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Ready-to-eat vegetables production with low-level water chlorination. An evaluation of water quality and of its impact on end products

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Abstract. *Introduction.* We evaluated the microbiological impact of low-level chlorination (1 ppm free chlorine) on the production of ready-to-eat (RTE) vegetables by monitoring the microbiological quality of irrigation and processing water in two production plants over a 4-season period, as well as the microbiological quality of unprocessed vegetables and RTE product. Water samples were also characterized in terms of some chemical and physico-chemical parameters of relevance in chlorination management. *Materials and methods.* Both producers use water with maximum 1 ppm free chlorine for vegetables rinsing, while the two processes differ by the number of washing cycles. *Results and conclusions. Salmonella* spp and *Campylobacter* spp were detected once in two different irrigation water samples out of nine from one producer. No pathogens were found in the vegetable samples. As expected, the procedure encompassing more washing cycles performed slightly better in terms of total mesophilic count (TMC) when comparing unprocessed and RTE vegetables of the same batch. However, data suggest that low-level chlorination may be insufficient in preventing microbial build-up in the washing equipment and/or batch-to batch cross-contamination.

Key words: ready-to-eat vegetables, water chlorination, microbiological decontamination.

Riassunto *(Produzione di vegetali di IV gamma con acqua clorata a bassa concentrazione. Valutazione della qualità dell'acqua e del suo impatto sul prodotto finito). Introduzione.* L'impatto dal punto di vista microbiologico della clorazione a bassa concentrazione (1 ppm cloro libero) nella produzione di vegetali di IV gamma è stato valutato mediante il monitoraggio annuale della qualità microbiologica delle acque di irrigazione e di processo, nonché dei vegetali materia prima e pronti al consumo, presso due impianti di produzione. Sono stati, inoltre, determinati alcuni parametri chimici e chimico-fisici rilevanti nella gestione della clorazione. *Materiali e metodi.* Entrambi i produttori utilizzano massimo 1 ppm di cloro libero nelle acque di lavaggio. Tuttavia, i processi adottati presso i due stabilimenti differiscono per il numero di cicli di risciacquo. *Risultati e conclusioni. Salmonella* spp e *Campylobacter* spp sono stati rinvenuti ciascuno in un campione di acqua di irrigazione su nove, presso uno dei due produttori. Non sono stati rilevati organismi patogeni nei campioni di vegetali. Confrontando i dati di conta mesofila totale di materia prima e prodotto finito dello stesso lotto, il processo comprendente un numero maggiore di cicli di risciacquo fornisce risultati migliori. Tuttavia, i dati ottenuti sembrano indicare che clorazione a bassa concentrazione potrebbe essere insufficiente ai fini della prevenzione della contaminazione delle apparecchiature di lavaggio e della contaminazione crociata tra lotti diversi di vegetali.

Parole chiave: vegetali pronti al consumo, clorazione dell'acqua, decontaminazione microbica.

INTRODUCTION

Ready-to-eat (RTE) vegetables consist of minimally processed produce, conveniently packaged and intended for consumption without further domestic processing (washing and/or cooking). These characteristics of RTE vegetables meet consumers demand for fresh, nutritious food that requires minimal preparation time. As a consequence, the market for such convenient food has been steadily expanding even as overall domestic consumption of fruits and vegetables decreases. For instance, an 8.3% increase in the consumption of RTE vegetables was observed [1] in Italy between January and October 2010, while, in the same period of time, consumption of fruits and vegetables decreased by 0.8%.

Because RTE vegetables are fresh products with limited shelf-life, that need to be stored under refrigeration, microflora may survive and grow on the product. This issue demands particular attention, since domestic washing and/or cooking, which nor-

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mally take care of microbial contamination of traditional fresh produce, are skipped in the case of RTE vegetables. Pathogen contamination (*Salmonella* spp, *Listeria monocytogenes*, *E. coli*, *Campylobacter* spp) may occur at any stage in the production process, since it may derive from poor quality of irrigation water, use of manure as fertilizer, or incorrect application of GMP and HACCP during processing. In fact, the presence and prevalence of pathogens in RTE salads [2-6], and the occurrence of epidemic outbreaks related to consumption of contaminated raw vegetables [7-21] are reported in the scientific literature. FAO/WHO Expert Consultation [22] identifies leafy green vegetables as the commodity group of highest concern from a microbiological safety perspective. In 2009, Italy received 98 notifications from European Commission's Rapid Alert System for Food and Feed [23], out of which 74 originated from other EU member States, while all others were issued by the Italian National Alert System. About 10% of the total alerts involved vegetable products, of which 4 were rocket and mixed salads contaminated with *Salmonella* spp [24].

Water can have a profound effect on hygiene and safety of the RTE product, since it can be considered as a "raw material" in irrigation, it removes dirt and cell exudates from harvested produce, and reduces the microbial population on the surface of vegetables. Specifically, in RTE vegetables processing, potable water should be used in the final rinsing step [25-28]. The effectiveness and necessity for extensive decontamination is under debate. In fact, shelf-life extension is not necessarily achieved by means of microbial decontamination [29]. Furthermore, decreasing the microbial population may favor the growth of competing pathogens. Current opinion [30] states that adding disinfectant products to water in vegetables processing should not be viewed as a means of sanitizing the product itself. In fact, several literature reports [29, 31-35] show that no advantage derives from adding disinfectants, in terms of total bacterial count after storage of the RTE product. A previous investigation [36] carried out in our laboratory on the microbiological quality of RTE vegetables from industrial plants using different processing strategies showed that focusing on high quality of raw material and process management, rather than counting heavily on sanitizing solutions, results in best end product quality. Adding disinfectants to water should, therefore, be rather viewed as a means of avoiding microbial contamination build-up in the facility, and cross-contamination between batches of processed vegetables. Cross-contamination occurrence in postharvest unit operation is well-documented [4].

Water disinfection through chlorination is a well-established, economical and simple way of keeping microbial contamination under control in various circumstances, including processing of RTE vegetables. However, the possibility of development of irritant gaseous chlorine in the working environment, and of formation of chlorinated organic by-products that may contaminate industrial effluents and RTE products [37] have prompted a number of studies on alternative sanitization methods [30, 32, 38-46]. Consequently, the use of chlorine is restricted by German law [47].

The efficacy of alternative disinfection methods being under debate, chlorination is still the method of choice, so that control of food quality has to deal with correct chlorination management [48, 49].

Water disinfection is usually carried out by addition of sodium hypochlorite (NaClO) solutions, gaseous chlorine (Cl_2), or chloride dioxide solutions (ClO2). Hypochlorous acid (HClO) resulting from pH-dependent NaClO hydrolysis or from Cl_2 disproportionation (1) is the most effective chlorine species.

$$Cl_2 + H_2O \rightarrow HOCl + Cl^- + H^+ \quad (1)$$

Based on reaction (1), addition of basic NaClO or acidic Cl_2 into water should be accompanied with pH control at 6.5 to 7.5 values, so as to maximize HOCl concentration and minimize dispersion of Cl_2, especially in poor-quality waters with high chloride concentrations. Optimal pH and low chlorides are, therefore, required for best disinfection with "active chlorine". Furthermore, water should be as free as possible from organic matter, which reacts rapidly with HOCl, possibly forming potentially harmful chlorinated organic substances, thus decreasing the amount of chlorine available for disinfection (free chlorine). Other oxidizable inorganic substances, such as ammonia, nitrite, iron and manganese also react with HOCl, [49], so that larger amounts of disinfectants need to be added in order to maintain the desired free chlorine level.

Typical chlorine concentrations in industrial processing of produce range from 50 to 350 ppm, depending on the vegetables and on the treatment type [48-50]. Such values are extremely high when compared to those recommended by WHO [51] for potable water, *i.e.* 0.1-0.3 ppm free chlorine, which is used in domestic processing of vegetables. High chlorine concentrations are justified in industrial vegetables processing by the fact that some microorganisms are poorly sensitive to chlorine. However, some vegetables undergo discoloration when exposed to high concentration chlorine, so that a compromise needs to be found. Furthermore, the effectiveness of sanitizing treatments can be limited towards internalized microorganisms.

In this framework, we report on our experience with two producers that adopt a milder approach for microbial stabilization in the production of RTE vegetables, by maintaining free chlorine in the 0.2-1 ppm range in processing water

Our study aims at evaluating the impact of low-level chlorination on the microbiological quality of water used in industrial vegetables processing for RTE production, and on product decontamination. To this end, the microbiological parameters that

were monitored in water samples were TBC at 22 °C; *Escherichia coli*, *Enterococci*, *Salmonella* spp, *Listeria monocytogenes* and *Campylobacter* spp, while TMC at 30 °C, *Salmonella* spp, *Listeria monocytogenes*, *Campylobacter* spp and *Escherichia coli* were determined on vegetable samples. Water samples were also characterized in terms of some physico-chemical parameters and ionic concentrations of relevance in chlorination management

MATERIALS AND METHODS

Beginning October 2009, a sampling campaign was undertaken at two different RTE vegetables production establishments located in central Italy (Lazio region). The activity of both producers (henceforth producer 1 and 2) encompasses all production steps, from field to RTE product, so that a complete evaluation of the production chain was performed. Each sampling comprised *(Figure 1)*: i) water used for irrigation (W1); ii) potable water entering the processing line (W2); iii) water from the final wash with chlorinated water (W3); iv) unprocessed vegetables ("raw materials") entering the processing line (RM); v) ready-to-eat eat products (RTE). Samples were collected on a monthly basis over a 4-season period, totalling 27 (nine W1, W2, W3) water samples and 18 (nine RM and RTE) vegetable samples from producer 1 and 24 (eight W1, W2, W3) water samples, and 16 (eight RM and RTE) vegetable samples from producer 2.

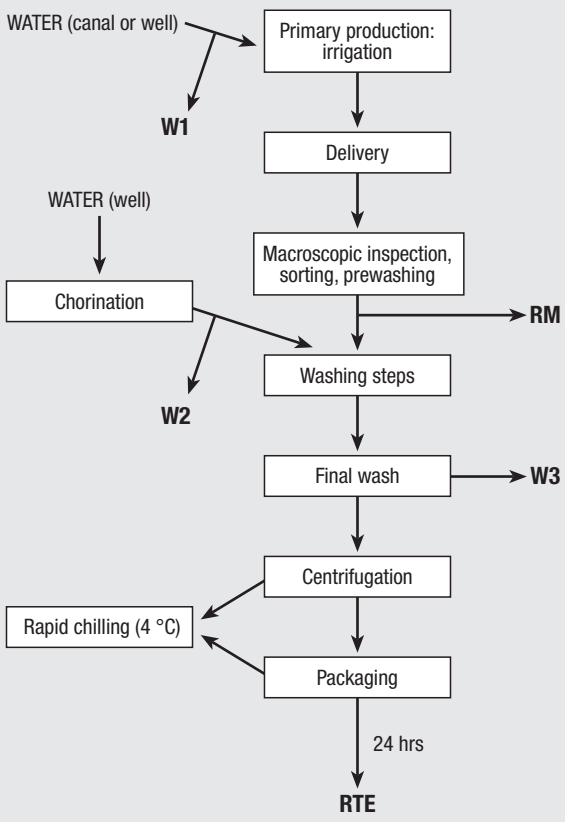

Fig. 1 | *Flow chart describing the main steps in the chain of production of RTE vegetables, and the identification of samples collected for the present study. The number of washing cycles varies between producers. The processing strategy for microbial stabilization may include, as in the case of producer 2, an additional step consisting of rapid chilling of vegetables in a cryogenic tunnel, prior to packaging. W1: irrigation water; W2: chlorinated water entering the processing line; W3: water from the final wash; RM: raw material,* i.e. *whole vegetables after macroscopic control, sorting, prewashing; RTE: ready-to-eat product 24 hrs after packaging.*

Producer 1

Broad-leaved endive was sampled from producer 1. "Use by" date as established by the producer is 7 days from packaging. Producer 1 utilizes surface water (canal) for irrigation. Processing water is drawn from a well and treated by maintaining 0.2-1 ppm of free chlorine throughout the process. Broad-leaved endive from the field (RM) undergoes a first wash with potable water, and is then transferred via a conveyor belt to a table where it is manually husked. Endive is then cut by an automated cutter under a laminar potable water flow, then transferred to a blowing washer. Contact time of vegetables with rinsing water is five minutes.

Producer 2

Rocket salad was sampled from producer 2. "Use by" date as established by the producer is 6 days from packaging. Producer 2 utilizes water from a well for irrigation. Processing water used for all washes is drawn from a well and treated, as declared by the producer, by maintaining 0.2-1 ppm of free chlorine. Rocket salad from the field (RM) undergoes a first spray wash on a conveyor belt feeding a blowing washer. Two further washing cycles are performed with closed pipe flumes and blowing washers. Contact time with rinsing water totals about 10 minutes. The product undergoes rapid cooling at +4 °C in a nitrogen cooling tunnel (Polar wind tunnel, Turatti, Italy) after centrifugation and before packaging.

Microbiological analysis of water and vegetables

All samples were transported at 4-6 °C to the laboratory. Water samples, RM vegetable samples (48 hours after harvesting), and the RTE vegetable samples (24 hours after packaging) were immediately analyzed.

The following parameters were determined on W1 and W3: TBC at 22 °C; *Escherichia coli*, *Salmonella* spp, *Listeria monocytogenes* and *Campylobacter* spp.

On W2: TBC at 22 °C, *E. coli*, enterococci, and pathogens Salmonella spp, *L. monocytogenes* and *Campylobacter* spp.

On RM and RTE samples, the following parameters were determined: TMC, *E. coli*, *Salmonella* spp, *L. monocytogenes*, and *Campylobacter* spp.

All microbiological media were from Oxoid (Cambridge, UK), unless otherwise specified.

The microbiological determinations on water samples were made using the membrane filtration method for the enumeration of *E. coli* and intestinal enterococci, according to the standard culture method (UNI EN ISO 9308-1/2002; UNI EN ISO 7899-2/2003).

The TBC on water samples was determined using the pour-plate method by inoculation in agar (Plate Count Agar) with a volume of 0.1 mL and 1 mL of potable water (W2). Due to heavier contamination of water samples W1 and W3, decimal dilutions were made in BPW (Buffered Peptone Water, Oxoid Cambridge, UK) and analyzed by the same method. Incubation conditions were 22 °C for 72h (UNI EN ISO 6222/2001).

The detection of *Salmonella* spp, *L. monocytogenes* and *Campylobacter* spp was carried out using a modified membrane filtration method: two liters of each water sample were filtered on 0.45 µm nitrocellulose membrane filter (Millipore, France), and the residue collected on the membrane was resuspended in 10 mL of the same water sample; the suspension obtained was shaken vigorously with a Vortex mixer for 3 min and than 1 mL was analyzed for detection of pathogens, according to their ISO methods (ISO 6579:2002; ISO 11290-1:1996; ISO 10272-1:2006).

Bacterial determinations on vegetables were carried out using the ISO culture methods for the enumeration of TMC and *E. coli* (ISO 4833:2003; ISO 16649-2:2001). The appropriate aliquots for analysis were obtained from 750 g of vegetables (either RM, or five bags of packaged salad) after careful mixing and homogenization.

For detection of pathogens *Salmonella* spp, *L. monocytogenes* and *Campylobacter* spp, twenty five grams of each sample were analyzed according to ISO culture methods (ISO 10272-1:2006, ISO 6579:2002 and ISO 11290-1:1996, respectively).

Salmonella spp and *L. monocytogenes* were also detected by PCR Bax System (DuPont-Qualicon, Geneva, Switzerland), a rapid molecular method that uses PCR technology for screening of pathogens in food and environmental samples, according to the manufacturer's instructions.

Chemical analysis of water

pH, conductivity, ammonium, nitrates and nitrites in water were determined upon arrival of the samples in the laboratory. 100 mL of each sample were transferred in PE bottles (Nalgene Labware, Thermofisher Scientific, USA), 1 mL of trace metal analysis grade nitric acid (Romil, Cambridge, UK) was added, and the resulting solutions were stored at 4 °C until metal analysis by Flame Atomic Absorption Spectrophotometry was carried out within one month from sampling.

pH (Hanna Instruments 8314 pH-meter) was determined after calibration with standards (Merck, Germany) at pH 4.0, 7.0, and 10.0.

Conductivity was measured with a conductivimeter (Hanna Instruments 8733) calibrated with a standard (Hanna Instruments, Italy) at 12880 µS/cm at 25 °C.

Spectrophotometrical assays were performed on a Beckman Coulter DU530 spectrophotometer. All solutions were prepared with distilled deionized water at 18.2 MΩ/cm (Sartorius Stedim arium 611 VF). All reagents were analytical grade and, unless otherwise specified, purchased from Carlo Erba (Italy), Merck (Germany), or Alfa Aesar (Germany). The following methods are adapted from *Standard methods for the examination of water and wastewater* [52].

Ammonia was determined by the phenate method after distillation in a Kjeldhal apparatus. Quantitation was performed against a standard solution at 0.2 mg/L, obtained by dilution of a certified 1000 mg/L ammonium standard (Merck, Germany).

Nitrites were determined by means of the Griess reaction. Quantitation was performed against a standard solution at 1 mg/L, obtained by dilution of a certified 1000 mg/L nitrite standard (Merck, Germany).

Nitrates were determined after reduction to nitrites with 5% Cd(OAc)2, 2 mL concentrated NH3, and 0.5 g powdered Zn. The resulting nitrites are determined with the Griess reaction.

Chlorides were determined by Mohr's argentometric method, *i.e.* by direct titration with 0.01 N silver nitrate standard solution (Panreac Quimica, Spain) at pH 7-10 (H_2SO_4 or NaOH can be used to adjust pH when necessary), in the presence of potassium chromate as indicator. Possible interference by sulphides was eliminated by treatment with hydrogen peroxide.

Fixed residue was determined by evaporating 100 mL water in a Teflon PFA capsule (Nalgene Labware, Thermofisher Scientific, USA), in a muffle furnace at 180 °C to constant weight.

Metals (Na, K, Ca, Mg, Mn, Fe) were determined by direct aspiration of the acidified water sample into the air-acetilene flame of a AAS (Perkin Elmer 4100 interfaced with Windows NT workstation equipped with AA Winlab Analysis software). The calibration curve was constructed prior to each analysis by means of standard solutions obtained by diluting 1000 mg/L commercial standard solutions (Merck, Germany).

RESULTS

Microbiological quality of water, raw materials, and RTE products

Microbiological data in *Tables 1 and 2* show that TBC ranges of W1 and of W2 are low. Some differences should be pointed out between canal water W1 from producer 1, and W1 from producer 2's well. In fact, the former exhibits a higher TBC than the latter, in association with presence of regulated microorganisms that, in some cases, are more numerous than established by the limits set by Italian law [53, 54] for treated wastewater used for irrigation: *Escherichia coli* higher than 100 cfu/100 mL in four samples out of nine (point values: 500, 538, 138, 1700 cfu/100 mL); *Salmonella* spp and *Campylobacter* spp which should be absent, are found in two different samples. It is worth noting that no pathogens or *E. coli* are found in W3, RM, and RTE

Table 1 | *Results of the microbiological analyses of W1, W2, W3 water samples and RM and RTE vegetable samples from producer 1*

Parameter (units)	Producer 1				
	W1 Average [range]	**W2 Average [range]**	**W3 Average [range]**	**RM Average [range]**	**RTE Average [range]**
TBC at 22 °C (cfu/ml)	5.5×10^4 [$5.0 \times 10^1 - 3.4 \times 10^5$]	3.0×10^1 [$0.0 - 1.7 \times 10^2$]	3.3×10^4 [$2.6 - 2.0 \times 10^5$]	n.d.	n.d.
TMC at 30 °C (cfu/g)	n.d.	n.d.	n.d.	2.1×10^9 [$2.1 \times 10^5 - 1.8 \times 10^{10}$]	9.9×10^8 [$7.5 \times 0^4 - 8.9 \times 10^9$]
Escherichia coli (cfu/100 mL – cfu/g)	3.4×10^2 [$0.0 - 1.7 \times 10^3$]	< 1	< 1	< 10	< 10
Enterococci (cfu/100 mL)	n.d.	< 1	n.d.	n.d.	n.d.
Salmonella spp (Presence/Absence in 2 L or 25 g)	Pres[a]	Abs	Abs	Abs	Abs
L. monocytogenes (Presence/Absence in 2 L or 25 g)	Abs	Abs	Abs	Abs	Abs
Campylobacter spp (Presence/Absence in 2 L or 25 g)	Pres[b]	Abs	Abs	Abs	Abs

RM: raw material; RTE: ready-to-eat; n.d.: not determined; [a]Salmonella *spp detected in one sample with the ISO method and confirmed by PCR-BAX;* [b]Campylobacter *spp detected in one sample with the ISO method.*

from producer 1, so that contamination of W1 within the ranges in *Table 1* does not seem to be carried over to the vegetables and to the processing water. No pathogens are found in W1 from producer 2. TBC is higher in W3 than in W2 at both producers. This may be attributed to microbial contribution from the vegetables being processed, to microbial build-up in the washing equipment, or to cross-contamination between batches. Data on microbial load differences between RM and RTE of the same batches *(Figure 2)* show a great variability at producer 1, where, in some cases, a reduction in the order of 3-4 log units was achieved, while on two occasions was the TMC higher in the RTE than in the RM samples. These data point at microbial build-up and/or cross contamination. As for producer 2, the microbial load in the RTE was always found comparable to or lower than the RM (less than 2 log units reduction at best, however). Therefore, a process encompassing multiple rinsing steps appears to be more reproducible in time. However, there is a possibility that microbial build-up and cross contamination may be liable for the limited reduction at producer 2.

Overall, when it comes to evaluating the impact of the procedures adopted by the two producers on the microbiological quality of the RTE, the outcome is quite similar. In fact *(Tables 1 and 2)*, the TMCs of RMs and of RTE products from both producers are in the same order of magnitude.

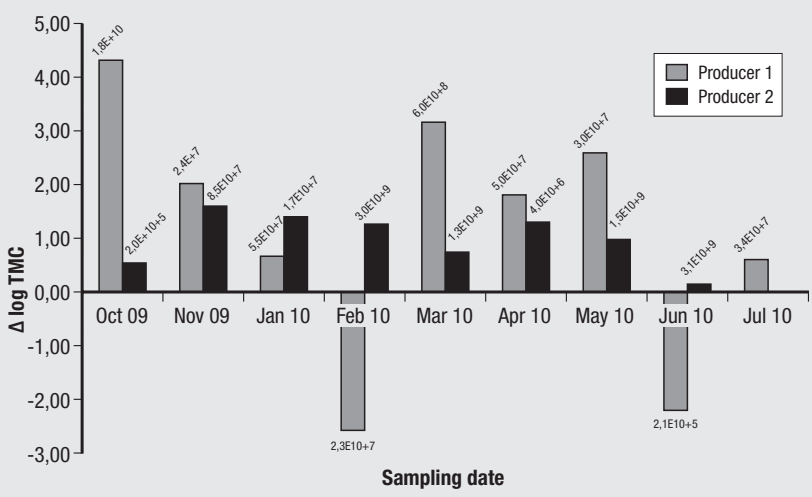

Fig. 2 | *Microbial load reduction in log units (log TMC of raw material – log TMC of RTE vegetable) at producer 1 (light bars) and producer 2 (dark bars). The labels on the bars of the histograms are the TMCs of the RMs.*

Table 2 | *Results of the microbiological analyses of W1, W2, W3 water samples and RM and RTE vegetable samples from producer 2*

Parameter (units)	Producer 2				
	W1 Average [range]	**W2 Average [range]**	**W3 Average [range]**	**RM Average [range]**	**RTE Average [range]**
TBC a 22 °C (cfu/ml)	2.1×10^2 [0.0 -1.6 x 10^3]	6.0 [0.0 - 1.9 x 10^1]	3.8×10^6 [0.0 - 3.0 x 10^7]	n.d.	n.d.
TMC a 30 °C (cfu/g)	n.d.	n.d.	n.d.	4.3×10^8 [2.0 x 105- 3.0 x10^9]	2.8×10^8 [5.3x104 - 2.1x10^9]
Escherichia coli (cfu/100 mL - ufc/g)	< 1	< 1	< 1	< 10	< 10
Enterococci (cfu/100 mL)	n.d.	< 1	n.d.	n.d.	n.d.
Salmonella spp (Presence/Absence in 2 L or 25 g)	Abs	Abs	Abs	Abs	Abs
L. monocytogenes (Presence/Absence in 2 L or 25 g)	Abs	Abs	Abs	Abs	Abs
Campylobacter spp (Presence/Absence in 2 L or 25 g)	Abs	Abs	Abs	Abs	Abs

RM: raw material; RTE: ready-to-eat. n.d.: not determined.

Table 3 | *Averages and ranges of the results of the physico-chemical analyses of W1, W2, and W3 water samples from producer 1 and producer 2*

Average [range]	Producer 1			Producer 2		
	W1	**W2**	**W3**	**W1**	**W2**	**W3**
pH	7.15 [6.93-7.5]	6.95 [6.78-7.02]	6.96 [6.50-6.96]	7.05 [6.87-7.29]	7.14 [7.00-7.14]	7.41 [7.00-7.85]
Conductivity (µS/cm)	1047 [489-1802]	1215 [700-2070]	1335 [593-2080]	397 [295-483]	1388 [694-2100]	1640 [760-2130]
Fixed residue at 180 °C (mg/L)	915 [572-1892]	806 [368-1600]	918 [762-1457]	162 [82-227]	849 [127-1182]	941 [666-1077]
N (Ammonium)(1) (mg/L)	0.21 [0.01-0.94]	0.08 [0.04-0.14]	0.12 [0.05-0.20]	0.02 [0.01-0.04]	0.15 [0.01-0.59]	0.16 [0.02-0.70]
N (Nitrate)(1) (mg/L)	1.53 [0.05-4.3]	0.10 [< 0.01-0.16]	1.38 [< 0.01-2.71]	5.52 [3.50-6.50]	0.94 [0.25-1.44]	0.71 [0.40-1.25]
N (Nitrite)(1) (mg/L)	0.02 [< 0.01-0.04]	0.01 [< 0.01-0.01]	0.01 [< 0.01-0.01]	< 0.01	< 0.01	< 0.01
Chloride (mg/L)	211.2 [40.8-398.1]	262.8 [85.4-500.6]	266.5 [83.3-496.3]	50.2 [17.5-73.4]	557.2 [170.16-961.0]	487.3 [226.2-858]
Sodium (mg/L)	111.2 [67.0-219.7]	135.5 [55.0-245]	121.9 [56.0-239]	9.4 [7.1-17.7]	192.9 [148-242]	216.3 [132-267]
Potassium (mg/L)	17.4 [8.0-27.2]	15.5 [12.2-21.3]	17.5 [13.1-21.7]	5.0 [4.0-7.1]	28.1 [24.1-30.2]	29.1 [26.6-32.5]
Iron (mg/L)	0.24 [0.08-0.46]	0.22 [0.07-0.41]	0.23 [0.07-0.61]	0.13 [< 0.02-0.48]	0.19 [0.02-0.56]	0.12 [0.03-0.20]
Manganese (mg/L)	0.05 [0.01-0.15]	0.04 [0.01-0.15]	0.03 [0.01-0.04]	0.01 [< 0.02-0.06]	0.20 [< 0.02-1.45]	0.19 [< 0.02-1.42]

(1) Ammonium, nitrate, and nitrite are expressed as mg/L of nitrogen in each form.

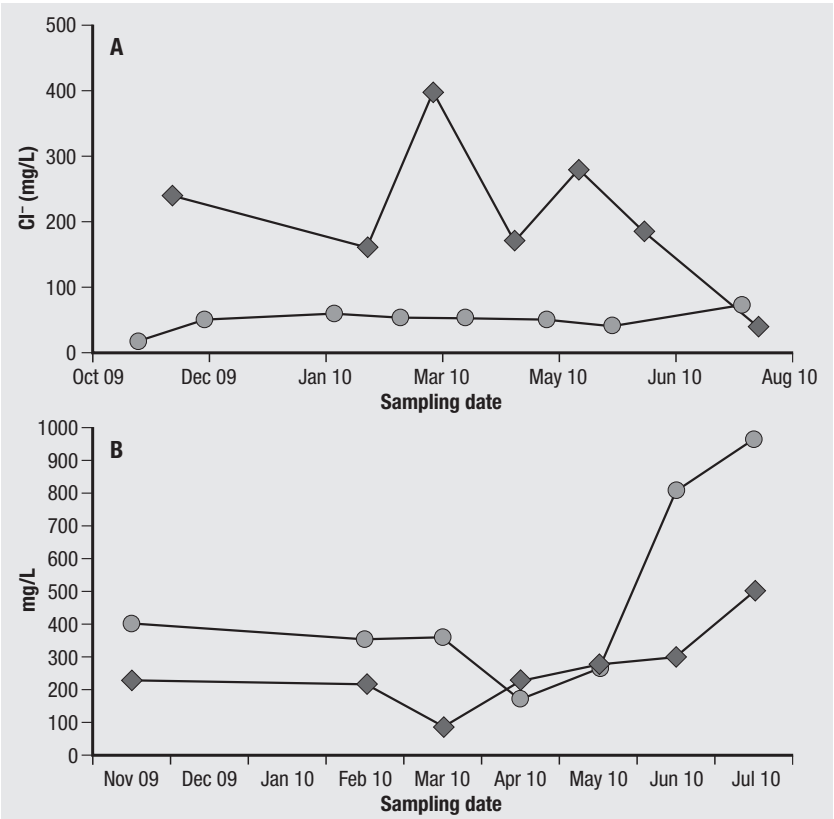

Fig. 3 | *Concentrations of chloride (producer 1: ◆; producer 2: ●) as a function of date of sampling in (A) irrigation water W1 and (B) water W2 feeding the processing line.*

Chemical characterization of irrigation and rinsing water

Table 3 summarizes averages and ranges of the results of the physico-chemical analyses of W1, W2, and W3 from both producers. The average chloride concentration in canal water W1 from producer 1 is higher than in W1 from producer 2's well. In fact, chloride concentrations *(Figure 3a)* in canal W1 from producer 1 are higher than the 250 mg/L limit Legislative Decree 152/2006. [53] in two out of seven determinations, with one value as high as 398 mg/L, while in W1 from producer 2 (well), chloride concentrations are less variable, and well below 250 mg/L. Nitrogen in the nitrite and nitrate forms *(Table 3)* in W1 from both producers are well below the limits set for potable water [55] of 0.5 mg/L and 50 mg/L, respectively. Fixed residue is higher in producer 1's W1 (canal water), than in W1 from producer 2's well.

As for W2 water samples, the outcomes of the chemical analyses *(Table 3)* are similar among the two producers, with small differences in pH (slightly more acidic at producer 1 than at producer 2) and ammonium. Chloride concentrations are consistently lower in W2 from producer 1 than from producer 2. In fact, all but one chloride concentration values in W2 from producer 2 are higher than 250 mg/L, *i.e.* the limit value for potable water [54], with a steep and continuous increase between April and July *(Figure 3b)*.

A comparison between microbial load reduction at producer 2 *(Figure 2)* and chloride concentration trend in W2 from the same producer *(Figure 3)* does not provide any evidence for correlating disinfection efficacy and chloride concentration [48, 49].

No difference is worth noting between W2 and W3 with respect to the physico-chemical parameters determined *(Table 3)*. In fact, W3 consists merely of W2 that has come into contact with the produce in the washing tanks, so that no difference was expected between these samples. Other chemical parameters may be considered (*i.e.* pesticides, fertilizers, disinfection by-products), the analysis of which is beyond the scope of this study, and deserves further investigation.

DISCUSSION

The frame of reference provided by current legislation and codes of practice for evaluating the quality of irrigation water is quite poor. Current Italian legislation [53, 54] focuses on the need for optimizing resources by utilizing, for irrigation purposes, treated wastewater, thus setting a mere "worst case scenario" with respect to water quality standards. It is worth noting that the chemical characteristics of water in the area where both producers are located is influenced by several factors, *i.e.* proximity to the marine coast, presence of underground thermal water, and seasonal trends in rainwater contribution (dry Spring-Summer season). The main chemical parameter that reflects such peculiarities is chloride concentration, which is considered of relevance to chlorination efficacy.

Chloride concentration in irrigation water from producer 2 (drawn from a well) was below the limits, and its microbiological quality was also good. Notwithstanding the differences in the quality of irrigation water between the two producers, pathogens were absent in the RMs from both, and TMCs were in the same range.

As for the different industrial procedures adopted by the two producers, the overall outcome is quite similar in terms of TMC. In fact, TMC ranges of RMs and of RTE products from both producers are in the same order of magnitude. No conclusions can be drawn on pathogens, since they were absent in all vegetable samples (raw material and RTE), as well as in all processing water samples. However, when looking closer at the production flow, rather than at overall performances, two observations can be made. First of all, microbial load reduction in the vegetables is not reproducible from batch to batch with the minimal process, while a more reproducible reduction is observed with the multiple washing process. Furthermore, there is some indication of microbial build-up in the washing apparatus and/or cross-contamination between batches, particularly at the producer adopting the minimal process. In conclusion, if we take TMC reduction as an indicator of the efficacy of the process, water chlorination at a low level (0.2-1 ppm) is adequate for RTE vegetables production, provided that the industrial process encompasses a sufficient number of washing cycles, with a sufficiently long contact times with chlorinated water. Furthermore, periodical sanitization of the washing apparatus is advisable in order to contrast microbial build-up and cross-contamination between batches. As for those chemical parameters that may be relevant for disinfection efficacy, both processes appeared insensitive to variations in chloride concentration.

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Conflict of interest statement

Authors report no financial relationships with commercial interests.

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