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SÉPARATION DES CASÉINES DU LAIT BOVIN PAR ELECTRO-ACIDIFICATION AVEC MEMBRANES BIPOLAIRES ET CARACTERISATION DES ISOLATS

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À mes parents,
Huguette et Jean-Claude

À mes deux trésors et demi,
Hélène et Solène

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Des isolats de haute pureté ont été produits par électroacidification avec membranes bipolaires (ÉAMBP.) Différents facteurs ont influencé les performances de la technologie : le débit, le type de lait, la permsélectivité des membranes cationiques. Le colmatage formé durant l'ÉAMBP est composé d'hydroxyde de calcium et de magnésium. L'acidification chimique et l'ÉAMBP présentent des différences dans les profils d'acidification ; le nombre de H^+ électrogénérés a fait ressortir l'importance des ions potassium pour assurer une meilleure efficacité énergétique. La meilleure combinaison est l'ajout de KCl à une force ionique de 0.5 M. Excepté pour l'isolat produit à une force ionique de 1M de $CaCl_2$, les isolats produits par ajout de sel à différentes forces ioniques présentent des propriétés fonctionnelles similaires entre elles, et comparables à celles d'isolats commerciaux et d'un isolat produit chimiquement. Un taux de déminéralisation de 75% influence la charge protéique et la concentration en lactose des isolats.

RÉSUMÉ LONG

Les objectifs principaux de cette étude étaient d'établir les conditions d'électroacidification optimales et de caractériser les isolats de caséines produits par électroacidification avec membranes bipolaires (ÉAMBP). Le but de ces travaux étant de permettre la validation de la méthodologie de l'ÉAMBP pour la séparation à l'échelle industrielle des protéines du lait.

Il ressort des résultats que des isolats de haute pureté contenant de 1.2 à 3.8% de cendres et de 85 à 98% de protéines ont été produits par ÉAMBP. La composition des profils moléculaires des surnageants a montré que les protéines sériques sont les seules protéines solubles restantes après électroacidification. Différents facteurs ont influencé les performances de la technologie : le débit, le type de lait et la permsélectivité des membranes cationiques. Les résultats obtenus sur la permsélectivité ont permis de comprendre la formation du colmatage des MEC et d'en identifier la nature.

Il est apparu que l'acidification chimique et l'ÉAMBP présentaient quelques différences dans les profils d'acidification ; les caséines électroacidifiées précipitant à un pH plus élevé que les caséines acidifiées chimiquement. L'évaluation du nombre de H^+ électrogénérés au cours de l'ÉAMBP a fait ressortir l'importance des ions potassium pour assurer une meilleure efficacité énergétique de l'ÉAMBP.

Au niveau de l'efficacité énergétique, nous avons mis en évidence que la meilleure combinaison est l'ajout de KCl à une force ionique de 0.5 M (diminution de l'énergie consommée de 45%). Pour les isolats produits par ajout de sel à différentes forces ioniques, nous avons démontré que ceux-ci présentaient des propriétés fonctionnelles similaires entre eux, excepté pour l'isolat produit par ajout de $CaCl_2$ à une force ionique de 1M. Pour les isolats produits après déminéralisation de la solution de lait écrémé, il est apparu qu'un taux de déminéralisation de 75% influençait la charge protéique ainsi que la concentration en lactose.

Enfin, les isolats produits par électroacidification avec membranes bipolaires avec différents types de sels et à différents niveaux de forces ioniques ajoutées, excepté pour l'ajout de $CaCl_2$ à une force ionique de 1 M, ont montré des propriétés physicochimiques et fonctionnelles similaires à celles d'isolats commerciaux et d'un isolat produit chimiquement.

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Les protéines du lait sont les protéines animales les plus anciennement et largement consommées par l'homme (Cheftel *et al.*, 1985). Elles représentent un quart des protéines alimentaires totales utilisées dans le monde industriel (Hambraeus, 1982). Leur importance nutritionnelle et technologique dans les produits agricoles et alimentaires motive depuis de nombreuses années l'étude de cette classe d'aliments et le développement de procédés industriels visant à les séparer ou à en améliorer les propriétés et la qualité. Les caséines représentent les principaux constituants protéiques du lait avec environ 80% des protéines totales. Les utilisations non alimentaires des caséines ont été, jusque vers les années 1950-1960, les principales formes de valorisation (Cayot et Lorient, 1998). Encore aujourd'hui, les caséines ont une grande importance économique car elles entrent dans la composition de colles, de peintures, de fibres textiles et peuvent être utilisées dans les industries du caoutchouc, du cuir et pharmaceutiques (Southward, 1989).

Les caséines du lait sont aussi très utilisées dans la fabrication de produits alimentaires pour leurs qualités nutritionnelles et leurs propriétés fonctionnelles. Cependant, la production industrielle de caséines de pureté élevée n'est possible que par une phase d'insolubilisation suivie d'une centrifugation pour séparer les caséines du lactosérum (Varnam et Sutherland, 1994). Il n'existe à l'heure actuelle que deux façons de produire des caséines : par acidification présure ou chimique. Pour la production de caséines acides, les trois procédures existantes sont basées sur la précipitation isoélectrique des caséines : par acidification chimique, physico-chimique ou par fermentation (Segalen, 1985; Southward, 1993; Varnam et Sutherland, 1994). Récemment d'autres méthodes appelées méthodes alternatives ont été proposées pour la production de caséines acides :

acidification du lait par échange ionique couplé à un acide (Salmon, 1983), par électrodialyse du lait écrémé suivi par une acidification (Laiteries Triballat, 1979) et enfin l'acidification par électrolyse des molécules d'eau à l'interface de membranes monopolaires (Bolzer, 1985).

Les techniques de séparation utilisées en industrie ont dans la majorité des cas le désavantage de générer de grands volumes d'effluents chimiques et d'engendrer des risques inhérents liés à la manipulation, au stockage et au transport des acides et des bases concentrés. Dans le cas des fermentations, il est nécessaire d'utiliser des ferments mixtes et de les changer régulièrement afin d'éviter les contaminations par les phages. Enfin, au cours de la fabrication des caséines présure, il y a dénaturation de la cas-κ, par formation du caséinomacropeptide et de la paracaséine-κ (Cayot et Lorient, 1998 ; Cheftel *et al.*, 1985).

Un procédé dérivé de l'électrodialyse (ÉD) et utilisant des membranes bipolaires a été développé récemment afin de précipiter les protéines de soya (Bazinet *et al.*, 1996 ; 1997c). Cette technologie appelée génériquement électrodialyse avec membranes bipolaires (ÉDMBP) ou plus spécifiquement électroacidification avec membranes bipolaires (ÉAMBP) est basée sur la production de protons par dissociation des molécules d'eau à l'interface d'une membrane bipolaire, membrane de type composite (Mani, 1991). Les protons ainsi produits peuvent migrer vers la cathode et servir à acidifier une solution de protéines. Ce procédé innovateur vise à précipiter les protéines pour les séparer des autres constituants et les isoler sans toutefois les dénaturer.

Ainsi, les objectifs principaux de cette étude seront : établir les conditions d'électroacidification optimales de séparation des caséines du lait, en termes d'efficacité énergétique et de cinétique de précipitation; caractériser les isolats de caséines produits par ÉAMBP. De façon plus générale, les résultats de cette recherche vont permettre l'acquisition de nouvelles connaissances sur le comportement électrochimique des protéines du lait. Le but éventuel de ces travaux sera de permettre de valider la méthodologie de l'électroacidification avec membranes bipolaires pour la séparation à

l'échelle industrielle des protéines ou des fractions protéiques du lait sans ajout chimique et cela par génération électrique contrôlée des espèces ioniques désirées.

CHAPITRE II

ÉTAT DE LA QUESTION ET OBJECTIFS POURSUIVIS

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LES CASÉINES DU LAIT BOVIN

La teneur moyenne en protéines d'un lait normal varie de 30 à 35 g/L, ce qui représente 95% de l'azote total présent dans le lait (Cheftel *et al.*, 1985). Les protéines du lait sont réparties en deux grandes catégories. La première catégorie est constituée des protéines solubles, dites protéines du lactosérum, qui ne précipitent pas lors de la coagulation du lait par ajout de présure ou acidification. Cette phase soluble est composée de protéines globulaires peu associées : β-Lactoglobuline (β-lg), α-lactalbumine (α-la), albumine de sérum bovin, immunoglobulines, protéose peptone et plusieurs enzymes ; elles représentent 14 à 24% des protéines totales du lait (Lorient, 1991; Swaisgood, 1982; Walstra et Jenness, 1984; Goff et Hill, 1993). La seconde est une phase micellaire constituée d'associations volumineuses des caséines α_{s1}, α_{s2}, β et κ responsables de l'aspect opalescent du lait ; elles représentent de 76 à 86% des protéines totales du lait.

Composition, structure et caractéristiques physico-chimiques des monomères

a) Caséine-α_{s1}

La caséine-α_{s1} (cas-α_{s1}) est présente dans le lait à une concentration de 12 à 15 g/L et représente 36% des protéines de la fraction caséinique. La chaîne polypeptidique compte

199 résidus d'acides aminés et la masse moléculaire de la protéine est comprise entre 23 614 et 22 068 Da (Lorient, 1991; Swaisgood, 1982). 15% des résidus d'acides aminés de la cas-α_{s1} forment des hélices α, 22% des feuillets β et 45% des courbures β (Farrell *et al.*, 1993). Cinq variants génétiques sont dénombrés à l'heure actuelle pour la cas-α_{s1} ; variants A, B, C, D et E (Whitney, 1988).

Comptant huit (α_{s1}-CN B-8P) ou neuf (α_{s1}-CN B-9P) résidus phosphoséryle, cette protéine est chargée très négativement (charge nette calculée de -20.6 mV à pH 6.6) (Swaisgood, 1982 ; Cheftel *et al.*, 1985; Mercier *et al.*, 1971). La molécule a donc un comportement dipolaire avec un pôle globulaire plutôt hydrophobe et un pôle chargé. Sur l'ensemble des résidus de sérine phosphate, sept se trouvent regroupés dans une portion (43-80) qui contient douze fonctions carboxyliques. Le segment 100-199 est quant-à lui très hydrophobe (7 des 9 résidus de tyrosine de la cas-α_{s1}, s'y trouvent), ce qui confère à la protéine une hydrophobicité globale élevée (4.89 kJ/résidu) (Swaisgood, 1982). L'absence de résidus Cys et la présence irrégulière de résidus Pro confèrent à cette protéine une structure tertiaire lâche (Mercier *et al.*, 1971; Cheftel *et al.*, 1985).

Les valeurs mesurées pour le volume spécifique et l'absorptivité à 280 nm sont respectivement 0.728 mL/g (Mc Kenzie et Wake, 1959) et 1.01 cm^2/g (Thomson et Kiddy, 1964; Thomson *et al.*, 1969).

Le pH isoionique (pH de la solution de protéines en l'absence d'électrolyte) des cas-α_{s1} se situe entre 4.91 et 5.05 (Swaisgood, 1982; Cheftel *et al.*, 1985). L'association de la cas-α_{s1} dépend fortement du pH et de la force ionique, ce qui indique que les interactions électrostatiques inter-protéines sont très importantes (Payens et Schmidt, 1966; Schmidt, 1970a, b). L'association est régie par les répulsions électrostatiques et les attractions liées aux liaisons hydrophobes et hydrogènes (Schmidt, 1970a; Aschaffenburg et Drewry, 1957). À pH neutre l'association des cas-α_{s1} est dépendante à la fois de la force ionique et de la température de la solution. À pH 8.0, l'association de la cas-α_{s1} est moins importante qu'à

pH 6.6, ce qui est en relation avec l'augmentation de charge négative. À pH 2.5, la charge électrique de la cas-α_{s1} est positive, et de fortes associations sont observées. La cas-α_{s1} se dissocie en monomère à des forces ioniques faibles 0.003-0.01 et au pH physiologique. À cause de sa stabilité marginale et de sa nature amphipathique, les dimensions moléculaires sont sensibles à la force ionique et si des monomères peuvent être observés à forte force ionique, ceux-ci seraient plus compacts (Swaisgood et Timasheff, 1968).

La présence d'un pôle hydrophile phosphorylé rend la cas-α_{s1} sensible à la présence des ions calcium; la cas-α_{s1} peut être précipitée à des niveaux très bas de Ca^{2+} (7 mM) (Swaisgood, 1982). En présence d'ions calcium, l'association de la cas-α_{s1} à pH 6.6 augmente considérablement et conduit à sa précipitation. L'adsorption de l'ion calcium sur la cas-α_{s1} résulte en la réduction de la charge électrique et la création de sites réactifs par lesquels l'agrégation peut s'initier (Parker et Dalgleish, 1977; Horne, 1979; Dalgleish et Parker, 1979; Dalgleish et Parker, 1980). La coagulation subséquente peut être décrite comme une réaction de condensation polyfonctionnelle (Parker et Dalgleish, 1977) qui est régie par la fonctionnalité du complexe Ca-α_{s1}--caséinate (Dalgleish et Parker, 1979) et par la charge électrique de ces complexes (Horne, 1979).

b) Caséine-α_{s2}

La concentration de la caséine- α_{s2} (cas- α_{s2}) dans le lait est de 3 à 4 g/L. La cas- α_{s2} représente 10% de la fraction caséinique. Le monomère a un poids moléculaire qui oscille entre 25150 et 25400 Da et sa chaîne polypeptidique est composée de 207 résidus d'acides aminés (Lorient, 1991; Swaisgood, 1982). Très peu d'informations sont disponibles sur la structure secondaire de la cas-α_{s2} ; cependant certaines considérations théoriques permettent d'avancer la valeur de 17% d'hélices α (Swaisgood, 1992). La cas-α_{s2} compte 4 variants génétiques; variants A, B, C et D (Whitney, 1988).

Malgré la présence de 2 pôles hydrophobes au sein de la molécule, celle-ci est la caséine la plus phosphorylée et la plus riche en résidus cationiques (Brignon *et al.*, 1977).

Les résidus phosphorylés (de 10 à 13 résidus) sont groupés en trois pôles (résidus 8-16, 56-61 et 129-133) alors que les parties hydrophobes se limitent aux portions 160-207 (séquence C-terminale) et 90-120 (séquence centrale). La valeur d'hydrophobicité calculée de 4.64 kJ/résidu, fait de la cas-α_{s2} la caséine la plus hydrophile. La charge nette calculée de la Cas-α_{s2} à pH 6.6 varie par conséquent entre -13.2 et -18 mV selon le variant génétique (Swaigswood, 1982; Cheftel *et al.*, 1985). D'autre part, les zones chargées négativement (N-terminale 1-68 ; charge nette à pH 6.6 : -21 mV) et les zones chargées positivement (C-terminale 160-207 ; charge nette à pH 6.6 : +9.5 mV) bien individualisées permettent de suggérer que les interactions électrostatiques sont un facteur important des caractéristiques structurales de la protéine et qu'elles dépendent du pH; il en résulte aussi une très grande sensibilité aux ions calcium (Brignon *et al.*, 1977; Swaigswood, 1982). La cas-α_{s2} est la caséine la plus sensible à la présence de calcium dans le milieu. La cas-α_{s2} a une capacité d'association à pH neutre et une sensibilité à la précipitation calcique similaires à la cas-α_{s1}. La présence de deux résidus cystéine au sein de la chaîne polypeptidique peut donner naissance à des dimères covalents de α_{s2} (Walstra et Jenness, 1984).

Le volume spécifique de la cas-α_{s2} est évalué à 0.72 mL/g et son absorption spécifique à 280 nm est estimée à 1.1 cm^2/g (Cheftel *et al.*, 1985).

Le pH isoionique des cas-α_{s2} se situe entre 5.19 et 5.39 (Swaigswood, 1982). La caséine-α_{s2} s'associe très fortement sans pour autant former des micelles. L'association de la cas-α_{s2} est maximale à pH 6.7 (20°C) et avec une force ionique de 0.2. Une hausse ou une baisse de la force ionique, dans ces mêmes conditions, conduit à une réduction de l'association (Snoeren *et al.*, 1980). Les réactions d'association des cas-α_{s2} sont liées à des interactions électrostatiques résultant de la répartition inégale des charges positives et négatives dans la séquence (Snoeren *et al.*, 1980). À force ionique basse, les associations sont peu nombreuses à cause des répulsions électrostatiques faibles entre toutes les molécules chargées négativement. Aux forces ioniques intermédiaires, cette répulsion devient moins importante et les interactions électrostatiques peuvent prendre place entre la

queue positive d'une molécule et le corps négatif d'une autre. Une élévation de la force ionique affaiblit ces interactions et les associations diminuent à nouveau (Schmidt, 1982; Snoeren *et al.*, 1980).

c) Caséine-β

Avec une concentration de 9 à 11 g/L dans le lait, la caséine-β (cas-β) est la deuxième plus importante protéine de la fraction caséinique (34%). La masse moléculaire moyenne du monomère est de 24000 Da (Lorient, 1991; Swaisgood, 1982). La chaîne polypeptidique de la cas-β contient 209 résidus d'acides aminés et elle est caractérisée par une forte teneur en résidus de proline (35 sur 209) répartis assez régulièrement mais de manière non répétitive le long de cette dernière. Cette richesse en résidus prolyle influe sur la structure spatiale de la protéine en raison des courbures que ces résidus induisent. La structure secondaire de la cas-β est donc peu ordonnée, ce qui lui confère une certaine sensibilité aux ions calcium et une flexibilité élevée. La cas-β contient 10% d'hélices α, 13% de feuillets β et 77% de structures non ordonnées (Cheftel *et al.*, 1985; Evans *et al.*, 1971). Pour la Cas-β, 7 variants génétiques sont connus; variants A^1, A^2, A^3, B, C, D et E (Whitney, 1988).

La cas-β avec 111 résidus hydrophobes est la plus hydrophobe des caséines (Ribadeau-Dumas *et al.*, 1972). L'hydrophobicité de la cas-β est de 5.58 kJ/résidu (Cayot et Lorient, 1998) et la charge nette de la molécule varie entre -8.2 et -12.8 mV (Swaisgood, 1982). C'est aussi la protéine dont la répartition en une zone hydrophobe (C-terminale) et une zone hydrophile (N-terminale) est la plus marquée. La séquence N-terminale (1-21) porte une charge nette d'environ –12 mV à pH 6.6, alors que le domaine hydrophobe (49-90 et 149-168) n'aurait pas de charge nette. Au total, le segment 49-209 est très peu chargé et très hydrophobe (Swaisgood, 1982). Les 5 résidus de phosphosérine que possède la cas-β et qui sont situés sur la seule zone 1-37 (4 sur la zone 1-21), ainsi que la présence de 7

groupes carboxyliques sur cette zone 1-21, expliquent la forte électronégativité de la zone N-terminale et donc son caractère très hydrophile.

Les valeurs mesurées expérimentalement de volume spécifique et d'absorption à 280 nm sont respectivement 0.741 mL/g (Noelken et Reibsteins, 1968) et 0.46 cm^2/g (Thompson et Pepper, 1964).

La Cas-β a un pH isoionique plus élevé que la cas-α_{s1}, avec une valeur de 5.35 mesurée expérimentalement pour le variant A (Ho et Waugh, 1965) et des valeurs calculées allant de 5.11 à 5.53 pour l'ensemble des variants génétiques (Swaisgood, 1982).

La cas-β est la caséine qui possède le pouvoir d'association inter-caséine-β le plus élevé et son association ne se produit pas si la portion hydrophobe de la molécule est clivée (Cheftel *et al.*, 1985). La balance hydrophile/hydrophobe de la cas-β a un rôle essentiel sur la structure tertiaire et les interactions hydrophobes qui permettent à cette protéine de s'associer avec d'autres (Kumosinski *et al.*, 1993 ; Mercier *et al.*, 1972 ; Takase *et al.*, 1980). La masse molaire du polymère est d'autant plus élevée que la force ionique, la température et la concentration en cas-β sont élevées (Payens et Vreeman, 1982). La température exerce une influence considérable sur les interactions hydrophobes; à 4°C, seuls des monomères sont présents, mais déjà à 8.5°C les associations sont considérables (Payens et Van Markwijk, 1963). La dépolymérisation à 4°C s'explique par la rupture de nombreuses interactions hydrophobes (Schmidt, 1982; Payens et Van Markwijk, 1963). Le degré de polymérisation à 20°C de la cas-β varie de 13 à 49 unités, pour des forces ioniques allant de 0.05 à 0.2 (Payens et Van Markwijk, 1963; Payens *et al.*, 1969; Evans *et al.*, 1971; Takase *et al.*, 1980); le poids molaire des micelles de cas-β reste donc sujet à controverse.

La Cas-β est moins sensible à la précipitation calcique que ne le sont les caséines-α_{s1} et -α_{s2}. La fixation de calcium et la précipitation à partir d'un seuil critique de

concentration en calcium sont moins marquées qu'avec la cas-α_{s1} et dépendent aussi du degré d'association de la protéine (Cheftel *et al.*, 1985).

d) Caséine-κ

Dans le lait de vache, la caséine-κ (cas-κ) se retrouve à une concentration de 3 à 4 g/L et représente 13% des protéines de la fraction caséinique (Lorient, 1991; Swaisgood, 1982). Elle a un poids moléculaire moyen de 19000 Da. Le monomère compte 169 résidus d'acides aminés sur sa chaîne polypeptidique. La structure secondaire de la cas-κ comprend 5 régions ordonnées en hélices α (23%), 7 régions en feuillets β (31%) et 10 régions en circonvolutions β (24%) (Loucheux-Lefebre *et al.*, 1978). Seulement deux variants sont dénombrés pour la cas-κ; variants A et B (Whitney, 1988).

La cas-κ est, après la cas-β, la plus hydrophobe des caséines. L'hydrophobicité de la cas-κ est de 5.12 kJ/résidu pour une charge nette à pH 6.6 variant de -3 à -3.9 mV (Cheftel *et al.*, 1985). Elle possède un caractère amphipolaire: sa partie N-terminale est hydrophobe alors que sa partie C-terminale est très hydrophile (charge nette -10 ou -11 mV à pH 6.6). La partie C-terminale (150-169) de la cas-κ contient une fraction glucidique composée de galactose, de N-acétylgalactosamine et d'acide N-acétylneuraminique. Les extrémités à tri- ou tétra-saccharides entraînent à elles seules une charge négative globale de -16 à -17 mV(Cheftel *et al.*, 1985; Hill et Wake, 1969). La cas-κ est la seule caséine glycosylée. Son niveau de glycosylation est variable, ce qui modifie son comportement électrophorétique (Cheftel *et al.*, 1985).

Dans le lait, un pont disulfure se forme au sein de la chaîne polypeptidique de la Cas-κ (Cys 11-Cys 88) sous l'action de la disulfhydryl-oxydase membranaire (Mercier *et al.*, 1973; Jolles *et al.*, 1972). Des polymères de cas-κ liés en leur sein par des ponts disulfures peuvent s'associer par des liens non covalents pour former des polymères encore

plus larges avec des poids moléculaires de 600000 à 650000 Da. Ces polymères sont très stables au pH physiologique du lait et ne sont pas dissociés par des changements de force ionique ou de température (Doi *et al.*, 1979; Talbot et Waugh, 1970). La valeur calculée pour le volume spécifique de la cas-κ est de 0.734 mL/g (Cheftel *et al.*, 1985), et la valeur mesurée expérimentalement pour l'absorption à 280 nm est de 0.95 cm^2/g (Talbot et Waugh, 1970).

Le pH isoionique de la Cas-κ varie de 5.37 à 5.64 selon les variants génétiques (Swaisgood, 1982; Ho et Waugh, 1965)

La cas-κ ne contient qu'un seul résidu phosphorylé (Ser-149), ce qui rend cette protéine peu sensible aux ions calcium. La cas-κ est capable de stabiliser jusqu'à 10 fois son propre poids de cas-α_s ou -β contre la précipitation calcique (Walstra et Jenness, 1984). Sa capacité à la stabilisation est perdue après clivage de la présure sur le lien Phe_{105}-Met_{106}, lors d'une coagulation par la présure, qui résulte en la formation d'une portion hydrophobe appellée para-caséine-κ (résidus 1 à 105) et d'une portion hydrophile (résidus 106-169). Le fragment hydrophile est référencé comme étant le glycomacropeptide de cas-κ (GMP) ou caséinomacropeptide (CMP) de masse molaire 33000 Da (Swaisgood, 1982).

Les micelles de caséines

Dans le lait, les caséines interagissent entre elles et avec des ions ou des sels pour former des complexes micellaires volumineux dont le diamètre peut varier de 20 à 600 nm en fonction de divers facteurs (espèce, race, stade de lactation...) (Cheftel *et al.*, 1985; Schmidt, 1982). La masse des micelles oscille entre 2108 et 2109 kDa (Cayot et Lorient, 1998).

a) Composition et structure de la micelle native

La plupart des caséines du lait sont sous la forme de particules colloïdales appelées micelles de caséines (Figure II-1). Ces micelles contiennent entre autres des caséines, du calcium, du phosphate, du citrate, des ions mineurs, de la lipase et du sérum de lait emprisonné (Walstra et Jenness, 1984). La micelle est un complexe calcium-caséinate-calcium-phosphate (Walstra et Jenness, 1984). Le ratio molaire des protéines à l'intérieur de la micelle au moment de leur sécrétion est approximativement α_{s1}:α_{s2}:β:κ = 4:1:4:1.3 (Walstra, 1990) ou environ 38% cas-α_{s1}, 10% cas-α_{s2}, 36% cas-β, et 13% cas-κ (Farrell,1988), mais ce ratio stoichiométrique peut varier entre les différentes submicelles (Cheftel *et al.*, 1985; Schmidt, 1982). Environ 7% de la matière sèche de la micelle est constituée de matériaux inorganiques, principalement du calcium et du phosphate (Walstra, 1990). 68% du calcium présent dans le lait (117 mg de calcium par 100 g de lait) est associé dans la micelle, soit environ 31 mg par gramme de caséine sèche. De la même façon 47% du phosphate présent dans le lait (203 mg de phosphate inorganique par 100 g de lait) est associé aux micelles de caséine. La micelle contient entre autres 0.4 g de citrate, 0.26 g de potassium, 0.11 g de magnésium et 0.11 g de sodium par 100 g de micelle sèche (Schmidt, 1982).

La structure en forme de framboise des micelles a été démontrée par microscopie électronique (Schmidt, 1982, Saito, 1973; Knoop *et al.*, 1973; Knoop *et al.*, 1979). Ce modèle s'appuie à la fois sur les structures primaires et sur les propriétés d'association des caséines. Les micelles de caséines sont formées d'un certain nombre de sous-unités sphérique, appelées submicelles, dont le diamètre varie de 8 à 20 nm (Schmidt *et al.*, 1973; Schmidt et Buchheim, 1970; Schmidt et Buchheim, 1976; Buchheim et Welsch, 1973). La taille des submicelles n'est pas uniforme et est gouvernée par la concentration en protéines, le pH , la force ionique et la température (Schmidt et Buchheim, 1976). Ces submicelles, d'un poids moléculaire de 250 à 2000 kDa (Goff et Hill, 1993), peuvent contenir entre 15 et 25 molécules de caseine (Walstra, 1990). Chaque submicelle résulte d'interactions hydro-

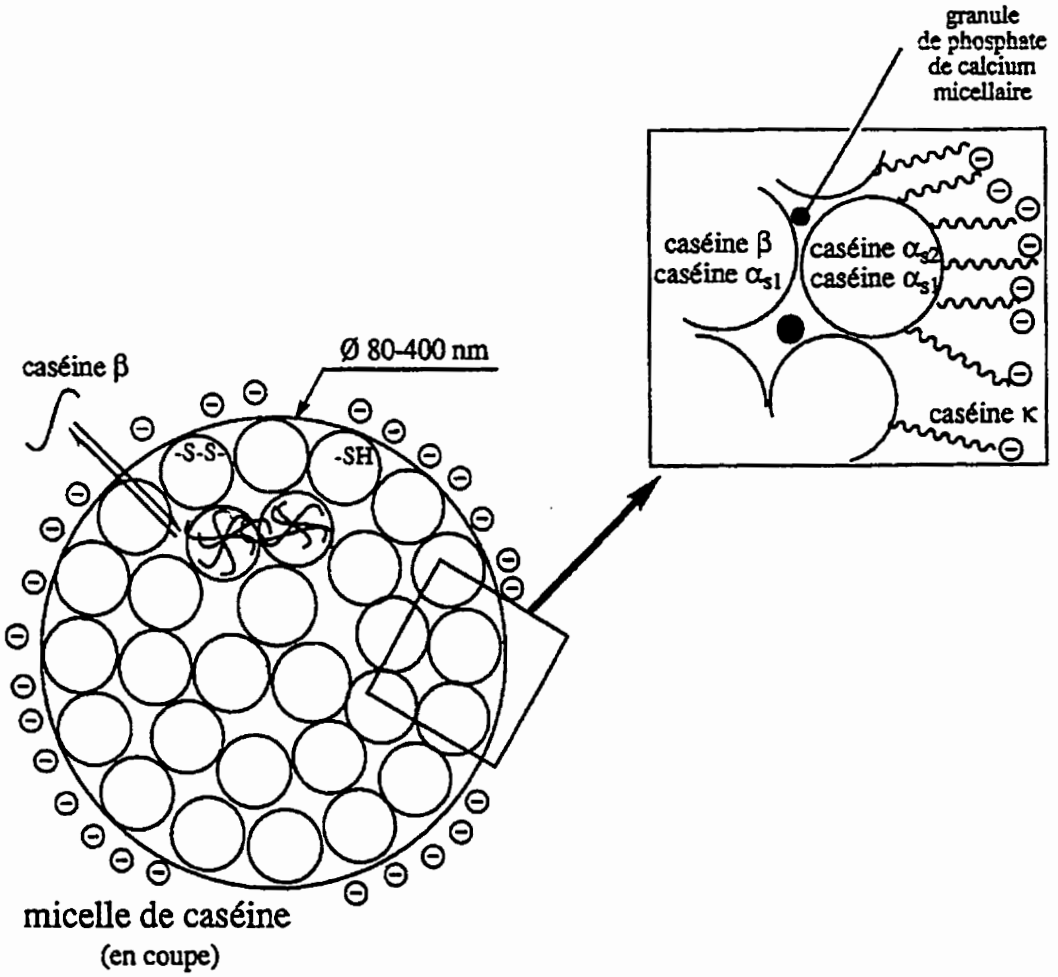

Figure II-1 : Modèle global d'une micelle de caséine (Source Cayot et Lorient (1998)).

phobes (Kumosinski *et al.*, 1996 ; Thurn *et al.*, 1987 ; Stothart, 1989) entre constituants; elle semble dépourvue de minéraux sauf en surface où les ions Ca^{2+} sont fixés sur les résidus phosphoséryl (Kakalis *et al.*, 1990). Les submicelles s'associent entre elles grâce au phosphate de calcium (groupes $Ca_9(PO_4)_6$ (Schmidt, 1982)) et de magnésium "colloïdal", ciment dans lequel le rapport (Ca+Mg)/P est égal à 1.58 (Schmidt, 1980; Schmidt, 1982; Cheftel *et al.*, 1985)(Figure II-2). Le phosphate de calcium colloïdal (PCC) joue par conséquent un rôle prépondérant dans le maintien de l'intégrité des micelles (Payens, 1979). La dégradation du PCC résulte en la dissociation des micelles en submicelles libres qui contiennent ni calcium ni phosphate organique (Lin *et al.*, 1972; Schmidt et Buchheim, 1970). La nature de la liaison entre le PCC et la caséine a été le sujet de nombreuses spéculations. Cependant, Schmidt (1982) a démontré que la liaison entre le PCC et la caséine est d'ordre électrostatique, le PCC étant chargé positivement et la caséine négativement.

Plusieurs observations expérimentales viennent suggérer que la caséine-κ serait surtout localisée à la surface des micelles et que les cas-α_s et -β seraient disposées au coeur de la micelle en association avec du phosphate de calcium colloïdal ; leur arrangement exact n'est cependant pas connu. Schmidt et Payens (1976) et Schmidt (1980) ont remarqué à plusieurs occasions que les petites micelles contenaient une proportion plus grande de cas-κ à comparer au plus grandes micelles, ce qui impliquerait que les submicelles riches en cas-κ occuperaient une position de surface alors que celles avec moins de cas-κ seraient situées au coeur de la micelle. D'autre part, les réactions de la β-lg avec la cas-κ lors du chauffage du lait, de même que la facilité de la chymosine à dégrader la cas-κ, ainsi que les interactions entre la cas-κ des micelles et le carraghenane, confirmeraient la position de surface de la cas-κ (Schmidt, 1982). Une confirmation de la localisation de la caséine κ à la surface de la micelle a été apportée par Kudo *et al.* (1979) par microscopie électronique après coloration spécifique des glucides. Dans les micelles de caséines, la cas-κ joue le rôle de macromolécule stabilisante avec sa partie para-caséine-κ qui est l'ancre et le macropeptide la partie stabilisante. Les autres caséines agiraient en tant que stabilisant, du

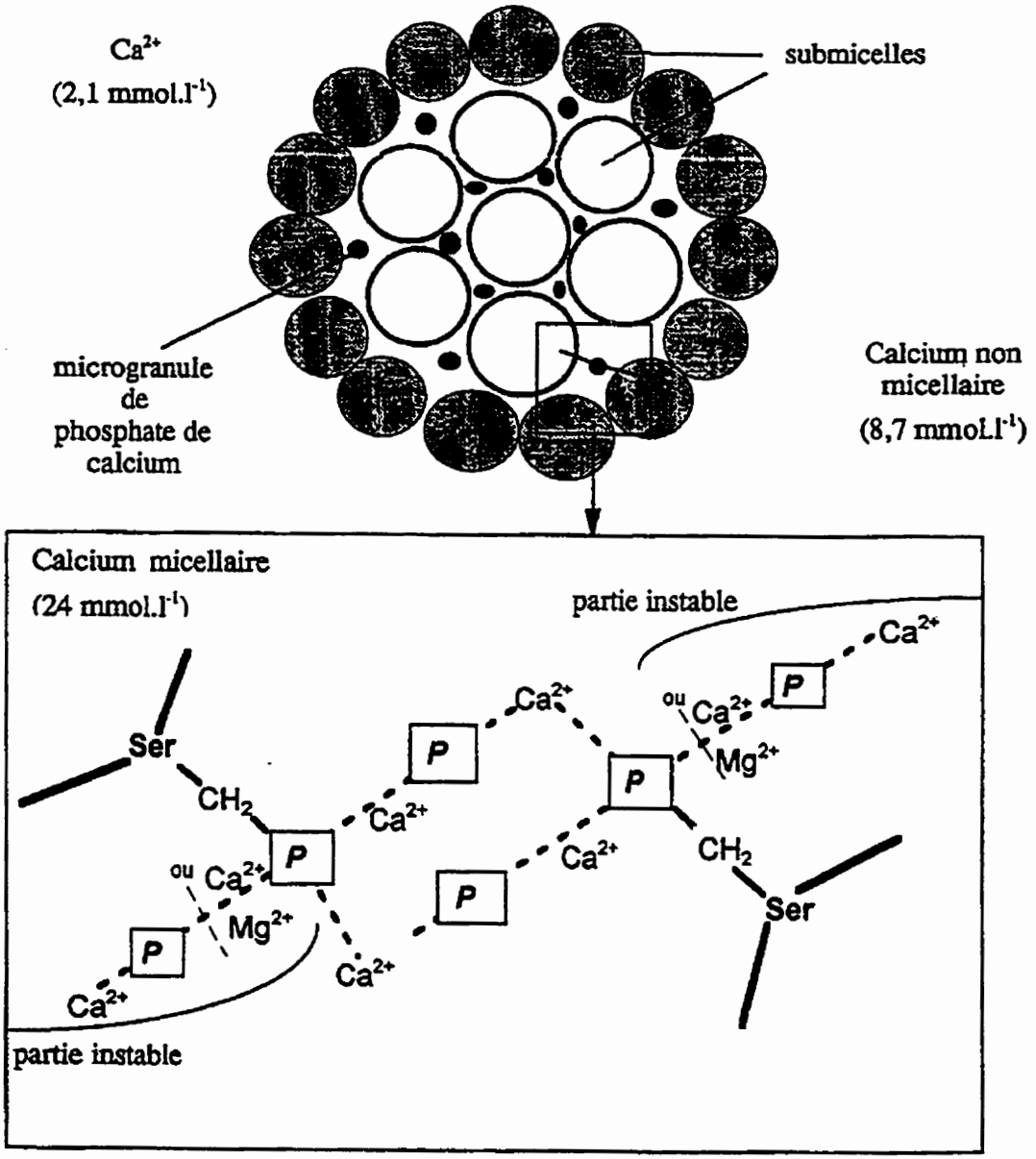

Figure II-2 : «Modèle de liaisons phosphocalciques reliant les caséines (les submicelles?) entre elles» (Source Cayot et Lorient (1998) d'après les modèles de Holt *et al.* (1989) *J. Dairy Res.* 56 : 411-416; Van Dijk (1990) *Neth. Milk Dairy J.* 44 : 65-81, 111-124, 125-141; et les données de Holt *et al.* (1986) *J. Dairy Res.* 53 : 557-572)

fait de la présence de régions très fortement hydrophiles le long de leurs chaînes peptidiques intercalées entre des parties hydrophobes; cependant leur sensibilité aux fortes concentrations de calcium rend cette fonction moins probable (Schmidt, 1982). Il semble cependant que la structure soit suffisamment lâche pour qu'une macromolécule aussi volumineuse que la carboxypeptidase A (enzyme sécrétée par le pancréas) puisse pénétrer dans la micelle et attaquer les caséines-α_s et -β (Ribadeau-Dumas et Garnier, 1970). De plus, la surface de la micelle est extrêmement perméable puisque les moindres modifications du milieu environnant permettent des échanges de minéraux ou de protéines entre le coeur de la micelle et ce milieu.

b) Stabilité de la micelle de caséines

La micelle est extrêmement stable dans certaines conditions de transformation, concentration, ultrafiltration et séchage (Schmidt, 1982), mais très instable en présence d'acides ou de chymosine (Walstra et Jenness, 1984).

Il est surprenant de constater que les micelles résistent fort bien à des températures supérieures à 100°C et à des traitements d'homogénéisation sévères, alors que de faibles variations de la composition du sérum peuvent les déstabiliser (Cheftel *et al.*, 1985).

Ali *et al.* (1980) ont montré que les micelles se dissocient partiellement en submicelles durant le stockage du lait au froid (4 à 7°C) en libérant jusqu'à 50% de la caséine-β en 24h. Par réchauffage, la cas-β se réassocie lentement à ces micelles, mais sa relocalisation aux sites initiaux n'a pas été confirmée.

La stabilité de la micelle à la chaleur dépend étroitement du milieu (pH, Ca^{2+}, autres protéines). Ainsi, la stabilité augmente lorsque la teneur en caséine-κ s'élève ou que celle en phosphate de calcium colloïdal diminue. Pendant le chauffage, la présence de β-lg induit

la formation d'un complexe avec les caséines-α_{s2} et -κ, ce qui améliore la stabilité des micelles vis-à-vis des protéases coagulantes. En ajustant le pH du milieu au point isoélectrique des caséines (pH 4.6 en moyenne), les attractions électrostatiques intra- et inter-protéique sont accrues, ce qui a pour effet de déstabiliser la phase minérale de la micelle (Cayot et Lorient, 1998). Le point isoélectrique est la valeur de pH à laquelle la charge nette des protéines est neutre, et résulte en une précipitation complète de ces dernières. La caséine isoélectrique déminéralisée devient insoluble. L'élimination du calcium par un agent complexant tel que le citrate, phosphate ou EDTA (Lin *et al.*, 1972), provoque une "transparisation" du milieu due à la destruction de la micelle de caséine. L'élimination d'une partie du calcium entraîne une perte de cas-β et une augmentation de la taille de la micelle. La micelle consisterait en un réseau de cas-α_s et -κ dans lequel la cas-β ne joue qu'un rôle de remplissage. L'augmentation de taille peut s'expliquer par le fait que les ions Ca^{2+} exercent un effet de contraction par formation de ponts ioniques.

Le retrait des ions calcium de la micelle cause la dissociation réversible de la cas-β et -κ des micelles sans destructuration de cette dernière, alors que l'ajout de calcium en excés favorise l'agrégation des composés micellaires (Yamauchi *et al.*, 1969 , Lin *et al.*, 1972). La solubilisation minérale à basse température serait responsable de la dissociation de la cas-β, ce qui indique sa responsabilité dans la stabilisation de la micelle (Rose, 1968; Lin *et al.*, 1972; Pierre et Brulé, 1981).

Fonctionnalité des caséines

Les propriétés fonctionnelles des caséines sont l'expression de leurs propriétés physico-chimiques permettant de contribuer aux caractéristiques désirées des aliments dans lesquels ces protéines sont incorporées. Les propriétés fonctionnelles des protéines alimentaires peuvent être classifiées en trois groupes majeurs : a) lés propriétés dépendantes des interactions protéine/eau: absorption et rétention d'eau, mouillabilité, gonflement, adhésion, dispersibilité, solubilité et viscosité ; b) les propriétés reliées aux interactions protéine/protéine : gelification, formation de structure variées telle que des fibres et des

pâtes protéiques ; c) les propriétés associées aux interactions protéines/surface ou interface : propriétés émulsifiantes et moussantes (Cheftel *et al.*, 1985).

Seules, la solubilité, la viscosité, et les propriétés d'émulsion et les propriétés moussantes seront considérées dans le cadre de cette étude.

a) Solubilité

La solubilisation correspond à la séparation des molécules de solvant et de protéines et à la dispersion des molécules de protéines dans le solvant avec un maximum d'interactions entre les protéines et le solvant. Pour être soluble une protéine doit par conséquent être capable de former des interactions avec le solvant (liens hydrogène, interactions dipôle/dipôle ou ioniques).

Deux méthodes standards sont utilisées pour mesurer la solubilité : l'indice de solubilité de l'azote (NSI = Nitrogen Solubility Index) (Anon, 1985a) et l'indice de dispersibilité des protéines (PDI = Protein Dispersibility Index) (Anon, 1985b). Dans les méthode NSI et PDI, le contenu en protéine d'un échantillon et de sa fraction soluble sont estimés par la détermination du contenu en azote par Kjeldahl, Léco ou d'autres méthodes spectrophotométriques, et la solubilité est exprimée par un simple pourcentage.

La solubilité est influencée par la force ionique, l'espèce ionique, le pH et la température. Un profil de solubilité typique des caséines montre que proche du point isoélectrique (pH 4.0-5.0), la forme acide des caséines est complètement insoluble, alors qu'aux valeurs de pH supérieures à 5.5, cette forme est convertie en caséinates (Na^+, K^+, Ca^{2+}) et est complètement soluble. L'addition progressive de sel augmente la solubilité de la protéine (effet de salting-in) ; après avoir atteint un maximum, la solubilité commence à diminuer (effet de salting-out). Ainsi, la solubilité du caséinate de sodium décroît aux valeurs de pH située de part et d'autre du point isoélectrique. À pH 7 ou 9, l'indice de solubilité est de 100% (10 g/L de protéines) mais, en présence de 1 M de chlorure de sodium, la solubilité décroît à 80%. En présence de la même concentration de sel, à pH 2 ou 3, la solubilité baisse de 90% à 75% (Courthaudon *et al.*, 1989). Les effets de salting-in

et salting-out dépendent très fortement du type de sel utilisé, comme cela est indiqué dans la série de Hofmeister (Kinsella, 1984). Ainsi, les caséinates de calcium sont moins solubles que les caséinates de sodium (Bastier *et al.*, 1993); la solubilité du caséinate de sodium peut diminuer à des forces ioniques inférieures à 0.2 M de NaCl. Les caséines sont étonnamment plus solubles à faible température. Celles-ci ont en effet tendance à moins s'associer entre elles lorsque la température diminue (interactions hydrophobes); leur solubilisation s'en trouve ainsi accrue (Kinsella, 1984). En général, la solubilité des protéines augmente pour une hausse de température comprise entre 0 et 40-50°C. Au-dessus de 40-50°C, le mouvement moléculaire devient suffisant pour briser les liaisons engagées dans la stabilisation des structures tertiaires et secondaires. Cette dénaturation est souvent suivie d'une agrégation, auquel cas la solubilité de la protéine diminue par rapport à celle de la protéine native (Cheftel *et al.*, 1985; Kinsella *et al.*, 1985).

b) Viscosité

La viscosité d'un fluide caractérise sa résistance à l'écoulement uniforme et sans turbulence. Le facteur principal influençant la viscosité de fluides protéiques est le diamètre apparent des molécules ou particules dispersées. Ce diamètre dépend des paramètres suivant : a) les caractéristiques intrinsèques de la molécule protéique, tels sa masse moléculaire, sa taille, son volume, sa structure et son asymétrie, ses charges électriques et sa facilité de déformation ; les facteurs environnementaux, tels le pH, la force ionique et la température, peuvent modifier ces caractéristiques suite au déplissement de la molécule ; b) les interactions protéines-solvant, lesquelles influencent le gonflement, la solubilité et l'espace d'hydratation hydrodynamique entourant la molécule ; c) les interactions protéines-protéines, lesquelles déterminent la taille des agrégats (Cheftel *et al.*, 1985; Cayot et Lorient, 1998).

Des viscosimètres à tube capillaire sont utilisés pour mesurer la viscosité de fluide à faible viscosité , alors que des viscosimètres rotationnels permettent la mesure de fluide protéique plus visqueux (Yamamoto *et al.*, 1986; Shoemaker *et al.*, 1992).

La viscosité d'une solution de caséinate de sodium est fortement dépendante du pH, avec un minimum à pH 7.0. À ce pH les charges électriques négatives entraînent un déplissement et une élongation supérieure des protéines. La viscosité des caséines est plus élevée à bas pH (2.5-3.5) qu'à pH neutre. La viscosité des solutions de caséinate de sodium est reliée de façon logarithmique à la concentration en protéines à cause d'une augmentation des interactions protéines/protéines. Les caséinates ont un comportement rhéologique pseudoplastique et agissent comme des systèmes thixotropiques à hauts taux de cisaillement, lorsque la baisse du coefficient de viscosité est réversible. Les cations présents ont un effet significatif sur la viscosité des caséinates, tout en étant étroitement liés au pH, à la température et à la concentration en protéines, car ils entraînent une rupture des liaisons hydrogènes (Mulvihill, 1992; Cheftel *et al.*, 1985). La viscosité d'un caséinate contenant 1% de calcium diminue fortement de façon curvilinéaire entre 30 et 38°C. Elle demeure constante jusqu'à environ 57°C ; la gélification des protéines survient au-delà de cette température à pH 5.4, mais pas à des valeurs de pH plus élevées. De bas niveaux de calcium augmentent la viscosité du caséinate de sodium au-dessus de pH 7.0, alors que la viscosité diminue en–dessous de pH 7.0 suite à la formation de micelles (Mulvihill, 1992).

c) Émulsion

Une émulsion est un système contenant deux phases liquides non-miscibles, l'une étant dispersée dans l'autre sous forme de gouttelettes dont le diamètre varie entre 0.1 et 50µm (Kinsella *et al.*, 1985). La phase présente sous la forme de gouttelettes est appelée la phase interne ou dispersée; la matrice dans laquelle les gouttelettes sont dispersées est la phase externe ou la phase continue.

De nombreuses méthodes telles que le changement de conductivité électrique (Webb *et al.*, 1970), la chute de courant électrique (Crenwelge *et al.*, 1974), ou encore la turbidité du milieu (Pearce et Kinsella, 1978; Cameron *et al.*, 1991) ont été employées pour l'étude des émulsions. Différents termes sont utilisés pour exprimer les résultats des études sur les propriétés émulsifiantes : la capacité émulsifiante, l'indice d'activité de l'émulsion,

la stabilité d'émulsion, l'aire interfaciale et la charge protéique. Seuls les trois derniers termes sont considérés dans cette étude.

La stabilité d'émulsion est l'aptitude d'une émulsion à ralentir, en cours de rupture, sa séparation en une couche aqueuse et/ou une couche de lipide ; elle est exprimée en m^2/mL d'émulsion. La capacité d'une protéine à stabiliser une émulsion est reliée à son abilité à s'adsorber à l'interface huile-eau. Elle est donc reliée à l'aire interfaciale qui peut être recouverte par la protéine (en m^2/mL d'émulsion). Enfin, la charge protéique correspond à la quantité de protéines adsorbées dans la phase huileuse d'une émulsion (en mg/m^2) (Vuillemard *et al.*, 1990 ; Mulvihill, 1992).

Les propriétés d'émulsion dépendent de la solubilité des protéines, de leur concentration, du pH, de la nature du sel et de la force ionique, et des modifications chimiques (Mohanty *et al.*, 1988; Blecker *et al.*, 1997). Les protéines non dissoutes contribuent très peu à la formation d'émulsion car les protéines doivent être en solution et migrer vers l'interface avant que leurs propriétés de surface entrent en jeu (Cheftel *et al.*, 1985). Le pH influe sur les propriétés d'émulsification des protéines de différentes façons. Certaines protéines, à leur point isoélectrique, sont modérément solubles et cela ne leur permet pas de contribuer à la stabilisation de la charge de surface des gouttelettes d'huile et par conséquent à former une émulsion. En revanche, la stabilité de l'émulsion dépend du pH et de la température du système colloïdal. À pH 6 et 8, l'émulsion faite à partir de caséinate de sodium possède une stabilité supérieure à celle obtenue avec un concentré de protéines sériques, quelle que soit la température alors qu'à pH 7 et à 65°C, le résultat est inverse (Cayot et Lorient, 1998). Les caséines, protéines flexibles et capables de se déplisser et de s'étaler lorsqu'elles sont en contact avec la surface lipidique, peuvent établir rapidement des interactions hydrophobes avec les gouttelettes lipidiques pour produire des films adsorbés ayant les propriétés viscoélastiques voulues et stabiliser efficacement les émulsions. Pour leur part, les protéines sériques globulaires possèdent une structure stable et une grande hydrophilicité de surface, ce qui en fait de médiocre agents émulsifiants à moins d'être au préalable déplissée par un traitement thermique modéré (Cheftel *et al.*, 1985).

d) Mousses

Une mousse est une dispersion de bulles de gaz dans une phase continue liquide (Phillips *et al.*, 1990; Britten et Lavoie, 1992). Dans de nombreux cas, le gaz est de l'air ou éventuellement du gaz carbonique, et la phase continue est une solution ou une suspension aqueuse contenant des protéines (Cheftel *et al.*, 1985).

Pour fabriquer une mousse, différentes méthodes sont possibles : faire barboter un gaz dans une solution protéique, procéder à une décompression de la solution en présence d'un faible volume d'air, secouer la solution en présence d'air dans un récipient clos, ou encore battre cette solution (battage) (Kinsella, 1984; Cayot et Lorient, 1998). L'évaluation de la propriété moussante se fait par la mesure de la capacité moussante et de la stabilité de la mousse. La capacité moussante est l'aptitude des protéines à générer de la mousse et se mesure par le volume de mousse produite. La stabilité moussante est l'abilité à maintenir la mousse et à résister à la rupture (Huang *et al.*, 1997). Le suivi du drainage au cours du temps est un paramètre hautement discriminant pour déterminer l'aptitude d'une protéine à retarder l'effondrement d'une mousse (Elizalde *et al.*, 1991). Le drainage est l'écoulement du liquide dispersant autour des bulles sous l'action de la pesanteur terrestre ou lors d'une centrifugation ménagée (Cayot et Lorient, 1998). La stabilité de la mousse peut aussi être suivie par une mesure de la taille des bulles ou par granulométrie laser (Kinsella, 1984).

Les propriétés moussantes dépendent et sont influencées par des facteurs intrinsèques comme la solubilité, la taille des protéines et leur concentration, la longueur et la constitution des chaînes d'acides aminés, ainsi que par des facteurs extrinsèques comme le pH, la température, la force ionique, la présence de sels ou d'additifs (Huang *et al.*, 1997; Britten et Lavoie, 1992; Bastiaens *et al.*, 1990). Une solubilité élevée des protéines est un prérequis à une bonne capacité et stabilité des mousses. Cependant, les particules protéiques insolubles peuvent avoir aussi un rôle bénéfique sur la stabilisation des mousses en augmentant probablement la viscosité de surface. Le drainage est d'autant plus faible que la concentration en protéine est forte, quel que soit le type de protéine (Britten et Lavoie, 1992). Les protéines sériques ralentissent moins efficacement le drainage que les

caséinates après 3 minutes de battage (Elizalde *et al.*, 1991), et jusqu'à une concentration protéique de 10% (P/V) à pH 7 et 5 minutes de battage (Britten et Lavoie, 1992). Généralement, les caséinates produisent des volumes de mousses élevés mais ces mousses sont moins stables que celles formées par des blanc d'œuf ou des concentrats de protéines du lactosérum. Les protéines globulaires de haute masse molaire résistent partiellement au déplissement en surface et produisent des films adsorbés épais ayant de bonnes propriétés rhéologiques de surfaces et des mousses stables. De plus, l'ovalbumine, qui représente 54% des protéines de l'albumen d'œuf, est très sensible à la dénaturation de surface, ce qui lui permet de stabiliser les mousses formées à froid (Cheftel *et al.*, 1985). Dans le cas des caséinates, le pH optimum pour la stabilisation des mousses se situe autour de pH 7. À ce pH, une augmentation de température n'affecte pas la stabilité. À pH 6 ou 8, la mousse est relativement stable à température ambiante mais pas à la chaleur. À pH 8, la stabilité pourrait être affectée par un manque de cohésion du film en raison des fortes répulsions électrostatiques. À pH 6, l'augmentation de température pourrait induire des agrégations de caséines par interactions hydrophobes (Cayot et Lorient, 1998). L'addition de sels aux pH éloignés du point isoélectrique améliore la stabilité des mousses de solution de caséinate, probablement en réduisant les répulsions électrostatiques. La cohésion du film s'en trouve renforcée, ce qui ralentit l'éclatement des bulles (Cayot et Lorient, 1998).

LES MÉTHODES DE SÉPARATION DES CASÉINES

De nombreuses méthodes permettent la séparation des deux fractions protéiques du lait : les méthodes classiques de précipitation, les méthodes électrophorétiques, le fractionnement chromatographique et les procédés à membrane (Tableau II-1). Les méthodes classiques de précipitation et les procédés à membrane sont utilisés actuellement en industrie pour la séparation ou le fractionnement car ils permettent des rendements en protéines bien plus important que ceux des deux autres méthodes. Les méthodes électrophorétiques et chromatographiques sont adaptées à l'analyse fine des composants protéiques du lait, mais ne sont pas utilisables à grande échelle. Au niveau industriel, seules les méthodes d'acidifications chimiques ou par la présure sont employées pour la production de caséines.

Tableau II-1 : Tableau non exhaustif des techniques de séparation des caséines.

	PRINCIPES	MÉTHODES	CONDITIONS	RÉFÉRENCES
MÉTHODES CLASSIQUES	Différence de solubilité dans différents systèmes de solvants	Ultracentrifugation	• Sédimentation des micelles de caséines par centrifugation à 40 000-50 000 g pendant 60 à 90 minutes à 37°C après ajustement du lait écrémé à 0.07 M $CaCl_2$ et à pH 6.6.	Mc Kenzie, 1971 Waugh *et al.*, 1962
		Précipitation fractionnée	• Fractionnement de la caséine entière en une phase soluble (caséine-κ) et une phase insoluble (caséines-α_s et β) à 0.25 M Ca^{2+}, à pH 7.0 et à 37°C.	Waugh et Von Hipel, 1956
		Solubilité différentielle	• Préparation de caséine-κ par précipitation des autres caséines dans une solution d'urée par ajustement à 12% d'acide trichloroacétique ou à pH 1.3-1.5 avec de l'acide sulfurique • Solubilisation des caséines dans l'urée 6.6 M à pH 7.0, précipitation du complexe α_s+κ par dilution à 4.6 M, et précipitation de la caséine-β par dilution à 1.7 M et à pH 7.0 • Isolation de la caséine-β par dispersion de caséine acide dans de l'urée à 3.3 M et à pH 4.6.	Swaisgood et Brunner, 1962 Zittle et Custer, 1963 Hipp *et al.*, 1952 Aschaffenburg, 1963.
MÉTHODES ÉLECTROPHORÉTIQUES	Différence de mobilité liée à la charge et à la taille des molécules	Électrophorèse avec ou sans sodium dodécyl sulfate	• Identification des protéines du lait par électrophorèse sur gel d'amidon ou de polyacryl-amide contenant de l'urée et du 2-mercaptoéthanol, en pH alcalin ou acide. • Analyse quantitative des caséines par couplage avec un densitomètre à balayage	Ribadeau-Dumas et Grappin, 1989 Trieu-Cuot, 1981 Mc Lean *et al.*, 1984
		«Isoelectric focusing» (IEF)	• Séparation et identification des différentes fractions caséiniques, par IEF sur gel de poly-acrylamide contenant des ampholytes, de l'urée à 7 M et de 2-mercaptoéthanol à 0.1%. • Phénotypage de l'ensemble des protéines du lait, sur gels de polyacrylamide, dans une solution d'urée 8M et de 2-mercaptoéthanol à 3%.	Trieu-Cuot et Gripon, 1981 Seibert *et al.*, 1985
		Électrophorèse bidimensionnelle sur gel	• Séparation de l'ensemble des caséines, de même que la paracaséine-κ, l'α-lactalbumine et la β-lactoglobuline.	Trieu-Cuot et Gripon, 1981 Miranda, 1983
MÉTHODES CHROMATOGRAPHIQUES	Différence de taille, de charge ou d'hydrophobicité	HPLC en phase inverse	• Séparation des caséines dans un gradient de tampon A (0.1% TFA/eau) de 63 à 33%, et de tampon B (0.096% TFA/acétonitrile 80%) de 37 à 57%..	Jaubert et Martin, 1992
		Chromatographie par échange d'ions	• Analyse quantitative des caséines du lait, sur D.E.A.E.-cellulose, par élution dans un gradient de NaCl et de tampon Tris à pH 8.6 contenant de l'urée à 6M • Préparation des variants A, B et C de caséine-β sur D.E.A.E.-cellulose dans un tampon imidazole de 0.01 M contenant de l'urée à 4.5 M et à pH 7 et ensuite dans un tampon Tris à pH 8.2.	Davies et Law, 1977 Garnier *et al.*, 1964
		Chromatographie d'affinité	• Purification de la caséine-κ sur gel thiol-sépharose en milieu urée (6M) à pH 7.0.	Chobert *et al.*,1981 Nijhuis et Klostermeyer, 1975
		Chromatographie liquide rapide des protéines (FPLC)	• Séparation et dosage des protéines du lait, dans un tampon Tris-HCl, pH 8.6, urée 3.3 M – β mercapto-éthanol.	Cheftel *et al.*, 1985
MÉTHODES MEMBRANAIRES	Différence de pression, taille des pores et nature des membranes	Microfiltration	• Isolation de la caséine-β par microfiltration de Ca-caséinate à 5°C • Isolation de la caséine-β par microfiltration de lait écrémé à 4°C entre pH 4.2 et 4.6 • Microfiltration de lait écrémé, sur membrane de 0.2 µm de diamètre pour la séparation des caséines et des protéines sériques.	Terré *et al.*, 1987 Maubois et Ollivier, 1992 Fauquant *et al.*, 1988
		Ultrafiltration	• Séparation d'une solution diluée de Na-caséinate en un perméat riche en cas-β et un rétentat riche en cas-α_s et κ	Murphy et Fox, 1990

Les caséines présures

Dans le cas des caséines présure, le mécanisme sous-jacent est identique à celui de la production d'un caillé fromager et dépend uniquement de la sensibilité du lien peptidique Phe_{105}-Met_{106} de la caséine-κ à l'hydrolyse par des protéinases acides, composants principaux de la présure (Varnam et Sutherland. 1994).

Dans le procédé de production de caséines présure, de la présure de veau ou microbienne (Southward et Elston, 1976) est ajoutée au lait écrémé dans un ratio de 1:4500 à 1:7000. Le mélange est maintenu à une température d'environ 30°C pendant une heure (Weal et Southward, 1974). Lorsque la coagulation a atteint le stade désiré, le gel est cassé par agitation pour permettre la formation de particules de caillé de taille optimale pour les étapes ultérieures du procédé. Par la suite, le mélange est chauffé à la vapeur à une température de 55°C avant de le séparer du lactosérum. Le coagulum est ensuite lavé plusieurs fois avec de l'eau dans des bassins (Jordan, 1983) ou des tours (Varnam et Sutherland, 1994) avant d'être égoutté par pressage mécanique (Munro et Vu, 1983) ou centrifugation (Munro *et al.*, 1980), et séché (Figure II-3).

Les caséines acides

Pour la production de caséines acides, les trois procédures existantes sont basées sur la précipitation isoélectrique des caséines soit par acidification chimique, échange ionique ou fermentation.

a) <u>Acidification chimique</u>

La production chimique de caséines par un acide minéral comprend le brassage de lait écrémé pasteurisé maintenu à une température de 20°C avec de l'acide chlorhydrique ou sulfurique dilué (0.5 N) pour baisser le pH jusqu'à une valeur de 4.6 (Figure II-3). Le

mélange est chauffé, par injection de vapeur, jusqu'à une température de 50-55°C afin de faciliter l'agglomération des particules de caséines. Après une courte période de résidence dans une ligne de chauffage et dans un bassin d'acidulation (période complétant l'acidification et initiant la synérèse)(Mulvihill, 1989), le caillé résultant est séparé du lactosérum, lavé et séché selon la procédure présentée pour la caséine présure et commune à tous les types de caséines. Au cours de la phase de déstabilisation acide, la formation d'un caillé ou d'un précipité avec des caractéristiques physiques optimales est essentielle afin de faciliter la séparation du lactosérum et les étapes suivantes de rinçage, égouttage, séchage et mouture. Ces caractéristiques optimales sont obtenues en utilisant le bon pH de précipitation et en ajustant la température pour contrôler les propriétés du caillé (Segalen, 1985; Southward, 1993; Varnam et Sutherland, 1994).

En Australie et en Europe, l'acide chlorhydrique est l'acide le plus employé comme agent de coagulation car ce dernier, en tant que sous-produit des industries chimiques, est disponible à un prix très abordable (Southward, 1993).

b) Acidification lactique

L'acidification d'un lait par une culture bactérienne est basée sur le phénomène naturel de fermentation par les bactéries lactiques, généralement la sous-espèce *Lactococcus lactis*, qui acidifient le milieu (Heap et Lawrence, 1984).

La précipitation de la caséine lactique est réalisée par l'ensemencement de lait écrémé pasteurisé avec des ferments de souches bactériennes mixtes ou multiples, suivie d'une incubation à 22-26°C. Durant la période d'incubation de 14-16 heures, les ferments transforment une partie du lactose en acide lactique. Au fur et à mesure que le pH diminue, le phosphate de calcium colloïdal fixé dans les micelles de caséine se dissout progressivement. Lorsque le pH avoisine le point isoélectrique des protéines, un coagulum ou réseau gelifié de caséines ayant une forte capacité de rétention d'eau se forme alors. Le

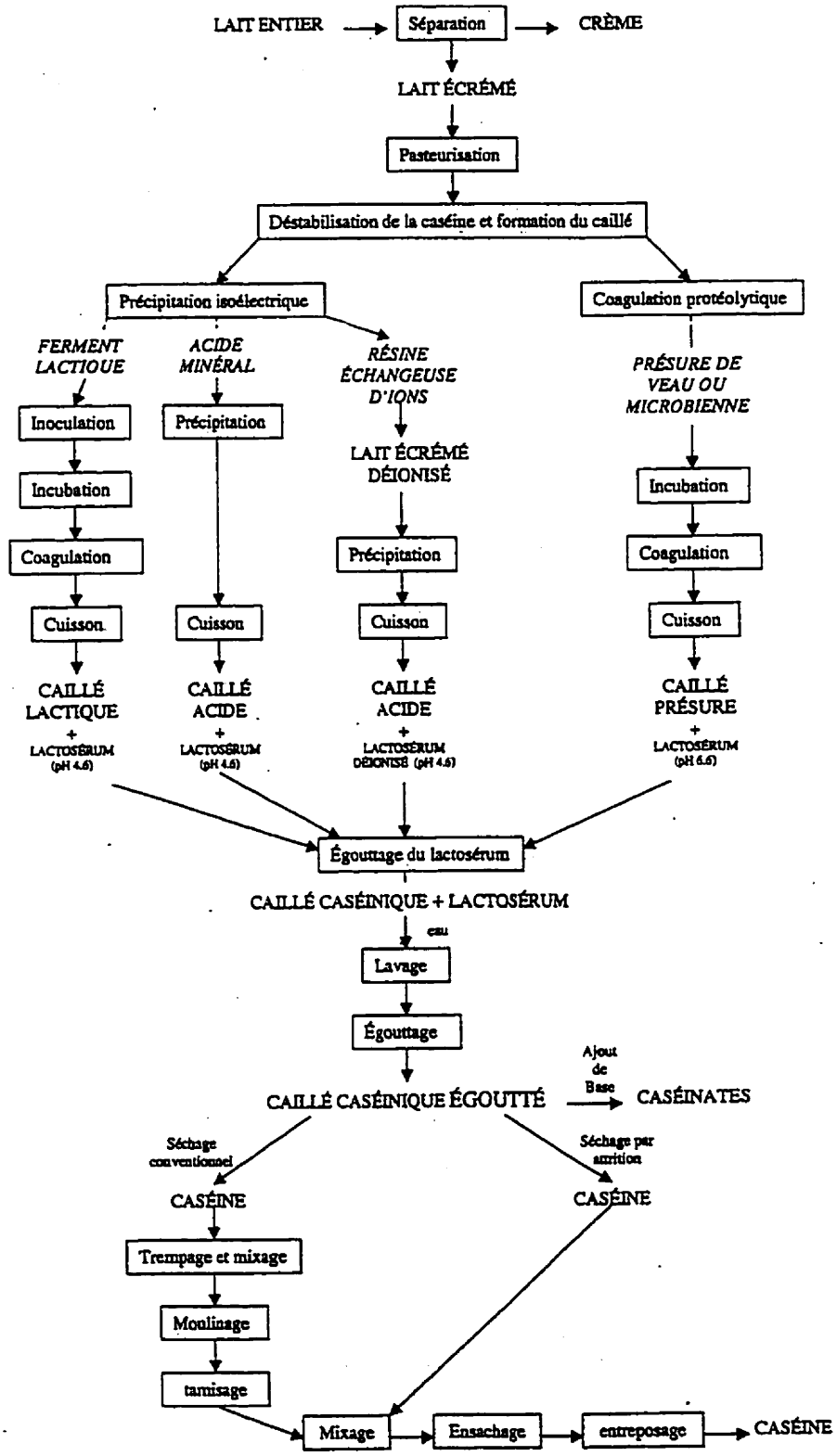

Figure II-3 : Schéma de fabrication des caséines et caséinates (Source Mulvihill (1989)).

coagulum peut ensuite être chauffé et suivre le schéma commun de préparation des caséines ou des caséinates (Segalen, 1985; Southward, 1993; Varnam et Sutherland, 1994) (Figure II-3).

c) Acidification par échange ionique

L'abaissement du pH par l'utilisation de résines échangeuses de cations, dans leur forme hydrogénée, se traduit par une perte des ions Ca^{2+}, Mg^{2+}, K^+ et Na^+ et un gain de protons H^+ provenant de la résine. Ainsi, le lait écrémé est acidifié et partiellement déminéralisé dans le même temps (Mulvihill, 1989).

Une partie du lait écrémé est acidifié à pH 2.2 par mélange avec une résine échangeuse de cations au sein d'une colonne de réaction maintenue à une température inférieure à 10°C. Le lait déionisé est mélangé avec du lait non traité dans des proportions qui permettent d'atteindre une valeur finale de pH conduisant à la précipitation. Le mélange est alors chauffé à la température de précipitation désirée pour induire la coagulation. Le coagulum est par la suite chauffé et préparé conventionnellement (Segalen, 1985; Southward, 1993; Varnam et Sutherland, 1994) (Figure II-3). Cette méthode a été développée à une échelle industrielle par la société Bridel (Rialland et Barbier, 1980) et serait appliquée dans au moins une de leurs usines en France (Pierre et Douin, 1984). Lorsque le procédé d'échange ionique est utilisé à la place de l'addition d'acide minéral, le lactosérum est dépourvu des anions acides, ce qui augmente sa valeur pour d'autres procédés (Rialland et Barbier, 1980). Ce procédé permettrait une augmentation de rendement en caséines de 3 à 5% par rapport aux procédés utilisant des acides minéraux. Cela serait lié à la co-précipitation des protéoses peptones qui sont normalement perdues dans les lactosérums produits lors des acidifications chimique et lactique (Pierre et Douin, 1984).

Les méthodes alternatives

Récemment, d'autres techniques ont été proposées pour la production de caséines acides : acidification du lait par échange ionique couplé à un acide (Salmon, 1983), par électrodialyse du lait écrémé à pH 5.0 suivi d'une acidification à pH 4.6 par un acide (Laiteries Triballat, 1979) ; par acidification à l'aide de protons générés par l'électrolyse des molécules d'eau à l'interface de membranes empilées au sein d'un système d'électrodialyse (ED) (Bolzer, 1985). Cette dernière méthode d'acidification, appelée procédé SAFIR, appliquée à un lait écrémé maintenu à une température de 4°C, permet le transfert et l'échange stoichiométrique des protons et des cations constitutifs du lait : Na^+, K^+, Ca^{2+} et Mg^{2+}. Une élimination des cations par électromigration apparaît de façon simultanée avec un abaissement du pH de la solution par les protons électrogénérés. Ainsi, dans ce procédé, un réservoir principal contenant des caséines à pH 2.5 est utilisé comme contrôleur de pH du procédé. Un volume calculé de lait est ajouté aux caséines acides pour atteindre un pH de 3.5. Le mélange est alors circulé dans la cellule d'ÉD, dont le pH de sortie est de l'ordre de 1.8 à 2.4. Une partie de cette solution caséinique très acide est ensuite utilisée pour maintenir le pH du réservoir principal, alors que le reste est envoyé vers les autres étapes du procédé de fabrication de caséines.

Les méthodes alternatives ont comme principal avantage de produire des lactosérums acides à teneur réduite en minéraux. Ce lactosérum est par conséquent plus facilement utilisable que les lactosérums acides produits par les procédés conventionnels (Southward, 1993).

Les techniques de production de caséine utilisées en industrie ont dans la majorité des cas le désavantage de générer de grands volumes d'effluents chimiques, dus à l'ajout de bases et d'acides durant les traitements, et d'engendrer des risques inhérents liés à la manipulation, au stockage et au transport des acides et des bases fortes. Dans le cas des fermentations, il est nécessaire d'utiliser des ferments mixtes et de les changer régulièrement afin d'éviter les contaminations par les phages. D'autre part, au cours de la fabrication des caséines présure, la cas-κ est dénaturée par formation de deux molécules, le caséino-

macropeptide et la para-caséine-κ (Cayot et Lorient, 1998 ; Cheftel *et al.*, 1985). Enfin pour ce qui est du procédé SAFIR, il a été démontré que la génération de protons à la surface des membranes anioniques et cationiques se fait avec une moins grande efficacité électrique que lors de l'utilisation dans l'électrodialyseur de membranes spécifiques bipolaires (Bazinet *et al.*, 1996 ;1997c).

L'ÉLECTRODIALYSE AVEC MEMBRANES BIPOLAIRES

Cette technologie est dérivée de l'électrodialyse avec membranes monopolaires. Les principales utilisations actuelles de l'électrodialyse avec membranes bipolaires, une technologie récente, sont orientées vers la génération de bases et d'acides (Mani, 1991 ; Nagasubramanian *et al.*, 1977 ; Mani *et al.*, 1988)

L'électrodialyse

a) Principe et généralités

L'électrodialyse (ÉD) est un procédé électrochimique basé sur la migration d'espèces ioniques (chargées électriquement) à travers des membranes monopolaires à perméabilités sélectives, sous l'influence d'un champ électrique créé par l'application d'une différence de potentiel entre deux électrodes plongées dans une solution aqueuse riche en espèces ionisées minérales ou organiques (Gardais, 1990 ; Chaput, 1979). La continuité électrique est assurée par le flux des ions (anions et cations) porteurs du courant.

Au début du vingtième siècle, l'utilisation de membranes neutres avec de fortes résistances électriques inhérentes rendait l'ÉD peu efficace au niveau énergétique et par conséquent relativement coûteuse à opérer. Ainsi, dans les années cinquante, la fabrication de membranes synthétiques échangeuses d'ions à faibles résistances électriques , par Juda

et McRae (1950) aux États-Unis et Kressman (1950) au Royaume-Uni, relança l'intérêt industriel pour l'ÉD. Aujourd'hui, l'utilisation de membranes sélectives performantes permet une plus grande versatilité de l'ÉD. Ainsi, l'ÉD est une technologie mature avec de nombreux systèmes en opération de par le monde. En Europe et au Japon, l'ÉD domine en temps que procédé de déminéralisation, respectivement pour le lactosérum et l'eau de mer, avec une capacité de l'ensemble des installation dépassant celle de l'osmose inverse et de la distillation (Shaposhnik et Kesore, 1997). L'ÉD est appliquée ou peut être appliquée à de nombreux systèmes pour la déminéralisation du lactosérum (Glassner, 1992; Houldsworth, 1980; Batchelder, 1986; Pérez *et al.*, 1994), des acides organiques (Jain et Reed, 1985) et des sucres (Maigrot et Sabates, 1890; Cassel et Kempe, 1894; Chaput, 1979), la séparation des acides aminés (Kikuchi *et al.*, 1995), la stabilisation des vins (Guérif, 1993; Audinos *et al.*, 1979, 1985; Escudier *et al.*, 1995), la déacidification des jus de fruits (Lamarche et Boulet, 1992) et la séparation des protéines (Jain, 1983 ; Reed, 1984; Amundson *et al.*, 1982; Slack *et al.*, 1986). Ces applications utilisent le principe de dilution-concentration par empilement de membranes au sein d'une cellule d'électrodialyse pouvant aller jusqu'à 300 membranes échangeuses d'ions monopolaires.

Dans un électrodialyseur, des membranes perméables aux anions et perméables aux cations, sont disposées alternativement dans des modules de type filtre presse (Figure II-4). Un cadre séparateur équipé d'un grillage, qui sert à la fois de support pour les membranes et de promoteur de turbulence, est placé entre chaque membrane. Le motif élémentaire ainsi constitué représente une cellule à deux compartiments : un compartiment de dilution et un de concentration. Un empilement de cellules forme un module. Aux extrémités de chaque module se trouvent les électrodes dans des compartiments à circuits indépendants. Chacun des compartiments ainsi formés, par la solution à traiter, et les solutions aux électrodes, a son circuit relié à son propre réservoir, dont le contenu est régulièrement remis en circulation par une pompe. Dans la pratique, un électrodialyseur est constitué d'un grand nombre de compartiments alimentés en série du point de vue électrique et en parallèle du point de vue hydraulique (Houldsworth, 1980).

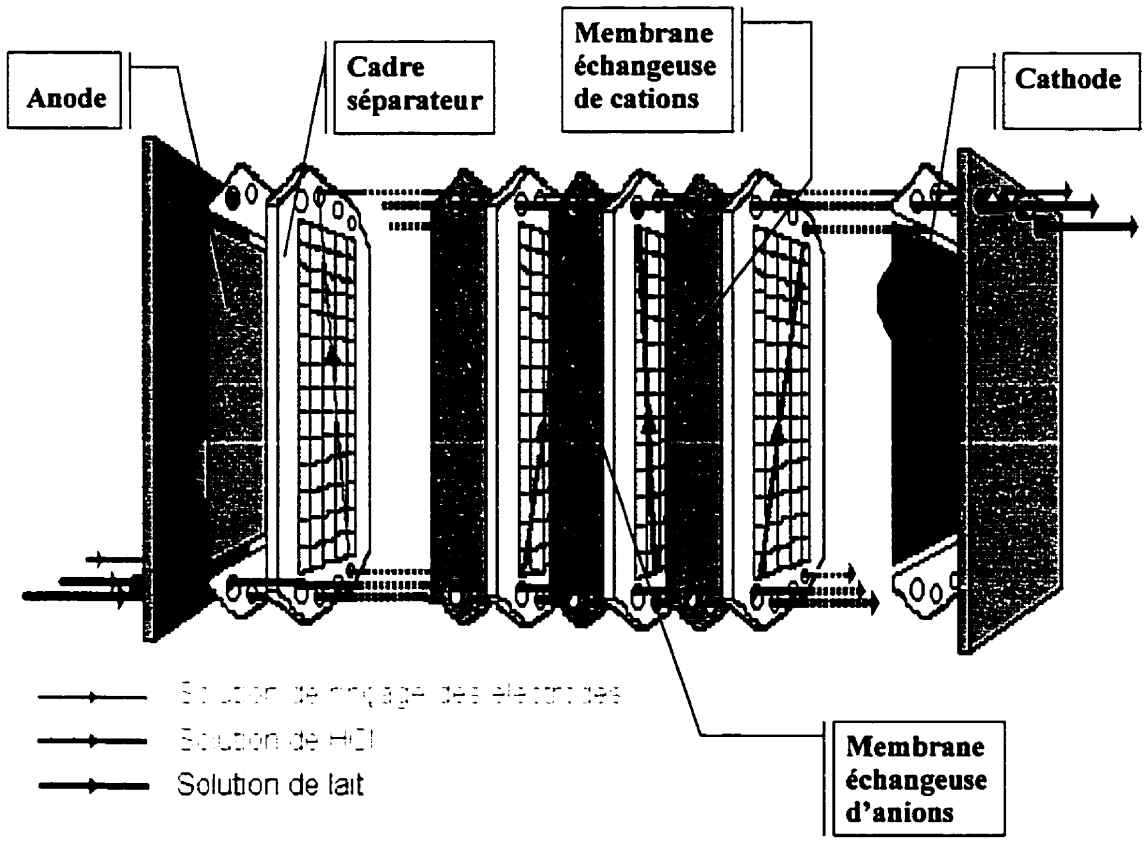

Figure II-4 : Principe de fonctionnement d'un électrodialyseur.

Du point de vue électrique, un module d'électrodialyse doit être considéré comme une résistance résultant d'une suite de résistances élémentaires montées en série et constituées par les membranes et les couches de concentrat et de diluat intermembranaires. Une partie de l'énergie mise en jeu pour l'électrodialyse se dissipe sous forme de chaleur, pouvant provoquer un échauffement important du produit et du matériel, lorsque l'intensité de travail est supérieure à celle préconisée par le fabricant ; elle est généralement bien plus élevée que celle correspondant à la valeur du courant limite (Mafard et Béliard, 1992). Le courant limite est la valeur maximale de courant affectée au transport des ions qu'un système d'électrodialyse peut supporter sans que n'apparaisse de dissociation des molécules d'eau.

b) Transfert de masse

Le transfert de masse au travers des membranes ioniques se fait en deux étapes (Korngold, 1984) : 1) la réduction de la concentration en sels dans la solution de diluat par électrotransport des ions de la couche limite proche de la membrane et 2) la diffusion des ions jusqu'à la couche limite partiellement désalinée.

Lors de la mise sous tension d'une cellule d'électrodialyse, il apparaît une variation du profil des concentrations aux abords des membranes, dans les couches limites relatives au diluat et au concentrat, entre la zone de turbulence des solutions et la membrane. Après un court régime transitoire, des gradients de concentration s'établissent dans les couches limites, où la diffusion fournit le flux complémentaire d'ions nécessaires au maintien d'un courant stationnaire (Figure II-5a).

Une augmentation de la tension appliquée aux bornes du module d'ÉD accroît la densité de courant et les gradients de concentration dans les couches limites, de manière à permettre un accroissement de la force motrice pour la diffusion. Le flux d'ions par électrotransport est aussi augmenté jusqu'à ce que la concentration des solutions dans les couches limites MEC-diluat et MEA-diluat approche zéro. La résistance des couches limites du diluat augmente fortement et la densité de courant plafonne à une valeur limite (Figure II-5b). Dans ces conditions le flux d'ions par diffusion est maximum.

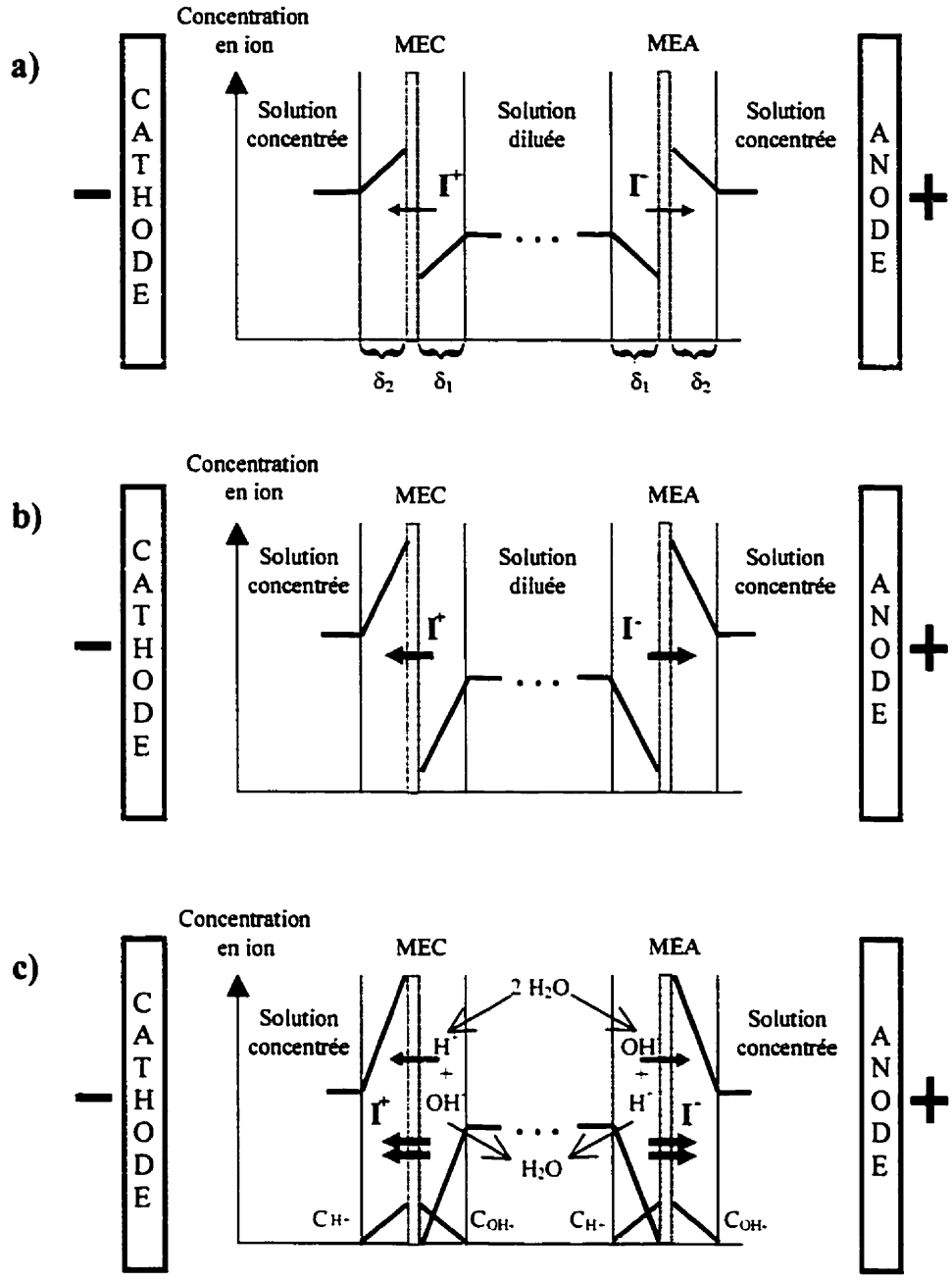

Figure II-5 : Phénomène de concentration de polarisation en électrodialyse (Source Bazinet *et al.* (1998c)): **a)** formation des gradients de concentration, **b)** atteinte de la densité de courant limite et, **c)** dépassement de la densité de courant limite et hydrolyse irréversible de l'eau.

Lorsque la valeur du flux par électrotransport est égale à la valeur du flux maximum par diffusion, l'unité d'électrodialyse fonctionne à son optimum de transfert de masse.

c) Densité de courant limite

Une augmentation subséquente de la tension aux bornes du module d'ED augmente la densité de courant et non plus le transfert de masse. La plus grande partie de ce courant additionnel sert alors à la dissociation des molécules d'eau (Brun, 1989; Korngold, 1984). En effet, les contre-ions nécessaires au transport dans la membrane doivent être générés au niveau des interfaces membranes-diluat par un mécanisme autre que la diffusion. Ainsi, la dissociation de l'eau libère des ions H^+ et OH^- immédiatement consommés par le transport ionique (Figure II-5c). Cela entraîne une forte augmentation de pH à l'interface MEC-diluat et MEA-concentrat, et une diminution proportionnelle, aux interfaces MEA-diluat et MEC-concentrat.

Dans la pratique de déminéralisation des solutions, les deux tiers de la densité de courant limite ne sont pas dépassés afin que cette densité limite ne soit pas atteinte en aucun point de l'électrodialyseur. Ainsi, l'électrodialyse est limitée par le transfert de masse, mais une augmentation de la vitesse d'écoulement accroît la densité de courant limite.

Les membranes ioniques

Les membranes échangeuses d'ions (ou sélectives aux ions), qui sont en fait des résines échangeuses d'ions laminées, sont au coeur du procédé d'ÉD.

a) Principe

Une membrane échangeuse d'ions (MEI) est constituée d'un matériau généralement macromoléculaire (squelette) portant des groupes ionisables (Figure II-6). Une fois imbibée

d'un solvant dissociant tel que l'eau, la membrane contient des ions fixes, solidaires du squelette, électriquement neutralisés par des charges mobiles de signes opposés, les ions compensateurs ou contre-ions (Bazinet *et al.*, 1996). Sous l'influence d'un courant électrique, les contre-ions peuvent s'échanger par diffusion entre la membrane et la solution adjacente (Gardais, 1990). Ils migrent d'un site vers un autre site au sein du réseau des groupements ioniques fixés sur la matrice de polymère et traversent la membrane. En parallèle, les co-ions, charges mobiles de même signe que celui des groupement ionisés fixés sur le squelette, sont rejetés du corps de la membrane ; cette répulsion électrostatique est appelée exclusion de Donnan (Donnan, 1911 ; Korngold, 1984).

b) Les membranes monopolaires

Il existe deux types principaux de MEI basés sur la nature des groupes fonctionnelles fixés sur la matrice de polymère : les membranes perméables aux cations appelées membranes échangeuses de cations ou cationiques (MEC) et des membranes perméables aux anions, appelées membranes échangeuses d'anions ou anioniques (MEA). Ces membranes sont des membranes monopolaires, c'est-à-dire perméable à un seul type d'ions. Les principaux groupements ioniques utilisés comme charges fixes dans les MEC sont des groupements sulfoniques ($-SO^{3-}$) et carboxyliques ($-COO^{-}$), et plus rarement des groupements arsonique ($-AsO_3^{2-}$) et phosphoriques ($-PO_3^{2-}$) (Juda et McRae, 1953; Evers, 1948; Bodamer, 1952; de Korosy et Schorr, 1963). Dans les MEA, les principales charges fixes sont des groupements alkyl ammonium ($-NR^{3+}$, $-NHR^{2+}$, $-NH_2R^{+}$), phosphnium ($-PR_3^{+}$) ou sulfonium ($-SR_2^{+}$) (Guérif, 1993; Hurwitz, 1999).

Les caractéristiques les plus importantes des MEI utilisées en ÉD sont une résistance électrique faible, une bonne permsélectivité aux cations et aux anions, de bonnes propriétés mécaniques, une bonne stabilité physique et une haute stabilité chimique (Korngold, 1984; Strathmann, 1990). Les propriétés des MEI sont déterminées par deux paramètres ; le type de la matrice polymèrique et le type et la concentration des groupes

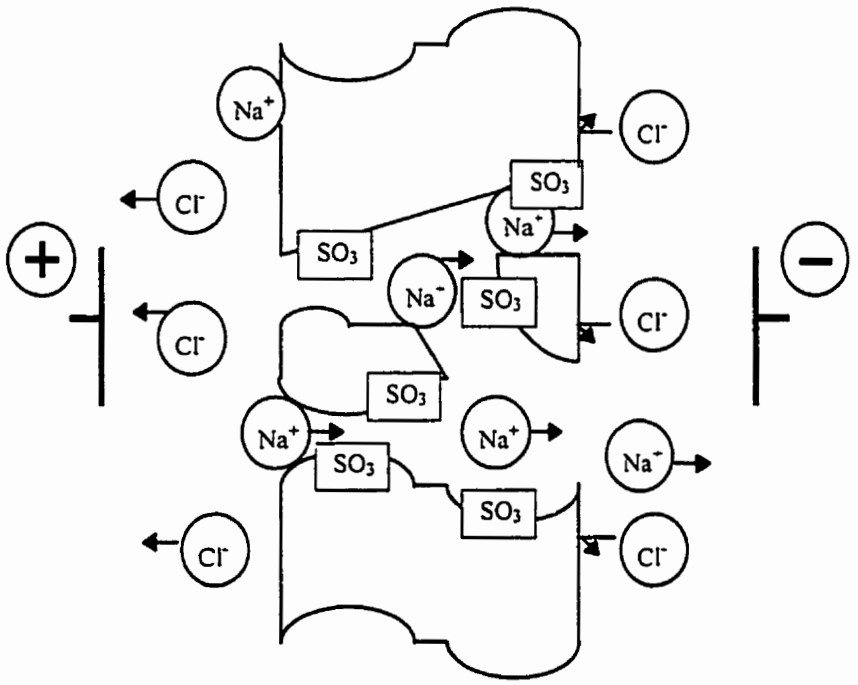

Figure II-6 : Principe de fonctionnement d'une membrane échangeuse de cations (Source Bazinet *et al.* (1996)).

ioniques fixés. La matrice de polymère détermine pour une grande part, la stabilité mécanique, chimique et thermique de la membrane. Très souvent la matrice de la MEI consiste en un polymère hydrophobe comme le polystyrène, le polyéthylène ou le polysulfone (Streat et Cloete, 1987). Bien que ces trois polymères soient insolubles dans l'eau et qu'ils montrent un bas niveau de gonflement, ils peuvent devenir solubles dans l'eau par l'introduction de groupements ioniques. Par conséquent, la matrice polymèrique de la MEI est très souvent composée de liens entrecroisés. Le degré d'entrecroisement des liens détermine alors pour une large part le degré de gonflement ainsi que la stabilité chimique et mécanique, en plus d'avoir un effet important sur la résistance électrique (Meares, 1983; Spiegler et Laird, 1980). Le degré de liens entrecroisés établit aussi le niveau de restriction aux ions de la matrice structurelle et sa porosité ou plus simplement la taille des pores (Streat et Cloete, 1987). La taille des pores des membranes commerciales peut varier de 10 à 100 Å suivant l'application visée. La taille des pores définit la perméabilité aux ions. Les membranes lâches ont une taille de pores de l'ordre de 100 Å permettant une perméabilité aux ions du même signe que ceux fixés sur la matrice plus élevées que celle des membranes serrées dont la taille des pores, de l'ordre de 20 Å, ne présente qu'une perméabilité minime aux co-ions. Une membrane typique d'ÉD a des pores d'environ 10 à 20 Å avec une capacité de 1.6 à 3.0 méq par gramme de résine sèche (Jain et Reed, 1985).

Les MEC employées généralement en électrodialyse sont des membranes monocouches. Des MEC particulières formées de trois couches ont été développées pour obtenir une permsélectivité aux ions monovalents. De chaques cotés de la couche échangeuse de cations, des polymères électrolytiques, avec une charge électrique différente de celle de la couche échangeuse de cations sont appliqués en surface. La permsélectivité au ions monovalents ainsi obtenue peut s'apparenter à un effet de filtration, car la similitude est frappante avec l'effet de piégeage des ions divalents par un filtre (Mizutani, 1990; Streat et Cloete, 1987).

Des efforts tout particuliers ont été portés sur le développement de membranes échangeuses d'ions ayant des tendances faibles au colmatage et à perméabilités hautement sélectives. Le colmatage donne lieu à une augmentation de la résistance (chute de courant), à une diminution du rendement (permsélectivité abaissée) et à une altération des membranes (réversible ou irréversible) (Ogata *et al.*, 1989 ; Davis *et al.*, 1997 ; Momose *et al.*, 1991). Le colmatage des MEI peut être de trois types : entartrage par formation d'hydroxyde d'ions divalents et trivalents, encrassement par des protéines ou des matières colloïdales ou empoisonnement des membranes par des agents tensio-actifs (Gavach, 1999). Momose *et al.* (1991) ont démontré, à l'aide d'un spectromètre par dispersion d'énergie des rayons X, la formation de complexes d'hydroxyde de magnésium et de calcium au sein et en surface de membranes utilisées dans l'industrie chlore-soude.

c) Les membranes bipolaires

Un nouveau type de membrane, appelée membrane bipolaire, est apparu commercialement à la fin des années 1980. Les membranes bipolaires réalisent la dissociation des molécules d'eau en présence d'un champ électrique (Mani, 1991) (Figure II-7). Elles sont conçues pour fonctionner dans des conditions de courant limite et nécessitent des valeurs de courant limite beaucoup plus faibles que celles des membranes monopolaires (Bazinet *et al.*, 1996).

Les membranes bipolaires sont dans la majorité des cas préparées en structure composite multicouche et formées de 3 régions : une couche échangeuse d'anions à permsélectivité élevée et ayant une excellente stabilité en solution alcaline; une couche échangeuse de cations de permsélectivité équivalente; une couche intermédiaire hydrophile, à leur jonction, avec des groupements ammonium tertiaire qui augmentent catalytiquement le taux de dissociation de l'eau (Strathmann *et al.*, 1993). Sous l'influence du champ électrique, l'eau diffuse à l'interface de la membrane où elle est dissociée en ions hydrogène (H^+) et hydroxyle (OH^-). Les ions H^+ et OH^- migrent respectivement à travers

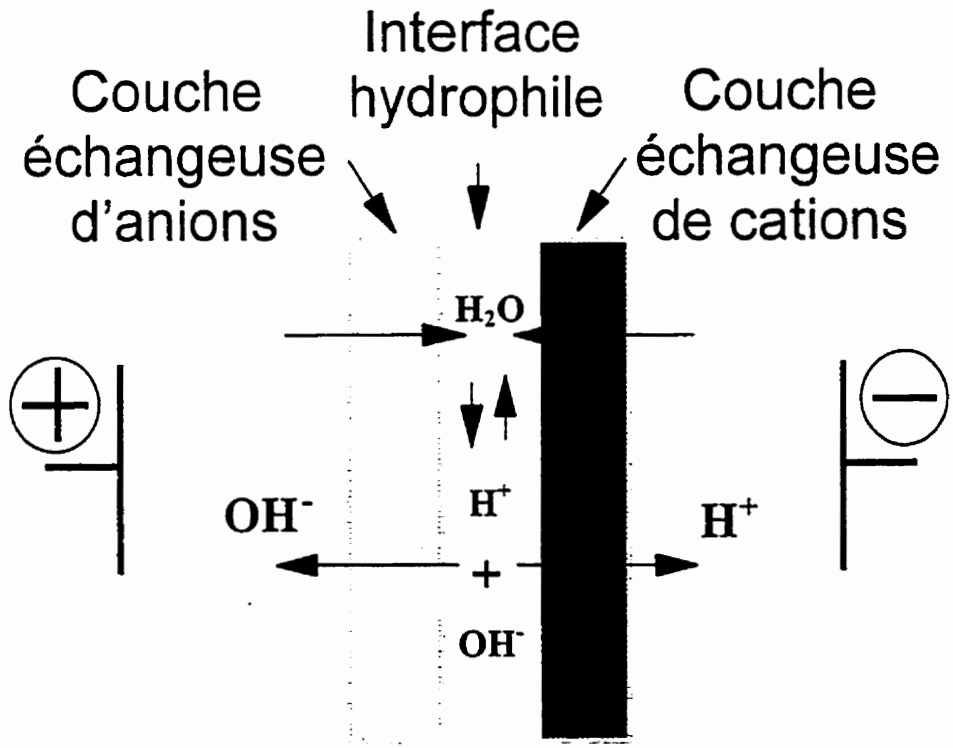

Figure II-7 : Principe de fonctionnement d'une membrane bipolaire (Source Bazinet *et al.* (1998c)).

les couches cationique et anionique vers les compartiments situés de part et d'autre de la membrane pour assurer le transport du courant (Mani, 1991).

Pour une efficacité optimale dans des applications pratiques, une membrane bipolaire doit présenter les caractéristiques suivantes : une résistance électrique faible à haute densité de courant, un taux de dissociation de l'eau élevé, un taux de transport des co-ions faibles, une forte sélectivité aux ions ainsi qu'une bonne stabilité thermique et chimique aux acides et bases forts. La faible résistance électrique des couches échangeuses d'anions et de cations des membranes bipolaires peut être obtenue par l'utilisation de groupements sulfoniques acides ($-SO_3^-$) pour la couche cationique et d'amines quaternaires ($-NH_3^+$) pour la couche anionique comme charges fixes à haute concentration dans la matrice de polymère. Pour minimiser la résistance de l'interface entre les deux couches de sélectivité opposée, cette interface doit avoir une épaisseur aussi mince que possible. Enfin, les groupes ioniques dans l'interface doivent avoir un taux de transfert des protons élevé et leur concentration doit être grande (Strathman *et al.*, 1993).

Applications de l'électrodialyse avec membranes bipolaires

L'électrodialyse avec membrane bipolaire (ÉDMBP) combine l'action de déminéralisation des membranes monopolaires et la particularité unique des membranes bipolaires à dissocier les molécules d'eau. L'ÉDMBP fonctionne dans deux sens : l'électroacidification (ÉAMBP) et l'électrobasification (ÉBMBP). Le pH d'une solution circulant à l'intérieur d'une cellule d'électrodialyse du côté cationique de la membrane bipolaire, où les H^+ sont générés, diminuera. De façon similaire, le pH d'une solution circulant du côté anionique de la membrane bipolaire, où les OH^- sont produits, augmentera.

a) Applications industrielles existantes ou potentielles

Les premières applications industrielles des membranes bipolaires ont été orientées vers la production d'acide et de base à partir d'une solution saline. L'alimentation d'un électrodialyseur avec une solution de sels et l'application d'une différence de potentiel aux bornes des électrodes conduisent à la conversion de la solution aqueuse de sels, tel que le chlorure de sodium, en la base NaOH et l'acide HCl (Figure II-8). Ainsi, les sels tels que le KF, KNO_3, NH_4Cl, Na_2SO_4, KCl, etc..., de même que des sels organiques, peuvent aussi être dissociés pour former les acides et les bases correspondants. Le principal produit industriel bénéficiaire de l'usage des membranes bipolaires est évidemment la soude caustique (Hurwitz, 1999). Cependant, si ce secteur économique reste déterminant pour le marché des membranes bipolaires, il faut aussi tenir compte de toute les applications importantes de ces membranes. La toute première installation industrielle utilisant les membranes bipolaires a été implantée en 1988, à l'usine Washington Steel en Pennsylvanie ; la régénération des acides HF et HNO_3 des bains de décapage d'acier inoxydable permettait la récupération totale des acides employés. Depuis 1994, une installation d'ÉDMBP a été établie à Batôn Rouge en Louisiane ; elle permet la régénération du KOH utilisé pour l'absorption d'HF gazeux, par réaction d'alkylation, dans les raffineries de pétrole. Il existerait à l'heure actuelle deux à trois installations en opération aux États-Unis, produisant des acides organiques et inorganiques spéciaux (Hurwitz, 1999). L'ÉDMBP permet aussi la récupération d'acide sulfurique et de soude caustique à partir de sulfate de sodium résiduaire dans les industries chimiques de la viscose et de la pâte à papier ainsi que la régénération d'acide chlorhydrique et de soude diluées pour les résines échangeuses d'ions (Hurwitz, 1999). Récemment, Boyaval *et al.* (1993), Siebold *et al.* (1995) et Alvarez *et al.* (1997) ont utilisés cette technologie pour les productions respectives à l'échelle laboratoire d'acides propionique, lactique et salicylique concentrés.

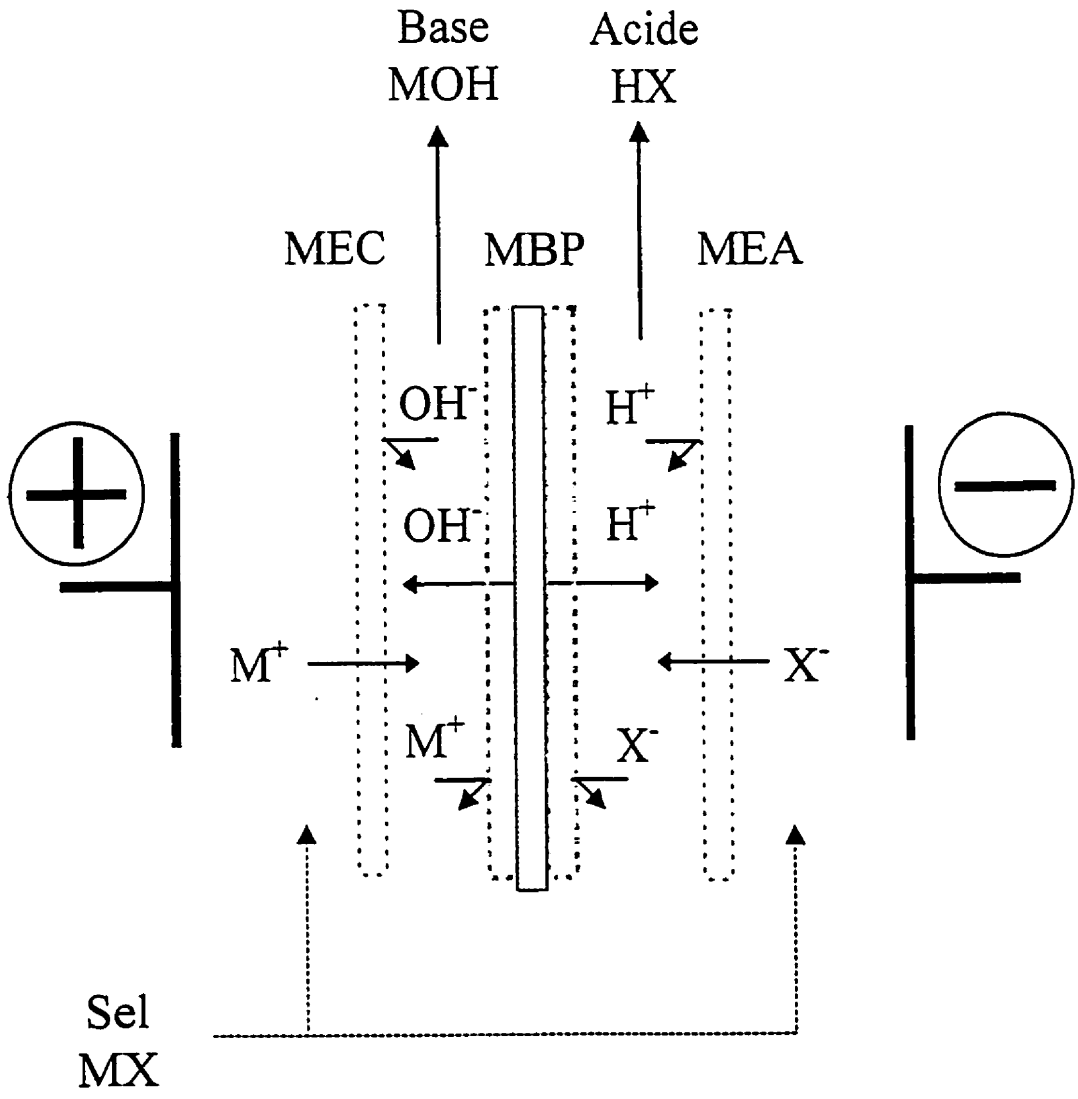

Figure II-8 : Schéma de fonctionnement d'une cellule d'électrodialyse pour la génération de base et d'acide à partir d'un sel (Source Bazinet *et al.* (1998a)).

b) Applications alimentaires

En 1990, Hatzidimitriu a breveté un procédé permettant l'ajustement du pH de fluide aqueux. Les revendications de ce brevet sont que ce procédé peut-être utilisé pour ajuster l'acidité de fluides alimentaires tels que les sirops de sucre, les jus de fruits et de légumes, les vins, ainsi que les sauces et pâtes de tomates, par électrodialyse au sein d'une cellule comprenant des paires de cellules composées de membranes bipolaires et de membranes sélectives aux ions monopolaires. Plus spécifiquement, Dykalo *et al.* (1996) ont breveté un procédé permettant de contrôler l'acidité des produits laitiers contenant une phase aqueuse. Ces deux applications, bien qu'utilisant directement la génération des protons produits à l'interface de la membrane bipolaire ont ouvert la voie à de nouvelles applications alimentaires au-delà de la production d'acide et de bases. Récemment, l'ÉDMBP a été appliquée au jus de pommes (Tronc *et al.*, 1997 ; 1998) et aux protéines de soya (Bazinet *et al.*, 1996 ; 1997c). Ces applications ne visent pas seulement le simple ajustement du pH mais aussi l'effet indirect de la dissociation des molécules d'eau sur le produit traité.

Inhibition du brunissement enzymatique du jus de pomme frais opalescent : La production de jus de haute qualité est rendue difficile par la présence de quantités importantes de polyphénols et de polyphénol oxydases (E.C.1.14.18.1, PPO). Ces composés fixés sur les particules en suspension et contenues dans le jus de pomme opalescent rendent ce dernier très sensible au brunissement enzymatique. Les réactions de brunissement enzymatique sont catalysées par la PPO et résultent en l'oxydation des composés phénoliques en ortho-quinones qui polymérisent par la suite en pigments complexes foncés (Macheix *et al.*, 1990).

Zemel *et al.* (1990) ont démontré que l'activité de la PPO pouvait être inhibée de façon irréversible par une diminution temporaire du pH à 2.0, à l'aide de HCl et par un réajustement du pH à sa valeur initiale en ajoutant une solution de NaOH. Ce traitement a

effectivement inhibé le brunissement enzymatique et stabilisé le jus de pomme, mais l'effet de dilution lié à l'ajout d'acide et de base et la formation de sels ($NaOH + HCl \rightarrow NaCl + H_2O$) ont rendu le jus impropre à la consommation.

Partant de ces observations, Tronc *et al.* (1997 ; 1998) ont utilisé les membranes bipolaires pour baisser le pH du jus de pomme opalescent. Le jus de pomme circulait du côté cationique de la membrane bipolaire, là où sont générés les ions H^+ ions (Figure II-9a). Le pH du jus de pomme a été abaissé temporairement de 3.5 à 2.0, à l'échelle laboratoire, à l'aide d'un électrodialyseur et à une intensité constante de 40 mA/cm^2. Cependant, l'ajout de sel (KCl) a été nécessaire pour atteindre la valeur de pH 2.0, la solution de jus de pomme n'étant pas assez riche en ions libres à mobilité élevée. Ainsi, le K^+ exogène permet l'accumulation des H^+ dans le jus en compensant la perte de K^+ du jus et en maintenant l'électroneutralité du milieu (Tronc *et al.*, 1998). La baisse du pH du jus à 2.0 a inhibé complètement l'activité de la polyphénol oxydase en comparaison avec un témoin (Tronc *et al.*, 1998). Suite à l'acidification, le pH du jus a été retourné à sa valeur initiale par l'introduction des OH^- produits par dissociation des molécules d'eau ; le jus a été mis a circulé du côté anionique de la membrane bipolaire, là où les OH^- sont produits (Figure II-9b). Le réajustement du pH a partiellement réactivé la PPO, mais l'inhibition du brunissement a été pour sa part complète et irréversible. Le traitement a permis d'obtenir un jus de pommes opalescent de couleur attrayante sans en altérer la saveur au cours de l'entreposage (Tronc *et al.*, 1997; 1998). Cette modification du pH aurait d'autres conséquences positives sur la stabilités du jus de pomme opalescent. La pectine méthyle estérase, responsable de précipités opalescent dans le jus, serait elle aussi affectée par le traitement d'ÉDMBP. De plus, les faibles pertes en ions divalents, tels que le Ca^{2+} et le Mg^{2+} par électromigration pourraient renforcer la stabilité des particules en suspension (Tronc, 1996). L'utilisation de l'ÉDMBP permet donc une stabilisation non thermique, de façon simple, efficace et applicable aux liquides alimentaires.

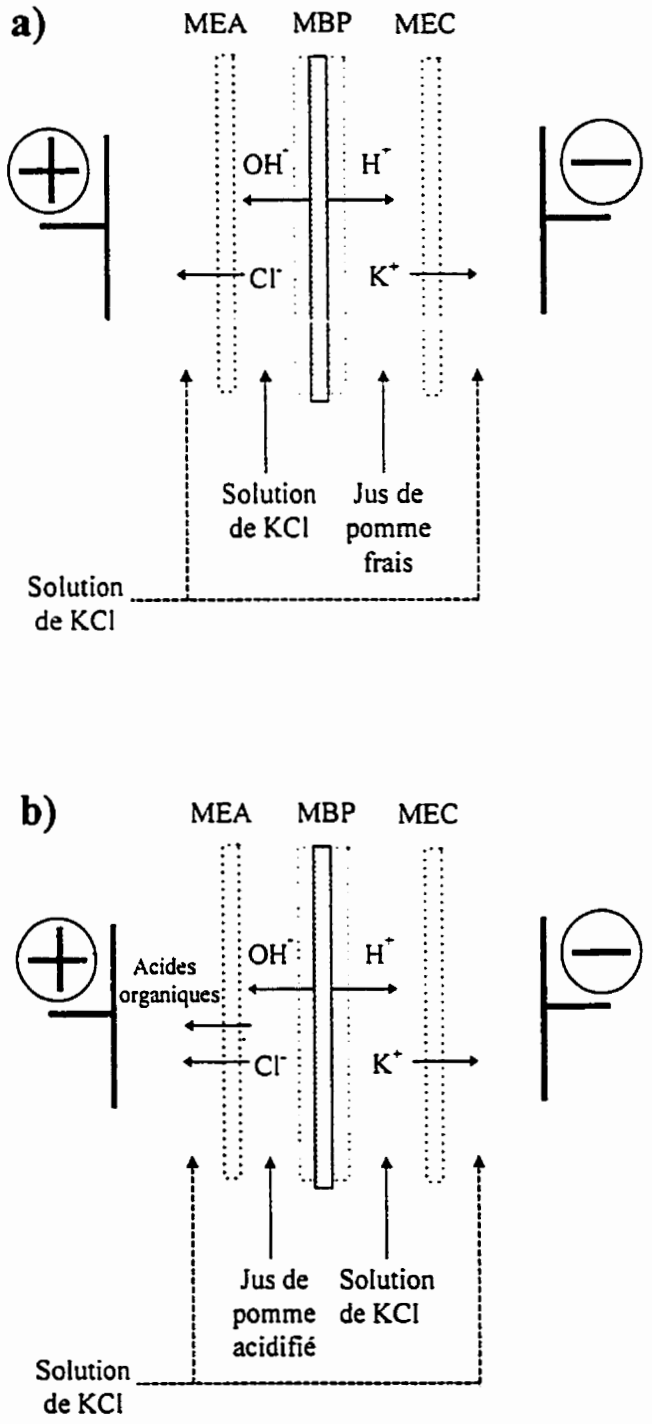

Figure II-9 : Configuration de la cellule d'électrodialyse utilisée pour l'inhibition du brunissement enzymatique du jus de pomme frais non-clarifié : **a)** phase d'électro-acidification et **b)** phase d'électrobasification (Source Bazinet *et al.* (1998a)).

Production d'isolats de protéines de soya : Les protéines de soya utilisées dans l'industrie alimentaire le sont majoritairement sous la forme d'isolats de protéines. La séparation par précipitation isoélectrique des protéines de soya à un pH compris entre pH 4.2 et 4.6 est reconnue comme le principal procédé industriel (Figure II-10).

Le procédé conventionnel comporte 5 étapes : l'extraction, la précipitation, le lavage, la resolubilisation et le séchage. Les flocons de soya dégraissés sont dissous dans de l'eau à pH 9 ± 2 dans des ratios allant de 6:1 à 20:1 et à une température inférieure à 80°C. La phase d'extraction dure généralement 30 minutes. Une fois l'extraction terminée, les protéines sont séparées par abaissement du pH à leur point isoélectrique, environ 4.5, avec de l'acide chlorhydrique concentré. La centrifugation permet de séparer le caillé protéique du surnageant (ou phytosérum) contenant les matières solubles et les composés à faibles poids moléculaires. Le caillé est alors lavé à l'eau pour ôter les impuretés solubles restantes. Une fois le caillé rincé, il est neutralisé par un ajout de soude caustique concentrée afin d'obtenir les protéines sous forme de protéinate soluble. Le produit final est séché par atomisation et empaqueté sous forme sèche (Bazinet *et al.*, 1998c).

Tous les isolats commerciaux de protéines de soya sont produits par précipitation acide. Les désavantages de cette méthode sont la dénaturation des protéines par des traitements acides et alcalins, un contenu élevé en cendres et une altération de la solubilité des protéines après réhydratation (Nash et Wolf, 1967). Localement, les fluctuations extrêmes de pH peuvent causer une dénaturation irréversible des protéines, altérant leur rendement de précipitation (Fisher *et al.*, 1986 ; Kilara et Sharkasi, 1986).

Dans ce contexte, une procédure utilisant l'ÉDMBP a été développée pour précipiter les protéines de soya (Bazinet *et al.*, 1996; 1997c). Dans ce procédé, les protons générés par la membrane bipolaire entrent en contact avec les protéines, tout en abaissant le pH de la solution à leur point isoélectrique, résultant en une séparation sélective de ces dernières. Une étape de centrifugation peut alors être employée, comme dans le procédé

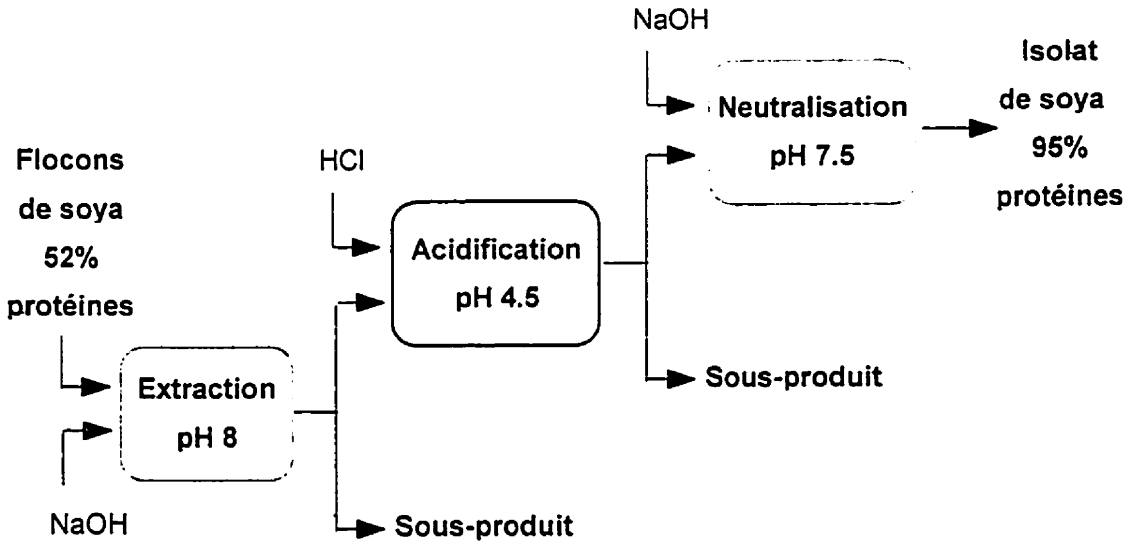

Figure II-10 : Procédé conventionnel de production des isolats de protéines de soya.

commercial de séparation des protéines. Pour abaisser le pH de la solution de protéines de soya, celle-ci a été circulée du côté cationique de la membrane bipolaire. Le pH de la solution protéique a été baissé de pH 8.0 à 4.5 dans une cellule de 100 cm^2 de surface effective d'électrode, à une intensité de courant de 25 mA/cm^2. L'atteinte du point isoélectrique par ÉAMBP a permis la préparation d'un isolat protéique (à 96% de protéines). Ces protéines ont présenté une qualité compositionnelle supérieure ou égale à celle de produits commerciaux de référence (SUPRO-515, SUPRO-500 et SUPRO-600) et des propriétés fonctionnelles comparables à ces derniers (Bazinet *et al.*, 1996, 1997c). L'ajout de sel (KCl) améliore l'efficacité énergétique du système par une diminution de la résistance électrique globale du système d'électrodialyse (Bazinet *et al.*, 1997a). En effet, l'ion K^+ est la principale espèce ionique à électromigrer afin de conserver l'électroneutralité de la solution protéique traitée par ÉAMBP; les ions K^+ migrés sont corrélés dans un ratio 1:1 avec le nombre de H^+ électrogénérés (Bazinet *et al.*, 1999a).

Avantages et inconvénients de l'ÉDMBP : L'ÉDMBP a de nombreux avantages par rapport aux procédés conventionnels de précipitation isoélectriques des protéines et de production de jus de pommes opalescent utilisés en industrie. L'ÉDMBP utilise l'électricité pour générer les espèces ioniques désirées pour acidifier ou alcaliniser de façon douce les solutions traitées. La dissociation de l'eau à l'interface de la membrane bipolaire se fait de façon continue tout en évitant les excès locaux d'acide. Dans l'appareil d'électrodialyse, le processus de dissociation des molécules d'eau permet non seulement l'acidification de la solution, mais aussi la basification dans le cas du jus de pommes ou du soya, ou la production d'une base en solution qui peut être utilisée pour l'étape d'extraction ou de neutralisation dans le cas du soya (Figure II-11). La génération in situ et la réutilisation des composés dangereux pour l'environnement (acides et bases) suppriment par conséquent les inconvénients et les risques liés au transport, au stockage, à l'utilisation et à l'élimination de ces matières. La consommation d'eau est diminuée par réutilisation d'une partie des effluents générés et la consommation énergétique est peu élevée (moins de 0.5 kWh/kg d'isolat produit) (Bazinet *et al.*, 1998c).

De plus, par l'utilisation à la base d'un système d'électrodialyse, système connu en industrie, cette technologie est facilement adaptable au niveau industriel. En effet, l'électrodialyse conventionnelle est une technologie déjà utilisée en industrie agro-alimentaire pour le traitement des sous-produits de l'industrie laitière et le dessalement des eaux saumâtres. Une cellule d'électrodialyse, de par les nombreux formats disponibles à l'heure actuelle sur le marché, peut être adaptée à tous les niveaux de production et par conséquent à toutes les tailles d'entreprises. Enfin, pour l'industriel producteur d'isolats de protéines de soya ou de jus de pommes, L'ÉDMBP a aussi pour avantage de s'insérer parfaitement dans le schéma du procédé conventionnel (Figure II-11). L'installation de cette technologie ne demanderait pas une reconceptualisation complète du procédé de production, mais seulement quelques modifications (Bazinet *et al.*, 1998a,c).

Les points faibles de l'ÉDMBP sont le prix élevé de l'équipement de base et la possibilité de colmatage à long terme. Cependant ces désavantages sont le résultat de la récence de cette technologie. Le développement de l'ÉDMBP et ces applications dans l'industrie alimentaire participeraient à diminuer le coût des membranes bipolaires et des électrodialyseurs. Le colmatage qui peut apparaître en ÉDMBP des protéines de soya n'est pas un colmatage des membranes comme celui qui est fréquemment rencontré en ultrafiltration ou en nanofiltration, mais principalement un colmatage des cadres séparateurs, résultant d'un design de cellule d'électrodialyse non optimal du point de vue hydrodynamique. Le colmatage est lié aux particules de caillé protéique en suspension qui s'agglomèrent entre elles dans le grillage des cadres séparateurs quand leur concentration devient trop élevée. Cependant une centrifugation en ligne permettrait de récupérer ces particules et de diminuer les problèmes de colmatage (Bazinet *et al.*, 1998a,c).

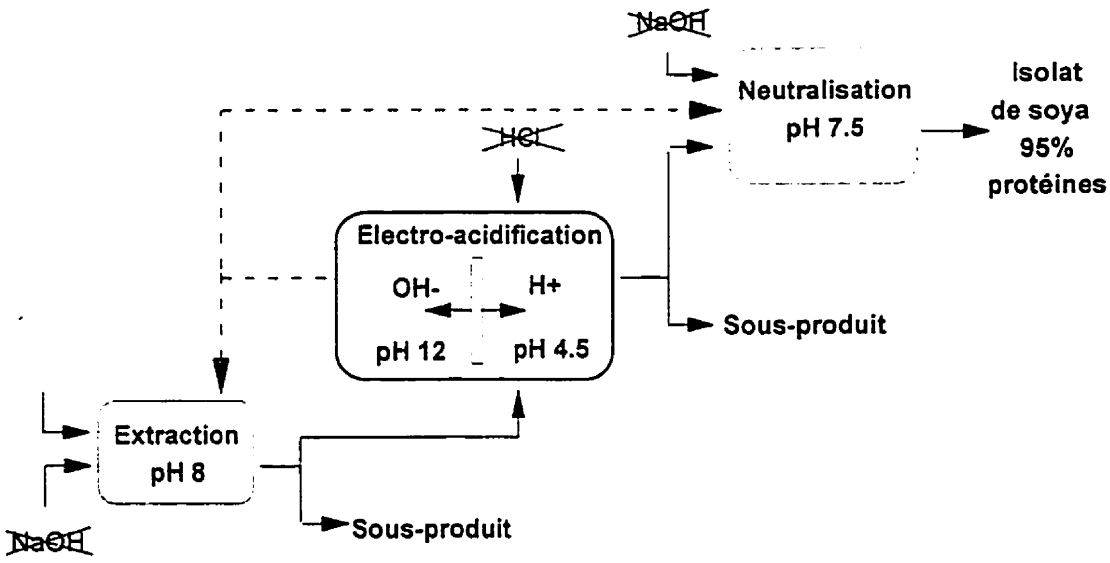

Figure II-11 : Procédé modifié de production d'isolats incorporant la phase d'électroacidification (Source Bazinet *et al.* (1998c)).

HYPOTHÈSE ET OBJECTIFS

Tel que présenté dans la revue de littérature, la force ionique, de même que la nature des sels présents en solution interfèrent sur les propriétés physicochimiques des caséines isolées et des micelles de caséines, ainsi que sur leur comportement en solution. Au niveau du système d'électrodialyse, les membranes ioniques présentent au sein de ce dernier vont, de part leur permsélectivité, permettre une migration sélective des ions en solution. Par contre, un éventuel colmatage, par des protéines ou des sels divalents ou trivalents, des membranes empilées dans l'électrodialyseur peut altérer le rendement énergétique et un changement de l'hydrodynamisme n'a plus d'impact sur l'efficacité globale du système.

Hypothèse

L'hypothèse du présent travail est la suivante : L'électroacidification avec membranes bipolaires permet de produire des isolats de caséine dont la fonctionnalité et la composition chimique dépendent des paramètres du milieu (force ionique et sels présents) et des paramètres électrodialytiques (permsélectivité des membranes cationiques et débit) qui ont également une influence sur les performances énergétiques du système d'électrodialyse et l'intégrité des membranes au cours de la séparation des caséines du lait écrémé.

Objectifs poursuivis

1) Valider la faisabilité de l'ÉAMBP pour la précipitation des caséines du lait et étudier l'effet du débit;

2) Étudier l'effet de la permsélectivité des membranes cationiques (MEC) mono- et multicouches sur la migration des cations du lait et la précipitation des protéines au cours du procédé d'ÉAMBP;

3) Étudier l'effet de la permsélectivité des MEC mono- et multicouches sur leur colmatage et identifier la nature du colmatage;

4) Comparer les acidifications chimiques et électrochimiques du lait reconstitué et identifier les éléments éventuels de différenciation entre les deux procédés;

5) Étudier l'équilibre ionique de la solution de lait électroacidifiée afin de :

a) Évaluer le nombre de H^+ électrogénérés au cours de l'ÉAMBP,

b) Identifier et quantifier les cations migrant au travers des membranes cationiques durant L'ÉAMBP du lait,

c) Comparer les cinétiques de migration des cations à celle des H^+ électrogénérés.

6) Étudier l'impact de la force ionique et de la nature des sels présents dans la solution de lait sur les performances de la technologie, la composition chimique et la fonctionnalité des isolats produits par ÉAMBP et, à cette fin :

Les principaux sous-objectifs sont les suivants :

a) Étudier l'effet de la nature des sels ajoutés et de la force ionique sur les performances énergétiques de l'ÉAMBP

b) Étudier l'effet de la nature des sels ajoutés et de la force ionique sur la composition chimique et la fonctionnalité des isolats produits par ÉAMBP

c) Comparer les qualités compositionnelle et fonctionnelle des différents isolats produits par ÉAMBP avec celles de standards commerciaux et chimiques.

7) Étudier l'effet du type de lait électroacidifié (lait en poudre reconstitué et lait frais non pasteurisé) sur les performances de l'ÉAMBP, la composition chimique et la fonctionnalité des isolats produits.

CHAPITRE III

PRODUCTION D'ISOLAT DE CASÉINE DE LAIT BOVIN PAR ÉLECTROACIDIFICATION AVEC MEMBRANES BIPOLAIRES

CHAPITRE III

PRODUCTION D'ISOLAT DE CASÉINE DE LAIT BOVIN PAR ÉLECTROACIDIFICATION AVEC MEMBRANES BIPOLAIRES

TRANSITION CONTEXTUELLE

Il ressort de la revue de littérature que l'électroacidification avec membranes bipolaires est une technologie récente et qu'elle n'a jamais été appliquée à la précipitation des protéines du lait. D'autre part, la dissociation des molécules d'eau à la surface des membranes monopolaires sous l'effet d'un champ électrique appliqué aux bornes d'une cellule d'électrodialyse étant affectée entre autres par le débit des solutions à traiter, les objectifs spécifiques de l'étude menée dans ce chapitre sont de valider la faisabilité de l'EAMBP pour la précipitation des caséines du lait et d'investiguer l'effet du débit.

Ce chapitre a fait l'objet d'un article intitulé **«Bipolar Membrane Electroacidification to Produce Bovine Milk Casein Isolate» publié dans *J. Agric. Food Chem.*, 1999, 47(12), 5291-5296** . Les auteurs sont : Laurent Bazinet (Candidat au Ph. D.: planification et réalisation des expériences, analyse des résultats et rédaction de l'article), François Lamarche (Co-directeur de thèse : supervision scientifique de l'étudiant, correction et révision du manuscrit), Denis Ippersiel (participation à la réalisation des expériences, correction et révision du manuscrit) et Jean Amiot (Directeur de thèse : supervision scientifique de l'étudiant, correction et révision du manuscrit).

RÉSUMÉ

L'électroacidification avec membranes bipolaires (ÉAMBP) a été étudiée précédemment et a été utilisée pour la précipitation isoélectrique des protéines du soya.

L'objectif de cette étude était de valider la faisabilité de l'ÉAMBP pour la précipitation des caséines du lait et d'investiguer l'effet du débit.

Des isolats de haute pureté contenant 1.23 et 2.00 % de cendres et 85.4 et 91.6% de protéines ont été obtenu avec des débits de 0.2 et 1.2 gal/min. La composition des profils moléculaires des isolats obtenus par HPLC montrent que seules les caséines sont précipitées. Cependant, sauf pour les courbes de précipitation protéiques, le débit n'a pas influencé la composition et la pureté finale des isolats. Ces résultats ont mis en évidence que l'ÉAMBP est un nouveau procédé alternatif pour la production d'isolat de caséine bovine de haute pureté.

Mots clés : Acidification électrochimique, Membrane bipolaire, Caséine, Lait, Précipitation.

ABSTRACT

Bipolar-membrane electroacidification (BMEA) has been developed previously (Bazinet et al., 1996, 1997a, b) and has been used for isoelectric precipitation of soybean proteins.

The purpose of this study was to validate the feasability of BMEA for the precipitation of milk casein and to investigate the effect of flowrate.

High purity isolates containing 1.23 and 2.00 % ash and 85.4 and 91.6% total protein were obtained with flowrates of 0.2 and 1.2 gal/min. The molecular composition profiles of the isolates obtained by HPLC showed that only caseins were precipitated. However, except for protein precipitation curves, the flowrate did not influence the final composition and purity of the isolates. These results showed that BMEA is a new alternative process for production of high purity bovine milk casein isolate.

Key words : Electrochemical acidification, Bipolar membrane, casein, Milk, Precipitation.

INTRODUCTION

Casein is extensively used in the manufacturing of food products for its nutritional quality and functional properties. However, highly pure casein can only be obtained by an insolubilisation step and centrifugation can then be used for a simple separation of the casein from whey (Varnam and Sutherland, 1994).

Two main types of casein usually produced in the industry are rennet and acid casein. In the case of rennet casein, the underlying mechanism is identical to that of the production of cheese curd and depends on the unique sensitivity of the Phe_{105}-Met_{106} bond of κ-casein to hydrolysis by acid proteinases, the active components of rennet. For acid casein production, the three main procedures used are based on isoelectric precipitation of casein by chemical, physico-chemical or fermentation acidification (Segalen, 1985; Southward, 1993; Varnam and Sutherland, 1994). Other techniques have been proposed for the production of acid casein : acidification of milk by ion-exchange plus acid (Salmon, 1983), electrodialysis of skim milk to pH 5.0 followed by acidification to pH 4.6 with acid (Laiteries Triballat, 1979) and acidification by water electrolysis at the surface of monopolar anion or cation exchange membranes stacked in an electrodialysis cell (Bolzer, 1985).

A procedure derived from electrodialysis and using bipolar membranes was developed in order to precipitate soybean protein (Bazinet *et al.*, 1996 ; 1997a, b; 1998b). Bipolar-membrane electroacidification (BMEA), uses a property of bipolar membranes to split water and the action of monopolar membranes for demineralization. When a current is passed across a bipolar membrane, electrical conduction is achieved by the transport of H^+ and OH^- ions generated by electro-dissociation of water (Mani, 1991). The protons thus generated can come into contact with the proteins, bringing them to their isoelectric point resulting in selective separation. Water dissociation may occurs with a lower efficiency, using monopolar membranes stacked in an electrodialysis cell (Bazinet *et al.*, 1996; Korngold, 1984; Davis *et al.*, 1997). This dissociation appears only when the current value exceeds the limiting current value (Brun, 1989; Korngold, 1984). Bipolar membrane are

made to be used above limiting current conditions and need a lower current to reach the limiting current value than monopolar membranes (Bazinet *et al.*, 1998c).

As the electrical water splitting in an electrodialysis cell at the surface of monopolar membrane is affected, among other factors, by the flowrate of the solution to be treated (Gardais, 1990; Klein *et al.*, 1987), this study is a part of a broader research project aimed at precipitation of bovine milk protein, without adding acids, by decreasing the pH through electrodialysis. Its specific objectives were to validate the feasability of BMEA for the precipitation of milk casein and to investigate the effect of flowrate. Both flowrates were compared in terms of electrodialysis cell parameters, percentage of proteins precipitated, protein composition profiles and chemical composition of isolates produced.

MATERIAL AND METHODS

Material

The raw material used in this study was commercial fresh pasteurized and homogenized skim milk (Quebon, Natrel, Longueuil, QC, Canada).

Methods

a) Electro-acidification cell

The electroacidification cell was the same as that used by Bazinet et al. (1997a, b) with four Neosepta CMX cationic membranes and three Neosepta BP-1 bipolar membranes (Tokuyama Soda Ltd., Tokyo, Japan). This arrangement defines three closed loops containing the milk solution, a 2 $g \cdot l^{-1}$ aqueous KCl solution and a 20 $g \cdot l^{-1}$ Na_2SO_4 solution. Each closed loop was connected to a separate external 600 mL double-jacket glass container (School, Duran, Germany), allowing for continuous recirculation. The electroacidification system did not allow for constant temperature control.

The anode/cathode voltage difference was supplied by a variable 0-100 V power source, Powerstat Model 236BU-2 (The Superior Electric Co., Bristol, CO). The electrolytes were circulated using three centrifugal pumps Model WMD-30RT-220 (Iwaki Walchem, Tokyo, Japan), and the flow rate was controlled using Model E-03248-58 flowmeters (Cole-Parmer Instrument Company, Vernon Hills, IL, USA). The anode, a dimensionally-stable electrode (DSA), and the cathode, a 316 stainless-steel electrode, were supplied with the MP cell.

b) Protocol

Electroacidification was performed in batch process using a current of 2.0 A, with electrolyte volumes of 1 litre. The electroacidification was stopped after the pH reached 4.0. The initial pH varied between 6.5 and 6.7. Two flowrates during electroacidification were tested (1.2 and 0.2 gpm) and three replicates of each condition were performed in this experiment.

During each treatment, 1.5 mL-samples of the milk solution were taken , in the outlet line outside of the BMEA cell and just before the milk reservoir, at every 0.2 pH unit decrease from initial pH (around 6.6) to pH 4.0. The time required to reach pH 4.0, the anode/cathode voltage difference, the conductivity and the temperature were recorded as the treatment progressed. The concentration of soluble protein was determined on freshly acidified 1.5 mL samples. At the end of each run, 500 mL of pH 4.0 milk were taken. These samples were centrifuged 10 minutes at 4°C, at 500 g (Centrifuge Model J2-21, rotor type JA-10, Beckman Instruments Inc., Palo Alto, CA). The precipitate was washed twice with distilled water, before being lyophilized for 48 hours at room temperature (Model Freezone 4.5, Labconco, Kansas City, MI). The lyophilized isolates were stored at 4 °C before total protein determination, protein composition profiles and ash content measurements were performed.

c) Analysis Methods

Ash Content: in accordance with method 930-30 (AOAC International, 1995b) approximately 1.5 g of lyophilized sample was added to the cooled crucibles, and the mass recorded. The sample was then ashed at 550 °C for 16 hours and weighed again when they reached room temperature.

Soluble protein and total protein determination : The protein concentration determination was done using an FP-428 LECO apparatus (LECO Corporation, Saint Joseph, MI). The instrument was calibrated each time with ethylenediaminetetraacetic acid (EDTA) as a nitrogen standard. A preliminary comparison of the LECO and Kjeldahl methods has demonstrated a very good correlation between both methods (R^2 = 0.986 and 0.996 for the high and low ranges of protein respectively)

For soluble protein determination the LECO conditions were the following :

Sample size : 75 mg

Analysis constants :	Oxidation Furnace temperature	900°C
	Oxidation standby temperature	650°C
	Purge cycles	3
	Minimum timeout	30
	Comparator level	1.00
	Loop select	Low range

Flow constants :	High	30 s
	High	30 s
	High	End

Gases : oxygen 99.99% and helium 99.99%

Calibration standard : 75 mg EDTA (n° 502-092, 9.56 ± 0.03 % Nitrogen, LECO Corporation, Saint Joseph, MI)

Conditions for total protein determination were the same as for soluble protein, except for loop select (High range), flow constant (high, 10 s; high, 30 s; high, end) and mass of EDTA (150 mg).

Molecular profiles : The chromatographic analysis of protein composition profile of the lyophilized protein isolate and skim milk was performed by reverse-phase HPLC according to Jaubert and Martin (1992). Separation of α_{s1}-, α_{s2}-, β- and κ-caseins was carried out on a 15-cm Vydac C4 column (Model 214 TP 5415, Vydac, Hesperia, CA) coupled with a Vydac Protein C4 guard column (Model 214 FSK 54, Vydac, Hesperia, CA). Elution at 1 mL/min. was achieved using a linear gradient from 37 to 57% solvent B (0.096% (V/V) trifluoroacetic acid in 80% (V/V) acetonitrile) and 63 to 43% solvent A (0.1 % (V/V) trifluoroacetic acid in water) for a total run time of 38 min at room temperature. The detection wavelength was 214 nm, and a 50 µL sample volume was injected.

Before analysis, freeze dried samples of isolates (55 mg) and lyophilized skim milk (LSM) (135 mg) were added to 4 mL of buffer solution (100 mM Tris-HCl, 8M urea, 1.3 % trisodium citrate, pH 7.0). The mixture was reduced in 10 mM DTT (1,4-dithiothreitol) and maintained for 1 h at 37 °C. Before injection, the homogenate sample was diluted 10 X with solvent A and filtered on 0.45 µm millipore filter.

d) Statistical Analyses

The duration of the electroacidification, the voltage, the conductivity and the percent soluble protein as a function of pH were subjected to a split-plot analysis of variance using SAS software (SAS, 1989). Regression equations and curve fitting were calculated for the voltage, duration and percent soluble proteins as a function of pH using SigmaPlot (version 3.0 for Windows, Jandel Scientific, Corte Madera, CA). The ash content and the percent total protein data were analyzed by an analysis of variance and examined by Duncan tests in order to determine the significance of differences between the different samples.

RESULTS AND DISCUSSION

After electroacidification of the skim milk solution, the final product was still milky. However, after the solution was left to settle, the precipitate was composed of small and soft clumps of casein, and the supernatant was clear. In the BMEA cell, a slight fouling appeared due to the formation of a casein network in the spacer turbulence promoter formed by a double layer of wire-mesh. The casein filled the 2 x 2 mm square of the turbulence promoter to form a soft white curd. However, the fouling did not alter the yield of casein recovered, since the curd was formed only at the end of the process, when all the casein was precipitated, by recirculation of these small particles. An on-line centrifuge should allow the recovery of the casein particles and decreased fouling.

Electro-acidification parameters : duration, anode/cathode voltage difference and conductivity

Results of the analysis of variance indicated that the flowrate had no significant effect on the duration of electro-acidification ($P>0.10$), on the anode/cathode voltage difference ($P>0.92$) and on the conductivity ($P>0.74$) during the electroacidification process. However, pH ($P<0.001$) and the dual interaction of pH and flowrate ($P<0.0009$) had a highly significant effect on the anode cathode voltage difference. The first-order linear regression calculated for the duration and the third-order regression curves calculated for the anode/cathode voltage difference as a function of pH produced coefficients of determination in the order of 0.950 to 0.999 .

a) Duration of electro-acidification

To decrease the pH of skim milk from 6.6 to 4.0, the duration was the same with 17.8 and 18.1 min at 1.2 and 0.2 gpm respectively (Figure III-1). The flowrate does not influence the duration of electroacidification. However, at the beginning of the process, the time to decrease the pH from 6.6 to 6.4 were different, with 2.1 and 3.4 min for 1.2 and 0.2 gpm respectively. This delay in acidification between 1.2 and 0.2 gpm was found from pH

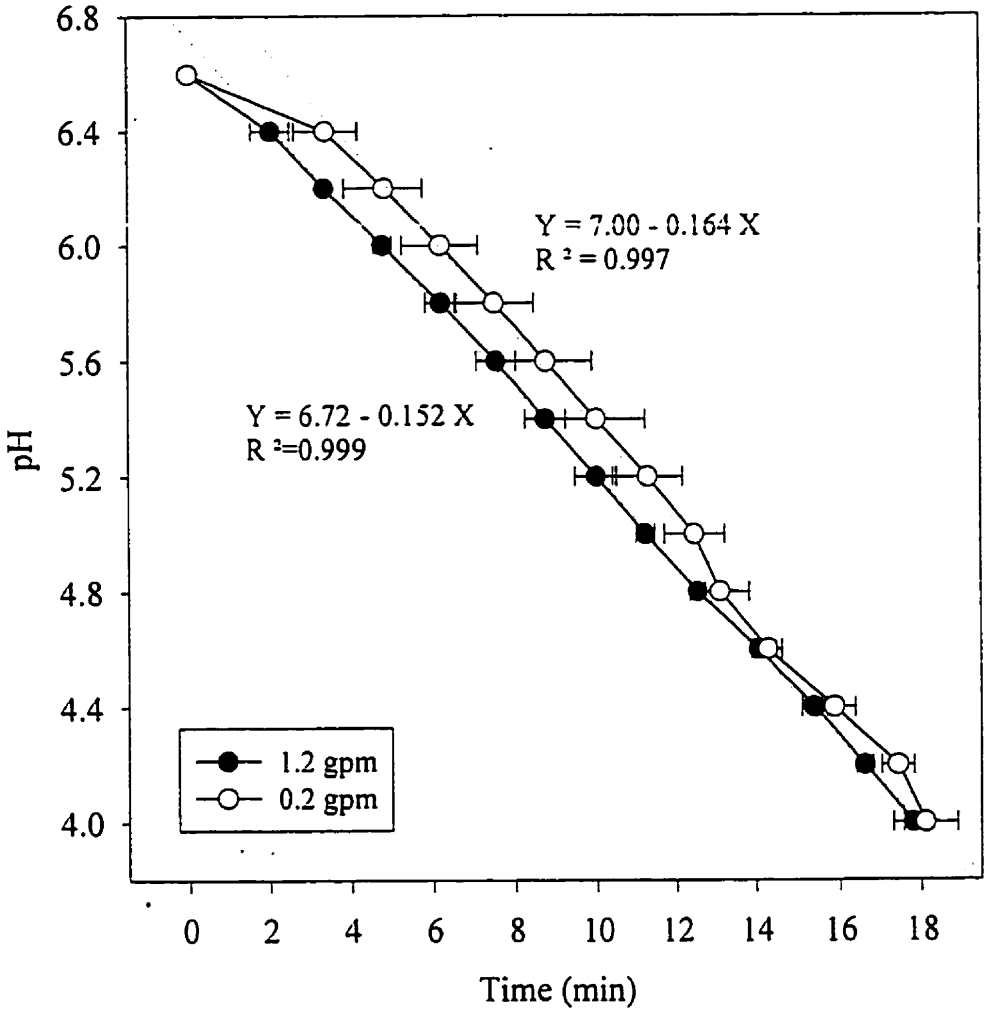

Figure III-1 : Effect of the flow rate, 1.2 and 0.2 gal/min, on the time required to decrease the pH by bipolar membrane electroacidification of a skim milk solution run at 2.0 A constant current.

6.4 to 5.0 and disappeared at pH 4.8. Considering that the system was at solution equilibration at pH 6.6, the acidification process was found to be linear in time, which was confirmed by the regression coefficients calculated in Figure III-1. From pH 6.4 to 4.0, delay in acidification was progressively diminished to disappear at the end of the process. This difference in time should be due to a faster circulation of the H^+ produced at the bipolar membrane at high flowrate, resulting in a better mixing of H^+ continuously produced with the milk protein solution in the bulk container. Moreover, the disappearance of delay in acidification between 1.2 and 0.2 gpm could be explained by a strong precipitation of milk protein between pH 5.0 and 4.8. These results are confirmed by the fact that the production of H^+, and consequently the time of acidification was previously shown to mainly depend on protein concentration and current density (Bazinet *et al.*, 1997a, b; 1998a ; Mani, 1991). When protein concentration and current density were maintained constant, the time of acidification was the same whatever the other conditions, except in the case of complete fouling of the electrodialysis cell spacers.

b) Anode cathode/voltage difference

At both flowrates, the evolution of anode/cathode voltage difference was the same : a drop followed by an increase (Figure III-2). During the acidification of skim milk, at 1.2 and 0.2 gpm respectively, the voltage dropped from 73.7 to 49.7 Volts and from 71.0 to 48.7 Volts, and then increases from 49.7 to 65.0 Volts and from 48.7 to 73 Volts. This phenomenon was previously observed by Bazinet *et al.* (1997a, b) on soyabean protein. The decrease in voltage would be the result of the higher conductivity of H^+ generated at the bipolar membrane in replacement of ions migrating across the cationic membrane, in order to maintain the electrical neutrality of the solution (Bazinet *et al.*, 1998b): The molar conductivity of H^+ is much higher than all the other ions (Brett and Oliveira-Brett, 1994). This replacement in part of the milk cations by H^+ in the protein solution, and the migration of these cations from the protein compartment to the KCl and Na_2SO_4 compartments across the cationic membrane, coupled in the KCl compartment with generation of OH^- at the anionic exchange layer of the bipolar membrane, induced a decrease in the overall resistance of the system, and consequently a decrease in anode/cathode voltage difference

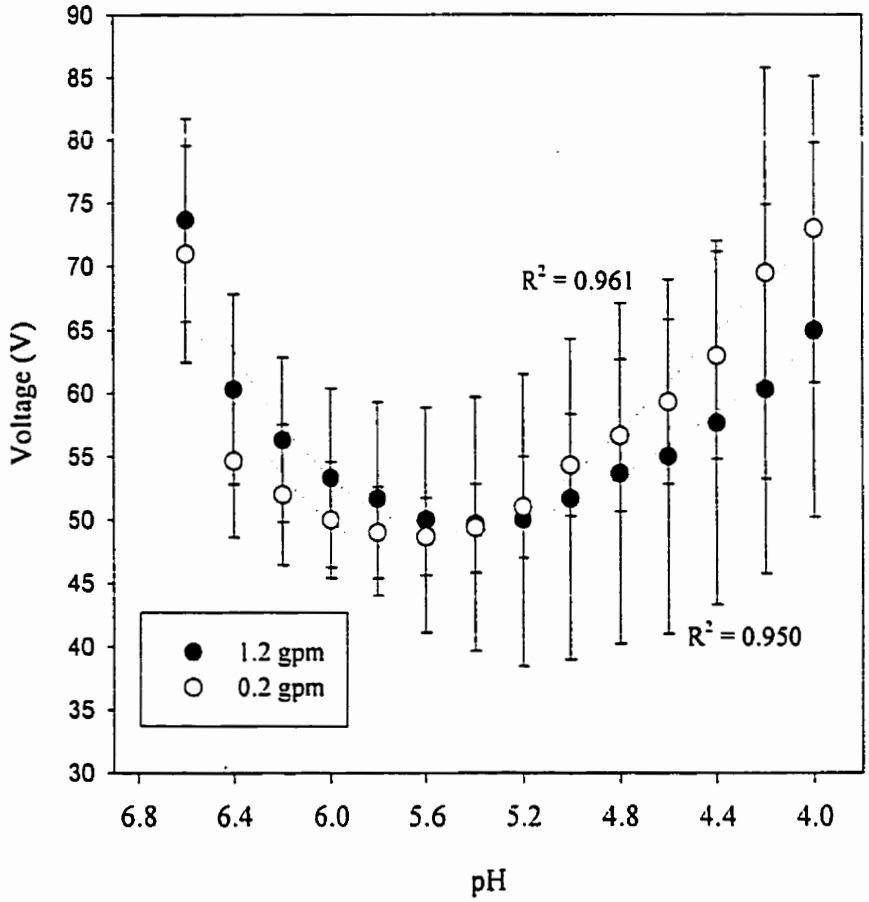

Figure III-2 : Effect of the flow rate, 1.2 and 0.2 gal/min, on the anode/cathode voltage difference during bipolar membrane electroacidification of a skim milk solution run at 2.0 A constant current.

(Bazinet *et al.*, 1998b). This phenomenon progressed during the entire process, but when the protein began to precipitate, the overall resistance of the system increased because of a slight fouling in the spacers of the cell.

c) Conductivity

Results of the statistic analysis indicate that the flowrate did not affect the conductivity (Figure III-3). The conductivity changed in the same way during electroacidification whatever the flowrate, as shown in Figure III-3. This demonstrates that conductivity at a level of 2.6-2.4 mS/cm does not appear to be a limiting factor in the electroacidification of milk protein.

The apparent lower decrease in conductivity, about 0.1-0.2 mS/cm during electroacidification was similar to the results obtained by Bazinet *et al.* (1997b) on soybean protein.

Soluble protein

The analysis of variance of the data shows that the flowrate ($P>0.50$) had no significant effect, while pH ($P<0.0001$) and the dual interaction of pH and flowrate ($P<0.0009$) had a highly significant effect on the amount of soluble proteins. The equations of the curves representing percentage of soluble proteins as a function of pH were calculated and modelled ; R^2 ranged between 0.993 and 0.997.

The flowrate had no effect on the amount of soluble protein which decreased from about 100% to 21.5-24.4% soluble protein (Figure III-4). However, the rate the decrease of soluble protein from pH 6.6 to 4.0 was different between the two flowrates. Thus, at the beginning of the procedure, from pH 6.6 to 5.6, soluble protein was the same for the both flowrates ranging between 93 and 100%. When the pH dropped from 5.6 to 5.2, at 0.2 gpm soluble protein decreased to 82.9 % at pH 5.4 and 70.9% at pH 5.2, while at 1.2 gpm soluble protein was constant at 93-100%. At pH 5.0, soluble protein was comparable for both flowrates at 31.3 and 33.2%. As the electroacidification continued from pH 4.8 to 4.0,

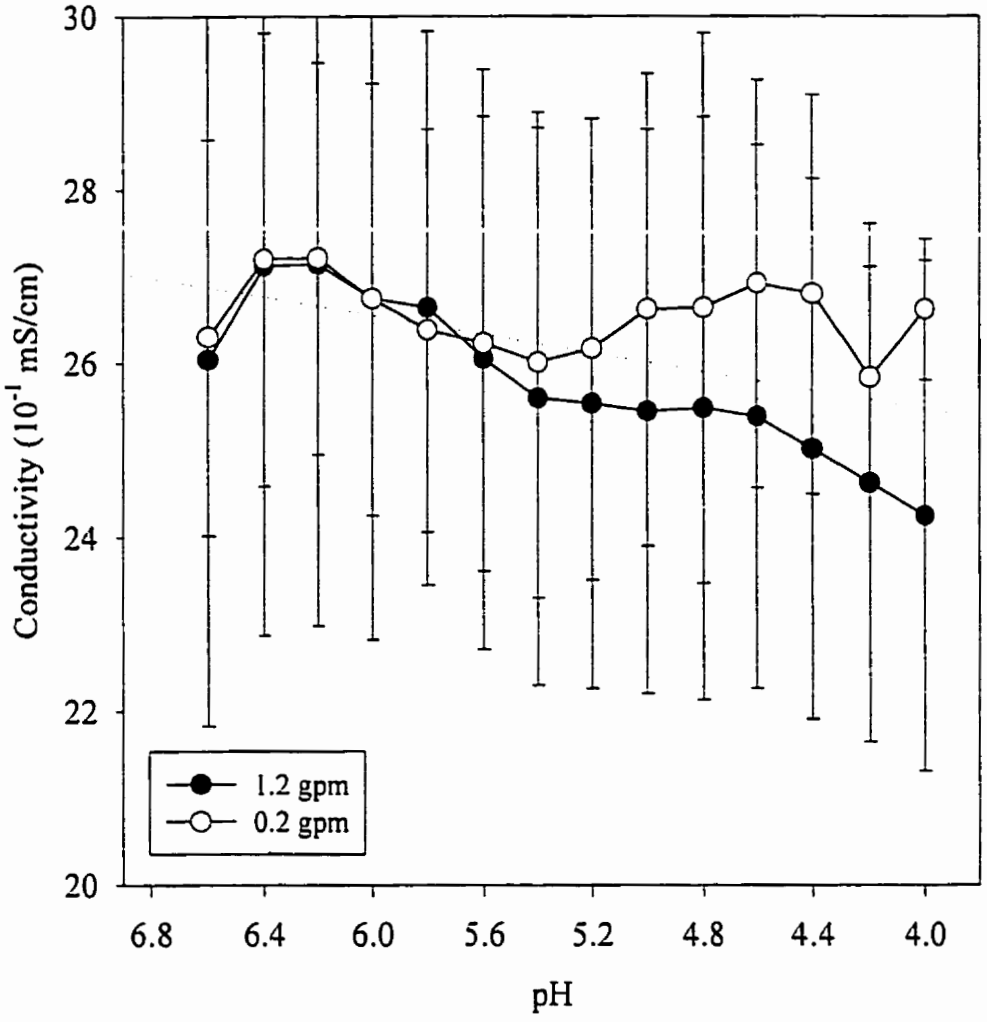

Figure III-3 : Effect of the flow rate, 1.2 and 0.2 gal/min, on the conductivity of skim milk solution during bipolar membrane electroacidification run at 2.0 A constant current.

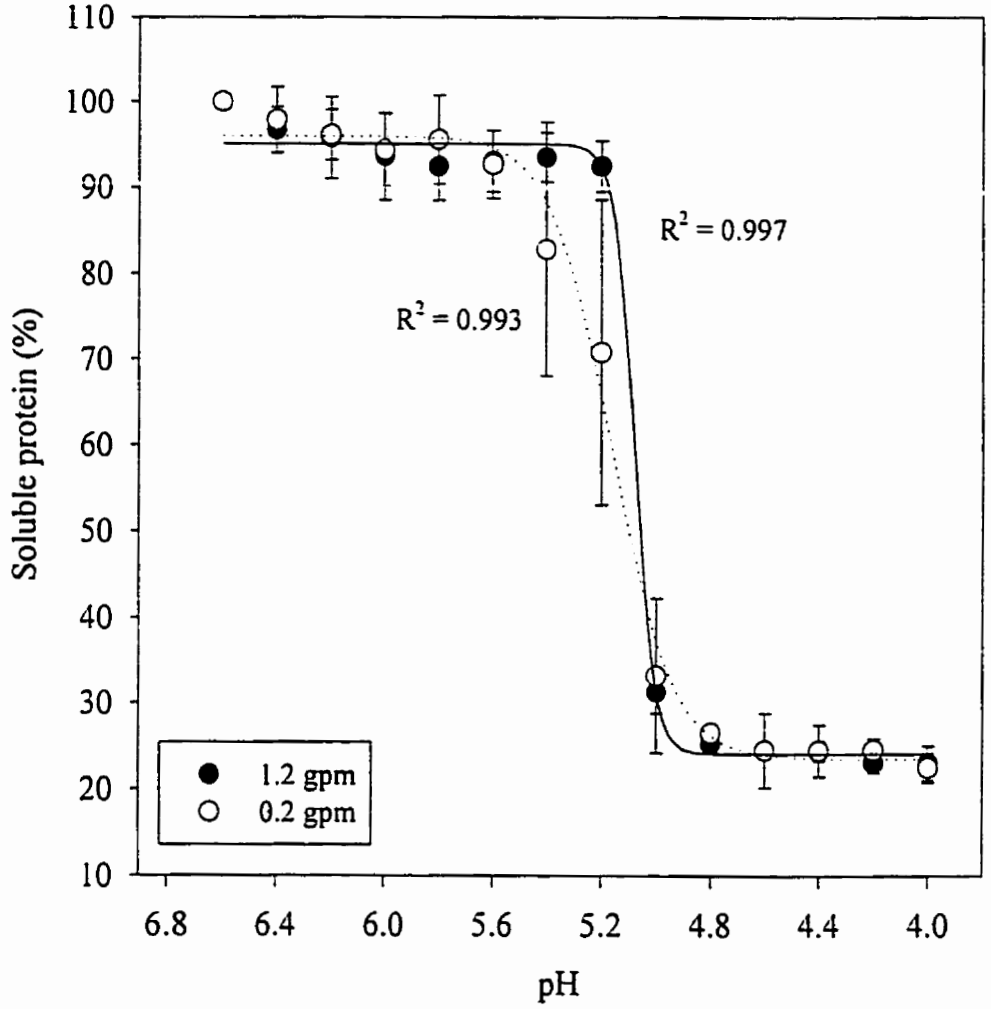

Figure III-4 : Effect of the flow rate, 1.2 and 0.2 gal/min, on the percentage of soluble proteins in the skim milk solution during bipolar membrane electroacidification run at 2.0 A constant current

soluble protein was constant at about 24% whatever the flowrate.

Decreasing the flowrate accelerated the precipitation of protein by increasing the residence time of the protein in the electrodialysis cell. Consequently, at 0.2 gpm, as the generation of H^+ was constant and as a lower volume of protein solution was in contact with more H^+, the pH decreased more rapidly and more protein precipitation occurred. Also, as protein was longer in contact with H^+ electrogenerated, a pH gradient occurred between the inlet of the electrodialysis cell, corresponding to the bulk container solution, and the outlet of the cell. At high flowrate, however, the shorter residence time was not sufficient to create a measurable pH gradient between the inlet and the outlet of the cell. Then, as the mixing between the outlet solution and the bulk solution was high, supplementary passes through the cell were required to obtain a pH close to the isoelectric point and to allow protein precipitation. The different evolution of soluble protein is confirmed by the calculations of the inflection points and the width of transition of the solubility curves : pH 5.08 and 0.04 pH unit, and pH 5.16 and 0.11 pH unit at 1.2 and 0.2 gpm respectively. The 24% soluble protein remaining would correspond to the whey proteins which are soluble in the pH range from 4.8 to 4.0. This is confirmed by percentages of whey protein in milk composition cited in the literature ranging from 14 to 24% (Cheftel *et al.*, 1985 ; Brunner, 1981 ; Swaisgood, 1982 ; Lorient, 1991).

Chemical composition

The chemical composition, in terms of ash content and total protein, of isolates obtained at pH 4.0 after electroacidification was compared to that of a lyophilized fresh skim milk (LSM).

The ash content of isolates was lower in comparison with LSM : 1.23 ± 0.01% and 2.00 ± 0.60 % for 0.2 and 1.2 gpm electrochemical isolates in comparison with 8.05 ± 0.01% for LSM. The ash content of LSM is in accordance with data found in the literature, 8.05 vs 7.9% (Hargrove and Alford, 1974; Bassette and Acosta, 1988 ; Renner *et al.*, 1996) while the ash content of the electroacidified isolate was lower than data cited in the literature, 2.0-3.8 and 8.0-10.5% for commercial casein and coprecipitated casein

respectively (Hargrove and Alford, 1974; Bassette and Acosta, 1988 ; Alais, 1984; Walstra and Jenness, 1984). This difference in ash content indicates a demineralization phenomenon acting during electroacidification. Indeed to maintain the milk solution electrically neutral, one cationic charge must cross the cationic membrane for each H^+ produced at the bipolar membrane (Bazinet *et al.*, 1997a, b ; 1999a).

The percentage of total protein is 2.3-2.5 times higher for electroacidified isolate in comparison with LSM. This result confirms the efficiency of BMEA for the precipitation and the separation of milk casein. Total protein in LSM is in accordance with the literature, 36.9 versus 36.2% for regular non-fat dried milk (Hargrove and Alford, 1974; Bassette and Acosta, 1988 ; Renner *et al.*, 1996), while the percent total protein of electroacidified isolates (91.6 ± 4.6 and 85.4 ± 4.1% for 0.2 and 1.2 gpm isolates respectively) is similar or slightly higher than data found in the literature, 83.0-88.5 and 83.0-85.0% for commercial casein and coprecipitated casein respectively (Hargrove and Alford, 1974; Bassette and Acosta, 1988; Alais, 1984). The difference in ash content could explain the difference in percent total protein observed for the isolates obtained by BMEA versus commercial isolates.

Molecular profile analysis

The comparison of the molecular profiles obtained by HPLC showed that BMEA allows the separation of high purity bovine milk casein (Figure III-5). Whey protein peaks, mainly α-lactalbumine and β-lactoglobulin, ranged from about 35 min. to 39 min. retention time, do not appear or appear in a very low quantity on the isolate profiles (Figure III-5). BMEA allows a good separation of casein from raw milk. Moreover, the flowrate does not influence the purity and the molecular profile of the final isolate obtained by BMEA.

CONCLUSION

Results obtained in this study show that BMEA is a new alternative process for production of high purity casein bovine milk isolate. Moreover, except for protein precipitation curves, flowrate does not influence the final composition and purity of the isolate

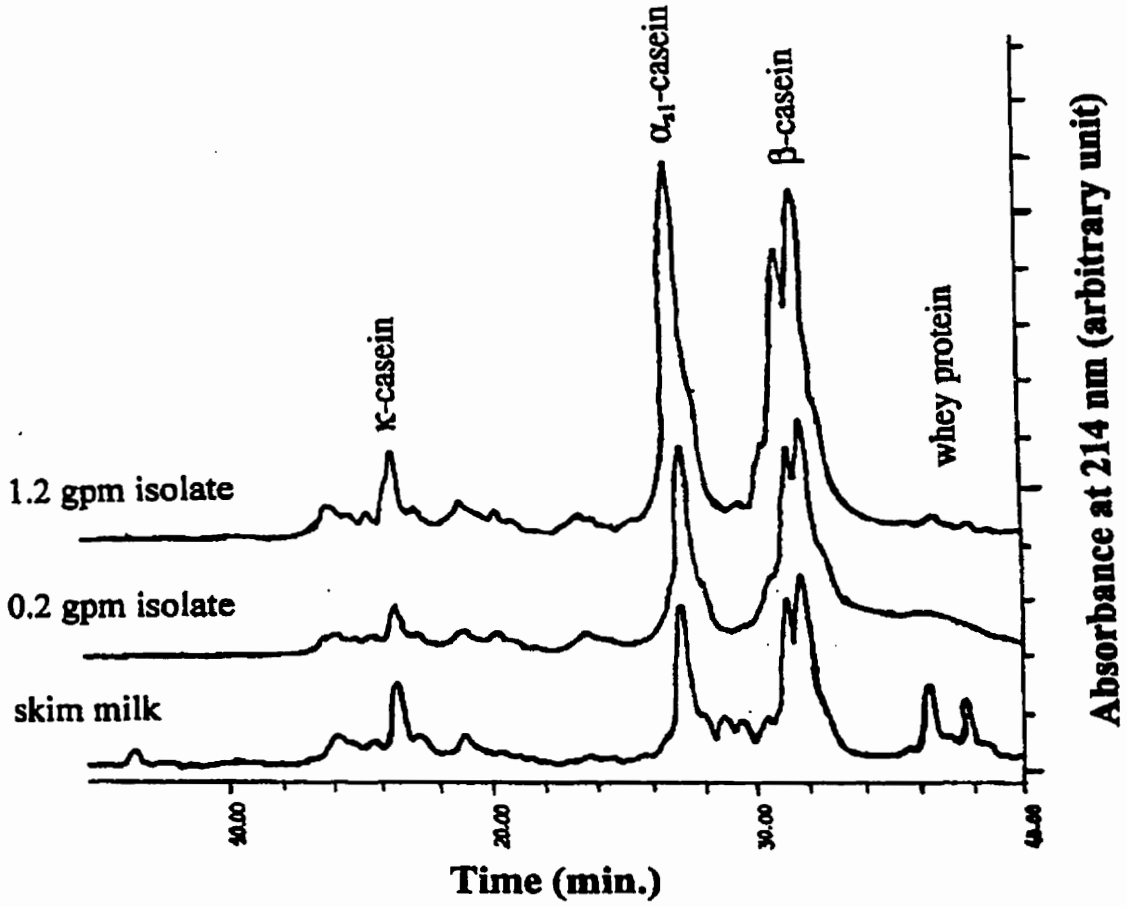

Figure III-5 : Reverse-phase HPLC chromatograms of skim milk and of isolates produced by bipolar membrane electroacidification of skim milk run at two different flow rates and at 2.0 A constant current.

The high purity of the isolate is explained by the demineralization process coupled with the action of bipolar membranes. Bipolar membrane decreases the pH, without adding acid, and cations migrate accross the cation exchange membrane (CEM) to decrease the ash content of the final product in order to maintain the electroneutrality of the milk solution.

However, a deposit, probably calcium hydroxide appears on the CEM side in contact with the base. The calcium ion migrating from the milk solution could precipitate with OH^- produced on the anionic side of the BMP to form a fouling complex on the CEM. Hence further research is needed to study the wide possibilities of BMEA and to understand the membrane phenomena associated with the migration of cations.

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ATTEINTE DES OBJECTIFS ET AVANCEMENT DES CONNAISSANCES

Les premiers travaux effectués dans ce chapitre ont permis de répondre à l'objectif concernant la validation de la faisabilité de l'ÉAMBP pour la précipitation des caséines du lait et l'étude du débit. Des isolats de haute pureté contenant 1.2-2.0% de cendres et 85-92% de protéines ont été produits par ÉAMBP. La composition des profils protéiques des surnageants a montré que seules les caséines avaient précipité. Cependant, excepté pour la cinétique de précipitation protéique, le débit (0.2 et 1.2 gal/min) n'a pas influencé la composition et la pureté finale des isolats.

Ces résultats ont contribué à l'avancement des connaissances sur l'ÉAMBP et la compréhension des phénomènes électrodialytiques. Ces travaux ont mis en évidence que

l'ÉAMBP est un nouveau procédé alternatif pour la production d'isolat de caséine bovine de haute pureté. Ils ont aussi concouru à la compréhension du mécanisme de formation du colmatage des promoteurs de turbulence par le caillé protéique au cours de l'ÉAMBP des protéines du lait.

CHAPITRE IV

EFFET DE LA PERMSÉLECTIVITÉ DES MEMBRANES CATIONIQUES SUR L'EFFICACITÉ DU PROCÉDÉ D'ÉLECTROACIDIFCATION AVEC MEMBRANES BIPOLAIRES ET LEUR COLMATAGE AU COURS DE L'ACIDIFICATION DU LAIT ÉCRÉMÉ

CHAPITRE IV

EFFET DE LA PERMSÉLECTIVITÉ DES MEMBRANES CATIONIQUES SUR L'EFFICACITÉ DU PROCÉDÉ D'ÉLECTROACIDIFCATION AVEC MEMBRANES BIPOLAIRES DU LAIT ÉCRÉMÉ ET LEUR COLMATAGE EN COURS DE PROCÉDÉ

TRANSITION CONTEXTUELLE

Dans le chapitre III, un dépôt suggéré être de l'hydroxyde de calcium a été observé sur la face des membranes cationiques (MEC) en contact avec la base au cours du traitement d'électroacidification. L'ion calcium migrant hors de la solution de lait précipiterait avec les OH^- produits sur la couche anionique de la membrane bipolaire et formeraient un colmatage complexe de la MEC. De plus, l'ajustement du pH du lait au point isoélectrique des caséines affecte la stabilité de la phase minérale des micelles. En effet, 68% du calcium présent dans le lait est associé aux micelles de caséines ; durant l'acidification, les micelles de protéines se brisent et libèrent le calcium dans le milieu. Dans ce contexte, les objectifs de l'étude menée dans ce chapitre sont (1) d'investiguer l'effet de la permsélectivité des MEC sur la migration des cations du lait et la précipitation des protéines au cours du procédé d'ÉAMBP et plus généralement sur l'efficacité globale de l'ÉAMBP, (2) d'étudier l'effet de la permsélectivité des MEC sur leur colmatage et (3) de suivre et d'identifier la nature du colmatage.

Ce chapitre a fait l'objet de deux articles : Le premier intitulé **«Effect of Cationic Membrane Permselectivity on the Efficiency of Skim Milk Electroacidification», publié dans *J. Agric. Food Chem.*, 2000, 48(6), 2595-2601**. Les auteurs sont : Laurent Bazinet (Candidat au Ph. D.: planification et réalisation des expériences, analyse des résultats et rédaction de l'article), François Lamarche (Co-directeur de thèse : supervision

scientifique de l'étudiant, correction et révision du manuscrit), Denis Ippersiel (participation à la réalisation des expériences, correction et révision du manuscrit), Behzad Mahdavi (révision du manuscrit) et Jean Amiot (Directeur de thèse : supervision scientifique de l'étudiant, correction et révision du manuscrit).

Le second ayant pour titre «**Effect of Membrane Permselectivity on the Fouling of Cationic Membranes during Skim Milk Electroacidification**», publié dans ***J. Membr. Sci.*, 2000, 174(1), 97-110**. Les auteurs sont : Laurent Bazinet (Candidat au Ph. D.: planification et réalisation des expériences, analyse des résultats et rédaction de l'article), Denis Ippersiel (participation à la réalisation des expériences, correction et révision du manuscrit), Diane Monpetit (réalisation des analyses élémentaire par microscopie électronique), Behzad Mahdavi (révision du manuscrit), Jean Amiot (Directeur de thèse : supervision scientifique de l'étudiant, correction et révision du manuscrit) et François Lamarche (Co-directeur de thèse : supervision scientifique de l'étudiant, correction et révision du manuscrit).

RÉSUMÉ (Premier article)

Une procédure développée pour la précipitation des protéines de soya, qui est dérivée de l'électrodialyse, a été testée pour la production de caséine acide. Cette technologie, appellée génériquement électrodialyse avec membranes bipolaires (ÉDMBP) ou plus spécifiquement électroacidification avec membranes bipolaires (ÉAMBP), utilise la propriété des membranes bipolaires a dissocier les molécules d'eau et l'action de déminéralisation des membranes échangeuses de cations (MEC). Cependant, le contenu minéral du lait est très sensible aux changements de pH et de force ionique. Dans le but de mieux comprendre l'effet des changements en minéraux du lait au cours de l'abaissement du pH et la déminéralisation du lait écrémé, les objectifs de cette étude étaient d'investiguer l'effet de deux différentes permsélectivités de membranes cationiques (membranes CSV et CMX) sur la migration des cations du lait écrémé et la précipitation des protéines au cours de l'ÉAMBP.

Les résultats ont montré que les deux membranes à permsélectivités différentes testées n'influencent pas l'efficacité finale de l'ÉAMBP et que l'ÉAMBP produit des isolats de caséine de lait bovin avec une pureté similaire ou supérieure (97-98% vs 93.4-96.7) à celle des isolats commerciaux, conséquence d'un contenu en cendres réduit par déminéralisation (1.2 vs 2.0-3.8%). Cependant, l'empilement des membranes CSV comme MEC semble stabiliser la conductivité de la solution de lait écrémé par une cinétique minérale de migration différente. Pour les deux membranes, les espèces ioniques migrant principalement sont les ions potassium, alors que les espèces secondaires sont le sodium et le calcium respectivement pour les membranes CMX et CSV. Cependant, la membrane CSV qui est vendue commercialement comme membrane permsélective aux ions monovalents, a été démontrée permsélective au calcium, qui est un ion divalent, au cours de l'EAMBP du lait écrémé.

Mots clés : Acidification électrochimique, Permsélectivité cationique, Caséine, Lait, Membrane bipolaire

ABSTRACT

A procedure developped for soybean protein precipitation which was derived from electrodialysis was tested for the production of acid casein. 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 demineralization action of cation-exchange membranes (CEM). However, as mineral salt content is very sensitive to ionic strenght and pH changes, the aim of this study was to better understand the effect of changes in mineral content during pH decrease and demineralization of skim milk. Our objectives were to investigate the effect of different cationic permselective membranes (CSV and CMX membranes) on skim milk cation migration and protein precipitation during BMEA

Results showed that the permselectivity of both membranes tested does not influence the final efficiency of BMEA and that BMEA produces bovine milk casein isolates with a similar or higher purity (97-98% vs 93.4-96.7) than those of commercial isolates, due to a reduced ash content (1.2 vs 2.0-3.8%) resulting from CEM demineralizing phenomenon. However, the stacking of CSV membranes as CEM seems to stabilized the skim milk solution conductivity by a differential mineral kinetic. For both membranes, the main ionic species to migrate was the potassium ions, while the secondary species were sodium and calcium for CMX and CSV membranes respectively. However, CSV membrane which is commercially sold as a monovalent permselective membrane, was demonstrated to be permselective to calcium which is a divalent ion during skim milk BMEA.

Key words : Electrochemical acidification, Bipolar membrane, Casein, Milk, Cationic permselectivity.

INTRODUCTION

Two main types of casein are usually produced in the dairy industry : rennet and acid casein. In the case of rennet casein, the underlying mechanism for purification is identical to that of production of cheese curd and depends on the unique sensitivity of the Phe_{105}-Met_{106} bond in κ-casein to hydrolysis by acid proteinases, the active components of rennet. For acid casein production, three main procedures exist, all based on isoelectric precipitation of casein use acidification by chemical, physico-chemical or fermentation (Segalen, 1985; Southward, 1993; Varnam and Sutherland, 1994).

A procedure developped for soybean protein precipitation (Bazinet *et al.*, 1996 ; 1997a,b; 1998b) which was derived from electrodialysis, was tested for the production of acid casein (Bazinet *et al.*, 1999c). 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.

A typical CEM for electrodialysis has a pore size of 10-20 Å with a capacity of 1.6-3.0 meq. per gram of dry weight of resin. The demineralization action of cation-exchange or cationic membranes (CEM) is closely linked to its internal structure. CEMs are made of a macromolecular material (skeleton) consisting of a hydrophobic polymer which carries ionizable groups. In a CEM, fixed anions are electronically neutralized by mobile cations in the interstices of the polymer. Under the influence of an electric field, cations move from one site to another in the network of anionic functional groups fixed on the skeleton and cross the membrane. Ions with the same charges as the ionized groups fixed on the polymer are rejected from the membrane core, as a result of an electrostatic repulsion called Donnan exclusion (Bazinet *et al.*, 1998a). The concentration of fixed anionic charges, as well as the degree of cross-linking of the CEM determine the ion permselectivity. The degree of cross-linking determines the tightness of the matrix structure, and thus its porosity or its pore size (Streat and Cloete, 1987). The CEM used in electrodialysis is generally a monolayer membrane. Some special three-layer CEMs have been developped in order to obtain a monovalent cation permselectivity. On each side of the CEM layer, electrolyte polymers

with a different electric charge are applied to the surface of the membrane. The monovalent cation permselectivity thus obtained is referred to as a filtering effect, because of its resemblance to the trapping of multivalent ions with a filter.

In milk, casein interacts with ions and salt, particularly calcium phosphate, to form voluminous micellar complexes with a diameter varying from 20 to 600 nm (Cheftel *et al.*, 1985; Schmidt, 1982). About 7% of casein micelle dry matter is constituted by inorganic material, mainly calcium and phosphate (Walstra, 1990). A total of 68 per cent of calcium present in milk (117 mg of calcium per 100 g of milk) is associated with the micelle, that is to say about 31 mg per gram dry micelle. The micelle also contains about 0.26 g of potassium, 0.11 g of magnesium and 0.11 g of sodium per 100 g dry micelle (Schmidt, 1982). By adjusting the pH of milk to the isoelectric point of casein, the intra- and inter-protein electrostatic attractions are increased, which affect the stability of the mineral phase of the micelle.

As the mineral salt content is very sensitive to ionic strength and pH changes, the aim of this study was to better understand the effect of changes in mineral content during pH decrease and demineralization of skim milk. Our objectives were to investigate the effect of cationic membrane permselectivity on skim milk cation migration and protein precipitation during BMEA and more generally on the global efficiency of BMEA. Two cationic membranes, with different permselectivity, were compared in term of electrodialysis cell parameters, percentage of proteins precipitated, ash content, mineral content, protein molecular profiles and chemical composition of isolates produced.

MATERIAL AND METHODS

Material

The raw material used in this study was fresh homogenized skim milk (Quebon, Natrel, Longueil, QC, Canada).

Methods

a) Electro-acidification cell

The module used was an MP type cell (100 cm^2 of effective electrode surface) from ElectroCell Systems AB Co. (Model MP cell, Täby, Sweden). The cell consists of eight compartments separated by four cationic membranes and three Neosepta BP-1 bipolar membranes from Tokuyama Soda Ltd. (Tokyo, Japan). This arrangement defines three closed loops containing the milk solution, a 2 g/L aqueous KCl solution and a 20 g/L Na_2SO_4 solution. Each closed loop was connected to a separate external reservoir, allowing for continuous recirculation (Bazinet *et al.*, 1997b).

The anode/cathode voltage difference was supplied by a variable 0-100 V power source, Powerstat Model 236BU-2 (The Superior Electric Co., Bristol, CO). The electrolytes were circulated using three centrifugal pumps Model XVB56C34F2012b-W (Marathon Electric, Wausau, WI), and the flow rate was controlled using Model FC-FI-C-3/8 Flowmeters (Filter-Chem, Alhambra, CA). The temperature of the electrolytes was maintained at 20°C by circulating water inside a stainless-steel coil immersed in each of the reservoirs. The anode, a dimensionally-stable electrode (DSA), and the cathode, a 316 stainless-steel electrode, were supplied with the MP cell.

b) Protocol

Electro-acidification was carried out in batch process using a current of 2.0 A, and after reaching 60 V, the voltage was maintained constant at 60 V, in order to not surpass the total power of the power supply. Electrolyte volumes of 4 litres were used for the Na_2SO_4 and KCl solutions while a 1.5 L volume was used for the milk solution. The electro-acidification was stopped after the pH reached 4.2. The initial pH varied between 6.5 and 6.7.

Two membranes were tested during electroacidification : the Neosepta CMX membrane from Tokuyama Soda Ltd., which is permeable to monovalent and divalent cations and the CSV membrane from Asahi Glass Co. Ltd, which is permeable to

monovalent cations only. Three replicates of each condition were performed in this experiment.

During each treatment, 1.5 mL samples and 30 mL samples of the milk solution were taken respectively every 0.4 and 0.8 pH unit decrease respectively from initial pH (around 6.6) to pH 4.2. In this experiment, milk was diluted (1.1 L milk and 0.4 L distilled water) in order to minimize the risk of spacer fouling. The time required to reach pH 4.2, the system resistance, the conductivity and the temperature were recorded as the treatment progressed. On freshly acidified 1.5 mL samples, the concentration of soluble protein was determined. The 30 mL samples were stored at –20°C before ash content, sodium, potassium and calcium concentrations, and molecular weight profiles were performed. At the end of each run, 500 mL samples of the pH 4.2 milk solution were taken. These samples were centrifuged for 10 minutes at 4°C, at 500 g (Centrifuge Model J2-21, rotor type JA-10, Beckman Instruments Inc., Palo Alto, CA), the precipitate was washed twice with distilled water, before being lyophilized for 24 hours at room temperature (Model Freezone 4.5, Labconco, Kansas City, MI). The lyophilized isolates were stored at 4°C before total protein determination, molecular profiles and ash content were performed.

c) Analysis Methods

pH measurement: The pH of the protein solution was measured with a pH meter Model Φ11 (Beckman Instruments Inc., Fullerton, CA)

System resistance: The system resistance was calculated, using Ohm's Law, from the voltage and the current intensity read directly from the indicators on the power supply .

Conductivity: A YSI conductivity meter Model 35 was used with a YSI immersion probe Model 3418, cell constant K= 0.1 cm^{-1} (Yellow Springs Instrument Co.,Yellowsprings, OH) to measure the conductivity of the protein solutions.

Moisture: Crucibles were washed beforehand in hydrochloric acid (HCl, 10%) for two hours, rinsed with deionized water and dried in a 550°C oven for one hour. They were

removed from the oven and placed in a dessicator for 30 minutes. For lyophilized isolates, approximately 2.5 g was added to the cooled crucibles, and the mass recorded. The samples were then dried in a vacuum oven at 100°C overnight. The samples were cooled in a dessicator for 30 minutes and weighed when they reached room temperature (AOAC International, 1995a).

Ash Content: Crucibles were washed beforehand in hydrochloric acid (HCl, 10%) for two hours, rinced with deionized water and dried in a 550 °C oven for one hour. They were removed from the oven and placed in a dessicator for 30 minutes. For liquid samples, 10 mL of milk solution was added to the cooled crucibles. The sample was dried overnight at 105°C and then ashed at 500°C for at least 16 hours. For lyophilized isolates, ash was determined on same portion as moisture. The sample was then ashed at 550°C. The samples were cooled in a dessicator for 30 minutes and weighed when they reached room temperature (AOAC International, 1995b,c).

Potassium, sodium and calcium concentration determination: Sodium and potassium concentration were determined by a flame emission spectrometric method (AOAC International, 1995d), while calcium concentration was measured by an atomic absorption spectrophotometric method (AOAC International, 1995e) with a Varian SpectrAA-100 (Malgruve, Victoria, Australia). The ion concentration was measured on an ashed sample diluted in 20 mL HCl 2N. A specific hollow cathode lamp (model 3UNX-Ca, Cathodeon Limited, Cambridge, UK) at 422.7 nm wavelength was used to determine calcium concentration in the solution. For flame emission, sodium and potassium concentrations were measured at 589 nm and 766.5 nm wavelengths respectively.

Total Protein determination: The protein concentration determination was obtained using a FP-428 LECO apparatus (LECO Corporation, Saint Joseph, MI) under the same conditions used by Bazinet *et al.* (1999c).

Molecular protein profiles: The chromatographic analysis of the molecular profile of the lyophilized protein isolate and skim milk samples was performed by reverse-phase

HPLC according to Jaubert and Martin (1992) under the same conditions used by Bazinet *et al.* (1999c).

d) Statistical Analyses

Time required for pH decrease, and measures of conductivity and of system resistance as a function of pH were subjected to an analysis of variance using SAS software (SAS, 1989) and linear regression was calculated using SigmaPlot (version 3.0 for Windows, Jandel Scientific, Corte Madera, CA). The concentration of soluble protein, the ash content, the concentration of Ca, Na and K ions in ash, and the percentage of κ-, α_{s1}-, α_{s2}-, β-casein and whey protein in milk solution as the pH decreased were analyzed with a split-plot analysis of variance, since the Huynh-Feldt condition was met (Huynh and Feldt, 1970). Regression contrasts were calculated for each univariate analysis of variance, using SAS software, to examine the effect of interaction between the variables. Duncan tests were used to determine the significance of the difference between both membranes. The total protein, ash content, the concentration of Ca, Na and K in ash, and the composition in κ-, α_{s1}-, α_{s2}-, β-casein and whey protein in the isolates produced with different membranes by BMEA were subjected to an analysis of variance and as well as Duncan tests in order to determine the significance of difference between isolates.

RESULTS AND DISCUSSION

Electro-acidification parameters : duration, system resistance and conductivity

Results of the analysis of variance indicated that the pH has a highly significant effect on the duration of BMEA ($P < 0.0001$) and on the system resistance ($P < 0.0004$), and the dual interaction of the pH and the membrane have a significant effect on the duration ($P<0.032$) of BMEA and on the conductivity ($p<0.0009$) of the milk solution. The regression calculated for the variables as a function of pH produced coefficients of determination ranging between 0.636 to 0.997.

a) System resistance

The evolution of the system resistance is the same whatever the permselectivity of the membrane, and it is characterized by a slight increase (Figure IV-1). The system resistance increased from 24.3 to 29.8 Ω and from 22.3 to 31.5 Ω for the CSV and CMX membranes respectively. This increase should be mainly due to a slight fouling of the spacers in the cell by the precipitated protein.

Indeed, as the mesh wire design of the spacer is fine, precipitated protein forms a slight deposit on the mesh, which increase the global system resistance. This result is in agreement with those of Bazinet *et al.* (1998b) obtained on soybean protein.

b) Duration

Time required to decrease the pH of a skim milk solution from 6.6 to 4.2 was 17.3 and 19.5 min for electroacidifications carried out with CMX and CSV membranes respectively (Figure IV-2). This is confirmed by the calculated slopes of -0.12 and -0.14 for CSV and CMX membranes respectively. The acidification of skim milk solution, during BMEA, whatever the membrane was carried out in a linear fashion, as confirmed by coefficients of determination of 0.991 and 0.997 calculated for CSV and CMX membranes respectively.

However the fact that the BMEA was not run at a constant current explains the difference observed in duration between the both membranes. The CSV membrane, is a cationic monovalent permselective membrane, and by its intrinsic selectivity, it slows down the crossing of divalent cations. As the BMEA was not carried out at constant current, the impact of membrane selectivity was even higher ; the CSV membrane increases the global resistance of the system by allowing mainly the migration of monovalent cations. This is confirmed by the different system resistance values discussed above (24.3 and 22.3 Ω , for CSV and CMX membranes respectively). Due to a higher resistance of the system, and at a constant voltage, the current intensity is lowered with CSV stacking and consequently, the number of H^+ electrogenerated is lowered. As the duration is directly linked to the quantity

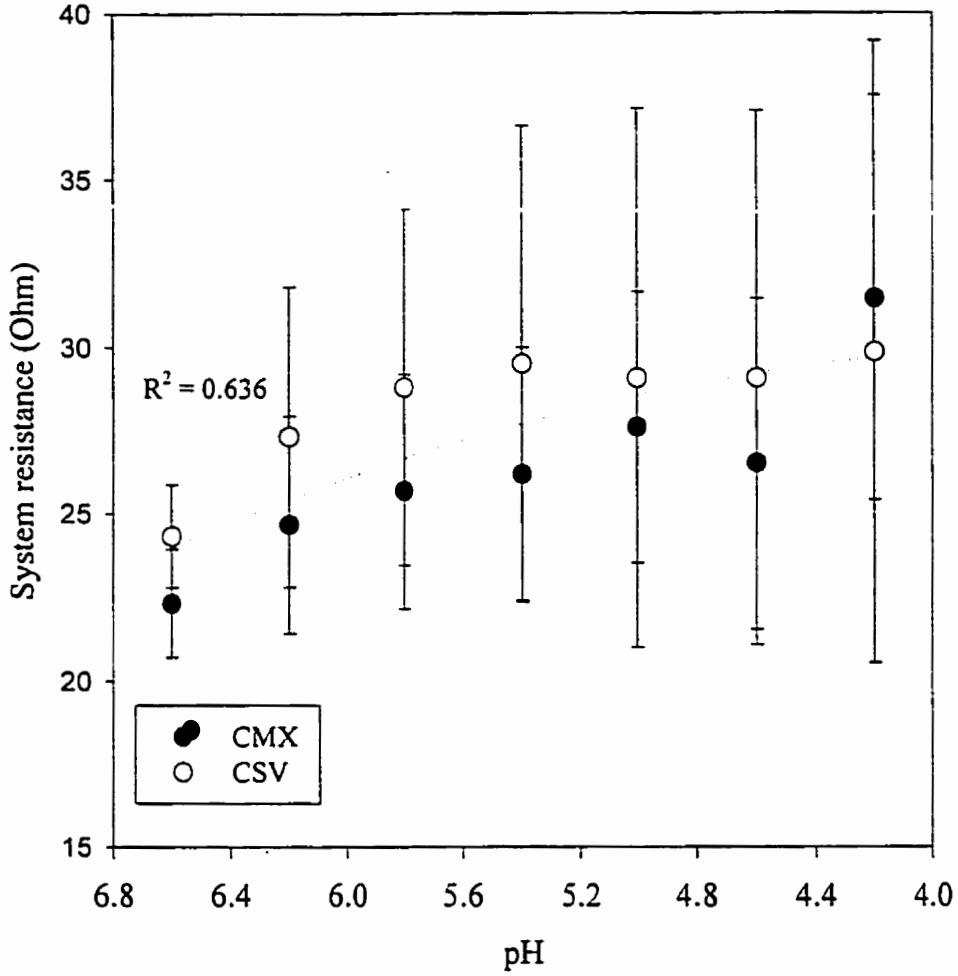

Figure IV-1 : Effect of the membrane permselectivity, CMX and CSV membranes, on the system resistance during bipolar membrane electroacidification of a skim milk solution run at 20°C.

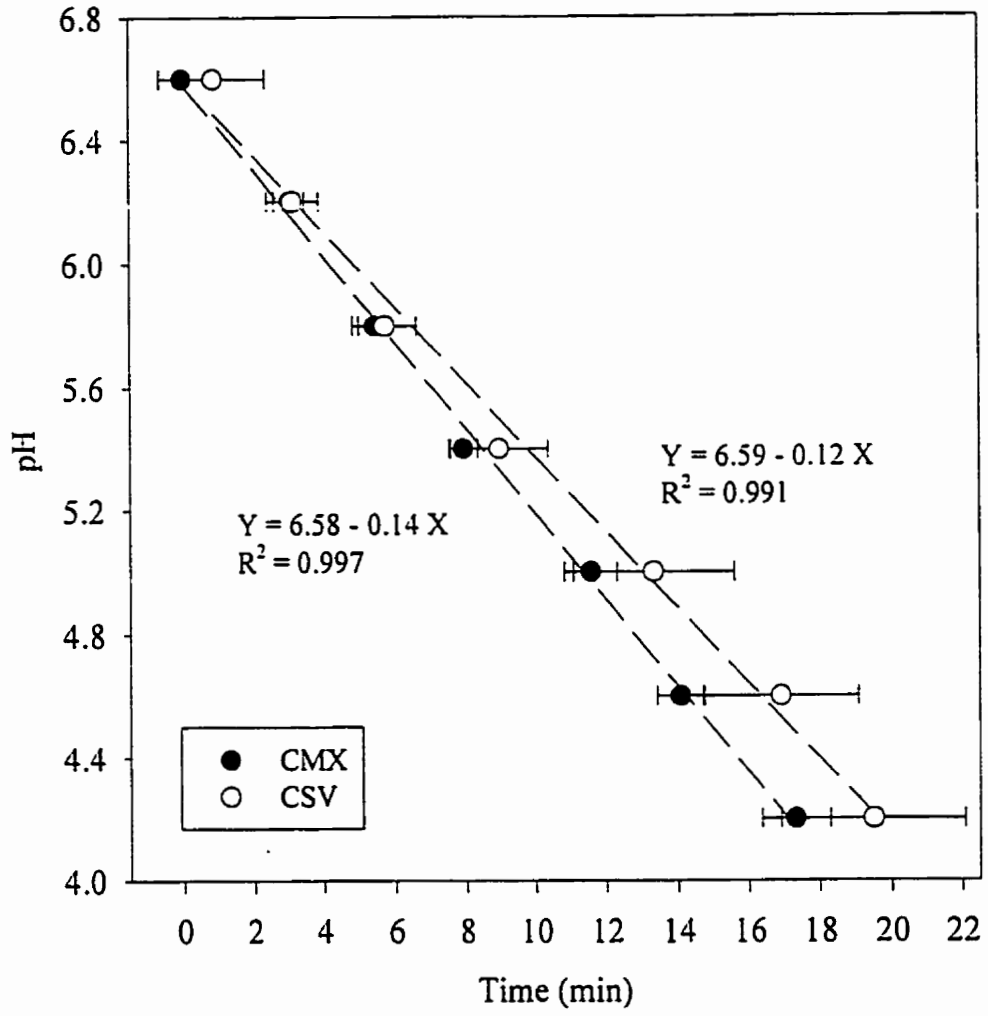

Figure IV-2 : Effect of the membrane permselectivity, CMX and CSV membranes, on the time to decrease the pH by bipolar membrane electroacidification of a skim milk solution run at 20°C.

of H^+ electrogenerated (Bazinet *et al.*, 1998b; 1999a), the duration of BMEA with CSV membrane is longer.

c) Conductivity

Results of the statistical analysis indicate that the evolution of the conductivity as pH decreases is different for both membranes (Figure IV-3). The conductivity changes differently during BMEA according to the membrane used : for the CSV membrane, the conductivity as characterized by a slight increase (or stabilization) from 2.6 to 2.7 mS/cm; while for the CMX membrane a decrease in conductivity was noted from 2.7 to 2.2 mS/cm. Decrease in conductivity obtained with the CMX membrane is in accordance with previous results obtained by Bazinet *et al.* (1999c). This demonstrates that the BMEA was carried out under excellent conductivity conditions, and that conductivity at a level of 2.2-2.7 mS/cm does not appear to be a limiting factor in BMEA of skim milk solutions.

Ash content

The analysis of variance of the data shows that the pH ($P<0.0001$) has a highly significant effect on ash content. The regression calculated for the ash content as a function of pH produced a coefficient of determination of 0.969.

As the pH decreased, the ash content (mg/10 mL) also decreased for both membranes tested (Figure IV-4). Thus, the average initial ash content was about 38.4 mg/10 mL at pH 6.6, and decreased linearly to 34.9, 29.4 and 25.7 at pH 5.8, 5.0 and 4.2 respectively : a de-ashing rate of 33.1%. This result agrees with the data in the literature (Bazinet *et al.*, 1997a; 1998b).

Bazinet *et al.* (1998b; 1999a) demonstrated that the quantity of H^+ necessary for electroprecipitation is related to the concentration of protein present in the solution. As the protein content was the same for both membranes, the same number of H^+ to be electrogenerated is consequently necessary. Hence, to obtain electrical neutrality of the skim milk solution, one cationic charge must cross the cationic membrane for each H^+

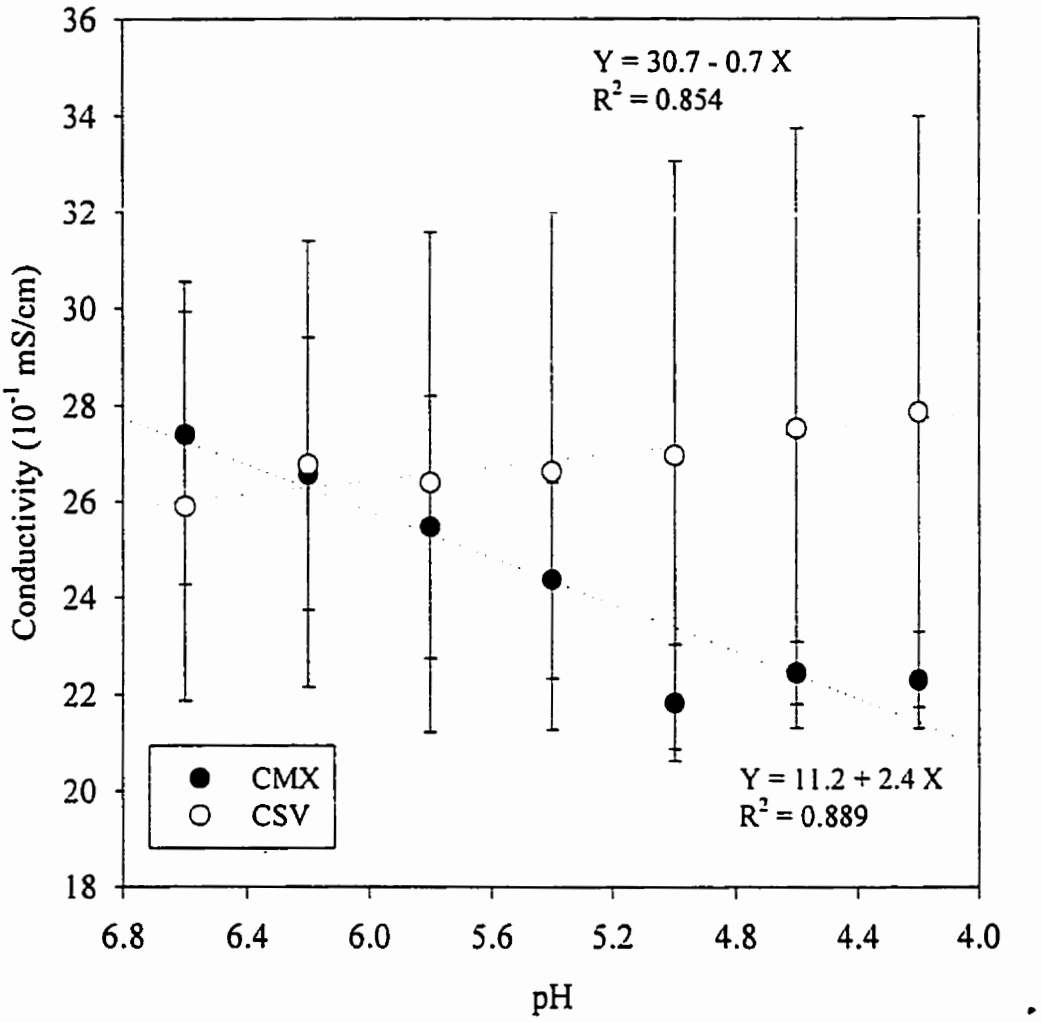

Figure IV-3 : Effect of the membrane permselectivity, CMX and CSV membranes, on the conductivity of the skim milk solution during bipolar membrane electroacidification run at 20°C.

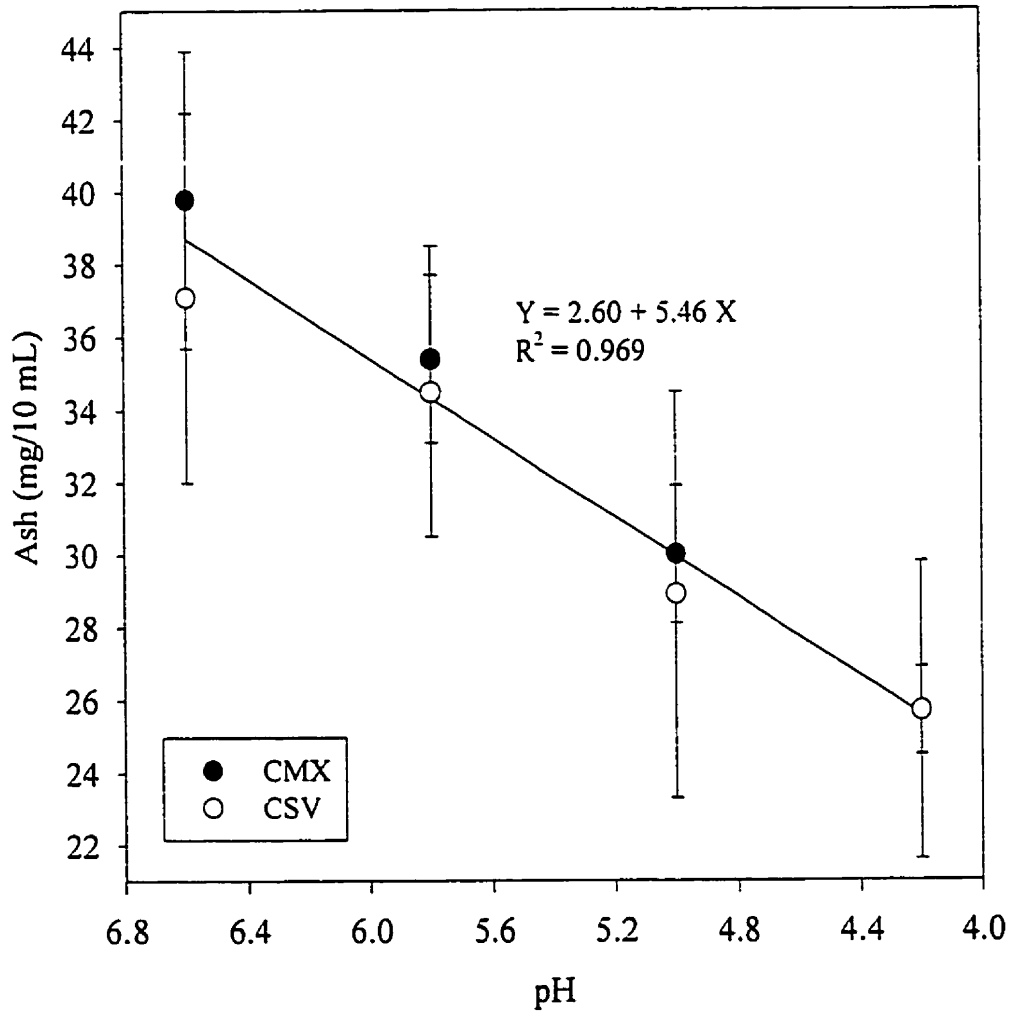

Figure IV-4 : Effect of the membrane permselectivity, CMX and CSV membranes, on the evolution of the ash content in the skim milk solution during bipolar membrane electroacidification run at 20°C.

produced at the BPM interface. This decreases the quantity of minerals, and therefore the overall ash content, by desalinization of the protein compartment (Bazinet *et al.*, 1997b; Houldworth, 1980 ; Chaput, 1979).

Concentration of calcium, sodium and Potassium :

The analyses of variance of the data show that the pH has a significant effect on calcium ($P<0.0001$) and potassium ($P<0.0001$) concentrations, as well as the dual interaction of pH and type of membrane on calcium ($P<0.0482$) concentration.

As the BMEA proceeded, the concentration of potassium, sodium and calcium decreased as a function of pH, but in a different way according to the membrane permselectivity (Figure IV-5). The potassium ion concentration decreased very rapidly, from 579.8 to 308.0 mg/L (-46.8% variation) from pH 6.6 to 4.2 respectively. The sodium ion decreased ($P=0.0598$) very slowly from 360.5 to 300.2 mg/L (-16.7% variation) from the beginning to the end of the BMEA run. The evolution of the calcium concentration was different according to the permselectivity of the membrane : with the CMX membrane, the calcium concentration in the milk solution decreased from 779.7 to 675.6 mg/L (-13.3% variation) from pH 6.6 to 4.2 respectively; while with the CSV membrane, the decrease was from 766.1 to 569.8 mg/L (-25.6% variation). Therefore, the calcium ion seems to migrate in a higher quantity with the CSV membrane than with the CMX membrane.

Furthermore, from pH 5.0 to 4.2, according to the membrane permselectivity, the three ions have a different evolution : the sodium migrates in a linear fashion during all the BMEA treatments with the CSV membrane while with the CMX membrane, its concentration drops drastically in this particular interval. In the same way, the calcium migrates linearly during BMEA with CMX membranes while with CSV membranes its concentration dropped. Contrary to both previous ions, migration of potassium seems to be reduced between pH 5.0 and 4.2 : with the CSV and the CMX membranes the concentration of potassium was stable, no change to the rate of migration of potassium ions. The significant decreases noted for the calcium and sodium ions for the CSV and CMX membranes respectively seems to compensate for the reduced migration rate of the potassium ions. In

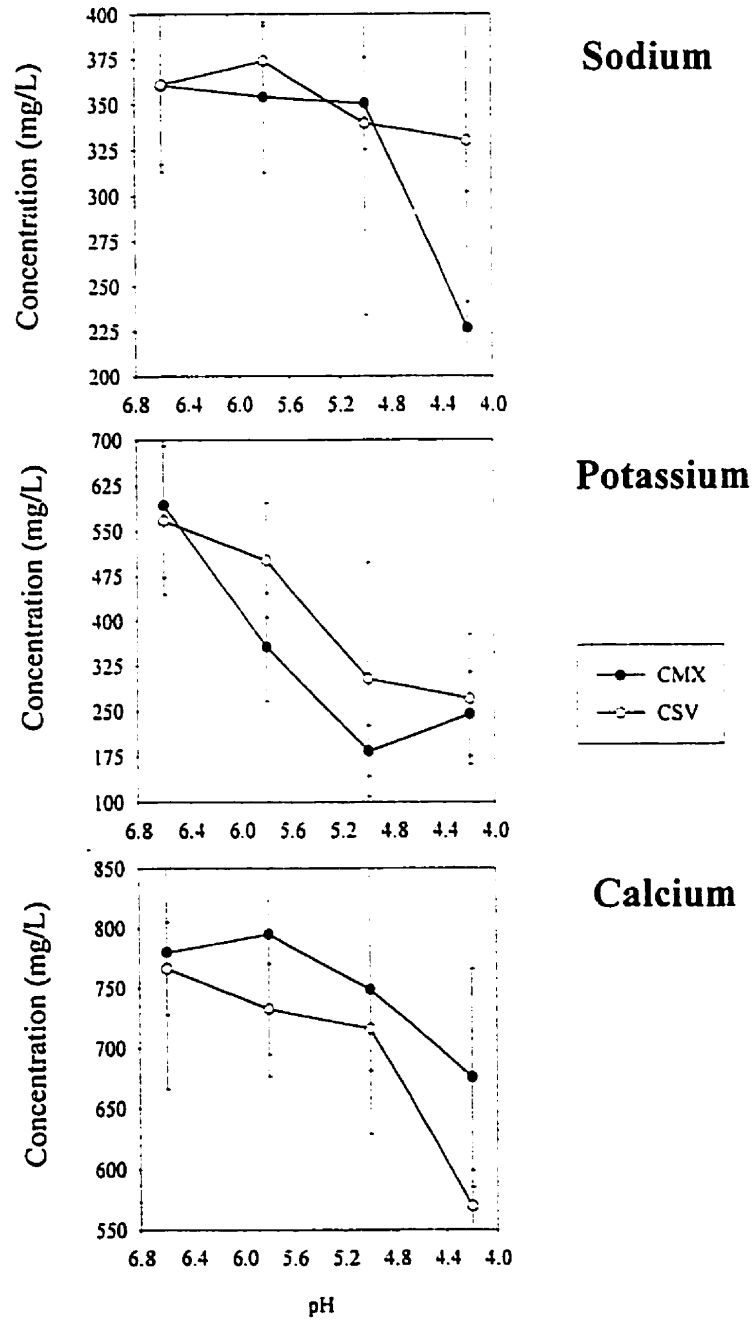

Figure IV-5 : Effect of the membrane permselectivity, CMX and CSV membranes, on the evolution of the sodium, potassium and calcium concentrations in the skim milk solution during bipolar membrane electroacidification run at 20°C.

addition, calcium, a divalent ion, migrated across the CSV membrane commercially sold as a monovalent permselective membrane.

These results confirm previous results indicating that monovalent ions seem to be much more mobile than divalent salts and that they can be easily removed from milk. Lower mobilities of the divalents ions and their abilities to form complexes with proteins would be involved (Young, 1974; Hiraoka *et al.*, 1979; Higgins and Short, 1980; Perez *et al.*, 1994).

However, in this experiment, sodium migrated more slowly than calcium. The migration rates of the different ions, in skim milk, is different according to the de-ashing rate, and their respective absolute conductivity and mobility values of 73.5 $S.cm^2/mol$ and 7.6 $cm^2/s.V$ for potassium, 50.1 $S.cm^2/mol$ and 5.2 $cm^2/s.V$ for sodium and 119.0 $S.cm^2/mol$ and 6.2 $cm^2/s.V$ for calcium (Hiraoka *et al.*, 1979; Brett and Oliveira-Brett, 1994). According to these conductivity and mobility values, calcium has the higher conductivity but a medium mobility due to its structure, the potassium has a medium conductivity and the higher mobility while the sodium has the lower conductivity and mobility. Moreover the mobility of calcium can be changed by formation of a complex with protein. Hiraoka *et al.* (1979) studied the evolution of the sodium, potassium and calcium concentration during demineralization of skim milk by electrodialysis. Potassium, sodium and calcium were shown to migrate at different rates from a 0 to 90% de-ashing rate. All the sodium and potassium were removed while about one-fourth of calcium was not yet removed. At low de-ashing rates, from 0 to about 30%, sodium and calcium de-ashing kinetics are inverted. Therefore, our experiment was carried out in the de-ashing rate range where the sodium and calcium kinetics are inverted, since the de-ashing rate calculated from ash data was about 33%.

Soluble protein :

The analysis of variance of the data shows that the pH ($P<0.0001$) has a significant effect on the percent soluble protein. A non-linear regression model of the percent soluble

protein as a function of pH (all membrane types averaged) was calculated and produced a good coefficient of determination ($R^2 = 0.995$).

The membrane permselectivity had no effect on the final percent soluble protein which decreased from about 100% to 25.3-25.6% soluble protein. Soluble protein decrease during the pH decrease from 6.6 to 4.2 was the same (Figure IV-6) and could be modeled as a sigmoidal curve. From pH 6.6 to 5.4, soluble protein was unchanged. When the pH dropped to 5.0, the percent soluble protein decreased to about 69%. As the BMEA continued from pH 4.6 to 4.2, the percent soluble protein was constant at about 25%.
The difference of cationic permselectivity of the membrane does not appear to have an effect on the percent soluble protein during BMEA of skim milk. The percent soluble protein of 25% obtained at the end of the run is in accordance with the literature (Brunner, 1981; Swaisgood, 1982; Cheftel *et al.*, 1985; Lorient, 1991). In this experiment, the percent soluble protein obtained at pH 5.0 is higher than that obtained in the previous experiment. In this experiment the skim milk was diluted with water (1.1 L skim milk and 0.4 L water). This dilution, in addition to the dead volume of the electrodialytic system (tubing, reservoir, flowmeter and pump), have decreased the ionic strength and the protein concentration of milk which are an important factor in protein precipitation (Cheftel *et al.*, 1985; Kinsella *et al.*, 1985). Bazinet *et al.* (1997a; 1998b) have demonstrated on soybean protein that the protein-solvent interactions are increased as well as is the solubility of the protein when ionic strength and protein concentration are lowered.

Molecular profiles :

The ANOVA showed a highly significant effect of pH on κ-cas ($P<0.0001$), α_{s2}-cas ($P<0.0001$), α_{s1}-cas ($P<0.0001$) and β-cas ($P<0.0001$), which were confirmed by Duncan tests. The pH was demonstrated to have no effect on whey proteins (α-la and β-lg) by both ANOVA results ($P>0.97$) and Duncan tests (Table IV-1).

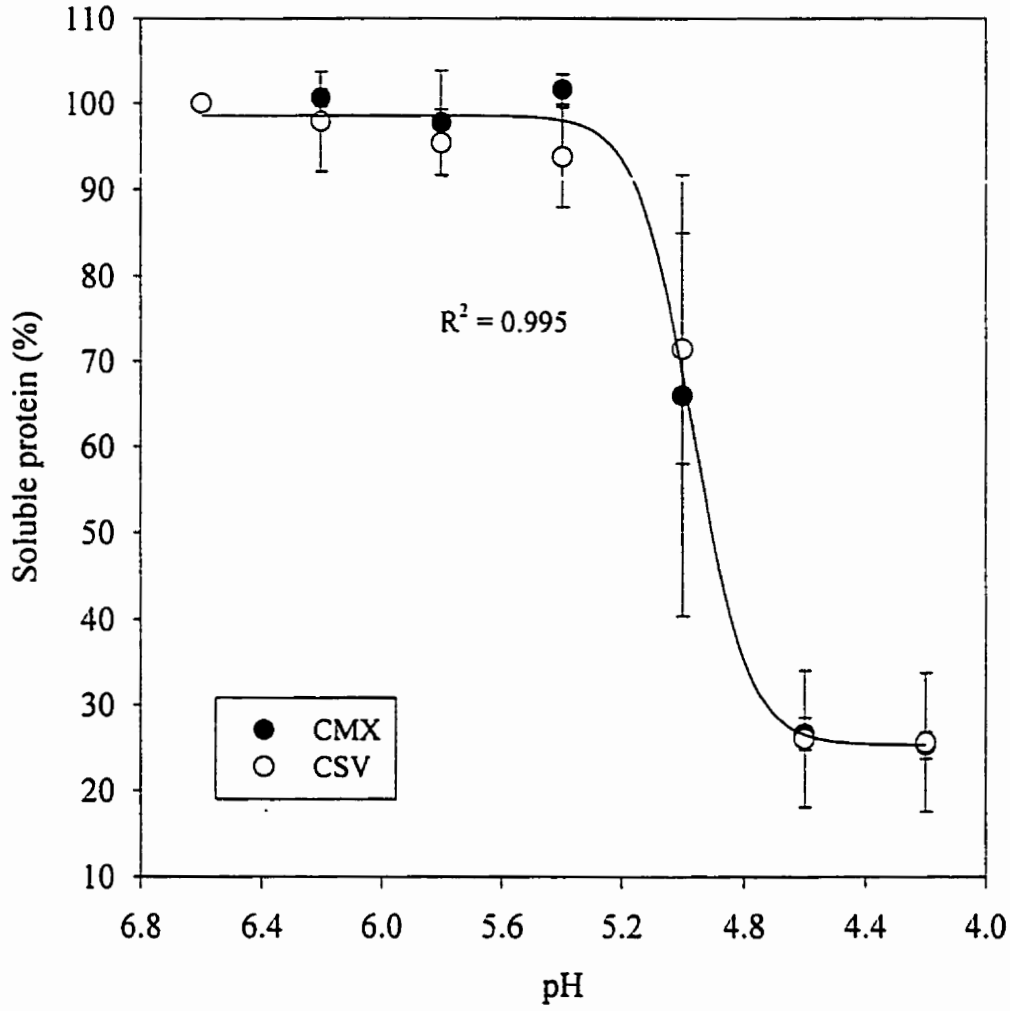

Figure IV-6 : Effect of the membrane permselectivity, CMX and CSV membranes, on the evolution of the percent soluble protein in the skim milk solution during bipolar membrane electroacidification run at 20°C.

Table IV-1 : Supernatant composition in each protein fraction during BMEA (data from both permselective membrane averaged).

pH	κ-casein	α_{s2}-casein	α_{s1}-casein	β-casein	Whey protein (α-la and β-lg)
6.6	12.0 a*	6.3 a	32.6 a	35.7 a	13.4 a
5.8	12.3 a	4.6 b	28.9 ab	30.8 ab	13.5 a
5.0	8.5 b	4.3 b	25.9 b	27.9 b	13.6 a
4.2	1.7 c	0.6 c	0.3 c	1.4 c	13.3 a

* Means within a column marked with different letters are significantly different ($P < 0.05$)

Membrane permselectivity had no effect on the evolution of each milk protein in the supernatant during BMEA of skim milk (Table IV-1). However, the evolution of the percentage of protein during pH decrease is different for each protein.

These results agree with previous data obtained for soluble protein, and give more information on the differential precipitation evolution of each protein. Moreover the initial casein percentages, transformed as percentage of caseinic fraction, are in accordance with values in the literature : κ-cas, 13.8 vs 13.9 %; α_{s2}-cas, 7.3 vs 10.7 %; α_{s1}-cas, 37.6 vs 38.7 %; and β-cas, 41.2 vs 36.6 % (Lorient, 1991; Swaisgood, 1982; Cheftel *et al.*, 1985; Whitney, 1988). In the same way, the percentage of whey protein (mainly α-la and β-lg) of 13.5 is also in accordance with the percentage of whey protein in milk composition cited in the literature, ranging from 14 to 24% of milk protein, BSA, proteose peptone and Ig included (Cheftel *et al.*, 1985 ; Brunner, 1981 ; Swaisgood, 1982 ; Lorient, 1991).

Chemical composition of isolates produced :

The chemical composition of isolates obtained at pH 4.2 after BMEA with both membranes was compared in terms of ash content, total protein and percentage of each protein fraction (Table IV-2).

Ash content of isolates was different for both membranes ($P<0.001$) : the CSV membrane reduced the migration of cations by electrodialysis phenomena in comparison with CMX membrane. The ash content of electroacidified isolate was lower than that of commercial casein, 2.0-3.8%, and coprecipitated casein, 8.0-10.5% (Hargrove and Alford, 1974; Bassette and Acosta, 1988; Alais, 1984; Walstra and Jenness, 1984). Demineralization phenomenon acting during electroacidification would therefore produce isolates with lower salt contents (Bazinet *et al.*, 1997a,b; 1999a).

The total protein content was shown to be the same for the permselectivity of both membrane ($P>0.69$) : 98% (both membrane averaged). The protein content, on a dry basis, of electroacidified isolates is slightly higher than that of commercial casein, 93.4-96.7%, and coprecipitated casein, 86.0-87.0% (Bassette and Acosta, 1988 ; Renner *et al.*, 1996;

Table IV-2 : Percent total protein, ash content and moisture of the isolates produced by BMEA with different permselective membranes.

	Total Protein (% dry basis)	Ash content (% dry basis)	Moisture (%)
CMX	97.8 ± 0.3 a*	0.98 ± 0.05 a	9.8 ± 4.2 a
CSV	98.2 ± 1.6 a	1.49 ± 0.01 b	4.9 ± 0.5 a

* Means within a column marked with different letters are significantly different ($P < 0.05$)

Alais, 1984; Walstra and Jenness, 1984). The difference in ash content may explain the difference in percent total protein observed for the isolates produced by BMEA versus commercial isolates.

The comparison of the molecular profiles obtained by HPLC and the statistical analysis results of each protein fraction showed that there was no difference between the two membranes (Figure IV-7 and Table IV-3). BMEA allows the separation of high purity bovine milk casein, and the permselectivity of both membranes tested does not influence the purity of the isolates produced.

CONCLUSION

We can conclude, from the data presented in this study, that the permselectivity of both membranes tested does not influence the final efficiency of BMEA. Moreover, these results confirm that BMEA allows the production of bovine milk casein isolates with a higher purity than those of commercial isolates, due to a lower ash content resulting from the CEM demineralizing phenomenon. However, the stacking of CSV membranes as CEM seems to stabilize the skim milk solution conductivity by differential mineral kinetics : for both membranes, the main ionic species to migrate was potassium, while the secondary species would be sodium and calcium for CMX and CSV membranes respectively.

An important point noted in this study, was that the CSV membrane, commercially sold as a monovalent permselective membrane, was demonstrated to be, during skim milk BMEA, permselective to calcium which is a divalent ion.

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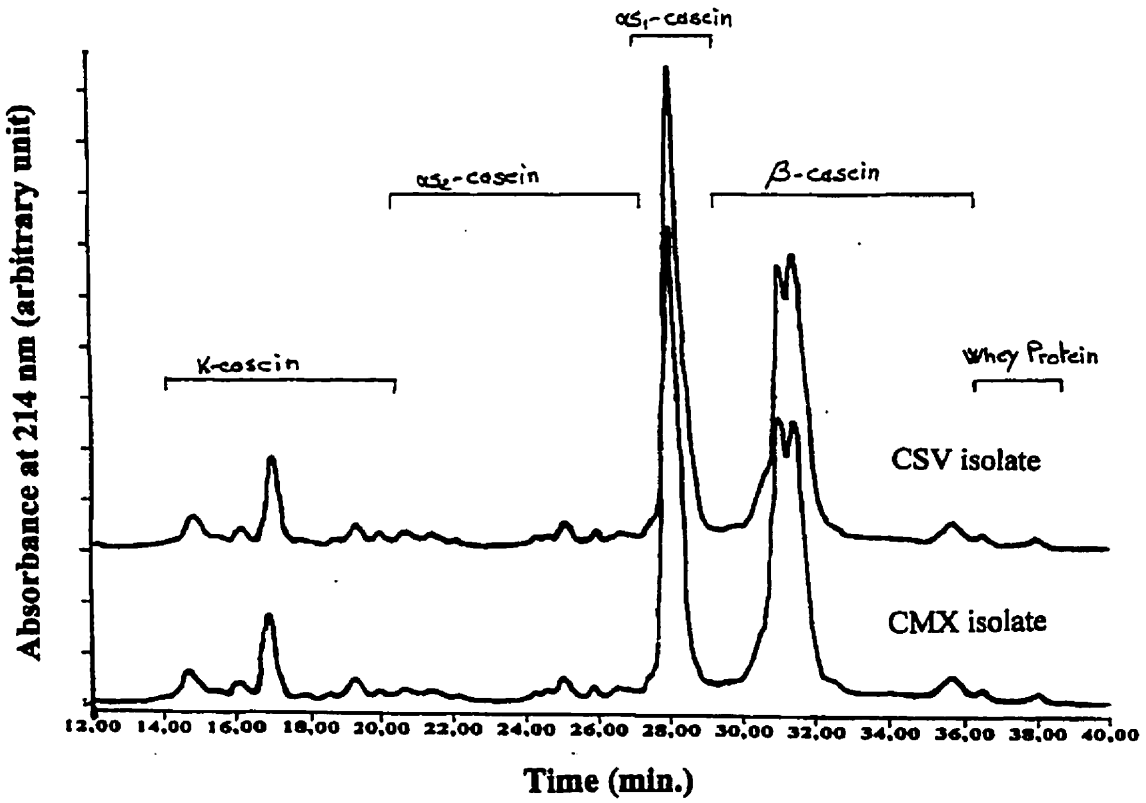


Figure IV-7 : Reverse-phase HPLC chromatograms of isolates produced by bipolar membrane electroacidification of skim milk run with CMX and CSV membranes at 20°C.

Table IV-3 : Percentage of each protein fraction in the isolates produced by BMEA with different permselective membranes.

	κ-casein	α_{s2}-casein	α_{s1}-casein	β-casein	Whey protein (α-la and β-lg)
CMX	11.3 ± 2.6 a*	6.5 ± 0.5 a	33.6 ± 1.5 a	47.3 ± 1.5 a	1.3 ± 0.1 a
CSV	13.1 ± 1.2 a	5.9 ± 0.2 a	34.8 ± 1.4 a	45.2 ± 2.6 a	1.1 ± 0.2 a

* Means within a column marked with different letters are significantly different ($P < 0.05$)

RÉSUMÉ (Deuxième article)

Une étude précédente sur le lait écrémé a démontré que l'électroacidification avec membarnes bipolaires (ÉAMBP) est une technologie qui peut être utilisée pour la précipitation isoélectrique des caséines du lait. Cependant, un dépôt suggéré être de l'hydroxyde de calcium, a été observé sur la face des membranes échangeuses de cations (MEC) en contact avec la base. Ainsi, les performances du procédé diminuent de même que la durée de vie des membranes. L'objectif de la présente étude est par conséquent d'évaluer deux types de MEC, avec des permsélectivités différentes, en terme d'efficacité électrodialytique et de paramètres de membranes.

Pour les deux types de membranes cationiques, les protéines sériques sont les seules protéines solubles restantes après électroacidification à pH 4.2 et les isolats de caséines produits (environ 97% de protéines) ont des profils protéiques moléculaires similaires. La migration des cations au travers de la membrane cationique a été influencée par la permsélectivité et les contenus en cendres des isolats produits ont été trouvés différents. La membrame CSV ralentit la migration des cations en comparaison avec la membrane CMX.

L'ÉAMBP peut être utilisée pour séparer les caséines du lait avec une pureté élevée, et la permsélectivité des deux membranes testées n'a pas influencée la pureté des isolats produits.

Mots clés : Acidification électrochimique, Caséine, Lait, Permsélectivité, Membrane cationique, Colmatage.

ABSTRACT

A previous study on skim milk demonstrated that bipolar-membrane electroacidification (BMEA) is a technology that can be used to produce isoelectric precipitation of casein from milk. However, a deposit, suspected to be calcium hydroxide, was observed on the cation-exchange membrane (CEM) side in contact with the base. Also, the performance of the process decreased as well as the lifetime of the membrane. The aim of the present study was therefore to evaluate two types of CEM with different permselectivity in term of electrodialysis efficiency and membrane parameters.

For both type of cationic membranes, whey proteins were the only soluble protein remaining after electroacidification down to pH 4.2, and casein isolates produced (about 97% protein) were found to have the same protein molecular profile. The migration of cations through the cationic membrane was found to be influenced by the permselectivity leading to isolates with different ash contents. The CSV™ membrane slowed down the migration of cations in comparison with CMX™ membranes.

BMEA can be used to separate high purity bovine milk casein, and the permselectivity of both membranes tested does not influence the purity of the isolates produced.

Key words : Electrochemical acidification, casein, milk, cationic membrane permselectivity, fouling.

INTRODUCTION

Casein is extensively used as an ingredient in food products for its nutritional value and functional properties. Pure casein is obtained by insolubilisation using rennet or acid. For acid casein production, the three main procedures are based on isoelectric precipitation by chemical, physico-chemical or fermentation acidification (Segalen, 1985; Southward, 1993; Varnam and Sutherland, 1994).

Recently, a procedure developped on soybean protein, bipolar-membrane electroacidification (BMEA) (Bazinet *et al.*,1996, 1997a,b; 1998b) and derived from electrodialysis, was tested for the production of acid casein (Bazinet *et al.*, 1999c). This technology, also based on isoelectric precipitation, was used to produce of bovine milk casein isolates with a similar or higher protein content (97-98% on a dry basis) than those of commercial isolates (93.4-96.7% on a dry basis) (Hargrove and alford, 1974 ; Bassette and Acosta, 1988 ; Alais, 1984 ; Walstra and Jenness, 1984), due to a lower ash content (1.2 vs 2.0-3.8%) resulting from a cation-exchange membrane (CEM) demineralization phenomenon (Bazinet *et al.*, 1999c). However, a deposit, suspected to be calcium hydroxide, was observed on the CEM side in contact with the base. The calcium ion migrating from the milk solution would precipitate with OH^- produced on the anionic side of the BPM and would form a fouling complex on the CEM (Bazinet *et al.*, 1999c). This phenomenon was not observed with soy isolates produced by BMEA, since the composition of the mineral content of soybean is different from milk; 50 % of the mineral salt content of soy protein concentrate is potassium (Waggle and Kolar, 1979 ; Pearson, 1983) while calcium represents only 5%. In milk, potassium and calcium represent 18.5 and 15.6% respectively of the total mineral content (White and Davies, 1958). In addition, by adjusting the pH of milk to the isoelectric point of caseins, the intra- and inter-protein electrostatic attractions are increased, affecting the stability of the mineral phase of the micelle (Graet and Brulé, 1993; Cayot and Lorient, 1998) : 68% of the calcium present in milk is associated with the micelle and during milk acidification, protein micelles break down releasing calcium (Cayot and Lorient, 1998; Schmidt, 1982).

The aim of this study is to investigate the effect of cationic membrane permselectivity on the fouling of cationic membrane, to follow the build-up and to identify the nature of the fouling. Two cationic membranes, with different permselectivity, were compared by measuring electrodialysis cell parameters, electroacidification efficiency and membrane parameters.

MATERIAL AND METHODS

Material

Commercial homogenized and pasteurized skim milk was used as raw material in this study (Quebon, Natrel, Longueuil, QC, Canada).

Methods

a) Electro-acidification cell

The electroacidification cell was the same as that used by Bazinet *et al.* (1997a,b) with four Neosepta CMX cationic membranes and three Neosepta BP-1 bipolar membranes (Tokuyama Soda Ltd., Tokyo, Japan). This arrangement defines three closed loops containing the milk solution, a 2 $g \cdot l^{-1}$ aqueous KCl solution and a 20 $g \cdot l^{-1}$ Na_2SO_4 solution. Each closed loop was connected to a separate external 600 mL double-jacket glass container (School, Duran, Germany), allowing for continuous recirculation. The electroacidification system was not equipped to maintain constant temperature (Bazinet *et al.*, 1999c).

b) Protocol

Two types of membranes were tested for electroacidification : Neosepta CMX membranes from Tokuyama Soda Ltd., which are permeable to monovalent and divalent cations and CSV membranes from Asahi Glass Co. Ltd (Tokyo, Japan), which are

permeable to monovalent cations only. Two replicates of three electroacidifications were performed. For each series of three BMEA, four membranes of the same permselectivity were cut from the same sheet, and always positioned at the same place in the cell stack, the same side always in contact with the same solution. Membranes #2 and 3 were in contact with the solution which becomes alkaline on one side, and the solution which turns acid on the other side (Figure IV-8).

The original membrane characteristics were measured on membrane #3, freshly cut from the sheet. The membrane characteristics were determined by measuring resistance and ion-exchange capacity. Then the membrane was soaked overnight in an HCl solution and the characteristics of the test membrane were determined once again. Membrane #2 was never cleaned and was called the control membrane. After each BMEA, the test membrane characteristics were measured and at the end of each three BMEA series, the control membrane characteristics were determined. In addition, the thickness of the control and test membranes were also measured. Potassium, sodium and calcium concentrations were determined in the HCl solution used to soak the test and control membranes and in the ash content of membranes, in order to follow the build-up and identify the nature of the fouling. Elemental analyses were performed on the CEM of ; the original membrane; the test membrane after three BMEA and HCl treatments; the control membrane after three BMEA; and the control membrane after three BMEA and HCl treatments. Only the side of the CEM in contact with the alkaline solution was analyzed.

Milk was diluted (1.1 L milk and 0.4 L distilled water) in order to minimize the risk of spacer fouling. During each BMEA, 1.5 mL-samples of the milk solution were taken every 0.4 pH unit, from the initial pH (around 6.6) down to pH 4.2. The time required to reach pH 4.2, the cell resistance, the conductivity of the milk solution and the temperature were recorded. On freshly acidified 1.5mL-samples at different pH, the concentration of soluble protein and the molecular profiles were determined. At the end of each run, 500 mL of sample of the milk solution at pH 4.2 was taken. These samples were centrifuged for 10 minutes at 4°C, at 500 g (Centrifuge Model J2-21, rotor type JA-10, Beckman Instruments Inc., Palo Alto, CA). The precipitate was washed twice with double-distilled water (Fistreem III Glass Still, Barnstead, Dubuque, IA) before being lyophilized for 24 hours at room temperature (Model Freezone 4.5, Labconco, Kansas City, MI). The lyophilized

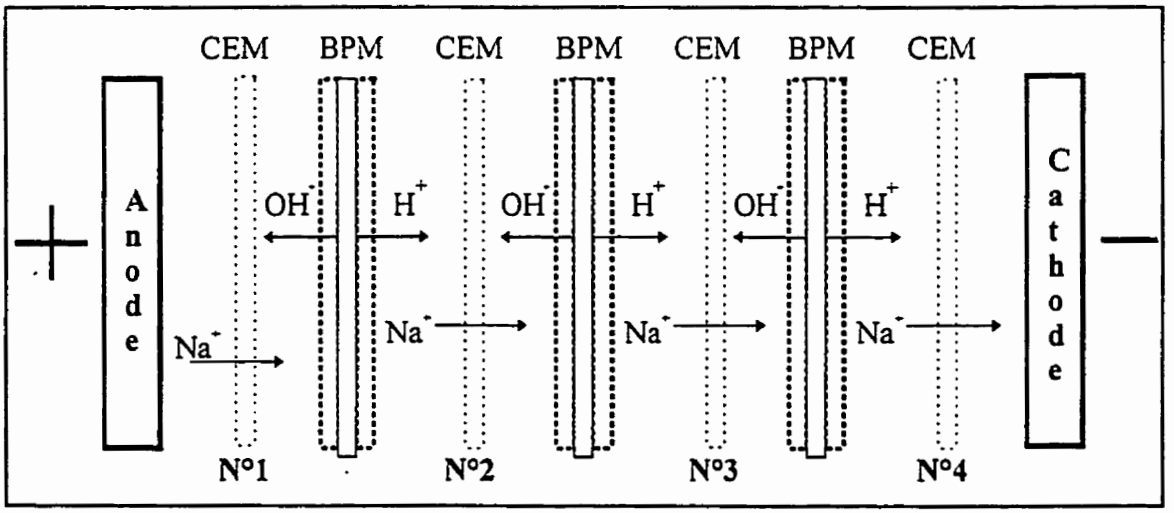


Figure IV-8 : Membrane stacking in a BMEA cell configuration during treatment of skim milk solution, run at 20 mA/cm^2 constant current or at 60 V constant voltage.

isolates were stored at 4°C before total protein determination, molecular profiles, ash content and calcium, sodium and potassium concentration analyses were performed.

c) Analysis Methods

Conductivity: A YSI conductivity meter Model 35 was used with a YSI immersion probe Model 3418, cell constant K= 0.1 cm^{-1} (Yellow Springs Instrument Co.,Yellowsprings, OH) to measure the conductivity of the protein solutions.

Membrane resistance: The membrane resistance was measured using specially designed pliers from the Association Montpellièraine pour le Développement des Procédés à Membranes (St. Jean de Védas, France) as follows :

(1) Pliers ends are plunged into a 0.1 N NaCl reference solution with the jaws wide apart for 1 minute, in order to bath the platine platinized electrodes fixed on each jaw. The measurement was performed on the same YSI conductivity meter as for the conductivity measurement. R_1, the reference solution resistance, was measured.

(2) The membrane was equilibrated beforehand for 10 minutes in the reference solution, and then introduced between the plier jaws and the resistance R_2 was measured. The membrane resistance (R_m) is given by : $R_m = R_2 - R_1$.

Six measurements of the reference solution resistance and six measurements at different spot on the membrane were performed, and the differences averaged to give the membrane resistance value.

Thickness: The thickness of the membrane was measured using a Mitutoyo Corp. digimatic indicator (Model ID-110 ME, Japan) and digimatic mini-processor (Model DP-1HS, Japan), specially devised for plastic film thickness measurements, with a resolution of 1 μm and a range of 10 mm. Ten measurements at different spots on the membrane were performed, and the differences averaged to give the membrane thickness value.

Ion-exchange capacity: The membrane ion-exchange capacity (IEC), corresponding to the number of active sites, was measured as follows :

(1) the membrane is soaked in a 1.000 N hydrochloric acid solution (HCl, VWR Scientific, West Chester, PA, USA) overnight on an orbital shaker (speed = 50 rotations per min.), in order to exchange the hydronium ions with the cations held by the negatively-charged sites of the CEM and to obtain the acidic form of the membrane.

(2) Afterward, the membrane is rinced with double-distilled water and soaked in a known volume of 0.100 N sodium hydroxide (NaOH, VWR Scientific) for 15 minutes on an orbital shaker (speed = 100 rotations per min.).

(3) The membrane is rinced again with double-distilled water. The rince water is mixed with the NaOH solution and then titrated with 0.500 N HCl solution.

(4) The IEC is calculated from the following equation :

$$IEC = \frac{(V_{NaOH} \times [NaOH]) - (V_{HCl} \times [HCl])}{M_{wm}}$$

IEC is the number of milliequivalents per gram of wet membrane, V_{NaOH} and [NaOH] the volume in milliliters and the concentration in normality of the NaOH solution in which the membrane is soaked, V_{HCl} and [HCl] the volume in milliliter and the concentration in normality of the HCl solution used to titrate the NaOH solution; M_{wm} is the mass of the wet membrane.

Moisture: The moisture was measured according to AOAC method no. 927-05 (AOAC international, 1995a).

Ash Content: The ash content was determined according to AOAC methods no. 930-30 and 945-46 (AOAC international, 1995b,c).

Potassium, sodium and calcium concentration determination: Sodium and potassium concentration were determined by the flame emission spectrometric method (AOAC international, 1995d), while calcium concentration was measured by the atomic absorption spectrophotometric method (AOAC international, 1995e) with a Varian SpectrAA-100 (Malgruve, Victoria, Australia).

Total Protein determination. The protein concentration determination was obtained using a FP-428 LECO apparatus (LECO Corporation, Saint Joseph, MI) under the same conditions used by Bazinet *et al.* (1999c).

Molecular protein profiles: The chromatographic analysis of the molecular profile of the lyophilized protein isolate and skim milk samples was performed by reverse-phase HPLC according to Jaubert and Martin (1992) under the same conditions used by Bazinet *et al.* (1999c).

Membrane surface elemental analysis : Elemental analyses were performed on an X-ray energy-dispersive spectrometer (EDS) (Oxford Link Isis, Oxford Instruments Microanalysis Group, Concord, MA) coupled to a scanning electron miscroscope (JMS-5600LV, Jeol, Boston, MA). The EDS conditions were 20 kV accelerating voltage, 15-mm working distance and a take off angle of 35°.

RESULTS AND DISCUSSION

Electroacidification parameters

a) Duration

Time required for the electroacidification to reach pH 4.2 was found to be significantly ($P<0.027$) longer for the CSV than for the CMX membrane (18.9 vs 23.0 minutes). However, the acidification of skim milk proceeded in a linear fashion

(determination coefficients of 0.995 and 0.996 for CSV and CMX membranes respectively).

These results agreed with those reported by Bazinet *et al.* (2000a) which showed a difference in BMEA duration, with however shorter durations of 17.3 and 19.5 min. for CSV and CMX membranes respectively. This difference could be explained by the membrane variability. In our previous work concerning the effect of permselectivity on the BMEA efficiency, the three repetitions for each membranes permselectivity were carried out with the same batch membrane, while in the present study, both repetitions of three BMEA were realized with two different batches of membranes.

Moreover, the BMEA was not run at constant current, which could explain the difference observed in duration between these membranes. The CSV, by its intrinsic selectivity, increased the global resistance of the system; therefore at a constant voltage, the current intensity was reduced. Consequently, the amount of electrogenerated H^+ is reduced. As the duration is directly linked to the amount of electrogenerated H^+, the duration of BMEA with the CSV membrane is longer (Bazinet *et al.*, 1997b;1999a).

b) Cell resistance

The cell resistance was calculated, using Ohm's Law, from the voltage and the current intensity read directly from the indicators on the power supply. The variation of the cell resistance between the beginning and the end of the BMEA was not influenced by the membrane permselectivity ($P>0.17$). The cell resistance (both membrane types averaged) varied from 28 to 41.5 Ω (a + 48.3% variation) (Figure IV-9). In addition, the cell resistance changes in an exponential fashion ($R^2 = 0.999$) : slowly from pH 6.6 to 5.0 (a + 3.1 Ω/pH unit increase) and very rapidly from pH 5.0 to 4.2 (a + 7.1 Ω/pH unit increase).

The high increase in resistance is due to the protein fouling of the spacer, as previously observed by Bazinet *et al.* (1999c). In fact, most of the protein in the skim milk solution was precipitated between pH 5.2 and 4.8, as confirmed by the soluble protein content (Figure IV-9).

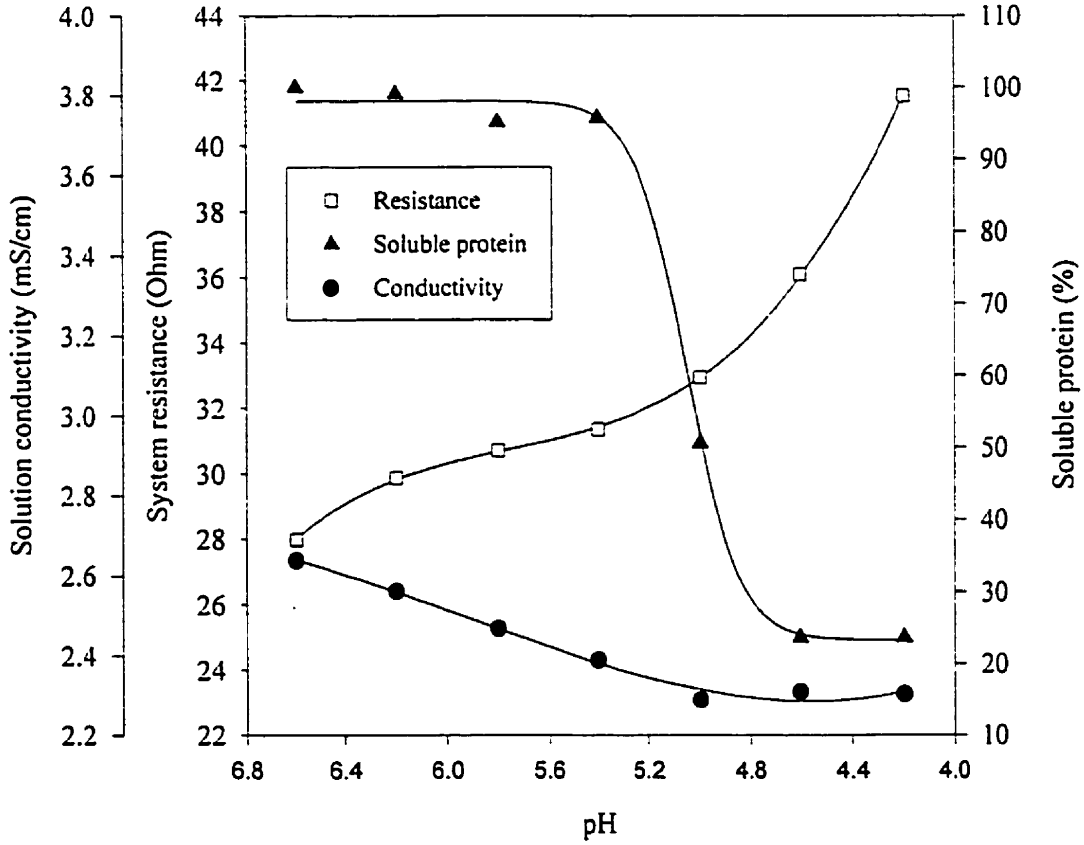


Figure IV-9 : Evolution of the system resistance, and the conductivity and the percent soluble protein of the skim milk solution during bipolar membrane electroacidification run at 20°C, and at 20 mA/cm^2 constant current or at 60 V constant voltage.

c) Conductivity of the skim milk solution

The membrane permselectivity had no effect on the variation of the conductivity of the skim milk solution from the beginning to the end of the BMEA ($P>0.13$). The milk solution conductivity decreased (both membrane types averaged) from 2.64 to 2.30 mS/cm (Figure IV-9). The regression curve calculated for the solution conductivity as a function of pH showed a slow linear decrease of conductivity from pH 6.6 to 5.0 and a stabilization of the conductivity between pH 5.0 and 4.2. The linear decrease is due to the demineralization effect of CEM during BMEA to maintain the solution electrically neutral as demonstrated by Bazinet *et al.* (1997a,b; 1998b) on electroacidification of soybean. However, for soybean protein precipitation, the conductivity decrease was linear. The non-linear decrease for milk could be related to micellar calcium released during milk protein precipitation. Indeed, 68% of the calcium in milk is bound in the micelle (Schmidt, 1982), and this calcium release acts in a manner opposite to the demineralization effect. This results in a stabilization of the conductivity.

Electroacidification efficiency and isolate composition

a) Soluble protein

Whey proteins were the only soluble proteins found at pH 4.2 for both types of cationic membrane ($P>0.96$). The non-linear regression model of soluble protein (%) as a function of pH (data from all membrane types averaged) gave a high coefficient of determination ($R^2 = 0.998$) (Figure IV-9).

The concentration of soluble protein (23%) obtained at the end of the run should correspond to whey proteins and minor proteins (Bazinet *et al.*, 1999c; Cayot and Lorient, 1998; Brunner, 1981; Swaisgood, 1982).

b) Chemical composition of isolates produced

The chemical composition of isolates obtained with BMEA for both membranes was compared in terms of ash and protein contents, percentage of each protein fraction; and calcium, sodium and potassium concentrations (Table IV-4).

The ash content of isolates was different for each membranes ($P<0.05$). The CSV membrane slowed down the migration of cations in comparison to the CMX membranes, as confirmed by the ash composition in cationic species : the ash content of isolates produced with CSV membranes showed higher sodium and potassium concentrations than those produced with the CMX membranes, while the calcium concentration was similar for both membranes (Table IV-4). The calcium concentration of the CSV isolate was surprising, because the calcium concentration should have been higher than that of CMX isolate, due to the CSV membrane intrinsic selectivity. Total protein content, calculated on a dry basis (Table IV-5), was shown to be the same for both membranes ($P>0.22$) : 97% (both membrane types averaged).

The comparison of the molecular profiles obtained by HPLC and the statistical analysis results of each protein fraction indicated that there was no difference between the membranes (Table IV-5). These results agreed with data obtained by Bazinet *et al.* (1999c). BMEA can be used to precipitate high purity bovine milk casein, and the permselectivity of both membranes tested does not influence the purity of the isolates produced.

Membrane parameters

a) Membrane resistance

Both membranes were demonstrated to have a different resistance originally ($P<0.0001$) with 3.8 and 9.8 $\mu\Omega$ for CMX and CSV respectively, which is in accordance with their physico-chemical structure (Figure IV-10). The evolution of the membrane resistance with time was the same whatever the membrane permselectivity, but the resistance values were higher for CSV. The original resistance value and the resistance values after each HCl treatment were found to be the same; about 4.0 and 9.9 $\mu\Omega$ for CMX and CSV respectively. For both membrane permselectivities, HCl treatment restores the original resistance of the membrane after BMEA. Hydrolysis of the deposit or/and salts by HCl was confirmed by pictures taken of the CSV membranes. Similarly, after each BMEA for the test membrane, or after three BMEA for the control membrane, the resistance of the

Table IV-4 : Ash content, moisture and concentration in calcium, sodium and potassium of the isolates produced by BMEA with two different types of permselective membranes.

	Ash content (% dry basis)	Moisture (%)	Sodium (mg/g ash)	Potassium (mg/g ash)	Calcium (mg/g ash)
CMX	1.00 ± 0.10 a*	6.4 ± 5.2 a	9.8 ± 2.9 a	10.9 ± 3.0 a	7.4 ± 3.4 a
CSV	1.26 ± 0.24 b	2.2 ± 2.5 a	15.1 ± 4.1 b	17.0 ± 4.5 b	8.5 ± 3.2 a

* Means within a column followed by different letters are significantly different ($P < 0.05$)

Table IV-5 : Percent total protein and percentage of each protein fractions of the isolates produced by BMEA with two different types of permselective membranes.

	Total Protein (% dry basis)	κ-casein (%)	α_{s2}-casein (%)	α_{s1}-casein (%)	β-casein (%)	Whey protein (α-la and β-lg) (%)
CMX	96.8 ± 1.5 a*	12.4 ± 2.1 a	5.8 ± 0.9 a	34.0 ± 1.3 a	46.6 ± 1.5 a	1.2 ± 0.2 a
CSV	97.2 ± 1.4 a	13.1 ± 0.8 a	5.5 ± 0.5 a	34.1 ± 1.0 a	46.1 ± 1.8 a	1.1 ± 0.2 a

* Means within a column followed by different letters are significantly different ($P < 0.05$)

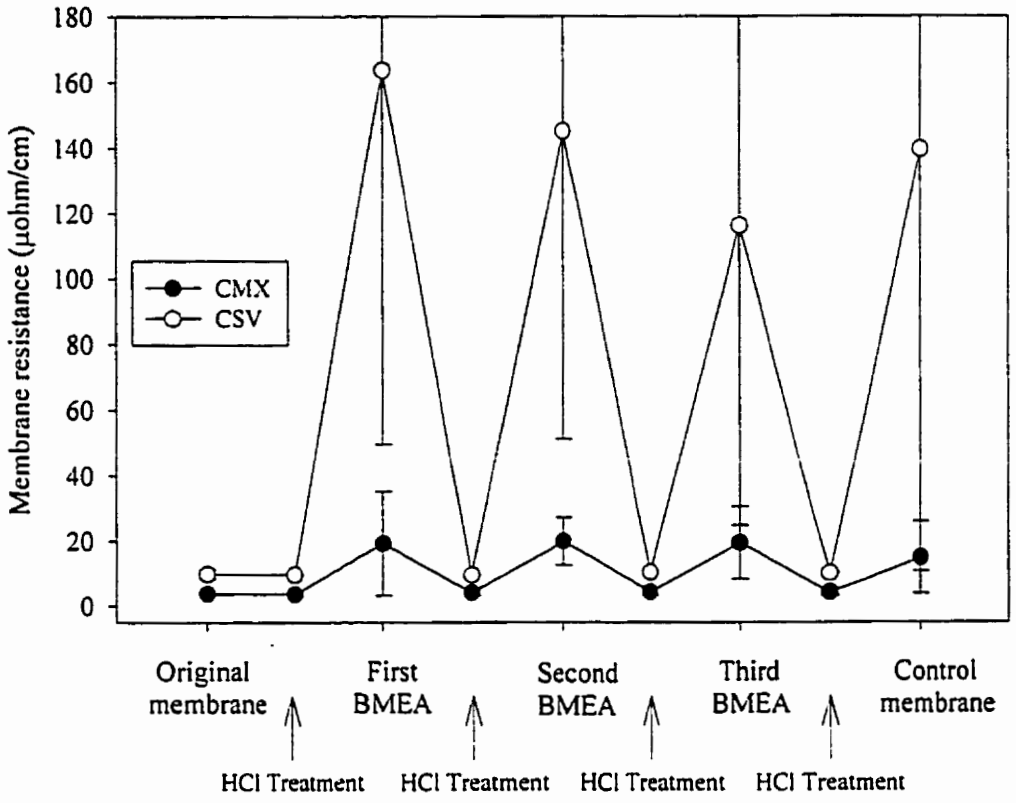


Figure IV-10 : Evolution of the CMX and CSV membrane resistance (original, test and control) during consecutive bipolar membrane electroacidifications of skim milk solution, run at 20°C, and at 20 mA/cm^2 constant current or at 60 V constant voltage.

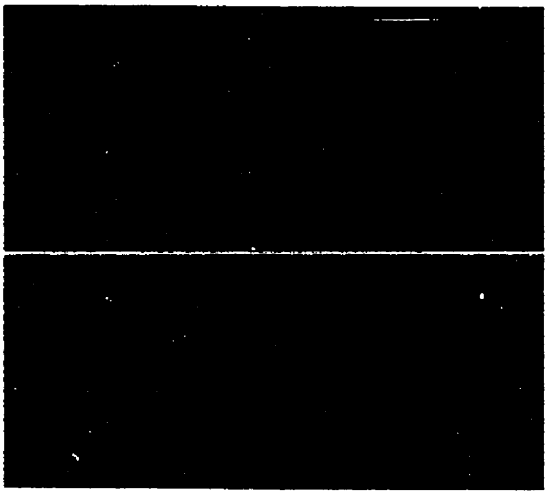
membrane was the same. However, these values were higher than the resistance of the respective original membranes : about 18.4 and 141.2 μΩ for CMX and CSV respectively. The BMEA affect greatly the resistance value of the membrane by forming a deposit on the sides of CEM as previously noted by Bazinet *et al.* 1999c; resistance values were 4.6 and 14.3 time higher for CMX and CSV respectively. This was confirmed by pictures comparing one original membrane to one membrane used for BMEA (Figure IV-11) : BMEA treatment leads to fouling of the CEM. The pictures do not really show a difference for CMX membranes used for three BMEA, and CMX membranes used during BMEA and then cleaned by HCl, but the cleaning effect of HCl on CSV membranes was very effective.

b) Membrane thickness

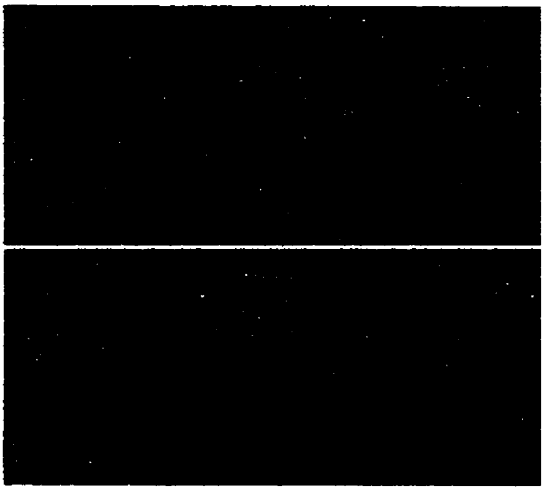
The thickness values measured for both membranes may explain the membrane resistance results. There is no difference in thickness for the CMX membrane at any place on the membrane where the measures were performed (outer edge and exposed portion of the membrane), or after different treatments (original, control and test) ($P>0.16$). The thickness of the CMX membrane was 0.1794 ± 0.0090 mm (Table IV-6). For the CSV membrane a difference in thickness was measured for the exposed portion of the control membrane in comparison with all othermeasurements : the thickness of the CSV membrane was 0.1054 ± 0.0069 mm, while a deposit of 0.0105 ± 0.0055 mm was formed on the exposed portion of the control membrane after only three BMEA.

c) Ion-exchange capacity

The IEC measured on both membranes confirmed the effect of BMEA on the filling of MEC active sites by ionic species or organic complexes. The CSV and CMX membranes were demonstrated to have similar original IECs while the IEC for CSV membranes after each HCl treatment were higher than values obtained for CMX membranes ($P<0.006$) (Figure IV-12). The evolution of the IEC in time was the same for both membranes. After each BMEA, for the test membrane, or after three BMEA for the control membrane, the IEC is equivalent to the original respective IEC values : IEC values were 0.14 and

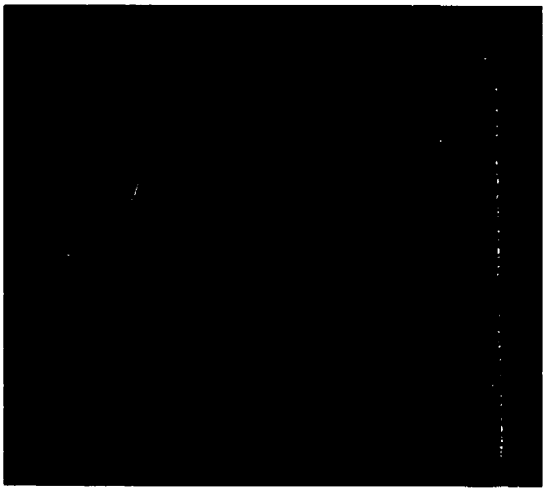


CMX control before HCl treatment



CMX original

CSV control before HCl treatment



CSV original

Figure IV-11 : Pictures of CMX and CSV membranes, before (original membrane) and after (control membrane) consecutive bipolar membrane electroacidifications of skim milk solution, run at 20°C, and at 20 mA/cm^2 constant current or at 60 V constant voltage.

Table IV-6 : Membrane thickness measured during the course of BMEA on the outer edge and on the exposed portion.

		Outer edge (mm)		Exposed portion (mm)	
CMX	Original	0.1822 ± 0.0004	a*	0.1809 ± 0.0020	a
	Test	0.1695 ± 0.0095	a	0.1866 ± 0.0045	a
	Control	0.1701 ± 0.0123	a	0.1874 ± 0.0039	a
CSV	Original	0.1052 ± 0.0007	a	0.1051 ± 0.0001	a
	Test	0.1048 ± 0.0001	a	0.1056 ± 0.0011	a
	Control	0.1063 ± 0.0001	a	0.1159 ± 0.0055	b

* Means for a given membrane followed by different letters are significantly different ($P < 0.05$)

0.26 meq. per gram wet membrane for the CMX and CSV membranes respectively. Both membranes exchange sites were saturated in their original form and after each BMEA. For the CMX membrane, the IEC was the same after HCl treatment following BMEA as that of the original membrane. For CSV membranes, Duncan's tests showed no statistical difference between the IEC after HCl treatment of the membrane after BMEA compared to the IEC of the original membrane. However, large standard deviations were obtained indicating an intrinsic membrane variability between each production batch, which was noted previously for BMEA duration measurements. However, the IEC of the acidic form of the CSV membrane after BMEA decreased slowly, which could confirm fouling of the membrane.

d) Ion fixation on the membrane

The concentration of sodium, potassium and calcium released by the membrane after HCl treatment was determined in order to follow a possible built-up of the membrane fouling (Davis *et al.*, 1997).

A higher concentration of sodium was released by the CSV membrane than the CMX membrane, whenever the HCl treatment was done ($P<0.04$) (Figure IV-13). In fact, the original CSV contained more sodium than the original CMX, 6.5 vs 4.1 meq./g wet membrane respectively. Moreover, the sodium released during HCl treatment of both membranes decreased slightly : for the CMX membranes, 4.1, 2.6 and 1.2 meq./g wet membrane were released by the original membrane, the membrane after the third BMEA and the control membrane respectively. Similarly, for the CSV membrane, the sodium concentration decreased from 6.5 to 3.4 and to 1.6 meq./g wet membrane for the original membrane, the membrane after the third BMEA and the control membrane.

For potassium, except for the CMX and CSV control membranes, the concentrations released after the HCl treatment were similar for all membranes at an average value of 0.074 meq./g wet membrane ($P<0.0007$) (Figure IV-13). For the control membranes, higher concentrations of released potassium were noted : 0.248 and 0.474 meq./g wet membrane for the CMX and CSV membranes respectively ($P<0.031$). Moreover, if the sum of the concentration released by the original membrane and the membrane after each BMEA is

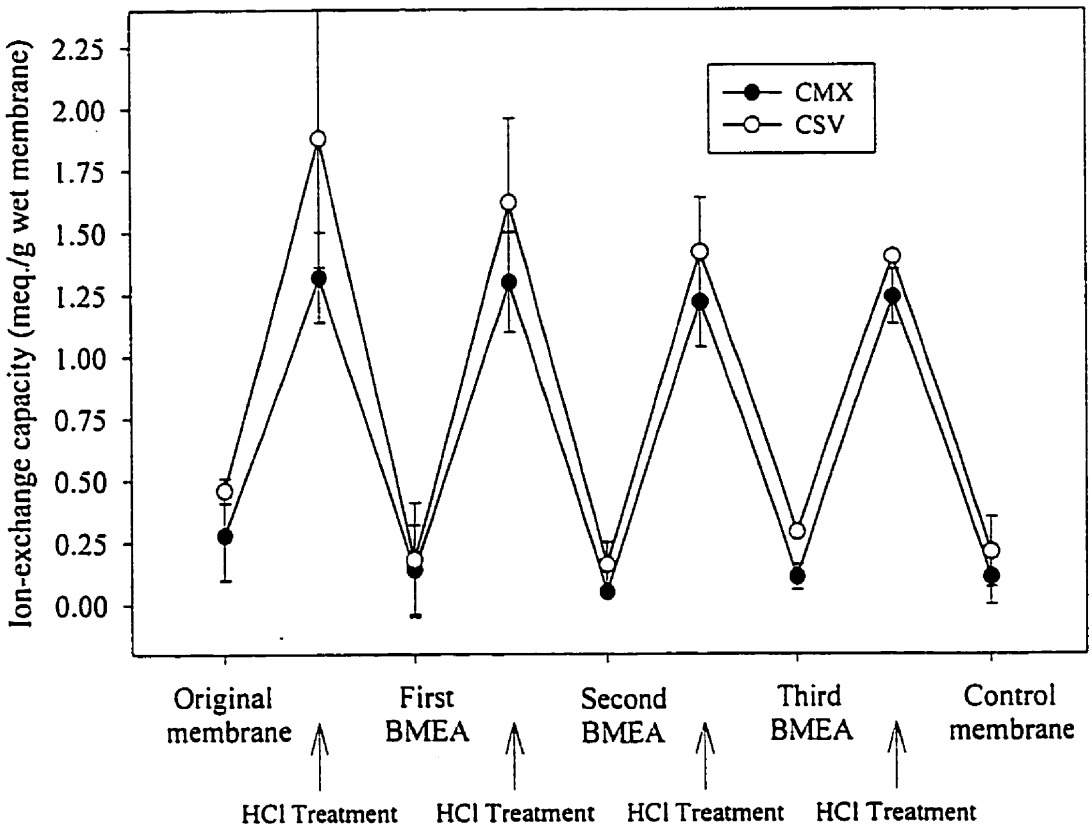


Figure IV-12 : Evolution of the CMX and CSV membrane ion-exchange capacity (original, test and control) during consecutive bipolar membrane electroacidifications of skim milk solution, run at 20°C, and at 20 mA/cm^2 constant current or at 60 V constant voltage.

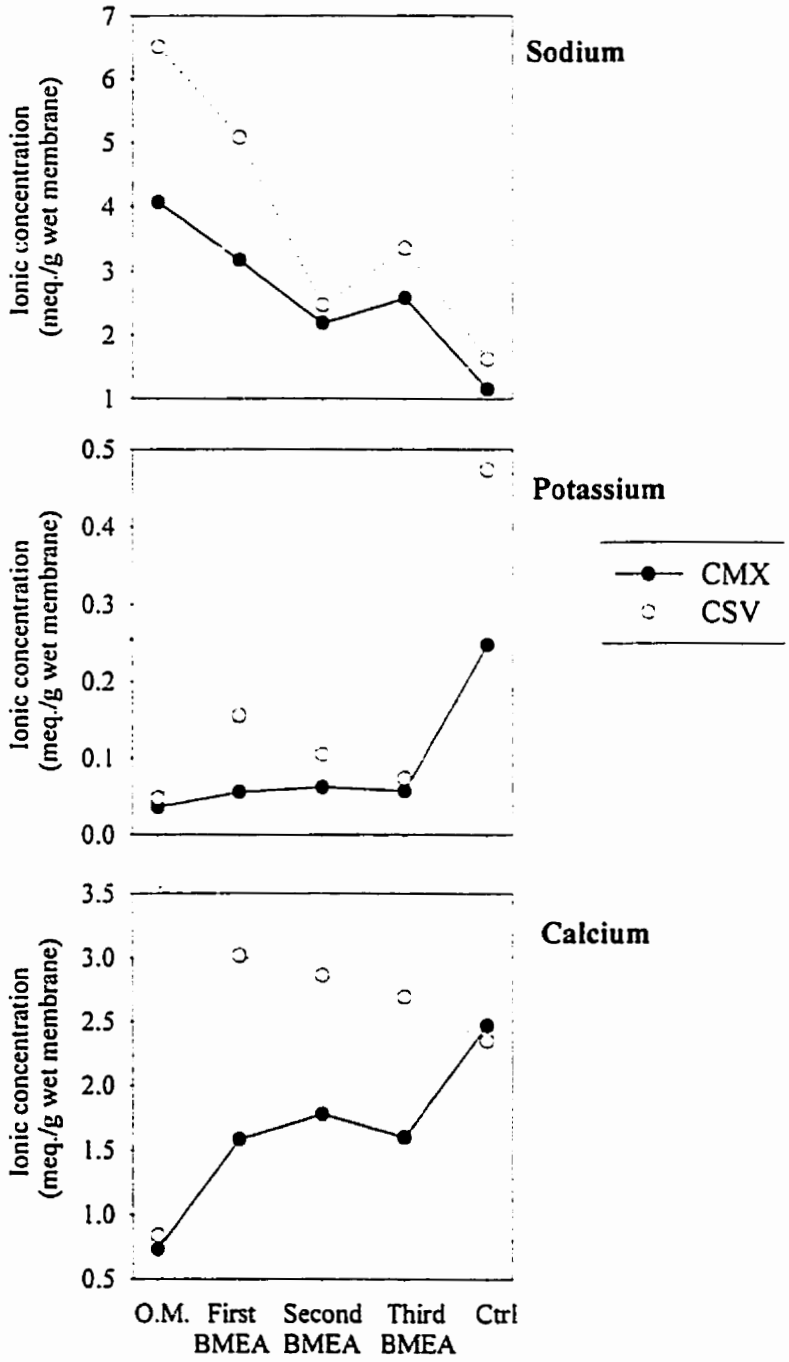


Figure IV-13 : Sodium, potassium and calcium concentrations released by CMX and CSV membranes (original, test and control) in the HCl bath before and after BMEA of skim milk solution, run at 20°C, and at 20 mA/cm^2 constant current or at 60 V constant voltage.

compared to the potassium concentration released by the control membrane, the figures are quite similar : 0.211 vs. 0.248 meq./g wet membrane for the CMX membrane and 0.383 vs 0.474 meq./g wet membrane for the CSV membrane. The potassium accumulated in the membranes and was released almost completely by the HCl treatment.

For calcium released during the HCl treatment, the tendancy for both membranes was the same ($P<0.031$) (Figure IV-13), except for the control membranes. The original membrane released a very small quantity of calcium, while for the membranes treated by HCl after each BMEA, the quantity is similar but higher than the amount released by the respective original membrane : 0.731 and 0.839 meq./g wet membrane for the CSV and CMX original membrane respectively compared to an average of 1.654 and 2.858 meq./g wet membrane for the CSV and CMX membranes treated after each BMEA ($P<0.026$). For the CMX control membrane, the calcium released was measured at 2.47 meq./g wet membrane, which was higher than that released by the membrane after each BMEA. For the CSV control membrane, the calcium released was 2.35 meq./g wet membrane, a concentration similar to that released by the membrane after each BMEA. Therefore, we concluded that calcium accumulates in the control membrane ; for CMX membranes, the HCl treatment released the majority of the calcium trapped in the membrane. However, for the CSV membrane, the calcium was not released completely.

In spite of its intrinsic structural characteristics, the CSV membrane could allow the passage, through the first multivalent ion rejection layer, of a part of the calcium ions of the solution, due to a possible saturation of the Donnan exclusion capacity. Following the migration of a some of calcium ions accross this first selective layer, these ions would migrate accross the non selective cation exchange layer and most ions would be trapped inside the CSV membrane : the repulsive effect of the second selective layer, combined with a precipitation with OH^- ions migrating from the sodium hydoxide solution would induce an irreversible calcium fouling of the membrane inner layer. This irreversible fouling by calcium hydroxide would explain that the IEC of the CSV membrane after HCl treatment decreases with BMEA treatment. Some of calcium ions that succeed in crossing the second multivalent ion rejection layer are precipitated with OH^- ions on the outer layer of the membrane. Results obtained by electronic microscopy (Figures IV-14 and 15) and by

photographs of the whole membranes (Figure IV-11) showed that the fouling of the outer layer was from calcium and magnesium, and both membranes (CSV or CMX) can be cleaned-up by a simple HCl treatment, to give a surface composition in ions comparable to the original membrane. The fouling formed at the outer layer of the CEM is therefore reversible.

Similar observations were made in the chlor-alkali industry, where magnesium and calcium cause problems by forming precipitates within the membrane. These cations migrate from the anolyte towards the catholyte and meet hydroxide ions back-migrating through the membrane and crystals of precipitates gradually grow with time. These precipitates will tend to block the passage of sodium ions through the membrane giving rise to higher resistances (Ogata *et al.*, 1989; Davis *et al.*, 1997; Momose *et al.*, 1991). Ogata *et al.* (1989) shown that the accumulation of impurities in the membrane in the chlor-alkali industry increased greatly at the time of significant change in the voltage. The brine impurity precipitates in the membranes and damages the membrane irreversibly. The precipitates in the membranes were identified as the respective hydroxides of alkaline earth metals found in the feed brine : calcium was the most harmful followed by magnesium, strontium, and barium.

Moreover, the hypothesis that some ions would be trapped irreversibly in the CSV and not in the CMX membrane was confirmed by ashing a piece of the second trial membranes. The ash content (on a dry basis) did not change for the CMX membrane (original membrane, test membrane after 3 BMEA and HCl treatment and control membrane after HCl treatment), about 8.9 ± 0.2 %, while for the CSV membrane the ash content increased from 7.8 ± 0.3 % to 8.3 ± 0.2 % for the original and the test membrane respectively and to 9.5 ± 0.7 % for the control membrane.

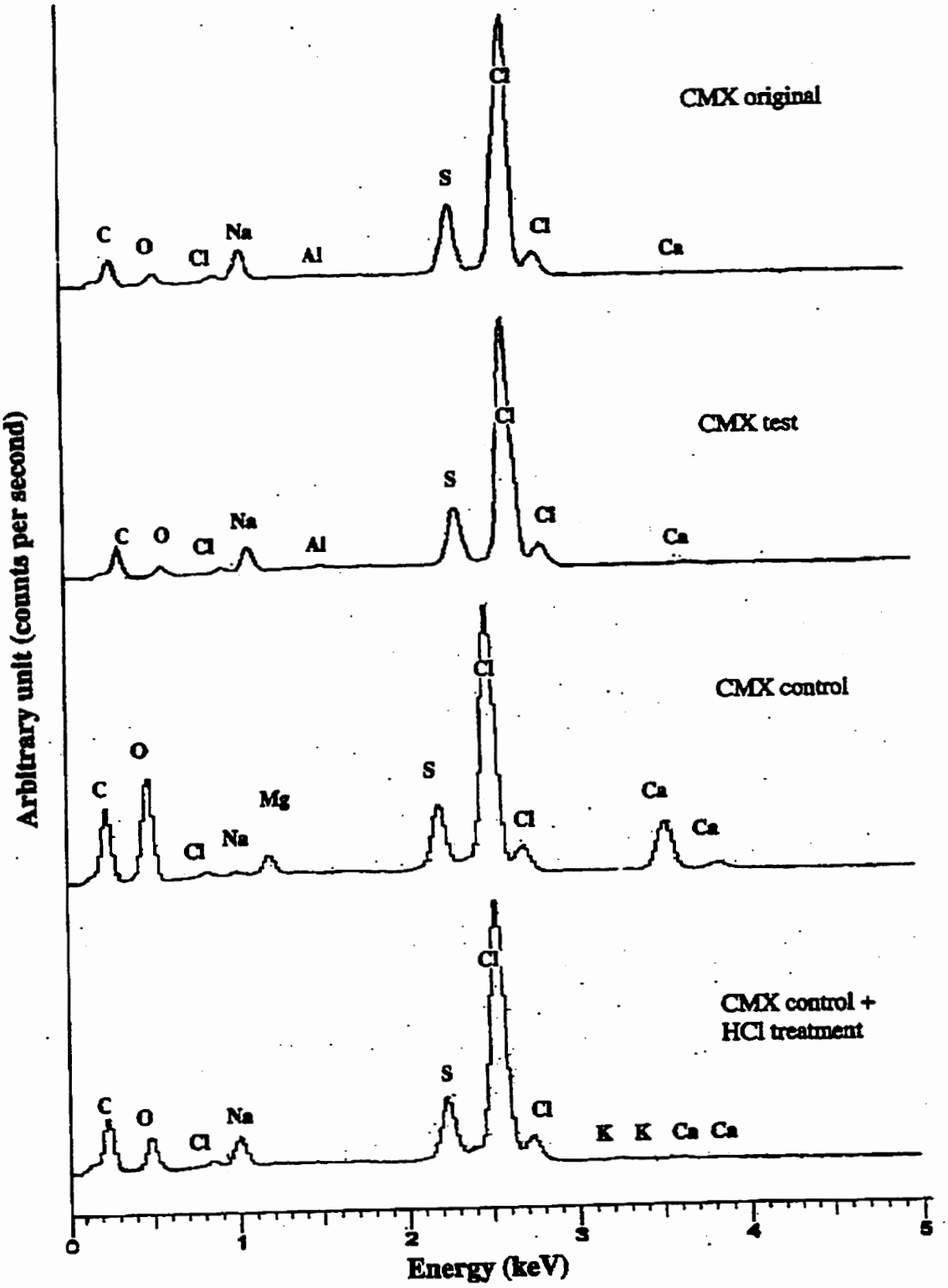


Figure IV-14 : Elemental analysis profiles of CMX membranes (original, test, control and control after HCl treatment).

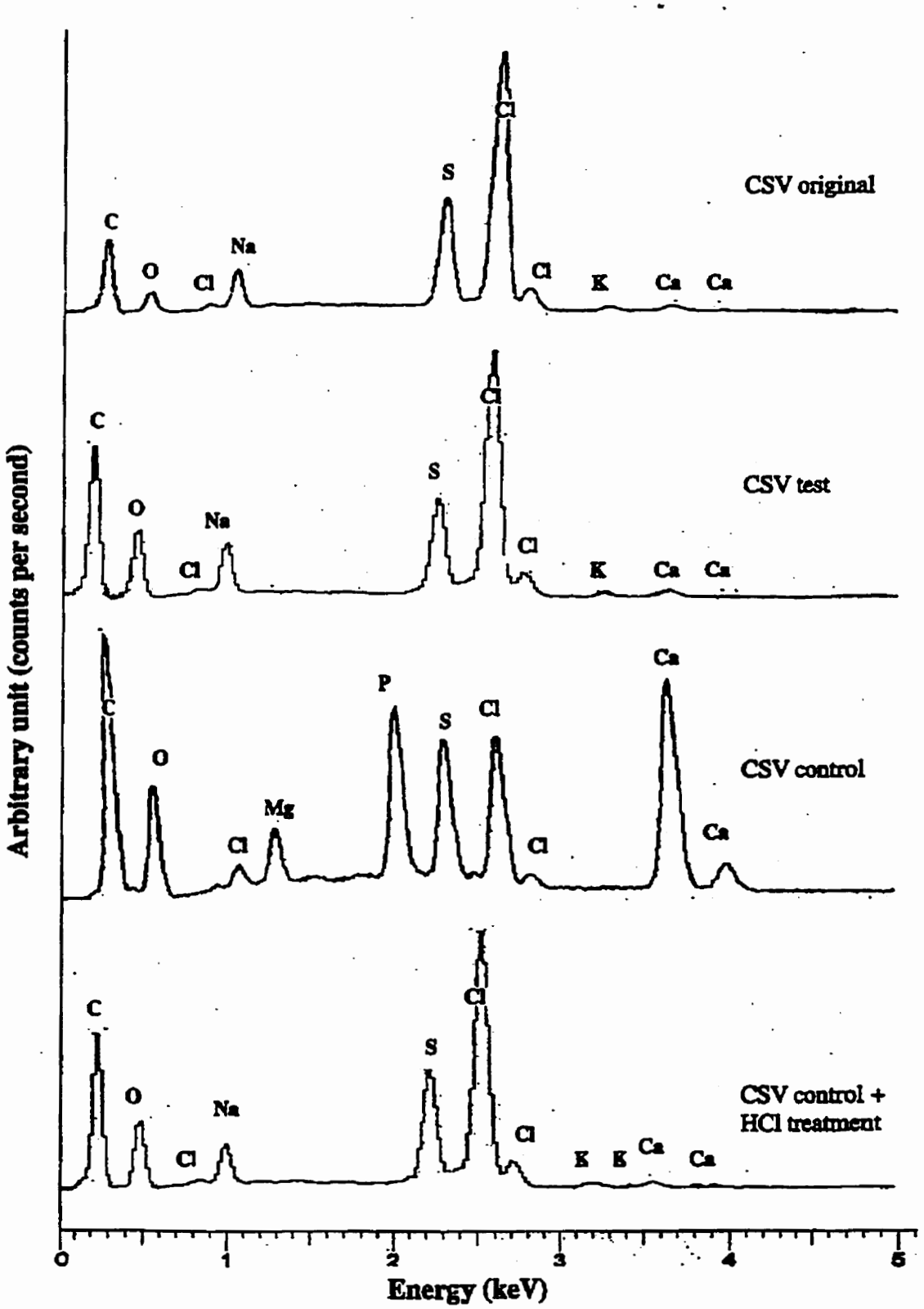


Figure IV-15 : Elemental analysis profiles of CSV membranes (original, test, control and control after HCl treatment).

CONCLUSION

From membrane parameters and surface elemental analysis, the membrane fouling of cation-exchange membrane was identified as precipitating calcium and magnesium hydroxides. The fouling formed at the outer layer of the CEM was reversible while the fouling of the membrane inner layer was irreversible.

Moreover, the high increase in cell resistance observed during BMEA would not be only the result of the protein fouling of the spacer, but also from the membrane fouling by calcium and magnesium hydoxide. This hydroxide fouling may have the most important effect on cell resistance since calcium and magnesium fouling increased the membrane resistance by a factor of 4.6 and 14.3 for CMX and CSV membranes respectively.

A further study aimed at decreasing the membrane fouling is currently under way to improve the efficiency of skim milk BMEA.

ACKNOWLEDGEMENTS

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ATTEINTE DES OBJECTIFS ET AVANCEMENT DES CONNAISSANCES

Les résultats obtenus dans ce chapitre ont permis d'atteindre l'objectif visant à étudier l'effet de la permsélectivité de membranes cationiques sur la migration des cations du lait et la précipitation des protéines au cours du procédé d'ÉAMBP. Ces résultats ont montré que pour les deux membranes de permsélectivités différentes testées, les ions potassium sont l'espèce ionique migrant principalement, alors que les espèces secondaires sont le sodium et le calcium respectivement pour la membrane permsélective aux cations mono- et divalents et la membrane permsélective uniquement aux cations monovalents. La migration des cations au travers de la membrane cationique a été influencée par la permsélectivité, et les contenus en cendres des isolats produits ont été trouvés différents.

Cependant, les cinétiques de précipitation des protéines au cours de l'électroacidification n'ont pas été influencées dans les deux cas et, pour les deux types de membranes cationiques, les protéines sériques ont été les seules protéines solubles restantes après électroacidification à pH 4.2; les isolats de caséines produits (environ 97% de protéines) ont des profils protéiques similaires.

Ces résultats ont aussi concouru à répondre à l'objectif concernant l'étude de l'effet de la permsélectivité des membranes échangeuses d'ions (MEC) sur leur colmatage et d'identifier la nature de ce colmatage. Les résultats obtenus dans cette partie de l'étude ont permis de comprendre la formation du colmatage des MEC (colmatages interne et de surface des membranes), de déterminer la réversibilité du colmatage (colmatages interne et de surface réversibles pour la membrane permsélective aux cations mono- et divalents, colmatage de surface reversible et colmatage interne irréversible pour la membrane permsélective aux cations monovalents) et enfin d'identifier la nature de ce colmatage (précipité d'hydroxyde de calcium et de magnésium). Ce colmatage a pour effet d'áugmenter la résistance électrique des membranes au cours du temps et ralentit la migration des espèces ioniques. Il est ressorti de ces travaux que la membrane permsélective aux cations monovalents ralenti la migration des cations en comparaison avec la membrane permsélective aux cations divalents et monovalents.

Ces résultats ont contribué à un avancement des connaissances sur la compréhension des phénomènes membranaires au cours de l'ÉAMBP. Les travaux ont permis de comprendre le mécanisme de formation du colmatage des MEC, d'en connaître sa réversibilité et enfin, d'en identifier la nature. Une information importante a été apportée par les travaux sur la permsélectivité des MEC : les membranes CSV vendues commercialement comme des membranes permsélectives aux cations monovalents, sont apparues, au cours de l'ÉAMBP du lait, permsélectives aux ions calcium qui sont pourtant des ions divalents.

CHAPITRE V

COMPARAISON DE L'ACIDIFICATION ÉLECTROCHIMIQUE ET CHIMIQUE DU LAIT ÉCRÉMÉ, ET MODÉLISATION DES ÉQUILIBRES CATIONIQUES AU COURS DE L'ÉLECTROACIDIFICATION

CHAPITRE V

COMPARAISON DE L'ACIDIFICATION ÉLECTROCHIMIQUE ET CHIMIQUE DU LAIT ÉCRÉMÉ, ET MODÉLISATION DES ÉQUILIBRES CATIONIQUES AU COURS DE L'ÉLECTROACIDIFICATION

TRANSITION CONTEXTUELLE

Dans les résultats obtenus dans les chapitres III et IV, les isolats de caséines produits par ÉAMBP, en comparaison avec les valeurs respectives des isolats commerciaux citées dans la littérature, semblent avoir une teneur en cendres inférieure (0.98-1.49 vs 2.0-3.8%) et une teneur en protéines totales similaire ou supérieure (97-98 vs 93.4-96.7%). Cependant, les isolats acidifiés chimiquement et électroacidifiés n'ont pas été préparés dans les mêmes conditions, ce qui rend la comparaison relative. De plus l'équilibre ionique dans le cas des deux procédés est différent : durant l'électroacidification les cations migrent au travers de la membrane cationique conduisant à une déminéralisation, alors qu'au cours de l'acidification chimique des ions supplémentaires sont apportés par ajout d'acide. Ainsi, les objectifs principaux de cette étude sont (1) de comparer les acidifications chimiques et électrochimiques afin d'identifier les éléments éventuels de différenciation entre les procédures, (2) d'évaluer le nombre de H^+ électrogénérés au cours de l'ÉAMBP, (3) d'identifier et quantifier les cations migrant au travers des membranes cationiques durant l'EAMBP du lait, et (4) de comparer les cinétiques de migration des cations à celle des H^+ électrogénérés.

Les résultats de ce chapitre ont fait l'objet de deux articles : Le premier intitulé «Comparison of Electrochemical and Chemical Acidification of Skim Milk» soumis pour publication dans *J. Food Sci.* Les auteurs sont : Laurent Bazinet (Candidat au Ph. D.: planification et réalisation des expériences, analyse des résultats et rédaction de l'article), François Lamarche (Co-directeur de thèse : supervision scientifique de l'étudiant, correction et révision du manuscrit), Denis Ippersiel (participation à la réalisation des

expériences, correction et révision du manuscrit), Christine Gendron (participation à la réalisation des expériences et révision du manuscrit), Behzad Mahdavi (révision du manuscrit) et Jean Amiot (Directeur de thèse : supervision scientifique de l'étudiant, correction et révision du manuscrit).

Le second ayant pour titre **«Cationic Balance in Skim Milk during Bipolar Membrane Electroacidification» publié dans *J. Membr. Sci.*, 2000, 173(2) 201-209**. Les auteurs sont : Laurent Bazinet (Candidat au Ph. D.: planification et réalisation des expériences, analyse et modélisation des résultats et rédaction de l'article), Denis Ippersiel (participation à la réalisation des expériences, correction et révision du manuscrit), Christine Gendron (participation à la réalisation des expériences et révision du manuscrit), Jocelyne Beaudry (analyse des minéraux par ICP), Behzad Mahdavi (révision du manuscrit), Jean Amiot (Directeur de thèse : supervision scientifique de l'étudiant, correction et révision du manuscrit) et François Lamarche (Co-directeur de thèse : supervision scientifique de l'étudiant, correction et révision du manuscrit).

RÉSUMÉ (Premier article)

L'objectif de cette étude était de comparer les acidifications chimiques et électrochimiques afin d'identifier les éléments éventuels de différenciation entre les procédures. Les résultats obtenus montrent que l'acidification chimique et l'ÉAMBP présentent quelques différences dans les profils d'acidification. Cette différence serait liée à un effet de salting-in découlant de l'ajout de sels, dans le cas de l'acidification chimique, opposé, dans le cas de l'ÉAMBP, à un retrait des sels par déminéralisation électrochimique, qui favoriserait la précipitation des protéines. Ainsi, à pH 4.6, toutes les caséines sont précipitées par ÉAMBP, alors qu'il reste quelques traces de ces protéines par acidification chimique. Cependant, la composition chimiques et le pourcentage de chacune des fractions protéiques entrant dans la composition des isolats produits ne semblent pas différer.

Mots clés : Électroacidification, Membrane bipolaire, Caséine, Acidification chimique, Isolat.

ABSTRACT

The purpose of this study was to compare chemical and electrochemical acidification, in order to identify differences between the two acidification procedures. The results reveal differences in the acidification profiles obtained with the two methods. Whereas chemical acidification showed a salting-in effect from addition of salts, BMEA removes salts through electrochemical demineralization, favouring protein precipitation. Hence, at pH 4.6, all the caseins were precipitated by BMEA, while some were not yet precipitated by chemical acidification. Nevertheless, the chemical composition and the casein fraction composition of the isolates were the same for both chemical and electrochemical acidification.

Key words : Electroacidification, Bipolar membrane, Casein, Chemical acidification, Isolate

INTRODUCTION

Milk proteins are the animal proteins that have been most widely consumed by humans for the longest time (Cheftel *et al.*, 1985). The nutritional and technological importance of milk proteins in the agricultural and food sectors, accounting for a quarter of all food proteins used in the industrial world, has made this food category a focus of research for many years and prompted efforts to develop industrial processes to separate the proteins or improve their properties and quality.

In the food industry, two main types of casein are produced: rennet caseins and acid caseins. The three main procedures employed to produce acid caseins are based on isoelectric precipitation of casein by chemical, physico-chemical or fermentation acidification (Segalen, 1985; Southward, 1993; Varnam and Sutherland, 1994). Other techniques have been proposed for the production of acid casein: acidification of milk by ion exchange plus acid (Salmon, 1983), electrodialysis of skim milk to pH 5.0 followed by chemical acidification to pH 4.6 (Laiteries Triballat, 1979), and acidification by water electrolysis at the surface of monopolar anion- or cation-exchange membranes stacked in an electrodialysis cell (Bolzer, 1985).

Bipolar-membrane electroacidification (BMEA), a technology that uses the property of bipolar membranes to split water and the action of monopolar membranes for demineralization, was recently applied to acid casein production (Bazinet *et al.*, 1999c). The casein isolates produced by BMEA (Bazinet *et al.*, 1999c) appear to have a lower ash content (0.98-1.49 vs 2.0-3.8%) and a similar or higher total protein content (97-98 vs 93.4-96.7%) (Hargrove and Alford, 1974; Bassette and Acosta, 1988; Alais, 1984; Walstra and Jenness, 1984; Renner *et al.*, 1996) than do commercial isolates.

The purpose of this study was to compare chemical and electrochemical acidification, in order to identify differences between the two acidification techniques. The two procedures were compared in terms of conductivity, percent protein precipitation and

molecular profiles observed during the process, as well as in terms of chemical composition and molecular profiles of the resulting isolates.

MATERIALS AND METHODS

Materials

The raw material used in this study was reconstituted milk (10% solids W/V) from low-heat skim milk powder, which was provided by Agropur (Granby, Quebec, Canada). The starting pH varied between 6.5 and 6.7. The averaged composition of the powdered milk was as follows (g/100g): total protein, 33.9; serum protein, 7.4; fat, 0.6; carbohydrates, 53.5; ash, 8.2; moisture, 3.8.

Methods

a) Electroacidification cell

A MP type electroacidification cell (100 cm^2 effective surface area) manufactured by ElectroCell (Täby, Sweden) was used according to the set-up described by Bazinet *et al.* (1999a)

b) Measurement of electroacidification parameters

During electroacidification, the milk solution pH was measured with a pH meter (Model φ 11, Beckman Instruments Inc., Fullerton, CA). The voltage was read directly from the indicator on the power supply (Powerstat Model 236BU-2, Superior Electric Co., Connecticut, USA). A YSI Model 35 conductivity meter was used with a YSI Model 3417 immersion probe, cell constant K= 1 cm^{-1} (Yellow Springs Instrument Co., Yellowsprings, OH) to measure the conductivity of the protein solutions.

c) Chemical analyses

Moisture and ash content were determined using AOAC methods No. 927-05 and No. 930-30 (AOAC, 1995a,b) respectively. The soluble protein and total protein concentrations were determined with an FP-428 LECO apparatus (LECO Corporation, Saint Joseph, MI) using the methods and parameters developed in an earlier study (Bazinet *et al.*, 1999c). Chromatographic analyses of the molecular profiles of the freeze-dried proteins and skim milk were performed by reverse-phase HPLC according to the method of Jaubert and Martin (1992) and applying the same conditions as Bazinet *et al.* (1999c).

d) Protocol

For the chemical acidifications, 250 mL samples of milk were used. HCl solutions of different normalities (1N and 2N) were added to acidify these milk solutions to a pH of 4.2. Electroacidification was performed in batch process using a constant current of 2 A, with electrolyte volumes of 3 litres. The electroacidification was stopped when the pH reached 4.2. The temperature was held constant at 20 ± 1°C during the electrochemical treatments and at 21 ± 1°C during the chemical acidifications. Three replicates of each of the acidification conditions were performed in this experiment.

During each treatment, milk solution samples were collected at every 0.4 pH unit decrease. The time required to reach pH 4.2, the voltage difference, and the conductivity were recorded throughout the treatment. The soluble protein concentration and the molecular profiles were determined on freshly acidified samples. At the end of each run, both chemical and electrochemical, 250 mL milk samples at pH 4.2 were collected. These samples were centrifuged for 10 minutes at 4°C, at 500 g (Model J2-21 centrifuge, JA-10 rotor type, Beckman Instruments Inc., Palo Alto, CA); the precipitate was rinsed twice with double-distilled water and then lyophilized for 24 hours (Freezone Model 4.5, Labconco, Kansas City, MI). The lyophilized isolates were stored at 4°C until total protein determination, molecular profile and ash content measurements were performed.

e) Statistical analyses

Data on the duration of electroacidification, the voltage, the conductivity and the percent soluble protein as a function of pH were subjected to multivariate analysis of variance using the SAS software (1989). The regression equations and curve fitting were computed for the voltage, duration, conductivity and percent soluble protein as a function of pH using SigmaPlot (version 3.0 pour Windows, Jandel Scientific, Corte Madera, CA). Multivariate analyses of variance were performed on the percentages of κ, α_{s1}, α_{s2} and β caseins and of whey proteins in the milk solutions collected as the pH dropped. The analysis of percent soluble protein and protein fraction composition was completed by Duncan tests to determine the significance of differences between samples taken at pH 4.6 and at pH 4.2, for the different acidification methods. Ash, total protein and the percentages of κ, α_{s1}, α_{s2} and β caseins and of serum proteins in the isolates were subjected to analysis of variance and Duncan tests to determine the significance of differences between the samples.

RESULTS AND DISCUSSION

Electroacidification parameters: duration, voltage and conductivity

The mean time required to electroacidify the three litres of reconstituted milk was 52.9 ± 2.2 minutes. Electroacidification occurred in a linear fashion, as shown by the slope of 22.1 (R^2 = 0.991) calculated for the regression line (Figure V-1). Since electroacidification was done at a constant current, the production of H^+ ions was constant, which is consistent with the results of earlier studies (Bazinet *et al.*, 1997a;1999a,b).

The voltage applied at the electrodes of the electrodialysis system remained constant initially with a mean value of 41.2 ± 2.7 volts, as pH decreased from 6.6 to 5.4; however the voltage then increased exponentially, reaching 83.4 ± 8.1 volts at pH 4.2 (Figure V-1).

This rise in voltage corresponded to a fouling of the cell spacers by precipitated proteins, as previously reported by Bazinet *et al.* (1997a). In fact, most of the milk proteins

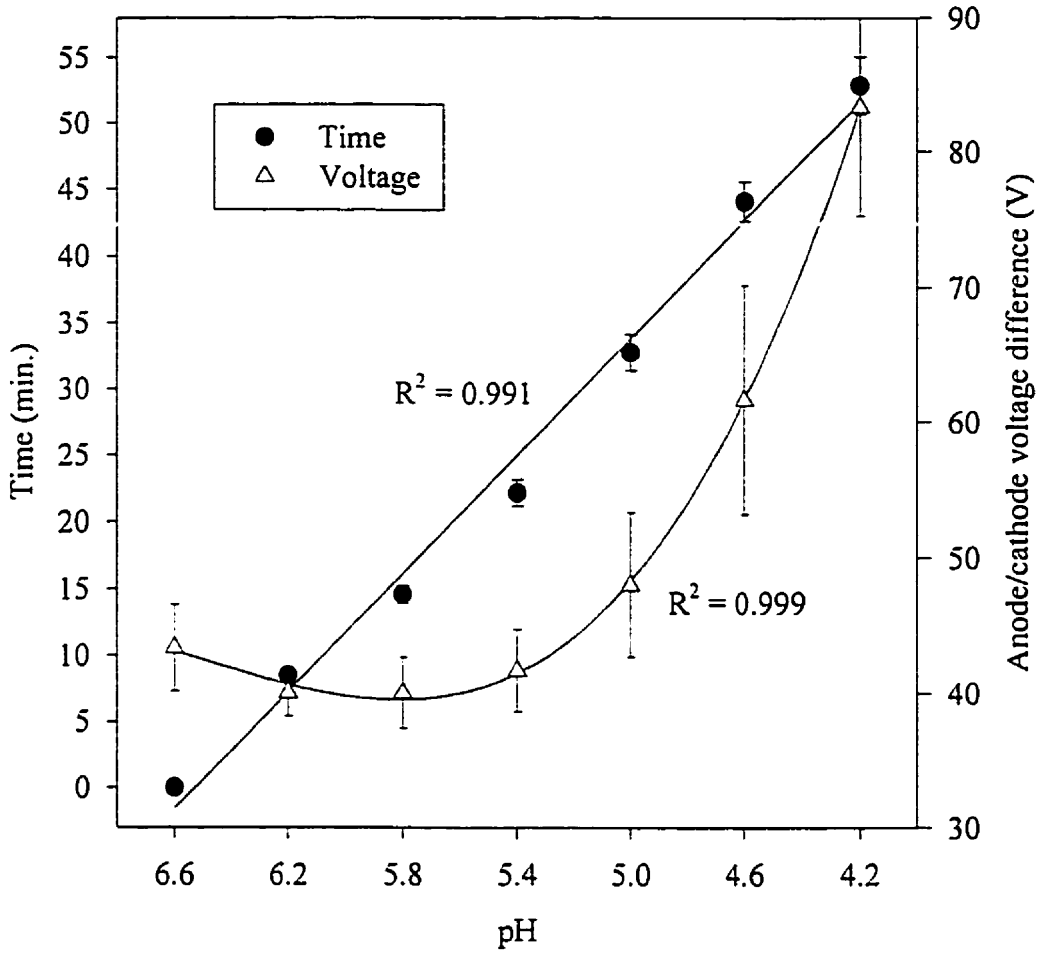

Figure V-1 : Electroacidification duration and voltage evolution during bipolar membrane electroacidification of skim milk solution, run at a cuurent density of 20 mA/cm^2.

precipitated out between pH 5.2 and 4.6. At lower pH values, the electrodialysis system merely stirs up the protein curd that forms in the spacer mesh.

The multivariate analysis of variance showed that the acidification method had a significant effect (1N HCl, 2N HCl and electroacidification) ($P<0.0001$) as did pH ($P<0.0001$), and that there was a dual interaction between pH and acidification method ($p<0.0001$), affecting conductivity. Linear regressions were calculated for the different acidification methods as a function of pH (R^2 varied between 0.983 and 0.991).

It therefore appears that the acidification method has a considerable effect on changes in conductivity as the pH decreases, with mean conductivity values (all pH values combined) for the milk solution being 6.6, 6.6 and 4.2 mS/cm respectively for solutions that were chemically acidified with 1N and 2N and those that were electroacidified (Figure V-2). The conductivity of solutions that were chemically acidified with 1N and 2N HCl rose by 5.15 and 5.1 mS/cm to 8.1 and 8.7 mS/cm respectively between pH 6.6 and 4.2, whereas the conductivity of electroacidified samples declined by 4.8 to 4.0 mS/cm over the same pH interval. The conductivity of acidified samples exhibited the same trend, regardless of the normality of the acid employed (R^2 = 0.983, Figure V-2). These major differences in conductivity relate to the fact that, during chemical acidification of the milk, Cl^- (molar conductivity of 76.4 $S.cm^2.mol^{-1}$) and H^+ (349.6 $S.cm^2.mol^{-1}$) ions are introduced into the solution when HCl is added, whereas during electroacidification demineralization of the solution occurs with the addition of H^+ ions , but there are no associated Cl^- ions (Bazinet *et al.*, 1998b; Lopez Leiva, 1988a,b; Pérez *et al.*, 1994).

Soluble protein

Based on the results of the multivariate analysis of variance, pH has a highly significant effect ($P<0.0001$) on the soluble protein concentration. Non-linear regressions were computed for the different acidification methods relative to pH, giving R^2 values varying from 0.996 to 0.998. To refine the results of the multivariate analysis of variance, Duncan tests were performed on the values obtained at pH 4.6 and pH 4.2 to identify significant differences between the acidification methods and the normality levels at these pH values.

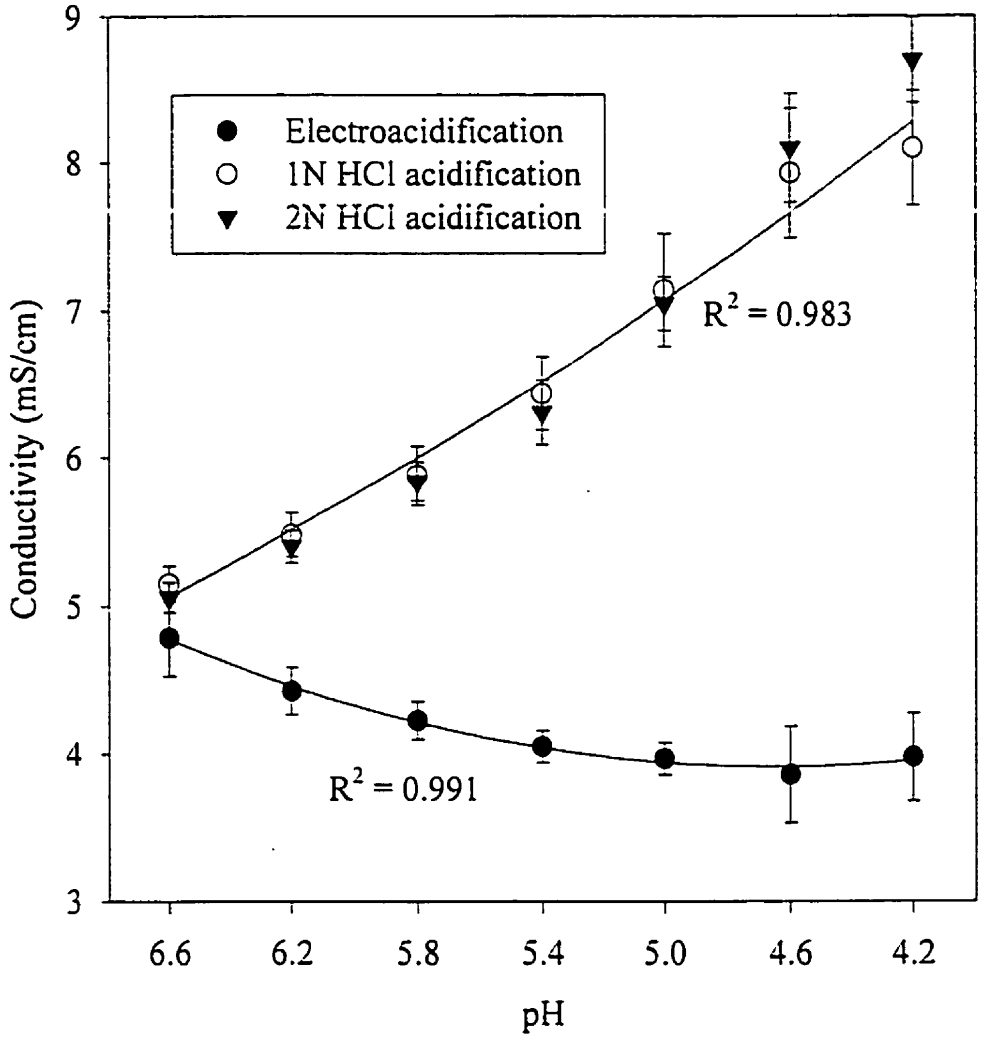

Figure V-2 : Evolution of skim milk solution conductivities during chemical (1N and 2N HCl) and electrochemical acidifications.

The change in percent soluble protein appears to be the same, regardless of the acidification method or the normality used (Figure V-3). In fact, between pH 6.6 and 5.0, percent soluble protein (for all acidification methods averaged) decreased slightly from 100 to about 93.6%, and then dropped sharply from 93.6 to 19.8% as the pH went from 5.0 to 4.6. After that, percent soluble protein remained stable at 19.8 and 19.0% as the pH dropped from 4.6 to 4.2. Thus, about 80% of the proteins are precipitated between pH 5.0 and 4.6, and this situation results in fouling of the spacers by proteins, which explains the increase in voltage observed previously. However, the Duncan test performed at pH 4.6 revealed a significant difference in percent soluble protein between the acidification methods or the level of normality employed, whereas the same test done at pH 4.2 showed no significant difference. At pH 4.6, percent soluble protein was 20.5, 21.7 and 17.3% respectively for the samples acidified chemically with 1N and 2N HCl and those that were electroacidified, whereas at pH 4.2 the mean percentage (for all acidification methods combined) was 19.0%. Thus, at pH 4.6, electroacidification should allow precipitation of all the proteins, in contrast with chemical acidification, where a lower pH value must be reached before the same result can be obtained. Complete chemical acidification of caseins should occur at about pH 4.5, which is intermediate between the values of the sampling done.

Finally, the milk solution undergoes a slight dilution during the electroacidification treatment, owing to the dead volume (about 500 mL of water) of the electrodialysis system (tubing, reservoir, flowmeter and pump). This dilution might have an effect on protein precipitation by decreasing the ionic strength of the solution and reducing the concentration of the milk solution (Riel, 1984; Bazinet *et al.*, 1997a). For example, an increase in the solubility of whole caseins following the addition of salts at a pH slightly lower than the isoionic point was observed by Sharp and Mc Inerney (1936) and by Ho and Waugh (1965) for α_{s1} caseins, as well as by Cayot *et al.* (1991) for β caseins. Ho and Waugh (1965) attributed this solubility increase to a salting-in effect associated with a decrease in electrostatic interactions within the protein (Creighton, 1984), which are maximal at the isoelectric point.

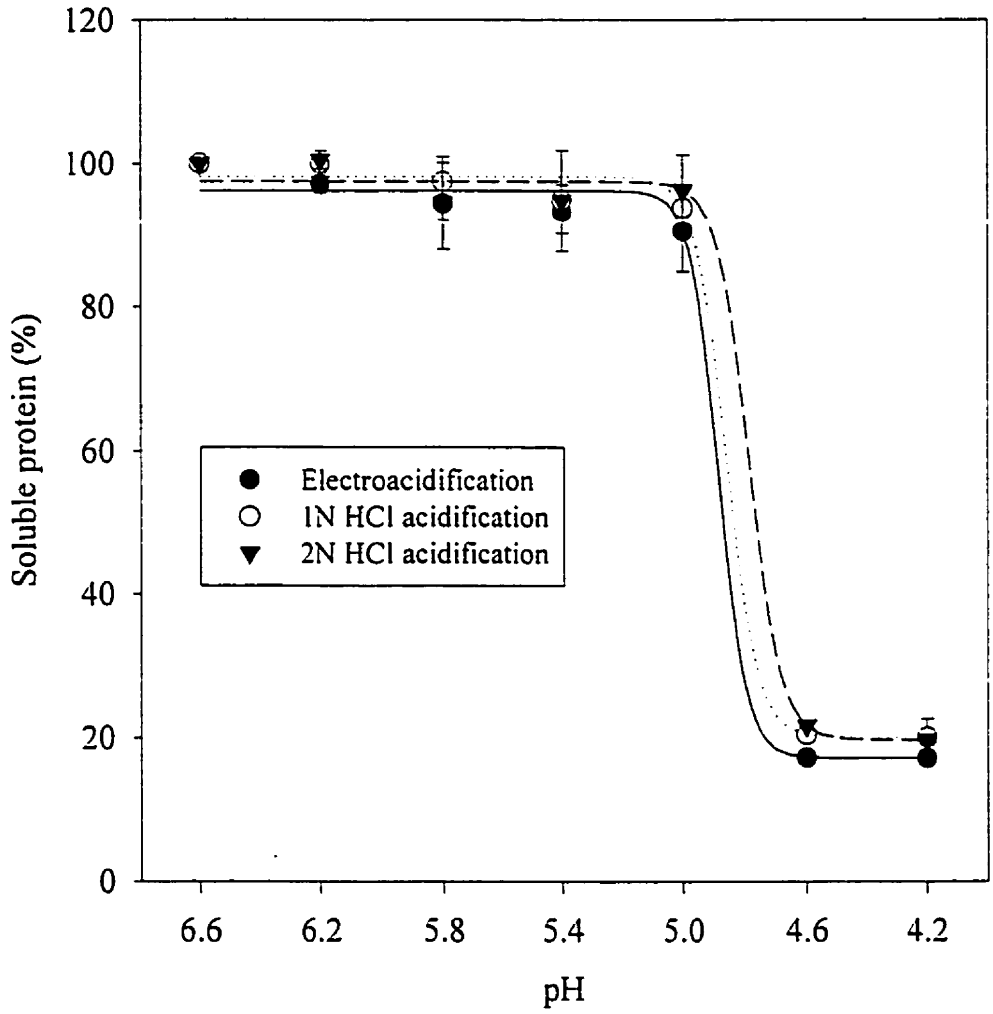

Figure V-3 : Evolution percent soluble protein of skim milk solution during chemical (1N and 2N HCl) and electrochemical acidifications

Molecular profiles

The multivariate analyses of variance dealing with the results of the different protein fractions of the milk showed that pH has a highly significant effect ($P<0.0001$) on the percentage of each of the protein fractions (κ casein, α_s casein, β casein and whey protein) compared with the starting composition of the milk supernatant. Duncan tests were carried out on the percent of each of the fractions in the supernatant at pH 4.6 in order to identify significant differences between the acidification methods.

The α_s and β casein fractions showed comparable trends (Table V-1), regardless of the acidification conditions. Between pH 6.6 and 5.4, these two protein fractions were fairly stable, accounting for 34 and 41.2% of the proteins in the supernatant respectively. The α_s and β casein concentrations decreased by 7.6 and 7.8% between pH 5.4 and 5.0, by 91.8 and 90.7% between pH 5.0 and 4.6, and by 1.5 and 0.6% between pH 4.6 and 4.2. Hence, most of the two protein fractions precipitated between pH 5.0 and 4.6. The κ casein concentrations decreased from 9.2 to 8.5% in the supernatant between pH 6.6 and 5.0, regardless of the acidification method and conditions. Between pH 5.0 and 4.6, the concentration of κ caseins then decreased to 87.3%, followed by a very small decline of 1.4% between pH 4.6 and 4.2. Most of the κ caseins were thus precipitated between pH 5.0 and 4.6. The mean concentration of serum proteins in the supernatant was 12.7% (all acidification methods and conditions averaged) from pH 6.6 to 5.4. Between pH 5.4 and 5.0, the serum protein concentration decreased by 7.9%; it then declined by 8.7 and 4.0% respectively as the pH went from 5.0 to 4.6 and then from 4.6 to 4.2. This drop in whey proteins appears to be linked to coprecipitation of α-la and β-lg with the caseins, owing to the formation of complexes during the drying treatment applied to the milk powder. Hence, during thermal processing the quantity of proteins in the whey associated with the caseins increases as heating intensity rises (Iametti *et al.*, 1993). The β-lg forms a complex with κ caseins via disulfide bond exchange at the natural pH of milk (Cayot and Lorient, 1998; Sawyer, 1969; Singh and Fox, 1987; Tessier and Rose, 1964). In addition, α-la appears to form complexes with β-lg. Furthermore, the thiol group of β-lg appears to catalyse the formation of disulphide bonds between α-la and κ casein (Cayot and Lorient, 1998;

Table V-1 : Evolution of protein fractions (κ-cas, α_s-cas, β-cas and whey proteins) in the supernatant.

	κ-casein (%)			α_s-casein (%)			β-casein (%)			Whey proteins (%)		
pH	BMEA	1N	2N	BMEA	1N	2N	BMEA	1N	2N	BMEA	1N	2N
6.6	9.3	8.9	9.5	35.2	35.0	34.8	42.7	42.6	42.5	12.9	13.5	13.1
6.2	8.7	9.5	9.1	33.9	32.8	34.2	40.9	39.0	41.9	12.7	12.4	12.5
5.8	8.5	8.5	8.9	34.2	34.1	33.9	41.1	41.4	41.5	12.5	13.0	12.6
5.4	8.1	7.9	9.0	32.8	32.3	33.9	39.7	39.5	41.6	12.3	12.3	12.8
5.0	8.0	8.0	9.4	29.6	32.6	32.0	35.4	39.7	39.0	10.6	12.5	11.9
4.6	0.3	0.5	0.4	0.0	0.6	0.2	0.2	0.7	0.8	10.5	10.4	11.0
4.2	0.2	0.3	0.3	0.0	0.0	0.0	0.1	0.0	0.0	9.8	10.0	10.5

Kinsella, 1984). The severity of the heat treatment used to produce milk powder explains why the decrease in whey proteins observed as pH decreased in this study was not observed in earlier research where the raw material used was freshly pasteurized milk (Bazinet *et al.*, 1999c).

The Duncan tests done at pH 4.6 revealed differences in supernatant composition (Table V-2). This trend was already noticeable at pH 5.0 (Table V-1), with a α_s casein value of 29.6 vs 32.6-32.0%, a β casein value of 35.4 vs 39.7-39.0%, and 10.6 for whey proteins vs 12.5-11.9%. Thus, the supernatant composition results obtained at pH 4.6 show that the supernatant of electroacidified milk had a higher whey protein content compared with the supernatants of chemically acidified solutions, with values of 95.0% and 87.2% respectively. In addition, superposition of the chromatograms obtained by HPLC (Figures V-4) revealed differences in the profiles at pH 4.6. At this pH value, there were still some unprecipitated α_s and β casein fractions in the chemical acidification treatments, whereas in the case of electrochemical acidification, there was no trace of these two casein fractions on the chromatogram. The difference observed between the molecular profiles confirms the results obtained previously for total protein and indicates a slight delay in precipitation of α_s and β caseins during the chemical acidifications. This precipitation difference may be due to a salting-in effect from the addition of salts (Ho and waugh, 1965) during chemical acidifications with HCl. By contrast, in electroacidification the removal of salts by electrochemical demineralization, combined with a dilution effect from the system's dead volume, appears to promote precipitation of the proteins. Indeed, at the isoelectric point (pH 4.5-4.7 for caseins), the proteins have a net charge of zero, and they precipitate due to the effect of hydrophobic interactions. Under these conditions, there are no electrostatic repulsions between the molecules. Hence, if the ionic strength is increased, as in the case of chemical acidifications, the salting-in effect of salts on hydrophobic interactions tends to slow the formation of aggregates and increase their solubility, leading to slower protein precipitation (Cheftel *et al.*, 1985; Creighton, 1984; Kinsella *et al.*, 1985). Electroacidification, on the other hand, appears to accelerate the formation of protein aggregates and their precipitation, by promoting hydrophobic interactions through a decrease in the salts that are present in solution.

Table V-2 : Percentage of each milk protein fractions in the supernatants sampled at pH 4.6 and produced in different acidification conditions (1N and 2N HCl, and electroacidification).

	HCl 1N	HCl 2N	Electroacidification
κ-casein (% total peak area)	3.89 a*	3.56 b	3.13 c
α_s -casein (% total peak area)	4.53 a	1.60 b	0.43 b
β-casein (% total peak area)	5.79 a	6.19 a	1.43 a
Whey proteins (% total peak area)	85.8 a	88.6 ab	95.0 b

* Means within a line marked with different letters are significantly different ($P < 0.05$)

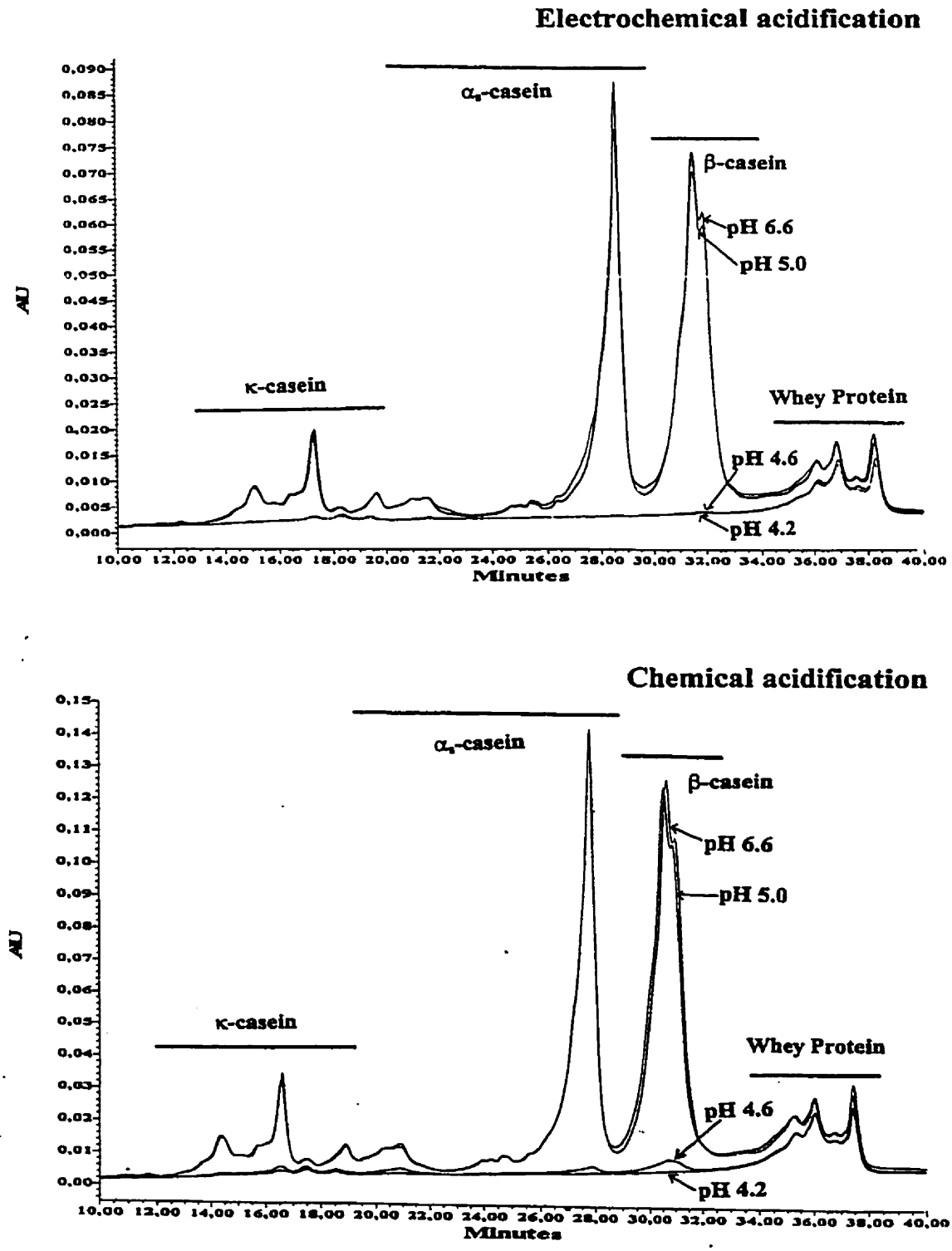

Figure V-4 : Reverse-phase HPLC chromatograms (214 nm) of skim milk supernatant sampled at different pH values during chemical and electrochemical acidifications.

Composition of the isolates

The chemical compositions of the isolates obtained by BMEA and by chemical acidifications were compared in terms of total protein and percent of each protein fraction (Table V-3).

The ash content of the isolates was the same regardless of the acidification method employed and the conditions ($P>0.51$). The total protein contents, computed on a dry basis, were also the same ($P>0.24$). A comparison of the molecular profiles obtained by HPLC and the statistical analysis conducted for each of the fractions showed that there was no difference between the acidification methods.

CONCLUSION

Based on the results of this study, there are a few differences in the acidification profiles obtained for chemical acidification and BMEA. The difference in the precipitation profile appears to be due to a salting-in effect from the addition of salts in chemical acidification. In contrast, with BMEA salts are removed through electrochemical demineralization, thereby favouring protein precipitation. Thus, at pH 4.6, all the caseins are precipitated with BMEA, whereas a few traces of these proteins remain when chemical acidification is employed.

However, at pH 4.2, the chemical composition and the percent of each of the protein fractions contained in the resulting isolates appear not to differ for BMEA and chemical acidification.

ACKNOWLEDGEMENTS

This study was made possible through financial assistance from Novalait Inc. (Quebec).

Table V-3 : Ash and protein contents of isolates produced by chemical and electrochemical acidifications.

	Ash (% dry weight)	**Total protein** (% dry weight)	**κ-casein** (% total peak area)	**α_s-casein** (% total peak area)	**β-casein** (% total peak area)	**Whey proteins** (% total peak area)
BMEA	1.20 ± 0.22 a*	91.9 ± 1.3 a	10.4 ± 0.4 a	40.6 ± 0.3 a	47.3 ± 0.3 a	1.7 ± 0.1 a
HCl 1N	1.28 ± 0.13 a	93.8 ± 0.3 a	10.6 ± 0.1 a	40.6 ± 0.4 a	47.2 ± 0.4 a	1.6 ± 0.2 a
HCl 2N	1.29 ± 0.16 a	93.6 ± 0.7 a	10.6 ± 0.3 a	40.7 ± 0.3 a	47.2 ± 0.2 a	1.5 ± 0.0 a

* Means within a column marked with different letters are significantly different ($P < 0.05$)

RÉSUMÉ (Deuxième article)

L'électroacidification avec membranes bipolaires (ÉAMBP) a été appliquée aux protéines du lait écrémé, afin de produire des isolats de caséines de pureté élevée. L'ÉAMBP utilise la propriété des membranes bipolaires a dissocier les molécules d'eau et l'action de déminéralisation des membranes monopolaires. Lorsqu'un H^+ est généré par la membrane bipolaire, une charge cationique inhérente à la solution en cours d'acidification doit traverser la membrane échangeuse de cations (MEC) pour conserver l'électroneutralité de la solution.

Le but de cette étude est de suivre la migration des charges au travers de la MEC pour contrebalancer les H^+ générés à la membrane bipolaire.

Les ions K^+ sont les ions prédominants à électromigrer de la solution de lait écrémé jusqu'à ce que pH 5.0 soit atteint. À ce point leur concentration devient insuffisante pour contrebalancer les H^+. Les autres cations présents remplacent partiellement les K^+ pour continuer à conserver l'électroneutralité de la solution. Il apparaît que les K^+ sont l'espèce ionique nécessaire pour assurer une bonne efficacité de l'ÉAMBP et pour limiter la migration non-désirée des H^+ au travers de la MEC. Ainsi, l'efficacité électrique de l'ÉAMBP est diminuée par une perte de H^+ électrogénérés, liée à un manque en ions suffisamment mobiles tels que les ions potassium. Par conséquent, l'ajout de potassium dans la solution de lait écrémé apparaît comme une nécessité pour obtenir une meilleure efficacité électrique.

Mots clés : Lait, Protéine, Membrane bipolaire, Électroacidification, Migration cationique, génération de H^+.

ABSTRACT

Bipolar membrane electroacidification (BMEA) technology has been applied to skim milk protein, in order to produce high purity casein isolates. BMEA uses a property of bipolar membrane to split water and the action of monopolar membranes for demineralization. When the H^+ are generated at the bipolar membrane, one cationic charge inherent to the solution being acidified must cross the cation exchange membrane (CEM) to keep the solution electrically neutral.

The purpose of this study was to monitor the migration of charge through the CEM to counterbalance H^+ generated at the bipolar membranes.

K^+ ions were identified as being the predominant ions that electromigrate from the skim milk solution until pH 5.0 is reached, at which point its concentration becomes insufficient to counterbalance H^+. The other cations partially replaced K^+ to assure the electroneutrality of the solution. It appears that K^+ is a necessary ionic species to ensure the best efficiency of the BMEA and to limit the non-desired migration of H^+ across the CEM. Therefore, the electrical efficiency of BMEA is decreased by a loss of electrogenerated H^+, due to a lack of sufficiently mobile ions such as potassium. Consequently, the enrichment of the skim milk with some potassium would be required in order to obtain a better electrical efficiency.

Key words : Milk ; Protein ; Bipolar-membrane electroacidification ; cation migration ; H^+ generation

INTRODUCTION

Bipolar membrane electroacidification (BMEA) uses a property of bipolar membrane to split water and the action of monopolar membranes for demineralization. When a current is passed across a bipolar membrane, electrical conduction is achieved by transport of H^+ and OH^- ions generated by electrodissociation of water. For a solution circulated in an electrodialysis cell on the cationic side of the bipolar membrane, where the H^+ are generated, the pH of the solution will decrease. In the same time, one cation of the solution being acidified must cross the cation exchange membrane to keep the solution electrically neutral, resulting in its demineralization. Bazinet *et al.* (1999a) have demonstrated, in the case of BMEA of soybean protein extract (SPE), that electromigration of the K^+, which is the predominant cation in SPE, is strongly correlated with the amount of electrogenerated H^+ in a 1:1 ratio.

Recently the BMEA technology has been applied to skim milk solutions, in order to produce high-purity casein isolates (1999c). The composition of the mineral content of soybean is, however, very different from milk : 50 % of the mineral salt content of SPE is potassium while calcium represents only 5% (Waggle and Kolar, 1979; Pearson, 1983). In milk, potassium and calcium represent 18.5 and 15.6% respectively of the total mineral content (White and Davies, 1958). In milk, casein interacts with ions or salt (particularly calcium phosphate) to form a voluminous micellar complex with a diameter which varies from 20 to 600 nm (Cheftel *et al.*, 1985; Schmidt, 1982). By adjusting the pH of milk to the isoelectric point of caseins, the intra- and inter-protein electrostatic attractions increase, which affect the stability of the mineral phase of the micelle (Graet and Brulé, 1993; cayot and Lorient, 1998) : 68% of the calcium present in milk is associated with the micelle and during milk acidification, the protein micelle breaks down, releasing calcium (Schmidt, 1982; Cayot and Lorient, 1998).

The purpose of this study is to identify the cations migrating across the cationic membranes during milk BMEA, to quantify them and to improve the BMEA of milk by determining the appropriate mineral balance. This study has four objectives: (1) to titrate

milk protein in order to determine the amount of H^+ necessary to decrease the pH of the solution, (2) to evaluate the amount of H^+ to be generated during BMEA by chemical titration and determination of the amount of electrical charge, (3) to measure the cations migrating during BMEA runs, in reference to previous work (Bazinet *et al.*, 2000b), and finally (4) to compare the amount of cations migrating relative to the amount of electrogenerated H^+.

MATERIAL AND METHODS

Material

The raw material used in this study was reconstituted milk (10% W/V) from low temperature spray-dried skim milk powder (Agropur, Granby, Canada). The average composition of the skim milk powder was the following (g/100g) : total protein, 33.9 ; whey protein, 7.4 ; fat, 0.6; carbohydrates, 53.5; ash, 8.2; moisture, 3.8.

Methods

a) Electroacidification cell

The module used was an MP type cell (100 cm^2 of effective surface) from the ElectroCell Systems AB Company (Täby, Sweden). The cell consists of a structure of nine compartments separated by four Neosepta CMX cationic membranes (Tokuyama Soda Ltd., Tokyo, Japan) and three Neosepta BP-1 bipolar membranes from Tokuyama Soda Ltd. This arrangement has the same configuration as that used by Bazinet *et al.* (1999a). The anode/cathode voltage difference was supplied by a variable 0-100 V power source Powerstat Model 236BU-2 (The Superior Electric Co., Bristol, CO). The three electrolytes: milk, KCl (5g/L) and Na_2SO_4 (20 g/L) were circulated using three centrifuge pumps Model XVB56C34F2012b-W (Marathon Electric, Wausau, WI), and the flow rate was controlled at 4.5 Lpm using Filter-Chem flowmeters Model FC-FI-C-3/8 (Alhambra, CA). The pH of the skim milk solution was measured with a Model Φ11 pH meter (Beckman Instruments

Inc., Fullerton, CA). The anode, a dimensionally-stable electrode (DSA), and the cathode, a 316 stainless steel electrode, were supplied with the MP cell.

b) Protocol

For chemical acidification, volumes of 250 ml of the skim milk solution were used. HCl solutions of 1 and 2 N were added to acidify the protein solution to pH 4.2.

Electroacidification was carried out in batch process at a constant current of 2 A (current density, 20 mA/cm^2), with electrolyte volumes of 6 litres for the Na_2SO_4 and KCl solution, and 3 litres for the milk solution (Bazinet *et al.*, 2000b). Electroacidification was stopped after pH 4.2 was reached. The initial pH of the reconstituted skim milk varied from 6.5 to 6.7. The temperature was maintained constant at 20 ± 1°C during electrochemical treatment and was stable at 21 ± 1°C during chemical acidifications. Three replicates of each experiment were performed in this study.

During each treatment, samples of the milk solution were taken at the following pH : initial pH (about 6.6), 6.2, 5.8, 5.4, 5.0, 4.6 and 4.2. During acidification treatment, the volume of HCl added was recorded at each sampling. During electrochemical acidification, the time required to reach pH 4.2 was recorded as well as the anode/cathode voltage difference, and the conductivity of the protein solution. The concentration of soluble protein (on the freshly acidified sample) and the concentrations of potassium, sodium, calcium and magnesium (on ash redissolved in 2N HCl) were determined, on each sample of milk solution.

c) Analysis Methods

Ash content : The ash content of the skim milk samples was determined according to the AOAC method no. 945-46 (AOAC, 1995c).

Soluble protein concentration : The protein concentration was determined using a FP-428 LECO apparatus (LECO Corporation, Saint Joseph, MI) following the same conditions and parameters used by Bazinet *et al.* (1999c).

Potassium, sodium, magnesium and calcium concentration measurements : Sodium, potassium, magnesium and calcium concentrations were determined by inductively coupled plasma (ICP, Optima 3300, dual view, Perkin-Elmer, Norwalk, CT). The wavelengths used to determine sodium, calcium, magnesium and potassium concentrations were 589.59, 422.67, 285.21 and 766.49 nm respectively. The analysis were carried out in radial view. Samples were prepared from known weight skim milk solution ash dissolved in 10 mL HCl (2N) and diluted with HCl (2N) to be within the calibration ranges for each cation.

RESULTS AND DISCUSSION

Chemical titration of milk protein solution

The volumes of HCl added to 250 mL of skim milk solution to reduce the pH from 6.6 to 4.2 were 15.1 ± 0.8 mL and 7.7 ± 0.2 mL respectively for 1 N and 2 N HCl. The amount of H^+ needed to decrease the pH of a protein solution and to overcome the protein buffering capacity is protein concentration dependent (cheftel *et al.*, 1985; Prakash and Narasinga Rao, 1990; Brunner, 1981).

Model curves of soluble protein concentration

The data obtained for the percentage of soluble protein as the pH decreases from 6.6 to 4.2, were fitted using Sigmaplot (Table V-4). The equations were calculated as sigmoidal curves as done by Bazinet *et al.* (1999a) :

$$S_p = b + \frac{a}{1 + \exp\left[-\left(\frac{pH_x - c}{w}\right)\right]} \qquad (1)$$

Table V-4 : Parameter calculated values of the sigmoidal soluble protein curves for electroacidification and 1N and 2N HCl chemical acidifications.

Acidification	Parameters	Calculated values
Electroacidification	*a*	79.0
	b	17.2
	c	4.88
	w	0.0449
	R^2	0.996
1 N HCl acidification	*a*	78.0
	b	20.1
	c	4.85
	w	0.0497
	R^2	0.997
2 N HCl acidification	*a*	77.8
	b	19.7
	c	4.78
	w	0.0551
	R^2	0.996

S_p is the percentage of soluble protein, pH_x the pH value ranging from pH 6.6 to 4.2, a the amplitude of the curve, b the percentage of soluble protein at the isoelectric point, c the center or point of inflection and w the width of the transition region of the sigmoidal curve.

The protein precipitation curves obtained during electroacidification and chemical acidification are similar, as confirmed by the comparable parameters obtained for all curves (Table V-4). The soluble fraction at pH 4.2 was composed mostly of whey proteins while the insoluble fraction was the milk caseins (Cheftel *et al.*, 1985; Swaisgood, 1982; Lorient, 1991). A trend in the extent of precipitation was observed : by acidification procedures 20.1 and 19.7% soluble protein were recovered in solution at the isoelectric point (about pH 4.6 for milk caseins), while by the electrochemical procedure 17.2% of the soluble protein was recovered. A decrease of the milk protein concentration and of the ionic strength of the milk solution related to the water containing-dead volume of the electrodialysis cell may be responsible for the difference recorded between both process.

Electrogenerated H^+

a) Chemical titration determination

Following the chemical precipitation of the milk caseins, the volumes of acid added were transformed into equivalent of H^+ added, expressed as mMole per gram of protein insolubilized at pH 4.2. As expected, the amount of H^+ added to precipitate one gram of protein at pH 4.2 is the same whatever the normality of HCl used (R^2=0.997) and this relationship between pH and H^+ added at a given pH could be represented by the following quadratic relation (Bazinet *et al.*, 1999a) :

$$H_{4.2} = 11.656 - 3.053\ pH_x + 0.195\ pH_x^2 \qquad (2)$$

$H_{4.2}$ is the number of H^+ added (by addition of HCl) per gram of protein insolubilized at pH 4.2 and pH_x the pH value ranging from pH 6.6 to 4.2.

By analogy, from equation (2), the amount of H^+ electrogenerated at a given pH to pH 4.2 to insolubilize a certain amount of protein can be obtained as follow (Bazinet *et al.*, 1999a) :

$$H_{e\ 4.2} = \left[0.195 \left(pH_x^2 - pH_i^2\right) - 3.053 \left(pH_x - pH_i\right)\right] A\ C_p \qquad (3)$$

$H_{e\ 4.2}$ is the number of mMole of H^+ electrogenerated per liter of protein solution, pH_x the pH value of the electroacidified protein solution, pH_i the initial pH value, C_p the percent total solids of the reconstituted skim milk, and A the percentage of total protein in the skim milk powder.

From equation (3) the amount of H^+ electro-generated to precipitate the respective protein amount at pH 4.2 was determined as shown in Figure V-5.

As the concentration of soluble protein varies with the pH value, at a given pH, the amount of H^+ added was calculated and expressed as mMole of H^+ per gram of protein precipitated at this pH. This amount was obtained by calculating the difference in mMole of H^+ between two pH values per gram of precipitated protein at pH 4.2 and dividing this value by the difference in precipitated protein in the same pH range: This calculation is related to the efficiency of added H^+, at a particular pH, to insolubilize protein. An increase in H^+ needed to precipitate one gram of protein represents a decrease in protein precipitation efficiency. The equation is :

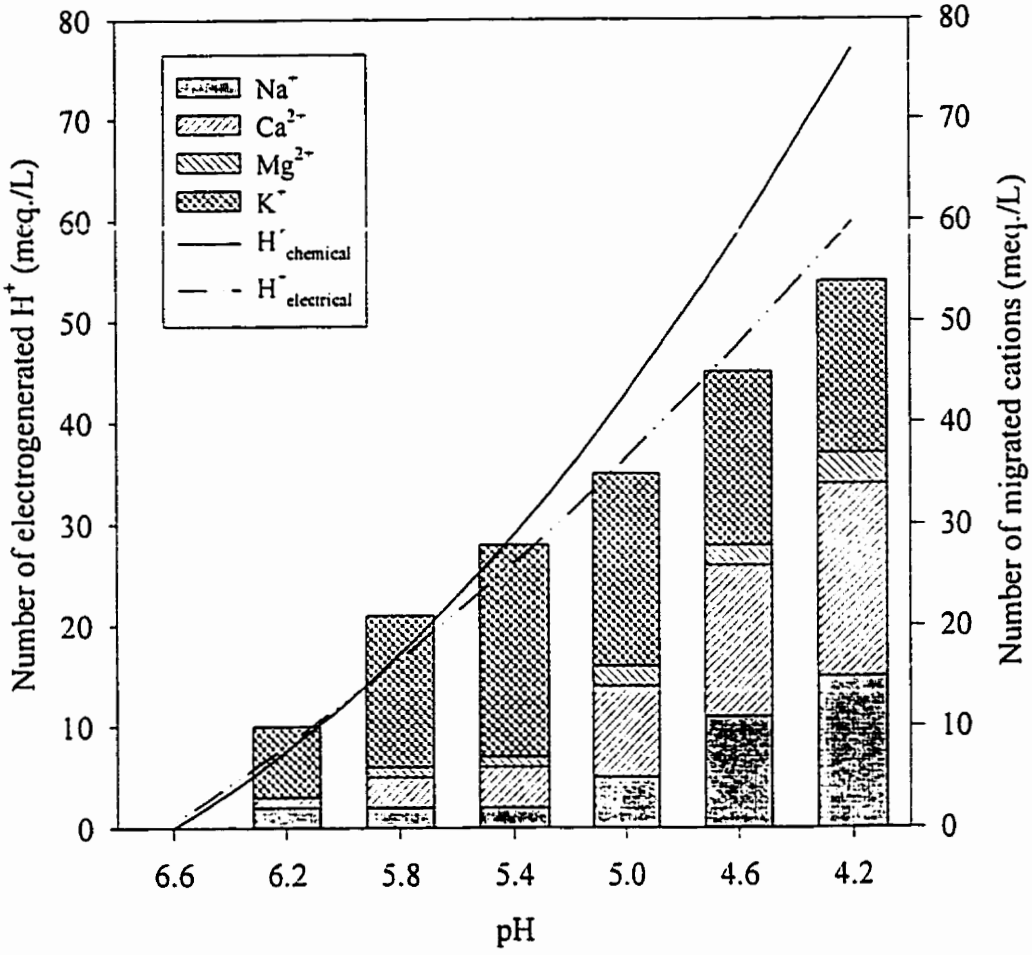

Figure V-5 : Comparison of the amount of electrogenerated H^+ (meq./L) and the amount of cations migrated (meq./L) during electroacidification of skim milk.

$$H_i = \frac{100\ A\ C_p \left[d_1 \left(pH_x - pH_{x-\delta x}\right) - d_2 \left(pH_x^2 - pH_{x-\delta x}^2\right)\right]}{A\ C_p \left[1 - \left(b + \frac{a}{1 + \Theta_{x-\delta x}}\right)\right] - A\ C_p \left[1 - \left(b + \frac{a}{1 + \Theta_x}\right)\right]} \quad (4)$$

with :

$$\Theta_{x-\delta x} = \exp\left(\frac{c - pH_{x-\delta x}}{w}\right)$$

and

$$\Theta_x = \exp\left(\frac{c - pH_x}{w}\right)$$

H_i is the number of mMole of H^+ added (by addition of HCl) per gram of protein insolubilized, pH_x the pH value, $pH_{x-\delta x}$ the pH_x value decreased by δx (both ranging from pH 6.6 to 4.2), C_p the percent total solids of the reconstituted skim milk, A the percentage of total protein in the skim milk powder, d_1 and d_2 are respectively the pH_x and pH_x^2 coefficients from equation (2), *a, b, c* and *w* are parameters from equation (1).

Simplification of equation (4) gives :

$$H_i = \frac{100 \left[\frac{d_1}{a} \left(pH_x - pH_{x-\delta x}\right) - \frac{d_2}{a} \left(pH_x^2 - pH_{x-\delta x}^2\right)\right] \left[1 + \Theta_x\right] \left[1 + \Theta_{x-\delta x}\right]}{\Theta_{x-\delta x} - \Theta_x} \quad (5)$$

Although, the sigmoïdal curve (eq. (1)) was different from the one calculated by Bazinet *et al.* (1999a) for the precipitation of soybean protein, the simplified equations for the amount of H^+ added per gram of protein insolubilized (eq. (5)) were the same for the soybean and milk protein. It appears that this equation could be generalized to every

sigmoïdal protein precipitation fitting in order to determine the amount of H^+ required (by addition of HCl) per gram of protein insolubilized and to determine the amount of electrogenerated H^+ by BMEA, for any running conditions.

Protein was not precipitated between pH ranging from 6.6 to 5.4. Consequently, the amount of H^+ added per gram of protein insolubilized obtained from equation (5) were limited to pH ranging from 5.4 to 4.6 in order to avoid an unrealistic deviation of the model. At the beginning of the protein precipitation from pH 5.4 to 5.0, a high amount of H^+, ranging between 8 and 30 mMole, is needed due to a low quantity of protein which may insolubilize in this range of pH. In fact, in this range of pH the percentage of soluble protein decreases slightly from 100 to 94.5% (Bazinet *et al.*, 1999c). The largest amount of protein is precipitated in the pH range of 5.0 to 4.6 (Bazinet *et al.*, 1999c). In this range, the relative efficiency of the H^+ added is the highest with about 0.6 mMole per gram insolubilized protein. These results agree with the literature indicating that milk casein completely precipitates in the pH range between 5.0-5.2 and 4.6-4.8 (Cheftel *et al.*, 1985; Cayot and Lorient, 1998; Lorient, 1991).

The amount of H^+ electro-generated can be calculated in mMole/g protein insolubilized, by introducing BMEA parameters from eq. (1) (79.0, 17.2, 4.88 and 0.0449) into eq. (5) (Table V-4). This produces the following equation :

$$H_{e\,l} = \frac{\left[3.865\left(pH_x - pH_{x-\delta x}\right) - 0.246\left(pH_x^2 - pH_{x-\delta x}^2\right)\right]\left[1 + \Theta_x\right]\left[1 + \Theta_{x-\delta x}\right]}{\Theta_{x-\delta x} - \Theta_x} \qquad (6)$$

with

$$\Theta_{x-\delta x} = \exp\left(\frac{4.88 - pH_{x-\delta x}}{0.0449}\right)$$

and

$$\Theta_x = \exp\left(\frac{4.88 - pH_x}{0.0449}\right)$$

$H_{e\ i}$ is the number of mMole of electrogenerated H^+ per gram of insolubilized protein, pH_x the pH value and $pH_{x-\delta x}$ the pH_x value decreased by δx (both ranging from pH 6.6 to 4.2).

The theoretical relative efficiency of the electrogenerated H^+ during BMEA can be calculated from equation (6). At the beginning of the precipitation, about 20 mMole of H^+ will be needed to render one gram of protein insoluble. The H^+ relative efficiencies are the highest in the same range of pH as chemical acidification, with amount of electrogenerated H^+ about 2.4-9.9 mMole : the maximum efficiency is at pH 4.8 with 0.3 mMole. The H^+ relative efficiency values are comparable for both chemical and electrochemical acidification, since the protein precipitation curves are similar.

b) Electrical determination

The mass equivalent transported by application of a direct electric current is given by Faraday's Law (Lopez-Leiva, 1988a; Bard and Faulkner, 1983):

$$\sum E_m = \frac{t\ I}{F} \qquad (7)$$

E_m is the equivalent mass transported, t the duration, I the current intensity and F the Faraday constant.

The amount of electricity necessary to produce a certain separation can also be calculated for a stack of N cell pairs by Faraday's Law (lopez-Leiva, 1988a). Equation (7) becomes :

$$\sum E_m = \frac{N \; t \; I \; \eta}{F} \qquad (8)$$

E_m is the equivalent mass transported, t the duration, I the current intensity, η the current efficiency coefficient and F the Faraday constant.

As the mass expressed in equivalents is related to the molar mass ($E_{mi} = n_i \; z_i$), equation (8) becomes :

$$\sum \; n_i \; z_i = \frac{N \; t \; I \; \eta}{F} \qquad (9)$$

n_i is the number of moles of species i, z_i the valence of the species i, N the number of cell pairs , t the duration, I the current intensity, η the current efficiency coefficient and F the Faraday constant.

Application of equation (9) to the production of H^+ (Mole/L) during electroacidification of skim milk solution with a electrodialysis stack containing N bipolar membrane gives the following equation :

$$H_{ee} = \frac{N \; t_x \; I \; \eta}{F \; V} \qquad (10)$$

H_{ee} is the amount of electrogenerated H^+ at time t_x (Mole/L), N the number of cell pairs , t_x the duration of electroacidification, I the current intensity, η the current efficiency coefficient, F the Faraday constant and V the volume of skim milk treated.

The BMEA was carried out on 3L skim milk, at a constant current of 2 A. The calculation was based on a 90% current efficiency coefficient, since 90-99% of the current is carried by the counter-ions across the membranes (Lopez-Leiva, 1988a). Then, the amount of electrogenerated H^+ at a given timepoint during BMEA can be calculated in Mole/L by simplification of equation (10) :

$$H_{ee} = 18.652.10^{-6}\, t_x \qquad (11)$$

H_{ee} is the amount of electrogenerated H^+ at time t_x (Mole/L) and t_x the duration of electroacidification (s).

Figure V-5 shows the result of the experimental values obtained for the amount of electrogenerated H^+ calculated from equation (11). For any pH value, the amount of electrogenerated H^+ estimated by calculation of electrical charge for a 90% current efficiency was very close to the amount of total migrated cations. In addition, the comparison between the amount of electrogenerated H^+ estimated by both calculation types showed a difference from pH 5.0 to pH 4.2. Before pH 5.0, both calculations gave similar responses. The difference in response, after pH 5.0, should be due to a salting in effect by addition of HCl which delayed the protein precipitation by 0.1-0.2 pH units (Bazinet *et al.*, 1999c). By chemical acidification, there was a simultaneous addition of H^+ and Cl^- which consequently increased the conductivity and ionic strength. These curve calculations should be considered as the upper and lower limits of the confidence interval.

Determination of migrated cations

As shown in Table V-5 and Figure V-5, from pH 6.6 to 5.4 during BMEA, the potassium is the predominant cation to migrate accross the CEM, with a decrease in its concentration of 76.8%. In the same range of pH the sodium, magnesium and calcium concentrations are constant at about 536, 89 and 1078 mg/L respectively. From pH 5.4 to 4.2, the sodium, magnesium and calcium concentrations decreased by 60.5, 33.7 and 31.7% respectively, while the potassium concentration in the skim milk solution increased by 61.0% (from 236 to 380 mg/L) in a linear fashion (12 mg/L per 0.1 pH unit decrease). The

Table V-5 : Changes in potassium, sodium, calcium and magnesium concentrations during bipolar membrane electroacidification of skim milk.

PH	Potassium (mg/L)	Sodium (mg/L)	Calcium (mg/L)	Magnesium (mg/L)
6.6	1017 ± 74 a*	567 ± 25 a	1121 ± 38 a	94 ± 4 a
6.2	692 ± 55 b	527 ± 35 a	1107 ± 45 a	92 ± 5 a
5.8	406 ± 89 c	526 ± 56 a	1055 ± 79 a	87 ± 7 a
5.4	236 ± 40 e	524 ± 26 a	1032 ± 65 ab	84 ± 5 ab
5.0	284 ± 23 ed	449 ± 45 b	943 ± 73 b	75 ± 7 bc
4.6	338 ± 15 cd	311 ± 48 c	829 ± 52 c	67 ± 4 cd
4.2	380 ± 15 c	212 ± 27 d	736 ± 54 c	60 ± 5 d

* Means within a column followed by different letters are significantly different ($P < 0.05$)

results obtained are in accordance with those of Bazinet *et al.* (2000a) and indicated that at the end of the BMEA there is a real increase of potassium ions in the skim milk solution. Moreover, these results confirmed that potassium is the first ion to migrate during BMEA (Bazinet *et al.*, 1999a), due to its higher electrical mobility and conductivity (Brett and Oliveira-Brett, 1994). It is also the predominant ion to leave the skim milk solution until a critical concentration of about 20% of its initial concentration was reached.

Comparison between generated H^+ and migrated cations during the electroacidification procedure

The values for migrated cations (mg/L) were normalized by calculating the variation of each cation concentration, for each pH interval, from the beginning to the end of the electroacidification at pH 4.2, and then normalized variation concentrations were expressed in meq./L. The sums of the contribution of each cation at different pH were compared to the amount of electrogenerated H^+ determined from equations (3) and (11) (Figure V-5). It appears that from pH 6.6 to 5.4, the H^+ are well counterbalanced by cation migrations, while from pH 5.0 to pH 4.2 there is a lack in cation migration. Moreover, this difference between electrogenerated H^+ and migrated cations increases as long as the BMEA runs. The main explanation of this phenomenon is the differential mobility and concentration of cations. At the beginning of the process, the potassium is the main ion to counterbalance the electrogenerated H^+ because of its highest mobility and relatively high concentration in skim milk. When its concentration is no longer sufficient, the other slower cations try to counterbalance the H^+. Their contribution is proportional to their mobility and valence, as shown previously (sodium, magnesium and calcium). However, these cations can not counterbalance completely the H^+ electrogenerated, as the H^+ have the highest mobility ; 7.6, 5.2, 11.0 and 6.2 $cm^2/s.V$ respectively for potassium, sodium, magnesium and calcium (Brett and Oliveira-Brett, 1994) compared to H^+ (36.2 $cm^2/s.V$). Therefore H^+ have to migrate across the cation-exchange membrane to ensure the electroneutrality. These migrated H^+ are lost for the process and decrease the electrical efficiency. Bazinet *et al.* (1999a) did not observed this phenomenon on soybean protein electroacidification due to the higher concentration in potassium ions in the raw material.

The increase in potassium observed in the skim milk after pH 5.0-5.4 was reached, would be explained by a leak of potassium across the bipolar membrane. Since the water molecules must enter the bipolar membrane, potassium ions are carried across the anionic layer of the BPM with water molecules, overriding the Donnan effect controlling the selectivity of the anionic layer, as the concentration in potassium on the anionic side of the BPM is high. Anion-exchange membranes are not completely selective to anions, and allow a small leakage of cations on the order of 1-2% (Davis *et al.*, 1997).

CONCLUSION

The different mathematical relations obtained from experimental values of H^+ added by chemical acidification allowed the determination of electrogenerated H^+ during the BMEA process in relation to the concentration of protein to be precipitated. These mathematical relationships can be easily generalized to sigmoidal protein precipitation curves.

Moreover, the electromigration of K^+ from the protein solution to the KCl compartment during BMEA has been demonstrated to be strongly correlated to the amount of H^+ electro-generated until its concentration becomes insufficient to counterbalance the H^+. It appears that K^+ is a necessary ionic species to ensure the best efficiency of the BMEA and to limit the non-desired migration of H^+ across the CEM and that other cations are not as successful in compensating for the lack of K^+ migration.

We can conclude from the data presented in this study that the electrical efficiency of BMEA is decreased by a loss of electrogenerated H^+, due to a lack of sufficiently mobile ions such as potassium. Consequently, the addition of a certain amount of potassium (about 7 to 25 meq/L) in the skim milk should be applicable in order to obtain a better electrical efficiency.

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ATTEINTE DES OBJECTIFS ET AVANCEMENT DES CONNAISSANCES

Dans ce chapitre, les résultats obtenus ont d'abord permis de répondre à l'objectif concernant la comparaison des acidifications chimiques et électrochimiques du lait et d'identifier d'éventuels éléments de différenciation entre les deux procédés. Ainsi, les résultats ont montré que l'acidification chimique et l'ÉAMBP présentent quelques différences dans les profils d'acidification; à pH 4.6, toutes les caséines sont précipitées par ÉAMBP, alors qu'il reste quelques traces de ces protéines par acidification chimique. Une hypothèse a été émise sur le fait que cette différence de précipitation serait liée à un effet de salting-in découlant de l'ajout de sels, dans le cas de l'acidification chimique, opposé dans le cas de l'ÉAMBP, à un retrait des sels par déminéralisation qui favoriserait la précipitation des protéines. Cependant, la composition chimique et le pourcentage de chacune des fractions protéiques entrant dans la composition des isolats produits, à pH 4.2, à la fois par ÉAMBP et par acidification chimique ont été démontrés équivalents.
De plus, les résultats obtenus dans ce chapitre, ont permis de répondre à l'objectif visant à étudier l'équilibre ionique de la solution de lait électroacidifiée. Les approches chimiques et électriques ont permis d'évaluer respectivement à 60 et 78 méq./L le nombre de H^+ électrogénérés au cours de l'ÉAMBP. Ensuite, les analyses par ICP ont contribué à l'identification et la quantification des cations migrant au travers des membranes cationiques durant l'ÉAMBP du lait ($K^+ >>> Ca^{2+} \geq Na^+ >> Mg^{2+}$). Finalement, les résultats obtenus ont permis de comparer les cinétiques de migration des cations à celles des H^+ électrogénérés ; les ions K^+ sont les ions prédominants à électromigrer de la solution de lait écrémé jusqu'à ce que pH 5.0 soit atteint. À ce point leur concentration devient insuffisante pour contrebalancer les H^+ électrogénérés et les autres cations présents ne remplacent que partiellement les K^+ pour continuer à conserver l'électroneutralité de la solution. Il est apparu que les K^+ sont l'espèce ionique nécessaire pour assurer une meilleure efficacité de l'ÉAMBP et pour limiter la migration non désirée des H^+ au travers de la MEC.

Ces résultats ont concouru à un avancement des connaissances sur la compréhension des phénomènes membranaires au cours de l'ÉAMBP. En effet, un modèle permettant de

déterminer le nombre de H^+ électrogénérés à l'interface des MBP a été établi. Ce modèle est apparu généralisable à tout type de protéine dont la courbe de précipitation est sigmoïdale. Ce modèle a aussi contribué à mettre en évidence le manque d'ions suffisamment mobiles tels que les ions potassium pour contrebalancer les H^+ électrogénérés et conserver l'électroneutralité de la solution de lait écrémé. Ce manque d'ions suffisamment mobiles aboutit donc à une migration non désirées d'une partie des H^+ électrogénérés, perdus pour l'ÉAMBP ; il en résulte une diminution de l'efficacité énergétique du procédé.

CHAPITRE VI

EFFET DE LA NATURE DES SELS AJOUTÉS ET DE L'AUGMENTATION DE LA FORCE IONIQUE DANS LA SOLUTION DE LAIT SUR LES PERFORMANCES DE LA TECHNOLOGIE, LA COMPOSITION CHIMIQUE ET LA FONCTIONNALITÉ DES ISOLATS PRODUITS PAR EAMBP

CHAPITRE VI

EFFET DE LA NATURE DES SELS AJOUTÉS ET DE L'AUGMENTATION DE LA FORCE IONIQUE DANS LA SOLUTION DE LAIT SUR LES PERFORMANCES DE LA TECHNOLOGIE, LA COMPOSITION CHIMIQUE ET LA FONCTIONNALITÉ DES ISOLATS PRODUITS PAR EAMBP

TRANSITION CONTEXTUELLE

Comme pour tout procédé électrochimique, les solutions traitées par ÉAMBP doivent posséder un contenu minéral relativement élevé pour permettre une conductivité électrique adéquate afin de diminuer la résistance globale de la cellule d'électrodialyse. Cette conductivité peut être obtenue par ajout d'une certaine quantité de sels dans la solution de lait écrémé comme suggéré au chapitre V pour améliorer l'efficacité énergétique du procédé. Les objectifs de cette étude sont donc (1) d'étudier l'effet de la nature des sels ajoutés et de l'augmentation de la force ionique sur les performances énergétiques de l'ÉAMBP, (2) d'étudier l'effet de la nature des sels ajoutés et de l'augmentation de la force ionique sur la composition chimique et la fonctionnalité des isolats produits par ÉAMBP et, (3) de comparer les qualités compositionnelle et fonctionnelle des différents isolats produits par ÉAMBP avec celles de standards commerciaux et chimiques.

Les résultats de ce chapitre ont fait l'objet de deux articles : Le premier intitulé «Effect of Added Salt and Increase in Ionic Strength on Skim Milk Electroacidification Performances» soumis pour publication dans *J. Dairy Res.* Les auteurs sont : Laurent Bazinet (Candidat au Ph. D.: planification et réalisation des expériences, analyse des résultats et rédaction de l'article), Denis Ippersiel (participation à la réalisation des expériences, correction et révision du manuscrit), Christine Gendron (participation à la réalisation des expériences et révision du manuscrit), Behzad Mahdavi (révision du

manuscrit), Jean Amiot (Directeur de thèse : supervision scientifique de l'étudiant, correction et révision du manuscrit), et François Lamarche (Co-directeur de thèse : supervision scientifique de l'étudiant, correction et révision du manuscrit).

Le second ayant pour titre «Effect of Added Salt and Ionic Strength on Physicochemical and Functional Properties of Casein Isolates Produced by Electroacidification» soumis pour publication dans *Int. J. Dairy Sci.* Les auteurs sont : Laurent Bazinet (Candidat au Ph. D.: planification et réalisation des expériences, analyse des résultats et rédaction de l'article), Christine Gendron (participation à la réalisation des expériences, analyse des propriétés fonctionnelles et révision du manuscrit), Denis Ippersiel (participation à la réalisation des expériences, correction et révision du manuscrit), Josée René-Paradis (analyse des propriétés fonctionnelles), Claudia Tétreault (analyse des propriétés fonctionnelles), Jocelyne Beaudry (analyse des minéraux sur ICP), Michel Britten (révision du manuscrit), Behzad Mahdavi (révision du manuscrit), Jean Amiot (Directeur de thèse : supervision scientifique de l'étudiant, correction et révision du manuscrit) et François Lamarche (Co-directeur de thèse : supervision scientifique de l'étudiant, correction et révision du manuscrit).

RÉSUMÉ (Premier article)

L'électroacidification avec membranes bipolaires (ÉAMBP), technologie utilisant la propriété des membranes bipolaires à dissocier les molécules d'eau et l'action déminéralisante des membranes cationiques, a été employée pour la production de caséines acides. Le but de cette étude était d'évaluer les performances de l'ÉAMBP dans différentes conditions de force ionique ($\mu_{ajoutee}$ = 0, 0.25, 0.5 et 1.0 M) et de sels ajoutés ($CaCl_2$, NaCl et KCl) afin d'établir la combinaison optimale des ces deux paramètres..

La meilleure alternative a été obtenue avec du KCl à une force ionique de 0.5 M ; elle a permis une diminution de la consommation énergétique de 45%. Cette amélioration de l'efficacité énergétique a été la résultante d'une baisse de tension, liée à une augmentation de la conductivité, couplée avec une compensation, par ajout de sel, du manque en ions suffisamment mobiles dans le lait écrémé.

Mots clés : Acidification électrochimique, Membrane bipolaire, Caséine, Sels, Force ionique.

ABSTRACT

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. The aim of this study was to evaluate the performance of BMEA in different conditions of ionic strength (μ_{added} = 0, 0.25, 0.5 and 1.0 M) and added salt ($CaCl_2$, NaCl and KCl).

The combination of KCl and μ_{added} = 0.5 M was the best combination with a 45% decrease in energy consumption. The better energy efficiency was the result of a decrease in the anode/cathode voltage difference, due to an increase of conductivity, coupled with the compensation, by addition of salt, for the lack in sufficiently mobile ions in the skim milk.

Key words : Electrochemical acidification, Bipolar membrane, casein, salt, ionic strength.

INTRODUCTION

A procedure developped for soybean protein precipitation (Bazinet *et al.*, 1996 ; 1997c), which was derived from electrodialysis, was tested for the production of acid casein (Bazinet *et al.*, 1999c). 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 products to be treated by BMEA must possess a relatively high mineral content to allow a good electrical conductivity in order to decrease the global resistance of the electrodialysis cell. Moreover, Bazinet *et al.* (2000c) recently demonstrated that the electrical efficiency of skim milk electroacidifiaction is decreased, because of a lack of sufficiently mobile ions such as potassium and consequently by 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. The role of intra- and intermolecular ionic bonds between caseins is not clear. However, 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 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 affect the stability of the mineral phase of the micelle (Cayot and Lorient, 1998). Furthermore, results obtained by Graet and Brulé (1993) have shown the effects of pH and ionic strength on the distribution of mineral salts in milk.

As the protein content is very 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. Performances of BMEA carried out at four ionic strengths (μ_{added} = 0, 0.25, 0.5 and 1.0 M) and with three added salts ($CaCl_2$, NaCl and KCl) were compared in terms of electrodialysis cell parameters, percentage of proteins precipitated, protein

molecular profiles, energy consumption and protein profiles of isolates produced in each set of conditions.

MATERIAL AND METHODS

Material

The raw material used in this study was reconstituted milk (10% W/V) from low temperature spray-dried skim milk powder (Agropur, Granby, Canada). The averaged composition of the skim milk powder was the following (g/100g) : total protein, 33.9 ; whey protein, 7.4 ; fat, 0.6; carbohydrates, 53.5; ash, 8.2; moisture, 3.8.

Methods

a) Électroacidification 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 N HCl solution and a 20 g/L Na_2SO_4 solution. Each closed loop was connected to a separate external reservoir, allowing for continuous recirculation (Bazinet *et al.*, 1997b).

b) Protocol

Electroacidification was carried out in batch process using a constant current of 2.0 A. Electrolyte volumes of 6 litres were used for the Na_2SO_4 and HCl solutions while a 3 L volume was used for the milk solution. The electroacidification was stopped when all the casein was precipitated (pH_c). As the pH_c values vary 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 4x3 factorial array was set up; 4 ionic strengths (μ_{added} = 0, 0.25, 0.5 and 1.0 M) and three different added salt ($CaCl_2$, NaCl and KCl) were tested. Two replicates of each combination of factors were performed in this experiment.

During each treatment, 1.5 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 molecular weight profiles were determined from the freshly acidified 1.5 mL samples,. At the end of each run, about 2.5 L samples of the pH_c milk solution were taken. These samples were centrifuged for 10 minutes at 4°C, at 500 g (Centrifuge Model J2-21, rotor type JA-10, Beckman Instruments Inc., Palo Alto, CA), the precipitate was washed twice with double-distilled water. The pH of the isolate was readjusted to pH 6.6 with 1 N NaOH to produce sodium caseinate, before being lyophilized for 24 hours at room temperature (Model Freezone 4.5, Labconco, Kansas City, MI). The lyophilized isolates were stored at 4°C prior to analysis.

c) Analysis methods

Soluble protein concentration measurement: The protein concentration was measured using a FP-428 LECO apparatus (LECO Corporation, Saint Joseph, MI) in the conditions used by Bazinet *et al.* (1999c).

Protein profiles: The chromatographic analysis of the molecular profile of the lyophilized protein isolate and skim milk samples was performed by reverse-phase HPLC according to Jaubert and Martin (1992) and applying the same conditions as Bazinet *et al.* (1999c).

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

$$E = \int_{t_0 : pH_{6.6} \text{ or relative initial pH}}^{t_e : pH_e} I/60 \times U \, dt$$

where: U = voltage (volts)

I = current (amperes)

t = time (minutes)

E = energy (joules)

RESULTS

BMEA parameters

a) Duration

The duration of the BMEA was the same for all salts (all ionic strength averaged) at 89.7 ± 29.3 minutes (P>0.1910) (Table VI-1). In addition as the ionic strength (all salts averaged) increased, the duration of the electroacidification increased as well (P<0.0001): for μ_{added} increasing from 0 to 1.0, the duration increased from 46.4 to 119.6 minutes respectively. As confirmed by the regression contrast, the duration increased in a quadratic fashion (P<0.0001) : the duration increased by 48.6% between μ_{added} 0 and 0.25 M, by 11.8% between μ_{added} 0.25 and 0.5 M and finally by 14.3% between μ_{added} 0.5 and 1.0 M.

b) Conductivity

Added salt and the ionic strength influenced the initial conductivity values of the skim milk solution (ionic strength added P<0.0001, salt P<0.0001, double interaction salt/ionic strength added P<0.0001)(Figure VI-1). However, conductivity was stable all through out the process whatever the salt and the level of ionic strength added. Initial conductivity of the skim milk solution varied with the concentration and the type of added

Table VI-1 : Effect of ionic strength added and type of salt added on the time of electroacidification (minutes).

	μ_{added} (M)			
	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

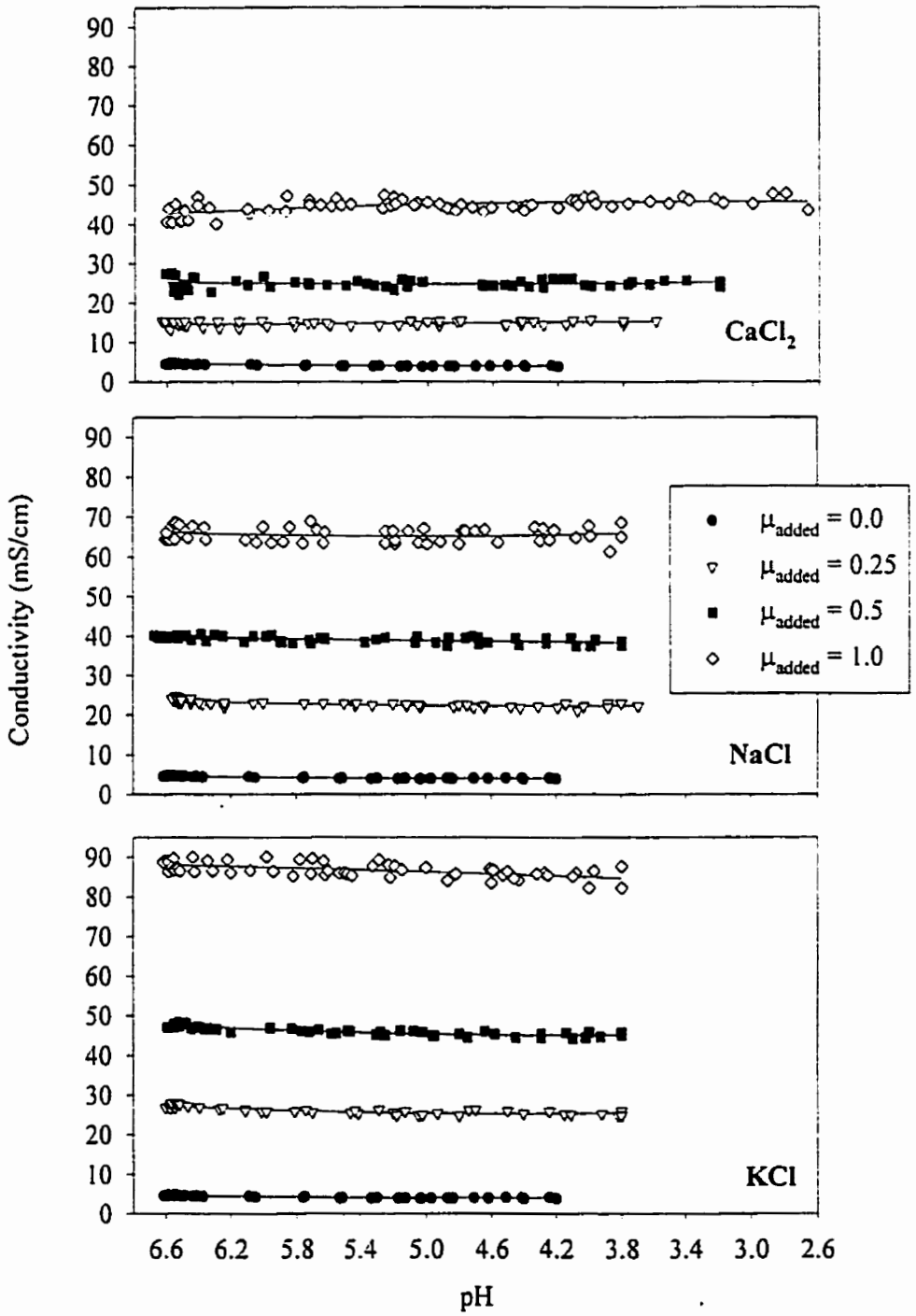

Figure VI-1 : Effect of added salts and ionic strength on conductivity of the milk solution during bipolar membrane electroacidification, run at a current density of 20 mA/cm^2.

salt : KCl from 4.7 to 87.8 mS/cm, NaCl from 4.7 to 65.4 mS/cm and $CaCl_2$ from 4.7 to 42.7 mS/cm.

c) 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 : 30.8, 18.0 and 17.4 volts for the $CaCl_2$, NaCl and KCl respectively. In addition, increasing the added ionic strength reduced the voltage variations between the beginning and the end of the BMEA in a quadratic fashion ($P<0.0002$) (Figure VI-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 finally from 17.3 to 8.3 volts between μ_{added} 0.5 and 1.0 M. The ionic strength added 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 $CaCl_2$, μ_{added}=0.5 M with a coefficient of 0.670. The evolution of soluble protein during BMEA of skim milk was different according to the salt added and the ionic strength level (Figure VI-3). The sigmoidal curve of the milk solution without salt added showed an inflection point at pH 4.94 which was the highest one in comparison with those of milk solutions with salt added. The inflection points of the curves obtained with addition of $CaCl_2$, NaCl and KCl (0.25, 0.5 and 1.0 M μ_{added} averaged) were pH 3.92, 4.18 and 4.33 respectively. Moreover, for each added salt, the soluble protein curves had different evolution according to the ionic strength added. The different evolution of soluble protein was confirmed by calculation of the inflection points and the width of transition of the solubility curves : for NaCl, the soluble protein curves were the same with inflection points at pH 4.21, 4.16 and 4.18 and width of transition of 0.08, 0.06 and 0.07 pH unit for μ_{added} of

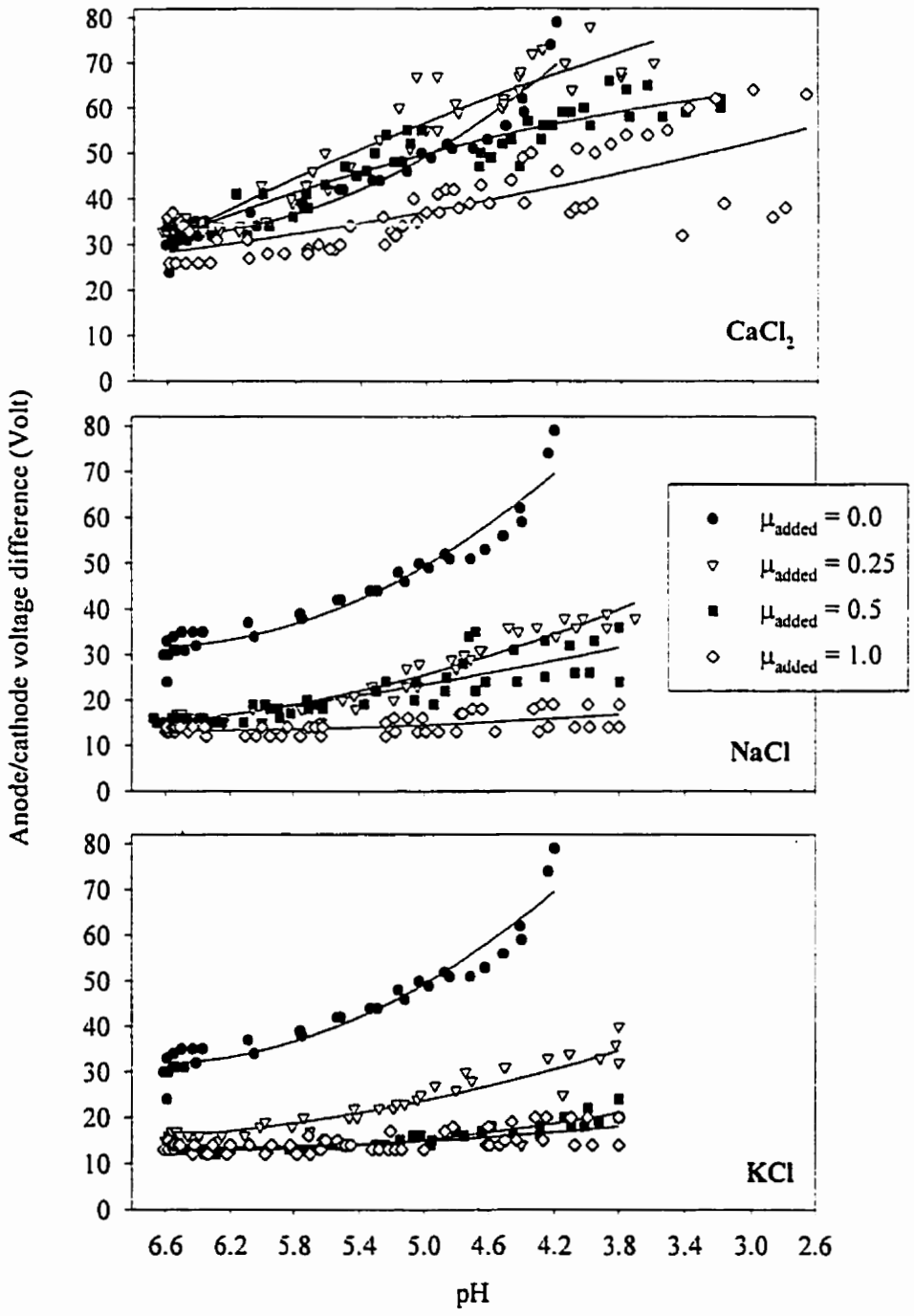

Figure VI-2 : Effect of added salts and ionic strength on anode cathode voltage difference during bipolar membrane electroacidification of skim milk, run at a current density of 20 mA/cm^2.

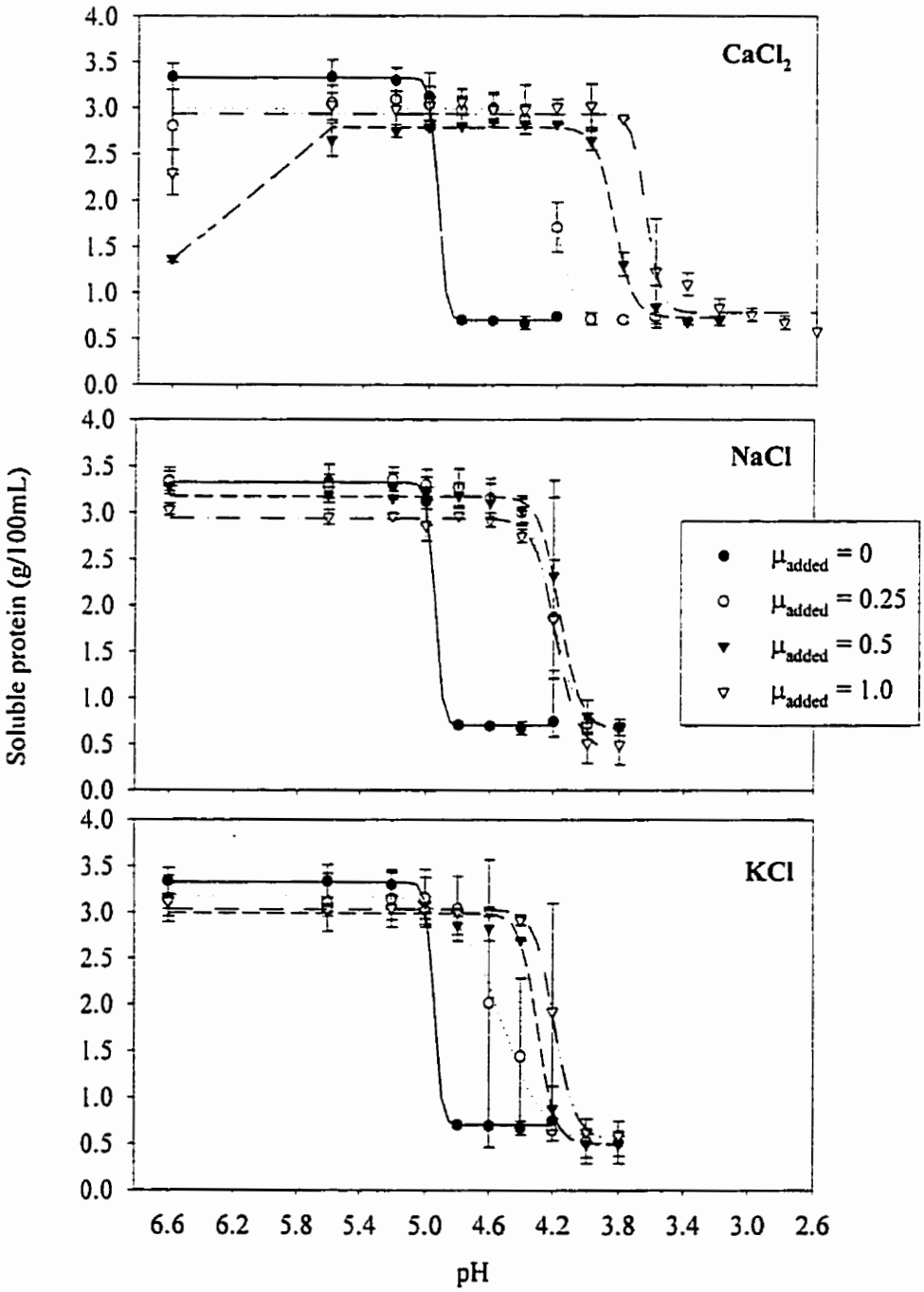

Figure VI-3 : Effect of added salts and ionic strength on soluble protein during bipolar membrane electroacidification of skim milk, run at a current density of 20 mA/cm^2.

0.25, 0.5 and 1.0 M respectively. In the case of $CaCl_2$, the delay in precipitation was increased with an increase in the ionic strength ; inflection points at pH 4.21, 3.92 and 3.65 and width of transition of 0.06, 0.004 and 0.04 pH unit for μ_{added} of 0.25, 0.5 and 1.0 M respectively. For the KCl, the delay in precipitation was increased with the increase in ionic strength, while the width of the transition of the solubility curve was decreased ; pH 4.51 and 0.14 pH unit, pH 4.29 and 0.05 pH unit, and pH 4.18 and 0.06 pH unit at μ_{added} of 0.25, 0.5 and 1.0 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 a μ_{added} = 0 and 0.5 M with addition of $CaCl_2$, and at μ_{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 VI-2 and 3). The precipitation of all casein fractions was delayed by increasing the ionic strength with $CaCl_2$ and KCl. Increasing the ionic strength with KCl did not affect the pH_c values; increasing the ionic strength with $CaCl_2$ resulted in lower pH_c values. In the case of NaCl addition, the percentage 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 higher 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 ± 0.5% and 46.1 ± 0.7% for α_s- and β-casein respectively (Table VI-4). However, the type of salt ($P<0.0151$ for the whey protein and $P<0.0057$ for κ-casein) and the added ionic strength

Table VI-2 : Effect of ionic strength added and type of salt added on the percentage of κ- and α_s-casein in the supernatant.

		μ_{added} (M)									
		No salt added	$CaCl_2$			NaCl			KCl		
		0	0.25	0.5	1.0	0.25	0.5	1.0	0.25	0.5	1.0
	pH										
	6.6	11.2 a*	9.5 a	4.5 bc	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 ab	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 ab	11.8 a	11.6 a	13.3 a	7.8 a	7.8 ab	12.2 a
	4.8	1.4 c	10.1 a	11.9 a	12.3 ab	11.0 a	10.6 a	10.7 a	7.7 a	8.1 a	11.7 a
	4.6	1.6 c	10.4 a	11.7 a	12.6 ab	11.0 a	9.9 ab	10.9 a	5.4 ab	7.9 ab	11.4 a
	4.4	1.3 c	9.0 a	11.6 a	13.2 ab	10.8 a	6.9 b	8.9 ab	3.6 bc	6.3 b	10.7 a
κ-cas	4.2	1.3 c	3.4 b	11.6 a	12.9 ab	6.2 b	3.0 c	5.7 bc	1.8 c	2.2 c	2.5 b
	4.0		1.7 c	10.6 a	12.5 ab	1.8 c	0.9 c	0.9 cd	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 bc	4.7 dc						
	3.4			1.6 c	1.8 de						
	3.2			1.1 c	1.6 de						
	3.0				0.7 e						
	2.8				0.4 e						
	2.6				0.1 e						
	pH										
	6.6	34.6 a	31.4 a	12.5 c	14.7 cd	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 ab	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 ab	27.3 ab	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 bc	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 ab	30.8 a	29.6 a	32.5 ab	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-cas	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 ab	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 ab	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 cde						
	3.4			0.3 d	4.1 de						
	3.2			0.7 d	2.0 e						
	3.0				1.2 e						
	2.8				0.1 e						
	2.6				0.0 e						

* Means within a column followed by different letters are significantly different ($P < 0.05$)

Table VI-3 : Effect of ionic strength added and type of salt added on the percentage of β-casein and whey protein in the supernatant.

		μ_{added} (M)									
		No salt added	$CaCl_2$			NaCl			KCl		
		0	0.25	0.5	1.0	0.25	0.5	1.0	0.25	0.5	1.0
	pH										
	6.6	38.5 a	34.5 ab	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 ab	42.9 a	42.1 a	40.0 a
	5.2	40.6 a	38.5 a	38.0 ab	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 ab	35.2 ab	43.2 a	39.7 a	38.8 ab	42.8 a	41.9 a	40.8 a
	4.8	0.1 c	36.3 ab	38.3 a	29.2 b	40.8 a	37.0 a	37.8 ab	39.9 a	38.8 a	38.5 ab
	4.6	0.0 c	36.1 ab	38.5 a	34.9 ab	38.3 a	33.2 a	36.8 ab	28.7 ab	36.3 a	37.9 ab
	4.4	0.0 c	29.3 b	37.6 ab	37.4 ab	35.8 a	21.1 b	33.8 b	16.7 b	30.5 a	35.1 b
β-cas	4.2	0.0 c	12.8 c	37.6 ab	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 ab	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 ab	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 dc						
	3.4			0.7 d	4.4 de						
	3.2			1.0 d	2.1 e						
	3.0				1.3 e						
	2.8				0.1 e						
	2.6				0.0 e						
	pH										
	6.6	13.7 ab	16.1 a	16.2 abc	16.9 abc	14.9 a	14.4 ab	15.1 a	15.2 ab	14.2 a	15.0 b
	5.6	14.1 ab	15.7 ab	16.3 abc	18.2 a	15.5 a	14.7 ab	14.6 a	15.7 a	14.2 a	15.1 b
	5.2	15.1 a	15.3 ab	16.1 abc	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 ab	15.8 ab	16.4 ab	17.7 ab	15.8 a	14.8 ab	14.6 a	16.4 a	14.6 a	16.7 a
	4.8	11.7 b	14.9 ab	16.3 abc	16.7 abc	15.4 a	14.6 ab	14.7 a	16.7 a	13.7 a	15.5 ab
	4.6	12.0 ab	15.4 ab	16.5 a	18.0 a	15.1 a	13.9 ab	14.2 ab	15.4 a	13.8 a	15.5 ab
	4.4	10.8 b	14.9 ab	15.7 abc	16.8 abc	15.2 a	13.0 b	14.0 ab	14.4 ab	14.0 a	14.8 b
Whey	4.2	10.9 b	13.6 b	15.6 abc	17.4 ab	14.2 a	12.5 bc	13.0 abc	13.0 bc	14.2 a	12.4 c
protein	4.0		11.4 c	15.0 abc	17.2 ab	12.1 b	10.1 cd	11.6 bc	12.9 bc	12.8 a	11.9 c
	3.8		9.4 d	13.9 abc	15.9 abc	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 abc	14.8 bcd						
	3.4			13.5 bc	13.9 cde						
	3.2			13.4 c	12.9 def						
	3.0				12.8 def						
	2.8				11.3 ef						
	2.6				10.9 f						

* Means within a column followed by different letters are significantly different ($P < 0.05$)

Table VI-4 : Effect of ionic strength added and type of salt added on the percentage of κ-casein, α_s-casein, β-casein and whey protein in the isolate produced by BMEA.

	μ_{added} (M)									
	No salt added	$CaCl_2$			NaCl			KCl		
	0	0.25	0.5	1.0	0.25	0.5	1.0	0.25	0.5	1.0
κ-cas	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-cas	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-cas	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

($P<0.0003$ 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.

For the effect of salt added (all μ_{added} averaged), the $CaCl_2$ increased the concentration of whey protein fraction and decreased the concentration of k-casein in comparison with NaCl and KCl which had similar values : about 1.8 and 11.6% for $CaCl_2$, 1.4 and 12.1% for NaCl, and 1.4 and 12.0% for KCl for whey protein and κ-casein fractions respectively.

For the effect of μ_{added} (all salts averaged), a 23.8% increase in concentration from 0 to 0.5 M μ_{added} and a 58.0% increase in concentration from 0.5 to 1.0 M μ_{added} were noted for whey protein fraction, while a 4.7% decrease from 0 to 0.25 M μ_{added} and a 6.4% increase in concentration from 0.25 to 1.0 M μ_{added} were obtained for κ-casein.

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 $CaCl_2$ (+188.3%) than for NaCl (+60.2%) and KCl (+38.5%). The concentration of κ-casein in the isolate produced by BMEA was similar whatever the ionic strength added for KCl (about 12.0 ± 0.1%), while for NaCl and $CaCl_2$ the concentration decreased from 0 to 0.25 M μ_{added} (-7.8 and -5.2% for $CaCl_2$ and NaCl respectively) and increased thereafter (+9.2 and +9.8% for $CaCl_2$ and NaCl respectively).

Energy efficiency of the process

The energy consumption (in kWh/kg of isolate) was first calculated with the time required for BMEA to decrease pH from 6.6, after readjustment of the pH following the addition of salt, to the pH_c value and thereafter with the relative time. The relative time is 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 salt, except for $CaCl_2$, resulted in a decrease in energy consumption (Table VI-5). For all μ_{added} averaged, 14.3 and 30.5 % decreases in energy consumption were obtained with addition of NaCl and KCl respecitvely, while a 182.9%

Table VI-5 : Effect of ionic strength added and type of salt added on the energy and relative energy consumption of electroacidification (kWh/kg of isolate).

		μ_{added} (M)			
		0	0.25	0.5	1.0
Energy	$CaCl_2$	0.720	2.112	2.358	1.642
	NaCl	0.720	0.687	0.561	0.603
	KCl	0.720	0.551	0.396	0.580
Relative Energy	$CaCl_2$	0.720	1.050	1.549	1.129
	NaCl	0.720	0.572	0.515	0.503
	KCl	0.720	0.551	0.396	0.554

increase was calculated with $CaCl_2$ addition. Moreover, according to the ionic strength and the salt added, the evolution of the energy consumption is different. With addition of NaCl and KCl, the energy consumption decreased by 4.6 and 23.5% respectively between μ_{added} = 0 and 0.25 M, thereafter the energy decreased by 18.3 and 28.1% respectively between μ_{added} = 0.25 and 0.5 M to finally increased by 7.4 and 46.5% between μ_{added} = 0.5 and 1.0 M. In the case of $CaCl_2$ addition, the energy consumption increase by 227.5% from 0 to 0.5 M and decreased drastically by 30.4% between μ_{added} = 0.5 and 1.0 M.

The values of energy consumption calculated with 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 μ_{added} = 0.25 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 30.9% from 0 to 1.0 M μ_{added} : the energy consumption decreased drastically between μ_{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 efficiency. In this experiment, althougth the conductivity of the skim milk solution was increased, the duration of the BMEA was increased, whatever the salt added. The conductivity was no longer the limiting factor. The limiting factor was the protein precipitation which is delayed by salting-in. Bazinet *et al.* (1997a), on BMEA of soybean protein, did not observed any difference in duration by increasing the salt concentration from 0.06 to 0.24 M of KCl. They showed that at 0.24 M of added KCl, the duration was the same as at 0.06 M KCl added, and that there is a slight delay, due to the salting-out effect, in the protein precipitation curve. However, results obtained for conductivity and anode/cathode voltage differences are in accordance with the data of Bazinet *et al.* (1997a) : the increase in added salt concentration increases the initial conductivity values and reduces the voltage rise at the end of the experiment.

Precipitation of protein during BMEA

For μ_{added} ranging from 0.25 to 1.0 M, except for the NaCl, the pH_c value, at which all the caseins are precipitated, decreased with an increase of the ionic strength by salting-in (Cheftel *et al.*, 1985; Kinsella *et al.*, 1985). In the case of NaCl addition, the different levels of μ_{added} had no effect on the soluble protein profile, however there is an effect of salting-in in comparison with soluble protein of skim milk without NaCl added. 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.* (1997a) on soybean 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 and 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 highest pH at which casein micelles coagulate decreased from 5.0 to 3.8 as $CaCl_2$ concentration was increased from 10 to 100mM (Bringe and Kinsella, 1987). 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 and 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 surround 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 pHi (acidic or basic) at 1% protein

concentration (w/v), but if the protein concentration was increased from 0.5 to 4% (w/v) at 0.5 M NaCl, the percentage of soluble proteins was strongly decreased.

Protein fraction 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.*, 2000b). The decrease in whey protein in the skim milk solution observed at the end of the process result likely of the co-precipitation of α-la and β-lg with κ-casein due to heating treatment prior to BMEA. During the drying treatment of milk, a complex of α-la and β-lg with κ-casein is formed (Cayot and Lorient, 1998). According to Iametti *et al.* (1993), the quantity of whey protein associated with caseins increases with the intensity of the thermal treatment. More precisely, during the thermal treatment, the β-lg forms a complex with the κ-casein via an exchange between sulfhydryl groups and disulfide bonds at the natural pH of the milk (Tessier and Rose, 1964; Sawyer, 1969; Singh and Fox, 1987; Cayot and Lorient, 1998). In addition, although the α-la does not form a complex with the κ-casein during thermal treatment, it forms a complex with the β-lg. Then the α-la could associate indirectly with the κ-casein. The thiol group of the β-lg could catalyze the formation of disulfide bonds between the α-la and the κ-casein (Kinsella, 1984; Cayot and Lorient, 1998). However, BMEA would not have such an effect.

The concentrations of the α_s- and β-casein fraction in the isolates were unchanged whatever the salt and the ionic strength added. Isolates produced by BMEA at 1.0 M μ_{added} were analyzed by LC/MS, and the whey protein fraction was identified as α-lactalbumin. $CaCl_2$ favoured the precipitation of whey protein fraction by BMEA. Mailliard and Ribadeau-Dumas (1988) demonstrated in their attempt to separate β-lg by salting-out from an acid whey retentate that between pH 3.0 and 4.0, at high salt concentrations (5% NaCl), the α-la 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 versus 5.2 $cm^2.s^{-1}.V^{-1}$). The better energy efficiency resulted from a decrease of the anode/cathode voltage difference, due to an increase of conductivity, coupled with the compensation, by addition of salt, of the lack in sufficiently mobile ions in the skim milk. This result was in accordance with previous results (Bazinet *et al.*, 2000b) suggesting the addition of salt in order to improve energy efficiency. However, in this study the amount of salt added, with addition of KCl at a μ_{added} = 0.5 M, 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.*,2000b). These results are not contradictory. The addition of 7-25 meq./L of K^+ to the skim milk solution was suggested in order to compensate for the lost of H^+. However, to improve the BMEA process, the results of Bazinet *et al.* (2000b) indicated adding 50 meq./L of K^+ and more. Furthermore, the kinetics of protein precipitation was influenced by the concentration of salt added in 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 to be generated.

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RÉSUMÉ (Deuxième article)

Une procédure développée pour la précipitation des protéines de soya et dérivée de l'électrodialyse a été employée pour la production de caséines acides à partir de lait écrémé reconstitué. L'objectif de cette étude était d'évaluer l'effet du type de sel ajouté ($CaCl_2$, NaCl et KCl) et de la force ionique de la solution de lait lait écrémé ($\mu_{ajoutée}$ = 0, 0.25, 0.5 and 1.0 M) sur la composition chimique et sur les propriétés physicochimiques et fonctionnelles des isolats produits par électroacidification. Ces isolats ont aussi été comparés avec trois isolats commerciaux et un isolat produit par acidification chimique.

Les isolats produits par électroacidification avec membranes bipolaires avec différents types de sels et à différents niveaux de force ioniques ajoutées, sauf avec $CaCl_2$ à $\mu_{ajoutée}$ de 1 M, montrent des propriétés physicochimiques et fonctionnelles similaires à celles de l'isolat produit chimiquement et des isolats commerciaux. Cependant, les différences observées entre les isolats au niveau de la capacité moussante et de l'activité émulsifiante seraient liées à une différence de composition minérale.

Mots clés : Acidification électrochimique, Membrane bipolaire, Caséine, Sels, Force ionique.

ABSTRACT

A procedure developed for soybean protein precipitation which was derived from electrodialysis was tested for the production of acid casein from reconstituted skim milk. The aim of this study was to evaluate the effect of type of added salt ($CaCl_2$, NaCl and KCl) and ionic strength of the skim milk solution (μ_{added} = 0, 0.25, 0.5 and 1.0 M) on the chemical composition and on the physicochemical and functional properties of electroacidified isolates produced. These isolates were compared with three commercial and one chemically-produced isolates.

The isolates produced by bipolar membrane electroacidification with different type of added salts and ionic strength, except at 1 M $CaCl_2$ μ_{added} showed physico-chemical and functional properties similar to the chemically-produced and commercial isolates, except for small differences in foaming properties and in emulsifying activity between isolates that might be the result of a different mineral content.

Key words : Electrochemical acidification, Bipolar membrane, casein, salt, ionic strength.

INTRODUCTION

Milk proteins account for about one-quarter of total dietary protein in the industrialized world and still cost less than proteins derived from eggs or meat (Hambraeus, 1982). In many cases, milk protein ingredients are promoted on the basis of their nutritional profiles, but their primary function is to impart specific functional properties to the finished product. Some dairy ingredients lack functionality as a consequence of the method of production. Relatively new fractionation and modification procedures have been developed to enhance the functionality of milk proteins (Modler and Jones, 1987).

Protein integrity can be affected during technological treatments. Changes in the surrounding media composition lead to modifications and to variation in protein functional properties (Cayot and Lorient, 1998). The functional properties are the macroscopic expression of the capacity of a protein to modify its conformation in response to an environmental change. Hence, an increase in ionic strength decreases the ionic interactions and consequently the solubility profile of the protein. Moreover, the presence of divalent ions, in high concentration, may lead to important aggregation during technological treatments with negative technological consequences (Cayot and Lorient, 1998).

Recently, bipolar membrane electroacidification (BMEA) has been applied to skim milk protein in order to produce acid casein isolates (Bazinet *et al.*, 1999c). Studies carried out on soybean have shown that the chemical composition of bipolar-membrane electrodialysed isolates was superior or equal to that of commercial standards, with functional properties comparable to these standards (Bazinet *et al.*, 1996; 1997c). Furthermore, in a previous study the addition of KCl in the milk solution at μ_{added} = 0.5 M allowed a 45% decrease in energy consumption (Bazinet *et al.*, 2000e).

The aim of this study was to evaluate the effect of increasing ionic strength of the skim milk solution (μ_{added} = 0, 0.25, 0.5 and 1.0 M) with different salts ($CaCl_2$, NaCl and KCl) during BMEA on the chemical composition, physico-chemical and functional

properties of BMEA isolates and to compare these isolates to commercial and chemically produced isolates.

MATERIAL AND METHODS

Material

Casein isolates (CAS) were produced by electroacidification. The electroacidifications described in a previous study were carried out under different conditions of added salt ($CaCl_2$, NaCl and KCl) and ionic strength of the skim milk solution (μ_{added} = 0, 0.25, 0.5 and 1.0 M) (Bazinet *et al.*, 2000e). The raw material used for BMEA was reconstituted milk (10% w/v) from low-temperature spray-dried skim milk powder (Agropur, Granby, Canada). Two replicates of each combination of added salts and ionic strength were performed in this experiment. At the end of each run, about 2.5 L samples of the electroacidified milk solution were collected. These samples were centrifuged for 10 minutes at 4°C, at 500 g (Centrifuge Model J2-21, rotor type JA-10, Beckman Instruments Inc., Palo Alto, CA), the precipitate was washed twice with double-distilled water, and the pH adjusted to 6.6 with 1N NaOH. The sodium caseinates (CAS) produced were lyophilised for 24 hours at room temperature (Model Freezone 4.5, Labconco, Kansas City, MI). The lyophilised CAS were stored at 4°C.

Three commercial sodium caseinates were purchased : New Zealand isolate from New Zealand Milk Products Inc. (Santa Rosa, CA), Sigma isolate from Sigma-Aldrich Canada Ltd (Oakville, ON), UFL isolate from Les Aliments UFL inc. (Montréal, QC).

A sodium caseinate was produced by chemical acidification with HCl (1.000 N, VWR Canlab, Ville Mont-Royal, QC) at the laboratory and prepared under the same conditions as the electrochemical CAS.

Methods

a) Protocol

Chemical analyses (protein, ash and lactose contents; moisture; sodium, calcium, magnesium and potassium concentrations) and physico-chemical (particle size and specific viscosity) and functional properties (foaming capacity and stability, emulsion stability, protein load, interfacial area, and solubility as a function of pH) were performed on each BMEA CAS in order to study the effect of type of added salt and ionic strength. BMEA CAS properties were also compared with commercial and chemically produced CAS. In addition, for each CAS, 250 mL of 5% (w/v) protein solution were dialysed (1/100) 18 hours against 0.01 M sodium phosphate buffer (pH 6.8), in order to reduce the influence of added salts on CAS functional properties. Chemical analyses, and physico-chemical and functional properties of each dialysed CAS were then repeated.

b) Analysis Methods

Protein content: protein content was determined, by FP-428 LECO apparatus (LECO Corporation, Saint-Joseph, MI) according to the conditions and parameters used by Bazinet *et al.* (1999c), on each of the BMEA, commercial and chemically produced CAS isolate powders.

Lactose concentration : 5% (w/v) protein solutions were acidified with 1.0 N hydrochloric acid to a pH below the isoelectric point. Samples were then centrifuged 15 minutes at 2000 g and 20°C in a Beckman GS-6 centrifuge (Beckman Instruments Inc, Mississauga, Ontario). 15µl of the 0.45 µm filtrated supernatant were injected on an Ion-300 column (Mandel Scientific CO., Rockwood, Ontario, Canada) connected to a HPLC (Waters Associates, Milford, MA) equipped with a UV detector (210 nm) (Model 490, Waters), and a refractive index detector (Model R410, Waters) according to the method of Doyon *et al* (1991). A 0.0054 N H_2SO_4 solution was used as the mobile phase at a flow rate

of 0.4 mL/minute. The concentration of lactose was determined using a commercial D-lactose solution (Sigma Chemical Co., St-Louis, MO) of known concentration.

Moisture : Water content was measured according to the AOAC method no.927-05 (AOAC International, 1995a).

Ash Content : Ash content was determined according to AOAC methods no.930-30 and 945-46 (AOAC International, 1995b,c).

Potassium, sodium, magnesium and calcium concentration measurements : Sodium, potassium, magnesium and calcium concentrations were determined by inductively coupled plasma (ICP, Optima 3300, dual view, Perkin-Elmer, Norwalk, CT). The wavelengths used to determine sodium, calcium, magnesium and potassium concentrations were 589.59, 422.67, 285.21 and 766.49 nm respectively (Bazinet *et al.*, 2000c). The analyses were carried out in radial view. Samples were prepared from known weight skim milk solution ash dissolved in 10 mL HCl (2N) and diluted with HCl (2N) to be within the calibration ranges for each cation.

Specific viscosity : 10 mL of 4% (w/v) protein solution was introduced into a calibrated viscometer size 100 (Cannon-Fenske RoutineViscosimeter, Cannon Instrument, VWR, Ville-Mont-Royal, Québec, Canada) placed at 25°C in a thermostated water bath. The time needed for the solution to flow through the thin capillary was measured precisely and divided by the time needed for double-distilled water to flow under the same conditions in order to give the relative viscosity (η_r)of the protein solution. The analysis was repeated 5 times for each solution. Specific viscosity was calculated from relative viscosity and protein concentration according to the following equation :

$$\eta_{sp} = \frac{\eta_r - 1}{[Prot]}$$

where η_{sp} is the specific viscosity (mL/g), η the relative viscosity of the protein solution and [Prot] the protein concentration of the solution (g/mL).

Interfacial Area (IA) of the Emulsions : 33% (v/v) oil emulsions were produced by mixing commercial corn oil (Mazola) and 4% (w/v) protein solution with a Polytron (model PT 10-35, probe PTA 10S, Kinematica AG, Littau, Switzerland) for 30 seconds at 9000 rpm, and homogenized at a pressure of 10000 Psi with an Emulsiflex-C5 homogeneisator (Avestin, Ottawa, ON). Interfacial area (IA) of the emulsions was calculated from the turbidity of diluted emulsions (Pearce and Kinsella, 1978) : emulsions were diluted to a final oil volume fraction of 6×10^{-5} in sodium phosphate buffer (0.01M, pH 7.0) containing 0.5% sodium dodecyl sulphate (SDS, Biorad Laboratories Canada Ltd, Mississauga, Ontario) according to Britten and Giroux (1993) ; optical density was measured in duplicate at 500 nm with a Beckman DU-640 spectrophotometer (Beckman Instruments Inc, Mississauga, Ontario). Calculations were performed according to the method of Cameron *et al.* (1991). Emulsion stability was measured by determining the IA of the emulsion stored 6 weeks at 4°C.

Protein Load of the Emulsions : Protein load was calculated from protein depletion in the serum phase after emulsion formation according to Britten and Giroux (1993). Serum phase was separated from the emulsion by centrifugation (25000g for 1 hour at 4°C) using a Beckman centrifuge (Model J2-21, rotor type JA 20-1). Protein was determined in the aqueous phase before and after emulsion formation using the Bradford's method (1976) calibrated with a bovine serum albumin (BSA) standard (Biorad Laboratories Canada LTD, Mississauga, ONT). Protein load results were expressed as mg/m^2. Protein concentration depletion in the aqueous phase was divided by the IA of the emulsion.

Foaming Properties : Foaming properties were measured according to Waniska and Kinsella (1979). 15 mL of 0.5% (w/v) protein solution was used. The solution in the column was sparged with nitrogen gas at a constant flow rate of 19 mL/min until foam volume reached 70 mL. Protein solution was added as required to maintain the volume constant at 15 mL. Time required to reach 55 mL of foam, and the volume of protein

solution added were recorded. At the end of the sparging, the volume of liquid drained from the foam after 2 minutes was measured. The analysis were done at room temperature and repeated 5 times for each solution.

Solubility profile : 0.2 N hydrochloric acid was added gradually to 250 ml of 2% (w/v) protein solution. 1.5 ml aliquots were taken at pH 6.6; 5.8; 5.4; 5.2; 5.0; 4.8; 4.6; 4.4; 4.2; 4.0 and centrifuged at 500g for 10 minutes at 4°C. Protein concentration was measured in the supernatant using the Bradford's method (1976). Non-linear regression equations were calculated according to Bazinet *et al.* (2000c) :

$$S_p = b + \frac{a}{1 + \exp\left[-\left(\frac{pH_x - c}{w}\right)\right]}$$

S_p is the percentage of soluble protein, pH_x the pH value ranging from pH 6.6 to 4.0, a the amplitude of the curve, b the percentage of soluble protein at the isoelectric point, c the center or point of inflection and w the width of the transition region of the sigmoidal curve.

Particle size : The particle size was determined, in duplicate on 0.5 to 1.0 mL of 0.5% (w/v) protein solution in 2 mL double distilled water, with a sub micron particle analyzer at 22°C (Beckman Coulter, Model N4MD, Miami, FL). Results are expressed in terms of mean diameter (nm).

c) Statistical Analyses

Using SAS software (SAS inc., 1989) the data from the compositional, physicochemical and functional analyses of isolates produced by BMEA were submitted to an analysis of variance with regression contrasts to examine the effect of interaction between the variables. The data from the compositional, physicochemical and functional analyses, except for solubility as a function of pH, for BMEA, commercial and chemical isolates were subjected to an analysis of variance, with a comparison of averages by

Duncan tests, in order to distinguish different groups between the samples. Non-linear regression equations were calculated for the data from the solubility as a function of pH using Sigmaplot (Version 3.0 for Windows, Jandel Scientific, Corte Madera, CA), and the model curve parameters were compared.

RESULTS AND DISCUSSION

Effect of added salts and ionic strength

a) Chemical composition

The analyses of variance showed a significant effect of ionic strength ($P<0.0001$, $P<0.0015$, $P<0.0005$, $P<0.0003$ and $P<0.0001$ for ash, lactose, protein, calcium and magnesium respectively) and type of added salts ($P<0.0285$, $P<0.0001$ and $P<0.0001$ for lactose, calcium and magnesium) on the lactose, ash, protein and mineral contents of the isolates produced by BMEA. The regression contrasts results demonstrated the significant double effects of the dialysis and ionic strength ($P<0.0001$, $P<0.0122$, $P<0.0001$ and $P<0.0004$ for ash, lactose, magnesium and calcium content respectively), dialysis and salts ($P<0.0001$ for magnesium and calcium), dialysis and salts ($P<0.0001$ for calcium) and the triple interaction of dialysis and added salts and ionic strength ($P<0.0159$ for calcium).

Protein : Protein content of the isolates decreased with an increase in ionic strength (Table VI-6). All added salts data averaged, the protein contents were 86.9, 80.9, 79.9 and 73.0 g/ 100g dry isolate at 0, 0.25, 0.5 and 1 M μ_{added}. After dialysis, protein content was adjust to 4% (w/v) so that lactose, ash and mineral content results were expressed as g/100g of protein to allow comparison.

Lactose : Lactose content was influenced by the type of the added salts (Table VI-6) : lactose contents (all dialysis and ionic strength data averaged) of CAS produced with addition of KCl and NaCl were similar with 5.51 and 5.72 g/100 g protein respectively, but different from CAS produced with $CaCl_2$ with 8.01 g/100 g protein.

Table VI-6 : Protein, ash and lactose content of BMEA, commercial and chemically produced isolates.

		Protein (% dry basis)	Ash (g/100 g protein)		Lactose (g/100 g protein)	
		not dialysed	not dialysed	dialysed	not dialysed	dialysed
	0.00 M	86.8 ab*	5.3 cd	0.056 c	6.8 bc	0.9 ab
	0.25 M	80.8 bc	10.2 bcd	0.057 bc	9.4 abc	1.2 ab
$CaCl_2$	**0.50 M**	78.5 bc	11.3 bcd	0.065 b	14.4 ab	1.3 ab
	1.00 M	68.0 d	23.7 a	0.090 a	19.7 a	2.1 a
	0.25 M	82.2 bc	8.3 cd	0.053 cd	10.0 abc	1.2 ab
NaCl	**0.50 M**	81.1 bc	10.8 bcd	0.054 cd	10.7 abc	1.2 ab
	1.00 M	76.0 bcd	16.1 abc	0.053 cd	10.0 abc	1.2 ab
	0.25 M	79.4 bc	9.1 cd	0.050 cde	10.6 abc	1.1 ab
KCl	**0.50 M**	80.0 bc	9.7 bcd	0.052 cde	7.9 abc	0.9 ab
	1.00 M	74.8 cd	20.4 ab	0.057 cb	10.9 abc	1.8 a
Chemical		83.2 bc	5.7 cd	0.052 cde	11.6 abc	1.1 ab
New Zealand		96.3 a	3.9 d	0.053 cd	0.0 c	0.1 b
Sigma		97.0 a	3.8 d	0.043 e	0.2 c	0.0 b
UFL		94.1 a	4.6 d	0.046 de	0.6 c	0.2 b

* Means within a column followed by different letters are significantly different ($P < 0.05$)

Ash :. Ash content increased with an increase in ionic strength (Table VI-6): all dialysis and added salts data averaged, the ash content were 2.66, 4.63, 5.32 and 10.07 g/100 g protein for 0, 0.25, 0.5 and 1 M respectively.

Sodium, potassium, calcium and magnesium concentrations :. For calcium, its final concentration in isolate always depended on the ionic strength and type of added salts (Table VI-7): as the ionic strength increased from 0 to 1 M (all data of added salts averaged), the final concentration of calcium increased by 5.7 times, but its concentration in the isolates was higher with addition of $CaCl_2$ (7.9 mg/100 g protein) than with NaCl (1.6 mg/100 g protein) and KCl (1.5 mg/100 g protein), all data of added ionic strength.

b) Physico-chemical and functional properties

Results of analyses of variance performed on emulsion interfacial area ($P>0.0683$), foaming capacity (min.) ($P>0.5489$), foaming stability ($P>0.3754$), protein load ($P>0.4929$) and emulsion stability ($P>0.8627$) showed no significant effect of dialysis, added salts and ionic strength. The analyses of variance showed a significant effect of dialysis ($P<0.0001$ for viscosity, foaming capacity (mL) and solubility, and $P<0.04$ for particle size), of ionic strength ($P<0.0001$, $P<0.0011$ and $P<0.0008$ for viscosity, particle size, solubility and foaming capacity (mL) respectively) and of added salts ($P<0.025$ and $P<0.0311$ for viscosity and foaming capacity (mL)) on some functional properties of the isolates produced by BMEA. The regression contrasts results demonstrated the significant double interaction between dialysis and ionic strength ($P<0.0001$ and $P<0.0008$ for viscosity and foaming capacity (mL)), added salts and ionic strength ($P<0.0006$, $P< 0.002$, $P<0.0001$ and $P<0.0003$ for viscosity, particle size, solubility and foaming capacity (mL) respectively) and dialysis and added salts ($P<0.0075$ for foaming capacity (mL)).

Particle size :. The particle size decreased from 291.2 to 234.6 for μ_{added} increasing from 0 to 0.5 M and thereafter increased to 263.0 at μ_{adedd} = 1 M (Table VI-8). More precisely (all dialysis data averaged), for the KCl and NaCl the particle size decreased in a similar manner with an increase in ionic strength from 0 to 1 M ; from 291.2 to 248.4 nm

Table VI-7 : Sodium, potassium, magnesium and calcium concentrations of BMEA, commercial and chemically produced isolates.

		Sodium (mg/100 g protein)		Potassium (mg/100 g protein)		Magnesium (mg/100g protein)		Calcium (mg/100g protein)	
		not dialysed	dialysed	not dialysed	dialysed	not dialysed	dialysed	not dialysed	dialysed
	0.00 M	1729 a*	15.7 a	53 b	0.54 a	8.9 bc	< dl** a	162 cd	1.3 c
	0.25 M	3139 a	11.8 ab	125 b	0.61 a	9.2 bc	< dl a	562 c	3.6 bc
$CaCl_2$	**0.50 M**	2544 a	12.3 ab	235 b	0.57 a	15.4 abc	0.06 a	1037 b	7.3 b
	1.00 M	3773 a	10.9 ab	625 b	0.73 a	30.3 a	< dl a	4972 a	19.5 a
	0.25 M	2649 a	12.5 ab	99 b	0.54 a	13.8 abc	< dl a	273 cd	1.7 c
NaCl	**0.50 M**	3061 a	14.9 ab	166 b	0.51 a	14.3 abc	0.02 a	293 cd	1.7 c
	1.00 M	5077 a	13.9 ab	279 b	0.89 a	16.5 abc	0.03 a	254 cd	1.9 c
	0.25 M	2299 a	13.8 ab	511 b	0.96 a	18.8 ab	< dl a	293 cd	1.6 c
KCl	**0.50 M**	1930 a	14.3 ab	712 b	0.70 a	17.3 abc	< dl a	274 cd	1.4 c
	1.00 M	2409 a	13.9 ab	4980 a	0.96 a	17.7 abc	< dl a	252 cd	1.7 c
Chemical		1441 a	13.5 ab	87 b	0.84 a	20.2 ab	0.06 a	425 cd	1.7 c
New Zealand		1112 a	15.2 ab	48 b	0.64 a	1.3 c	< dl a	20 d	1.1 c
Sigma		1054 a	11.2 ab	27 b	0.51 a	1.4 c	< dl a	51 d	0.7 c
UFL		1275 a	10.6 b	42 b	1.17 a	7.0 bc	< dl a	198 cd	0.5 c

* Means within a column followed by different letters are significantly different ($P < 0.05$).

** dl = detection limit

Table VI-8 : Viscosity and particle size of BMEA, commercial and chemically produced isolates

		Particle size (nm)		Specific viscosity (mL/g of protein)	
		not dialysed	dialysed	not dialysed	dialysed
	0.00 M	291.5 a*	291.0 ab	56.98 a	60.62 b
	0.25 M	249.0 b	259.0 abcd	37.88 cd	53.60 b
$CaCl_2$	**0.50 M**	158.8 d	185.0 d	25.91 e	48.88 b
	1.00 M	240.2 b	341.5 a	37.17 cd	79.43 a
	0.25 M	268.0 ab	278.0 abc	45.63 bc	56.84 b
NaCl	**0.50 M**	256.5 ab	269.2 abc	34.03 de	57.69 b
	1.00 M	237.5 b	262.0 abcd	34.82 de	51.20 b
	0.25 M	271.8 ab	277.5 abc	42.47 cd	57.14 b
KCl	**0.50 M**	258.0 ab	280.0 abc	38.59 cd	58.48 b
	1.00 M	242.5 b	254.2 bcd	32.88 de	52.69 b
Chemical		268.5 ab	285.8 ab	47.59 abc	57.46 b
New Zealand		267.5 ab	270.5 abc	56.57 a	55.11 b
Sigma		197.8 c	198.2 cd	55.04 ab	55.73 b
UFL		292.0 a	286.2 ab	53.35 ab	63.26 ab

* Means within a column followed by different letters are significantly different ($P < 0.05$)

(-14.7%) and 291.2 to 249.7 nm(-14.2) respectively. With an addition of $CaCl_2$, the particle size decreased from 291.2 to 171.9 nm (-41.0%) between 0 and 0.5 M μ_{added} and increased back to 290.8 nm at 1M m_{added}. Dialysis increased the particle size of the isolates from 247.4 to 269.7 nm (all data of added salts and ionic strength averaged). The calcium has therefore a major effect on the particle size.

Specific viscosity : All data of type of added salts and ionic strength averaged, dialysis increased the viscosity of the protein solution from 38.6 to 55.6 mL/g of protein (Table VI-8). The averaged viscosity was the same for the isolates electroacidified with addition of KCl and NaCl with respective values of 47.0 and 46.7 mL/g of protein, while the averaged viscosity for isolates produced with addition of $CaCl_2$ was lower at 43.7 mL/g of protein. More precisely, the viscosity was influenced by the type of added salt and the ionic strength ; the viscosity of isolates electroacidified with addition of NaCl and KCl decreased similarly by 26.9 and 27.2% respectively with an increase in ionic strength from 0 to 1 M, while the viscosity for isolates produced with addition of $CaCl_2$ decreased by 36.4% with an increase in ionic strength from 0 to 0.5 M, and increased by 28.2% between 0.5 and 1 M μ_{added}. In addition, although the viscosity values were increased by dialysis, the dialysis stabilized the viscosity obtained at the different ionic strength values (38.7 vs 10.6% variation between 0 and 1 M μ_{added}).

Solubility : Dialysis changed the solubility curve by precipitating proteins at higher pH values : in fact the inflection points of all the model curves were increased (Table VI-9). The addition of KCl and NaCl during BMEA treatment influenced the solubility of the protein solution in a similar way whatever the ionic strength. All dialysis and ionic strength data averaged, the curve parameters were the same (Table VI-9) for both added salts ; soluble protein at isoelectric point, amplitude, inflection point and width of the curves were 0.314 vs 0.250, 16.8 vs 16.5, 5.15 vs 5.14 and 0.101 vs 0.108 respectively for NaCl and KCl. With an addition of $CaCl_2$, the solubility profiles were different according to the ionic strength ; for ionic strength ranging from 0 to 0.5 M, the curves were similar among each others with curve parameters comparable to that of monovalent salts (Table VI-9). However the solubility profile at 1 M μ_{added} was different ; soluble protein at isoelectric

Table VI-9 : Model curve parameters of solubility as a function of pH of BMEA, commercial and chemically produced isolates

		not dialysed					dialysed				
		soluble protein at pH_i	amplitude	Center	Width	R^2	soluble protein at pH_i	amplitude	Center	Width	R^2
$CaCl_2$	**0.00 M**	0.033	14.9	5.04	0.101	0.995	0.181	17.7	5.18	0.102	0.997
	0.25 M	0.168	16.3	4.98	0.058	0.997	0.213	16.8	5.12	0.095	0.999
	0.50 M	0.239	16.8	5.06	0.087	0.999	0.289	17.3	5.10	0.094	0.998
	1.00 M	0.790	6.3	4.99	0.010	0.962	0.905	11.5	5.21	0.013	0.982
NaCl	**All ionic strength averaged**	0.198	14.7	4.97	0.078	0.985	0.314	16.8	5.15	0.101	0.994
KCl	**All ionic strength averaged**	0.140	15.2	4.98	0.075	0.977	0.250	16.5	5.14	0.108	0.986
Chemical		0.112	14.8	5.01	0.073	0.996	0.104	15.4	5.05	0.148	0.960
New Zealand		0.116	18.7	5.16	0.118	0.997	0.690	17.0	5.19	0.013	0.991
Sigma		-0.019	18.1	5.10	0.121	0.996	0.159	18.0	5.16	0.083	0.998
UFL		-0.115	17.6	5.06	0.110	0.993	0.149	17.1	5.18	0.099	0.999

point, amplitude, inflection point and width of the curves were 0.227 vs 0.905, 17.3 vs 11.5, 5.13 vs 5.21 and 0.097 vs 0.013 respectively for averaged μ_{added} parameters from 0 to 0.5 M and 1 M. Calcium at high concentration has a major effect on the solubility profile of protein.

Foaming properties : Foaming capacity, expressed in minute to reach the top of the column, was the same whatever the type of added salt and ionic strength. Dialysis did not change these values (Table VI-10). Foam stability was also not influenced by dialysis. However, the foaming capacity, expressed in mL of protein solution added to maintain the foam level at the top of the column, was influenced by dialysis, salt and ionic strength (Table VI-10). Dialysis decreased the quantity of protein solution to be added from 15.0 to 13.1 mL. The quantity of protein solution to be added was different according to the type of salt added during BMEA treatment ; the quantity was the same for NaCl and KCl with respective values of 14.5 and 14.2 mL, while with the addition of $CaCl_2$, the quantity was lower than that of both monovalent salts with 13.6 mL to be added. More precisely, ionic strength acted in concert with the type of added salt to influence the quantity of protein solution to be added. For NaCl and KCl, whatever the ionic strength the quantity of solution was the same with 14.3 and 14.1 mL respectively. For the addition of $CaCl_2$ the quantity was similar as for both monovalent salts, with 14.4 mL to be added for μ_{added} ranging from 0 to 0.5 M, while at μ_{added} of 1 M the quantity to be added decreased to 11.5 mL. In addition, whatever the ionic strength, the dialysis has a more important effect on the quantity of protein solution to be added for the isolates prepared from electroacidification with addition of KCl (-19.2%) and NaCl (-16.3%) than for $CaCl_2$ (-6.0%).

Emulsifying properties : Emulsifying properties of the electroacidified isolates in different conditions of added salt and ionic strength were unchanged (Table VI-11). Averaged protein load was 19.0 mg/m^2, the averaged interfacial area was 0.63 m^2/mL of emulsion and averaged emulsion stability was 0.9 m^2/mL of emulsion.

Table VI-10 : Foaming properties of BMEA, commercial and chemically produced isolates.

		Foaming capacity (min.)		Foaming capacity (mL)		Foam stability (mL)	
		not dialysed	dialysed	not dialysed	dialysed	not dialysed	dialysed
	0.00 M	4.94 a*	3.24 a	13.94 b	13.65 a	3.60 ab	4.03 a
	0.25 M	3.08 b	3.80 a	16.18 a	13.55 a	4.80 a	3.66 ab
$CaCl_2$	**0.50 M**	3.39 ab	3.60 a	14.82 ab	14.15 a	4.09 ab	3.89 a
	1.00 M	3.70 ab	3.22 a	11.08 c	11.85 a	3.33 b	3.60 ab
	0.25 M	3.80 ab	3.67 a	15.89 a	13.10 a	4.04 ab	3.86 a
NaCl	**0.50 M**	3.10 b	3.65 a	15.36 a	13.80 a	4.51 ab	3.97 a
	1.00 M	4.97 a	3.64 a	16.13 a	12.75 a	3.16 b	3.84 a
	0.25 M	3.60 ab	3.54 a	16.21 a	12.05 a	4.27 ab	3.64 ab
KCl	**0.50 M**	4.41 ab	3.28 a	15.39 a	13.30 a	3.70 ab	4.09 a
	1.00 M	2.99 b	3.58 a	15.49 a	12.70 a	4.42 ab	3.89 a
Chemical		3.95 ab	3.27 a	13.73 b	12.30 a	4.02 ab	3.72 ab
New Zealand		3.60 ab	3.47 a	11.24 c	9.97 ab	3.68 ab	3.25 ab
Sigma		3.28 ab	3.71 a	11.82 c	10.14 ab	3.82 ab	3.17 ab
UFL		3.59 ab	3.62 a	11.30 c	6.45 b	3.81 ab	2.53 b

* Means within a column followed by different letters are significantly different ($P < 0.05$)

Table VI-11 : Emulsifying properties of BMEA, commercial and chemically produced isolates

		Interfacial area (m^2/ mL emulsion)		Protein load (mg/m^2)		Emulsion stability (m^2/mL emulsion)	
		not dialysed	dialysed	not dialysed	dialysed	not dialysed	dialysed
	0.00 M	0.65 bcd*	0.57 ab	28.2 a	17.4 ab	1.17 a	1.00 a
	0.25 M	0.73 ab	0.59 ab	27.6 a	14.5 bc	0.58 a	1.02 a
$CaCl_2$	**0.50 M**	0.51 e	0.60 ab	22.3 a	18.7 ab	0.57 a	1.09 a
	1.00 M	N/A	0.64 ab	N/A	24.8 a	N/A	
	0.25 M	0.65 bcd	0.63 ab	21.6 a	14.3 bc	0.93 a	1.10 a
NaCl	**0.50 M**	0.61 bcde	0.64 ab	21.0 a	13.7 bc	0.72 a	1.12 a
	1.00 M	0.68 abc	0.62 ab	28.3 a	16.9 ab	0.74 a	1.11 a
	0.25 M	0.69 abc	0.55 ab	21.0 a	6.9 c	0.98 a	1.18 a
KCl	**0.50 M**	0.67 abc	0.62 ab	13.9 a	15.6 b	0.79 a	1.08 a
	1.00 M	0.59 cde	0.71 a	20.9 a	14.2 bc	1.03 a	0.94 a
Chemical		0.79 a	0.62 ab	23.9 a	12.5 bc	0.47 a	1.22 a
New Zealand		0.58 cde	0.63 ab	9.0 a	15.5 b	0.59 a	1.12 a
Sigma		0.59 cde	0.46 b	8.9 a	18.5 ab	0.70 a	1.05 a
UFL		0.53 de	0.63 ab	14.7 a	10.7 bc	0.41 a	1.00 a

* Means within a column followed by different letters are significantly different ($P < 0.05$)

It appears that the chemical composition of the isolates produced by BMEA varied according mainly to the ionic strength of the skim milk solution treated. At high concentration of added salt, whatever the salt, there was a retention of salts and lactose in the isolates decreasing the total protein content of these isolates. As the isolates were washed in the same conditions whatever the ionic strength, these washing conditions in the case of high salt concentration isolates were not sufficient to allow diffusion of most of the salts and lactose from the coagulum. Moreover, the concentration of lactose in the isolates increased with the ionic strength ; ionic strength might influence porosity and particle size of coagulum which affect contact with washing solution, and effectiveness of lactose removal. Many authors have pointed out that processing variables such as heat treatment, number of washes to which the curd is subjected and the content of iron influence the quality of the rennet casein (Munro *et al.*, 1980; McDowell *et al.*, 1976; Weal and Southward, 1974). Moreover, Munro *et al.* (1980) noted that poorly washed casein contains a very high lactose content. Zadow (1971) reported the results of a theoritical study of factors that affect the efficiency of casein washing. He found that for a 99% efficency of lactose removal, during subsequent washing, the lactose content of the wet curd increased from 0.12 to 0.80% while at 98% efficiency, the lactose content increased from 0.24 to 1.60% over the same range of whey removal.

The type of salt and the ionic strength influenced some functional properties and the structure of the sodium caseinate produced by BMEA. The KCl and NaCl addition during BMEA, from 0 to 1 M μ_{added} decreased the viscosity of the protein solution but the foaming and emulsifying properties and also the particle size and the solubility profile were unchanged. The $CaCl_2$ acted in a different way from both previous monovalent salts. It decreased the particle size and the viscosity from 0 to 0.5 M μ_{added}, and for 0.5 to 1 M μ_{added} increased back both factors. The solubility profile of the isolate produced with addition of $CaCl_2$, from μ_{added} 0 to 0.5 M was similar to those from both monovalent salts, while at μ_{added} of 1 M the solubility was decreased. By addition of $CaCl_2$, whatever the ionic strength, the emulsifying and foaming properties were unchanged except for the foaming capacity which was decreased.

These results agree with data in the literature. As the isolate produced by BMEA with addition of 1 M $CaCl_2$, showed a high calcium content after dialysis, this caseinate could be comparable to a Ca/Na caseinate. This isolate presented the functional and physicochemical properties of a Ca/Na mixed caseinate. It is frequently recognized that Ca or mixed caseinates form colloidal dispersions in water rather than solutions, with a lower solubility than that of Na and K caseinates (Modler, 1985). Na and K caseinates are supposed to be completely soluble at pH> 5.5 (Kinsella *et al.*, 1989). Mulvihill and Fox (1989) reported that Ca caseinate exists in water as large aggregate (artificial casein micelles) that are stable at pH >5.5, if the calcium concentration is not excessive. The dialysis of isolates produced with calcium allowed the production of Na caseinate except in the case of 1 M μ_{added}, which explain that isolates produced with addition of 0.25 and 0.5 M $CaCl_2$ μ_{added} had similar physico-chemical and functional properties than isolates produced with addition of NaCl and KCl. In addition, Bastier *et al.* (1993) showed that the calcium content was negatively correlated to the solubility index, rate of water absorption and apparent viscosity. However, the difference in solubility at the isoelectric point observed for the isolate produced by addition of 1 M $CaCl_2$ during BMEA could be related to the higher whey protein content measured by Bazinet *et al.* (2000e) in this isolate in comparison with the others. Moreover, the particle size of mixed caseinate was demonstrated to be negatively correlated to the water absorption capacity (Bastier *et al.*, 1993). Water absorption was governed mainly by the amount and type of cation bound to the protein constituents. In aqueous saline solution (free of protein), Ca ions have a higher primary hydration numbers than Na ions, but within caseinates, Ca ions would be expected to absorb less water due to their chelating and cross-linking effects (Rüegg and Morr, 1984; Bastier *et al.*, 1993).

In such conditions the dialysis of BMEA isolates was necessary and has been very efficient, except in the case of the isolate produced with addition of 1 M $CaCl_2$ μ_{added}, to sufficiently decreased the salt and lactose concentrations of the isolates. In order to compare their functional properties with those of commercial and chemical isolates, BMEA isolates had therefore to be dialyzed.

Comparison of BMEA isolates with commercial and chemically produced isolates :

a) Chemical composition

The analyses of variance of the data from the lactose ($P<0.0001$) and ash content ($P<0.0001$), and those of potassium ($P<0.0001$), and calcium ($P<0.0001$) indicated at least one significant difference between the isolates.

Lactose : Two main groups could be distinguished (Table VI-6), indicating a difference in final lactose concentration : the three commercial isolates had similar lactose content at 0.1 g/100 g protein while chemical and BMEA isolates had higher lactose concentration at about 1.3 g/100g protein. In the second group of BMEA and chemical isolates, the 1 M KCl and $CaCl_2$ conditions gave the highest concentrations in lactose.

Ash : Four main groups could be distinguished (Table VI-6), indicating a difference in ash content : the Sigma and UFL isolates had the lowest ash content (0.045 g/100g protein); the 1 M $CaCl_2$ isolate has the highest ash content (0.090 g/100g protein); the 0.5 M $CaCl_2$ isolate has an intermediate ash content (0.065 g/100g protein); the other isolates appeared to have the same ash content at about 0.054 g/100g protein.

Sodium, potassium, calcium and magnesium concentrations :. Three main groups could be distinguished (Table VI-7), indicating a difference in calcium final concentration : the 1 M $CaCl_2$ isolate has the highest calcium content (19.5 mg/100 g protein; the 0.5 and 0.25 M $CaCl_2$ had intermediate calcium concentrations (7.3 and 3.6 mg/100 g protein respectively) ; the other isolates appeared to have the same calcium concentration at about 1.4 mg/100g protein.

b) Physico-chemical and functional properties

The analyses of variance of the data from the particle size ($P<0.0004$), viscosity ($P<0.0001$), foaming capacity (mL) ($P<0.0001$), solubility ($P<0.0001$) and interfacial area

($P<0.0225$) indicated at least one significant difference between the isolates. No significative differences were observed between the isolates for the foaming capacity (min.) ($P>0.3170$), foam stability ($P>0.1660$), protein load ($P>0.6105$), and emulsion stability ($P>0.1637$).

Particle size :.Three main groups could be distinguished (Table VI-8), indicating a difference in particle size : the 1 M $CaCl_2$ isolate has the highest particle size (341.5 nm), the Sigma and 0.5 M $CaCl_2$ had the lowest particle size (191.6 nm), and all the other isolates with similar intermediate particle size (about 274.0 nm).

Specific viscosity : Two main groups could be distinguished (Table VI-8): the 1 M $CaCl_2$ isolate has the highest viscosity (79.4 mL/g), while all other isolates had similar viscosity of about 56.0 mL/g. Moreover, it appeared that the 1 M KCl and NaCl, and 0.5 M $CaCl_2$ had the lowest viscosity of the second group.

Solubility : Three main groups could be distinguished (Table VI-9): the 1 M μ_{added} $CaCl_2$ isolate with the lower solubility (amplitude of 11.5 mg/mL and soluble protein at the pH_i of 0.905 mg/mL), the chemical isolate with intermediate solubility (amplitude of 15.4 mg/mL, and soluble protein at the pH_i of 0 mg/mL), and all other isolates with higher solubility (averaged amplitude of 17.2 mg/mL and soluble protein at pH_i of 0.280 mg/mL).

Foaming properties : The foaming capacity expressed in minute was the same for all the isolates with a duration of 3.63 minutes (Table VI-10). In the same way the foam stability was similar for all the isolates with a quantity of protein solution to be withdrawn of 3.80 mL. However, the UFL isolate has a lower foam stability in comparison with the others. For the foaming capacity (mL), three main groups could be distinguished : the UFL isolate which has the lowest foaming capacity (6.45 mL), the Sigma and New Zealand isolate with an intermediate foaming capacity (10.05 mL), and all the other isolates with an averaged foaming capacity of 13.0 mL.

Emulsifying properties : The protein load and the emulsion stability were the same for all isolates with respective averaged values of 17.7 mg/m^2 and 0.90 m^2/mL of emulsion (Table VI-11). For the interfacial area, three main groups could be distinguished : the Sigma isolate has the lowest interfacial area (0.46 m^2/mL of emulsion), the 1 M KCl isolate has the highest interfacial area (0.71 m^2/mL of emulsion), and all other isolates with intermediate and similar interfacial area (0.61 m^2/mL of emulsion).

Although the chemical composition of the isolates were different, three main groups could be defined based on all data concerning the functional properties : the first group is composed of the 1 M $CaCl_2$ μ_{added} isolate with high viscosity, low solubility, high interfacial area and intermediate foaming properties; the second group is composed of the UFL and Sigma isolates with intermediate viscosity, high solubility, high foaming properties, and intermediate emulsifying properties; the third group is composed of all other isolates with intermediate viscosity, high solubility, intermediate foaming and emulsifying properties.

An interesting complementary information from this study is that the salt concentration did not influenced any of the functional and physicochemical properties of the isolates. In fact, except for foaming capacity expressed in milliliter of added protein solution and viscosity, all other functional properties were unchanged by the ionic strength and the type of added salt, before and after dialysis. Viscosity was influenced by the concentration of salts, due to a different rate of water absorption, and foaming capacity expressed in milliliter was dependant on the surface viscosity of the protein solution during foam formation (Robin *et al.*, 1993; Cayot and Lorient, 1998).

In conclusion, the isolates produced by BMEA at different added salts and ionic strength, except at 1 M $CaCl_2$ μ_{added}, showed similar physicochemical and functional properties than the chemical and New Zealand isolates. However, the small differences in foaming properties in comparison with the UFL isolate and in interfacial area in comparison with the Sigma isolate should be the resultant of a different mineral content, coming from a different isolate preparation procedure.

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ATTEINTE DES OBJECTIFS ET AVANCEMENT DES CONNAISSANCES

Dans ce chapitre , l'objectif visant à étudier l'impact de l'augmentation de la force ionique et de la nature des sels présents dans la solution de lait sur les performances de la technologies, la composition chimique et la fonctionnalité des isolats produits par ÉAMBP a été vérifié.

Au niveau de l'efficacité énergétique, il a été démontré que l'augmentation de la force ionique par ajout de sel jusqu'à 0.5 M, excepté pour l'ajout de $CaCl_2$, permettait une diminution de l'énergie électrique consommée alors qu'au delà l'efficacité diminuait. Cependant la meilleure combinaison a été l'ajout de KCl à une force ionique de 0.5 M : elle a permis une diminution de l'énergie consommée de 45% par rapport à une solution traitée sans ajout de sel. Cette amélioration de l'efficacité énergétique a été la résultante d'une baisse de tension, liée à une augmentation de la conductivité, couplée avec une compensation, par ajout de sel, du manque en ions suffisamment mobiles dans le lait écrémé.

Pour les isolats produits par ajout de sel à différentes forces ioniques, il a été montré que ceux-ci présentaient des propriétés fonctionnelles similaires entre eux, excepté pour l'isolat produit par ajout de $CaCl_2$ à une force ionique de 1M. Cependant, les petites différences observées entre les isolats non dialysés, au niveau de la capacité moussante et de l'activité émulsifiante seraient liées à une différence de composition minérale. Leurs compositions chimiques ont en effet été affectées par les différents niveaux d'ajout de sel, suite à un lavage non efficace des isolats.

Enfin, il est ressorti de ces résultats que les isolats produits par électroacidification avec membranes bipolaires avec différents types de sels et à différents niveaux de forces ioniques ajoutées, excepté pour l'ajout de $CaCl_2$ à une force ionique de 1M, montrent des

propriétés physicochimiques et fonctionnelles similaires à celles d'un isolat produit chimiquement et des isolats commerciaux.

Ces résultats ont permis un avancement des connaissances sur la compréhension de l'effet de l'ajout de sels et de leur nature et sur la précipitation des protéines au cours de l'ÉAMBP. Les travaux sur les sels ont contribué à déterminer la combinaison optimale concernant la nature du sel et la force ionique à ajouter à la solution de lait pour améliorer les performances énergétiques du système d'ÉAMBP. De plus, il est apparu que la nature des sels et la force ionique ont un effet marqué sur la précipitation des caséines du lait en cours de procédé : les sels divalents (calcium) retardent la précipitation des caséines par un effet de salting-in plus marqué que pour les sels monovalents (NaCl et KCl). Il ressort aussi de ces résultats que la présence des sels n'influencent pas les tendances observées au niveau des propriétés fonctionnelles et physicochimiques des isolats produits par ÉAMBP. En effet, les tendances des sels et des forces ioniques sont similaires avant et après dialyse des solutions d'isolats protéiques.

CHAPITRE VII

EFFET DE LA DIMINUTION DE LA FORCE IONIQUE DANS LA SOLUTION DE LAIT SUR LES PERFORMANCES DE LA TECHNOLOGIE, LA COMPOSITION CHIMIQUE ET LA FONCTIONNALITÉ DES ISOLATS PRODUITS PAR EAMBP

CHAPITRE VII

EFFET DE LA DIMINUTION DE LA FORCE IONIQUE DANS LA SOLUTION DE LAIT SUR LES PERFORMANCES DE LA TECHNOLOGIE, LA COMPOSITION CHIMIQUE ET LA FONCTIONNALITÉ DES ISOLATS PRODUITS PAR EAMBP

TRANSITION CONTEXTUELLE

Il ressort de la revue de littérature que la production d'un lactosérum déminéralisé, sous produit de la précipitation des caséines du lait, est avantageuse pour son utilisation ultérieure. Ainsi, les objectifs de cette étude sont d'étudier (1) l'effet de la diminution de la force ionique sur la faisabilité, les performances énergétiques du procédé et les cinétiques de précipitation des protéines au cours de l'EAMBP, et (2) l'effet de la diminution de la force ionique sur la composition chimique et la fonctionnalité des isolats produits par EAMBP.

Les résultats de ce chapitre ont fait l'objet d'un article ayant pour titre «Effect of demineralisation prior to bipolar membrane electroacidification» soumis pour publication dans *Int. J. Dairy Sci.* Les auteurs sont : Laurent Bazinet (Candidat au Ph. D.: planification et réalisation des expériences, analyse des résultats et rédaction de l'article), Christine Gendron (participation à la réalisation des expériences, analyse des propriétés fonctionnelles et révision du manuscrit), Denis Ippersiel (participation à la réalisation des expériences, correction et révision du manuscrit), Josée René-Paradis (analyse des propriétés fonctionnelles), Claudia Tétreault (analyse des propriétés fonctionnelles), Jocelyne Beaudry (analyse des minéraux sur ICP), Michel Britten (révision du manuscrit), Behzad Mahdavi (révision du manuscrit), Jean Amiot (Directeur de thèse : supervision scientifique de l'étudiant, correction et révision du manuscrit), François Lamarche (Co-

directeur de thèse : supervision scientifique de l'étudiant, correction et révision du manuscrit).

RÉSUMÉ

L'objectif de cette étude était d'évaluer l'effet de la baisse du contenu minéral du lait écrémé, par électrodialyse (ÉD) avant électroacidification, sur les performances du procédé et sur la composition chimique et les propriétés fonctionnelles et physicochimiques des isolats produits par ÉAMBP.

La phase de déminéralisation du lait écrémé par ÉD a été très efficace. Cependant, les paramètres d'électroacidification ont été très fortement influencés par le niveau de déminéralisation de la solution de lait : l'efficacité énergétique a été diminuée avec une augmentation du niveau de déminéralisation et il a été possible de réaliser des électroacidifications à très faibles conductivités. D'autre part, les isolats produits par ÉAMBP après déminéralisation à différents niveaux par ÉD ont montré des compositions chimiques similaires, exceptés pour les contenus en lactose et en potassium à 75% de déminéralisation. Ces isolats, excepté pour la charge protéique à 75% de déminéralisation, ont présenté des propriétés physicochimiques et fonctionnelles semblables, quelquesoit le niveau de déminéralisation.

Mots clés : Acidification électrochimique, déminéralisation par électrodialyse, cinétique de précipitation, propriétés fonctionnelles, isolats de caséine.

ABSTRACT

The aim of this study was to evaluate the effect of decreasing mineral content of skim milk by electrodialysis (ED) prior to electroacidification by bipolar membrane (BMEA) on the performance of the process, the chemical composition and the physicochemical and functional properties of the isolates produced.

ED used to demineralize the skim milk solution was very efficient. However, the electroacidification parameters were influenced by the demineralisation level of the skim milk solution : the energy efficiency was decreased with an increase in demineralisation but it was still possible to performed BMEA at a very low conductivity level. Moreover, the isolates produced by BMEA after electrodialysis demineralization at different rate showed similar chemical composition, except on potassium and lactose contents for 75% demineralized isolate. These isolates, except on protein load for 75% demineralization rate, showed similar physicochemical and functional properties, whatever the demineralization rate.

Key words : Electrochemical acidification, electrodialysis demineralization, precipitation kinetic, functional properties, casein isolate.

INTRODUCTION

Hydrochloric acid is commonly used for casein production since the acid is available as a relatively inexpensive by-product of the chemical industry (Southward, 1993). Other techniques have been proposed for the production of acid casein. Acidification of milk by ion-exchange plus acid (Salmon, 1983) or by ion-exchange alone (Rialland and barbier. 1980) has been developed in France. A proposed alternative involves electrodialysis of skim milk to pH 5.0, followed by acidification to pH 4.6 with acid (Laiteries Triballat, 1979). A specific advantage of these methods is the production of acid whey with reduced mineral content. This acid whey is more readily used than acid whey produced by the normal acidification process and may increase its value for further processing (Southward, 1993; Mulvihill, 1989).

More recently, bipolar membrane electroacidification (BMEA) has been used for isoelectric precipitation of skim milk protein and production of isolates (Bazinet *et al.*, 1999c). BMEA uses the a property of bipolar membrane to split water and the action of monopolar membranes for demineralization. According to results obtained by Bazinet *et al.* (1999c, 2000c), the skim milk solution is demineralized at 30-40% of the initial level during the process. Howevèr, a higher demineralization rate of the whey would be of great interest for the dairy industry and for its use in such application as infant formula.

The aim of this study was therefore to evaluate the effect of decreasing mineral content of skim milk by electrodialysis prior to electroacidification on the performance of the process and on the chemical composition and the physicochemical and functional properties of isolates produced by BMEA.

MATERIAL AND METHODS

Material

The raw material used in this study was reconstituted milk (10% w/v) from low temperature spray-dried skim milk powder (Agropur, Granby, Canada). The averaged

composition of the skim milk powder was the following (g/100g) : total protein, 33.9 ; whey protein, 7.4 ; fat, 0.6; carbohydrates, 53.5; ash, 8.2; moisture, 3.8.

Methods

a) ED cell

The ED cell and stack system were the same that those used by Bazinet *et al.* (1999b) with 10 AR-103-QZL-388 anionic membranes (Ionics Inc., Watertown, MA), 9 CR-64-LMP-401 and 2 CR-61-AZL-389 cationic membranes (Ionics Inc., Watertown, MA). This arrangement sets up 3 circuits: the skim milk (4L); the concentrate, a 0.1 N KCl solution (6L); the electrolyte, a 20 g/L Na_2SO_4 (6L). The flow rate of the skim milk solution was controlled at 1.6 $L.min^{-1}$. The anode of the module was made of platinum-plated niobium and the cathode was a plate of stainless steel 316.

b) BMEA cell

The module 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 (3L), a 0.25 N HCl solution (6L) and a 20 g/L Na_2SO_4 solution (6L). Each closed loop was connected to a separate external reservoir, allowing for continuous recirculation (Bazinet *et al.*, 1997b). The anode, a dimensionally-stable electrode (DSA), and the cathode, a 316 stainless-steel electrode, were supplied with the MP cell.

c) Protocol

Electrodialysis was performed in batch process using a current of 2.0 A. After reaching 30 V, the voltage was maintained constant at 30 V, in order to limit water splitting (Bazinet *et al.*, 1999b). The initial pH varied between 6.5 and 6.7. Electro-acidification was carried out in batch process using a current of 2.0 A; after reaching 90 V, the voltage was

maintained constant at 90 V in order to not surpass the total power of the power supply. The electro-acidification was stopped when all the caseins were precipitated (pH_c). As the pH_c values vary with the demineralisation level, the respective values of pH_c were determined in a preliminary study. Four demineralisation levels were tested during electroacidification : 0, 25, 50 and 75%. Three replicates of each condition were performed in this experiment.

1.5 mL samples of the milk solution were taken at the beginning and at the end of the ED process, at the beginning of the BMEA process and at every 0.2 pH unit decrease during electroacidification from pH 5.8 to the pH_c value. The time required to demineralize at the desired level by ED and to reach the pH_c value by BMEA were recorded, as well as the conductivity of the skim milk solution as the treatments progressed. The concentration of soluble protein was determined on freshly acidified 1.5 mL samples. At the end of each BMEA, about 2.5 L samples of the electroacidified milk solution were taken. These samples were centrifuged for 10 minutes at 4°C, at 500 g (Centrifuge Model J2-21, rotor type JA-10, Beckman Instruments Inc., Palo Alto, CA); the precipitate was washed twice with double-distilled water, and the pH adjusted to 6.6 with 1N NaOH. The sodium caseinates (CAS) produced were lyophilised for 24 hours at room temperature (Model Freezone 4.5, Labconco, Kansas City, MI). The lyophilized CAS were stored at 4°C before chemical composition and physicochemical and functional properties were performed.

d) Analysis Methods

System resistance : The system resistance was calculated using Ohm's Law, from the voltage and the current intensity read directly from the indicators on the power supply .

Conductivity : A YSI conductivity meter Model 35 was used with a YSI immersion probe Model 3418 with cell constant K= 1 cm^{-1} (Yellow Springs Instrument Co.,Yellowsprings, OH) to measure the conductivity of the protein solutions.

Energy and relative energy consumption : The voltage as a function of time multiplied by the current, was integrated to determine the energy consumption. (Sappino *et al.*, 1996; Pérez *et al.*, 1994; Bazinet *et al.*, 1999b).

Protein content: Protein content of 1.5 mL samples of freshly acidified milk and of CAS isolate powder was determined using FP-428 LECO apparatus (LECO Corporation, Saint-Joseph, MI), according to the conditions and parameters used by Bazinet *et al.* (1999c).

Lactose concentration : 5% (w/v) protein solutions were acidified with 1.0 N hydrochloric acid to a pH below the isoelectric point. Samples were then centrifuged 15 minutes at 2000 g and 20°C in a Beckman GS-6 centrifuge (Beckman Instruments Inc, Mississauga, Ontario). 15µl of the 0.45 µm filtrated supernatant were injected on an Ion-300 column (Mandel Scientific CO., Rockwood, Ontario, Canada) connected to a HPLC (Waters Associates, Milford, MA) having a UV detector (210 nm) (Model 490, Waters), and a refractive index detector (Model R410, Waters) according to the method of Doyon *et al* (1991). A 0.0054 N H_2SO_4 solution was used as mobile phase at a flow rate of 0.4 mL/minute. The concentration of lactose was determined using a commercial D-lactose solution (Sigma Chemical Co., St-Louis, MO) of known concentration.

Ash Content : Ash content was determined according to the AOAC methods no.930-30 and 945-46 (AOAC International, 1995b,c).

Potassium, sodium, magnesium and calcium concentrations : Sodium, potassium, magnesium and calcium concentrations were determined by inductively coupled plasma (ICP, Optima 3300, dual view, Perkin-Elmer, Norwalk, CT). The wavelengths used to determine sodium, calcium, magnesium and potassium concentrations were 589.59, 422.67, 285.21 and 766.49 nm respectively (Bazinet *et al.*, 2000c). The analysis were carried out in radial view. Samples were prepared from known weight skim milk solution ash dissolved

in 10 mL HCl (2N) and diluted with HCl (2N) to be within the calibration ranges for each cation.

Protein profile : The chromatographic analysis of molecular profile of the lyophilized protein isolate and skim milk samples was performed by reverse-phase HPLC according to Jaubert and Martin, in the conditions used by Bazinet *et al.* (1999c).

Specific viscosity : 10 mL of 4% (w/v) protein solution was introduced into a calibrated viscometer size 100 (Cannon-Fenske RoutineViscosimeter, Cannon Instrument, VWR, Ville-Mont-Royal, Québec, Canada) placed at 25°C in a thermostated water bath. The time needed for the solution to flow through the thin capillary was measured precisely and divided by the time needed for double-distilled water to flow in the same conditions in order to give the relative viscosity (η_r)of the protein solution. The analysis was repeated 5 times for each solution. Specific viscosity was calculated from relative viscosity and protein concentration according to the following equation :

$$\eta_{sp} = \frac{\eta_r - 1}{[Prot]}$$

η_{sp} the specific viscosity (mL/g), η the relative viscosity of the protein solution and [Prot] the protein concentration of the solution (g/mL).

Interfacial area (IA) of the emulsions : 33% (v/v) oil emulsions were produced by mixing commercial corn oil (Mazola) and 4% (w/v) protein solution with a Polytron (model PT 10-35, probe PTA 10S, Kinematica AG, Littau, Switzerland) for 30 seconds at 9000 rpm and homogenized at a pressure of 10000 Psi with an Emulsiflex-C5 homogeneisator (Avestin, Ottawa, ONT). Interfacial area (IA) of the emulsions was calculated from the turbidity of diluted emulsions (Pearce and Kinsella, 1978). Emulsions were diluted to a

final oil volume fraction of 6 x 10^{-5} in sodium phosphate buffer (0.01M, pH 7.0) containing 0.5% sodium dodecyl sulphate (SDS, Biorad Laboratories Canada Ltd, Mississauga, Ontario) according to Britten and Giroux (1993). Optical density was measured in duplicate at 500 nm with a Beckman DU-640 spectrophotometer (Beckman Instruments Inc, Mississauga, Ontario). IA was calculated according to the method of Cameron *et al.* (1991). Emulsion stability was measured by determining the IA of the emulsion stored 6 weeks at 4°C.

Protein load of the emulsions : Protein load was calculated from protein depletion in the serum phase after emulsion formation according to Britten and Giroux (1993). Serum phase was separated from the emulsion by centrifugation (25000g for 1 hour at 4°C) using a Beckman centrifuge (Model J2-21, rotor type JA 20-1). Protein was determined in the aqueous phase before and after emulsion formation using the Bradford's method (1976) calibrated with a bovine serum albumin (BSA) standard (Biorad Laboratories Canada LTD, Mississauga, ONT). Protein load results were expressed as mg/m^2. For that purpose, protein concentration depletion in the aqueous phase was divided by the IA of the emulsion.

Foaming properties : Foaming properties were measured according to Waniska and Kinsella (1979). 15 mL of 0.5% (w/v) protein solution was used. The solution in the column was sparged with nitrogen gas at a constant flow rate of 19 mL/min until foam volume reached 70 mL. Protein solution was added as required to maintain the volume constant at 15 mL. Time required to reach 55 mL of foam, and the volume of protein solution added were recorded. At the end of the sparging, the volume of liquid drained from the foam after 2 minutes was measured. The analysis were done at room temperature and repeated 5 times for each solution.

Solubility profile : 0.2 N hydrochloric acid was added gradually to 250 ml of 2% (w/v) protein solution. 1.5 ml aliquots were taken at pH 6.6; 5.8; 5.4; 5.2; 5.0; 4.8; 4.6; 4.4; 4.2; 4.0 and centrifuged at 500g for 10 minutes at 4°C. Protein concentration was measured

in the supernatant using the Bradford's method (1976). Non-linear regression equations were calculated according to Bazinet *et al.* (2000c) :

$$S_p = b + \frac{a}{1 + \exp\left[-\left(\frac{pH_x - c}{w}\right)\right]}$$

S_p is the percentage of soluble protein, pH_x the pH value ranging from pH 6.6 to 4.0, a the amplitude of the curve, b the percentage of soluble protein at the isoelectric point, c the center or point of inflection and w the width of the transition region of the sigmoidal curve.

e) Statistical Analyses

Using SAS software (SAS inc., 1989) the data from the compositional, physicochemical and functional analyses of CAS produced by BMEA were submitted to an analysis of variance with regression contrasts to examine the effect of the demineralization treatment prior to BMEA. Repeated measure analysis of variance were performed for soluble protein and protein fractions as treatment progressed and for isolate solubility profile. Linear regression equations for the duration and the conductivity of the skim milk solution as the ED and BMEA progressed, and non-linear regression equations for the solubility as a function of pH, were calculated using Sigmaplot (Version 2.01 for Windows, Jandel Scientific, Corte Madera, CA).

RESULTS AND DISCUSSION

Electrodialytic parameters : duration, conductivity, resistance and energy consumption

a) Duration

The duration of the global process (BMEA + ED) was different according to the demineralisation level ($P<0.0187$) (Figure VII-1). The time to reach pH_c increased in a

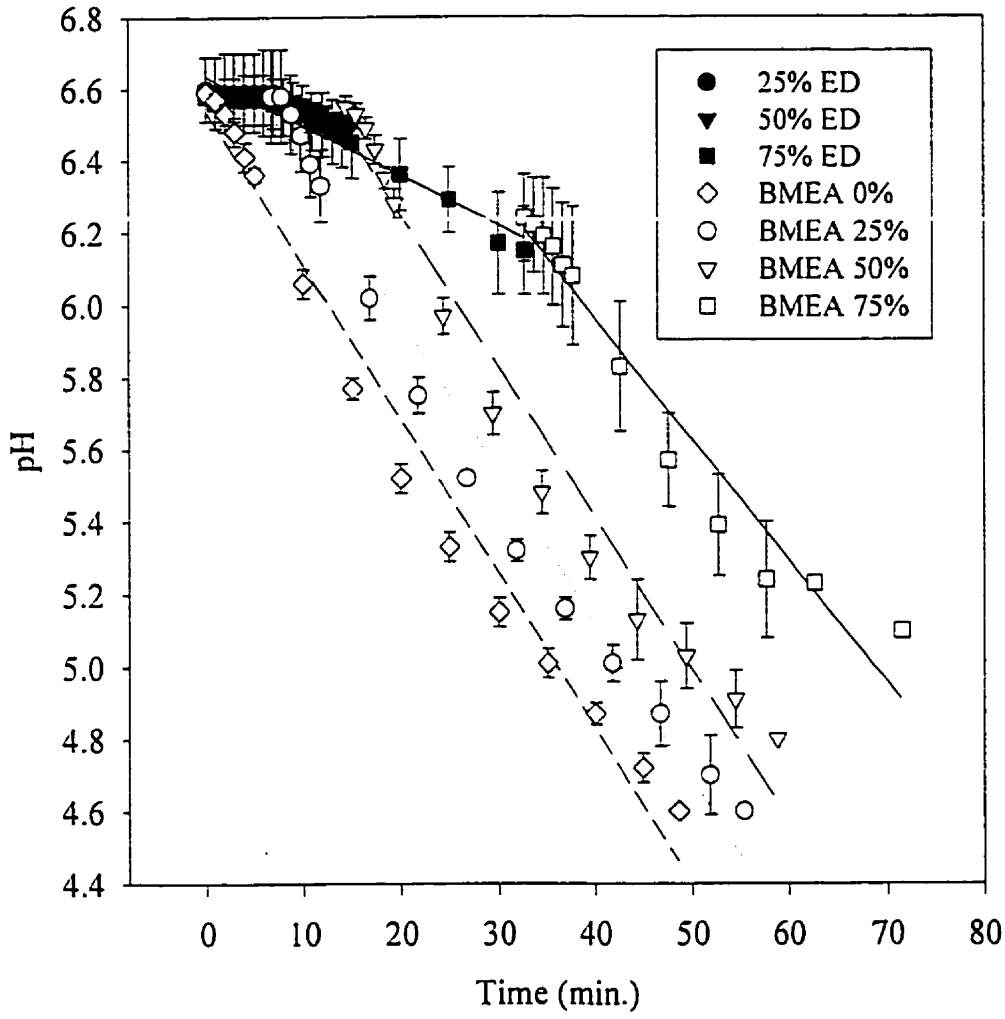

Figure VII-1 : Effect of demineralization rate on the duration of ED phase, BMEA phase and global process (ED + BMEA).

linear fashion with an increase in demineralisation : 48.7, 55.3, 58.8 and 71.5 minutes for 0, 25, 50 and 75% demineralisation level respectively. Moreover, according to the linear coefficients calculated for the BMEA phase, the BMEA has been carried out in similar way whatever the demineralisation level.

b) Conductivity of the skim milk solution

As expected, the variation in conductivity of the skim milk solution from the beginning to the end of the global process was influenced by the demineralisation level ($P<0.0001$) (Figure VII-2): the variation in conductivity increased in a linear fashion from 0.7 to 3.9 mS/cm with an increase in demineralisation from 0 to 75%. During the ED phase, the conductivity of the milk solution decreased in a similar way whatever the demineralisation level with an averaged linear coefficient of – 0.134 (R^2 = 0.937) while during BMEA phase the conductivity decrease was lower due to an increase in demineralisation, and disappear at 75%. Hence, during BMEA, the conductivity decreased by 13.10^{-3} and 2.10^{-3} mS/cm.min for 0% and 75% demineralised milk respectively.

c) Global resistance of the cell

As expected, the variation of the cell resistance during BMEA phase was influenced by the demineralisation level ($P<0.0172$) (Figure VII-3). The resistance variation increased from 14 to 23.3 Volts with an increase in demineralisation from 0 to 25% and stabilised at 24 Volts thereafter. In addition, during the ED phase, resistance of the ED cell increased in a similar way whatever the demineralisation level, with an averaged linear coefficient of 0.754 (R^2 = 0.985). During BMEA, the resistance increase was higher by an increase in demineralisation to stabilize at 50 and 75% demineralisation. Hence, during BMEA, the resistance increased by 256.10^{-3} to 525.10^{-3} Ω/min from 0% to 50% demineralisation to stabilised at 0.568 Ω/min for 75% demineralised milk.

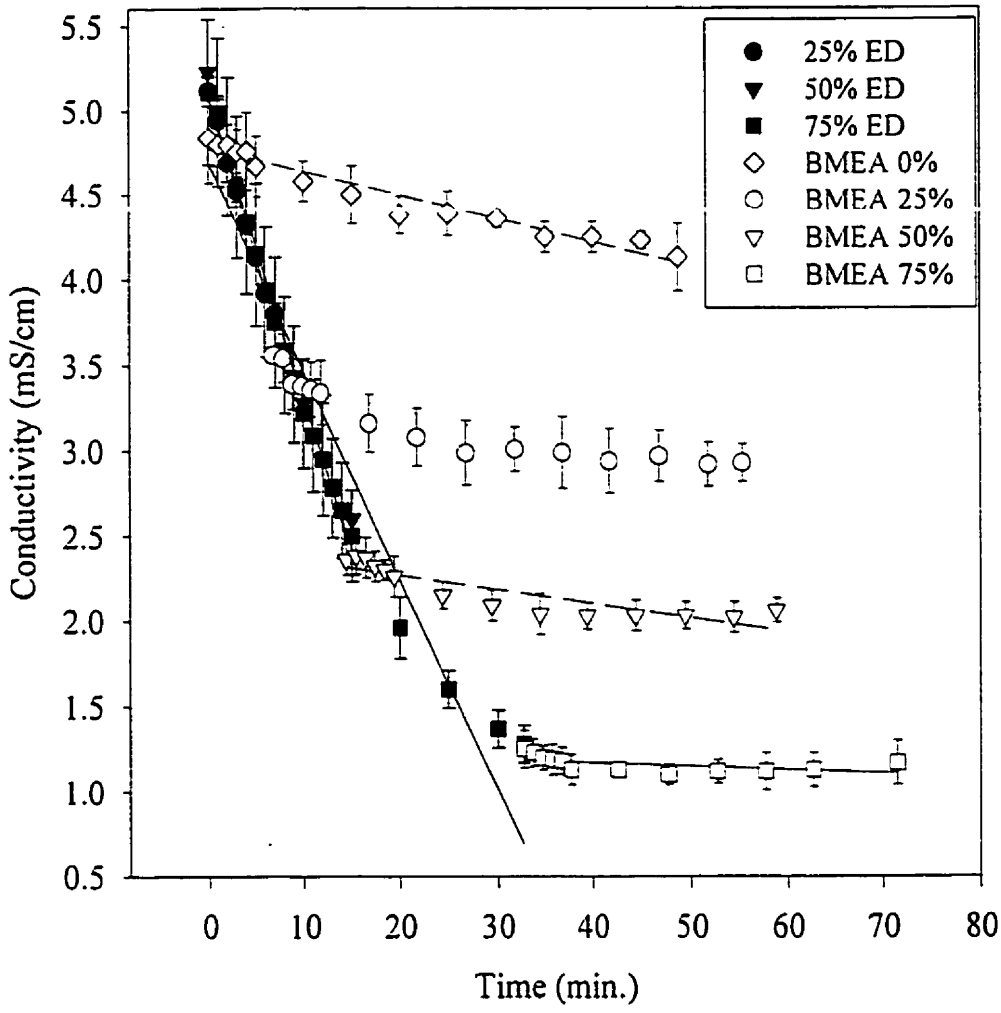

Figure VII-2 : Evolution of the skim milk conductivity during ED and BMEA phases carried out at four demineralisation rates.

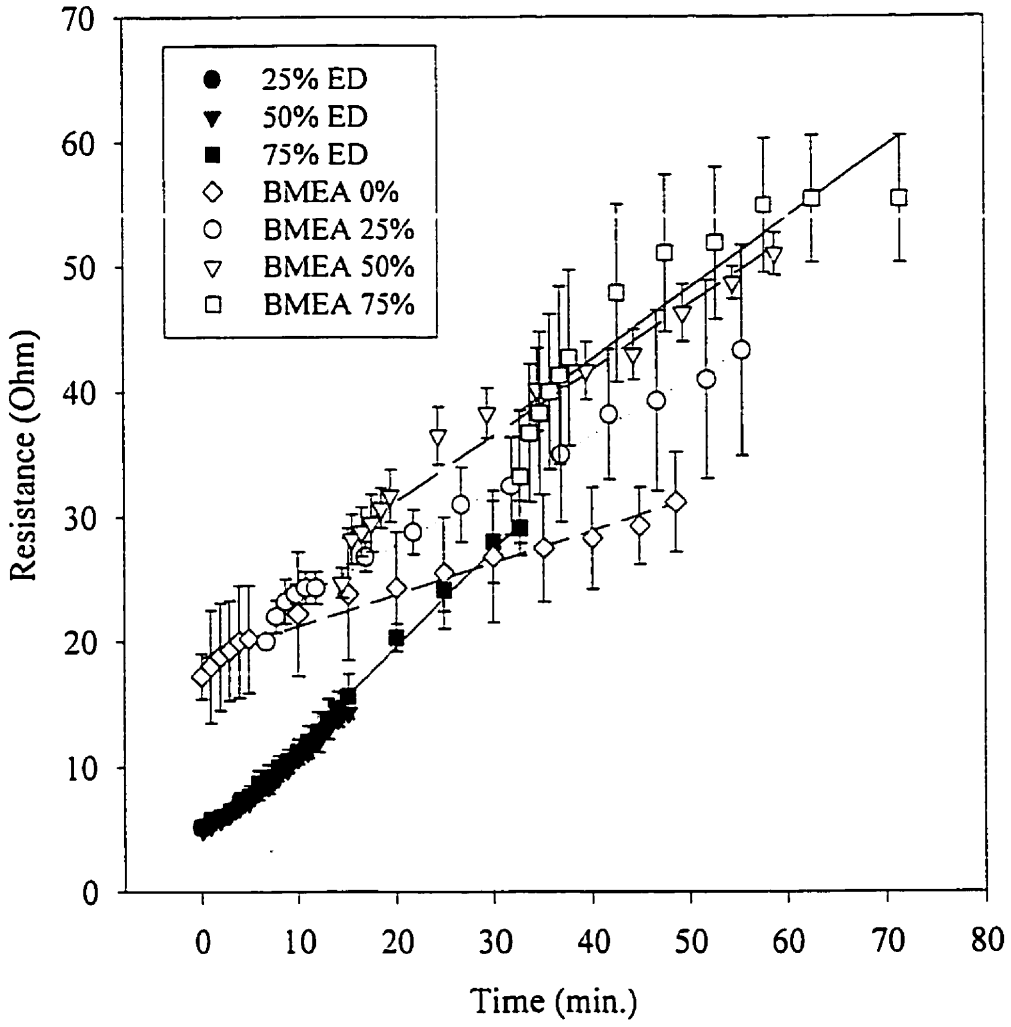

Figure VII-3 : Evolution of the global cell resistance during ED and BMEA phases carried out at four demineralisation rates.

d) Energy and relative energy consumption

During the first 18.7 minutes of the ED phase, the energy needed at one time was the same whatever the demineralisation level (R^2 = 0.985). After 18.7 minutes of ED, particularly at 75% demineralisation, the energy needed at one time decreased constantly. In fact, after 18.7 minutes of demineralisation the maximum voltage of the power supply was reached, due to an increase in resistance, and the voltage was fixed at 90 Volts. Consequently since the current intensity decreased, the energy needed at one time decreased also.

During BMEA, the increase in energy needed at one time varied by 1.03 to 1.5 J/min with an increase in demineralisation from 0 to 25% and stabilised at 1.4 J/min for 50% demineralisation; afterwards, for 75% demineralisation BMEA, the energy needed at one time could be considered stable with a linear coefficient of – 0.11 J/min.
As expected, the relative energy consumption expressed in kWh/kg of isolate produced increased in a linear fashion by 200% with an increase in demineralisation from 0 to 75%.

ED to demineralize the skim milk solution was very efficient. However, the electroacidification parameters were strongly modified and related to the demineralisation level of the skim milk solution. These results were in accordance with data in the litterature : as the demineralisation progresses, the resistance of the system increased; high demineralisation level was related to a higher energy consumption and a higher electrical efficiency factor (Pérez *et al.*, 1994; Bazinet *et al.*, 1999b; Hiroaka *et al.*, 1979). Moreover, a pH decrease was observed during ED phase when the demineralisation level was over 25%. Delbeke (1975) observed the same phenomenon during different demineralization levels of cheese whey : decreases of 0.39 and 1.38 pH unit were obtained with 70 and 90% demineralisation respectively. Perez *et al.* (1994), with demineralized whey permeates and retentates obtained by ultrafiltration using electrodialysis, observed similar decreases in pH ranging from 0.13 to 0.55 pH unit for 40 to 65% demineralisation rate. This change in pH during demineralization can be explained as follow : at an early period of demineralization, the chlorine and potassium ions are mainly removed and later the remaining phosphoric acid radicals or calcium and magnesium ions are mainly removed. However, since their

hydration radius is large, it is difficult for these ionic species to pass through the membrane. For these reasons, water is dissociated into OH^- and H^+, the OH^- easily passing through the membrane but the H^+ remaining in the deashing solution, resulting in a lowering in pH (Hiraoka *et al.*, 1979). Nevertheless, it was possible to performe BMEA at a very low conductivity level, but of course the energy efficiency was decreased with an increase in the demineralisation level.

Precipitation kinetics during acidification

The repeated measure analysis of variance of the data showed that the pH ($P<0.0001$) and the double interaction pH/demineralization rate ($P<0.0001$) had a significant effect on the precipitation of milk proteins. The non-linear regression sigmoidal curves produced coefficients of determination ranging from 0.984 to 0.999 (Table VII-1).

Soluble protein evolution during pH decrease revealed differences between the different demineralization rate. From pH 6.6 to 5.4, the percent soluble protein was the same, ranging from 95-100% whatever the demineralization rate. At pH 5.2, the 75% demineralized milk showed the lower soluble protein with 25% followed by the 50, 25 and 0% demineralized milk with respective soluble protein of 95, 100 and 100%. As the electroacidification progressed from pH 5.2 to 5.0, 75 and 50% demineralized milk showed similar low percent soluble protein with 20.7 and 23.1% respectively, while the 25 and 0% demineralized milk had soluble protein values of 29.5 and 98.1% respectively. At pH 4.8 and after, the soluble protein was similar for the demineralized and non-demineralized milk with an averaged value of 20.1%. The demineralization rate did not influence the final precipitation extent of protein. These different precipitation kinetics were confirmed by model sigmoidal curves : for 0, 25, 50 and 75% demineralization rate the inflection points were respectively pH 4.96, 5.06, 5.09 and 5.29.

The difference observed between milk electroacidified at different demineralization rate can be explained mainly by an increase in electrostatic repulsion (salting-in) due to a decrease in ionic strength. Delay in protein precipitation was observed previously between the chemical and electrochemical acidification and should be due to a salting-in effect by

Table VII-1 : Parameter calculated values of the protein precipitation kinetics obtained for chemical and electrochemical acidifications of 0, 25, 50 and 75% demineralized skim milk.

		0%	25%	50%	75%
BMEA	*Amplitude*	79.8	80.9	79.1	76.7
	Soluble Protein at pH_i	20.6	20.2	18.5	20.7
	Inflection point	4.96	5.06	5.09	5.29
	Transition width	0.0115	0.0309	0.0324	0.0315
	R^2	0.998	0.998	0.984	0.993
Chemical acidification	*Amplitude*	78.6	81.5	80.9	77.5
	Soluble Protein at pH_i	19.7	17.7	17.2	20.9
	Inflection point	4.89	4.91	5.05	5.13
	Transition width	0.0442	0.0419	0.0346	0.0262
	R^2	0.995	0.998	0.998	0.999

addition of HCl (Bazinet *et al.*, 2000b). Therefore, to confirm that observation milk solutions demineralized at the different rates were chemically acidified. For each demineralization rate, a delay in precipitation was observed (Table VII-1): the inflection points calculated by model sigmoidal curves for the chemical acidifications were 4.89, 4.92, 5.05 and 5.13 for 0, 25, 50 and 75% demineralization rates respectively. As hypothesised by Bazinet *et al.* (2000b) for HCl chemical acidification, the salts added would conduct to a salting-in effect, and consequently to a delay in precipitation, while in the case of electroacidification, the migration of salts from the protein solution by electrochemical demineralisation would favour the precipitation of proteins.

Protein profile during acidification

The repeated measure analysis of variance showed a highly significant effect of pH on κ-casein ($P<0.0001$), α_s-casein ($P<0.0001$), β-casein ($P<0.0001$) and whey protein concentrations ($P<0.0001$), and of double interaction pH/demineralization rate on κ-casein ($P<0.0001$), α_s-casein ($P<0.0001$) and β-casein ($P<0.0001$).

For casein fractions, their percentages in the supernatant decreased as the acidification progressed, and were influenced by the demineralization rate. The precipitation of these fractions took place at higher pH value when the demineralization rate was increased. For whey protein fraction, its percentage was stable until the casein precipitated and that a small part of the whey protein fraction co-precipitated with the casein. The precipitation of this small part with casein would also be influenced by the demineralization rate since the probability level of the double interaction pH/demineralization rate ($P>0.0540$) was close to the 5% acceptance level. This co-precipitation was the result of the precipitation of a β-lg--α-la--κ-casein complex formed during pasteurization heat treatment (Cayot and Lorient, 1998; Singh and Fox, 1987).

These results were in accordance with previous results obtained for the precipitation kinetics and gave more informations on each casein fraction precipitation.

Isolate composition

The analyses of variance showed no significant effect of demineralization rate on protein (P>0.4731), ash (P>0.2831), magnesium (P>0.3688) and calcium (P>0.0908) concentrations while a significant effect was showed on lactose (P<0.0179), potassium (P<0.0019) and sodium (P<0.0331) concentrations. The analyses of variance showed no significant effect of demineralization rate on percent κ-casein (P>0.1406), α_s-casein (P<0.1265), β-casein (P>0.2046) and whey protein (P<0.3764) fractions.

The protein and ash content of isolates produced in the different conditions were the same with respective values of 92.0 ● 2.1 and 4.45 ± 0.44% (table VII-2). In the same way, the mineral composition of the isolates in magnesium and calcium were the same whatever the conditions in which they were produced with respective averaged concentrations of 9.1 ± 3.0 and 239 ± 77 mg/100g protein for magnesium and calcium (Table VII-2). The lactose content was decreased by 58.9% with an increase in demineralization from 0 to 75%. Sodium and potassium concentrations first decreased by 13.5 and 46.1% respectively with an increase in demineralization rate from 0 to 50%, and thereafter increased by 34.8 and 33.4% respectively with a 75% demineralization rate (Table VII-2). In the isolates produced after BMEA of the different demineralized skim milk solutions, the percent of κ-casein, α_s-casein, β-casein and whey protein fractions were the same with respective values of 14.9, 37.3, 47.6 and 0.2 % total peak area (table VII-2).

The protein content of the isolates was not influenced by the demineralization rate, and monovalents cations, which are the more mobile ions, were removed efficiently from the skim milk solution. However, sodium and potassium content increased in the isolate after a demineralization rate higher than 50%. Moreover, the efficiency of lactose removed during curd washing was increased with an increase in demineralization rate. During the precipitation of the casein relatively large amounts of lactose are trapped within the curd and this prevents their removal during washing of the curd (Zadow, 1971). Sufficient holding time during each washing stage is therefore required to allow diffusion of the lactose from the curd into the wash water (Mulvihill, 1989). Since the size of the casein

Table VII-2 : Chemical composition of casein isolates produced at different demineralization rates.

	0%	25%	50%	75%
Protein (% dry weight)	92.1 ± 0.9	92.1 ± 2.1	93.4 ± 1.3	90.5 ± 3.3
Ash (g/100 g protein)	4.52 ± 0.25	4.24 ± 0.13	4.22 ± 0.24	4.85 ± 0.75
Lactose (g/100 g protein)	4.07 ± 0.75	2.19 ± 0.94	1.72 ± 0.25	1.67 ± 0.94
Sodium (mg/100 g protein)	1333 ± 137	1335 ± 18	1153 ± 127	1555 ± 179
Potassium (mg/100 g protein)	39 ± 3	26 ± 6	21 ± 3	28 ± 3
Magnesium (mg/100 g protein)	9 ± 3	7 ± 3	11 ± 1	9 ± 4
Calcium (mg/100 g protein)	197 ± 40	177 ± 40	306 ± 24	279 ± 105
κ-casein (% total peak area)	14.7 ± 0.3	14.4 ± 1.0	16.6 ± 2.4	13.9 ± 0.5
α_s-casein (% total peak area)	37.3 ± 0.1	37.0 ± 0.2	36.8 ± 1.0	37.9 ± 0.5
β-casein (% total peak area)	48.0 ± 0.4	48.1 ± 0.5	46.6 ± 1.5	47.8 ± 0.6
Whey protein (% total peak area)	0.0 ± 0.0	0.5 ± 0.5	0.0 ± 0.0	0.4 ± 0.4

micelles was reduced during demineralization (Kimura et al., 1991), lactose diffusion during the washing of the curd was facilitated and could explain the lower content of lactose for the 75% demineralized BMEA isolate.

Isolate physicochemical and functional properties

Results of analyses of variance showed that the viscosity ($P>0.5334$), interfacial area ($P>0.1638$), emulsion stability ($P>0.0651$), foaming capacity expressed in minute ($P>0.4292$), foaming capacity expressed in milliliter of protein solution added ($P>0.4578$) and foam stability ($P>0.8521$) were unchanged whatever the demineralization rate. The analyses of variance showed a significant effect of demineralization rate on protein load ($P<0.0015$). The repeated measure analysis of variance indicated no significant effect of demineralization rate ($P>0.1960$) on solubility of isolate produced as a function of pH.

Except for protein load, the emulsifying properties of the isolates produced from milk solutions demineralized at different rates were unchanged (Table VII-3). Averaged interfacial area was 0.617 ± 0.027 m^2/mL, and averaged emulsion stability was 1.04 ± 0.17 m^2/mL. The protein load of the isolates produced at different demineralization rate increased in an quadratic fashion ($P<0.0032$) ; the protein load was stable between 0 and 25, with respective values of 10.5 and 10.0 mg/m^2, increased slightly to 11.5 mg/m^2 from 25 and 50% demineralization, to reached a value of 25.9 mg/m^2 at 75% demineralization. The demineralization rate would allow a more complete unfolding of the protein.

These results were in accordance with previous data obtained for isolate produced by BMEA (Bazinet *et al.*, 2000f). Demineralization by electrodialysis prior to bipolar membrane electroacidification, except on protein load, did not influence the functional and physichochemical properties of isolates produced.

Table VII-3 : Physico-chemical and functional properties of casein isolates produced at different demineralization rates, and relative energy consumption.

		0%	25%	50%	75%
Viscosity (mL/g)		61.2 ± 5.1	60.4 ± 5.7	73.5 ± 21.0	60.8 ± 10.5
Foaming capacity (min.)		3.51 ± 0.31	3.20 ± 0.26	3.19 ± 0.28	3.62 ± 0.55
Foaming capacity (mL added)		13.3 ± 0.6	13.2 ± 0.5	13.8 ± 0.2	13.7 ± 0.7
Foam stability (mL recovered)		3.5 ± 0.3	3.7 ± 0.4	3.7 ± 0.7	3.5 ± 0.3
Protein load (mg/m^2)		10.6 ± 2.2	10.0 ± 0.9	11.5 ± 1.3	25.9 ± 0.1
Interfacial area (m^2/mL emulsion)		0.63 ± 0.02	0.59 ± 0.02	0.63 ± 0.03	0.62 ± 0.02
Emulsion stability (m^2/mL emulsion)		1.01 ± 0.09	1.01 ± 0.01	1.42 ± N/A	0.92 ± 0.22
Solubility as a function of pH	*Amplitude*	95.5	99.7	96.5	94.3
	Soluble Protein at pH$_I$	0.82	0.22	-0.04	0.72
	Inflection point	4.97	4.98	5.01	5.00
	Transition width	0.085	0.074	0.081	0.082
	R^2	0.991	0.994	0.995	0.987
Energy (kWh/kg of isolate)		1.02	1.61	1.92	3.08

CONCLUSION

The ED phase to demineralize the skim milk solution was very efficient. However, the electroacidification parameters were modified by the demineralisation level of the skim milk solution : the energy efficiency was decreased with an increase in demineralisation but it was still possible to performed BMEA at a very low conductivity level.

The difference observed between milk electroacidified at different demineralization rate can be explained mainly by an increase in electrostatic repulsion due to a decrease in ionic strength. These results confirm that in the case of HCl chemical acidification, the salts added would conduct to a salting-in effect, and consequently to a delay in precipitation, while in the case of electroacidification, the migration of salts from the protein solution by electrochemical demineralisation would favour the precipitation of proteins.

The isolates produced by BMEA after electrodialysis demineralization at different rate showed similar chemical composition, except on potassium and lactose contents for 75% demineralized isolate. These isolates, except on protein load for 75% demineralization rate, showed similar physicochemical and functional properties, whatever the demineralization rate.

ATTEINTE DES OBJECTIFS ET AVANCEMENT DES CONNAISSANCES

Dans ce chapitre, l'objectif visant à étudier l'impact de la baisse de la force ionique dans la solution de lait sur les performances de la technologies, la composition chimique et la fonctionnalité des isolats produits par ÉAMBP a été atteint.

La diminution de la force ionique de la solution de lait écrémé, par déminéralisation électrodialytique, a abouti à une augmentation de l'énergie consommée : motamment une consommation énergétique triplée par déminéralisation à 75% de la solution de lait électroacidifiée.

Cependant, la déminéralisation de la solution de lait écrémé avant un traitement par électroacidification a eu un impact plus conséquent sur les propriétés fonctionnelles et la

composition chimique des isolats que l'ajout de sel. Pour les isolats produits après déminéralisation de la solution de lait écrémé, il est apparu que jusqu'à un taux de déminéralisation de 50%, les propriétés fonctionnelles et la composition chimique des isolats produits étaient similaires, mais qu'à 75% de déminéralisation, la charge protéique avait triplé et la concentration en lactose avait baissé de moitié.

Ces résultats ont permis un avancement des connaissances sur la compréhension de l'effet du retrait des sels et sur la précipitation des protéines au cours de l'ÉAMBP. Les résultats sur la déminéralisation des solutions avant ÉAMBP ont démontré tout d'abord que, contrairement à toutes attentes et aux résultats obtenus au niveau de la modélisation des H^+ électrogénérés, il est possible d'effectuer une ÉAMBP à de très faibles concentrations en ions présents. De plus, le suivi des cinétiques de précipitation lors de l'ÉAMBP des solutions de lait déminéralisées à différents taux et la comparaison à l'acidification chimique ont concouru à la confirmation de l'hypothèse émise au chapitre VI sur la différence observée entre les profils moléculaires des surnageants des deux procédés d'acidification. L'ÉAMBP permet une précipitation des caséines du lait à un pH plus élevé que l'acidification chimique; plus la concentration en sels de la solution de lait diminue, plus le pH de précipitation des protéines s'élève et se rapproche du point isoionique des caséines. Ainsi, la différence de précipitation est effectivement liée à un effet de salting-in découlant de l'ajout de sels, dans le cas de l'acidification chimique, opposé dans le cas de l'ÉAMBP, à un retrait des sels par déminéralisation qui favorise la précipitation des protéines du lait.

CHAPITRE VIII

EFFET DU TYPE DE LAIT SUR LA COMPOSITION CHIMIQUE ET LA FONCTIONNALITÉ DES ISOLATS PRODUITS AU COURS D'ACIDIFICATIONS CHIMIQUES ET ÉLECTROCHIMIQUES

CHAPITRE VIII

EFFET DU TYPE DE LAIT SUR LA COMPOSITION CHIMIQUE ET LA FONCTIONNALITÉ DES ISOLATS PRODUITS AU COURS D'ACIDIFICATIONS CHIMIQUES ET ÉLECTROCHIMIQUES

TRANSITION CONTEXTUELLE

Dans les chapitres V et VI, nous avons observé que des protéines lactosériques se retrouvaient dans les isolats produits par EAMBP de lait écrémé reconstitué à partir de poudre séchée par atomisation à basse température. Ces protéines proviendraient de la précipitation de complexes formés au cours du traitement de séchage entre les caséines-κ, la β-lactoglobuline et l'α-lactalbumine. La présence de ces complexes protéiques dans la poudre de lait écrémé pourrait influer directement sur la cinétique de précipitation des caséines au cours de l'EAMBP et indirectement par la présence de protéines sériques dans les isolats produits par acidification qui interagiraient sur les propriétés physicochimiques et fonctionnelles de ces derniers. Par conséquent, les objectifs de ce chapitre sont de comparer (1) les acidifications chimique et électrochimiques de lait frais et reconstitué en terme de paramètres électrodialytiques, de cinétique de précipitation au cours de l'acidification, et (2) la composition chimique et les propriétés physicochimiques et fonctionnelles des isolats produits.

Les résultats de ce chapitre ont fait l'objet d'un article ayant pour titre «Comparison between reconstituted and fresh skim milk chemical and electrochemical acidifications» soumis pour publication dans *J. Agric Food Chem.* Les auteurs sont : Laurent Bazinet (Candidat au Ph. D.: planification et réalisation des expériences, analyse des résultats et rédaction de l'article), Christine Gendron (participation à la réalisation des expériences, analyse des propriétés fonctionnelles et révision du manuscrit), Denis Ippersiel (participation à la réalisation des expériences, correction et révision du manuscrit), Josée

René-Paradis (analyse des propriétés fonctionnelles), Claudia Tétreault (analyse des propriétés fonctionnelles), Jocelyne Beaudry (analyse des minéraux sur ICP), Michel Britten (révision du manuscrit), Behzad Mahdavi (révision du manuscrit), Jean Amiot (Directeur de thèse : supervision scientifique de l'étudiant, correction et révision du manuscrit) et François Lamarche (Co-directeur de thèse : supervision scientifique de l'étudiant, correction et révision du manuscrit).

RÉSUMÉ

Le but de cette étude était de comparer les acidifications chimiques et électrochimiques de laits frais et reconstitué en terme de paramètre électrodialytiques, de cinétique de précipitation, et de composition chimique, de propriétés physicochimiques et fonctionnelles des isolats produits par ÉAMBP.

Les paramètres électrodialytiques n'ont pas été influencé par le type de lait lorsque ceux-ci ont été comparés sur une même base de protéines et de cendres. La différence de cinétique de précipitation observée entre les deux laits, quelque soit la procédure d'acidification employée, s'explique principalement par une différence de contenu en sels.

Les isolats produits par ÉAMBP, excepté au niveau de la capacité moussante, ont montré des propriétés physicochimiques et fonctionnelles similaires à celles des isolats chimiques. Le facteur principal influençant la composition et les propriétés fonctionnelles des isolats serait le (ou les) prétraitement(s) subit par le lait avant l'acidification : ce facteur aurait un impact sur les propriétés fonctionnelles bien supérieur à celui du traitement d'acidification.

Mots clés : Acidification électrochimique, acidification chimique, lait écrémé frais, lait écrémé reconstitué, isolat, propriétés fonctionnelles.

ABSTRACT

The aim of this study was to compare the electrochemical and chemical acidification of reconstituted and fresh skim milk in terms of electrodialytic parameters, precipitation kinetics, chemical composition, and physicochemical and functional properties of isolates produced by BMEA.

The electrodialytic parameters were not influenced by the type of milk when both milk were compared on a similar protein and salt content. The difference in precipitation kinetic observed between both milk, whatever the acidification procedure can be explained mainly by a difference in salt content.

Isolates produced by BMEA, except on foaming capacity, showed similar physicochemical and functional properties than the isolates chemically produced. The main factor affecting the composition and the physichochemical and functional properties was the pre-treatments of milk prior to acidification : they have a higher impact on the functional properties than the acidification treatment itself.

Key words : Electrochemical acidification, chemical acidification, isolate, functional properties, fresh skim milk, reconstituted skim milk.

INTRODUCTION

Thermal treatments were recognized to denature whey proteins during production of milk powder. During the thermal treatment, β-lg forms a complex with κ-casein via an exchange between sulfhydryl groups and disulfide bonds at the natural pH of the milk (Tessier and Rose, 1964; Sawyer, 1969; Singh and Fox, 1987; Cayot and Lorient, 1998). Althought α-la does not form a complex with κ-casein during thermal treatment, α-la forms a complex with β-lg and consequently could associate indirectly with the κ-casein. The α-la and κ-casein association is catalyzed by the thiol group of β-lg (Kinsella , 1984; Cayot and Lorient, 1998). The quantity of whey protein associated with caseins increases with the intensity of the thermal treatment (Iametti *et al.*, 1993) and was proposed to estimate the severity of milk thermal treatment (Buchheim *et al.*, 1994).

Bazinet *et al.* (2000b) had observed some whey protein in isolates produced by BMEA of skim milk reconstituted from low heat spray dried powder. Moreover they demonstrated that bipolar membrane electrochemical acidification (BMEA) of reconstituted skim milk was similar to chemical acidification in terms of molecular profile, but different in precipitation kinetics : caseins were precipitated at a higher pH value by BMEA. The association of whey protein with casein in skim milk powder could influence the precipitation kinetic of protein during BMEA process and the physicochemical and functional properties of the casein isolates (CAS) produced.

Therefore, the aim of this study was to compare the electrochemical and chemical acidification of reconstituted and fresh skim milk in terms of electrodialytic parameters, precipitation kinetics, chemical composition, and physicochemical and functional properties of isolates produced by BMEA.

MATERIAL AND METHODS

Material

The reconstituted skim milk (10% w/v) was from low temperature spray-dried skim milk powder (Agropur, Granby, Canada). The averaged composition of the powder was the following (g/100g) : total protein, 33.9 ; ash, 8.2; moisture, 3.8.

Raw milk from bulk tank was skimmed, homogenized but non-pasteurized (Agropur, Granby, Canada). Fresh milk was always used the day after being processed. The averaged composition of fresh milk was the following (g/100 mL) : total protein, 3.26 ; ash, 0.65.

Methods

a) BMEA 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 (3L), a 0.25 N HCl solution (6L) and a 20 g/L Na_2SO_4 solution (6L). Each closed loop was connected to a separate external reservoir, allowing for continuous recirculation (Bazinet *et al.*, 1997b). The anode, a dimensionally-stable electrode (DSA), and the cathode, a 316 stainless-steel electrode, were supplied with the MP cell.

b) Protocol

For chemical acidification, skim milk solution volumes of 3 L were used. HCl (1.000 N, VWR Canlab, Ville-Mont-Royal, QC) was added to acidify the milk solution to pH 4.4. Electro-acidification was carried out in batch process using a current of 2.0 A. Electroacidification was stopped at pH 4.4. The initial pH of the reconstituted and fresh

milk varied from 6.5 to 6.7. Three replicates of each milk electrochemical and chemical acidification were performed in this experiment.

1.5 mL samples of milk solution were taken at the beginning of the acidifications and every 0.2 pH unit decrease during acidifications from pH 5.6 to 4.4. The time required to reach pH 4.4, the anode/cathode voltage difference during BMEA, as well as the conductivity of the skim milk solution were recorded as the acidifications progressed. On freshly acidified 1.5 mL samples, the concentration of soluble protein in the supernatant of centrifuged samples was determined. At the end of each acidification, 2.5 L samples of the pH 4.4 milk solution were collected. These samples were centrifuged for 10 minutes at 4°C, at 500 g (Centrifuge Model J2-21, rotor type JA-10, Beckman Instruments Inc., Palo Alto, CA), the precipitate was washed twice with acidified double-distilled water, and the pH adjusted to 6.6 with 1N NaOH. The sodium caseinates (CAS) produced were freeze-dried for 24 hours at room temperature (Model Freezone 4.5, Labconco, Kansas City, MI). The lyophilized CAS were stored at 4°C before chemical composition, and physicochemical and functional properties were performed.

c) Analysis Methods

Anode/cathode voltage difference: The voltage was read directly from the indicators on the power supply.

Conductivity: A YSI conductivity meter Model 35 was used with a YSI immersion probe Model 3418, cell constant K= 1 cm^{-1} (Yellow Springs Instrument Co.,Yellowsprings, OH) to measure the conductivity of the protein solutions.

Protein content: Protein content of 1.5 mL samples of freshly acidified milk and of CAS isolate powder was determined by FP-428 LECO apparatus (LECO Corporation, Saint-Joseph, MI), according to the conditions and parameters used by Bazinet *et al.* (1999c).

Lactose concentration : 5% (w/v) protein solutions were acidified with 1.0 N hydrochloric acid to a pH below the isoelectric point. Samples were then centrifuged 15 minutes at 2000 g and 20°C in a Beckman GS-6 centrifuge (Beckman Instruments Inc, Mississauga, Ontario). 15µl of the 0.45 µm filtrated supernatant were injected on an Ion-300 column (Mandel Scientific CO., Rockwood, Ontario, Canada) connected to a HPLC (Waters Associates, Milford, MA) having a UV detector (210 nm) (Model 490, Waters), and a refractive index detector (Model R410, Waters) according to the method of Doyon *et al* (1991).

Moisture : Moisture was measured according to the AOAC method no.927-05 (AOAC International, 1995a).

Ash Content : Ash content was determined according to the AOAC methods no.930-30 and 945-46 (AOAC International, 1995b,c).

Potassium, sodium, magnesium and calcium concentration measurements : Sodium, potassium, magnesium and calcium concentrations were determined by inductively coupled plasma (ICP, Optima 3300, dual view, Perkin-Elmer, Norwalk, CT). The wavelengths used to determine sodium, calcium, magnesium and potassium concentrations were 589.59, 422.67, 285.21 and 766.49 nm respectively. The analysis were carried out in radial view. Samples were prepared from known weight skim milk solution ash dissolved in 10 mL HCl (2N) and diluted with HCl (2N) to be within the calibration ranges for each cation.

Protein profile : The chromatographic analysis of protein profile of the freeze-dried protein isolate and supernatant of centrifuged skim milk samples was performed by reverse-phase HPLC according to Jaubert and Martin, in the conditions used by Bazinet *et al.* (1999c).

Specific viscosity : 10 mL of 4% (w/v) protein solution was introduced into a calibrated viscometer size 100 (Cannon-Fenske RoutineViscosimeter, Cannon Instrument, VWR, Ville-Mont-Royal, Québec, Canada) placed at 25°C in a thermostated water bath. The time needed for the solution to flow through the thin capillary was measured precisely and divided by the time needed for double-distilled water to flow in the same conditions in order to give the relative viscosity (η_r)of the protein solution. The analysis was repeated 5 times for each solution. Specific viscosity was calculated from relative viscosity and protein concentration according to the following equation :

$$\eta_{sp} = \frac{\eta_r - 1}{[Prot]}$$

η_{sp} the specific viscosity (mL/g), η the relative viscosity of the protein solution and [Prot] the protein concentration of the solution (g/mL).

Interfacial area (IA) of the emulsions : 33% (v/v) oil emulsions were produced by mixing commercial corn oil (Mazola) and 4% (w/v) protein solution with a Polytron (model PT 10-35, probe PTA 10S, Kinematica AG, Littau, Switzerland) for 30 seconds at 9000 rpm and homogenized at a pressure of 10000 Psi with an Emulsiflex-C5 homogeneisator (Avestin, Ottawa, ONT). Interfacial area (IA) of the emulsions was calculated from the turbidity of diluted emulsions (Pearce and Kinsella, 1978). Emulsions were diluted to a final oil volume fraction of 6×10^{-5} in sodium phosphate buffer (0.01M, pH 7.0) containing 0.5% sodium dodecyl sulphate (SDS, Biorad Laboratories Canada Ltd, Mississauga, Ontario) according to Britten and Giroux (1993). Optical density was measured at 500 nm with a Beckman DU-640 spectrophotometer (Beckman Instruments Inc, Mississauga, Ontario). Calculations were performed according to the method of Cameron *et al.* (1991). Emulsion stability was measured by determining the IA of the emulsion stored 6 weeks at 4°C.

Protein load of the emulsions : Protein load was calculated from protein depletion in the serum phase after emulsion formation according to Britten and Giroux (1993). Serum phase was separated from the emulsion by centrifugation (25000g for 1 hour at 4°C) using a Beckman centrifuge (Model J2-21). Protein was determined in the aqueous phase before and after emulsion formation using the Bradford's method (1976) calibrated with a bovine serum albumin (BSA) standard (Biorad Laboratories Canada LTD, Mississauga, ONT). Protein load results were expressed as mg/m^2. For that purpose, protein concentration depletion in the aqueous phase was divided by the IA of the emulsion.

Foaming properties : Foaming properties were measured according to Waniska and Kinsella (1979). 15 mL of 0.5% (w/v) protein solution was used. The solution in the column was sparged with nitrogen gas at a constant flow rate of 19 mL/min until foam volume reached 70 mL. Protein solution was added as required to maintain the volume constant at 15 mL. Time required to reach 55 mL of foam, and the volume of protein solution added were recorded. At the end of the sparging, the volume of liquid drained from the foam after 2 minutes was measured. The analysis were done at room temperature and repeated 5 times for each solution.

Solubility profile : 0.2 N hydrochloric acid was added gradually to 250 ml of 2% (w/v) protein solution. 1.5 ml aliquots were taken at pH 6.6; 5.8; 5.4; 5.2; 5.0; 4.8; 4.6; 4.4; 4.2; 4.0 and centrifuged at 500g for 10 minutes at 4°C. Soluble protein concentration was determined in the supernatant using the Bradford's method (1976). Non-linear regression equations were calculated according to Bazinet *et al.* (2000c) :

$$S_p = b + \frac{a}{1 + \exp\left[-\left(\frac{pH_x - c}{w}\right)\right]}$$

S_p is the percentage of soluble protein, pH_x the pH value ranging from pH 6.6 to 4.0, a the amplitude of the curve, b the percentage of soluble protein at the isoelectric point,

c the center or point of inflection and *w* the width of the transition region of the sigmoidal curve.

Particle size : The particle size was determined on 0.5 to 1.0 mL of 0.5% (w/v) protein solution in 2 mL double distilled water, with a sub micron particle analyzer at 22°C (Beckman Coulter, Model N4MD, Miami, FL). Results are expressed in terms of mean diameter (nm).

d) Statistical analyses

Using SAS software (SAS inc., 1989), repeated measure analysis of variance were performed for conductivity and soluble protein as treatment progressed. The time to reach pH 4.4 and the voltage variation between the beginning and the end of the BMEA were subjected to Student t-test. Data from the compositional, physicochemical and functional analyses of isolates produced by chemical and electrochemical acidifications were submitted to an analysis of variance. HPLC data of milk sample collected during acidification were analyzed by repeated measure analysis of variance. Linear regression equations for the duration and the conductivity of the skim milk solution as the acidification progressed and non-linear regression equations for the precipitation kinetic and the solubility as a function of pH using Sigmaplot (Version 2.01 for Windows, Jandel Scientific, Corte Madera, CA) were calculated.

RESULTS AND DISCUSSION

Electrodialytic parameters : duration and anode cathode/voltage difference

a) Duration

The duration of the BMEA was different according to the type of milk ($P<0.0251$). The time to reach pH 4.4 was longer for reconstituted milk (RM) than for fresh milk (FM) with

respective duration of 51.4 ± 1.8 and 47.2 ± 1.0 minutes. The small difference observed was due to an averaged 0.38 g/mL protein difference between RM and FM initial protein content, since the duration of BMEA was previously shown to be protein concentration dependent (Bazinet *et al.*, 1997a).

b) Anode/cathode voltage difference

The variation of voltage during the process was the same for both milk at 25.2 ± 9.0 ($P>0.4528$). The voltage increased linearly from 21.9 to 47.0 Volts ($R^2 = 0.970$) as the treatment progressed. The type of milk treated did not influence the hydrodynamic of the electrodialysis cell.

Electrodialytic parameters were not influenced by the type of milk when both milks were compared on a similar protein and salt content. The fact that the RM milk was heat treated during spray-drying did not influence the electroacidification process. Moreover, electroacidification results obtained on RM were in accordance with previous results obtained by Bazinet *et al.* (2000b).

Conductivity

The repeated measure analysis of variance of the data showed that the pH ($P<0.0001$) and the double interaction pH/acidification type ($P<0.0001$) had a significant effect on the conductivity, whatever the milk type.

During chemical acidification of milk solution, the conductivity increased from 5.2 to 8.4 $mS.cm^{-1}$, and decreased from 4.7 to 4.0 $mS.cm^{-1}$ in electroacidification (all milk type averaged). Similar conductivity variations were observed by Bazinet *et al.* (2000b).

Precipitation kinetics during acidification

The percent soluble protein in the supernatant of samples collected as the skim milk was acidified was analyzed by repeated measure analysis of variance. The analysis showed

that the pH ($P<0.0001$), the double interactions pH/milk type ($P<0.0130$) and pH/acidification type ($P<0.0001$) and the triple interaction pH/milk type/acidification type ($P<0.0287$) had a significant effect on the precipitation of milk proteins. The non-linear regression sigmoidal curves produced coefficients of determination ranging from 0.953 to 0.999.

A comparison of the soluble protein evolution during pH decrease revealed a difference between the chemical and electrochemical acidification with an influence of the milk type (Figure VIII-1). From pH 6.6 to 5.2, the percent soluble protein was the same at about 100% whatever the acidification and milk type. From pH 5.2 to 5.0, the electroacidified reconstituted milk (EARM) showed the lowest soluble protein with 69.3% followed by the chemically acidified fresh milk (CAFM), the chemically acidified reconstituted milk (CARM) and the electrochemically acidified fresh milk (EAFM) with respective soluble protein of 83.8, 89.5 and 93.5%. As the acidification progressed from 5.0 to 4.8, both milk electroacidification showed similar low percent soluble protein with values of 22.6 and 21.6 respectively for EARM and EAFM, while the CARM and CAFM had different values of 81.5 and 52.7% respectively. At pH 4.6, the difference decreased and disappeared at pH 4.5 : hence, at pH 4.6, the CARM had a different soluble protein value of 30.3% in comparison with 22.6, 21.8 and 21.2% soluble protein for the other milk and acidification treatment. From pH 4.5 to 4.4, the percent soluble protein was similar whatever the acidification and milk type at 20.5 and 20.8% respectively. The acidification procedures and the milk type did not influence the final precipitation extent of protein, but the electroacidified protein precipitated at higher pH value than chemically acidified proteins. These different precipitation kinetics were confirmed by model sigmoidal curves : for chemical acidification the inflection points for RM and FM were respectively pH 4.71 and 4.83, while for the electroacidification the inflection points of the curves were at 4.99 and 4.92 respectively for RM and FM.

The difference observed between both milk, whatever the acidification procedure can be explained mainly by a difference in salt content. In fact, according to the averaged compositional analysis of both milk presented in the material part, the ash content of the

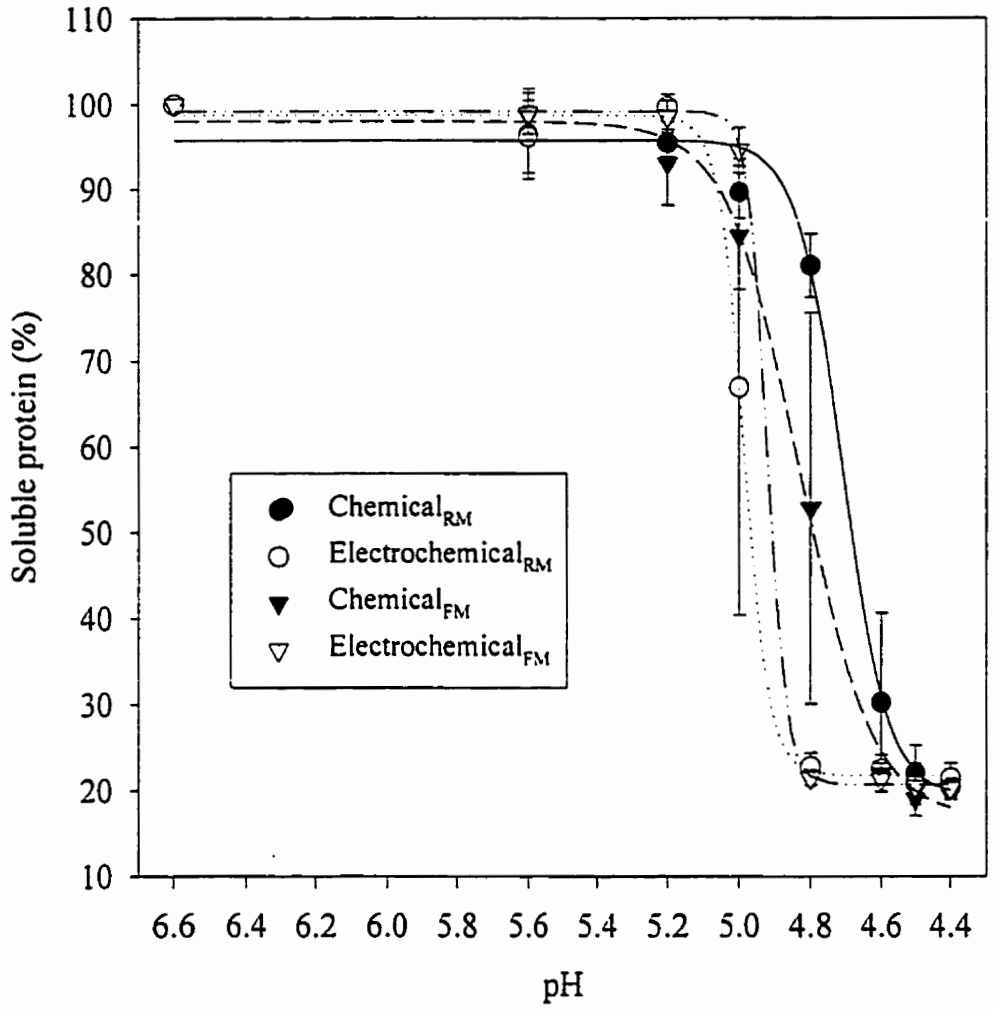

Figure VIII-1 : Evolution of soluble protein during chemical and electrochemical acidifications of reconstituted (RM) and fresh (FM) skim milk.

FM was 20.7% lower than that of RM : the lower the ash content of FM was, the lower the ionic strength and consequently the higher the electrostatic repulsion. An increase in electrostatic repulsion, by decreasing ionic strength, resulted in a loss of solubility at a higher pH value (Kinsella *et al.*, 1985; Cheftel *et al.*, 1985). In the case of RM the higher salt concentration resulted in a delay in protein precipitation by salting-in effect.
Moreover, the delay in protein precipitation observed between the chemical and electrochemical acidification should be due to a salting-in effect by addition of HCl. According to Bazinet *et al.* (2000b), in the case of HCl chemical acidification, the salts added would conduct to a salting-in effect, and consequently to a delay in precipitation, while in the case of electroacidification, the migration of salts from the protein solution by electrochemical demineralisation would favour the precipitation of proteins.

Protein profile during acidification

The percent initial peak area of each milk fractions in the supernatant of samples collected as the skim milk was acidified was analyzed by repeated measure analysis of variance. The analysis of variance showed a highly significant effect of pH on κ-casein ($P<0.0001$), α_s-casein ($P<0.0001$) and β-casein ($P<0.0001$) and of double interaction pH/acidification procedure on κ-casein ($P<0.0024$), β-casein ($P<0.0453$). No significant difference was observed for whey protein ($P>0.1478$).

For κ- and β-casein fractions, their percent initial peak area decreased as the acidification progressed, and were influenced by the acidification procedure : the precipitation of both fractions by electroacidification took place at a higher pH value (pH 4.8) than by chemical acidification (pH 4.5). For α_s-casein fraction, its percent initial peak area decreased with lowering pH, but was not influenced by the type of milk and the acidification procedure. However, a difference between the acidification procedure, comparable to both previous casein fractions, could be considered as real due to a probability level ($P>0.0961$) close to the 5% acceptance level. For whey protein fraction, its percent initial peak area was constant whatever the pH, the milk type and the acidification procedure. However, a small decrease in whey protein percentage would appeared at the

end of the acidification with RM, although the statistical analysis showed no significant difference between milk type, due to large standard deviations.

These results were in accordance with previous results obtained for the precipitation kinetics during acidification and gave more informations on each casein fraction precipitation.

Isolate composition

The analyses of variance showed no significant effect of milk type and acidification procedures on protein ($P>0.9881$), Lactose ($P>0.7865$), ash ($P>0.9772$) and sodium ($P>0.7628$), potassium ($P>0.8159$), magnesium ($P>0.2892$) and calcium concentration (0.2594) concentrations. The analyses of variance showed a significant effect of milk on percent α_s-casein ($P<0.0016$) and whey protein ($P<0.0043$) fractions, while no significant difference was observed for κ-casein ($P>0.4053$) and β-casein ($P>0.2526$) fractions.

The protein, ash and lactose content of isolates produced in the different conditions were the same with respective values of 93.0 ± 2.9, 4.11 ± 0.37 and 3.42 ± 2.59 % respectively (Table VIII-1). Mineral composition of the isolates were the same whatever the conditions in which they were produced with respective averaged concentrations of 1461 ± 197, 48 ± 21, 6 ± 6 and 112 ± 67 mg/100 g of protein for sodium, potassium, magnesium and calcium (Table VIII-1). In the isolates produced after chemical and electrochemical acidification of RM and FM, the percent of κ-casein and β-casein fractions were the same with respective values of 11.7 and 45.9% of the total isolate proteins (Table VIII-1). The percent of α_s-casein fraction of FM was higher than that of RM (42.4 vs 40.8% respectively) while the percent whey protein fraction of FM was lower than that of RM (0.35 versus 1.0% respectively). Less whey proteins were recovered in the FM and consequently the percent of α_s-casein was positively influenced. This confirm the fact that a part of whey proteins are denaturated by thermal treatment during spray-drying of skim milk for production of powder.

Table VIII-1 : Chemical composition of isolates produced by chemical and electrochemical acidifications of reconstituted and fresh skim milk.

	Fresh milk		Reconstituted milk	
	Chemical Acidification	Electrochemical Acidification	Chemical Acidification	Electrochemical Acidification
Protein (% dry weight)	92.8 ± 4.0	93.6 ± 2.0	92.7 ± 2.4	92.9 ± 4.5
Ash (g/100 g protein)	4.12 ± 0.60	4.03 ± 0.30	4.13 ± 0.24	4.17 ± 0.48
Lactose (g/100 g protein)	4.20 ± 4.63	2.08 ± 0.52	4.11 ± 2.82	3.32 ± 1.72
Sodium (mg/100 g protein)	1646 ± 539	1532 ± 231	1429 ± 65	1506 ± 330
Potassium (mg/100 g protein)	55 ± 25	41 ± 12	58 ± 28	48 ± 29
Magnesium (mg/100 g protein)	11 ± 15	3 ± 2	10 ± 7	8 ± 7
calcium (mg/100 g protein)	180 ± 195	68 ± 22	152 ± 84	147 ± 73
κ-casein (% total peak area)	11.6 ± 0.6	11.4 ± 0.6	12.3 ± 0.9	11.7 ± 0.5
α_s-casein (% total peak area)	42.5 ± 1.0	42.4 ± 0.6	41.3 ± 0.3	40.3 ± 0.3
β-casein (% total peak area)	45.5 ± 0.6	45.9 ± 0.9	45.5 ± 1.1	46.8 ± 0.6
Whey protein (% total peak area)	0.4 ± 0.3	0.3 ± 0.2	0.9 ± 0.3	1.1 ± 0.4

Isolate physicochemical and functional properties

Results of analyses of variance showed that the viscosity (P>0.7903), particle size (P>0.2643), protein load (P>0.5359), interfacial area (P>0.8636) and emulsion stability were unchanged whatever the milk type and acidification procedure (Table VIII-2). The analyses of variance showed a significant effect of milk type (P<0.0028 for foaming capacity expressed in milliter of protein solution added, P<0.0001 for foaming capacity expressed in minute and P<0.0216 for foam stability), acidification procedure (P<0.0202 for foaming capacity in mL) and double interaction milk/acidification procedure (P<0.0395 for foaming capacity in mL). The repeated measure analysis of variance indicated a significant effect of pH (P<0.0001) and double interaction pH/milk type (P<0.0001) on solubility of isolate produced as a function of pH.

a) Foaming properties

The foaming capacity expressed in milliliter is an indicator of the foam density : the higher the quantity of protein solution added was, the higher the density of the foam. The foam density of isolates from FM was lower than those from RM (8.6 vs 12.9 mL added). Compared to chemical acidification, electroacidification produced isolates with lower foam densities (9.3 vs 12.2 mL added) (Table VIII-2). However, EAFM and CARM showed the lowest and highest foam density respectively, while EARM and CAFM had similar foam density.

The foaming capacity expressed in minute is an indicator of the foam development rate : the lower the time value was, the more rapidly the foam was developped. The foam development rate of FM was lower than that of RM (4.6 vs 3.4 minutes). Compared to chemical acidification, electroacidification produced isolates with lower foam development rates (4.2 vs 3.8 minutes)(Table VIII-2).

The foam stability was influenced by the milk type with 6.5 and 4.0 mL recovered for FM and RM respectively.

Table VIII-2 : Physico-chemical and functional properties of isolates produced by chemical and electrochemical acidifications of reconstituted and fresh skim milk.

	Fresh milk		Reconstituted milk	
	Chemical Acidification	Electrochemical Acidification	Chemical Acidification	Electrochemical Acidification
Viscosity (mL/g)	57.5 ± 3.0	57.4 ± 8.2	59.1 ± 10.0	62.4 ± 2.2
Particle size (nm)	290 ± 15	293 ± 3	274 ± 8	281 ± 20
Foaming capacity (min.)	4.27 ± 0.23	4.95 ± 0.54	3.37 ± 0.12	3.43 ± 0.16
Foaming capacity (mL added)	11.3 ± 0.4	6.0 ± 3.1	13.1 ± 0.7	12.7 ± 1.2
Foam stability (mL recovered)	7.8 ± 1.1	5.2 ± 2.9	4.0 ± 0.0	4.0 ± 0.2
Protein load (mg/m^2)	24.3 ± 4.2	21.3 ± 5.6	27.1 ± 5.3	25.4 ± 3.2
Interfacial area (m^2/mL emulsion)	0.569 ± 0.017	0.600 ± 0.025	0.594 ± 0.039	0.608 ± 0.107
Emulsion stability (m^2/mL emulsion)	0.407 ± 0.017	0.341 ± 0.025	0.338 ± 0.130	0.411 ± 0.169

b) Solubility

As expected, the solubility of the isolates (all milk type and acidification procedure averaged) decreased with a decrease in pH. Moreover, the solubility profile of the isolates was influenced by the milk type (Figure VIII-2). The isolates produced from FM precipitated at a higher pH value than isolates produced from RM. These results were confirmed by similar inflection points for the model curves calculated for both milk : pH 5.10 and 5.17 for RM and FM respectively. In addition, according to the model curve there was three times more soluble protein in the pH range of insolubilisation for FM than for RM isolate : 1.490 vs 0.598 % respectively.

It appears from the isolate physicochemical and functional properties analyses that the foaming properties and the solubility as a function of pH were affected by the type of milk and the acidification procedure. The isolates from reconstituted milk had better foam stability and foam development rate, and higher foam density than fresh milk. During foam formation, it was observed that fresh milk formed bigger bubbles than reconstituted milk, which bursted more easily. These differences in functional properties between fresh and reconstituted skim milk should be due to the heat treatment by spray drying for production of powder. This is in accordance with the data in the litterature. El-Samragy *et al.* (1993a) has demonstrated the effect of heat treatment on the foaming capacity of skim milk retentate powders ; an increase in temperature decreased the foaming capacity of the protein solution at pH 7.0 and 6.7 in comparison with a non heat-treated skim milk. Moreover, they showed that the solubility index was significantly affected by the more severe heat treatment (85°C) perhaps because of protein unfolding and aggregation (El-Samragy *et al.*, 1993b). Heat treatment induces the formation of complex between β-lactoglobulin, α-lactalbumin and κ-casein which precipitates during acidification and result in higher proportion of whey protein in the isolates produced with reconstituted skim milk : the sole difference between reconstituted and fresh skim milk isolate chemical composition was whey protein content (1.0 vs 0.35% total peak area respectively) These whey proteins would change the functional properties of the final isolates. Denatured whey proteins are recognized to have a higher water binding capacity than casein (Kinsella et al., 1985) which

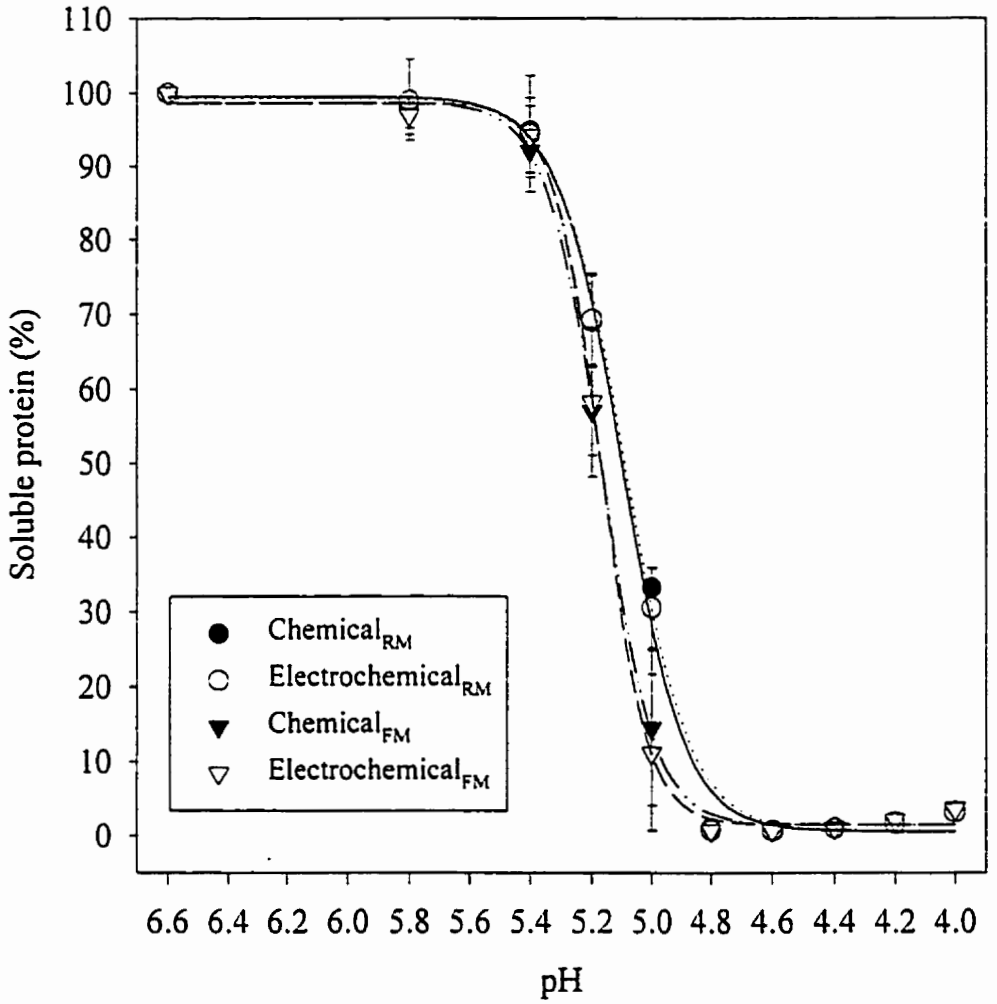

Figure VIII-2 : Solubility profiles of isolates produced by chemical and electrochemical acidifications of reconstituted (RM) and fresh (FM) skim milk.

explains the higher foam density and stability of reconstituted milk. In manufacture of co-precipitates, the process cause heat denaturation of the whey proteins (Southward and Aird, 1978). 4 to 15% of whey proteins are altered (Modler, 1985), so they precipitate with the casein at pH 4.6 but dissolve completely with the casein when alkali is added to pH 6.6-7.0 (Connolly, 1983) : as a consequence, the content of whey proteins in casein co-precipitate was about 10 to 15% (Koning *et al.*, 1970). Co-precipitates have solubility characteristics similar to caseinates, but caseinates generally give higher foam volume but produce less stable foams than whey protein concentrates. The whipping and foaming properties of soluble co-precipitates were generally slightly better than those of sodium caseinate though not as good as those of egg albumen (Southward and Goldman, 1978).

In conclusion, the isolates produced by BMEA, except on foaming capacity, showed similar physicochemical and functional properties than the isolates produced chemically. The main factor affecting the composition and the physichochemical and functional properties was the pre-treatment of milk prior to acidification (heating, spray-drying) : they have a higher impact on the functional properties than the acidification treatment.

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ATTEINTE DES OBJECTIFS ET AVANCEMENT DES CONNAISSANCES

Cette dernière partie du travail expérimental a permis de répondre à l'objectif concernant l'étude de l'effet de la nature du lait. Il a été démontré d'une part que le type de lait (lait en poudre reconstitué et lait frais non pasteurisé) a eu un effet sur les performances électrodialytiques du système, mais que cet effet était principalement lié à une différence de teneur minérale et protéique. D'autre part, le type de traitement de chaleur subi par le lait a influencé certaines propriétés fonctionnelles par la présence de protéines sériques dans les

isolats produits, tant par acidification chimique que par électroacidification. Enfin, il a été démontré que les isolats produits par ÉAMBP, excepté au niveau de la capacité moussante, avaient des propriétés fonctionnelles et physicochimiques similaires à celles des isolats produits chimiquement.

Ces résultats ont permis un avancement des connaissances sur la compréhension de l'effet de la nature du lait et sur la précipitation des protéines au cours de l'ÉAMBP. Les résultats sur les propriétés fonctionnelles et physicochimiques ont démontré que le facteur principal affectant la composition et la fonctionnalité des isolats produits était les prétraitements du lait avant électroacidification.

CHAPITRE IX

DISCUSSION GÉNÉRALE

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Suite aux résultats présentés dans ce travail, il est possible d'affirmer que :

- le débit (paramètre électrodialytique) n'influe pas sur la composition chimique des isolats et les performances du système d'électrodialyse;
- la permsélectivité des MEC (paramètre électrodialytique) n'a pas d'effet sur l'efficacité finale de l'ÉAMBP, en terme de précipitation des caséines, mais qu'elle a un impact sur les performances de la technologie en terme d'efficacité énergétique et de durée de vie des MEC, par l'encrassement interne irréversible de la membrane permsélective aux cations monovalents;
- la permsélectivité des MEC influe sur la teneur finale en cendres des isolats produits par ÉAMBP;
- les sels présents en solution et principalement le potassium contribuent à l'efficacité du système en contrebalançant pour les H^+ électrogénérés afin de conserver l'électroneutralité de la solution. Par conséquent, l'ajout de potassium dans la solution de lait écrémé apparaît comme une nécessité pour obtenir une meilleure efficacité électrique;
- la nature des sels ajoutés et de la force ionique a un impact sur l'efficacité énergétique du système soit en l'augmentant ou en la diminuant;
- la composition chimique des isolats produits par ÉAMBP avec ajout de sels est influencée par la force ionique et la nature des sels, affectant par conséquent leurs propriétés physicochimiques et fonctionnelles;
- la déminéralisation des solutions de lait avant électroacidification démontre que la fonctionnalité et la composition chimique des isolats de caséines produits par ÉAMBP sont influencées par la baisse de force ionique;
- la fonctionnalité et la composition chimique des isolats ne sont que très peu influencée par le traitement d'électroacidification en comparaison avec une acidification chimique, alors que les prétraitements tels que le chauffage aurait un impact majeur;

• enfin, les performances de l'ÉAMBP ne sont pas affectées par le type de lait traité.

Par conséquent, l'hypothèse de départ semble être confirmée : l'électroacidification avec membranes bipolaires permet de produire des isolats de caséine dont la fonctionnalité et la composition chimique dépendent des paramètres du milieu (force ionique et sels présents) et des paramètres électrodialytiques (permsélectivité des membranes cationiques et débit) qui ont également une influence sur les performances énergétiques du système d'électrodialyse et l'intégrité des membranes au cours de la séparation des caséines du lait écrémé

L'ensemble des résultats obtenus permet de dire que l'ÉAMBP est un nouveau procédé alternatif pour la production d'isolat de caséine bovine de haute pureté. De plus par l'utilisation à la base d'un système d'électrodialyse (ÉD), connu en industrie, cette technologie serait facilement transférable au niveau industriel. Une cellule d'ÉD, de par les nombreux formats disponibles à l'heure actuelle sur le marché, peut être adaptée à tous les niveaux de production et par conséquent à toutes les tailles d'entreprises. De plus, cette technologie apparaît comme une avancée technologique car elle permet la production de base très pure *in situ*, réutilisable pour la resolubilisation des caséines, en parallèle avec l'électroacidification de la solution de lait ainsi que la production de lactosérum déminéralisé, plus facilement transformable que les lactosérums acides produits par les procédés conventionnels. La génération *in situ* et la réutilisation des composés dangereux pour l'environnement (acides et bases) suppriment les inconvénients et les risques liés au transport, au stockage, à l'utilisation et à l'élimination de ces matières.

Les principales limites de l'ÉAMBP du lait sont le prix élevé de l'équipement de base et les susceptibilités de colmatage à long terme. Deux types de colmatage existent : le colmatage des cadres séparateurs et le colmatage des membranes. Le colmatage des cadres séparateurs est lié aux micelles de caséines en suspension qui s'agglomèrent entre eux pour former un réseau protéique dans le grillage des cadres séparateurs par recirculation et accumulation au sein de la cellule de cette masse de protéines insolubilisées. Ce colmatage a pour inconvénient de faire augmenter le voltage appliqué aux bornes de la cellule et de diminuer l'efficacité énergétique du système. Cependant, une centrifugation en ligne à

l'entrée et/ou à la sortie de la cellule d'électrodialyse permettrait de limiter cette formation non désirée au sein du système. De même, le fait de travailler avec du lait très acide comme dans le cas du procédé SAFIR, où un volume calculé de lait est ajouté aux caséines acides (pH de l'ordre de 1.8 à 2.4) pour atteindre un pH de 3.5, diminuerait fortement les possibilités de colmatage par le réseau protéique, inexistant à bas pH. Ainsi, une partie de cette solution caséinique très acide serait utilisée pour maintenir le pH d'un réservoir principal alors que le reste de la production serait transformé en caséines. En cours de procédé, un autre type de colmatage apparaît, beaucoup plus insidieux et pernicieux que le premier : le colmatage des membranes cationiques. La formation d'hydroxyde de calcium et de magnésium en cours d'ÉAMBP aussi bien en surface qu'à l'intérieur des MEC diminue la durée de vie de ces membranes et l'efficacité énergétique du système à court terme. Une étude effectuée sur les membranes cationiques utilisées pour les essais du chapitre VI, non inclus dans ce manuscrit laisse toutefois suggérer qu'un nettoyage régulier des membranes lors d'une procédure de nettoyage en place serait efficace pour éviter la formation de ce colmatage.

L'application industrielle de l'ÉAMBP pour la production de caséines ne semble pas être adaptée pour le moment à la production laitière québécoise et plus généralement canadienne. En effet, au Québec et au Canada la majorité du lait est transformé en lait de consommation ou en fromage. La production de caséine pour la fabrication d'isolat ne serait pas viable car il n'existe pas au Québec et au Canada d'usine de transformation du lait en isolat de caséine et parce que le prix de vente du lait sous forme de lait de consommation ou de fromage est plus élevé. Cependant, dans des pays déjà producteurs de caséines ou de caséinates comme la Nouvelle-Zélande, la France ou les Etats-Unis, les avantages de L'ÉAMBP pourraient être intéressants. À la vue des résultats, les conditions optimales de l'ÉAMBP incluraient l'ajout de KCl à une concentration de l'ordre de 0.5 M, afin d'obtenir la meilleure efficacité énergétique du procédé. Cependant, le fait de rajouter des sels ne semble pas économiquement judicieux. En effet, l'augmentation du coût énergétique de 5 à 15 cents par kilogramme d'isolat de caséine suite à une déminéralisation par électrodialyse de 75% de la solution de lait de départ serait largement compensée par la production d'un lactosérum final déminéralisé à plus de 80%. Ce lactosérum pourrait être

utilisé directement sans autre traitement pour d'autres applications comme les laits maternisés. Cela éviterait l'utilisation de procédé coûteux comme l'échange ionique ou l'osmose inverse pour une déminéralisation inférieure ou équivalente. Dans ce contexte, le couplage de l'ÉAMBP avec un électrodialyseur conventionnel deviendrait un procédé très compétitif, qui utiliserait les forces de chacunes des deux technologies et permettrait l'utilisation ou la réutilisation de chacun des fluides utilisés tout en limitant les rejets ou les sous-produits.

Ce travail a par conséquent permis de valider la méthodologie de l'électroacidification avec membranes bipolaires pour la séparation à l'échelle industrielle des protéines du lait sans ajout chimique grâce à la génération électrique contrôlée des espèces ioniques désirées.

CHAPITRE X

CONCLUSION GÉNÉRALE

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Il ressort des résultats que des isolats de haute pureté peuvent être produits par ÉAMBP et que les protéines sériques sont les seules protéines solubles restantes après électroacidification. Différents facteurs influencent les performances de la technologie en terme de cinétique de précipitation protéique et d'éfficacité énergétique: le débit, le type de lait, la permsélectivité des membranes cationiques, la nature des sels et la force ionique de la solution. Les résultats obtenus sur l'étude de la permsélectivité ont permis de comprendre la formation du colmatage des MEC et d'identifier ce précipité comme étant composé d'hydroxyde de calcium et de magnésium. Le nombre de H^+ électrogénérés au cours de l'ÉAMBP a été évalué à 60-78 méq./L de solution de protéines à 10% de solide et il a été démontré que les K^+ sont l'espèce ionique nécessaire pour limiter la migration non désirée des H^+ au travers de la MEC. Ainsi, au niveau de l'efficacité énergétique, la meilleure combinaison est l'ajout de KCl à une force ionique de 0.5 M : elle a permis une diminution de l'énergie consommée de 45% par rapport à une solution traitée sans ajout de sel. Enfin, il est ressorti de ces résultats que les isolats produits par électroacidification avec membranes bipolaires avec différents types de sels et à différents niveaux de forces ioniques ajoutées, exceptés pour les isolats produits par l'ajout de $CaCl_2$ à une force ionique de 1M et déminéralisé à 75%, montrent des propriétés physicochimiques et fonctionnelles similaires entre eux et à celles d'un isolat produit chimiquement et des isolats commerciaux.

Deux problèmes peuvent apparaître limitatifs pour l'application au niveau industriel de cette technologie : le colmatage des cadres séparateurs et le colmatage des membranes. Au cours du procédé d'ÉAMBP, le caillé protéique formé peut aboutir à un colmatage des cadres séparateurs de la cellule par recirculation et accumulation, au sein des promoteurs de turbulence des cadres séparateurs, des protéines agglomérées. Ce colmatage a pour

inconvénient de faire augmenter le voltage appliqué aux bornes de la cellule et de diminuer l'efficacité énergétique du système. En cours de procédé, un autre type de colmatage apparaît beaucoup plus insidieux et pernicieux que le premier : le colmatage des membranes cationiques. La formation d'hydroxyde de calcium et de magnésium en cours d'ÉAMBP aussi bien en surface qu'à l'intérieur des MEC diminue la durée de vie de ces membranes et l'efficacité énergétique du système à court terme.

Il existe cependant des solutions pour remédier au colmatage des cadres séparateurs et des membranes cationiques en cours d'ÉAMBP. Ainsi, une centrifugation en ligne à l'entrée et/ou à la sortie de la cellule d'électrodialyse permettrait de limiter cette formation non désirée au sein du système. De même, le fait de travailler avec du lait très acide comme dans le cas du procédé SAFIR, où un volume calculé de lait est ajouté aux caséines acides pour atteindre un pH de 3.5, diminuerait fortement les possibilités de colmatage par le réseau protéique, inexistant à bas pH. Afin de pallier la formation d'hydroxyde de calcium et de magnésium au cours de l'ÉAMBP aussi bien en surface qu'à l'intérieur des MEC, un nettoyage acide régulier des membranes lors d'une procédure de nettoyage en place serait efficace.

À la lumière des travaux, et étant donné l'intérêt potentiel de certains pays producteurs de caséines tels que la Nouvelle-Zélande, la France et les Etats-Unis, il serait intéressant d'effectuer une étude économique des coûts. En parallèle avec l'étude économique, une optimisation du procédé pourrait être effectuée en s'attardant sur la diminution de la résistance de la cellule d'ÉAMBP ou plus spécifiquement, sur l'agencement des membranes au sein de la cellule et l'épaisseur des cadres séparateurs. De plus, dans le but de compléter cette étude tant au niveau de la compréhension globale de cette technologie qu'au niveau de la mise en évidence de son potentiel en temps que procédure industrielle, il serait intéressant de travailler sur l'effet de la température, tant pour la production de co-précipités que pour la séparation éventuelle de la caséine-β. Enfin, pour parachever les travaux, une étude de la composition du lactosérum et des propriétés fonctionnelles et physicochimiques des protéines sériques s'avère importante.

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