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ARTICLE

Composition of Phenolic Compounds and Glycoalkaloids α-Solanine and α-Chaconine during Commercial Potato Processing

Jens Mäder,*,† Harshadrai Rawel,‡ and Lothar W. Kroh†

†Department of Food Analysis, Institute of Food Technology and Food Chemistry, Berlin University of Technology, Gustav-Meyer-Allee 25, D-13355 Berlin, Germany, and ‡Institute of Nutritional Science, University of Potsdam, Arthur-Scheunert-Allee 114-116, D-14558 Nuthetal

The influence of a commercial production process for dehydrated potato flakes on the content of free phenolic compounds, total phenolics, and glycoalkaloids in potatoes during the subsequent processing steps was determined. Processing byproducts, such as potato peel (steam peeling), mashed potato residues, and side streams (blanching and cooking waters), have also been investigated. A high-performance liquid chromatography (HPLC) method was developed to separate and quantify caffeic acid, gallic acid, ferulic acid, *p*-coumaric acid, *p*-hydoxybenzoic acid, protocatechuic acid, vanillic acid, catechin, and three isomers of caffeoylquinic acid: chlorogenic, neochlorogenic and cryptochlorogenic acid. Determination of the glycoalkaloids α-solanine and α-chaconine was performed by using a high-performance thin-layer chromatography (HPTLC) method. The deliverables reveal that processing potatoes to potato flakes remarkably diminishes the content of the analyzed compounds, mainly due to peeling and leaching. The influence of thermal exposure is less significant. About 43% of the initial phenolic acids and 10% of the glycoalkaloids remain after processing. The results of the total phenolic content assay by Folin−Ciocalteu reagent are proportional to the content of phenolic compounds determined by HPLC. Steam peeling has a higher influence on glycoalkaloid losses compared to that on phenolics. The highest amounts of phenolic compounds and glycoalkaloids were found in peeling byproduct. During processing, the amount of chlorogenic acid decreased, whereas the concentration of neochlorogenic acid increased due to isomerization. The impact of the results on potato processing technology is discussed.

KEYWORDS: Potato flakes; phenolic acids; glycoalkaloids; chaconine; solanine; potato processing; TLC; total phenolics; chlorogenic acid

INTRODUCTION

Besides starch recovery, the production of drum-dried potato products is the most important industrial process for the dehydration and preservation of potatoes. Drum-dried potatoes are marketed as potato flakes for mashed potato products as well as intermediate material for French fries, potato chips, and many other convenience products. Worldwide, the potato (*Solanum tuberosum* L.) is one of the leading vegetable processing tubers, and it supplies remarkable portions of phenolic compounds, predominantly in the peel and adjoining tissues. These components help constitute defense mechanisms against plant diseases and herbivores. In nutrition, they have received considerable attention as they contain potentially protective factors against cancer and heart diseases, basically because of their potent antioxidative properties (*1*). **Figure 1** shows the chemical structures of the analyzed phenolic compounds in potatoes. Several researchers have set up analytical methods for the determination of phenolic compounds and total phenolic acids in raw and processed potatoes (*2*−*5*). However, information on the changes and losses of bioactive compounds during the particular processing steps of the whole processing chain of potato flakes is not available.

Steroidal glycoalkaloids are another important class of biologically active compounds in potatoes. They influence the flavor of fresh and processed potatoes from a bitter taste toward a burning sensation at higher concentrations (*6*) and are regarded to be toxic to humans in concentrations above 200 mg/kg of fresh weight of potatoes (*7*). α-Chaconine and α-solanine are the major potato glycoalkaloids, with regard to their contribution to total glycoalkaloid content as well as their bioactivity (*8*). They both consist of a side chain of three glycosyl residues attached to the 3-OH position of the steroidal part, solanidine (**Figure 2**). The ratio of the most biologically active α-chaconine to α-solanine depends on several factors (e.g., cultivar, growing conditions) and is reported to range from 1.8:1 to 7:1 for fresh unpeeled potatoes (*9*−*11*) and up to 4.1:1 in genetically modified potatoes (*12*).

*Author to whom correspondence should be addressed (telephone +49-30-314-72595; fax +49-30-314-72823; e-mail j.maeder@tu-berlin.de).

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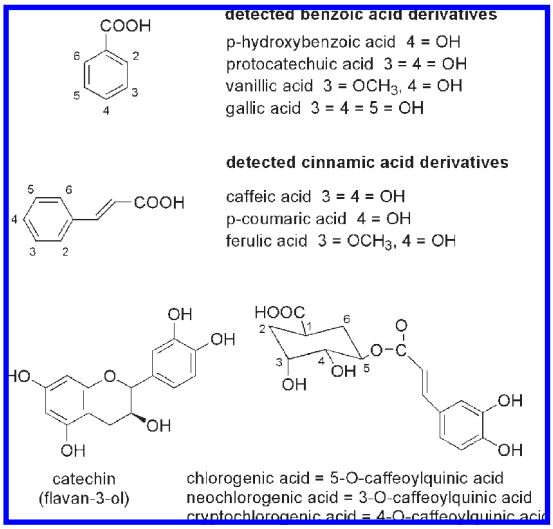

Figure 1. Structures of main potato phenolic compounds analyzed.

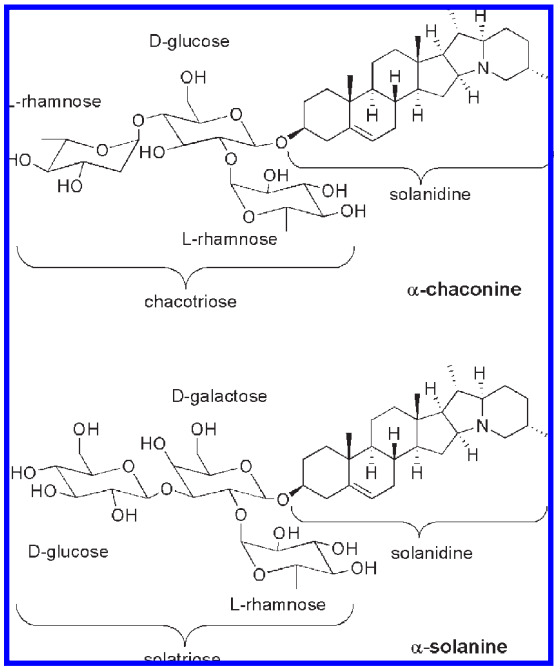

Figure 2. Chemical structures of the potato glycoalkaloids α-chaconine and α-solanine.

The potato peel and its extract contain phenolic acids and have a high antioxidant activity together with good antimicrobial properties (*13*−*16*). Therefore, peeling waste from potato processing has a potential in industrial polyphenol exploitation (*17*−*19*). Furthermore, potato polyphenols are important for the quality and processing of food, in particular with regard to their flavor induction (astringency) and capacity to promote discolorations such as enzymatic browning reactions (*20*−*22*). Often, for potato processors, possible beneficial effects of potato polyphenols are contrary to their negative effects regarding undesirable color inductions. Despite technical advances in potato processing over the past few decades, significant losses of phenolic compounds still occur during the commercial preparation of dehydrated potato flakes. The objective of the present research work is to quantify valuable and less valuable constituents in processing stages and byproducts of potato processing. The concentrations of different isomers of chlorogenic acid were investigated as well.

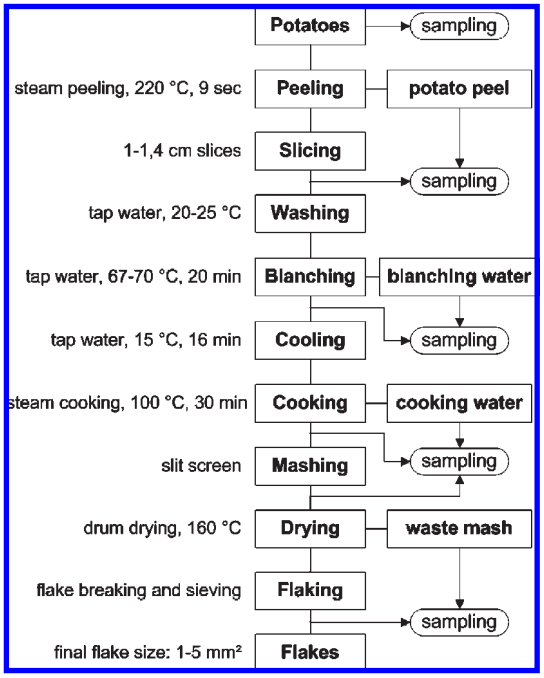

Figure 3. Flow diagram of industrial processing of potatoes to potato flakes including process parameters and sampling stages (*26*).

MATERIALS AND METHODS

Reagents and Chemicals. Acetic acid, *p*-coumaric acid, methanol, and methylene chloride were purchased from VWR International (Darmstadt, Germany). Folin−Ciocalteu's phenol reagent, antimony(III) chloride, chlorogenic acid, gallic acid, caffeic acid, and ferulic acid were purchased from Fluka (Taufkirchen, Germany). α-Chaconine, α-solanine, protocatechuic acid, vanillic acid, (+)-catechin, sodium carbonate, and *n*-heptane were purchased from Roth (Karlsruhe, Germany).

Industrial Potato Processing, Sampling, and Sample Preparation. For reasons of comparability, the use of a single potato cultivar during the whole sampling process was chosen. Potato tubers of the cultivar 'Karlena', a widely used cultivar for potato flake production grown under conventional farming, were processed within 2 days after harvesting. Potato flake production took place under industrial conditions in a potato-processing plant in Mecklenburg, Western Pomerania, Germany. The probed process steps were raw (washed), steam-peeled (sliced), water-blanched, steam-cooked, mashed, and flaked after drum-drying. The process residues and process waters including potato peel, mashed potato residue, blanching water and cooking water were also analyzed. A part of the initially used potatoes was labeled by the addition of food coloring in order to locate the same charge throughout the whole process. **Figure 3** shows the commercial potato flake process including the sampling stages and a short description of the processing conditions. The potato samples were placed into polyethylene bags, immediately immersed in a bed of dry ice for 2 h, and stored at −69 °C. After the deep-frozen samples had been crushed to fragments of approximately 5 mm^3, the samples were lyophilized for 48 h and stored at −30 °C until analysis. Directly after sampling, the process waters were filtered through a 3hw folded filter (Sartorius, Göttingen, Germany) and stored at −69 °C until analysis.

All samplings and subsequent procedures were performed at least twice for duplicate determinations. Dry weights of all samples were measured to obtain comparable results.

Extraction of Phenolic Compounds for HPLC and Folin−Ciocalteu Assay. The extraction was performed with an ASE 200 accelerated solvent extractor (Dionex, Sunnyvale, CA). One and a half grams of the freeze-dried and ground samples was mixed with ASE adsorbent (Isolute HM-N, Biotage AB, Uppsala, Sweden) in 11 mL extraction cells and extracted three times with methanol/water (70:30, v/v). The extraction conditions were as follows: 75 °C, 1500 psi, preheat 5 min, heat 5 min, static 2 min, 5 cycles per vial, respectively. The extracts were each filled to 20 mL,

combined, and centrifuged (13000*g*). The supernatant was filtered, adequately diluted with appropriate mobile phase, and used for HPLC.

For Folin−Ciocalteu assay (*23*), 1 mL of the supernatant was brought to pH 7, diluted with appropriate distilled water, and treated with an ascorbate oxidase spatula (Roche, Mannheim, Germany) for 10 min to eliminate interfering ascorbic acid.

Extraction of Glycoalkaloids for HPTLC. Two and a half grams of the freeze-dried and ground samples was added to 15 mL of methanol/acetic acid (95:5, v/v) and blended at 65 °C for 5 min at a rotational speed of 15000 rpm by an Ultra-Turrax T 25 homogenizer (IKA, Staufen, Germany; dispersing tool: S 25 N-25 F, 15). After the dispersing tool had been rinsed with 5 mL of the extraction agent, the suspension was centrifuged at 3500*g* and the supernatant was decanted. The residue was repeatedly extracted three more times according to the procedure described above. The collected supernatants were evaporated under vacuum at 40 °C; the residue was redissolved in 5 mL methanol/acetic acid (99:1, v/v) and filtered before the analysis was performed.

HPLC Analysis of Phenolic Compounds. The following method was applied for the quantification of phenolic compounds and chlorogenic acid isomers. As external standards for the total phenolic assay and for the qualitative and quantitative characterization of the phenolic compounds, stock solutions of (+)-catechin, chlorogenic acid, caffeic acid, gallic acid, vanillic acid, *p*-coumaric acid, ferulic acid, protocatechuic acid, and *p*-hydroxybenzoic acid in methanol/water (70:30, v/v) were used.

For identification of chlorogenic acid isomers, isomerization of chlorogenic acid to neo- and cryptochlorogenic acid was performed according to the procedure of Maslak (*24*), with some adaptations: 5 mg of chlorogenic acid was dissolved in 25 mL of distilled water. Isomerization was started by the addition of 25 mL of 0.2% aqueous sodium carbonate. After 0, 5, and 30 min, the reaction was stopped by the addition of acetic acid (10%) and methanol to an aliquot of the reaction solution. The final ratio of the reaction solution/acetic acid/methanol was 4:1:5 (v/v/v), respectively.

HPLC analysis of phenolic compounds was carried out in an LC 10 AS/SPD-10 A VP system (Shimadzu, Duisburg, Germany). The column used was a 150 mm × 4.6 mm i.d., 3 μm, Prontosil 120-3-C18 (Bischoff, Leonberg, Germany). The detection wavelengths were 280 and 325 nm. The injection volume was 20 μL. The flow rate was kept at 1 mL/min, and the column temperature was 40 °C. Eluent A was acetic acid (2% w/w), and eluent B was methanol. The gradient used was (A/B) 80/20−65/35 (in 20 min), 32/68 (in 2 min), 32/68 (3 min), 80/20 (in 3 min), and 80/20 (12 min). The retention times of the nine standards and the chlorogenic acid isomers on the HPLC column used were as follows: gallic acid, 3.96 min; neochlorogenic acid, 5.50 min; protocatechuic acid, 6.98 min; catechin, 8.45 min; cryptochlorogenic acid, 9.49 min; chlorogenic acid, 10.40 min; vanillic acid, 11.97 min; *p*-hydroxybenzoic acid, 12.80 min; caffeic acid, 14.43 min; ferulic acid, 20.82 min; *p*-coumaric acid, 22.47 min. Additionally, the identity of the sample peaks was confirmed with the standard substances using a HPTLC method (*25*).

Determination of Total Phenolic Content. Total phenolics were determined spectrophotometrically using Folin−Ciocalteu's reagent according to the method reported elsewhere (*23*) with some modifications. Two hundred microliters of the ASE extract or the process water was placed in standard cuvettes, 1 mL of Folin−Ciocalteu reagent (previously diluted 1:9 (v/v) with distilled water) and 800 μL of Na_2CO_3 (7.5%, w/v) were added, and then the solutions were mixed, allowed to stand for 30 min, and used for photometric determination of total phenolics at a wavelength of 765 nm (photometer Bio-Tek Instruments, Uvikon XL). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per 100 g of dry substance or milligram of 100 mL for process waters.

HPTLC Analysis of Glycoalkaloids. A HPTLC procedure for the qualification and quantification of potato glycoalkaloids was used as described previously (*26*). As external standards, stock solutions of α-chaconine and α-solanine in methanol/acetic acid (99:1, v/v) were prepared.

RESULTS AND DISCUSSION

Analytical Aspects. *Extraction Method.* The described accelerated solvent extraction method gave excellent yields of minimum 94% recovery for all phenolic compounds and samples after the third extraction cycle. The temperature of 75 °C in combination with the high pressure resulted in exhaustive cell disruption, which was also shown by microscopy.

Analysis of Phenolic Compounds. The HPLC method that we developed to assign and quantify the mentioned phenolic compounds and chlorogenic acid isomers gave well-separated chromatographic peaks for all standard substances as well as for the sample compounds. The Folin−Ciocalteu method for the determination of total phenolics showed sufficient accuracy and repeatability for all analyzed samples if performed according to the procedure described. If ascorbate oxidase is not added prior to analysis, overestimation of the total phenolic content occurs due to interference with ascorbic acid (*27*), especially contained in raw and initially processed potatoes.

Analysis of Glycoalkaloids. The method for the determination of the glycoalkaloids α-solanine and α-chaconine during potato processing has shown to be selective, robust, and fast. The method is preferred against HPLC, because up to 16 samples can be applied and separated in a single run on one HPTLC plate. Consequently, all processing stages and byproducts from one batch can be monitored and traced simultaneously within one analysis (*26*).

Isomerization of Chlorogenic Acid. During alkaline treatment of chlorogenic acid with aqueous sodium carbonate as described above, first cryptochlorogenic acid and subsequently neochlorogenic acid are formed (*24*). The first sample (0 min) gave only a single signal for chlorogenic acid. After 5 min, an additional strong signal for cryptochlorogenic acid and a small peak for neochlorogenic acid were observed. After 30 min, the neochlorogenic acid signal was the dominating peak. The elution sequence on the used reversed-phase column is neochlorogenic, cryptochlorogenic, and chlorogenic acid.

Influence of Processing on Phenolic Compounds and Glycoalkaloids. *Processing and Phenolic Compounds.* The contents of total phenolics, free phenolic compounds, and glycoalkaloids in subsequent potato-processing stages in samples of the cultivar 'Karlena' are listed in **Table 1**. All data are referred to corresponding dry matter for reasons of comparability. To roughly estimate the concentrations in fresh or rather reconstituted mashed potatoes (ready for consumption), the values have to be divided by 5. All analyzed samples were taken subsequently during a typical industrial production process for potato flakes. The potatoes utilized for processing were a representative and ordinary charge with an average content of green and bruised tubers. It must be pointed out that the individual samples were from different potatoes but all from one cultivar (Karlena) and the batch originating from the same acre. This sampling during a running industrial process is believed to be responsible for the comparatively high standard deviations of some data.

Chlorogenic acid isomers are the dominant phenolic acids in potatoes, followed by caffeic acid. Except for peels, all other analyzed phenolic compounds could be found in only some samples at low amounts or traces just above the limit of quantification. Chlorogenic acid can therefore be regarded as the leading substance representing the phenolic compounds in potatoes. According to the literature, the ratios and concentrations of the three detected chlorogenic acid isomers in raw potatoes vary widely (*2*, *28*, *29*), but it is generally accepted that chlorogenic acid is the main isomer in potatoes. The initial chlorogenic acid content (total of chlorogenic acid isomers, in mg/100 g of dry weight) of 148.1 is reduced to 92.0 by steam peeling, to 86.6 by blanching, and to 73.2 by subsequent cooking. The following processing steps, mashing and drying, do not significantly affect the concentrations of chlorogenic acids and caffeic acid.

Table 1. Average Concentrations of Total Phenolics, Glycoalkaloids, and Free Phenolic Compounds at Different Processing Stages and Byproducts

	raw[b]	peeled[b]	blanched[b]	cooked[b]	mashed[b]	dried[b]	peels[b]	waste mash[b]	blanching water[c]	cooking water[c]
totals phenols GAE[a] (Folin−Ciocalteu)	183.0 ± 2.8	130.4 ± 14.4	129.6 ± 10.6	101.6 ± 7.1	105.0 ± 1.2	107.1 ± 7.2	2700.7 ± 6.2	109.5 ± 10.5	16.8 ± 0.05	5.5 ± 0.1
α-solanine	7.8 ± 0.49	1.3 ± 0.09	1.0 ± 0.08	1.0 ± 0.07	0.9 ± 0.06	0.9 ± 0.06	224 ± 18.9	1.6 ± 0.09	5.4 ± 0.75	3.05 ± 0.91
α-chaconine	21.6 ± 1.57	5.4 ± 0.47	2.4 ± 0.19	2.7 ± 0.23	2.3 ± 0.19	2.0 ± 0.13	669 ± 54.6	3.3 ± 0.26	21.3 ± 3.26	5.9 ± 1.82
neochlorogenic acid	66.3 ± 0.97	56.0 ± 2.13	52.8 ± 0.22	48.1 ± 1.53	50.1 ± 1.6	49.4 ± 0.71	91.2 ± 32.7	49.2 ± 3.81	4.2 ± 1.29	1.7 ± 0.55
cryptochlorogenic acid	10.0 ± 0.55	3.1 ± 0.24	3.6 ± 0.07	6.1 ± 0.53	5.36 ± 0.06	4.5 ± 0.21	163.3 ± 6.6	6.4 ± 0.66	1.6 ± 0.05	0.32 ± 0.02
chlorogenic acid	71.8 ± 1.39	32.9 ± 2.67	30.2 ± 0.33	19.0 ± 1.36	18.1 ± 0.65	17.3 ± 0.17	1.468.1 ± 39.3	23.9 ± 2.66	4.3 ± 0.05	1.1 ± 0.12
caffeic acid	20.3 ± 1.18	2.0 ± 0.2	1.2 ± 0.03	1.1 ± 0.12	1.1 ± 0.15	1.1 ± 0.09	172.4 ± 3.25	1.1 ± 0.16	0.18 ± 0.04	0.17 ± 0.09
catechin	1.3 ± 0.05	nd	nd	nd	1.5 ± 0.14	1.1 ± 0.07	nd	nd	0.16 ± 0.10	nd
vanillic acid	1.0 ± 0.02	0.78 ± 0.2	nd	0.35 ± 0.04	nd	nd	22.4 ± 2.54	0.46 ± 0.03	0.07 ± 0.07	0.02 ± 0.009
p-hydroxybenzoic acid	nd	nd	nd	nd	nd	nd	7.84 ± 1.27	nd	nd	nd
coumaric acid	nd	nd	nd	nd	nd	nd	1.6 ± 0.99	nd	0.007 ± 0.001	0.007 ± 0.002
protocatechuic acid	nd	nd	nd	nd	nd	nd	7.6 ± 0.57	0.90 ± 0.09	nd	0.02 ± 0.006
gallic acid	0.95 ± 0.08	nd	nd	nd	nd	nd	nd	nd	nd	nd
ferulic acid	0.46 ± 0.1	0.44 ± 0.08	nd	nd	nd	nd	3.9 ± 2.51	nd	0.02 ± 0.02	0.02 ± 0.015

[a] Total phenols are expressed as gallic acid equivalents (GAE). [b] Mean ± SD (n = 2; in mg/100 g of dry wt). [c] Mean ± SD (n = 3; in mg/100 mL).

Figure 4. Effect of industrial potato processing on loss of free phenolic acids, glycoalkaloids, and total phenolics.

Processing and Glycoalkaloids. Peeling and blanching are predominantly responsible for glycoalkaloid losses. Steam peeling reduces the glycoalkaloid content (total of α-chaconine and α-solanine, in mg/100 g of dry weight) from 29.4 to 6.7; the content is reduced to 3.4 by the subsequent blanching step. Cooking, mashing, and drying have no significant effect on the glycoalkaloid concentration. The final concentration of total glycoalkaloids in potato flakes was 2.9 mg/100 g of dry weight, resulting in a concentration of 0.58 mg/100 g of fresh weight for reconstituted flakes. No health risk can be expected by the commercial processing of average and not excessive greened potatoes. The ratio of α-chaconine to α-solanine was between 4.1:1 and 2.2:1 for all of the analyzed samples.

Comparison of Phenolics and Glycoalkaloids. The effect of industrial potato processing on the loss of free phenolic acids, total phenolics, and total glycoalkaloids is shown in **Figure 4**. The percentage decrease based on 100% reveals that the losses of glycoalkaloids by peeling are much higher than the losses of phenolic acids and total phenolics, which is explainable by the exceptionally high content of glycoalkaloids in peels. Nevertheless, steam peeling is responsible for the highest losses of all the analyzed compounds within this study. Blanching has a remarkable influence on the content of phenolic acids and glycoalkaloids due to leaching over a period of 20 min and more. Steam cooking has no significant effect on glycoalkaloid concentration because they are stable during heating. In comparison, phenolic acids are more heat sensitive during cooking. The terminal mashing and drying steps do not significantly effect the concentrations of all analyzed compounds. One explanation is the formation of rigid cell structures due to retrogradation during the cooling step and water evaporation during drum-drying. The rigid structures are more resistant to the extraction procedures. The relatively gentle drum-drying method with contact times of only a few seconds can be regarded as an additional reason. In the final potato flake product, between 43% (phenolic acids, determined by HPLC) and 59% (total phenolics, determined by Folin−Ciocalteu method) of the phenolic compounds remain. In contrast, only 10% of the initial concentration of glycoalkaloids can be found in the end product.

Chlorogenic Acid Isomers during Processing. During processing, the amount of chlorogenic acid decreased, whether the proportion of neochlorogenic acid increased (**Figure 5**). This isomerization reaction is also described for sweet potatoes (*30*). However, the reaction mechanism of chlorogenic

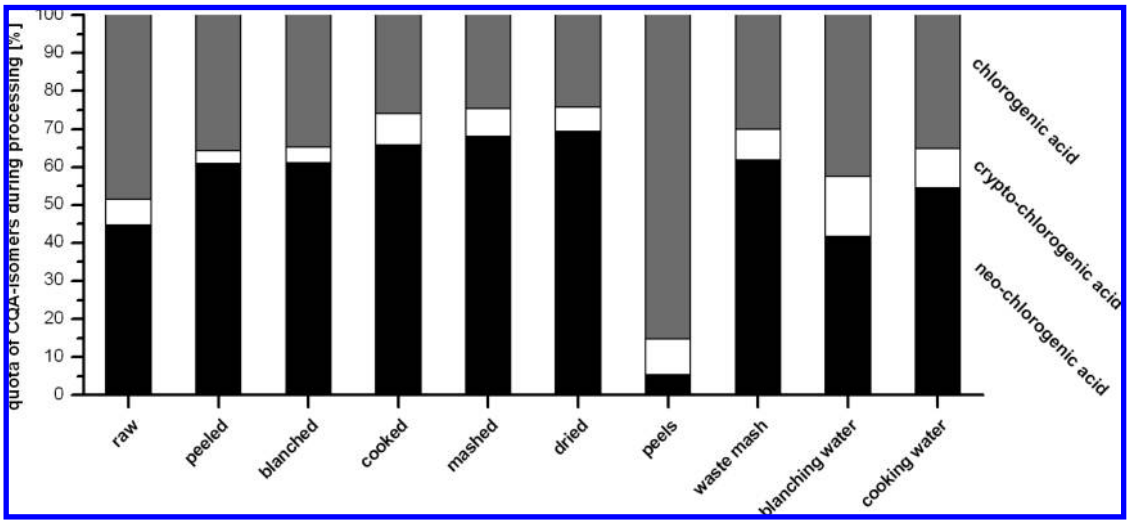

Figure 5. Amounts of chlorogenic acid (gray), neochlorogenic acid (black), and cryptochlorogenic acid (white) during industrial potato processing.

acid isomerization is not confirmed. The amount of cryptochlorogenic acid was relatively stable during the whole process.

The distribution of chlorogenic acid isomers in the peel was different compared to that inside the tuber. Eighty-five percent of the free chlorogenic acid in the peel was represented by chlorogenic acid.

Phenolic Compounds and Glycoalkaloids in Byproducts. **Table 1** reveals that the rejected waste mash has about the same glycoalkaloid and phenolic acid composition as mashed and dried potatoes. Its high starch content and the low amount of toxic glycoalkaloids are ideal for the utilization as fodder, for ruminants as well as for nonruminants, due to the pulped starch fraction. The amount of rejected waste mash is more than 10% of the initially used potatoes. The other important fraction of the pasty byproduct is the potato peel. Peeling losses range from 6 to 10%, referred to fresh weight. The potato peel is rich in phenolic acids (total = 1.976 mg/100 g of dry weight, determined by HPLC) but not usable as fodder due to its high content of toxic glycoalkaloids (total = 893 mg/100 g of dry weight). When used as a sole fodder source, it would exceed by far the concentration limit set for humans and animals (*7*, *31*). It could, however, be of interest if mixed with other feed or used as a possible source material for the extraction and utilization of natural antioxidants.

The concentration of the phenolic compounds in blanching water (10.5 mg/100 mL of total phenolic acids; HPLC) is >3 times higher as compared to that in the cooking water; the trend was also confirmed by the Folin−Ciocalteu assay (**Table 1**). During blanching, the potatoes are immersed in water, which causes leaching of the phenolics and glycoalkaloids, whereas during the subsequent cooking process after cooling, the potatoes are subjected to a steam treatment at atmospheric pressure. The resulting condensed water can be presumed to be responsible for the observed differences in the content of the bioactive compounds.

In conclusion, this study shows for the first time the changes in the content of the two important bioactive substance classes as influenced by the processing conditions on a large commercial scale. In the literature, some relevant studies have also been conducted documenting the effect of different processing steps such as peeling (*32*) or peeling and cooking (*33*). These papers illustrate the decrease of the chlorogenic acids in the range of 51% and in the case of glycolakaloids of about 58% during peeling and further 22% as observed during cooking. These results are only comparable to a certain extent (**Figure 3**) because they refer to different cultivars and single steps on laboratory-scale processing without considering the preceding and subsequent treatment the potatoes are expected to undergo.

One of the current projects includes the optimization of the extraction of the valuable phenolic compounds from the blanching water and mixtures originating from the peeling process combined with blanching water. One further goal is the investigation of the fate of the glycosidically bound phenolic acids during potato processing by means of preliminary hydrolysis and enzyme treatment. Another research area that also needs to be addressed is the investigation of additional different potato cultivars, especially those used in industrial processing, to gain more representative data about the content and behavior of potato phenolics and glycoalkaloids during commercial potato processing.

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