(RESEARCH ARTICLE)

Statistical methods and its applications in Whey protein binding (*Bos Taurus)*

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Abstract

Milk is made up of two proteins, such as casein (80%) and whey (20%). These proteins, which include membrane globular proteins, glycoproteins, and lipoproteins, are mostly found in the outer layer of milk fat globule membranes. WP can be separated from the casein in milk or formed as a byproduct of cheese making and contains all nine essential amino acids with a low lactose content. Whey protein has many health benefits, including improving strength and body composition, promoting weight loss, boosting metabolism, stifling hunger, maintaining good health, avoiding nourishing the bones, reducing inflammation, promoting quick wound healing, lowering blood pressure, repairing and preserving muscle tissues *etc*. Many dairy and food-based industries eagerly explore the accurate retention time for the formation of health related new byproducts. As of date, there are very few studies available in the world literature citation database and too many micro and macro studies on WP are currently being undertaken globally, they provide insight into the binding of particular amino acids (Seq. peptides) and their accurate lag time of binding to the formation of "helixes and sheaths. In this paradigm, statistical tool are very important to determine average time and WP algorithms for analysis of seq amino acids and the metabolic pathway's action. Overall, the present research attempts to extrapolate the Seq peptide's binding capacity and its retention time for amino acids in order to fill the aforementioned research gap. For the model demonstration we used the commonly cited whey protein peptide (Seq) NCBS, which was considered to prove the hypothesis. The Markova two state random and Monte Carlo simulation (superimposed artificial neural network) models were formulated to extract transient probabilities and retention times of Seq peptides. As per the results, the binding capacity was found to be excellent with a good retention time (5 to 11.50 minutes) in cross-linked and unlinked cases (10 to 13.50 minutes). Bioinformatics, the dairy industry, and life scientists will find these more recent discoveries to be very helpful for measuring likelihoods and transient probabilities of binding.

Keywords: MCM –montecarlo simulation model; ANN-artificial neural work; Whey Protein; Seq peptides; Protein index

1. Introduction

The *Bostaurus*milk constituents (80%) casein and 20%whey protein (WP), are a group of soluble proteins comprising approximately 20%of the protein content of cow's milk and are usually isolated from the liquid portion of milk during cheese making followed by filtration and drying. The WP fraction is made-up of several proteins, including *lactoglobulin*, *lactalbumin*, *immunoglobulins,* and *glycomacropeptides,* which have a range of varied properties. They are high quality proteins containing all the essential and non essential amino acids (nAA) and are relatively rich in the branched chain amino acids (BCAA) of *leucine*, *isoleucine,* and *valine*. A number of different classes of WP products are commercially available and include the filtrates, whey protein concentrates (WPC), protein isolates (WPI) filtered, then enzymatically digested, and WP hydrolysate (WPH). These differ in terms of nutrient profile, with WPI and WPH containing the most protein in commercially available products, typically 85–95 g of protein per 100 g of product, and the highest concentration of BCAA in commercially available WP and indeed in all milk proteins (Almeida et al., 2016) . The

consumption of whey protein is commonly associated with bodybuilding and sports nutrition, where it is used as a supplemental protein source to enhance muscle protein synthesis. Biochemically, it is embedded in an acyclic network of heavy and light chain *immunoglobulins*(*IGs*), *bovine serum albumin* (*BSA*), *lactoferin* (LF), *and lactoperoxidase glycomacropeptide* (GMP) with low molecular weights (Aaludatt et al., 2012; Abrahao et al.,2012). Given the emerging evidence suggesting a potential role of dairy proteins in improved vascular function (VF), it was examined in a number of studies to determine the short- and long-term effects on the vascular system. As per the biochemical reaction, the potent intracellular antioxidant glutathione and in one-carbon metabolism contain 3–4 times more bioavailable cysteine than other proteins (Amalfitano et al., 2019; Barac et al., 2011). Cysteine is a very important amino acid for the biosynthesis process of glutathione (tripeptide with antioxidants), anticarcinogen and immune stimulatory properties (Boye et al., 2012; Brodkorb et al., 2016). A major health benefit of WP is to develop strength and body composition, promote weight loss by boosting metabolism, suppressing appetite, maintaining a good metabolism, nourishing bones, reducing inflammation, promoting rapid healing of wounds, lowering blood pressure, reducing hunger, repairing and preserving muscle tissues, *etc.* (Brodkorb et al., 2016; Chen et al., 1998). Nowadays, the WP can rarely use degenerative diseases like cancer and various surgical operations. It acquires rehabilitative benefits like autoimmune disorders and develops immunity for immunocompromised (AIDS/HIV) patients; it can show undesirable effects that will be combated with increased intake of WP. In terms of realistic insight, it is one of the important bioavailability sources for maintaining a better gut health system (Croy et al., 1980; Corrochano et al., 2019). Currently, numerous micro and macro research studies on WP have been conducted worldwide, and they have also explored the binding of specific amino acids (peptides) and the retention time of WP binding to form ' helix and sheath. In this paradigm, very limited studies have developed algorithms (separate) and formulated the bindings of amino acid acyclic networks and their binding capacity. This driven algorithm will help the food and dairy technologist explore the whey protein by-products in the form of capsules and oral administration (neutracuticales) for the geriatric population and growing children, and we can also use it for treating patients for terminal illnesses (cancer), , HIV/AIDS, and other immunocompromised diseases. During the pandemic hit of SARS-CoV-2, too-many medical and nutritional specialists have admitted (WP) whey protein for the development of gutmicobia (immunity). As of the calendar year, numerous studies have been conducted on whey protein (WP) with amino acid binding and its usage, but in the case of human health intervention, a large number of studies will be necessary to describe the overall wellbeing of whey protein for the eradication of different diseases and ailments in humans. In this paradigm, the present study attempts to address the importance of whey protein by reviewing published articles worldwide. This basic systematic analysis review will help us to describe the most important health benefits and also know the retention time for whey protein binding with different peptide sequences. This newer research (evidence-based approach) will help the researcher to extend the basic and advanced (nutritional) translation health studies.

2. Material and methods

2.1. StudyIntervention

Table 1 Different accession data base sequences used for model demonstration

Accession number	bp (basepair)	Software	Linear DNA/miRNA
NG_028350.2; GI: 345110543	1502	FASTA	Linear DNA
AY267466.1;GI: 34398413	4,058	BLAST/FASTA	Linear DNA
GJ062448.1;GI:270038557	5,008,217	FASTA	Linear DNA
GK000016.2; GI: 257481329	81,724,687	FASTA	Linear DNA
NM 001080219.1; GI: 122692296	1,749	FASTA	Linear DNA

Source: https://www.ncbi.nlm.nih.gov/nuccore

Whey protein isolates (WPI) are the liquid by-product that can be further processed into spray dried products like whey protein concentrates (WPC) , whey protein isolates (WPI), or whey protein hydrolysate (WPH)..The whey isolates have had their base component (water) removed and are generally considered almost lactose and cholesterol free , typically containing at least (90%) protein. , were isolated by two methods: (i) ion exchange and (ii) membrane filtration. The whey protein concentration (WPC) was prepared by three various methods common to the dairy manufacturing industry and concentrated to (80%) total solids . Whey protein concentration lactic acid (WPC-L) was produced from milk using LAB cultures to convert lactose to lactic acid to lower the Ph to 4.6 in order to precipitate the casein. WPC-LR was produced using a combination of rennet and LAB .The rennet enzyme mixture is inactivated during processing, and whey protein concentration mineral acid (WPC-MA) was produced using mineral acid, sulfuric or hydrochloric , to

lower the ph (∼ 4.6) in order to precipitate the casein. The study was conducted based on the meta-analysis reports (effective population size was determined). Most of the cited *Bostaurus* protein accessions were downloaded from the NCBI virtual platform (website).

2.2. Model formulation

2.2.1. Markova random two state models of peptide sequence

The Markova (two state) random model was used to derive probabilities for the most cited peptide sequences (S_1, S_2, S_3.. S_n),, and each of the peptide sequences was assigned numerical values to form an accession number. These sets of accession numbers are called "tags" or alphabetic symbols (represented). The Markov chain model (MCM) was formulated by Monte-Karlo simulation techniques to quantify retention time and binding capacity of different WP peptides , thatwe have predicted clear sequences of binding with particular amino acids (determining the current state). The state of the sequences was taken before defining the current state of random variables (peptide sequences) . This postulate (model) does not affect the future action of hidden state variables driven by MCM. All possible state variables were coded as [1, 2,...n], numeral one indicates the lower binding of amino acids, and numeral two (coded) indicates the strong binding of AA for the formation of WP . The probability of binding of a particular AA (P = 0.20) for the formation of WP is extracted from the regression model by bootstrapping techniques (model building).

2.3. Model optimization

The MCM model was superimposed on the artificial neural network (ANN) for obtaining promising transient probabilities of retention time (peptide sequences) and the nature of the Seq peptide. However, during the process of binding WP, enormous amounts of retrieving energy were released (energy pathway) for the synthesis of different essential amino acids. Regarding the ambient pathway on WP, we need better optimisation techniques for accurate extrapolation. During the first stage of model formulation, we defined the state variables from the substitution method of MCM , the binding capacity and likelihood were efficiently determined by iteration techniques (bootstrap); and we also derived the specific amino acids in order to estimate the marginal difference between (retention time) the binding capacity of peptides. Further, we formulated a machine learning superimposed (MLS) model by Thompson iteration method for prediction of accurate retention time and labeling of various amino acids (AA) for the creation of the Seq Peptide Library (model estimation purpose). Based on the above intervention, the MLS algorithms are very essential components for the prediction of unobservable amino acids from the labeled data. Reviewed from previous literature, the researchers estimated the retention time of WP by traditional methods like stochastic gradient descent with different momentum, Ada Grad, RMS Prop, Adam Optimizer) etc. The above formulated models show various analytical gaps because they are based on simple extrapolation techniques for noisy data. In this research, we reduced the noisy data by smoothing the MLS by different iterations, , the sequence peptide data was derived closer to the original function and connected to the different kinds of probability distributions. Simultaneously, we assigned the weighted averages to each Seq peptide based on the different eclectic charges and protein indexes models were estimated by standard softwares and correlated with Protein index (defined the sequences in $'\mu'$)

$$\mu_t = \beta\mu_{t-1} + (1-\beta)S_t \quad (1.1)$$

$$\mu_{t-1} = \beta\mu_{t-2} + (1-\beta)S_{t-1} \quad (1.2)$$

$$\mu_{t-2} = \beta\mu_{t-3} + (1-\beta)S_{t-2} \quad (1.3)$$

μ = Peptide sequence, time taken for biding, β = parameter constant, S = noisy

$$\mu_t = \frac{\mu_t}{1-\beta^t} \quad (1.4)$$

The proteomics of WP has frequently been described as protein expression from gene transcription and translation (it is the broad study of proteins). This cutting-edge technology was used to identify the pathway of nucleotides and amino acid binding (AAb), as well as the diversity of AA and the effect of interaction between different biochemical pathways. These underlying biological processes (Eymoori et al., 2007; El Hiani et al., 2019; Dalsgaard et al., 2007) are very useful for the researcher to formulate products. Too many food technology industries (FTI) have used rationalised bioinformatics based on proteomic research (knowing the pathways) to formulate dairy food products by utilising different biochemical methods. So far, numerous studies have conveyed different pathways of whey protein (WP) for regulating to identify the most promising AA for the completion of biochemical processes. Many R&D institutes have developed health quotient scores for dairy products and correlate algorithms of biochemical pathways. Since the

binding of amino acids in whey protein is the essential component used to investigate homeostatic effects in humans, WP will support the study of the quality of life (QOL) of individuals to formulate nutritional studies around the world. In fact, proteomics is a more direct way to study biochemical processes than genomics and transcriptomics. Proteomics will be widely used to estimate milk components and its protein profile. The pathway of unknown amino acid binding of whey protein is described in Figure 1. All Seq peptide accession analysis was carried out by 'FASTA' software based on parent proteins and their composition. The protein index was developed as per the SOP.
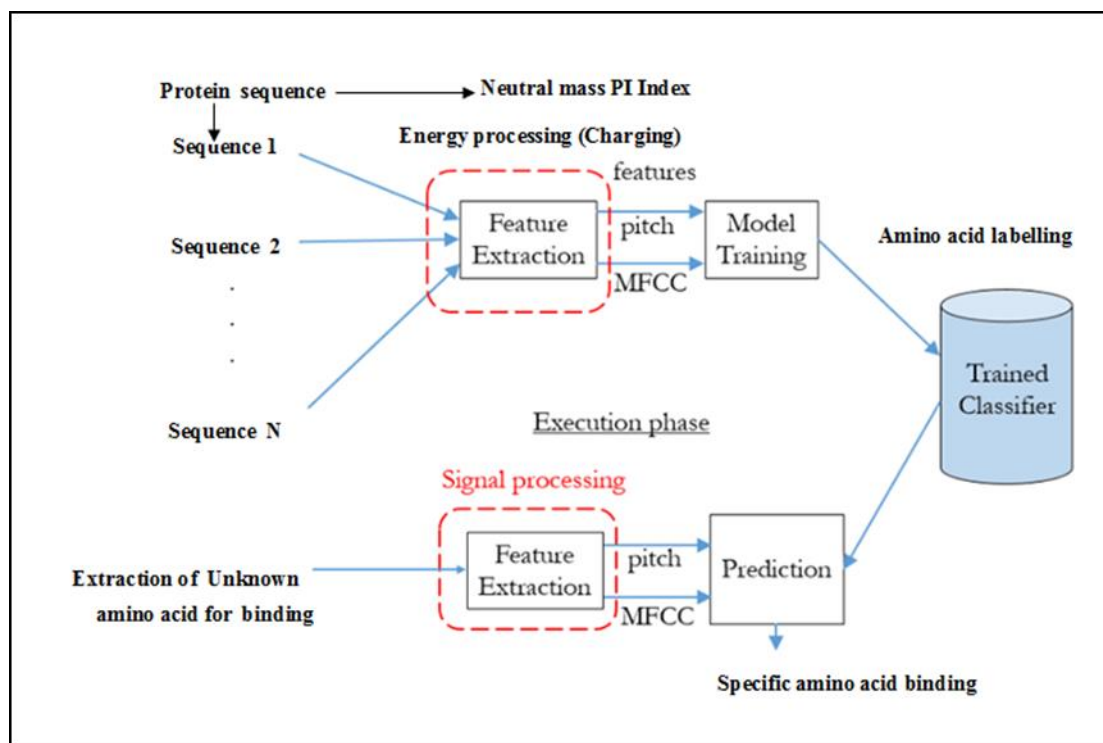

Figure 1 Flow chart for identification of unknown Amino acid binding for whey protein

We formulated stochastic matrix by using regression estimator P- value (P = 0.20) with latent p value is (1- p) = 0.80, assumed that 0.50 (50%) random state variable converges for Seq peptide completed binding.

$$P = \begin{bmatrix} \text{State} & 1 & 2 \\ 1 & 0.50 & 0.50 \\ 2 & 0.80 & 0.20 \end{bmatrix} \qquad (1.5)$$

For binding state, we solved the eqn $(1.1) X * P = X$; and $x_1 + x_2 = 1$

$$X = [x_1, x_2]P = \begin{bmatrix} 0.50 & 0.50 \\ 0.80 & 0.20 \end{bmatrix} ie => [x_1, x_2] \begin{bmatrix} 0.50 & 0.50 \\ 0.80 & 0.20 \end{bmatrix} = [x_1, x_2]$$

$$x_1(0.50) + x_2(0.80) = x_1; x_1(0.50) + x_2(0.80) = x_2$$

$$x_1 + x_2 = 1 \text{ag in we simplified}; x_1(-0.50) + x_2(0.80) = 0$$

$$x_1(0.50) + x_2(-0.80) = 0; x_1 + x_2 = 1$$

$$[x_1, x_2] = [0.615, 0.384] \text{ from stochastic matrix eqn} \qquad (1.6)$$

An initial state $I = [0, 0.20]$. The value of (P= 0.20) was determined by the MLS model (X= neutral mass of each peptide sequence and Y= PI); $\hat{Y} = 8.66 - 0.0025 X'$ P=0.20

Probability of the state after 'I' process is as follows

$$I * P = [0, 0.20] * \begin{bmatrix} 0.50 & 0.50 \\ 0.80 & 0.20 \end{bmatrix} = [16.0 , 4.0]$$

$$I * P^2 = 1 * P^1 * P = [16.0 , 4.0] * \begin{bmatrix} 0.50 & 0.50 \\ 0.80 & 0.20 \end{bmatrix} = [11.2 , 8.8]$$

$$I * P^3 = 1 * P^2 * P = [11.2 , 8.8] \begin{bmatrix} 0.50 & 0.50 \\ 0.80 & 0.20 \end{bmatrix} = [12.64 , 7.36]$$

2.3.1. Denturation of Whey protein (Kinetic analysis) Model

In order to thermal Denturation of WP , it was determined by using different following mathematical eqn (rate of change of time) $\frac{dC_t}{dt} = K_n C^n_t$ (1.7)

For $n \neq 1$, this eqnwill yields $\left(\frac{C_t}{C_o}\right)^{1-n} = 1 + (n-1)K_n C^{n-1}_0 t$ (1.8)

Where n =1 this eqn yields $l_n\left(\frac{C_t}{C_o}\right) = -K_n C_0^{n-1} t$ (1.9)

Where n = reaction order$C_0 (g, L^{-1})$ = concentration of protein before heat treatment. $C_t (g, L^{-1})$ = Concentration of native protein@ time t(s)and $K_n (g^{1-n}L^{-1}s^{-1})$ = rate constant Eqn (1.8 and1.9) were used to calculate the reaction order 'n' at different temperature of WP .The rate constant K_n wascalculated$\left(\frac{C_t}{C_o}\right)^{1-n}$ and itwas plottedagainstat thetime 't'. The temperature dependence rate with constant K_n has modelled by Arrhenius eqn (1.10)

$$l_n(K_n) = l_n(K_{n0}) - \frac{E_\alpha}{RT}$$ (1.10)

Where $K_n$frequency factor $(g^{1-n}L^{-1}s^{-1}) = E_\alpha$ = activation energy(J mol⁻¹),R = universal gas constant, T = Absolute temperature

3. Results

Table 1 Whey Protein Bio-informatics (Seq peptide sequencing)

Peptide sequence	Parent protein	Composition	Neutral mass	PI
Low bindings peptide sequences				
VIESPPEIN	*K-casein*	C (44) H(70) N (10) O (15) S (0)	996.51	3.79
VEELKPTPEGDLEIL	*β-lactoglobulin*	C (75) H (122) N (16) O (26) S (0)	1680.88	3.91
TPVVVPPFLQPEVM	*β-casein*	C (74) H(115) N(15) O (18) S(1)	1551.53	4.00
ELKPTPEGDLEIL	*β-lactoglobulin*	C(65) H(106) N(14) O (22) S(0)	1452.77	4.00
LVRTPEVDDE	*β-lactoglobulin*	C(49) H(79) N(13) O (19) S(0)	1171.57	4.16
VRTPEVDDE	*β-lactoglobulin*	C(43) H(68) N(12) O (18) S(0)	1058.48	4.16
TPVVVPPLQPE	*β-casein*	C(55) H (88) N (12) O (15) S(0)	1174.65	4.59
Mediumbinding sequences				
HHKAPGPEDSLHEQ	*Folate receptor-α*	C (67) H (98) N (22)O (22) S (0)	1580.83	5.75
DTDYKKY	*β-lactoglobulin*	C (42) H (59) N (9)O (14)S (0)	931.42	5.96
SWMHQPHQPLPPTVM	*β-casein*	C (81) H (118) N (22) O (19) S (2)	1784.84	6.66
WMHQPHQPLPPTVM	*β-casein*	C (78) H (113) N (21) O (17) S (2)	1697.81	6.92

DHKSEEDKHLKIR	*Osteopontin-k*	C (69) H (113) N (23) O (22) S (0)	1633.85	6.93*
Strongbinding sequences				
YWLAHK	*α-lactalbumin*	C (41) H (54) N (10) O (7) S (0)	816.42	8.60*
EKTKIPAVF	*β-lactoglobulin*	C (49) H (79) N (11) O (12) S (0)	1031.6	8.63*
KILDKVGIN	*α-lactalbumin*	C (45) H (80) N (12) O (12) S (0)	998.61	8.59*
KTKIPAVF	*β-lactoglobulin*	C (44) H (72) N (10) O (9) S (0)	902.55	10.0**

Despite the complete exploration of the protein sequences of whey protein , the seq peptide had a higher neutral mass index and protein index (PI) with alphabetic expression (EKTKIPAVF , parent protein *β-lactoglobulin* ,neutral mass index 1031.60 , PI 8.63 ; similarly 'KILDKVGIN' - *α-lactalbumin* neutral mass index was 998.61, and KTKIPAVF, - *β-lactoglobulin* neutral mass index 902.55, and PI was 10.0) .The present results show that , the above Seq peptide has relatively higher expression and binding capacity and is found to be excellent with retention times ranging from 5 to 11.50 minutes) in cross-link and 10 to 13.50 minutes) , unlinked. It was found to be statistically significant, with R^2 (%) = 0.88.
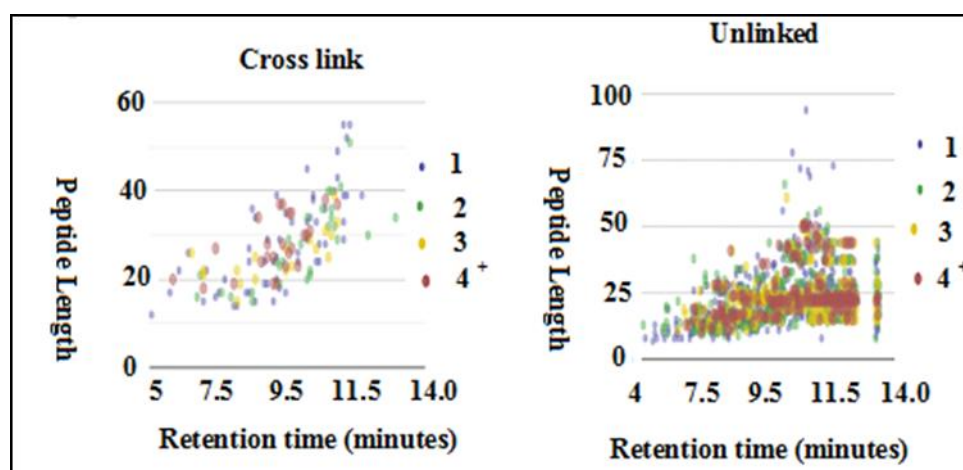

Figure 2 Scattered plot of Peptide length v/s retention time with cross and unlinked

Figure (2) shows the X axis (peptide length) and Y axis (retention time) to express the binding of peptides with different PI-charges (shows different colours 1 to 4[+]) . As per the scattered graph , all observed values (peptide length) have an ordinate between 5.0 to 11.75 minutes (retention time).The peptide length (unlinked) vs. retention time was correlated based on the machine learning superimposed (MLS) scattered plot, , the results show more retention time in unlinked Seq peptides (range between 4.0 to 13.75 minutes).In a similar concordance study conducted by (Kersey et al. (2004), he opined that , the genomic sequences of several eukaryotes , their proteomes remain relatively poorly defined. Information about proteins identified by different experimental and computational methods is stored in different databases, meaning that no single resource offers full coverage of known and predicted proteins . The IPI (International Protein Index) has addressed these issues and offers complete, non redundant data sets representing the human , mouse, and rat proteomes , built from the Swiss Prot , Tr EMBL , Ensembl, and Ref Seq data bases . (Sara Pegolo et al., 2020) Potential functional relationships among milk protein fractions in dairy cattle were formulated in a structural equation model (SEM). GWAS provide a decomposition of total SNP effects into direct effects and mediated by traits that are upstream in phenotypic networks (fitted a mixed Bayesian multi trait genomic model). This model infers the genomic correlations among 6 milk nitrogen fractions (CN), namely κ-, β-, α S_1-, and α S_2-CN, and two whey proteins, namely *β-lactoglobulin (β-LG) and α-lactalbumin (α-LA)*, in a population of 989 Italian Brown Swiss cows. The information acquired might be leveraged for the setting of optimal management and selection strategies aimed at improving milk quality and technological characteristics in dairy cattle. (Amalfitano et al., 2019). Together with whey proteins, CNs are important sources of biologically active peptides characterised by different physiological properties and nutritional implications (Silva and Malcata, 2005). Whey protein concentrate (WPC) and hydrolysate (WPH) are protein ingredients used in sports and medical formulations (Julie et al., 2016). Whey is generally considered a dietary protein supplement that can provide antimicrobial activity, immune modulation, improve muscle strength and body composition, and protect against cardiovascular disease and osteoporosis (Abraho et al.,2005)

Table 2 Whey Protein Peptide sequencing with different Charge correlation

Peptide sequence	Charge									
	+1	**+2**	**+3**	**+4**	**+5**	**+6**	**+7**	**+8**	**+9**	**+10**
HHKAPGPEDSLHEQ	1581.74	791.37	527.92	396.19	317.15	264.46	226.83	198.60	176.64	159.08
TPVVVPPFLQPEVM	1552.84	776.93	518.29	388.97	311.37	259.65	222.70	194.99	173.43	156.19
VEELKPTPEGDLEIL	1681.89	841.45	561.30	421.23	337.18	281.15	241.13	211.12	187.77	169.10
LVRTPEVDDE	1172.58	586.79	391.53	293.90	235.32	196.27	168.37	147.45	131.18	118.16
ELKPTPEGDLEIL	1453.78	727.39	485.26	364.20	291.56	243.14	208.55	182.60	162.43	146.28
DHKSEEDKHLKIR	1634.86	817.93	545.63	409.47	327.78	273.32	234.41	205.24	182.55	164.39
EKTKIPAVF	1032.61	516.81	344.87	258.91	207.33	172.94	148.38	129.96	115.63	104.17
TPVVVPPLQPE	1175.67	588.34	392.56	294.67	235.94	196.78	168.82	147.84	131.22	118.47
KILDKVGIN	999.62	500.31	333.88	250.66	200.73	167.44	143.67	125.83	111.96	100.87
VIESPPEIN	997.52	499.26	333.18	250.14	200.31	167.09	143.37	125.57	111.73	100.66
YWLAHK	817.44	409.22	273.15	205.11	164.29	137.08	117.64	103.06	91.72	82.65
KTKIPAVF	903.57	452.29	301.86	226.65	181.52	151.43	129.94	113.83	101.29	91.26
SWMHQPHQPLPPTVM	1785.86	893.43	595.96	447.22	357.98	298.48	255.99	224.11	199.32	179.49
DTDYKKY	932.44	466.72	311.48	233.86	187.29	156.25	134.07	117.44	104.50	94.15
VRTPEVDDE	1059.50	530.25	353.84	265.63	212.70	177.42	152.22	133.32	118.62	106.86
WMHQPHQPLPPTVM	1698.82	849.92	566.95	425.46	340.57	283.98	243.55	213.23	189.65	170.79

Between and within the sequences were tested by one way -ANOVA, df (9,159), $Fisher_{Cal}$ = 86.63** ,p<0.001
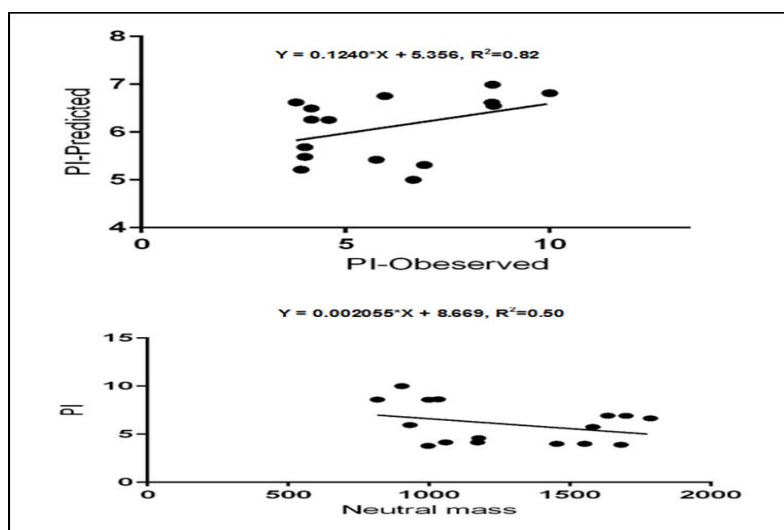
Figure 3 Scattered plot of PI observed and predicted values of Whey

The observed PI and neutral mass index were tested by regression smoothing techniques, and the results show that both the observed and predicted PI are strongly correlated with binding capacity (R^2 =0.82) .However, in the case of neutral mass with PI , binding capacity shows a very low correlation because of the non-homology of amino acids in whey (R^2 =0.50).

4. Discussion

The present study discusses the salient findings of the binding capacity of whey protein (WP) and its retention times (low, medium, medium and strong) of amino acids. This is the newer area to explore the research on whey protein (WP) to explore the bioactive linkage of peptides (BAP) that are encrypted in the acyclic structure of whey protein, including the protein index (PI) of each peptide array. All of dairy protein has derived different peptides with semi structured bonding to form food proteins, commonly referred to as bioactive peptides (BAP), *i.e.,* protein fragmentation, that have a positive influence on physiological and metabolic functions or conditions of the body and may have ultimate beneficial effects on human health . The bioactive peptide typically contains 2–20 amino acids. In our study, we extrapolated peptide sequences of whey protein, and peptide arrays like 'EKTKIPAVF' (*β-lactoglobulin*) ,'KILDKVGIN' (*α-lactalbumin*) and 'KTKIPAVF'(*β-lactoglobulin*) were found to have the highest protein index (PI) withIQR range between (7-8.63). The above peptide array shows the strong binding capacity of whey protein (WP) in *Bostaurus*milk. The retention time of bindings is 50–137.50 seconds), it there is a strong association for both cross-linked and unlinked peptides ($R^2 =$ 0.82). Many studies suggest that the diverse peptide array and potential role of peptide link and PI in enhancing good binding between hydrophobic ligands (*ie* retinol and long chain fatty acids),enzyme regulation, and the development of passive immunity in the paediatric and geriatric populations However, the bioactive peptide (BAP) will support studies of the gut (human physiology) and pathways of signal cells in the process of cell division. The present study is not intended to indicate that health benefits have been convincingly demonstrated in humans , but rather for consistency with its use in the scientific literature based on the evidence derived largely from isolated cell systems and animal studies. To continue to show that consumption of dairy foods is linked to beneficial or neutral associations with risk of hypertension, cardiovascular disease (CVD), and type-2 diabetes, an examination of the research on dairy protein derived peptides (cross- and unlinked) in relation to these three conditions of public health concern is of interest. (Pace et al., 2011) opined that , the three BCAAs leucine , isoleucine, and valine are the essential amino acids found in both whey protein and casein, with the rich source of the two being whey protein at approximately 17 g per 100g of total protein. Administration of BCAA has been demonstrated to alter cell signaling, particularly the mechanistic target of rapamycin (mTOR), which plays a key role in both nutrient sensing and cell growth and proliferation (El Hiani et al., 2019) .Circulating BCAA concentrations have been positively correlated with CVD risk, including hypertension, in a number of cohorts (Tobias et al., 2018 and Yamaguchi et al, 2017) . In addition, while BCAA intake was positively associated with hypertension in an Iranian cohort (Teymooriet al, 2017), twin studies in a UK cohort demonstrated an association between BCAA dietary intake and reduced twin risk of hypertension, reduced risk of insulin resistance, and metabolites such as salphahydroxyisovalerate, which are highly associated with diposity (Jeenings et al., 2016). (Tatiana Moro et al., 2019) Plasma BCAA and phenylalanine concentrations are the main indicators for ingestion of WPH and whey, which increased plasma valine for 60 minutes, leucine for 90 minutes , isoleucine for 60 minutes, and phenylalanine for 45 minutes post ingestion (p 0.05).Plasma amino acid concentrations did not differ between the lineages. Only valine tended to be slightly higher at 15 minutes WPH compared with after whey ingestion (p 0.001). In our study, we are not to test ingestion because of analytical limitations. WPC is the least processed and is 35–80%) protein by weight, whereas WPI is further processed to remove fat and carbohydrates to produce a product that is (90%) protein by weight (Croissant et al., 2005) WPH is enzymatically and acid-pretreated to reduce particle size and is the most digestible protein of the three variants. Whey protein typically comprises an abundance of amino acids (building blocks of protein). (Nancy Auestad et al., 2021) WP have gained extensive use for anabolic recovery, generating maximum response with the least total protein and least calories, which are important considerations for weight management, conditions of limited food intake such as illness or ageing, or post exercise recovery, including muscle building use for athletes. The importance of the *mTOR* signal and the high concentration of leucine in whey protein (WP) can only be appreciated in the context of daily protein turnover. Every day, adults need to make 250–300 g) of new protein to repair and replace existing proteins. This process occurs with continuous cycling between the synthesis and breakdown of proteins, and while skeletal muscle accounts for approximately (50%) of total body protein , it receives only about (25%) of the newly formed protein. The model-based study was done by (Carvlho et al., 2012 ;Tavareset al., (2013). The whey protein derived peptides have been proposed to have anti-inflammatory properties, and Swiss mouse models have demonstrated the ability of WPH when dosed at 300 mg/kg body weight to reduce markers of inflammation as measured by examining oedema levels and leucocyte activity.

5. Conclusion

An overall summing of the results concludes that whey proteins a commonly consumed protein irrespective of age group and a significant source of high-quality protein. The retention time and sequences of peptides for binding are essential, unobservable components for dairy specialists. In the above study, we derived the WP retention time and categorization of peptides in a sequential manner. As we noticed from the study, peptide sequences had relatively higher expression and excellent binding capacity, with retention times ranging from 5 to 11.50 minutes in cross-linked and unlinked cases.

Moreover, as WP are an especially rich source of BCAAs, including leucine, future areas of study should include the impact of this higher usage of Whey on different diseases and also clarify the effect of the risk of unlinked amino acids. The machine learning superimposed (MLS) model (used for estimation of likelihood calculation) is most robust for determining sequence peptide looping. This model is very useful for bioinformatics, dairy chemists, and life scientists for the accurate estimation of likelihoods and transient probabilities.

Compliance with ethical standards

Acknowledgments

Disclosure of conflict of interest

There is no conflict of interest between any extramural or Institutional funding agencies.

Statement of ethical approval

Only secondary Seq Peptide secondary data are used in the current investigation. There is no ethical clearance concern.

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