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Impact of Fermentation on Phenolic Compounds in Leaves of Pak Choi (*Brassica campestris* L. ssp. *chinensis* var. *communis*) and Chinese Leaf Mustard (*Brassica juncea* Coss)

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Four different cultivars of Chinese *Brassica* vegetables (two pak choi cultivars and two Chinese leaf mustard cultivars) were fermented according to a traditional Chinese method called pickling. The plant material was investigated before and after the fermentation procedure to determine the qualitative and quantitative changes in its polyphenols. A detailed description of the identified phenolic compounds of leaf mustard by HPLC-ESI-MS^n is presented here for the first time, including hydroxycinnamic acid mono- and diglycosides (gentiobioses) and flavonoid tetraglycosides. Flavonoid derivatives with a lower molecular mass (di- and triglycosides) and aglycones of flavonoids and hydroxycinnamic acids were detected in fermented cabbages compared to the main compounds detected in nonfermented cabbages (tri- and tetraglycosides of flavonoids and hydroxycinnamic acid derivatives of malic acid, glycoside, and quinic acid). During the fermentation process, contents of flavonoid derivatives and some hydroxycinnamic acid derivatives were found to decrease. Some marginal losses of polyphenols were observed even in the kneading step of the plant material prior to the fermentation procedure. The antioxidative potential of fermented cabbages was much higher compared to that of nonfermented cabbages in the TEAC assay, but not observable in the DPPH assay. The increase of the antioxidative potential detected in the TEAC assay was attributed to the qualitative changes of polyphenols as well as other reductones potentially present.

KEYWORDS: Chinese *Brassica* vegetables; polyphenols; kaempferol tetraglycosides; Chinese traditional fermentation; antioxidative potential

INTRODUCTION

Brassica vegetables play an important role in the human diet. In China, cabbages are consumed as fresh or fermented products. In the traditional Chinese fermentation procedure of "pickling," salted cabbage plants are withered, kneaded, and stored under pressure in clay pots for several weeks. The fermentation process is similar to the one used in traditional sauerkraut production from white cabbages, but without starter culture. Several bacteria, particularly lactic acid bacteria, are responsible for the fermentation process resulting in the formation of lactic acid and acetic acid, which causes a decrease in pH (*1–3*).

Due to their phytochemical content, *Brassica* vegetables exert beneficial effects on human health (*4, 5*). Flavonoids present in vegetables and fruits were first shown to play a role in the prevention of coronary heart diseases by researchers in The Netherlands (Zutphen Elderly Study). Fermented Chinese cabbage (Chinese cabbage kimchi) was observed to induce anticancerogenic effects in human gastric and colon cancer cell cultures (*6*).

Recently, Rochfort et al. (*7*) and Harbaum et al. (*8*) described the phenolic composition of different pak choi cultivars. Several polyphenols, such as flavonoid glycosides and hydroxycinnamic acid derivatives, were identified. The major flavonoids in Chinese cabbage are kaempferol and isorhamnetin derivatives (*7, 8*). In leaf mustard, the presence of isorhamnetin-diglucoside was reported (*9*). However, detailed data about the phenolic composition of leaf mustard cultivars are not available in the extant literature.

During the fermentation process of cabbages, the total glucosinolate content decreases (*5, 10*), and ascorbigen, which possesses high antioxidative potential, is formed as the major degradation product of the glucosinolate glucobrassicin (*11*). The ascorbic acid content was found to decrease during the

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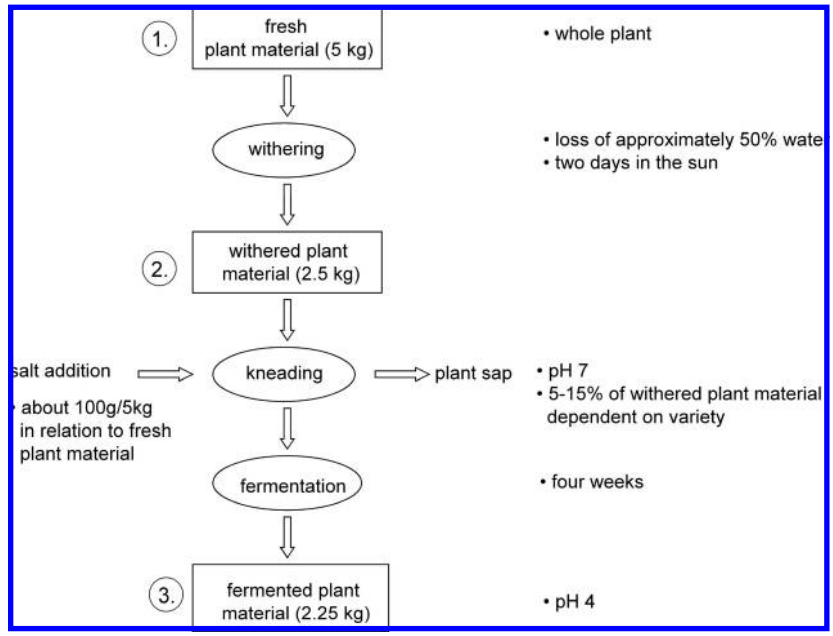

Figure 1. Flowchart of the fermentation process of Chinese cabbages.

fermentation (*12*). Little is known about the impact of fermentation on phenolic compounds, and the results are controversial. Chun et al. (*12*) reported that the total phenolic content is lower in drained processed cabbages versus raw cabbages. However, using the Folin−Ciocalteu assay, Ciska et al. (*13*) detected higher total phenolic content in extracts of sauerkraut samples including the plant sap compared with white nonfermented cabbage extracts. Another study found constant kaempferol concentrations in cabbage throughout the fermentation process (*5*). The concentration levels were determined on the basis of the analysis of flavonoid aglycones after the hydrolysis reaction. Qualitative changes in the polyphenol pattern of the individual glycosylated and acylated derivatives have not been investigated.

In the fermentation of olives, Romero et al. (*14*) observed qualitative changes in polyphenols resulting from the degradation of glycoside linkages to aglycones. Changes in the glycoside and organic acid residues are of interest with respect to bioavailability and metabolism in humans (*15*).

The aim of this study was to investigate in detail the impact of fermentation on the qualitative and quantitative polyphenol composition in the plant material of different cultivars of Chinese *Brassica* vegetables by HPLC with diode array detection (DAD) and HPLC with electrospray ionization mass spectrometry (ESI-MS^n).

MATERIALS AND METHODS

Chemicals. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (Sigma), acetonitrile (HPLC grade, Fisher Scientific), ascorbic acid (Carl Roth GmbH), ascorbigen, diphenylpicrylhydrazyl (Sigma), caffeic acid (Carl Roth GmbH), chlorogenic acid (Sigma), ferulic acid (Carl Roth GmbH), Folin−Ciocalteu reagent (Merck), formic acid (Carl Roth GmbH), kaempferol-3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside [isolated compound according to the method of Harbaum et al. (*8*)], kaempferol-3-*O*-glucoside (Sigma), kaempferol-7-*O*-neohesperidoside (Sigma), methanol (HPLC grade, Fisher Scientific), oxalic acid dihydrate (Carl Roth GmbH), metaphosphoric acid (Fluka), PBS buffer (Fluka), potassium peroxodisulfate (Fluka), sinapic acid (Carl Roth GmbH), sodium hydrogencarbonate (Carl Roth GmbH), sodium hydroxide (Carl Roth GmbH), trifluoroacetic acid (Carl Roth GmbH), and Trolox (Fluka) were used.

Plant Material and Sampling. Two pak choi cultivars (*Brassica campestris* L. ssp. *chinensis* var. *communis* cv. Hangzhou You Dong Er and cv. Shanghai Qing) and two leaf mustard cultivars (*Brassica juncea* Coss cv. Xue Li Hong and cv. Bao Bao Qing Cai) were cultivated in greenhouses in Germany and under field conditions in China. The plants were harvested (at 10 weeks in China; at 6 weeks in Germany), and a portion of the plant material was subjected to liquid N_2 and lyophilized to obtain a sample of nonfermented plant material as a reference. The plant material from each of the different cultivars was sampled in duplicate ($n = 2$) in Germany and in triplicate ($n = 3$) in China.

Fermentation Process. The traditional Chinese pickling method for fermenting cabbages is schematically presented in **Figure 1**. The harvested plant material was put in the sun for 2 days to reduce its water content by approximately 50% (withering). Afterward, the plants were kneaded with salt (100 g/5 kg of fresh weight) until sap leaked from the plant material. The wet plant material was fermented under pressure in clay pots for 4 weeks under ambient conditions. The fermentation procedure in China as well as in Germany was done in a single pot for each cultivar. Furthermore, the plant sap released during the kneading process was collected. Samples (Germany, $n = 2$; China, $n = 3$) were collected after withering and after fermentation to monitor the phenol changes during the storage stages. Differences in repetitions resulted from different amounts of cultivated plant material and pot volumes in China and Germany. The samples were freeze-dried and stored for further analysis. Additionally, the plant sap that was extruded in the kneading step was analyzed.

Extraction. The extraction of all freeze-dried samples (resulting from experiments in China as well as Germany) were conducted in Germany under the same laboratory conditions: The extraction procedure was carried out in duplicate according to the method of Harbaum et al. (*8*). Freeze-dried plant material (ca. 0.3 g) was extracted four times (1 × 4 mL and 3 × 2 mL) with acidic aqueous methanol (containing 1% metaphosphoric acid and 0.5% oxalic acid dihydrate) facilitated by ultrasonication (1 min for each step) and centrifuged. The collected supernatants of each extraction step were made up to 10 mL and filtered. Oxalic acid and metaphosphoric acid were used in the extraction solvent for the further determination of the ascorbic acid content in the extracts. The ascorbic acid content could be eliminated as interfering compounds in Folin−Ciocalteu assay and assays for the determination of antioxidative potential.

Total Phenolic Content (TPC). The TPC was determined according to the method of Singleton and Rossi (*16*) and modified as follows: 0.5 mL of extract was added to 2.5 mL of 0.03 M NaOH solution and 0.5 mL of methanol; 0.25 mL of 1:1 with distilled water diluted Folin−Ciocalteu reagent was added. After 5 min of reaction time, 0.5 mL of 5% sodium hydrogencarbonate solution was added. After 1 h

of incubation in the dark, the absorption was measured at 725 nm. Calibration was carried out by using different concentrations of gallic acid (0.02–0.4 mg/mL).

HPLC Analysis of Polyphenols. Quantitative HPLC analysis of polyphenols in the obtained exctracts was carried out on a HP1100 HPLC (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector according to the method of Harbaum et al. (*8*). Separation was carried out on a 250 × 4 mm i.d., 5 μm, RP-18 Nucleodur column with an 8 × 4 mm Nucleodur guard column at 20 °C. Eluent A consisted of 0.15% trifluoroacetic acid in water and eluent B of 100% acetonitrile at a flow rate of 0.9 mL/min. Gradient elution started with 10% B for 9 min, reaching 12.5% B after 13 min and 14% B at 19 min. It remained at 14% B until 35 min, reaching 26% B after 60 min, 70% B after 75 min, and 10% B after 77 min until 80 min. Compounds were detected and quantified by UV absorption at 330 nm. Injection volume was set to 25 μL. Kaempferol-3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside, which was obtained by the isolation procedure as presented by Harbaum et al. (*8*), was used as a reference compound for quantification (detection limit = 0.002 mg/mL). The quantification of hydroxycinnamic acids was carried out relative to external standard sinapic acid (detection limit = 0.001 mg/mL), and further calculations were performed utilizing the molecular weight of the main identified hydroxycinnamic acid compound sinapoylmalate: molecular weight factor (mwf) = *M*[sinapoylmalate = 340]/*M*[sinapic acid = 224] (*8*).

HPLC Analysis of Ascorbic Acid. The quantitative content of ascorbic acid in the extract was determined by HPLC-DAD to calculate its influence on Folin−Ciocalteu assay and antioxidative capacity assays. The methanolic extracts (50%) were diluted 10-fold with water containing 1% metaphosphoric acid and 0.5% oxalic acid dihydrate and directly analyzed by HPLC-DAD. HPLC analysis was performed on a Nucleodur 250 × 4 mm i.d., 5 μm C18 column equipped with an 8 × 4 mm guard column. Injection volume was set to 20 μL. Eluent A consisted of 0.15% trifluoroacetic acid and eluent B of 100% acetonitrile at a flow rate of 0.7 mL/min. The gradient was as follows: 0 min, 0% B; 6 min, 8% B; 10 min, 90% B; 11 min, 90% B; 13 min, 0% B. Detection was carried out at 245 nm (t_R = 5.9 min).

HPLC-ESI-MS^n. Qualitative HPLC-ESI-MS^n analysis was carried out according to the method of Harbaum et al. (*8*). The HPLC system was coupled to an Agilent 1100 series LC/MSD trap with electrospray ionization. The HPLC conditions were the same as described above except for eluent A, which contained 0.1% formic acid instead of trifluoroacetic acid. The nebulizer pressure was 60 psi and the nitrogen flow rate 10 L/min at a drying temperature of 350 °C. Mass scans were performed from *m*/*z* 50 to 2000 in the negative ionization mode. Helium was used as the collision gas for the fragmentation of the isolated compounds in the ion trap. The detection conditions were as follows: capillary, 3500 V; skimmer, –40 V; cap exit, –158.5 V; Oct1DC, 12 V; Oct2DC, 2.45 V; trap drive level, 45.0; OctRF, 150 Vpp; lens 1, 5.0 V; lens 2, 60 V. MS^n experiments were carried out by isolation and fragmentation procedure of detected ions.

Antioxidative Assays. The DPPH assay was carried out by mixing 1 mL of 0.3 mmol/L 2,2-diphenyl-1-picrylhydrazyl (DPPH radical) in methanol with 1.95 mL of 25% aqueous methanol containing 0.1 M PBS buffer to obtain a final 50% methanolic solution. An initial reading was carried out at 516 nm (initial absorbance approximately 1.3). Afterward, 50 μL of the sample was added. The absorbance was measured after 10 min of reaction time. Using Trolox as the calibration standard, the antioxidative potential was calculated as Trolox equivalents.

The Trolox equivalent antioxidant capacity (TEAC) assay was performed according to the method of Re et al. (*17*). ABTS (8 mmol/L) and 3 mmol/L potassium peroxodisulfate ($K_2S_2O_8$) were dissolved in water and allowed to stand for approximately 12 h to generate the ABTS radical. The stock radical solution was diluted with 0.1 M PBS buffer solution to an initial absorbance of approximately 1.3 at 734 nm. The diluted radical solution (2.95 mL) was used for the initial reading. Fifty microliters of the sample was added. Absorbance was measured again after 20 min of reaction time. Known concentrations of Trolox were used for calibration, and the antioxidant capacity was expressed as Trolox equivalents.

Statistical Analysis. Statistical analysis was carried out by SPSS 15.0 (one-way ANOVA; Bonferroni $p < 0.05$).

RESULTS AND DISCUSSION

The effect of pickling, a traditional Chinese fermentation procedure, on the phenolic composition of pak choi leaves (*B. campestris* L. ssp. *chinensis* var. *communis*) and Chinese leaf mustard (*B. juncea* Coss) was investigated.

Identification of Polyphenols. Polyphenols were extracted from the plant material and identified by HPLC with coupled ESI-MS^n and UV spectra of the DAD.

The main polyphenols detected in the four cultivars of pak choi (cv. Hangzhou You Dong Er and cv. Shanghai Qing) and leaf mustard (cv. Xue Li Hong and cv. Bao Bao Qing Cai) before fermentation were glycosylated kaempferol derivatives and hydroxycinnamic acid derivatives of malate, quinic acid, and glycoside. The presence of these compounds was reported earlier for pak choi cultivars (*7, 8*). The main flavonoids in pak choi are kaempferol-3-*O*-diglucoside-7-*O*-glucoside derivatives acylated with different hydroxycinnamic acids (*8*). Leaf mustard cv. Xue Li Hong possessed the greatest number of identified polyphenol derivatives, including flavonoid derivatives, which had not been detected in Chinese leaf mustard or pak choi before (compounds **A6**, **A10**, **A11**, **A16**, **A17**, **A18**, and **A20**; see **Tables 1** and **2**). **Figure 2A** and **Table 1** present all tentatively detected compounds in the four cultivars. The fragmentation procedure of compounds **A6**, **A10**, **A11**, **A16**, **A17**, **A18**, and **A20** (**Table 2**) shows the loss of 4 × 162 amu (atomic mass unit) and was attributed to acylated flavonoid tetraglucosides with sinapic acid, ferulic acid, caffeic acid, and hydroxyferulic acid, which have been reported in white cabbages (*18*), cauliflower (*19*), or turnip tops (*20*). In the case of compound **A10** ($[M - H]^-$ at *m*/*z* 1095), 5 × 162 amu was split off in three subsequent fragmentation steps, which is indicative of the loss of a glycoside in position 7 of the aglycone (MS^2 at *m*/*z* 933), caffeic acid (MS^3 at *m*/*z* 771), and a triglycoside moiety [MS^4 at *m*/*z* 285 (90% relative intensity)]. In contrast, a mass of *m*/*z* 1095 (as presented for compound **A10**) was attributed to a kaempferolpentaglucoside in the *Brassica* vegetables tronchuda cabbage (*21*), cauliflower (*19*), and broccoli (*22*).

Furthermore, new hydroxycinnamic acid diglycosides of hydroxyferulic acid, sinapic acid, and caffeic acid were tentatively identified by HPLC-ESI-MS^n (compounds **A31**, **A32**, **A36**; **Table 1**). A detailed fragmentation pattern is presented in **Table 3**. According to the similarity of the fragmentation pattern as presented by Price et al. (*23*) and Harbaum et al. (*8*), these compounds were identified as gentiobiose derivatives esterified with two or three hydroxycinnamic acids. Additionally, the monoglycoside hydroxyferuloylglycoside (compound **A3**) was detected in pak choi and leaf mustard. This compound was also identified in *Arabidopsis* (*24*) and showed the same MS^n fragmentation pattern as described in the present study. The occurrence of hydroxyferulic acid as a residue of polyphenols in *Brassica* vegetables was identified for the flavonoid kaempferol-3-*O*-hydroxyferuloylsophoroside-7-*O*-glucoside by Harbaum et al. (*8*) and verified by NMR for pak choi. Therefore, hydroxyferulic acid is also assigned as a hydroxycinnamic acid moiety for compounds **A3**, **A31**, and **A36**.

Changes in the Polyphenol Pattern during Fermentation. Freeze-dried plant material of two pak choi cultivars and two leaf mustard cultivars, grown in Germany and China, was extracted before withering (1), after withering (2), and after the pickling procedure (3) to characterize the changes in polyphenols (**Figure 1**). No qualitative changes were observed during

Table 1. Retention Times t_R of Tentatively Identified Phenolic Compounds, Their Negative *m/z* Values $[M - H]^-$ of the Detected Molecular Masses, and Their Occurrence in Extracts of Chinese *Brassica* Vegetables Cultivated in China and Germany

				occurrence before fermentation[d]				occurrence after fermentation[d]			
no.	t_R (min)	*m/z* $[M - H]^-$	structure assignment	HYDE	SQ	XLH	BBQC	HYDE	SQ	XLH	BBQC
A1	8.4	353	monocaffeoylquinic acid[a]	X[e]	X	X	(X)	(X)	(X)	(X)	(X)
A2	9.7	341	caffeoylglycoside[a]	(X)	(X)	(X)	(X)	–	–	–	–
A3	10.6	371	hydroxyferuloylglycoside[b]	–	(X)	X	(X)	–	–	–	–
A4	12.9	337	coumaroylquinic acid[a]	X	X	X	X	(X)	(X)	(X)	(X)
A5	13.8	337	coumaroylquinic acid[a]	X	X	X	X	(X)	(X)	(X)	(X)
A6	14.7	1125	kaempferolhydroxyferuloyltetraglucoside[c]	–	–	(X)	–	–	–	–	–
A7	15.1	771	kaempferol-3-*O*-diglucoside-7-*O*-glucoside[a]	X	X	X	X	–	–	–	–
A8	16.1	367	feruloylquinic acid[a]	(X)	(X)	(X)	(X)	(X)	(X)	(X)	–
A9	16.1	353	monocaffeoylquinic acid[a]	(X)	(X)	(X)	(X)	–	–	–	–
A10	16.7	1095	kaempferolcaffeoyltetraglucoside[c]	–	–	(X)	–	–	–	–	–
A11	17.0	1125	kaempferolhydroxyferuloyltetraglucoside[c]	–	–	(X)	–	–	–	–	–
A12	17.3	963	kaempferol-3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside[a]	X	X	X	X	–	–	–	–
A13	19.1	355	feruloylglycoside[a]	(X)	(X)	(X)	(X)	–	–	–	–
A14	19.3	933	kaempferol-3-*O*-caffeoyldiglucoside-7-*O*-glucoside[a]	X	X	X	X	–	–	–	–
A15	20.1	385	sinapoylglycoside[a]	X	X	X	X	(X)	(X)	(X)	(X)
A16	20.5	1139	kaempferolsinapoyltetraglucoside[c]	–	–	(X)	–	–	–	–	–
A17	21.4	1139	kaempferolsinapoyltetraglucoside[c]	–	–	(X)	–	–	–	–	–
A18	21.8	1109	kaempferolferuloyltetraglucoside[c]	–	–	(X)	–	–	–	–	–
A19	22.1	977	kaempferol-3-*O*-sinapoyldiglucoside-7-*O*-glucoside[a]	X	X	X	X	–	–	–	–
A20	23.5	1109	kaempferolferuloyltetraglucoside[c]	–	–	(X)	–	–	–	–	–
A21	23.9	609	kaempferol-3-*O*-glucoside-7-*O*-glucoside[a]	X	X	X	X	–	–	–	–
A22	23.9	947	kaempferol-3-*O*-feruloyldiglucoside-7-*O*-glucoside[a]	X	X	X	X	–	–	–	–
A23	25.8	639	isorhamnetin-3-*O*-glycoside-7-*O*-glycoside[a]	X	X	X	X	–	–	–	–
A24	25.8	917	kaempferol-3-*O*-coumaroyldiglucoside-7-*O*-glucoside[a]	X	X	X	X	–	–	–	–
A25	27.3	295	caffeoylmalate[a]	X	X	X	X	(X)	–	(X)	–
A26	29.4	325	hydroxyferuloylmalate[a]	X	X	X	X	–	(X)	(X)	(X)
A27	42.5	279	coumaroylmalate[a]	(X)	(X)	(X)	(X)	–	–	–	–
A28	47.0	609	kaempferoldiglucoside[a]	(X)	–	(X)	–	(X)	(X)	(X)	(X)
A29	47.4	309	feruloylmalate[a]	X	X	X	X	X	(X)	X	X
A30	48.4	339	sinapoylmalate[a]	X	X	X	X	X	(X)	X	X
A31	53.6	739	sinapoylhydroxyferuloyldiglycoside[b]	–	–	(X)	–	–	–	–	–
A32	54.9	709	sinapoylcaffeoyldiglycoside[b]	–	–	(X)	–	–	–	–	–
A33	56.8	447	kaempferolglucoside[a]	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
A34	57.6	477	isorhamnetinglycoside[a]	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
A35	59.9	753	disinapoyldiglycoside[a]	(X)	(X)	(X)	(X)	–	–	–	–
A36	60.4	945	disinapoylhydroxyferuloyldiglycoside[b]	–	–	(X)	–	–	–	–	–
A37	61.2	723	sinapoylferuloyldiglycoside[a]	(X)	(X)	(X)	(X)	–	–	–	–
A38	62.4	693	diferuloyldiglycoside[b]	–	–	(X)	–	–	–	–	–
A39	65.2	959	trisinapoyldiglycoside[a]	(X)	(X)	(X)	(X)	–	–	(X)	–
A40	65.7	929	disinapoylferuloyldiglycoside[a]	(X)	(X)	(X)	(X)	–	–	(X)	–

[a] Identified according to the procedure of Harbaum et al. (*8*) for pak choi cultivars. [b] Hydroxycinnamic acid derivatives in leaf mustard cv. Xue Li Hong; for the detailed fragmentation pattern, see **Table 3**. [c] Flavonoid glycosides in leaf mustard cv. Xue Li Hong; for the detailed fragmentation pattern, see **Table 2**. [d] Cultivars: HYDE, Hangzhou You Dong Er; SQ, Shanghai Qing; XLH, Xue Li Hong; BBQC, Bao Bao Qing Cai. [e] X, predominantly significant contents; –, not detected; (X), marginal contents or not detected in all samples.

withering (not shown). After fermentation, however, marked differences in the polyphenol spectrum were found (see HPLC chromatograms in **Figure 2**). **Table 4** presents the negative *m/z* values $[M - H]^-$ of the main de novo detected molecular masses and main fragments MS^n after the fermentation procedure conducted for each cultivar. The detected molecular masses were lower in fermented cabbage than in nonfermented cabbage (**Tables 1** and **4**). Most of the detected molecular masses in fermented cabbage were found as fragment masses MS^n in the nonfermented cabbages (*8*). This indicates the degradation of the compounds by fermentation into smaller molecules, for example, the loss of one or more glucose moieties. The sugar linkage at position 7 of the flavonoid aglycone was particularly prone to cleavage during fermentation. The most de novo formed compounds after fermentation were detected in the cv. Xue Li Hong. This cultivar also possessed the most complex spectrum before fermentation, with several kaempferol tetraglucosides (**Tables 1** and **2**). Some compounds in cv. Xue Li Hong formed by fermentation were kaempferol triglycosides, exhibiting a fragmentation pattern $[M - H]^-$ to MS^3 (**Table 4**) comparable to the fragmentation pattern MS^2 to MS^4 of proposed kaempferol tetraglycosides in nonfermented cabbage (**Table 2**). This led to the assumption that the kaempferol triglycosides in the fermented cabbage had been formed by the degradation of kaempferol tetraglycosides. However, the retention times and fragmentation patterns of the observed kaempferol triglycosides in fermented plants differed from those of nonfermented cabbages [see **Tables 2** and **4** and Harbaum et al. (*8*)]. Each of the compounds **B5**, **B6**, **B9**, and **B12** showed the loss of 486 amu (3 × 162 amu) for the fragmentation step MS^2 [771] to MS^3 [285]. These data enabled the tentative identification of the triglucoside moiety at position 3 of the kaempferol aglycone in *Brassica* vegetables for tetraglycosides in nonfermented plants and triglycosides (acylated kaempferol-3-*O*-triglucosides) in fermented cabbages (cv. Xue Li Hong) according to the literature (*19, 20*). In contrast, the triglycosides in nonfermented cabbages were identified as acylated kaempferol-3-*O*-diglucoside-7-*O*-glucosides [sophoroside moiety at position 3, as presented by Harbaum et al. (*8*)].

Compounds **B7**, **B10**, **B11**, **B13**, and **B14** are acylated kaempferol diglucosides, which appear to be generated from triglucosides in nonfermented cabbages [**Table 1** and Harbaum et al. (*8*)] due to the absence of acylated diglucosides in untreated plant material. The fragmentation pattern $[M - H]^-$

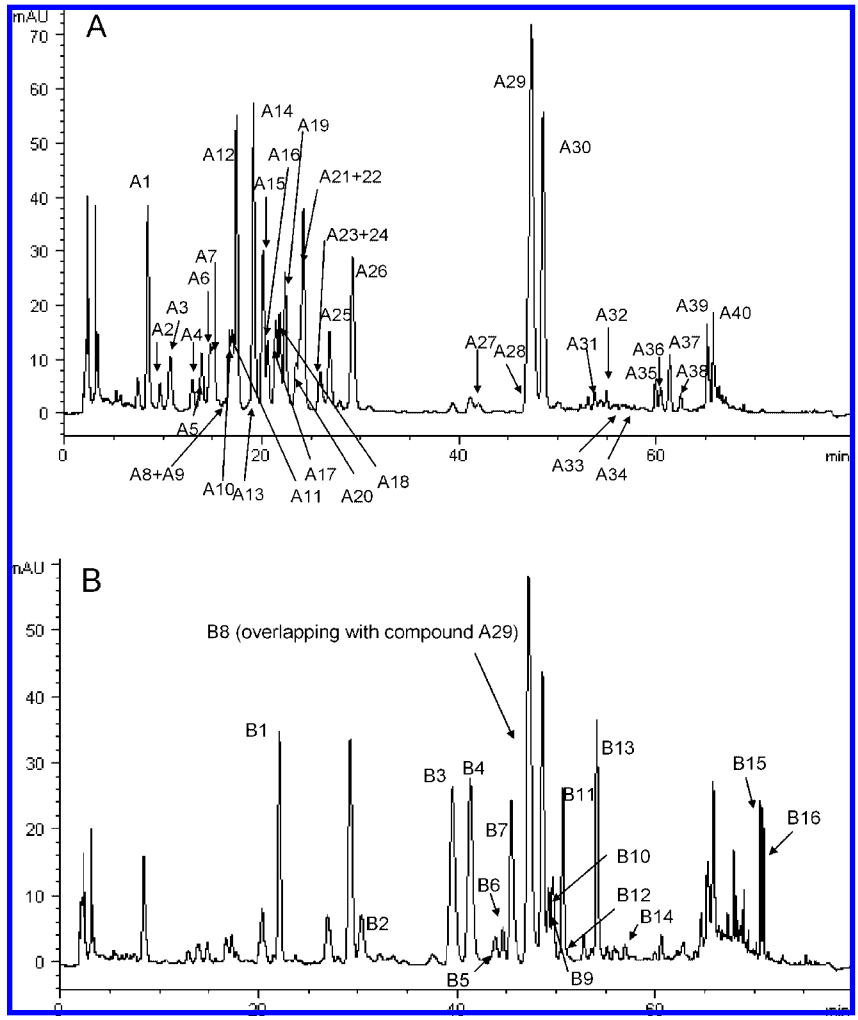

Figure 2. HPLC chromatograms of aqueous methanolic extract of nonfermented (before withering) (**A**) and fermented (**B**) leaf mustard cv. Xue Li Hong cultivated in Germany, monitored at 330 nm (for numbering, see **Tables 1** and **4**).

Table 2. Negative *m*/*z* Values $[M - H]^-$ of the Detected Molecular Masses of Tentatively Identified Flavonoid Tetraglycosides in Extract of Leaf Mustard (cv. Xue Li Hong) and the Main Fragments MS^n

		m/*z* of the main fragments by ESI-MS^n (relative intensity)		
no.	*m*/*z* $[M - H]^-$	MS^2 $[M - H]^-$	MS^3 $[MS^2$ (100%)]	MS^4 $[MS^3$ (100%)]
A6	1125	963 (100), 771 (4)	963 (11), 785 (10), 771 (100), 609 (1), 591 (2)	609 (14), 591 (94), 393 (12), 327 (25), 285 (100), 257 (11), 255 (22)
A10	1095	933 (100), 771 (8), 447 (1)	933 (2), 771 (100), 609 (2), 591 (2), 285 (2)	609 (31), 591 (100), 393 (30), 309 (14), 285 (90), 284 (31), 283 (15), 257 (15), 255 (12)
A11	1125	963 (100), 771 (11)	963 (2), 785 (2), 771 (100)	651 (12), 609 (42), 429 (60), 357 (12), 327 (10), 309 (9), 285 (100), 284 (26), 283 (10), 255 (19)
A16	1139	1121 (2), 977 (100), 785 (1)	785 (24), 771 (44), 753 (100), 591 (1), 463 (2)	609 (7), 591 (100), 461 (35), 393 (67), 327 (22), 285 (58), 284 (6)
A17	1139	977 (100), 771 (1)	785 (91), 771 (100), 753 (38), 591 (6)	609 (30), 591 (100), 489 (10), 461 (12), 429 (8), 327 (14), 285 (40), 284 (52), 255 (5)
A18	1109	947 (100), 771 (1)	785 (64), 771 (100), 753 (35), 591 (11)	609 (23), 591 (69), 489 (13), 393 (11), 327 (17), 285 (100), 284 (32), 257 (25), 255 (8)
A20	1109	947 (100), 771 (2)	785 (19), 771 (100), 753 (20), 609 (2)	609 (100), 446 (21), 429 (86), 327 (20), 285 (86), 284 (63), 255 (22)

to MS^3 of diglucosides in fermented cabbages (**Table 3**) is comparable to the fragmentation pattern of triglucosides (MS^2 to MS^4) in nonfermented cabbages, as shown by Harbaum et al. (*8*). In general, lower retention times were found for more highly glycosylated polyphenols (particularly flavonoids) than for polyphenols with a lower degree of glycosylation and aglycones (**Tables 1** and **4**). Aglycones of flavonoids and hydroxycinnamic acids do not normally occur in fresh or untreated plant material, but these compounds do occur in fermented cabbages. Moreover, some compounds such as hydroxycinnamic acid derivatives were not fully degraded by the fermentation procedure. These compounds are also indicated in **Table 1**. Proportions of sinapoylmalate and feruloylmalate remain unchanged during fermentation; that is, sinapoylmalate and feruloylmalate are also the major hydroxycinnamic acids in addition to the aglycones of sinapic acid and ferulic acid in

Table 3. Negative *m/z* Values $[M - H]^-$ of the Detected Molecular Masses of Tentatively Identified Hydroxycinnamic Acid Derivatives in Extract of Leaf Mustard (cv. Xue Li Hong) and the Main Fragments MS^n

		m/z of the main fragments by ESI-MS^n (relative intensity)		
no.	*m/z* $[M - H]^-$	MS^2 $[M - H]^-$	MS^3 $[MS^2$ (100%)]	MS^4 $[MS^3$ (100%)]
A3	371	232 (15), 209 (100), 191 (58)	195 (4), 194 (100), 165 (66), 150 (43)	
A31	739	721 (2), 533 (2), 515 (100), 275 (3), 233 (2), 209 (1)	515 (39), 275 (63), 209 (50), 194 (23), 191 (100), 176 (26), 165 (25)	
A32	709	691 (2), 515 (3), 503 (4), 499 (1), 485 (100), 245 (4)	383 (8), 245 (12), 179 (20), 175 (29), 161 (100)	
A36	945	721 (100), 515 (10)	529 (23), 515 (100), 511 (6)	499 (24), 275 (67), 247 (33), 209 (100), 191 (96), 165 (62)
A38	693	499 (100), 259 (6), 247 (1)	259 (40), 217 (22), 200 (15), 193 (100), 179 (7), 174 (70)	

fermented cabbages. Other presented hydroxycinnamic acid compounds occurred mostly in trace amounts after the fermentation procedure. However, flavonoid derivatives identified in untreated plant material completely disappeared by fermentation. Overall, the degradation products, which were formed by fermentation, were found to be the same in plants fermented in Germany and China.

Quantitative Changes of Polyphenols. Quantitative changes in polyphenolic compounds at different stages of the Chinese cabbage fermentation procedure (**Figure 1**) are presented in **Tables 5** and **6**. The contents in milligrams per gram of dry matter (dm) of flavonoids and hydroxycinnamic acids determined by HPLC decreased predominantly after fermentation, as presented exemplarily for compounds **A12** and **A30** (as described above, **Table 1**). Compound **A30** (sinapoylmalate) amounted to approximately 50% of the original content after fermentation (mean value; degradation ranged from 20 to 100%). The total content of hydroxycinnamic acids in micromoles per gram of dm did not change significantly in the withering stage or during the fermentation procedure, except for cv. Shanghai Qing in Germany and cv. Xue Li Hong in China. This led to the assumption that changes of polyphenol contents (in milligrams per gram of dm) were rather due to the split-off of moieties (such as sugars and organic acids) than to the complete degradation of hydroxycinnamic acids. Furthermore, compound **A12** (kaempferol-3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside) disappeared completely during fermentation due to the split-off of moieties, such as sugar at position 7, as presented for the qualitative polyphenol changes. However, losses of total flavonoid content in micromoles per gram of dm were observable for all cultivars fermented in Germany and China, except for cv. Hangzhou You Dong Er fermented in Germany. This led to the assumption that the flavonoid contents had not only been changed by the cleavage of moieties but also degraded by fermentation.

Table 7 presents the contents in the plant sap after the kneading of the withered plant material. The sap is a highly concentrated solution of plant juice (particularly caused by withering). A marginal loss of polyphenols was observed after the kneading step in the sap, which represents only 5–15% of the withered plant material, as shown for the fermentation procedure in Germany (**Figure 1**).

In addition, the quantitative changes were investigated by a spectrophotometric method (a screening method for the determination of TPC: Folin−Ciocalteu assay). The values for the TPC were in the same range as presented for cabbages in the literature (*12, 25*), considering the use of different calibration standards such as gallic acid or catechol.

The TPC values were higher after withering and fermentation than before withering (**Tables 5** and **6**), particularly with respect to the fermentation procedure in China (significant for all cultivars). Ciska et al. (*13*) also detected higher total phenolic content (8.25 mg/g of dm) in extracts of sauerkraut than in extracts of white cabbage (5.72 mg/g of dm). The increase of the values may be due to the release of bound sugar moieties and the formation of free hydroxyl groups. Reducing sugars have been reported to interfere with the determination of polyphenols in the Folin−Ciocalteu assay (*26, 27*). Furthermore, the possible cleavage of cell wall bound phenols during the fermentation procedure has been discussed in relation to the changes of TPC values (*13*).

The TPC were lower in the present study (for uncorrected and corrected values for the content of ascorbic acid), as reported for other Asian vegetables [approximately 6.2 mg/g of dm for mustard and 9.2 mg/g of dm for cabbage, expressed as catechol equivalents (*25*), as well as 11.9 mg/g for Chinese cabbage (*28*)].

Changes in Antioxidative Capacity Caused by Fermentation. Several studies have investigated the antioxidative potential of fruits and vegetables. However, a comparison of the detected antioxidative potential with literature data is difficult due to the different assays used, and the assays were often modified by the authors, for example, reaction times or different calibration standards (Trolox, vitamin C) (*29, 30*). The results in this study are in the same range for nonfermented cabbages in Germany (**Table 5**) as described in the literature. Bahorun et al. (*28*) reported the highest antioxidant capacity of Chinese cabbage (approximately 36.8 μmol/g of dm TEAC); cabbage showed medium values (17.3 μmol/g of dm). Cao et al. (*31*) determined an antioxidative capacity of 32 μmol/g of dm, expressed as Trolox equivalents (ORAC assay), for cabbages. Proteggente et al. (*32*) detected approximately 49.2 μmol/g of dm (Trolox equivalents) for green cabbages, 29.5 μmol/g for cauliflower, and 64.8 μmol/g for broccoli. However, the antioxidant capacity of nonfermented plants in China was higher (approximately double that of plants cultivated in Germany). In the present study, the cv. Xue Li Hong (nonfermented plants) possessed the highest antioxidative capacity among the plants cultivated in Germany [TEAC, 53.51 μmol of Trolox/g of dm; and DPPH, 20.61 μmol of Trolox/g of dm (both values without ascorbic acid)] as did the cv. Bao Bao Qing Cai (TEAC, 96.63 μmol of Trolox/g) and the cv. Xue Li Hong (DPPH, 39.64 μmol of Trolox/g) in China.

No significant tendency in changes during fermentation was observed for the antioxidative capacity determined by DPPH assay. However, a higher antioxidant capacity was shown in the literature for sauerkraut (31 μmol of Trolox/g) compared to white cabbage (25 μmol of Trolox/g) and is also in accordance with the data from the TEAC assay in the present study, which showed predominantly significantly higher values after fermen-

Table 4. Retention Times t_R of Tentatively Identified Phenolic Compounds, Their Negative *m/z* Values $[M - H]^-$ of the Detected Molecular Masses, the Main Fragments MS^n, and Their Occurrence in Extracts of Chinese *Brassica* Vegetables after Fermentation Procedure in China and Germany

						occurrence in cultivar[a]			
no.	t_R(min)	*m/z* $[M - H]^-$		*m/z* of the main fragments by ESI-MS^n (relative intensity)	structure assignment	HYDE	SQ	XLH	BBQC
B1	22.0	209	MS^2 $[M - H]^-$	195 (20), 194 (100), 166 (12), 165 (46), 150 (27)	hydroxyferulic acid	(X)[b]	–	(X)	(X)
B2	30.3	771	MS^2 $[M - H]^-$	609 (16), 591 (100), 489 (8), 393 (14), 327 (15), 285 (86), 284 (29), 257 (13), 256 (4), 212 (10)	kaempferoltriglucoside	–	–	(X)	–
			MS^3 $[MS^2$ (100%)]	563 (25), 429 (100), 411 (44), 393 (64), 339 (77), 327 (30), 309 (58), 257 (25)					
B3	39.6	193	MS^2 $[M - H]^-$	179 (16), 178 (61), 150 (31), 149 (100), 135 (10), 134 (45)	ferulic acid	X	(X)	X	X
B4	41.3	223	MS^2 $[M - H]^-$	209 (9), 208 (100), 179 (41), 164 (34)	sinapic acid	X	(X)	X	X
B5	43.8	963	MS^2 $[M - H]^-$	785 (2), 771 (100), 753 (1), 609 (2)	kaempferolhydroxyferuloyltriglucoside	–	–	(X)	–
			MS^3 $[MS^2$ (100%)]	651 (9), 609 (33), 591 (6), 447 (6), 429 (37), 327 (16), 309 (20), 285 (100), 284 (26), 257 (8), 255 (15)					
B6	44.5	947	MS^2 $[M - H]^-$	785 (64), 771 (100), 753 (41), 609 (1), 591 (9)	kaempferolferuloyltriglucoside	–	–	(X)	–
			MS^3 $[MS^2$ (100%)]	609 (13), 591 (81), 327 (14), 285 (100), 284 (23), 257 (25), 255 (20)					
B7	45.4	801	MS^2 $[M - H]^-$	624 (8), 610 (18), 609 (100), 429 (3), 285 (8)	kaempferol-3-*O*-hydroxyferuloyldiglucoside	X	X	X	X
			MS^3 $[MS^2$ (100%)]	489 (8), 447 (13), 429 (82), 339 (7), 327 (7), 309 (9), 285 (100), 284 (55), 257 (9), 256 (2), 255 (23)					
B8	47.0	609	MS^2 $[M - H]^-$	489 (6), 447 (11), 429 (71), 327 (9), 309 (7), 286 (10), 285 (100), 284 (54), 257 (4), 256 (2), 255 (10)	kaempferoldiglucoside	–	(X)	(X)	(X)
B9	49.2	977	MS^2 $[M - H]^-$	785 (16), 771 (100), 753 (15), 609 (2), 591 (1), 429 (2)	kaempferolsinapoyltriglucoside	–	–	(X)	–
			MS^3 $[MS^2$ (100%)]	609 (53), 447 (15), 429 (55), 339 (8), 327 (44), 309 (10), 285 (100), 284 (12)					
B10	49.8	771	MS^2 $[M - H]^-$	610 (7), 609 (100), 591 (2), 447 (1), 429 (6), 285 (9), 284 (4), 257 (1), 255 (2)	kaempferol-3-*O*-caffeoyldiglucoside	X	X	X	X
			MS^3 $[MS^2$ (100%)]	429 (43), 339 (13), 285 (57), 284 (100), 283 (35), 257 (53), 255 (36), 227 (23)					
B11	50.7	815	MS^2 $[M - H]^-$	624 (11), 623 (65), 610 (14), 609 (100), 591 (34), 447 (1), 429 (8), 285 (8), 284 (12), 257 (2), 255 (4)	kaempferol-3-*O*-sinapoyldiglucoside	X	X	X	X
			MS^3 $[MS^2$ (100%)]	447 (9), 429 (94), 339 (6), 327 (20), 285 (100), 284 (61), 257 (15), 255 (17)					
B12	52.6	947	MS^2 $[M - H]^-$	785 (15), 771 (100), 753 (16), 609 (1)	kaempferolferuloyltriglucoside	–	–	(X)	–
			MS^3 $[MS^2$ (100%)]	651 (9), 610 (43), 609 (92), 447 (6), 429 (70), 309 (10), 286 (17), 285 (100), 284 (39), 283 (11), 256 (5)					
B13	54.0	785	MS^2 $[M - H]^-$	624 (10), 623 (53), 610 (19), 609 (100), 593 (13), 591 (14), 429 (5), 299 (6), 285 (8), 284 (9), 255 (4)	kaempferol-3-*O*-feruloyldiglucoside	X	X	X	X
			MS^3 $[MS^2$ (100%)]	489 (4), 447 (10), 429 (63), 339 (3), 309 (10), 285 (100), 284 (78), 257 (12), 255 (22)					
B14	56.0	755	MS^2 $[M - H]^-$	610 (21), 609 (100), 591 (23), 429 (5), 285 (5)	kaempferol-3-*O*-coumaroyldiglucoside	(X)	(X)	(X)	(X)
			MS^3 $[MS^2$ (100%)]	489 (20), 429 (63), 285 (100), 284 (51), 257 (10), 255 (14)					
B15	70.5	285	MS^2 $[M - H]^-$	285 (100), 257 (3), 229 (3), 213 (3), 151 (10)	kaempferol	X	X	X	X
B16	70.8	315	MS^2 $[M - H]^-$	301 (5), 300 (100), 272 (1)	isorhamnetin	(X)	(X)	(X)	(X)

[a] Cultivars: HYDE, Hangzhou You Dong Er; SHQ, Shanghai Qing; XLH, Xue Li Hong; BBQC, Bao Bao Qing Cai (results for plants cultivated in Germany and China). [b] X, detected; (X), marginal contents or not detected in all samples; –, not detected.

Table 5. Quantitative Changes in Polyphenol Contents by Fermentation Procedure at Different Storage Stages (Fermentation in Germany, Plants from Greenhouses)

		TPC		hydroxycinnamic acids[b]		flavonoids[b]		DPPH		TEAC	
cultivar[a]	storage stage	mg/g of dm[c,d]	mg/g of dm[c,e]	compd **A30** (mg/g dm)[f]	total (μmol/g of dm)[g]	compd **A12** (mg/g of dm)[h]	total (μmol/g of dm)[h]	μmol/g of dm[d,i]	μmol/g of dm[e,i]	μmol/g of dm[d,i]	μmol/g of dm[e,i]
HYDE	nonwithered	3.58	3.06	1.07 ± 0.06	8.68 ± 0.95	0.47 ± 0.01	4.69 ± 0.50	28.04	19.47	60.99	51.76
	withered	3.84	3.64	0.92 ± 0.07	6.78 ± 0.63	0.28 ± 0.09	3.17 ± 0.72	20.57	16.28	86.01	81.54
	fermented	4.91	4.91	0.65 ± 0.03	7.12 ± 0.30	nq[j]	3.62 ± 0.23	21.23	21.23	116.13	116.13
SQ	nonwithered	2.89	2.73	0.83 ± 0.11	7.34 ± 0.47	0.59 ± 0.15	6.45 ± 0.91	21.99	17.29	47.86	42.84
	withered	3.67	3.34	1.03 ± 0.21	8.81 ± 1.87	0.51 ± 0.08	6.88 ± 1.62	23.78	18.38	67.11	61.29
	fermented	3.34	3.34	nq	0.97 ± 0.29	nq	1.96 ± 0.30	12.08	12.08	75.52	75.52
XLH	nonwithered	3.23	3.14	0.43 ± 0.01	7.23 ± 0.21	1.35 ± 0.08	8.07 ± 0.22	23.16	20.61	56.16	53.51
	withered	4.72	4.64	0.59 ± 0.05	7.58 ± 0.19	1.30 ± 0.11	7.59 ± 0.01	25.44	23.25	75.29	73.04
	fermented	4.28	4.16	0.30 ± 0.01	6.03 ± 0.26	nq	5.75 ± 0.13	19.41	16.96	81.06	78.53
BBQC	nonwithered	2.99	2.72	0.54 ± 0.06	5.46 ± 0.12	1.49 ± 0.06	8.25 ± 0.35	23.26	18.63	52.07	47.15
	withered	4.28	4.01	0.73 ± 0.07	7.47 ± 0.96	1.69 ± 0.34	8.58 ± 1.02	25.28	20.88	76.32	71.63
	fermented	4.88	4.76	0.37 ± 0.03	5.41 ± 0.60	nq	3.84 ± 0.31	21.83	19.35	86.00	83.43

[a] Cultivars: HYDE, Hangzhou You Dong Er; SHQ, Shanghai Qing; XLH, Xue Li Hong; BBQC, Bao Bao Qing Cai. [b] Mean values and mean deviation of each storage stage ($n = 2$). [c] Folin−Ciocalteu test, expressed as gallic acid equivalents. [d] Values including ascorbic acid. [e] Values without ascorbic acid. [f] Expressed as sinapoylmalate equivalents [calibrated as sinapic acid equivalents and further calculated by mwf = 340/224 as presented by Harbaum et al. (*8*)]. [g] Expressed as sinapic acid equivalents. [h] Expressed as kaempferol-3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside equivalents. [i] Expressed as Trolox equivalents. [j] nq, not quantified.

Table 6. Quantitative Changes in Polyphenol Contents by Fermentation Procedure at Different Storage Stages (Fermentation in China, Plants from Field Cultivation)

			hydroxycinnamic acids		flavonoids			
cultivar[a]	storage stage[b]	TPC[c,d] (mg/g of dm)	compd **A30** (mg/g of dm)[e]	total (μmol/g of dm)[f]	compd **A12** (mg/g of dm)[g]	total (μmol/g of dm)[g]	DPPH (μmol/g of dm)[d,h]	TEAC (μmol/g of dm)[d,h]
HYDE	nonfermented	3.78	0.81 ± 0.05	8.40 ± 0.45	0.67 ± 0.10	11.24 ± 2.79	26.57	61.98
	fermented	6.31*	0.65* ± 0.01	8.16 ± 0.42	nq[i]	4.95* ± 0.22	30.89	84.53*
SQ	nonfermented	3.89	0.84 ± 0.24	9.88 ± 2.31	0.88 ± 0.33	16.71 ± 2.16	30.49	89.77
	fermented	7.07*	0.51 ± 0.09	7.82 ± 1.05	nq	6.45* ± 0.06	33.69	131.47*
XLH	nonfermented	4.51	0.57 ± 0.24	6.96 ± 1.99	0.71 ± 0.23	12.87 ± 1.80	39.64	86.56
	fermented	6.66*	0.19* ± 0.02	3.44* ± 0.18	nq	2.76* ± 0.46	34.56	116.26*
BBQC	nonfermented	5.03	0.86 ± 0.20	8.41 ± 1.60	2.32 ± 0.39	18.35 ± 0.85	32.25	96.63
	fermented	8.26*	0.55 ± 0.06	7.14 ± 0.89	nq	4.13* ± 0.60	38.41	126.11*

[a] Cultivars: HYDE:, Hangzhou You Dong Er; SHQ, Shanghai Qing; XLH, Xue Li Hong; BBQC, Bao Bao Qing Cai. [b] Significant differences between the mean values of each storage stage ($n = 3$) are indicated by an asterisk (*). [c] Folin−Ciocalteu test, expressed as gallic acid equivalents. [d] Directly measured values, ascorbic acid only present in traces in extracts. [e] Expressed as sinapoylmalate equivalents [calibrated as sinapic acid equivalents and calculated by mwf = 340/224 as presented by Harbaum et al. (*8*)]. [f] Expressed as sinapic acid equivalents. [g] Expressed as kaempferol-3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside equivalents. [h] Expressed as Trolox equivalents. [i] nq, not quantified.

Table 7. Contents of Hydroxycinnamic Acids and Flavonoids in Sap after Kneading (Fermentation in Germany, Cultivated under Greenhouse Conditions)

cultivar[a]	hydroxycinnamic acids (μmol/mL)[b]	flavonoids (μmol/mL)[c]
HYDE	0.50	0.16
SQ	0.36	0.11
XLH	1.03	0.66
BBQC	0.61	0.34

[a] Cultivars: HYDE, Hangzhou You Dong Er; SQ, Shanghai Qing; XLH, Xue Li Hong; BBQC, Bao Bao Qing Cai. [b] Expressed as sinapic acid equivalents. [c] Expressed as kaempferol-3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside equivalents.

Table 8. Correlation between the Contents of Polyphenols and Antioxidative Capacity in Samples of Plant Material in Germany and China

	relationship R^2			
	nonwithered and withered		nonwithered, withered, and fermented	
	DPPH	TEAC	DPPH	TEAC
HPLC[a]	0.6200	0.4222	0.1611	0.0096
TPC[b]	0.3583	0.6993	0.4338	0.7467

[a] Correlation of contents in μmol/g (sum of hydroxycinnamic acids and flavonoids) with corrected values of TEAC and DPPH by ascorbic acid. [b] Correlation of uncorrected contents of Folin−Ciocalteu values by ascorbic acid with uncorrected values of TEAC and DPPH by ascorbic acid.

tation (fermentation procedure in China in particular, **Table 6**). Additionally, the relative influence of ascorbic acid in the DPPH assay (approximately 25% of the activity can be attributed to ascorbic acid, **Table 5**) is higher compared to the TEAC assay. Ascorbic acid, an important constituent of cabbages, influences the antioxidative potential (*27*).

The relationship R^2 values between the total contents of polyphenols (TPC and HPLC) and the antioxidative capacities (TEAC and DPPH) are presented in **Table 8**. The HPLC values (sum of hydroxycinnamic acids and flavonoids) were correlated with the corrected values of DPPH and TEAC assay by ascorbic acid content. The TPC were correlated with DPPH and TEAC values, all without correction for ascorbic acid content. There

is a correlation between the detected antioxidative capacity of both assays (DPPH and TEAC) and the detected total phenolic contents (Folin−Ciocalteu assay) in plant material, which did not change significantly when the fermented samples were considered (**Table 8**). The correlations between the contents determined by HPLC and the antioxidant capacity become stronger when the fermented samples are excluded [**Table 8**; $R^2 = 0.6200$ (DPPH, without fermented samples) and 0.4222 (TEAC, without fermented samples)]. In turn, the relationship is weaker when fermented plant samples are included [**Table 8**; $R^2 = 0.1611$ (DPPH, all samples) and 0.0096 (TEAC, all samples)].

The formation of free hydroxyl group(s) by linkage cleavages of polyphenol derivatives, for example, flavonoids, is one factor that might explain the higher antioxidative capacity in the TEAC assay as well as the increasing TPC values in the fermentation procedure compared to the quantitative content of hydroxycinnamic acids and flavonoids detected by HPLC-DAD. The TEAC values for available standard compounds were exemplarily determined to investigate the influence of the molecular structure on the antioxidative potential. The determined TEAC values for standard compounds relative to Trolox were as follows: sinapic acid, 2.2; caffeic acid, 1.1; chlorogenic acid, 1.2; ferulic acid, 2.9; kaempferol-7-*O*-neohesperidoside, 3.7; kaempferol-3-*O*-glucoside, 4.2; and kaempferol-3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside, 1.9. The increasing values of the antioxidative potential of the less glycosylated kaempferol derivatives confirm the possible influence of the free hydroxyl groups. Additionally, Rice-Evans et al. (*33*) detected an antioxidative potential of 4.7 for quercetin aglycone (relative TEAC value to Trolox) and 2.4 for rutin (quercetin-3-*O*-glucorhamnoside), which verifies the influence of substituents at the hydroxyl groups of the aglycone. Amic et al. (*34*) also reported that the occurrence and number of hydroxyl groups affect the radical scavenging activity of the polyphenol structure (*34*). However, there is rather no influence of substituents esterified to the carboxyl group of hydroxycinnamic acids as presented for caffeic acid aglycone (1.1 Trolox equiv) and the monocaffeoylquinic acid standard compound chlorogenic acid (1.2 Trolox equiv).

The high correlation of TEAC with TPC may be an indication of the similarity of the reactivity of the extracts in both assays; however, the correlation is lower for DPPH and TPC. All assays are predominantly based on electron transfer reactions (*35*). Slight differences in the antioxidative capacity of DPPH values compared to the great changes in TEAC values (as a result of the fermentation procedure) could be an indication of the generated compounds' various abilities to react with radicals such as $ABTS^{\bullet}$ and DPPH. Overall, the results require careful interpretation. It is possible that the higher antioxidative potential after withering and fermentation is also influenced by the formation of other reductones, for example, reducing sugars, lactic acid, or the cleavage of phenols bound to the cell wall, as annotated by Ciska et al. (*13*). These compounds may also react in different ways in the TPC and TEAC assays than in the DPPH assay. Ascorbigen, which is generated by the fermentation procedure from glucosinolates (*11*), was not detected in extracts produced from plant material of the withering and fermentation steps.

Microorganisms and endogenous enzymes are responsible for the fermentation process and the changes of quality in vegetables such as cabbage. The decrease of the pH from 7 to 4 during the fermentation process can possibly be attributed to the formation of lactic acid. During the fermentation of Chinese cabbage, the occurrence of and changes in bacterial endospores, lactic acid bacteria, enterobacteriaceae, and fungi were reported (*2*). Maifreni et al. (*36*) reported the spontaneous fermentation of turnips (*Brassica rapa*), a process caused by naturally occurring microorganisms: 225 different lactic acid bacteria strains and 63 yeasts were isolated. *Lactobacillus* spp. (heterofermentative population) and *Pediococcus* spp. (homofermentative) constituted the major bacteria populations and *Candida* spp. the main yeast.

The qualitative changes in polyphenols during fermentation, such as cleavages of linkages, could be an indication of esterase and glucosidase activity during the fermentation process, which may cause the cleavage of sugar linkages and organic acids. It was reported that the lactic acid bacteria *Lactococcus lactis* exhibit esterase activity (*37*).

During the fermentation process, the microorganisms use different organic substrates for their own metabolic processes. Glucose and fructose were fermented by *Leuconostoc mesenteroides* into lactate, ethanol, acetate, and mannitol (*38*). These products are also described in the fermentation of sauerkraut by lactic acid bacteria and in the metabolism of all fermentable sugars (*3*).

In general, the salt concentration, fermentation time, and temperature are the important factors affecting the quality of fermented vegetables (*39*). The salt and acid concentrations influence the growth of microorganisms as well as enzymatic activities.

The degradation of glycoside linkages (e.g., by fermentation) may cause a different bioavailability compared to phenolic compounds in nonfermented Chinese cabbages. Previously, it was shown that the bioavailability of less glycosylated flavonoids such as quercetinmonoglucoside is higher than that of the quercetin aglycone as well as the more highly glycosylated quercetin derivatives (*40*).

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