization is run at a temperature of 60−90 °C. It should be conducted in possibly the lowest temperature and the shortest time to destroy pathogenic microflora and inhibit the activity of detrimental enzymes while simultaneously preserving flavor and nutritive values of the products. However, a number of nutritive and therapeutic constituents, including vitamins, amino acids, and enzymes valuable to health, are lost in that process. Undoubtedly, valuable components occurring in fermented cabbage also include products of glucobrassicin degradation that have been proven to display anticarcinogenic potential.

The aim of this study was to investigate the effect of the pasteurization process on contents of biologically active products of glucobrassicin degradation, i.e., ascorbigen, indole-3-carbinol, indole-3-acetonitrile, and 3,3′-diindolylmethane, in fermented cabbage. Thus far, however, there has been little discussion on that matter, although commercial fermented cabbage is usually a pasteurized food product.

MATERIALS AND METHODS

Fermented cabbage used in the study was obtained through spontaneous fermentation of white cabbage (*Brassica oleracea* L. var. *capitata* cv. Kamienna Glowa), as previously described.[15] The total and volatile acidity of juice accounted for 1.26 and 3.72, respectively, and were consistent with recommendations of the Polish Standard.[20] Taste, aroma, and hardness of shreds were typical and indicative of a good quality of fermented cabbage.

Pasteurization of Fermented Cabbage. Drained fermented cabbage (400 g) and 45 mL of juice drained earlier from cabbage were placed in 0.5 L twist-type jars. The jars were pasteurized for 5, 10, 15, 20, 25, and 30 min in a water bath at a temperature of 80 °C. Once pasteurization has been completed, the samples of cabbage were transferred to a 1000 mL beaker and immediately cooled in a mixture of water and ice. Afterward, the cabbage was filtered off on a Buchner funnel. The filtrate of cabbage was then transferred to a measuring flask, which was filled with water up to the volume of 100 mL. The pasteurization process was repeated 3 times, with the initial material always being averaged fermented cabbage from the same batch.

Standards. Indole-3-carbinol and indole-3-acetonitrile were purchased from Merck, Darmstadt, Germany.

Synthesis of Ascorbigen. Ascorbigen was synthesized from indole-3-carbinol and ascorbic acid according to Kiss and Neukom,[21] as described earlier.[15] High-performance liquid chromatography (HPLC) purity of the compound was determined to be 99.6%.

Synthesis of 3,3′-Diindolylmethane. 3,3′-Diindolylmethane was prepared from indole and formaldehyde according to Buskov et al.,[22] and the structure confirmation of the resultant compound was carried out using the gas chromatography−mass spectrometry (GC−MS) method according to Chevolleau et al.,[23] as described earlier.[18] HPLC purity of the compound was 99.7%.

HPLC Analysis. Ascorbigen, indole-3-carbinol, indole-3-acetonitrile, and 3,3′-diindolylmethane were analyzed with HPLC, as described earlier by Ciska et al.[18] Briefly, duplicate samples of fermented cabbage were homogenized with water. The following solvents were applied for the extraction of individual compounds from cabbage homogenate: ascorbigen, acetone followed by ethyl acetate; indole-3-carbinol, methyl *tert*-butyl ether (TMBE); and indole-3-acetonitrile and 3,3′-diindolylmethane, methylene chloride. Samples of juice were analyzed directly using the HPLC technique, for ascorbigen, or once extracted with respective, aforementioned solvents, for indole-3-carbinol, indole-3-acetonitrile, and 3,3′-diindolylmethane. The HPLC analyses were conducted using a RP-18 column, with the elution method with mobile phases in the gradient system: ascorbigen, 10% acetonitrile in 0.1 M ammonium acetate buffer (pH 5.7) (A) and 80% acetonitrile in 0.1 M ammonium acetate buffer (pH 5.7) (B); indole-3-carbinol, indole-3-acetonitrile, and 3,3′-diindolylmethane,

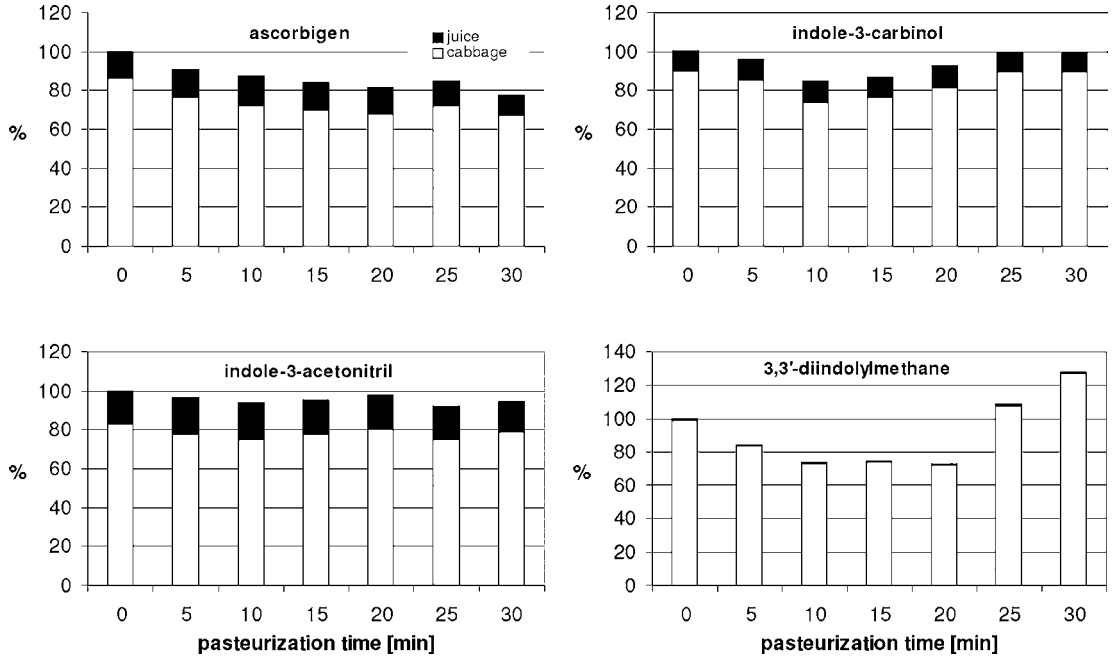

Figure 1. Relative content of glucobrassicin degradation products in pasteurized fermented cabbage and juice expressed as a percentage of their content in non-pasteurized fermented cabbage and fermented cabbage juice.

10% acetonitrile (A) and 80% acetonitrile (B). Ascorbigen was detected with the use of an ultraviolet (UV) detector at UV at $\lambda = 280$ nm, while the other compounds were analyzed with a fluorescence detector at excitation = 285 nm and emission = 340 nm.

Statistical Analysis. Data obtained were subjected to the analysis of variance (ANOVA). Statistically significant differences between the results were tested with the Fisher's protected least significant difference (LSD) test, at $p \leq 0.05$. The statistical analysis was performed using software package StatSoft, Inc., version 8.0, Tulsa, OK.

RESULTS AND DISCUSSION

In the reported study, we have monitored the effect of pasteurization of fermented cabbage on contents of glucobrassicin degradation products, including ascorbigen, indole-3-carbinol, indole-3-acetonitrile, and 3,3′-diindolylmethane. These compounds are synthesized in the early stage of cabbage fermentation, during which glucobrassicin is subject to complete hydrolysis.[15] Indole-3-carbinol and indole-3-acetonitrile are direct products of glucobrassicin hydrolysis. In the acidic medium, indole-3-carbinol readily undergoes further reactions; e.g., its condensation (dimerization) results in the synthesis of 3,3′-diindolylmethane, while ascorbigen is generated upon its reaction with ascorbic acid.

Results referring to contents of ascorbigen, indole-3-carbinol, indole-3-acetonitrile, and 3,3′-diindolylmethane in fermented cabbage and juice, before and after pasteurization, are presented in Table 1. Values provided for juice at "0 min" refer to contents of the compounds examined in juice that was used to pour the cabbage before the pasteurization process. Hence, this study is the first ever to show that products of glucobrassicin degradation occur not only in fermented cabbage but also in juice produced during fermentation.

In cabbage intended for pasteurization, ascorbigen appeared to be the predominating compound. Its content reached ca. 9.5 μmol/100 g of FW and was 90 times higher than that of indole-3-carbinol, being a precursor of ascorbigen. In turn, the content of 3,3′-diindolylmethane was 13 times lower than that of the free monomer. Both contents and ratios of the compounds analyzed in the cabbage used for pasteurization are consistent with results obtained in our previous studies,[15,18] as well as with findings published by Aleksandrova et al.[17] In juice, the ratios of ascorbigen and indole-3-carbinol were similar to those noted in cabbage, whereas 3,3′-diindolylmethane was detected in trace quantities below 0.001 μmol/100 g of FW.

The effect of the pasteurization process on contents of individual compounds in the pasteurized products was diversified. Pasteurization resulted in a decrease in the content of ascorbigen, with the greatest loss of this compound in cabbage (ca. 12%) occurring during the first 5 min of pasteurization (Table 1). The content of ascorbigen in cabbage pasteurized for 5−30 min reached from 80 to 90% (Figure 1).

3,3′-Diindolylmethane content during pasteurization changed to a higher extent. A statistically significant loss of that compound by over 25% was observed already after 10 min of pasteurization (Figure 1). In turn, pasteurization spanning for more than 20 min caused an increase in the mean content of 3,3′-diindolylmethane in cabbage. As a consequence, after 30 min of pasteurization, its content increased by ca. 30% compared to the non-pasteurized cabbage (Figure 1).

The process of pasteurization had no significant effect on contents of indole-3-acetonitrile and indole-3-carbinol in the cabbage nor did it affect contents of the compounds analyzed in juice.

The impact of the pasteurization process on contents of glucobrassicin degradation products in fermented cabbage has never been studied before. Studies with fermented cabbage have mainly been focused on the analysis of glucosinolate degradation products in raw fermented cabbage produced as a result of spontaneous fermentation or the fermentation process with starter cultures. In addition, data are lacking on the contents of glucosinolate degradation products in fresh vegetables subjected to hydrothermal treatment. Owing to those reasons, results achieved in this study will, out of necessity, be discussed only based on findings from our earlier

study addressing the boiling of fermented cabbage.[18] Still, it needs to be emphasized that, in the spontaneously fermented cabbage, there are no non-hydrolyzed glucosinolates.[15]

Generally, tendencies of changes in the concentrations of ascorbigen and 3,3′-diindolylmethane during pasteurization were similar to those observed during cooking fermented cabbage.[18] Both of these processes, i.e., cooking and pasteurization, are hydrothermal processes differing in temperature. Losses of ascorbigen during cooking, because of thermal hydrolysis, accounted for 30% after 10 min of boiling and 70% after 30 min of boiling. The decrease in the ascorbigen content was accompanied by a drastic increase in the content of 3,3′-diindolylmethane, observed as soon as after the first minutes of boiling. In cabbage boiled for 40 min, the increase in the 3,3′-diindolylmethane content was over 5-fold. In the course of the pasteurization process, the losses of ascorbigen did not exceed 20%. It is common knowledge that the rate of a chemical reaction depends upon the temperature. Hence, it may simply be speculated that, during pasteurization, the hydrolysis of ascorbigen proceeds at a lower rate than during cabbage boiling. The slower hydrolysis of ascorbigen resulted in a lower concentration of indole-3-carbinol released from ascorbigen and, as a result, lower gains of 3,3′-diindolylmethane (a product of indole-3-carbinol condensation) observed only at extended pasteurization periods. It is difficult to provide an explicit explanation of the diminished 3,3′-diindolylmethane content within the first 10 min. The elucidation of this phenomenon would require determining the contents of all potential products of indole-3-carbinol condensation and secondary products of 3,3′-diindolylmethane condensation with indole-3-carbinol. The feasibility of the formation of such oligomers, particularly tri- and tetrameters, at pH 3−4 has been indicated by results reported by Grose and Bjeldanes[24] and Buskov et al.[22] The formation of higher indole-3-carbinol oligomers could also explain the tendency for a diminishing content of indole-3-carbinol observed during the first 10 min of pasteurization, despite the decrease of ascorbigen content by as much as 15% (Figure 1).

The different behavior of glucobrassicin degradation products during pasteurization and boiling of fermented cabbage is also due to the specific character of both of those processes. The appropriate temperature of pasteurized cabbage placed in a jar is reached gradually as a result of impaired heat exchange. Therefore, changes in the contents of the analyzed compounds proceeding during pasteurization will be noticed already after a respectively long time, when the whole bulk of cabbage will reach the desired temperature. Both processes, i.e., pasteurization and boiling, also differ in terms of the effectiveness of compound extraction from cabbage. The free immersion of cabbage in boiling water triggers an immediate extraction, being especially effective in the case of the most polar compound, ascorbigen. The extraction-induced loss of this compound to the stock within the first 5 min of boiling accounted for over 60%.[18] In turn, pasteurization of cabbage spanning for even 30 min did not cause any losses of the analyzed compounds as a result of their extraction to juice.

Results obtained in this study demonstrate that pasteurization affected contents of glucobrassicin degradation products in fermented cabbage only to a small extent. The total content of the compounds analyzed in cabbage pasteurized for 10−30 min decreased by ca. 20%, and the losses were due to thermal degradation of ascorbigen predominating in cabbage. Pasteurization conducted even for a relatively long time, i.e., 30 min, and a temperature of 80 °C did not evoke any significant changes in the contents of indole-3-acetonitrile and idole-3-carbinol, although the latter compound was subject to partial dimerization to 3,3′-diindolylmethane at elongated pasteurization periods. The pasteurization of fermented cabbage did evoke statistically significant changes in the contents of the analyzed compounds in juices, although a descending tendency was observed in ascorbigen content.

Pasteurization is the final stage in the industrial production of fermented cabbage; hence, the experimental data presented in this paper for the first time ever and pointing to the stability of glucobrassicin degradation products during that process are of key significance to a consumer, owing to the anticarcinogenic effect of these compounds. It ought to be emphasized, however, that the conditions of pasteurization conducted in our experiment on the laboratory scale differ from the course of industrial pasteurization. Therefore, although promising, the results obtained should be completed with data referring to pasteurization being the final stage of the industrial production of fermented cabbage.

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REFERENCES

(1) Gronowitz, S.; Svensson, L.; Ohlson, R. Studies of some nonenzymic reactions of progoitrin. *J. Agric. Food Chem.* **1978**, *26*, 887−890.

(2) Gil, V.; MacLeod, A. J. The effects of pH on glucosinolate degradation by a thioglucoside glucohydrolase preparation. *Phytochemistry* **1980**, *19*, 2547−2551.

(3) Gil, V.; MacLeod, A. J. Studies on glucosinolate degradation in *Lepidium sativum* seed extracts. *Phytochemistry* **1980**, *19*, 1369−1374.

(4) Cole, R. A. Epithiospecifier protein in turnip and changes in products of autolisis during ontogeny. *Phytochemistry* **1978**, *17*, 1563−1565.

(5) Nugon-Baudon, L.; Rabot, S. Glucosinolates and glucosinolate derivatives: Implications for protection against chemical carcinogenesis. *Nutr. Res. Rev.* **1994**, *7*, 205−231.

(6) Bailey, G. S.; Williams, D. S. Potential mechanism for food-related carcinogens and anticarcinogens. *Food Technol.* **1993**, *47*, 105−118.

(7) Verhoeven, D. T. H.; Verhagen, H.; Goldbohm, R. A.; Van Brandt, P. A.; Van Poppel, G. A review of mechanisms underlying anticarcinogenicity by *Brassica* vegetables. *Chem.-Biol. Interact.* **1997**, *103*, 79−129.

(8) Das, S.; Tuagi, A. K.; Haur, H. Cancer modulation by glucosinolates: A review. *Curr. Sci.* **2000**, *79*, 1665−1671.

(9) Talalay, P.; Fahey, J. W. Phytochemicals from cruciferous plants protect against cancer by modulating carcinogen metabolism. *J. Nutr.* **2001**, *31* (Supplement 11), 3027S−3033S.

(10) Johnson, I. T. Glucosinolates in the human diet. Bioavailability and implications for health. *Phytochem. Rev.* **2002**, *1*, 183−188.

(11) Song, L.; Thornalley, P. J. Effect of storage, processing and cooking on glucosinolate content of *Brassica* vegetables. *Food Chem. Toxicol.* **2007**, *45*, 216−24.

(12) Ciska, E.; Kozlowska, H. The effect of cooking on the glucosinolates content in white cabbage. *Eur. Food Res. Technol.* **2001**, *212*, 582−587.
(13) Fenwick, G. R.; Heaney, R. K.; Mullin, W. J. Glucosinolates and their breakdown products in food and food plants. *Crit. Rev. Food Sci. Nutr.* **1983**, *18*, 123−194.
(14) Gliszczynska-Swiglo, A.; Ciska, E.; Pawlak-Lemanska, K.; Chmielewski, J.; Borkowski, T.; Tyrakowska, B. Changes in the content of health-promoting compounds and antioxidant activity of broccoli after domestic processing. *Food Addit. Contam.* **2006**, *23*, 1088−1098.
(15) Ciska, E.; Pathak, D. Glucosinolate derivatives in stored fermented cabbage. *J. Agric. Food Chem.* **2004**, *52*, 7938−7943.
(16) Ciska, E.; Martyniak-Przybyszewska, B.; Kozlowska, H. Content of glucosinolates in cruciferous vegetables grown at the same site for two years under different climatic conditions. *J. Agric. Food Chem.* **2000**, *48*, 2862−2867.
(17) Aleksandrova, L. G.; Karolev, A. M.; Preobrazhenskaya, M. N. Study of natural ascorbigen and related compounds by HPLC. *Food Chem.* **1992**, *45*, 61−69.
(18) Ciska, E.; Verkerk, R.; Honke, J. Effect of boiling on the content of ascorbigen, indole-3-carbinol, indole-3-acetonitrile, and 3,3′-diindolylmethane in fermented cabbage. *J. Agric. Food Chem.* **2009**, *57*, 2334−2338.
(19) Tolonen, M.; Taipale, M.; Viander, B.; Pihlava, J. M.; Korhonen, H.; Ryhänen, E. L. Plant-derived biomolecules in fermented cabbage. *J. Agric. Food Chem.* **2002**, *50*, 6798−6803.
(20) Polska Norma. *Kapusta Kwaszona PN-72, A77700*.
(21) Kiss, G.; Neukom, H. Über die struktur des ascorbigens. *Helv. Chim. Acta* **1966**, *49*, 989−992.
(22) Buskov, S.; Hasselstrøm, J.; Olsen, C. E.; Sørensen, H.; Sørensen, J. C.; Sørensen, S. Supercritical fluid chromatography as a method of analysis for the determination of 4-hydroxybenzylglucosinolate degradation products. *J. Biochem. Biophys. Methods* **2000**, *43*, 157−174.
(23) Chevolleau, S.; Gasc, N.; Rollin, P.; Tulliez, J. Enzymatic, chemical, and thermal breakdown of 3H-labeled glucobrassicin, the parent indole glucosinolates. *J. Agric. Food Chem.* **1997**, *45*, 4290−4296.
(24) Grose, K. R.; Bjeldanes, L. F. Oligomerization of indole-3-carbinol in aqueous acid. *Chem. Res. Toxicol.* **1992**, *5*, 188−193.