J. Product. & Dev., 27(1): 1- 13 (2022)

PRODUCTION OF FUNCTIONAL MILK DRINK BY USING SOME VITAL MATERIALS FROM WHEY

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ABSTRACT

Cocoa drink fortified with biopeptides is made from whey (GMP), which has a high nutritional value. One of the most important results obtained was a simple and inexpensive method and the use of whey and sodium caseinate are cheap raw materials to isolate this compact(GMP).

The glycomacropeptide is rapidly absorbed, thus increasing the absorption of essential elements of the body such as iron, zinc and calcium.And the drink in which whey cheese is used as an aqueous solution contains a higher percentage of protein

***Conclusively**, It is possible to use glycomacropeptide obtained from whey as a substance that increases the nutritional value of healthy drinks or dairy products and the use of whey resulting from the manufacture of cheese, which is a by- product and is thrown into sewers and drains, despite the fact that it contains high-value nutritional compounds.*

Key words: Glycomacropeptide .cheese whey. sialic acid.

INTRODUCTION

In the middle of the twentieth century, scientists first found evidence about the presence of protein bound sialic acid in milk (György *et al.*, 1954) while working on a variant of Lactobacillus bifidus. They observed that human and cow milk contain some bifido-factor which is not destroyed or altered in its activity even by autoclaving, although the ash was found to be inactive. However, it was the pioneering work of French workers (Delfour *et al*., 1965) who for the first time established that milk contains a sialic acid bound protein called kappa-casein (κ-CN). They also reported that glycomacropeptide (GMP), a sialic acid rich peptide, is formed by the cleavage of κ-CN between Phe105-Met106 by the action of rennet (chymosin) during the manufacture of cheese. It took many more years when GMP was taken as a new product opportunity.

The GMP preparations until then were not pure enough to replace synthetic amino acid mixtures in the management of phenylketonuria and severe liver disease (Marshall, 1991). concentrations of GMP also exist in bovine milk. However, GMP released from casein is almost ten times higher than free GMP in mature milk (Furlanetti and Prata, 2003). GMP constitutes 20–25% of total proteins in whey products viz., whey powder, whey protein isolates (WPI), whey protein concentrates (WPC) *etc.*, manufactured from cheese whey (Farías *et al*., 2010). It is recognized as a bioactive peptide and is thought to be an ingredient with a potential use in functional foods, and thus great interest has been generated for its isolation. The composition of GMP is variable and depends on the particular whey source and the fractionation technology employed in its isolation (Martín–Diana *et al.*, 2006).

Earlier, attempts were mainly made to review the isolation and biological properties of GMP (Brody 2000; El-Salam *et al*., 1996; Thomä-Worringer *et al*., 2006).

Therefore, this article aims to discuss the chemical and functional properties of GMP and its role in the detection methods for checking cheese whey adulteration in milk and milk products. Some of the recent concepts used for the isolation of GMP from cheese whey are also discussed. According to Neemila *et al.* (2013).

MATEERIALS AND METHODS

Milk : Fresh buffalo Skim milk was obtained from the Sakha station. **Rennet:** It was obtained from chr, Hansen lab (Copenhagen Denmark).
Sodium caseinate : From the local market.
Sweet cheese whey: It is contain (T.S 7.08%, T.P 1.37%, Lactose 5.05% and Ash 0.53%).
Permeate: It is contain (T.S 5.35%, T.p 0.18%, Lactose 4.8% and Ash 0.47%).
S**ucrose:** It was obtained the local market .
N-acetylneuraminic acid: for the standard curve of sialic acid, was obtained from sigma, in powder form.
Cacao powder: It was obtained from the local market (T.S82.5%, T.P 8.7%, T. Carbohydrate70.4, lipids 3.4%).
pH : Value was determined using a digital pH meter (Jenway 3505 pH meter)
Total solids, Total protein and ash were determined according to AOAC (2007).
Sialic acid determination: Sialic acid was determined according to Lacomba *et al*.(2010) (Periodate- Resorcinol method).

The reagents used for the quantitative assay: 0.04 M periodic acid (H_5IO_6). 0.6 gr of resorcinol (Fisher Certified Reagent) in solution containing 60 ml of 28% HCl, 40 ml of water and 25µmoles of $CuSO_4$, 95% tert – butyl alcohol. The reagents were prepared fresh daily from stock 0.4M periodic acid and 6% resorcinol solution. The reagents were stable to storage at -19^{0}C in the dark expect the tert ‗ butyl alcohol which was maintained at room temperature.

Sialic acid determination by HPLC:

GMP was hydrolyzed with 0.1N H_2SO_4 at 80 ^{0}C for one hour .for hydrolyzates of other carbohydrate, 2N H_2SO_4 at 100 ^{0}C was used, the hydrolyzates were filtered through 0.22 mm Millipore membrane filter (water Millipore, USA) and 20ml were injected into HPLC.

***HPLC of carbohydrates*:**

HPLC waters Associates equipped with (sugar peak)
Column (300 x 605 mm) at 90 ^{0}C, Multisolvent Delivery system 600E and baseline 815chromatography, work station, 20 µl of samples were injected into HPLC. The detection was carried out using water 410 Refraction Index detector. the absorbed sugars were eluted isocratic all using the mobile phase calcium disodium EDTA and flow rate 0.5ml / min , and quantitative determination was carried and using the baseline815 data system.

Determination of amino acid profile of GMP:

The amino acid contents of GMP were determined using HPLC-Pico- Tag method described in Millipore cooperative (1987).

A GMP sample corresponding to 40mg protein and hydrolyzed with 7.5 ml of 6N HCL at110 ^{0}C for 24 hours.

The sample was made up to25ml with HPLC grade water and filtered through 0.45 mm Millipore membrane.

Sample dehydration was coined out by placing 10ml in a vial and dries up in waters Pico-Tag workstation (waters, USA) for10-15min at 250 military. Then 30 ml of a drying solution (a mixture of 200 ml methanol, 0.2 N sodium acetate acid 100ml triethylamine) was added to the sample and dried again in workstation.

The sample was then derivatized by adding 30ml of freshly prepared reagent(50ml of phenyliso thiocyante (PITCO in 350 methanol) and the reaction was carried out of 20 min and then dehydrated in the workstation for15 min .30 ml of methanol was added and the tubes were redried again and 250 ml of sample diluents (water, USA)were added
vortexed and transferred to injection vials20mlof the sample were injected in Mail pore Reversed phase C4 , amino acid column.

Preparation of cheese whey to separate GMP:

Fresh buffalo skim milk was coagulated using rennet. The rennet casein curd was separated and the whey obtained was treated by combined acid and heat treatment to separate whey protein according to EL- Gazzar (1973). And there is no effect, heat and acid on GMP. Then the supernatant which was separated by centrifugation (6000 r.p.m-30 min) was concentrated by Rotary Evaporator.

Preparation of GMP by using sodium caseinate as a raw material:

For preparing rennet – casein curd sodium caseinate was dissolved in warm water at 50 ^{0}C ,the resultant solution(4%) was cooled to 37 ^{0}C and maintained constant at that temperature then adjusting the PH to 6.4.

Rennet was added followed by hydrolyzing for 15 min .then calcium chloride and lactic acid of 88% purity was added to precipitate calcium phosphate followed by mixing well. Then centrifuged to separate the precipitate.

The supernatant obtained was adjusted to PH 4.8 and heated at 90^0 C for 10min .to in activate the enzyme , then cooled to 40^0 C and concentrated the liquid which contains GMP by Rotary Evaporator. The method described by Dosako *et al*. (1991).

Preparation of cacao drink:

Different ratio of concentrated GMP prepared from sweet cheese whey (5, 10, 15% w/v), cacao (3, 5, 7% w/v) and 2% sugar were mixed well dissolved either in sweet cheese whey or permeate and continued to 100 ml. Then heated at 80 ^{0}C for 15min and cooled in water bath to 5 ^{0}C the product was stored at 4 ^{0}C in refrigerator.

Sensory evaluation:

Samples were sensory evaluated by regular taste panel 10 staff members of dairy Department in animal production research institute. Using scoring points, 50 points for flavor, 25 points for colour and 25 points for appearance. according to Shahani *et al*. (1979).

Statistical analysis:

Data achieved were statistically analyzed by the Genial Linear Model (GLM) procedure of SAS (2001).

RESULTS AND DISCUSSION

Simple and inexpensive methods for preparing glycomacropeptide (GMP):

Many researchers and health experts suggested that glycomacropeptide

(GMP) appears to have many health benefits beyond nutrition and consider it a functional food researchers have indicated that GMP can be used as a medical foodstuff for individuals suffering from phenylketonuria (PKU). Patients with PKU cannot digest phenylalanine, an essential phenolic amino acid, due to a defect in the enzyme hydroxylase. GMP is interesting in that it is naturally low in phenylalanine and has the potential to be used as a protein source in PKU diets.

Generally the separation methods of GMP grouped in four groups, based on ion-exchange chromatography, gel filtration chromatography, ultra filtration and hydrophobic interaction chromatography.

However, these procedures are laboratory methods and not feasible for large scale manufacture, either because the materials are not food grade, the procedures are difficult to scale up and would generate much wastewater, or recovery is low (Nakano and Ozimek, 1999 and Nakano , 2000)

The worldwide availability of whey makes it the preferred starting material for preparing GMP As recovery of GMP from cheese whey is receiving much attention as an ingredient for special uses as a mean to modify the functional properties of whey protein concentrates (Veith and Reynolds, 2004). In this work two methods for preparing GMP from sweet cheese whey and also from sodium caseinat as a raw material were carried out.

a: *Using cheese whey to prepare concentrate glycomacropeptide*:

Cheese whey was a starting material to prepare GMP by combined acid and heat treatment to separate B -lactoglobulin and a-lactalbumin and they are removed by centrifugation. The supernatant obtained was considered as the GMP solution.lt was concentrated by vacuum evaporation.

Table (1) presented the composition of the GMP concentrate produced. Results showed that the total protein content ranged from 2.40 to 2.20 with an average value for- protein content in GMP concentrate of 2.30%. This may indicate that GMP prepared contained proteins other than GMP. These results agreed with those obtained by Walstra and Jennies (1984) who reported that proteose and peptone are Known to remain into solution after the removal of acid heat denatured whey proteins.

Ash content of the concentrate GMP solution reached an average of 3.61% (Max.3.80-Min.3.42).As for the carbohydrate content reached 34.11%. This seemed high and that was due to the lactose content beside other sugars.

Table (1): The composition of concentrate GMP prepared from cheese whey and from sodium casinate

Composition	GMP1			GMPII		
	Max.	Min.	Average	Max.	Min.	Average
pH	5.4	4.8	5.1±0.06 ^{aA}	4.9	4.7	4.8± 0.09 ^{bA}
T.S%	45.00	43.90	44.45±0.04 ^{aC}	23.09	21.91	22.50± 0.01 ^{bA}
T.P%	2.4	2.2	2.3±0.03 ^{aB}	4.53	4.21	4.37± 0.08 ^{bB}
T.carbohydrate%	38.01	36.21	37.11±0.05 ^{aA}	7.11	6.65	6.88±0.05 ^{bA}
Ash%	4.52	4.30	4.41± 0.01 ^{aB}	8.41	8.33	8.37±0.06 ^{bA}
Sialic acid (gr/L)	4.65	3.91	4.28±0.05 ^{aA}	4.09	4.11	4.1±0.03 ^{aB}

Each analysis was carried out in duplicate
I: GMP prepared from cheese whey
II: GMP prepared from sodium casinate

Sialic acid content of GMP

Sialic acid is a key component of both human milk oligosaccharides and neural tissues may be a conditional nutrient during periods of rapid brain growth (Bing *et al.*, 2007). GMP contains almost all the sialic acids (N-acetylneuraminic acid) in casein. Therefore, the determination of sialic acid can be taken as a measure of GMP (Warren, 1959). Also, Nakano and Ozimek (1999) reported that the determination of sialic acid is important to estimate the concentration of GMP. N-actylnuraminic acid (NANA). Its molecular weight is 309.28 gr/M ($cnH_{19}N0_{9)}$

The quantitative determination of sialic acid in dairy products was modified by introducing a periodate oxidation step prior to heating with resorcinol reagent in comparing with the other methods used which need hydrolysis and the determination was spectrophotometricaly at 630 nm. The periodate-resorcinol method was substantially more sensitive than the resorcinol procedure. It was not affected by lipids, amino acids or sugars, and was applied to detecttotal sialic acids (Locomba *et al.*, 2010**).**

The concentration of sialic acid content in the sample was calculated according to the standard curve and the equation. Y=2.34+455X

y and X represented the concentration of sialic acid (pg /0.5ml) and the absorbance at 630 nm, respectively.

Recovery of sialic acid

Recovery was carried out by adding known concentration of the standard solution of sialic acid (NANA) to the tested sample and calculated by the equation **(**AOAC, 2002)

$$R\ \% = (Cs - Cp/Ca) .100$$

Where R (%) is the percent recovery of added standard, C_s is sialic acid concentration in the sample, C_p is sialic acid concentration in the original

sample and C_a is sialic acid concentration of the standard solution of sialic acid.

The recovery was carried out every assay run to correct the results obtained. It was found that the recovery of the sialic acid added to the sample ranged in between 86-91 % with an average of 88.5 %.

Table (5) represented the average value of sialic acid content in the concentrate GMP prepared from cheese whey. It reached 4.28 gr /L.

B-using sodium caseinate as a raw material:

In case of using sodium caseinate as a raw material for preparing rennet casein curd by enzymatic milk coagulating treatment with rennet. Then removing the resulting coagulum (rennet- casein) by centrifugation. The supernatant was hence free of whey proteins and lactose. The supernatant was concentrated by Rotarotry evaporator.

It was observed that the protein content of the concentrate GMP obtained reached an average of 4.37% (Table 1). This value seemed high; it was due to the decomposition casein fragments other than K- Casein. GMP which remained in the supernatant. That agreed with Dosako *et al*. (1991) who reported that the increase in protein content in GMP returned to the decomposition of casein fragment. The average value of sialic acid content in the concentrate GMP from sodium caseinate reached 4.10 g/L, and the ash content was 8.37%.

The carbohydrate reached about 6.88%. Lieste and Knorad (1996) mentioned that the ratio of glycosylated GMP to non-glycosylated raised and that increased the carbohydrate content in GMP. Also, Kawasaki *et al*. (1992) reported that most GMP was glycosylated.

Amino acid contents of the concentrated GMP prepared from cheese whey and sodium caseinate.

Table (2) Showed the amino acid contents of the prepared GMP. Results are agreed with Yun *et al* . (1996).

As GMP is rich in acidic amino acids (Glu), low in aromatic amino acids (Tyr, Phe and in Sulfur amino acids, Meth). Glutamic acid and Threonine were present in the highest amounts followed by Isoleucine and proline, followed by Aspartic, Valine and Serine. Also, the low content of phenylala nine was observed.

Producing cacao drinks fortified with glycomacropeptide using cheese whey or permeate as aqueous solutions.

Advances in dairy technology have made it possible to supplement infant formula or some functional foods with specific bovine milk proteins fractions, that to raise its biological activity or improve absorption of nutrients (Shannon

Table(2): Amino acids content of the concentrate GMP prepared(mg/100ml)

A.A	T I (mg/100ml)	T II (mg/100ml)
Ala	152	240
Arg	72	97.9
Asp	191	201.6
Glu	304	414.1
Gly	27	131
His*	6	90.5
Isol * *	208	213.7
Lea **	60	180.2
Lys *	150	210.7
Meth*	48	150.7
Phe*	12	28.8
Pro	160	208.2
Thr*	211	39.5.7
Tyr	15	132
Ser	103	150.1
Val * *	120	194.6

T l=A.A GMP from cheese whey, TII =A.A GMP from sodium caseinate, A.A:Amino acids
*=Essential amino acids , **=Branched chaine amino acid

et al., 2003). They found that the two proteins have such biological activity were glycomacropeptide, a carbohydrate rich casein peptide, which increases absorption of calcium, iron, or zinc, and a-lactalbumin, a major protein for promoting a plasma amino acids pattern, and increases calcium and zinc absorption.

Using cheese whey and permeate as aqueous solutions:

Cheese whey is an important potential-protein source. The whey protein although small in amount, is of the highest quality and most easily digested (El-Gazzar, 1973). It is particularly valuable because it supplies the body with all essential amino acids which present in whey protein in suitable proportion

to support normal growth and production. Whey cheese is still a challenge to food technologists.

The relatively low total solids content only about 6% and low ratio between protein and lactose 0.14/70 resulted higher drying cost. Cheese whey contains other values nutrients as minerals and vitamins. For the above mentioned importance of cheese whey and whey protein we are planning to find out the suitability for using cheese whey and permeate (is ultra filtered (UF) milk), permeate which composed of water, lactose, some minerals and non protein compounds as an aqueous solution to produce cacao drink fortified with GMP prepared (section I) and that would be a valuable nutritive and functional drinks for both enfant and adult .Different treatments were carried out beside the control which contained cheese whey or permeate plus sugar and cacao powder and without GMP. Among these treatments two treatments were acceptable and gained the highest score for panel, test while the others gained lesser scores which reached 60 out of 100 degrees.

The two treatments were:

a- cheese whey cacao drink fortified with 10%concentrate GMP (w/v) +2% sucrose (w/v) +3% cacao powder(w/v).This treatment gained 90 out of 100 degrees.

b-Permeate cacao drink fortified with 15% GMP (w/v) +2% sucrose (w/v) +4% cacao powder. This treatment gained 80 out of 100 degrees.

Results in Tables (3 and 4) presented the composition of the cheese whey cacao drink fortified with GMP at rate of 10% w/v and cacao powder 3% w/v. The protein content in 100g of the drink reached about 2.87%, total carbohydrate11.01%. Total ash 0.81 %and fat content was 0.10% while in case of using permeate instead of cheese whey the protein content was 1.60%, total carbohydrate 12%, ash 0.76 and fat reached 0.12%. It was observed that the drink of cheese whey contained higher amount of protein than drink of permeate that retuned to cheese whey protein which increased the value of protein and also the ash content. Concerning the total calories in cheese whey cacao drink and permeate cacao drink were 56.42 and 55.48 calories per 100gr respectively.

Sialic acid content:

Sialic acid content for Tl and TII were 51.2 mg/100g and 29.1 mg/100 respectively. The determination was carried out by using HPLC. lt was observed that value of sialic acid in treatment(l) is more than treatment (II). That may be due to using cheese whey in treatment(l) as an aqueous solution.

Table (3): The chemical composition gr/100gr sample and calories per/100gr of the Cheese whey cacao drink.

Composition	Value
PH	6.0
Total solids %	14.88
Total Protein %	2.87
Total carbohydrate %	11.01
Total ash %	0.89
Fat %	0.10
Sialic acid mg/100g	51.2
Calories from protein	11.48
Calories from carbohydrate	44.04
calories from fat	0.90
Total calories	56.42

Average of three replicates

Table (4): The chemical composition gr/100gr sample and calories per/100gr of the permeate cacao drink.

Composition	Value
PH	6.0
Total solids %	13.49
Total protein %	1.60
Total carbohydrate %	12.00
Total ash	0.76
Total fat %	0.12
Sialic acid mg /1 OOg	29.1
Calories form protein	6.40
Calories from carbohydrates	48.00
Calories from fat	1.08
Total calories	55.46

Average of three replicates

Amino acid contents:

Table (5) presented the amino acids content of cheese whey cacao drink fortified with glycomacropeptide (10% w/v) treatment I (Tl) and permeate cacao drink fortified with (15% w/v) glycomacropeptide treatment II (T II).Data obtained showed that the highest value was for Alanine follow by Threonine, Glutamic acid, Arginine, valine, Lysine, Leucine and Isoleucine

Table (5): Amino acid contents (A.A) of cheese whey cacao drink (I) and permeate cacao drink (ll).mg/100ml

A.A	I	II
Ala	270	130
Arg	205	62
Asp	128	87
Glu	210	100
Gly	110	88
His*	140	62
Isleu * *	150	50
Leu * *	175	68
Lys *	190	82
Meth*	101	60
Phe*	103	70
Pro	105	26
Thr*	220	86
Tyr	122	78
Ser	90	60
Val * *	201	98

A.A=Amino acid , l=A.A GMP from cheese whey, II=A.A GMP from sodium caseinate
*=Essential amino acids
**=Branched chain amino acids

and the lowest value obtained was for serine (Tl). However in treatment II in which permeate was used as an aqueous solution, data obtained showed that the highest value was for Alanine, followed by Glutamic acid and the lowest was for Iso-leucine

Conclusively, it is possible to use glycomacropeptide obtained from whey as a substance that increases the nutritional value of healthy drinks or dairy products and the use of whey resulting from the manufacture of cheese, which is a by- product and is thrown into sewers and drains, despite the fact that it contains high-value nutritional compounds.

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انتاج مشروب لبني وظيفي باستخدام بعض المواد الحيوية من الشرش

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تم تصنيع مشروب كاكاو مدعم بالبيبتيدات الحيوية من الشرش (الجليكوماكروبيبتيد) والتي لها قيمة غذائيه عالية.

وكان من اهم النتائج التي تم الحصول عليها وهي التوصل الى طريقة بسيطة وغير مكلفة واستخدام فيها مواد تعزل هذا المركب من الشرش او ماده كازينات الصوديوم كمواد خام رخيصه.

وان الجليكوماكروبيبتيد سريعه الامتصاص فبذلك تزيد من امتصاص عناصر اساسيه للجسم مثل الحديد والزنك والكالسيوم . وان المشروب المستخدم فيه شرش الجبن كمحلول مائي يحتوي على نسبه اعلى من البروتين

التوصية :- انه يمكن استخدام الجليكوماكروبيبتيد التي يتم الحصول عليها من الشرش كمادة ترفع من القيمة الغذائية للمشروبات الصحية او منتجات الالبان .

واستخدام الشرش الناتج من صناعة الجبن والذي يعتبر منتج ثانوي ويلقى في البالوعات والمصارف بالرغم من احتوائه على مركبات غذائية عالية القيمة .