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PROTEINS IN SALTED WHEY

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The whey proteins are precipitated by heating salted whey to 85°C at pH 5.5 and 6.8. The product contains ; in an average, 15.89% N, 0.11% P, 0.41% Cl and 0.24% amino N.

The acid hydrolysis of whey proteins contained ; 0.39% glycine, 2.51% alinine, 2.81% valine, 26.77% leucine, 10.44% therionine, 10.03% aspartic acid, 16.55% glutamic acid, 0.92% methionine, 2.55% arginine, 7.62% lycine, 3.86% phenylalanine, 3.26% histidine, and 1.24% proline. The resin hydrolysis of the whey proteins give ; 0.19% glycine, 1.86% alanine, 2.25% valine, 17.68% leucine, 2.84% therionine, 1.51% aspartic acid, 1.91% glutamic acid, 0.85% methionine, 2.69% arginine, 0.89% lycine, 2.46% pheny lalanine, 0.93% histidine, and 1.27% proline. The resin hydrolyzates contains less amounts of amino acids than acid hydrolyzates.

Whey is considered as a waste in the manufacture of cheese. It contains, however, two important milk proteins, namely, albumin and globulin.

Attention has been paid to the preparation of whey proteins either as a heat denatured or as undenatured protein. The resulting protein precipitate is separated from the whey and washed and then either dried directly or treated before drying with alkaline salts to render the product more soluble. This research deals with the preparation of whey proteins from salted whey and analysis the product for N, P, Cl, amino acid N, and amino acid content.

Materials and Methods

Preparation of total whey proteins

Salted rennet whey was adjusted to pH 6.4 with 1N HCl, and the precipitated casein was discarded. The filtrate was adjusted to pH 5.5 with 3N NH_4OH, heated to 85°C for 1 h. and the precipitated albumin was collected. The filtrate was made alkaline, pH 6.8, with 3N NH_4OH and heated again to 85°C for 30 min to precipitate the globulins. The first and second precipitates were combined and washed thoroughly with distilled water, and dried with alcohol, and ether.

Chemical analysis

Nitrogen was determined by the micro Kjeldahl method as described by Ling (1956), phosphorus was estimated spectrophotometrically as described by Snell and Snell (1949) and chlorides were determined volumetrically

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as described by Sanders (1939). Dry weight and sah content were determined according to the method of Chibnall *et al* (1943), while the amino acid N was determined following the method of Pyne (1932).

Acid hydrolysis : the acid hydrolysis of whey proteins was carried out according to the method of Paulson et al (1953), 0.2 g of proteins were refluxed with 20 ml of 6N NCl for 20 h. The hydrolytic product was stirred with 100 mg charcoal and then filtered. The charcoal was washed several times with 0.15N NH_4OH and the combined filtrate was evaporated to dryness under vacuum, and the residue was dissolved in 100 ml of 10% isopropanol.

Resin hydrolysis : The resin hydrolysis of whey proteins was carried out according to the methods of Paulson et al (1953), 0.25 g of proteins were refluxed with 25 ml of 0.05N NCl and 0.2 g of dried resins 50 W. The hydrolytic product was treated exactly the same as in the acid hydrolysis.

Quantitative determination of amino acids by paper chromatography The ascending technique for paper chromatography was used throughout all the experiments. Two separate solvent systems were used to develop the chromatograms. Butanol : acetic acid : water (4 : 1 : 5) (solvent A), and methyl ethyl ketone : pyridine : water (7 : 1.5 : 1.5) (solvent B). The chromatograms were developed twice successively to improve the resolution of the amino acids. With solvent A and B the chromatograms were developed into two solvent systems according to the pattern recorded in table 2. The chromatograms were sprayed with 0.1% ninhydrin in butanol and 1% copper nitrate solution as described by Bode *et al.* (1952). The coloured spots were cut, put in a clean test tube and then extracted with 5 ml methanol. The optical density of the extract was then measured spectrophotometrically at 515 mu wavelength. The concentration of the amino acids was calculated from standard curves.

Quantitative determination of tryptophan and tyrosin

Determined spectrophotometrically as described by Goodwin and Morton (1946).

Quantitative determination of cystine : 0.2 g of whey proteins were weighed in a test tube, 2 ml of 57% hydroiodic acid were added and the tube was sealed and then heated at 100°C for 24 h. The hydrolyzates were transferred to a 25 ml volumetric flask and made up to volume by water. Cystine was determined in the hydrolyzate colorimetrically by the method of Kassel and Brand (1938).

Results and Discussion

1. *Analysis of whey proteins*

Results in table 1 showed that the N contents of the whhey proteins varied from 15.68 % with an average of 15.89%. The phosphosrus contents ranged from a minimum of 0.08% to a maximum of 0.13%, while the mean value was 0.11%. The maximum Cl content was found to be 0.74%

and the minimum was 0.22%, while the mean value was 0.41%. The amino acid nitrogen contents ranged from 0.16% to 0.44% with a mean value of 0.24%.

2. *The amino acids composition of whey proteins*

(*a*) *Paper chromatography of amino acids*: It was noticed that running the chromatograms of amino acids obtained by acid and resin hydrolysis of whey proteins for 18 to 20 h. in solvent systems: butanol-acetic acid-water (solvent A) and for 12 h. in methyl ethyl ketone-pyridine-water (solvent B), gave a poor separation. However, running the chromatograms of the two solvent systems for another period after drying gave better separation. Table 2, shows the amino acids patterns of whey proteins in (solvent A) and (solvent B) arranged in a descending order to their position on the chromatogram. The first spot in the amino acid pattern of whey proteins had relative movement of arginine. The second spot in solvent A had the relative movement of lysine either in the acid or resin hydrolysis. The third spot in solvent A had the relative movement of histidine in the acid and in the resin hydrolysis. The fourth spot in solvent A had the same movement of both glycine and aspartic acid in the acid hydrolysis and in the resin hydrolysis. The fifth spot in solvent A had the same movement of both glutamic and therionine in the acid hydrolysis and resin hydrolysis. The sixth, seventh and eighth spots in solvent A had the same movement of alamine, proline, and tyrosine respectivaly. Valine and methionine were found to occupy the ninth spot in solvent A in the resin hydrolysis, phenylalanine was placed in the tenth spot. Leucine was found to occupy the eleventh spot in solvent A.

As presented in table (2), the first spot in solvent B had the same movement of arginine. The second spot had the same movement of both lysine and aspartic acid. The third spot had the relative movement of glutamic acid. The fourth and fifth spot had the relative movement of histidine and glycine respectively. The sixth spot had the relative movement of both alanine and therionine in the acid hydrolysis, and resin hydrolysis. The proline was found to occupy the seventh spot in solvent (B). Valine was found in the eighth spot in solvent (B). Methionine was found to occupy the ninth spot in solvent (B). The tenth spot in solvent B had the rdlativem movement of tyrosine. The phenylalanine was found to occupy the eleventh spot in solvent (B). The twelveth spot had similar movement to leucine.

(*b*) *The amino acid contents*

1. *The mono-amino-mono-carboxylic contents of the whey proteins* The glycine content of whey proteins in acid hydrolysis ranged from 0.13 % to 0.74 % with an average of 0.39 %, table (3). The corresponding values in resin hydrolysis had a smaller range than acid hydrolysis since they varied from 0.13 to 0.29% with a mean value of 0.19%, table (4).

TABLE 1.—Standard deviations and standard errors of TN, amino acid N, P and Cl contents of whey proteins.

	Min	Max	Mean	S.D.	S.E.
T.N.	15.68	16.08	15.89	0.15	0.05
Amino acid N . . .	0.16	0.44	0.24	0.11	0.04
P	0.08	0.13	0.11	0.02	0.01
Cl	0.22	0.44	0.41	0.20	0.07

TABLE 2.—The amino acids pattern of whey proteins arranged in an increasing order to their mobility on an ascending filter paper chromatogram.

No.	Solvent (A)	Solvent (B)
1	Arginine	Arginine.
2	Lysine	Lysine-Aspartic.
3	Histidine	Glutamic.
4	Glycine-Aspartic	Histidine.
5	Glutamic-Therionine	Glycine.
6	Alanine	Alanine-Therionine.
7	Proline	Proline.
8	Tyrosine	Valine.
9	Valine-Methionine	Methionine.
10	Phenylalanine	Tyrosine.
11	Leucine	Phenylalanine.
12	—	Leucine.

The alanine content of whey proteins in acid hydrolysis ranged from 2.25 to 3.17% with a mean value of 2.51% table (3) On the other hand, the alanine contents of whey proteins in resin hydrolysis were less than that in acid hydrolysis. The values ranged from 0.11 to 2.52%, and thus gave a lower mean of 1.86%, table (4).

TABLE 3.—MAXIMUM, MINIMUM, MEAN, STANDARD DEVIATIONS AND STANDARD ERRORS OF AMINO ACID CONTENT OF WHEY PROTEINS (ACID HYDROLYSIS).

Amino acids	Min	Max	Mean	S.D.	S.E.
I. *Mono-Amino Mono-Carboxylic Acid*					
Glycine	0.13	0.74	0.39	0.205	0.07
Alanine	2.25	3.17	2.51	0.338	0.12
Valine	2.03	3.47	2.81	0.525	0.19
Leucine	23.16	30.56	26.77	3.095	1.09
II. *Hydroxy-Amino Acid*					
Therionine	7.97	12.30	10.44	1.560	0.55
III. *Dicarboxylic Acid*					
Aspartic	11.93	9.56	10.03	0.758	0.27
Glutamic	13.99	22.02	16.55	2.715	0.96
IV. *Sulphur Containing Amin Acid*					
Cysteine-Cystine	1.69	8.64	5.87	2.677	0.95
Methionine	0.43	1.71	0.92	0.386	0.13
V. *Basic Amino Acid*					
Arginine	2.15	3.17	2.55	0.579	0.20
Lycine	7.14	8.31	7.62	0.424	0.15
VI. *Aromatic Heterocyclic*					
Tryptophane	0.86	3.17	2.30	—	—
VII. *Aromatic Amino Acid*					
Phenyl Alanine	2.27	4.50	3.86	0.754	0.27
Tyrosine	1.24	5.21	3.62	—	—
VIII. *Hetero Cyclic Acid*					
Histidine	1.79	4.77	3.26	0.585	0.30
Proline	0.48	2.11	1.24	0.371	0.13

The alanine content of whey proteins in acid hydrolysis ranged from 2.25 to 3.17% with a mean value of 2.51%table (3). On the other hand the alanine contents of whey proteins in resin hydrolysis were less than that in acid hydrolysis. The values ranged trom 0.11 to 2.52%, and thus gave a lower mean of 1.86%, table (4).

TABLE 4.—MAXIMUM, MINIMUM, MEAN, STANDARD DEVIATIONS AND STANDARD ERRORS OF AMINO ACID CONTENT OF WHEY PROTEINS (RESIN HYDROLYSIS).

Amino acids	Min	Max	Mean	S.D.	S.E.
I. *Mono-Amino Mono-Carboxylic Acid*					
Glycine	0.13	0.29	0.19	0.05	0.02
Alanine	0.11	2.52	1.86	1.05	0.37
Valine	1.62	2.94	2.25	0.39	0.14
Leucine	10.51	22.22	17.68	3.91	0.14
II. *Hydroxy-Amino Acid*					
Therionine	2.00	3.30	2.84	0.50	0.18
III. *Dicarboxylic Acid*					
Aspartic	0.18	3.18	1.51	1.16	0.41
Glutamic	0.45	4.91	1.91	1.53	0.54
IV. *Sulphur Containing Amino-Acid*					
Cysteine-Cystine	—	—	—	—	—
Methionine	0.40	1.54	0.85	0.36	0.13
V. *Basic Amino Acid*					
Arginine	2.43	3.02	2.69	0.25	0.09
Lycine	0.46	1.34	0.89	0.29	0.11
VI. *Aromatic Heterocyclic*					
Tryptophane	—	—	—	—	—
VII. *Aromatic Amino Acid*					
Phenyl Alanine	1.29	3.16	2.46	0.67	0.24
Tyrosine	—	—	—	—	—
VIII. *Heterocyclic Acid*					
Histidine	0.37	1.80	0.93	0.59	0.21
Proline	0.48	2.41	1.27	0.66	0.23

The valine content of whey proteins in acid hydrolysis ranged from 2.03 to 3.47%with a mean value of 2.81%, table (3). Resin hydrolysis however, showed lower mean value of 2.25% with a range of 1.62 to 2.94%, table (4).

Hihger values that found in both methods for valine were reported by Joshi and Raj (1954). They found that it ranged from 5.37 to 5.40% for whey proteins of buffalo milk.

The leucine contents of the whey proteins in acid hydrolysis ranged from 23.16 to 30.56% with a mean value of 26.77%, table (3) while in the resin hydrolysis the range was less, being from 10.5 to 22.22% with a coresponding lower mean value of 17.68%, table (4).

Lower values for leucine however, were found by joshi and Raj (1954), namely 9.44 to 9.55% for whey protein of buffalo milk.

II.—*The hydroxy-amino acid contents of the whey proteins*

The mean values of therionine content of whey proteins in acid hydrolysis was higher than that in resin hydrolysis, being 10.44, and 2.84%, table 3 and 4 respectively. Accordingly, its values ranged from 7.97 to 12.3% in the acid hydrolysis and from 2.00 to 3.30% in the resin hyrdrolysis.

Joshi and Raj (1954), reported that the therionin content of whey protein of buffalo milk ranged from 4.62 to 4.67%, this finding was lower than the therionine content in the resin hydrolysis found in this study.

III.—*Dicarborylic acids contents of the whey proteins*

The mean value of aspartic acid in acid hydrolysis wss 10.03%, table (3) which was higher than that found in resin hydrolysis, being 1.51%. It ranged from 11.93 to 9.56% in the acid hydrolysis which was high in range than resin hydrolysis, being 0.18 to 3.18%, table (4).

The mean value of glutamic acid in hydrolysis was 16.55%, table (3) which was higher than that found in resin hydrolysis, being 1.91%. It ranged from 13.99 to 22.02% in the acid hydrolysis which was high in range than resin hydrolysis, being 0.45 to 4.91%, table (4).

IV.—*Sulphur-contatining amino-acid contents of the whey proteins*

The acid hydrolysis of proteins is known to cause destruction of cystine, according separate hydrolysis with hydroiodic acid was used for the determination of cystine. Block and Balling (1944), had showed that the hydroiodic acid hydrolyzate gave the most reliable results for cystine in proteins. The cystine-cysteins content was ranged from 1.69 to 8.63% with a mean value of 5.87%, table (3).

The methionine content of whey proteins in acid hydrolysis ranged from 0.43 to 1.71%, table (3) with a mean value of 0.92%. In resin hydrolysis to the methionine content had nearly the same values as in acid hydrolysis since it ranged from 0.40 to 1.54% with a mean value of 0.85%, table (4). Joshi and Raj (1954), however, found higher values of methionine content of the total whey protein of buffalo milk, namely 2.77, and 2.82%.

V.—*Basic amino acid contents of the whey proteins*

The arginine contents of the whey proteins in th acid hydrolysis ranged from 2.15 to 3.17% with mean value of 2.55%, table (3). In resin hydrolysis the arginine contents had nearly the same values as in acid hydrolysis since it

ranged from 2.43 to 3.02% with a mean value of 2.69%, table (4). There values were close to that reported by Joshi and Raj (1954), for total whey proteins of buffalo milk, being 2.43 to 3.02%.

The mean value of lycine in acid hydrolysis was 7.62%, table (3) which was higher than that found in resin hydrolysis, being 0.89%, table (4),. It ranged from 7.14 to 8.31% in the acid hydrolysis which was higher in range than resin hydrolysis, bing 0.46 to 1.34%. Joshi and Raj (1954), reported that the lycine contents of the total whey proteins of buffalo milk ranged from 7.01 to 7.071%, nearly the same as in acid hydrolysis.

VI.—*The aromatic heterocyclic acid contents of the whey proteins*

The tryptophan contents of the whey proteins determined spectrophotometrically in the protein as such, after dissolving it in alkaline. The tryptophan contents ranged from 0.86 to 3.17% with a mean value of 2.3%, table (3). These values were higher than that reportd by Joshi and Rag (1954), for the total whey proteins of buffalo milk, bing 1.36 to 1.42%.

VII.—*The aromatic amino acid contents of the whey proteins*

The tyrosine content of the whey proteins determined spectrophotometrically in the protein as such, after dissolving it in 0.1N NaOH using ultraviolet absorption. The tyrosine contents ranged from 1.24% to 5.21 % with a mean value of 3.62%, table (3).

The mean value of phynylalaine in acid hydrolysis was 3.86%, table (3) which was higher than that found in resin hydrolysis, being 2.46%, table (4). It ranged from 2.27 to 4.50% in the acid hydrolysis which was higher in range than resin hydrolysis, being 1.29 to 3.16%. Joshi and Raj (1954), reported higher values than found in both methods for phenylalaine. They found that it ranged from 4.56 lto 4.57% for total whey proteins of buffalo milk.

VIII.—*Heterocyclic acid contents of the total whey proteins*

The histidine contents of the whey proteins in acid hydrolysis ranged from 1.79 to 4.77% with a mean value of 3.26%, table (3), while in the resin hydrolysis the range was less, being 0.37 to 1.80% with a correspondingly lower mean value of 0.93%, table (4). Joshi and Raj (1954), reported that the contents of whey proteins of buffalo milk ranged from 1.99 to 2.02%, this finding was lower than the hidstindine contents in acid hydrolysis and higher than the histidine contents in the resin hydrolysis found in this text.

The protein contents of the whey proteins in acid hydrolysis ranged from 0.48 to 2.11% with a mean value of 1.24%, table (3). In resin hydrolysis the proline contents had nearly the same values as in acid hydrolysis since it ranged from 0.48 to 2.41% with a mean value of 1.27%, table (4).

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بروتينات الشرش الملح

دكتور ابراهيم الدسوقى رفعت ـ محمد عبد الجليل خورشيد
دكتور عبد الحميد الخوفى ـ دكتور محمد الحسينى عبد السلام

الملخص

رسبت بروتينات الشرش الملح على درجتين pH ٦٫٨ ، pH ٥٫٥ باستعمال درجة حرارة ٨٥°م وكان الناتج فى المتوسط يحتوي على ١٥٫٨٩٪ نيتروجين ١١٫٠٪ فوسفور ٠٫٤١٪ كلوريد ٢٤٪ نتروجين أحماض أمينية .

هذا وقد أعطى التحليل الحمضى لبروتينات الشرش النسب الآتية من الأحماض الأمينية وهى ٣٩٫٠٪ جليسين ، ٢٫٥١٪ الانين ، ٢٫٨١٪ فالين ، ٢٦٫٧٧٪ ليوسين ، ١٠٫٤٤٪ ثريونين ، ١٠٫٠٣٪ حامض اسبارتك ، ١٦٫٥٥٪ حامض جلوتامك ، ٩٢٪ مثيونين ٢٫٥٥٪ ارجنتين ٧٫٦٢ ليسين ، ٣٫٨٦٪ فينيل الانين و ٣٫٢٦٪ هيستيدين ١٫٢٤٪ برولين .

وعموما كانت البروتينات المتحللة بواسطة المبادلات الايونية تحتوى على نسب أقل من الاحماض الامينية .

أما تحليل البروتينات بواسطة استعمال المبادلات الايونية اعطت النسب الآتية : من الأحماض الامينية ١٫١٩٪ جليسين ، ١٫٨٦٪ الانين ٢٫٥٢٪ فالين ، ١٧٫٦٨٪ ليوسين ٢٫٨٤٪ ثريونين ، ١٫٥١٪ حامض اسبارتك ، ١٫٩١٪ حامض جلوتامك ، ٠٫٨٥٪ مثيونين ، ٢٫٦٩٪ أرجنين ، ٠٫٨٩٪ ليسين ، ٢٫٤٦٪ فنيل الانين ، ٠٫٩٣٪ هيستيدين ، ١٫٢٧٪ برولين .

وعموما كانت البروتينات المتحللة بواسطة المبادلات الايونية تحتوى على نسب أقل من الأحماض الامينية .

* معمل الصناعات الغذائية والألبان ـ المركز القومى للبحوث بالدقى ج.ع.م.