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ORIGINAL PAPER

Antibacterial and antifungal activity of water-soluble extracts from Mozzarella, Gouda, Swiss, and Cheddar commercial cheeses produced in Canada

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Abstract The aim of this study was to evaluate the antibacterial and antifungal activities of water-soluble extracts (WSEs) from different types of cheeses against several food-borne pathogens. A total of five commercial cheeses manufactured in Canada were selected namely Mozzarella, Gouda, Swiss, and old and medium Cheddar. WSEs were ultrafiltrated through 10 kDa cutoff membranes and desalted using Sep-Pak cleanup column. Resulting peptide fractions were subject to physico-chemical characterization and assessment for their antimicrobial activity against bacteria (*Listeria ivanovii*, *Listeria monocytogenes*, *Escherichia coli* MC4100, and *E. coli* O157:H7) and filamentous fungi (*Aspergillus*, *Mucor*, *Fusarium*, and *Penicillium*). Mozzarella and Gouda WSEs were the most active and inhibited with *L. monocytogenes* significantly, with respective reductions of 3.83±0.15 and 2.93± 0.33 log. After desalting and organic acids removal, Mozzarella and Gouda WSEs produced 3-log reductions of *L. ivanovii* and *E. coli* MC4100, with minimal inhibitory concentration (MIC) values ranging 8.5–17 $mg.mL^{-1}$. At a concentration of 34 $mg.mL^{-1}$, all cheese peptidic WSEs induced a delay in spore germination. All WSEs were equally active against *Fusarium* sp., with a minimal concentration of 17 $mg.mL^{-1}$. Gouda, Mozzarella, and medium Cheddar WSE were the strongest inhibitors in the case of *Aspergillus versicolor* and *Mucor racemosus* (17 $mg.mL^{-1}$), whereas these spores were less sensitive to old Cheddar and Swiss WSE (34 $mg.mL^{-1}$). This study demonstrates that peptidic WSEs of commercial cheeses manufactured in Canada exhibit antibacterial and antifungal activities, which may offer a promising alternative for purposes of food preservation.

Keywords Cheese · Antibacterial activity · Antifungal activity · Water-soluble peptidic extract

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1 Introduction

Dairy proteins are a natural source of bioactive peptides encrypted within primary amino acid sequences and released upon enzymatic digestion during food processing or gastrointestinal transit (Möller et al. 2008). A growing number of such peptides are being identified in dairy protein hydrolysates and fermented dairy products and shown to have opioid, immunomodulatory, antimicrobial, or antihypertensive properties (El-Salam and El-Shibiny 2012). Particularly, dairy antimicrobial peptides (AMP) are promising alternative to satisfy consumer demands for safe, ready-to-eat, extended shelf-life, fresh-tasting, and minimally processed foods without chemical additives (Théolier et al. 2014a). Recently, MilkAMP, a database dedicated to dairy AMPs was created containing valuable microbiological and physicochemical information about dairy AMPs (Théolier et al. 2014b). Besides, the history of the consumption and the "generally recognize as safe" status of milk proteins is an asset and satisfies both consumers and industry.

Cheeses are a rich source of bioactive peptides, as they possess high protein amounts that can be hydrolysed by different enzymes existing endogenously in milk or released by present lactic acid bacteria (LAB) (Hernández-Ledesma et al. 2011). During ripening and maturation, starter and nonstarter bacteria can influence widely cheese quality by altering rheological characteristics or microbial composition, and thus can enrich cheeses with bioactive peptides (Settanni and Moschetti 2010; Jardin et al. 2012). As several factors could affect the intensity of proteolysis, different cheeses are likely to vary in term of peptide composition and bioactivity (Fox and McSweeney 2004).

There are few studies of the antimicrobial effects of cheeses and we have little means of predicting the impact of antimicrobial fractions produced by bacteria in cheeses or by enzymatic hydrolysis of dairy proteins (Losito et al. 2006; Rizzello et al. 2005). Rizzello et al. (2005) have demonstrated that water-soluble extracts (WSEs) of Italian cheeses contain several AMPs with inhibitory activity against Gram-positive and Gram-negative bacterial species, including spoilage and food-borne pathogenic bacteria. Further evidence for the release of short AMPs by fermentation of milk caseins by LAB has been provided by Hayes et al. (2006). More recently, several antilisterial peptides were identified in Asiago d'Allevo and Emmental de Savoie cheeses (Lignitto et al. 2012; Nguyen Thi et al. 2014). Similarly, Meira et al. (2012) have evaluated WSE of ovine cheeses (Feta, Roquefort, Pecorino, Sardo and Cerrillano) produced in Uruguay and Brazil for their antioxidative, antimicrobial, and ACE-inhibitory properties. None of these WSE show antimicrobial activity. Thus, composition and levels of antimicrobial compounds in different type of cheeses are likely to vary considerably.

The aim of the present study was to evaluate the antibacterial and antifungal activities of WSEs from different types of cheeses against several food-borne pathogens. A total of five commercial cheeses manufactured in Canada were selected namely Mozzarella, Gouda, Swiss, and old and medium Cheddar. WSEs were fractioned, biochemically characterized, and assessed for antimicrobial activity against bacteria (*Listeria ivanovii*, *Listeria monocytogenes*, *Escherichia coli* MC4100, and *E. coli* O157:H7) and filamentous fungi (*Aspergillus*, *Mucor*, *Fusarium*, and *Penicillium*).

2 Material and methods

2.1 Preparation of water-soluble extracts (WSEs)

Five cheese varieties, namely Swiss (Selection Eco; Metro, Toronto, ON, Canada), Gouda (Selection Eco), hard Mozzarella (Saputo, Montréal, QC, Canada) and old and medium Cheddar (Cracker Barrel; Kraft, Don Mills, ON, Canada) were purchased from a local supermarket. The procedure described by Kuchroo and Fox (1982) was followed with some modifications. Samples (300 g) were grated and dispersed in distilled water (1/3, *w*/*w*) using an Ultra-Turrax (Janke & Kunkel, Staufen, Germany) for 10 min at room temperature. Cheese homogenates were then stirred for 1 h at 40 °C. The resulting suspensions were centrifuged at 3,000× *g* for 30 min at 4 °C then filtered on Whatman no 2 paper. The supernatants were subjected to ultrafiltration (10 kDa cut-off; Millipore, Bedford, MA, USA) and the resulting permeate was lyophilized. The lyophilized material was dissolved in sterile water, filtered on 0.22 μm membrane (Sigma Chemical Co., St. Louis, MO, USA), concentrated and stored at −20 °C until testing. Triplicate independent batches/lots of each commercially produced cheese were used for preparation of WSEs. For salts and lactic acid removal, WSEs were individually cleaned by passage over a Sep-Pak C18 cleanup column (Waters Corporation, Millford, MA, USA).

2.2 Chemical analysis of the extracts

The pH of WSEs was measured using an IQ150 pH meter (IQ Scientific Instruments Inc, Carlsbad, CA, USA). Dry extract and moisture were determined using a drying oven (100 °C) and mineral content was determined by ashing the dry extract at 550 °C. Lipid content was determined using the method of Bligh and Dyer (1959). Carbohydrates were measured using the colorimetric method of Dubois et al. (1956) and water-soluble nitrogen concentrations were determined using a Leco FP-528 nitrogen analyzer (Leco Corp., St. Joseph, MI, USA). Each test was done in triplicate. Concentrations of lactic, acetic, propionic, butyric, iso-butyric, valeric, and isovaleric acids in WSEs were determined using a Waters chromatograph connected to a differential refractometer Waters 410 and equipped with an ION-300 ion exclusion column (300×7.8 mm) (Interaction Chemicals, Mountain View, CA, USA). Sulfuric acid (H_2SO_4, 5 mM) was used as mobile phase and flowed at 0.4 ml.min^{-1} and 37 °C.

2.3 Bacterial strains, molds, and culture conditions

L. ivanovii HPB28, *L. monocytogenes* Scott A3, *E. coli* MC4100, *E. coli* O157:H7 ATCC 35150 as bacteria and *Fusarium* sp. LMA-590, *Mucor racemosus* LMA-425, *Aspergillus versicolor* LMA-370, *Penicillium camemberti* ATCC 4845, and *Penicillium commune* LMA-212 as fungus were used as target microorganisms for the microbial growth inhibition assays. The bacteria were all grown in Tryptic Soy Broth with 0.6% (*w*/*v*) yeast extract (TSB; Difco Laboratories, Sparks, MD, USA). *L. ivanovii* and *L. monocytogenes* were incubated aerobically at 30 °C and *E. coli* strains were incubated aerobically at 37 °C. All the fungi were grown aerobically on potato dextrose agar (PDA; Difco) and were incubated at 25 °C for 1 week.

2.4 Determination of antimicrobial activity

Minimal inhibitory concentrations were determined by critical dilution method. This bioassay was done using polystyrene micro-assay plates (96-well Microtest, Becton Dickinson Labware, Sparks, MD, USA) as described by Hammami et al. (2009). Briefly, microplates loaded with twofold serial dilutions of WSE (starting at 34 $mg.mL^{-1}$) in TSB were seeded with approximately 1×10^4 CFU per well using log-phase culture diluted in TSB to $0.5–1.0\times10^6$ $CFU.mL^{-1}$. Microplates were incubated at 30 °C (or 37 °C for *E. coli*) for 24 h and absorbance at 595 nm was measured hourly using an Infinite® F200 PRO photometer (Tecan US inc., Durham, NC, USA). Minimal inhibitory concentration (MIC) was expressed in milligram per milliliter and corresponds to the lowest concentration that limited the development of turbidity after 16 to 20 h.

Microbial counts were determined after 24 h for the highest concentration (34 $mg.mL^{-1}$) of WSE. For fungi, TSB was replaced by PDB and wells were seeded with approximately 1×10^4 $spores.mL^{-1}$ prior to incubation at 25 °C for 48 h. Dichloran Rose Bengale Chloramphenicol Agar (DRBC agar, EMD Chemicals, Gibbstown, NJ, USA) was used for viability count after 48 h.

2.5 Statistical analysis

All values are means of triplicate (mean ± SD). Bacterial cell counts were log 10-transformed and tested for normality using the Shapiro-Wilk method. Data were subjected to ANOVA using the GLM procedure of SAS (SAS Institute, Inc., Cary, NC, USA). A multiple comparison test (LSD) was used to test the significant differences between the treatment means ($P\leq0.01$).

3 Results

3.1 Water-soluble extract production and physicochemical characterization

Table 1 shows the starter cultures, curdling temperatures, and ripening times involved in the production of Gouda, Swiss, Mozzarella, and medium and old Cheddar cheeses. These parameters have a major impact on the quality and the quantity of fat, carbohydrate, and protein in cheeses (Fox and McSweeney 2004). The WSE preparation procedure was designed to recover and concentrate nitrogenous compounds smaller than 10 kDa. The analysis of the different extracts is shown in Table 2. Before purification, they were composed mainly of nitrogenous compounds (52.04±2.13%) and mineral salts (40.62±2.25%), while fat and carbohydrates were present at much lower concentrations (less than 4%). The natural organic acids in WSE are lactic and propionic acid. Lactic acid was present in amount between 0.49 and 0.06 M in all cheeses WSE with minimal and maximal values in old Cheddar and Swiss, respectively. Propionic acid was identified in Swiss-type cheese only at 0.32 M. Cheese WSEs presented similar pH values which ranged from 5.23 to 5.82 (Table 1). Lactic and propionic acid were active against all the tested strains with MICs values of 0.06 and 0.12 M, respectively. Noticeably, no organic acids were detected in different peptidic

Table 1 Characteristics of the cheese varieties tested in this study

Cheese	Starter[a]	Curdling temperature[a] (°C)	pH[b]	% Moisture[c]
Medium Cheddar	*Lactococcus lactis* ssp. *lactis*, *L. lactis* ssp. *cremoris* and *Streptococcus salivarius* spp. *thermophilus* (Bissonnette et al. 2000; Sheehan et al. 2005)	38	5.23	36
Old Cheddar	*L. lactis* ssp. *lactis*, *L. lactis* ssp. *cremoris* and *S. salivarius* spp. *thermophilus* (Bissonnette et al. 2000)	38	5.25	36
Gouda	*L. lactis ssp. lactis* and *L. lactis ssp. cremoris*, *L. lactis ssp. lactis biovar diacetylactis* and *Leuconostoc spp.* (Ayad et al. 2001; Exterkate and Alting 1995)	35–38	5.47	41
Swiss	*Lactobacillus helveticus*, *Streptococcus thermophilus* (ratio 1:1) and *Propionibacterium freudenreichii* (Reinbold 1972; Jenkins et al. 2002)	51–58	5.82	40
Mozzarella	*S. thermophilus, L. helveticus* or *L. delbrueckii subsp. bulgaricus* (ratio 2:1 or 3:1) (Kindstedt and Fox 1993)	40–44	5.31	42

[a] Theoretical data

[b] Experimental values

[c] According manufacturers

extracts after cleanup with Sep-Pak column, and mineral salts concentration was dropped (about 4%).

3.2 Antibacterial activity

Growth inhibitions in the presence of each WSE are summarized in Table 3. In the absence of WSEs, bacterial counts after 24 h were 10.70±0.02 log CFU.mL^{-1} for *L. ivanovii*, 9.56±0.12 log CFU.mL^{-1} for *L. monocytogenes*, 10.43±0.05 log CFU.mL^{-1} for *E. coli* MC4100, and 10.00±0.01 log CFU.mL^{-1} for *E. coli* O157:H7 (data not shown). Before purification, in the presence of 34 mg.mL^{-1} of WSE, *L. ivanovii* counts were decreased by more than four log cycles for medium Cheddar, Gouda, and Mozzarella and about 1 log CFU.mL^{-1} for old Cheddar and Swiss. Although Gouda and Mozzarella WSE inhibited *L. monocytogenes* significantly, with respective reductions of 2.93±0.33 and 3.83±0.15 log, extracts of the other cheeses did not inhibit this strain significantly (less than 1-log reduction). Reductions of almost four log cycles were obtained for *E. coli* MC4100 in the presence of Gouda and Mozzarella WSE and close to 1.5 log cycles in the presence of Cheddar and Swiss WSE. *E. coli* O157:H7 was also inhibited by Mozzarella WSE (reduction of 2 log CFU.mL^{-1}) and to a lesser extent by medium Cheddar and Gouda (1.42±0.24 and 1.35±0.25 log CFU.mL^{-1}). With WSE from Old Cheddar and Swiss cheese, a 1-log reduction of *L. ivanovii* and *E. coli* MC4100 was obtained, while no significant reduction (less than 1-log) was observed with

Table 2 Chemical composition of the water-soluble extracts of five cheeses varieties

		Concentration ($mg.mL^{-1}$)				
	Cheeses	Mozzarella	Gouda	Swiss[a]	Old Cheddar[a]	Medium Cheddar[a]
Before Sep-Pak purification	Dry content	147.59±2.42	155.40±8.08	176.06±0.00	170.63±2.41	184.48±11.19
	Mineral salts (ash)	55.13±0.50	57.95±0.67	62.37±0.00	65.12±0.54	58.20±0.37
	Fat	6.35±0.71	5.21±0.13	3.36±0.15	6.42±0.13	5.43±0.61
	Carbohydrate	9.37±2.65	4.13±2.44	1.77±0.18	3.12±1.47	8.87±5.90
	Water-soluble nitrogen	68.35±1.45	68.07±2.56	77.07±0.83	88.00±1.92	81.28±0.39
	Total (ash+fat+CHO+N)	139.21	135.37	144.57	162.65	153.78
	% identified dry matter	94.32	87.11	82.11	95.33	83.36
	Lactic acid (M)	0.18±0.02	0.32±0.09	0.29±0.01	0.40±0.05	0.06±0.00
	Propionic acid (M)	–	–	0.32±0.07	–	–
After Sep-Pak purification	Dry content	82.78±1.02	78.25±6.65			
	Mineral salts (ash)	4.26±0.21	3.94±0.31			
	Fat	2.25±1.32	1.78±1.63			
	Carbohydrate	2.56±1.05	1.13±0.44			
	Water-soluble nitrogen	67.85±0.63	67.99±3.12			
	Total (ash+fat+CHO+N)	76.92	74.84			
	% identified dry matter	92.92	95.64			

Values presented are the means of three replicates ± standard error of the mean

[a] Purification was not realized because of a lack of activity before organic acids and salts removal

Table 3 Logarithmic reductions of the growth of *L. ivanovii*, *L. monocytogenes*, *E. coli* MC4100, and *E. coli* O157:H7 in the presence of 34 mg.mL^{-1} of each water-soluble extract

Bacterial strains		Log NC/NI[a]				
		Mozzarella	Gouda	Swiss	Old cheddar	Medium Cheddar
Before purification	*L. ivanovii* HPB28	4.66±0.28*	4.46±0.59*	1.42±0.04*	1.19±0.24*	3.05±0.22*
	L. monocytogenes Scott A3	3.83±0.15*	2.93±0.33*	0.52±0.01	0.68±0.64	0.59±0.50
	E. coli MC4100	3.67±0.33*	3.93±0.50*	1.59±0.24*	1.49±0.08*	1.40±0.06*
	E. coli O157:H7 ATCC 35150	2.01±0.11*	1.35±0.25	0.43±0.29	0.77±0.33	1.42±0.24*
After purification	*L. ivanovii* HPB28	3.36±0.52*	3.05±0.22*			
	L. monocytogenes Scott A3	0.31±0.11	0.02±0.13			
	E. coli MC4100	2.88±0.18*	3.02±0.51*			
	E. coli O157:H7 ATCC 35150	1.08±0.03*	NI			

Values (mean ± SD, n=3) are significantly different (* P<0.01) from the mean for control

NI no inhibition

[a] NC and NI are the 24 h increase in the number of colony-forming units in wells without and with peptidic extract, respectively

L. monocytogenes and *E. coli* O157:H7. With medium Cheddar WSE, a 4-log reduction of *L. ivanovii* was obtained while only a moderate or weak inhibition of *E. coli* and *L. monocytogenes* was obtained. It is noteworthy that Gouda and Mozzarella WSE strongly affected cell viability, with Mozzarella WSE being the most active against *E. coli* O157:H7 (1-log versus 2-log reduction).

After removal of organic acids and salts, Mozzarella and Gouda peptidic extracts always exhibited a significant bacterial activity (Fig. 1). The curves obtained illustrate a dose-dependent inhibition. MICs values are presented in Table 4. *L. ivanovii* was inhibited by 8.50 mg.mL^{-1} for Gouda and Mozzarella. *L. monocytogenes* was the less sensitive among the four bacterial strains, with no activity of any of the five extracts. *E. coli* MC4100 showed MIC values similar to those of *L. ivanovii* for Mozzarella extract although for Gouda extract the MIC was 17 mg.mL^{-1}. In contrast, *E. coli* O157:H7 presents weak sensitivity and its partial inhibition requiring at least 34 mg.mL^{-1} of Mozzarella or Gouda WSE. This time, in the presence of 34 mg.mL^{-1} of peptidic extract, reductions by more than 3 log CFU.mL^{-1} were obtained for Gouda and Mozzarella against *L. ivanovii*. Gouda and Mozzarella WSE demonstrated activity against *L. monocytogenes* although they did not inhibit this strain significantly (less than 1-log reduction). *E. coli* MC4100 counts were decreased by almost three log cycles for Mozzarella and Gouda. *E. coli* O157:H7 was also inhibited by Mozzarella peptidic extract (reduction of 1 log CFU.mL^{-1}) but not by Gouda (no reduction). In contrast, peptidic

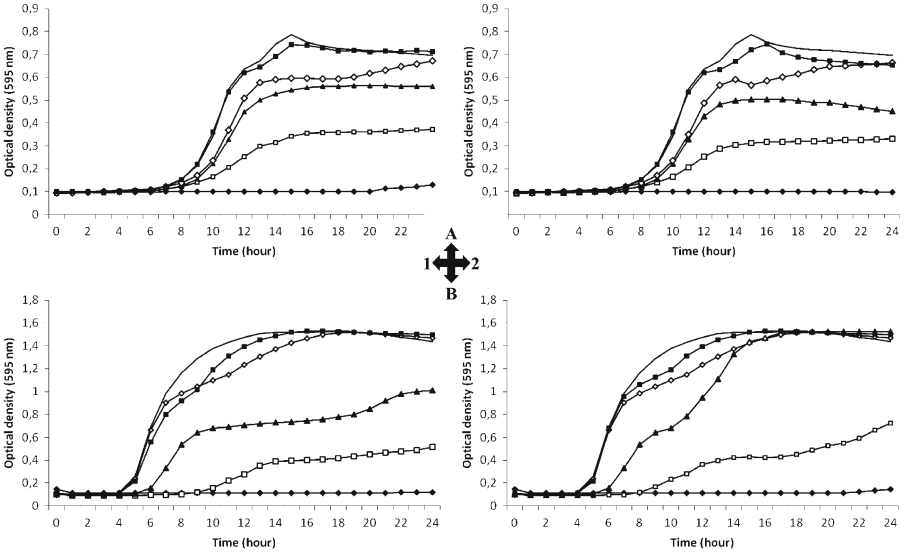

Fig. 1 Growth of *Listeria ivanovii* (**a**) and *Escherichia coli* (**b**) in the presence of WSE of Mozzarella (*1*) and Gouda (*2*) in tryptic soy broth. Concentrations (mg/mL) of extract were 34 (*solid diamond*), 17 (*open square*), 8.5 (*solid triangle*), 4.25 (*open diamond*), 2.13 (*solid square*), and 0 (none)

extracts of Swiss and Cheddar cheeses did not exhibit antibacterial activity (data not shown).

3.3 Antifungal activity

The same purified WSEs were tested for antifungal activity using agar plates. Hyphal growth was not inhibited in the presence of cheese WSEs (data not shown). However, after organic acids and salts removal, at a concentration of 34 mg.mL^{-1}, all cheese WSEs induced a delay in spore germination, as shown in Table 5. All WSEs were equally active against *Fusarium* sp., with a minimal concentration of 17 mg.mL^{-1}. Gouda, Mozzarella, and medium Cheddar WSE were the strongest inhibitors in the case of *A. versicolor* and *M. racemosus* (17 mg.mL^{-1}), whereas these spores were less sensitive to old Cheddar and Swiss WSE (34 mg.mL^{-1}). The lowest concentration values causing delay in germination were obtained against *Penicillium* strains, with

Table 4 Minimal inhibitory concentrations (MIC) of water-soluble extracts of cheeses (after purification) in the case of food-borne bacteria

Bacterial strain	Minimal inhibitory concentration (mg.mL^{-1})	
	Mozzarella	Gouda
L. ivanovii HPB28	8.50	8.50
L. monocytogenes Scott A3	>34	>34
E. coli MC4100	8.50	17
E. coli O157:H7 ATCC 35150	34	34

Table 5 Minimal inhibitory concentrations of water-soluble extracts of cheeses (after purification) causing a delay in spore germination of food-borne molds

Fungal strain	Minimal inhibitory concentration ($mg.mL^{-1}$)				
	Old Cheddar	Swiss	Medium Cheddar	Gouda	Mozzarella
A. versicolor LMA-370	34	34	17	17	17
M. racemosus LMA-425	34	34	17	17	17
Fusarium sp. LMA-590	17	17	17	17	17
P. camemberti ATCC 4845	34	8.5	34	8.5	8.5
P. commune LMA-212	8.5	8.5	34	8.5	8.5

Swiss, Gouda, and Mozzarella WSE being the most active (8.50 $mg.mL^{-1}$). *P. commune* was the most sensitive mold to all WSEs except for medium Cheddar, which exhibited a lower inhibitory concentration value (17 $mg.mL^{-1}$) against *A. versicolor*, *M. racemosus*, and *Fusarium* sp. It was observed that WSEs delayed germination significantly but did not affect spore viability (data not shown).

4 Discussion

Compared to other biological activities, the antimicrobial activities of peptides isolated from cheese or other dairy products have not been widely reported. Several authors have isolated and purified AMP from pure whey proteins (Salami et al. 2010; Almaas et al. 2011; Demers-Mathieu et al. 2013; Théolier et al. 2013), but the same procedures performed on whole food matrices have so far failed to corroborate these results. Rizzello et al. (2005) and Losito et al. (2006) demonstrated the presence of casein-derived antimicrobial peptides in Italian cheeses, but no antimicrobial peptides derived from whey proteins were identified. However, these studies suggest that known antibacterial peptides derived from caseins should be found in cheese WSE. In our case, peptide fractions used during this study contain both whey- and casein-derived substances. These peptidic fractions also contain only small amounts of mineral salts (ash) and no organic acids. However, antimicrobial tests performed with salt concentrations at 10 $mg.mL^{-1}$ did not show antimicrobial activity against the tested strains (data not shown). Thus, the observed antimicrobial activity was likely due to peptides. The cheeses in this work were all made from cow milk using similar manufacturing processes. So, differences between them stem primarily from the amount and type of starter culture used and ripening time as previously observed (Lignitto et al. 2012; Nguyen Thi et al. 2014).

In comparison with previous works, MIC values of active extracts were low, indicating a high specific antimicrobial activity. Rizzello et al. (2005) did not see any activity for WSE in the range of concentrations up to 60 $mg.mL^{-1}$. Fractionating of these WSEs revealed antimicrobial activities with MIC values lower than 200 $\mu g.mL^{-1}$ (Rizzello et al. 2005). Further peptide purification led to higher antibacterial activities with MIC values a thousand times lower. In the present study, peptidic fractions prepared from both Mozzarella and Gouda manufactured in Canada were active at lower concentration

(34 mg.mL^{-1}) and may therefore be considered more potent. These two extracts presented strain dependent activity and reached MIC values against *E. coli* strains and *L. ivanovii* HPB28. These results suggest different mechanisms of action against the tested strains. This can be due to the production of various antimicrobial substances (e.g., lactic acid, bacteriocins) by fermented bacteria and/or because of the inhibitory action of milk-derived antimicrobial peptides. However, in accordance with the Canadian regulation, no identified bacteriocins are present in cheeses. Besides, this study report on antimicrobial activity against Gram-negative bacteria, which are usually resistant to bacteriocins from dairy microorganisms. Thus, the observed activity was probably due to a range of different peptide sequences. In our study, all the tested cheeses were made from cow milk and it is likely that the same peptides may be present in other WSE, such as observed by Losito et al. (2006). Further investigations are currently in progress in order to determine sequences and mode of action of identified peptides.

To our knowledge, no antifungal peptides derived from cheese WSE have been reported in the literature. However, hydrolysis of some whey proteins has been shown to release peptides with both antibacterial and antifungal properties (Bellamy et al. 1994; Lahov and Regelson 1996; van der Kraan et al. 2005; Wakabayashi et al. 1996). Furthermore, pure lactoferrin and lactoferricin B have been shown to inhibit filamentous fungi (Bellamy et al. 1994; Wakabayashi et al. 1996). Working with crude fractions gives a less clear picture of antimicrobial activity than pure peptides, since peptide mixtures may also stimulate bacterial cell growth. For example, using WSE fractionated with a 5-kDa cutoff membrane, Pritchard et al. (2010) demonstrated very different activities in the permeate and retentate, one antibacterial and the other increasing the growth of bacterial cells. Rizzello et al. (2005) showed earlier that although a total of five Italian cheeses contained antibacterial peptides, only two crude fractions were active among the nine tested. The absence of measurable activity in crude extracts therefore does not negate the presence of antimicrobial peptides in those fractions.

This paper is the first report of antifungal activity in water-soluble extracts of cheeses. All five WSEs delayed the germination of spores of food-borne molds to a significant degree. Cheese WSE may offer a promising alternative for purposes of food preservation. Further studies in progress are aiming to identify and examine the mode of action of the active agents in these extracts within the scope of challenge tests in foodstuffs.

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