



Risk profiling of wash waters in vegetable processing industry towards possible allergen carry-over



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ABSTRACT

The carry-over of food allergens via reuse of water in the food industry deserves more attention. The lack of quantitative knowledge on this topic, hampers reliable estimations of the potential risk for the produce processing industry. Wash water samples from three vegetable processing industries were collected and significant protein concentrations were determined in the water (0–596 µg/ml). The influence of several product and process parameters on the protein carry-over from the vegetable to the wash water was studied. The type of process, batch or semi-continuous, had an impact on the protein carry-over, as well as the degree of cutting and the surface area of the vegetable. It was shown that the protein carry-over to the wash water is higher at acid pH. In the vegetable processing industry acid is often added to the wash water to prevent enzymatic browning in e.g. celeriac.

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1. Introduction

In the vegetable processing industry, wash water is often reused for environmental and economic reasons. This means that several batches of vegetables are washed in the same water or that water is recirculated without thorough purification or treatment. Reusing water may cause several food safety hazards, which need to be included in the food safety management system of a company.

Many studies discussed the chemical and microbiological quality of wash waters. However, when it comes to *reuse* of wash water, the information is scarce. The (few) published articles discussing reuse or recirculation of wash water in fresh produce generally focus on cross-contamination of microorganisms and the chemical quality of the wash water (e.g. different methods for water disinfection) (Gil, Selma, Lopez-Galvez, & Allende, 2009; Holvoet, Jaxsens, Sampers, & Uyttendaele, 2012; Lopez-Galvez et al., 2012; Luo, 2007; Ölmez & Kretzschmar, 2009; Selma, Allende, Lopez-Galvez, Conesa, & Gil, 2008). Moreover, in the legislation and guideline documents, an attempt is made to define microbial and/or chemical criteria for the reuse of water. However, water reuse also implies a risk for carry-over of allergens. Only one paper discusses the possible carry-over of allergens via wash waters (Kerkaert, Jaxsens, Van De Perre, & De Meulenaer, 2012). The lack of scientific knowledge on carry-over of allergens via reuse of wash water is a critical gap in allergen management.

Food allergy is an abnormal immunological response to a food component (Johansson et al., 2001). Celery is one of the fourteen food

ingredients listed in the EU Directive on allergen labeling (2007/68/EC). Usage of celery as an ingredient must be indicated on the label of a foodstuff in the EU. Yet, nothing is stated about unintentionally added allergens (via cross-contamination) in the directive. However, food producers are responsible for producing food safe for all consumers through EU Regulation 178/2002.

According to the European Food Safety Authority (EFSA, 2004), celery is a frequent cause of pollen-related food allergy, particularly in European countries. About 30–40% of patients with food allergy in Switzerland and France are sensitized to celery root (EFSA, 2004), and it is the most frequent cause of food anaphylaxis in Switzerland (Wang, Li, Yuan, Wu, & Chen, 2011). In a study of André, Andre, Colin, Cacaraci, and Cavagna (1994), conducting 60 cases of severe reactions to food over a period of 9 years (1984–1992) in France, celery was shown to be responsible for 30% of the anaphylactic reactions. Furthermore, in Germany, 70% of patients with a pollen-related food allergy have a positive skin prick test or RAST to celery (Jankiewicz et al., 1996). Six celery proteins have been described as allergens, Api g 1–6 (IUIS Allergen Nomenclature Sub-Committee, n.d.), Api g 1 being the major allergen (Hoffmann-Sommergruber et al., 1999). Their molecular weights range between 7 and 58 kDa (IUIS Allergen Nomenclature Sub-Committee, n.d.). The MWCO of ultrafiltration, commonly used in wastewater treatment, is 1–100 kDa (Dow Water, n.d.), meaning the celery allergens would probably be retained by finer ultrafiltration. Thermal processing of celery has an impact on the allergenicity of the major allergenic protein Api g 1, whereas the profilin Api g 4 is less affected, and the carbohydrate determinants are heat stable (Ballmer-Weber et al., 2002; Jankiewicz et al., 1997). The allergenicity of celery is, thus, not completely reduced by heat treatment (Ballmer-Weber et al., 2002). No further vegetables are included in the EU Directive on allergen labeling (2007/68/EC). However, several other vegetable

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proteins have been described as allergens in the literature, e.g. carrot (Ballmer-Weber et al., 2001; Moreno-Ancillo, Gil-Adrados, Cosmes, Dominguez-Noche, & Pineda, 2006), green beans (Pastorello et al., 2010; Zoccatelli et al., 2010) and lettuce (Hartz et al., 2007; San Miguel-Moncin et al., 2003). The only treatment for allergic patients is complete avoidance (van Putten et al., 2006), which means correct labeling of food products is of great importance for the allergic consumer.

The general lack of knowledge on allergen carry-over via wash water and wash water reuse, hampers reliable estimations of the potential risk for the produce processing industry, such as fresh-cut, frozen or canned vegetables. Thus, Kerkaert et al. (2012) calculated that the threat of a potential carry-over of allergenic proteins from celery to wash water and from the water to other vegetables is indeed relevant.

Consequently, a database concerning the carry-over of (allergenic) proteins in industrial wash waters in the vegetable processing industry is developed. The impact of several process and product parameters on the protein content of the wash waters is investigated. The evaluated process parameters are batch or semi-continuous processing, the number of washing steps and the pH of the wash water. Evaluated product parameters are the processing level of the vegetable, the surface area of the vegetable and the impact of blanching of the vegetable as heat treatment. The insight in the impact of these parameters on the possible transfer of (allergenic) proteins to wash water will lead to a better established allergen management and risk estimation of the reuse of wash water. In a following work, the protein transfer from the wash water to a second batch of vegetables will be studied, and the potential risk of cross-contamination for sensitive consumers estimated.

2. Materials and methods

2.1. Sampling of industrial wash waters

Samples were collected from three Belgian vegetable processing industries. Two companies produce chilled fresh-cut vegetables with means of batch processing. The third company produces frozen fresh-cut vegetables (blanched depending on the type of vegetable) with semi-continuous processing. Sampling of the wash water was carried out before adding vegetables into the production line (blank water sample) and at the end of a production batch (worst case water sample). The samples were collected in plastic bottles and stored at -18°C until analyzed.

2.1.1. Batch processing

Sampling was performed for each vegetable (i.e. cabbage, carrot, celeriac, iceberg lettuce, and white part of leek) or vegetable mix (i.e. lettuce line: several kinds of lettuce washed after each other; lettuce mix: mixture of several types of lettuce washed simultaneously; mussel vegetables: celery and onion; soup vegetables a: leek, carrot and celery; soup vegetables b: white part of leek, celery, onion; wok vegetables: ten different vegetables) on two different production days to get insight into the variability of the protein concentration in the water. Samples were collected from one, two or three wash water baths depending on the production line (Fig. 1). The total number of samples from the batch processes was 112. In the two companies, no water purification takes place before the water is reused for a second batch of vegetables.

2.1.2. Semi-continuous processing

Samples were taken once in one or several production units (i.e. pre-washing, washing, water transport, blancher, cooling) for each vegetable (i.e. carrot, cauliflower, celeriac, zucchini, green beans, onion, peas

and spinach). The total number of samples from the semi-continuous process was 26. In the semi-continuous process two kinds of water were used, i.e. recup and process water (Fig. 2). The company has a water treatment installation where the recup water underwent anaerobic and aerobic digestion and sand filtration, and for the process water additional ultrafiltration, reversed osmosis and UV treatment was applied. The recup water was used for the initial washing step aimed at removing soil and physical dirt. The process water is potable water and was used for the final washing step and in the cooling section of the blancher. The water exiting the cooling section is recirculated to the pre-heating section of the blancher. The water leaving the pre-heating section is collected in a reservoir together with water from the water transport section. The water in the reservoir is recycled in the water transport section, and excess reservoir water goes to the water treatment installation.

2.2. Analysis of industrial wash waters

After sample collection, samples were frozen at -18°C until analysis. Thawing was performed over night at room temperature. The water samples were filtered over a tea strainer to get rid of residual vegetable pieces. The pH of the samples was monitored using a LAB 850 pH-meter (Schott Instruments, LSB, Kontich, Belgium) and the crude and non-protein content was analyzed with the AOAC International Official Method 981.10 (1981), as described by Kerkaert et al. (2012). Prior to determination of the protein content, the samples were concentrated by evaporation, resulting in a detection limit (LOD) of $11\text{ }\mu\text{g protein/ml}$ wash water for the Kjeldahl method. 50 or 80 ml of sample, for the crude and non-protein content determination respectively, was evaporated until 1–5 ml was left for destruction. All samples were analyzed in duplicate and the conversion factor 6.25 was used to convert nitrogen to protein. The net protein content was obtained by subtracting the non-protein content from the crude protein content (Fig. 3).

2.3. Database, additional data collection on washing processes and statistical analysis

The net protein concentrations of the blank water samples (before adding vegetables into the production line) were subtracted from the net protein concentrations of the worst case water samples (at the end of a production batch) to obtain the final net protein concentrations (carry-over from the vegetables to the wash water) (Fig. 3). All protein concentrations mentioned in the Results section are final net protein concentrations, unless else mentioned.

The measured pH and final net protein concentrations were compiled in a database together with specific process information. This additional information on the washing processes was collected by interviewing the quality or production manager of the companies. The questions asked were related to the product and process parameters, e.g.

- Process chart, i.e. number of wash baths (batch process), type of unit operations (semi-continuous process)
- Vegetable mass and volume of wash baths (batch process), or vegetable and water flow (semi-continuous process), to be able to calculate the vegetable-to-wash-water ratio for the different batches
- Vegetable cutting (intact, peeled/scraped, size of pieces or grating)
- Contact time between the vegetable and the wash water.

The influence of different process and product parameters on the protein carry-over from the vegetable to the wash water was

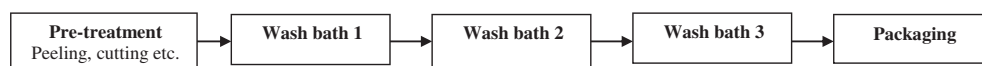


Fig. 1. Overview of the batch process. Water samples were taken in the different wash baths (differing number of wash baths were present on different production lines).

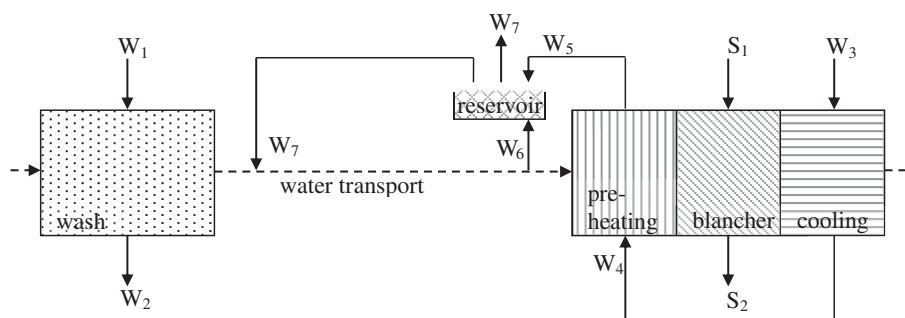


Fig. 2. Overview of the semi-continuous process. Water samples were taken in the wash section, water transport section, and in the cooling section of the blancher. *Wash section* W_1 : Recup water (less purified water) is used in the initial washing steps. The last washing step is carried out with process water (potable water). W_2 : The water exiting the wash section is not recirculated, but continues to the water treatment installation. *Blancher section* W_3 : Process water (potable water) enters the cooling section of the blancher. W_4 : Water used in the cooling section goes to the pre-heating section of the blancher. *Water transport* W_5 : Water exiting the pre-heating section of the blancher is collected in a reservoir. W_6 : Water exiting the water transport section goes to the same reservoir. W_7 : Water from the reservoir is recycled to the water transport section. Excess water goes to the water treatment installation.

statistically evaluated by dividing the individual measurements into groups depending on what parameter to be evaluated. For batch processing, the influence of the number of wash baths, the number of washing steps and the vegetable cut were evaluated. For semi-continuous processing the surface area of the vegetable (area/mass) and blanching were studied. The influence of the wash water pH was evaluated for both batch and semi-continuous processing. The influence of the type of process was assessed as well.

Several statistical tests were used to identify significant differences between the different categorical product or process parameters. When two groups were compared, the parametric *t*-test or the nonparametric Mann–Whitney *U* test was used, depending on if the subdivided data could be approximated to a normal distribution or not. The normality of the data was evaluated with the mean, 5% trimmed mean, median, skewness and kurtosis, the Kolmogorov–Smirnov and Shapiro–Wilk test, box plots and normal QQ-plots. If the data in the two groups were paired and showed a non-normal distribution, the nonparametric Wilcoxon test was used. When three groups were compared analysis of variance (ANOVA) was performed. If the assumptions underlying the ANOVA were not met, the nonparametric Kruskal–Wallis test was carried out in a following step. If the Kruskal–Wallis test showed significance, the nonparametric Dunn's test was used for pairwise multiple comparison of means of ranks to assess which group(s) differed significantly from the other. The relationship between two continuous

variables was analyzed using simple linear regression. The Spearman's rank correlation coefficient was used to evaluate the correlation between two variables. The statistical processing of the data was performed with the software SPSS Statistics 19.

3. Results

3.1. Characterization of the wash waters: pH of the wash waters and protein carry-over from the vegetable to the wash water

For batch processing, the pH of the blank wash waters (before adding vegetables into the production line) was neutral to slightly basic (pH 7.2–8.3), except for celeriac, carrot and cabbage (Table 1). For most vegetables, the pH slightly increases in the samples at the end of the production batch compared to the blank water samples. In the semi-continuous process, the pH of the blank wash waters was neutral to slightly basic (pH 7.1–8.3). Depending on the vegetable, the pH increases or decreases (sometimes more than one pH unit) in the samples at the end of the production batch compared to blank (Table 2).

Significant concentrations of protein were determined in the wash waters. For the two batch processes, the concentrations ranged between 0 and 287 $\mu\text{g}/\text{ml}$ wash water (Table 3), and for the semi-continuous process between 0 and 596 $\mu\text{g}/\text{ml}$ (Table 4). The difference in protein concentration between the two samplings of the same vegetable on

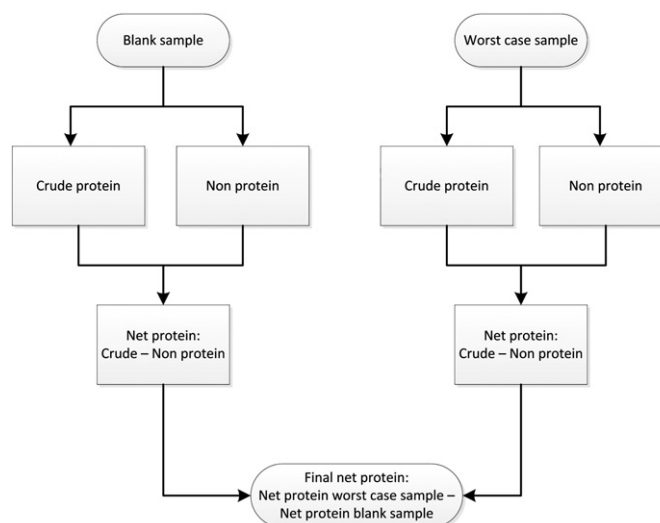


Fig. 3. Overview of the stepwise calculations to end up with the final net protein concentrations. Blank (water before vegetable washing) and worst case water samples (at the end of a production batch) were sampled in the industry. The crude and non-protein concentration of these were determined. The net protein content of the blank and worst case samples was determined by subtracting the non-protein concentration from the crude protein concentration. The final net protein concentration was assessed by subtracting the net blank protein concentration from the net worst case concentration. This final concentration equals the carried-over protein from the vegetable to the wash water.

two different production days was surprisingly high (sometimes more than tenfold, Table 3), indicating a high variability in protein carry-over depending on the production moment.

3.2. Range of process parameters in the investigated industrial processes

For the batch process, the volume of the wash baths varied from 150 to 1800 l and the total production mass/batch ranged between 150 and 5800 kg. This resulted in vegetable-to-wash-water ratios between 0.2 and 5.1 kg vegetable/l wash water for the different wash baths and batches. For the semi-continuous process, the water flow ranged between 2 and 17.4 m³/h, and the vegetable flow between 3.5 and 14 ton/h, which resulted in vegetable-to-wash-water ratios between 0.2 and 1.8 kg/l. In order to obtain comparable data for the statistical analysis, the concentrations (μg protein/ml wash water) were recalculated (to μg protein/g washed vegetable) with means of the respective vegetable-to-wash-water ratio.

3.3. Influence of process and product parameters on the protein carry-over in a batch process

The influence of the *number of wash baths* on the protein carry-over was investigated. A comparison between the absolute protein content in a wash bath (g protein, considering the volume of the wash baths) showed that there was no significant difference between the first and the second wash bath. This counts for the two batch processing industries together, as well as for the two industries separately ($\alpha = 0.05$, Wilcoxon rank-sum test).

The influence of the *number of washing steps* on the carry-over of protein was studied. When comparing the number of wash baths, the total volume of the wash baths was taken into account, whereas the vegetable-to-wash-water ratio was taken into consideration when examining the number of washing steps. There was no significant difference between the protein concentration (μg protein/g vegetable, the vegetable-to-wash-water ratio taken into account) between the first and the second washing step. This counted for both industries together, as well as for the two industries separately ($\alpha = 0.05$, Wilcoxon rank-sum test).

The influence of *processing level of the vegetable* (vegetable cut) on the protein carry-over to the wash water was evaluated. A distinction

Table 1

The wash water pH of the blank and used water samples from the batch process. The data points represent mean values $\pm$ standard deviation of two independent samplings. If no standard deviation is indicated, only one sampling was carried out. pH blank water: pH of water before washing. pH used water: pH of water after vegetable washing, at the end of the production batch.

	Wash bath	pH blank water	pH used water
Cabbage	1	7.8 $\pm$ 0.6	5.8 $\pm$ 0.7
	2	4.1 $\pm$ 0.1	7.1 $\pm$ 0.1
Carrot	1	3.6 $\pm$ 0.1	4.1 $\pm$ 0.0
Celeriac	1	3.4 $\pm$ 0.0	4.6 $\pm$ 0.1
Iceberg lettuce	1	7.8 $\pm$ 0.1	8.4 $\pm$ 0.2
	2	7.8 $\pm$ 0.0	8.5 $\pm$ 0.1
Lettuce mix	1	7.6 $\pm$ 0.0	8.4 $\pm$ 0.1
	2	7.9 $\pm$ 0.1	8.3
Lettuce line	1	7.5 $\pm$ 0.4	7.2 $\pm$ 1.6
	2	3.5	4.9 $\pm$ 0.6
	3	7.6 $\pm$ 0.1	6.4 $\pm$ 3.0
Mussel vegetables	1	7.2	8.0
	2	7.4	8.0
Soup vegetables a	1	8.3	7.1
	2	7.6	7.7
Soup vegetables b	1	7.6 $\pm$ 0.2	8.4 $\pm$ 0.4
	2	7.8 $\pm$ 0.8	8.3 $\pm$ 0.2
White part of leek	1	7.7 $\pm$ 0.8	8.8 $\pm$ 0.2
	2	7.8 $\pm$ 0.6	8.5 $\pm$ 0.1
Wok vegetables	1	7.8 $\pm$ 0.2	7.5 $\pm$ 1.1
	2	8.0 $\pm$ 0.8	7.6 $\pm$ 0.8

Table 2

The wash water pH in the semi-continuous process during washing and during cooling after vegetable blanching. The data points represent mean values $\pm$ standard deviation of two independent samplings. If no standard deviation is indicated, only one sampling was carried out. Type of blank water: Indicates type of blank water (recup or process water) used in the different process steps. pH used water: pH of water after vegetable washing, at the end of the production batch. Recup water: Recup water used for initial washing steps. Process water: Potable water.

	Process step	Type of blank water	pH used water
Recup water	–	–	8.3 ^a
Process water	–	–	7.7 $\pm$ 0.6 ^a
Carrot	Washing	Process	5.4 $\pm$ 0.8
	Cooling	Process	8.0
Cauliflower	Washing	Recup	6.2
	Cooling	Process	7.4
Celeriac	Washing	Process	6.6
Green bean	Washing	Recup	7.6
	Cooling	Process	7.2 $\pm$ 0.04
Onion	Transport	Process	6.6
Pea	Washing	Recup	4.6
	Cooling	Process	7.6
Spinach	Washing	Process	8.9
	Cooling	Process	8.5
Zucchini	Washing	Process	4.9

^a Blank water, not used water.

was made between sliced or chopped vegetables, and vegetables that were peeled or scraped and grated. Logically, a higher degree of processing would give a higher protein carry-over. This hypothesis was confirmed ($0.01 < P < 0.05$) with the Mann–Whitney *U* test (one-tailed). The medians for the two groups were 14.8 and 62.1 μg protein/g for the ‘sliced and chopped’ and ‘scraped and grated’ vegetables, respectively.

To analyze the influence of the *wash water pH* on the protein carry-over from the vegetable to the wash water, the data were divided into three groups: slightly acid (pH 4.1–6.0), neutral (pH 6.1–8.0) and slightly basic (pH 8.1–9.0). The difference in carry-over between the slightly acid and slightly basic group was determined to be significant ($0.01 < P < 0.05$, Kruskal–Wallis test followed by Dunn’s test on mean ranks). The median protein concentrations in the different pH groups

Table 3

Protein concentration ($\mu\text{g}/\text{ml}$ wash water) of the wash waters per washing step for the two batch processes. All data are final net protein concentrations, meaning the net protein concentration in the blank wash water has been subtracted from the net protein concentration of the worst case water sample. Two samplings were performed for most vegetables. The mean $\pm$ standard deviation represents two independent measurements of one sampling. <LOD: Lower than detection limit. /: Not analyzed. Empty cells: These vegetables are not washed a second or third time.

	Sampling	Washing step		
		1	2	3
Cabbage	1	7.9 $\pm$ 10.2	17.5 $\pm$ 10.4	
	2	72.5 $\pm$ 42.9	61.6 $\pm$ 56.4	
Carrot	1	39.2 $\pm$ 59.0		
	2	287.3 $\pm$ 22.1		
Celeriac	1	103.7 $\pm$ 19.7		
	2	113.3 $\pm$ 7.3		
Iceberg lettuce	1	20.2 $\pm$ 3.1	18.6 $\pm$ 7.6	
	2	56.9 $\pm$ 3.1	43.2 $\pm$ 16.3	
Lettuce line	1	36.1 $\pm$ 8.3	37.8 $\pm$ 9.9	46.8 $\pm$ 1.9
	2	1.1 $\pm$ 5.9	5.7 $\pm$ 3.7	<LOD
Lettuce mix	1	0.5 $\pm$ 6.9	24.6 $\pm$ 3.9	
	2	96.3 $\pm$ 13.0	/	
Mussel vegetables	1	18.2 $\pm$ 3.6	14.7 $\pm$ 3.6	
Soup vegetables a	1	91.9 $\pm$ 17.3	46.5 $\pm$ 12.0	
Soup vegetables b	1	1.1 $\pm$ 3.1	16.7 $\pm$ 14.4	
	2	46.5 $\pm$ 21.9	21.6 $\pm$ 3.6	
White part of leek	1	<LOD	<LOD	
	2	<LOD	<LOD	
Wok vegetables	1	98.4 $\pm$ 17.9	15.8 $\pm$ 4.9	
	2	57.4 $\pm$ 12.1	119.9 $\pm$ 5.0	

Table 4

Protein concentration ($\mu\text{g}/\text{ml}$ wash water) of the wash waters per washing step for the semi-continuous process. All data are final net protein concentrations, meaning the net protein concentration in the blank wash water has been subtracted from the net protein concentration of the worst case water sample. Only one sampling was carried out for most vegetables. The mean $\pm$ standard deviation represents two independent measurements of one sampling. <LOD: Lower than detection limit. /: Not analyzed. Empty cells: These vegetables are not blanched.

	Sampling	Process step	
		Washing/water transport	Cooling (after blancher)
Carrot	1	183.2 $\pm$ 16.6	8.4 $\pm$ 9.5
	2	198.0 $\pm$ 6.8	/
Cauliflower	1	314.7 $\pm$ 43.5	21.5 $\pm$ 2.9
Celeriac	1	3.2 $\pm$ 2.9	/
Green bean	1	189.9 $\pm$ 11.6	6.2 $\pm$ 4.4
	2	/	16.3 $\pm$ 2.9
Onion	1	542.0 $\pm$ 44.9	
Pea	1	596.2 $\pm$ 91.7	<LOD
Spinach	1	8.9 $\pm$ 3.8	4.3 $\pm$ 4.3
Zucchini	1	531.0 $\pm$ 46.0	

were slightly acid 62.1, neutral 21.9 and slightly basic 13.8 μg protein/g washed vegetable.

3.4. Influence of process and product parameters on the protein carry-over in a semi-continuous process

To study the influence of the wash water pH on the protein carry-over to the wash water in a semi-continuous process, the data were divided into two pH groups: slightly acid (pH 4.1–6.0) and neutral to slightly basic (pH 6.1–9.0). A trend towards a difference in protein carry-over was observed ($0.05 < P < 0.1$, Mann–Whitney U test). The slightly acid pH group contained more protein (median 1073.2 μg protein/g vegetable) than the neutral to slightly basic pH group (median 271.3 μg protein/g vegetable).

Simple linear regression was used to analyze the influence of the vegetable surface area (mm^2/g) on the protein carry-over to the wash water. The data set contained naturally small vegetables, i.e. pea, as well as vegetables that had been cut in small pieces. The following vegetables and cuts were present. Peas and spinach were unprocessed, carrot and celeriac were peeled, zucchini and onion were cut into cubes of 6 mm, whereas cauliflower was cut into pieces of 38–45 mm, and the edges of the green beans had been cut off. Simple linear regression indicated that a larger surface area resulted in a higher protein carry-over to the wash water: *protein concentration (μg carried over protein/g washed vegetable) = $84.4 + 0.98 \times (\text{surface area})$* , $R^2 = 0.64$. The intercept was not significantly different from zero ($P > 0.05$). The slope was determined significant ($0.01 < P < 0.05$).

The influence of blanching on the protein carry-over to the cooling water after the blancher was analyzed. The protein concentration in the washing and water transport steps were statistically compared with the protein concentration of the cooling section in the blancher. The protein concentration was significantly higher in the washing and water transport steps (median 338.8 μg protein/g vegetable) than in the cooling section of the blancher (median 16.1 μg protein/g vegetable) ($0.01 < P < 0.05$, Mann–Whitney U).

3.5. Influence of the type of process on the protein carry-over to the wash water

The protein concentration in the first wash bath of the batch processes was compared with the protein concentration of the washing and transport steps of the semi-continuous process. It was proven that the protein concentration in the wash water of the semi-continuous process ($480.3 \pm 488.8 \mu\text{g}/\text{g}$) was significantly higher than in the batch processes ($41.5 \pm 30.9 \mu\text{g}/\text{g}$) ($0.01 < P < 0.05$, t -test). Only the

highest concentration of the two samplings of the different vegetables in the batch process was used in this test.

4. Discussion

Significant protein concentrations were determined in the various wash waters from the three vegetable processing industries. This confirms the findings of Kerkaert et al. (2012) that protein carry-over from the vegetable to the wash water occurs. The differing quantity of protein in the wash waters can be associated with several product and process parameters, of which several were evaluated in this research. The difference in protein concentration between two samplings of the same vegetable on the same processing line on two different production days, is however harder to explain. Here, product and process parameters such as wash water pH and cutting of the vegetable are basically the same. The time between the duplicate samplings ranged between three to six weeks. Biological materials like vegetables show differences in texture depending on preharvest environmental, cultural, physiological and genetic factors (Sams, 1999). Moreover, vegetables are continuously changing postharvest due to continued transpiration, causing water loss, and active metabolism (Galindo, Herppich, Gekas, & Sjöholm, 2004). The heterogeneity between the duplicates could be attributed to a combination of these factors. Nevertheless, it can be concluded that processing of fresh-cut vegetables is a heterogeneous process, which makes it less easy to handle when it comes to risk management in general and of allergens in particular (Luning et al., 2011).

No significant difference in protein carry-over between the first and the second wash bath could be shown, neither for the absolute protein content (volume of wash bath taken into account), nor for the protein concentration (the vegetable-to-wash-water ratio taken into consideration) for the batch process. A lower protein concentration in the second wash bath would be expected due to washing out of cell juices and further dilution. However, when the vegetables are transferred from the first to the second wash bath they bring water, including proteins, from the first wash bath with them into the second bath. An additional explanation might be due to the turgor and homeostasis. The broken cells (where the vegetable has been cut) will be refilled with cell fluid from surrounding cells since the vegetable material attempts to maintain the turgor and homeostasis, and the broken cells will continue to leach cell contents into the wash water.

A higher degree of processing of the vegetable resulted in a significantly higher protein carry-over to the wash water. Two facts support the theory that a higher degree of processing of the vegetable results in more protein carry-over. (1) The more cuts, the more destroyed/wounded cells come in contact with the wash water and the cell contents leaks out. (2) The higher the degree of processing of the vegetable, the higher area-to-volume ratio of the product. The higher area-to-volume ratio, the more contact between the vegetable and the wash water, and hence the higher the chance of protein leaching to the wash water. Furthermore, a larger surface area results in more protein carry-over to the wash water (semi-continuous processing). In the data set, vegetables with a naturally large surface area, e.g. peas, were represented, as well as vegetables that obtain a large surface area by processing (e.g. cutting and grating). The more processing of a vegetable, the larger the area-to-volume ratio/surface area gets.

It was not possible to determine any trend of more or less protein carry-over for different vegetable types (e.g. leafy vegetables compared to root crops) with the collected data. The washed vegetables are characterized by different protein contents, ranging from 0.8 to 5.2% protein (average $1.7 \pm 1.1\%$), for the different vegetables (DTU National Food Institute, 2009; Health Canada, n.d.; National Food Agency, n.d.; Rijksinstituut voor Volksgezondheid en Milieu, 2011; United States Department of Agriculture, 2011). Carrot and celery have the lowest protein content, and peas the highest. The protein content of celeriac equals the average. No correlation between the protein content of the vegetable and the protein concentration in the wash water could be

shown (semi-continuous process, Spearman's rank correlation $r_s = 0.000$, $P > 0.05$).

The wash water samples from the vegetable processing industries were characterized by different pH. The slightly basic pH of the blank wash waters can be explained by the addition of hypochlorite as a technical agent to prevent microbial growth in the washing water (Barrera, Blenkinsop, & Warriner, 2012; Vandekinderen et al., 2009). The acid pH of the blank wash waters of celeriac, carrot and cabbage can be attributed to the addition of ascorbic, citric or other acid to prevent discoloring of the vegetable through enzymatic browning (Altunkaya & Gokmen, 2009; Jaxsens, Devlieghere, Van der Steen, & Debevere, 2001). Since industrial vegetable washing is performed at acidic to slightly alkaline pH, the influence of the pH on the protein carry-over to the wash water was studied.

The higher protein carry-over to the wash water at acid pH (for batch processing as well as semi-continuous processing) was unexpected since generally proteins dissolve better at alkaline pH. Some possible reasons for the higher carry-over at acid pH can be given. Pectin's main constituent, D-galacturonic acid, has a pK_a value of 3.5 (Kohn & Kovac, 1978; McFeeters, 1986). The experimental pK_a values of the free carboxyl groups of polygalacturonic acid lay in the range 3.6 to 4.1 when the polygalacturonic acid is titrated from the fully protonated form to the completely deprotonated form (Cesaro, Ciana, Delben, Manzini, & Paoletti, 1982; McFeeters, 1986). At low pH pectin will thus be protonated and unavailable for ionic binding with cations (McFeeters, 1986) such as proteins, hence facilitating protein solubility. Furthermore, several studies have shown that cell wall-loosening enzymes have an acidic optimum pH (Rayle & Cleland, 1992). It can be questioned if the buffer can enter the apoplast through the waxy cuticle (Rayle & Cleland, 1992), however, when the vegetables have been cut the cuticular barrier is ruptured and the buffer could enter the apoplast.

Significantly higher protein concentrations were determined in the washing and water transport steps compared to the cooling section of the blancher in the semi-continuous process. During blanching loss of cell solutes takes place due to diffusion and expulsion of cell sap as turgor is lost on cell death (Selman, Rice, & Abdulrezzak, 1983; Selman & Rolfe, 1979). Blanching generates changes in the texture of the vegetable through gelification, solubilization and degradation of the cell wall pectins (Neri, Hernando Hernando, Perez-Munuera, Sacchetti, & Pittia, 2011). The enzymatic and chemical changes of the pectins during blanching increase their water holding capacity, and pectin gelation follows when the phase transition temperature is reached (Neri et al., 2011; Plat, Benshalom, Levi, Reid, & Goldschmidt, 1988). The gel formation might be the reason why only low protein concentrations were detected in the cooling section.

The higher concentration of proteins in the wash waters of the semi-continuous process compared to the batch processes could be attributed to several factors. The contact time between the vegetable and the wash water differed between the three companies. In the batch processes, the vegetables are in contact with the wash water for 1 to 1.5 min per wash bath, which results in 3–4.5 min in total. In the semi-continuous process on the other hand, the contact time between the vegetable and the wash water is 15–30 min depending on the vegetable. The vegetables might be exposed to more physical damage in a semi-continuous process, containing unit operations such as cyclone and peddle washers, compared to wash baths and conveyor belts in the two batch processes. For batch processing, wash baths are filled with fresh water and waste water is discharged after one or several batches. In the semi-continuous process the water is recirculated in several stages and no complete refreshment of the processing line is conducted at a daily basis. Although fresh water is supplied and excess used water is discharged from recirculation reservoirs in the process, accumulation of proteins could take place. Despite the listed differences between the two kinds of processing, it can be stated that for the three fresh-cut vegetable processing lines investigated, the protein concentrations present in the wash waters were significantly higher in the semi-continuous process.

Methods for detection of celery protein and DNA have been developed, e.g. an ELISA with LOQ 5.6 µg celery protein/g food sample (Wang et al., 2011) and a PCR method with LOQ 5–10 µg celery seed/g sample (Hupfer, Waiblinger, & Busch, 2007). Nevertheless, detection of the total protein content of the wash waters was preferred in this study due to several reasons. In this work wash waters from washing of several different kinds of vegetables were studied, thus, a different detection method would then be needed for every vegetable. The use of the technical agent hypochlorite in the wash water may cause degradation of several amino acids (Cucu, Devreese, et al., 2013; Kerkaert et al., 2011), consequently making ELISA detection unreliable. Blanching was applied in one of the studied washing processes and the result of ELISA and PCR detection methods are often erroneous when the food matrix has been heated (Cucu, Jaxsens, & De Meulenaer, 2013).

In conclusion, industrial vegetable wash waters contain significant protein concentrations. Several product and process parameters have an influence on the protein carry-over from the vegetable to the water. The type of process, batch or semi-continuous, showed a great influence on the protein content of the wash water, as well as the degree of vegetable cutting. The smaller the vegetable (the more cut and/or the larger surface area) the higher the protein carry-over to the wash water. Another risk factor to be considered is the pH of the wash water, since higher protein concentrations were determined after washing at acid pH. This is an important finding since celeriac is usually washed at acid pH to prevent enzymatic browning. The database with protein carry-over from vegetables to the wash water will be used in further research to estimate the carry-over to a following batch of vegetables, and to evaluate the potential risk for sensitive consumers, in the framework of a science-based allergen management for the vegetable processing industry.

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