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Influence of Thermal Treatment on Color, Enzyme Activities, and Antioxidant Capacity of Innovative Pastelike Parsley Products

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ABSTRACT: Conventional spice powders are often characterized by low sensory quality and high microbial loads. Furthermore, genuine enzymes are only inhibited but not entirely inactivated upon drying, so that they may regain their activity upon rehydration of dried foods. To overcome these problems, initial heating was applied in the present study as the first process step for the production of innovative pastelike parsley products. For this purpose, fresh parsley was blanched (80, 90, and 100 °C for 1−10 min) and subsequently comminuted to form a paste. Alternatively, mincing was carried out prior to heat treatment. Regardless of temperature, the color of the latter product did not show any change after heating for 1 min. With progressing exposure time the green color turned to olive hues due to marked pheophytin formation. Inactivation of genuine peroxidase (POD) and polyphenol oxidase (PPO) was achieved at all temperature−time regimes applied. In contrast, the parsley products obtained after immediate water-blanching were characterized by brighter green colors and enhanced pigment retention. With the exception of the variants water-blanched at 80 °C, POD and PPO were completely inactivated at any of the thermal treatments. Furthermore, in water-blanched samples, antioxidant capacities as determined by the TEAC and FRAP assays were even enhanced compared to unheated parsley, whereas a decrease of phenolic contents could not be prevented. Consequently, the innovative process presented in this study allows the production of novel herb and spice products characterized by improved sensory quality as compared to conventional spice products.

KEYWORDS: *parsley, Petroselinum crispum (Mill.) Nym ex A. W. Hill, blanching, chlorophyll, color, peroxidase, polyphenol oxidase, Folin−Ciocalteu, TEAC, FRAP*

INTRODUCTION

Parsley (*Petroselinum crispum* (Mill.) Nym ex A. W. Hill), an Apiaceous plant, belongs to the widely used herbs and spices. In conventional spice production, fresh plant material is dried after harvest and subsequently comminuted, provoking considerable losses of volatiles and pigment degradation, which results in unappealing color and flavor appearance.[1] Furthermore, herbs and spices are commonly burdened with high microbial loads, thus posing a potential risk for human consumption. Although dried spices are almost nonperishable goods due to their low water activity, microbial populations may grow rapidly upon rehydration of the spices. Accordingly, microbial counts of fresh products may dramatically increase until consumption. Moreover, endogenous enzymes affecting product quality, such as peroxidases (POD), polyphenol oxidases (PPO), chlorophyllases, and proteinases, are inhibited only in the dry state but are not entirely inactivated. Consequently, such enzymes may regain their activity upon rehydration of dried foods and adversely affect color, taste, and texture properties of the products.[2]

Peroxidases (EC 1.11.1.7) are ubiquitous in nature, decomposing hydrogen peroxide in the presence of a hydrogen donor. They exhibit the highest thermal stability among all enzymes in plants, and their inactivation is therefore often used as an indicator of blanching processes.[3] Chlorophylls of parsley leaves were suggested to be degraded by peroxidase activity. Enzymatic degradation was reported to be an indirect process, where apigenin, the major flavonoid of parsley, is first oxidized, which subsequently degrades chlorophylls.[4]

PPO is characterized by two different reactions. Cresolase (EC 1.14.18.1) activities catalyze the hydroxylation of monophenols in the presence of molecular oxygen, yielding the corresponding *o*-dihydroxyl derivatives. The oxidation of *o*-dihydroxyl phenols in the presence of molecular oxygen generating the corresponding *o*-quinones is catalyzed by catecholase (EC 1.10.3.2) activities, which marks the first step of enzymatic browning.[3,5]

Chlorophylls are the predominant pigments of green plants and, consequently, are a major quality attribute of vegetable products.[6] Since bright green colors are associated with freshness, discoloration may lead to consumer rejections.[7] The loss of green color is mainly attributed to chlorophyll degradation. Thermal degradation of chlorophylls *a* and *b* results in color changes from bright green to olive brown due to the formation of pheophytins *a* and *b* as a consequence of the release of the Mg atom.[6,8] In contrast, the loss of the phytol moiety, catalyzed by chlorophyllase (EC 3.1.1.14) activities, thus forming water-soluble chlorophyllides, is the first step of chlorophyll degradation in senescent leaves, with the removal of the Mg atom from chlorophyllide being the second step in nonthermal degradation of chlorophylls.[9]

Recently, an alternative process for the production of high-quality spices has been developed to overcome the afore-

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mentioned drawbacks of conventional spice production. For this purpose, the plant material was immediately blanched after harvest and subsequently processed into a paste. Alternatively, mincing was performed prior to thermal treatment. Due to the initial thermal treatment of the plant material, the spice powders were characterized by significantly reduced microbial loads and brighter colors compared to conventional spices.[10] Furthermore, a complete inactivation of PPO was achieved, while POD was only partially inactivated.[11]

Since spice powders have low sensory quality and are difficult to handle in industrial food processing due to lump and dust formation, the development of pumpable pastelike herb and spice products should provide significant advantages. Furthermore, energy demanding processes such as drying and cryogenic milling are required to obtain mostly hygroscopic powders. Therefore, the objective of the present study was to apply an initial heating step instead of drying as a first step for the production of innovative pastelike parsley products, which have not been studied so far. By omitting drying and cryogenic milling, the process is expected to save energy, thus contributing to the improvement of energy efficiency. Therefore, parsley was minced and subsequently heated in the present study. Alternatively, blanching prior to mincing was applied to study the effect of earlier enzyme inactivation. Investigations were undertaken to optimize the initial heating step with regard to its impacts on color and chlorophyll retention, enzyme activities, total phenolic contents, and antioxidant capacity.

MATERIALS AND METHODS

Raw Material. Parsley [*P. crispum* (Mill.) Nym ex A. W. Hill cv. 'Bukett'] was purchased from a wholesale in Stuttgart, Germany.

Chemicals. Tropolone, PVPP (polyvinylpolypyrrolidone), ABTS [2,2′-azinobis-(3-ethylbenzothiazoline)-6-sulfonate diammonium salt], ABAP [2,2′-azo-bis(2-amidinopropane) hydrochloride], TPTZ [2,4,6-tris(2-pyridyl)-*s*-triazine], and $FeCl_3 \cdot 6\ H_2O$ were purchased from Fluka (Steinheim, Germany). BHT (2,6-di-*tert*-butyl-*p*-cresol) and BHA (3-*tert*-butyl-4-hydroxyanisol) were from Sigma-Aldrich (Steinheim, Germany). Celite 545 was obtained from Carl Roth (Karlsruhe, Germany). Folin−Ciocalteu reagent, methanol, acetone, and petroleum ether were purchased from VWR (Darmstadt, Germany). Chlorophyll *a*, chlorophyll *b* (Sigma-Aldrich, St. Louis, MO), gallic acid, and Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) purchased from Sigma-Aldrich (Steinheim, Germany) were used for external calibration. All other solvents and reagents were of analytical or HPLC grade and were from Merck (Darmstadt, Germany). Deionized water was used throughout.

Process for the Production of Pastelike Parsley Products. Amounts of 500 g of freshly harvested parsley were washed and ground for 5 min using a bowl chopper (MADO Garant, Dornhan, Germany) after addition of 1 L distilled water. The resulting products with a particle size ranging from 0.16 to 3.15 mm were immediately heated in a hermetically sealed 3 L pilot-plant scale Type EL 3 reaction vessel (ESCO-Labor, Riehen, Switzerland) under stirring at 80 and 90 °C for 1, 5, 7, and 10 min and at 100 °C for 5, 7, and 10 min, respectively (process variant I). The vessel was equipped with a reflux cooling system to avoid the evaporation of water and losses of volatiles. Set temperatures were achieved after approximately 2−4 min. After heat treatment the pastelike products were cooled to 22 °C.

Alternatively, the plant material was blanched prior to grinding (process variant II). Aliquots of 300 g of washed parsley were blanched at 80 and 90 °C for 1, 5, 7, and 10 min and at 100 °C for 1, 5, 7, and 10 min in a water bath. Furthermore, steam-blanching at 100 °C for 1, 5, 7, and 10 min was performed using a boiler. The blanched material was subsequently cooled in ice−water, drained, and minced for 5 min to form a pastelike product using the aforementioned chopper.

An unheated control sample was prepared for each process variant. For this purpose, parsley was minced applying the conditions described above.

Characterization and Quantitation of Individual Chlorophylls. Chlorophyll extraction was performed as previously described.[12] Sample preparation was carried out under dim light, and amber glassware was used to avoid chlorophyll degradation. All determinations were carried out in duplicate. Parsley paste (1 g for process variant I and 0.5 g for process variant II) was homogenized for 10−20 s using an ultra-Turrax (Janke & Kunkel, Stauffen, Germany) after adding 0.25 g of Celite 545, 0.25 g of calcium carbonate, and 30 mL of cold methanol. After filtering the homogenized suspensions through a sintered glass funnel (porosity 2, 40−100 μm) under reduced pressure, the recovered solids were successively re-extracted two or three times with ca. 30 mL of cold methanol. Extraction was carried out until the residue was colorless. The combined methanolic extracts were transferred to a 500 mL amber glass separatory funnel with 40 mL of petroleum ether (extra pure; bp 40−60 °C), containing BHT (100 mg/L) and BHA (100 mg/L) as antioxidants and 30 mL of NaCl (10%, w/w) to prevent formation of emulsions. Chlorophylls were extracted from the hydroalcoholic phase four times with 20 mL of petroleum ether each until this phase was yellowish. The pooled organic phases were dried with 2 g of sodium sulfate and evaporated in vacuo. The residue was dissolved in 5 mL of 2-propanol, membrane-filtered (0.2 μm), and transferred to amber glass vials. The extracts were stored at −80 °C until HPLC-DAD and LC/MS analyses.

HPLC analyses were performed as reported previously[13] using a model 2690 Waters separation module equipped with an autosampler injector, a model Jetstream 2 plus Waters column oven, and a model 2966 Waters UV−visible photodiode array detector controlled by a Millenium 32 (version 3.2) workstation (Waters, Milford, MA). Chlorophylls were separated on a 150 × 3.0 mm i.d., 3 μm particle size, analytical scale YMC C30 reversed-phase column (Wilmington, MA). The mobile phase consisted of methanol/methyl *tert*-butyl ether (MTBE)/water (81:15:4, v/v/v; eluent A) and methanol/MTBE/water (4:92:4, v/v/v; eluent B). The modified gradient used was as follows: 0% B to 40% B (30 min), 100% B isocratic (5 min), 100% B to 0% B (5 min), 0% B isocratic (5 min). Total run time was 40 min. The injection volume was 7 μL. Chlorophylls were monitored at 660 nm (chlorophyll *a* and its derivatives) and 644 nm (chlorophyll *b* and its derivatives) at a flow rate of 0.42 mL/min. Additionally, UV/vis spectra were recorded in the range of 200−700 nm.

Individual compounds were quantitated using calibration curves of the corresponding standard compounds. When reference compounds were not available, the calibration of structurally related substances exhibiting the corresponding chlorophyll backbone was used including a molecular weight correction factor.[14] Concentrations of stock solutions were determined spectrophotometrically using the absorption coefficients A_{1cm}^{1mM} of 82 at 660 nm for chlorophyll *a* and 56 at 644 nm for chlorophyll *b* in diethyl ether (Sigma-Aldrich, St. Louis, MO).

LC−MS analyses were carried out using an Agilent HPLC 1100 series (Agilent, Waldbronn, Germany) equipped with ChemStation software, a model G1322A degasser, a model G1312A binary pump, a model G1313A autosampler, a model G1316 column oven, and a model G1315A DAD system. The analyses were performed using the column and mobile phase described above. The HPLC system was coupled online to a Bruker (Bremen, Germany) model Esquire 3000+ ion trap mass spectrometer fitted with an atmospheric pressure chemical ionization (APCI) source. Data acquisition and processing were performed using Esquire Control software. Positive ion mass spectra of the column eluate were recorded in the range of m/z 50−1000 at a scan speed of 13 000 Th/s (peak width 0.6 Th, full width at half-maximum). Nitrogen was used both as drying gas at a flow rate of 4.0 L/min and as the nebulizing gas at a pressure of 50 psi. The nebulizer temperature was set at 350 °C, and a potential of 2779 kV was used on the capillary. Corona was set at 4000 nA in the positive ion mode, and the vaporizer temperature was set at 400 °C. Helium was used as the collision gas for collision-induced dissociation (CID) at a pressure of 4.0×10^{-6} mbar. CID spectra were obtained with an

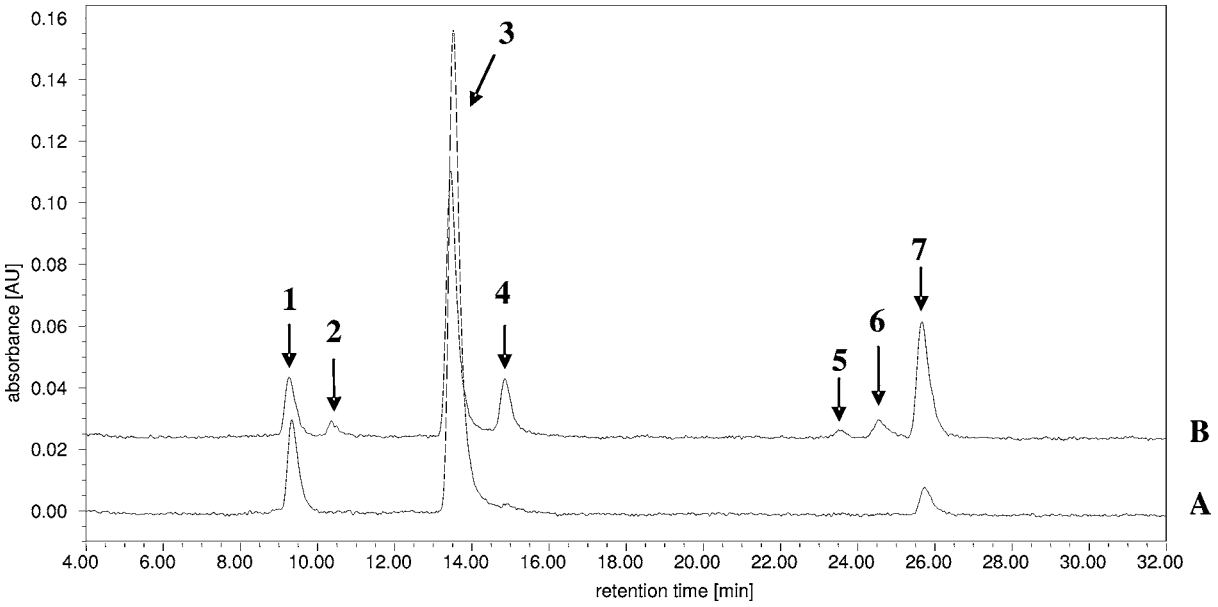

Figure 1. HPLC-DAD chromatograms (660 nm) of chlorophylls and their derivatives in unheated (A) and steam-blanched (B) parsley. For peak assignment see Table 1.

isolation width of 6.0 Th for precursor ion selection and fragmentation amplitude of 1.0 V.

Color Measurement. Color measurements were performed using a CR-300 chroma meter (Minolta, Osaka, Japan) immediately after processing. The instrument was calibrated with a standard white tile (L^* = 97.43, a^* = −0.01, b^* = 1.64). A glass Petri dish containing the samples was placed below the light source. Chroma [$C^* = (a^{*2} + b^{*2})^{1/2}$] and hue angle [$h° = (\arctan b^*/a^*)$] were calculated from CIE a^* and b^* using illuminant D_{65} and a 10° observer angle. All determinations were carried out in quintuplicate.

Enzyme Extraction. The procedure of POD and PPO extraction was carried out as reported previously with some modifications.[11] Aliquots of the paste (2 g for process variant II, 4 g for process variant I) were mixed with 0.7 g of PVPP and 25 g of cooled McIlvaine buffer (pH 6.5), consisting of 30% 0.1 M citric acid and 70% 0.2 M disodium hydrogen phosphate solution (v/v), and stirred for 2 h at 4 °C. The suspension was centrifuged at 25 000*g* for 20 min at 4 °C. After centrifugation, the supernatant was filtered through a Macherey-Nagel MN 615 1/4 paper (Düren, Germany) and adjusted to 20 g with McIlvaine buffer. The clear supernatant was kept at 4 °C until POD and PPO activity assays were performed on the same day. All extraction protocols were carried out in duplicate.

Quantitation of Peroxidase and Polyphenoloxidase Activity. POD and PPO activities were determined as described earlier.[11] Enzyme activity was calculated from the slope of the linear regression curve of the absorbance−time plot. For blank correction, the slopes of the sample blank (McIlvaine buffer instead of paste) and a reagent blank (McIlvaine buffer without tropolone and H_2O_2) were subtracted.

Quantitation of Total Phenolic Content and Total Antioxidant Capacity. For the extraction of phenolic compounds, approximately 1 and 2 g of the products for process variants II and I, respectively, were exactly weighed and extracted with 20 mL of 50% aqueous methanol under nitrogen atmosphere using an ultrasonic bath for 15 min. The supernatant was decanted, 20 mL of acetone/water (70:30, v/v) was added to the residue, and the extraction was repeated. After filtration through a Macherey-Nagel MN 615 1/4 paper (Düren, Germany), the organic extracts were combined and evaporated to dryness in vacuo at 30 °C. The residue was dissolved in acetone/water (70:30, v/v) and adjusted to a volume of 10 mL. All determinations were carried out in duplicate. The extracts were stored at −20 °C until analysis.

Total phenolic contents were estimated using the Folin−Ciocalteu colorimetric method.[15] Briefly, 100 μL of the sample extract was mixed with 900 μL of distilled water and 100 μL of Folin−Ciocalteu reagent. After vortexing, the reaction was allowed to proceed for 3 min before adding 800 μL of sodium carbonate (75 g/L). After a reaction time of 1 h, absorbance was measured photometrically at 720 nm and 25 °C. Gallic acid was used as standard, and the results were expressed as gallic acid equivalents (GAE) in mmol/kg DM for the pastelike products.

Total antioxidant capacity (TEAC assay) was carried out by an improved $ABTS^{\bullet-}$ method.[16] Briefly, the diammonium salt of ABTS was dissolved in a pH 7.4 phosphate buffer (Na_2HPO_4/KH_2PO_4 containing NaCl) at a concentration of 20 mM. Then 0.5 mL of this solution was mixed with 100 mL of a 2.5 mM ABAP solution in phosphate buffer and heated at 60 °C for 15 min. Aliquots of 40 μL of the sample extract were added to an aliquot of 2000 μL of the ABTS solution and the components were mixed thoroughly. Absorbance was registered at 734 nm and 25 °C after a reaction time of exactly 6 min. Calibration curves were established with the vitamin E analogue Trolox. Antioxidant capacity was expressed as Trolox equivalents (TE) in mmol/kg dry matter (DM) for the pastelike products.

The FRAP assay was performed as reported previously[17] with some modifications. The FRAP reagent was prepared by mixing 25 mL of sodium acetate buffer (pH 3.6) with 2.5 mL of a TPTZ solution dissolved in diluted hydrochloric acid, and 2.5 mL of an aqueous solution of $FeCl_3$. 1500 μL of freshly prepared TPTZ reagent were mixed with 300 μL of sample extract. Following thorough mixing, absorbance was measured at 593 nm and 25 °C after exactly 4 min. Results of the FRAP assay were expressed as Trolox equivalents (TE) in mmol/kg DM.

All photometric assays were performed using a Cary 100 spectrophotometer (Varian, Victoria Mulgrave, Australia) equipped with a Varian Cary temperature controller and Varian Cary Win UV 2.0 software.

Determination of Dry Matter (DM). All samples were lyophilized for 72 h using a Lyovac GT4 freeze-dryer (AMSCO Finn-Aqua; Hürth, Germany). Dry matter contents were calculated on the basis of the sample weights prior to and after freeze-drying.

Statistical Analysis. Significant differences (α = 0.05) were determined using the Tukey test for differences between independent samples. Data evaluation was performed using the SAS software package (SAS Institute, Cary, NC; v. 9.1).

Table 1. UV/Vis Spectra and Mass Spectrometric Characteristics of Chlorophylls and Their Derivatives in Pastelike Parsley Products

compd	t_R, min	identity	HPLC-DAD, λ_{max}, nm	$[M + H]^+$, m/z	HPLC–APCI(+)MS^n experiment, m/z (% base peak)
1	9.3	chlorophyll *b*	468, 604, 652	907	MS^2 [907]: 629 (100), 597 (61), 569 (61), 541 (19) MS^3 [907 → 629]: 569 (100), 597 (98), 541 (44)
2	10.4	chlorophyll *b*′	467, 605, 652	907	MS^2 [907]: 629 (100), 569 (63), 597 (49), 630 (22) MS^3 [907 → 629]: 569 (100), 541 (66), 597 (47)
3	13.5	chlorophyll *a*	430, 620, 661	893	MS^2 [893]: 615 (100), 555 (80), 556 (64), 583 (64), 616 (38) MS^3 [893 → 615]: 555 (100), 583 (45), 481 (28), 584 (21)
4	14.9	chlorophyll *a*′	430, 620, 661	893	MS^2 [893]: 615 (100), 555 (96), 614 (54), 583 (43) MS^3 [893 → 615]: 555 (100), 583 (50), 481 (37)
5	23.5	pheophytin *b*	435, 596, 661	885	MS^2 [885]: 607 (100), 547 (59), 857 (23) MS^3 [885 → 607]: 547 (100), 579 (32), 519 (17)
6	24.6	pheophytin *a*′	409, 610, 667	871	MS^2 [871]: 593 (100), 533 (32), 594 (30) MS^3 [871 → 593]: 533 (100), 534 (26)
7	25.7	pheophytin *a*	409, 610, 667	871	MS^2 [871]: 593 (100), 533 (51), 594 (22) MS^3 [871 → 593]: 533 (100), 460 (8)

RESULTS AND DISCUSSION

Due to lump and dust formation, handling and dosing of conventional spice powders are hindered in industrial food processing. Furthermore, endogenous enzymes, such as peroxidases and polyphenol oxidases, are only transiently inhibited, but not completely inactivated. Consequently, they may regain their activity upon rehydration of dried foods, resulting in significant quality deterioration.[2] Therefore, early heating was attempted as the first step of processing parsley into an innovative pastelike product both to reduce microbial loads and to inactivate endogenous quality deteriorating enzymes.

Analysis and Characterization of Individual Chlorophylls. The chromatograms of chlorophylls and their derivatives in unheated and blanched pastelike parsley products are illustrated in Figure 1 with peak assignment being specified in Table 1. Due to coelution of chlorophylls and carotenoids, Aman et al.[18] developed a method for the separation of these pigments using high-speed counter-current chromatography (HSCCC). This method allowed the quantitation both of carotenoids and chlorophylls in a single run. For the sole determination of chlorophylls by HPLC, the chromatographic runs may be monitored at 660 nm for chlorophyll *a* and its derivatives and at 644 nm for chlorophyll *b* and its derivatives to exclude interference of carotenoids.[19] Characterization of chlorophyll *a* (m/z 893) and *b* (m/z 907) eluting after 13.5 and 9.3 min was based on the comparison of retention times, UV/vis spectra, and MS data with those of standard substances. Chlorophyll *a* is characterized by a methyl group at position C7 (B ring), whereas chlorophyll *b* exhibits a formyl group at this position resulting in a molecular weight difference of 14 Da (Figure 2).

All other compounds were characterized by comparing their MS characteristics with literature data. Similar fragmentation patterns were observed for all chlorophyll derivatives. As can be seen from Table 1, by using APCI in the positive ionization mode, mass spectra of these compounds were mainly characterized by their prominent $[M + H]^+$ and $[M + H - C_{20}H_{38}]^+$ ions. The neutral loss of a phytadiene fragment (278 Da) in the MS^2 experiment was due to the elimination of the alkyl chain from the phytol moiety.[20] Furthermore, a characteristic loss of 60 Da from the $[M + H - C_{20}H_{38}]^+$ ion was observed in the MS^3 experiment through the loss of the carbomethoxy group and an H atom from the E ring.[21]

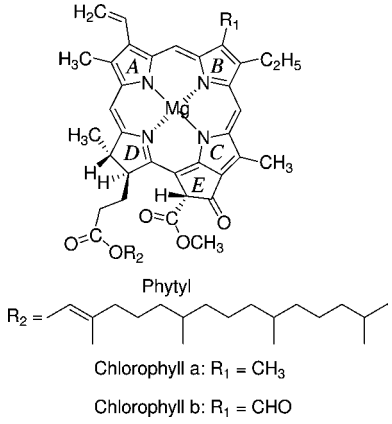

Figure 2. Structures of chlorophyll *a* and *b*.

Compound **5** revealing an ion at m/z 885 was tentatively assigned to pheophytin *b* due to the difference of 22 Da between compound **5** and chlorophyll *b*. This indicates Mg^{2+} substitution of the chlorophyll by H^+. Additionally, compound **7** produced an $[M + H]^+$ ion at m/z 871 with identical molecular weight difference of 22 Da compared to chlorophyll *a*. Therefore, compound **7** was tentatively assigned to pheophytin *a*. Compounds **2**, **4**, and **6** exhibited UV/vis spectra, $[M + H]^+$ ions, and fragmentation patterns identical to those of chlorophyll *b*, chlorophyll *a*, and pheophytin *a*, respectively. This indicated the presence of chlorophyll *b*′, chlorophyll *a*′, and pheophytin *a*′, the C-10 epimers of the aforementioned compounds.

Chlorophyll Stability during Processing. Chlorophyll *a* and *b* were the predominant compounds in all control samples besides minor amounts of pheophytin *a* as an indicator of beginning leaf senescence (Figures 3 and 4). Since mincing resulted in decompartmentalization of individual cells, acids were released from the vacuoles, thus provoking the formation of magnesium-deficient derivatives. Furthermore, in non-thermally treated samples (control), the exchange of the magnesium by two protons may be catalyzed by the enzyme magnesium dechelatase.[22] In contrast, pheophytin *b*, which would have been expected according to these findings, could not be detected. Furthermore, unheated samples obtained according to process variant I revealed low contents of chlorophyll *a*′. It is assumed that this epimer is an artifact

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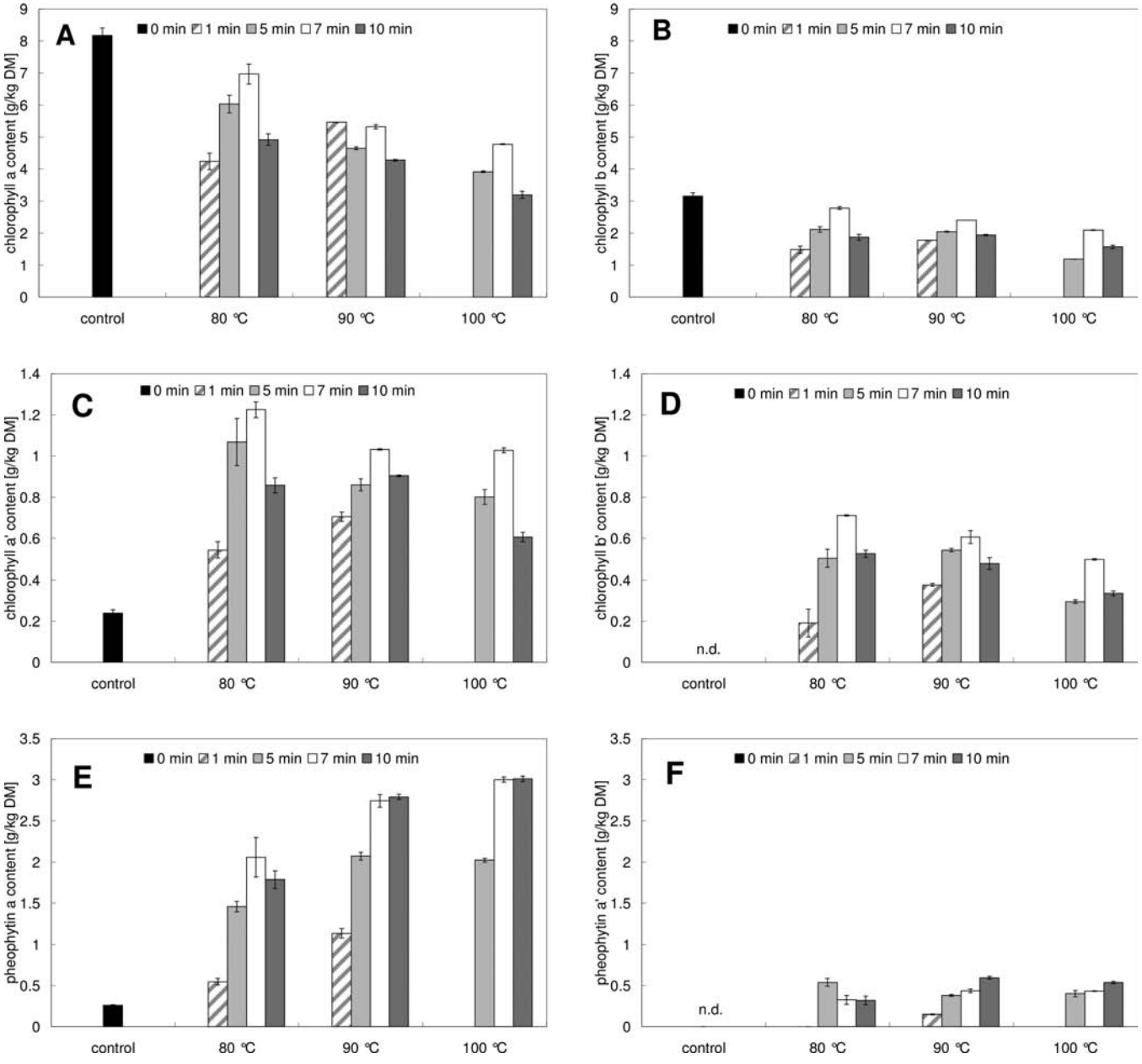

Figure 3. Contents of chlorophyll *a* (A), chlorophyll *b* (B), chlorophyll *a*′ (C), chlorophyll *b*′ (D), pheophytin *a* (E), and pheophytin *a*′ (F) in pastelike parsley products obtained according to process variant I. Columns and bars represent mean ± standard deviation (n = 2).

formed as a result of methanolic chlorophyll extraction, which has also been reported for hogweed and barley leaves.[23] Greater quantities of chlorophyll *a*′ and chlorophyll *b*′ were detected in all samples after heating, indicating that thermal treatment markedly enhances epimerization. This is in agreement with studies of spinach chlorophylls also revealing epimerization upon thermal treatment.[20]

Thermal treatment according to both process variants resulted in significant losses of chlorophyll *a* and *b*, however, to various extents. For products obtained according to process variant I, chlorophyll *a* and chlorophyll *b* losses ranged from 15 to 61% and 12 to 62%, respectively. Upon blanching (process variant II), losses were in the range of 4−45% for chlorophyll *a* and 1−39% for chlorophyll *b*. These differences indicate blanching to be the more preservative procedure, since less destruction of tissue may better protect secondary metabolites from degradation. The decreases of chlorophyll *a* and *b* contents were attributed to degradation of both compounds to pheophytin *a* and *b*, respectively.[24] Highest amounts of pheophytin *a* were found when the samples were boiled and steam-blanched. In samples of process variant I only traces of pheophytin *b* were detected in all samples except after 1 min of treatment under any condition.

Upon heating of minced parsley, chlorophyll *a* and *b* degradation followed the same trends at all time−temperature regimes. As can be deduced from Figure 3, levels of chlorophyll *a* and *b* decreased with increasing temperature and heating time. Expectedly, pheophytin *a* contents increased with more rigorous thermal treatment. In contrast, even after extended heating only traces of pheophytin *b* were observed. Chlorophyll *a* has been reported to be degraded to pheophytin *a* at higher conversion rates than chlorophyll *b* to pheophytin *b*.[25] Additionally, chlorophyll *a* is known to exhibit greater susceptibility to "pheophytinization" than chlorophyll *b*.[6]

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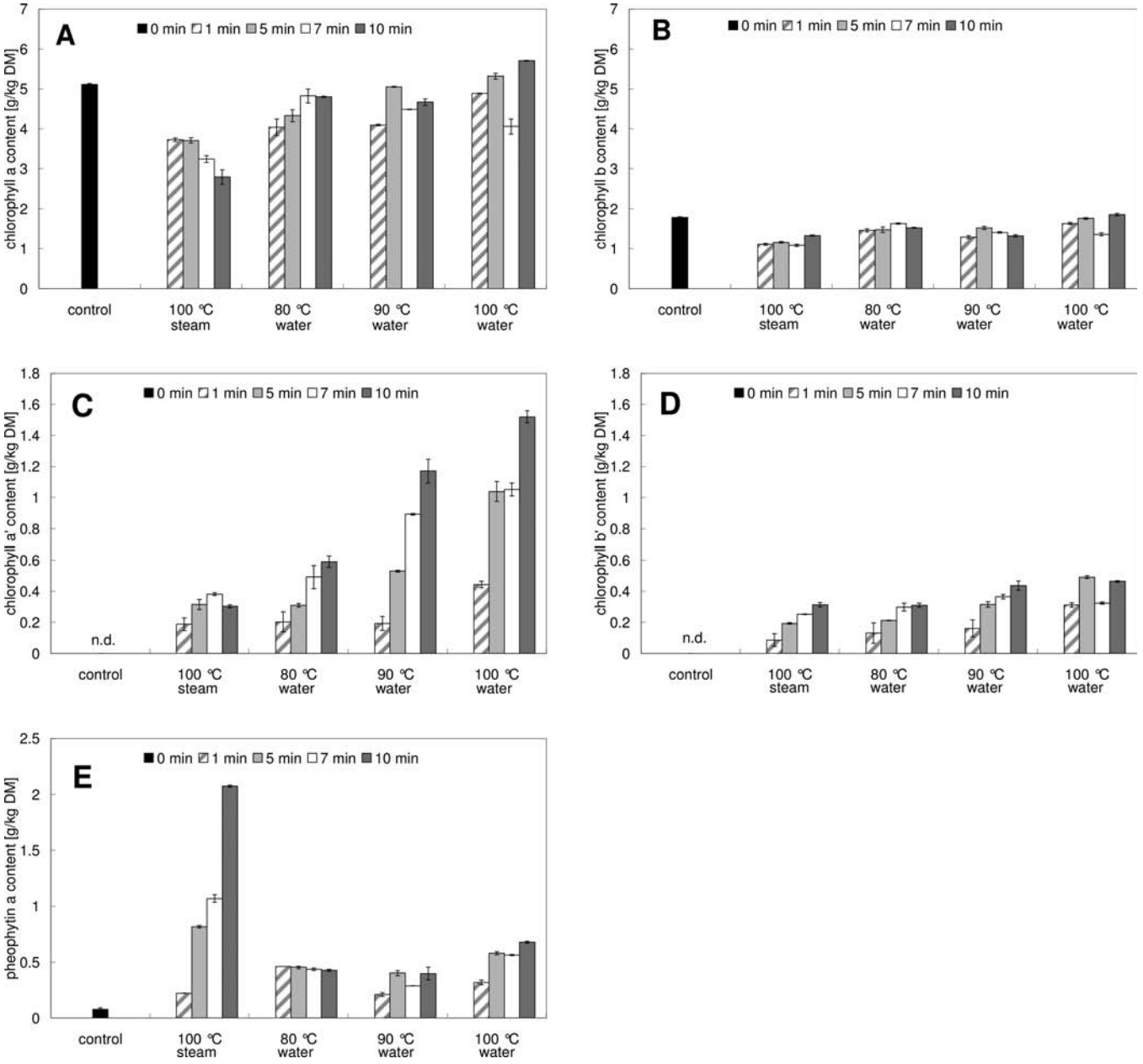

Figure 4. Contents of chlorophyll *a* (A), chlorophyll *b* (B), chlorophyll *a*′ (C), chlorophyll *b*′ (D), and pheophytin *a* (E) in pastelike parsley products obtained according to process variant II. Columns and bars represent mean ± standard deviation (n = 2).

These observations may explain the low levels of pheophytin *b* in pastelike parsley products.

Steam-blanching resulted in great losses of chlorophyll *a* and *b* as well as in enhanced formation of pheophytin *a* (Figure 4), which is in agreement with the results of a study on chlorophyll stability in spinach leaves.[26] Maximum pheophytin *a* amounts were found after steam-blanching for 10 min. It has been reported that volatile organic acids are readily lost upon blanching in boiling water, whereas these acids are retained upon steam treatment, thus leading to enhanced pheophytin formation.[27] In contrast, water-blanching resulted in lower pheophytin *a* formation. However, significantly higher levels were observed with increasing blanching time at 90 and 100 °C. After steam-blanching (except for 1 min) and water-blanching for 7 and 10 min, traces of pheophytin *b* were detected.

Interestingly, the levels of chlorophyll *a* and *b* were higher after water-blanching at 100 °C for 5 and 10 min, respectively, than in the nonblanched controls, which is probably due to enhanced extractability of the pigments, since heating is known to enhance the disruption of plant cells and tissues, thus facilitating the release of pigments.[7]

After steam- and water-blanching, the occurrence of chlorophyll *a*′ and *b*′ was observed, which is in accordance with literature reporting the formation of both isomers to be induced by mild heat treatment, such as blanching.[8] Greater quantities of these isomers were detected after extended heating. Similar results were observed after prolonged blanching of spinach.[26] However, since light absorption properties of the isomers are equal to those of chlorophyll *a* and *b*, their formation is not associated with a change of color.[28]

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Table 2. Color Characteristics of Pastelike Parsley Products Obtained According to Process Variant I (n = 5)[a]

process parameter	L^*	a^*	B^*	C^*	h°
control (unheated)	26.4 ± 1.1 d	−11.3 ± 0.3 gh	16.1 ± 0.5 ab	19.6 ± 0.6 ab	125.1 ± 0.3 a
80 °C, 1 min	29.4 ± 1.4 a	−12.0 ± 0.4 h	16.8 ± 0.5 a	20.7 ± 0.6 a	125.6 ± 0.6 a
80 °C, 5 min	28.4 ± 0.6 abc	−10.2 ± 0.2 f	15.8 ± 0.4 abc	18.8 ± 0.4 bc	122.9 ± 0.6 b
80 °C, 7 min	28.1 ± 0.8 abcd	−9.8 ± 0.4 ef	15.7 ± 0.9 abc	18.5 ± 0.9 bc	121.9 ± 0.9 bc
80 °C, 10 min	27.7 ± 0.9 abcd	−9.2 ± 0.4 de	15.5 ± 0.7 abc	18.0 ± 0.8 bc	120.7 ± 0.3 c
90 °C, 1 min	28.8 ± 1.0 ab	−11.1 ± 0.6 g	15.2 ± 0.9 abc	18.8 ± 1.1 bc	126.0 ± 0.5 a
90 °C, 5 min	28.4 ± 1.2 abc	−9.0 ± 0.5 cd	14.6 ± 0.5 bcd	17.2 ± 0.7 cd	121.5 ± 0.5 c
90 °C, 7 min	27.4 ± 1.0 bcd	−8.8 ± 0.3 cd	14.9 ± 0.7 bcd	17.3 ± 0.7 cd	120.7 ± 0.4 c
90 °C, 10 min	28.5 ± 0.7 abc	−8.2 ± 0.2 c	15.5 ± 0.6 abc	17.5 ± 0.6 cd	118.0 ± 0.5 de
100 °C, 5 min	28.4 ± 0.9 abc	−8.7 ± 0.5 cd	15.5 ± 1.2 abc	17.8 ± 1.2 cd	119.2 ± 0.8 d
100 °C, 7 min	27.6 ± 0.6 abcd	−7.4 ± 0.3 b	14.4 ± 1.0 cd	16.2 ± 1.0 de	117.3 ± 0.8 e
100 °C, 10 min	26.8 ± 0.8 cd	−5.7 ± 0.3 a	13.6 ± 0.9 d	14.7 ± 0.9 d	113.0 ± 1.0 f

[a]Different letters (vertically) indicate significant differences ($p < 0.05$).

Table 3. Color Characteristics of Pastelike Parsley Products Obtained According to Process Variant II (n = 5)[a]

process parameter	L^*	a^*	b^*	C^*	h°
control (unheated)	25.3 ± 0.8 f	−9.2 ± 0.5 a	12.2 ± 0.6 f	15.2 ± 0.7 i	127.0 ± 0.2 g
100 °C, steam, 1 min	27.8 ± 0.8 bcd	−13.6 ± 0.3 fghi	15.3 ± 0.5 cde	20.5 ± 0.5 defgh	131.6 ± 0.5 bc
100 °C, steam, 5 min	28.5 ± 0.4 bc	−11.8 ± 0.4 c	15.3 ± 0.7 cde	19.4 ± 0.8 efgh	127.7 ± 0.6 g
100 °C, steam, 7 min	28.7 ± 0.4 abc	−12.1 ± 0.3 cd	17.0 ± 0.7 ab	20.8 ± 0.7 cdefg	125.5 ± 0.5 h
100 °C, steam, 10 min	28.4 ± 0.3 bc	−10.4 ± 0.3 b	16.2 ± 0.7 abc	19.3 ± 0.8 fgh	122.9 ± 0.3 i
80 °C, water, 1 min	30.1 ± 1.0 a	−14.8 ± 0.6 j	17.4 ± 1.3 a	22.8 ± 1.4 a	130.6 ± 1.0 cdef
80 °C, water, 5 min	29.1 ± 1.1 ab	−14.6 ± 0.2 j	17.3 ± 0.6 a	22.7 ± 0.6 ab	130.2 ± 0.7 def
80 °C, water, 7 min	28.9 ± 0.9 abc	−13.2 ± 0.5 efg	16.0 ± 0.6 abc	20.7 ± 0.8 cdefg	129.5 ± 0.5 f
80 °C, water, 10 min	29.2 ± 0.7 ab	−13.4 ± 0.5 efgh	16.1 ± 0.8 abc	21.0 ± 0.9 bcdef	129.9 ± 0.7 ef
90 °C, water, 1 min	29.3 ± 0.6 ab	−14.8 ± 0.3 j	17.5 ± 0.5 a	23.0 ± 0.5 a	130.2 ± 0.4 def
90 °C, water, 5 min	27.4 ± 0.7 cde	−14.4 ± 0.4 hij	16.6 ± 0.8 abc	21.9 ± 0.8 abcd	130.9 ± 0.7 cde
90 °C, water, 7 min	26.6 ± 1.0 def	−12.9 ± 0.7 def	15.0 ± 1.1 cde	19.8 ± 1.3 efgh	130.8 ± 0.7 cde
90 °C, water, 10 min	25.3 ± 0.4 f	−12.6 ± 0.2 cde	14.3 ± 0.3 de	19.0 ± 0.3 gh	131.3 ± 0.2 cd
100 °C, water, 1 min	28.8 ± 0.8 abc	−14.5 ± 0.6 ij	17.1 ± 0.9 ab	22.4 ± 1.0 abc	130.3 ± 0.6 def
100 °C, water, 5 min	26.2 ± 0.1 ef	−14.0 ± 0.2 ghij	15.7 ± 0.2 bcd	21.1 ± 0.3 bcde	131.8 ± 0.2 abc
100 °C, water, 7 min	25.7 ± 0.4 f	−13.1 ± 0.3 ef	14.1 ± 0.6 de	19.3 ± 0.7 efgh	132.8 ± 0.5 ab
100 °C, water, 10 min	25.5 ± 0.2 f	−12.8 ± 0.2 def	13.8 ± 0.3 ef	18.8 ± 0.3 h	132.9 ± 0.4 a

[a]Different letters (vertically) indicate significant differences ($p < 0.05$).

Compared to air-dried basil, the chlorophyll retention in the parsley products obtained in the present study was improved. After drying of raw basil leaves at 50 °C for 4.5 h, chlorophyll *a* and chlorophyll *b* losses amounting to 90% and 70%, respectively, were reported.[29]

Color Stability during Processing. The raw material and processed parsley significantly differed in their color. The color of blanched parsley exhibited a more intense green color, which was brighter than that of the unheated control and samples heated after grinding (process variant I). These findings were in agreement with the measured color values.

As can be seen from Table 2, pastelike parsley products obtained according to process variant I revealed a significant increase of a^* values (decrease of greenness) following more rigorous thermal treatments. In contrast, b^* values remained virtually unchanged independent of the time−temperature regimes applied. Only heating for 7 and 10 min at 100 °C resulted in a slight decrease of b^* and thus in a decrease of yellowness. L^* values tended to increase, translating into bleaching compared to the unheated control.

Parsley paste revealed increased greenness, yellowness, and lightness after steam- and water-blanching compared to the untreated sample (Table 3). Only samples obtained after extended blanching at 90 and 100 °C in water did not show brightening compared to the unheated sample. C^* values showed the same tendency as b^* values for both process variants.

Hue angles increased upon water-blanching and 1 min of steam-blanching, indicating a more intense green color as compared to the control samples. While hue angles of water-blanched samples remained constant independent of temperature, h° values decreased upon steam-blanching. Enhanced yellowish tones were observed with progressing heating time and temperature in samples heated after grinding, probably due to pheophytin formation. This is underlined by the fact that major chlorophyll losses were observed in samples heated at 100 °C (Figure 3).

Higher color saturation (C^*) as a result of higher absolute values of a^* and b^* after blanching is assumed to be due to minor chlorophyll degradation as compared to process variant I as well as to different chlorophyll conversion rates. Blanching went along with a significant increase in chlorophyll a' and b' levels, which did not cause color changes. Their contents were higher in water-blanched samples than in samples treated with steam. Accordingly, the latter showed lower color saturation values. Moreover, tissue softening and release of intercellular air and of dissolved gases due to blanching may have an impact on surface reflectance and depth of light penetration into tissues as

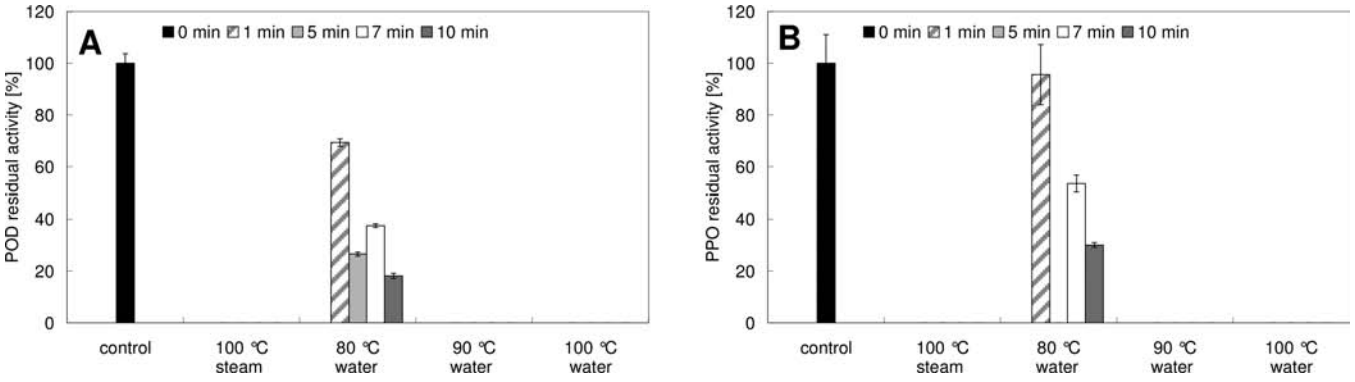

Figure 5. POD (A) and PPO (B) residual activity in pastelike parsley products obtained according to process variant II. Columns and bars represent mean ± standard deviation (n = 2).

reported for green vegetables.[30−32] Therefore, the color of blanched parsley appeared brighter. In contrast, color saturation and hue angle values of air-dried basil were lower, thus representing a yellow-green color.[29]

Effects of Heating on Activities of Quality Deteriorating Enzymes. The control samples of process variant I and II exhibited POD activities of 157.5 ± 7.8 and 202.6 ± 7.5 nkat/g DM, respectively. Heat treatment following grinding of parsley (process variant I) resulted in complete POD inactivation under any of the thermal conditions. Steam-blanching also strongly affected the POD activity of parsley, since complete POD inactivation was achieved within 1 min. Water-blanching at 90 and 100 °C also permitted complete inactivation (Figure 5). In contrast, samples blanched in water at 80 °C for 1 min only showed a 31% reduction of POD activity, with residual activities still ranging from 18 to 38% after 5−10 min of treatment. This is in according with a study assessing enzyme inactivation in paprika and chili after immediate thermal treatment of the fresh plant material, where a complete inactivation of POD could not be achieved either even when applying rigorous time−temperature regimes. POD residual activities in this study ranged from 1.8 to 3.5% for paprika and from 1.9 to 3.3% for chili powder.[11]

The PPO activity of the control sample for process variants I and II were 150.7 ± 14.1 and 94.3 ± 6.5 nkat/g DM, respectively. The inactivation of PPO followed the same trends as observed for POD. Samples obtained according to process variant I, the steam-blanched samples, and samples blanched with water at 90 and 100 °C did not show any residual PPO activity. In contrast, blanching in water at 80 °C was insufficient for PPO inactivation with the exception of blanching for 5 min (Figure 5). Up to 1 min of water-blanching at 80 °C did not even reveal significant differences from the untreated control. PPO of parsley appeared to be more heat resistant than the PPO of chili and paprika, which was inactivated after water-blanching for 10 min at 80 °C.[11]

In summary, blanching at 80 °C was insufficient for both PPO and POD inactivation. The target temperatures for heating the pastelike products in the reaction vessel according to process variant I were achieved within approximately 2−4 min. Thus, the heating-up period contributed to the overall thermal enzyme inactivation, and cooling of the pastes to 22 °C after heat treatment further prolonged overall heating time. In contrast, blanching did not require a heat-up phase, and rapid cooling was carried out in ice water. Therefore, it is assumed that insufficient enzyme inactivation upon water-blanching at 80 °C was due to the shorter residence time compared to process variant I. Furthermore, enzyme inactivation of the minced parsley was facilitated due to the decompartmentalization of cells. The significant differences in enzyme activities of the control samples may result from different enzyme extractability as a result of varying extent of cell disintegration.

Total Phenolic Contents and Total Antioxidant Capacity as Affected by Processing. The control samples of both process variants showed total phenolic contents of 80.82 ± 0.26 and 87.34 ± 2.56 mmol of GAE/kg DM, respectively (Tables 4 and 5). These results were in the range of data reported in the literature.[33,34]

Table 4. Total Phenolic Contents (Folin−Ciocalteu Assay) and Antioxidant Capacity (FRAP and TEAC Assays) of Pastelike Parsley Products Obtained According to Process Variant I (n = 2)[a]

process parameter	total phenolic content, mmol GAE/kg DM	FRAP assay, mmol TE/kg DM	TEAC assay, mmol TE/kg DM
control (unheated)	80.8 ± 0.3 a	7.4 ± 0.0 f	81.8 ± 1.8 bc
80 °C, 1 min	75.8 ± 1.3 ab	14.7 ± 1.2 bcd	86.6 ± 0.2 ab
80 °C, 5 min	67.8 ± 4.0 bcd	19.6 ± 1.1 a	80.9 ± 2.5 bc
80 °C, 7 min	75.4 ± 0.8 ab	16.8 ± 0.5 abc	85.2 ± 1.0 abc
80 °C, 10 min	64.1 ± 4.4 cd	14.1 ± 0.4 bcd	76.1 ± 4.0 c
90 °C, 1 min	75.7 ± 1.3 ab	15.9 ± 1.2 bc	86.6 ± 1.2 ab
90 °C, 5 min	70.8 ± 0.1 abc	10.2 ± 1.2 ef	91.6 ± 1.6 a
90 °C, 7 min	63.6 ± 0.6 cd	14.2 ± 0.3 bcd	81.0 ± 1.6 bc
90 °C, 10 min	50.0 ± 0.2 e	13.7 ± 0.1 cd	64.1 ± 0.1 d
100 °C, 5 min	58.9 ± 2.3 de	11.6 ± 0.4 de	76.1 ± 0.6 c
100 °C, 7 min	62.9 ± 7.0 cd	16.9 ± 0.9 abc	76.2 ± 5.7 c
100 °C, 10 min	66.0 ± 1.0 bcd	17.2 ± 1.3 ab	78.4 ± 0.6 bc

[a]GAE gallic acid equivalent, TE trolox equivalent, DM dry matter. Different letters (vertically) indicate significant differences (p < 0.05).

A significant decrease was observed after blanching and heating for at least 5 min. In general, phenolic contents were higher in pastes heated after grinding than in products obtained from the blanched material. At 90 °C, the contents decreased progressively with heating time (process variant I). Water-blanching at 80 and 90 °C and steam-blanching for 1 min did not bring about changes in the total phenolic contents compared to the control sample. Applying more rigorous time−temperature regimes during water-blanching resulted in a decrease of total phenolic contents. This was attributed to thermal degradation of phenolic compounds as well as

Table 5. Total Phenolic Contents (Folin−Ciocalteu Assay) and Antioxidant Capacity (FRAP and TEAC Assays) of Pastelike Parsley Products Obtained According to Process Variant II (n = 2)[a]

process parameter	total phenolic content, mmol GAE/kg DM	FRAP assay, mmol TE/kg DM	TEAC assay, mmol TE/kg DM
control (unheated)	87.3 ± 2.6 a	8.4 ± 0.1 cd	70.7 ± 0.3 efg
100 °C, steam, 1 min	89.2 ± 5.6 a	36.7 ± 6.1 a	107.7 ± 4.7 a
100 °C, steam, 5 min	49.1 ± 2.4 ef	9.3 ± 0.5 cd	74.1 ± 1.0 def
100 °C, steam, 7 min	59.6 ± 1.8 cd	10.5 ± 0.5 bcd	85.9 ± 1.9 bc
100 °C, steam, 10 min	59.7 ± 0.1 cd	12.6 ± 1.3 bcd	93.3 ± 2.2 b
80 °C, water, 1 min	83.5 ± 3.8 a	29.6 ± 0.2 a	94.2 ± 1.7 b
80 °C, water, 5 min	71.9 ± 0.2 b	15.5 ± 2.0 bc	84.6 ± 1.2 bcd
80 °C, water, 7 min	57.8 ± 2.6 cde	17.8 ± 2.7 b	68.8 ± 4.3 fg
80 °C, water, 10 min	52.0 ± 1.9 def	10.5 ± 0.5 bcd	71.4 ± 2.5 efg
90 °C, water, 1 min	87.5 ± 0.7 a	15.8 ± 1.3 bc	106.3 ± 1.8 a
90 °C, water, 5 min	52.3 ± 2.2 def	14.9 ± 1.5 bc	84.0 ± 0.3 bcd
90 °C, water, 7 min	45.0 ± 3.4 fg	12.4 ± 0.4 bcd	62.3 ± 3.1 gh
90 °C, water, 10 min	34.6 ± 2.6 hi	7.1 ± 0.5 d	53.8 ± 5.4 hi
100 °C, water, 1 min	64.1 ± 0.7 bc	15.0 ± 1.7 bc	81.7 ± 2.5 cde
100 °C, water, 5 min	47.4 ± 0.5 fg	14.5 ± 1.2 bcd	64.6 ± 1.0 fgh
100 °C, water, 7 min	39.1 ± 2.0 gh	12.2 ± 2.0 bcd	57.0 ± 4.3 h
100 °C, water, 10 min	29.1 ± 2.1 i	8.7 ± 0.5 cd	43.6 ± 2.9 i

[a]GAE gallic acid equivalent, TE trolox equivalent, DM dry matter. Different letters (vertically) indicate significant differences ($p < 0.05$).

leaching.[35] The latter effect may also be the reason for the lower phenolic contents of the water-blanched samples compared to the samples heated after mincing.

The antioxidant capacity of pastelike parsley products was evaluated using the ABTS discoloration method and the FRAP assay. The effects of thermal treatments on the antioxidant capacity of ground parsley as evaluated by the TEAC assay were marginal (Table 4). Only when the samples were heated at 90 °C for 5 and 10 min were significant differences observed compared to the unheated control. In contrast, the samples ground prior to heating showed higher antioxidant capacities than the unheated product when evaluated using the FRAP assay. The difference between both assays can be ascribed to varying assay conditions and diverse reaction mechanisms. The FRAP assay is based on the ability of phenolic compounds under acidic conditions to reduce Fe^{3+} to Fe^{2+} and the formation of a colored Fe^{2+} complex. In contrast, the TEAC assay is carried out at neutral pH and based on the discoloration of the $ABTS^{\bullet-}$ radical by phenolic compounds.[36]

Water-blanching for 1 and 5 min and steam-blanching resulted in higher antioxidant capacities as determined by the TEAC assay compared to the unheated control. With prolonged blanching at a distinct temperature, antioxidant capacity of the water-blanched products decreased. In contrast, antioxidant capacity measured by the FRAP assay was significantly increased upon steam-blanching for 1 min and water-blanching at 80 °C for 1 and 7 min. Maximum total phenolic contents and antioxidant capacity as evaluated by the TEAC and FRAP assays were observed after steam-blanching for 1 min.

The increase of phenolic contents and antioxidant capacity upon heating was probably due to the enhanced release of phenolic and antioxidant components as a result of thermal degradation of cell walls and subcellular compartments.[37] Thus, improved extractability of these compounds was presumably achieved. Moreover, it is also assumed that the formation of strong radical-scavenging antioxidants as well as of novel antioxidant compounds may increase antioxidant capacity.[37,38] Synergistic and additive effects of phenolic compounds may further enhance the total antioxidant activities.[39] Furthermore, immediate thermal inactivation of oxidative enzymes was reported to improve the retention of antioxidants.[35] However, the contribution of each of these factors to increased phenolic contents and antioxidant capacity still remains to be elucidated.

A poor linear correlation between antioxidant activity and total phenolic content was obtained (R^2 = 0.6794 for Folin and TEAC assay; R^2 = 0.2442 for Folin and FRAP assay). This may be attributed to different reaction conditions of the Folin−Ciocalteu and the FRAP and TEAC assays, respectively. Furthermore, phenolic and other antioxidant compounds are known to exhibit differing reactivities to the reacting agent.[36]

In conclusion, comminution of parsley and subsequent short-time heating (1 min) did not cause significant color losses. Prolonged heating resulted in marked pheophytin formation accompanied by a shift toward olive colors of the pastelike products. Antioxidant capacity as evaluated by the TEAC assay and total phenolic contents tended to decrease upon heating, whereas the antioxidant potential as measured by the FRAP assay increased. Inactivation of POD and PPO was achieved at any of the time−temperature regimes applied. In contrast, both short-time and extended water-blanching of uncomminuted parsley and subsequent comminution resulted in improved green color compared to the control sample. Water-blanching for 1 min at 100 °C was sufficient for complete inactivation of POD and PPO. Blanching resulted in bright green colors, indicating marginal chlorophyll loss. Under these conditions, antioxidant capacity was even enhanced, while a loss of total phenols supposedly caused by leaching was inevitable. Comparing both process variants, water-blanching appeared to be more suitable since contents of secondary plant metabolites were better retained. Therefore, this process variant proved to be most appropriate as an initial step for the production of pastelike parsley products. Subsequent processing steps required for the production of microbiologically safe herb and spice products characterized by improved sensory quality are the subject of our current research.

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