Controlling (bio)chemical conversions of health-related plant-based compounds by processing: the case of Brussels sprouts and leek

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Abstract

Brussels sprouts and leek are suppliers of multiple health-related compounds, yet understudied on how processing impacts their concentration. In this study, the plant tissue structure was altered by processing to direct endogenous enzyme-substrate interactions. For this, both conventional and novel processing techniques were employed. Heating intact vegetables prior to high shear mixing resulted in relatively good preservation of health-related compounds, attributed to a prompt inactivation of endogenous enzymes. Oppositely, high shear mixing prior to heating facilitated over 82% conversion for both vitamin C, S-alk(en)yl-L-cysteine sulfoxides (ACSOs) and glucosinolates. Pulsed electric field (PEF) treatment prior to heating, resulted in ±25% ACSOs conversion, yet no glucosinolates conversion. PEF treatment effectiveness is attributed to substrate-enzyme localization in the matrix and cell size. Vitamin K1 remained relatively stable during all treatments. It was postulated that higher concentrations of vitamin C protect carotenoids against oxidation pointing to interaction effects among health-related compounds.

Keywords

Vitamin C; Vitamin K1; Carotenoids; Glucosinolates; S-alk(en)yl-L-cysteine sulfoxides; Pulsed electric fields

Abbreviations

AA: ascorbic acid

ACSOs: S-alk(en)yl-L-cysteine sulfoxides

DHAA: dehydroascorbic acid

DW: dry weight

FW: fresh weight

HM: Heat-Mix

MIH: Mix-Incubation-Heat

PEF : pulsed electric field

PIHM: PEF-Incubation-Heat-Mix

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1. Introduction

Brussels sprouts (*Brassica oleracea* var. *gemmifera*) and leek (*Allium ampeloprasum* var. *porrum*) are important crops for the worldwide agricultural sector and in particular in Belgium. In 2020, leek was the most produced outdoor vegetable for the Belgian fresh market, Brussels sprouts were ranked seventh (VLAM, 2021). Moreover, brassica vegetables were the fourth most produced vegetables worldwide in 2021. Alliaceous vegetables were ranked second (Statista, 2022). Although both vegetables encompass such a significant part of the Belgian and worldwide vegetable production, they are understudied within their families on how different processing techniques impact their health-related compounds concentration profile.

Just like other *Brassica* and *Allium* species, Brussels sprouts and leek are rich sources of vitamins and phytochemicals. For instance, Brussels sprouts are well-known for their high concentration of vitamin C (EFSA, 2013). Vitamin C comprises both forms: ascorbic acid (AA) and dehydroascorbic acid (DHAA), which are readily interconverted. AA can also be converted to DHAA by endogenously present ascorbic acid oxidase. Both forms have vitamin C function, yet DHAA is less stable and easily degrades into 2,3-diketogulonic acid and other degradation products, so losing its health-contributing function (Belitz et al., 2004). Furthermore, Brussels sprouts and leek are also rich in lipid-soluble vitamins, such as vitamin K1. In plant cells, vitamin K1 is strongly bound to chloroplasts (EFSA, 2017).

Next, phytochemicals are secondary plant metabolites produced by environmental changes as attractants or protectants. They contribute to human health by management and prevention of illnesses and diseases (Monjotin et al., 2022). Some phytochemicals are present in nearly all plants (e.g. carotenoids), while others are family specific (e.g. glucosinolates, S-alk(en)yl-L-cysteine sulfoxides). Like most green leafy vegetables, Brussels sprouts and leek are rich in carotenoids. These lipid-soluble antioxidants accumulate in the chloroplasts with a relatively constant chloroplast carotenoid composition within different plants: 25-30% β-carotene, 40-50% lutein, 15% violaxanthin and 15% neoxanthin. Moreover, β-carotene is well-known for its provitamin A activity (Britton et al., 2009). Furthermore, glucosinolates are typically present in species of the *Brassica* family, such as Brussels sprouts. Glucosinolates can be converted by the endogenous enzyme, myrosinase, into various health-related breakdown products, such as isothiocyanates. Isothiocyanates are assigned health-promoting as they possess anti-inflammatory properties and play a role in the modulation of xenobiotic metabolism (Oliviero et al., 2018). Oppositely, S-alk(en)yl-L-cysteine sulfoxides (ACSOs) are typically present in *Allium* species, such as leek. They can be converted into several health-related organosulfur compounds by the endogenous enzyme alliinase (Putnik et al., 2019). Nevertheless, enzymatic conversions of glucosinolates and ACSOs into their respective health-related products can only occur upon cell disruption. Glucosinolates are located in the vacuoles of S-cells, where they are

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physically separated from myrosinase, which is located in the vacuole of adjacent myrosin cells (Oliviero et al., 2018). However, it is also assumed that some cells contain both glucosinolates and myrosinase in separated subcellular compartments (Koroleva & Cramer, 2011). ACSOs and alliinase are located in the same cells, yet in different cell organelles: ACSOs are present in the cytoplasm while alliinase is compartmentalized in the vacuole (Rose et al., 2005). It can be questioned to what level the health potential of these phytochemical conversions (glucosinolates, ACSOs) can be tailored by targeted processing. For example, (partial) tissue disruption can differentiate enzyme-substrate interactions.

Tissue/cell disruption, however, can also lead to the degradation of health-related compounds. In the case of vitamin C, upon cell disruption, cell wall bound ascorbic acid oxidase can come into contact with AA, which is located in mitochondria, cytosol, vacuoles, chloroplasts and cell walls (Smirnoff, 1996). Conversion of AA into the unstable DHAA form leads to facilitated degradation of vitamin C, e.g. during thermal treatment. In case of the lipid-soluble compounds, carotenoids and vitamin K1, cell disruption can increase exposure to oxygen, which can in turn lead to non-enzymatic oxidation. Carotenoids could undergo enzyme-catalyzed oxidative breakdown as well due to reactive oxygen species formed upon unsaturated fatty acids oxidation by lipoxygenase following cell disruption (Britton et al., 2009). In green leaves, lipoxygenase is also located in the chloroplasts, bound to the thylakoid membranes (Hatanaka, 1993). Overall, the potential formation of health-related compounds (e.g. isothiocyanates, organosulfur compounds) upon cell disruption must be balanced against potential losses of other health-related compounds (e.g. vitamin C, carotenoids, vitamin K1).

Although the impact of processing (mostly heat treatments) is already widely evaluated on individual bioactive compounds, such as vitamin C (Soares et al., 2017), glucosinolates (Oliviero et al., 2018), ACSOs (Putnik et al., 2019) or carotenoids (Britton et al., 2009), comparing different processing techniques and sequences leading to different levels of cell disruption, received much less attention. Since targeted processing including cell disruption can stir enzymatic conversions, more research is needed to explore its potential, especially for nutrient-rich vegetables such as leek and Brussels sprouts. Additionally, since vegetables are delivery systems of a whole range of health-related compounds, studying the processing impact on multiple health-related compounds simultaneously is very relevant. Lastly, little information is present on the impact of any kind of processing on vitamin K1 in vegetables.

Generally, plant tissue structure can be disrupted mechanically, for example by high shear mixing vegetables into a puree. Such mechanical disruption will largely influence both the macro- and microstructure of vegetables. Recently, a less invasive processing method came to the attention:

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pulsed electric field (PEF). This technique allows to retain the macrostructure while altering the microstructural properties via electroporation by applying short pulses of low to high (0.1-40 kV/cm) electric field intensity (Morales-de la Peña et al., 2021). In other words, some microstructural modifications can be introduced in the plant material, while the tissue remains intact (i.e. solid plant tissue). Until now, research mainly focused on applying PEF at high field strength as an alternative for conventional preservation processes involving heat treatment (e.g. safe, high-quality foods) and medium field strength to improve the efficiency of certain industrial processes (e.g. extraction yields). However, interest in PEF applications at low field strength is growing as it has the potential to stimulate the biosynthesis of secondary metabolites in plants (Morales-de la Peña et al., 2021). In the current work, it was hypothesized that through the creation of pores in cell membranes, contact between substrate and enzyme could be facilitated without altering the vegetable macrostructure.

Therefore, the current work intended to control the (bio)chemical conversions of health-related components in Brussels sprouts and leek by targeted processing conditions. Three different treatment conditions were designed with the aim to induce different levels of (bio)chemical reactivity in both Brussels sprouts and leek. *(i)* **Heating** of the intact vegetable (pieces), with the aim to induce prompt inactivation of the enzymes while retaining the macro- and microstructure (target: least reactivity). *(ii)* high shear **mixing** followed by incubation, allowing enzymatic conversions and obtaining a disintegrated vegetable tissue (i.e. puree) (target: maximal reactivity). *(iii)* **PEF treatment at low electric field strength** (1.01 kV/cm) followed by incubation, enabling enzymatic conversions by electroporation of the cell membrane, inducing a change in a microstructural property in the intact material (target: intermediate reactivity).

2. Material and Methods

2.1 Materials

Two batches of Brussels sprouts were harvested from local fields (Staden and Houthulst, Belgium) in January 2021 and kindly donated by Ardo (Ardooie, Belgium). In April 2021, two batches of leeks were harvested from a local field (Ardooie, Belgium) and kindly donated by d'Arta (Ardooie, Belgium). For Brussels sprouts, the Cryptus variety (15-25 cm diameter) was selected, while for leek the Pluston variety (3-5 cm diameter, 40% white and 60% green) was chosen because of their industrial relevance. Both matrices were washed, stored (3 °C, dark), and processed (Section 2.2) within one week after harvesting. The raw samples (considered as reference samples) were pooled over different plant commodities per batch (at least 1 kg Brussels sprouts and 4 leeks) and snap-frozen with liquid nitrogen on the day of harvest and stored at -40°C until analysis. All chemicals used were analytical or HPLC grade. Analytical standards ascorbic acid, menaquinone, phylloquinone, methiin, and alliin were

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purchased from Sigma-Aldrich (Overijse, Belgium). Analytical standards echinenone, violaxanthin, neoxanthin, lutein, and β-carotene were acquired from CaroteNature (Münsingen, Switzerland). Analytical standards glucobrassicanapin, glucoerucin, glucoiberin, gluconapin, gluconasturtiin, glucoraphanin, glucotropaeolin, neoglucobrassicin, progoitrin, sinigrin, 4-methoxyglucobrassicin, and glucobrassicin were purchased from Extrasynthese (Genay, France).

2.2 Processing conditions

With the aim to control the (bio)chemical conversions of health-related components in Brussels sprouts and leek, three different treatment conditions were executed on two independent batches of Brussels sprouts or leek (n=2 for both matrices) (Fig. 1). For each treatment condition, in total, at least 5 kg of Brussels sprouts or 18 leek stems were processed and pooled per batch. To obtain this volume of processed material, multiple repetitions of each treatment were needed (at least 8 repeated treatments per condition per batch).

2.2.1 Thermal treatment

Brussels sprouts versus leek were thermally treated in vacuum bags placed in a water bath equilibrated at 95°C until a peroxidase negative result was obtained, which was 15 min for Brussels sprouts and 18 min for leek (data not shown). Peroxidase is the most heat-stable enzyme present in these vegetables, so the negative test was linked with complete inactivation of endogenous enzymes (FAO, 1995). Afterward, the vegetables were cooled in an ice bath for 10 min until refrigerated temperature. Next, the vegetables were high shear mixed into a puree using a Thermomix (Vorwerk, Wuppertal, Germany) at 10700 rpm for 1 min. Demineralized water was added to create an edible puree. A 1:1 ratio was chosen for Brussels sprouts and a 2:3 ratio for leek. Finally, the puree was frozen and stored at -40°C until analysis. This type of vegetable puree, according to the above-described sequence of processing steps, will be termed “Heat-Mix” in the following sections (Fig. 1).

2.2.2 Mechanical disintegration treatment

Brussels sprouts or leek were high shear mixed with demineralized water in the same ratios as described in Section 2.2.1. It was arbitrarily chosen to keep the puree at room temperature for 1 hour to allow potential enzymatic conversions, keeping in mind industrial relevance. After this incubation step, the puree was thermally treated in a Thermomix (Vorwerk, Wuppertal, Germany) at 95°C while gently stirring, to stop the endogenous enzymatic activity. Both purees gave a peroxidase negative result (FAO, 1995) after a treatment of 11 min and more (data not shown). Consequently, a thermal treatment time of 11 min was chosen. Afterward, the puree was cooled in an ice bath for 10 min until refrigerated temperature. Next, the puree was frozen and stored at -40°C until analysis. This type of

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vegetable puree, according to the above-described sequence of processing steps, will be termed “Mix-Incubation-Heat” in the following sections (Fig. 1).

2.2.3 Pulsed electric field (PEF) treatment

The PEF treatment was executed in a batch pulsed electric field unit (Cellcrack III, Elea, German Institute for Food Technologies, Quackenbrück, Germany). The treatment chamber had a volume of 12.2 L and an interelectrode distance of 29.4 cm. The treatment medium was standardized tap water (0.27 g NaCl/L and 40.13 mg $CaCl_2.H_20$/L dissolved in Milli-Q water) (Willemsen et al., 2017), having a conductivity of 600 µS/cm at room temperature. The total mass in the treatment chamber was 5 kg and the weight ratio vegetable to medium was 1:7. Intact Brussels sprouts were used and leeks were cut axial in half to fit into the treatment chamber. To allow complete immersion of the vegetables during treatment, a float was used on top of the medium. Both vegetables were exposed to 30 pulses with a pulse amplitude of 30 kV, resulting in a field strength of 1.01 kV/cm. The total applied specific energy was 2.7 kJ/kg. The pulse width was 225 ± 19 µS and the pulse frequency was 2 Hz. In the preliminary tests, the efficiency of the PEF processing conditions was checked through evaluation of the conductivity increase of MQ-water (initial conductivity of 2.5 ± 1.6 µS/cm) when PEF-treated vegetables remained submerged for up to 16h after treatment. Since effective PEF treatment causes membrane permeabilization, ion leakage should result in a conductivity increase in the medium (Ersus & Barrett, 2010). For both Brussels sprouts and leek, a plateau in conductivity increase was reached around 30 pulses, resulting in a conductivity increase of 706% for Brussels sprouts and 678% for leek (Fig. A in Supplementary Material). Immediately after PEF treatment, the vegetables were taken out of the treatment medium and kept for 1h at room temperature to facilitate potential enzymatic conversions. Next, these PEF-treated and incubated vegetables were thermally treated in vacuum bags to inactivate all enzymes. Subsequently, they were high shear mixed into a puree to prepare the sample for further analyses. Heating and high shear mixing was done in the same conditions as described in Section 2.2.1. Finally, the puree was frozen and stored at -40°C until analysis. This type of vegetable puree, according to the above-described sequence of processing steps, will be termed “PEF-Incubation-Heat-Mix” in the following sections (Fig. 1).

2.3 Microscopy

The microstructure of each vegetable puree was evaluated using an Olympus BX-51 microscope (Olympus Optical Co. Ltd., Tokyo, Japan). Representative micrographs were taken using Cell Image analysis software (Soft Imaging System, Germany).

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2.4 Analysis of (precursors of) health-related compounds

2.4.1 Sample preparation before analysis

As a preparatory step before analysis, (processed) Brussels sprouts and leek were freeze-dried. Therefore, the raw frozen Brussels sprouts and leek were ruptured and further high shear mixed in a frozen state (Grindomix GM200, Retsch GmbH, Haan, Germany) to avoid enzymatic activity. The frozen powder was then freeze-dried (Alpha 2-4 LSC plus, Christ, Osterode, Germany) and stored at -40°C. All processed vegetable samples (purees) were also freeze-dried (Alpha 2-4 LSC plus, Christ, Osterode, Germany).

2.4.2 Vitamin C content

The total vitamin C content was determined based on the protocol of Verbeyst et al. (2013). First, 200 mg of freeze-dried powder was ball-milled for 30 sec at 0.30 s^{-1} (MM400, Retsch GmbH, Haan, Germany) to completely open the cell structure. Three independent aliquots from the freeze-dried, ball milled powder were taken to perform an independent extraction on each of them, and this was for each independent batch (n=2). The powder was dissolved in 20 mL extraction buffer of pH 2.0 (1% m-phosphoric acid and 0.5% oxalic acid in Milli-Q water) and centrifuged for 30 min at 18900 g at 4°C (Sigma 4-16KS centrifuge, Sigma, Osterode am Harz, Germany). Extraction was performed at low pH and on ice to limit enzymatic conversions of raw samples during extraction as much as possible. After centrifugation, 5 mL of supernatant was brought to pH 3.5. One part of the supernatant was diluted (1:2) in phosphate buffer of pH 3.5 (20 mM NaH_2PO_4 and 1mM Na_2EDTA in Milli-Q water) and filtered (Chromafil A-45/25, Macherey-Nagel, Düren, Germany). The ascorbic acid content of this solution was analyzed by HPLC. The second part of the supernatant was diluted (1:2) in tris(2-carboxyl-ethyl) phosphine (TCEP) solution of pH 3.5 (2.5 mM TCEP in phosphate buffer) to enable the reduction of dehydroascorbic acid to ascorbic acid. After centrifuging for 15 min at 24°C at 19900 g (Microfuge 22R, Beckman Coulter Inc, Indianapolis, IN, USA), the solution was filtered and analyzed by HPLC for the total ascorbic acid (total vitamin C) content. The difference in the amount of ascorbic acid between those two solutions indicates the amount of dehydroascorbic acid in the sample. HPLC analysis was carried out by a Prevail C18 column (250×4.6 mm, 5 µm particle diameter, Theale, Reading, England) with a corresponding guard column, coupled to an HPLC with a diode array detector (DAD) set at 245 nm (1200 Series, Agilent Technologies, Diegem, Belgium). An isocratic elution buffer (1 mM Na_2EDTA and 10 mM CH_3COONH_4 in Milli-Q water, pH 3.0, 0.8 ml/min) was used. For all analyses, 20 µL was injected. A standard curve of ascorbic acid dissolved in extraction buffer was used for quantification.

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2.4.3 Vitamin K1 and carotenoid content

Vitamin K1 and carotenoids were extracted based on the protocol of Lee et al. (2020). This method does not include a saponification step, which is beneficial for retaining xanthophylls (Rodriguez-amaya, 2001). Firstly, 200 mg of freeze-dried powder was ball milled for 30 sec at 0.30 s^{-1} (MM400, Retsch GmbH, Haan, Germany). Three independent aliquots of each independent batch (n=2) from the freeze-dried, ball-milled powder were taken to perform an independent extraction on each of them. The powder was dissolved in 1 mL extraction solvent (100% ethanol containing 0.1% w/v butylhydroxytoluene) by end-over-end rotation for 15 min at 4 °C. Menaquinone and echinenone were used as internal standards for vitamin K1 and the carotenoids, respectively. These standards were dissolved in the extraction solvent and added at the start of the extraction procedure. After centrifugation of 5 min at 20°C at 12300 g (Microfuge 22R, Beckman Coulter Inc, Indianapolis, IN, USA), the supernatant was collected. The extraction was repeated two times more as mentioned above after adding 1 mL extraction solvent. In total three supernatants were combined. The extraction of lipophilic compounds was checked for completeness during preliminary experiments, which was obtained after three extraction cycles (data not shown). After filtering the supernatant (Chromafil PET-20/25 filter, Macherey-Nagel, Düren, Germany), the extract was concentrated in a rotational vacuum concentrator (RVC 2-91, Martin Christ, Osterode am Harz, Germany) for 1h30 at 37°C and analyzed by HPLC. The extraction was performed under dimmed red light to limit light exposure. HPLC analysis was based on Vervoort et al. (2011). Briefly, analyses were carried out using a YMC C30 analytical column (150x4.6mm, 3µm particle size, YMC CO., LTD., Kyoto, Japan) and corresponding guard column, coupled to an HPLC and diode array detector (1200 Series, Agilent Technologies, Diegem, Belgium). Wavelengths of maximal absorbance of the compounds in ethanol were set at 248 nm (vitamin K) (Indyk et al., 2003), 440 nm (violaxanthin and neoxanthin), 445 nm (lutein), 450 nm (β-carotene) and 461 nm (echinenone) as described before (Britton et al., 1995). A gradient elution set at 1 ml/min was used: 0-2 min: methanol 95% and Milli-Q water 5%, 2-10 min: methanol 95%, MTBE (methyl-t-butyl ether) 0-3% and Milli-Q water 5-2%, 10-20 min: methanol 95-78.3%, MTBE 3-21% and Milli-Q water 2-0.7%, 20-23 min: methanol 78.3-50%, MTBE 21-50% and Milli-Q water 0.7-0%, 23-27 min: methanol 50% and MTBE 50%, 27-27.1 min: methanol 95%, and Milli-Q water 5%, 27.1-32min: methanol 95% and Milli-Q water 5%. Standard curves of phylloquinone, violaxanthin, neoxanthin, lutein and β-carotene were used for quantification.

2.4.4 Glucosinolate content

Glucosinolates can be converted into a variety of health-related products (Oliviero et al., 2018). Therefore, precursor (7 major compounds for Brussels sprouts) degradation is considered as a

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measure for health-related products formation. To study the glucosinolates profile of the different Brussels sprout samples, analyses were performed based on the protocol of Sun et al., (2016) with minor adaptations. Three independent aliquots from the freeze-dried powder were taken to perform an independent extraction on each of them for each independent batch (n=2). Briefly, glucosinolates were extracted by the addition of 5 mL 60:40 methanol:water (extraction solvent) to 300 mg freeze-dried Brussels sprouts powder. The mixture was vortexed, sonicated for 60 min on ice, and centrifuged for 15 min at 5000 g at 4 °C. After filtering and diluting the supernatant, extracts were analyzed using UHPLC-TOF-MS (Acquity UPLC coupled to a Synapt G2 S, Waters, Milford, Massachusetts, USA), equipped with an Acquity C18 column (2.1 x 100 mm, 1.7 µm particle diameter) (Waters, Milford, Massachusetts, USA) kept at 50 °C. The injection volume was 1 µL. A gradient elution set at 0.4 ml/min was used: 0-2 min: H_2O + 0.1% acetic acid 100%, 2-9.55 min: methanol 0-38%, H_2O + 0.1% acetic acid 100-62%, 9.55-10 min: methanol 38-95%, H_2O + 0.1% acetic acid 62-5%, 10-10.5 min: methanol 95%, H_2O + 0.1% acetic acid 5%, 10.5-14 min: methanol 95-0%, H_2O + 0.1% acetic acid 5-100%. For quantification, matrix-matched standard curves of glucobrassicanapin, glucoerucin, glucoiberin, gluconapin, gluconasturtiin, glucoraphanin, glucotropaeolin, neoglucobrassicin, progoitrin, sinigrin, 4-methoxyglucobrassicin, and glucobrassicin were used.

2.4.5 S-alkenyl cysteine sulfoxide (ACSO) content

ACSOs can be converted into a variety of health-related products (Putnik et al., 2019). Therefore, precursor (2 major compounds for leek) degradation is considered a measure of health-related product formation. The ACSOs content of leek samples was determined as described by Bernaert et al. (2012). Three independent aliquots from the freeze-dried powder were taken to perform an independent extraction on each of them for each independent batch (n=2). Therefore, 100 mg freeze-dried leek powder was dissolved in O-carboxymethyl)hydroxylamine hemihydrochloride (OCMHA) solution of 1.1g/L. After 10 min of shaking, the solution was centrifuged at 3946 g for 10 min. The supernatant was then diluted (1:5) in 0.1% formic acid solution. After filtering, 5 µL of the extract was injected in an UHPLC-MS/MS system (Acquity UPLC coupled to a Xevo XS, Waters, Milford, Massachusetts, USA) equipped with a Cortecs column (2.1 x 150 mm, 2.7 µm particle diameter) (Waters, Milford, Massachusetts, USA) kept at 30 °C. A gradient elution set at 0.4 ml/min was used: 0-1 min: H_2O + 0.1% formic acid 5%, acetonitrile + 0.1% formic acid 95%, 1-10 min: H_2O + 0.1% formic acid 5-60%, acetonitrile + 0.1% formic acid 95-40%, 10-11 min: H_2O + 0.1% formic acid 60%, acetonitrile + 0.1% formic acid 40%, 11-15 min: H_2O + 0.1% formic acid 60-5%, acetonitrile + 0.1% formic acid 40-95%. Matrix-matched standard curves of methiin and alliin were used for quantification (an analytical standard of isoalliin is commercially not available at this moment).

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2.5 Data analysis

Data analysis was performed using the statistical software JMP (JMP Pro 16.0.0., SAS Institute Inc., Cary, NC, USA). Each treatment was analyzed in triplicate (n=3, analytical error) per batch (n=2, experimental error). To verify significant differences between each treatment and the raw material, a Student's t-test was used with a significance level of $\alpha = 0.05$. To determine significant differences among the mean values of the treated samples, ANOVA with a Tuckey's HSD test was performed with a significance level of $\alpha = 0.05$.

3. Results and discussion

3.1 Characterization of Brussels sprouts and leek starting material

Raw Brussels sprouts (85.3 ± 0.1 % water content) and leek (88.4 ± 0.3 % water content) were characterized for the health-related compounds of interest in this study. The results obtained are shown in Table 1 (Brussels sprouts) and Table 2 (leek). The vitamin C content measured in Brussels sprouts was in line with the range of 76-192 mg/100g fresh weight (FW) reported by Biondi et al. (2021). In most cultivated crops, the amount of DHAA is less than 10% of the total vitamin C present (Lee & Kader, 2000), as was the case here. Vitamin K1 and the total carotenoid content of Brussels sprouts were similar to the amounts determined by Lee et al. (2020). Furthermore, the most abundant glucosinolates were sinigrin (43%), glucoiberin (21%), progoitrin (13%), and glucobrassicin (10%), which were also detected by Cieślik et al. (2007) but in different ratios.

The vitamin C content in leek (Table 2) was within the range described by Shelke et al. (2020). To the best of our knowledge, no studies were found reporting the vitamin K1 and carotenoid content of leek. However, the carotenoid ratios were slightly different for leek and Brussels sprouts, with their most abundant carotenoid lutein and violaxanthin (about 33% and 36% of the total carotenoid concentration, respectively), which is different from the average chloroplast carotenoid pattern of 45% lutein and 15% violaxanthin presented before (Britton et al., 2009). However, β-carotene and neoxanthin concentrations for both vegetables were within the range of the average chloroplast carotenoid pattern. Concerning the ACSOs present in leek, Bernaert et al. (2012) reported an isoalliin and methiin concentration of 873-5319 mg/100g dry weight (DW) and 124-1552mg/100 g DW, respectively. The 92:8 isoalliin:methiin ratio in this study was also in line with the ratio reported by Bernaert et al. (2012).

Generally, minor differences in concentrations of health-related compounds were observed for the raw matrices as compared to other studies on diverse *Allium* and *Brassica* species. This may be because of differences in vegetable variety, harvesting period, climate, etc. (Rodriguez-amaya, 2001;

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Bernaert et al., 2012). Overall, the results confirmed that Brussels sprouts contain high amounts of vitamin C, whereby consumption of 100 g raw Brussels sprouts can provide the Average Requirement for men and women (EFSA, 2013). Besides, both matrices can be considered as a rich source of vitamin K1 (100 g raw Brussels sprouts and leek can each provide 429% and 286% respectively of the Adequate Intake for adults) (EFSA, 2017).

3.2 Impact of processing on the microstructure of Brussels sprout and leek purees

Tissue disruption by high shear mixing was performed at different stages during the treatments, resulting in various microstructures of the final purees (Fig. 2). High shear mixing performed *prior* to heating caused large extents of cell breakage and thus released intracellular material, next to the presence of some cell clusters (Fig. 2 (B, E)). When high shear mixing was executed *after* heating, cells predominantly separated rather than broke which was attributed to the fact that heating induced softening of the cell-cell adhesion (Waldron et al., 1997), resulting in large abundancies of individual intact cells in the final purees (Fig. 2 (A, C, D, F)). Although the effect of the PEF treatment on the microstructure cannot be visualized microscopically, it was demonstrated that the PEF treatment could create pores in the cell membranes by measuring a conductivity increase as explained in Section 2.2.3. It was assumed that membrane permeabilization could enable enzymatic conversions to a certain extent.

3.3 Impact of processing on health-related compounds in Brussels sprout purees

In this study, three different treatment types were performed (Section 2.2). All treatments affected the degree of retaining health-related compounds in Brussels sprout purees (Fig. 3).

3.3.1. Vitamin C

Enzymatic conversion of the stable AA form to the unstable DHAA form by ascorbic acid oxidase can eventually lead to a faster loss of its health-contributing function (Belitz et al., 2004). Therefore, this enzymatic conversion should be limited. Inactivating endogenous enzymes by the Heat-Mix treatment resulted in relatively good retention of vitamin C (Fig. 3A). The ratio AA:DHAA remained constant.

In contrast, the Mix-Incubation-Heat treatment showed a large decrease in total vitamin C (Fig. 3A) and a slightly higher DHAA proportion of the remaining vitamin C (from around 3.2 % to 8.9 %). This is most likely the consequence of cell disruption, enabling ascorbic acid oxidase to convert AA into its less stable DHAA form. Upon heating, DHAA could then hydrolyze rapidly and irreversibly to 2,3-diketogulonic acid (Belitz et al., 2004). However, not all vitamin C was converted/degraded during this treatment. It is assumed that the incubation time (1 hour) was not sufficient to completely convert all AA, since ascorbic acid oxidase in broccoli was reported to have approximately the same activity at

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room temperature as at 50°C (Munyaka et al., 2010). In addition, the presence of intact cells even after processing (Fig. 2B) should not be forgotten.

Regarding the results of the PEF-Incubation-Heat-Mix treated Brussels sprouts (Fig. 3A), the decrease in vitamin C content is about 18% more than the Heat-Mix treatment and about 57% less than the Mix-Incubation-Heat treatment. In this way, it can be postulated that the PEF treatment contributed to some extent to ascorbic acid conversion by releasing ascorbic acid oxidase due to the permeabilizing effect of the PEF treatment. Similar effects were observed by Leong et al. (2015) in carrot puree.

3.3.2 Glucosinolates

Upon cell disruption at neutral pH, glucosinolates can be converted by myrosinase into health-related isothiocyanates (Oliviero et al., 2018). Fig. 3B shows that the Heat-Mix treatment did not affect the total glucosinolate content, indicating the heat inactivation of myrosinase prior to cell disruption.

A similar observation was made for the PEF-Incubation-Heat-Mix treatment (Fig. 3B). This treatment had no significantly different effect compared to the Heat-Mix treatment, indicating that the PEF treatment did not facilitate glucosinolates conversion by myrosinase. Since glucosinolates and myrosinase are located in different cell types of different sizes (Oliviero et al., 2018), electroporation of the cell membranes induced by the PEF treatment was most probably not sufficient to allow substrate-enzyme conversions. Additionally, it is also known that the effectiveness of the PEF treatment depends on the cell size (Janositz & Knorr, 2010). More specifically, smaller cells/cell organelles require higher field strengths than larger cells/cell organelles to initiate membrane permeabilization.

Furthermore, the Mix-Incubation-Heat treatment (Fig. 3B) caused a nearly complete conversion of total glucosinolates. This was attributed to the cell disruption by high shear mixing, enabling myrosinase to convert glucosinolates, as glucosinolates and myrosinase are located in the vacuoles of different cell types (Oliviero et al., 2014). The formation of isothiocyanates was qualitatively confirmed by volatile profile analyses in a parallel study by Delbaere et al. (2022). Additionally, other glucosinolate conversion products such as nitriles were also detected (Delbaere et al., 2022). Since the pH of the Brussels sprout puree was around 6.5, it is assumed that predominantly isothiocyanates were formed. Bhat & Vyas (2019) also reported an increasing myrosinase activity at higher concentrations of AA, acting as a cofactor for myrosinase. Since Brussels sprouts showed a high AA concentration (Table 1), cell disruption could make more AA available for the enzyme, enhancing its activity.

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3.3.3 Carotenoids and vitamin K1

Violaxanthin and neoxanthin are not shown in Fig. 3C as both carotenoids were completely degraded in all treatments. The thermolability of carotenoid epoxides was described before (Britton et al., 2009). The Heat-Mix treatment showed no significant losses of lutein and β-carotene (Fig. 3C).

Furthermore, no significant difference between the Heat-Mix treatment and the PEF-Incubation-Heat-Mix treatment was observed for lutein and β-carotene, indicating that the creation of pores by the PEF treatment did not influence the stability of lutein and β-carotene (Fig. 3C).

Moreover, about 63% lutein and β-carotene were degraded in the Mix-Incubation-Heat treatment. Since high shear mixing resulted in a notable amount of cell breakage (Fig. 2B), it was assumed that the intracellular material was more exposed to oxygen and light during incubation as well as heat in the consecutive step, causing significant degradation of both carotenoids. Additionally, processed-induced contact of unsaturated fatty acids and lipoxygenase can also be a cause of carotenoid degradation (Britton et al., 2009).

Results in Fig. 3D confirm the stability of vitamin K1 as approximately 76 to 86% of vitamin K1 was retained in all treatments. The Mix-Incubation-Heat treatment showed slightly more degradation, most probably due to the higher exposure to light and oxygen. Furthermore, PEF treatment did not have an impact on vitamin K1 stability.

3.4. Impact of processing on health-related compounds in leek purees

All treatments: “Heat-Mix” (HM), “Mix-Incubation-Heat” (MIH) and “PEF-Incubation-Heat-Mix” (PIHM) affected the degree of retaining health-related compounds in leek purees (*Allium* family) (Fig. 4). Similar trends were observed compared to Brussels sprout purees (*Brassica* family) (Fig. 3). However, the matrix effect of the vegetables also had an impact on the behavior of different health-related compounds during processing.

3.4.1 Vitamin C

The stability of vitamin C in processed leek (Fig. 4A) was different from the trends observed in processed Brussels sprouts (Fig. 3A) for Heat-Mix and PEF-Incubation-Heat-Mix treatment. Although the inactivation of endogenous enzymes by the Heat-Mix treatment also resulted in a relatively good retention of vitamin C in leek, about 81%, it was less than for Brussels sprouts (93%). This could be attributed to the difference in heat impact for both vegetables (Section 2.2.1). Moreover, ascorbic acid stability is also dependent on ascorbic acid concentration (Oey et al., 2006). Since ascorbic acid concentration in leek was about half the amount compared to Brussels sprouts (Table 1; Table 2), this could also have played a role in vitamin C retention in leek.

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The effect of PEF treatment was less outspoken for leek than for Brussels sprouts. Huelin & Stephens, (1947) showed that ascorbic acid oxidase activity was far less in onion (*Allium* family*)* compared to cabbage (*Brassica* family). Taking into account this information and the much lower ascorbic acid concentration for leek than for Brussels sprouts (Table 1; Table 2), it can therefore be assumed that membrane permeabilization in leek is not sufficient to achieve enzymatic conversion of ascorbic acid. A larger degree of substrate-enzyme contact seems necessary to have an ascorbic acid conversion, which was the case for Mix-Heat treatment (about 70% ascorbic acid conversion).

3.4.2 ACSOs

Upon cell disruption, ACSOs can be converted into health-related organosulfur compounds by the endogenous enzyme alliinase (Putnik et al., 2019). This conversion is favored as it exploits the health potential of leek. During the Heat-Mix treatment (Fig. 4B), ACSOs remained relatively stable. When the tissue structure was disrupted by high shear mixing (MIH), about 90% ACSOs could be converted by alliinase. In contrast to the glucosinolates in Brussels sprout (*Brassica* family), the PEF-Incubation-Heat-Mix treatment lowered the ACSOs concentration in leek puree (*Allium* family). As ACSOs and alliinase are physically separated by the tonoplast membrane of the vacuole, electroporation of this membrane by the PEF treatment seems to have enabled contact between substrate and enzyme. Moreover, more effective PEF treatment for leek than for Brussels sprouts was attributed to a much larger cell size (Fig. 2) (Janositz & Knorr, 2010) and also confirmed by higher conductivity increase after PEF treatment (Section 2.2.3). However, still 63.1 ± 5.4% of ACSOs remained unconverted. Next to PEF treatment intensity and cell wall integrity, the reaction time at room temperature can also play a role, since the optimal alliinase temperature of different *Allium* species is around 40°C (Krest et al., 2000).

3.4.3 Carotenoids and vitamin K1

An overall larger degradation of the lutein and β-carotene was observed in Heat-Mix treated leek (Fig. 4C) compared to Heat-Mix treated Brussels sprouts (Fig. 3C). Previous results (Table 2) showed that leek contained more than triple the amount of carotenoids but only half the amount of vitamin C compared to Brussels sprouts (Table 1). Therefore, these results confirm that carotenoid stability can be promoted in the presence of vitamin C (Jomova & Valko, 2013). For the Mix + Heat treatment, a rather equal carotenoid degradation was observed in leek compared to Brussels sprouts. As nearly all AA was converted in this treatment, few was left to contribute to carotenoid protection and thus retention. Furthermore, the impact of the PEF-Incubation-Heat-Mix treatment also differed from the impact of the Heat-Mix treatment in leek (Fig. 4C). This was not the case in Brussels sprout puree (Fig. 3C). This can be attributed to the differences in cell size and/or chloroplast size of Brussels sprouts and leek (Fig. 2C,F), which determines the effectiveness of the PEF treatment (Janositz & Knorr, 2010).

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As PEF treatment can increase carotenoid extractability (studied on spinach with field strengths from 3.3 to 26.7 kV/cm) (Zhang et al., 2017), it can be assumed that more indirect degradation of carotenoids by the enzyme lipoxygenase could take place and/or that carotenoids were more susceptible to the heating and high shear mixing afterward. Overall, vitamin K1 (Fig. 4D) remained relatively stable during all treatments with a retention of 75 to 85%.

4. Conclusion

In this study, three different treatment types were performed to introduce distinct levels of (bio)chemical reactivity in Brussels sprouts and leek with the aim to impact their health-related compounds profile. In the "Heat-Mix" treatment, most health-related compounds remained relatively stable, except for violaxanthin and neoxanthin which were completely degraded. The presence of vitamin C promoted the stability of carotenoids against oxidation. The "Mix-Incubation-Heat" treatment was able to facilitate the enzymatic conversion of vitamin C, glucosinolates as well as ACSOs (over 82% for each compound). This treatment had a considerable negative effect on carotenoid stability. However, vitamin K1 remained relatively stable. Next to both conventional processing treatments, a more novel processing technique was employed. The "PEF-Incubation-Heat-Mix" treatment at low field strength (1.01 kV/cm) had a different result for the two vegetables. PEF treatment was able to facilitate the enzymatic conversion of ACSOs in leek (*Allium* family), which was not the case for glucosinolates in Brussels sprouts (*Brassica* family). This was attributed to the differences in location of substrates and enzymes in the plant tissues as well as cell sizes. Leek cells were much larger than Brussels sprout cells, which could explain the slightly enhanced PEF treatment effectiveness. PEF treatment of higher intensity could potentially accomplish contact between glucosinolates and myrosinase across cells. However, more intense treatments could induce higher losses of other health-related compounds and thus must be carefully investigated. Overall, this study proved that different process-induced structural properties resulted in different enzymatic reactivities and as a result in distinct concentrations of different health-related compounds. Therefore, it is important to evaluate the impact of traditional as well as innovative processing on multiple health-related compounds regarding the possible synergistic effect health-related compounds can have. Moreover, it could be of interest to quantify the stability of enzymatic conversion products of glucosinolates and ACSOs as affected by processing techniques to better assess the health potential of vegetable products.

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References

Belitz, H.-D., Grosch, W., & Schieberle, P. (2004). Food Chemistry. In *Springer*. https://doi.org/10.1007/978-3-662-07279-0_20

Bernaert, N., Goetghebeur, L., De Clercq, H., De Loose, M., Daeseleire, E., Van Pamel, E., Van Bockstaele, E., & Van Droogenbroeck, B. (2012). Influence of cultivar and harvest time on the amounts of isoalliin and methiin in leek (Allium ampeloprasum var. porrum). *Journal of Agricultural and Food Chemistry*, *60*(44), 10910–10919. https://doi.org/10.1021/jf302132a

Bhat, R., & Vyas, D. (2019). Myrosinase: insights on structural, catalytic, regulatory, and environmental interactions. *Critical Reviews in Biotechnology*, *39*(4), 508–523. https://doi.org/10.1080/07388551.2019.1576024

Biondi, F., Balducci, F., Capocasa, F., Visciglio, M., Mei, E., Vagnoni, M., Mezzetti, B., & Mazzoni, L. (2021). Environmental conditions and agronomical factors influencing the levels of phytochemicals in brassica vegetables responsible for nutritional and sensorial properties. *Applied Sciences (Switzerland)*, *11*(4), 1–21. https://doi.org/10.3390/app11041927

Britton, G., Liaaen-Jensen, S., & Pfander, H. (1995). *Carotenoids Volume 1B: Spectroscopy*. Birkhäuser Verlag.

Britton, G., Liaaen-Jensen, S., & Pfander, H. (2009). *Carotenoids Volume 5: Nutrition and Health*. Birkhäuser Verlag. https://doi.org/10.1007/978-3-7643-7501-0

Cieślik, E., Leszczyńska, T., Filipiak-Florkiewicz, A., Sikora, E., & Pisulewski, P. M. (2007). Effects of some technological processes on glucosinolate contents in cruciferous vegetables. *Food Chemistry*, *105*(3), 976–981. https://doi.org/10.1016/j.foodchem.2007.04.047

Delbaere, S. M., Bernaerts, T., Vangrunderbeek, M., Vancoillie, F., Hendrickx, M. E., Grauwet, T., & Loey, A. M. Van. (2022). The Volatile Profile of Brussels Sprouts (Brassica oleracea Var . gemmifera) as Affected by Pulsed Electric Fields in Comparison to Other Pretreatments , Selected to Steer (Bio) Chemical Reactions. *Foods*, *11*(2892), 1–17. https://doi.org/https://doi.org/10.3390/foods11182892

EFSA. (2013). Scientific Opinion on Dietary Reference Values for vitamin C. *EFSA Journal*, *11*(11), 1–68. https://doi.org/10.2903/j.efsa.2013.3418

EFSA. (2017). Dietary reference values for vitamin K. *EFSA Journal*, *15*(5), 78. https://doi.org/10.2903/j.efsa.2017.4780

Ersus, S., & Barrett, D. M. (2010). Determination of membrane integrity in onion tissues treated by pulsed electric fields: Use of microscopic images and ion leakage measurements. *Innovative Food Science and Emerging Technologies*, *11*(4), 598–603. https://doi.org/10.1016/j.ifset.2010.08.001

FAO. (1995). *Fruit and vegetable processing: Chapter 9: Vegetable specific processing technologies*. FAO Agricultural Service Bulletin No.119. https://www.fao.org/3/v5030e/v5030e0q.htm#:~:text=The heat treatment to destroy,are presented in section 9.2

Hatanaka, A. (1993). The biogeneration of green odour by green leaves. *Phytochemistry*, *34*(5), 1201–1218. https://doi.org/0031-9422/93

Huelin, F. E., & Stephens, I. M. (1947). The enzyme-catalysed oxidation of ascorbic acid in fruit and vegetable suspensions. *Australian Journal of Biological Sciences*, *1*(1), 58–64. https://doi.org/10.1071/BI9480058

Indyk, H. E., Shearer, M. J., & Wollard, D. C. (2003). *Encyclopedia of food science and nutrition. Vitamin K. Properties and determination* (Volume 9). Elsevier Science Ltd. https://doi.org/10.1016/B0-12-227055-X/01263-3

Janositz, A., & Knorr, D. (2010). Microscopic visualization of Pulsed Electric Field induced changes on plant cellular level. *Innovative Food Science and Emerging Technologies*, *11*(4), 592–597. https://doi.org/10.1016/j.ifset.2010.07.004

Jomova, K., & Valko, M. (2013). Health protective effects of carotenoids and their interactions with other biological antioxidants. *European Journal of Medicinal Chemistry*, *70*(November 2017),

Accepted

102–110. https://doi.org/10.1016/j.ejmech.2013.09.054
Koroleva, O. A., & Cramer, R. (2011). Single-cell proteomic analysis of glucosinolate-rich S-cells in Arabidopsis thaliana. *Methods*, *54*(4), 413–423. https://doi.org/10.1016/j.ymeth.2011.06.005
Krest, I., Glodek, J., & Keusgen, M. (2000). Cysteine sulfoxides and alliinase activity of some allium species. *Journal of Agricultural and Food Chemistry*, *48*(8), 3753–3760. https://doi.org/10.1021/jf990521+
Lee, H. W., Zhang, H., Liang, X., & Ong, C. N. (2020). Simultaneous determination of carotenoids, tocopherols and phylloquinone in 12 Brassicaceae vegetables. *Lwt*, *130*(May), 109649. https://doi.org/10.1016/j.lwt.2020.109649
Lee, S. K., & Kader, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology*, *20*(3), 207–220. https://doi.org/10.1016/S0925-5214(00)00133-2
Leong, S. Y., Oey, I., Clapperton, D., Aganovic, K., & Toepfl, S. (2015). Chilling prior to low intensity pulsed electric field processing improved vitamin C stability of carrot purée (Daucus carota cv. Nantes). *International Journal of Food Science and Technology*, *50*(8), 1757–1763. https://doi.org/10.1111/ijfs.12836
Monjotin, N., Amiot, M. J., Fleurentin, J., Morel, J. M., & Raynal, S. (2022). Clinical Evidence of the Benefits of Phytonutrients in Human Healthcare. *Nutrients*, *14*(9), 1–54. https://doi.org/10.3390/nu14091712
Morales-de la Peña, M., Rábago-Panduro, L. M., Soliva-Fortuny, R., Martín-Belloso, O., & Welti-Chanes, J. (2021). Pulsed Electric Fields Technology for Healthy Food Products. *Food Engineering Reviews*, *13*(3), 509–523. https://doi.org/10.1007/s12393-020-09277-2
Munyaka, A. W., Makule, E. E., Oey, I., Van Loey, A., & Hendrickx, M. (2010). Thermal Stability of l-ascorbic acid and ascorbic acid oxidase in broccoli (Brassica oleracea var. italica). *Journal of Food Science*, *75*(4), 336–340. https://doi.org/10.1111/j.1750-3841.2010.01573.x
Oey, I., Verlinde, P., Hendrickx, M., & Van Loey, A. (2006). Temperature and pressure stability of L - ascorbic acid and / or [6s] 5-methyltetrahydrofolic acid : A kinetic study. *European Food Research and Technology*, *223*, 71–77. https://doi.org/10.1007/s00217-005-0123-x
Oliviero, T., Verkerk, R., Van Boekel, M. A. J. S., & Dekker, M. (2014). Effect of water content and temperature on inactivation kinetics of myrosinase in broccoli (Brassica oleracea var. italica). *Food Chemistry*, *163*, 197–201. https://doi.org/10.1016/j.foodchem.2014.04.099
Oliviero, Teresa, Verkerk, R., & Dekker, M. (2018). Isothiocyanates from Brassica Vegetables—Effects of Processing, Cooking, Mastication, and Digestion. In *Molecular Nutrition and Food Research* (Vol. 62, Issue 18). Wiley-VCH Verlag. https://doi.org/10.1002/mnfr.201701069
Putnik, P., Gabrić, D., Roohinejad, S., Barba, F. J., Granato, D., Mallikarjunan, K., Lorenzo, J. M., & Bursać Kovačević, D. (2019). An overview of organosulfur compounds from Allium spp.: From processing and preservation to evaluation of their bioavailability, antimicrobial, and anti-inflammatory properties. In *Food Chemistry* (Vol. 276, pp. 680–691). Elsevier Ltd. https://doi.org/10.1016/j.foodchem.2018.10.068
Rodriguez-amaya, D. B. (2001). A Guide to Carotenoid Analysis in Foods. In *International Life Sciences Institute*.
Rose, P., Whiteman, M., Moore, K., & Zhun, Y. (2005). Allium : the chemistry of potential therapeutic agents. *Nat. Prod. Rep.*, *22*, 351–368. https://doi.org/10.1039/b417639c
Shelke, P. A., Rafiq, S. M., Bhavesh, C., Rafiq, S. I., Swapnil, P., & Mushtaq, R. (2020). Leek (Allium ampeloprasum L.). In G. Nayik & A. Gull (Eds.), *Antioxidants in Vegetables and Nuts - Properties and Health Benefits* (pp. 309–332). Springer Nature Singapore Pte Ltd. https://doi.org/10.1007/978-981-15-7470-2
Smirnoff, N. (1996). Function and metabolism of ascorbate acid in plants. *Annals of Botany*, *78*, 661–669. https://doi.org/0305-7364/96/120661
Soares, A., Carrascosa, C., & Raposo, A. (2017). Influence of Different Cooking Methods on the Concentration of Glucosinolates and Vitamin C in Broccoli. *Food and Bioprocess Technology*,

Accepted

10(8), 1387–1411. https://doi.org/10.1007/s11947-017-1930-3

Statista. (2022). *Global production of vegetables in 2020, by type (in million metric tons)*. Statista: Agriculture: Farming. https://www.statista.com/statistics/264065/global-production-of-vegetables-by-type/

Sun, J., Zhang, M., & Chen, P. (2016). GLS-finder: A platform for fast profiling of glucosinolates in brassica vegetables. *Journal of Agricultural and Food Chemistry*, *64*(21), 4407–4415. https://doi.org/10.1021/acs.jafc.6b01277

Verbeyst, L., Bogaerts, R., Van der Plancken, I., Hendrickx, M., & Van Loey, A. (2013). Modelling of Vitamin C Degradation during Thermal and High-Pressure Treatments of Red Fruit. *Food and Bioprocess Technology*, *6*(4), 1015–1023. https://doi.org/10.1007/s11947-012-0784-y

Vervoort, L., Van Der Plancken, I., Grauwet, T., Timmermans, R. A. H., Mastwijk, H. C., Matser, A. M., Hendrickx, M. E., & Van Loey, A. (2011). Comparing equivalent thermal, high pressure and pulsed electric field processes for mild pasteurization of orange juice: Part II: Impact on specific chemical and biochemical quality parameters. *Innovative Food Science and Emerging Technologies*, *12*(4), 466–477. https://doi.org/10.1016/j.ifset.2011.06.003

VLAM. (2021). *Groentenproductie 2011-2020: Belgische vermarktbare productie (in ton) - per soort voor de versmarkt*. VLAM Kennisbank. https://www.vlaanderen.be/vlam/kennisbank?f%5B0%5D=facet_sector%3A53

Waldron, K. W., Smith, A. C., Parr, A. J., Ng, A., & Parker, M. L. (1997). New approaches to understanding and controlling cell separation in relation to fruit and vegetable texture. *Trends in Food Science and Technology*, *8*(7), 213–221. https://doi.org/10.1016/S0924-2244(97)01052-2

Willemsen, K. L. D. D., Panozzo, A., Moelants, K., Debon, S. J. J., Desmet, C., Cardinaels, R., Moldenaers, P., Wallecan, J., & Hendrickx, M. E. G. (2017). Physico-chemical and viscoelastic properties of high pressure homogenized lemon peel fiber fraction suspensions obtained after sequential pectin extraction. *Food Hydrocolloids*, *72*, 358–371. https://doi.org/10.1016/j.foodhyd.2017.06.020

Zhang, Z. H., Wang, L. H., Zeng, X. A., Han, Z., & Wang, M. S. (2017). Effect of pulsed electric fields (PEFs) on the pigments extracted from spinach (Spinacia oleracea L.). *Innovative Food Science and Emerging Technologies*, *43*(April), 26–34. https://doi.org/10.1016/j.ifset.2017.06.014

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639 **Tables**

640 **Table 1.** Health-related compound concentrations in raw Brussels sprouts ± standard errors (n = 2;

641 DW: dry weight; FW: fresh weight).

Component	mg/100g DW	mg/100g FW
Vitamins		
Total Vitamin C	668.1 ± 13.1	98.2 ± 1.9
Ascorbic acid	646.6 ± 14.9	95.1 ± 2.2
Dehydroascrobic acid	21.5 ± 1.8	3.2 ± 0.3
Vitamin K1	1.8 ± 0.0	0.3 ± 0.0
Carotenoids		
Violaxanthin	4.4 ± 0.8	0.7 ± 0.1
Neoxanthin	1.6 ± 0.3	0.2 ± 0.0
Lutein	3.4 ± 0.6	0.5 ± 0.1
Beta-carotene	2.8 ± 1.4	0.4 ± 0.2
Glucosinolates		
Total glucosinolates	944.4 ± 74.3	138.8 ± 10.9
Synigrin	403.8 ± 26.0	59.4 ± 3.8
Glucoiberin	194.1 ± 35.6	28.5 ± 5.2
Progoitrin	126.9 ± 2.8	18.7 ± 0.4
Glucobrassicin	90.7 ± 3.2	13.3 ± 0.5
Gluconapin	59.1 ± 3.7	8.7 ± 0.5
Glucoraphanin	33.2 ± 8.1	4.9 ± 1.2
4-Methoxyglucobrassicin	32.6 ± 0.5	4.8 ± 0.1

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644 **Table 2.** Health-related compound concentrations in raw leek ± standard errors (n = 2; DW: dry weight;

645 FW: fresh weight).

Component	mg/100g DW	mg/100g FW
Vitamins		
Total Vitamin C	350.6 ± 23.0	40.7 ± 2.7
Ascorbic acid	318.3 ± 18.0	37.0 ± 2.1
Dehydroascorbic acid	32.4 ± 5.0	3.8 ± 0.6
Vitamin K1	1.7 ± 0.0	0.2 ± 0.0
Carotenoids		
Violaxanthin	10.7 ± 0.3	1.2 ± 0.0
Neoxanthin	5.6 ± 0.3	0.7 ± 0.0
Lutein	13.1 ± 0.8	1.5 ± 0.1
Beta-carotene	10.8 ± 0.4	1.3 ± 0.0
ACSOs		
Total ACSOs	1314.0 ± 54.0	152.7 ± 6.3
Isoalliin	1211.5 ± 34.5	140.8 ± 4.0
Methiin	102.6 ± 19.5	11.9 ± 2.3

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Figures

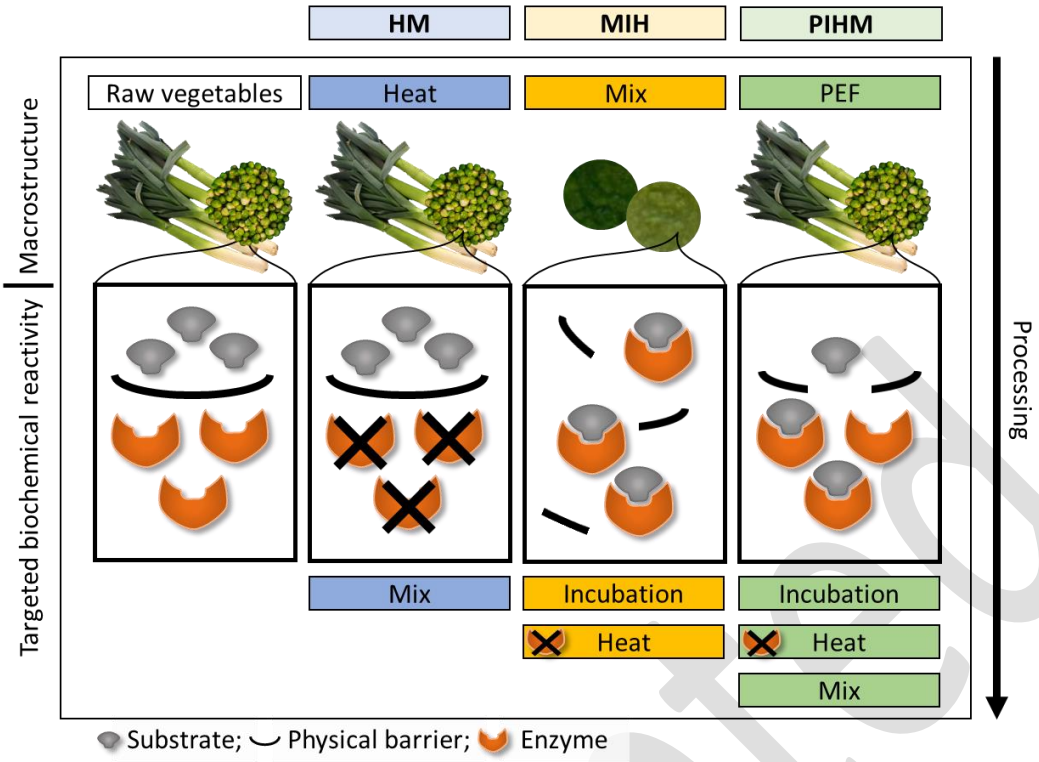


Figure 1. Conceptual drawing on the reasoning behind the applied processing conditions (PEF: pulsed electric field; HM: Heat-Mix; MIH: Mix-Incubation-Heat; PIHM: PEF-Incubation-Heat-Mix). In case of the raw vegetables and after heating, the physical barrier represents the intact cell membrane and cell wall. After high shear mixing, no physical barrier is present as the cell membrane and cell wall are disrupted. In case of the PEF treatment, cell wall can form a physical barrier while the cell membrane is partially disrupted (electroporated).

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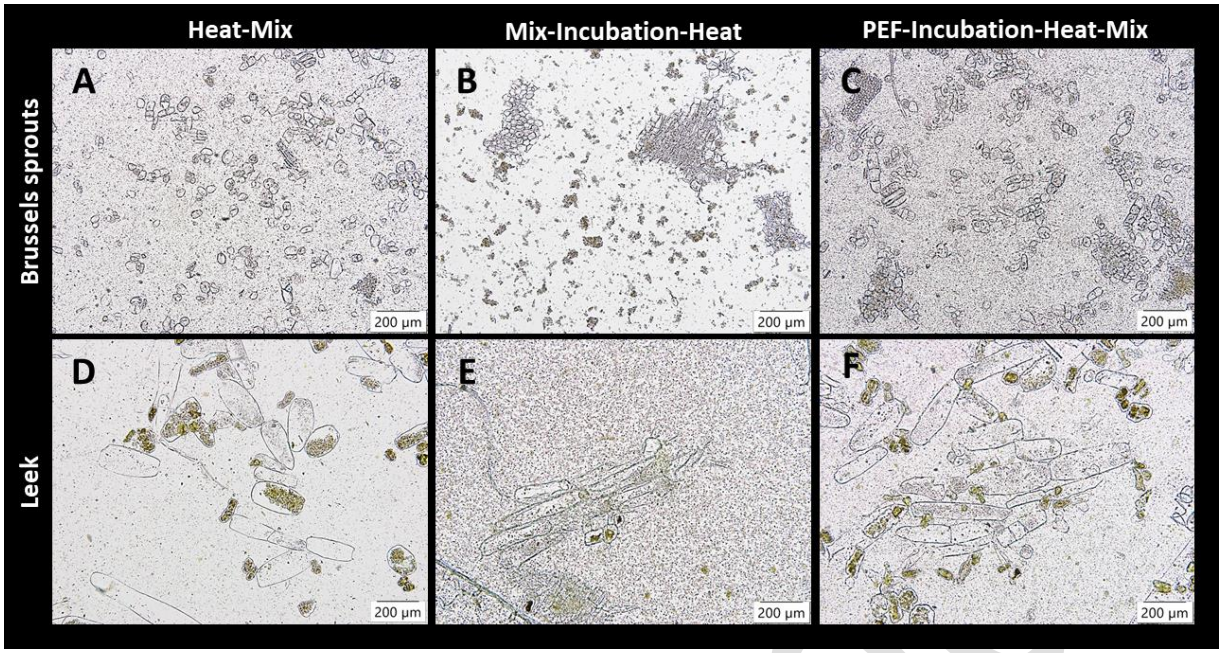
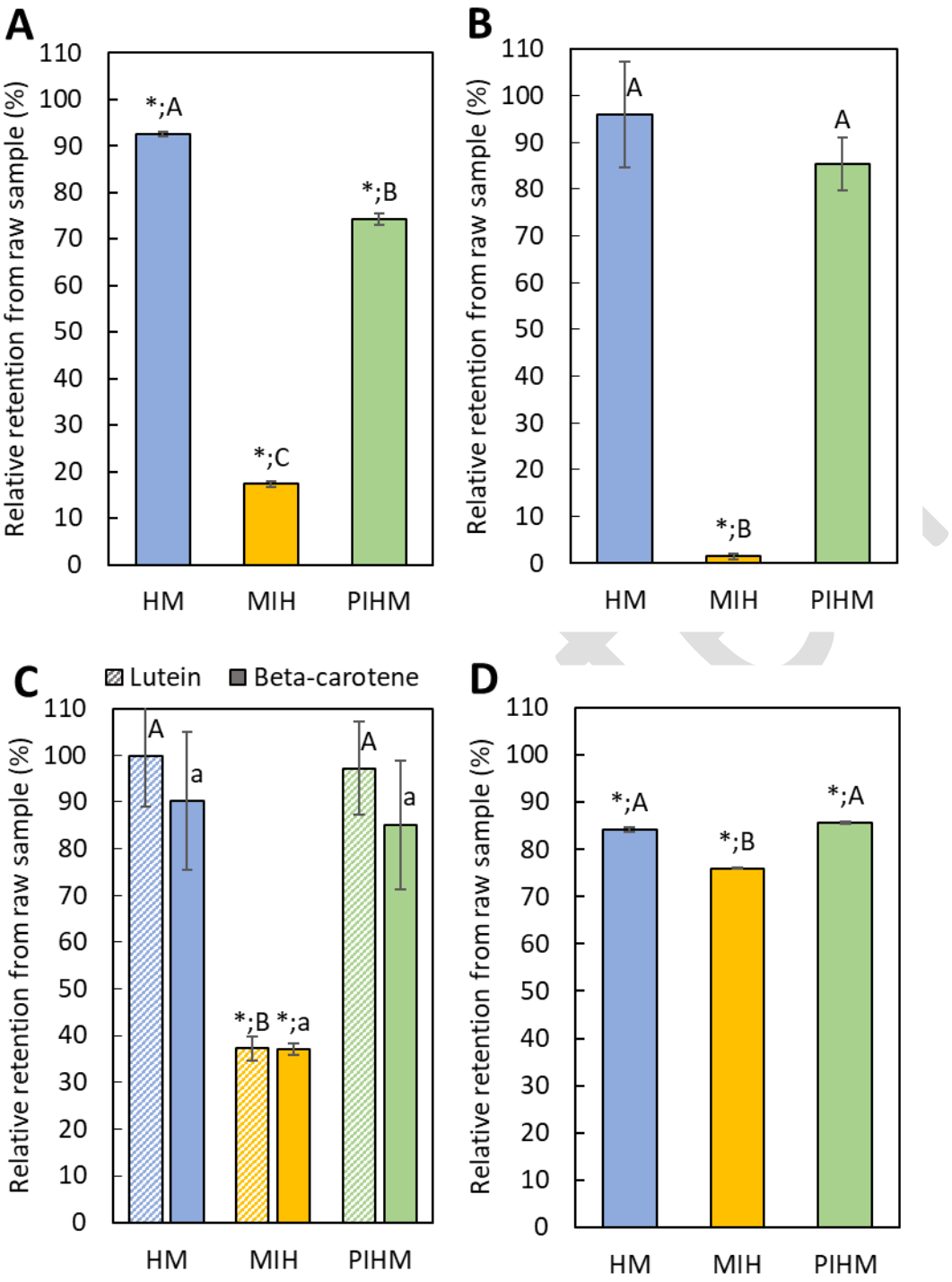


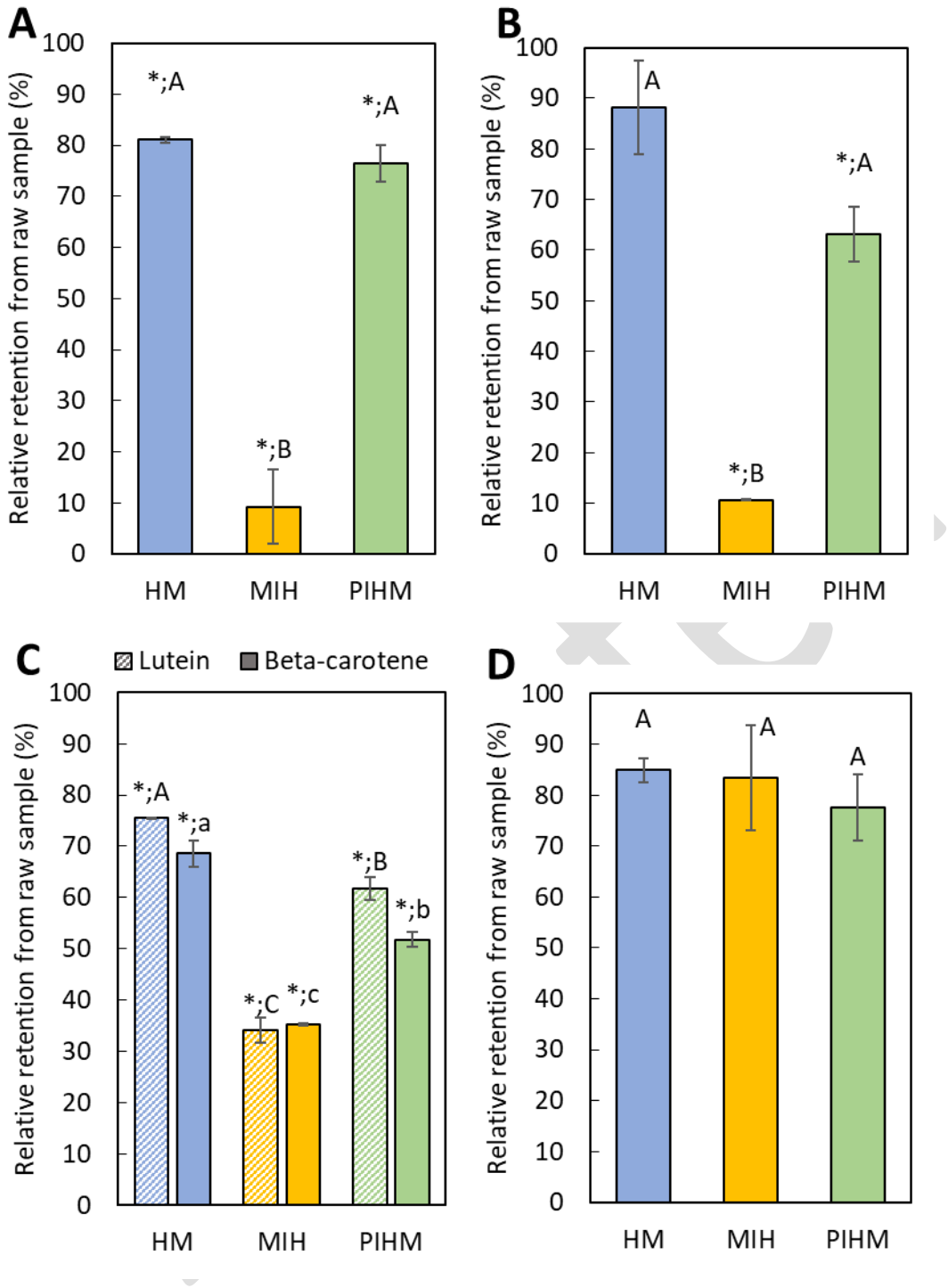
Figure 2. Representative micrographs of differently processed (A-B-C) Brussels sprout purees and (D-E-F) leek purees at a 10x magnification.

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660 **Figure 3.** Relative health-related compound concentrations in differently processed Brussels sprout
661 purees: (A) total vitamin C; (B) total glucosinolates; (C) carotenoid species; and (D) vitamin K1
662 evaluated after treatment (Heat-Mix (HM); Mix-Incubation-Heat (MIH); and PEF-Incubation-Heat-Mix
663 (PIHM)). Asterisks indicate significant differences between raw material (Table 1) and each treatment
664 ($p < 0.05$) (n=2). Different letters indicate significant differences between all treatments ($p < 0.05$)
665 (n=2).



666

667 **Figure 4.** Relative health-related compound concentrations in differently processed leek purees: (A)
668 total vitamin C; (B) total ACSOs; (C) carotenoid species; and (D) vitamin K1 evaluated after treatment
669 (Heat-Mix (HM); Mix-Incubation-Heat (MIH); and PEF-Incubation-Heat-Mix (PIHM)). Asterisks indicate
670 significant differences between raw material (Table 2) and each treatment ($p < 0.05$) (n=2). Different
671 letters indicate significant differences between all treatments ($p < 0.05$) (n=2).