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Separation of Casein Glycomacropeptide from Whey: Methods of Potential Industrial Application

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Abstract: Casein glycomacropeptide (GMP) is a casein derived peptide found in sweet whey. Interest in GMP has increased recently as a result of the discovered biological activity and its potential uses as a special and dietetic foods. Different methods have been developed for large scale separation of GMP from whey. These methods depend on the heat stability and solubility of GMP, its low molecular weight (8000 Dalton) compared to other whey protein, its characteristic negative charge even at low pH of 3.5 and its association dissociation behaviour at different pH values. The present review describe the different methods developed for the separation of GMP from whey grouped into four groups namely; methods based on heat precipitation of other whey proteins; methods based on membrane filtration, methods based on ion-exchange chromatography and combined ultrafiltration and ion-exchange chromatography. Out of the different approaches for the recovery of GMP from whey, the use of Ultrafiltration (UF) offer the advantage of simplicity and that other whey proteins can be recovered with the least changes in their properties. However, the retained GMP is less pure than that prepared by the more sophisticated ion exchange chromatography. Changing, the conditions of UF separation may improve the purity of the product.

Key words: Casein glycomacropeptide, ultrafiltration, whey protein, membrane filtration, ion-exchange chromatography

Introduction

Casein glycomacropeptide (GMP) is a casein derived peptide found in whey. When milk is coagulated with chymosin during cheese making, the κ-fraction is hydrolysed into two peptides. The larger peptide containing amino acid residues 1-105 is called para-κ-casein which is insoluble and becomes part of the cheese curd. The smaller peptide containing the amino acid residues 106-169 of κ-casein which is soluble and becomes part of the whey proteins.

GMP is a heterogeneous fraction containing peptides having the same peptide chain but with variable carbohydrate and phosphorus contents. The functionality and biological activity of this fraction has been attributed to its carbohydrate moiety.

Interest in GMP has increased recently as a result of the discovered biological activity and its potential uses as a special and dietetic foods (Abd El-Salam *et al.*,1996; Clare, 1998; Brody, 2000).

GMP is present in significant quantities in whey from cheeses made by rennet coagulation. It is estimated that GMP occures in sweet whey at about 1.2-1.5 g L^{-1} which constitutes between 15-25% of proteins in this whey (Regester and Smithers, 1991). Recovery of GMP from whey is receiving much attention as an ingredient for special uses and as a mean to modify the functional properties of whey protein concentrates (Veith and Reynolds, 2004).

Different methods have been developed for the preparation of GMP which uses casein or cheese whey as a source for this fraction. However, the worldwide availability of whey (IDF, 2005), makes it the preferred starting material for this purpose.

The aim of this review is to describe different methods that have been developed for large scale separation of GMP from whey.

Methods Based on Heat Precipitation of Other Whey Proteins

A process for commercial production of κ-casein glycomacropeptides from partially delectosed whey protein concentrate was described by Berrocal and Neeser (1991). WPC (75%) protein) was reconstituted in distilled water at the ratio of 10%, w/w, 0.15% $CaCl_2$ was added and pH was adjustment to 6.0. The whey protein solution was heated (90°C/20 min) and the resulting gel was broken, removed from the aqueous phase and washed with water. The aqueous phase and combined washings were then concentrated by ultrafiltration. Ethanol was then added to the retentate and pH was adjusted to 4.5 to precipitate the contaminating proteins. The filtrate was then freeze dried to yield GMP. They claimed a purity of 84%.

Neilsen and Tromholt (1994) heated a slurry (8% protein) of whey protein concentrate (Lactoprodan-80; 80% protein) at 90°C for 15 min, cooled to 50°C and the pH was adjusted to 4.5 with HCl. The filtrate was concentrated by ultrafiltration using membrane with molecular cut off value of 100,000 Dalton. The permeate was then concentrated by hyperfiltration and spray dried. They claimed that the phenylalanine content of the product was one third the phenylalanine content in the raw material which they considered satisfactory.

Methods Based on Membrane Filtration

Tanimoto ***et al.*** (1991) patented a method for the recovery of casein glycomacropeptide (GMP) based on ultrafiltration (UF) of cheese whey at variable pH values. The pH value of whey was adjusted to 3.5±0.2. They said that the control of whey pH was essential step for the recovery of GMP. At pH 4 or higher the GMP tended to aggregate and was difficult to pass the UF membrane pores. On the other hand at pH 3 or less, the sialic acid which is an integral part of the GMP became unstable decreasing the physiological effectiveness of the obtained GMP. After adjusting the pH value, whey was ultrafiltered using membranes of molecular cut-off of 20,000 Dalton. Ultrafiltration was carried out at 50°C to maximum concentration, followed by diafiltration in order to increase the recovery of GMP in permeate. The obtained permeate contained the GMP, while other whey proteins were concentrated in the retentate. The pH of permeate was then adjusted to pH higher than 4 and re-ultrafiltered using membrane of molecular cut-off of 20,000 Dalton or less. Under these conditions aggregated GMP can be recovered, concentrated, desalted and then spray dried or freeze dried. They gave several examples for the preparation of GMP from whey or whey protein concentrate as raw material. However, the reported yield of GMP was very low (about 1% of the theoretical yield) from whey compared to that from whey protein concentrate (about 25% of the theoretical yield). However, in both cases they claimed 80% purity of the obtained GMP.

Chatterton and Holst (2002) reported a modified procedure of the previous patent for the isolation of GMP from whey protein concentrate. These modifications were:

- Ultrafiltration was carried out at low temperature i.e., 15°C instead of 50°C used in the previous patent.
- The use of a starting solution with higher protein content i.e., 7.5% instead of 2% protein solution in the previous patent.

- Coating the membrane with a suspension of calcium phosphate before ultrafiltration. They claimed that this process improve the permeability of GMP.
- The use of membrane filter of molecular cut-off of 20,000 and 5,000 Dalton for the first and second step respectively. They claimed that the membrane filter was more durable than that used in the previous patent.

According to this patent, a solution of WPC in de-ionized water (7.5% protein) was adjusted to pH 3.0 and 15°C was ultrafiltered, diafiltered with cold de-ionized water using spiral wound UF module with membrane having molecular cut-off of 20,000 Dalton coated before ultrafiltration with a suspension of calcium phosphate. The pH of the permeate was then adjusted to pH 6.7 and 15°C and then ultrafiltered using a membrane with molecular cut-off of 5,000 Dalton. The retentate was then desalted by diafiltration using de-ionized water at 15°C. The concentrate was then spray dried. It had a 79.5% protein with 90% of it being GMP. However, no amino acid analysis was given for the product.

Methods Based on Combined Ultrafiltration and Ion Exchange Chromatography

Kawasaki *et al.* (1994a) described a method for the separation of GMP from whey using a combination of ion exchange chromatography and ultrafiltration. They said that cheese whey, rennet casein whey and whey protein concentrates can be used as a starting raw material. The method can be summarized in the following steps:

1. The pH of the starting raw material was adjusted according to the cation exchanger used. When an anion exchanger having a carboxy methyl groups (e.g., CM-Sepadex C-50) is used the pH valueis adjusted to 3-4.5 and when the used cation exchanger has diethyl amino groups (e.g., DEAE-Cellulose) the pH value is adjusted to 6-7. A cation exchanger having sulphone groups or quaternary amine group (Indion S2) can also be used at pH 3.0.
2. The whey was then treated with the cation exchanger either by stirring (for example 25 g CM-Sephadex C-50 swelled in water at 40 C was added to 10 L of Gouda cheese whey and stirred for 20 hours).or through column packed with the same amount of exchanger at a flow rate of 0.5 L h^{-1}. Under these conditions, whey proteins other than GMP were retained on the exchanger, while the unbound GMP was found in the filtrate.
3. The pH of the filtrate containing the GMP was adjusted to 7.0 and then concentrated by ultrafiltration at 50°C using membrane having a molecular cut-off of 20,000 Dalton and desalted by diafiltration.
4. The concentrated GMP solution is then spray dried or freeze dried.
5. They claimed a purity of 80-88% of the obtained GMP. However, the recovery of GMP was small compared to the theoretical yield.

Kawasaki *et al.* (1994b) described a method based on the preferential retention of GMP on anion exchanger, while other whey proteins remain unbound in solution. Subsequently, bound GMP was eluted from the exchanger, concentrated, desalted by ultrafiltration and diafiltration and then dried by spray or freeze drying. They gave an example for the use of this method in which 10 kg of Gouda cheese whey was adjusted to pH 3.0 using HCl. The acidified whey was then passed through a column packed with 25 g of DEAE-Sepadex A-50 at a flow rate of 0.5 L h^{-1}. The column was then washed with water and the GMP was then eluted from the column using 10 L of 1 M sodium chloride. The

collected elute was ultrafiltered at 50°C using membrane having a molecular cut-off of 20,000 Dalton, desalted by diafiltration and the concentrate was freeze dried. They claimed a purity of 87% of the obtained GMP.

Ayers *et al*. (2003) described a process for isolating glycomacropeptide low in phenylalanine content (<0.5% w/w) from whey protein concentrate (WPC). Initially a 10% solution of WPC (80% protein content) was adjusted to pH 4.75 using 10% HCl and then mixed with anion exchanger (Quaternary amino-cellulose) in a bed of water. The pH was maintained at 4.75 whilst continuing the mixing for 30 min at 10°C. The GMP depleted solution was then drained and the anion exchanger was then washed with distilled water. The GMP was then retained from the anion exchanger using 55 mM sodium chloride solution at pH 2.0. The crude GMP solution at pH 2.0 and 55 mM NaCl was passed through a column of SP (sulphopropyl-cellulose) which had been adjusted to pH 2.0 perior to setting of the column. The elute was flushed through the column with water. The GMP solution was further purified by one of the following methods:

1. Concentration of the GMP solution (adjusted to pH 7.0) by ultrafiltration (5-6 folds using membrane with molecular cut-off of 10,000 Dalton. The retentate was acidified to pH 4.0 with HCl and left to stand while some of the precipitated impurities agglomerated and removed by centrifuging. The supernatant was neutralized, dialyzed and freeze dried.
2. The GMP solution which passed the cation exchanger was made 175 mM in sodium chloride, the pH was adjusted to 8.5 and then passed through anion exchanger (QA-cellulose) which had been previously stirred in 175 mM NaCl solution adjusted to pH 8.5. Chromatograhy was carried out at 10°C and the last part of the GMP solution was flushed through the column with 175 mM NaCl. The GMP solution was adjusted to pH 6.7, ultrafiltered and diafiltered (3 times) until the conductivity of the permeate was 2 mS. A final concentration stage was carried out to increase the concentration of the retentate to a refractive index corresponding to 16° Brix. The GMP retentate was then spray dried to give a product containing 82% protein (dry basis) and phenyl alanine content of 0.24%.

Methods Based on Ion Exchange Chromatography

One of the early methods described for the preparation of GMP used a special medium called Spherosil QMA (Skudder, 1983, 1985). This medium consists of highly porous silica spheres to which a copolymer of styrene vinyl triethoxysilane carrying a strong base group of -N $(CH_3)_3Cl$- is grafted. When whey was passed through this medium at pH 5.0, the GMP was selectively adsorbed and even displaced other whey proteins initially adsorbed on the column. The adsorbed GMP can then be recovered from the medium by elution with dilute HCl or NaCl solutions. This method has been covered by a patent (Burton and Skudder, 1987). Spherosil has the advantage that it is non-biodegradable and does not swell or contract with changes in the ionic environment. However, Spherosil is expensive and the amino acid profile of GMP prepared by this method (Marshall, 1991) suggested the presence of other protein contaminants.

Outinen *et al*. (1995) developed a simple method for isolating a peptide fraction, consisting largely of GMP from emmental cheese whey. The whey was first clarified by microfiltration, its pH was adjusted to 5.0 and then passed through a polystyrene basic anion exchange resin column (V_{whey}: V_{resin} = 6.7). The GMP which was selectively adsorbed on the column was released with dilute NaCl solution, desalted, concentrated by ultrafiltration and dried. Other whey components remained

intact. About 70% of GMP originally present in whey were recovered in the prepared GMP fraction by the outlined method with a yield of 253 mg from 200 mL of clarified whey. They claimed a purity of 70-80% of the prepared GMP.

Erdman and Neuman (1999) used a polystyrene weak anion exchange resin in the alkaline form to capture GMP from acidified whey solution.

Nakano and Ozimek (1999) purified GMP from the non-dialyzable fraction of sweet whey by anion exchange chromatography on DEAE-Sephacel at pH 6.4 and 3.0. They reported that chromatography at pH 3 gave GMP fraction of high purity and yield which they considered as a simple method that can be applied for large scale production of GMP.

Nakano and Ozimek (2000) considered that dialysis of whey to be important to maximize the yield of GMP by chromatography on DEAE-Sephacel. They found that only highly sialylated GMP accounting for approximately 55% of the total sialic acid content was absorbed on the anion exchanger from non-dialyzable sweet whey.

Xu *et al*. (2000), reported that casein glycomacropeptide was selectively adsorbed from Cheddar cheese whey at pH 4.7 on a polystyrene anion exchange resin IRA93. The adsorbed material was then released with dilute NaCl solution, desalted and concentrated by ultrafiltration using Amicon YM 100 membrane. However, no figure was given for the purity of the prepared fraction.

Etzel (2001) invented a process for producing a substantially pure κ-casein macropeptide from whey. The method in based on two steps. In the first step, the GMP was recovered from whey using strong anion exchanger. The crude GMP was then purified using a metal affinity chromatography. As an example 5 L of Mozzarella cheese whey were adjusted to pH 5.0 and then passed through a water jacketed column packed with 500 mL of a quaternary amino ethyl cellulose ion exchanger (QAE). Chromatography was carried out at 40°C in upflow mode at a flow rate of 75 mL/min. The column was then washed with 160 mL water to remove contaminants and the GMP was then eluted with 1650 mL of 0.5 M sodium chloride. The elute was adjusted to pH 7.15 using 1 M sodium hydroxide. The crude GMP solution was then chromatographed on iminodiacetic acid agarose beads containing immobilized Cu sup2+ metal ion, in upflow mode at 22°C. The beads were washed with 50 mL 0.02 M sodium phosphate, 0.5 M sodium chloride pH 7.15. The effluent up to this point was the substantially purified GMP product.

Doultani *et al*. (2003) used cation exchange resin to recover Whey Protein Isolates (WPI) from sweet whey and the effluent was fed to an anion exchanger resin to recover glycomacropeptide (GMP). They reported that nearly all of the major whey proteins (α-lactalbumin, β-lactoglobulin, immunoglobulin G and serum albumin) and about half of the total Kjeldahl nitrogen were recovered by the cation exchange resin. The anion exchange resin recovered nearly all the GMP from the effluent of the cation exchanger. They considered this method as the first process to simultaneously manufacture WPI and GMP from a single stream of whey increasing the value obtained from whey.

Futrure Trends

Membrane filtration offer a simple and feasible methods for the preparation of GMP especially that these methods are used widely now by the dairy industry. However, the purity of GMP prepared by these methods need further improvement. This can be achieved by selecting more appropriate membranes and changing the filtration conditions.

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