Effect of added salt and increase in ionic strength on skim milk electroacidification performances

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Summary. Bipolar-membrane electroacidification (BMEA) technology, which uses the property of bipolar membranes to split water and the demineralization action of cation-exchange membranes (CEM), was tested for the production of acid casein. BMEA has numerous advantages in comparison with conventional isoelectric precipitation processes of proteins used in the dairy industry. BMEA uses electricity to generate the desired ionic species to acidify the treated solutions. The process can be precisely controlled, as electro-acidification rate is regulated by the effective current density in the cell. Water dissociation at the bipolar membrane interface is continuous and avoids local excess of acid. In-situ generation of dangerous chemicals (acids and bases) reduces the risks associated with the handling, transportation, use and elimination of these products. The aim of this study was to evaluate the performance of BMEA in different conditions of added ionic strength ($\mu_{added} = 0$, 0·25, 0·5 and 1·0 M) and added salt ($CaCl_2$, NaCl and KCl).The combination of KCl and $\mu_{added} = 0{\cdot}5$ M gave the best results with a 45% decrease in energy consumption. The increased energy efficiency was the result of a decrease in the anode/cathode voltage difference. This was due to an increase of conductivity, produced by addition of salt, necessary to compensate for the lack of sufficiently mobile ions in the skim milk. However, the addition of salts, irrespective of type or ionic strength, increased the required operation time. The protein profile of isolates were similar under all experimental conditions, except at 1·0 M-$CaCl_2$.

Keywords: Bipolar-membrane electroacidification, cation valence, acid casein, ionic strength, milk.

Two main types of casein are usually produced by the dairy industry: rennet and acid casein. However, there are several disadvantages associated with these procedures. For example, the production of large volumes of chemical effluents, due to the addition of bases and acids during treatments, together with the inherent risks

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linked to handling, stocking and transportation of concentrated bases and acids. In the case of acid casein production by fermentation, it is necessary to use mixed starter culture and to change them regularly in order to avoid contamination by phage. Furthermore, during rennet casein production, κ-casein is denatured by formation of caseinomacropeptide and para-κ-casein (Cheftel *et al.* 1985; Cayot & Lorient, 1998).

A procedure developed for soyabean protein precipitation (Bazinet *et al.* 1997*a*), which was derived from electrodialysis, was tested for the production of acid casein (Bazinet *et al.* 1999). This technology, generically termed bipolar-membrane electrodialysis or more specifically bipolar-membrane electroacidification (BMEA), uses the property of bipolar membranes to split water and the action of cation-exchange membranes (CEM) to demineralize. As in any electrochemical process, the materials to be treated by BMEA must possess a relatively high mineral content to provide a good electrical conductivity in order to reduce the global resistance of the electrodialysis cell. Moreover, Bazinet *et al.* (2000*a*) demonstrated that the electrical efficiency of skim milk electroacidification is decreased, due to a lack of sufficiently mobile ions such as potassium resulting in a loss of electrogenerated H^+. They suggested adding a certain amount of salt to the skim milk in order to obtain a better electrical efficiency.

In milk, electrostatic interactions between amino acid chains and ions in solution give a strong structural stability to protein. There are numerous sites for potential ionic bonds between the casein molecules, and these sites would play a major role in sub-unit interactions (Farrell, 1988). Calcium and phosphate ions are critical for micelle stability. By adjusting the pH of milk to the isoelectric point of casein, the intra- and inter-protein electrostatic interactions are increased, which affects the stability of the mineral phase of the micelle (Cayot & Lorient, 1998). Furthermore, results obtained by Graet & Brulé (1993) have shown the effects of pH and ionic strength on the distribution of mineral salts in milk.

As milk proteins are sensitive to ionic strength and pH changes, the aim of this study was to evaluate the performance of BMEA under different conditions of ionic strength and added salt. BMEA performance was evaluated in terms of electrodialysis cell parameters, energy consumption, percentage of protein that was precipitated, and the protein profile of isolates produced under each set of conditions.

materials and methods

The material used in this study was reconstituted milk (100 g/l) from low temperature spray-dried skim milk powder (Agropur, Granby, Québec, Canada). The average composition of 100 g of the skim milk powder was: 33·9 g total protein (including 7·4 g whey protein), 0·6 g fat, 53·5 g carbohydrates, 8·2 g ash, and 3·8 g moisture.

Electroacidification cell

The module used was an MP type cell (100 cm^2 of effective electrode surface) from ElectroCell Systems AB Co. (Täby, Sweden). This arrangement defines three closed loops, separated by cationic and bipolar membranes (Tokuyama Soda Ltd, Tokyo, Japan) containing the milk solution, a 0·25 M-HCl solution and a solution of 20 g Na_2SO_4/l (Fig. 1). Each closed loop was connected to a separate external reservoir, allowing continuous re-circulation (Bazinet *et al.* 1997*b*).

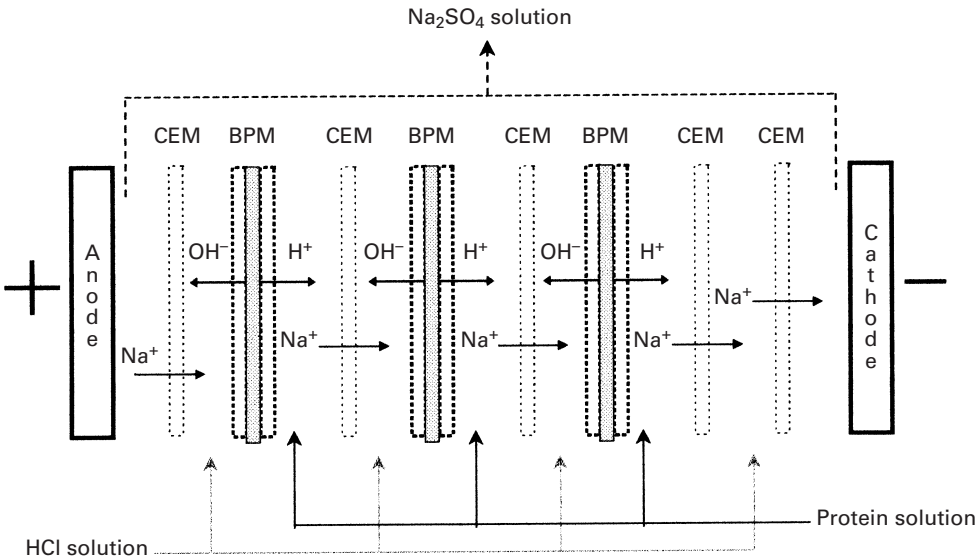

Fig. 1. Bipolar membrane (BPM) and cation exchange membrane (CEM) stack configuration used for the electroacidification of 3 litres reconstituted skim milk.

Table 1. *Effect of ionic strength (μ_{added} 0, 0·25, 0·5 and 1·0 M) and type of salt added ($CaCl_2$, NaCl and KCl) on the time (min) required to decrease pH from 6·6 to the pH where all the casein of the skim milk solution was precipitated by bipolar membrane electroacidification, run at a constant current density of 20 mA/cm²*

(Values are means ± SD, $n = 2$)

	μ_{added} (M)			
Salt	0	0·25	0·5	1·0
$CaCl_2$	46·4 ± 0·8	97·3 ± 3·9	106·6 ± 17·0	134·9 ± 34·3
NaCl	46·4 ± 0·8	93·6 ± 3·5	105·7 ± 10·4	106·9 ± 7·4
KCl	46·4 ± 0·8	80·2 ± 10·5	95·1 ± 3·9	116·9 ± 3·9

Protocol for preparation of sodium caseinate by electroacidification

Electroacidification was carried out in batch process using a constant current density of 20 mA/cm². Electrolyte volumes of 6 litres were used for the Na_2SO_4 and HCl solutions while a 3 litre volume was used for the milk solution. The flow rate of each solution was controlled at 4·5 litres/min. The electroacidification was stopped when all the casein was precipitated (pH_c). As pH_c values varied with the type of added salt and ionic strength, the respective values of pH_c were determined in a preliminary study for each combination of salt and ionic strength.

A 3 × 4 factorial array was set up; three different added salts ($CaCl_2$, NaCl and KCl) were tested, each at four ionic strengths ($\mu_{added} = 0$, 0·25, 0·5 and 1·0 M). Two replicates of each combination of factors were performed in this experiment. Since, the initial pH of the protein solution varied according to the added salt and its concentration and in order to compare the BMEA process on the same basis, the pH of the solution after addition of salt was readjusted to the initial pH of the reconstituted milk at pH 6·6 with 1·0 M-NaOH. However, the pH after addition of salt (without readjustment to pH 6·6) was noted to calculate the relative energy

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consumption of the process. The gain in pH decrease by addition of salt would be of interest in an industrial process.

During each treatment, 3·0 ml samples of the milk solution were taken at pH 6·6, 5·6, 5·2 and thereafter at every 0·2 pH unit decrease until the pH_c value was reached. The time required to reach the pH_c value, the anode/cathode voltage difference, the conductivity and the temperature were recorded as the treatment progressed. The concentration of soluble protein and the protein profiles were determined from the freshly acidified 1·5 ml samples. At the end of each run, about 2·5 litre samples of the pH_c milk solution were taken. These samples were centrifuged at 500 ***g*** and 4 °C for 10 min (Model J2-21 Centrifuge; Beckman Instruments, Palo Alto, CA, USA). The precipitate was washed twice with double-distilled water. The isolate was re-adjusted to pH 6·6 with 1 M-NaOH to produce sodium caseinate, before being lyophilized. The lyophilized isolates were stored at 4 °C prior to analysis.

Product analysis and process evaluation

Soluble protein. The protein concentration was measured using an FP-428 LECO apparatus (LECO, Saint Joseph, MI, USA) as described by Bazinet *et al*. (1999). This combustion method is based on the Dumas nitrogen determination.

Protein profiles. Chromatographic analysis of the lyophilized protein isolate and skim milk supernatant sampled at different pH during BMEA was performed by reversed-phase HPLC as described by Jaubert & Martin (1992) and using the same conditions as Bazinet *et al*. (1999).

Energy consumption. The energy consumption for each treatment was determined to measure the electrical efficiency of the procedure (Lopez Leiva, 1988; Pérez *et al*. 1994). The voltage as a function of the time multiplied by the current was integrated according to the following equation:

$$E = \int_{t_0:\,pH_{44}\text{ or relative initial pH}}^{t_x:pH_c} I/60 \times V\,dt$$

where: V = voltage (volts); I = current (amperes); t = time (min); E = energy (joules).

RESULTS

BMEA parameters

Duration. At constant ionic strength the type of added salt used did not influence the duration of BMEA ($P > 0{\cdot}191$) (Table 1). In addition as the ionic strength (all salts averaged) increased, the duration of the electroacidification increased ($P < 0{\cdot}0001$): for μ_{added} increasing from 0 to 1·0 M, the duration increased from 46·4 to 119·6 min respectively. As confirmed by the regression contrast, the duration increased in a quadratic fashion ($P < 0{\cdot}0001$): by 48·6 % between μ_{added} 0 and 0·25 M, by 11·8 % between μ_{added} 0·25 and 0·5 M and by 14·3 % between μ_{added} 0·5 and 1·0 M.

Conductivity. Initial conductivity of the skim milk solution varied with the concentration and the type of salt added (salt type $P < 0{\cdot}0001$, ionic strength added $P < 0{\cdot}0001$, double interaction salt/ionic strength added $P < 0{\cdot}0001$; Table 2). However, conductivity was stable throughout the process whatever the salt and the level of ionic strength added.

Anode/cathode voltage difference. According to the added salt and ionic strength the initial voltage applied differed due to the intrinsic conductivity of the salt, as previously shown for the conductivity: the initial averaged voltage values were 30·8,

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Table 2. *Effect of ionic strength (μ_{added} 0, 0·25, 0·5 and 1·0 M) and type of salt added ($CaCl_2$, NaCl and KCl) on the conductivity level (mS/cm) of the skim milk solution during bipolar membrane electroacidification, run at a constant current density of 20 mA/cm²*

(Values are means ± SD, $n = 2$)

	μ_{added} (M)			
Salt	0	0·25	0·5	1·0
$CaCl_2$	4·2 ± 0·3	14·8 ± 0·6	25·0 ± 1·2	44·7 ± 1·8
NaCl	4·2 ± 0·3	22·8 ± 0·8	39·1 ± 0·9	65·6 ± 1·9
KCl	4·2 ± 0·3	26·2 ± 1·0	46·2 ± 1·1	86·7 ± 1·9

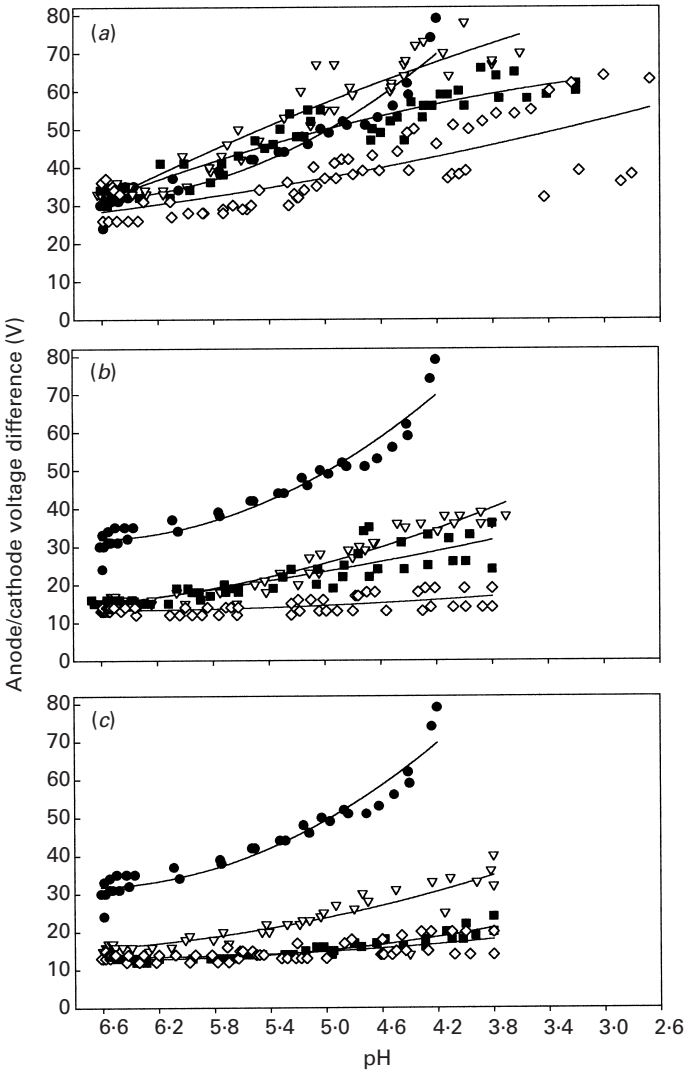

Fig. 2. Effect of (*a*) $CaCl_2$, (*b*) NaCl and (*c*) KCl added to reconstituted skim milk at ionic strength levels of ●, 0; ▽, 0·25; ■, 0·5 and ◇, 1·0 M, on anode/cathode voltage difference during bipolar membrane electroacidification, run at a constant current density of 20 mA/cm².

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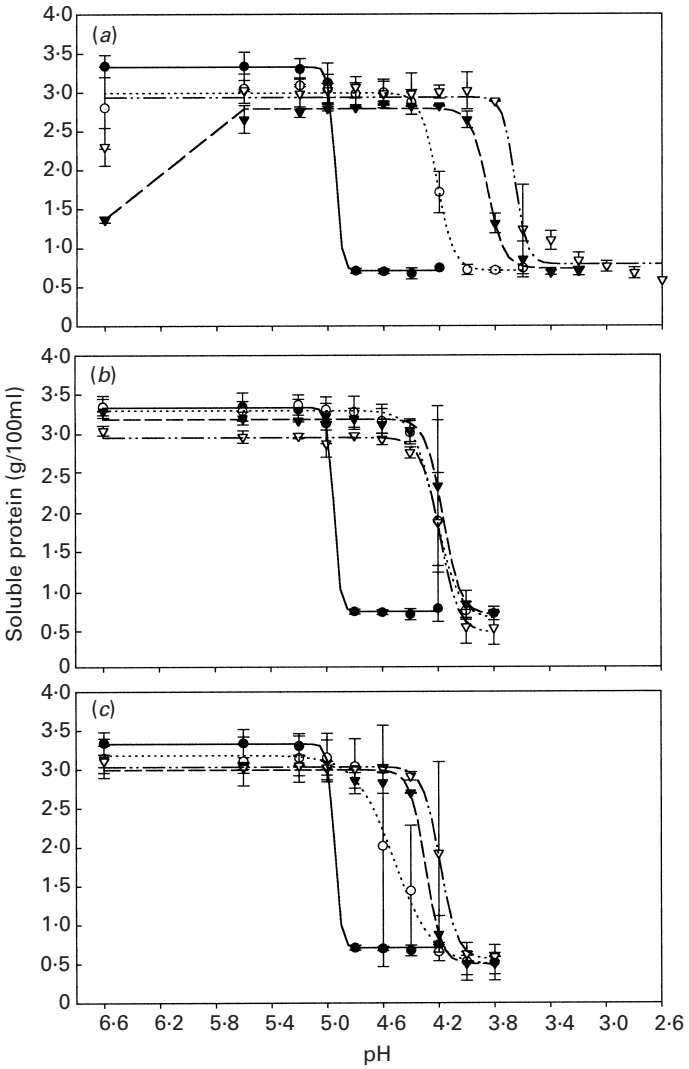

Fig. 3. Effect of (*a*) $CaCl_2$,(*b*) NaCl and (*c*) KCl added to reconstituted skim milk at ionic strength levels of ●, 0; ○, 0·25; ▼, 0·5 and ▽, 1·0 M, on soluble protein during bipolar membrane electroacidification, run at a constant current density of 20 mA/cm^2.

18·0 and 17·4 volts respectively for the $CaCl_2$, NaCl and KCl respectively (Fig. 2). In addition, increasing the added ionic strength reduced the voltage variations between the beginning and the end of the BMEA in a quadratic fashion ($P < 0{\cdot}0002$) (Fig. 2) for all salts averaged, the voltage variation decreased from 42 to 26·5 volts between μ_{added} 0 and 0·25 M, from 26·5 to 17·3 volts between μ_{added} 0·25 and 0·5 M and from 17·3 to 8·3 volts between μ_{added} 0·5 and 1·0 M. Increasing the ionic strength decreased the voltage variation due to an increase in ionic species available to migrate across the CEM.

Soluble protein

The equations of the curves representing the changes in percentage of soluble proteins as a function of pH produced coefficients of determination ranging from 0·963 to 0·999, except for $CaCl_2$, $\mu_{added} = 0{\cdot}5$ M with a coefficient of 0·670. The change

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Table 3. *Effect of ionic strength (μ_{added} 0, 0·25, 0·5 and 1·0 M) and type of salt added ($CaCl_2$, NaCl and KCl) on the percentage of κ- and α_s-casein (CN) in the supernatant sampled at different pH during bipolar membrane electroacidification of reconstituted skim milk, run at a constant current density of 20 mA/cm²*

(Values are means, $n = 2$)

		Type and amount of added salt								
			$CaCl_2$ (μ_{added}; M)			NaCl (μ_{added}; M)			KCl (μ_{added}; M)	
	pH	None	0·25	0·5	1·0	0·25	0·5	1·0	0·25	0·5	1·0
	6·6	11·2^{a}	9·5^{a}	4·5$^{b, c}$	5·8^{c}	12·5^{a}	11·1^{a}	11·6^{a}	8·2^{a}	9·2^{a}	12·3^{a}
	5·6	11·2^{a}	10·3^{a}	10·4^{a}	13·8^{a}	12·1^{a}	11·5^{a}	11·1^{a}	8·1^{a}	8·6^{a}	11·8^{a}
	5·2	11·2^{a}	10·8^{a}	11·8^{a}	13·5$^{a, b}$	12·0^{a}	11·3^{a}	11·2^{a}	7·7^{a}	8·7^{a}	11·9^{a}
	5·0	9·8^{b}	10·7^{a}	11·3^{a}	13·5$^{a, b}$	11·8^{a}	11·6^{a}	13·3^{a}	7·8^{a}	7·8$^{a, b}$	12·2^{a}
	4·8	1·4^{c}	10·1^{a}	11·9^{a}	12·3$^{a, b}$	11·0^{a}	10·6^{a}	10·7$^{a, b}$	7·7^{a}	8·1^{a}	11·7^{a}
	4·6	1·6^{c}	10·4^{a}	11·7^{a}	12·6$^{a, b}$	11·0^{a}	9·9$^{a, b}$	10·9$^{a, b}$	5·4$^{a, b}$	7·9$^{a, b}$	11·4^{a}
	4·4	1·3^{c}	9·0^{a}	11·6^{a}	13·2$^{a, b}$	10·8^{a}	6·9^{b}	8·9$^{a, b}$	3·6$^{b, c}$	6·3^{b}	10·7^{a}
κ-CN	4·2	1·3^{c}	5·4^{b}	11·6^{a}	12·9$^{a, b}$	6·2^{b}	3·0^{c}	5·7$^{b, c}$	1·8^{c}	2·2^{c}	2·5^{b}
	4·0		1·7^{c}	10·6^{a}	12·5$^{a, b}$	1·8^{c}	0·9^{c}	0·9$^{c, d}$	1·1^{c}	1·0^{d}	0·7^{b}
	3·8		1·3^{c}	5·3^{b}	10·2^{b}	1·5^{c}	0·3^{c}	0·4^{d}	0·8^{c}	0·8^{d}	0·3^{b}
	3·6		1·6^{c}	2·6$^{b, c}$	4·7$^{d, c}$						
	3·4			1·6^{c}	1·8$^{d, e}$						
	3·2			1·1^{c}	1·6$^{d, e}$						
	3·0				0·7^{e}						
	2·8				0·4^{e}						
	2·6				0·1^{e}						
	6·6	34·6^{a}	31·4^{a}	12·5^{c}	14·7$^{c, d}$	32·6^{a}	33·2^{a}	35·2^{a}	33·7^{a}	33·4^{a}	32·7^{a}
	5·6	34·9^{a}	34·2^{a}	26·5^{b}	35·2^{a}	32·3^{a}	33·2^{a}	33·6^{a}	34·4^{a}	34·0^{a}	32·3^{a}
	5·2	35·6^{a}	33·4^{a}	33·5$^{a, b}$	34·2^{a}	33·3^{a}	35·1^{a}	34·1^{a}	33·8^{a}	35·1^{a}	32·2^{a}
	5·0	29·5^{b}	35·2^{a}	33·5$^{a, b}$	27·3$^{a, b}$	33·7^{a}	34·2^{a}	34·2^{a}	34·6^{a}	33·3^{a}	33·3^{a}
	4·8	0·0^{c}	32·7^{a}	33·8^{a}	19·8$^{b, c}$	31·7^{a}	32·4^{a}	33·2^{a}	33·4^{a}	32·3^{a}	32·0^{a}
	4·6	0·1^{c}	33·2^{a}	34·2^{a}	29·6$^{a, b}$	30·8^{a}	29·6^{a}	32·5$^{a, b}$	24·3^{a}	31·1^{a}	32·1^{a}
	4·4	0·0^{c}	27·7^{a}	33·8^{a}	32·3^{a}	29·3^{a}	19·8^{b}	29·2^{b}	14·3^{b}	26·7^{a}	30·1^{a}
α_s-CN	4·2	0·0^{c}	13·9^{b}	34·9^{a}	35·1^{a}	14·5^{b}	6·1^{c}	17·2^{c}	2·0^{c}	8·4^{b}	4·0^{b}
	4·0		0·1^{c}	32·7$^{a, b}$	34·0^{a}	0·4^{c}	0·0^{c}	0·6^{d}	0·2^{c}	0·1^{b}	0·2^{b}
	3·8		0·0^{c}	12·1^{c}	30·2$^{a, b}$	0·1^{c}	0·0^{c}	0·0^{d}	0·1^{c}	0·1^{b}	0·0^{b}
	3·6		0·0^{c}	3·9^{d}	12·2$^{c, d, e}$						
	3·4			0·3^{d}	4·1$^{d, e}$						
	3·2			0·7^{d}	2·0^{e}						
	3·0				1·2^{e}						
	2·8				0·1^{e}						
	2·6				0·0^{e}						

^{a,b,c,d,e} Values in the same column without a common superscript were significantly different: $P < 0·05$.

in soluble protein during BMEA of skim milk was dependent upon the type of salt added and the ionic strength level (Fig. 3). The sigmoidal curve of the milk solution without salt added showed an inflection point at pH 4·9 which was higher than that of any milk solution to which salt was added. The pH at inflection was highest for KCl followed by NaCl and $CaCl_2$ at each level of ionic strength. Moreover, for each added salt, the soluble protein curves differed according to the ionic strength added. This can be shown by comparison of the inflection points and the width of transition of the solubility curves. The curves for μ_{added} of 0·25, 0·5 and 1·0 M-NaCl (Fig. 3*b*) all had the same inflection point at pH 4·2 and similar width of transition values (0·08, 0·06 and 0·07 pH units respectively). In the case of $CaCl_2$ (Fig. 3*a*), the delay in precipitation increased as ionic strength was increased (μ_{added} of 0·25, 0·5 and 1·0 M), with inflection points at pH 4·2, 3·9 and 3·7 and width of transition values of 0·06, 0·004 and 0·04 pH unit respectively. For the KCl (Fig. 3*c*), the delay in precipitation

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Table 4. *Effect of ionic strength (μ_{added} 0, 0·25, 0·5 and 1·0 M) and type of salt added ($CaCl_2$, NaCl and KCl) on the percentage of β-casein (β-CN) and whey protein in the supernatant sampled at different pH during bipolar membrane electroacidification of reconstituted skim milk, run at a constant current density of 20 mA/cm²*

(Values are means, $n = 2$)

		Type and amount of added salt								
			$CaCl_2$ (μ_{added}; M)			NaCl (μ_{added}; M)			KCl (μ_{added}; M)	
	pH	None	0·25	0·5	1·0	0·25	0·5	1·0	0·25	0·5	1·0
β-CN	6·6	38·5^{a}	34·5$^{a, b}$	13·9^{c}	17·5^{c}	41·4^{a}	38·5^{a}	40·5^{a}	42·3^{a}	42·6^{a}	40·4^{a}
	5·6	39·2^{a}	38·4^{a}	32·5^{b}	41·2^{a}	41·4^{a}	38·3^{a}	38·6$^{a, b}$	42·9^{a}	42·1^{a}	40·0^{a}
	5·2	40·6^{a}	38·5^{a}	38·0$^{a, b}$	40·9^{a}	43·1^{a}	42·0^{a}	39·3^{a}	42·2^{a}	43·0^{a}	39·6^{a}
	5·0	32·5^{b}	39·0^{a}	37·9$^{a, b}$	35·2$^{a, b}$	43·2^{a}	39·7^{a}	38·8$^{a, b}$	42·8^{a}	41·9^{a}	40·8^{a}
	4·8	0·1^{c}	36·3$^{a, b}$	38·3^{a}	29·2^{b}	40·8^{a}	37·0^{a}	37·8$^{a, b}$	39·9^{a}	38·8^{a}	38·5$^{a, b}$
	4·6	0·0^{c}	36·1$^{a, b}$	38·5^{a}	34·9$^{a, b}$	38·3^{a}	33·2^{a}	36·8$^{a, b}$	28·7$^{a, b}$	36·3^{a}	37·9$^{a, b}$
	4·4	0·0^{c}	29·3^{b}	37·6$^{a, b}$	37·4$^{a, b}$	35·8^{a}	21·1^{b}	33·8^{b}	16·7^{b}	30·5^{a}	35·1^{b}
	4·2	0·0^{c}	12·8^{c}	37·6$^{a, b}$	39·9^{a}	17·0^{b}	6·5^{c}	19·9^{c}	2·5^{c}	9·8^{b}	5·0^{c}
	4·0		0·3^{d}	34·7$^{a, b}$	38·9^{a}	0·6^{c}	0·2^{c}	0·8^{d}	0·3^{c}	0·2^{b}	0·4^{d}
	3·8		0·2^{d}	12·1^{c}	33·9$^{a, b}$	0·3^{c}	0·0^{c}	0·0^{d}	0·0^{c}	0·1^{b}	0·2^{d}
	3·6		0·0^{d}	4·0^{d}	11·2$^{d, c}$						
	3·4			0·7^{d}	4·4$^{d, e}$						
	3·2			1·0^{d}	2·1^{e}						
	3·0				1·3^{e}						
	2·8				0·1^{e}						
	2·6				0·0^{e}						
Whey protein	6·6	13·7$^{a, b}$	16·1^{a}	16·2$^{a, b, c}$	16·9$^{a, b, c}$	14·9^{a}	14·4$^{a, b}$	15·1^{a}	15·2$^{a, b}$	14·2^{a}	15·0^{b}
	5·6	14·1$^{a, b}$	15·7$^{a, b}$	16·3$^{a, b, c}$	18·2^{a}	15·5^{a}	14·7$^{a, b}$	14·6^{a}	15·7^{a}	14·2^{a}	15·1^{b}
	5·2	15·1^{a}	15·3$^{a, b}$	16·1$^{a, b, c}$	18·2^{a}	15·6^{a}	16·4^{a}	14·7^{a}	15·4^{a}	14·2^{a}	15·1^{b}
	5·0	13·3$^{a, b}$	15·8$^{a, b}$	16·4$^{a, b}$	17·7$^{a, b}$	15·8^{a}	14·8$^{a, b}$	14·6^{a}	16·4^{a}	14·6^{a}	16·7^{a}
	4·8	11·7^{b}	14·9$^{a, b}$	16·3$^{a, b, c}$	16·7$^{a, b, c}$	15·4^{a}	14·6$^{a, b}$	14·7^{a}	16·7^{a}	13·7^{a}	15·5$^{a, b}$
	4·6	12·0$^{a, b}$	15·4$^{a, b}$	16·5^{a}	18·0^{a}	15·1^{a}	13·9$^{a, b}$	14·2$^{a, b}$	15·4^{a}	13·8^{a}	15·5$^{a, b}$
	4·4	10·8^{b}	14·9$^{a, b}$	15·7$^{a, b, c}$	16·8$^{a, b, c}$	15·2^{a}	13·0^{b}	14·0$^{a, b}$	14·4$^{a, b}$	14·0^{a}	14·8^{b}
	4·2	10·9^{b}	13·6^{b}	15·6$^{a, b, c}$	17·4$^{a, b}$	14·2^{a}	12·5$^{b, c}$	13·0$^{a, b, c}$	13·0$^{b, c}$	14·2^{a}	12·4^{c}
	4·0		11·4^{c}	15·0$^{a, b, c}$	17·2$^{a, b}$	12·1^{b}	10·1$^{c, d}$	11·6$^{b, c}$	12·9$^{b, c}$	12·8^{a}	11·9^{c}
	3·8		9·4^{d}	13·9$^{a, b, c}$	15·9$^{a, b, c}$	12·0^{b}	9·7^{d}	10·7^{c}	11·3^{c}	11·9^{a}	10·7^{d}
	3·6		10·3^{d}	15·0$^{a, b, c}$	14·8$^{b, c, d}$						
	3·4			13·5$^{b, c}$	13·9$^{c, d, e}$						
	3·2			13·4^{c}	12·9$^{d, e, f}$						
	3·0				12·8$^{d, e, f}$						
	2·8				11·3$^{e, f}$						
	2·6				10·9^{f}						

^{a,b,c,d,e,f} Values in the same column without a common superscript were significantly different: $P < 0·05$.

was increased with the increase in ionic strength; pH 4·5, 4·3 and 4·2 at μ_{added} of 0·25, 0·5 and 1·0 M respectively.

Protein profiles during BMEA and of isolates

The linear equations of the curves representing the changes in percentage of each protein fraction as a function of pH produced coefficients of determination ranging from 0·744 to 0·961, except for whey protein fraction, at $\mu_{added} = 0$ and 0·5 M with addition of $CaCl_2$, and at $\mu_{added} = 0·5$ M with addition of KCl, with coefficients of 0·663, 0·552 and 0·105 respectively. Added salt and ionic strength influenced the evolution of the percentage of each protein fraction in the supernatant (Tables 3 and 4). The precipitation of all casein fractions was delayed by increasing the ionic strength with $CaCl_2$ and KCl. Increasing the ionic strength from 0·25 to 1·0 M with KCl did not affect the pH_c values; increasing the ionic strength with $CaCl_2$ resulted

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Table 5. *Effect of ionic strength (μ_{added} 0, 0·25, 0·5 and 1·0 M) and type of salt added ($CaCl_2$, NaCl and KCl) on the percentage of κ-casein (κ-CN), α_s-casein (α_s-CN), β-casein (β-CN) and whey protein in the isolate produced by bipolar membrane electroacidification of reconstituted skim milk, run at a constant current density of 20 mA/cm²*

(Values are means ± SD, $n = 2$)

	Type and amount of added salt									
		$CaCl_2$ (μ_{added}; M)			NaCl (μ_{added}; M)			KCl (μ_{added}; M)		
	None	0·25	0·5	1·0	0·25	0·5	1·0	0·25	0·5	1·0
κ-CN	12·0 ± 0·1	11·1 ± 0·1	11·2 ± 0·4	12·1 ± 0·1	11·4 ± 0·7	12·3 ± 0·2	12·5 ± 0·3	11·9 ± 0·1	12·1 ± 0·1	12·0 ± 0·2
α_s-CN	40·7 ± 0·1	41·3 ± 0·4	40·0 ± 0·7	39·5 ± 0·2	40·3 ± 0·7	39·6 ± 1·3	40·5 ± 0·2	41·0 ± 0·1	40·7 ± 0·1	40·1 ± 0·8
β-CN	46·1 ± 0·1	46·3 ± 0·4	47·3 ± 1·1	45·0 ± 0·1	47·0 ± 1·2	46·8 ± 1·4	45·1 ± 0·8	45·8 ± 0·1	45·8 ± 0·2	46·4 ± 0·5
Whey protein	1·2 ± 0·0	1·3 ± 0·1	1·5 ± 0·1	3·3 ± 0·2	1·3 ± 0·1	1·4 ± 0·3	1·9 ± 0·7	1·2 ± 0·1	1·4 ± 0·1	1·6 ± 0·5

Table 6. *Effect of ionic strength (μ_{added} 0, 0·25, 0·5 and 1·0 M) and type of salt added ($CaCl_2$, NaCl and KCl) on the energy and relative energy consumption (kW.h/kg of isolate) during bipolar membrane electroacidification of reconstituted skim milk, run at a constant current density of 20 mA/cm²*

	μ_{added} (M)			
	0	0·25	0·5	1·0
Energy				
$CaCl_2$	0·7	2·1	2·4	1·6
NaCl	0·7	0·7	0·6	0·6
KCl	0·7	0·6	0·4	0·6
Relative energy				
$CaCl_2$	0·7	1·0	1·5	1·1
NaCl	0·7	0·6	0·5	0·5
KCl	0·7	0·6	0·4	0·6

in lower pH_c values. In the case of NaCl addition, the percentages of each casein fraction precipitated at pH 4·4 and 4·2 were the same for μ_{added} of 0·25 and 1·0 M, but lower than that of 0·5 M μ_{added}. The NaCl appears to have a salting-out effect at 0·5 M.

As confirmed by Duncan tests ($P < 0·05$), a part of the whey protein was precipitated with the casein fraction.

In the isolates produced by BMEA, the concentrations of the major protein fractions of milk, α_s- and β-caseins, were the same whatever the salt and the ionic strength added ($P > 0·0847$ and $P > 0·1303$ for the α_s-casein and β-casein respectively): $40·4 \pm 0·5$% and $46·1 \pm 0·7$% for α_s- and β-casein respectively (Table 5). However, the type of salt ($P < 0·0151$ for the whey protein and $P < 0·0057$ for κ-casein) and the added ionic strength ($P < 0·0003$ for the whey protein and $P < 0·03$ for the κ-casein) influenced the final concentration of whey protein and κ-casein in the isolate composition.

$CaCl_2$ was found to increase the concentration of whey protein fraction and decreased the concentration of κ-casein in comparison with NaCl and KCl which had similar values. For the effect of μ_{added}, from 0 to 0·5 M there was no real significant difference between $CaCl_2$, NaCl and KCl, while at 1·0 M $CaCl_2$ there was a significantly higher whey level in the isolate.

For the interaction salt added/ionic strength added ($P < 0·014$ for whey protein fraction and $P < 0·0481$ for κ-casein), the concentration of whey protein in the isolate increased with an increase in μ_{added}, and this increase from 0 to 1 M μ_{added} was higher for $CaCl_2$ (approximately +190%) than for NaCl (+60%) and KCl (+40%). The concentration of κ-casein in the isolate produced by BMEA was similar whatever the ionic strength added for KCl (12%), while for NaCl and $CaCl_2$ the concentration decreased from 0 to 0·25 M μ_{added} (approximately −8 and −5% for $CaCl_2$ and NaCl respectively) and increased thereafter (approximately +9 and +10% for $CaCl_2$ and NaCl respectively).

Energy efficiency of the process

The energy consumption (in kW.h/kg of isolate) was first calculated from the time required for BMEA to decrease pH from 6·6 (after re-adjustment of the pH following the addition of salt) to the pH_c value and thereafter from the relative time. The relative time was the time required for BMEA to decrease the pH from the pH value obtained after the addition of salt (without readjustment to 6·6) to the pH_c.

Addition of $CaCl_2$ caused a large increase in energy consumption (Table 6), while

addition of NaCl and KCl resulted in a slight drop. Energy consumption was affected by both the ionic strength and the salt added. With addition of monovalent salts (NaCl and KCl), the energy consumption decreased between $\mu_{added} = 0$ and 0·5 M and finally increased between $\mu_{added} = 0{\cdot}5$ and 1·0 M. In the case of the divalent salt ($CaCl_2$) addition, the energy consumption greatly increased from 0 to 0·5 M and decreased drastically between $\mu_{added} = 0{\cdot}5$ and 1·0 M.

The values of energy consumption calculated from the relative times gave similar results for KCl, due to a very low pH decrease by this monovalent ion. For $CaCl_2$ addition, the results for the energy consumption obtained for $\mu_{added} = 0{\cdot}25$ and 0·5 M were lower but the trends in energy consumption were the same as for the previous calculations. For NaCl, the relative energy consumption decreased by approximately 30 % from 0 to 1·0 M μ_{added}: the energy consumption decreased drastically between $\mu_{added} = 0$ and 0·25 M and stabilized thereafter.

DISCUSSION

BMEA parameters

During electrochemical processes, such as BMEA, high conductivities are necessary to increase the current effectiveness. In this experiment, although the conductivity of the skim milk solution was increased, the duration of the BMEA was increased, whichever salt was added. The conductivity was no longer the limiting factor. The limiting factor was the protein precipitation which was delayed by salting-in. Bazinet *et al.* (1997*b*), on BMEA of soyabean protein, did not observe any difference in duration due to increasing the salt concentration from 0·06 to 0·24 M-KCl. They showed that the duration remained the same following addition of 0·24 M-KCl, or 0·06 M-KCl and that there is a slight delay, due to the salting-out effect, in the protein precipitation curve. However, the results obtained for conductivity and anode/cathode voltage differences in the present study are in accordance with the data of Bazinet *et al.* (1997*b*) i.e. the increase in added salt concentration increased the initial conductivity values and reduced the voltage rise at the end of the experiment. The voltage rise at the end of the experiment was due to a slight protein fouling of the spacers in the cell.

Precipitation of protein during BMEA

Except for the NaCl, the pH_c value, at which all the caseins were precipitated, decreased with an increase in ionic strength by salting-in (Cheftel *et al.* 1985; Kinsella *et al.* 1985). This confirms the results obtained for the BMEA parameters and particularly the increase in time required to reach pH_c value, as the pH_c was reached at a lower pH value. The results obtained for the KCl are in accordance with the data obtained by Bazinet *et al.* (1997*b*) for soyabean protein electroacidification. Repulsive hydration forces between proteins and protein solubility are minimal at the isoelectric pH, unless the net charge on the proteins is controlled in part by highly hydrated cations such as Mg^{2+}, Ca^{2+} and Na^+ (Pashley & Israelachvili, 1984). In the latter situation, coagulation occurs when the H^+ concentration is high enough to replace the hydrated cations. The more Ca^{2+} present, the higher the H^+ concentration required to cause coagulation. Thus the pH at which casein micelles coagulate decreased from 5·0 to 3·8 as $CaCl_2$ concentration was increased from 10 to 100 mM (Bringe & Kinsella, 1987). Moreover the difference observed between $CaCl_2$ and the other salts was probably due to the role of Ca^{2+} in stabilizing micelles; an increase in the Ca^{2+} concentration in solution prevents migration of bound calcium from the

micelle, which is normally complete at pH 5, thus maintaining micelle structure till lower pH is reached. Strange *et al.* (1994), observed that at pH levels above the isoionic point the pH of all casein solutions measured decreased upon addition of NaCl, indicating an exchange between Na^+ and H^+. Ho & Waugh (1965) also noted a decrease in pH when KCl was added to isoionic α_s-casein and attributed this decrease to the binding of K^+. However, this decrease in pH may also be attributed to replacement of H^+ from the diffuse, positively charged electronic layer which surrounds the negatively charged protein with Na^+ (Bull, 1943).

Courthaudon *et al.* (1989) showed that for an ionic strength increased by addition of NaCl concentration ranging from 0 to 1 M, the protein solubility was enhanced in the pH range of the isoelectric point by decreasing the electrostatic attractions and competition between Na^+ and protons which causes a salting-in effect. Moreover, they observed that the solubility was slightly decreased for the pH far from the pH_i (acidic or basic) at 10 g/l protein concentration, but if the protein concentration was increased from 5 to 40 g/l at 0·5 M-NaCl, the percentage of soluble proteins was strongly decreased.

Protein profiles

The results obtained on protein profiles of milk proteins during BMEA agree with data for soluble protein, and give more information on the differential precipitation evolution of each protein. The precipitation of a part of the whey protein was observed in previous results obtained on skim milk powder (Bazinet *et al.* 2000*b*). The decrease in whey protein in the skim milk solution observed at the end of the process probably results from the co-precipitation of α-lactalbumin and β-lactoglobulin with κ-casein due to heating treatment prior to BMEA (Kinsella, 1984; Cayot & Lorient, 1998). However, BMEA would not have such an effect.

The concentrations of the α_s- and β-casein fractions in the isolates were unchanged whatever the salt and the ionic strength added. Isolates produced by BMEA at 1·0 M μ_{added} were analysed by liquid chromatography/mass spectrometry, and the whey protein fraction was identified as α-lactalbumin. $CaCl_2$ favoured the precipitation of whey protein fraction by BMEA. Mailliard & Ribadeau-Dumas (1988) demonstrated, in their attempt to separate β-lactoglobulin by salting-out from an acid whey retentate, that between pH 3·0 and 4·0, at high salt concentrations (5% NaCl), the α-lactalbumin was precipitated and was predominant in the precipitate.

Energy efficiency of BMEA of skim milk

Adding NaCl and KCl at μ_{added} of 0·25 and 0·5 M increased the energy efficiency of BMEA. The combination of KCl and μ_{added} = 0·5 M was the best combination with a 45% decrease in energy consumption, since the potassium ion mobility is higher than that of sodium ion (7·6 *v.* 5·2 $cm^2.s^{-1}.V^{-1}$). Better energy efficiency resulted from a decrease in anode/cathode voltage difference, due to increased conductivity achieved by addition of salt, which was necessary because of the lack of sufficiently mobile ions in the skim milk. This result was in accordance with previous results (Bazinet *et al.* 2000*a*) suggesting the addition of salt in order to improve energy efficiency. However, in this study the amount of salt added (e.g. 0·5 M-KCl), corresponds to an addition of 500 mEq/l of skim milk, which is different from the 7–25 mEq/l value estimated by difference between cations migrated and electrical and chemical calculations (Bazinet *et al.* 2000*a*). These results are not contradictory. The addition of 7–25 mEq./l of K^+ to the skim milk solution was suggested in order

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to compensate for the loss of H^+. However, to improve the BMEA process, the results of Bazinet *et al.* (2000*a*) indicated adding 50 mEq./l of K^+ and more. Furthermore, the kinetics of protein precipitation was influenced by the concentration of salt added to the skim milk solution. Since the kinetics of the protein precipitation was delayed by addition of salts, a lower pH value has to be reached, and consequently, a higher number of H^+ must be generated.

BMEA has numerous advantages in comparison with conventional isoelectric precipitation of proteins used in the dairy industry. The process can be precisely controlled, as electroacidification rate is regulated by the effective current density in the cell. The water consumption is lower by reuse of a part of the effluent generated. Energy consumption is low (less than 1·0 kW.h/kg of isolate produced). Moreover, by the use of an electrodialytic system, which is well-known in industry, BMEA technology can easily be transferred to the industrial scale and installation would not need a complete change of the production process, but only some modifications (Bazinet *et al.* 1998).

The major disadvantage of BMEA is the high cost of equipment. The development of this technology, and its application in the dairy industry, and more generally in the food industry, would help to minimize the cost of the bipolar membranes and electrodialysis cells. Two problems limit the application of the BMEA at an industrial level: the fouling of spacers and the fouling of cationic membranes. During BMEA process, the protein curd formed can lead to a fouling of the cell spacers by re-circulation and accumulation of protein aggregates in the turbulence promoters of the spacers. This fouling decreases the energy effectiveness of the electrodialysis system. However, an on-line centrifugation at the inlet and/or outlet of the cell would limit the problem. Furthermore, working with acid milk, as in the SAFIR process (Bolzer, 1985), would drastically decrease the possibility of fouling by the protein network, which does not exist at low pH. In the SAFIR process a calculated volume of milk is added to acid casein (pH $\approx$ 1·8 to 2·4) to adjust the pH of the whole solution at pH 3·5. A part of the very acid casein solution would be used to maintain the pH of the main reservoir while the other part would be for the production of casein.

Long-term processing of milk leads to a more problematic type of fouling i.e. fouling of the cationic membrane. Formation of calcium carbonate occurs during BMEA on the surface of the membrane in contact with the base electrogenerated but also inside the membrane and on the surface of the membrane in contact with the milk solution (Bazinet *et al.* 2000*c*). Such fouling does not appear in the case of soyabean since calcium is present at a lower concentration. This decreases the long-term life of the membrane and increases the energy used by the system in the short-term. A study carried out in our laboratory has shown that an acid clean of the cell *in situ* would efficiently remove or prevent the formation of a calcium carbonate fouling.

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