foamed better at low pH and relatively high solids concentration.

CONCLUSION

The physical–chemical properties of whey protein preparations appear to have large effects upon their foaming properties. Kinds and amounts of carbohydrates, salts, and lipids affect both foam volume and stability. The effect of pH appears extremely complex and dependent upon several other variables. If the food system permits, pH manipulation may improve foaming. Unless lipid contents are very low, foaming properties are markedly improved by a mild denaturation heat treatment.

Future work needs to be done to elucidate further the complex relationships between the variables affecting foaming. Until this is done, the foaming capacity of whey proteins intended for a food system can only be evaluated in a system which closely simulates it.

Whey proteins clearly possess different foaming properties than egg albumen. Their capacity for foam formation has been adequately demonstrated. The challenges now are to utilize them for their unique properties and to utilize them at their maximum potential.

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Received for review May 23, 1978. Accepted September 22, 1978. Presented at the 174th National Meeting of the American Chemical Society, Division of Agricultural and Food Chemistry, Chicago, IL, Aug 1977.

Use of Whey Proteins for Supplementing Tortilla

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Although the average Mexican diet represents adequate protein intake, additional proteins, such as those obtained from whey sources, would be highly desirable. A simple technique, applicable to small industries, is proposed, consisting of coagulating by heat at pH 4.5, siphoning off the lactose supernatant, decanting into suspended sacks, and drying in a forced air oven at 40–50 °C. The protein concentrate obtained had 54.7% protein, 25.6% fat, 9.9% moisture, 1.3% ash, and 8.7% nonnitrogenous material. The protein efficiency ratio (PER) was 3.28. Enriched tortilla (65% corn protein + 35% whey protein) had a PER of 2.16 in comparison to 2.11 for casein and was well accepted according to panel tests.

International statistics point out that Mexico has a daily protein availability per capita of some 68 g of total protein, of which 18 g are animal protein (Narayana, 1973). However, Mexico is an unevenly developed country, with deficient communications, so that an average figure does not reflect reality. National statistics (Ramirez et al., 1971) distinguish four main types of nutrition areas: good, medium, bad, and very bad. Whereas the first two types show acceptable figures, the last two areas have daily per capita intakes of 1895–2124 kcal, 50–56 g of total protein, and 8–10 g of animal protein, respectively.

Animal protein production is deficient in Mexico. Cheese production, for example, is only about 77 000 metric tons per year (Anuario Estadistico, 1976). Statistical figures for whey production are not available, but, assuming that 8 kg of whey is produced per kilogram of cheese, we estimate a yearly production of some 600 000 tons of whey. Estimating a yield of about 2 kg of crude protein for each ton of liquid whey, the above figure means some 1200 tons of high-quality protein.

There is little information about the use of whey in Mexico. The big cheese plants dry the whey, but it appears that in rural areas whey is generally fed directly to domestic animals; discharge into rivers also seems to be common.

The object of this work is to utilize whey protein recovered under Mexican conditions for human consumption. The process for protein recovery should fulfill the following criteria: (a) the process must be a very simple one, applicable to small-scale industry and to unskilled labor; (b) the recovered protein should be used for enriching a traditional basic food.

Protein recovery may be performed by a heat coagulation process of sweet whey. This protein could be added to tortilla, which is a staple food in the diet of rural populations in Mexico.

EXPERIMENTAL SECTION

We used as raw material cheddar cheese whey of pH 6.0–6.5 and applied a heat coagulation technique at pH 4.5. The scheme of the process is shown in Figure 1. The pH was adjusted by acidifying approximately one-fourth

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0021-8561/79/1427-0668$01.00/0

Table I. Main Composition of Raw Material and Heat Coagulated Whey Protein Concentrates

	moisture, %	protein (N × 6.38)	fat, g/100 g	nonnitrogenous material (by difference)	ash, %
cheddar cheese whey	92.0	1.20	0.73	5.57	0.58
whey protein concentrate separation					
centrifuging	6.9	61.90	31.80	0	0.98
filtering	5.6	54.30	34.30	4.83	0.88
siphoning	9.9	54.70	25.57	8.70	1.30

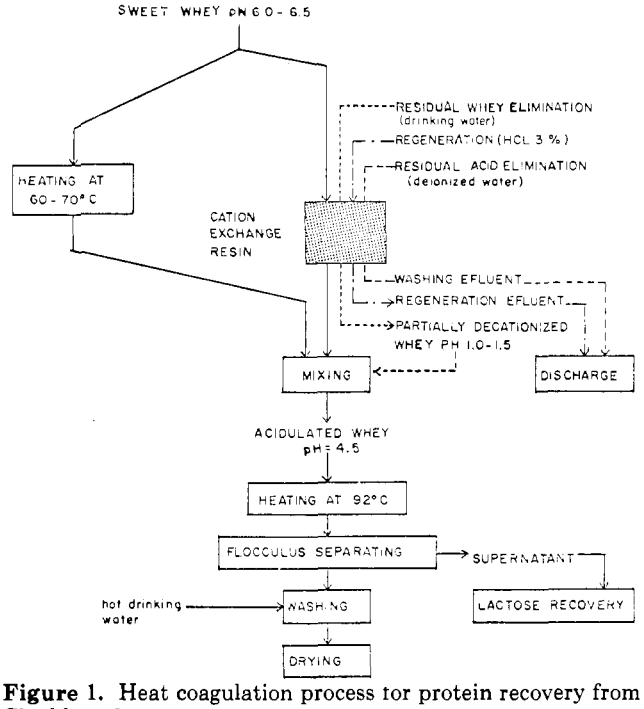

Figure 1. Heat coagulation process for protein recovery from Cheddar whey.

to one-fifth of the whey by means of a strong cation-exchange resin (Dowex 50 × 8, 20–50 mesh) in the hydrogen cycle. The remainder of the whey was heated to 60–70 °C. After adding enough of the acidified whey to obtain a pH of 4.5, the total mixture was heated to 90 °C. The protein precipitate was allowed to stand about 20 min and, depending on the whey volume, was separated by one of the following three methods: (1) for 2–3 L, centrifuging at 3500 rpm for 15 min; (2) for 50 L, filtering with a filter press (Columbia, Model 8) at 4 atm using filter paper sheets S 934; (3) for 300 L, siphoning off the supernatant whey and allowing the flocculus to drain in sacks hung up overnight. In all three cases, the precipitate was dried in a forced air oven at 40–50 °C.

Analytical Methods. Moisture, fat, protein, and ash were determined in the protein concentrates according to "Official Methods of Analysis" (AOAC, 1970). Nonnitrogenous material, considered to be lactose, was determined by difference. Amino acids were analyzed by ion-exchange chromatography utilizing a Technicon NC II P automatic amino acid analyzer in the Applied Biochemistry Laboratory of the University of Nancy (Racotta et al., 1978). The analysis of tryptophan was performed by the Ehrlich method with *p*-aminobenzaldehyde, as described by Spies and Chambers (1949). The available lysine was determined colorimetrically with 2-chloro-3,5-dinitropyridine (Tsai et al., 1972).

Protein efficiency ratio (PER) was determined with weanling male albino rats individually caged and housed in a temperature-controlled room. Synthetic diets were prepared (10% protein) and fed to three groups of eight rats each for 28 days. One diet contained casein as the reference protein, the second contained the whey protein concentrate that was separated by siphoning, and the third contained a mixture of 65% corn meal protein + 35% whey protein. The last two diets were prepared in the form of tortillas. The tortillas were prepared in the usual manner (Bressani et al., 1958).

Organoleptic Tests. For organoleptic evaluation, the same whey protein concentrate was incorporated into tortilla "masa" in two proportions: 65 or 75% corn meal and 35 or 25% whey protein, respectively.

Several blind triangle tests were performed comparing these enriched tortillas with common tortillas as reference; the judges were separated from one another while they evaluated the samples.

The judges scored the samples in the following way: I like it very much; I like it; I do not like it or dislike it; I dislike it; I dislike it very much. These qualitative statements were translated into the numerical scores 10, 7.5, 5, 2.5, and 0, respectively. There were 128 evaluations involving 13 persons of different degrees of education. Statistical evaluations for significance were carried out by the Kruskall–Wallis test.

RESULTS AND DISCUSSION

Process for Protein Recovery. We chose a type of sweet whey for our experiments because the protein yield is better (Racotta et al., 1972; Racotta, 1976) and we wished to recover lactose from the supernatant (Figure 1).

The cation-exchange resin was used for two reasons: first, the isoelectric point could be attained without the addition of external acid, and, second, the ash content of the final product would be reduced from the original 7% (dry basis) to about 1% (dry basis) (Table I). If practical considerations do not permit the use of an ion-exchange resin, the protein can be recovered by simple heat coagulation. The yield in this case was about 45%, instead of the 55% attained by heat coagulation at pH 4.5 (Racotta, 1976). This last figure is in agreement with the yield obtained by other workers under similar conditions (Jelen et al., 1973).

The protein and fat content of the final products were not influenced very much by the separation method used (Table I). The separation was intended to be as rapid as possible, in order to eliminate lactose from the protein coagulum and hence to prevent the Maillard reaction during drying. Filtering and siphoning, which are both time consuming, did not succeed in eliminating the lactose.

Amino Acid Composition. Aminograms of the raw material and of the three types of whey protein concentrates showed that in all concentrates the amino acid content was quite similar (Table II). The whey protein concentrates were all richer in cystine and tryptophan; the leucine content was also somewhat increased. The other essential amino acids were present in adequate amounts, especially lysine. However, lysine was only 50% available in the case of the material obtained by filtering. When the available lysine content is compared to the carbohydrate content of the whey protein concentrates (Table I), only the centrifuged concentrate had no nonnitrogenous

Table II. Comparative Aminograms of Cheddar Cheese Whey and Various Heat Coagulated Whey Protein Concentrates (g/100 g of protein)

amino acid	cheddar cheese whey	whey protein concentrates[a] 1	2	3
aspartic acid	9.7	11.2	11.7	10.2
threonine	6.5	5.4	4.6	5.2
serine	4.9	4.0	4.2	4.6
glutamic acid	17.2	16.9	17.3	15.9
proline	5.3	4.7	5.6	4.5
glycine	2.1	2.0	2.2	2.2
alanine	4.8	5.2	5.2	5.1
cystine	0.6	1.5	1.1	2.2
valine	6.6	6.2	6.1	7.0
methionine	2.2	2.6	2.0	2.2
isoleucine	6.1	5.1	5.2	5.5
leucine	10.3	11.6	11.8	12.8
tyrosine	2.6	3.0	3.1	3.1
phenylalanine	3.8	3.4	3.4	3.6
lysine	10.3	10.4	9.3	8.9
histidine	2.0	2.1	2.1	1.8
arginine	2.6	2.9	2.9	3.2
tryptophan[b]	1.6	2.1	2.5	2.6
available lysine[b]	6.75	7.43	3.90	8.86

[a] 1, centrifuging; 2, filtering; 3, siphoning. [b] Determined by colorimetric method.

material present. The low content of available lysine in the filtered concentrate may be due to acceleration of the Maillard reaction caused by the 4 atm pressure used during preparation. This phenomenon will be the object of further studies.

The whey protein concentrate that was separated by siphoning was chosen for further experiments because technology for its production was applicable under conditions of small-scale industry and unskilled labor, and the available lysine content was good. A comparison among the essential amino acid contents of tortilla, whey protein concentrate, whey-supplemented tortilla, and the WHO/FAO pattern showed that supplementation with whey protein concentrate raised the level of essential amino acids to equal or better those of the WHO/FAO pattern in all cases except lysine (Table III). In the two supplemented tortillas, 65 and 75% of the final protein was supplied by corn, the rest of the proteins being supplied by whey protein. In terms of ingredient proportions, this corresponded to 88 and 59 g of whey protein concentrate per kilogram of final mixture, respectively. The 65:35 mixture was designed in an attempt to match the WHO/FAO scoring pattern. The 75:25 mixture was only studied as an alternative for the 65:35 mixture, in case the flavor of the whey should be perceived by the judges when the samples were tasted.

Examination of the chemical scores of supplemented tortillas showed that our whey protein concentrate contained enough tryptophan to counteract the tryptophan deficiency in tortilla. In the case of lysine, however, the chemical score could be raised to only 88 and 75, respectively. The ideal supplementation proved to be 35% whey protein to 65% corn meal protein, and, therefore, the biological test was performed on this mixture.

Nutritional Value. The supplementation of corn protein with 35% whey protein succeeded in doubling the PER value of tortillas and raised its value to equal that of casein (Table V). It is important to note that this striking effect is due to an addition in terms of ingredients, of only 88 g of whey protein concentrate per kilogram of final mixture, and seems not to be affected by the relatively low chemical score of lysine (Table IV).

Literature values for PER and net protein utilization (NPU) of whey protein concentrates prepared by different means were compared to values we obtained in our studies (Table VI). The NPU value for our filtered whey protein concentrate was determined in a previous work (Racotta, 1976). From the figures of Sinnamon (1975), and those of the present work, it can be seen that the PER values of heat coagulated concentrates were lower (3.05 and 3.28) than in the case of ultrafiltered (4.01) and gel filtered (4.29) concentrates. Nevertheless, the protein quality in all cases was superior to that of casein. The NPU values were similar for all the whey protein concentrates except for the roller-dried sample. Ultrafiltration and gel filtration were the best methods for keeping intact the functional properties of the whey proteins, such as foaming, water absorption, etc. (Morr, 1976). However, under Mexico's conditions, a simple process should be used, so the product isolated by the heat coagulation method, which was adequate technically, was also satisfactory nutritionally.

Literature values for the PER's of several cereal products supplemented with whey protein were compared to results we obtained with out supplemented tortilla (Table VII). The supplementations are shown in terms of ingredients. The increases in PER seem to be more striking than in our work. However, it should be remembered that our whey protein concentrate was obtained by thermocoagulation and hence, had a slightly lower PER value (Table VI). Sinnamon (1975) also used heat coagulated whey proteins, but his figure referred to pure whey protein and not to the total concentrate. In our concentrate, only 54.7% corresponded to pure protein (Table I), so we can conclude that our results show good agreement with the literature data.

Organoleptic Assay. The results of the sensory evaluations showed that there was no significant difference between flavor scores of the two kinds of tortilla compared to scores of the reference tortilla (Table VIII).

Table III. Essential Amino Acids Levels in Tortilla, Whey Protein Concentrate, and Supplemented Tortilla (g/100 g of protein)

amino acid	WHO/FAO scoring pattern (1973)	tortilla[a]	whey protein concentrate (siphoning)	supplemented tortilla 65:35	75:25
isoleucine	4.0	4.1	5.5	4.6	4.5
leucine	7.0	13.2	12.8	13.1	13.1
lysine	5.5	2.5	8.8[b]	4.7	4.1
phenylalanine + tyrosine	6.0	8.1	6.7	7.6	7.7
methionine + cystine	3.5	4.0	4.4	4.1	4.1
threonine	4.0	4.1	5.2	4.5	4.4
tryptophan	1.0	0.6	2.6	1.3	1.1
valine	5.0	5.3	7.0	5.9	5.7

[a] Taken from Cravioto and Cervantes (1965). [b] Given as available lysine.

Table IV. Chemical Score of Amino Acids in Tortilla, Whey Protein Concentrate, and Supplemented Tortilla

amino acid	tortilla	whey prot. concentrate[a]	suppl. tortilla 65:35	suppl. tortilla 75:25
Ile	102	137	115	113
Leu	189	183	187	187
Lys	45	161	88	75
Phe + Tyr	134	112	126	128
Met + Cys_2	115	125	117	117
Thr	102	130	112	110
Trp	60	260	130	110
Val	106	140	118	114

[a] Siphoning.

Table V. Protein Efficiency Ratio of Casein, Whey Protein, Tortilla, and Supplemented Tortilla

protein source	PER, x + SD	PER, % of casein PER
casein	2.11 ± 0.43[a]	100
whey protein concentrate	3.28 ± 0.23[b]	155
tortilla	1.20[c]	57
35% whey prot. suppl. tortilla	2.16 ± 0.37	102

[a] P = 0.000 001 with respect to casein. [b] P = 0.000 002 with respect to casein. [c] Taken from Cravioto and Cervantes (1965).

Table VI. Comparison between Literature and Personal Data for Protein Efficiency Ratio and Net Protein Utilization

product	PER	NPU
whey proteins, gel filtration[a]	4.01 ± 0.29	94.0 ± 2.2
whey proteins, ultrafiltration[a]	4.29 ± 0.20	84.8 ± 7.1
whey proteins, ultrafiltration[b]	2.98	
whey proteins, heat coagulation[c]	3.05	
whey proteins, roller dried[d]		78
whey proteins, olivet atomization[d]		94
whey proteins, heat coagulation	3.28 ± 0.23[e]	95.2 ± 0.7[f]

[a] Taken from Forsum (1974). [b] Taken from McDonough et al. (1976). [c] Taken from Sinnamon (1975). [d] Taken from Kunachowicz et al. (1974). [e] Present work. [f] Taken from Racotta (1976).

Table VII. Protein Efficiency Ratio of Some Vegetable Proteins Supplemented with Whey Proteins

product	PER	author
common macaroni	0.70	Sinnamon (1975)
macaroni + 7% WP[a]	2.41	
wheat	0.5	Forsum et al. (1973)
wheat + 8% WPC[b]	2.5	
maize	1.2	Forsum (1974)
maize + 6% WPC	4.11	
soy	1.5	
soy + whey (9:1)	2.0	Hutton (1975)
soy + whey (3:1)	2.9	
soy + whey (1:1)	3.0	
commercial white bread	0.75	Bates et al. (1974)
bread + 32 b. wt. whey	1.04	
wheat flour	0.45	Abrahamson et al. (1974)
88% wheat + 12% WPC	3.4	
tortilla	1.20	present work
tortilla + 8.8% WPC	2.16	

[a] WP = whey proteins. [b] WPC = whey protein concentrate.

It can be concluded from the data presented here that the enrichment of tortilla protein with 35% heat coagulated whey protein had a beneficial nutritional effect and did not cause any unacceptable flavor change.

Table VIII. Taste Panel Test Results[a]

judge no.	common tortilla	median values from 0 to 10: enriched tortilla with 35%	enriched tortilla with 25%
1	7.50	7.50	7.50
2	7.50	7.50	3.75
3	6.25	6.25	5.00
4	7.50	2.50	
5	5.00	7.50	7.50
6	5.00	5.00	7.50
7	6.87	7.50	3.75
8	5.00	5.00	7.50
9	5.00	7.50	5.00
10	7.50	5.00	2.50
11	7.50	6.25	7.50
12	7.50	7.50	6.25
13	7.50	7.50	7.50
median	7.50	7.50	6.90

[a] Kruskall-Wallis test ("rank variance analysis"): H = 1.006, P = 0.6.

ACKNOWLEDGMENT

The authors acknowledge the Physiology Department of the Biological Sciences School for supplying the animals and allowing the use of the temperature controlled chamber. We want also to thank Radu Racotta for his valuable advice. The whey was kindly furnished by the Kraft Food S.A. Co. from Mexico.

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Received for review May 23, 1978. Accepted March 6, 1979. Presented at the 174th National Meeting of the American Chemical Society, Division of Agricultural and Food Chemistry, Chicago, IL, Aug 1977. V.R. is a fellow of DEDICT-COFAA.

Lactose Chemistry

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The importance of lactose in the overall problem of whey utilization and disposal has led to greater interest in all aspects of lactose chemistry. Three general areas of research are discussed—complexing with metal ions, adsorption of volatile compounds, and development of efficient crystallization processes. Anhydrous forms of lactose have been shown to adsorb a large variety of volatile compounds. Within a homologous series, the amount adsorbed increased with increase in chain length. Heats of adsorption and structure of volatile compounds were used to explain mode of adsorption by different forms of lactose. CaO can be used to precipitate sucrose from molasses (Steffen process) and has been shown to remove lactose from whey, lactose being more readily complexed than sucrose under similar conditions. By proper control of the reaction, over 90% of the lactose can be recovered as an insoluble calcium–lactose complex. Lactose was shown to combine with many cations in a one-to-one ratio, but no complexing could be demonstrated with K^+ and NH_4^+. With the commercialization of reverse osmosis and ultrafiltration processes, the feasibility of using the permeate for crystallization of lactose is being investigated, but calcium phosphate is a problem. Attempts are being made to improve the lactose recovery process.

Lactose, the carbohydrate of milk, is except for milkfat produced by a few breeds, the major constituent in milk. It normally forms over 50% of the solids in the skimmilk portion and an even greater portion of the solids in whey. Thus, in whey utilization and disposal we are concerned largely with lactose utilization and disposal, which accounts for much of the increased interest in lactose in recent years. The other major reason for increased interest in lactose is the problem of lactose intolerance (Rosensweig, 1975).

The importance of lactose in the overall problem of whey utilization and disposal has led to greater interest in all aspects of lactose chemistry. Developments in three general areas of research are reviewed in this paper: adsorption of volatiles on lactose, complexing of metal ions with lactose, and crystallization processes for recovery of lactose.

ADSORPTION OF FLAVORS

Lactose has served as an extender for spices and volatile aromas (Reger, 1958). At one time it was used in the manufacture of instant coffee to adsorb volatiles during roasting and drying. When this lactose was incorporated into the coffee powder the flavor was improved. Some other food uses of lactose are based on its ability to accentuate flavors in conjunction with its low sweetness level. However, there has been a lack of experimental evidence to illustrate this point.

In an attempt to measure flavor adsorption quantitatively, Nickerson and Dolby (1971) measured adsorption of diacetyl by lactose and other selected sugars. They found that the form (type) of lactose had a marked influence on its adsorption capacity. Regular α-hydrate powder, for example, adsorbed much less diacetyl than did the anhydrous forms. Converting the hydrate to the anhydrous forms of α- or to β-lactose increased the quantity of diacetyl adsorbed under a standard set of conditions.

Lee et al. (1975) investigated adsorption by stable anhydrous α-lactose of three homologous series of normal aliphatic alcohols, methyl esters, and methyl ketones. The amount adsorbed varied greatly, but was related linearly with the increase in chain length within a homologous series. Adsorbed in greatest quantity were the alcohols, followed by the methyl esters and, then the methyl ketones (Figure 1). Adsorption was from a flowing gas stream at 25 °C with nitrogen as the carrier gas. As the boiling point within a homologous series increased, the amount adsorbed also increased (Figure 2).

McMullin et al. (1975) expanded the number of organic compounds to include aldehydes and hydrocarbons as well as alcohols, esters, and ketones, and measured the heats of adsorption when these compounds were adsorbed by stable anhydrous α-lactose. The heats of adsorption were calculated from the retention times measured at five different temperatures. A plot of ln t_{corr} against reciprocal absolute temperature yields a straight line, and the heat of adsorption can be calculated from the slope of the line (Gale and Beebe, 1964). Heats of adsorption on lactose ranged from about 6 to 18 kcal/mol for the compounds studied (Figure 3). The functional group of a molecule was important in determining the heat of adsorption, with alcohols having considerably higher heats than the other

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0021-8561/79/1427-0672$01.00/0